EVIDENCE-BASED ADVANCE AND MANAGEMENT OF ADVERSE EVENTS OF IMMUNOTHERAPY FOR CANCER

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REVIEW
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Advances in Targeted Therapy and Immunotherapy for Non-small Cell Lung Cancer Based on Accurate Molecular Typing

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The essence of precision medicine is to achieve the goal of "individualized treatment" through genotyping of patients and targeted therapy. At present, the pathogenic genes of non-small cell lung cancer (NSCLC) have been studied most thoroughly and targeted therapy based on genotyping has been the most successful. This paper focuses on the precision treatment of NSCLC based on genotyping, comparing gene detection methods and summarize the latest progress of NSCLC immunotherapy.

Keywords: NSCLC, targeted therapy, immunotherapy, EGFR, PD-L1, ALK, ROS1

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INTRODUCTION

Fundamentally, cancer is a genetic disease caused by gene variations or epigenetic alterations. However, even the same tumor can be caused by different genetic variations, which is the heterogeneity of the tumor. Even patients with the same pathological type of tumor may respond differently to the treatment because of heterogeneity. Precision medicine is an individualized medical model based on the rapid development of genome sequencing technology and the vigorous rise of biological information and big data science. According to the different molecular types of patients, different treatment regimens are the most reliable methods to improve response and reduce adverse reactions. With the continuous progress of biological detection technology, the cost of human gene molecular typing is becoming lower while the accuracy is getting higher, and individualized treatment is gradually becoming reality.

In recent years, the incidence and mortality of lung cancer have shown a sharp rise in the world. What's more, lung cancer is the most common cause of cancer death worldwide, with 1.38 million people dying every year, accounting for 18.2% of the total cancer deaths (Jemal et al., 2011). It is also the cancer with the highest morbidity and mortality in China, approximately 781,000 new cases and 626,000 deaths had been reported in 2014 (Chen et al., 2014). Based on the data from the Global cancer statistics 2018, it shows that among the males, incidence rate of the NSCLC is 223.0 per 100,000 and mortality rate is 166.6 per 100,000. Besides, in the female, the incidence rate is 182.6 per 100,000 (Bray et al., 2018). Non-small cell lung cancer (NSCLC) accounts for about 80–85% of lung cancer, and its clinical manifestations are complex and diverse. There are many risks associated with surgical management of advanced NSCLC (Zhang et al., 2017a), drug therapy for advanced NSCLC is safer than surgical treatment (Nie et al., 2012). Patients with the same pathological type of NSCLC may have different responses to the same anticancer drug. At present, the pathogenic genes of NSCLC have been studied most thoroughly and targeted therapy based on genotyping has been the most successful. The purpose of this article is to review the accurate

treatment of advanced NSCLC based on genotyping, including targeted therapy, immunotherapy, and comparison of several common detection methods.

OVERVIEW OF TARGETED THERAPY OF NSCLC

Chemotherapy was the most important treatment for stage III and IV NSCLC patients until targeted therapy was well developed. The generally acknowledged third-generation new chemotherapy drugs combined with platinum regimen have an overall effect resulted in a significant improvement in survival (HR, 0.79; 95% CI, 0.62–1.00; $p = 0.05$) and a 5 years survival improvement of 11% (67% with chemotherapy vs. 56% with observation) (Nagasaka and Gadgeel, 2018), however, the median survival period is only 8–10 months (Li and Liu, 2018). Meanwhile, chemotherapy drugs cannot differentiate tumor cells and normal cells while working, the treatment related adverse reactions are dramatically strong therefore being feared by patients.

It was not until the emergence of targeted therapy based on molecular typing that the survival period of patients with advanced NSCLC was improved to several years, such as the second generation ALK-TKI alectinib (Alecensa) achieved the PFS of first-line NSCLC patients with ALK fusion up to 34.8 months (Peters et al., 2017), and the adverse reactions were greatly reduced, such as the adverse events of grade 3 or higher was lower with the third generation EGFR-TKI osimertinib (Tagrisso, 23%) than with platinum-pemetrexed (47%) (Mok et al., 2017; Peters et al., 2017).

The discovery of NSCLC targeted therapy is an event of necessity in contingency. At the end of 2003, researchers from Dana-Farber and Massachusetts general hospital in the United States simultaneously found high remission rates in some NSCLC patients using tyrosine kinase inhibitors (TKIs), and these patients' high remission rates were confirmed to be the result of EGFR gene mutation (Kris et al., 2003). The first drug, bevacizumab, was approved by the FDA in 2004 for the treatment of advanced colorectal cancer (Herbst et al., 2018). By 2009, the first large randomized controlled study, IPASS, demonstrated that gefitinib significantly prolongs PFS in lung cancer patients with EGFR mutations related to carboplatin-paclitaxel (hazard ratio for progression or death, 0.48; 95% CI, 0.36–0.64; $P < 0.001$) (Mok et al., 2009). An important advance in the management of advanced stage NSCLC occurred in 2015, when the US FDA approved the ICB nivolumab for the treatment of patients whose disease progressed during or after platinum-based therapy, heralding a new era in the management of lung cancer (Herbst et al., 2018). Since then, a series of genes related to the pathogenesis and treatment of NSCLC have been discovered, and a variety of targeted drugs and detection methods have been developed, changing the patterns of advanced NSCLC treatment thoroughly. The latest NSCLC guideline, 2019 v3, published by national comprehensive cancer network (NCCN) suggests that 9 genes related to targeted therapy should be detected, including EGFR, KRAS, HER2, ALK, ROS1, MET, BRAF, RET, and NTRK.

Here we use the timeline to show the development of targeted therapies and immunotherapies for the treatment of NSCLC over two decades **Figure 1** (Herbst et al., 2018).

EGFR

As the first therapeutic target discovered, EGFR has been the most thoroughly studied and the most successful. Based on recent studies, EGFR is the most common driving gene in NSCLC in Asia-Pacific and Russian, with an incidence of 49.3% (Han et al., 2017). Mutation types mainly include single nucleotide variation (SNV), insertion, deletion and copy number variation (CNV). The variations were mostly concentrated in exons 18–21, and the responses of exon 19 and 21 to EGFR-tyrosine kinase inhibitor (EGFR-TKI) were generally better than exons 18 and 20. The most common sensitive mutations are the deletion of amino acids at 747–750 of exon 19 (19Del) and the L858R mutation of exon 21, so the use of first-generation EGFR-TKI, namely gefitinib (Iresa), erlotinib (Trockai) and ecotinib (Kemet sodium), can be considered. Afatinib (Giotrif), the second generation of EGFR-TKI, is an irreversible inhibitor with two targets, EGFR and HER2. It is especially applicable for patients with EGFR-TKI resistance caused by HER2 mutation. Afatinib is effective for certain types of rare EGFR mutations and has been approved by FDA for use with rare EGFR mutations: G719X, L861Q, and S768I (Yang et al., 2015).

Drug resistance is almost inevitable after 8–14 months of the first or second-generation EGFR-TKI treatment (Maemondo et al., 2010; Mitsudomi et al., 2010; Tan et al., 2016). The reasons for drug resistance are varied. The mutation of T790M of EGFR exon 20 is the most common cause of drug resistance, accounting for about 50–60% (Kobayashi et al., 2005; Oxnard et al., 2011; Sequist et al., 2011; Yu et al., 2013). In addition, downstream KRAS, BRAF and other activation mutations, HER2 mutation, MET amplification lead to bypass activation, PTEN lost, and transformation to small-cell lung cancer (SCLC), which are also the mechanisms of acquired drug resistance.

If T790M mutation is detected, we can switch to the third-generation EGFR-TKI osimertinib. Studies have shown that after the first or second -generation EGFR-TKI resistance caused by T790M mutation, the survival period of 7.6m can still be obtained by using osimertinib. AURA3 studies in NSCLC patients with EGFR-T790M mutations showed significantly longer PFS with osimertinib compared with permetrexine (Mok et al., 2017). The FLAURA study showed that regardless of whether T790M mutations were detected, the PFS of the first-line treatment group with osimertinib reached 18.9 months, while the median PFS of the first-line standard treatment of the first-generation EGFR-TKI was only 10.2 months, and patients with osimertinib had a high safety (Soria et al., 2018). Thus, first-line use of osimertinib may have a longer FPS than switching to osimertinib after the resistance of first-generation EGFR-TKI.

Taking targeted drug osimertinib as an example, in NSCLC patients with epidermal growth factor receptor (EGFR) T790M mutation, the median duration of progression-free survival (PFS) was significantly longer with osimertinib than with platinum-pemetrexed (10.1 vs. 4.4 months; HR: 0.30; 95% CI, 0.23–0.41;

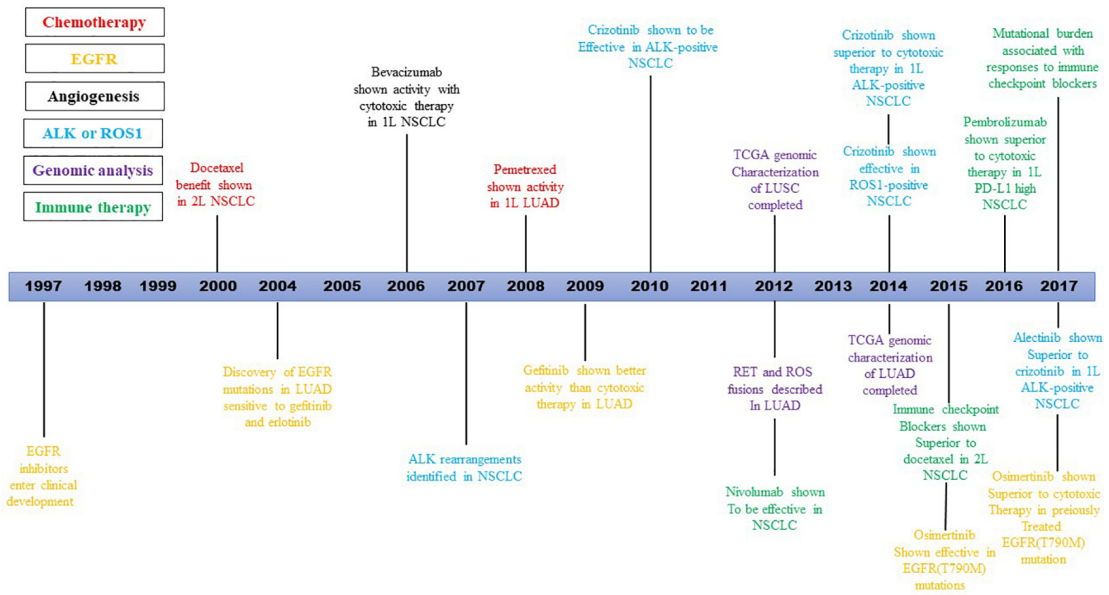

FIGURE 1 | Timeline illustrating the development of targeted therapies and immunotherapies for the treatment of NSCLC over two decades.

$P < 0.001$); the objective response rate (ORR) were 71% vs. 31% (odds ratio, 5.39; 95% CI, 3.47–8.48; $P < 0.001$) (Mok et al., 2017).

Unfortunately, even if the initial treatment is very effective, patients still have to face drug resistance after months of using osimertinib. The mutation of C797S of EGFR exon 20 is the most common cause of acquired drug-resistance of osimertinib. Geoffrey and his team found out that non-invasive genotyping of cell-free plasma DNA (cfDNA) is a useful biomarker for prediction of outcome from osimertinib (Oxnard et al., 2016). If C797S and T790M are on different chromosomes (trans-configuration), it will still be sensitive to the combination of the first and third-generation EGFR-TKI (Arulananda et al., 2017). On the other hand, if these two mutations are in the same chromosomes (cis-configuration), EGFR-TKI should no longer be used (Goldberg et al., 2018). Interestingly, NSCLC patients with C797S/T790M/19Del or L858R (triple-mutation) are resistance to EGFR-TKIs, but sensitive to ALK-TKI brigatinib (Alunbrig) (Uchibori et al., 2017).

It has been reported that EGFR L718Q, G796D, L844V, et al. mutations are also an acquired drug-resistance site to osimertinib and first-generation EGFR-TKI, but is sensitive to afatinib (Liu et al., 2017; Zheng et al., 2017). Moreover, EGFR is expressed in many normal epithelial tissues and in many human cancers, including those of colon and rectal. For instance, cetuximab also received approval by the FDA for the treatment of head and neck cancer (Altaha and Abraham, 2007).

ALK

Anaplastic lymphoma kinase (ALK) fusion accounts for 3–5% of NSCLC, which is the second largest mutant gene found after EGFR gene. ALK is most common fused with echinoderm microtubule-associated protein-like 4 (EML4). EML4 is located at P21 of human chromosome 2, while ALK is located at P23. These two genes form a new gene EML4-ALK through inversion fusion. EML4-ALKfusion has more than 21 different forms according to the location of fracture. The sensitivity of different fusion forms to ALK-TKI was also different. The response rate and PFS of patients fused with exon 13 of EML4 gene and exon 20 of ALK gene to crizotinib (Xalkori) were significantly higher than those of other fusion types (Yoshida et al., 2016).

It was once thought that there was exclusion between ALK fusion and EGFR activating mutations (Crystal and Shaw, 2011), which was actually a misunderstanding. Both EGFR and ALK are strong driving genes, as a result, the presence of either of them is sufficient to form tumors, so the presence of them has been rarely observed before. As the data accumulated, some scientists felt obliged to investigate for the patients' samples did show these two genetic variants coexist (Wen et al., 2016).

PROFILE series of clinical research evidence all confirmed that compared with chemotherapy, both the response rate and disease control time of crizotinib were significantly better (Blackhall et al., 2017). The response rate of crizotinib for ALK fusion positive patients could reach 60∼80%, while that of chemotherapy drugs was only about 30∼40%. Crizotinib is one of the most significant clinical benefits drugs in targeted therapy of lung cancer.

Unfortunately, after initial response to crizotinib, tumors inevitably relapse and end up with drug-resistant symptoms like brain metastasis. In order to overcome crizotinib resistance, alectinib, ceritinib (Zykadia), brigatinib and other second-generation ALK-TKIs were developed, which are more potent and brain-penetrable (Duruisseaux et al., 2017). Surprisingly, all three second-generation ALK inhibitors achieved significant improvements in the first line NSCLC patients with ALK positive. Next generation agents (alectinib and brigatinib) revealed significant improvement in PFS (HR 0.50 [0.43, 0.57;

$p < 0.00001$]), ORR (OR 1.57 [1.21, 2.04; $p = 0.0006$]) in comparison to crizotinib and yielded better response intracranially than crizotinib in terms of objective response rate (OR 5.87 [3.49, 9.87; $p < 0.00001$]) and time to CNS progression (HR 0.25 [0.13, 0.46; $p < 0.0001$]) (Khan et al., 2018; Paik and Dhillon, 2018). What's more, brigatinib received granted accelerated approval by the United States Food and Drug Administration. In ASCEND-4 study (Soria et al., 2017), the mPFS of ceritinib is 16.6 months. In ALTA-1L study (Camidge et al., 2018), the estimated 12 months PFS rate, brigatinib and crizotinib achieved 67 and 43%, respectively. Especially in ALEX study, alectinib was head-to-head compared with crizotinib in first-line advanced ALK-positive NSCLC (Peters et al., 2017). The updated follow-up results was reported in 2018 ASCO conference. The mPFS of first-line alectinib was 34.8 months, compared with 10.9 months for crizotinib, which was amazing 3 times longer. An mPFS of nearly 3 years is enough to allow patients to live for 4–5 years or more, making lung cancer turn into a chronic disease. In addition, significant differences were observed between the two treatment groups regardless of whether the patient's baseline was associated with brain metastasis. Alectinib was also significantly superior to crizotinib in efficacy duration analysis. Safety data of alectinib is also excellent, and the incidence of serious adverse events remains low even after long-term use. The excellent efficacy and safety of alectinib may be related to its special molecular structure (Kinoshita et al., 2012). The drug structure determines the selectivity and affinity with the target. The higher the selectivity, the higher the safety and efficacy. Alectinib also has excellent blood-brain barrier permeability, which means better treatment and even prevention of brain metastases, and therefore longer PFS. Overall, ceritinib, brigatinib and alectinib have become the first-line treatment scheme recommended in NCCN guidelines for ALK fusion positive patients.

However, very similar to the case of EGFR-TKI, the second-generation ALK-TKIs also face resistance problems. Mutations in the ALK tyrosine kinase domain, such as G1202R, may affect the binding of ALK-TKIs, leading to drug resistance (Gainor et al., 2016). Fortunately, for patients with alectinib resistance, we can also choose the third-generation ALK-TKI lorlatinib (Lorbrena), which is specifically designed for drug resistance mutation sites and has a high blood-brain barrier permeability. By virtue of data already disclosed in the phase I/II studies (Basit et al., 2017; Shaw et al., 2017), lorlatinib has been approved by FDA as a breakthrough drug, and the phase III CROWN study is ongoing. Entrectinib was shown to be well tolerated and active against those gene fusions in solid tumors, including in patients with primary or secondary CNS disease (Drilon et al., 2017).

ROS1

Human ROS1 gene is a transmembrane tyrosine kinase gene of insulin receptor family, located at q21 of chromosome 6. ROS1 rearrangement represents a new and unique molecules subtype of NSCLC, whose frequency is 1–2%, and is common in young non-smoking female lung adenocarcinoma patients. However, ROS1 fusion occurs in up to 5% of patients with negative EGFR and ALK. Due to the low occurrence probability of ROS1 fusion in lung cancer patients, this molecular subtype was often neglected in the past in clinical practice, and patients with ROS1 fusion were often treated with standard regimens such as chemotherapy for first-line. With the development of gene sequencing technology, ROS1 fusion has been truly demonstrated to clinicians.

ROS1 rearrangement is mainly concentrated in exon 32–36, and at least 9 different fusion types have been found in NSCLC, among which CD74-ROS1 and SLC34A2-ROS1 are common (Jun et al., 2012).

Crizotinib and ceritinib have been shown to be effective in ROS1-positive NSCLC patients. A study published in 2014 showed that in NSCLC patients with ROS1 fusion using crizotinib showed amazing anti-tumor activity, with the objective response rate (ORR) being 72% and median duration of response (mDOR) being 17.6 months (Shaw et al., 2014). But for crizotinib, the first-generation ALK inhibitor, which can cause relapse of CNS metastases can be overcome by newer ROS1 inhibitors (Dong et al., 2016).

Similarly, NSCLC patients with ROS1 fusion who are treated with crizotinib may develop drug resistance, especially CNS metastases. Common drug resistance mutations include G2032R and D2033N of ROS1, which can be overcome by cabozantinib and lorlatinib (Katayama et al., 2015; Zou et al., 2015). A newer inhibitors, entrectinib (RXDX-101), is a ROS1, Pan-TRK, and ALK inhibitor with activity in multiple molecularly defined cancer indications. Entrectinib has extremely high anti-tumor activity against cell lines dependent on the pharmacological targets of this drug *in vitro*, which has shown great promise in phase I/II clinical trials (Ardini et al., 2016). One of the major differences between entrectinib and crizotinib is that crizotinib cannot normally penetrate the brain and CNS, and entrectinib has shown effective CNS activity.

BRAF

BRAF gene mutation is not only one of the mechanisms of EGFR-TKI resistance, but also an important driver gene and target of targeted therapy. BRAF mutations are found in 1.5–3.5% of NSCLC and cause downstream activation of the MAPK signaling pathway (Leonetti et al., 2018). V600E is the most common mutation. Selective BRAF inhibitors, such as dabrafenib (Tafinlar) and trametinib (Mekinist) has been recommended for the first-line and second-line treatment of advanced NSCLC in the NCCN guidelines.

The ORR of dabrafenib monotherapy for NSCLC patients with BRAF-V600E is 33% (95% CI, 23–45%) (Planchard et al., 2016b). In previously untreated metastatic NSCLC patients with BRAF-V600E, dabrafenib combined with trametinib achieved an ORR of 64% (95% CI, 46–79%), with 2 (6%) patients achieving a CR and 21 (58%) a PR (Planchard et al., 2017). Analogously, in pretreated patients the ORR was 63.2% (95% CI, 49.3–75.6%) (Planchard et al., 2016a).

Vemurafenib (Zelboraf) was approved by the FDA for the treatment of unrespectable and metastatic malignant melanoma with BRAF-V600E mutation in 2011. In the VE-BASKET study, vemurafenib was used for NSCLC patients with BRAF-mutant, the ORR was 42% (95% CI, 20–67%). The median PFS was 7.3 months (95% CI, 3.5–10.8%). The 12 months rate of PFS and

OS were 23% (95% CI, 6–46%) and 66% (95% CI, 36–85%), respectively (Hyman et al., 2015).

MET

c-MET is a kind of transmembrane receptors with independent phosphorylation activity, encoded by mesenchymal epithelial transition (MET) gene (Gherardi et al., 2012). The hepatocyte growth factor (HGF) is the specificity ligand of c-MET (Naldini et al., 1991). MET gene amplification, or c-MET protein overexpression, is one of the causes of resistance in NSCLC patients with the first or second-generation EGFR-TKI, accounting for about 5% of resistances (Engelman et al., 2007; Cappuzzo et al., 2009; Yu et al., 2013). Therefore, some researchers believe that the combination of c-MET inhibitor and EGFR-TKI will be a new idea to overcome drug resistance.

There is also a special mutation form of MET genes: exon 14-skipping (Awad et al., 2016). The Cancer Genome Atlas (TCGA) showed that MET exon 14-skipping were present in about 4% (10/230) of lung adenocarcinoma, leading to partial or complete skipping deletion of MET exon14 at mRNA level (Cancer Genome Atlas Research Network, 2014). The NCCN guidelines recommended crizotinib for patients with c-MET protein overexpression, MET gene amplification, and MET exon 14-skipping mutation in NSCLC patients.

NTRK

Rearrangements including NTRK1, NTRK2, and NTRK3 are in approximately 2–3% NSCLC patients without other driving genes, such as EGFR, KRAS, EML4-ALK, and ROS1 (Ricciuti et al., 2017).

On November 26, 2018, the US FDA accelerated the approval of larotrectinib (LOXO-101, Vitrakvi) for the treatment of locally advanced or metastatic solid tumors in adults and children with neurotropic tyrosine kinase receptor (NTRK) gene fusion, regardless of the region of cancer occurrence.

It is the first broad-spectrum cancer targeting drug that has been approved by the FDA, which target at NTRK fusion mutations and regardless of cancer type. It have been reported in almost every cancer, and shown to be effective in 17 childhood and adult tumors (Berger et al., 2018).

According to the latest data released at the annual meeting of the European society of oncology (ESMO) in October 2018, larotrectinib was able to achieve 80% objective response rate (ORR) in 55 patients with NTRK fusion cancer that could be measured by RECIST 1.1 criteria. It is noteworthy that larotrectinib showed very consistent results in various cancer types.

Entrectinib was also effective for NTRK fusion. In the studies of ALKA-372-001 and STARTRK-1, the ORR of 5 cases with NTRK fusion was 100%, including 3 cases of brain metastasis (Drilon et al., 2017). Results of phase II study STARTRK-2 have been published on 2018 ESMO congress. The result shows that, regardless of whether brain metastases, 54 NTRK fusion patients (sarcoma 24%, NSCLC 19%, sample secretory breast carcinoma 13%, breast cancer 11%, colorectal cancer 7%, bile duct carcinoma and other gynecological tumor, neuroendocrine carcinoma and salivary gland carcinoma and pancreatic cancer) treated by entrectinib all have gain benefit: ORR 57.4%, mDOR 10.4 months, mPFS 11.2 months, and mOS 20.9 months.

Others

In recent years, due to the progress of molecular subtyping technology, people have gained a new understanding of the biological mechanism of the occurrence and development of NSCLC. Several specific subtypes of driving genes, such as HER2, RET, KRAS, have been discovered, and corresponding targeted drugs have been developed, thus paving the way for the era of personalized medicine of NSCLC.

The incidence of RET fusion in lung adenocarcinoma is unknown and may be anywhere between 0.4 and 2% (Smit, 2017). The drugs target at RET fusion are much less effective than EGFR-TKI or ALK-TKI, and combination therapies may be the key to improving response in the future.

Human epidermal growth factor receptor 2 (HER2) is expressed in many cancers, including NSCLC. HER2 amplification has been reported to occur in up to 13–22.8% of NSCLC (Yu et al., 2013; Ko et al., 2018). Afatinib could be a useful therapeutic agent as HER2-targeted therapy for patients with NSCLC harboring HER2 alterations (Torigoe et al., 2018).

KRAS is a driving gene with very high mutation frequency in NSCLC and colorectal cancer, accounting for 3% in western population and 6% in Asian population (Domagala et al., 2012). KRAS has a high mutation frequency in many other tumor species. However, unfortunately, there are currently no targeted drugs specifically targeting KRAS mutations approved in list. KRAS mutation may affect the efficacy of EGFR-TKI, but increase the efficacy of immunotherapy.

OVERVIEW OF DETECTION METHOD

With the continuous progress of biological detection technology, the acquisition of human gene molecular typing is becoming more convenient and easier, and accurate treatment is gradually becoming the reality.

According to the different detection objects, there are many detection methods: ARMS, NGS, ddPCR, and FISH are targeted at DNA; RNA was targeted by RNA-Seq and RT-PCR, and IHC for protein. If the amount of tumor tissue is too small or unable to obtain tissue, we can supplement with liquid biopsy through blood, hydrothorax or ascites, pericardial effusion, cerebrospinal fluid and other specimens (Zhang et al., 2017b).

ARMS

The amplification refractory mutation system (ARMS) based on fluorescence quantitative PCR technology has become the mainstream technology of gene mutation detection due to its simple operation, high specificity, high sensitivity and good repeatability, which is suitable for hospital laboratory to carry out test by itself. ARMS can be used to detect gene mutations in tumor tissues and peripheral blood, but the prerequisite is to specify the specific site to be detected in advance and design the corresponding PCR primer. Therefore, ARMS is only applicable

to the detection of known mutation sites and cannot be used for the detection of unknown sites.

NGS

High-throughput sequencing, or next-generation sequencing (NGS), because of its low cost, large throughput, high accuracy and rich information content, has become a very important role in the study of genome, transcriptome and epigenetics. NGS mainly includes the whole genome sequencing (WGS), whole exome sequencing (WES), as well as the targeted region sequencing (TRS) or cancer gene panel (CGP). Recently, NGS is used to sequence circulating tumor DNA (ctDNA), as a kind of liquid biopsy. ctDNA is derived from necrotic tumor cells, apoptotic tumor cells, circulating tumor cells and exosomes produced by tumor cells. Although ctDNA samples are relatively simple to obtain, they are extremely low in content, accounting for 0.01∼1.00% of cell free DNA (cfDNA) in plasma. It was not until the emergence of NGS technology that the difficulty of detecting mutations from extremely low abundance samples was solved.

The types of mutations detected by NGS mainly include SNV, insertion, deletion, CNV, etc., including known and unknown mutation forms, which can provide much more information than ARMS. However, in terms of laboratory hardware setting and personnel qualification, it has higher requirements than conventional PCR laboratories. Therefore, it cannot be widely used in more hospitals at present, and third-party testing institutions are more likely to provide testing services.

FISH

Fluorescence *in situ* hybridization (FISH), can be used for the detection of CNV and gene fusion frequently. The number and location of the corresponding genes can be clearly displayed under the microscope by fluorescent probe labeling, and the CNV and fusion can be determined by this method. FISH is the gold standard for the detection of gene fusion. Taking ALK fusion as an example: two probes, red and green, are designed to mark the two ends of ALK gene, respectively, once ALK gene is broken and rearranged or inverted, red and green signals will be separated under the microscope; while those that are not broken will show yellow fluorescence signals. FISH can detect whether a gene has been rearranged or reversed, but it cannot determine where it has been broken or fused with which gene. NGS detects gene fusion to identify the site of rupture and to identify which genes are fused. However, about 10% of fusion fracture sites were located in introns or not contained by probes, so NGS could not detect them. Therefore, NGS combined with FISH will help to determine the final result.

ddPCR

Droplet digital PCR (ddPCR), as the third generation of PCR technique usually used in ctDNA liquid biopsy, gain a great of attention in the field of clinical diagnosis because of its high sensitivity. By using the micro-PCR amplification system, absolute quantification can be achieved, which overcomes the difficulty of ctDNA not easy to be amplified due to its low content. Same as ARMS, ddPCR can only detect known sites, but it is fast and sensitive, with almost no detection threshold. ddPCR combined with NGS cannot only quickly check whether there are common mutations, but also examine whether there are rare mutations in a wide range.

Due to its high sensitivity, relatively non-invasive and absolute quantification, ddPCR has been used for dynamic monitoring of mutant copy number, which can be used for real-time monitoring of the relationship between tumor progression, drug resistance and gene evolution.

IHC

Genes ultimately function through proteins, so detection of proteins is of more concern. Immunohistochemistry (IHC) is the detection of protein level, which can detect protein deficiency, overexpression, fusion and so on. Surgical tissue, fine needle aspiration biopsy tissue, pleural or ascites centrifugal precipitates can all be used for the detection of immunohistochemistry.

The test results of DNA level and protein level sometimes do not match. Amplification of a gene usually implies overexpression of the corresponding protein, such as androgen receptor (AR). On the contrary, when the AR protein is overexpressed, but the AR gene is not necessarily amplified, because there are many regulatory mechanisms from DNA to protein. Similarly, if the promoter is methylated, then even if the DNA sequencing is normal, the corresponding protein may be missing. There is also a weak relationship between some genes and protein expression, such as CD274 gene and PD-L1 protein, and there is almost no relationship between the expression level of PD-L1 and the copy number of CD274.

OVERVIEW OF IMMUNE CHECKPOINT INHIBITORS

The cause of the tumor is related to immune system oversight. All malignancies originate from genetic mutations, including germline and somatic mutations. According to the genetic central dogma, the changes in DNA ultimately cause changes in proteins. The altered proteins (tumor proteins) are immunogenic (neoantigens). A large number of neoantigens, caused by genetic and epigenetic changes, can induce an immune response, but tumors can induce tolerance between tumors and specific T cells by up-regulating ligands of inhibit receptors, ultimately resisting immune attacks (Mellman et al., 2011). According to the Cancer immunoediting theory first proposed by Dunn et al. (2002), the immune response has three stages of elimination, equilibrium and escape, and it is regulated by various stimulant and inhibitory factors (Dunn et al., 2002). Tumor immunotherapy is to activate the body's auto defense mechanism or give exogenous substances to regulate the immune response to tumor, and stimulate the immune cells to identify, inhibit and kill tumor cells (Dong et al., 2018). The immune escape mechanism of tumors plays an important role in the occurrence and development of tumors (Siegel et al., 2017).

Currently, inhibitors of two checkpoints, cytotoxic T lymphocyte antigen – 4, (CTLA-4), programmed cell death protein – 1 (PD-1)/programmed cell death ligand 1 (PD-L1),

have been approved to be listed. PD-1 inhibitors include nivolumab, pembrolizumab, and PD-L1 inhibitors include atezolizumab, durvalumab, avelumab, etc.

Immunotherapy often produces some magical results and has aroused great interest among oncologists worldwide. However, not every patient can achieve this effect, and some patients may even progress faster after receiving immunotherapy. MDM2/4 amplification, chromosome 11q13 (CCND1, FGF3, FGF4, and FGF19) amplification and EGFR amplification may be associated with hyperprogression in immunotherapy (Kato et al., 2017). Molecular typing is also needed to determine which patients are suitable for use, and biomarkers are used to screen out which patients can use immunotherapy.

PD-L1

PD-1 is expressed in T cells, natural killer cells (NK), monocytes and B cells. PD-L1 is mainly expressed on the surface of tumor cells and in the tumor microenvironment. When PD-1 binds to PD-L1, T cells are inhibited. PD-L1 on the tumor cell membrane can be abnormally up-regulated and inhibit the activation of T lymphocytes, leading to tumor immune escape (Haanen and Robert, 2015). The PD-L1 expression level is the first biomarker approved by FDA. Although there were some differences reported in the results of clinical trials in which different PD-1/PD-L1 inhibitors were involved, the benefit of immunotherapy was significantly higher in those with high expression of PD-L1 than in those with low expression (Dong et al., 2018).

IHC is the gold standard for detecting the expression rate of PD-L1 protein. There are two platforms (Dako, Ventana) and four testing kits (28-8, 22C3, SP263, SP142) used for PD-L1 detecting.

TMB

Tumor mutation burden (TMB) is also able to identify candidate patients who will benefit from immunotherapy. TMB is defined as the number of non-synonymous somatic mutations per million bases, excluding germline mutations. Therefore, TMB describes the stability of the genome. The higher the TMB is, the more mutations will be, as a result there will be more new antigens, more immunogenicity, and better effect of immunotherapy.

NGS is the only method for TMB detection. TMB measured by WES is the gold standard, but it is costly, time-consuming and inconvenient for clinical application. The TMB measured by TRS (CGP) avoids these problems successfully and has been widely carried out in clinical practice. The question then becomes how to define "TMB-H." When different regions were selected for sequencing, the TMB value obtained was inconsistent and an accepted cutoff value could not be determined. A smart solution is to compare the ordering of the absolute value of CGP-TMB instead of comparing the absolute value directly. The CGP-TMB values of the same tumor species detected by the same detection institution can be sorted, with the highest 25% defined as "high," the middle 50% defined as "medium," and the lowest 25% defined as "low," which can also be divided into three equal parts. Blood-based TMB (bTMB) has also been shown to be useful in screening people for immunotherapy (Gandara et al., 2018).

In October 2018, based on the results of Checkmate-227 (Hellmann et al., 2018) and Checkmate-026 (Carbone et al., 2017), the NCCN issued 2019 v1.0 guidelines for NSCLC, recommending TMB for the first time to identify lung cancer patients who are suitable for the combination of "nivolumab + ipilimumab" and "nivolumab." But it also points out that there is no consensus on how to measure TMB.

MMR and MSI

Mismatch-repair (MMR) deficiency and microsatellite-instability (MSI) can predict the therapeutic effect of tumor immunotherapy (Le et al., 2015). MSI is major caused by MMR- deficient (dMMR), and we detect MSI to determine whether the function of MMR is proficient (pMMR). Therefore, like TMB, MSI, and MMR are indicators of genomic stability. dMMR enables tumors to synthesize new antigens with potential immunogenicity, thus causing an immune response (Colle et al., 2017).

The gold standard for MMR detection is IHC to detect whether four MMR proteins (MLH1, MSH2, MSH6, PMS2) are expressed. There are two possible reasons for the non-expression of MMR protein. 1. MMR gene variation; 2. Methylation of MMR gene promoter. The first cause can be also detected by NGS, but the second can only be confirmed by detecting promoter methylation (McCarthy et al., 2018).

MSI can be measured by PCR or NGS. PCR-MSI is easy to operate, and has been carried out in many hospitals, but only five sites have been detected so far. Compared with PCR, NGS detection has a wider range and can avoid detection omission effectively (Hempelmann et al., 2018). The sensitivity and specificity of CGP-MSI based on NGS can reach 93.1–96.6% and 97.2–100%, respectively (Salipante et al., 2014; Hempelmann et al., 2018). In addition to improved sensitivity, NGS-MSI testing offers several advantages over PCR-MSI methods. (1) They do not require matched non-tumor tissue; (2) Interpretation is streamlined and semiautomated (Hempelmann et al., 2018).

However, dMMR or MSI is a low-probability event in NSCLC.

SUMMARY

Tumor-targeted therapy is to determine the treatment method for specific driving gene mutations by detecting whether there are gene mutations or gene spectrum changes in tumors that lead to tumor growth. In order to determine whether patients can use targeted therapeutic drugs, genetic molecular typing is needed first.

In the selection of gene detection technology, it is necessary to select appropriate detection technology according to its corresponding target to ensure the accuracy and reliability of detection results.

NSCLC has the most thorough research and the most sufficient evidence in driving gene molecular typing. Accurate treatment of NSCLC patients can only be achieved by using the technology and accurate detection. We hope that in the future, more detection technologies, more driving genes and more effective targeted drugs will emerge, so that NSCLC will become a controllable chronic disease.

AUTHOR CONTRIBUTIONS

QZ put forward the content of the paper. JD wrote the manuscript. BL, DL, and DH literature and clinical data were reviewed.

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Safety and Efficacy of Anti-PD-1 Monoclonal Antibodies in Patients With Relapsed or Refractory Lymphoma: A Meta-Analysis of Prospective Clinic Trails

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Background: Immune checkpoint inhibition therapy with monoclonal antibody against programmed cell death protein 1 (PD-1), including nivolumab and pembrolizumab, has demonstrated powerful clinical efficacy in the treatment of advanced cancers. However, there is no evidence-based systematic review on the safety and efficacy of anti-PD-1 antibody in treating lymphoma.

Methods: To evaluate the safety and efficacy of nivolumab/pembrolizumab, we analyzed clinical trials from PUBMED, EMBASE, and The Cochrane Library. For safety analysis, the incidence and exhibition of any grade and grade ≥3 adverse events (AEs) were evaluated. Overall response rate (ORR), 6-month progression-free survival (PFS) and 6-month overall survival (OS) were calculated for efficacy analysis.

Results: Overall ten studies and 718 patients (114 non-Hodgkin lymphomas, 604 Hodgkin lymphomas) were enrolled, including 4 phase I studies and 6 phase II studies. The pooled incidences of any grade and grade ≥3 adverse events (AEs) were 74 and 24%, respectively. Drug-related deaths occurred in two patients. The most common any grade AEs were fatigue (14.91%), rash (14.8%), hypothyroidism (13.77%), platelet count decreased (13.54%), pyrexia (13%). The most common grade ≥3 AEs were neutropenia (4.79%), pneumonitis (3.58%), rash (3.38%), and leukopenia (3.31%). Fatigue ($p = 0.0072$) and rash ($p = 0.0078$) in any grade AEs were less observed in patients treated with pembrolizumab than nivolumab. The pooled ORR, PFS rate and OS rate were 58, 73, and 96%, respectively. The ORR in patients with Hodgkin lymphomas (HL) was higher than patients with non-Hodgkin lymphomas (NHL) (69.08 vs. 30.77%, $p < 0.0001$). However, there was no significant difference of efficacy between nivolumab and pembrolizumab.

Conclusions: Nivolumab and pembrolizumab have promising outcomes with tolerable AEs and drug-related deaths in patients with relapsed or refractory lymphoma. Pembrolizumab caused less any grade AEs like fatigue and rash than nivolumab. Patients with HL got better response than NHL.

Keywords: anti–PD-1 monoclonal antibodies, nivolumab, pembrolizumab, relapsed or refractory lymphoma, safety, efficacy

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INTRODUCTION

Programmed cell death protein 1 (PD-1) is an immune checkpoint receptor mainly expressed on activated T cells, natural killer cells, and B cells (Ishida et al., 1992). The PD-L1 and PD-L2 are its known ligands, which interact with PD-1 on T cells and prevent T-cell activation and proliferation. PD-L1 is expressed on macrophages and it can be upregulated in some tissues and tumors in answer to IFN-γ and other inflammatory factors (Dong et al., 2002; Yamazaki et al., 2002; Taube et al., 2012). While, PD-L2 is expressed on macrophages and dendritic cells (Tseng et al., 2001; Ishida et al., 2002). Besides PD-1, PD-L1 can combine with CD80/B7-1 (Butte et al., 2007; Park et al., 2010) and PD-L2 can incorporate with RGMb (Xiao et al., 2014); these may cause the differences in response and immune-related adverse events (AEs) between anti-PD-1 and anti-PD-L1 antibodies.

Combination chemotherapy can cure most patients with classic Hodgkin lymphomas (cHL). However, for patients who failed to treatment (refractory cHL) or regained the disease soon (relapsed cHL), immunotherapy can be an appropriate option. CHL's typical feature is the existence of the malignant Hodgkin Reed Sternberg (HRS) cells surrounded by an inflammatory immune infiltrate. Meanwhile, PD-L1 expression was upregulated in cHL via JAK2-STAT signaling with near universal genetic amplification of the 9p24.1 locus (Green et al., 2010). Among the non-Hodgkin lymphomas (NHL), the overexpression of PD-L1 is also identified in many cases (Chen et al., 2013). Therefore, the anti-PD-1 antibody can be a potential therapy for patients with lymphoma.

The US Food and Drug Administration (FDA) currently approved two anti-PD-1 antibodies, including pembrolizumab and nivolumab. Pembrolizumab is a fully humanized IgG4 kappa isotype anti-PD-1 monoclonal antibody. Nivolumab is a fully human IgG4 anti-PD-1 monoclonal antibody. Clinic trials with other anti-PD-1 antibodies and anti-PD-L1 antibodies are ongoing, the results have not been publicated.

In recent years, immunotherapy with PD-1 blockage or PD-L1 blockage were successfully used in many cancers, including melanoma, non-small cell lung cancer, renal cell carcinoma, ovarian cancer, lymphoma, et al. (Sunshine and Taube, 2015). However, the efficacy of anti-PD-1 in lymphoma ranged widely. Additionally, the adverse events (AEs) with checkpoint inhibition is not related to traditional therapy, such as nausea, vomiting, hair loss, etc., but relates to several autoimmune side effects. However, there is no systematic review to evaluate the safety and efficacy of anti-PD-1 antibody in treating lymphoma. Therefore, this meta-analysis was to assess the safety and efficacy of anti-PD-1 antibody in patients with lymphoma, offering evidence-based references for clinicians.

METHODS

Literature Search

We obeyed the Preferred Reporting Items for Systematic reviews and Meta-Analyses (PRISMA) guidelines. We searched PUBMED, EMBASE, and The Cochrane Library to identify the relevant studies up to March 2018. We used a combination of terms: "pembrolizumab/ lambrolizumab/ Keytruda/ MK-3475" OR "Nivolumab/ MDX-1106/ ONO-4538/ BMS-936558/ Opdivo" AND "lymphoma."

Inclusion and Exclusion Criteria

Studies had to meet the following criteria: (1) prospective trials concerning the efficacy or safety of nivolumab/pembrolizumab on patients with relapsed or refractory lymphoma. (2) articles reporting any of the data: ORR, 6-month PFS rate, 6-month OS rate, and drug-related AEs.

Exclusion criteria: (1) articles not association with our topics; (2) studies without usable data; and (3) retrospective or observed studies, letters, editorials, case reports, and reviews.

Data Extraction and Quality Control

The eligible studies were reviewed and extracted data by two authors independently. We extracted first author, published year, ClinicalTrials.gov number, phase, study design, treatment, disease, number of patients, age, prior systemic treatment regimens, ORR, 6-month PFS rate, 6-month OS rate, any grade AEs, grader ≥3 AEs, and drug-related deaths. The methodological index for non-randomized studies (MINORS) (Slim et al., 2015) was used to evaluate the methodological quality of the included articles. MINORS contained 12 items, the first eight being specifically for non-comparative studies. The items including a stated aim of the study, the inclusion of consecutive patients, prospective collection of data, endpoint appropriate to the study aim, unbiased evaluation of endpoints, follow-up period appropriate to the major endpoint, loss to follow up not exceeding 5% and prospective calculation of the sample size. Each item was scored from 0 to 2; 0 indicates that it was not reported, one represented that it was reported inadequately, and 2 revealed that it was reported adequately.

Statistical Analysis

The primary outcome for efficacy was ORR; secondary outcomes were 6-month PFS and 6-month OS. For safety analysis, the incidence and exhibition of any grade and grade ≥3 AEs were evaluated. In each trial, objective response rate (ORR) = [(complete responses + partial responses) ÷ total no. of patients] × 100. Heterogeneity among studies was detected with a forest plot and the inconsistency statistic (I^2). A random-effect model was used when potential heterogeneity existed (I^2 >50%); otherwise, the fixed-effect model was employed. The Metaprop module in the R-3.3.2 statistical software package was used to analyze the efficacy and safety. Subgroup analysis was performed to solve heterogeneity. Sensitivity analysis was carried out by using different effect models. No dose effect was considered. $P < 0.05$ suggested statistically significant.

RESULTS

Study Selection

The search strategy produced a total of 443 records; 41 studies were removed after duplication; 391 studies were excluded. Finally, ten studies were enrolled after removing one study with

combined therapy (Ansell et al., 2015; Armand et al., 2016, 2018; Lesokhin et al., 2016; Younes et al., 2016; Chen et al., 2017; Ding et al., 2017; Maruyama et al., 2017; Zinzani P. et al., 2017; Zinzani P. L. et al., 2017). **Figure 1** showed the procedure of study selection.

Study Characteristics

Table 1 showed the characteristics of the included studies. The included studies were published from 2015 to 2018. We included a total of 10 studies, 762 patients, of which 114 patients were NHL [9 CLL with Richter transformation, 105 primary mediastinal large B-cell lymphomas (PMBCL)], 604 patients were HL, 17 patients were leukemia, and 27 patients were multiple myeloma. Altogether 384 patients' mean ages were <50 years, while 79 patients' mean ages were >50 years. We assessed AEs, ORR, PFS and OS only in patients with lymphoma. There were 4 phase I studies and 6 phase II studies. Patients in 5 studies used nivolumab and five studies received pembrolizumab. Two studies were dose-escalation, cohort expansion studies, three studies were multicohort studies, and five studies were single-arm trails. Patients received nivolumab intravenously at a dose of 1 or 3 mg/kg every 2 weeks. Pembrolizumab were given 10 mg/kg every 2 weeks or 200 mg every 3 weeks. Drug-related deaths occurred in two patients; one received nivolumab with pneumonitis/ARDS, one treated with pembrolizumab observed with Pseudomonas sepsis.

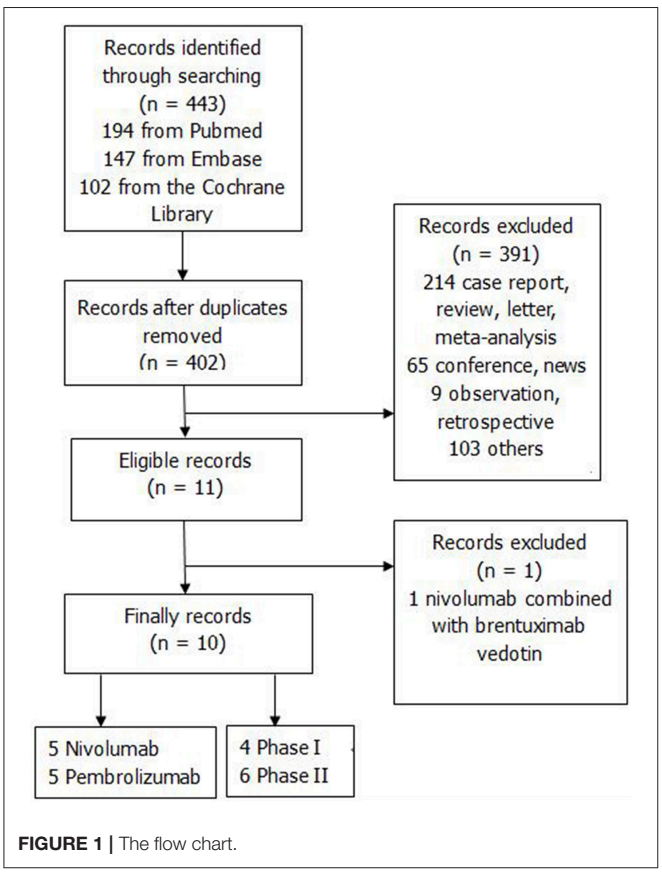

FIGURE 1 | The flow chart.

Safety

Overall eight studies were included to assess the pooled incidence of any grade (74%, 95%CL: 62%−84%) and grade ≥3 (24%, 95%CL: 17%−34%) AEs (**Figure 2**). There was no significant difference in the total risk of AEs between the nivolumab and pembrolizumab. The most common any grade adverse event was fatigue (14.91%, 10.27%−21.13%). Other common drug-related any grade AEs were rash (14.8%), hypothyroidism (13.77%), platelet count decreased (13.54%), pyrexia (13%), cough (11.56%), pruritus (10.81%), and nausea (10.16%). Neutropenia was the most common grade ≥3 AEs (4.79%). Another common severe AEs were pneumonitis (3.58%), rash (3.38%), and leukopenia (3.31%). We also compared nivolumab with pembrolizumab in patients with lymphoma, the incidences of any grade fatigue ($p = 0.0072$) and rash ($p = 0.0078$) were lower in pembrolizumab group than those patients with nivolumab. More details were exhibited in **Table 2**.

Efficacy

The pooled ORR, 6-month PFS rate and 6-month OS rate were performed to evaluate the efficacy of nivolumab or pembrolizumab treated lymphoma. We enrolled all ten studies to analyze ORR, five studies to evaluate PFS and five studies to assess OS. The pooled ORR, PFS rate and OS rate were 58% (95%Cl: 47%−69%), 73% (95%Cl: 68%−78%), and 96% (95%Cl: 92%−98%), respectively. There were no significant differences in ORR between patients' mean age >50 years (46%, 16%−79%) and < 50 years (62%, 50%−73%). PFS and OS between patients' mean age >50 and <50 years did not analyze due to limitation numbers. Meanwhile, the ORR, PFS and OS between nivolumab and pembrolizumab had no significant differences. While, the ORR in patients with HL was higher than patients with NHL (69.08 vs. 30.77%, $p < 0.0001$). The PFS and OS could not be subgrouped by HL and NHL. These results were exhibited in **Figure 3**.

Study Quality

Two studies without full text can't evaluate totally. The two items including unbiased evaluation of endpoints and prospective calculation of the sample size were not reported. The overall score was high. Therefore, the overall quality of the included studies was satisfactory (**Table 3**).

DISCUSSION

Anti-PD-1 antibodies are rapidly developed in recent decades. FDA has approved two anti-PD-1 antibodies, including pembrolizumab and nivolumab. However, the AEs may be different between pembrolizumab and nivolumab. Meanwhile, the efficacy of these two anti-PD-1 antibodies in lymphoma ranged widely.

This meta-analysis included overall ten prospective studies with 718 patients with lymphomas, including 114 patients with NHL and 604 patients with HL, to assess the safety and efficacy. The pooled incidence of AEs of any grade reached 74%, while grade ≥3 was only 24%. However, there were two patients occurred drug-related death. Approximately 58% of patients

TABLE 1 | The characteristics of included studies.

No.	Study	Clinic trials gov.	Phase	Study design	Treatment	Disease	No. of patients	Ages(years), medium (range)	Median prior systemic treatment regimens	Drug-related adverse events(any grade, n)	Drug-related adverse events (grade≥3, n)	Drug-related deaths(n)	ORR(%)	6-month PFS rate(%)	6-month OS rate(%)
NIVOLUMAB															
1	Lesokhin et al. (2016)	NCT01592370	I	dose-escalation, cohort-expansion	nivolumab, 1 or 3 mg/kg every 2 weeks	relapsed or refractory NHL/MM	82:27 MM;31 B-NHL; 23 T-NHL; 1 CML	MM: 63(32–81) B-NHL: 65(23–74) T-NHL:61(30–81)	3(1–12)	51	18	1(pneumonitis/ ARDS)	3.70%; 22.22%; 0	–	–
2	Ansell et al. (2015)	NCT01592370	I	dose-escalation, cohort-expansion	nivolumab, 1 or 3 mg/kg every 2 weeks	relapsed or refractory HL	23	35(20–54)	–	18	5	0	87%	86%	–
3	Maruyama et al. (2017)	JapicCTI-142755	II	multicenter, single-arm	nivolumab, 3 mg/kg every 2 weeks	relapsed or refractory HL	17	63(29–83)	3 (2–5)	17	4	0	75%	60%	100%
4	Younes et al. (2016)	NCT02181738	II	multicentre, multicohort, single-arm	nivolumab, 3 mg/kg every 2 weeks	relapsed or refractory HL	80	37 (28–48)	4 (4–7)	71	20	0	66%	77%	99%
5	Armand et al. (2018)	CheckMate 205	II	multicohort, single-arm	nivolumab 3 mg/kg every 2 weeks	relapsed/ refractory cHL	243	–	–	–	–	0	69%	–	–
PEMBROLIZUMAB															
1	Armand et al. (2016)	NCT01953692	Ib	multicohort	pembrolizumab, 10 mg/kg every 2 weeks	relapsed or refractory HL	31	32(20–67)	–	21	5	0	65%	69%	100%
2	Chen et al. (2017)	NCT02453594	II	multicenter single-arm	pembrolizumab, 200 mg once every 3 weeks	relapsed/ refractory HL	210	35(18–76)	4(1–12)	–	–	0	69%	72.40%	99.50%
3	Ding et al. (2017)	NCT02332980	II	single-arm	pembrolizumab, 200 mg every 3 weeks	relapsed and transformed CLL	25: 16 relapsed or refractory CLL; 9 RT	69(46–81)	4(1–10)	25	15	1(Pseudomonas sepsis)	0; 44%	–	57.14%; 71.43%
4	Zinzani et al. (2018)	NCT01953692	Ib	multicenter, international, multicohort	pembrolizumab, 10 mg/kg every 2 weeks(10); 200 mg every 3 weeks(8)	relapsed/ refractory primary mediastinal large B-cell lymphoma (rrPMBCL)	18	30(22–62)	4(2–6)	11	2	0	41%	–	–
5	Zinzani P. L. et al. (2017)	NCT02576990	II	two -cohort, multicenter	pembrolizumab 200 mg IV every 3 weeks	relapsed/ refractory primary mediastinal large B-cell lymphoma (rrPMBCL)	33	32(20–58)	3(1–5)	19	6	0	35%	–	–

CTC for AE version, Common Terminology Criteria for Adverse Events version; n/a, non-available. HL, Hodgkin's lymphoma; NHL, non-Hodgkin's lymphoma; MM, multiple myeloma; T-NHL, T cells non-Hodgkin's lymphoma; B-NHL, B cells non-Hodgkin's lymphoma; CML, chronic myeloid leukemia; CLL, chronic lymphocytic leukemia; RT, Richter transformation.

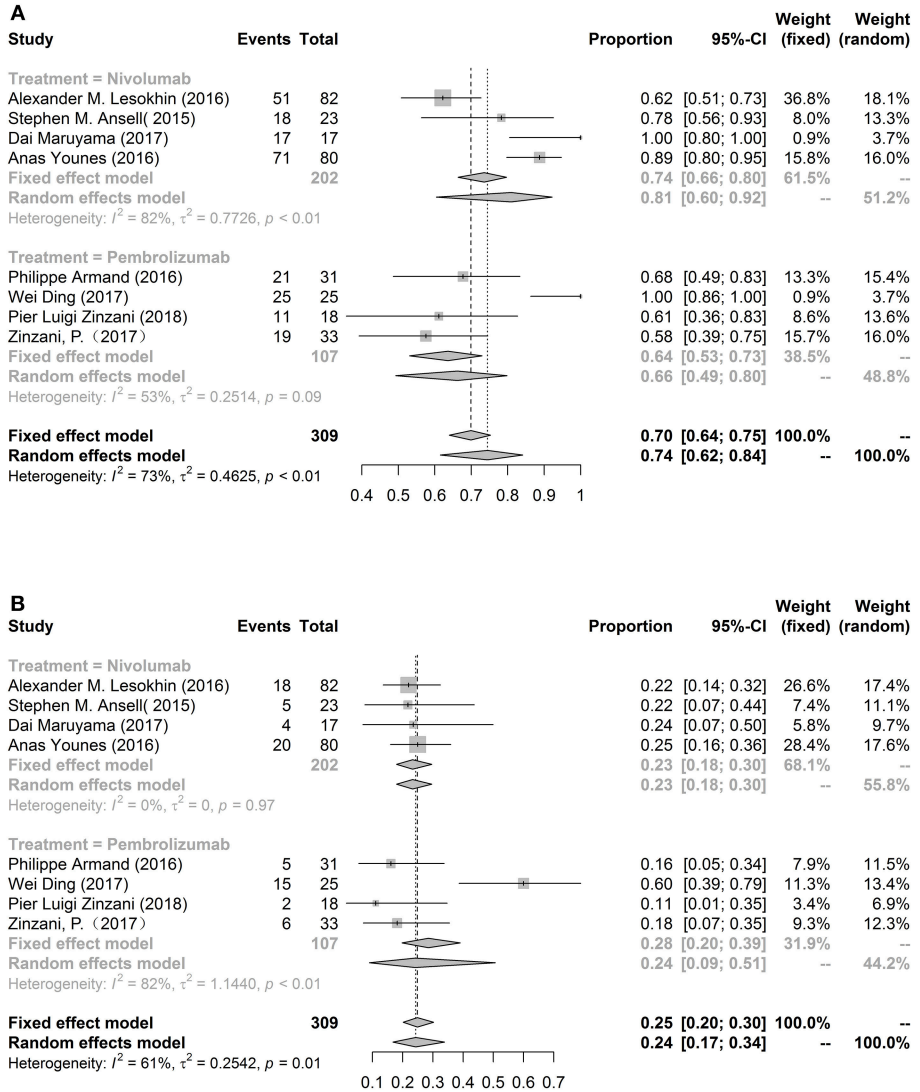

FIGURE 2 | The forest plot of pooled incidence of AEs in any grade **(A)** and grade ≥3 **(B)**.

gained complete response or partial response. Meanwhile, 73% of patients' diseases remained stable for half a year, and 96% of patients survived for half a year.

Immune-related adverse events caused by blockage of the PD-1 pathway can affect almost any organ, mainly mediated by T cells (Weber et al., 2015b). B cells secreting antibodies (Good-Jacobson et al., 2016) and granulocytes secreting inflammatory mediators and cytokines (Zitvogel and Kroemer, 2012; Good-Jacobson et al., 2016) may also develop immune-related adverse events. We found that the most common any grade adverse event were fatigue, rash, hypothyroidism, platelet count decreased, pyrexia, cough, pruritus, and nausea. The severe AEs over 3% were neutropenia, pneumonitis, rash, and leukopenia. In advanced melanoma, fatigue (19–21%, 34%), diarrhea (14–17%, 11–19%), pruritus (14%, 16–19%), rash (13–15%, 9–22%), arthralgia (9–12%, 6–8%), vitiligo (9–11%, 5–11%) and hypothyroidism (9–10%, 4–9%) were most common for any grade in pembrolizumab (Robert et al., 2015b) and nivolumab (Larkin et al., 2015; Robert et al., 2015a; Weber et al., 2015a), respectively. In advanced lung cancer, fatigue (14%, 16%), diarrhea (8%, 8–10%), pruritus (11%, 6–8%), rash (10%, 4–11%), arthralgia (9%, 5%), hypothyroidism (8%, 4–7%) and pneumonitis (8%, 4–7%) were most common for any grade in pembrolizumab (Garon et al., 2015; Herbst et al., 2016) and nivolumab (Borghaei et al., 2015; Brahmer et al., 2015; Rizvi et al., 2015), respectively. Therefore, the safety of anti-PD-1 antibodies were similar between the different cancers.

Many clinic trials reported fatigue as one of the AEs with anti-PD-1 antibodies (Brahmer et al., 2015; Rizvi et al., 2015). While, it was generally mild and not related to other systemic symptoms. We reported the maculopapular rash was most commonly. Additionally, rarer rashes including lichenoid

TABLE 2 | The incidence of adverse events in all grade or grade ≥3.

AEs	Treatment	Any grade						Grade ≥3					
		Included study	Event	Total patients	Pooled rate (95%CI)	Model	*P*-value	Included study	Event	Total patients	Pooled rate (95%CI)	Model	*P*-value
GENERAL													
Fatigue	Nivolumab	4	40	202	0.2018 [0.1514; 0.2636]	Fixed	**0.007**	4	0	202	0.0121 [0.0030; 0.0471]	Fixed	0.2200
	Pembrolizumab	4	28	284	0.1024 [0.0715; 0.1445]	Fixed		3	3	253	0.0280 [0.0044; 0.1595]	Random	
	Overall	8	68	486	0.1491 [0.1027; 0.2113]	Random		7	3	455	0.0245 [0.0113; 0.0524]	Fixed	
Pyrexia	Nivolumab	3	16	122	0.1675 [0.0486; 0.4422]	Random	0.442	3	1	122	0.0367 [0.0105; 0.1199]	Fixed	0.49
	Pembrolizumab	3	26	253	0.1030 [0.0711; 0.1471]	Fixed		3	2	253	0.0208 [0.0073; 0.0579]	Fixed	
	Overall	6	42	375	0.1300 [0.0728; 0.2213]	Random		6	3	375	0.0263 [0.0118; 0.0575]	Fixed	
Chills	Pembrolizumab	3	9	266	0.0386 [0.0202; 0.0727]	Fixed		2	0	235	0.0067 [0.0009; 0.0463]	Fixed	
Asthenia	Pembrolizumab	2	5	241	0.0288 [0.0064; 0.1199]	Random		1	1	33	0.0303		
RESPIRATORY													
Pneumonitis	Nivolumab	2	12	162	0.0629 [0.0129; 0.2571]	Random	0.859	2	5	162	0.0370 [0.0155; 0.0860]	Fixed	0.853
	Pembrolizumab	2	4	49	0.0840 [0.0319; 0.2037]	Fixed		1	1	33	0.0303		
	Overall	4	15	211	0.0942 [0.0574; 0.1508]	Fixed		3	6	195	0.0358 [0.0161; 0.0775]	Fixed	
Embolism	Nivolumab	2	1	162	0.0138 [0.0035; 0.0535]	Fixed		2	1	162	0.0138 [0.0035; 0.0535]	Fixed	
Cough	Nivolumab	1	2	23	0.0879			1	0	23	0		
	Pembrolizumab	2	19	235	0.1297 [0.0236; 0.4793]	Random		2	1	235	0.0091 [0.0023; 0.0357]	Fixed	
	Overall	3	21	258	0.1156 [0.0346; 0.3226]	Random		3	1	258	0.0107 [0.0031; 0.0364]	Fixed	
Upper respiratory tract infection	Nivolumab	2	5	97	0.0603 [0.0010; 0.8038]	Random		2	0	97	0.0130 [0.0018; 0.0871]	Fixed	
	Pembrolizumab	1	13	210	0.0619			1	0	210	0		
	Overall	3	18	307	0.0780 [0.0147; 0.3243]	Random		3	0	307	0.0074 [0.0015; 0.0358]	Fixed	
Dyspnea	Nivolumab	1	3	80	0.0375			1	1	80	0.0125		
	Pembrolizumab	3	17	266	0.0951 [0.0172; 0.3873]	Random		2	6	235	0.0424 [0.0024; 0.4524]	Random	
	Overall	4	20	346	0.0764 [0.0202; 0.2489]	Random		3	7	315	0.0298 [0.0036; 0.2068]	Random	
SKIN													
Rash	Nivolumab	4	35	202	0.1927 [0.1146; 0.3057]	Random	**0.008**	4	5	202	0.0366 [0.0175; 0.0749]	Fixed	0.651
	Pembrolizumab	2	18	235	0.0766 [0.0488; 0.1183]	Fixed		2	1	235	0.0145 [0.0006; 0.2577]	Random	
	Overall	6	53	437	0.1480 [0.0869; 0.2408]	Random		6	6	437	0.0338 [0.0176; 0.0639]	Fixed	
Pruritus	Nivolumab	4	24	202	0.1403 [0.0707; 0.2593]	Random		4	0	202	0.0121 [0.0030; 0.0471]	Fixed	
	Pembrolizumab	1	8	210	0.0381			1	0	210	0		
	Overall	5	32	412	0.1081 [0.0509; 0.2152]	Random		5	0	412	0.0087 [0.0025; 0.0296]	Fixed	
GASTROINTESTINAL													
Decreased appetite	Nivolumab	2	9	162	0.0532 [0.0160; 0.1621]	Random	0.645	2	0	162	0.0061 [0.0009; 0.0420]	Fixed	
	Pembrolizumab	2	4	49	0.0844 [0.0320; 0.2045]	Fixed		1	0	18	0		
	Overall	4	13	211	0.0701 [0.0410; 0.1172]	Fixed		3	0	180	0.0099 [0.0020; 0.0476]	Fixed	

(Continued)

TABLE 2 | Continued

AEs	Treatment	Any grade						Grade ≥3					
		Included study	Event	Total patients	Pooled rate (95%CI)	Model	*P*-value	Included study	Event	Total patients	Pooled rate (95%CI)	Model	*P*-value
Diarrhea	Nivolumab	4	19	202	0.0959 [0.0620; 0.1455]	Fixed	0.9710	4	0	202	0.0121 [0.0030; 0.0471]	Fixed	0.853
	Pembrolizumab	4	26	284	0.0969 [0.0667; 0.1387]	Fixed		3	2	253	0.0142 [0.0050; 0.0398]	Fixed	
	Overall	8	45	486	0.0965 [0.0727; 0.1269]	Fixed		7	2	455	0.0134 [0.0058; 0.0306]	Fixed	
Nausea	Nivolumab	2	13	103	0.1262 [0.0747; 0.2054]	Fixed	0.305	2	0	103	0.0113 [0.0016; 0.0761]	Fixed	0.958
	Pembrolizumab	4	23	284	0.1069 [0.0547; 0.1983]	Random		3	0	253	0.0106 [0.0021; 0.0509]	Fixed	
	Overall	6	36	387	0.1016 [0.0741; 0.1380]	Fixed		5	0	356	0.0109 [0.0031; 0.0369]	Fixed	
Vomiting	Nivolumab	1	6	80	0.0750			1	0	80	0		
	Pembrolizumab	3	13	266	0.0533 [0.0311; 0.0899]	Fixed		2	0	235	0.0067 [0.0009; 0.0463]	Fixed	
	Overall	4	19	346	0.0594 [0.0381; 0.0913]	Fixed		3	0	315	0.0065 [0.0013; 0.0318]	Fixed	
Constipation	Nivolumab	2	7	97	0.0746 [0.0359; 0.1484]	Fixed	0.134	2	0	97	0.0130 [0.0018; 0.0871]	Fixed	
	Pembrolizumab	2	8	241	0.0349 [0.0175; 0.0683]	Fixed		1	0	210	0		
	Overall	4	15	338	0.0495 [0.0300; 0.0807]	Fixed		3	0	307	0.0074 [0.0015; 0.0358]	Fixed	
Stomatitis	Nivolumab	2	4	105	0.0456 [0.0171; 0.1157]	Fixed		2	2	105	0.0229 [0.0057; 0.0873]	Fixed	
HEPATIC													
Lipase increased	Nivolumab	2	5	105	0.0514 [0.0215; 0.1179]	Fixed		4	18	428	0.0454 [0.0288; 0.0710]	Fixed	
AST increased	Nivolumab	1	4	80	0.0500			1	2	80	0.0250		
	Pembrolizumab	1	2	31	0.0645			1	1	31	0.0323		
	Overall	2	6	111	0.0544 [0.0246; 0.1159]	Fixed		2	3	111	0.0272 [0.0088; 0.0810]	Fixed	
ALT increased	Nivolumab	1	3	80	0.0375			2	9	323	0.0279 [0.0146; 0.0528]	Fixed	
	Pembrolizumab	1	2	31	0.0645			1	1	31	0.0323		
	Overall	2	5	111	0.0465 [0.0195; 0.1070]	Fixed		3	10	354	0.0283 [0.0153; 0.0518]	Fixed	
ENDOCRINE													
Hypothyroidism	Nivolumab	2	7	40	0.1789 [0.0493; 0.4780]	Random	0.251	2	0	40	0.0241 [0.0034; 0.1520]	Fixed	0.463
	Pembrolizumab	3	33	259	0.1279 [0.0924; 0.1745]	Fixed		2	1	228	0.0098 [0.0025; 0.0385]	Fixed	
	Overall	5	40	299	0.1377 [0.1025; 0.1826]	Fixed		4	1	268	0.0132 [0.0043; 0.0403]	Fixed	
NEURAL													
Headache	Nivolumab	2	6	97	0.0844 [0.0080; 0.5126]	Random		2	0	97	0.0130 [0.0018; 0.0871]	Fixed	
	Pembrolizumab	1	13	210	0.0619			1	0	210	0		
	Overall	3	19	307	0.0768 [0.0246; 0.2155]	Random		3	0	307	0.0074 [0.0015; 0.0358]	Fixed	
Back pain	Nivolumab	2	4	97	0.0544 [0.0113; 0.2244]	Random		2	0	97	0.0130 [0.0018; 0.0871]	Fixed	0.669
	Pembrolizumab	1	4	210	0.0190			2	1	241	0.0132 [0.0007; 0.2022]	Random	
	Overall	3	8	307	0.0358 [0.0119; 0.1026]	Random		4	1	338	0.0184 [0.0059; 0.0559]	Fixed	
Myalgia	Nivolumab	2	8	97	0.0838 [0.0424; 0.1587]	Fixed		2	0	97	0.0130 [0.0018; 0.0871]	Fixed	
	Pembrolizumab	1	5	210	0.0238			1	0	210	0		

(Continued)

TABLE 2 | Continued

AEs	Treatment	Any grade						Grade ≥3					
		Included study	Event	Total patients	Pooled rate (95%CI)	Model	*P*-value	Included study	Event	Total patients	Pooled rate (95%CI)	Model	*P*-value
	Overall	3	13	307	0.0552 [0.0216; 0.1343]	Random		3	0	307	0.0074 [0.0015; 0.0358]	Fixed	
Arthralgia	Nivolumab	1	11	80	0.1375			1	0	80	0.0062		
	Pembrolizumab	1	8	210	0.0381			1	1	210	0.0048		
	Overall	2	19	290	0.0742 [0.0201; 0.2388]	Random		2	1	290	0.0069 [0.0017; 0.0270]	Fixed	
HEMATOLOGIC													
Anemia	Nivolumab	2	8	162	0.0507 [0.0256; 0.0982]	Fixed	0.955	2	3	162	0.0331 [0.0124; 0.0850]	Fixed	0.945
	Pembrolizumab	2	11	235	0.0579 [0.0005; 0.8856]	Random		2	5	235	0.0296 [0.0003; 0.7551]	Random	
	Overall	3	16	317	0.0576 [0.0098; 0.2741]	Random		3	8	317	0.0292 [0.0046; 0.1631]	Random	
Neutropenia	Nivolumab	2	10	162	0.0670 [0.0364; 0.1203]	Fixed	0.649	3	12	405	0.0322 [0.0183; 0.0558]	Fixed	0.243
	Pembrolizumab	3	14	253	0.0558 [0.0333; 0.0921]	Fixed		4	14	286	0.0685 [0.0216; 0.1969]	Random	
	Overall	5	24	415	0.0602 [0.0407; 0.0883]	Fixed		7	26	691	0.0479 [0.0234; 0.0953]	Random	
Lymphopenia	Nivolumab	3	8	185	0.0474 [0.0239; 0.0921]	Fixed		3	5	185	0.0276 [0.0115; 0.0647]	Fixed	
Platelet count decreased	Nivolumab	2	5	103	0.0553 [0.0038; 0.4766]	Random		2	0	103	0.0113 [0.0016; 0.0761]	Fixed	
	Pembrolizumab	1	11	25	0.4400			1	5	25	0.2000		
	Overall	3	16	128	0.1354 [0.0225; 0.5159]	Random		3	5	128	0.0414 [0.0034; 0.3530]	Random	
Leukopenia	Nivolumab	2	6	162	0.0390 [0.0176; 0.0841]	Fixed		2	3	162	0.0331 [0.0124; 0.0850]	Fixed	

Bold values indicates the classification of the adverse events.

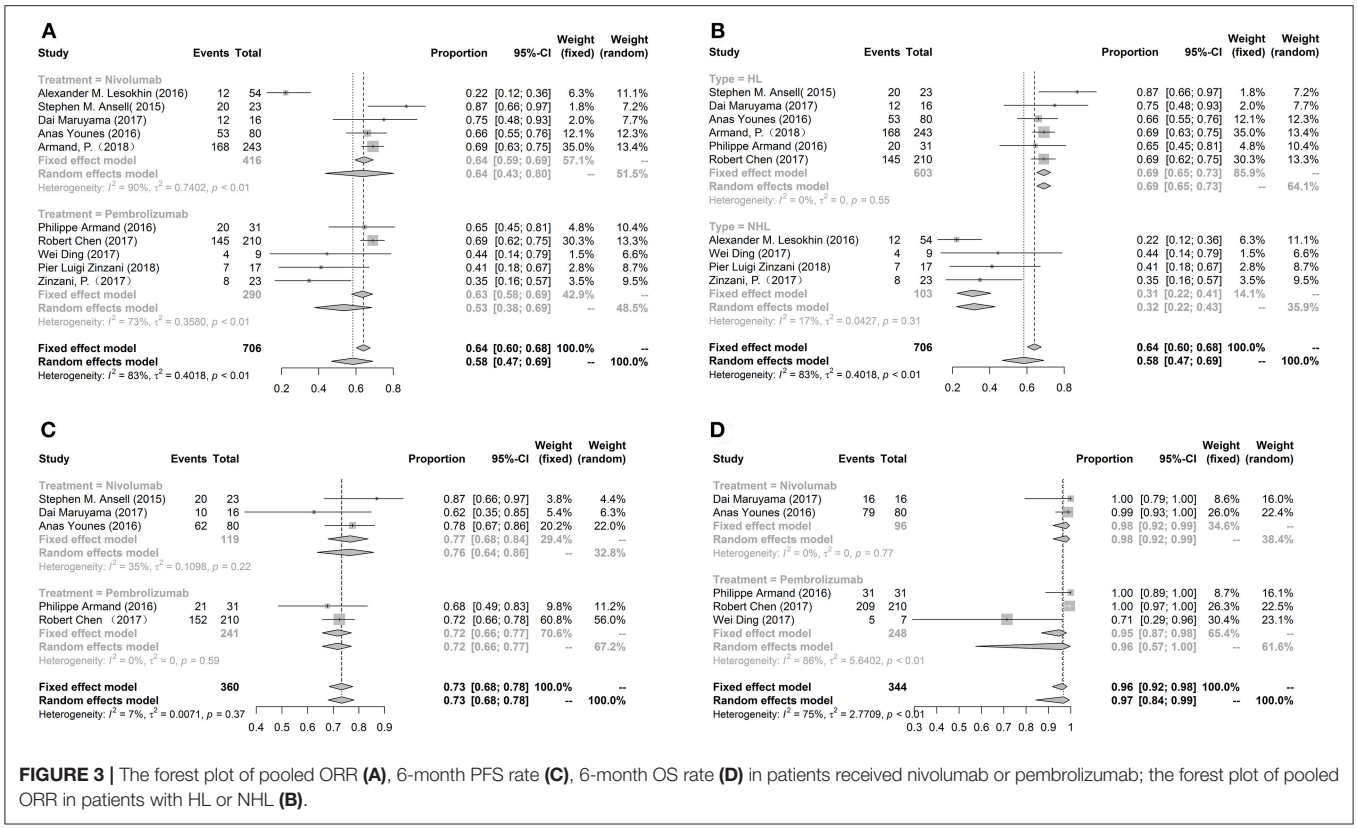

FIGURE 3 | The forest plot of pooled ORR **(A)**, 6-month PFS rate **(C)**, 6-month OS rate **(D)** in patients received nivolumab or pembrolizumab; the forest plot of pooled ORR in patients with HL or NHL **(B)**.

TABLE 3 | The scores of MINORS.

References	1	2	3	4	5	6	7	8	Total
Lesokhin et al. (2016)	2	2	2	2	0	2	2	0	12
Ansell et al. (2015)	2	2	2	2	0	2	2	0	12
Maruyama et al. (2017)	2	2	2	1	0	2	2	0	11
Younes et al. (2016)	2	2	2	2	0	2	2	0	12
Armand et al. (2018)	2	–	–	2	–	2	–	–	6
Armand et al. (2016)	2	2	2	2	0	2	2	0	12
Chen et al. (2017)	2	2	2	2	0	2	2	0	12
Ding et al. (2017)	2	2	2	1	0	2	2	0	11
Zinzani et al. (2018)	2	2	2	1	0	2	2	0	11
Zinzani P. L. et al. (2017)	2	–	–	2	–	–	–	–	4

(Joseph et al., 2015), bullous pemphigoid (Carlos et al., 2015), Stevens-Johnson syndrome, and toxic epidermal necrolysis (Postow, 2015) were also described and may be life-threatening. Immune-modulating medications like corticosteroids were usually utilized to treat the rash. Pyrexia was described in multiple immunotherapy, including cancer vaccines, adoptive T-cell therapy, chimeric antigen receptor T cells, and antibodies (Weber et al., 2015b). The cytokine release and nonspecific activation of an immune response may cause this AEs (Schwartz et al., 2002). Antipyretics such as acetaminophen or nonsteroidal anti-inflammatory drugs may solve the problem. Hypothyroidism was another common AEs, which can be managed with thyroid hormone replacement. Pneumonitis was common both in any grade and severe AEs. If pneumonitis grade >1, infectious diseases physicians and pulmonologist should exclude infectious etiologies, and oral or intravenous corticosteroids may be needed. Diarrhea and nausea are most commonly AEs in gastrointestinal disorders. Mild diarrhea can be cured with diet and antidiarrheal medications including atropine and oral diphenoxylate hydrochloride (Postow, 2015). Worsening or persistent diarrhea for more than 3 days should consider an infectious cause. Therefore, early detect and properly manage these immune-related AEs are very important. Additionally, the trails compared AEs of anti-PD-1 and traditional therapy should be performed to find an optimal treatment.

Arthritis, myositis, sicca syndrome, vasculitis were common AEs for anti-PD-1 antibodies in the type of rheumatology. Several studies suggested that patients with underlying autoimmunity, including rheumatic diseases, can be effectively treated by immune checkpoint inhibitors, but 1/3 of patients may occur the outbreak of underlying diseases (Johnson et al., 2016; Maul et al., 2016; Menzies et al., 2017). Therefore, rheumatologists and oncologists were needed to care of such patients and to explore the potential mechanisms of these complications (Calabrese and Mariette, 2018).

Former study (Lee et al., 2016) found that nivolumab and pembrolizumab combine with similar areas, but another study (Tan et al., 2017) suggested that the two antibodies bind to

completely different areas of PD-1. The pembrolizumab mainly binds to the C'D loop of PD-1, while nivolumab primarily binds to the N-loop, which is not involved in recognition of PD-L1. We found no difference in ORR, PFS and OS. However, the incidences of any grade AEs like fatigue and rash were lower in pembrolizumab than nivolumab, consistent with the previous study with lymphoma (Xu-Monette et al., 2017) and with advanced melanoma (Spain et al., 2016). This difference may because the different structures which the anti-PD-1 agents bind to play a different role in downstream cytokine signaling. Therefore, more randomized controlled trials are needed to detect the difference of safety between two agents, and further basic experiments are needed to explore the potential mechanism.

Generally, the expression of PD-1 is usually elevated on tumor-infiltrating T cells (TILs) in lymphomas, especially observed in HL (Yamamoto et al., 2008; Muenst et al., 2009) than in NHL (Ahearne et al., 2014; Kiyasu et al., 2015; Kwon et al., 2016). Similarly, we showed that the ORR in patients with HL was higher than NHL. It may suggest that the anti-tumor activity is an association with PD-1 expression. Additionally, PD-L1/PD-L2 expression often increased in cHL (97%) (Roemer et al., 2016) and PMBCL (70%) (Green et al., 2010) because of copy-number gain or amplification of 9p24.1. Meanwhile, Epstein-Barr virus (EBV) infection also may lead PD-L1 overexpression in HL (Kieser et al., 1997; Green et al., 2012; Ok et al., 2013). Therefore, anti-PD-1 antibodies inhibited PD-L1/PD-L2 binding to PD-1, increasing the anti-tumor activity of T cells in HL. We could not evaluate the differences of PFS and OS between HL and NHL due to the limitation of study number. However, some studies showed that high expression of PD-1 on TILs was related to poor prognosis (OS) (Muenst et al., 2009) and disease-specific survival (Greaves et al., 2013). Therefore, more randomized controlled trials are needed to detect the difference of efficacy between HL and NHL.

Previous study (Georgieva et al., 2018) has demonstrated that first-line pembrolizumab for non-small cell lung cancer may be cost-effective in the US but not the UK, in spite of very similar incremental cost-effectiveness ratios values in both countries. Therefore, the cost must be considered to use anti-PD-1 antibodies for patients.

Our study has several limitations. First, the study number was limited, which may make the data skewed. Second, there were only phase I/II studies without double-blinded RCT, which may lead the potential performance bias. Third, the survival time and PFS time didn't present individually, so we can't perform the survival analysis.

In conclusion, this meta-analysis demonstrated that nivolumab and pembrolizumab have potential effects of ORR, 6-month OS rate and 6-month PFS rate, while the AEs and drug-related deaths were tolerable in patients with relapsed or refractory lymphoma. We also demonstrated that pembrolizumab had a lower risk of AEs than nivolumab, and patients with HL had a better ORR than NHL. Further researches with these novel drugs are needed to compare with traditional therapy for patients with relapsed or refractory lymphoma.

AUTHOR CONTRIBUTIONS

HZ collected, analyzed the data and wrote the article. XF and QL collected data, prepared the pictures and tables. TN provided the idea and modified the article. All authors read and approved the final manuscript.

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REVIEW
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A Review of Efficacy and Safety of Checkpoint Inhibitor for the Treatment of Acute Myeloid Leukemia

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Immune checkpoint inhibitors (ICIs) as positive modulators of immune response have revolutionized the treatment of cancer and have achieved impressive efficacy in melanoma and numerous solid tumor malignancies. These agents are being investigated in acute myeloid leukemia (AML) to further enhance response rate as induction therapy and to improve relapse-free survival (RFS) post chemotherapy and bone marrow transplantation. PD-1 and CTLA-4 are the two most actively investigated checkpoint receptors, which play a role in different stages of anti-tumor immune response. This study reviews data from ongoing phase I, II clinical trials evaluating PD-1 and CTLA-4 inhibitors on AML patients and discusses especially efficacy and adverse events as well as prospects of these drugs in treating AML. Single anti-PD-1 monoclonal antibody infusion shows rather modest clinical efficacy. While combinations of PD-1 inhibitor with hypomethylating agents (HMAs) represent encouraging outcome for relapsed/refractory (R/R) AML patients as well as for elderly patients as first-line therapy option. Adding PD-1 inhibitor to traditional induction therapy regimen is also safe and feasible. CTLA-4 inhibitor ipilimumab exhibits specific potency in treating relapsed AML patients with extramedullary disease in later post-transplantation stage. In terms of side effects, irAEs found in these trials can mostly be appropriately managed with steroids but are occasionally fatal. More rationally designed combinational therapies are under investigation in ongoing clinical trials and will further advance our understanding of checkpoint inhibitors as well as lead us to the most appropriate application of these agents.

Keywords: checkpoint inhibitor, acute myeloid leukemia, safety, efficacy, immunotherapy

Abbreviations: Allo-SCT, Allogeneic hematopoietic stem cell transplantation; AML, Acute myeloid leukemia; ASCT, Autologous stem cell transplant; CR, Complete remission; CRi, CR with incomplete count recovery; CRp, CR with incomplete platelet recovery; CTLA-4, Cytotoxic T-lymphocyte-associated protein 4; EFS, Event-free survival; GVHD, Graft-versus-host disease; GVL, Graft-versus-leukemia; HMA, Hypomethylating agents; ICIs, Immune checkpoint inhibitors; irAEs, Immune-related adverse events; MHC, Major histocompatibility complex; MRD, Minimal residual disease; ORR, Overall response rate; OS, Overall survival; PD-1, Programmed-death 1; PR, Partial remission; R/R, Relapsed/refractory; SD, Stable disease; TCR, T-cell receptor; Treg cells, Regulatory T cells.

INTRODUCTION

Acute myeloid leukemia (AML) is a form of cancer originated from malignant clonal stem cells in bone marrow marked by heterogenous clinical outcome due to the complexity of its molecular and cytogenetic architecture (Dohner et al., 2015). For a long period of time, the treatment options for AML are limited to chemotherapy and hematopoietic stem cell transplantation. However, despite the progression in remission rate with many newly approved chemo-drugs, there are still a bunch of problems that need to be solved regarding treatment efficacy of AML, such as resistance to chemotherapy, relapse after transplantation, and non-tolerance of older patients to high-intensity chemotherapy. Thus, there is a desperate need for innovative approaches. In recent years, with the deepened understanding of the role of immune evasion in tumor maintenance as well as development of immunotherapy, the great wave of antibody therapy is refactoring the field of cancer treatment. Among various immunotherapy approaches, using checkpoint inhibitors to block inhibitory molecules on T cell surface thus reversing T cell from "exhausted" state to "activated" state to kill tumor cells has proven to be a promising option. Following the success of immune checkpoint inhibitors (ICIs) in solid tumors such as melanoma and non-small cell lung cancer, these drugs are being explored in hematopoietic malignancies including AML (Hodi et al., 2010; O'Day et al., 2010; Rizvi et al., 2015). The inhibition of CTLA-4 and PD-1 are the two most commonly used clinical strategies as immune checkpoint blockade. As proven by the efficacy of allogeneic hematopoietic stem cell transplantation (allo-SCT), leukemia is the typical immune responsive tumor type. Besides, leukemia cells express high level of checkpoint inhibitor receptors for sharing an immune cell lineage, making them potential targets for this treatment (Vollmer et al., 2003; Whiteway et al., 2003; Graf et al., 2005).

IMMUNITY AND TUMOR/ACUTE MYELOID LEUKEMIA

The immune system helps to defend the body against foreign invaders such as bacteria and tumor cells by distinguish between self and non-self. This complex while delicate system plays an essential role in anti-tumor response. Under normal physiological conditions, immune system could recognize a wide variety of neo-antigens expressed on the surface of tumor cells caused by genetic abnormalities (Desrichard et al., 2016). Aside from fusion proteins and mutated proteins, immune system can also recognize the products of non-mutated genes that are preferentially expressed by tumor cells. The effective anti-tumor response contains three main steps (Mellman et al., 2011). Firstly, antigen presenting cells (APCs) such as dendritic cells ingest the antigens, fragment them into antigen peptides, and display them on the surface of the cell joined together with major histocompatibility complex (MHC) molecules. Next, these APCs roam to lymphoid tissues where T cell resides. By recognizing specific peptide-MHC complex, accompanied by costimulatory signals, T cells are activated into effector T cells, which mainly are CD8 positive subpopulation that are capable of attacking infected cells or tumor cells. Finally, the tumor-immune response happens when activated effector T cells infiltrate the tumor bed.

Activating the immune system either passively or spontaneously has long been a goal in cancer treatment for therapeutic benefit. Extraordinary effort has been made throughout history in cancer immunotherapy. On the one hand, doctors fed the patients with anticancer monoclonal antibodies or clear the leukemia cells by the graft-versus-leukemia (GVL) effect when patients receive allogeneic bone marrow transplantation (Ruggeri et al., 2002; Dougan and Dranoff, 2009). These were potent measures for a variety of hematological malignancies as well as solid tumors. On the other hand, scientists tried to provoke spontaneous anti-tumor immunity. Coley, the so-called "father of immunotherapy," tried to treat his patient with "Coley's toxins"—the two dead bacteria, *Streptococcus pyogenes* and *Serratia marcescens*—by causing inflammation and destroying tumor cells through activated antibacterial cells. Though this formula remained controversial in the medical field due to the infection risk, Coley's work showed the possibilities of immunotherapy in cancer, thus leading cancer treatment into a new era (Nossal, 1993).

Among various methods of cancer immunotherapy, inhibiting the immune suppression that contributes a large part to sustaining tumor is of great concern. Cancer cells escape from attacks from immune system by a variety of mechanisms that influence different stages of cancer-immune response circuit. By releasing several kinds of mediators, adenosine for instance, tumors could suppress T-cell activation and enable expansion of regulatory T cells (Treg cells) whose function is to oppose the activity of effector T cells (Ohta, 2016). Another mechanism of tumor to prevent T-cell activation is related to the co-stimulatory signals. Cancer cells with high expression of CTLA4 negatively modulate activated T cells through competitively binding to co-stimulatory molecules on T cell surface (Walunas et al., 1994). Tumor cells can also downregulate their MHC molecule expression to avoid T cell recognition. Up-regulation of several inhibitory molecules such as PD-1 on the surface of tumor cells could cause T-cell anergy or exhaustion after engagement of their ligands on T cells. Based on above mechanisms, several kinds of targeted immunotherapies are under testing, including monoclonal antibodies, immune adjuvants, cytokines, and ICIs. To achieve deeper remission in AML patients, bone marrow transplantation is an effective treatment. Despite the high response rate in some patients, there are still a group of them suffering from disease relapse after transplantation. Studies found that patients with graft-versus-host disease (GVHD) were 2.5 times less likely to relapse compared with those without (Weiden et al., 1979). Lower relapse rate was observed in patients without GVHD who received allografts than those who received identical twin transplants. These results supported an anti-leukemia effect of allogeneic grafts independent of GVHD and suggested the possibility and rationality of boosting immune system to treat AML (Horowitz et al., 1990).

CHECKPOINT INHIBITION IN ACUTE MYELOID LEUKEMIA: PRECLINICAL EVIDENCES

Blockade of PD-1 in Acute Myeloid Leukemia

TCR (T-cell receptor)-mediated T cell activation is regulated by co-signaling molecules expressed on T cells, which can be divided into two classes: co-inhibitor and co-stimulator, based on their functional outcome. The balance between positive and negative adjustment of T cell activation relies on spatial and temporal expression of the co-stimulator and co-inhibitor ligands on tumor cells and antigen-presenting cells (Chen, 2004). PD-1 as an inhibitory checkpoint receptor is expressed on activated T cells, B cells, and myeloid cells. As a co-inhibitory molecule, PD-1 could lead to the attenuation of TCR-mediated signal after the engagement with its ligand PD-L1 (B7-H1) expressed on the surface of tumor cells or antigen-presenting cells in the tumor microenvironment (Freeman et al., 2000) (**Figure 1**). Recent studies suggest a novel mechanism that tumor cells might evade host immune attack through increased expression of PD-L1 (Dong et al., 2002). In tumor immune response, up-regulated PD-L1 molecule on tumor cell surface mediates T-cell anergy or exhaustion (Butte et al., 2007; Francisco et al., 2009). This up-regulation is possibly a result from pro-inflammatory cytokines such as interferon-γ produced by tumor infiltrating inflammatory cells (Dong et al., 2002).

Studies on murine models show the importance of PD-1/PD-L1 pathway in immune evasion in hematological malignancies and provide a rationale for targeting this pathway in clinical trial for leukemia patients. Scientists found that PD-L1 expression was up-regulated on C1498 (a murine AML cell line) when growing *in vivo*. PD-1 knockout mice could generate stronger immune response when transferred with C4198 and bore lower leukemia burden as well as showing longer survival. After using the antibody for PD-L1, similar results were obtained (Zhang et al., 2009). Another study on murine model found that co-expression of PD-1 and Tim-3 on CD8+ T cells increased during AML progression, and instead of blocking single pathway, combined PD-1/PD-L1 and Tim-3/galectin-9 blockade led to the reduction of tumor burden and lethality (Zhou et al., 2011). Treg cells play a negative part in anti-tumor immune response. In a systematic model of murine AML, tumor progression contributed to accumulation of regulatory T cells and elevation of expression of PD-1 molecules on CD8+ T cells in the tumor microenvironment. AML-associated Treg cells could suppress the function ability of activated CD8+ T cell. Using anti-PD-1 treatment on mice model prolonged the survival of CD8+ T cells at tumor sites, which led to tumor burden decrease and long-term survivors. Treg cell depletion following PD-1/PD-L1 blockade showed better therapeutic outcome. These data indicated a new approach of PD-1/PD-L1 blockade together with Treg cell depletion for treating AML patients by improving anti-tumor activation of AML-associated CD8+ T cell (Zhou et al., 2010).

Increasing data have shown a higher expression of PD-L1 in AML cells in some patients. And the expression level of PD-L1 was closely related to disease relapse, which was regarded as an independent negative prognostic factor (Chen et al., 2008). In order to illustrate the significance of checkpoint inhibitor expression level in tumor microenvironment, Daver and his partners performed 17-color multi-parameter flow-cytometry on bone marrow aspirates from 74 AML patients. Thirty-six of them were untreated AML patients and the rest were relapsed ones. This study showed that compared to healthy controls, PD-1 expression level was significantly higher in all T cell subpopulations both in untreated cohort ($P < 0.05$) and relapsed group ($P < 0.006$) (Daver et al., 2016). Other researchers found PD-1 expression level both on CD8+ and CD4+ T cell increased significantly at relapse stage after stem cell transplantation (Schnorfeil et al., 2015).

Blockade of CTLA-4 in Acute Myeloid Leukemia

CTLA-4 is a surface molecule expressed on activated T cells that regulates and mediates inhibitory signal to T cells. Sharing similar structure with its homologous T-cell co-stimulatory protein CD28 and with higher affinity to their common ligands, it competitively binds to CD80 and CD86 expressed by APCs thus resulting in negative effector T cell activation (**Figure 2**). CTLA-4 is an important mediator of self-tolerance and tolerance to tumor antigens. Treg cells often express high level of CTLA-4 and this could partly explain its suppressive function (Takahashi et al., 2000).

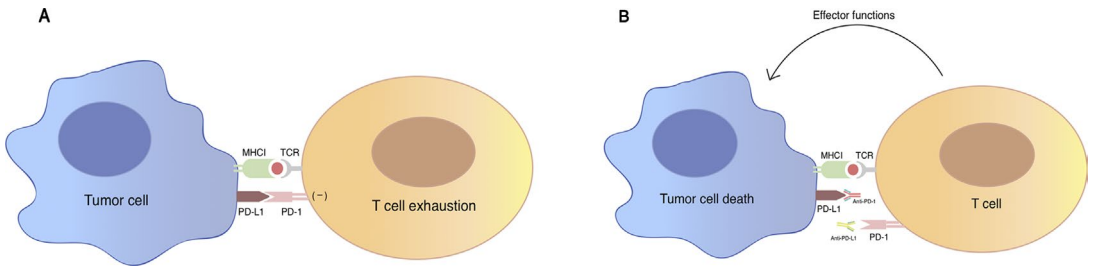

FIGURE 1 | An illustration of PD-1/PD-L1 mediated immune tumor response. **(A)** PD-1 is a co-inhibitory molecule expressed on T cell, B cells, and myeloid cells. Binding of PD-1 to its B7 family of ligands PD-L1 on tumor cells results in suppression of proliferation and immune response of T cell, which are described as the "exhaustion" state of T cell. Activation of PD-1/PD-L1 signal pathway serves as a major mechanism of immune evasion by tumor cells. **(B)** Antibody blockade of PD-1 and PD-L1 reverses this process and enhances anti-tumor immune response. TCR, T-cell receptor; MHC, major histocompatibility complex; PD-1: programmed cell death protein 1; PD-L1: programmed death-ligand 1.

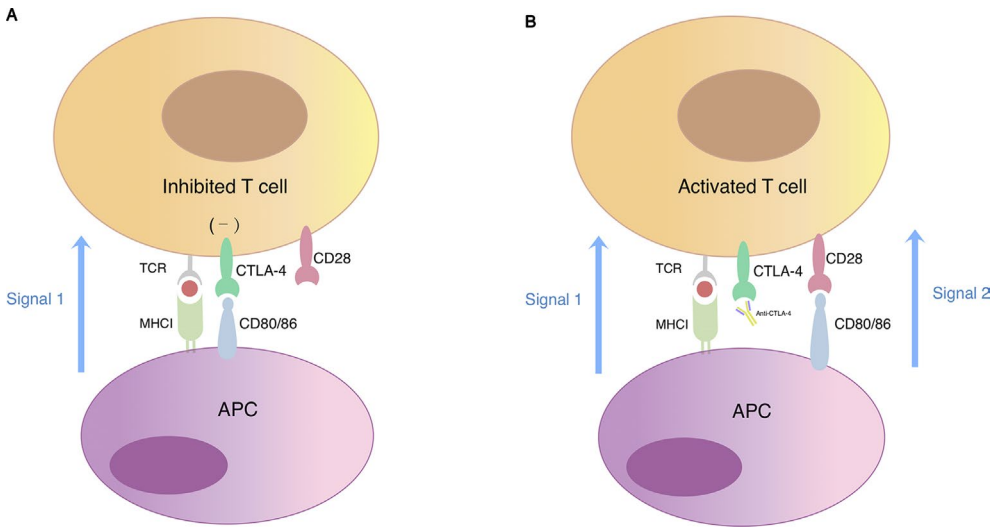

FIGURE 2 | T cell activation regulated by CTLA-4 and CD28. **(A)** Simultaneous recognition of a specific major histocompatibility complex (MHC)–peptide complex by the T cell receptor (TCR) and of CD80/CD86 by the co-stimulatory receptor CD28 results in T cell activation. Cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) is a CD28 homologue expressed on the surface of T lymphocytes with higher affinity for CD80/CD86. When CTLA-4 competitively binds to CD80/CD86, signal 2 required for T cell activation reduces, which eventually leads to T cell anergy. **(B)** The blockade of CTLA-4 signaling restores signal 2 in response to binding of CD28 with CD80/CD86 thus promoting T cell activation and proliferation.

In an AML mouse model, persistent leukemic cells showed more resistance to specific cytotoxic T cells and presented higher expression level of PD-1 and CD80. Blocking of these PD-1 or CTLA-4/CD80 interaction could enhance CTL-mediated killing of persistent cells *in vitro* and prolonged mice survival *in vivo* (Saudemont and Quesnel, 2004). By analyzing AML patient samples, scientists found that 80% of AML samples tested at diagnosis constitutively expressed CTLA-4 and that CTLA-4 blockade might be a way to induce killing of leukemic cells through apoptosis (Pistillo et al., 2003; Laurent et al., 2007).

CTLA-4 blockade also plays a part in eliminating minimal residual disease (MRD) in AML. Dr. Saudemont found that when mice with residual disease were treated with anti-CTLA4 monoclonal antibody, persistent leukemic cells could be further cleared by enhanced CTL-mediated killing (Saudemont and Quesnel, 2004).

In a murine model, Dr. Blazar found that graft-versus-host effect was enhanced by anti-CTLA4 antibody infusion in the early course of post-bone marrow transplantation, which mainly depended on CD28. However, in the later course of post-transplantation stage, CTLA-4 blockade produced limited GVHD but augmented GVL effect of donor lymphocytes against host-derived leukemic cells (Blazar et al., 1999).

CHECKPOINT INHIBITION THERAPY IN THE CLINIC

PD-1 Inhibition

The PD-1 inhibitors that are actively investigated in clinical trials include pidilizumab, nivolumab, pembrolizumab, durvalumab, and atezolizumab.

Nivolumab

Nivolumab is a human IgG4 anti-PD-1 monoclonal antibody. It is used as a first-line treatment for metastatic melanoma in combination with ipilimumab and as a second-line treatment for squamous non-small cell lung cancer as well as renal cell carcinoma (Johnson et al., 2015; Sundar et al., 2015). In 2016, the FDA approved nivolumab for patients with relapsed or progressed classical Hodgkin's lymphoma after stem cell transplantation.

Aside from single agent approaches, scientists are trying to find novel therapeutic combinations of ICIs with other drugs to achieve better clinical outcome.

An interesting find is that epigenetic drugs could modulate the expression of checkpoint molecules on tumor-immersed lymphocytes as well as tumor cells. By treating MOLT-4 cells (a lymphatic leukemia cell line) with different concentration of 5-azacytidine, Zhang et al. found that PD-1 expression was positively related to the concentration of 5-azacytidine. This team demonstrated that PD-1 over-expression on lymphocytes was caused by the demethylation of promoter by 5-azacytidine, and changing the methylation state of PD-1 genes to recover T cell function could be a novel treatment direction (Zhang et al., 2011). Hypomethylating agent (HMA) 5-azacytidine was used as a standard regimen in treating older AML patients (Kantarjian et al., 2012). Yang et al. (2014) found that PD-1 as well as its two ligands PD-L1 and PD-L2 were up-regulated on CD34+ cells in patients with myeloid leukemia and their over-expression may contribute to treatment resistance to azacytidine. These evidences lead to several clinical trials combining epigenetic therapy with PD-1/PD-L1 blockade to improve response and survival rate in AML.

In an open-label, phase II study, Dr. Daver assessed the efficacy of combination therapy of nivolumab and azacytidine in R/R AML

patients and the results were quite encouraging (Daver et al., 2019). This study enrolled 70 AML patients who previously received therapies including HMA. Among the 70 patients, the overall response rate was 33% including 16 (24%) patients who achieved complete remission (CR)/CR with incomplete blood count recovery (CRi)/partial remission (PR) and 7 of them reaching the standard of hematologic improvement. Six patients (9%) remained on study for over 6 months without either remission or clinical deterioration. The remaining 41 (58%) patients showed no response to therapy. Compared with historical controls in the entire population, the ORR of this study was higher with 33% versus 20%. In the subgroup of patients who did not receive HMA prior treatment, the superiority of new regimen was even more evident with ORR at 52% to 22%. The median overall survival (OS) was also higher in novel treatment group with 6.3 months versus 4.6 months (P = 0.013). Similarly, the event-free survival (EFS) was longer (4.2 vs 2.2 months). As for toxicities, grade 2 and grade 3–4 irAEs were observed in eight (11%) and eight (11%) patients respectively, which was similar to that observed in solid tumors. Among the patients with grade 2–4 side effects, pneumonitis was the most common with nine patients who suffered from such episodes. The rest included nephritis in six patients, skin rash related to immune response in three patients, and transaminitis in two. Steroids took effect on 88% of the patients who suffered from drug-related toxicities, and these 14 patients took on nivolumab treatment safely later on. Two patients died due to irAEs, both of which were refractory to steroids as well as subsequent infliximab therapy. Majority of the irAEs happened in the first 8 weeks after initial treatment of nivolumab. By performing multiparameter flow cytometry on bone marrow aspirates pre-therapy and on-therapy, they found that CD3+ and CD8+ T cells in the pre-therapy bone marrow aspirates were the best predictors of response, with the cut-off rate at 13.2% and 4.01%, respectively. These were well-recognized biomarkers in other solid tumors. CTLA-4 expression level on effector CD4+ and CD8+ T cells was increased in bone marrow aspirate samples from patients who showed no response to the treatment compared with responders. This indicated that the up-regulation of CTLA-4 was a potential mechanism of resistance to PD-1 blockade in non-responders, which had been seen in the therapeutic process in most solid tumors.

Another batch of enrolling cohorts conducted by the same team focused on frontline AML patients older than 65 years. In a 2017 ASH abstract, Daver et al. reported the preliminary results. Ten patients were treated with the combination of nivolumab and azacytidine with a median age of 75. Nine of them are evaluable for response: two CR, three CRp (CR with incomplete platelet recovery), one PR, one stable disease (SD) > 6 months, and two NR (no response) (Daver et al., 2017).

One year later, on the 60th ASH meeting, Dr. Daver reported their encouraging early findings on the study of treating salvage 1–2 R/R AML patients with nivolumab, azacytidine, and ipilimumab (NCT02397720) (Daver et al., 2018). Among the 14 evaluable patients, 43% of them achieved CR/CRi/CRp (n = 6). The median overall survival time for all patients was not reached and the projected 1-year overall survival rate was 58%.

On the same meeting, Dr Rita Assi and his colleagues reported their findings in a phase II study of accessing the addition of nivolumab to standard frontline therapy in patients with AML (NCT02464657) (Assi et al., 2018). This study enrolled 42 AML patients and 2 high-risk MDS patients with a median age of 54. Most of them were diagnosed with *de novo* AML (73%) and the remaining were therapy-related AML (7%) and high-risk myelodysplastic syndrome (4%). Nineteen patients had adverse genetic risk. Among the 44 evaluable patients, the ORR was 77% including 63% CR and 14% CRi. Thirty-four patients achieved CR or CRi, and among them, 18 patients were MRD (minimal residual disease) negative at the time of response. Nine of the remaining responders became MRD negative during additional follow-up at 1 to 3 months of nivolumab therapy. The median relapse free survival for patients who achieved response was 18.5 months and the median overall survival was 18.54 months. There was a trend of improved median OS when compared with a historical cohort of patients treated with cytarabine and idarubicin alone (mOS = 13.2 m). Concerning drug toxicities, the grade 3–4 adverse events were observed in six patients, including the rash found in two patients, colitis in two patients, and pancreatitis and transaminitis in one patient, respectively. Grade 3/4 cholecystitis in one patient possibly attributed to nivolumab. These events could be reversed by drugs. Eighteen patients proceeded to allo-SCT; 13 of them developed GVHD (grade I/II in 8, grade III/IV in 5). Eight patients with GVHD responded to treatment quite well. This group also performed multicolor flow cytometry studies and evidences showed that the co-expression of PD-1 and TIM3 (P = 0.04) on CD4-positive effector T cells in bone marrow was higher among non-responders compared with those who achieved remission, which indicated that up-regulation of TIM3 may contribute to drug resistance through some mechanism.

Using nivolumab in post-transplantation setting showed limited efficacy. Davids et al. (2018) reported severe adverse events in their phase I/Ib study on evaluating the safety of nivolumab in patients with relapsed hematological malignancies after allo-SCT. In the study, 28 patients were treated, with 11 relapsed AML patients. The median time post-transplantation was 21 months. Twenty-two patients were treated with 0.5 mg/kg nivolumab after two patients of first cohort (n = 6) on 1 mg/kg resulted in dose-limiting toxicity. However, accrual was terminated due to early GVHD and severe irAEs. Two patients developed grade III GVHD (liver and gut) together with grade 3 elevated bilirubin (n = 1) and grade 3 transaminitis (n = 1). Both of these two patients died from complications of GVHD. On the 0.5 mg/kg cohort, 10 patients (45%) had new onset or worsening GVHD. Other irAEs included grade 4 lipase elevation and grade 3 hypotension. Only one patient with AML achieved PR.

Eric et al. demonstrated the result of interim assessment on six patients with relapsed hematological malignancies treated with nivolumab after allo-SCT (Wong et al., 2018). Patients received 3 mg/kg nivolumab for up to 48 weeks. The median time from allo-SCT to first nivolumab administration was 25.5 months. Among the six patients, two AML patients showed no response with one participant achieving initial blast reduction (from 21% to 13%) but deteriorated in the end. Two patients developed grade III GVHD within the first 2 weeks after nivolumab treatment.

A number of trials evaluating nivolumab as a single agent in controlling AML and eliminating MRD are recruiting patients (NCT02275533, NCT02532231). **Table 1** lists currently active clinical trials of PD-1/PD-L1 inhibitors in AML.

TABLE 1 | Ongoing clinical trials of immune checkpoint blockade in AML.

Setting	Target	Drug	NCT number	Phase of study	Study population	Therapy regimen	Objective	Status
AML and high-risk MDS	PD-1	Nivolumab	02464657	Phase I/II	AML or high-risk MDS	Nivolumab + idarubicin + cytarabine, single arm	MTD, EFS	Recruiting
Remission maintenance	PD-1	Nivolumab	02275533	Phase II	AML in remission	Single agent, two arms	PFS; OS, toxities	Recruiting
	PD-1	Nivolumab	02532231	Phase II	AML in remission, high risk for relapse	Single agent, single arm	RFS (time frame 6 months)	Recruiting
	PD-L1	Atezolizumab	03154827	Phase Ib/II	AML (>60 years) in remission	Atezolizumab + BL-8040	RFS (time frame: up to 5 years)	Recruiting
R/R AML	PD-1	Nivolumab	02397720	Phase II	R/R AML or elderly *de novo* AML patients	Azacitidine+nivolumab or azacitidine+nivolumab+ipilimumab, two arms	MTD, ORR of nivolumab with azacitidine, adverse event	Recruiting
	PD-1	Pembrolizumab	02768792	Phase II	R/R AML	Pembrolizumab after high-dose cytarabine as induction therapy	OR (CR/Cri); toxicities	Recruiting
	PD-1	Pembrolizumab	02845297	Phase II	R/R AML or elderly *de novo* AML patients	Pembrolizumab following azacitidine, single arm	MTD; ORR (CR, CRi)	Recruiting
	PD-1	Pembrolizumab	02996474	Phase I/II	R/R AML	Pembrolizumab and decitabine	Feasibility; efficacy	Active, not recruiting
	PD-1	Pembrolizumab	03291353	Early phase I	Refractory AML	Single agent, single arm	Adverse event; RR, OS	Recruiting
	PD-1/ TIM-3	PDR001/MBG453	03066648	Phase I	R/R AML or *de novo* AML not suitable for standard therapy	Decitabine+PDR001 or decitabine+MBG453 or decitabine+PDR001+MBG453 or MBG453 alone or PDR001+MBG453	Safety, DLT	Recruiting
	CTLA-4	Ipilimumab	01757639	Phase I	R/R AML	Single agent, single arm	DLT, T-reg cell percentages; efficacy, PFS, OS	Completed
	CTLA-4	Ipilimumab	02890329	Phase I	R/R AML or elderly *de novo* AML	Ipilimumab and decitabine	MTD; clinical response	Recruiting
High risk or old age not eligible transplant	PD-1	Pembrolizumab	02708641	Phase II	Elderly AML (>60 years) not eligible for transplantation	Single agent, single arm	Time to relapse; OS	Recruiting
	PD-1	Pembrolizumab	02771197	Phase II	High-risk AML not eligible for transplant	Pembrolizumab following lymphodepletion therapy (fludarabine+melphalan), single arm	2-year relapse risk; safety	Recruiting
	PD-L1	Durvalumab	02775903	Phase II	Elderly AML (>=65 years) not eligible for transplantation	Durvalumab+azacitidine	ORR (CR/CRi)	Active, not recruiting
	CTLA-4	Ipilimumab	00039091	Phase I	AML in remission, not eligible for transplant	Single agent, single arm	Toxicities; ORR	Terminated
Post transplant	PD-1	Pembrolizumab	03286114	Phase I	AML relapse after allo-SCT	Single agent, single arm	Clinical benefit; response rate	Recruiting
	PD-1	Pembrolizumab	02981914	Early phase I	AML relapse after allo-SCT	Single agent, single arm	Adverse event; duration of response	Recruiting
	PD-1	Ipilimumab+ nivolumab	02846376	Phase I	AML after allo-SCT	Ipilimumab or nivolumab or ipilimumab+nivolumab	Safety (DLT); toxicities	Recruiting
	PD-1	Ipilimumab or nivolumab	01822509	Phase I/Ib	AML relapse after allo-SCT	Ipilimumab or nivolumumab, single arm	MTD, adverse events; RR, PFS, OS	Active, not recruiting
	PD-1	Ipilimumab or nivolumab	03600155	Phase I	High-risk AML or relapsed AML after allo-SCT	Nivolumab or ipilimumab or nivolumab+ipilimumab	MTD, DLT; ORR, DFS, OS	Recruiting
	CTLA-4	Ipilimumab	00060372	Phase I	AML after allo-SCT	Single agent, single arm	Safety dose	Completed
	CTLA-4	Ipilimumab	01919619	Early phase I	AML after allo/auto-SCT	Ipilimumab+lenalidomide	Toxicity rate (time frame: 28 days)	Recruiting

AML, acute myeloid leukemia; MDS, myelodysplastic syndrome; MTD, maximum tolerated dose; EFS, event-free survival; PFS, progression-free survival; OS, overall survival; RFS, recurrence-free survival; ORR, overall response rate; RR, response rate; DLT, dose limiting toxicity; DFS, disease-free survival; allo-SCT, allogeneic stem-cell transplantation; CR, complete remission; CRi, complete remission with incomplete hematologic recovery.
Objectives are listed as "primary; secondary" outcome measures.

Pembrolizumab

Another PD-1 blockade drug is pembrolizumab (formerly known as MK-3475 or lambrolizumab), an IgG4 isotype antibody. The FDA initially approved it in treating metastatic melanoma, and this drug was further approved to be used on unresectable or metastatic solid tumor with certain genetic anomalies (Syn et al., 2017).

Based on the previous study results, Dr. Joshua F. Zeidner conducted a multicenter phase II study to evaluate clinical outcome of the administration of pembrolizumab after high-dose cytarabine salvage chemotherapy (NCT02768792) (Zeidner et al., 2018). His group reported their early findings of this ongoing study on the 60th ASH meeting. Twenty-six R/R AML patients with median age of 54 had been evaluated for response and safety; 46% (n = 12) of the patients were in genetic adverse group according to ELN-risk standard. The overall response rate was 42% with nine CR/CRi (35%), one PR, and one MLFS (morphologic leukemia free state). Five of nine CR/CRi patients were MRD negative by standard monitoring. Four patients proceeded to allo-SCT in CR (n = 3) and MLFS (n = 1). Steroid responsive-grade II acute and moderate chronic GVHD was observed in two (50%) of them post-transplantation. With a median follow-up of 10.8 months, the median OS was 10.5 months. Most frequently observed grade 3 irAEs included hepatitis (n = 2), rash (n = 2), and epigastric pain of liver mass-lymphocytic infiltrate (n = 1). All the above events responded quite well to steroid treatment or resolved spontaneously without pharmaceutical intervention. Peripheral blood analysis revealed an increased diversity of TCR Vβ repertoire on CD8+ T cells in those who responded to PD-1 blockade therapy compared with non-responders. RNA-seq data from different cell fraction of bone marrow revealed specific gene expression profile correlated with response to therapy and these biomarkers were present prior to therapy.

Preliminary results of a single center, single arm trial of pembrolizumab (200 mg/m^2) on day 1 in every 3-week cycle in R/R AML patients followed by decitabine (20 mg/m^2) on days 8–12 and days 15–19 for 8 cycles were reported on the 60th ASH meeting (NCT02996474) (Lindblad et al., 2018). Ten patients with median age of 62 were enrolled, 7 with refractory disease and 3 with relapsed AML. Of the 10 evaluable patients, the ORR was 20% with one patient achieving MRD-negative CR and another one meeting the criteria of MLFS. With a median follow-up of 13 months to date, the mOS was 7 months. irAEs included grade 4 hypotension observed in one patient, grade 3 bilirubin elevation (n = 1), and diarrhea (n = 1). Two patients suffered from hypothyroidism (<grade 3) and another patient developed central diabetes insipidus that possibly attributed to pembrolizumab.

Pembrolizumab is also tested in post-transplantation setting in a prospective clinical trial. Justin Kline et al. (2018) reported an ongoing study of pembrolizumab for treatment of relapse of disease following allogeneic hematopoietic cell transplantation (NCT02981914). Eleven patients with hematopoietic malignancies including eight AML and three lymphoma were included. Out of these patients, seven were evaluable for response. AML patients showed modest response to pembrolizumab with two patients who had stable disease and another two who experienced disease progression. irAEs of any grade were observed in 63% of the patients. Grades 3–4 irAEs were reported in three patients with pneumonitis (n = 2) and hyperthyroidism (n = 1), which occurred within 3–6 weeks after pembrolizumab administration. These adverse events were resolved after pembrolizumab discontinuation and corticosteroid treatment.

Pidilizumab

CT-011 (Pidilizumab) is a humanized IgG1 monoclonal antibody that interacts with PD-1 to positively modulate antitumor immune response of T cells.

The interaction of this drug with PD-1 expressed on lymphocytes stimulates T cell activity and prolongs effector T cell survival. In a phase I clinical trial conducted by Berger et al., this drug was administered to patients with advanced stage hematological malignancies including eight AML patients, four of which had accepted allo-SCT previously. The result was rather modest with only one AML patient achieving a minimal response presented by a drop in peripheral blasts percentage from 50% to 5% at day 21 after administration of CT-011. This patient eventually had disease progression 61 weeks after initial treatment. No treatment-related toxicities were observed. The most frequent adverse event observed in the study is diarrhea, which occurred in one AML patient, but it may have resulted from GVHD instead of drug treatment. Another female patient received allo-SCT 8 weeks before enrollment, who was treated with the lowest dose of drug due to her early sign of GVHD. This patient eventually died from grade IV GVHD and persistent leukemia. It was unclear whether the deterioration of her disease was related to CT-011. Another three AML patients died due to serious adverse events, which were believed to be related with fulminated resistant leukemia (Berger et al., 2008). **Table 2** shows a summary of efficacy of ongoing clinical trials using checkpoint inhibitors in AML patients.

CTLA-4 Inhibition

For patients with AML, allogeneic transplantation is a curative treatment option. Even so, there are still a portion of patients who would go through disease relapse after transplantation. The main mechanism for this therapy is contributed both by preparative regimen and more importantly by the immunologic GVL effect (Horowitz et al., 1990). Tumor cells escaping from the donor immune system contribute to relapse after allo-SCT. Based on evidences observed in murine model, CTLA-4 blockade to treat late relapse after transplantation by augmenting GVL effect seems a rational attempt.

Ipilimumab is a human IgG1 monoclonal antibody that antagonizes CTLA-4. It was first approved by the FDA for treating melanoma. This antibody has been explored in several solid tumors such as non-small cell lung cancer, small cell lung cancer, and bladder cancer.

The study evaluating ipilimumab on hematological malignancies conducted by Bashey enrolled 29 patients who underwent allo-SCT due to some certain malignancies but relapsed more than 90 days after last transplantation (Bashey et al., 2009).

TABLE 2 | Efficacy data of immune checkpoint inhibition in AML.

Agent	Pathway	Study design	Trial regimen	Study population	Response state	Overall survival	Comments
Pidilizumab	PD-1	Phase I	Single arm monotherapy	N = 8	Minimal response in 1 AML	NR	Limited efficacy as a single agent on AML, safe and tolerable dose as 0.2–6 mg/kg for advanced hematologic malignancies.
Nivolumab	PD-1	Phase II	Nivolumab+azacytidine in R/R AML	N = 70	ORR = 33% (CR/CRi = 15, PR = 1, HI = 7)	6.3m	Encouraging response rate and overall survival especially in salvage 1 (mOS = 10.6 months) and HMA naïve group (ORR = 52%)
Nivolumab	PD-1		Nivolumab+azacytidine in frontline elderly AML	N = 10	ORR = 60% (CR/CRp = 5, PR = 1)	NR	This trial is still enrolling
Nivolumab	PD-1	Phase II	Nivolumab, azacytidine, and ipilimumab on salvage 1–2 R/R AML	N = 14	ORR = 43% (CR/Cri/CRp)	NR	Projected 1 year os is encouraging at 58%. This trial is still enrolling.
Nivolumab	PD-1	Phase II	Nivolumab plus "3+7" standard therapy in AML	N = 42	ORR = 77% (CR = 28, CRi = 6)	18.5m	Addition to (I+A) induction is safe and feasible. Post-transplant severe GVHD is not significantly increased and is manageable.
Nivolumab	PD-1	Phasel/Ib	Single arm in relapsed AML after allo-SCT	N = 11	PR in one AML patients	NR	Severe GVHD and irAEs occurred early and efficacy is modest.
Pembrolizumab	PD-1	Phase II	Pembrolizumab after HiDAC in R/R AML	N = 26	ORR = 42% (CR/CRi = 9, PR = 1, MLFS = 1)	10.5m	Pembrolizumab is well-tolerated in this setting. Response rate is encouraging without additive toxicities after HSCT.
Pembrolizumab	PD-1	Phasel/II	Pembrolizumab followed by decitabine	N = 10	ORR = 20%	7 months	This first proof of principle study demonstrates the feasibility of the combination of pembrolizumab and decitabine in relapsed/refractory adult AML patients.
Pembrolizumab	PD-1		Pembrolizumab for relapsed AML after allo-SCT	N = 8	No patients showed response	NR	Treatment with pem in the post-alloSCT disease relapse setting is feasible, but can induce early and severe irAEs, for AML patients this regimen is less effective.
Ipilimumab	CTLA-4	Phasel/Ib	Ipilimumab for R/R AML after allo-SCT	N = 12	ORR = 42%	With median follow up of 15 months, 12 month OS was 49%	CTLA-4 blockade was a feasible approach for the treatment of patients with relapsed hematologic cancer after transplantation. Complete remissions with some durability were observed, especially in extramedullary AML.

NR, not reported; ORR, over all response rate; OS, overall survival; CR, complete remission; CRi, complete remission with incomplete hematologic recovery; CRp, CR with incomplete platelet recovery; PR, partial response; HI, hematologic improvement; MLFS, morphological leukemia-free state; HMA, hypomethylating agents; HiDAC, high-dose cytarabine.

Patients were required to have not experienced grade III or IV acute GVHD and to be off immunosuppressive medications for more than 6 weeks before enrollment. They received ipilimumab as single infusion at dose between 0.1 and 3 mg/kg. Most of the patients in this cohort suffered from Hodgkin's disease, and two AML patients were included. Median donor T cell chimerism on the day of ipilimumab infusion was 100%. Three patients demonstrated objective disease response but does not include any AML patients. Organ-specific immune irAEs were seen in four patients (14%) including grade 3 arthritis, grade 2 hyperthyroidism, and recurrent grade 4 pneumonitis. Dose-related grade 3 adverse events were anemia, thrombocytopenia, and neutropenia/fever, and grade 4 infection was observed. Most of grade 1 and 2 toxicities showed no clear relationship with the studied drug. No patient developed

TABLE 3 | Immune-related adverse event rates associated with ICIs in acute leukemia.

	Nivolumab (0.5–3 mg/kg) (Daver et al., 2019; Davids et al., 2018; Assi et al., 2018)	Pembrolizumab (200 mg/m²) (Justin Kline et al., 2018; Lindblad et al., 2018; Zeidner et al., 2018)	Ipilimumab (0.1–10 mg/kg) (Bashey et al., 2009; Davids et al., 2016)
	≥Grade 3 (%)	≥Grade 3 (%)	≥Grade 3 (%)
Pneumonitis	1	18	3.4–4.5
Rash	4.5	7.6	
Pruritus	3		
Transaminitis	2–4		3.4
Colitis	1–4.5		4.5
Pancreatitis	2		
Elevated bilirubin	4	10	
Fatigue	1		
Hepatitis		7.6	
Hypotension		10	
Diarrhea		10	
Hyperthyroidism		9–14	
Arthritis			3.4

grade III or IV acute GVHD after ipilimumab alone. One AML patient treated at the dose level of 0.1 mg/kg developed grade 3 polyarthropathy clinically consistent with rheumatic arthritis and achieved complete regression of her symptom after being treated with corticosteroid.

In a phase I/Ib, open label, multicenter study of treating patients with relapsed hematological malignancies after allo-SCT with ipilimumab, 28 patients were enrolled who received two different dosages of ipilimumab (3 or 10 mg/kg) including 12 AML patients (Davids et al., 2016). The median time from transplantation to drug treatment was 22.5 months and median pretreatment T cell chimerism was 99%. Objective response was only observed in the cohort of patients who were treated on drug dose of 10 mg/kg with seven patients reaching the criteria for response. All responders had baseline donor T cell chimerism in the blood of 99% or higher, suggesting the important role of donor T cell in antitumor activity. Complete response was observed in five patients (23%), including three patients with leukemia cutis, one patient with myeloid sarcoma, and another one with AML developed from smoldering myelodysplastic syndrome with bone marrow involvement. With a median follow-up of 15 months, the 1-year survival rate was 49% and four patients who had a response continued to have a durable remission for more than 1 year. Toxicities were not specifically reported on AML cohort. On patients treated with 10 mg/kg ipilimumab, GVHD was observed in 3 out of 22 patients, including 2 cases of chronic GVHD of the liver and 1 case of grade II acute GVHD of the gut. All of these events were resolved with glucocorticoids but precluded further ipilimumab administration. Immune-related adverse events occurred in three patients including grade 2 immune thrombocytopenia, grade 3 colitis, and grade 2–4 pneumonitis, which responded to glucocorticoids. The incidences of grade 3 and 4 irAEs are listed in **Table 3**. One patient died of grade 3 colitis and grade 4 pneumonitis eventually. Exploratory studies were conducted to identify some possible predictors for response. Response was associated with *in situ* infiltration of CD8+ T cells as well as enrichment of effector T cell subsets.

CONCLUSIONS

Checkpoint inhibition treatment for AML is no doubt a major breakthrough. Preliminary data from ongoing clinical trials are promising especially for combination of PD-1 inhibitor nivolumab with HMAs with significantly higher response rate compared with historical control. In AML patients with extramedullary disease who relapsed post-transplantation, CTLA-4 inhibitor ipilimumab as a single agent shows a particular benefit. Due to the limited size of the early phase of clinical trials, more data are needed before we can better interpret these positive data and the response improvements observed in these trials need further validation. Despite the promising outcome from clinical trials, the introduction of checkpoint inhibitors is associated with unique irAEs, which are mostly reversible but can occasionally be fatal. Compared with toxicity resulting from conventional chemotherapy, immune-related irAEs caused by checkpoint inhibitors usually have a delayed onset and prolonged duration as well as a different toxicity profile (Fehrenbacher et al., 2016; Puzanov et al., 2017). Early recognition and proper intervention with immune suppression strategy, which is appropriate to affected organs, are key factors for effective management of irAEs. The areas of substantial interest for future study would be better innovative combinations to modulate immunologic targets and defining of biomarkers to select AML patients who are most likely to benefit from checkpoint inhibition therapy. Data from ongoing clinical trials emerging in the near future will guide further development of these agents while helping us gain understanding of how to minimize the risk of immune-related toxicities.

AUTHOR CONTRIBUTIONS

This study was DL's original idea. DL also reviewed the literature and contributed to the manuscript writing and editing. TN mentored and contributed to the writing and editing of the manuscript. MW reviewed the literature and contributed to the manuscript writing and editing. YL contributed to the writing and editing of the manuscript. JL edited and proofread the manuscript.

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REVIEW
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Management of Adverse Events in Cancer Patients Treated With PD-1/PD-L1 Blockade: Focus on Asian Populations

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The interaction between programmed cell death protein 1 (PD-1) and its ligand programmed death-ligand 1 (PD-L1) induces exhaustions of cytotoxic lymphocytes in the tumor microenvironment, which facilitates tumor immune evasion. PD-1/PD-L1 blockade therapy, which prevents the receptors and ligands from binding to each other, disrupts the T-cell exhaustion signaling, thereby increasing antitumor immunity. Inspiringly, it has revolutionized the treatment of many different types of cancers including non-small-cell lung carcinoma, melanoma, lymphoma, and so on. However, with the intention of generating an antitumor immune response, PD-1/PD-L1 blockade may also lead to a spectrum of side effects. The profile of adverse events (AEs) of PD-1/PD-L1 blockade is not exactly the same with other immune checkpoint blockades, such as blockade of cytotoxic T-lymphocyte-associated protein 4. Although cutaneous, gastrointestinal, and pulmonary systems are common victims, AEs of PD-1/PD-L1 blockade might occur in any other organ system of the human body. These toxicities can be life-threatening if not managed promptly, and proper treatment intervention is imperative for optimal control and prevention of severe damage. Currently, clinical practice for the management of AEs in PD-1/PD-L1 blockade remains sporadic and variable. The majority of initial clinical trials were carried out in Caucasians. The trials of multiple races usually included a small portion of Asian participants, and results were calculated and interpreted for the entire included subjects without any race-specific conclusions. Therefore, the information on PD-1/PD-L1 blockade in Asians is far from systematic or comprehensive. Recently, as the results of clinical trials of anti-PD-1/PD-L1 agents in Asian populations have been gradually released, we summarized current evidence with a specific focus on the Asian population, hoping to outline strategies and offer guidance on the management of AEs in cancer patients treated with PD-1/PD-L1 blockade in the Asian world.

Keywords: programmed cell death protein 1, programmed death-ligand 1, adverse event, Asian, cancer, immunotherapy

BACKGROUND

Overview of Programmed Cell Death Protein 1/Programmed Death-Ligand 1 Blockade

Programmed cell death protein 1 (PD-1), also known as cluster of differentiation 279 (CD279), is a protein expressed on the surface of cells. The principal ligand of PD-1, programmed death-ligand 1 (PD-L1), also known as B7-H1 or CD274 (Ishida et al., 1992), is frequently expressed within the tumor microenvironment, including in cancer cells, antigen presenting cells (APCs), tumor-infiltrating macrophages, T cells, B cells, dendritic cells, and mesenchymal stem cells (Weber, 2010; Pardoll, 2012). Interacting with its cell surface ligands, PD-1 negatively regulates the effector phase of T-cell responses (Blank et al., 2004) (**Figure 1**). Through multiple mechanisms including simultaneous proapoptotic effects in cytotoxic T cells and antiapoptotic effects in regulatory T cells, PD-1 downregulates the immune system and promotes self-tolerance. This regulates the immune system's response to the cells and prevents the immune system from killing tumor cells in the human body (Syn et al., 2017).

Immune checkpoint inhibitors (ICIs) blocking the interaction of PD-1 and PD-L1 significantly enhance T-cell function and therefore exert antitumor activity (Brahmer et al., 2012). By now, several anti-PD-1/PD-L1 antibodies have been developed, including nivolumab, pembrolizumab, cemiplimab, and camrelizumab (anti-PD-1 antibodies) as well as atezolizumab, durvalumab, and avelumab (anti-PD-L1 antibodies). The efficacies of these anti-PD-1/PD-L1 agents have been proven across various cancer types, such as melanoma (Hamid et al., 2013; Robert et al., 2015; Weber et al., 2015b), non-small-cell lung cancer (NSCLC) (Nishio et al., 2017), and Hodgkin lymphoma (Maruyama et al., 2017). Several agents have been approved by the US Food and Drug Administration (FDA) and the European Medicines Agency because of their great performance over conventional treatments in malignancies.

Adverse Events in Cancer Patients Treated With Programmed Cell Death Protein 1/Programmed Death-Ligand 1 Blockade

Immune checkpoints are an essential component of the immune system. They function in a delicate organism of self-regulation to avoid excessively activated or even deleterious immune responses (Postow et al., 2012). Among the immune checkpoints, the PD-1/PD-L1 pathway is a crucial regulator in balancing the activation and tolerance of T cells (Okazaki and Honjo, 2006). The basic idea of PD-1/PD-L1 blockade is to block the interaction of PD-1 on T cells and PD-L1 on tumor cells, which offers tumor cells additional resistance to T-cell-mediated apoptosis, thus preventing cancer cells from defending themselves against antitumor immune responses (Azuma et al., 2008).

However, PD-L1 also exists in noncancer tissues, such as pancreatic islets, heart, endothelium, small intestine, and many other tissues yet to be discovered (Simeone and Ascierto, 2012). In preclinical researches, PD-1-deficient mice exhibited systemic lupus erythematosus-like disease (Nishimura et al., 1999), lupus-like arthritis, glomerulonephritis (Nishimura et al., 1999), and cardiomyopathy (Nishimura et al., 2001). Moreover, the polymorphism in PD-1 has been associated with autoimmune diseases in humans (Prokunina et al., 2002). The above evidence indicated that blockade of the PD-1/PD-L1 pathway may induce

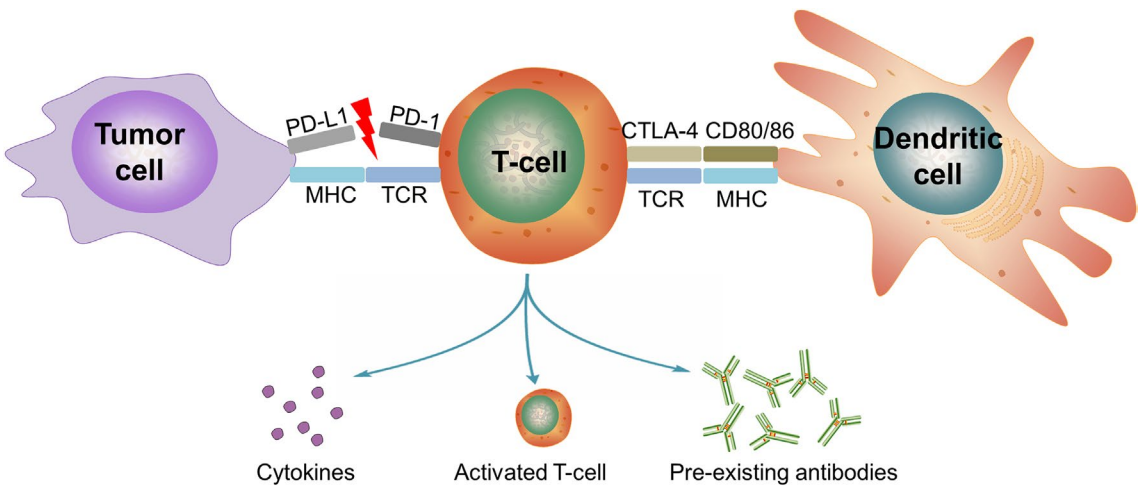

FIGURE 1 | Possible mechanisms of immune-related adverse events in cancer patients treated with PD-1/PD-L1 blockade. PD-L1 is expressed in tumor cells. After prolonged activation, PD-1 is upregulated in T cells and binds to its ligands on tumor cells or other immune cells to dampen an ongoing immune response. Anti-PD-1/PD-L1 therapy blocks this inhibitory signaling, thereby provoking the immune response to tumor. Possible mechanisms of immune-related adverse events with PD-1/PD-L1 blockade include 1) off-target effects of T cell-mediated immunity in healthy tissue, such as in myocarditis and pneumonitis; 2) increased preexisting autoantibodies, such as in arthritis and thyroid toxicity; and 3) increased inflammatory cytokines (Calabrese et al., 2018; Postow et al., 2018). (PD-1, programmed cell death protein 1; PD-L1, programmed death-ligand 1; CTLA-4, cytotoxic T-lymphocyte-associated protein 4; TCR, T-cell receptor; MHC, major histocompatibility complex).

autoimmune disease and systemic inflammation (**Figure 1**). As confirmed in clinical trials, a spectrum of immune-related adverse events (irAEs) has emerged because of the turbulence in immunomodulation accompanying PD-1/PD-L1 blockade (Postow et al., 2018), despite favorable efficacy in suppressing tumors.

Uniqueness of Immune-Related Adverse Events in Cancer Patients Treated With Programmed Cell Death Protein 1/ Programmed Death-Ligand 1 Blockade

IrAEs are defined as any AE associated with exposure to immunotherapy and with an immune-mediated mechanism. Upon the diagnosis of an irAE, infections and other definite etiologies should be ruled out (Sgambato et al., 2016). Anti-cytotoxic T-lymphocyte-associated protein-4 (CTLA-4) and anti-PD-1/PD-L1 agents are both ICIs that form the new generation of immunotherapy and share a similar background in drug development. Nevertheless, the profile of AEs in patients treated with PD-1/PD-L1 blockade is not exactly the same with those treated with other ICIs such as CTLA-4 blockade.

PD-1 bears homology to CTLA-4 but provides distinct immune-inhibitory signals. More specifically, PD-1 impedes the activity of effector T cells in the effector phase, whereas CTLA-4 regulates T-cell function in an earlier activation phase. Moreover, PD-1 is expressed in various types of cells including T cells, B cells, natural killer cells, and macrophages. Unlike PD-1, the expression of CTLA-4 is confined to T cells (Dong et al., 2002; Fanoni et al., 2011; Ribas, 2012). As described previously, the PD-1 receptor is crucially involved in peripheral tolerance, PD whereas CTLA-4 is pivotal in central tolerance and control (Sharon et al., 2014).

In preclinical animal models, the autoimmune phenotypes were different between PD-1-deficient mice and CTLA-4-deficient mice (Nishimura et al., 1999, Nishimura et al., 2001). In the PD-1 knockout mice, strain- and organ-specific autoimmunity was demonstrated in a modest later-onset model compared with early lethality in CTLA-4 knockout mice (Nishimura et al., 1999, Nishimura et al., 2001). As shown in clinical trials, PD-1/PD-L1 blockade is associated with a different spectrum of irAEs from anti-CTLA-4 therapy. As an example, nivolumab has been associated with a unique spectrum of pneumonitis (Postow et al., 2012). Another example is that colitis is more frequently seen in patients treated with ipilimumab (CTLA-4 blockade) than in patients treated with anti-PD-1 therapy (Agarwala, 2015).

Although PD-1/PD-L1 blockade reveals comparatively fewer and milder toxic effects than those for CTLA-4 blockade (Brahmer et al., 2010; Ribas, 2012; Zumelzu et al., 2018), the definite incidence of AEs in patients with PD-1/PD-L1 blockade was high, and some high-grade AEs can be lethal. Therefore, the current review aimed to summarize the recent updates in the management of AEs in patients under PD-1/PD-L1 blockade. Up to now, majority of AE experience in patients treated with ICIs comes from clinical trials in the Western world. As phased results of clinical trials of PD-1/PD-L1 blockade in Asians are being published, the current review focuses on the profile of the Asian world in the field of the management of AEs.

ADVERSE EVENTS OF PROGRAMMED CELL DEATH PROTEIN 1/PROGRAMMED DEATH-LIGAND 1 BLOCKADE IN ASIAN POPULATIONS

Among the PD-1/PD-L1 inhibitors, nivolumab, pembrolizumab, atezolizumab, avelumab, and durvalumab have been approved by the FDA in the United States for the treatment of cancers. The majority of initial clinical trials were carried out in Caucasians. Large multicenter trials with patients of mixed races usually included a small portion of Asian participants, and results such as response rate, survival, and incidence of AEs were calculated and interpreted for the entire included subjects. Therefore, the information of PD-1/PD-L1 blockade in Asians is far from systematic or comprehensive. By now, the results of trials on nivolumab (Hamanishi et al., 2015; Kang et al., 2017; Kudo et al., 2017; Maruyama et al., 2017; Nishio et al., 2017; Yamazaki et al., 2017), camrelizumab (Fang et al., 2018; Huang et al., 2018, Huang et al., 2019; Mo et al., 2018), pembrolizumab (Shimizu et al., 2016; Tahara et al., 2018; Nishio et al., 2019), avelumab (Doi et al., 2018), and atezolizumab (Mizugaki et al., 2016) in Asian populations have been published (**Table 1**). Here, the most common treatment-related AEs (TRAEs) of any grade and TRAEs of grades 3–5 reported in the above articles were summarized per organ system (**Figure 2**). To explore whether the profile of AEs in Asian patients is similar to those in studies carried out in Caucasians or mixed races, we searched the Pubmed database and extracted the incidence of each reported AE from available results of clinical trials (**Supplementary Table 1**). The search terms "(PD-L1 OR) AND trial" were used, and the last search date was April 6, 2019. We also manually screened the references of related studies to avoid omissions. The inclusion criteria of studies include the following: a) clinical trials of cancer patients treated with PD-1/PD-L1 blockade published in English; b) studies reporting the incidence of AEs of any system. Accordingly, clinical trials with null information on the prevalence of AEs and case reports of rare AEs were excluded. Single-center studies with patients of Western origin, multicenter studies of patients from mixed Western origins, and large multicenter trials with patients of mixed races, which included a portion of Asian participants, were classified as "Western/international" studies. Single-center studies with patients of Asian origin and multicenter studies of patients from mixed Asian origins were classified as "Asian" studies. We displayed the top 60 AEs of any grade (**Figure 3**) and AEs of grades 3–5 (**Figure 4**) with a heatmap. The incidence of each AE was compared between Asian and Western/international populations, and selected AEs with significantly different incidences between groups were shown (**Figure 5**). Hierarchical clustering analysis was performed based on the incidence of AEs by using the pheatmap package (https://cran.r-project.org/web/packages/pheatmap/index.html, version 1.0.12). The AEs with different prevalences between Asian and Western/international populations were depicted by violin plots. Statistical analysis was performed using the Wilcoxon test in ggpubr package (https://cran.r-project.org/web/packages/ggpubr/index.html, version 0.2). P values <0.05 were considered statistically significant. The features of AEs in Asian patients treated with PD-1/PD-L1 blockade are discussed per agent below.

TABLE 1 | Incidence of AEs in published results of clinical trials of anti-PD-1/PD-L1 monotherapy in Asian populations.

Year	Trial number	Country/ Region	Agent	Cancer	Phase	Sample size	Rate of AE	Rate of TRAE	Rate of irAE	Treatment interrupted because of AE	Treatment discontinued because of AE	Common types of AE
2017	JapicCTI-142422 (Kudo et al., 2017)	Japan	Nivolumab	Esophageal carcinoma	2	65	85%	60%	NA	23%	11%	Diarrhea, appetite decrease, constipation
2017	JapicCTI-142533 (Yamazaki et al., 2017)	Japan	Nivolumab	Melanoma	2	24	91.7%	83.3%	NA	8.3%	8.3%	Vitiligo, pruritus, hypothyroidism, malaise
2017	JapicCTI-142755 (Maruyama et al., 2017)	Japan	Nivolumab	Hodgkin lymphoma	2	17	100%	NA	NA	41.2%	NA	Pyrexia, pruritus, rash
2016	JapicCTI-132073 (Nishio et al., 2017)	Japan	Nivolumab	NSCLC	2	76	NA	84.2%	NA	NA	15.8%	Malaise, pyrexia, rash, appetite decrease
2015	UMIN000005714 (Hamanishi et al., 2015)	Japan	Nivolumab	Ovarian cancer	2	20	NA	95%	NA	NA	11%	AST increase, hypothyroidism, lymphocytopenia
2017	NCT02267343 (Kang et al., 2017)	Japan, South Korea, Taiwan	Nivolumab	Gastric and gastroesophageal junction cancer	3	330	91%	43%	NA	NA	2.7%	Pruritus, diarrhea, rash, fatigue
2018	NCT02721589 (Fang et al., 2018)	China	Camrelizumab	Nasopharyngeal carcinoma	1	93	NA	97%	NA	12.9%	2.2%	Reactive capillary hemangiomas, fatigue, hypothyroidism
2018	NCT02742935 (Mo et al., 2018)	China	Camrelizumab	Solid tumors	1	36	97.2%	88.9%	86.1%	NA	2.8%	Reactive capillary hemangiomas, pruritus, fatigue
2018	NCT02742935 (Huang et al., 2018)	China	Camrelizumab	Esophageal carcinoma	1	30	NA	83.3%	83.3%	6.7%	0	Reactive capillary hemangiomas, pruritus, hypothyroidism
2019	NCT02742935 (Huang et al., 2019)	China	Camrelizumab	Gastric and gastroesophageal junction cancer	1	30	100%	100%	93.3%	NA	NA	Reactive capillary hemangiomas, pruritus, fatigue
2016	NCT01840579 (Shimizu et al., 2016)	Japan	Pembrolizumab	Solid tumors	1	10	NA	80%	40%	NA	0	Nausea, malaise, pyrexia
2018	NCT02007070 (Nishio et al., 2019)	Japan	Pembrolizumab	NSCLC	1b	38	NA	87%	24%*	NA	11.1%	Malaise, diarrhea, maculopapular rash
2018	NCT01848834 (Tahara et al., 2018)	Japan, South Korea, Taiwan	Pembrolizumab	Head and neck squamous cell carcinoma	1b	26	NA	62%	19%	NA	3.8%	Fatigue, appetite decrease, hypothyroidism, rash
2016	JapicCTI-132208 (Mizugaki et al., 2016)	Japan	Atezolizumab	Solid tumors	1	6	100%	NA	NA	50%	0	Rash, increased AST, ALT, and ALP, headache
2018	NCT01943461 (Doi et al., 2018)	Japan	Avelumab	Solid tumors	1	17 (dose-escalation cohort)	94.1%	64.7%	11.8%	NA	0	Infusion-related reaction, rash maculopapular, stomatitis
2018	NCT01943461 (Doi et al., 2018)	Japan	Avelumab	Solid tumors	1	40 (dose-expansion cohort)	100%	80%	12.5%	NA	10%	Infusion-related reaction, pruritus, pyrexia
2019	NCT02836795 (Tang et al., 2019)	China	Toripalimab	Melanoma and urologic cancer	1	36	100%	100%	NA	16.7%	14%	Hyperglycemia, proteinuria, rash
2019	NCT03114683 (Shi et al., 2019)	China	Sintilimab	Classical Hodgkin lymphoma	2	92	100%	93%	54%	NA	3%	Pyrexia, hypothyroidism, increased TSH

PD-1, programmed cell death protein 1; PD-L1, programmed death-ligand 1; NSCLC, non-small-cell lung cancer; AE, adverse event; TRAE, treatment-related adverse event; irAE, immune-related adverse event; AST, aspartate aminotransferase; ALT, alanine aminotransferase; ALP, alkaline phosphatase; TSH, thyroid-stimulating hormone; NA, not available.

**rate of irAE plus infusion reaction.*

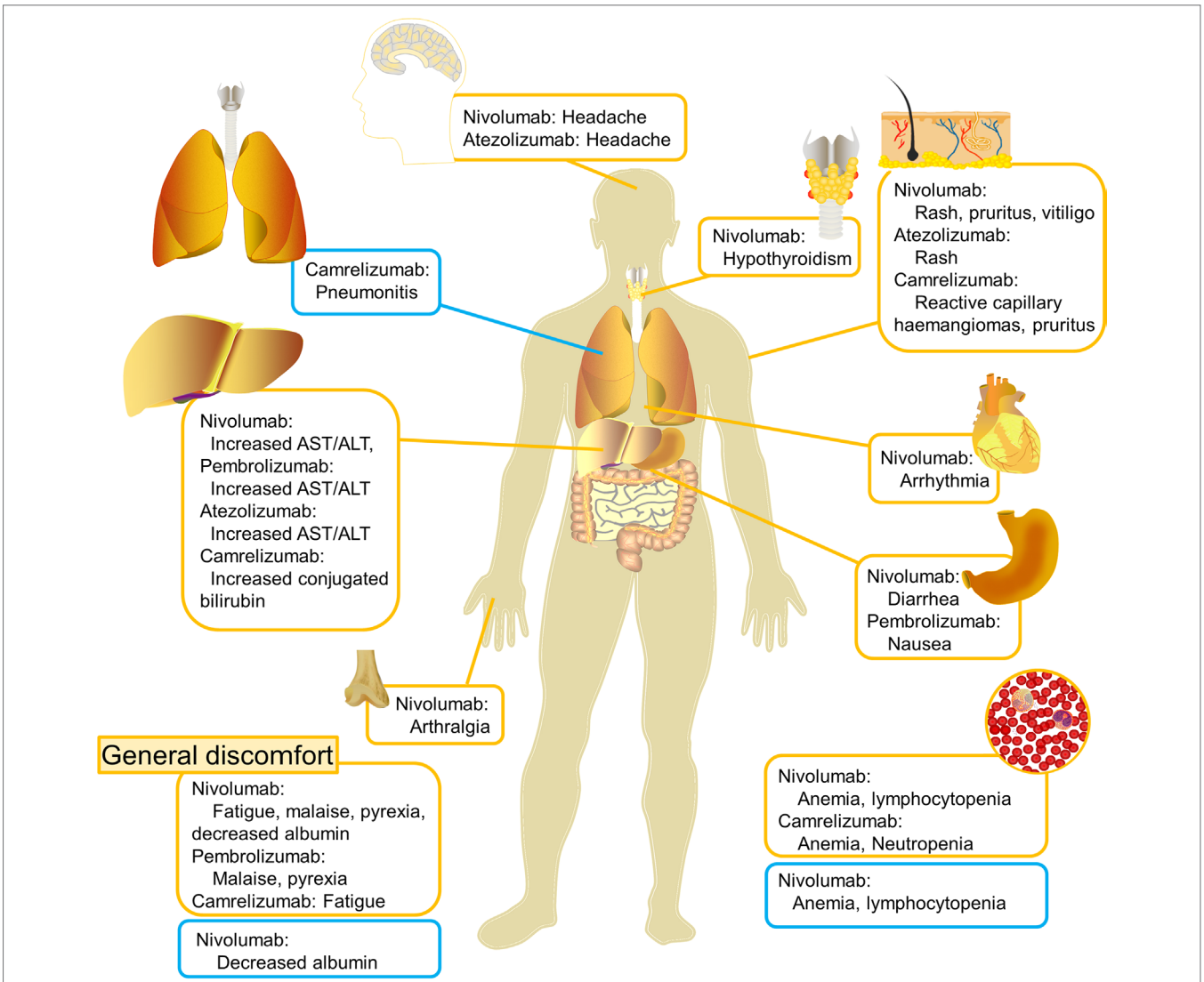

FIGURE 2 | Clinical spectrum of treatment-related adverse events reported in clinical trials of major PD-1/PD-L1 blockade in Asian populations. The most common adverse events (AEs) of any grade per organ system were dermatological toxicities, hepatic toxicities, endocrinopathies, and general disorders. The incidence of pulmonary toxicities was lower, but it is the most common reason for a serious AE. (Orange text boxes: treatment-related AEs of any grade observed in ≥20% of patients in clinical trials; blue text boxes: treatment-related AEs of grades 3–5 observed in ≥5% of patients in clinical trials, unless a case of onset <2 in trials with a small sample size; AST, aspartate aminotransferase; ALT, alanine aminotransferase.)

Nivolumab

Nivolumab (BMS-936558/ONO-4538) is a fully human monoclonal immunoglobulin G4 (IgG4) antibody inhibitor of PD-1 (Hardy et al., 1997). Nivolumab has been approved by FDA as monotherapy in unresectable or metastatic melanoma, metastatic NSCLC, advanced renal cell carcinoma, locally advanced or metastatic urothelial carcinoma, recurrent or metastatic head and neck squamous cell carcinoma, relapsed or refractory classical Hodgkin lymphoma, microsatellite instability-high or mismatch repair deficient metastatic colorectal cancer, or hepatocellular carcinoma that has been previously treated with sorafenib and in combination with ipilimumab in unresectable or metastatic melanoma (Bristol-Myers, 2019). In a recent meta-analysis, hypothyroidism, pneumonitis, colitis, and hypophysitis were several of the most common irAEs of any grade. Among them, pneumonitis was the most common serious AE (Baxi et al., 2018).

In Asians, results of phase 2 and 3 trials of nivolumab have been published (Hamanishi et al., 2015; Kang et al., 2017; Kudo et al., 2017; Maruyama et al., 2017; Nishio et al., 2017; Yamazaki et al., 2017). In a phase 2 trial of 65 patients with esophageal squamous cell carcinoma in Japan, the most common AEs were diarrhea, appetite decrease, constipation, rash, and fatigue, the majority of which resolved with drug discontinuation and/or supportive care. Twenty-six percent of patients developed grade 3–4 AEs, and 17% developed serious AEs. Serious AEs that occurred in this group of patients included lung infection, dehydration, and interstitial lung disease (ILD) (Kudo et al., 2017). In a phase 2

FIGURE 3 | Heatmap of the incidence of the top 60 most common AEs of any grade in cancer patients treated with PD-1/PD-L1 blockade. Fifteen clinical trials in Asian patients and 69 trials in Western or mixed international population of PD-1/PD-L1 blockade monotherapy (including nivolumab, pembrolizumab, atezolizumab, avelumab, durvalumab, and camrelizumab) were included. APTT, activated partial thromboplastin time; ALP, alkaline phosphatase; GGT, gamma-glutamyltransferase; ALT, alanine aminotransferase; AST, aspartate aminotransferase.)

trial of nivolumab in Japanese patients with previously untreated advanced melanoma, AEs were reported in 91.7% of patients. Serious AEs that occurred were colitis, abnormal hepatic function, renal impairment, and pleural effusion. The case of colitis was the only TRAE of grade ≥3. These patients experienced two episodes of colitis. The first episode of colitis alleviated with the treatment with corticosteroids and suspension of nivolumab but relapsed 5 months later when nivolumab was discontinued (Yamazaki et al., 2017). As with two other phase 2 trials of nivolumab in Japanese patients with classical Hodgkin lymphoma (Maruyama et al., 2017) and NSCLC (Nishio et al., 2017), no treatment-related death was observed. The results of a phase 3 study of patients with advanced gastric or gastroesophageal junction cancer refractory to, or intolerant of, at least two previous chemotherapy regimens have been recently published (Kang et al., 2017). The study included 330 patients in the nivolumab group and 161 in the placebo group from Japan, South Korea, and Taiwan. TRAEs were observed in 43% of patients in the nivolumab group and 27% of patients in the placebo group. Five (2%) patients in the nivolumab group and two (1%) patients in the placebo group died from TRAE (Kang et al., 2017). Generally, the safety profile of nivolumab in Asians was similar to what was reported in previous large international studies (Robert et al., 2015; Weber et al., 2015b).

Camrelizumab

Camrelizumab (SHR-1210) is a selective, humanized, high-affinity IgG4-κ monoclonal antibody against PD-1 developed by Jiangsu Hengrui Medicine (Huang et al., 2019). All the trials of camrelizumab with published data were performed in China (Fang et al., 2018; Huang et al., 2018, Mo et al., 2018; Huang et al., 2019).

The results of a large phase 1 clinical trial (NCT02742935) have been reported in esophageal carcinoma (Huang et al., 2018), nasopharyngeal carcinoma (Fang et al., 2018), and gastric cancer (Huang et al., 2019). The antitumor efficacy was promising. Unlike a varied spectrum of AEs in different types of cancers with other PD-1 inhibitors, the most common TRAEs in different cancer types were concentrated in reactive capillary hemangiomas (RCHs), pruritus, hypothyroidism, fatigue, and hypothyroidism. The safety profile of camrelizumab in Asian patients was similar to that of other PD-1 inhibitors (Huang et al., 2018). Although more than 80% of participants experience

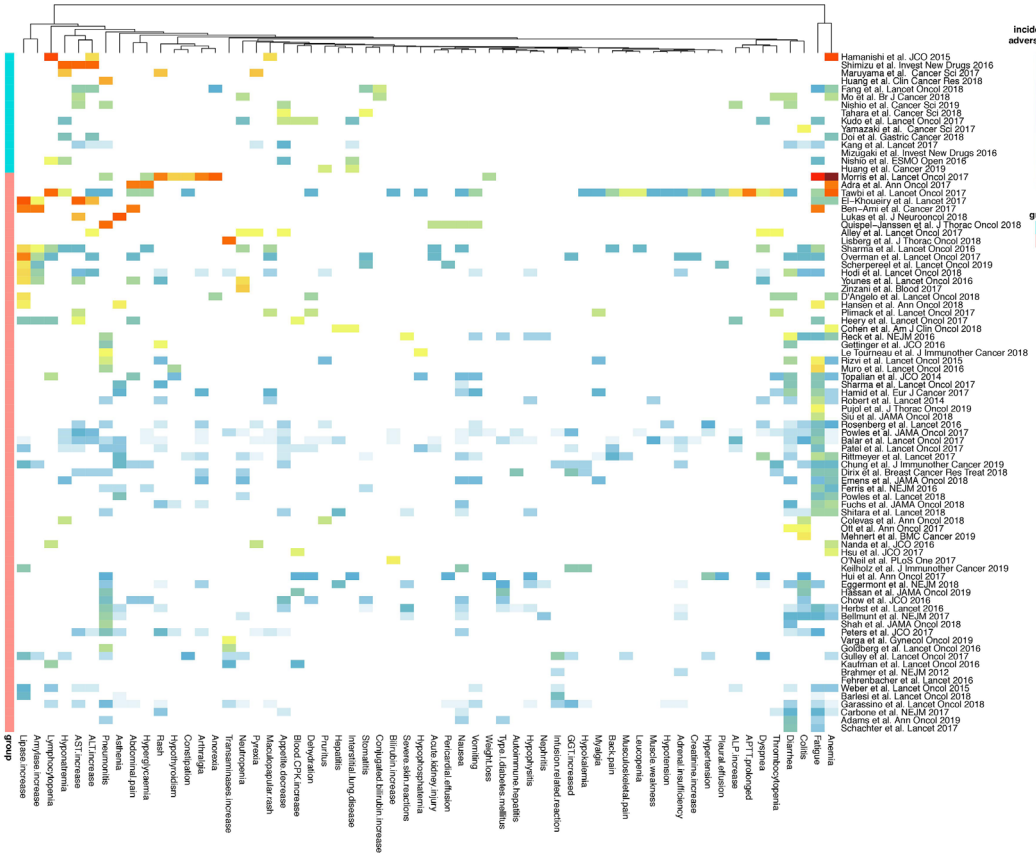

FIGURE 4 | Heatmap of the incidence of the top 60 most common AEs of grades 3–5 in cancer patients treated with PD-1/PD-L1 blockade. Fifteen clinical trials in Asian patients and 70 trials in Western or mixed international population of PD-1/PD-L1 blockade monotherapy (including nivolumab, pembrolizumab, atezolizumab, avelumab, durvalumab, and camrelizumab) were included. (APTT, activated partial thromboplastin time; ALP, alkaline phosphatase; GGT, gamma-glutamyltransferase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; CPK, creatinine phosphokinase.)

AEs of any grade, most AEs were grade 1 or 2 and could be well managed with supportive care or medical therapy.

Notably, RCH was seen in a large portion of patients, involving 76.7% of patients with advanced esophageal carcinoma (Huang et al., 2018), 88% of patients with nasopharyngeal carcinoma (Fang et al., 2018), 86.6% of patients with advanced gastric and gastroesophageal junction cancer (Huang et al., 2019), and 83.3% of patients with solid tumors (including NSCLC, breast cancer, colorectal cancer, and hepatocellular carcinoma) (Mo et al., 2018). This phenomenon has never been observed in other PD-1/PD-L1 inhibitors.

RCH is a type of reactive hyperproliferative vascular response. The clinical manifestations of RCH triggered by camrelizumab include red papules or macules with clear boundaries. It can be disseminated all over the body, and the most frequently involved areas are trunks, upper extremities, head, and neck (Huang et al., 2018). RCH emerged at a median time of 23 days after usage of camrelizumab (Mo et al., 2018). A common complication of RCH is bleeding, and a rare complication is ulceration (Teng et al., 2019), which calls for extra supportive care.

Despite the high incidence, the severity of camrelizumab-associated RCH was mostly grade 1, and none of the patients terminated therapy because of this AE. Symptoms of RCH could be spontaneously mitigated during the treatment, but complete regression only occurred after discontinuation of camrelizumab (Huang et al., 2018, Huang et al., 2019).

The mechanism of RCH in patients receiving camrelizumab is yet elucidated. Potential explanations include the imbalance of receptor/receptor–ligand interactions with upregulation of vascular proliferative proteins (Piguet and Borradori, 2002; Teng et al., 2019). Another experimental study using human receptor proteome screening indicated that camrelizumab mediated off-target binding to human receptors, such as vascular endothelial growth factor receptor 2 (VEGFR2), which might thereby drive hemangioma development *via* vascular endothelial cell activation (Finlay et al., 2019). Interestingly, RCH was less frequently seen in patients of advanced hepatocellular carcinoma, gastric, or esophagogastric junction cancer who received camrelizumab in combination with apatinib (Xu et al., 2019). Moreover, the incidence of RCH dropped to 22%, and severity was lower in patients treated with a combinatory regimen of camrelizumab and gemcitabine plus cisplatin. This might be explained that the chemotherapy inhibited the hyperproliferation of endothelial cells (Fang et al., 2018). Currently, the phase 3 trial of camrelizumab

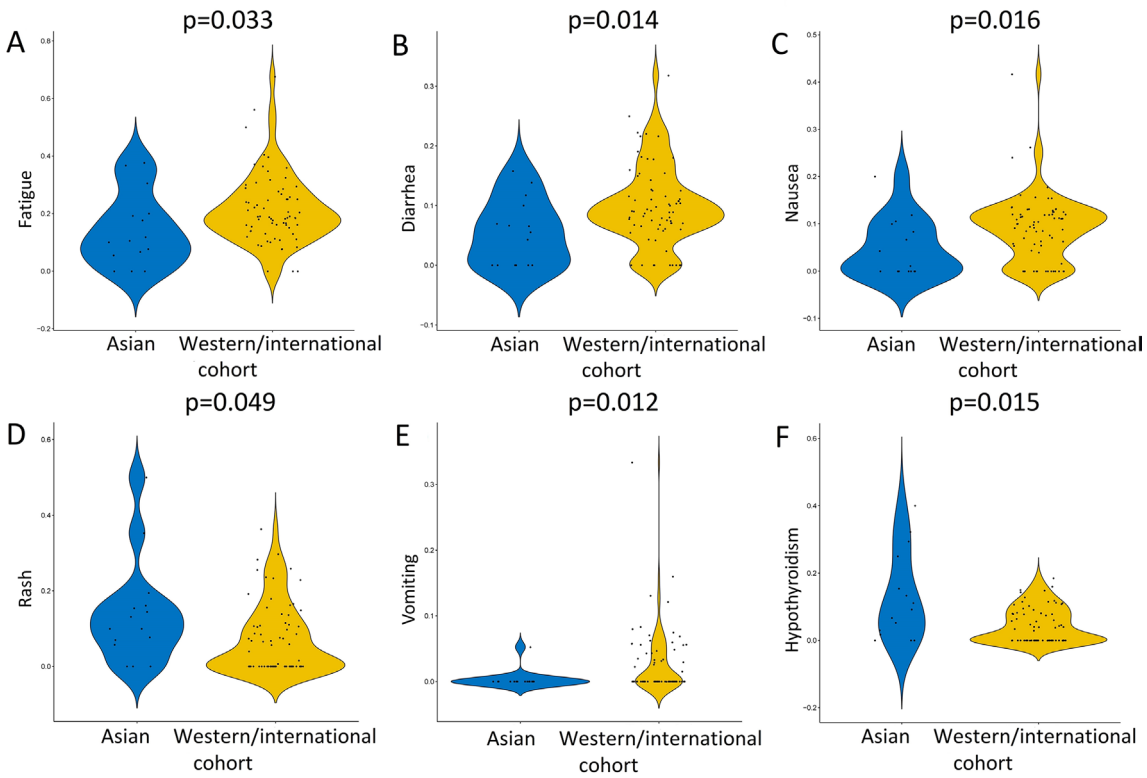

FIGURE 5 | Selected adverse events with different incidences between Asian populations and Western/international populations in cancer patients treated with PD-1/PD-L1 blockade. The adverse events (AEs) of any grade with different prevalences between Asian populations and Western/international populations include fatigue **(A)**, diarrhea **(B)**, nausea **(C)**, rash **(D)**, vomiting **(E)**, hypothyroidism **(F)**, ALT increase, asthenia, dizziness, fever, adrenal insufficiency, hyponatremia, lipase, malaise, and reactive capillary hemangiomas. The AEs of grades 3–5 with different prevalences between Asian populations and Western/international populations include fatigue, nausea, interstitial lung disease, lipase increase, hyponatremia, and increase in conjugated bilirubin. The comparative analysis was only performed in AEs with at least one event in both Asian patients and Western/international patients.

is ongoing, and the mechanism of this particular AE shall be further explored for potential drug refinement to prevent unwanted properties.

Pembrolizumab

Pembrolizumab (MK-3475), previously known as lambrolizumab, is a highly selective IgG4-κ humanized isotype monoclonal antibody against PD-1. It is designed to prevent Fc-mediated antibody-dependent cellular cytotoxicity, thus avoiding cytotoxic effects of the antibody when it binds to the T cells (Hamid et al., 2013).

In a phase 1 trial of pembrolizumab in the treatment of 10 Japanese patients with advanced solid tumors including NSCLC, melanoma, and breast cancer, grade 3 alanine transaminase (ALT) elevation, grade 3 aspartate transaminase (AST) elevation, grade 1 pneumonitis, and grade 1 thyroid-stimulating hormone (TSH) elevation were reported as irAEs (Shimizu et al., 2016). One patient with advanced NSCLC developed grade 3 ALT elevation, grade 3 AST elevation, and grade 1 pneumonitis simultaneously on day 42 and further developed grade 3 hyponatremia after termination of pembrolizumab (Shimizu et al., 2016). In a phase 1b study (KEYNOTE-012) in Asia-Pacific patients with advanced head and neck squamous cell carcinoma, two (8%) patients experienced serious TRAEs, one of which was a grade 2 ILD that resulted in drug discontinuation (Tahara et al., 2018).

As seen from published data, the safety profile of pembrolizumab in Asian populations is generally similar to that in non-Asian patients (Shimizu et al., 2016). However, results of large trials are needed to validate the conclusion, considering the small sample size of existing trials in Asians.

Other Programmed Cell Death Protein 1/ Programmed Death-Ligand 1 Blockades

Among various anti-PD-1/PD-L1 agents, another two agents with published data of trials in Asians are atezolizumab and avelumab (Mizugaki et al., 2016; Doi et al., 2018). Atezolizumab (MPDL3280A) is a human IgG1 monoclonal anti-PD-L1 antibody. Because it does not block the interaction of PD-1 and its second ligand PD-L2, the immune homeostasis is maintained theoretically (Chen et al., 2012). In a phase 1 study of monotherapy with atezolizumab in Japanese patients with advanced solid tumors, all six patients experienced AEs, and half of the patients developed AEs that led to suspension of atezolizumab, including influenza-like illness and increased alkaline phosphatase. Still, all events were grade 1 or 2, and no death occurred (Mizugaki et al., 2016).

Avelumab, another human IgG1 monoclonal anti-PD-L1 antibody, has also been tested in Asian populations. In a phase 1 trial of avelumab in Japanese patients with advanced solid tumors, the most common AEs were infusion-related reactions (IRRs) and rash in the dose-escalation cohort and IRRs and pruritus in the dose-expansion cohort (Doi et al., 2018). In more recent phase 1b studies in Europe and the United States, less cutaneous but more general toxicities such as fatigue, chills, and diarrhea were observed. Nevertheless, IRRs remain the dominant AE across populations (Disis et al., 2019; Hassan et al., 2019).

Apart from the above, the safety profiles of novel agents such as sintilimab (Shi et al., 2019) and toripalimab (JS001) (Tang et al., 2019) have also been reported in Asians. Moreover, a number of large trials of anti-PD-1/PD-L1 monotherapy or combinatory therapy with chemotherapy and/or targeted therapy are ongoing. A growing body of evidence is expected to contribute to the profile of AEs of PD-1/PD-L1 blockade in Asian populations.

MANAGEMENT OF IMMUNE-RELATED ADVERSE EVENTS IN CANCER PATIENTS TREATED WITH PROGRAMMED CELL DEATH PROTEIN 1/PROGRAMMED DEATH-LIGAND 1 BLOCKADE

The incidence of any grade irAEs in clinical trials in Asian populations reportedly is as low as 12% to as high as greater than 90% (Doi et al., 2018; Huang et al., 2019). For some PD-1 inhibitors, the frequency, but not the type, of irAE may increase with dose (Barbee et al., 2015). The profile of irAEs varies among different types of malignancies. A possible explanation is that the irAEs may be associated with the sites of action or sites with T-cell aggregation (Barbee et al., 2015).

In clinical trials, the severity of AEs was evaluated and reported using the Common Terminology Criteria for Adverse Events, which grades AEs on a scale of 1 for mild events that do not need intervention to 5 for death related to the AE (U.S. Department of Health and Human Services, 2018). Although precise practice protocols vary with irAE and anti-PD-1/PD-L1 agent, the American Society of Clinical Oncology has provided general recommendations for irAEs with ICI therapy: for grade 1 AEs, continue therapy with close monitoring; for grade 2 AEs, suspend the therapy and consider resuming when symptoms and/or laboratory values revert to grade ≤1. Corticosteroids may be administered as appropriate; for grade 3 AEs, suspend the therapy and initiate high-dose corticosteroids. If symptoms do not improve within 2–3 days, infliximab may be offered as appropriate; for grade 4 AEs, permanently discontinue the therapy, with the exception of endocrinopathies that have been controlled by hormone replacement (Brahmer et al., 2018). AEs related to PD-1/PD-L1 blockade are generally of low grade (grade 1–2) (Lu et al., 2015). With prompt and proper management, most grade 1–2 AEs can be resolved within a relatively short time (Brennan et al., 2010). However, serious AEs can always be fatal. Therefore, close and continuous monitoring, early recognition, and proper intervention of AEs with rapid onset and poor outcomes are paramount for clinical management. Patient/family member education on self-monitoring should also be involved (Champiat et al., 2015). Currently, prophylaxis against irAEs is not routinely recommended (Barbee et al., 2015).

Because irAEs with PD-1/PD-L1 blockade affect a wild spectrum of body systems, the management of these toxicities requires the collaborative efforts of a multidisciplinary team, including oncologists, pathologists, radiologists, dermatologists, endocrinologists, pulmonologists, neurologists, rheumatologists, gastroenterologists, and the nursing team (Brahmer et al., 2018).

Pulmonary Toxicity

Pneumonitis is the leading pulmonary toxicity among irAEs with ICI treatment. PD-1/PD-L1 blockade-related pneumonitis is caused by off-target effects against the normal lung parenchyma. In a real-world retrospective study of nivolumab/pembrolizumab monotherapy in Asian patients with NSCLC, grade 4 pneumonitis with subsequent mortality was the most serious AE, which occurred in 3.8% (3/74) of patients (Lin et al., 2018). In another retrospectively study of 123 patients with NSCLC treated with nivolumab or pembrolizumab in Japan, 18 patients (14.6%) experienced anti-PD-1-related pneumonitis, of which four (3.3%) were grade ≥3 (Jodai et al., 2018). It has been observed in less than 10% of patients receiving PD-1/PD-L1 inhibitors, but it can quickly escalate and is one of the major causes of treatment-related death (Naidoo et al., 2015). Compared with PD-1 inhibitors, severe pneumonitis is less seen with PD-L1 inhibitors (Kong and Flynn, 2014). Of the two ligands of PD-1, PD-L1 is distributed in a broad spectrum of tissues, whereas PD-L2 is limited primarily to dendritic cells (Latchman et al., 2001). Lung tissue expresses PD-L1 and contains activated alveolar macrophages. Therefore, it is likely that anti-PD-1 antibodies remove the inhibitory signals that control tissue proliferation and cytokine production in the lung, whereas anti-PD-L1 antibodies preserve the ligation between PD-1 and PD-L2 (Kong and Flynn, 2014). Moreover, pneumonitis is more commonly observed in patients with NSCLC (Lu et al., 2015) possibly because of difficulties in differentiating pulmonary symptoms and radiographic manifestations caused by treatment from those by disease progression (Lu et al., 2015). The risk factors for drug-related pneumonitis include preexisting ILD (Yamaguchi et al., 2018b) and preexisting pulmonary fibrosis (Jodai et al., 2018; Yamaguchi et al., 2018b). In a previous study in Japanese patients, male gender and smoking history were suggested to be potential risk factors for nivolumab-related pneumonitis (Kato et al., 2017).

Clinical manifestations of pneumonitis range from asymptomatic isolated radiographic abnormalities to a mimic of severe bacterial pneumonia (Sgambato et al., 2016). Onset time of pneumonitis also varies, with the reported range from a few days to over 2 years after treatment initiation (Naidoo et al., 2015; Jodai et al., 2018). Once the patient presents with new pulmonary symptoms, such as cough and shortness of breath, pneumonitis should be suspected (Sgambato et al., 2016). Standard diagnostic algorithms recommend radiologic investigation by chest computed tomography scan. Lung testing, bronchoscopy, and consultations from Infectious Diseases and

Pulmonology can be considered in cases of grade ≥2 pneumonitis (Chow, 2013; Sgambato et al., 2016). Differential diagnosis can be a clinical enigma here, and diseases such as infection, early pulmonary edema, congestive heart failure, pulmonary embolus, immune-related tumor inflammation, and tumor progression should all be taken into consideration (Sano et al., 2016; Boyer and Palmer, 2018). Management is guided by clinical symptoms (Topalian et al., 2012; Li et al., 2015). Symptomatic pneumonitis should be monitored daily, and administration of moderate doses (1–2 mg/kg) of prednisone slowly tapered for at least 4 weeks is recommended (Chow, 2013). For patients with severe pneumonitis, a high dose of intravenous steroids (such as 2 mg/kg of methylprednisone) is recommended. Additional immunosuppression with infliximab, mycophenolate mofetil, or cyclophosphamide is reasonable (Postow, 2015). Oxygen and ventilatory support should be applied as appropriate (Chow, 2013). In case the patient's symptoms are aggressive and severe but differential diagnosis fails between immune-related pneumonitis and immune reactions against tumor cells, management of immune-related pneumonitis should be the priority because immunosuppressant therapy including corticosteroids for irAEs does not affect tumor response (Weber et al., 2015a).

Dermatologic Toxicity

Dermatologic toxicity is the most common irAE for ICIs (Postow et al., 2015). It occurs in 30–40% of patients treated with anti-PD-1 antibodies, which is comparatively less than the incidence in patients treated with ipilimumab (40–50%) (Belum et al., 2016; Wills et al., 2018). Generally, dermatologic toxicities triggered by anti-PD-1 antibodies are milder and with later onset compared with those triggered by ipilimumab (Palmieri and Carlino, 2018).

The mechanism of PD-1/PD-L1 blockade-induced dermatologic AEs is speculated to be the T-cell homeostasis within the skin, thereby causing self-directed cytotoxic and inflammatory reactions (Okiyama and Katz, 2014). Of note, the combination of nivolumab and radiotherapy might be a risk factor for severe dermatologic AEs, as recently reported in a 77-year-old Japanese patient with advanced melanoma (Tanita et al., 2018) and a 60-year-old Chinese patient with advanced squamous cell lung cancer (Zhao et al., 2018).

Lichenoid reactions, eczema, vitiligo, and pruritus are the most commonly reported dermatologic toxicities after anti-PD-1 monotherapy (Collins et al., 2017). Less common manifestations include lichenoid dermatitis (Joseph et al., 2015), bullous pemphigoid (Carlos et al., 2015), Sweet's syndrome (Naidoo et al., 2015), and follicular or urticarial dermatitis (Naidoo et al., 2015). Rash and pruritus are two leading AEs of the dermatologic system with PD-1/PD-L1 blockade in Asian trials (**Table 1**). Rash in patients treated with PD-1 inhibitors usually presents as maculopapular lesions on the trunk and extremities within the first few weeks of treatment initiation (Sibaud et al., 2016). A standard workup of dermatological lesions include a comprehensive skin examination, elucidation of prior history of dermatologic conditions, laboratory evaluation of renal and hepatic function panel, and serum levels of tryptase and IgE, as indicated. Skin biopsy should also be considered in selected cases (Wills et al., 2018). Histologic findings might vary among types of immune-related dermatitis. Generally, it often reveals an interface, perivascular and periadnexal lymphocytic dermatitis, with few plasma cells and eosinophils (Naidoo et al., 2015). In a case series of pembrolizumab and nivolumab-induced rash, histopathologic tests revealed perivascular, periadnexal lymphocytic infiltrates with scattered eosinophils (Cramer and Bresalier, 2017).

PD-1/PD-L1 blockade can be continued with caution for grade ≤2 dermatologic AEs. However, consider interrupting it in case the AE does not resolve to grade ≤1 within 1–2 weeks (Haanen et al., 2017). Mild dermatologic AEs can be treated with topical corticosteroids (such as betamethasone or fluocinonide) and oral antipruritic agents (such as antihistamines) (Palmieri and Carlino, 2018; Wills et al., 2018). In case of pruritus involvement, supportive care such as cold compresses and oatmeal baths might alleviate symptoms (Sgambato et al., 2016). Although dermatologic irAEs are usually mild to moderate in severity, rare exfoliative conditions such as Stevens-Johnson syndrome/toxic epidermal necrolysis (Chirasuthat and Chayavichitsilp, 2018) have been observed in Asian patients and can be fatal (Puzanov et al., 2017). In such cases, PD-1/PD-L1 blockade should be permanently discontinued. The patient should be hospitalized immediately. Dermatologic consultation for intravenous corticosteroids, maintenance of fluids, electrolyte monitoring, and appropriate wound care are required (Lu et al., 2015; Zimmermann et al., 2017).

Endocrinopathy

ICI-related endocrinopathies may affect any axis of the endocrine system, including the pituitary, thyroid, adrenals, and pancreas (Sznol et al., 2017). Specifically, hypophysitis, thyroiditis, hypothyroidism, hyperthyroidism, and Grave's disease have been seen in ICI therapy (Barroso-Sousa et al., 2018). As previously regarded, hypophysitis occurs mainly with CTLA-4 inhibitors or combinatorial ICIs; dysthyroidism is predominant with PD-1/PD-L1 blockade (Myers, 2018). This is confirmed by the endocrinopathy profile in Asian populations. As observed in early-phase trials in Japan (Hamanishi et al., 2015; Yamazaki et al., 2017), China (Huang et al., 2018), and Asia-Pacific regions (Tahara et al., 2018), hypothyroidism was one of the top 3 most common AEs with anti-PD-1/PD-L1 therapies. In a real-world retrospective study of monotherapy with nivolumab or pembrolizumab in patients with NSCLC in Taiwan, abnormal thyroid function was the most common adverse effect (5/74, 6.5%). Among them, three patients developed hypothyroidism and two developed hyperthyroidism (Lin et al., 2018). In a real-world prospective study in 66 Japanese patients who received nivolumab, destructive thyroiditis was the most frequent endocrine irAE induced, with the onset time from 9 to 60 days (median, 35 days). In addition, patients with positive antithyroglobulin antibodies and/or anti-thyroid peroxidase antibodies at baseline were prone to develop destructive thyroiditis after initiation of nivolumab (Kobayashi et al., 2018). In PD-1/PD-L1 blockade-related irAEs, rare observed endocrinopathies include primary

adrenal insufficiency, insulin-dependent diabetes mellitus (type I), hypercalcemia, and hypoparathyroidism (Wills et al., 2018). Specifically, fulminant type 1 diabetes has recently been discovered as an important subtype, especially in East Asia. It accounts for approximately 20% of acute-onset type 1 diabetes in Japan (Imagawa et al., 2000; Matsuura et al., 2018).

The symptoms of immune-related endocrinopathies are usually nonspecific, such as fatigue, headache, and nausea, which are especially common for cancer patients (Geukes Foppen et al., 2017; Sosa et al., 2018). Therefore, laboratory monitoring of endocrine function is a fundamental method in the diagnosis. Imaging tests, such as magnetic resonance imaging, are indicated in selected cases (Postow, 2015). A characteristic of immune-related endocrinopathies is that the development of disease is typically irreversible (Myers, 2018). Fortunately, endocrinopathies could be easily managed with hormone supplementation or replacement, such as levothyroxine for a hypothyroid status (Postow et al., 2015). For symptomatic hypophysitis, adrenal crisis, or severe thyrotoxicosis, such as thyroid storm, short-term high-dose corticosteroids are required (Illouz et al., 2017; Brahmer et al., 2018). As majority of immune-related endocrinopathies can be treated successfully with hormone replacement, anti-PD-1/PD-L1 therapy is not usually discontinued under the premise of close monitoring of treatment response and endocrine functions (Naidoo et al., 2015; Sgambato et al., 2016).

Gastrointestinal Toxicity

Diarrhea and colitis account for the most gastrointestinal toxicities with PD-1/PD-L1 blockade across populations (Kudo et al., 2017). Upon the diagnosis of immune-related diarrhea, infection with *Clostridium difficile* or other pathogens shall be ruled out. For mild diarrhea, oral hydration, electrolyte substitution, and antimotility agents (such as loperamide) can be adopted with close monitoring (Haanen et al., 2017; Puzanov et al., 2017; Brahmer et al., 2018). In case antimotility agents are not appropriate, consider low-dose systemic corticosteroids or local budesonide (Haanen et al., 2017; Brahmer et al., 2018). In clinically serious cases, anti-PD-1/PD-L1 agents should be discontinued, and the patients can be hospitalized for intravenous corticosteroids (such as prednisone 1 mg/kg daily). If no response to corticosteroids is observed, or the condition relapses after corticosteroids, additional immunosuppression with anti-tumor necrosis factor agents (such as a single dose of infliximab 5 mg/kg) can be considered (Lu et al., 2015; Haanen et al., 2017; Prieux-Klotz et al., 2017; Brahmer et al., 2018).

Renal Toxicity

Renal AEs related to PD-1/PD-L1 blockade are comparatively less common in Asian populations. In a multicenter phase 2 study of nivolumab in Japanese patients with advanced or recurrent NSCLC, renal toxicities were reported in 5.3% (4/76) of patients (Nishio et al., 2017). In other trials of anti-PD-1/PD-L1 antibodies in Asians, treatment-related renal AEs were scarcely seen. Very rare cases reported in Asian patients also involved acute granulomatous tubulointerstitial nephritis (Nakatani et al., 2018) and minimal change in the disease. The patients are usually asymptomatic despite an elevated creatinine identified from routine laboratory tests. Therefore, frequent monitoring of renal function indexes is recommended throughout the entire process of anti-PD-1/PD-L1 treatment. If immune-related nephropathy is suspected, renal biopsy might be considered for definite diagnosis unless contraindicated (Boussiotis, 2016). For severe immune-related kidney injury, potential nephrotoxic agents shall be avoided, and corticosteroids and discontinuation of anti-PD-1/PD-L1 therapy are recommended (Boussiotis, 2016). In case of renal function recovery, anti-PD-1/PD-L1 therapy can be reintroduced with caution (Nakatani et al., 2018).

Ocular Toxicity

Ocular irAEs occur in <1% of patients receiving ICIs (Antoun et al., 2016), and common ocular manifestations include episcleritis, conjunctivitis, and uveitis (Antoun et al., 2016). In clinical trials in Asians, uveitis was seen in 3% (1/38) in a phase 1b study (KEYNOTE-025) of pembrolizumab in Japanese patients with previously treated PD-L1-positive advanced NSCLC (Nishio et al., 2019). In a phase 1 study of camrelizumab in Chinese patients with advanced solid tumors, conjunctivitis was observed in one of 12 patients who received intravenous camrelizumab at 60 mg but not at higher dosage levels (4-week interval after first dose followed by a 2-week schedule) (Mo et al., 2018). Moreover, in a multicenter phase 2 study of nivolumab in Japanese patients with relapsed or refractory classical Hodgkin lymphoma, cataract was seen in 11.8% (2/17) of patients (Maruyama et al., 2017). For any visual complaints during anti-PD-1/PD-L1 therapy, ophthalmologic assessment including dilated fundoscopy and slit lamp examination should be performed promptly (Puzanov et al., 2017). Mild ocular irAEs may resolve spontaneously or can be treated with topical corticosteroids, whereas oral or systemic corticosteroids are indicated for more severe cases (Kumar et al., 2017). An ocular condition that calls for extra attention is immune-related uveitis, which is rare but may result in irreversible visual loss if not properly managed (Wang et al., 2019). A case report presented a 64-year-old Chinese female who developed grade 4 panuveitis with bilateral serous retinal detachment after treatment with nivolumab for metastatic renal cell carcinoma (Wang et al., 2019). In that patient, pulsed intravenous methylprednisolone and oral prednisone improved visual acuity and retinal detachment. However, uveitis relapsed 2 weeks after reinitiation of nivolumab. In the end, intravitreal injection of dexamethasone implant, but not the periorbital injection of steroid or the steroid eye drops, was effective to control the posterior uveitis and serous retinal detachment (Wang et al., 2019).

Immune-Related Adverse Events in Other Organ Systems

Theoretically, any organ system of the body can be affected with irAEs. In Asian cancer patients treated with anti-PD-1/PD-L1 antibodies, other sporadically reported AEs include neuroskeletomuscular toxicities such as neuromyelitis optica spectrum disorder (Narumi et al., 2018), akathisia (Reyes

et al., 2016), and myasthenia gravis (Mitsune et al., 2018); cardiotoxicities such as acute coronary syndrome (Wang et al., 2017), fulminant myocarditis (Yamaguchi et al., 2018a), sick sinus syndrome (Hsu et al., 2018a), and rhabdomyolysis (Chen et al., 2018); coagulopathies such as acute thrombosis (Kunimasa et al., 2018) and Trousseau's syndrome (Horio et al., 2018); and rheumatologic toxicities such as inflammatory arthritis (Inamo et al., 2018).

MANAGEMENT OF PATIENTS WITH PREEXISTING INFECTIOUS CONDITIONS

There have been concerns whether PD-1/PD-L1 blockade exacerbates preexisting conditions that were well maintained without turbulence in immune homeostasis (Mitsune et al., 2018). In Asian patients with cancers, a particular condition that needs extra consideration is the preexisting chronic infection of certain viruses. Hepatitis B virus (HBV) infection is a major public health problem globally. As reported by the Polaris Observatory Collaborators, Asian (Central, East, and Southeast) and Sub-Saharan Africa are two major regions with the highest prevalence of HBV (Collaborators, 2018). In China, despite the drop of incidence and mortality of HBV infection, thanks to a national program for HBV immunization, China certainly confronts the largest number of patients with HBV infection in the world for the size of the population. Chronic HBV infection remains a prominent cause of liver cancer in China (Xiao et al., 2019). Immune dysregulation modulates the entire process of HBV-associated liver diseases from hepatitis to HBV-related hepatocellular carcinoma (HCC) (Li et al., 2016; Cho et al., 2017; Trehanpati and Vyas, 2017). In chronic viral hepatitis, the extended upregulation of PD-1 and CTLA-4 is associated with T-cell exhaustion and persistent viral infection, suggesting that the expressions of immune inhibitory factors are positively associated with the chronicity of viral disease (Shun et al., 2019). Currently, immunotherapy that inhibits immune checkpoint pathway is being tested as a new approach for the cure of HBV (Shire, 2017). And in HBV-related HCC or HBV carriers with cancer, ICIs might benefit both virus relapse and tumor progression theoretically (Shun et al., 2019). In a study of HCC patients treated with tremelimumab (a CTLA-4 inhibitor) in combination with ablation, five patients with hepatitis B were enrolled. In these patients, quantitative hepatitis B antigen was found to decrease over time in all patients, and no viral reactivation was seen (Duffy et al., 2017). However, in a retrospective study of ICI in Taiwan, 12 patients were hepatitis B carriers. Among them, one patient contracted hepatitis. Later, the patient was suspected of hepatitis B recurrence and resistance to entecavir. Hence, the original drug was switched to tenofovir (Hsu et al., 2018b).

Here, a similar situation involves the infection of tuberculosis (TB). In a recent case report, pulmonary TB of a 65-year-old Chinese female was activated after administration of pembrolizumab for metastatic melanoma. Immunotherapy was suspended, and anti-TB drugs were administered, followed by pembrolizumab (He et al., 2018).

As the contrainteraction among viruses, cancers, and PD-1/PD-L1 inhibitors remains unclear, physicians shall bear in mind the reactivation of latent infection and opportunistic infection as potential AEs when managing cancer patients with PD-1/PD-L1 blockade, especially for patients from endemic areas (Lee et al., 2016; Reungwetwattana and Adjei, 2016). At present, carriers of viruses such as HBV, TB, and HIV were routinely excluded from clinical trials. Therefore, there is a lack of information for anti-PD-1/PD-L1 treatment in patients with existing infectious conditions. With limited regulations for clinical practice, screening for major viruses such as HIV, TB, and HBV (especially for patients with HCC) according to the prevalence before initiation of PD-1/PD-L1 blockade is encouraged (Reungwetwattana and Adjei, 2016; He et al., 2018).

LIMITATIONS

Limitations of the statistical analyses performed in this review shall be addressed. First, the incidence of AEs included for statistical analyses comes from studies on different anti-PD-1/PD-L1 agents. The dosages and the frequencies of administration can be inconsistent among studies with the same therapeutic agent. Moreover, the studies were performed in patients with different types of cancers, and significant heterogeneity across studies may exist. Second, the standard definitions of AE, irAE, or TRAE were yet to be established. Thus, the definitions adopted in the studies might be inconsistent or even subjective to the investigators. Therefore, the results of statistical analysis in this review shall be interpreted with caution. While it depicted a generally different profile of AEs between Western/international patients and Asian patients treated with PD-1/PD-L1 blockade, the exact prevalence of certain AEs shall be determined by clinical studies with large sample sizes.

CURRENT CHALLENGES AND FUTURE PERSPECTIVES

While PD-1/PD-L1 blockade is revolutionizing the treatment in oncology, it leads to a new spectrum of AEs. In the field of the management of AEs with immunotherapy, the balance between control of irAEs and maintenance of antitumor effect has been a recent research focus. Because PD-1/PD-L1 blockade works by enhancing antitumor immunity, it has been wondered whether treatment of irAEs by immunosuppression would impair the antitumor efficacy of PD-1/PD-L1 blockade. In several retrospective studies, irAEs were associated with favorable clinical outcomes including tumor response (Ishihara et al., 2017; Kim et al., 2018) and survival (Teulings et al., 2015; Yamazaki et al., 2017). Nevertheless, the results remain controversial (Weber et al., 2017), and it cannot be ruled out that nonresponder patients discontinued PD-1/PD-L1 blockade before the onset of irAEs (Yamazaki et al., 2017). Therefore, studies exploring the exact relationships between treatment of irAEs and clinical outcomes are needed to select the right time for immunosuppressive intervention of AEs and to obtain a balance between minimal

toxicity and optimal antitumor efficacy. In addition, there have been few studies focusing on the management strategies for AEs with PD-1/PD-L1 blockade. Current management is mainly based on the guidelines developed for other ICIs such as CTLA-4 inhibitors, and lots of ambiguities remain. In the future, experimental studies and clinical studies with large sample sizes may further elucidate the mechanism and reveal the characteristics of AEs in patients treated with PD-1/PD-L1 blockade. Potential research interests might include prophylaxis of AEs and individualized dosing regimens of PD-1/PD-L1 blockade.

By now, only a small number of clinical trials in Asian populations have reported outcomes, and included patients were mainly from Japan, China, and South Korea. The profile of AEs in Asians needs to be further depicted in the future. As concluded in the current review, the characteristics of AEs in Asian populations might be different from those in Western patients with cancers. Along with the growing body of information of AEs with PD-1/PD-L1 blockade, tools of precision medicine shall be applied to determine the optimal management strategy of AEs in cancer patients of different races or other characteristics. Moreover, guidelines that adapt to types of AEs in certain populations shall be refined and updated pertinently.

AUTHOR CONTRIBUTIONS

JY and HS conceptualized this review. JY wrote and edited the manuscript. JY and XH created the figures. QL, JJ, and HS revised and edited the manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fphar.2019.00726/full#supplementary-material

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The Relative Risk of Immune-Related Liver Dysfunction of PD-1/PD-L1 Inhibitors Versus Chemotherapy in Solid Tumors: A Meta-Analysis of Randomized Controlled Trials

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Background: Immune checkpoint inhibitors (ICIs) have made a significant breakthrough in the treatment of solid tumors; however, their use also generates unique immune-related adverse effects (irAEs). Here, we performed a systematic review and meta-analysis to assess the risk of immune-related liver dysfunction between in patients treated by programmed death 1 (PD-1)/programmed death ligand 1 (PD-L1) inhibitors exclusively and chemotherapy.

Methods: A comprehensive search of multiple databases identified eligible studies, including randomized controlled trials (RCTs) with PD-1/PD-L1 inhibitors exclusively and chemotherapy in patients with different solid tumors was carried out. The elevations of alanine aminotransferase (ALT) and aspartic aminotransferase (AST) were used to evaluate liver dysfunction. The relative risk (RR) and 95% confidence intervals (CI) were calculated and analyzed by Review Manager 5.3 and STATA version 12.0 statistical software.

Results: After screening and eligibility assessment, a total of 5638 patients from 12 RCTs were included in our meta-analysis. In comparison with chemotherapy, patients treated with PD-1/PD-L1 inhibitors exclusively showed an increased incidence of all-grade ALT/AST elevations (ALT: RR, 1.52, 95% CI, 1.09–2.13; $p = 0.01$; AST: RR, 1.96, 95% CI, 1.37–2.81; $p = 0.0002$). Patients receiving PD-1 inhibitors showed the significantly higher risk of all-grade ALT/AST elevations incidence than those receiving chemotherapy (ALT: RR, 1.47; 95% CI, 1.05–2.07; $p = 0.03$; AST: RR, 1.90, 95% CI, 1.32–2.73; $p = 0.0005$). However, no significant difference was found between PD-L1 inhibitor and chemotherapy group. Moreover, for non-small cell lung cancer (NSCLC) and urothelial carcinoma (UC), patients treated with PD-1/PD-L1 inhibitors exclusively exhibited a significant higher risk of all-grade ALT elevation incidence (NSCLC: RR, 1.92; 95% CI, 1.23–3.02; $p = 0.004$; UC: RR, 3.36; 95% CI, 1.12–10.06, $p = 0.03$) and all-grade AST elevation incidence

(NSCLC: RR, 2.37; 95% CI, 1.45–3.87, $p = 0.0005$; UC: RR, 4.47; 95% CI, 1.30–15.38, $p = 0.02$) than chemotherapy.

Conclusions: The meta-analysis confirms that PD-1/PD-L1 inhibitors exclusive pose an increased risk of immune-related liver dysfunction than chemotherapy. PD-1/PD-L1 blockade in NSCLC and UC increase the risk of immune-related liver dysfunction, but not in melanoma (MM) and head-neck squamous cell carcinoma (HNSCC).

Keywords: immune checkpoint inhibitors, PD-1/PD-L1 inhibitors, immune-related adverse events, liver dysfunction, Nivolumab, Pembrolizumab, Atezolizumab

INTRODUCTION

Immune checkpoint blockade has become a most recent frontline of cancer treatment, since it significantly prolongs survival with fewer side effects compared with traditional chemotherapy (Gong et al., 2018). Despite the impressive antitumor immune response induced by the immune checkpoint inhibitors (ICIs), by blocking the negative immune regulatory mechanism that are normally vital for maintaining immunologic homeostasis, these agents also lead to autoimmune-like toxicities termed immune-related adverse events (irAEs) (Jing et al., 2016, Davies and Duffield, 2017). IrAEs are quite different both in mechanism and management of adverse effects induced by chemotherapy (Sznol et al., 2017), they most commonly include pruritus, diarrhea, rash, colitis, endocrine dysfunction, nephritis, liver dysfunction, and pneumonitis. Among these irAEs, immune-related liver dysfunction is usually asymptomatic and has only been discovered in routine liver function examination. Thus, it is usually ignored by clinicians. However, this liver dysfunction tends to present with higher severity and may be fatal. Explosive hepatitis with jaundice and liver failure has been reported in the treatment of Ipilimumab, highlighting the need for seriously attention (Chmiel et al., 2011). To date, clinical experience, especially the identification and therapy, has still been very scarce.

According to the permission of Food and Drug Administration (FDA), ICIs are mainly used in patients with advanced cancer or metastatic tumor. Improving the quality of life was considered as important as the prolongation of survival in these patients. Therefore, pursuing a balance between toxicity and curative effect of treatment became crucial for decision making. The side effect of traditional cytotoxicity chemotherapy was well known by plenty through clinical experience. It is urgent to compare the toxicity of ICI therapy with chemotherapy. Furthermore, with the outstanding clinical outcome of ICI treatment, the use of ICIs is expanding rapidly. It is necessary to improve our understanding about this specific side effect.

This meta-analysis was designed to determine the risk of immune-related liver dysfunction by evaluated the elevations of alanine aminotransferase (ALT) and aspartic aminotransferase (AST) in patients with solid tumors treated with programmed death 1 (PD-1)/programmed death ligand 1 (PD-L1) inhibitors exclusively or chemotherapy.

METHODS

Search Strategy

Original articles were from the following databases: the Embase, Medline, Web of Science, and PubMed (up to December 31, 2018). Studies on the risk of immune-related liver dysfunction in PD-1/PD-L1 inhibitors therapies exclusive versus chemotherapy were searched. The following keywords and corresponding Medical Subject Heading terms were used for analyses: "ICIs," "immune checkpoint inhibitors," "Nivolumab," "Pembrolizumab," "Atezolizumab," "PD-1 inhibitor," "PD-L1 inhibitor," "cancer," "tumor," "carcinoma," "phase II," and "phase III".

Selection and Exclusion Criteria

Studies meeting the following criteria were included in our meta-analysis: 1) phase II/III randomized controlled trials (RCTs) with primary endpoints, such as overall survival (OS), progression-free survival (PFS), or objective response rate (ORR); 2) histologically confirmed solid carcinomas; 3) random assignment of participants to treatment with single-agent PD-1/PD-L1 inhibitors or chemotherapy; 4) information of immune-related liver dysfunction for all-grade (1–5) and high-grade (3–5). Two independent reviewers screened the studies based on the key terms contained in the titles and abstracts. Then, the full texts of all potentially eligible studies were assessed. The references of relevant studies were also revised to identify other suitable studies. Letters, expert opinions, case reports, reviews, articles without available data, and duplicate publications were excluded.

Data Extraction

Two independent investigators performed data extraction and evaluated the identified studies by using a patient, intervention, comparison, and outcome (PICO) chart (Huang et al., 2006). Discrepancies between the two reviewers were resolved by a third reviewer. The following information was recorded from the selected studies: first author's name, year of publication, trial phase, type of solid tumors, the primary endpoint, therapeutic regimen, number of patients in the PD-1/PD-L1 inhibitors treatment or control group, number of patients enduring immune-related liver dysfunction of all-grade (1–5; recorded according to Version 4 of the Common Terminology Criteria for

Adverse Events of the National Cancer Institute) and high-grade (3–5) (Basch et al., 2014).

Statistical Analysis

The data analysis, including the comparison of the incidence and relative risk (RR) of liver dysfunction between PD-1/PD-L1 inhibitors exclusive and chemotherapy, was performed using Review Manager 5.3 (Cochrane Collaboration 2014, Nordic Cochrane Center, Copenhagen, Denmark) and STATA version 12.0 statistical software (STATA Corporation, College Station, TX, USA). The RR and the corresponding 95% confidence intervals (CIs) were calculated in patients assigned to PD-1/PD-L1 inhibitors exclusively compared with those assigned to chemotherapy in the same trial. RR >1.0 indicates a higher risk or higher incidence of liver dysfunction in patients treated with PD-1/PD-L1 inhibitors exclusively than those treated with chemotherapy. For the calculation of the RR, random or fixed-effect models were used, depending on the heterogeneity of included studies. The Q test and I^2 statistics were used to assess the heterogeneity among the RCTs. When substantial heterogeneity ($p > 0.05$ or $I^2 < 50\%$) was not observed, the pooled estimate was calculated based on the fixed-effect model. When substantial heterogeneity ($p < 0.05$ or $I^2 > 50\%$) was observed in the analysis, the random-effect model was used for the meta-analysis (Higgins et al., 2003, DerSimonian and Laird, 2015). Sensitivity analysis was performed by deleting one study at a time to determine if the results would be affected by a single study, particularly facing with a suspicious result or considerable heterogeneity. Subgroup analysis was conducted according to different PD-1/PD-L1 inhibitors and different types of cancer to explore the source of heterogeneity. We evaluated potential publication bias using the Begg's and Egger's tests with funnel plots (Begg and Mazumdar, 1994, Sterne et al., 2000). A two-tailed p value < 0.05 was considered statistically significant.

Quality Assessment

To assess the risk of bias for the included studies, the Cochrane risk of bias tool was used. This tool assesses each trial for selection bias (including both random sequence generation and allocation concealment), performance bias (blinding of participants and personnel), detection bias (blinding of outcome assessment), attrition bias (incomplete outcome data), reporting bias (selective reporting), and other bias (Higgins et al., 2011). Trials with more than two and four high-risk components were considered to have a moderate and high risk of bias, respectively.

RESULTS

Search Results and Study Characteristics

Among the 236 studies included in our database, after duplication removal, a total of 12 studies were selected (Borghaei et al., 2015, Brahmer et al., 2015, Caroline et al., 2015, Robert et al., 2015, Weber et al., 2015, Fehrenbacher et al., 2016, Ferris et al., 2016, Herbst et al., 2016, Bellmunt et al., 2017, Carbone et al., 2017). Nine of the 12 studies came from the United States and three from France. The patients enrolled in the 12 studies are all Caucasian population. Selection process and exclusion reasons are shown in **Figure 1**. A total of 5638 patients (PD-1/PD-L1 inhibitors: 3040; chemotherapy: 2598) were included in the analysis from six nivolumab trials, three pembrolizumab trials, and one atezolizumab trial. Tumor types tested in these studies included non-small cell lung cancer (NSCLC) (n = 5), melanoma (MM) (n = 3), urothelial carcinoma (UC) (n = 1), and head-neck squamous cell carcinoma (HNSCC) (n = 1). Two of the studies involved three-arm trials, in which two doses of pembrolizumab arms were compared with chemotherapy treatment. The baseline characteristics of each trial are outlined in **Table 1**.

The Cochrane risk of bias tool was used to evaluate the quality of each study. As shown in **Figures 2**, **3** the overall risk of bias was assessed as low risk, and all included studies were qualified.

RR of ALT and AST Elevations Incidence by Treating With PD-1/PD-L1 Inhibitors or Chemotherapy

Patients treated with PD-1 inhibitor showed a significantly higher risk of all-grade ALT and AST elevations incidence than those treated with chemotherapy (ALT: RR, 1.47; 95% CI, 1.05–2.07; $p = 0.03$; AST: RR, 1.90; 95% CI, 1.32–2.73; $p = 0.0005$, respectively) (**Figures 4**, **5**). However, no significant difference in the risk of all-grade ALT or AST elevations incidence was found between PD-L1 inhibitor (atezolizumab) and chemotherapy (ALT: RR, 5.70; 95% CI, 0.70–46.76; $p = 0.10$; AST: RR, 5.70; 95% CI, 0.70–46.76; $p = 0.10$, respectively). Moreover, there was neither significant difference in the pooled RR of high-grade ALT elevation (PD-1 inhibitor: RR, 1.39; 95% CI, 0.64–3.05; $p = 0.41$; PD-L1 inhibitor: RR, 6.66; 95% CI, 0.35–127.69; $p = 0.21$) nor AST elevation (PD-1 inhibitor: RR, 1.67; 95% CI, 0.66–4.22; $p = 0.28$; PD-L1 inhibitor: RR, 6.66; 95% CI, 0.35–127.69; $p = 0.21$) between patients treated with PD-1/PD-L1 inhibitors and chemotherapy.

Subgroup Analysis of ALT and AST Elevations Incidence by Drug

In comparison with chemotherapy, patients receiving pembrolizumab achieved a significantly higher risk of all-grade ALT and AST elevations incidence (ALT: RR, 1.61; 95% CI, 1.01–2.58; $p = 0.05$; AST: RR, 2.15; 95% CI, 1.28–3.61; $p = 0.004$, respectively) (**Figures 6**, **7**), but only the risk of all-grade AST elevation incidence was significantly increased in nivolumab subgroup (RR, 1.69; 95% CI, 1.01–2.81; $p = 0.04$). Furthermore, we found no significant differences between nivolumab or pembrolizumab and chemotherapy in pooled RR of high-grade ALT elevation (nivolumab: RR, 1.45; 95% CI, 0.54–3.89; $p = 0.47$; pembrolizumab: RR, 1.31; 95% CI, 0.36–4.73; $p = 0.68$) and AST elevation (nivolumab: RR, 1.98; 95% CI, 0.58–6.82; $p = 0.28$; pembrolizumab: RR, 1.35, 95% CI, 0.33–5.43; $p = 0.68$).

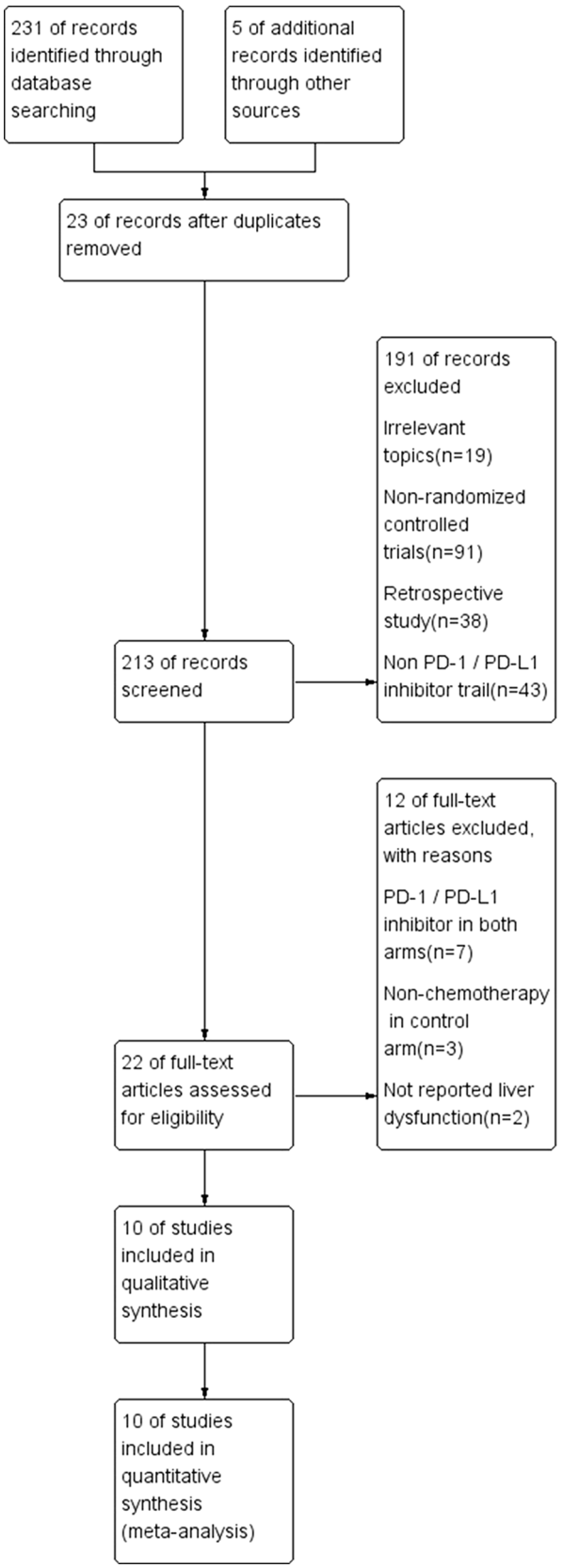

FIGURE 1 | A flowchart describing the RCT selection process.

The overall heterogeneity of incidence for all-grade ALT elevation was tiny in the nivolumab subgroup, low in the PD-1 inhibitor subgroup, and moderate in the pembrolizumab subgroup (nivolumab: $I^2 = 0\%$, $p = 0.58$; PD-1: $I^2 = 27\%$, $p = 0.19$; pembrolizumab: $I^2 = 58\%$, $p = 0.05$, respectively). Additionally, regarding high-grade ALT and all-grade AST elevation incidence, a small heterogeneity was observed in the nivolumab subgroup (ALT: $I^2 = 0\%$, $p = 0.60$; AST: $I^2 = 5\%$, $p = 0.39$, respectively), the pembrolizumab subgroup (ALT: $I^2 = 8\%$, $p = 0.36$; AST: $I^2 = 0\%$, $p = 0.43$, respectively), and the PD-1 inhibitor subgroup (ALT: $I^2 = 0\%$, $p = 0.63$; AST: $I^2 = 0\%$, $p = 0.48$, respectively). Of note, for high-grade AST elevation incidence, we not only found a small heterogeneity in the PD-1 inhibitor subgroup ($I^2 = 6\%$, $p = 0.39$) and the nivolumab subgroup ($I^2 = 0\%$, $p = 0.56$) but also a moderate heterogeneity in the pembrolizumab subgroup ($I^2 = 44\%$, $p = 0.15$). The fixed-effect model was used for the RR analysis of all- and high-grade ALT and AST elevations incidence, due to an overall lack of heterogeneity within the included studies.

As shown in **Tables 2**, **3**, **4**, the sensitivity analysis was performed to detect whether the results could have an impact on the PD-1 (grades 1–5 ALT elevation) subgroup ($I^2 = 27\%$), the pembrolizumab (grades 1–5 ALT elevation) subgroup ($I^2 = 58\%$), and the pembrolizumab (grades 3–5 AST elevation) subgroup ($I^2 = 44\%$), respectively.

Subgroup Analysis of ALT and AST Elevations Incidence by Cancer Type

As shown in **Figure 8**, the risk of all-grade ALT elevation incidence significantly increased in patients with NSCLC and UC treated by PD-1/PD-L1 inhibitors than chemotherapy (NSCLC: RR, 1.92; 95% CI, 1.23–3.02; $p = 0.004$; UC: RR, 3.36; 95% CI, 1.12–10.06; $p = 0.03$), but did not change significantly in patients with MM and HNSCC (MM: RR, 0.95; 95% CI, 0.52–1.73; $p = 0.86$; HNSCC: RR, 0.31; 95% CI, 0.05–1.85; $p = 0.20$). Additionally, with respect to high-grade ALT elevation, treatment with PD-1/PD-L1 inhibitors did not significantly increase the pooled RR of ALT elevation incidence in patients suffering from NSCLC (RR, 2.28; 95% CI, 0.81–6.44; $p = 0.12$) and UC (RR, 6.71; 95% CI, 0.35–129.29; $p = 0.21$).

Compared with chemotherapy, significant higher risk of all-grade AST elevation incidence was observed in patients with NSCLC (RR 2.37, 95% CI, 1.45–3.87, $p = 0.0005$) and UC (RR 4.47, 95% CI, 1.30–15.38, $p = 0.02$) treated with PD-1/PD-L1 inhibitors exclusively (**Figure 9**). However, no significant difference of all-grade AST elevation incidence was found in patients with either MM (RR, 1.38; 95% CI, 0.76–2.54; $p = 0.29$) or HNSCC (RR, 0.47; 95% CI, 0.07–3.30; $p = 0.45$). Furthermore, in regard to high-grade AST elevation, NSCLC patients treated with PD-1/PD-L1 inhibitors showed a significantly higher RR of AST elevation incidence (RR, 3.52; 95% CI, 1.02–12.18; $p = 0.05$) than those treated with chemotherapy, but this difference was not observed in UC patients (RR, 12.46; 95% CI, 0.71–220.13; $p = 0.09$).

A small overall heterogeneity of all-grade ALT and AST elevations incidence was found in both the MM subgroup (ALT: $I^2 = 9\%$, $p = 0.35$; AST: $I^2 = 2\%$, $p = 0.38$, respectively) and the

TABLE 1 | Characteristics of the eligible RCTs.

Study[year]	Country	Study type	Histology	Endpoint	Treatment arms	patients	ALT (G1-5)	ALT (G3-5)	AST (G1-5)	AST (G3-5)
Brahmer et al. (2015)	USA	RCT III	NSCLC	OS	nivolumab 3 mg/kg q2w	131	2	0	2	0
					DOX 75 mg/m^2 q3w	129	1	1	1	1
Borghaei et al. (2015)	USA	RCT III	NSCLC	OS	nivolumab 3 mg/kg q2w	287	9	0	9	1
					DOX 75 mg/m^2 q3w	268	4	1	2	0
Herbst1 (2016)	USA	RCT III	NSCLC	OS	pembrolizumab 2 mg/kg q2w	339	16	2	10	2
					DOX 75 mg/m^2 q3w	309	4	0	3	0
Herbst2 (2016)	USA	RCT III	NSCLC	OS	pembrolizumab 10 mg/kg q2w	343	8	1	7	0
					DOX 75 mg/m^2 q3w	309	4	0	3	0
Fehrenbacher et al. (2016)	USA	RCT II	NSCLC	OS	atezolizumab 1200 mg q3w	142	6	3	6	3
					DOX 75 mg/m^2 q3w	135	1	0	1	0
Carbone et al. (2017)	USA	RCT III	NSCLC	OS	nivolumab 3 mg/kg q2w	267	19	7	23	7
					chemotherapy control	263	14	2	12	1
Weber et al. (2015)	USA	RCT III	MM	ORR	nivolumab 3 mg/kg q2w	268	7	2	11	1
					chemotherapy control	102	1	0	2	0
Robert et al. (2015)	France	RCT III	MM	OS	nivolumab 3 mg/kg q2w	206	3	2	2	1
					dacarbazine 1000 mg/m^2 q3w	205	3	1	4	1
Schachter1 (2015)	France	RCT III	MM	OS	pembrolizumab 10 mg/kg q2w	278	12	0	14	0
					chemotherapy control	256	9	2	6	2
Schachter1 (2015)	France	RCT III	MM	OS	pembrolizumab 10 mg/kg q3w	277	4	1	6	1
					chemotherapy control	256	9	2	6	2
Bellmunt et al. (2017)	USA	RCT III	Urothelial Ca	OS PFS	pembrolizumab 200 mg q3w	266	14	3	14	6
					chemotherapy control	255	4	0	3	0
Ferris et al. (2016)	USA	RCT III	head neck	OS	nivolumab 3 mg/kg q2w	236	2	1	2	0
					chemotherapy control	111	3	1	2	0

NSCLC, non-small cell lung cancer; MM, melanoma; Urothelial Ca, urothelial carcinoma; head neck, head-neck squamous cell carcinoma. DOX, docetaxel; PFS, progression-free survival; OS, overall survival; ORR: objective response rate. Both Herbst1 and Herbst2 belong to Herbst et al 2016. And both Schachter1 and Schachter2 belong to Schachter et al 2015. Herbst1, pembrolizumab 2mg/kg q2w; Herbst2, pembrolizumab 10mg/kg q2w; Schachter1, pembrolizumab10mg/kg q2w; Schachter2, pembrolizumab 10mg/kg q3w.

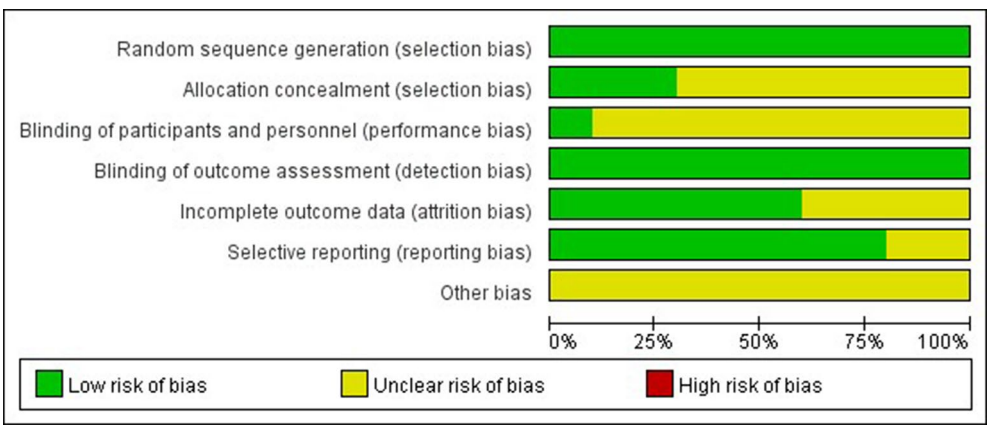

FIGURE 2 | Risk of bias summary. Bar chart comparing the percentage risk of bias for each included RCT. Low risk of bias (green), high risk of bias (red), and unclear risk of bias (yellow).

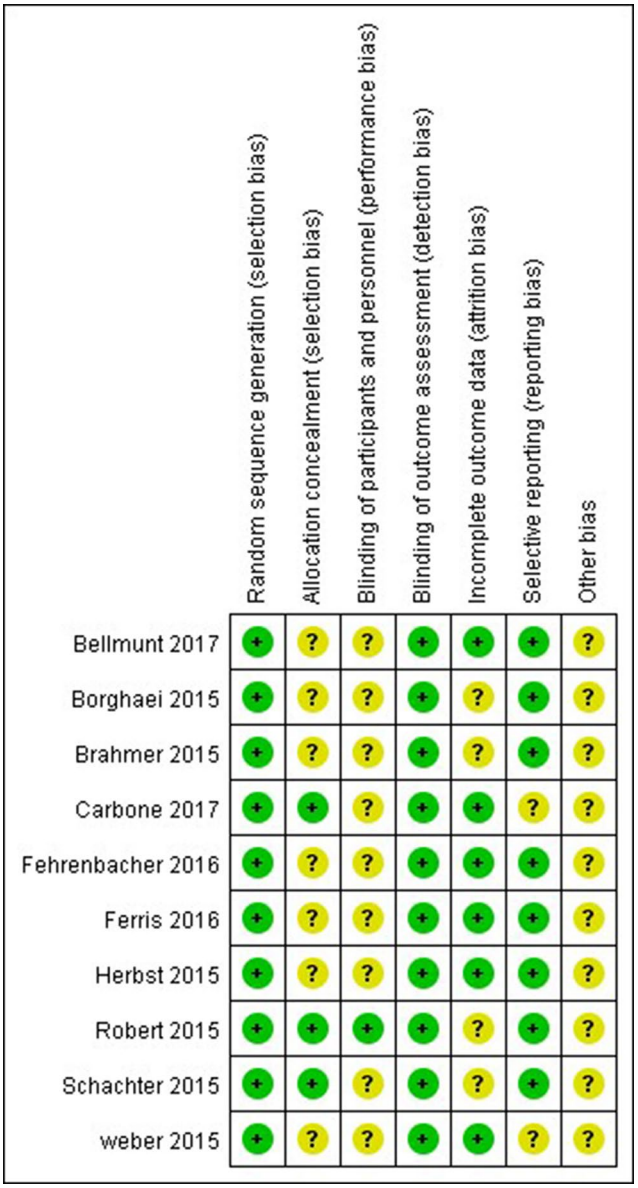

FIGURE 3 | Risk of bias summary. Risk of bias for each included RCT, representing low risk of bias (+), high risk of bias (–), and unclear risk of bias (?).

NSCLC subgroup (ALT: I^2 = 0%, p = 0.62; AST: I^2 = 0%, p = 0.87, respectively). As to high-grade ALT and AST elevations incidence, we also observed a tiny heterogeneity in the MM subgroup (ALT: I^2 = 0%, p = 0.58; AST: I^2 = 0%, p = 0.83, respectively) and the NSCLC subgroup (ALT: I^2 = 0%, p = 0.56; AST: I^2 = 0%, p = 0.60, respectively).

Analysis of Publication Bias

We used Egger's test and Begg's test conducted in STATA 12.0 software to assess the publication bias of the included literatures. As shown in **Table 5**, all the p values were > 0.05 after two tests. In addition, the funnel plots for a relative risk of all- and high-grade ALT/AST elevations showed that each trail was arranged symmetrically on either side of the funnel (**Figures 10–13**). Collectively, there was no significant publication bias in our meta-analysis.

DISCUSSION

Currently, ICIs have gathered a great deal of attention as a novel promising antitumor therapy, with PD-1 or PD-L1 inhibitor demonstrating remarkable antitumor immune responses, overturning tumor-induced immune tolerance and improving survival rate of patients with malignant tumors after surgery, radiotherapy, or chemotherapy (Hodi et al., 2010, De et al., 2017). PD-1/PD-L1 inhibitors, such as nivolumab, pembrolizumab, and atezolizumab, have been approved by Food and Drug Administration (FDA) for the treatment of various advanced solid tumors, including NSCLC, UC, MM, and HNSCC; new indications are expected to rise further. With the increasing application of these agents, more and more irAEs were observed in clinic practice (Davies and Duffield, 2017, Wang et al., 2019). Among these irAEs, immune-related liver dysfunction is very insidious and usually discovered by elevations of ALT and AST in routine liver function tests instead of by clinical symptoms. It is worth noting that this autoimmune-mediated disorder may progress and even be life-threatening (Chmiel et al., 2011). Currently, both ICIs and chemotherapy are approved treatment for advanced cancer. Although for advanced cancer treatment, a lot of times, reducing the toxicity was considered as important as prolongation of survival, especially for palliative treatment in some very late stage cancer. Therefore, determining the liver dysfunction in patients with solid tumors treated with PD-1/PD-L1 inhibitors exclusively or chemotherapy is needed for informed treatment decisions.

Previous studies have demonstrated that chemotherapy has long been related to serious adverse events, whereas PD-1/PD-L1 inhibitors are generally safer than chemotherapy in most toxic events for patients (Khan et al., 2018, Luo et al., 2018). However, some recent studies have suggested that patients treated with PD-1/PD-L1 inhibitors exclusively have a higher risk of increasing the incidence of certain irAEs, such as pneumonia, colitis, and hyperthyroidism, in comparison with chemotherapy (O'Kane et al., 2017, Ma et al., 2018, Su et al., 2018). In present study, our results confirmed that patients receiving PD-1 inhibitor exclusively increased the risk of both all-grade ALT and AST elevations incidence than chemotherapy. In comparison with previous investigations, our result is more convinced with a larger number of recruited clinical trials. In addition, our study showed that there was no high-grade ALT or AST elevation found in patients treated with PD-1/PD-L1 inhibitors exclusively than chemotherapy, which provided more details of toxicity of ICIs to clinician for making treatment selection. Taken together, our finding suggested that more attention needs to be paid on advanced cancer patients with liver dysfunction, when considering treating by ICIs.

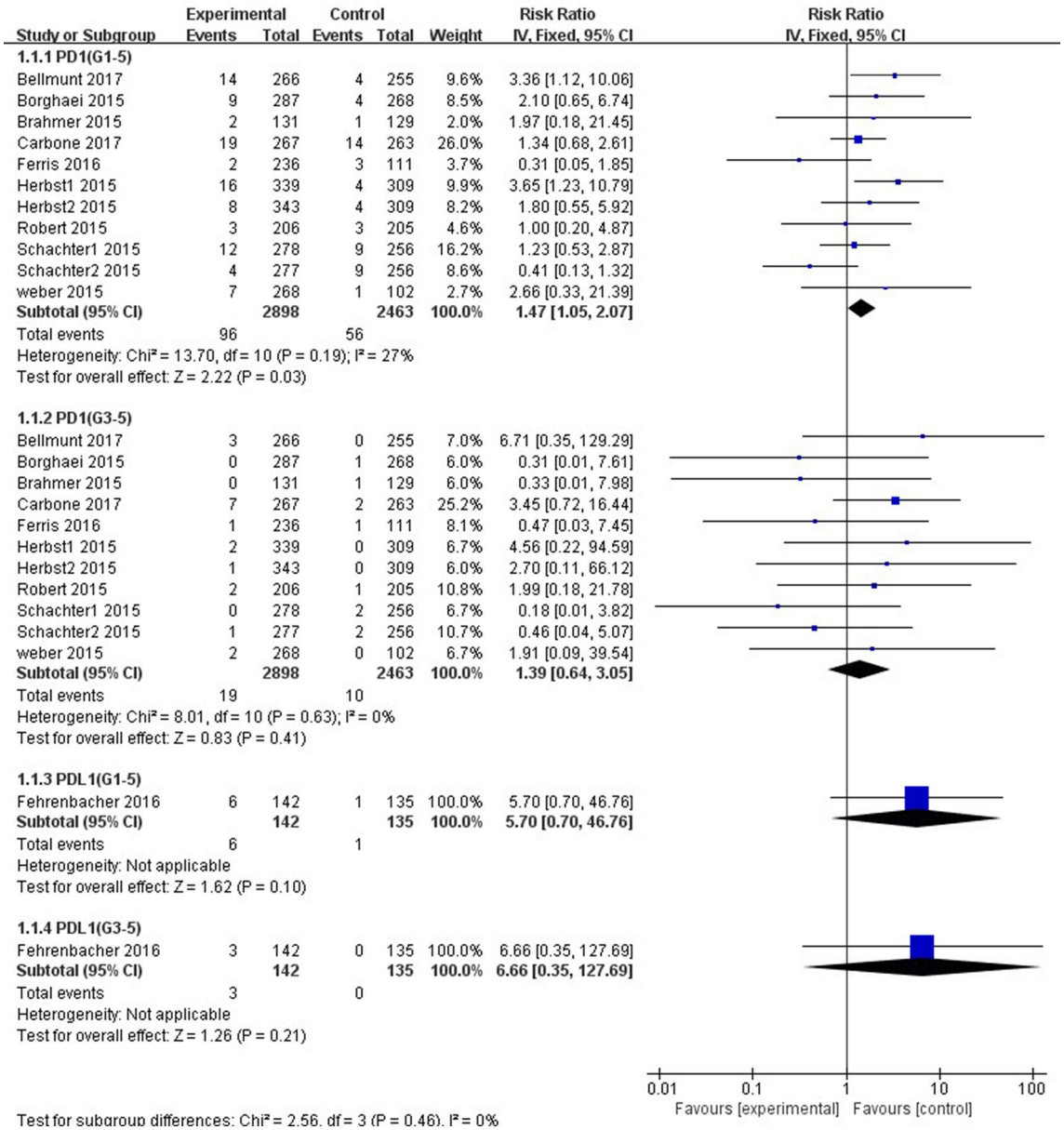

FIGURE 4 | Forest plot analysis for ALT elevation with PD-1/PD-L1 inhibitors versus chemotherapy. G1-5: grades 1–5; G3-5: grades 3–5.

A newly published meta-analysis has demonstrated that patients treated with PD-1 inhibitor were more likely to have a higher mean incidence of grade 3 or higher adverse events than treated with PD-L1 inhibitor (Wang et al., 2019). Interestingly, our study found similar results; PD-1 inhibitor was associated with increased ALT and AST elevations incidence compared with PD-L1 inhibitor. PD-1 is known to have two ligands, PD-L1 (B7-H1/CD274) and PD-L2 (B7-DC/CD273) (Zak et al., 2017), whereas PD-L1 inhibitor only blocks binding to PD-1 (Philips and Atkins, 2015). Therefore, PD-1 inhibitor may block more level of checkpoint signaling than PD-L1 inhibitor (Friedman et al., 2016, Postow et al., 2018). It is noticeable, without well-designed clinical trials to compare the adverse events between PD-1 inhibitor and PD-L1 inhibitor, interpretation of these results need to be made carefully. On the other hand, our meta-analysis also revealed that although both nivolumab and pembrolizumab belong to PD-1 inhibitor, pembrolizumab caused more risk of ALT and AST elevations incidence when compared with nivolumab. It has been shown that nivolumab and pembrolizumab have no overlapping binding regions on the PD-1 protein (Tan et al., 2017), suggesting that the mechanism of action may be different in these two inhibitors. These differences in PD-1 binding sites between nivolumab and pembrolizumab may account for the different risk of ALT and AST elevations incidence. In this regard, our research may provide a basis for clinicians to recommend proper medications for patients.

TABLE 2 | Sensitivity analysis for ALT elevation (Grade1-5) in patients treated with PD-1 inhibitor versus chemotherapy.

Removed study	Trails	Heterogeneity		RR (95% CI)	P
		P	I^2		
All Study	11	0.19	27%	1.47 (1.05–2.07)	0.03
Bellmunt et al. (2017)	10	0.26	20%	1.35 (0.94–1.93)	0.10
Borghaei et al. (2015)	10	0.15	32%	1.42 (1.04–2.03)	0.05
Brahmer et al. (2015)	10	0.14	34%	1.46 (1.04–2.06)	0.03
Carbone et al. (2017)	10	0.14	34%	1.52 (1.02–2.26)	0.04
Ferris et al. (2016)	10	0.30	16%	1.56 (1.10–2.26)	0.01
Herbst1 (2016)	10	0.30	16%	1.33 (0.93–1.91)	0.12
Herbst2 (2016)	10	0.14	34%	1.44 (1.01–2.06)	0.04
Robert et al. (2015)	10	0.14	34%	1.50 (1.06–2.12)	0.02
Schachter1 (2015)	10	0.14	33%	1.52 (1.05–2.21)	0.03
Schachter2 (2015)	**10**	**0.47**	**0%**	**1.66 (1.16–2.37)**	**0.005**
Weber et al. (2015)	10	0.15	33%	1.45 (1.02–2.04)	0.04

The bold text indicates that this study is the main source of heterogeneity in the subgroup.

TABLE 3 | Sensitivity analysis for ALT elevation (Grade1-5) in patients treated with pembrolizumab versus chemotherapy.

Removed study	Trails	Heterogeneity		RR (95% CI)	P
		P	I^2		
All Study	5	0.05	58%	1.61 (1.01–2.58)	0.05
Bellmunt et al. (2017)	4	0.06	60%	1.36 (0.81–2.30)	0.24
Herbst1 (2016)	4	0.07	57%	1.33 (0.79–2.25)	0.28
Herbst2 (2016)	4	0.02	69%	1.58 (0.94–2.63)	0.08
Schachter1 (2015)	4	0.04	67%	1.82 (1.03–3.20)	0.04
Schachter2 (2015)	**4**	**0.35**	**9%**	**2.10 (1.26–3.51)**	**0.005**

The bold text indicates that this study is the main source of heterogeneity in the subgroup.

TABLE 4 | Sensitivity analysis for AST elevation (Grade3-5) in patients treated with pembrolizumab versus chemotherapy.

Removed study	Trails	Heterogeneity		RR (95% CI)	P
		P	I^2		
All Study	5	0.15	44%	1.35 (0.33–5.43)	0.68
Bellmunt et al. (2017)	**4**	**0.31**	**14%**	**0.68 (0.14–3.34)**	**0.63**
Herbst1 (2016)	4	0.10	56%	0.97 (0.20–4.67)	0.97
Herbst2 (2016)	4	0.15	44%	1.35 (0.33–5.43)	0.68
Schachter1 (2015)	4	0.20	38%	2.30 (0.48–11.07)	0.30
Schachter2 (2015)	4	0.12	52%	2.33 (0.42–13.00)	0.33

The bold text indicates that this study is the main source of heterogeneity in the subgroup.

TABLE 5 | Evaluation of publication bias with Begg's and Egger's tests.

	Trails	Heterogeneity		RR (95% CI)	Begg's test		Egger's test	
		P	I^2		Z	P	T	P
ALT elevations (G1-5)	12	0.17	28%	1.52 (1.09–2.13) p = 0.01	0.07	0.945	0.28	0.785
ALT elevations (G3-5)	12	0.62	0%	1.54 (0.72–3.29) p = 0.26	0.89	0.373	–1.09	0.301
AST elevations (G1-5)	12	0.48	0%	1.96 (1.37–2.81) p = 0.0002	0.21	0.837	–0.11	0.912
AST elevations (G3-5)	12	0.41	3%	1.89 (0.78–4.57) p = 0.16	0.36	0.721	–0.73	0.486

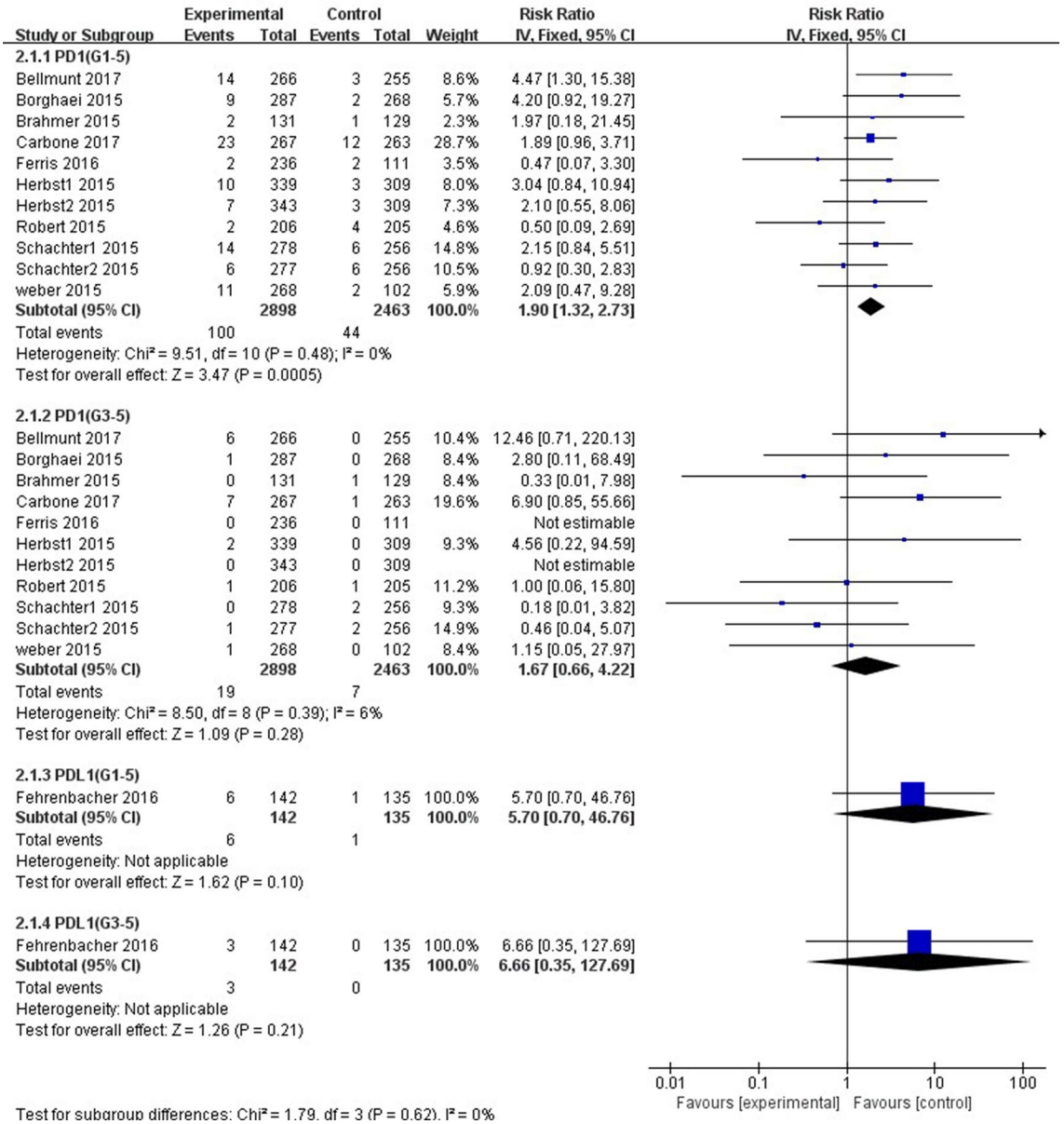

FIGURE 5 | Forest plot analysis for AST elevation with PD-1/PD-L1 inhibitors versus chemotherapy. G1-5: grades 1–5; G3-5: grades 3–5.

Previous study indicated that the incidence of irAEs was different in patients with different solid tumors (Wang et al., 2019). Similarly, our results showed that the risk of ALT and AST elevations incidence was significantly higher in patients with NSCLC compared to patients with other tumors. To date, the mechanism by which this result occurs has not been well illustrated. Several studies have reported a high expression of PD-1 in NSCLC. It is also confirmed that the expression of PD-1 was related to the negative regulation of anti-tumor immune response in NSCLC (Konishi et al., 2004, Ji et al., 2016). In addition, the FDA has approved pembrolizumab as a first-line treatment for NSCLC with high PD-1 expression (> 50%) (Reck et al., 2016). Our meta-analysis suggested it may be that NSCLC cells up-regulated more PD-1, therefore, when PD-1/PD-L1 inhibitors block the binding of these receptors to their ligands, the inhibitory signals are strongly eliminated and the host's anti-tumor response is more likely to be effectively enhanced (Rizvi et al., 2015). At the same time, normal liver tissue cells also suffer more attacks, resulting in an increased risk of ALT and AST elevations incidence in patients with NSCLC. Another reason may be that the chemotherapy regimens and doses of NSCLC are different from those of other tumor types (NSCLC: Docetaxel 75 mg/m^2 every 3 weeks; MM: dacarbazine 1000 mg/m^2 every 3 weeks; HNSCC: methotrexate or docetaxel; UC: paclitaxel, docetaxel, or vinflunine), which may cause differences in the overall original data and final results of this meta-analysis.

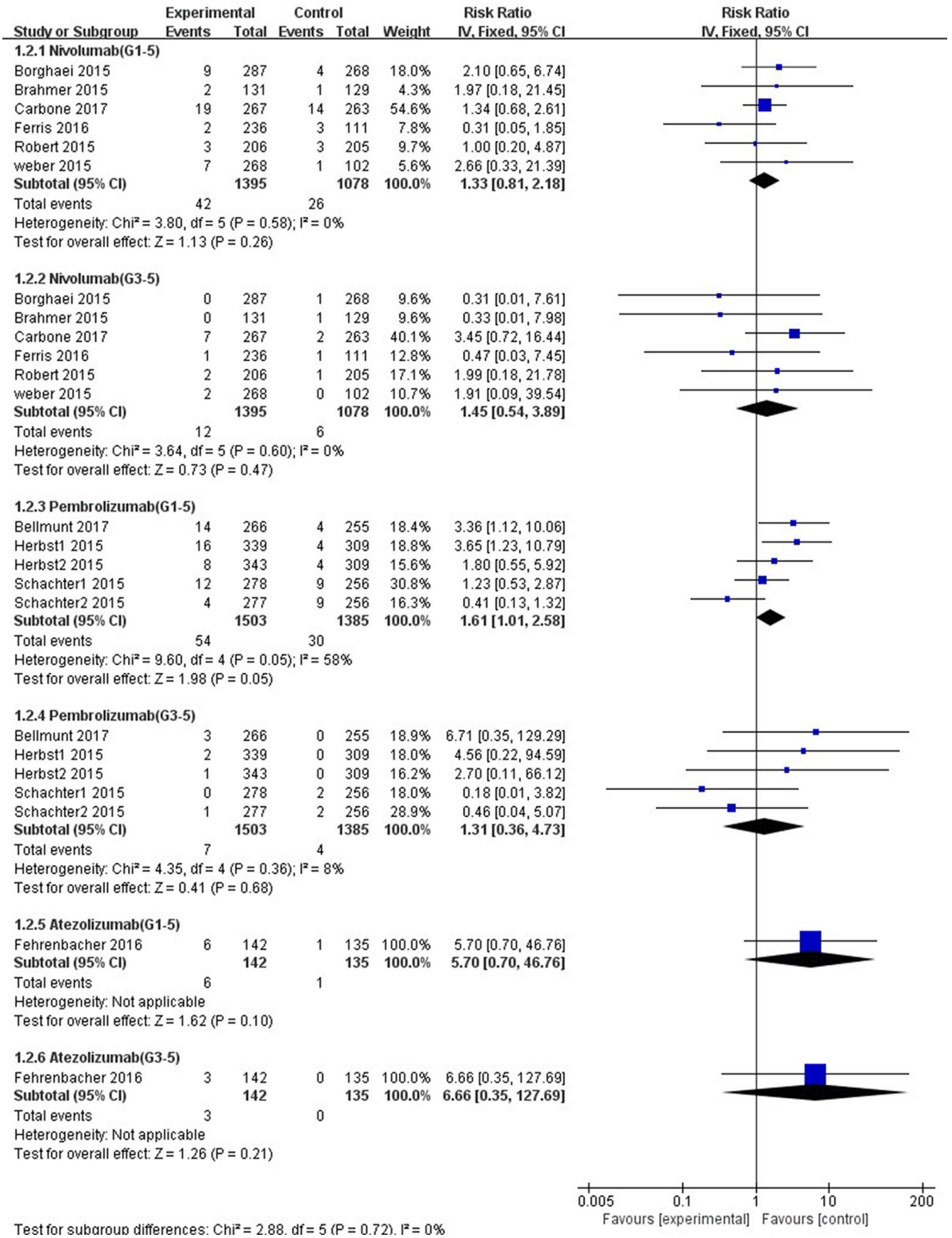

FIGURE 6 | Forest plot analysis for ALT elevation with different type of immune checkpoint inhibitors (nivolumab, pembrolizumab, and atezolizumab) versus chemotherapy. G1-5: grades 1—5; G3-5: grades 3—5.

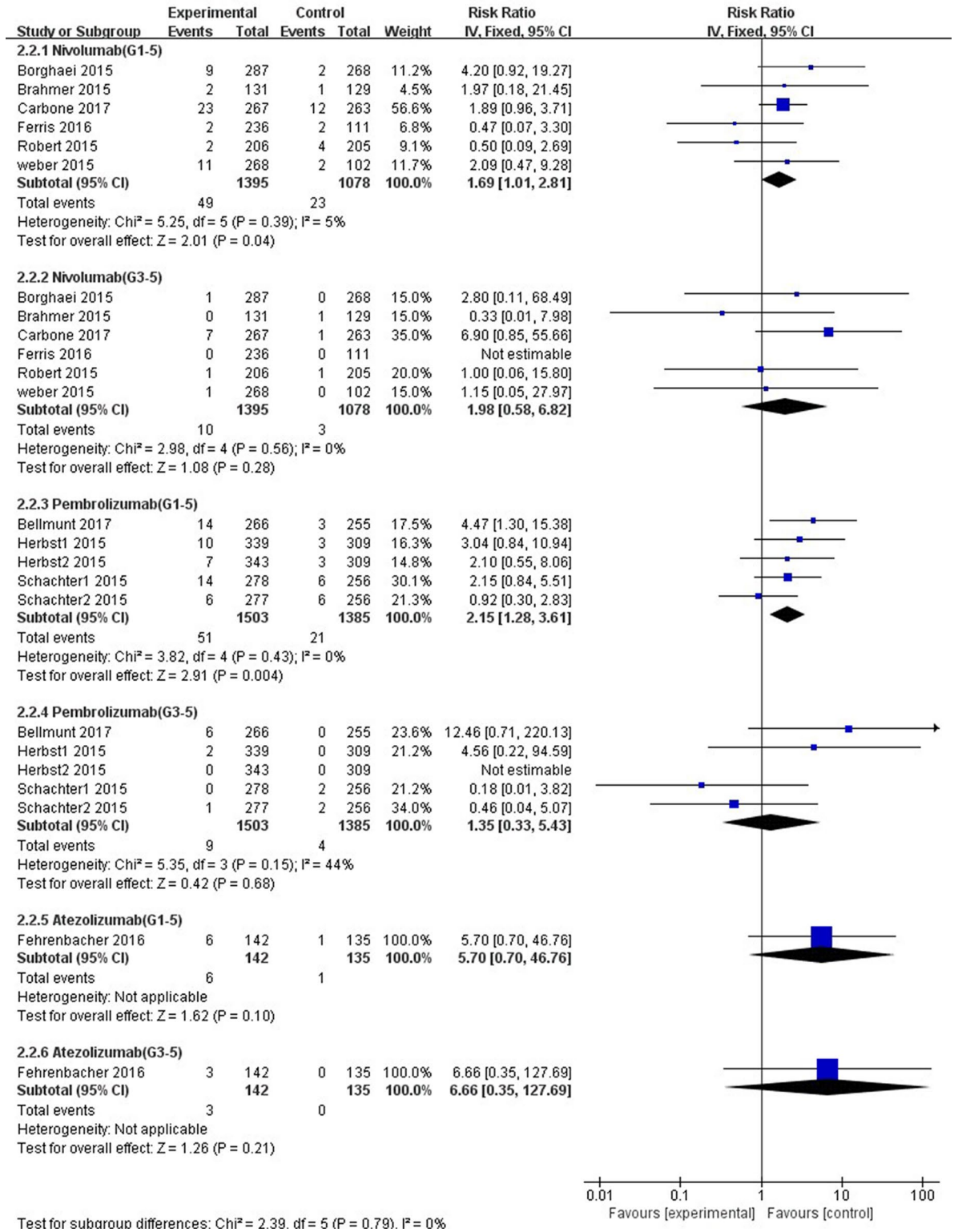

FIGURE 7 | Forest plot analysis for AST elevation with different type of immune checkpoint inhibitors (nivolumab, pembrolizumab, and atezolizumab) versus chemotherapy. G1-5: grades 1—5; G3-5: grades 3—5.

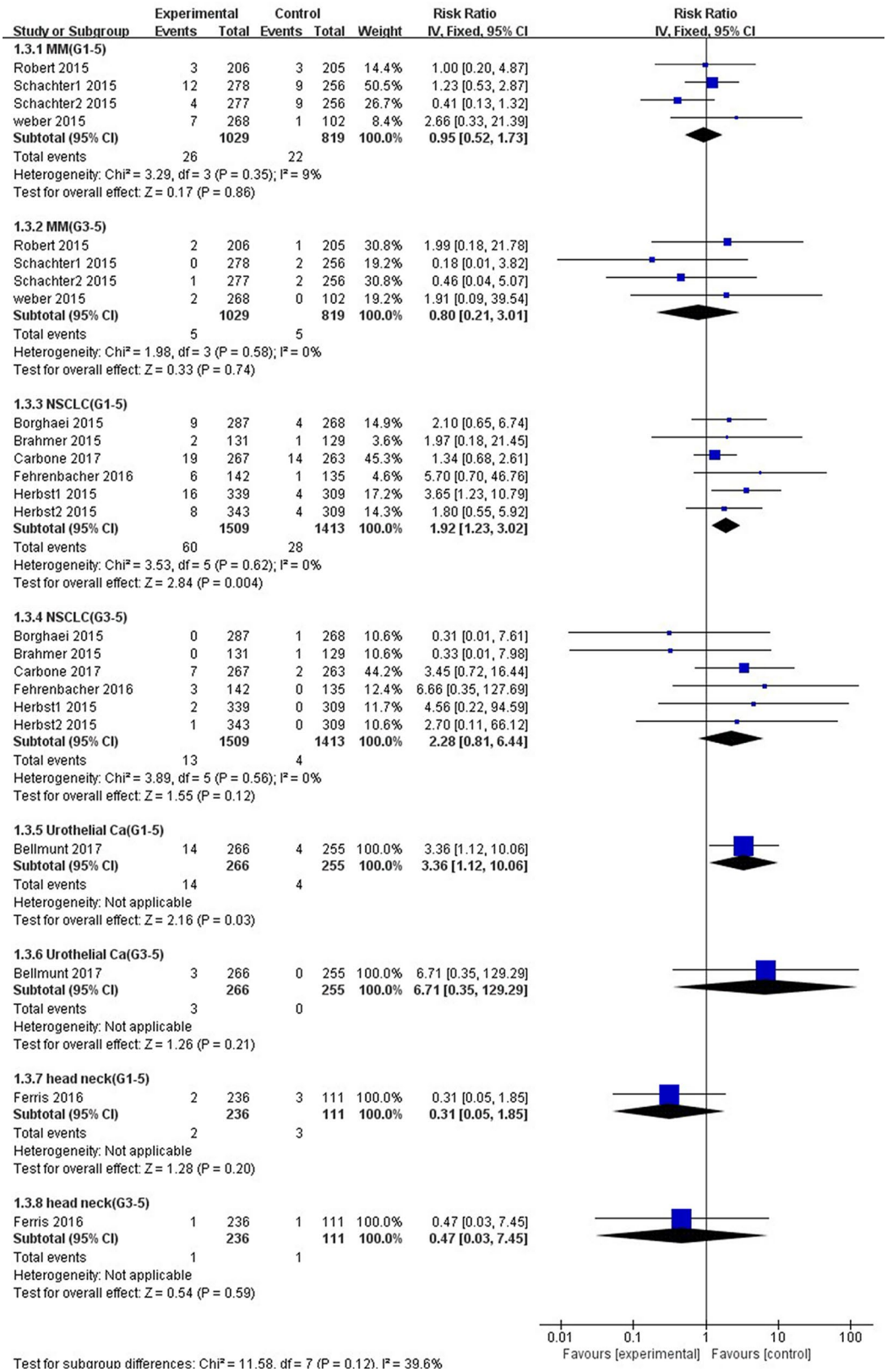

FIGURE 8 | Forest plot analysis for ALT elevation in different cancers with PD-1/PD-L1 inhibitors versus chemotherapy. MM: melanoma; NSCLC: non-small cell lung cancer; Urothelial Ca: urothelial carcinoma; head neck: head-neck squamous cell carcinoma. G1-5: grade 1–5; G3-5: grade 3–5.

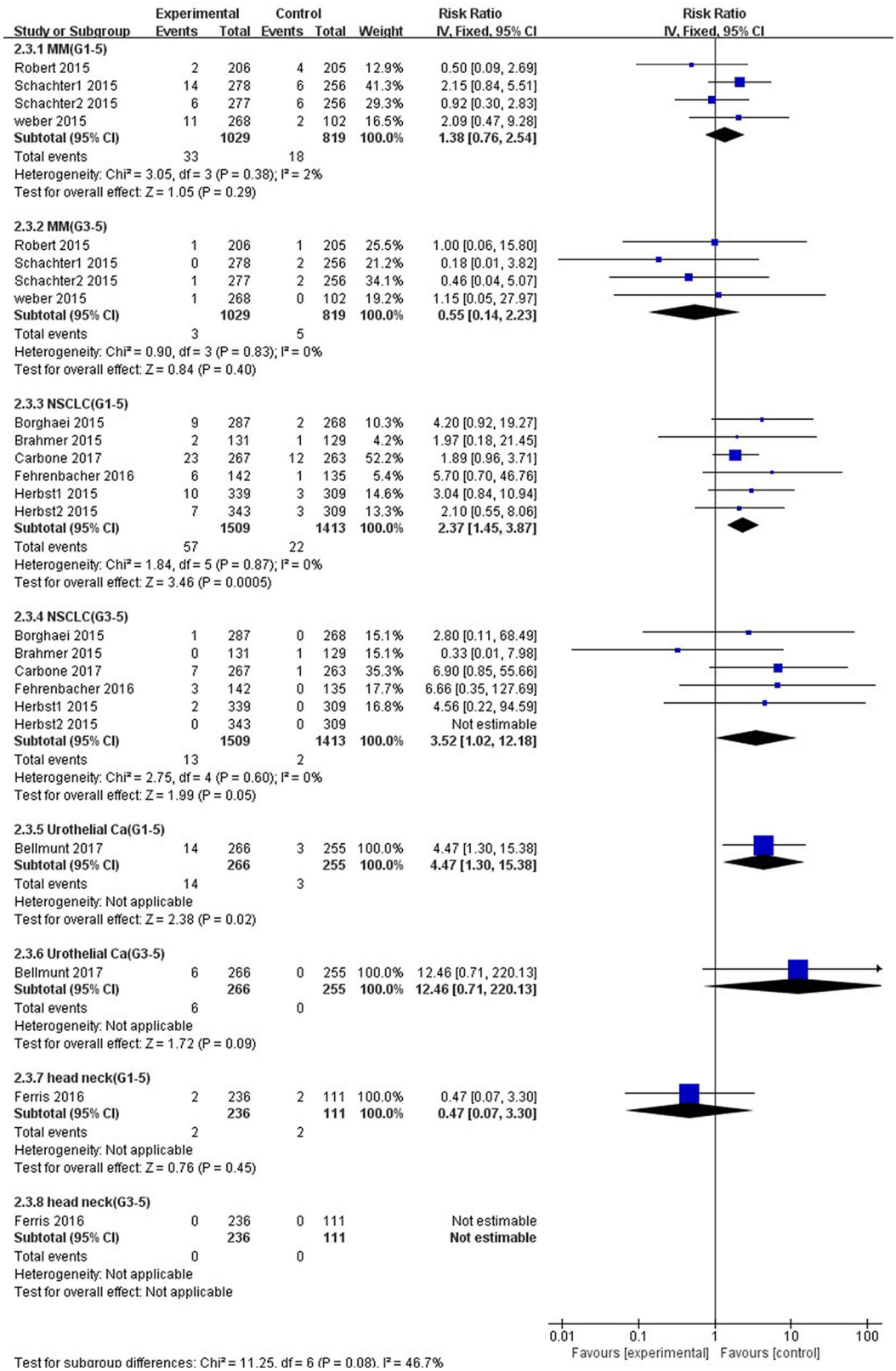

FIGURE 9 | Forest plot analysis for AST elevation in different cancers with PD-1/PD-L1 inhibitors versus chemotherapy. MM: melanoma; NSCLC: non-small cell lung cancer; Urothelial Ca: urothelial carcinoma; head neck: head-neck squamous cell carcinoma. G1-5: grade 1–5; G3-5: grades 3–5.

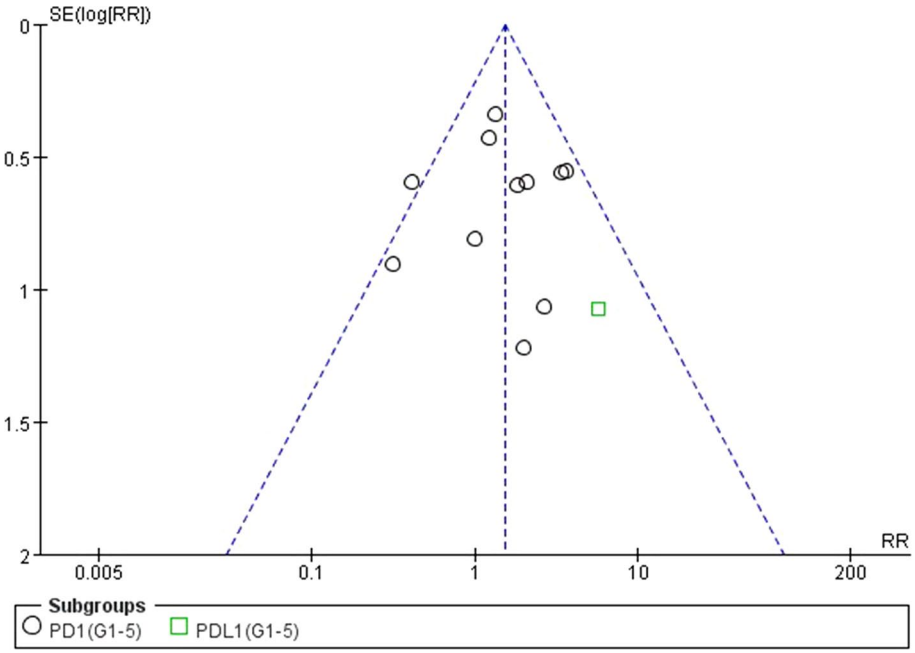

FIGURE 10 | Funnel plot for ALT elevation (grades 1–5) in patients treated with PD-1 antibodies versus chemotherapy therapy.

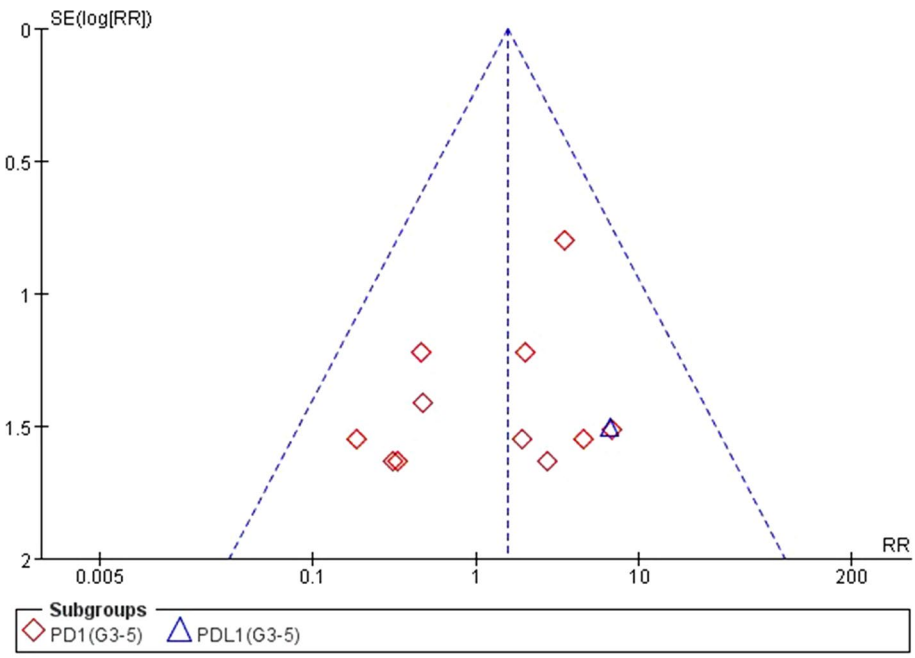

FIGURE 11 | Funnel plot for ALT elevation (grades 3–5) in patients treated with PD-1 antibodies versus chemotherapy therapy.

Therefore, our results suggested that the risk of immune-related liver dysfunction incidence depends on the cancer type, and it provided the evidence for clinicians to make the appropriate treatment selection for patients with advanced cancer.

In general, relatively small heterogeneity was observed in our meta-analysis. It is logical, given that the diagnosis of ALT and AST elevations is established on the basis of liver function examination, thus, there are no subjective factors to influence the results. Our meta-analysis based on published data itself inevitably has some limitations. First, the results described in this meta-analysis are subject to the limitations of the selected individual clinical trials, this study is influenced by all the biases

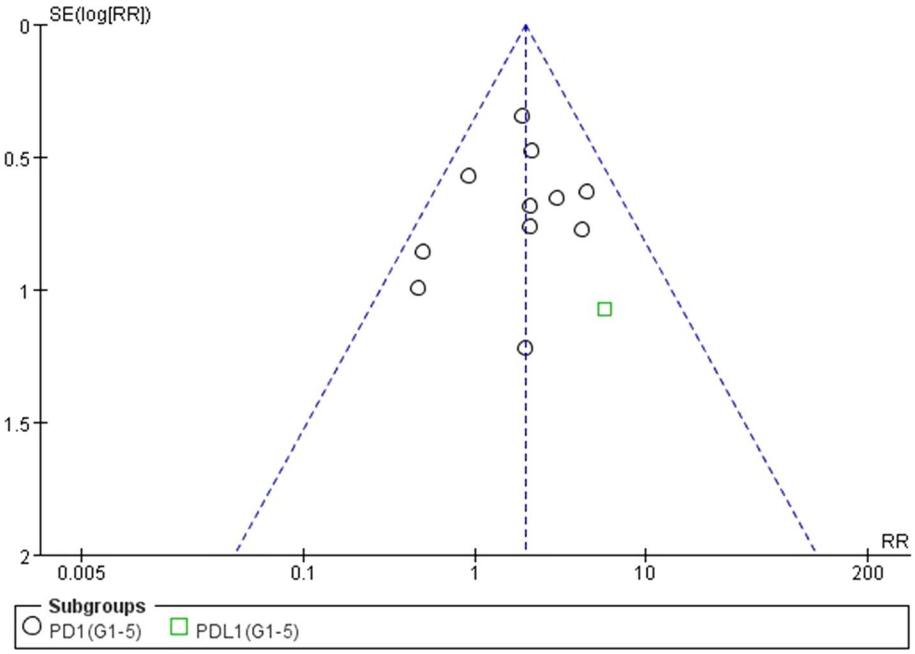

FIGURE 12 | Funnel plot for AST elevation (grades 1–5) in patients treated with PD-1 antibodies versus chemotherapy therapy.

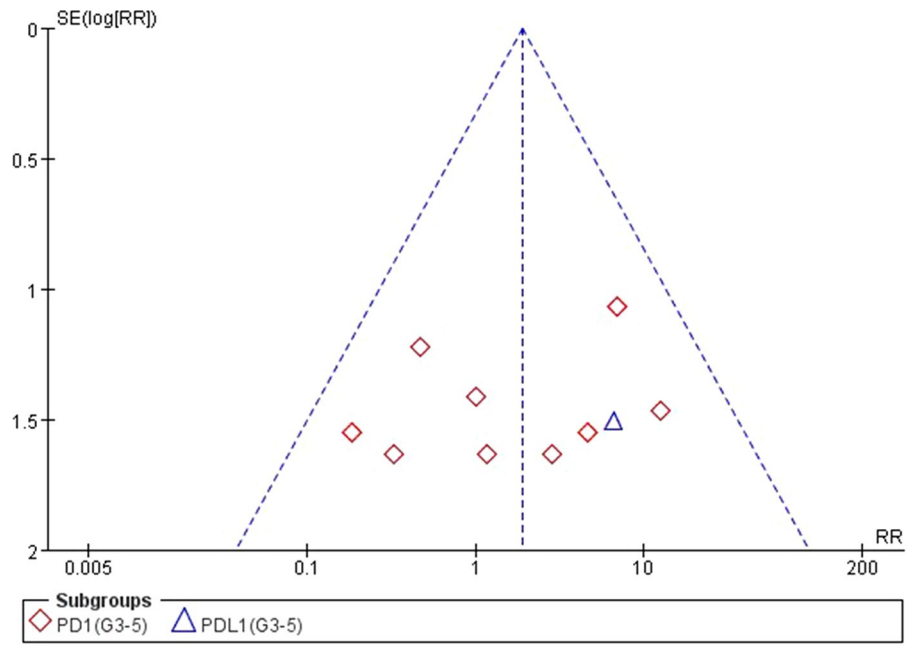

FIGURE 13 | Funnel plot for AST elevation (grades 3–5) in patients treated with PD-1 antibodies versus chemotherapy therapy.

and errors of the original investigators. Second, given that the diagnostic criteria of ALT/AST elevations were identical for all recruited trials, the liver dysfunction may occur on account of not only drug-induced liver injury but also cancer itself, it is hard to avoid the bias of individual selection. Lastly, there are some questions that remained unclear, such as those for the two PD-1 inhibitors, nivolumab and pembrolizumab. Our results showed that only pembrolizumab caused more risk of ALT elevation than chemotherapy.

Overall, although ICIs have made great breakthroughs in the treatment of multiple types of tumors, our meta-analysis indicated that ICIs could significantly increase the risk of liver dysfunction

when compared with traditional chemotherapy, especially in the NSCLC patients treated with pembrolizumab. This suggests that clinicians need to pay more attention to avoid this risk and focus on the guidelines and expert consensus on management protocols for this rare but potentially serious liver dysfunction (Haanen et al., 2017, Puzanov et al., 2017, Brahmer et al., 2018).

CONCLUSION

To sum up, PD-1 inhibitor posed an increased risk of immune-related liver dysfunction compared with chemotherapy. In PD-1 inhibitor, our meta-analysis concluded that pembrolizumab is more likely to cause an increased risk of immune-related liver dysfunction than nivolumab. Moreover, the risk of immune-related liver dysfunction in NSCLC is higher than in other tumor types with the treatment of PD1/PD-L1 inhibitors. Immune-related liver dysfunction, although relatively rare in irAEs, still requires clinicians to pay closely attention, and timely formulate corresponding prevention and response strategies, as well as appropriate management measures. Although ensuring the medication is more reasonable and effective, it is necessary to further reduce the possible liver dysfunction. We expect that further research on the molecular mechanisms of immune-related liver dysfunction will provide help to prevent and mitigate this adverse event for patients with advanced cancer.

DATA AVAILABILITY

All datasets generated for this study are included in the manuscript/supplementary files.

AUTHOR CONTRIBUTIONS

Conception and design: SD and SL. Provision of study material or patients: XS and JL. Collection and/or assembly of data: SD and QY. Data analysis and interpretation, Manuscript writing, final approval of manuscript, and equally accountable for all aspects of the work: a authors.

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The Top 100 Highly Cited Original Articles on Immunotherapy for Childhood Leukemia

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Background: Childhood leukemia is one of the most common cancers in children. As a potential treatment for leukemia, immunotherapy has become a new research hotspot. This research aimed at exploring the status and trends of current researches on immunotherapy for childhood leukemia through bibliometric analysis.

Methods: The Institute for Scientific Information Web of Science core collection database was searched for articles on immunotherapy and childhood leukemia using a computer. Time period for retrieval was from the beginning of the database to June 15, 2019. The top 100 highly cited articles were selected to extract their information on publication year, authors, title, publication journal, number of citations, author's affiliations, country, and so on. These general information and bibliometric data were collected for analysis. VOSviewer software was used to generate a figure for keywords' co-occurrence network and a figure for researcher's coauthorship network that visualized reference and cooperation patterns for different terms in the 100 articles.

Results: The number of citations in the top 100 articles ranged from 17 to 471. These articles were published in 52 different publications. The top four journals in terms of the number of our selected articles were *Leukemia* (11 articles), *Blood* (10 articles), *Bone Marrow Transplantation* (6 articles), and *Clinical Cancer Research*. The most frequently nominated author was T. Klingebiel from Goethe University Frankfurt, and of the top 100 articles, 12 listed his name. These top 100 articles were published after the year 2000. Most of these articles were original (67%). The United States and Germany were the major countries researching immunotherapy for childhood leukemia and made significant contributions to the combat against the disease. Adoptive immunotherapy and stem cell transplantation appeared more frequently in keywords.

Conclusions: This study analyzed the top 100 highly cited articles on immunotherapy for childhood leukemia and provided insights into the features and research hotspots of the articles on this issue.

Keywords: childhood leukemia, immunotherapy, bibliometrics, Web of Science, VOSviewer

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INTRODUCTION

Leukemia is a malignant clonal disease of hematopoietic stem cells (Greaves, 2016). It is estimated that this disease will see more than 0.4 million new cases and 0.3 million related deaths worldwide, according to the GLOBOCAN 2018 (Bray et al., 2018). And new leukemia cases account for 31% of new patients of childhood malignancies (Ward et al., 2014). Most children with leukemia show rapid onset (Miranda-Filho et al., 2018). Due to the complexity in leukemia typing and prognosis, there is no one-size-fits-all treatment for the disease (Pui et al., 2015; Lam et al., 2017). At present, main treatment methods for the disease contain the following types: chemotherapy (Saygin and Carraway, 2017), radiation therapy (Simone et al., 2012), targeted therapy (Mugoni et al., 2019), immunotherapy (Acheampong et al., 2018), stem cell transplantation (Cornelissen and Blaise, 2016), and the like. After reasonable and comprehensive treatment, the prognosis of leukemia has been greatly improved (Marcos-Gragera et al., 2017). A considerable number of patients can be cured or reach long-term stability (Pui et al., 2003; Lennmyr et al., 2019).

Immunotherapy, a new treatment for cancer, can help the immune system fight cancer (Majzner et al., 2017). Over the past few decades, immunotherapy has developed targeting cancer at a striking rate (Foster and Maude, 2018). There are many immunotherapies for leukemia, such as chimeric antigen receptor (CAR) T-cell therapy, bispecific T-cell engager (BiTE) therapy, and antibody-drug conjugates (ADCs) (Aldoss et al., 2017; June et al., 2018; Liu et al., 2019). Chimeric antigen receptor T-cell technology is the most influential immunotherapy for childhood leukemia in the last decade (Gardner et al., 2017). This measure uses autologous T cells to attack malignant cells (Wang et al., 2017). Studies have shown that CAR T cells are effective in inducing remission among leukemia patients and thus provide valuable opportunities for subsequent transplantation, finally achieving durable remission (Pan et al., 2019). Evidence also has shown that CAR–T-cell therapy can achieve fine effects among patients with recurrent B-cell malignancies or those facing relapse after cord blood transplant, with fewer complications (Fan et al., 2017). Bispecific T-cell engager therapy is also a new advance in immunotherapy for childhood leukemia. Preclinical studies have shown that BiTE can realize antileukemia function by targeting T cells and $CD33^+$ monocyte myelogenous suppressor cells, recruiting and expanding autologous T cells and inducing acute myeloid leukemia -blasts lysis (Krupka et al., 2016; Jitschin et al., 2018). Antibody-drug conjugate therapy, another immunotherapy, has been widely concerned. In this approach, cytotoxic molecules could bind to antibodies, and then the antibodies specifically bind to specific tumor antigens, and the cytotoxic molecules would be endocytosed into cells, thereby killing tumor cell from inside (Foster and Maude, 2018). An *in vitro* experiment showed that ADCs could improve the antiproliferation and cytotoxicity of human acute lymphoblastic leukemia cell lines (Hicks et al., 2019). In the mouse model of leukemia, ADC treatment could significantly improve survival rate without overt toxicity or adverse effects (McGinn et al., 2017). As an effective antileukemia immunotherapy, ADC has been assessed for its safety and efficacy in leukemia patients (Li et al., 2018).

Immunotherapy is important in treating childhood leukemia, but there is no bibliometric analysis on researches in this field. The purpose of this study was to use bibliometric methods to analyze the top 100 highly cited articles on immunotherapy for childhood leukemia, hoping to have a better understanding of current situation and trend of those researches through analyzing their main characteristics.

MATERIALS AND METHODS

Data Sources

Literature on immunotherapy for childhood leukemia was retrieved from Institute for Scientific Information (ISI) website of the Science Core Collection Database of Henan University from the beginning of the database to April 30, 2019 (updated to June 15, 2019). *Childhood*, *pediatric*, *leukemia*, and *immunotherapy* were used as search terms. Retrieved documents were arranged in descending order according to the number of citations, and the top 100 most cited articles were finally obtained.

Data Extraction

The top 100 most frequently cited articles were selected, and the following information was extracted from them: the number of citations, the names of the authors, authors' affiliations, country, publication year, article title, article type, journal, Web of Science categories, quartile in category, and impact factor of the journal (2017 edition of Journal Citation Reports).

Two independent researchers evaluated each identified article to identify articles involving immunotherapy for childhood leukemia, regardless of article type. If there were different opinions, a third reviewer would be consulted, and consensus was thus achieved through discussion.

Statistical Analysis

Microsoft Excel 2013 software was used for descriptive statistical analyses, including those on publication year, author, author affiliation, country, journal, and citation number. VOSviewer 1.6.8 (van Eck and Waltman, 2010) was used to draw figures for keyword co-occurrence network and coauthored network, so as to implement network visualization analysis. In network visualization, each circle and label represented a keyword or researcher, and the size of circles represented the frequency of occurrence. The larger the circle was, more frequently the circle-represented body appeared. Circles adopting different colors in graph represented different clusters. Lines between two circles indicated that two keywords or researchers appeared together. The thicker the lines were, more frequently they appeared together. More relevant two keywords or researchers were, closer two circles located. The minimum number of co-occurrences was adjusted according to graphic results.

RESULTS

Characteristics of Included Studies

A total of 360 articles were retrieved from the Web of Science Core Collection Database to introduce immunotherapy for childhood leukemia. Articles are listed in descending order

according to the cited frequency; the top 100 articles with the highest cited frequency are selected. All of the top 100 highly cited articles were published between 2000 and 2018, about two to nine articles each year. Of the articles, 99 were published in journals and 1 in a book. These articles were in 19 categories, including hematology, oncology, and immunology. The 100 articles were published by researchers from 23 countries, most from the United States, Germany, Italy, and Japan (**Figure 1**). The 100 articles were published in 52 publications, 7 of which were not included in the 2017 edition of Journal Citation Reports. The quartile in category was distributed in Q1–Q4; impact factors of the journals ranged from 0.698 to 32.621, and basic information of the journals is shown in **Table 1**. The average and median number of citations, range of citation number, and interquartile range of the 100 articles were 53.2, 35.5, 17 to 471, and 35.25, respectively. The articles contained 67 original articles, 23 reviews, 6 conference abstracts, 2 letters, 1 editorial material, and 1 book chapter. The information of the top 100 highly cited articles is listed in **Supplementary Table S1**.

The Top 10 Authors

Among the 100 articles, the researchers who published most articles were T. Klingebiel from Goethe University Frankfurt, Germany, reaching a total of 12 articles, while the second and third authors came from the same university. Of the top 10 authors, nine were from Germany, four from the United States, three from Japan, and one from Italy (**Table 2**).

The Top 10 Institutions

Like Eberhard Karls University of Tubingen and National Institutes of Health, the University of Pennsylvania, produced 10% of the top 100 articles, followed by the Goethe University Frankfurt (8%), Johns Hopkins University (8%), and National Cancer Institute (8%) (**Table 3**).

The Top 10 Articles

The top 10 highly cited articles contained four original articles, five reviews, and one book chapter (**Table 4**). They were from the United States, Italy, Germany, Japan, and Australia. Of the 10 articles, 6 were published in journals, which ranked Q1 in the 2017 edition of Journal Citation Reports, and 1 in that ranking Q3, while journals publishing the other 3 were not included in the 2017 edition of Journal Citation Reports. The categories of these journals involved blood, tumors, pediatrics, and the like. The top 10 articles covered the updating of treatments for childhood leukemia (Rodriguez-Galindo et al., 2003; Pui et al., 2011; Locatelli et al., 2012; Barrett et al., 2014), different mechanisms of immunotherapy (Sotillo et al., 2015; Fry et al., 2018), WT1 targeted therapy (Sugiyama, 2001; Rosenfeld et al., 2003), a case series report (Diak et al., 2010), and a clinical study (Dagher et al., 2002).

Keyword Co-Occurrence Network Visualization

VOSviewer software was used to draw a figure for keyword co-occurrence network, setting the minimum number of occurrences at 3. As shown in **Figure 2**, circles representing keywords such as *acute lymphoblastic-leukemia*, *bone-marrow-transplantation*, *acute myeloid-leukemia*, *versus-host-disease*, and *stem cell transplantation* are larger than others, indicating that these keywords appeared more frequently. Blinatumomab, a BiTE drug, has been extensively studied.

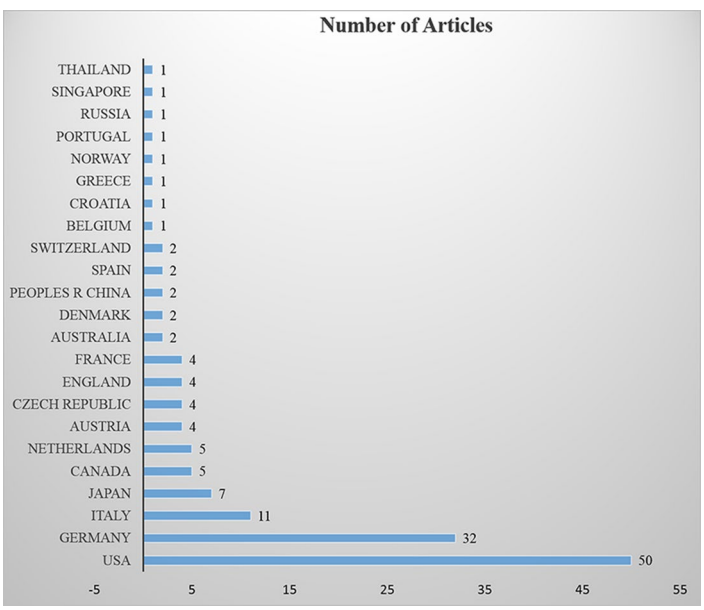

FIGURE 1 | Countries from which the 100 most highly cited articles originated and the number of our collected articles from each of those countries.

TABLE 1 | Journals publishing the top 100 most highly cited articles[#].

Journal	No. of articles	Quartile in category	Impact factor	Citation count	Country
Leukemia	11	Q1	10.023	487	England
Blood	10	Q1	15.132	495	USA
Bone Marrow Transplantation	6	Q1	4.497	257	England
Clinical Cancer Research	6	Q1	10.199	364	USA
British Journal of Haematology	4	Q1	5.128	134	USA
Biology of Blood And Marrow Transplantation	3	Q1	4.484	97	USA
Journal of Clinical Oncology	3	Q1	26.36	570	USA
Klinische Padiatrie	3	Q4	0.698	126	Germany
Blood Cells Molecules and Diseases	2	Q4	1.836	175	USA
Cancer Genetics and Cytogenetics	2	—	—	134	USA
Cancer Research	2	Q1	9.13	74	USA
Haematologica—The Hematology Journal	2	—	—	67	Italy
International Journal of Hematology	2	Q3	1.942	170	USA
Journal of Immunology	2	Q2	4.539	54	USA
Medical and Pediatric Oncology	2	—	—	231	USA
Pediatric Blood & Cancer	2	Q1	2.646	74	USA
PLoS One	2	Q1	2.766	74	USA
Therapeutic Advances in Hematology	2	—	—	81	England
Angewandte Chemie—International Edition	1	Q1	12.102	34	Germany
Annals of Oncology	1	Q1	13.93	28	England
Annual Review of Medicine	1	Q1	14.97	197	USA
Arthritis and Rheumatism	1	—	—	151	USA
Biomarkers	1	Q3	1.976	40	England
Cancer Cell	1	Q1	22.844	30	USA
Cancer Discovery	1	Q1	24.373	251	USA
Cancer Immunology Immunotherapy	1	Q2	4.225	21	USA
Cancer Journal	1	Q2	3.519	17	USA
Cancer Journal from Scientific American	1	—	—	18	USA
Clinical Immunology	1	Q2	3.557	20	USA
Current Medicinal Chemistry	1	Q2	3.469	19	United Arab Emirates
Current Opinion in Hematology	1	Q2	2.821	19	USA
Current Opinion in Immunology	1	Q1	7.932	89	England
Cytotherapy	1	Q1	3.993	19	England
Discovery Medicine	1	Q3	2.398	24	USA
Drug Discovery Today	1	Q1	6.848	25	England
Expert Review Of Hematology	1	Q3	1.937	26	England
Frontiers in Immunology	1	Q1	5.511	20	Switzerland
Frontiers in Pediatrics	1	Q2	2.335	27	Switzerland
Haematologica	1	Q1	9.09	20	Italy
Immunological Reviews	1	Q1	9.217	36	USA
Immunology Letters	1	Q3	2.436	51	Netherlands
International Journal of Cancer	1	Q1	7.36	26	USA
Journal of Allergy and Clinical Immunology	1	Q1	13.258	80	USA
Journal of Oncology Pharmacy Practice	1	Q3	1.908	17	England
Leukemia Research	1	Q3	2.319	19	England
Molecular Immunology	1	Q2	3.188	20	England
Nature Medicine	1	Q1	32.621	123	USA
Nature Reviews Clinical Oncology	1	Q1	24.653	52	USA
Oncologist	1	Q1	5.306	19	USA
Oncotarget	1	—	—	31	USA
Pediatric Hematology and Oncology	1	Q3	1.154	23	USA
Science Translational Medicine	1	Q1	16.71	64	USA

[#]*Data from the 2017 edition of Journal Citation Reports.*

Researcher Coauthored Network Visualization

VOSviewer software was used to analyze coauthorship network of authors, with the minimum number of coauthors at 2, and figure for coauthorship network was drawn (**Figure 3**). Accordingly, circles representing T. Klingebiel, P. Bader, and P. Lang are larger than others, and connection lines between them were denser and thicker, indicating that they contributed to more collaborating articles and had closer relation.

DISCUSSION

In this study, we identified and analyzed the top 100 highly cited articles in the field of immunotherapy for childhood leukemia.

TABLE 2 | Top 10 authors most frequently appearing in the articles.

Rank	Author	Number of articles	Affiliation	Country
1	Klingebiel T	12	Goethe University Frankfurt	Germany
2	Bader P	10	Goethe University Frankfurt	Germany
3	Lang P	8	Eberhard Karls University of Tubingen	Germany
4a	Gruhn B	6	Friedrich Schiller University of Jena	Germany
4b	Grupp Sa	6	University of Pennsylvania	USA
4c	Handgretinger R	6	Eberhard Karls University of Tubingen	Germany
4d	Niethammer D	6	Osaka University	Japan
4e	Sugiyama H	6	Osaka University	Japan
9	Kreyenberg H	5	Goethe University Frankfurt	Germany
10a	Barrett Dm	4	University of Pennsylvania	USA
10b	Brown P	4	Johns Hopkins University	USA
10c	Dilloo D	4	Heinrich Heine University Dusseldorf	Germany
10d	Kremens B	4	University of Duisburg Essen	Germany
10e	Locatelli F	4	University of Pavia	Italy
10f	Mackall Cl	4	Stanford University	USA
10g	Oka Y	4	Osaka University	Japan
10h	Zintl F	4	Friedrich Schiller University of Jena	Germany

TABLE 3 | Institutions contributing to the 100 most highly cited articles.

Institution name	Country	Number of articles
Eberhard Karls University of Tubingen	Germany	10
National Institutes of Health	USA	10
University of Pennsylvania	USA	10
Goethe University Frankfurt	Germany	8
Johns Hopkins University	USA	8
NIH National Cancer Institute	USA	8
Children's Hospital of Philadelphia	USA	7
Johns Hopkins Medicine	USA	7
St. Jude Children's Research Hospital	USA	7
Osaka University	Japan	6

Through bibliometric analysis, the status and characteristics of publications in this field were explored, including publication journals, research institutions, authors, and other information. The trends of the most frequently cited articles in this field have been clarified, which provided ideas and directions for researchers.

According to publication years of the top 100 highly cited articles, every year could see two to nine articles highly cited, and only one-fourth of those articles were published in the past 5 years. Perhaps because immunotherapy is an emerging approach, a large amount of preclinical and clinical researches are still in progress. Among the top 100 articles, most were from European and American countries, few from Asia. Reason for such a phenomenon possibly is that leukemia has higher incidence in Europe and the United States. According to statistics, the risk of leukemia is 10 to 20 times higher in Europe and the United States than in Asia (Yang et al., 2015). Therefore, many research institutions in Europe and the United States have been exploring in this field. According to the number of citations, the most frequently cited articles in the top 100 ones were cited 471 times. Compared with other features (Liao et al., 2016; Wang et al., 2019), such figure was not large, probably because research in this field is still in the initial stage, and our research topic involved only the blood system and immunotherapy. However, considering physical, psychological, and financial burden from leukemia on the patients (Bosshard et al., 2018) and enormous potential of immunotherapy in treating this disease, researches in this area are important.

A total of 52 journals were involved in this study. The journals were arranged in descending order according to the number of the top 100 highly cited articles they published. The journals were divided into three groups, each with the same number of the articles, and then the number of journals in the three groups was 4, 14, and 34, respectively, approximate to $1:3^1:3^2$. The distribution of these publications was consistent with Bradford's Law (Bradford, 1985). Of the top 100 highly cited articles, 66% were published in Q1 (2017 edition of the journal citation report), 9% in Q2 and Q3, separately, and 5% in Q4. Most of them were published in journals possessing high impact factor, while these journals are often subscribed by more researchers, and high-quality research results face more opportunities to be cited (Callaham et al., 2002).

According to the results of keyword co-occurrence, the top 100 highly cited articles covered various aspects of immunotherapy for childhood leukemia, such as leukemia type, immunotherapy type, immunotherapy mechanism (Foster and Maude, 2018), immunotherapy experiments *in vitro*, animal experiments *in vitro* (McGinn et al., 2017; Hicks et al., 2019), preclinical studies, stem cell transplantation, changes in survival time after immunotherapy, and leukemia recurrence. According to the figure for keywords co-occurrence network, we can intuitively observe links between keywords and analyze hot topics of the researches. In recent years, a variety of immunotherapies have been approved for clinical leukemia treatment (Kantarjian et al., 2017; Kantarjian et al., 2018; Mueller et al., 2018; Jabbour et al., 2019). Growing preclinical studies have also been used to explore new immunotherapies for different types of leukemia.

From the figure for coauthorship network, coauthorship between authors and comparisons on the number of published

TABLE 4 | Top 10 most highly cited articles[$].

Author	Title	Year	Journal	Quartile in category	Impact factor	Citation count	Article type	Country
Pui CH	Biology, risk stratification, and therapy of pediatric acute leukemias: an update	2011	*Journal of Clinical Oncology*	Q1	26.36	471	Review	USA
Sotillo E	Convergence of acquired mutations and alternative splicing of CD19 enables resistance to CART-19 Immunotherapy	2015	*Cancer Discovery*	Q1	24.373	251	Article	USA
Barrett DM	Chimeric antigen receptor therapy for cancer	2014	*Annual Review of Medicine*	Q1	14.97	197	Book Chapter	USA
Diak P	Tumor necrosis factor alpha blockers and malignancy in children forty-eight cases reported to the Food and Drug Administration	2010	*Arthritis and Rheumatism*	—	—	151	Article	USA
Fry TJ	CD22-targeted CAR T cells induce remission in B-ALL that is naive or resistant to CD19-targeted CAR immunotherapy	2018	*Nature Medicine*	Q1	32.621	123	Article	USA
Rodriguez-Galindo C	Treatment of Ewing sarcoma family of tumors: current status and outlook for the future	2003	*Medical and Pediatric Oncology*	—	—	129	Review	USA
Locatelli F	How I treat relapsed childhood acute lymphoblastic leukemia	2012	*Blood*	Q1	15.132	112	Review	Italy and Germany
Sugiyama H	Wilms' tumor gene WT1: its oncogenic function and clinical application	2001	*International Journal of Hematology*	Q3	1.942	108	Review	Japan
Rosenfeld C	WT1 in acute leukemia, chronic myelogenous leukemia and myelodysplastic syndrome: therapeutic potential of WT1 targeted therapies	2003	*Leukemia*	Q1	10.023	103	Review	USA and Austria
Dagher R	Pilot trial of tumor-specific peptide vaccination and continuous infusion interleukin-2 in patients with recurrent Ewing sarcoma and alveolar rhabdomyosarcoma: an inter-institute NIH study	2002	*Medical and Pediatric Oncology*	—	—	102	Article	USA

[$]*Data from the 2017 edition of Journal Citation Reports.*

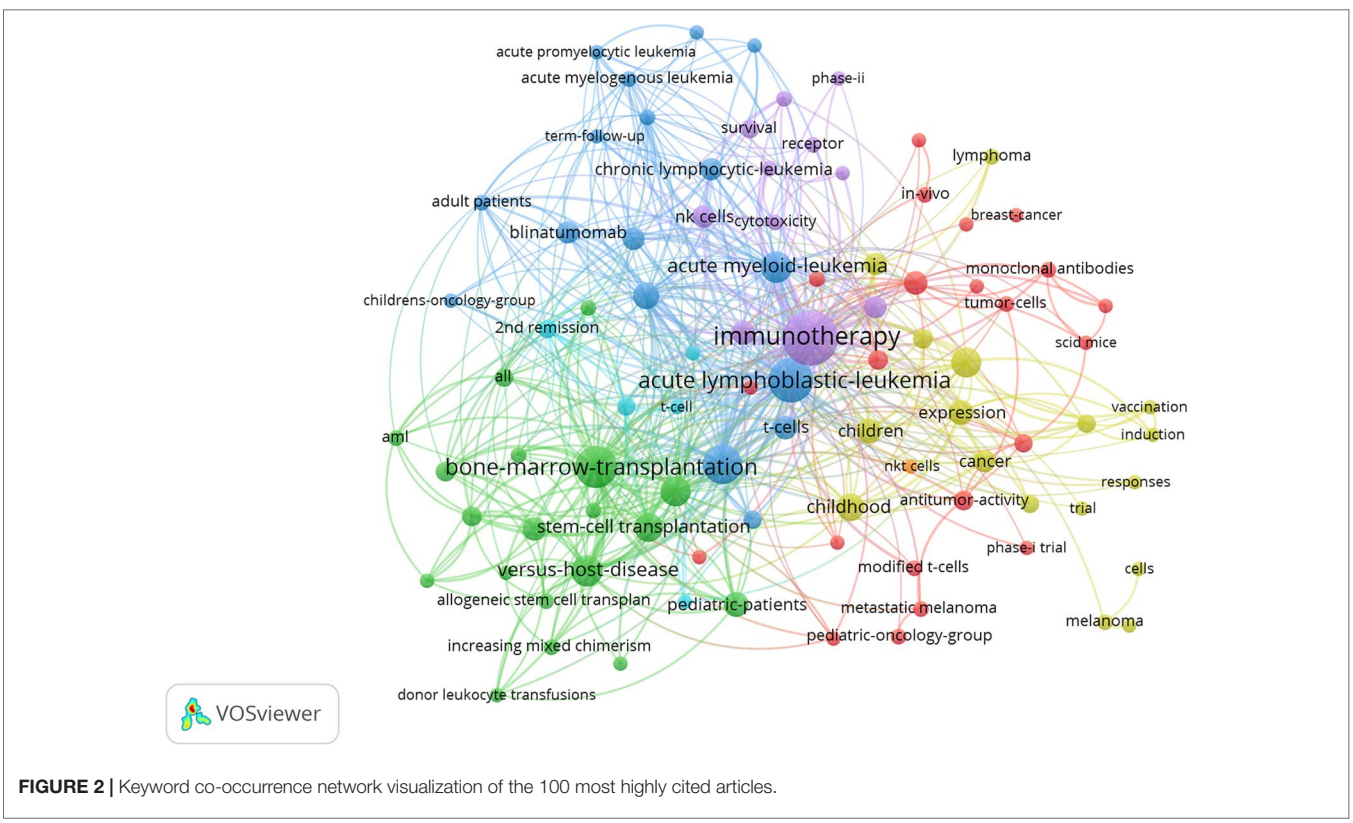

FIGURE 2 | Keyword co-occurrence network visualization of the 100 most highly cited articles.

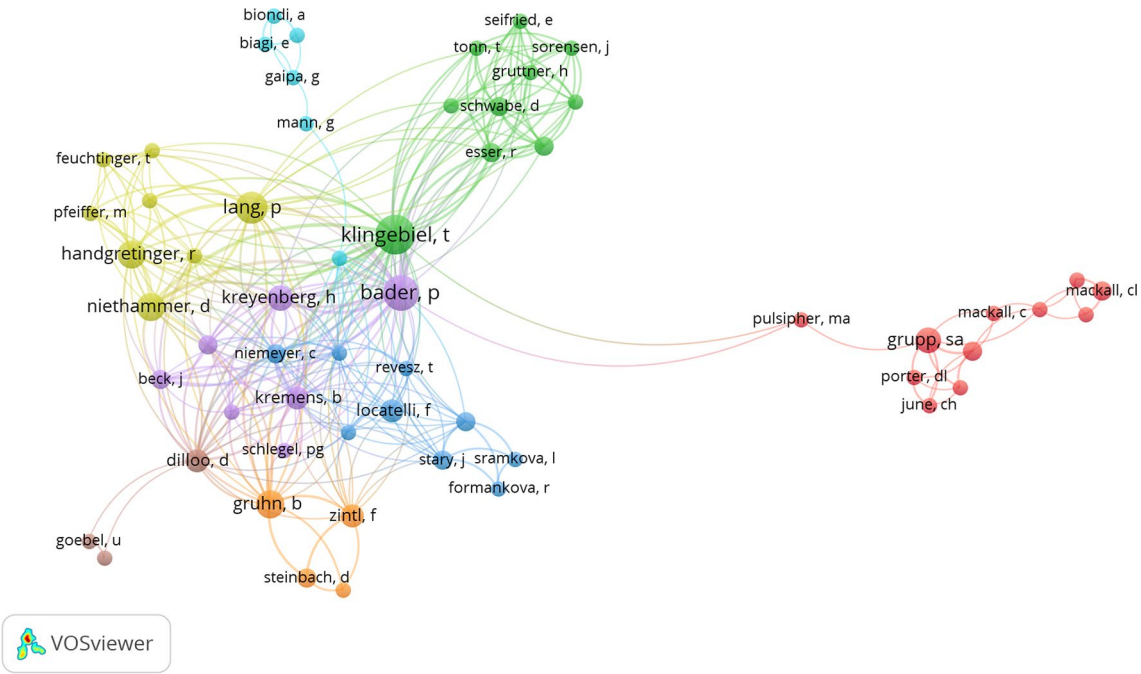

FIGURE 3 | Author coauthorship network visualization of the 100 most highly cited articles.

articles can be visually observed. After multiple times of drawing network figure, the minimum times for author appearing was set at 2. Even so, most researchers were not presented in network diagram because they did not meet the conditions. Researchers in this field are scattered, and only few of them have published more articles. Researchers such as T. Klingebiel, P. Bader, P. Lang, and B. Gruhn from Germany were in close contact with other researchers in this field and have published more articles.

This study still had some limitations. On the one hand, some of the influential articles may be omitted. We only searched the ISI Web of Science core collection database, and articles in other sources such as PubMed and Scopus might be missed, so our final results may be affected by such operation (Riggs et al., 2017). On the other hand, the number of citations is only an index to evaluate academic influence of articles. Higher impact factors of journals, greater academic influence of authors in this field, Open Access publishing, higher visibility of institutions, and long publication time of literature may all have positive impact on the number of citations for articles (Calver and Bradley, 2010; Zhang and Poucke, 2017). Besides, the quality of the top 100 articles was not assessed, so the quality of the documents may be different. This may affect the interpretation of the results.

CONCLUSIONS

In this study, we analyzed the top 100 highly cited articles on immunotherapy for childhood leukemia *via* bibliometrics. Most of the articles were published after the year 2000 on top journals in the field of oncology and hematology. Adoptive immunotherapy and stem cell transplantation are the main topics in these articles, which contributed to the development and optimization of immunotherapy. The United States and European countries such as Germany and Italy were major original countries in this research field. Professor T. Klingebiel from Goethe University Frankfurt represented a leader in the field of immunotherapy for childhood leukemia. This report provided insights into the features and research hotspots of highly cited articles on immunotherapy for childhood leukemia.

DATA AVAILABILITY

All datasets generated for this study are included in the manuscript/**Supplementary Files**.

AUTHOR CONTRIBUTIONS

Y-HJ designed this study. QZ and B-HL performed search and collected data. Q-QZ re-checked data. Z-MZ performed analysis, Z-HZ rechecked. QZ wrote the manuscript. Y-HJ reviewed the manuscript.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fphar.2019.01100/full#supplementary-material

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Safety and Efficacy of Therapeutic Cancer Vaccines Alone or in Combination With Immune Checkpoint Inhibitors in Cancer Treatment

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Therapeutic cancer vaccines have proven to seldom induce dramatic clinical response when used alone, and therefore, they are being studied in combination with additional treatment modalities to achieve optimal treatment activities. Growing preclinical data show that combining vaccines and immune checkpoint inhibitors (ICIs) can prime intensified immunogenicity and modulate immunosuppressive tumor microenvironment. Herein, we focus on the safety and efficacy of approved and promising cancer vaccines alone or combined with ICIs in the treatment of several malignancies. Generally, the majority of clinical trials support the concept of synergy that combination therapy of vaccines and ICIs holds maximized potential to improve clinical outcomes. Importantly, the combination has acceptable safety and minimal additional toxicity compared with single-agent vaccines or ICIs. Additionally, the potential strategies of combining personalized tumor vaccines with ICIs will become priority option and future direction of vaccine development and application and the urgent need to develop effective biomarkers to screen appropriate patient populations and predict response to combination therapy.

Keywords: cancer vaccine, immune checkpoint inhibitor, combination therapy, neoantigen, immunotherapy

INTRODUCTION

Cancer immunotherapy, including cancer vaccines, immune checkpoint inhibitors (ICIs), and adoptive cell therapy, represents a scientific breakthrough in the treatment of various malignancies (Kirkwood et al., 2012). Cancer vaccines are designed to specifically target tumor antigens and provoke host immune system to selectively fight against cancer cells (Melief et al., 2015). Currently, multiple cancer vaccine platforms have been developed, including peptide- or protein-based vaccines, oncolytic virus– or recombinant virus–vectored vaccines, dendritic cell (DC) vaccines, engineered cellular vaccines, and idiotype vaccines (Schlom, 2012; Guo et al., 2013). Generally, the majority of vaccines are well tolerated and have limited toxicity (Gatti-Mays et al., 2017). Unfortunately, with the recent failure of phase III clinical trial, vaccines as monotherapy have been shown to produce only modest or negative survival benefits (Hu et al., 2018; Gulley et al., 2019). Hence, combining

therapeutic cancer vaccines with additional treatment modalities has been explored, as an approach to augment immune responses and treatment activities.

Malignant tumors may evade immune surveillance by utilizing inhibitory immunoregulatory mechanisms, especially immune checkpoint receptor pathways. ICIs can enhance antitumor immune response by blocking these negative regulation signaling and have revolutionized the treatment landscapes of different tumor types such as melanoma, lung, renal cell, and bladder cancers (Hodi et al., 2010; Topalian et al., 2012). Nonetheless, ICIs do not appear to achieve clinical improvement in some other malignancies, for example, prostatic and pancreatic cancers, and less than 20% of unselected patient response to single-agent ICI (Royal et al., 2010; Beer et al., 2016; Strauss et al., 2016). In addition, ICI therapy also induces inflammatory responses and toxicity referred to as immune-related adverse events (irAEs), which may affect multiple organs and range from mild and manageable to life-threatening (Champiat et al., 2015; Puzanov et al., 2017).

Recently, growing preclinical and clinical researches have tended to combining therapeutic cancer vaccines and ICIs to explore the synergistic effects. Herein, we focus on the safety and efficacy of approved (sipuleucel-T and talimogene laherparepvec [T-VEC]) and promising cancer vaccines alone or combined with ICIs (cytotoxic T-lymphocyte-associated protein 4 [CTLA-4] and, programmed cell death 1 [PD-1] and its ligands [PD-L1]) for the treatment of several malignancies. We highlight the enormous potential of personalized cancer vaccines in combination with ICIs, which can produce complete tumor regression in several studies, and hope to provide theoretical foundations and innovative ideas for the development and application of cancer vaccines in clinical settings.

THERAPEUTIC CANCER VACCINES

Currently, the Food and Drug Administration (FDA) has approved two therapeutic cancer vaccines: sipuleucel-T for metastatic castration-resistant prostate cancer (mCRPC) based on modest improvement in overall survival (OS), and T-VEC for unresectable advanced melanoma based on partial improvement in OS and durable response rate (DRR) (Kantoff et al., 2010a; Andtbacka et al., 2015). There is also a promising cancer vaccine PROSTVAC, but the ultimate outcome from phase III clinical trial has proven to be a failure (Gulley et al., 2019).

Sipuleucel-T

Sipuleucel-T is an infusional autologous DC vaccine, generated by incubating patient's peripheral blood mononuclear cells (PBMCs) with the recombinant protein PA2024, composed of prostate acid phosphatase (PAP) fused to granulocyte–macrophage colony-stimulating factor (GM-CSF), which was FDA approved for asymptomatic or minimally symptomatic mCRPC in 2010 (Kantoff et al., 2010b). Immunological analysis demonstrated an increase in PAP-specific T cells and activated lymphocytes recruitment into the tumor microenvironment (TME) following vaccination (Fong et al., 2014). Remarkably, sipuleucel-T also elicits humoral immune response to nontargeted tumor antigens, known as antigen cascade and associated with improved clinical outcomes (Guha et al., 2015).

There are three randomized phase III trials to evaluate the safety and efficacy of sipuleucel-T. The pivotal IMPACT trial enrolled 512 patients randomized (2:1) to receive sipuleucel-T or placebo administered three intravenous infusions at 2-week intervals. The study demonstrated a 4.1-month median survival improvement (25.8 vs. 21.7 months) and an extended 3-year survival (31.7% vs. 23.0%) in sipuleucel-T group compared with placebo (Kantoff et al., 2010a). Common adverse events (AEs) included chills (54.1%), pyrexia (29.3%), headache (16%), and influenza-like illness (9.8%), primarily occurring within 1 to 2 days after infusion. Most AEs were mild to moderate (grades 1–2), and no treatment-related autoimmune complications were reported. The integrated analysis of two other clinical trials (D9901 and D9902A) showed a relative reduction of 33% in the risk of death for sipuleucel-T arm compared to placebo (Higano et al., 2009). However, sipuleucel-T vaccination did not prolong the time to disease progression and induce survival benefit without tumor shrinkage or prostate-specific antigen (PSA) declines (Kantoff et al., 2010b).

T-VEC

T-VEC is an intralesional oncolytic viral vaccine created by genetically engineered herpes simplex virus type 1, in which partial viral genes (ICP34.5 and ICP47) are deleted and replaced by a gene encoding GM-CSF (Liu et al., 2003). The modified virus infects both cancerous and healthy cells but only selectively replicate within tumors, causing the cells to swell and finally be lysed to release tumor-associated antigens (TAAs) (Kohlhapp and Kaufman, 2015). Meanwhile, vaccine viruses also utilize the translation mechanism of cancer cells to secrete GM-CSF, attracting DCs to the TME and stimulating them to present TAA (Toda et al., 2000). In 2015, T-VEC was approved by FDA for the treatment of unresectable nodal, cutaneous, and subcutaneous lesions in recurrent melanoma.

In a phase III OPTiM study, patients (n = 436) with unresected stages III to IV melanoma were randomly assigned (2:1) to intralesional T-VEC or subcutaneous GM-CSF. Compared to GM-CSF, T-VEC significantly improved DRR (16.3% vs. 2.1%) and overall response rate (26.4% vs. 5.7%) and resulted in a trend toward prolonged median OS (23.3 vs. 18.9 months; $P = 0.051$) (Andtbacka et al., 2015). The subgroup of patients with stages IIIB to IVM1a melanoma or treatment-naive disease achieved greater benefit from T-VEC. The most common AEs were fatigue (50%), chills (49%), pyrexia (43%), nausea (36%), and flu-like symptoms (30%). Only grades 3 to 4 AE in ≥2% of patients was cellulitis (2.1%), but no fatal treatment-related AEs occurred (Andtbacka et al., 2015). Furthermore, T-VEC also conduced a complete resolution in 22% of uninjected nonvisceral lesions, as well as 9% of visceral lesions, suggesting that it can generate systemic antitumor immunity to induce tumor regression distant from injection site (Andtbacka et al., 2016).

PROSTVAC

PROSTVAC (PSA-TRICOM) is a recombinant poxviral vectors vaccine, composed of heterologous prime-boost regimen: the vaccinia priming vaccine and the fowlpox boosting vaccine, which contains human PSA as encoded antigen and a triad of immune costimulatory molecules designated TRICOM: B7.1 (CD80), LFA-3 (CD58), and ICAM-1 (CD54) (Madan et al., 2009). In a previous phase II randomized trial, PROSTVAC prolonged median OS by 8.5 months (25.1 vs. 16.6 months) and improved 3-year survival (30% vs. 17%) in mCRPC compared with placebo (Kantoff et al., 2010a). Unfortunately, in the subsequent larger phase III study, no effective treatment had activities on primary endpoint—median OS; Criteria for futility were met, and ultimately the trial was terminated early (Gulley et al., 2019). Most frequently reported AEs were injection-site reactions (62%) as expected; common non–injection site events and cardiac-related events were fatigue (21%) and arrhythmias (1.4%), respectively. The majority of AEs (>75%) were mild (grade 1), and all serious treatment-related AEs occurred in less than 1% of patients. PROSTVAC is capable of increasing tumor-infiltrating lymphocytes (TILs) and generating specific immune responses against PSA and cascade antigens (Gulley et al., 2014). Combination therapy of PROSTVAC and ICI is currently being investigated in other clinical trials.

RATIONALE FOR COMBINATION IMMUNOTHERAPY

Growing preclinical data and clinical trials have shown that combination therapy of vaccines and ICIs can trigger intensified immunogenicity and also improve immunosuppressive TME, increasing efficacy than either treatment alone (Pardoll, 2012; Karyampudi et al., 2014). Here, we provide a brief summary of the rationale for combination immunotherapy.

Intensified Immunogenicity

Several studies have shown that ICI therapy alone has impressive activity in tumors with previous tumor-infiltrating immune response, for example, non–small cell lung cancer (NSCLC) and melanoma (Brahmer et al., 2015; Robert et al., 2015). However, ICIs are regretfully ineffective in nonimmunogenic tumors such as prostatic and pancreatic cancers for the lack of underlying immune recognition (Royal et al., 2010; Beer et al., 2016). Cancer vaccines can generate tumor-specific T cells in periphery or *in situ* tumors and are capable of driving these activated peripheral T cells into the TME leading to increased TILs (Fong et al., 2014; Gulley et al., 2014). Moreover, vaccine-mediated tumor cell death leads to the release of more cascade antigens and induces stronger immune responses specific to antigens not contained within the vaccine, a phenomenon referred to as antigen cascade or epitope spreading (Gregor et al., 2004; Guha et al., 2015). Thus, the hypothesis was proposed that greater efficacy of ICI treatment may be achieved by optimizing tumor immunogenicity or host immune responses with vaccines.

Improved Immunosuppressive TME

A major challenge for cancer vaccines is that despite the activation of tumor-specific immune responses, immunosuppressive TME restricts effector T-cell function (Thompson et al., 2007; Ahmadzadeh et al., 2009). CTLA-4 is mainly expressed on T helper cells and regulatory T cells (Tregs), mediating inhibitory effects during antigen presentation in periphery by interaction with ligands CD80 or CD84 (Baxter and Hodgkin, 2002; Pardoll, 2012). CTLA-4 inhibitors can directly block these negative signalings to enhance vaccine-induced tumor-specific T cells. CTLA-4 blockade also impacts on Tregs to increase the proportion of effector T cells to Tregs in the TME, which shifts intratumoral balance from immune suppression toward permissive status (Quezada et al., 2006; Liakou et al., 2008). PD-1 plays a critical inhibitory role in modulating the proliferation and cytolytic function of tumor-specific T cells *via* interaction with the ligand PD-L1. Blockade of PD-1 can prevent the senescence of vaccine-activated T cells in the TME, thereby prolonging antitumor activity of effector T cells and can restore the down-regulation of cytokine (interleukin 2, interferon γ [IFN-γ], and tumor necrosis factor α) to promote the cytotoxic effects (Wang et al., 2009; Postow et al., 2015). Taken together, ICIs may enhance and maintain vaccine-induced immune responses by favorably altering immunosuppressive TME and blocking these negative regulations.

CANCER VACCINES AND ICI COMBINATIONS

Based on above considerations, a host of clinical trials have been completed or are currently underway. Although many combination studies are in early phases, most of them support the concept of synergy that combining ICIs and therapeutic cancer vaccines has the potential to improve clinical outcomes.

Combining Anti–CTLA-4 and Vaccines

Ipilimumab Plus T-VEC

The phase II trial evaluated ipilimumab combined with T-VEC versus ipilimumab alone for unresectable stage IIIB to IV melanoma patients (n = 198). T-VEC was given intratumorally at first dose ≤4 ml × 10^6 pfu/ml, after 3 weeks at subsequent doses ≤4 ml × 10^8 pfu/ml every 2 weeks; ipilimumab 3 mg/kg was intravenously administered every 3 weeks for up to four doses (Puzanov et al., 2016). The objective response rate (ORR) was significantly higher in combination therapy than ipilimumab alone (39% vs. 18%). Moreover, 52% of patients treated with the combination and 23% of patients who received ipilimumab alone had a decrease in uninjected visceral lesions. Frequently occurring AEs for the combination were fatigue (59%), chills (53%), diarrhea (42%), pruritus (40%), and rash (39%), and incidence rates of grade ≥3 AEs in the combination and ipilimumab alone were 45% and 35%, respectively. Three patients with combination therapy had fatal AEs, but none were treatment related (Chesney et al., 2018). These data indicated that the combination had enhanced antitumor activity without additional toxicity compared to ipilimumab alone.

Ipilimumab Plus Sipuleucel-T

A small phase I trial of sipuleucel-T in combination with dose-escalation ipilimumab included nine men with docetaxel-naive progressive mCRPC. Subjects received three doses of sipuleucel-T every 2 weeks, immediately followed by low-dose ipilimumab 1 mg/kg given intravenously for a total of one, two, or three doses every 3 weeks (Scholz et al., 2017). Three patients died of disease progression. For six survivors, the median survival has surpassed 50.5 months compared with 35 months in phase III trials of enzalutamide or abiraterone. Tumor-specific antibodies directed at PAP and PA2024 demonstrated a significant increase after sipuleucel-T vaccination and a further elevation after ipilimumab treatment (Ku et al., 2018). There was no unexpected toxicity from combination therapy, and AEs of sipuleucel-T were consistent with previous reports. Ipilimumab led to only a transient grade 1 rash and resolved without additional treatment. Promising survival data and immunological properties in this study support further clinical trials of the combination in larger patient populations and higher doses of ipilimumab.

Ipilimumab Plus PROSTVAC

The phase I trial assessed dose-escalation ipilimumab combined with fixed-dose PROSTVAC for patients (n = 30) with mCRPC. PROSTVAC was subcutaneously given at prime doses of 2×10^8 pfu/ml, with subsequent monthly at boost doses of 1×10^9 pfu/ml. Intravenous ipilimumab was administered at doses of 1, 3, 5, and 10 mg/kg on the same day as vaccine. Median OS with the combination in all dose cohorts was 31.3 months, and for patients receiving ipilimumab 10 mg/kg, it was 37.2 months, remarkably longer than historical controls of PROSTVAC or ipilimumab alone (Singh et al., 2015). In total, 58% (14/24) of chemotherapy-naive patients had PSA declines from baseline, and 25% of them had PSA decreases of more than 50% (Jochems et al., 2014). The combination did not exacerbate irAEs associated with ipilimumab, and no dose-limiting toxicity (DLT) was recorded. Grades 1 to 2 injection-site reactions were most common AEs, and rash was frequently reported irAEs mostly occurred in patients treated with ipilimumab 10 mg/kg. Grades 3 to 4 irAEs were observed in eight patients (27%), including rash, diarrhea, colitis, and endocrine events, requiring replacement hormones or supportive measures (Madan et al., 2012). These findings are particularly notable, given that ipilimumab alone has yet to show clinical benefit in mCRPC.

Ipilimumab Plus GVAX

GVAX is an engineered cellular vaccine derived from allogeneic cancer cells transfected with GM-CSF, which has been shown to induce durable and specific antitumor immune responses (Lutz et al., 2011). A phase I trial of fixed-dose GVAX plus dose-escalation ipilimumab was conducted in chemotherapy-naive mCRPC. All patients (n = 28) received GVAX intradermally at a priming dose of 5×10^8 cells with subsequent injections at a dose of 3×10^8 cells every 2 weeks for 24 weeks and intravenous ipilimumab at extended doses of 0.3, 1, 3, and 5 mg/kg every 4 weeks. The study demonstrated >50% PSA declines from baseline in 25% (7/28) of patients, and four patients obtained stable disease measured by bone scan (Gerritsen et al., 2008). Most common AEs (>30%) were grades 1 to 2 injection-site reactions, fatigue, fever, influenza-like symptoms, and rash. At 5 mg/kg dose level, one patient underwent grade 4 sarcoid alveolitis defined as DLT. Other grade 3 irAEs included hypophysitis and hepatitis, both related to ipilimumab and responding to hormone replacement therapy (Eertwegh et al., 2012). Overall, irAEs with the combination appeared to be manageable.

Another phase Ib trial evaluated ipilimumab with or without GVAX in previously treated advanced pancreatic adenocarcinoma. Patients (n = 30) were randomized (1:1) to receive intravenous ipilimumab 10 mg/kg alone or intradermal GVAX at doses of 5×10^8 cells with subsequent ipilimumab 10 mg/kg. Compared with ipilimumab alone, the combination had prolonged disease stabilization (31, 71, and 81 weeks for three patients vs. 7 and 22 weeks for two patients), improved 1-year survival (27% vs. 7%), and a trend of favorable median OS (5.7 vs. 3.6 months; $P = 0.072$) (Le et al., 2013). CA19-9 biochemical responses were observed in 47% (7/15) of patients with combination therapy, whereas none in ipilimumab alone. Most common AEs in combination therapy were grades 1 to 2 injection-site reactions, rash, fatigue, fever, and influenza-like illness. Similar to previous ipilimumab reports, 20% of patients experienced grades 3 to 4 irAEs including rash, colitis, pneumonitis, and nephritis. All irAEs responded to steroids with the exception of nephritis requiring hemodialysis (Le et al., 2013). Further researches on the combination of ICIs and GVAX in the treatment of mCRPC or pancreatic cancer are warranted.

Ipilimumab Plus Peptide Vaccine

The efficacy of ipilimumab plus peptide vaccination (gp100) was explored in progressive stage IV melanoma patients (n = 56), who received two different doses of ipilimumab concomitantly with gp100 vaccination. The study demonstrated a durable objective response correlating with autoimmunity and tumor regression (Attia et al., 2005). Unfortunately, in pivotal phase III study for previously treated advanced melanoma, ipilimumab combined with gp100 was negative. Patients (n = 676) were randomly assigned (3:1:1) to ipilimumab plus vaccine, ipilimumab alone, or vaccine alone. Gp100 emulsified with incomplete Freund's adjuvant (IFA) was subcutaneously injected, and ipilimumab 3 mg/kg was given intravenously every 3 weeks for up to 3 months. No difference in median OS was detected between the combination and ipilimumab alone (10 vs. 10.1 months; $P = 0.76$). The best ORR was 10.9% in ipilimumab alone compared to 5.7% in combination arm (Hodi et al., 2010). The irAEs were similar in ipilimumab with or without vaccine, which most often affected skin and gastrointestinal tract. Although four patients required infliximab for grades 3 to 4 diarrhea or colitis, most of irAEs are reversible with corticosteroids or hormone replacement therapy. Ultimately, these data did not indicate any improved clinical outcome of ipilimumab plus peptide vaccine.

Other studies evaluated ipilimumab combined with peptide vaccines (MART-1/gp100/tyrosinase with Montanide ISA 51 VG) as adjuvant setting in high-risk resected stages IIIC to IV melanoma. In first single-arm trial, patients (n = 19) received three different doses of ipilimumab with multipeptide. The study showed that response rate to specific peptides (47%) was higher

than previous reports, and disease relapse rate was lower in patients with autoimmunity (Sanderson et al., 2005). Subsequently, another phase II trial enrolled 75 patients randomized (2:1) to receive extended-dose ipilimumab (3 or 10 mg/kg) every 6 to 8 weeks, along with subcutaneous immunizations of peptide vaccines. Although activated T cells increased over time after vaccination, only 25% of patients had immune responses to specific multipeptide. Autoimmune evidence positively correlating with improved relapse-free survival (RFS) was observed in 37% of patients, but the combination failed to generate additional benefits (Sarnaik et al., 2011). The AEs with the combination are generally reversible, and there were no treatment-related deaths. Frequently occurring grades 3 to 4 AEs were diarrhea, colitis, and hypopituitarism, which occurred in 29% of patients. All required tapering doses of systemic steroids, and most patients returned to normal within 3 months. In brief, adjuvant ipilimumab plus peptide vaccine following resection of high-risk melanoma had no impressive clinical activity.

Combining Anti–PD-1/PD-L1 and Vaccines

Pembrolizumab Plus T-VEC

The phase Ib trial evaluated pembrolizumab plus T-VEC for the treatment of unresectable stages IIIB to IV melanoma. Patients (n = 21) received T-VEC at initial dose of 4 ml × 10^6 pfu/ml, followed 3 weeks later at full dose of 4 ml × 10^8 pfu/ml every 2 weeks. Pembrolizumab 200 mg was administered intravenously coinciding with subsequent doses of T-VEC (Long et al., 2015). The confirmed ORR was 62%, about twice as shown in phase III study of pembrolizumab (34%) and T-VEC (26%), and complete response rate for per immune-related response criteria was 33%. An increase in lymphocytes infiltration, PD-L1 protein, and IFN-γ gene expression was observed in patients responded to combination therapy. The combination did not increase toxicity of monotherapy, with fatigue (62%), chills (48%), fever (43%), rash (33%), and arthralgia (33%) as the most common AEs. Only one grade 1 AEs associated with the combination resulted in hospitalization, while other grades 3 to 4 AEs were solely due to pembrolizumab (Ribas et al., 2017). Subsequently, the further phase III KEYNOTE-034 trial of systemic administration of pembrolizumab with intralesional injection of T-VEC is ongoing (NCT02263508).

Similarly, the phase Ib study evaluated pembrolizumab combined with T-VEC in patients (n = 36) with advanced squamous cell carcinoma of the head and neck. T-VEC was injected intralesionally at first dose of 8 ml × 10^6 pfu/ml, then at subsequent doses of 8 ml × 10^8 pfu/ml every 3 weeks. Intravenous pembrolizumab 200 mg was administered every 3 weeks (Harrington et al., 2017). Preliminary data from this study showed that the ORR was 16.7% (six patients with five subjects PD-L1 positive), and disease control rate was 38.9% (14 patients with 11 subjects PD-L1 positive). The most common AEs for the combination were pyrexia (36.1%), dyspnea (33.3%), and fatigue (25.0%). Grades 3 to 4 AEs were observed in 24 patients (66.7%), of which two (5.6%) and one (2.8%) patients discontinued treatment attributed to T-VEC and pembrolizumab, respectively. In one patient, DLT occurred: fatal arterial hemorrhage (Harrington et al., 2018). But overall, combination therapy was considered to have manageable safety, with amended protocol to exclude patients who received the neck reirradiation or at high risk of arterial hemorrhage (Harrington et al., 2017).

Nivolumab Plus Peptide Vaccine

In the phase I trial, therapeutic efficacy of nivolumab with or without multipeptide vaccines was assessed in ipilimumab-refractory and -naive melanoma. Patients (n = 90) with unresectable stages III to IV melanoma were treated with extended dose of nivolumab (1, 3, or 10 mg/kg) with or without peptide vaccines (MART-1/NY-ESO-1/gp100 with Montanide ISA 51 VG) (Kudchadkar et al., 2012). For both ipilimumab-refractory and -naive subjects, the RECIST response rates were 25%, and nivolumab-induced durable responses for up to 140 weeks. Combination therapy was well tolerated and safe, and no treatment-related death occurred. The common AEs were fatigue and injection-site reaction, most of which were mild to moderate and easy to manage. Other grade 3 irAEs (optic neuritis, fever, pneumonitis, and rash) can be resolved by prednisone taper as described previously for nivolumab. However, immunoassay demonstrated no increased responses in patients' PBMC to multipeptide at all doses and finally confirmed that peptide vaccines failed to improve clinical efficacy of nivolumab (Weber et al., 2013).

The same group conducted the phase I trial of nivolumab plus multipeptide vaccines as adjuvant setting in resected stages IIIC to IV melanoma. Patients (n = 33) were treated with extended dose of nivolumab (1, 3, or 10 mg/kg) plus peptide vaccines (MART-1/NY-ESO-1/gp100 with Montanide ISA 51 VG) every 2 weeks for 24 weeks, followed by nivolumab alone every 3 months for up to 2 years (Gibney et al., 2015). Estimated median RFS was 47.1 months, extremely beneficial compared with historical median RFS (12–21 months) (Hsueh et al., 2002; Sosman et al., 2011). The median OS was not reached with median follow-up of 32.1 months, and relapse rate at that time significantly decreased to 30.3%. Most common AEs (>40%) were injection-site reaction, fatigue, rash, pruritus, nausea, and arthralgia. Treatment-related grade 3 AEs included hypokalemia, rash, enteritis, and colitis, and only one toxicity meeting the DLT criteria was colitis. All related AEs responded to systemic management of steroids and supportive care (Gibney et al., 2015). This study suggested that nivolumab plus peptide vaccines can produce immunologic activity and promising survival as adjuvant therapy for high-risk advanced melanoma.

Emerging Progress in Combination Strategy

Lately, the combination of ICIs with antigen-presenting cell administration, especially DC vaccines, has been explored as an encouraging therapeutic strategy. The phase II study investigated ipilimumab combined with TriMixDC-MEL, created by autologous DCs electroporated with synthetic mRNA, in pretreated advanced melanoma (Wilgenhof et al., 2016). Patients (n = 39) were administered TriMixDC-MEL subcutaneously and intravenously plus ipilimumab 10 mg/kg every 3 weeks for

four doses, followed by nivolumab maintenance every 3 months. The disease control rate was 51% at 6 months, and ORR with the combination was 38%, which was higher than ipilimumab monotherapy (10%–15%). Tumor responses included eight complete and seven partial responses, half of which are ongoing after median follow-up of 3 years. The most common AEs (>30%) consisted of injection-site reactions, influenza-like illness, dermatitis, and chills, and no treatment-related deaths occurred. A total of 14 patients (36%) underwent grades 3 to 4 events, but most AEs were reversible by using established treatment algorithms (Wilgenhof et al., 2016).

Other studies undertook ICIs combined with intratumoral injection of innate immune activators, particularly Toll-like receptor 9 (TLR9) agonist, as a potential approach to improve clinical benefits. The phase I trial of tremelimumab plus subcutaneous administration of TLR9 agonist (CPG 7909) in stage IV melanoma or other advanced solid tumors demonstrated durable (>170 days) partial responses in 12% (2/17) of the patients with good tolerability (Millward et al., 2013). Another phase Ib study evaluated pembrolizumab plus intratumoral SD-101, a synthetic CpG oligonucleotide as TLR9-stimulating factor, in unresectable or metastatic melanoma. Among nine anti–PD-1 therapy-naive patients, the ORR was 78%, and 1-year progression-free survival rate was 88%. Combination therapy induced increased TILs in the TME and durable tumor responses in uninjected visceral lesions. SD-101 vaccination most often led to transient grades 1 to 2 injection-site reactions and influenza-like illness, and combination therapy had minimal additional toxicity relative to pembrolizumab alone (Ribas et al., 2018). Likewise, at the 2018 American Association for Cancer Research Annual Meeting, preliminary data from phase Ib trial of pembrolizumab plus intratumoral TLR9 agonist CMP-001, a CpG-A oligodeoxynucleotide packaged in virus-like particles, demonstrated a remarkable improvement in ORR of 33% for advanced melanoma previously resistant to anti–PD-1 therapy (Milhem et al., 2018).

CHALLENGES AND FUTURE PERSPECTIVES

Management of Combination-Related AEs

Recently, dual checkpoint blockade (combining ipilimumab and nivolumab) has demonstrated improved response rates in advanced melanoma and NSCLC; however, the benefit comes with drawbacks of additional toxicity (Antonia et al., 2015; Larkin et al., 2015). In contrast, observed toxicity with the combination of ICIs and cancer vaccines was within previously described spectrum of AEs for monotherapy ICI or vaccine, and no novel-toxicity was reported. Vaccination most often led to mild to moderate (grade 1 or 2) injection-site reactions, pyrexia, fatigue, and flu-like symptoms, appearing as transient symptoms at the early stage (Kantoff et al., 2010b; Andtbacka et al., 2015). Clinical toxicity related to ICIs covers a series of tissue-specific inflammatory events known as irAEs, which affect but are not limited to skin (rash, pruritus), gastrointestinal (diarrhea, colitis), endocrine (thyroiditis, hypophysitis), lung (pneumonitis), kidney (nephritis), and liver (hepatitis) (Weber et al., 2012; Kyi and Postow, 2016). Although severe irAEs may result in prolonged hospitalizations and even fatalities, the frequency of grade 3 or higher irAEs for combination therapy does not increase compared with ICI or vaccine alone.

The management of combination-related AEs is similar to that of immunotherapy alone, the majority of irAEs with the combination of ICIs and cancer vaccines are reversible when treatment is discontinued and/or managed with standard immunosuppressive algorithms such as steroids, and on occasion infliximab for refractory diarrhea or hepatitis (Weber et al., 2012; Champiat et al., 2015; Kyi and Postow, 2016). Details about management strategies of specific irAEs have been comprehensively reviewed (Champiat et al., 2015; Puzanov et al., 2017), and we highlight the importance of early recognition and prompt intervention. The median remission time for endocrine-related toxicity is longer, requiring continued but not necessarily permanent hormone replacement therapy; long-term effects of combination therapy and whether different ranges of irAEs will exhibit during chronic exposure have yet to be observed (Weber et al., 2012; Kyi and Postow, 2016). Additionally, irAEs are dose dependent and appear to be correlated with improved median OS, but are not a prerequisite for therapeutic efficacy. These results are based on retrospective analysis of small samples and so warrant further clinical exploration (Eertwegh et al., 2012; Owen et al., 2017).

Optimization of Vaccine Platforms

Despite the limited efficacy of vaccine monotherapy, cancer vaccines as key components of combination therapy can generate tumor-specific immune responses associated with survival (Sheikh et al., 2013). There are several key considerations for vaccine design needed to be emphasized. Accumulating evidence (e.g., the failure of gp100 peptide) indicated that immune responses elicited by peptide vaccines may be transient or of low magnitude and insufficient to enhance the efficacy of ICIs (Slingluff, 2011; Hirayama and Nishimura, 2016), while peptide-loaded autologous DC vaccines with strong immunogenicity and well tolerance have demonstrated remarkable clinical activities when combined with ICIs (Gatti-Mays et al., 2017). Besides, vaccines emulsified with IFA may lead to tumor-specific T-cell sequestration, dysfunction, and eventually apoptosis at injection site instead of destroying tumors, which is another major cause of peptide–IFA vaccine failure (Hailemichael et al., 2013). Thus, combination strategies are being optimized by applying suitable vaccine preparations and adjuvants, for example, DCs, viral vectors, or TLR agonists acting on innate immunity (Hailemichael et al., 2018).

Another vital factor in vaccine design is the selection of antigen targets. The majority of identified tumor antigens are self-antigens with lower affinity for TCR molecules inducing less robust clinical responses, and targeting these antigens may result in increased toxicity (Cloosen et al., 2007; Collins et al., 2018). Conversely, neoantigens derived from somatic mutations with minimal central immune tolerance and theoretical limited toxicity have become an optimal strategy for vaccine

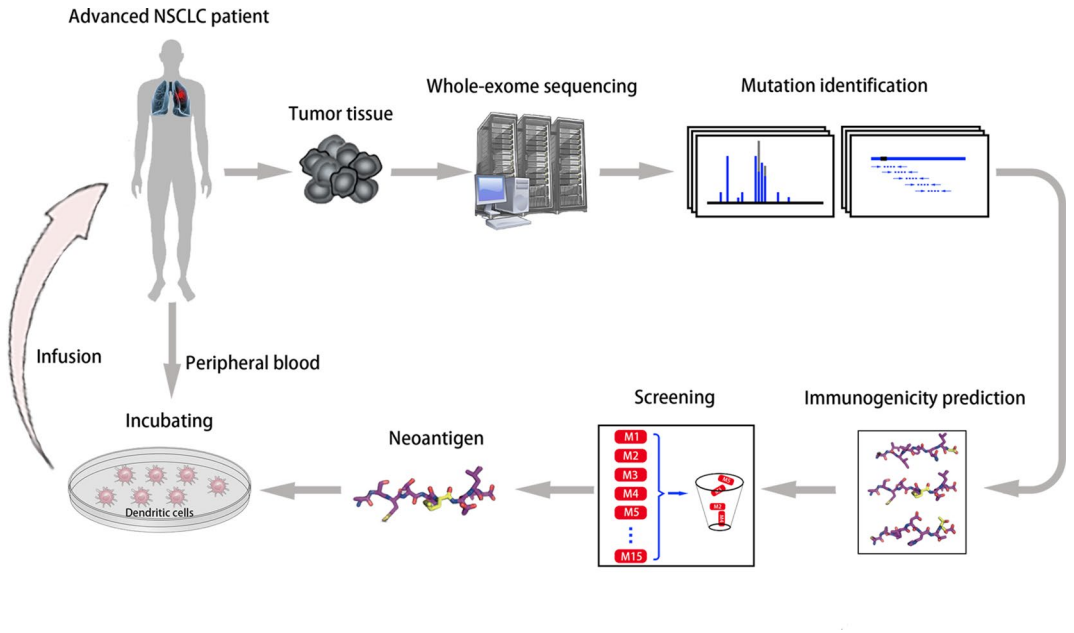

FIGURE 1 | Neoantigen-primed personalized DC vaccines for refractory NSCLC. We utilized whole-exome sequencing of tumor tissues, computational epitope prediction, and immunological approaches to screen for neoantigens from individual patients, and then infused autologous DCs pulsed with neoantigen-derived peptides into each patient (ClinicalTrials.gov Identifier: NCT02956551).

development (Stone et al., 2015; Hirayama and Nishimura, 2016). We conducted a phase I trial of neoantigen-primed DC vaccines for individualized treatment of refractory NSCLC (NCT02956551, **Figure 1**). As of May 2019, the study enrolled 11 patients, eight of whom finally received vaccination. Preliminary data demonstrated good tolerance, with only one patient developing a rash. Seven patients obtained stable disease with median progression-free survival of 5.7 months (range, 3.8–10.0 months) (Ding et al., 2019). Notably, two independent small-scale phase I studies of neoantigen-targeted personalized vaccines showed that three patients received vaccination plus ICIs, and all experienced complete tumor regression (Ott et al., 2017; Sahin et al., 2017). These findings indicate that "precise target" tumor vaccines combined with ICIs will become a priority candidate for antitumor therapy.

Time Sequence and Clinical Settings

Different combination strategies of vaccines and ICIs may have dissimilar ideal schedule. Checkpoint receptors change after vaccination in a time-dependent manner; namely, CTLA-4 expression decreased significantly 7 days after T-cell activation, whereas PD-1 expression persistently increased for a longer period (Fend et al., 2017). Studies showed that CTLA-4 blockade restrained tumor growth most availably when administered 1 day after vaccination, while administration on the same day did not produce antitumor activities. Anti–PD-1 treatment was most effective when administered 7 days after vaccination (Rojas et al., 2015; Fend et al., 2017). In another research, anti–CTLA-4 administration on the day of vaccination, or 1 day after instead of before, can maximize intratumoral $CD8^+$ T cell infiltration and tumor-specific lysis (Wada et al., 2013). However, other evidence indicated that administration of CTLA-4 and PD-1 blockade prior to vaccination still reduced tumor progression and improved long-term survival (Espenschied et al., 2003; Ali et al., 2016). Preclinical studies on time sequence of combination therapy are yet to entirely consistent, and predicting their manner of translation in clinical settings is difficult.

Furthermore, preclinical studies showed that combining vaccines and ICIs did not improve survival in prophylactic murine model (immunization before tumor inoculation), but did extend survival in therapeutic model, may be owing to epitope spreading caused by immunogenic cell death after initial vaccination (Davila et al., 2003; Williams et al., 2013). As above, combination therapy appeared to improve clinical outcomes in adjuvant postoperative therapy. Patients had preexisting tumors and often for years or may remain microscopic metastases after surgery, which provided antigens to prime underlying immune responses (Gibney et al., 2015; Morse and Lyerly, 2015). The Cancer Vaccine Consortium recommended the introduction of therapeutic cancer vaccines in early-stage and/or low-volume disease, but fortunately, combination therapy with ICIs may extend the scope of vaccine application to advanced or metastatic clinical settings (Finke et al., 2007; Dillman, 2017).

Biomarkers for Combination Therapy

The selection of appropriate patient population for immunotherapy is all important, but to date, no effective predictive biomarkers have been found. Consistent data

suggest that PD-L1 expression alone is insufficient to predict response to immunotherapy, that is, negative PD-L1 staining does not preclude the response (Weber et al., 2013; Shen and Zhao, 2018). Besides, the expression of PD-L1 in the TME is dynamic adaptive changes, while detection of PD-L1 expression in pretreatment biopsy only provides single static assessments (Sawada et al., 2015; Boussiotis, 2016). Recent studies showed that mismatch repair deficiency and high mutational burden may generate neoantigens and increase tumoral immunogenicity, which have become new biomarkers for response to ICI treatment (Snyder et al., 2014; Le et al., 2017).

However, the value of predictive biomarkers may observably change with combination therapy of vaccines and ICIs. Immunological analysis of nivolumab plus vaccines demonstrated a remarkable increase in peripheral Tregs and decrease in antigen-specific T cells in nonresponders and those with progressive disease (Weber et al., 2013). In adjuvant setting, a trend toward lower baseline peripheral Tregs and myeloid-derived suppressor cells was observed in nonrelapsing patients, and PD-L1 expression was not associated with RFS (Gibney et al., 2015). Similarly, in the study of ipilimumab combined with vaccine, the frequency of Tregs increased in patients with progressive disease, resulting in a shorter survival (Santegoets et al., 2013). Significantly improved OS was seen in patients with pretreatment high levels of $CD4^+CTLA\text{-}4^+$, $CD4^+PD\text{-}1^+$, and differentiated $CD8^+$ T cells or low levels of Tregs and differentiated $CD4^+$ T cells (Santegoets et al., 2013). All these findings highly implicated that depletion of Tregs may be one of the key factors to enhance therapeutic efficacy of the combination.

CONCLUSION

Cancer vaccines monotherapy produce only modest clinical benefits, but as key components of combination therapy, they can generate tumor-specific immune responses associated with survival. Many combination studies are in early phases, most of which support that combining ICIs and cancer vaccines holds maximized potential to improve clinical outcomes. Importantly, the combination has minimal additional toxicity compared to single-agent vaccines or ICIs. Personalized cancer vaccines have become a priority option for vaccine design, and potential strategies of combining these "precise target" vaccines with ICIs lack full testing but hold great promise. Moreover, the selection of appropriate patient population for immunotherapy is all important, but to date, no single immunology or tumor characteristic is sufficient to predict response to combination therapy and warrants further study.

AUTHOR CONTRIBUTIONS

J-YL and JZ contributed conception and overall idea of the study; JZ wrote the first draft of the manuscript; YC and Z-YD wrote sections of the manuscript. All authors contributed to manuscript revision, read, and approved the submitted version.

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SYSTEMATIC REVIEW
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Adverse Events of Concurrent Immune Checkpoint Inhibitors and Antiangiogenic Agents: A Systematic Review

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Background: Immune checkpoint blockade has revolutionized the treatment of multiple malignancies. Currently, however, the effect is not universal, with objective response rates (ORR) of about 15–25%, and even lower for some cancers. Abnormal vasculature is a hallmark of most solid tumors and plays a role in immune evasion. Growing body of evidence suggests that vascular normalization and immune reprogramming could operate synergistic effect, resulting in an enhanced therapeutic efficacy. However, the benefit of antitumor efficacy must be weighed against the risk of added toxicity. In this systematic review, we summarize severe toxicity observed in such a kind of combination regimen.

Methods: PubMed and Embase were searched for English references published up to May 31, 2019, with MeSH and keywords search terms of immune checkpoint inhibitors (ICIs) and antiangiogenic agents approved for using in solid tumors. Studies performing concomitant use of ICIs and antiangiogenic agents, and also reporting severe treatment-related adverse events (trAEs) (≥grade 3), were included for further analysis.

Results: A total of 32 studies including a total of 2,324 participants were analyzed. Limited available data suggests that both antiangiogenic monoclonal antibodies (mAbs) and tyrosine kinase inhibitors (TKIs) show potential risk of increasing treatment-related toxicity when combined with ICIs. Overall, the total incidence of severe adverse events (AEs) associated with ICIs plus mAbs (44.5%) is lower than that of ICIs plus TKIs (60.1%). However, the trAEs observed in combination therapy are mostly consistent with the known safety profiles of corresponding monotherapy, and they seem to be largely related to antiangiogenic agents, rather than a true immune-related adverse event (irAE) predominantly due to ICIs. The majority of trAEs are intervened by holding ICI treatment and adding corticosteroids, as well as reducing dose or adjusting administration frequency of the antiangiogenic drugs.

Conclusions: Concurrent use of ICIs and antiangiogenic agents shows potential treatment-related toxicity. Further research is required to compare the efficacy and safety of the combination regimen and corresponding monotherapy and identify predictive biomarkers, as well as explore dose, duration, and sequencing schedules of drugs.

Keywords: immune checkpoint inhibitor, antiangiogenic monoclonal antibody, tyrosine kinase inhibitor, concurrent therapy, treatment-related adverse event, immune-related adverse event, systematic review

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INTRODUCTION

Interventions for local advanced or metastatic solid tumors have evolved rapidly in recent years, among which immune checkpoint blockade therapy may be the most notable strategy (Pardoll, 2012; Hoos, 2016; Papaioannou et al., 2016). Indeed, immune checkpoint inhibitors (ICIs) targeting the cytotoxic T lymphocyte-associated protein 4 (CTLA-4) and programmed cell death protein 1 (PD-1), a T-cell immune checkpoint receptor, or its ligand PD-L1 may be effective for various types of cancer and have brought significant improvements in clinical prognosis (Hodi et al., 2010; Herbst et al., 2014; Ansell et al., 2015; Sharma and Allison, 2015). However, these therapies benefit just a few of patients, with objective response rates (ORR) of about 15–25%, and even lower for pancreatic carcinoma, prostate cancer, ovarian carcinoma, triple negative breast cancer, and microsatellite stable colorectal cancer. It may be attributed to insufficient abundance of tumor neoantigens, tumor heterogeneity, and genetic variation among individuals. Besides, acquired tumor resistance of ICIs is also a challenge (Ma et al., 2016; Wang and Wu, 2017). Therefore, it is necessary to seek combination therapy strategy which can activate anti-tumor immunity and enhance treatment efficacy.

Researches have identified that abnormal tumor vasculature in the tumor microenvironment (TME) not only fuels tumor progression but also has a negative impact on the effectiveness of all types of anticancer therapies, especially immunotherapy. Elevated interstitial fluid pressure of the TME caused by the leaky nature of tumor vessels and dysfunctional lymphatic drainage, along with low expression level of cell adhesion molecules, such as vascular cell adhesion protein 1 (VCAM1) and intercellular adhesion molecule 1 (ICAM1), limits the entry of drugs and the trafficking of immune effector cells into tumors (Griffioen et al., 1996; Buckanovich et al., 2008; Jain, 2013). Besides, angiogenic molecules presenting in the TME, such as vascular endothelial growth factor (VEGF), act as a mediator of tumor-associated immunosuppression. Firstly, VEGF directly prevent mobilization, trafficking, development, proliferation, and effector function of CD8-positive cytotoxic T lymphocytes (CTLs) (Ohm and Carbone, 2001; Voron et al., 2015). Secondly, VEGF could promote the recruitment and proliferation of immunosuppressive cells, including regulatory T (Treg) cells, myeloid-derived suppressor cells (MDSCs), and M2-like tumor-associated macrophages (TAMs) (Terme et al., 2013; Chaudhary et al., 2014; Maenhout et al., 2014). Thirdly, maturation and antigen presentation of dendritic cells (DCs) might be suppressed by elevated VEGF (Gabrilovich et al., 1996; Gabrilovich et al., 1998). Thus, strategies inducing vascular normalization may restore immune cell functions and help to attenuate the immunosuppression of the TME, thereby improve the activity of immunotherapy. For example, sunitinib could increase T-cell and B-cell levels and decrease PD-1 expression in tumor-infiltrating T-cells as well as inhibit MDSCs and Treg cells into tumor (Heine et al., 2011; Voron et al., 2015). Bevacizumab and pazopanib could increase the infiltration or activity of CD8-positive and CD4-positive T-cells and enhance the maturation of DCs (Elamin et al., 2015; Zizzari et al., 2018). However, recent studies have also shown that an adaptive immunosuppression caused by the up-regulation of PD-L1 in endothelial cells (ECs) and tumor cells after antiangiogenic therapies limits the activity of antiangiogenesis (Allen et al., 2017). It suggests that combination of antiangiogenesis and immune checkpoint blockade targeting PD-1/PD-L1 may be a good choice. More interestingly, bioinformatic analyses revealed that gene expression features related to vascular normalization correlate with immunostimulatory pathways, especially the activation and infiltration of T-cells. As a result, activating of CD4-positive T-cells by ICIs promoted the normalization of tumor vessels in return (Tian et al., 2017). Therefore, it demonstrates that vascular normalization and immune reprogramming have synergistic effect, which provides a basis for the rationality of the combination of ICIs and antiangiogenic agents.

Indeed, preclinical evidences have confirmed the efficacy of these combination regimens (Yasuda et al., 2013; Motoshima et al., 2015; Du Four et al., 2016; Kimura et al., 2018; Laubli et al., 2018). For instance, in a mouse model of colon adenocarcinoma, treatment with axitinib led to an improved T-cell response, and it resulted in a synergistic therapeutic efficacy when combined with anti-PD-1 antibody (Laubli et al., 2018). On the basis of preclinical data, these combination therapies have been tested in dozens of clinical trials, which reported promising outcomes in patients with metastatic melanoma, non-squamous non-small-cell lung carcinoma (NSCLC), and renal cell carcinoma (RCC). Among them, IMpower150 trial showed that atezolizumab plus chemotherapy plus bevacizumab significantly improved progression-free survival (PFS) and overall survival (OS) of patients with metastatic non-squamous NSCLC, regardless of mutational status and checkpoint expression of tumor (Reck et al., 2019). Similarly, in other two phase 3 trials on the first-line treatment of advanced or metastatic RCC, concomitant use of pembrolizumab and axitinib improved OS, PFS, and ORR over the standard of care (Rini et al., 2019a), while combining avelumab with axitinib improved PFS and ORR (Motzer et al., 2019).

However, despite the enhanced anti-tumor efficacy, the combination treatment is not without challenge, including the risk of added toxicity and increasing of immune-related adverse events (irAEs). As is well known, toxic effects associated with ICIs manifesting with autoimmune-like side-effects are commonly seen in the skin, gastrointestinal tract, pulmonary, hepatic, renal, nervous, hematologic, cardiovascular, and endocrine systems (Gordon et al., 2017; Puzanov et al., 2017). Likewise, antiangiogenic monoclonal antibodies (mAbs) and small-molecule tyrosine kinase inhibitors (TKIs), the two main types of antiangiogenic agent, also have diverse adverse effects, mainly including hypertension, arterial thromboembolic events, proteinuria, bowel perforation, reversible posterior leukoencephalopathy syndrome, wound complications, and hemorrhage (Chen and Cleck, 2009). At present, there is no systematic analysis of the toxicity of such a kind of combination. This review will focus on the severe treatment-related adverse events (trAEs) and irAEs of the concomitant use of ICIs and antiangiogenic agents.

MATERIAL AND METHODS

Search Strategy and Eligibility

The study was performed according to the "PRISMA" statement. Search was done on 31 May 2019. PubMed and Embase databases were searched for relevant literatures published in English using MeSH and keywords "nivolumab," "pembrolizumab," "atezolizumab," "avelumab," "ipilimumab," "durvalumab," "immune checkpoint inhibition" or "immune checkpoint inhibitors," combined with "bevacizumab," "ramucirumab," "anlotinib," "apatinib," "axitinib," "cabozantinib," "cediranib," "fruquintinib," "lenvatinib," "motesanib," "nintedanib," "pazopanib," "regorafenib," "sorafenib," "sunitinib," "vandetanib," "aflibercept," or "endostar." Studies included in this review were limited to clinical trial of any phase, retrospective study, or case report involving adult patients with solid tumors. Only original articles were included. Duplicates, conference abstracts or poster presentations, commentaries, reviews, and secondary reporting of clinical trials were excluded.

Studies involving concurrent treatment of ICIs and antiangiogenic agents were eligible. The study should properly describe the safety of the combination treatment. Studies not describing toxicity or the timing of antiangiogenic therapy in relation to ICIs were excluded. AEs should be assessed according to the National Cancer Institute Common Terminology Criteria for Adverse Events (CTCAE). If not, authors rated them accordingly. When more than one article reported the same trial, the most recent data was used. When patients in case report were from the same cohort of a clinical trial and were reported with the same AEs, the case report was excluded. All relevant articles underwent evaluation for eligibility by two independent authors (LG and XY) and then were verified by senior author (HZ and CY). Titles and abstracts were preliminary screened. Subsequently, full-text reading was used to check whether the study met inclusion or exclusion criteria.

Data Extraction

Two authors (LG and XY) collected all data for included studies. Data was sought on authors, year of publication, study type, number of patients, as well as the type, dose, and treatment duration of ICIs and antiangiogenic agents. Tumor types and stages, follow-up time, toxicity, and management were also collected. Only grade 3–5 trAEs and irAEs were included for analysis.

RESULTS

Included Studies and Overview

We initially identified a total of 1,883 references from database search. There were 348 papers excluded due to duplication, and the remaining 1,535 references were read with title and abstract. Subsequently, 104 relevant articles were further assessed for eligibility by full-text reviewing. Finally, 32 articles meeting the inclusion criteria were included into this systematic review (**Figure 1**). Among them, there were 17 prospective studies (n = 2186), 5 retrospective studies (n = 104), and 10 case reports (n = 34), with the median number of patient as 70 per study. Studies of

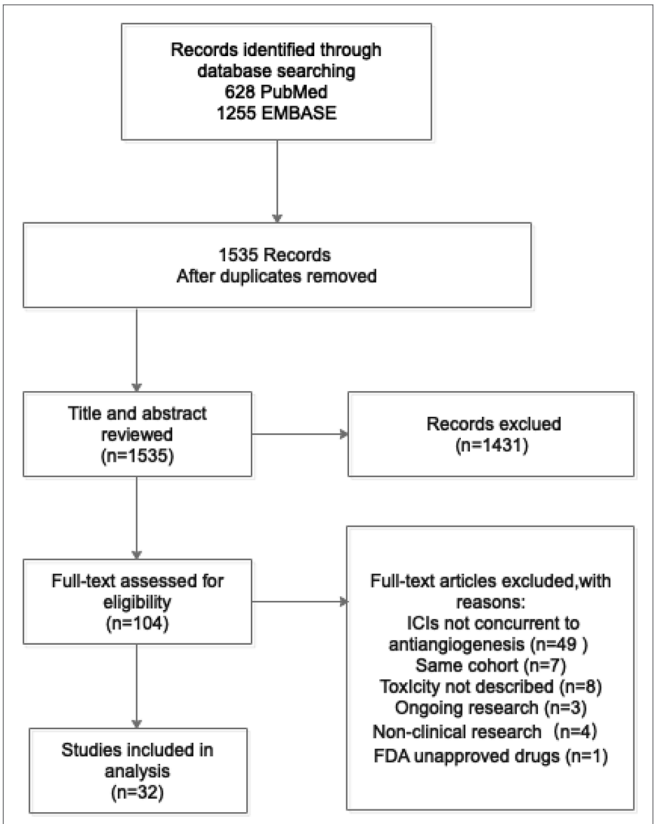

FIGURE 1 | Search flow diagram.

the combination of ICIs and anlotinib, fruquintinib, motesanib, nintedanib, regorafenib, vandetani, or aflibercept were not found. The concurrent use of ICIs and mAbs was reported in 15 studies (**Table 1**), while concurrent use of ICIs and TKIs was in 17 studies (**Table 2**).

The reported treatment-related toxicities of included studies were listed in **Table 3**. When ICIs combined with mAbs (n = 1166), severe toxicity reported as grade 3/4 and grade 5 AEs was observed in 501 (43%) and 18 (1.5%) patients, respectively (**Figures 2A, B**), while for ICIs plus TKIs (n = 1158), grade 3/4 and grade 5 AEs were in 687 (59.3%) and 9 (0.8%) patients, respectively (**Figures 2A, C**). Overall, the total incidence of severe trAEs associated with ICIs plus mAbs was lower than that of ICIs plus TKIs (**Figures 2B, C**).

Toxicity of Concurrent ICIs and Antiangiogenic mAbs (Bevacizumab and Ramucirumab)

Anti-CTLA-4 (Ipilimumab)

One prospective study (Hodi et al., 2014) and one case series (Carter et al., 2016) were identified (**Table 1**), examining concurrent ipilimumab and bevacizumab in melanoma and glioblastoma, respectively. The median dose of bevacizumab ranged from 7.5 to 15 mg/kg, and the dose of ipilimumab was 3 or 10 mg/kg every 3 weeks. Hodi et al. reported a total of

TABLE 1 | Included articles with concurrent ICIs and antiangiogenic mAbs.

Authors	Study year	Study type	Patients (n)	Compounds and dosage	Treatment timing	Primary tumor	Follow-up (median time)	Toxicity (≥3)
Wallin et al.	2016	Prospective	10	Bevacizumab 15 mg/kg i.v./3 w * 1 cycle, and then atezolizumab 20 mg/kg i.v., bevacizumab 15 mg/kg i.v./3 w	Renal cell carcinoma (RCC)	Advanced; metastatic	17.2 months	Y
McDermott et al.	2018	Phase 2 trial	101	Atezolizumab 1,200 mg i.v., bevacizumab 15 mg/kg i.v./3 w	RCC	Advanced; metastatic	20.7 months	Y
Rini et al.	2019	Phase 3 trial	451	Atezolizumab 1,200 mg i.v., bevacizumab 15 mg/kg i.v./3 w	RCC	Advanced; metastatic	15 months	Y
Reck et al.	2019	Phase 3 trial	394	Atezolizumab + bevacizumab + carboplatin + paclitaxel (atezolizumab 1,200 mg i.v., bevacizumab 15 mg/kg i.v./3 w)	Non-squamous non-small-cell lung cancer (NSCLC)	Chemotherapy-naïve metastatic	19.6 months	Y
Wu et al.	2017	Case report	1	Pembrolizumab + bevacizumab + cisplatin + gemcitabine (pembrolizumab 1 mg/kg i.v., bevacizumab 4 mg/kg i.v.)	Urothelial carcinoma (UC)	Recurrent	NR	N
Gadgeel et al.	2018	Phase 1 trial	24	Pembrolizumab + bevacizumab + carboplatin + paclitaxel (pembrolizumab 2(n = 11) or 10 mg/kg (n = 13) i.v., bevacizumab 15 mg/kg i.v./3 w * 4 cycles, and then pembrolizumab + bevacizumab for 2 years)	Non-squamous NSCLC	Stage IIIB/IV without EGFR mutations or ALK translocations	16.4 months	Y
Blumenthal et al.	2016	Retrospective	10	Pembrolizumab 150 mg i.v., bevacizumab, dosage NR i.v.,/3 w	Central nervous system (CNS) tumor	Recurrent	NR	N
Kurz et al.	2018	Retrospective	28	Pembrolizumab 2 mg/kg i.v./3 w (n = 19), or nivolumab 3 mg/kg i.v./2 w (n = 12), bevacizumab 10 mg/kg i.v./2 w (n = 28)	High-grade gliomas (HGGs)	Recurrent	NR	N
Mantica et al.	2018	Retrospective	43	Nivolumab 3 mg/kg i.v./2 w, bevacizumab, dosage NR	HGGs	Advanced	6.4 months	Y
Kanda et al.	2016	Phase 1b trial	6	Nivolumab+paclitaxel+carboplatin+ bevacizumab (nivolumab 10 mg/kg i.v., bevacizumab 15 mg/kg i.v./3 w * 6 cycles, and then pembrolizumab + bevacizumab maintain)	Non-squamous NSCLC	Stage IIIB without indication for definitive thoracic radiotherapy; stage IV; recurrent	7.54 months	Y
Normann et al.	2019	Prospective	5	Nivolumab 3 mg/kg i.v./2 w, bevacizumab dosage NR	Platinum resistant ovarian cancer	Recurrent	30 weeks	Y
Shirali et al.	2016	Case report	1	Nivolumab 3 mg/kg i.v., bevacizumab 15 mg/kg i.v./3 w)	NSCLC	Progression	NR	Y
Hodi et al.	2014	Prospective	46	Ipilimumab 10 mg/kg i.v./3 w *4 cycles, and then 10 mg/kg i.v./12 w + bevacizumab 7.5 mg/kg (cohort 1) or 15 mg/kg (cohort 2) i.v./3 w; ipilimumab 3 mg/kg i.v./3 w *4 cycles, and then 3 mg/kg i.v./12 w + bevacizumab 7.5 mg/kg (cohort 3) or 15 mg/kg (cohort 4) i.v./3 w	Melanoma	Unresectable stage III; stage IV	17.3 months	Y
Carter et al.	2016	Case series	20	Ipilimumab 3 mg/kg i.v./3 w *4 cycles, and then 3 mg/kg i.v./12 w, bevacizumab 10 mg/kg i.v./2 w	Glioblastoma	Grade IV disease or recurrent astrocytoma (grade II); progression or after first-line therapy	≥12 weeks	Y
Arkenau et al.	2018	Phase 1 trial	26	Pembrolizumab 200 mg i.v. d1, ramucirumab 8 mg/kg i.v. d1, d8/3 w	Biliary tract cancer (BTC)	Advanced; metastatic	15.7 months	Y

32 grade 3/4 trAEs in 13 patients, including one grade 4 hepatic and two grade 4 proteinuria. Among them, grade 3/4 trAEs in four cohorts were 5 (15.6%), 11 (34.4%), 6 (18.8%), and 10 (31.3%), respectively (**Table 3**). It seemed that the incidence of severe trAEs tended to elevate with the increase dose of bevacizumab. But it did not seem to increase the incidence of dermatologic or gastrointestinal side effects such as colitis, which were more concerning for ipilimumab treatment (Hodi et al., 2014). In addition, one case series reporting the combination regimen in glioblastoma observed seven grade 3 trAEs. However, all immune-related toxicities were manageable with corticosteroids, without diagnosing of endocrinopathies (Carter et al., 2016).

TABLE 2 | Included articles with concurrent ICIs and TKIs.

Authors	Study year	Study type	Patients (n)	Compounds and dosage	Primary tumor	Treatment timing	Follow-up (median time)	Toxicity (≥3)
Atkins et al.	2018	Phase 1b trial	52	Axitinib 3,5 or 7 mg p.o. bid continuously, (median dose: 8.8 mg/day), pembrolizumab 2 mg/kg i.v. d8/3 w	RCC	Advanced	20.4 months	Y
Rini et al.	2019	Phase 3 trial	429	Axitinib 5 mg (2–10 mg) p.o. bid continuously, pembrolizumab 200 mg i.v./3 w	RCC	Advanced, recurrent	12.8 months	Y
Wilky et al.	2019	Phase 2 trial	33	Axitinib 5 mg (2–10 mg) p.o. bid continuously, pembrolizumab 200 mg i.v. d8/3 w up to 2y	Sarcomas, including alveolar soft-part sarcoma (ASPS)	Advanced; metastatic	14.7 months	Y
Choueiri et al.	2018	Phase 1b trial	55	Axitinib 5 mg p.o. bid, d1–7 (lead-in period), axitinib 5 mg p.o. bid continuously, avelumab 10 mg/kg i.v./2 w	RCC	Advanced	52.1 weeks	Y
Motzer et al.	2019	Phase 3 trial	434	Axitinib 5 mg p.o. bid, avelumab 10 mg/kg i.v./2 w	RCC	Advanced	11.6 months	Y
Qiao et al.	2018	Case report	1	Pazopanib + pembrolizumab + RAK cells (pazopanib 200 mg p.o. qd for 2 days, 400 mg qd for 5 days, then 600 mg qd up to now, pembrolizumab 100 mg i.v./3 w)	Primary hepatic angiosarcoma (PHA)	Advanced	About 15 months	N
Amin et al.	2018	Phase 1 trial	20(P+N)33(S+N)	Pazopanib 800 mg p.o. qd, nivolumab 2 mg/kg i.v./3 w; sunitinib 50 mg p.o. qd/4 weeks on and 2 weeks off, nivolumab 2 mg/kg i.v./3 w	RCC	Advanced	27.1 months (P+N); 50 months (S+N)	Y
Paoluzzi et al.	2016	Retrospective	18	Pazopanib 400–800 mg p.o. qd, nivolumab 3 mg/kg i.v./2 w	Sarcomas	Relapsed metastatic; unresectable	≥13 months	Y
Yu-Li Su et al.	2017	Case report	1	Pazopanib 400 mg p.o. qd continuingly, nivolumab 3 mg/kg i.v./2 w	RCC	Metastatic	≥4 months	N
Chen et al.	2017	Case report	1	Sorafenib 200 mg p.o. bid, pembrolizumab 2 mg/kg i.v. d1/3 w (4 w starting in cycle 3)	Hepatocellular carcinoma (HCC)	End-stage	NR	N
Feng et al.	2017	Case series	6	Sorafenib 200 mg p.o. bid, nivolumab 3 mg/kg i.v. d1/3 w	HCC	Advanced	NR	N
Mahmoud et al.	2016	Case report	1	Sunitinib 50 mg p.o. qd/4 weeks on and 2 weeks off, nivolumab NR	RCC	Metastatic	≥11 months	N
Lee et al.	2017	Phase 1 trial	14	Cediranib 20/30 mg p.o. qd, + durvalumab 10 mg/kg i.v./2 w; cediranib 20 mg p.o. qd/5 days on and 2 days off, + durvalumab 1,500 mg i.v./4 w	Solid tumors	Recurrent; metastatic	NR	Y
Zhao et al.	2019	Case report	1	Apatinib 500 mg p.o. qd, nivolumab 3 mg/kg i.v./2 w	Liver carcinosarcoma	Advanced	About 15 months	Y
Makker et al.	2019	Phase 2 trial	53	Lenvatinib 20 mg p.o. bid, pembrolizumab 200 mg i.v./3 w	Endometrial cancer	Metastatic	13.3 months	Y
Iyer et al.	2018	Retrospective	12	Lenvatinib 20 mg p.o. bid, pembrolizumab 200 mg i.v./3 w	Anaplastic thyroid carcinoma (ATC)	Progression	13.74 months (8.14 + 5.6)	Y
Bhat et al.	2019	Case report	1	Cabozantinib, nivolumab, dosage NR	RCC	Metastatic	NR	N

In summary, limited available data indicated potential toxicity of concurrent ipilimumab and bevacizumab. But at least, it did not seem to increase the incidence of some special interest irAEs. Data on the combination of ipilimumab and ramucirumab is lacking.

Anti-PD-1 (Pembrolizumab and Nivolumab)

For pembrolizumab, there were two phase 1 trials (Arkenau et al., 2018; Gadgeel et al., 2018), two retrospective studies (Blumenthal et al., 2016; Kurz et al., 2018), and one case report (Wu et al., 2017). Among them, four were concerning combined with bevacizumab, and one was with ramolumab. In the study of Gadgeel et al. where 24 patients with advanced non-squamous NSCLC received concurrent pembrolizumab, bevacizumab, and chemotherapy, grade 3 trAEs occurred in 10 (42%) patients, which was similar to patients treated without bevacizumab [10 (40%)]. But the grade 3 irAEs (colitis, pneumonitis, and pancreatitis) and infusion reaction occurred in five (20.8%) and one (4%) patients treated with or without addition of bevacizumab (**Table 3**) (Gadgeel et al., 2018). In the two retrospective studies, concomitant use of pembrolizumab and

TABLE 3 | Treatment-related toxicity as observed within the included articles.

Antiangiogenic agents	ICIs	Study	Median treatment duration	Patients (n)	Grade 3 (n) trAE/irAE	Grade 4 (n) trAE/ irAE	Grade 5 (n) trAE/irAE	Total toxicity (≥3)	Management
Bevacizumab				1,140	494		18	512	
	Ipilimumab	Hodi et al.	NR	46	ALT (n = 2), AST (n = 2), abdomen pain (n = 2), adrenal insufficiency (n = 2), allergic reaction (n = 1), colitis (n = 2), endocrine-other (n = 1), fatigue (n = 1), head or headache (n = 1), hemorrhage-other (n = 1), hepatic-other (n = 1), hypertension (n = 4), hyponatremia (n = 2), lipase (n = 2), lymphopenia (n = 1), mucostomatitis by exam, oral cavity (n = 1), rash or desquamation (n = 2), thrombosis or thrombus or embolism (n = 1), vascular-other (n = 1). Among them, 5 trAEs were observed in cohort 1, 8 were in cohort 2, 6 were in cohort 3, and 10 in cohort 4.	Hepatic-other (n = 1), proteinuria (n = 2) All above were observed in cohort 2 Number of patient was 13 (grade 3/4)	0	13	NR
		Carter et al.	65% patients complete four cycles	20	Diarrhea (n = 1), abscess formation (dental, uterine, diverticular) (n = 3), intracerebral bleed (n = 1), pulmonary embolism (n = 2)	0	0	7	Three abscess were managed surgically; corticosteroids (diarrhea), dosage NR; NO discontinued treatment.
	Pembrolizumab	Gadgeel et al.	Pemb: 10 doses (30 weeks)	24	Thrombocytopenia (n = 1), neutrophil count decreased (n = 1), white blood cell count decreased (n = 2)/colitis (n = 1), pneumonitis (n = 1), pancreatitis (n = 1). Grade 3 trAEs occurred in 10 (42%) and 10 (40%) patients with or without bevacizumab, respectively. Grade 3 irAEs and infusion reactions occurred in 5 (20.8%) and 1 (4%) patients with or without bevacizumab, respectively.	0	0	10	Discontinuation: pembrolizumab 2 mg/kg group (n = 2, 18%); 10 mg/kg group (n = 3, 23%)
		Blumenthal et al.	Pemb: 3 doses (9 weeks)	10	NR	NR	NR	0	Steroids weaned off or minimal 2 mg/d
		Wu et al.	11 cycle (about 7.7 months)	1	NR	NR	NR	0	A mild immune-related skin was resolved completely with anti-histamines.
	Pembrolizumab or nivolumab	Kurz et al.	NR	28	0	0	0	0	On steroids when pembrolizumab initiated: n = 17 (55%), dosage NR Discontinuation: n = 1 (3%)
	Nivolumab	Mantica et al.	8 cycle (about 16 weeks)	43	Pneumonitis (n = 1) / irAEs (including colitis and pneumonitis): n = 3	Pneumonitis (n = 2), colitis (n = 1)	0	4	Discontinuation: n = 4 (8%)
		Kanda et al.	NR	6	White blood cell count decreased (n = 3), neutrophil count decreased (n = 6), lymphocyte count decreased (n = 1), anemia (n = 1), platelet count decreased (n = 2), febrile neutropenia (n = 1)/select adverse events (those with a potential immunologic cause) (n = 0); number of patient was 6.		0	6	No discontinuation. NR

(Continued)

TABLE 3 | Continued

Antiangiogenic agents	ICIs	Study	Median treatment duration	Patients (n)	Grade 3 (n) trAE/irAE	Grade 4 (n) trAE/ irAE	Grade 5 (n) trAE/irAE	Total toxicity (≥3)	Management
		Normann et al.	Bev: 16 weeks Nivo: 12 weeks	5	Hepatitis (n = 1) There was a tendency toward increased toxicity when using concomitant bevacizumab [2 (40%) of 5 vs. 1 (11%) of 9].	0	Intestinal perforation (n = 1); Believed to cause by bevacizumab	3	Grade 2 events continued treatment after administration of steroids (dosage NR) Discontinuation because of nivolumab: n = 2 (14%)
		Shirali, et al.	10 months	1	Acute interstitial nephritis (n = 1)	NR	NR	1	Hospitalization: methylprednisolone 125 mg i.v. for 3 days, followed by prednisone 60 mg/d p.o., which was tapered over the next month.
	Atezolizumab	Wallin et al.	Atez: 15.9 months	10	Hypertension (n = 3), acute respiratory failure (n = 1), hypercalcemia (n = 1), abdominal pain (n = 1)/n = 0		0	6	NR
		McDermott et al.	Bev: 10.3 months Atezb: 11.8 months	101	Fatigue (n = 2), diarrhea (n = 4), nausea (n = 1), palmar–plantar erythrodysaesthesia syndrome (PPE) (n = 2), decreased appetite (n = 2), stomatitis (n = 2), headache (n = 1), arthralgia (n = 1), proteinuria (n = 8)/elevated liver enzymes or hepatitis (n = 4). TrAEs significantly increased with addition of bevacizumab (40 vs. 17%), but frequencies of irAEs were similar (5 [5%] of 101 vs. 3[3%] of 103).		Intracrinal hemorrhage (n = 1)	41	Discontinuation: n = 9 (9%) Dose modification or interruption: n = 61 (60%)
		Rini et al.	12 months	451	Hypertension (n = 63), fatigue (n = 6), hypothyroidism (n = 1), diarrhea (n = 7), proteinuria (n = 15), rush (n = 3), arthralgia (n = 10), decreased appetite (n = 2), nausea (n = 1), stomatitis (n = 2), mucosal inflammation (n = 1), anemia (n = 1), thrombocytopenia (n = 3), neutropenia (n = 2)/rush (n = 3), hypothyroidism (n = 1), hyperthyroidism (n = 1), LFT abnormalities (n = 13), colitis (n = 4), pneumonitis (n = 4). Frequency of trAEs was lower than that of sunitinib [182 (40%) of 451 vs. 240 (54%) of 446].		Cerebral infarction (n = 1, with known hypercholesterolaemia), intracranial hemorrhage (n = 1, following a fall), adrenal insufficiency (n = 1, with a history of coronary artery disease and myocardial infarction), multiple organ dysfunction syndrome (n = 1, following a post-radiation ulcer with cecum perforation), sepsis (n = 1, following pneumonia)	187	Discontinuation:treatment regimen n = 24 (5%), any treatment component n = 53 (12%) Systemic corticosteroids: n = 74 (16%) High-dose systemic corticosteroids (prednisone ≥40 mg/d or equivalent): n = 42 (9%)

(Continued)

TABLE 3 | Continued

Antiangiogenic agents	ICIs	Study	Median treatment duration	Patients (n)	Grade 3 (n) trAE/irAE	Grade 4 (n) trAE/ irAE	Grade 5 (n) trAE/irAE	Total toxicity (≥3)	Management
		Reck et al.	Bev: 6.7 months Atez: 8.2 months	394	Peripheral neuropathy (n = 11), nausea (n = 15), fatigue (n = 13), anemia (n = 24), decreased appetite (n = 10), diarrhea (n = 11), neutropenia (n = 54), hypertension (n = 25), arthralgia (n = 3), asthenia (n = 5), epistaxis (n = 4), vomiting (n = 6), decreased platelet count (n = 20), myalgia (n = 2), thrombocytopenia (n = 16), proteinuria (n = 10), decreased neutrophil count (n = 34), rush (n = 5), stomatitis (n = 4), febrile neutropenia (n = 33), decreased white blood cell count (n = 13), decreased weight (n = 4), alt increased (n = 4), dehydration (n = 8), AST increased (n = 4), leukopenia (n = 7), hypokalemia (n = 7), pulmonary embolism (n = 7), hyponatremia (n = 8), pneumonia (n = 7), pneumonitis (n = 4), colitis (n = 5), transaminases increased (n = 4), cerebrovascular accident (n = 1), sepsis (n = 1)/rash (n = 9), hepatitis (laboratory abnormalities) (n = 16), hypothyroidism (n = 1), hyperthyroidism (n = 1), pneumonitis (n = 6), colitis (n = 5), hepatitis (diagnosis) (n = 4), adrenal insufficiency (n = 1), pancreatitis (n = 2), hypophysitis (n = 1), nephritis (n = 1), ocular inflammatory toxicity (n = 1), myositis (n = 1), encephalitis (n = 1), meningoencephalitis (n = 1); information was from an article reporting the same trial (Socinski et al., 2018). TrAEs elevated with addition of bevacizumab or atezolizumab (56.7 vs. 43%, 56.7 vs. 48.5%). But the addition of bevacizumab did not significantly increased irAEs (12.5 vs. 9.5%).		Febrile neutropenia (n = 3), hemoptysis (n = 3), pulmonary hemorrhage (n = 2), cerebrovascular accident (n = 1), aortic dissection (n = 1), intestinal obstruction (n = 1). Information was from an article reporting the same trial (Socinski et al., 2018). Treatment-related death elevated with addition of bevacizumab (2.8 vs. 1%), but the addition of atezolizumab did not significantly increased it (2.8 vs. 2.3%).	234	Discontinuation or interruption No dose reduction for atezolizumab or bevacizumab Steroids, dosage NR
Ramucirumab				26	7		0	7	
	Pembrolizumab	Arkenau et al.	Ramu: 9 weeks Pemb: 9.3 weeks	26	Hypertension (n = 5), alanine aminotransferase increased (n = 1), aspartate aminotransferase increased (n = 1)	0	0	7	Discontinuation: n = 1 (3.8%)
Apatinib				1	1		0	1	
	Nivolumab	Zhao et al.	About 7 months	1	Elevated aminotransferases (n = 1)	NR	NR	1	Discontinued and received liver-protecting drugs with magnesium isoglycyrrhizinate injection and transmetil for 3 weeks.
Axitinib				1003	594		8	602	

(Continued)

TABLE 3 | Continued

Antiangiogenic agents	ICIs	Study	Median treatment duration	Patients (n)	Grade 3 (n) trAE/irAE	Grade 4 (n) trAE/ irAE	Grade 5 (n) trAE/irAE	Total toxicity (≥3)	Management
	Pembrolizumab	Atkins et al.	14.5 months	52	Fatigue (n = 5), diarrhea (n = 5), hypertension (n = 12), increased alanine aminotransferase concentration (n = 4), decreased appetite (n = 1), nausea (n = 1), palmar–plantar erythrodysaesthesia (n = 2), increased aspartate aminotransferase concentration (n = 2), weight decreased (n = 2), proteinuria (n = 1), oral pain (n = 1), headache (n = 2), vomiting (n = 1), dizziness (n = 1)/diarrhea (n = 4), increased alanine aminotransferase concentration (n = 2), increased aspartate aminotransferase concentration (n = 2), fatigue (n = 2), weight decreased (n = 1), colitis (n = 1), lymphocyte count decreased (n = 1)	Hyperuricemia (n = 1)/ hyperuricemia (n = 1)	0	34	Axitinib dose modification + symptomatic treatment: axitinib starting dose: 5 mg bid; dose level-1: 3 mg bid; dose level-2: 2 mg bid; permanently discontinued. For pembrolizumab: hold treatment until toxicity was <grade 2; discontinue if toxicity does not resolves within 12 weeks of last dose or inability to reduce corticosteroids to 10 mg or less of prednisone or equivalent per day within 12 weeks; permanently discontinue
		Rini et al.	Pemb+axi: 8.3 months Pemb: 9.2 months Axi: 9.6 months	429	Diarrhea (n = 31), hypertension (n = 91), hypothyroidism (n = 1), fatigue (n = 10), palmar–plantar erythrodysesthesia (n = 22), alanine aminotransferase increased (n = 52), dysphonia (n = 1). Aspartate aminotransferase increased (n = 29), decreased appetite (n = 9), nausea (n = 2), proteinuria (n = 11), stomatitis (n = 3), mucosal inflammation (n = 4), pruritus (n = 1), arthralgia (n = 3), hyperthyroidism (n = 4), asthenia (n = 6), rash (n = 1), dysgeusia (n = 1), vomiting (n = 1), platelet count decrease (n = 1), anemia (n = 1), neutrophil (n = 1), neutrophil count decreased (n = 1)/hypothyroidism (n = 1), hyperthyroidism (n = 5), adrenal insufficiency (n = 3), hepatitis (n = 10, pneumonitis (n = 2), thyroiditis (n = 1), colitis (n = 8), severe skin reactions (n = 5), infusion reactions (n = 1), nephritis (n = 1), hypophysitis (n = 4), myasthenic syndrome (n = 2), myositis (n = 1), myocarditis (n = 2), pancreatitis (n = 2), type 1 diabetes mellitus (n = 1)		Myasthenia gravis (n = 1), myocarditis (n = 1), necrotizing fasciitis (n = 1), pneumonitis (n = 1)/ myasthenia gravis (n = 1), myocarditis (n = 1), pneumonitis (n = 1) Incidence of treatment-related death was lower than that of sunitinib [4(0.9%) vs. 7 (1.6%)].	270	Interruption: n = 267 (62.2%) Discontinuation of both pembrolizumab and axitinib: n = 35 (8.2%) Dose reduction of axitinib: n = 86 (20%) Steroids, dosage NR
		Wilky et al.	NR	33	Oral mucositis (n = 1), nausea or vomiting (n = 2), diarrhea (n = 1), abdominal pain or dyspepsia (n = 1), hypertension (n = 5), hemoptysis (n = 1), pneumothorax (n = 1), seizures (n = 2)/hyperglycemia (n = 1), autoimmune hepatitis (n = 1), autoimmune colitis (n = 1), autoimmune arthritis (n = 2)	Elevated ALT, AST, or AP (n = 1), hypertriglyceridemia or hyperlipidemia (n = 1)	0	16	Axitinib dose modification + symptomatic treatment: axitinib starting dose: 5 mg bid. If grade 2 or greater toxicity, dose level-1: 4 mg bid; dose level-2: 3 mg bid; dose level-3: 2 mg bid; permanently discontinued. Steroids and discontinuation of study treatment: n = 3 (9%). One patient with autoimmune arthritis was also given methotrexate and hydroxychloroquine.

(Continued)

TABLE 3 | Continued

Antiangiogenic agents	ICIs	Study	Median treatment duration	Patients (n)	Grade 3 (n) trAE/irAE	Grade 4 (n) trAE/ irAE	Grade 5 (n) trAE/irAE	Total toxicity (≥3)	Management
	Avelumab	Choueiri et al.	Axi: 66.6 weeks Ave: 66.0 weeks	55	Diarrhea (n = 2), hypertension (n = 16), fatigue (n = 2), PPE syndrome (n = 4), ALT increased (n = 4), rush (n = 1), AST increased (n = 1), amylase increased (n = 3), decreased appetite (n = 1), mucosal inflammation (n = 1), infusion-related reaction (n = 1), lipase increased (n = 1), nausea (n = 1), arthralgia (n = 1), weight decreased (n = 1), proteinuria (n = 2), hypophosphatemia (n = 2), blood triglycerides increased (n = 1), dehydration (n = 1), pain in extremity (n = 1), drug eruption (n = 1), dyslipidemia (n = 1), urticaria (n = 1), venous thrombosis (n = 1)/ rash (n = 2), hepatitis (n = 2), colitis (n = 1)	Amylase increased (n = 1), lipase increased (n = 3), hematoma (n = 1), pulmonary embolism (n = 1)	Myocarditis (n = 1)	33	Dose interruption of avelumab: n = 1 (1.8%) Discontinuation of avelumab: n = 7 (13%) Discontinuation of axitinib: n = 4 (7%) Dose reductions of axitinib: n = 28 (51%) Steroids, dosage NR
		Motzer et al.	Axi: 9.0 months Ave: 8.6 months	434	Diarrhea (n = 22), hypertension (n = 106), fatigue (n = 13), PPE syndrome (n = 25), dysphonia (n = 2), nausea (n = 3), hypothyroidism (n = 1), stomatitis (n = 8), decreased appetite (n = 7), chills (n = 1), mucosal inflammation (n = 5), alanine aminotransferase increased (n = 21), rash (n = 2), dyspnea (n = 6), arthralgia (n = 1), infusion-related reaction (n = 7), aspartate aminotransferase increased (n = 12), weight decreased (n = 7), vomiting (n = 1), asthenia (n = 5), thrombocytopenia (n = 1), anemia (n = 1), neutropenia (n = 1)/n = 39, events NR		Sudden death (n = 1), myocarditis (n = 1), necrotizing pancreatitis (n = 1)/n = 0	249	Discontinuation of both avelumab and axitinib: n = 33 (7.6%) Dose reduction of axitinib: n = 183 (42.2%) High-dose glucocorticoids (≥40 mg total daily dose of prednisone or equivalent): n = 48 (11.1%)
Cabozantinib				1	0		0	0	
	Nivolumab	Bhat et al.	NR	1	NR	NR	0	0	NR
Cediranib				14	7		0	7	
	Durvalumab	Lee et al.	>15 months	14	(1) Once-daily cediranib: lymphopenia (n = 1), anemia (n = 2), nausea (n = 1), diarrhea (n = 3), colitis (n = 1), fatigue (n = 1), headache (n = 1), hypertension (n = 3), pulmonary thromboembolism (n = 1), pulmonary hypertension (n = 1). Number of patient was 7; (2) intermittent cediranib:fatigue (n = 1)	(1) Once-daily cediranib: lymphopenia (n = 1), pulmonary thromboembolism (n = 1); (2) intermittent cediranib: hypertension (n = 1)	NR	7	Discontinued or dose reduced of daily cediranib: n = 7 (87.5%) Systemic corticosteroids, dosage NR

(Continued)

TABLE 3 | Continued

Antiangiogenic agents	ICIs	Study	Median treatment duration	Patients (n)	Grade 3 (n) trAE/irAE	Grade 4 (n) trAE/ irAE	Grade 5 (n) trAE/irAE	Total toxicity (≥3)	Management
Lenvatinib				58	40		1	41	
	Pembrolizumab	Makker et al.	NR	53	Fatigue (n = 3), diarrhea (n = 4), palmar–plantar erythrodysesthesia syndrome (n = 3), hypertension (n = 18), proteinuria (n = 1), oral pain (n = 1), dehydration (n = 1), increased aspartate aminotransferase (n = 1), anemia (n = 1), hyponatremia (n = 2), increased lipase (n = 1), increased alanine aminotransferase (n = 1), prolonged electrocardiogram qt interval (n = 1), hypocalcaemia (n = 1), acute kidney injury (n = 2), pulmonary embolism (n = 2), syncope (n = 2), adrenal insufficiency (n = 1), cardiac failure (n = 1), colitis (n = 1), dysarthria (n = 1), hypertensive encephalopathy (n = 1), ischemic colitis (n = 1), neutropenia, pancreatitis (n = 1), retinal vein occlusion (n = 1), small intestinal obstruction (n = 1), upper abdominal pain (n = 1)/n = 30, irAEs (including skin, endocrine, gastrointestinal, pulmonary, hepatic, and renal adverse events), but grade NR	0	Intracranial hemorrhage (n = 1)	37	Discontinued: n = 5 (9%) High-dose glucocorticoids (≥40 mg/d of prednisone or equivalent): n = 3 (10%)
		Iyer et al.	5.6 months	5	Fatigue (n = 1), hypokalemia (n = 1), weakness (n = 1), altered mental status (n = 1), hypophosphatemia (n = 1)/2 patients had mild irAEs, including a grade 2 hepatic	0	0	4	Grade 2 colitis: n = 1(20%), budesonide, dosage NR, and continued pembrolizumab Grade 2 hepatitis: n = 1(20%), high dose of prednisone, and discontinued pembrolizumab.
Pazopanib				40	18		0	18	
	Pembrolizumab	Qiao et al.	About 15 months	1	NR	NR	NR	0	NR
	Nivolumab	Amin et al.	Pazo: 13.9 months Nivo: 15.1 months	20	Fatigue (n = 3), diarrhea (n = 4), hypertension (n = 2), increased alt (n = 4), increased AST (n = 4), hypothyroidism (n = 1), arthralgia (n = 1)/endocrine (n = 2), gastrointestinal (n = 4), hepatic (n = 4)		0	14	Discontinuation: n = 5 (25%) Systemic corticosteroid: n = 12(60%), including prednisone [n = 11 (55%)], dexamethasone [n = 2 (10%)], and methylprednisolone [n = 2 (10%)].

(Continued)

TABLE 3 | Continued

Antiangiogenic agents	ICIs	Study	Median treatment duration	Patients (n)	Grade 3 (n) trAE/irAE	Grade 4 (n) trAE/ irAE	Grade 5 (n) trAE/irAE	Total toxicity (≥3)	Management
		Paoluzzi et al.	Nivo: 8 cycles (16 weeks)	18	AST elevation (n = 1), ALT elevation (n = 3), alkaline bilirubin elevation (n = 2), pneumonitis (n = 1), colitis (n = 1). The total number of patient who suffered grade 3/4 trAEs was 4. The frequencies of trAEs significantly increased with addition of pazopanib [4 (22%) of 18 vs. 0 (0%) of 10].	Bilirubin elevation (n = 1), AST elevation (n = 1)	0	4	Discontinuation of both nivolumab and pazopanib: n = 4 (22%), among which, two patients restarted on treatment with both drugs, while one patient restart pazopanib only High-dose steroids (prednisone 1 mg/kg/daily), with a slow taper over about 2 months: n = 3 One patients needed intubation.
		Yu-Li Su et al.	4 months	1	NR	NR	NR	0	NR
Sorafenib				7	0		0	0	
	Pembrolizumab	Chen et al.	NR	1	NR	NR	NR	0	To avoid tumor rupture, the schedule of pembrolizumab was changed to every 4 weeks starting in cycle three.
	Nivolumab	Feng et al.	Nivo: 7.1 cycles	6	NR	NR	NR	0	NR
Sunitinib				34	27		0	27	
	Nivolumab	Amin et al.	Suni: 28 months Nivo: 45.1 months	33	Fatigue (n = 3), diarrhea (n = 3), nausea (n = 1), hypertension (n = 6), decreased appetite (n = 1), increased alt (n = 6), increased AST (n = 3), blood creatinine increased (n = 2), vomiting (n = 1)/ skin (n = 2), gastrointestinal (n = 3), hepatic (n = 8), renal (n = 4), pulmonary (n = 1)		0	27	Discontinuation of both nivolumab and sunitinib: n = 13(39.4%) Systemic corticosteroid n = 13 (39.4%) (prednisone, dexamethasone, and methylprednisolone)
		Mahmoud et al.	Suni: ≥11 months Nivo: ≥8 months	1	NR	NR	0	0	NR

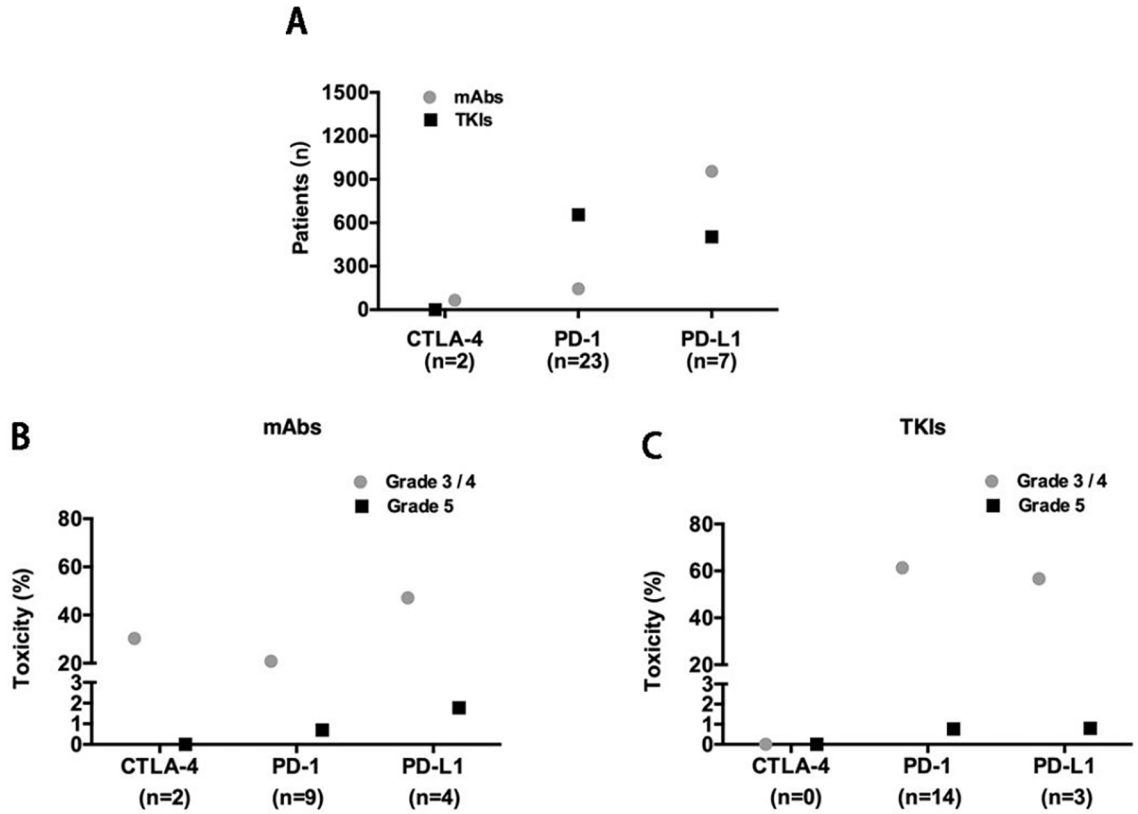

FIGURE 2 | (A) Included studies and patients; X-axis: n, number of included studies. Severe trAE evaluation of concurrent use of antiangiogenic mAbs **(B)** or TKIs **(C)** with each class of ICIs.

bevacizumab in recurrent central nervous system (CNS) tumor was well tolerated, with no significant toxicity (Blumenthal et al., 2016; Kurz et al., 2018). The only one case report by Wu et al., 2017 observed no grade 3–5 trAEs in one patient with urothelial carcinoma (UC) receiving such a concurrent regimen (Wu et al., 2017). There was only one phase 1 trial, treating a total of 26 patients with concurrent pembrolizumab and ramucirumab for advanced or metastatic biliary tract cancer (BTC). A total of seven (27%) grade 3 trAEs of 26 patients were observed, with hypertension accounting for 71% (**Table 3**) (Arkenau et al., 2018).

As for nivolumab, two retrospective studies on recurrent high-grade gliomas (HGGs) (Kurz et al., 2018; Mantica et al., 2018), one phase 1b trial and one case report on non-squamous NSCLC (Kanda et al., 2016; Shirali et al., 2016) and one prospective trial on platinum resistant ovarian cancer (Normann et al., 2019) were identified (**Table 1**). In the two retrospective studies (n = 71 patients), a total of four (5.6%) patients experienced grade 3/4 trAEs, among which there were three cases of irAEs including colitis and pneumonitis (**Table 3**) (Kurz et al., 2018; Mantica et al., 2018). The phase 1b trial of concurrent nivolumab, bevacizumab, and chemotherapy for NSCLC patients observed a total of 14 grade 3/4 trAEs of 6 patients. However, all of them were hematological AEs, and no grade 3/4 irAEs were reported (Kanda et al., 2016). The prospective trial by Normann et al. observed a grade 3 hepatitis, and one death (grade 5) of intestinal perforation which was believed to be caused by bevacizumab in recurrent ovarian cancer patients. They also found that there was a tendency to increase toxicity when using concomitant nivolumab and bevacizumab [2 (40%) of 5 vs. 1 (11%) of 9] (**Table 3**) (Normann et al., 2019). Besides, Shirali, et al. reported one event of grade 3 acute interstitial nephritis in a progressive NSCLC patient treated with concurrent nivolumab and bevacizumab (Shirali et al., 2016).

In summary, although the available data was limited, it suggested that concurrent use of pembrolizumab/nivolumab and bevacizumab is relatively safe. The data on the combination of pembrolizumab/nivolumab and ramucirumab is insufficient for conclusions.

Anti-PD-L1 (Atezolizumab)

Bevacizumab was combined with atezolizumab in four prospective studies (Wallin et al., 2016; McDermott et al., 2018; Reck et al., 2019; Rini et al., 2019b). Three were associated with unresectable or metastatic RCC, and one was about chemotherapy-naïve metastatic non-squamous NSCLC (**Table 1**). The median follow-up ranged from 15 to 20.7 months. In total, 468 severe trAEs (≥grade 3), including 17 treatment-related deaths (grade 5), were reported of 956 patients (**Table 3**).

But our search did not find studies on concurrent atezolizumab and ramucirumab.

In patients with RCC, grade 3/4 trAEs were observed in 228 (40.6%) of 562 patients, and also 6 (1.1%) grade 5 trAEs were reported, consisting of 2 intracranial hemorrhage, 1 cerebral infarction, 1 adrenal insufficiency, 1 multiple organ dysfunction syndrome, and 1 sepsis (**Table 3**). An early clinical trial reported six grade 3/4 trAEs, but none of them were deemed related to atezolizumab (Wallin et al., 2016). McDermott et al. found that concurrent atezolizumab and bevacizumab led to a significantly increase of the incidence of grade 3–5 trAEs (40 *vs*. 17%), but the incidence of irAEs was similar (5 *vs*. 3%) (McDermott et al., 2018). The phase 3 trial of Rini et al. observed that patients given atezolizumab plus bevacizumab had lower frequency of grade 3/4 trAEs than that of sunitinib (40 *vs*. 54%) (**Table 3**) (Rini et al., 2019b). Regarding NSCLC, the only one phase 3 trial suggested that when adding atezolizumab to bevacizumab and chemotherapy, grade 3/4 trAEs and treatment-related death (grade 5) slightly elevated, while the increase degree was higher when adding bevacizumab to atezolizumab and chemotherapy. However, the addition of bevacizumab did not significantly increase the incidence of irAEs (12.5 *vs*. 9.5%) (**Table 3**) (Reck et al., 2019). Information about the AEs was from an article that reporting the same trial (Socinski et al., 2018).

In summary, concurrent atezolizumab and bevacizumab might increase trAEs, but not irAEs. In addition, no unexpected patterns of toxicity emerged in the combination therapy. Data about the combination of atezolizumab and ramucirumab is not available.

Toxicity of Concurrent ICIs and Antiangiogenic TKIs (Apatinib, Axitinib, Cabozantinib, Cediranib, Lenvatinib, Pazopanib, Sorafenib, Sunitinib)

Anti-PD-1 (Pembrolizumab, Nivolumab)

The concomitant use of pembrolizumab and antiangiogenic TKIs was examined in seven studies (Chen et al., 2017; Atkins et al., 2018; Iyer et al., 2018; Qiao et al., 2018; Makker et al., 2019; Rini et al., 2019a; Wilky et al., 2019), which were highly diverse in research and tumor types (**Table 2**). In a phase 1b study, where a total of 52 RCC patients received concurrent pembrolizumab and axitinib, grade 3/4 trAEs were observed in 34 patients. The most common trAEs, such as diarrhea (8%) and elevations in liver-enzyme levels (8%), seemed to be largely related to axitinib rather than a true irAE predominantly due to pembrolizumab (Atkins et al., 2018). Similarly, the phase 3 trial by Rini et al. reported 270 (51%) grade 3 or higher toxicities of 429 patients (**Table 3**), which were as expected on the basis of the known profiles of each drug. Although there were four (0.9%) patients died from trAEs, none of them related to hepatic adverse that might be more challenging due to the overlapping toxicities of axitinib and pembrolizumab. Moreover, combined group had fewer treatment-related death than sunitinib [4 (0.9%) *vs*. 7 (1.6%)] (Rini et al., 2019a). We also found a phase 2 trial of this combined regimen for soft-part sarcoma. A total of 16 grade 3/4 trAEs occurred in 33 patients, and grade 3/4 irAEs in 5 (15%) patients (Wilky et al., 2019). Two studies examining concurrent pembrolizumab and lenvatinib were identified. In a phase 2 trial of metastatic endometrial cancer, Makker et al. observed 36 (68%) patients with grade 3 trAEs and a grade 5 intracranial hemorrhage. Among them, there were 30 irAEs in total, but the grade was not described in detail (**Table 3**) (Makker et al., 2019). One retrospective study for progressive anaplastic thyroid carcinoma (ATC) reported four grade 3 trAEs of five patients and some mild irAEs (such as grade 2 hepatitis) (Iyer et al., 2018). Two case reports about concurrent pembrolizumab and pazopanib for primary hepatic angiosarcoma (PHA) (Qiao et al., 2018) and pembrolizumab plus sorafenib for HCC (Chen et al., 2017) did not observe any significant toxicity.

Regarding nivolumab, there were three studies examining concurrent nivolumab and pazopanib (Paoluzzi et al., 2016; Amin et al., 2018; Yu-Li Su, 2018), two for combining with sunitinib (Mahmoud et al., 2016; Amin et al., 2018), and one for combining with apatinib (Zhao et al., 2019), cabozantinib (Bhat et al., 2019) or sorafenib (Feng et al., 2017), respectively (**Table 2**). Zhao et al. observed grade 3 elevated aminotransferases in a patient with advanced liver carcinosarcoma treated with nivolumab plus apatinib (Zhao et al., 2019), while Bhat et al. did not observe severe trAEs in a patients treated with nivolumab plus cabozantinib (Bhat et al., 2019). In the phase 1 trial of Amin et al. 27 (81.8%) and 14 (70.0%) patients in arms nivolumab plus sunitinib and nivolumab plus pazopanib, respectively, experienced grade 3/4 trAEs, and 18 (55%) and 10 (50%) patients, respectively, experienced grade 3/4 irAEs (**Table 3**). The rate of arm nivolumab plus pazopanib was higher than that of arm nivolumab or pazopanib monotherapy in previous reports (Amin et al., 2018. Paoluzzi et al. reported 10 grade 3/4 trAEs in 4 (22.2%) patients receiving concomitant nivolumab and pazopanib, but no grade 3/4 trAEs occurred in nivolumab monotherapy group (Paoluzzi et al., 2016). Yu-Li Su et al. observed no toxicity after treatment of concurrent nivolumab and pazopanib in a patient with metastatic RCC (Yu-Li Su, 2018). Feng et al. analyzed nivolumab combined with sorafenib for advanced HCC in six patients and observed no severe toxicity (Feng et al., 2017), while Mahmoud et al. did not observe severe trAEs in a patient treated with nivolumab plus sunitinib (Mahmoud et al., 2016).

In summary, data on the toxicity of concurrent anti-PD-1 antibody and TKIs was conflicting. Some severe trAEs of the combination seemed to be largely related to TKIs, rather than a true irAE predominantly due to anti-PD-1 monotherapy. However, most studies were early phase clinical trials or case report, not randomized controlled studies with a large population, so the data is insufficient for conclusions.

Anti-PD-L1 (Avelumab, Durvalumab)

Two prospective studies (Choueiri et al., 2018; Motzer et al., 2019) evaluated concurrent use of avelumab and axitinib on advanced RCC. A total of 282 (57.7%) in 489 patients experienced grade 3–5 trAEs, of which the most frequent were diarrhea, hypertension, fatigue, palmar–plantar erythrodysesthesia syndrome, and changes of liver enzymes (**Table 3**). In addition, in the phase 1b trial of Choueiri et al.

where 55 patients received avelumab plus axitinib, one patient developed a fatal treatment-related autoimmune myocarditis (Choueiri et al., 2018). In the prospective phase 3 trial of Motzer et al., three (0.7%) treatment-related deaths were attributed to sudden death, myocarditis, and necrotizing pancreatitis, respectively (Motzer et al., 2019) (**Table 3**). However, the trAEs observed with combination therapy were generally consistent with the known safety profiles of monotherapy. No new toxicities were reported.

Currently, only one phase 1 trial, treating a total of 14 patients with concurrent durvalumab and cediranib for several recurrent or metastatic solid tumors, was found (**Table 2**). Lee et al. observed 19 grade 3/4 trAEs occurred in 7 patients. In durvalumab plus intermittent cediranib, the severe AEs were only one grade 3 fatigue and one grade 4 hypertension. In contrast, daily cediranib with durvalumab was not well tolerated (**Table 3**) (Lee et al., 2017).

In summary, the very small number of patients treated with avelumab plus axitinib or durvalumab plus cediranib and lack of compared monotherapy group make it difficult to draw conclusions about their safety.

Management of irAEs

In the included literatures, holding the ICI treatment was the first thing for managing grade 3/4 irAEs, and most of studies did not reduce the dose of ICIs, with an exception for one. In the case, a patient with HCC had a low-grade fever relating to remarkable tumor necrosis. Thus, to avoid tumor rupture, the schedule of pembrolizumab was changed to every 4 weeks (Chen et al., 2017). Besides, high-dose corticosteroids (including prednisone, methylprednisolone, and dexamethasone) were the first line for treating irAEs, and often effective in alleviating symptoms (**Table 3**). As some severe trAEs that were largely related to the addition of antiangiogenic agents, reducing or holding dose, as well as adjusting administration frequency of the antiangiogenic drugs, were the other common ways to deal with treatment-related toxicity (**Table 3**) (Carter et al., 2016; Lee et al., 2017; Amin et al., 2018; Atkins et al., 2018; Choueiri et al., 2018; Motzer et al., 2019; Rini et al., 2019a; Wilky et al., 2019). In addition, the rest of trAEs were managed with symptomatic treatment such as drugs or surgery (**Table 3**).

DISCUSSION

In this review, we demonstrated the risk of added toxicity of concurrent ICIs and antiangiogenic agents, but there are not abundant of data from multi-institutional randomized controlled trials (RCTs) to draw an exact conclusion. From the available data, bevacizumab and axitinib were the most commonly used antiangiogenic agents for concomitant treatment. For other antiangiogenic drugs, available safety information is primarily based on small, retrospective single institution experiences, and even case report. In terms of tumor types, the three most numerous studies on concurrent ICIs and antiangiogenic agents were RCC, non-squamous NSCLC, and CNS tumors (including glioblastoma) (**Tables 1** and **2**). However, the combination of the two types of therapies is indeed a research hotspot at present, with a huge amount of ongoing trials (**Table 4**).

Usually, immune checkpoint blockade treatment is associated with multitude and atypical types of tumor responses and has specific toxicity profiles which are termed irAEs (Wolchok et al., 2009; Gordon et al., 2017). In general, within the first 3–4 months of treatment, 80% patients may experience irAEs (Chen et al., 2015; Michot et al., 2016). Because of the different functions of CTLA-4 and PD-1/PD-L1, the types and frequency of irAEs related to various checkpoint inhibitors were different (Michot et al., 2016). Anti-CTLA-4 antibodies mostly affect the skin (44%) and the gastrointestinal tract (35%), whereas the endocrine (6%) and hepatic (5%) systems are rarely affected (Boutros et al., 2016; Cousin and Italiano, 2016; Eggermont et al., 2016). The side effects of anti-PD-1/PD-L1 antibodies are less frequent and less severe than those of anti-CTLA-4 antibodies (Champiat et al., 2016; Puzanov et al., 2017). The main AEs of PD-1 and PD-L1 blocking agents are pneumonia, myalgia, hypothyroidism, arthralgia, and vitiligo (Boutros et al., 2016; Cousin and Italiano, 2016). In this review, the frequencies, types, and severities of irAEs that mentioned in most of studies were consistent with previous data for ICI treatment alone, and trAEs of combination regimen were largely consistent with the known safety profiles of each monotherapy. Besides, the data of included literatures suggested that some severe trAEs of the concurrent treatment were largely related to the addition of antiangiogenic agents, rather than a true irAEs caused by ICIs (Hodi et al., 2014; Atkins et al., 2018; Socinski et al., 2018; Reck et al., 2019). In addition, frequency of severe trAEs in ICI plus TKI groups was a little higher than that of ICIs plus mAbs groups, which may be explained by the multiple targets of TKIs. The toxicities consist of not only AEs related to the blockade of VEGR/VEGFR pathway but also AEs caused by additional targets inhibition (Chen and Cleck, 2009; Qin et al., 2019). For example, sunitinib (targeting VEGFR-1/2, PDGFR-α/β, Flt-3, and c-kit) is known to cause both neutropenia and thrombocytopenia as a result of VEGF inhibition and simultaneous inhibition of c-kit (Demetri et al., 2006; Chen and Cleck, 2009). Similarly, anemia and decrease of both platelet and neutrophil counts were observed in an included study of concurrent avelumab and axitinib (targeting VEGFR-1–3, PDGFR, and c-kit) (Matias et al., 2017; Rini et al., 2019a). Therefore, the selection of optimal components for combination therapy is worthy of further research.

In general, most irAEs are mild and manageable, although a few patients treated with ICIs develop severe irAEs (grade 3/4), even immune-related death (grade 5). Recommendations on the management of irAEs have been published as the guidelines in Europe and the United States (Puzanov et al., 2017; Brahmer et al., 2018; Haanen et al., 2018). Firstly, successful management of irAEs requires standardize grading based on the common terminology criteria for adverse events (CTCAE 4.0) grading. As for intervention, patients with grade 1 irAEs can continue immunotherapy, except

TABLE 4 | Parts of ongoing phase 2/3 clinical trials of ICIs combined with antiangiogenic agents.

ICIs	Antiangiogenic agents	Primary tumor	Status and end points	ClinicalTrials.gov identifier
Nivolumab	Bevacizumab	Glioblastoma	Phase 2: recruiting (OS, ORR, DOR, and PFS)	NCT03452579
	Ramucirumab	Mesothelioma, malignant	Phase 2: recruiting (ORR, AEa, PFS, and OS)	NCT03502746
	Axitinib	Renal cell carcinoma	Phase 2: recruiting (AEs, ORR, DOR, PFS, OS, PD-L1 expression, and tumor infiltrating lymphocyte assessments, pharmacodynamic effect of study treatment including cytokines)	NCT03172754
	Cabozantinib	Renal cell carcinoma	Phase 3: recruiting (PFS, OS, ORR, AEs, SAEs)	NCT03141177
	Lenvatinib	Advanced hepatocellular carcinoma	Phase 2: recruiting (ORR, AEs, SAEs, TTP, PFS, OS, and translational research)	NCT03841201
	Regorafenib	Advanced and metastatic solid tumor	Phase 1/2: recruiting (RD, MTD, ORR, PFS, DCR, OS, and AEs)	NCT03406871
	Sunitinib	Soft tissue sarcoma, bone sarcoma	Phase 1/2: recruiting (PFSR, OS, ORR, immune response, tumor response, AEs, and clinical outcome)	NCT03277924
	Sorafenib	Hepatocellular carcinoma	Phase: recruiting (MTD, ORR, DOR, AEs, irAEs, OS, and PFS)	NCT03439891
(Nivolumab + ipilimumab)	Cabozantinib	Genitourinary tumors	Phase 2: recruiting (ORR, DOR, PFS, OS, CBR, AEs, and effects of treatment in patients with bone-only disease)	NCT03866382
	Nintedanib	Non-small-cell lung cancer metastatic	Phase 1/2: recruiting (MTD, ORR, DCR, OS, and PFS)	NCT03377023
SHR 1210 (anti-PD-1 mAb)	Apatinib	Gastric cancer and HCC	Phase 1/2: recruiting (OSR, tumor control rate, DCR, DOR, and AEs)	NCT02942329
Pembrolizumab	Bevacizumab	Colorectal cancer, metastatic cancer	Phase 2: recruiting (ORR, PFS, OS, and AEs)	NCT03475004
	Ramucirumab	Head and neck squamous cell carcinoma	Phase 1/2: recruiting (RP2D, ORR, AEs, DOR, PFS, OS, and changes in quality of life)	NCT03650764
	Apatinib	Advanced urothelial carcinoma, advanced MSI-H or dMMR solid tumors, advanced gastric or gastroesophageal junction (GEJ) adenocarcinoma	Phase 1/2: recruiting (DLTs, ORR, and PFS)	NCT03407976
(Pembrolizumab+ D-CIK)	Axitinib	Renal cancer metastatic	Phase 2: recruiting (ORR, PFS, OS, DOR, the quality of life, and AEs)	NCT03736330
	Anlotinib	Advanced solid tumor	Phase 2/3: recruiting (PFS, ORR, DCR, and OS)	NCT03975036
	Cabozantinib	Advanced metastatic melanoma	Phase 1/2: not yet recruiting (DLTs, ORR, DCR, PFS, and OS)	NCT03957551
	Lenvatinib	Thyroid gland carcinoma	Phase 2: recruiting (CR, AEs, PFS, OS, AEs, and biomarker levels)	NCT02973997
	Regorafenib	Metastatic colorectal cancer	Phase 1/2: not yet recruiting (DLTs, PFS, and OS)	NCT03657641
	Sunitinib	Thymic carcinoma	Phase 2: recruiting (ORR, AEs, OS, PFS, and PD-L1 expression)	NCT03463460
	Sorafenib	Hepatocellular carcinoma	Phase 1b/2: recruiting (ORR, OS. TTP, change in functional activity of effector T cells, and levels of immunosuppressive cell PFS)	NCT03211416
Atezolizumab (MPDL3280A)	Bevacizumab+chemotherapy	Ovarian cancer	Phase 3: recruiting (efficacy, TSST, OS, and AEs)	NCT02891824
	Ramucirumab	Non-small-cell lung cancer	Phase 2: recruiting (OS, CBR, and irAEs)	NCT03689855
	Cabozantinib	Hepatocellular carcinoma	Phase 3: recruiting (PFS and OS)	NCT03755791
Avelumab	Ramucirumab++paclitaxel	Gastroesophageal junction Adenocarcinoma/adenocarcinoma of the stomach	Phase 2: recruiting (OSR, OS, PFS, PFSR, DOR, ORR et al.)	NCT03966118
	Axitinib	Non-small-cell lung cancer; urothelial cancer	Phase 2: recruiting (ORR, TTR, tumor tissue biomarker status, ADA, DOR, PFS, Cmax of axitinib or avelumab, OS et al.)	NCT03472560
	Regorafenib	Metastatic solid tumors	Phase 1/2: recruiting (pharmacokinetics, RP2D, antitumor activity, MTD, DLT, toxicity, ORR, PFS, and blood biomarkers et al.)	NCT03475953
Durvalumab	Bevacizumab	Hepatocellular carcinoma	Phase 3: recruiting (RFS, OS, RFS24 h/36 h, TTR)	NCT03847428
	Pazopanib	Sarcoma	Phase 2: not yet recruiting (progression free rate: antitumor efficacy)	NCT03798106
MEDI4736 (anti-PD-L1 mAb)	Cediranib	Colorectal neoplasms; breast neoplasms	Phase 1/2: recruiting (RP2D and ORR)	NCT02484404

for some neurologic, hematologic, and cardiac toxicities. Holding ICI treatment should be considered for most grade 2 irAEs until symptoms and/or laboratory values reduce to grade 1 or less and then treat them with locally or orally small doses of corticosteroids (0.5–1 mg/kg/d of prednisone or equivalent). For grade 3 irAEs, discontinuation of the ICI therapy and giving moderate to high-dose corticosteroids (prednisone 1–2 mg/kg/d or methylprednisolone i.v. 1 to 2 mg/kg/d) are recommended. Resuming treatment should be caution depending on the risk/benefit ratio. Regarding to life-threatening events (grade 4), hospitalization and high-dose corticosteroids (methylprednisolone i.v. 1-2 mg/kg/d) or other immunosuppressive measures (infliximab 5 mg/kg) are necessary. And ICI treatment should be permanently discontinued (Champiat et al., 2016; Puzanov et al., 2017; Brahmer et al., 2018). In the included studies, most of immune-related toxicities of the concurrent treatment were managed *via* holding ICI treatment and adding corticosteroids. Reducing dose or adjusting the administration frequency of the antiangiogenic drugs was also used to alleviate some symptoms of trAEs (**Table 3**). However, the information concerning the new advances and management of irAEs are limited.

Recently, irAEs were considered as therapy-induced loss of tolerance, similar to autoimmune disorders (Boutros et al., 2016; Postow et al., 2018; Pauken et al., 2019). Thus, the known risk factors for autoimmunity may also predict the risk of irAEs. Hoefsmit et al. 2019 searched for susceptible loci associated with various autoimmune diseases and pooled them in groups most likely to be associated with ICIs-induced irAEs (Hoefsmit et al., 2019), which may help to screening out patients with pre-existing subclinical autoimmune disorders or susceptibility to autoimmune diseases and guide physicians in a more refined and personal manner. Besides, depending on the degree of similarity between irAEs and autoimmune disorders, we can find reference in therapies developed for autoimmunity to manage irAEs (Pauken et al., 2019). For example, anti-TNF-α antibodies are usually used to treat steroid-refractory inflammatory bowel disease and could also alleviate ICIs-induced colitis (Dougan, 2017). Also, experts in autoimmune disorders should be involved in the care of cancer patients receiving ICIs. In addition, studies have found that gut microbiome is not only associated with the efficacy of immunotherapy but also with some specific irAEs, such as colitis (Osman and Luke, 2019). Thus, the ability to predict which patient has a high risk of developing ICI-induced colitis is very valuable to clinicians who have to weigh the potential risks and benefits of ICI therapy. Regarding to the combination of ICIs and antiangiogenic agents, the problem also includes the dose, optimal duration of treatment, and sequencing of each therapy. As is well known, anti-VEGF therapies have the window of normalization (Winkler et al., 2004; Huang et al., 2012), with the dose and duration time of antiangiogenic agents being the key modulating factors (Huang et al., 2012; Chaudhary et al., 2014). High dose or long duration time of antiangiogenic therapy are associated with aggressive ablation of the vasculature, leading to higher degree of hypoxia and immunosuppression (Huang et al., 2012; Allen et al., 2017). Thus, reducing the dose of antiangiogenic agents has been taken into account in the design of some clinical trials with the combination of ICIs (Fukumura et al., 2018). Besides, as vascular normalization can enhance delivery and distribution of ICIs in the tumor tissues, the low dose of ICIs may help to reduce the incidence and severity of irAEs (Fukumura et al., 2018). In addition, identification of predictive or prognostic markers is also expected to help screening suitable patients in order to prevent unnecessary side effects of combination therapy. Previous study found that expression level of PD-L1 was a predictive marker of the response to immunotherapy and also a negative prognostic marker in RCC patients receiving VEGF-targeted therapy (Shin et al., 2015). Angiopoietin 2 (ANG2), a vessel-destabilizing ligand of TIE2 and a critical regulator of blood vessel maturation, is a potential biomarker of resistance to anti-VEGF therapy (Bauerschlag et al., 2013; Jain, 2014; Labussiere et al., 2016). At the same time, evidence showing high serum level of ANG2 was inversely correlated with treatment response and prognosis of ICI treatment in metastatic melanoma patients (De Palma and Jain, 2017). Therefore, it is not so sensible to provide such a combined strategy for this kind of patients.

The current review has limitations that the number of available studies, especially RCTs, is insufficient. Even for some drugs, the data is lacking. These may partly due to the fact that many studies assessing the combination treatment of ICIs and antiangiogenic agents are still ongoing for this emerging area of research. Besides, the information about the new advances and management of irAEs in the included studies are limited. In addition, the review mainly focuses on three well known immune checkpoints, CTLA-4, PD-1, and PD-L1. However, identification of better biomarkers or therapeutic agents aimed at improving the clinical response in refractory patients and reducing irAEs is also necessary, which has led to the development of "next-generation" ICIs, such as T cell immunoglobulin mucin 3 (TIM-3), lymphocyte activation gene 3 (LAG-3), T-cell immunoreceptor with immunoglobulin and ITIM domains (TIGIT), indoleamine-2,3-dioxygenase 1 (IDO1), and so on (Mazzarella et al., 2019). Hundreds of registered past and ongoing clinical trials investigate the mechanism and efficiency of "next-generation" ICIs either as monotherapy or combining with other ICIs (Mazzarella et al., 2019; Tundo et al., 2019). Therefore, updated information is still required in the future.

CONCLUSION

In summary, concurrent ICIs and antiangiogenic agents show potential treatment-related toxicity. Further research is required to compare the efficacy and safety of the combined regimen and the corresponding monotherapy. It is also necessary to explore dose, duration, and sequencing schedule of drugs, as well as identify predictive or prognostic biomarkers.

AUTHOR CONTRIBUTIONS

LG, XY and HZ conceived and designed the study. LG and XY screened, extracted the data, and wrote the manuscript. HZ and CY contributed to the revise of the manuscript.

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frontiers in Pharmacology

REVIEW
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Immune Checkpoint Inhibitor Toxicity in Head and Neck Cancer: From Identification to Management

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Benefiting from the continuously clarifying underlying biology of immune checkpoints and ligand–receptor interactions, the emergence of new anticancer treatment strategy, immunotherapy has shown substantial benefits on several liquid and solid tumors. Immune checkpoint inhibitors (ICIs) can block the negative regulatory components and enhance the T cell function, thus leading to prominent anticancer activity. On account of their promising effect on various malignancies shown in clinical trials, ICIs have been considered to be the most potent anticancer agents in the near future. Head and neck cancer is the seventh most common neoplasm worldwide, and the gross 5-year survival rate was only 60%. Managing locoregionally advanced, recurrent, or metastatic head and neck tumors is still a challenging problem for both oncologists and surgeons. Recent clinical trials employing the immune-modulating antibodies that target cytotoxic T-lymphocyte-associated antigen-4 (CTLA-4) and programmed cell death 1 (PD-1) herald a new era of anticancer therapy. However, like all other anticancer drugs, ICIs also have side effects while upregulating the immune system to enhance antitumor response, which were known as immune-related adverse events (irAEs). Generally, most irAEs were transient, but sometimes they can cause serious organ dysfunction, even fatal. In addition, due to the distinct anatomical feature, advanced head and neck tumors often affect the upper aerodigestive tract and cause serious dyspnea or dysphagia. Toxicities of ICIs may be more lethal for such patients. Thus, with the increasing application of anti-checkpoint agents in head and neck cancer, there is urgent need to ascertain the safety of this novel treatment strategy. Here, we compile this review of existing clinical trials on the toxicity of ICIs during cancer treatment. The particular clinical manifestation, characteristics of complication development in fatal cases, and the management strategies were discussed. This may provide vital information for future oncology trials and clinical practice.

Keywords: head and neck cancer, immunotherapy, immune checkpoint inhibitors, immune-related adverse events, programmed cell death 1, programmed death-ligand 1, cytotoxic T-lymphocyte-associated antigen-4

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INTRODUCTION

Head and neck cancer is the seventh most common neoplasm worldwide, and the gross 5-year survival rate was only 60% (Torre et al., 2015). Managing locoregionally advanced, recurrent, or metastatic head and neck tumors is still a challenging problem for both oncologists and surgeons. In recent years, the development of immune checkpoint inhibitors (ICIs) has demonstrated a

significant anticancer activity in different types of malignancies, including head and neck cancer. Treatment with ICIs improved the overall survival in patients with head and neck squamous cell carcinoma (HNSCC) and improved quality of life compared with the concurrent chemotherapy and radiation therapy (Porceddu and Haddad, 2017; Ribas and Wolchok, 2018).

Physiologically, immune checkpoint proteins are responsible for regulating immune tolerance and avoiding excessive immune injury. One of the main causes of the recurrence and metastasis of HNSCC is tumor-induced immune evasion, which is partially mediated by T cell-suppressive immune checkpoint (Ferris, 2015). ICIs facilitate endogenous anticancer activity by removing the inhibition signals and enhancing the activity of T cells. Currently, cytotoxic T-lymphocyte-associated antigen 4 (CTLA-4) and programmed cell death 1 (PD-1) are the ICIs that cause the most clinical interest. CTLA-4 is expressed in activated $CD8^+$ T cells and is involved in the regulation of the early state of T cell activation. Furthermore, CTLA-4 mainly provides significant negative signals to inhibit the activation of T cells and weaken the anti-tumor immune response. PD-1 can be expressed on both the T and B cell's surface. When PD-1 binds to its ligands PD-L1 or PD-L2, it releases inhibitory signals to T cells and decreases the downstream signal transmission through the PI3K pathway, resulting in the inhibited activation and proliferation of T cells (Stambrook et al., 2017; Szturz and Vermorken, 2017).

Nivolumab (an anti PD-1 agent) was the first ICI approved by the FDA for HNSCC therapy in 2016, based on results from the CheckMate141 and KEYNOTE-012 trial (Alfieri et al., 2018; Yang et al., 2018). Currently, the ICIs that have been tested on HNSCC include PD-1 (nivolumab and pembrolizumab), PD-L1 (atezolizumab, durvalumab, and avelumab), and CTLA-4 (ipilimumab and tremelimumab) (Alsaab et al., 2017; Szturz and Vermorken, 2017; Dogan et al., 2018; Gong et al., 2018).

Despite their therapeutic promise and benefits, treatments with ICIs are associated with the onset of immune-related adverse events (irAEs) on account of facilitating autoimmune activity not only against tumor cells but also any organs of the body. In fact, during HNSCC treatment, ICIs may lead to significant morbidity or, in rare cases, mortality. Identification of the side effects and prompt treatment are crucial for patients receiving these agents (Saba et al., 2017; Pauken et al., 2019). Based on all the existing eight clinical trials of ICI use in HNSCC, here, we conducted this review and summarized all the irAEs, the fatal complications, and the management strategies.

CLINICAL CHARACTERISTICS OF IRAES AND MANAGEMENT—GENERAL PRINCIPLES

After a comprehensive retrieval of online databases including Pubmed, ISI, and clinicaltrials.gov, there were eight clinical trials that use ICI agents to treat HNSCC. General information on these trials is shown in **Table 1**. According to the eight trails, irAEs seem to most commonly involve the skin, gastrointestinal tract, endocrine glands, pulmonary and musculoskeletal. The incidence of irAEs (of any grade) among patients taking ICI in HNSCC ranges from 57% to 67%, and the most common irAEs of all grades were pruritus/rash, diarrhea, and hypothyroidism. More serious irAEs occur less frequently; grades 3–4 occur in 8–17% of patients treated with ICI agents. A list of the current ICIs and their common associated irAEs in HNSCC is shown in **Table 2**.

In general, management of moderate to severe irAEs relies on the use of corticosteroids, other immunomodulatory agents, supportive care, and treatment interruption (Spain et al., 2016; Haanen et al., 2017; Puzanov et al., 2017; Brahmer et al., 2018; Kottschade, 2018; Williams et al., 2019). The Common Terminology Criteria for Adverse Events (CTCAE) is the standard assessment used in clinical trials to grade the severity of adverse events. Generally, grade 1–2 irAEs are mild to moderate, do not require hospitalization, and should be treated symptomatically. Grade 3–4 irAEs are severe to life-threatening conditions, which require hospitalization to observe patients closely. Systemic steroid administration and permanent discontinuation of ICI therapy may be required. Grade 5 refers to death related to adverse events.

TABLE 1 | Overview of the included clinical trials of ICIs in HNSCC.

NCT number	Study type	No. of patients	ICI dose	RR (%)	Median OS (months)
02105636 (Checkmate-141)	Randomized phase III	240	Nivolumab 3 mg/kg, every 2 weeks	13.3	7.5
01848834 (Keynote-012)	Phase Ib	192	Pembrolizumab 10 mg/kg, every 2 weeks OR Pembrolizumab 200 mg every 3 weeks	18	8
02252042 (Keynote-040)	Randomized phase III	247	Pembrolizumab 200 mg every 3 weeks	14.6	8.4
02255097 (Keynote-055)	Phase II	171	Pembrolizumab 200 mg every 3 weeks	16	8
01375842	Phase Ia	32	Atezolizumab 15/20 mg/kg every 3 weeks	21	6
01693562	Phase I/II	62	Durvalumab 10 mg/kg every 2 weeks	6.5	8.4
02207530	phase II	112	Durvalumab 10 mg/kg every 2 weeks	16.2	7.1
02319044	Randomized phase II	133	Durvalumab (20 mg/kg every 4 weeks) + tremelimumab (1 mg/kg every 4 weeks)	7.8	7.6
		67	Durvalumab (10 mg/kg every 2 weeks)	9.2	6
		67	Tremelimumab (10 mg/kg every 4 weeks	1.6	5.5

NCT, national clinical trial; ICIs, immune checkpoint inhibitors; RR, response rate; OS, overall survival; HNSCC, head and neck squamous cell carcinoma.

TABLE 2 | List of current ICIs and their associated common toxicities in HNSCC therapy.

Drug class	Drug name	No. of trials mentioned	Adverse events (%)
PD-1 inhibitors	Nivolumab	*n* = 1 (Ferris et al., 2018)	Dermatological (15.7%), Hypothyroidism (6.3%), Diarrhea (6.8%)
	Pembrolizumab	*n* = 4 (Chow et al., 2016; Seiwert et al., 2016; Bauml et al., 2017; Cohen et al., 2019)	Hypothyroidism (9–16%), Dermatological (8–19%), Diarrhea (6–8%)
PD-L1 inhibitors	Atezolizumab	*n* = 1 (Colevas et al., 2018)	Dermatological (16%), Diarrhea (9%)
	Durvalumab	*n* = 3 (Siu et al., 2018; Segal et al., 2019; Zandberg et al., 2019)	Diarrhea (5.4–10.8%), Dermatological (6.3–13%), Hypothyroidism (3.2–10.8%)
CTLA-4 inhibitors	Tremelimumab	*n* = 1 (Siu et al., 2018)	Diarrhea (15.4%), Dermatological (12.3%)
PD-L1 + CTLA-4 inhibitors	Durvalumab + Tremelimumab	*n* = 1 (Siu et al., 2018)	Diarrhea (14.3%), Hypothyroidism (8.3%)

PD-1, programmed death receptor-1; PD-L1, programmed death-ligand 1; CTLA-4, cytotoxic T-lymphocyte-associated protein 4; ICIs, immune checkpoint inhibitors; HNSCC, head and neck squamous cell carcinoma.

COMMON IRAES AND THE RELEVANT MANAGEMENT IN HNSCC

Dermatological Adverse Events

Dermatologic toxicities are one of the most reported irAEs associated with immune checkpoint inhibitors and occur in 8–19% of HNSCC patients treated with ICI agents (Chow et al., 2016; Seiwert et al., 2016; Bauml et al., 2017; Colevas et al., 2018; Ferris et al., 2018; Siu et al., 2018; Cohen et al., 2019; Segal et al., 2019; Zandberg et al., 2019). The majority of cases are usually low grade, ranging from pruritus and rash to dermatitis. However, serious skin reaction is less common (grades 3–4 are less than 2%). Cases of Stevens–Johnson syndrome are practically notable, and one case resulted in a treatment-related death, with pembrolizumab treatment (Cohen et al., 2019).

Skin biopsy is useful to rule out any other etiologies, such as an infection and drug interaction or other autoimmune blistering dermatoses. Grade 1–2 rashes (macules/papules covering less than 10% or 10–30% of body surface area) can be treated with topical emollients, oral anti-histamine, and topical steroids; consider initiating prednisone 0.5–1 mg/kg in grade 2 skin reaction, with tapering over at least for 4 weeks. For grade 3 skin reaction (> 30% BSA), withhold the ICI therapy and manage with systemic high-dose steroid prednisone 1–2 mg/kg (or equivalent), with tapering over at least for 4 weeks. For grade 4 (life-threatening) skin reactions such as Stevens–Johnson syndrome/toxic epidermal necrolysis, treatment consists of permanent discontinuation of ICI therapy, supportive care, and intravenous steroid. Intravenous immunoglobulin or cyclosporine is considered in severe or corticosteroid-unresponsive cases.

Endocrine Adverse Events

Endocrine toxicity is a common side effect of ICI therapy in HNSCC patients. The most frequent endocrinopathies are hypothyroidism, hyperthyroidism, and hypophysitis. Rare cases of primary adrenal insufficiency, hypercalcemia, and immune-related type 1 diabetes mellitus, leading to hyperglycemia and diabetic ketoacidosis, have been reported (Chow et al., 2016; Seiwert et al., 2016; Bauml et al., 2017; Colevas et al., 2018; Ferris et al., 2018; Siu et al., 2018; Cohen et al., 2019; Segal et al., 2019; Zandberg et al., 2019). Routine monitoring of thyroid function tests, hormone levels testing, and glucose level before starting therapy and before each dose are required. Consultation with an endocrinologist is recommended in all cases of suspected endocrinopathies.

Hypothyroidism is the most common endocrinopathy and occurs in higher incidence with anti-PD-1/PD-L1 agents in HNSCC patients (6.8–16%), and the majority of cases are with mild symptoms. For patients treated with tremelimumab (CTLA-4 inhibitors), the incidence rate of hypothyroidism is less than 2% (Siu et al., 2018).

Typically, hypothyroidism [elevated thyroid-stimulating hormone (TSH), normal or low FT4] presents with nonspecific symptoms such as fatigue, asthenia, cold intolerance, and dry skin. The management of hypothyroidism consists of thyroid hormone replacement (levothyroxine), usually starting with 0.8–1.0 μg kg^{-1} day^{-1} (Ma et al., 2019) and supportive care. ICI therapy is withheld in severe cases until symptoms resolve to baseline with appropriate supplementation.

Hyperthyroidism was reported with low frequency (about 3%) and may present with a new onset of atrial fibrillation, heat intolerance, and weight loss. Cardiovascular and neurological symptoms are relieved by a beta-blocker (atenolol 25–50 mg/day) and supportive care. In severe cases, the ICI therapy is withheld until symptoms resolve to baseline. In the most recent study of MA and colleagues, high-dose glucocorticoids (HDGs) did not improve the outcome of ICI-related thyroid disorders; therefore, routine use of HDGs in patients with suspected thyroid disorders such as thyrotoxicosis is not recommended. In addition, they suggested using HDGs in patients who present with symptoms of thyroid storm or in patients with cardiac disease (Ma et al., 2019).

The incidence of hypophysitis in HNSCC patients treated with ICI agents is less than 1%. Hypophysitis patients will present with headache, fatigue, and with multiple hormone deficiencies

[adenocorticotropic hormone (ACTH), TSH, follicle-stimulating hormone, luteinizing hormone, growth hormone, and prolactin]. The diagnosis of hypophysitis is confirmed with pituitary magnetic resonance imaging, which shows the enlargement of the pituitary and thickening of the stalk. Laboratory studies distinguish hypophysitis from primary adrenal insufficiency (low cortisol and high ACTH) and primary hypothyroidism (high TSH and low FT4). Tissue biopsy is the definitive diagnosis for lymphocytic hypophysitis. Treatment consists of long-term hormone replacement with supportive care and withholding ICI therapy. In severe symptoms, treatment is with the high-dose corticosteroid prednisone 1–2 mg/kg (or equivalent), with tapering over at least for 4 weeks.

Gastrointestinal Adverse Events

Diarrhea (an increase in the frequency of stools) is one of the more frequently observed irAEs with ICIs, and the majority of cases are mild. The incidence of diarrhea/colitis is higher in patients receiving the durvalumab + tremelimumab combination arm (14.3%) or in the tremelimumab arm (16.9%), whereas patients treated with anti PD-L1 alone experience less frequent cases (5.4–10.4%) (Siu et al., 2018). Moreover, the incidence of all grade diarrheas is lower with anti PD-1 drugs, less than 8% (Chow et al., 2016; Seiwert et al., 2016; Bauml et al., 2017; Ferris et al., 2018; Cohen et al., 2019). This gave us an implication that HNSCC patients are more prone to develop diarrhea during CTLA-4 therapy.

Symptoms of colitis are diarrhea accompanied with abdominal pain and, occasionally, rectal bleeding. Severe colitis can be a life-threatening condition and result in intestinal perforation. The upper gastrointestinal (GI) tract is less commonly affected. Symptoms such as dysphagia and epigastric pain have been reported (Chow et al., 2016; Seiwert et al., 2016; Bauml et al., 2017; Siu et al., 2018; Cohen et al., 2019; Zandberg et al., 2019).

A stool analysis, including bacterial cultures to exclude other etiologies such as infections with *Clostridium difficile* or other bacterial or viral pathogens, is required. Colonoscopy or sigmoidoscopy and abdominal CT scan are helpful in patients with bloody diarrhea or severe diarrhea (≥7 stool per day over baseline). Upper endoscopy is indicated in patients with upper GI symptoms.

Grade 1–2 diarrhea is managed with supportive care and antidiarrheal medication. Patients whose diarrhea progresses and/or is grade 3 or higher should withhold ICI therapy and treat with prednisone 1 mg/kg or equivalent, with tapering over at least for 4 weeks. In severe or life-threatening enterocolitis, the ICI therapy should be discontinued permanently and a high dose of corticosteroids given, prednisone 1–2 mg/kg (or equivalent), with tapering over for 4–6 weeks. In addition, infliximab (anti-TNFα monoclonal antibody) 5–10 mg/kg is recommended for patients not improved with steroids or to use mycophenolate mofetil if infliximab cannot be used.

Hepatic Adverse Events

Hepatitis is observed in 1–8% of HNSCC patients treated with immune checkpoint inhibitors, with severe adverse events (grades 3–5) occurring in 1–3%. Most patients present with asymptomatic elevation of aspartate aminotransferase and alanine transferase, but may present with hyperbilirubinemia, jaundice, and fatigue in advanced cases.

Liver function tests, liver enzyme test, and viral hepatitis serology are recommended prior to initiating ICI therapy. Biopsy and radiological tests could be considered to rule out other etiologies. Imaging may be helpful to rule out disease progression. Management consists of withholding ICI administration and prompt treatment with corticosteroid for moderate cases. For grade 3 or higher, treat with a high dose of corticosteroid, prednisone 1–2 mg/kg (or equivalent), with tapering over at least for 4 weeks, and ICI therapy should be permanently discontinued if there is no improvement with corticosteroids and liver function still elevated. In addition, mycophenolate mofetil IV 1 g twice a day is suggested for cases refractory to steroids. However, infliximab not given to patients with elevated AST/ALT since infliximab can cause hepatic injury.

Pulmonary Adverse Events

Pneumonitis is a noninfectious inflammation of the lung which occurs in 1–4% of HNSCC patients receiving ICI therapy. This condition can be severe and life-threatening. The most common symptom of pneumonitis is dyspnea (shortness of breath), which may be accompanied by dry cough and hypoxia. The incidence of pneumonitis is lower in HNSCC patients compared to patients with non-small cell lung cancer (NSCLC) (Suresh et al., 2018).

In general, lung biopsy and bronchoscopy might be indicated to exclude infection and other causes. Patients with suspected pneumonitis should undergo a CT scan. Imaging usually shows interstitial infiltrates and ground-glass opacities. In a recent study, Colen et al. reported several radiomic features that can be used to predict patients at risk for immunotherapy-induced pneumonitis, such as the maximum relevance and minimum redundancy feature selection method, anomaly detection algorithm, and leave-one-out cross-validation which identified radiomic features that were significantly different (Colen et al., 2018).

The American Society of Clinical Oncology (ASCO) guideline recommends withholding ICI therapy for any grade pneumonitis (Brahmer et al., 2018). For grade 2 pneumonitis, treat with systemic steroids, prednisone 1–2 mg kg^{-1} day^{-1} (or equivalent), and empirical antibiotics in the case of infections and withhold ICI therapy. Grade 3 or higher pneumonitis treatment consists of permanently discontinuing the use of ICI therapy and using a high dose of intravenous corticosteroids, (methyl)prednisone 1–2 mg kg^{-1} day^{-1} with additional immunosuppression (infliximab 5 mg/kg or mycophenolate mofetil IV 1 g twice a day or IVIG for 5 days or cyclophosphamide). Steroids should be tapered slowly over weeks.

Rheumatologic/Musculoskeletal Adverse Events

Musculoskeletal side effects are commonly seen with the ICI therapy trials. Arthralgia and myalgia are the most common and

occur in 2–6% percent in the HNSCC patients treated with ICI agents (Chow et al., 2016; Seiwert et al., 2016; Bauml et al., 2017; Colevas et al., 2018; Siu et al., 2018; Segal et al., 2019; Zandberg et al., 2019). The incidence of grade 3 or higher was rare with musculoskeletal irAEs. One case of grade 3 musculoskeletal pain had been reported with pembrolizumab therapy (Seiwert et al., 2016).

Early rheumatologic consultation is advised. The diagnostic workup should include complete rheumatologic and neurological history and examination including muscle strength. Autoimmune blood panel and inflammatory markers such as erythrocyte sedimentation rate and C-reactive protein may be considered for inflammatory arthritis (Brahmer et al., 2018).

Manage arthralgia and myalgia with analgesia with paracetamol and/or NSAIDS. For moderate symptoms, treat with low-dose prednisolone 10–20 mg/day or equivalent for 4–6 weeks and withhold ICI therapy. For severe symptoms, withhold ICI therapy and treat with high-dose corticosteroid prednisone 1–2 mg/kg (or equivalent). In severe arthritis, anti-TNFα therapy should be initiated. Plasmapheresis and intravenous immunoglobulin therapy or other immunosuppressant therapy may be considered for severe myositis cases.

LESS COMMON IRAES AND THE RELEVANT MANAGEMENT IN HNSCC

Besides the common irAEs mentioned above, ICI toxicity can also affect other body organs, including the neurologic, cardiovascular, and renal systems, based on the existing clinical trials. The incidence of these adverse effects may be relatively low, but sometimes the consequences can be extremely serious and even lethal (**Table 3**).

Neurologic irAEs remain an uncommon toxicity, and the incidence of grade 3 or higher is less than 1% in HNSCCs. A range of neurologic events have been described, which include Guillain–Barré syndrome, encephalitis, and peripheral neuropathy. One case of severe adverse event (grades 3–5), Guillain–Barré syndrome, has been reported with pembrolizumab (Cohen et al., 2019). Diagnosis of neurologic toxicity comes from nerve conduction studies, lumbar puncture, and spine/brain MRI. Significant neurological toxicity should be managed with high-dose steroid and withholding the ICI therapy. For progressive Guillain–Barré syndrome, intravenous immunoglobulin (0.4 g kg^{-1} day^{-1} for 5 days) or plasmapheresis should be initiated. Frequent neurologic evaluation and pulmonary function monitoring are recommended. Patients with peripheral neuropathy may be offered low-dose prednisolone 0.5–1 mg/kg, GABA agonist (e.g., pregabalin or duloxetine) and withhold the ICI therapy. Patients with aseptic meningitis or encephalitis are managed with methylprednisolone 1–2 mg/kg and empiric antiviral (IV acyclovir).

TABLE 3 | List of uncommon iRAEs of ICIs in HNSCC therapy.

Organs	Disease	Drug classes	Incidence of grade 3/4 toxicity
Neurologic	Guillain–Barré syndrome	PD-1	≤1%
Cardiovascular	Congestive cardiac failure, atrial fibrillation, and cardiac tamponade	PD1 and PD-L1	2–3%
Renal	Nephritis	PD-L1	≤1%

PD-1 programmed death receptor-1; PD-L1 programmed death-ligand 1; ICIs immune checkpoint inhibitors; HNSCC head and neck squamous cell carcinoma; iRAEs immune-related adverse events.

Cardiovascular irAEs are uncommon and seen in less than 3% of HNSCC patients on ICI therapy and can be associated with general myositis. Several cases of cardiac tamponade, arrhythmias, and congestive heart failure were reported (Chow et al., 2016; Seiwert et al., 2016; Bauml et al., 2017; Colevas et al., 2018; Siu et al., 2018; Cohen et al., 2019; Segal et al., 2019; Zandberg et al., 2019). Diagnosis of cardiac toxicity can be established by electrocardiogram, cardiac biomarkers, and cardiac magnetic resonance imaging. Early consultation with a cardiologist is recommended. Management consists of a high dose of intravenous corticosteroids, prednisone 1–2 mg kg^{-1} day^{-1}, with additional immunosuppression (e.g., infliximab 5 mg/kg, intravenous immunoglobulin or mycophenolate mofetil) and withholding the ICI therapy.

Renal irAEs are less common and occur in less than 3% of patients on ICI therapy. One case of grade 3 nephritis has been reported with durvalumab therapy (Segal et al., 2019). Serum creatinine should be monitored prior to starting therapy and before each dose. Renal biopsy can be considered to rule out other causes. For moderate nephritis cases, withhold ICI therapy and treat with prednisone 0.5–1 mg/kg or equivalent, with tapering over at least for 6 weeks. In severe or life-threatening nephritis, the ICI therapy should be discontinued permanently and a high dose of corticosteroids is given, prednisone 1–2 mg/kg or equivalent, with tapering over for 4–6 weeks. In addition, dialysis may be required for patients with severe renal failure.

IRAE-RELATED DEATH IN HNSCC

The overall incidence of irAE-related death is low, but does occur at a rate of 0.3% to 1.03% (Wang et al., 2018). In a recent review by Jiang et al., the most common CTLA-4 treatment-related death was gastrointestinal toxicity, and the most PD-1 treatment-related death was pulmonary toxicity (Jiang et al., 2019). In our analysis, we observed six fatal irAEs in HNSCC. Three deaths were reported in patients treated with pembrolizumab and two deaths were reported with nivolumab therapy; one death was reported with combination therapy (durvalumab + tremelimumab). Detailed information was listed in **Table 4**.

Two patients died of treatment-related pneumonitis: one in the CheckMate-141 trial and another in the single-arm phase II KEYNOTE-055 trial (Bauml et al., 2017; Ferris et al., 2018). Pneumonitis is less common irAEs, but it is one of the most common causes of ICI-related deaths. In addition, the incidence of treatment-related death is higher with anti-PD-1 therapy and typically occurs later than other irAEs. In a phase II/III study

TABLE 4 | Baseline characteristics of death cases and involved clinical trials.

Involved organs	Treatment-related death	NCT no.	ICI	Dose of ICI
Pulmonary (*n* = 3)	Pneumonitis (*n* = 2)	02105636 (*n* = 1)	Nivolumab	3 mg/kg every 2 weeks
		02255097 (*n* = 1)	Pembrolizumab	200 mg every 3 weeks
	ARF (*n* = 1)	02319044 (*n* = 1)	Durvalumab + Tremelimumab	Durvalumab (20 mg/kg every 4 weeks) + tremelimumab (1 mg/kg every 4 weeks)
Dermatological (*n* = 1)	SJS (*n* = 1)	02252042 (*n* = 1)	Pembrolizumab	200 mg every 3 weeks intravenously
Gastrointestinal (*n* = 1)	LIP (*n* = 1)	02252042 (*n* = 1)	Pembrolizumab	200 mg every 3 weeks
Endocrine (*n* = 1)	Hypercalcemia (*n* = 1)	02105636 (*n* = 1)	Nivolumab	3 mg/kg every 2 weeks

NCT, national clinical trial; ICI, immune checkpoint inhibitor; ARF, acute respiratory failure; SJS, Stevens–Johnson syndrome; LIP, large intestine perforation.

on the efficacy and safety of pembrolizumab in patients with advanced NSCLC, three cases of pneumonitis-related deaths (3 of 682 NSCLC patients) were reported in Herbst's study (Herbst et al., 2016).

Treatment-related death, Stevens–Johnson syndrome (SJS), occurred in one patient treated with pembrolizumab in the KEYNOTE-040 trial (Cohen et al., 2019). SJS is a severe life-threatening cutaneous adverse reaction, which is, in most cases, drug-induced. Patients present with purpuric rashes with blisters, oral mucositis, and conjunctivitis. SJS involves <10% body surface area skin detachment. Super infection, massive fluid losses, and electrolyte imbalances can lead to death (Harr and French, 2010; Plachouri et al., 2019; Woolum et al., 2019).

Incidence of anti-PD-1/PD-L1 perforating colitis is less frequent compared to anti-CTLA-4 treatment-associated perforated colitis. However, treatment-related death occurred in one patient treated with pembrolizumab (large intestine perforation induced by colitis) in the KEYNOTE-040 trial (Cohen et al., 2019).

One patient died from treatment-related acute respiratory failure in the combination therapy arm (durvalumab + tremelimumab) in the phase II CONDOR randomized clinical trial. The primary cause of death was squamous cell carcinoma disease progression (Siu et al., 2018). In the CheckMate 141 Study, one patient in the nivolumab group died from treatment-related hypercalcemia (Ferris et al., 2018).

The risk of fatal irAEs is very low and typically occurs in the early phase during treatment. It is vital for the clinician to be aware of these potential lethal complications. Early recognition, proper intervention, and long-term monitoring of potential fatal adverse events may be effective in preventing treatment-related death.

FUTURE PERSPECTIVE AND ONGOING CLINICAL TRIALS FOR THE TREATMENT OF HNSCC

Although ICIs have been approved by the FDA as the second-line treatment of recurrent and/or metastatic HNSCC, the relatively high rate of irAEs and the low response rate call for new immunotherapy strategies, either in monotherapy or in combination with existing ICIs. As of August 2019, there are six ongoing clinical trials evaluating ICIs in HNSCC (**Figure 1**). Pembrolizumab and Atezolizumab are now undergoing phase 2 and phase 3 trials. Notably, the remaining four trials were designed as ICIs in combination with other agents, including SNS-301, Cetuximab, FT500, and BMS986205.

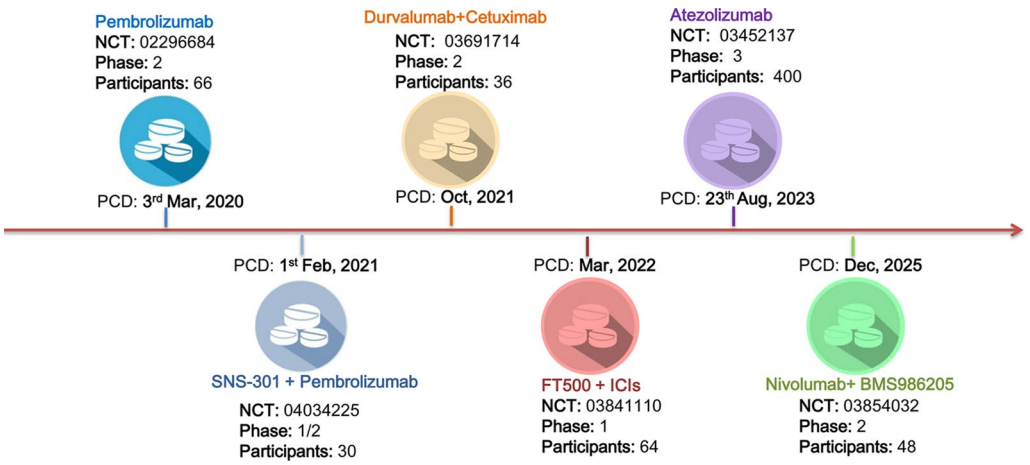

FIGURE 1 | Ongoing clinical trials for the treatment of head and neck squamous cell carcinoma (HNSCC). There were six registered ongoing trials as of August 2019. Four of them were designed in combining intervention strategy. The other two were undergoing phase 2 and phase 3 trials for Pembrolizumab and Atezolizumab in HNSCC therapy, respectively. *PCD* primary completion date.

SNS-301 is a cancer vaccine that targets human aspartate β-hydroxylase (ASPH), which had been found overexpressed in many solid tumors, but would not be expressed in human after fetal development. A phase I study in prostate cancer had confirmed the safety and tolerability of SNS-301. The ASPH-specific immune activity had also been established in this study, which provided the foundation for phase II study in solid and hematological tumors. Cetuximab is a well-known anti-EGFR monoclonal antibody that has been widely used in HNSCC. A recent study published by Andre et al. suggested that cetuximab plus monalizumab, a humanized anti-NKG2A antibody, had a promising response rate (31%) and common irAEs (ranging from 10% to 17%) compared to current ICIs in HNSCC therapy. The involved mechanism had also been suggested as monalizumab can enhance natural killer (NK) cell activity against tumor cells and rescue $CD8^+$ T cell function (Andre et al., 2018). FT500, a NK cell product derived from the clonal master iPSC line, may overcome the multiple mechanisms of ICI resistance, including recognition and lysis of tumor cells upon the downregulation of HLA-1 on tumor cells. In AACR 2018, Bjordahl and his colleagues reported that FT500 can facilitate the T cell recruitment and enhance T cell activation (Bjordahl et al., 2018). BMS986205 is a novel enzymatic-targeted drug that belongs to the indolamine-2,3-dioxygenase-1 (IDO-1) inhibitors, which can restore the differentiation of T cells and downregulate the immunosuppressive effect of kynurenine. Combining IDO-1 inhibitors with anti-PD1/PD-L1 was shown effective in many kinds of solid tumors. In a phase 1/2a trial, the treatment-related adverse events range from 6.8% to 18.2%, and there were no grade 4 or 5 adverse events (Zhu et al., 2019).

The irAEs and the response rates differing from various tumor types are the main challenges for the first-generation immunotherapy. Fortunately, the safety and efficiency of ICIs in different tumors are now getting more distinct. Moreover, combining ICIs with other agents, including but not limited to anti-ASPH vaccine, anti-EGFR monoclonal antibody, IDO-1 inhibitors, and NK cell products, may shed light to complement current HNSCC immunotherapy.

CONCLUSIONS

Overall, our results did not show higher rates or severity of irAEs in HNSCC patients compared with other malignancies. The most common reported adverse events are dermatologic, endocrine, and gastrointestinal. Higher rates of endocrine disorders are associated with anti-PD-1 therapy, whereas gastrointestinal toxicities are more common with CTLA-4 inhibitor administration. In addition, pneumonitis seems to be associated with a higher risk of ICI treatment-related death in HNSCC. This raised a claim that clinicians must maintain sharp vigilance on the respiratory symptoms and early intervention should be taken once the pneumonitis was suspected.

General management of irAEs includes treatment interruption, immunosuppression or immunomodulatory agents, and hormone replacement. Glucocorticoid therapy was commonly used for moderate to severe irAEs. But it must be noted that routine use of HDGs may not be suitable for all adverse events, including but not limited to ICI-related thyroid disorders.

As the use of immunotherapy increases, patients receiving ICI therapy are at risk of developing irAEs that may lead to severe or fatal toxicities. Knowledge of these adverse events and the management algorithm discussed in this paper will provide an important tool for clinicians and for future oncology trials.

AUTHOR CONTRIBUTIONS

The original idea of this study was from HW. HW and AM reviewed the literature and contributed equally to the manuscript writing and editing. JZ instructed the whole manuscript formation. SL and JL contributed to the literature retrieval and review. DL and HY contributed to the manuscript editing and proofreading.

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SYSTEMATIC REVIEW
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The Differences in the Safety and Tolerability of Immune Checkpoint Inhibitors as Treatment for Non–Small Cell Lung Cancer and Melanoma: Network Meta-Analysis and Systematic Review

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Background: Immune checkpoint inhibitors (ICIs) have evolved for the treatment of solid tumors. In addition to the efficacy of ICIs for cancer, the adverse events (AEs) of ICIs are also noteworthy for gradually more extensive clinical use.

Objective: To conduct a systematic review and network meta-analysis to evaluate the treatment-related AEs that occurred in clinical trials using different kinds of ICIs, to explore the differences in AEs among ICIs for treating non–small cell lung cancer (NSCLC) and melanoma, and to compare select immune-related AEs.

Methods: PubMed, EMBASE, Cochrane Library, ClinicalTrials.gov, and other available sources were systematically searched for published reports up to January 1, 2019. Two reviewers independently selected reports about phase II/III randomized controlled trials to compare among ICIs and between ICIs and chemotherapy. After the bias assessment of all included trials, a Bayesian network meta-analysis was performed. The primary outcomes were any-grade and high-grade treatment-related AEs from all ICIs. The secondary outcomes were AEs in patients with NSCLC and melanoma and the presence of the select AEs pneumonitis/pneumonia and colitis.

Results: Eighteen randomized controlled trials containing 11,223 patients with NSCLC or melanoma were included. A total network meta-analysis was conducted. The meta-analysis showed that atezolizumab 1,200 mg and pembrolizumab 2 mg/kg every 3 weeks were generally more tolerable than other ICIs. ICI combined with chemotherapy might suggest a higher risk of treatment-related AEs than monotherapy with a single ICI, except durvalumab and ipilimumab. In the NSCLC subgroup, pembrolizumab was associated with a higher risk of high-grade AEs than nivolumab. In addition, ICIs (nivolumab, atezolizumab, and avelumab) led to a lower risk of any/high-grade treatment-related AEs than traditional chemotherapy and ICI combination chemotherapy. However, ICIs did not present preferable safety and tolerability compared to chemotherapy in

treating melanoma. Compared with chemotherapy, nivolumab, durvalumab, two ICIs, and ICI combined chemotherapy led to more pneumonitis/pneumonia. However, when treating NSCLC, different types of ICIs did not differ significantly regarding the incidence of pneumonitis/pneumonia. A combination of nivolumab and ipilimumab had the highest risk for colitis, while pembrolizumab and atezolizumab had a lower possibility than the other ICIs.

Conclusion: Atezolizumab 1,200 mg and pembrolizumab 2 mg/kg every 3 weeks were ordinarily safer than other ICIs. When treating NSCLC, nivolumab had the lowest risk; when treating melanoma, pembrolizumab had the lowest toxicity.

Keywords: immune checkpoint inhibitors, non–small cell lung cancer, melanoma, network meta-analysis, treatment-related adverse events

INTRODUCTION

Since ipilimumab, an anti–cytotoxic T lymphocyte-associated antigen 4 (CTLA-4) therapy, was approved by the Food and Drug Administration (FDA) in 2011, remarkable progress has been made in immunotherapy. As the first approved checkpoint inhibitor, ipilimumab is indicated only for melanoma. Another checkpoint inhibitor against the programmed death 1(PD-1)/programmed death ligand 1 (PD-L1) has also shown prominent success for patients with advanced solid tumors. In the National Comprehensive Cancer Network guidelines for non–small cell lung cancer (NSCLC) (Ettinger et al., 2017), an update focusing on targeted therapies and immunotherapies has been added to change the recommended therapy. The FDA suggested pembrolizumab as a first-line treatment for patients with PD-L1 expression levels ≥50% based on Keynote-024 (Brahmer et al., 2017). The indications of PD-1/PD-L1 were amplified after numerous clinical trials were completed and reported. Among these inhibitors, nivolumab and pembrolizumab alone and in combination with other agents have obtained approval by the FDA for melanoma and NSCLC monotherapy. Currently, ongoing clinical trials are focused on both PD-1 (nivolumab and pembrolizumab) and PD-L1 (atezolizumab, durvalumab, and avelumab) for different indications.

Compared with standard chemotherapy, immune checkpoint inhibitors (ICIs) showed great clinical benefits in prolonging the overall survival and progression-free survival for patients with solid tumors (Borghaei et al., 2015; Herbst et al., 2016; Sharma et al., 2016; Rittmeyer et al., 2017). This result was also indicated by evidence-based medical research (Zoratti et al., 2019; Frederickson et al., 2019). Along with the prominent efficacy of ICIs, adverse events (AEs) are gradually becoming concerns. In comprehensive real-world clinical use, chemotherapy has been clearly established as a general treatment with unequivocal benefits and survival advantages. Compared with traditional chemotherapy, ICIs can be taken as new administrations for advanced cancers with less toxicity and AEs. When the efficacy data on survival outcomes are reported in clinical trials and real-world practices, the understanding of the toxicities of immunotherapy needs to be expanded to establish better treatment options for advanced cancers. As inhibitors of immune checkpoints, CTLA-4 and PD-1/PD-L1 normally prevent the overactivation of the immune system and maintain the immune balance inside the body (Pardoll, 2012). This immune mechanism results in the toxicity reaction known as immune-related AEs, and classical chemotherapy toxicities also happen during treatment. Most AEs occur acutely and can be treated with steroids in 1 to 7 days (Johnson et al., 2018).

Acknowledging the AEs caused by ICIs is necessary for better clinical management. In a study by Wang W. et al. (2017), the risk of hepatotoxicity related to ICIs was demonstrated. Wang W. et al. (2017) reported that CTLA-4 inhibitors may lead to a high risk of hepatotoxicity, while PD-1 inhibitors had a low risk. The study by Nishijima et al. (2017) systematically reviewed the safety and tolerability of PD-1/PD-L1 inhibitors in advanced cancer and concluded that PD-1/PD-L1 inhibitors were overall better tolerated than chemotherapy. However, these studies did not compare the total immune-related or any treatment-related AEs. Direct meta-analyses were limited to the control group, which might overlook safety comparisons among different control arms in different clinical trials. Therefore, in this research, we conducted a systematic review and a network meta-analysis of randomized controlled trials (RCTs) to evaluate the AEs and toxicity among various ICIs and standard chemotherapy. As a previous trial conducted by Hellmann et al. (2018) showed, a combination of ipilimumab and nivolumab had a high response rate for NSCLC. However, it is difficult to acquire an integrated picture of AEs from RCTs when ICIs are indicated in two different cancers.

The purpose of this study was to systematically review and conduct a network meta-analysis on the safety and toxicity of different ICIs in treating NSCLC and melanoma. The risks for select specific treatment-related AEs (colitis and pneumonitis/pneumonia) were also compared among these different treatment patterns.

METHODS

Systematic Review

The present report was performed according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses

(PRISMA) guidelines and the PRISMA extension statement for network meta-analysis (Hutton et al., 2015). Two authors searched PubMed, EMBASE, Cochrane Library, and ClinicalTrials.gov independently for articles published between January 2000 and January 2019 with the following MeSH terms: "CTLA-4," "PD-1," "PD-L1," "ipilimumab," "atezolizumab," "nivolumab," "durvalumab," "pembrolizumab," and "avelumab" (**Supplement Figure 1**). Only RCTs were included. We also searched abstracts from the American Society of Clinical Oncology, and abstracts without full text were eliminated. The two reviewers assessed the screening results and made the final inclusion decisions. The references of relevant studies were also reviewed to include additional studies.

Study Selection

Only randomized controlled clinical trials were included. Articles that met the following criteria were included: (a) phase II or phase III clinical trials on patients with NSCLC or melanoma; (b) studies with outcomes reporting of the rates of any all-grade and high-grade (3–4) AEs or treatment-related AEs that led to discontinuation or treatment-related death; and (c) at least one ICI as the intervention. It has been proven that autoimmune AEs occur, such as colitis, pneumonitis, skin AEs, endocrine dysfunction, and hepatitis (Johnson et al., 2018). It was also observed that, when treated with ipilimumab, patients had a higher risk for colitis than when treated with PD-1/PD-L1 inhibitors (Wang D. et al., 2017). The incidence of ICI-related pneumonia was also higher in the treatment of NSCLC than in the treatment of melanoma (Nishino et al., 2016). To explore the differences between the incidences of colitis and pneumonitis/pneumonia when patients were treated with ICIs, subgroup analyses of these two select AEs were conducted.

Data Extraction

Two researchers (Q-QC and J-YD) independently conducted the data extraction. The following data were summarized: first author, title, year of publication, study ID, tumor site, trial phase, treatments, median follow-up time, version of the Common Terminology Criteria for Adverse Events, any AEs, treatment-related AEs, specific AEs, specific treatment-related AEs, treatment-related AEs leading to discontinuation, and treatment-related deaths.

Quality Assessment

The qualities of the trials were ranked by the Jadad scale based on the original article, updated references and **Supplementary Materials** (**Figure 2**), the presence of sequence generation, allocation concealment, blinding, and incomplete and selective reporting (Jadad et al., 1996). When assessing the quality, a score of 2 was assigned for appropriate random sequence generation, accurate allocation concealment, and an appropriate description of blinding, and a score of 1 was assigned when there was incomplete and selective reporting. All disagreements in the study selection, data extraction, and quality assessment were discussed for consistency.

Statistical Analysis

The primary objective of this article was to compare the toxicity and AEs among all ICIs and standard chemotherapy. Additionally, the differences in AEs between patients with NSCLC and melanoma were studied. Pairwise meta-analysis (PWMA) was applied for direct evidence that was pooled in random-effects models if heterogeneity existed ($P < 0.05$).

A total network was built containing all the included trials, and both direct and indirect comparisons were conducted. The consistency between the direct and indirect evidence was statistically confirmed by node-splitting analyses. The incidence of specific treatment-related toxicity, relative risk (RR) for any AEs, and odds ratio for high-grade AEs were calculated with 95% confidence intervals. When treatment-related AEs were not observed in the original studies, a relative index of any AEs that occurred during treatment was taken as a replacement. Heterogeneity among the trials was verified by the Cochrane Q statistic and quantified with the I^2 index (Higgins et al., 2003). When eminent heterogeneity was not shown ($P > 0.05$), pooled odds ratios/RRs and their 95% confidence intervals were reported in a fixed-effects model; otherwise, a random-effects model was applied.

Subgroups were created based on the cancer site, specific treatment-related AEs, and different ICIs. All analyses involved the use of the packages "gemtc" and "pcnetmeta" in R v3.5.1, and PWMA was conducted in Review Manager v5.3.

RESULTS

Search Results and Eligible Trials

The selection and exclusion criteria of the study are presented in **Figure 1**. A total of 631 studies were identified, of which 41 potential articles were reviewed intensively as full text. Finally, 18 randomized clinical trials, with a total of 11,223 patients, were incorporated in this network meta-analysis. In total, 11,018 patients had reported AE analyses in these original studies. The characteristics of these 18 trials are demonstrated in **Table 1**, among which 11 RCTs (Borghaei et al., 2015; Brahmer et al., 2015; Fehrenbacher et al., 2016; Herbst et al., 2016; Rittmeyer et al., 2017; Barlesi et al., 2018; Gandhi et al., 2018; Paz-Ares et al., 2018; Socinski et al., 2018; Antonia, 2019) compared ICIs to treat NSCLC, and seven trials (Larkin et al., 2015; Postow et al., 2015; Ribas et al., 2015; Robert et al., 2015; Schachter et al., 2017; Weber et al., 2017; Larkin et al., 2018) focused on melanoma. Nivolumab was used in eight trials, and the most common dosage was 3 mg/kg every 2 weeks intravenously. Another strategy was combining nivolumab 1 mg/kg with ipilimumab 3 mg/kg. Five RCTs containing pembrolizumab compared 2 or 10 mg/kg every 2 or 3 weeks with standard chemotherapies. Ribas et al. (2015), Herbst et al. (2016), and Schachter et al. (2017) also explored the outcomes when the dosage changed. Ipilimumab was indicated only for melanoma, and Larkin et al. (2015) and Postow et al. (2015) compared ipilimumab in different dosages with ipilimumab combined with nivolumab. Atezolizumab 1,200 mg was compared with docetaxel or used in combination therapy to treat NSCLC (Fehrenbacher et al., 2016; Rittmeyer et al., 2017; Socinski et al., 2018).

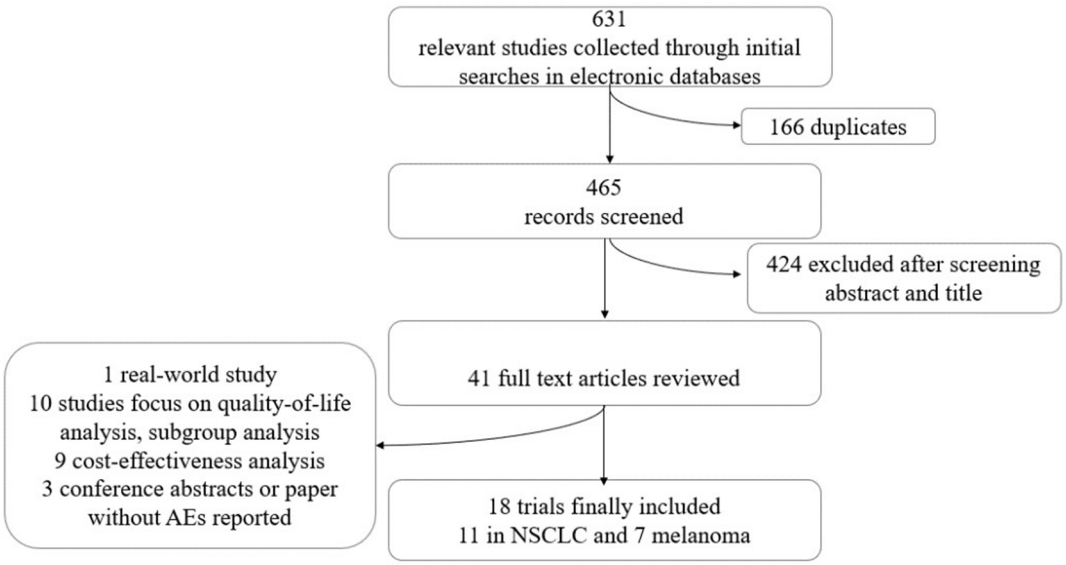


FIGURE 1 | Flowchart of select of included trials in network meta-analysis.

Detailed characteristics of the included trials are shown in **Table 1**. The modified Jadad scores indicated that almost all data included in this network meta-analysis (NMA) were from high-quality studies, with only one study that had the lowest score of 3. All trials were randomly designed, but only eight (44.44%) demonstrated the generation of random sequences, and there was no selective or incomplete outcomes reporting.

Network Geometry

Figure 2 presents two network diagrams illustrating the whole network: a total network meta-analysis and a comparison among different ICIs. The cancer-based analysis is presented in **Supplement Figure 3.** Chemotherapy was the most common control group, and this group had the largest proportion of patients.

Network Meta-Analysis for Treatment-Related AEs

All relative outcomes of any-grade or high-grade treatment-related AEs in the NMA are presented in **Supplement Figure 4.** Compared with chemotherapy, nivolumab 3 mg/kg, atezolizumab 1,200 mg, and pembrolizumab 2 or 10 mg/kg every 3 weeks had a lower risk of high-grade AEs. When ICI was combined with chemotherapy, the risk of suffering from high-grade treatment-related AEs was higher than that with nivolumab 3 mg/kg, atezolizumab 1,200 mg, pembrolizumab 2 or 10 mg/kg, ipilimumab 3 mg/kg every 3 weeks, or avelumab 10 mg/kg every 2 weeks. This finding might imply that monotherapy with some ICIs was more tolerable than ICI combination chemotherapy, but there was no evidence of superiority between chemotherapy and combination therapy. In the comparison of ICI combination chemotherapy with nivolumab 1 mg/kg plus ipilimumab 3 mg/kg, no significant differences were observed.

Figure 3A shows the results of the network meta-analysis based on different ICIs. The network meta-analysis demonstrated a significantly higher risk of all AEs with ICI plus chemotherapy than with nivolumab, atezolizumab, pembrolizumab, and avelumab. In other words, monotherapy with ICIs led to a lower risk of suffering from AEs than combination therapy with any ICIs, except durvalumab or ipilimumab, and this finding was consistent with the outcomes regarding high-grade treatment-related AEs. In the analysis of high-grade AEs, nivolumab and pembrolizumab were more tolerable than chemotherapy, regardless of dosage. The safety ranking for any-grade AEs is as follows: avelumab (40%), atezolizumab (32%), pembrolizumab (22%), nivolumab (23%), ipilimumab (21%), nivolumab plus ipilimumab (11%), chemotherapy (46%), durvalumab (20%), and ICI plus chemotherapy (71%); this ranking was mainly the same as the ranking for high-grade AEs. The possibility of avelumab becoming the safest ICI was 40%, and ICI plus chemotherapy had a 71% probability of being the least tolerant.

Subgroup Analysis Between NSCLC and Melanoma

The patients were divided into NSCLC and melanoma subgroups. Group NSCLC involved 11 original studies with 7,033 patients, while the melanoma group involved 4,190 patients from seven articles. **Figure 3B** shows that the risk of both any-grade and high-grade treatment-related AEs was lower with nivolumab, atezolizumab, and avelumab than with ICI combination chemotherapy and traditional chemotherapy. Pembrolizumab was superior to ICI combination chemotherapy but not to traditional chemotherapy. The results for high-grade AEs remained roughly identical with those for any-grade AEs, with the exception of pembrolizumab. Pembrolizumab also showed a lower risk than traditional

TABLE 1 | Characteristics of 18 studies.

	Author, year	ID	Trial phase	Masking	Total N	Follow-up time (mo)	Inventions	Analyzed patients	CTCAE version	Discontinuation*
NSCLC										
1	(Brahmer et al., 2015)	Checkmate017	III	Open-label	272	UK	Nivolumab 3 mg/kg q 2 weeks	135	4.0	4
							docetaxel 75 mg/m^2 q 3 weeks	137		13
2	(Govindan et al., 2017)	Checkmate026	III	Open-label	541		Nivolumab 3 mg/kg q 2 weeks	267	4.0	26
						UK	Platinum-based chemotherapy q3 weeks	263		35
3	(Borghaei et al., 2015)	Checkmate057	III	Open-label	582	14.5	Nivolumab 3 mg/kg q 2 weeks	287	4.0	14
							Docetaxel 75 mg/m^2 q 3 weeks	268		40
4	(Rittmeyer et al., 2017)	OAK	III	Open-label	850	21	atezolizumab 1,200 mg	425	4.0	46
							docetaxel 75 mg/m^2 q 3 weeks	425		108
5	(Fehrenbacher et al., 2016)	POPLAR	II	Open-label	287	14.8	Atezolizumab 1,200 mg	144		2
							Docetaxel 75 mg/m^2 q 3 weeks	143		24
6	(Socinski et al., 2018)	IMPOWER150	III	Open-label	787	15.4	Atezolizumab + bevacizumab + carboplatin plus paclitaxel (ABCP)	393	4.0	128
							Bevacizumab + carboplatin + paclitaxel (BCP group)	394		98
7	(Herbst et al., 2016)	Keynote010	II/III	Open-label	1,034	10.4	Pembrolizumab 2 mg/kg q 3 weeks	339	4	15
							Pembrolizumab 10 mg/kg q 3 weeks	343		17
							Docetaxel 75 mg/m^2 q 3 weeks	309		31
8	(Gandhi et al., 2018)	Keynote189	III	Double-blind	616	10.5	Pembrolizumab + pemetrexed + platinum-based drug	405	4	112
							Placebo + pemetrexed + Platinum-based drug	202		30
9	(Paz-Ares et al., 2018)	Keynote407	III	Double-blind	559	7.8	Pembrolizumab 200 mg + chemotherapy	278	4.03	37
							Placebo + chemotherapy	280		34
10	(Antonia et al., 2017)	PACIFIC	III	Double-blind	713	14.5	Durvalumab 10 mg/kg q 2 weeks	475	4.03	73
							Placebo	234		23
11	(Barlesi et al., 2018)	JAVELIN Lung 200	III	Open-label	792	18.3	Avelumab 10 mg/kg q 2 weeks	393	4.03	28
							Docetaxel 75 mg/m^2 q 3 weeks	365		51
Melanoma										
12	(Larkin et al., 2018)	Checkmate037	III	Open-label	405	24	Nivolumab 3 mg/kg q 2 weeks	268	4.0	13
							Chemotherapy	102		11
13	(Robert et al., 2015)	Checkmate066	III	Double-blind	418	16.7	Nivolumab 3 mg/kg q 2 weeks	206	4.0	14
							Dacarbazine 1,000 mg/m^2 q 3 weeks	205		24

(*Continued*)

TABLE 1 | Continued

	Author, year	ID	Trial phase	Masking	Total N	Follow-up time (mo)	Inventions	Analyzed patients	CTCAE version	Discontinuation*
14	(Larkin et al., 2015)	Checkmate067	III	Double-blind	945	9	Nivolumab 1 mg/kg + ipilimumab 3 mg/kg	313	4.0	24
							Nivolumab 3 mg/kg q 2 weeks	313		114
							Ipilimumab 3 mg/kg q 3 weeks	311		46
15	(Postow et al., 2015)	Checkmate069	II	Double-blind	142	24.6	Nivolumab 1 mg/kg + Ipilimumab 3 mg/kg	94	4.0	44
							Ipilimumab 3 mg/kg q 3 weeks	46		8
16	(Weber et al., 2017)	Checkmate238	III	Double-blind	906	19.5	Nivolumab 3 mg/kg q 2 weeks	452	4.0	35
							Ipilimumab 10 mg/kg q 3 weeks	453		189
17	(Ribas et al., 2015)	Keynote 002	II	Double-blind	540	10	Pembrolizumab 2 mg/kg q 3 weeks	180	4.0	4
							Pembrolizumab 10 mg/kg q 3 weeks	181		13
							Chemotherapy	179		10
18	(Schachter et al., 2017)	Keynote006	III	Open-label	834	22.9	Pembrolizumab 10 mg/kg, q 2 weeks	278	4.0	19
							Pembrolizumab 10 mg/kg, q 3 weeks	277		30
							Ipilimumab q 3 weeks	256		23

**Discontinuation for treatment-related Aes CTCAE, Common Terminology Criteria for Adverse Events; UK, unknown; q 2 weeks, every 2 weeks; q 3 weeks, every 3 weeks.*

chemotherapy, but pembrolizumab was related to a higher risk of high-grade AEs than nivolumab. Unexpectedly, durvalumab showed intolerability in terms of high-grade AEs, even more so than ICI combination chemotherapy. In the melanoma subgroup, ICIs did not show better safety or more tolerability than chemotherapy, which is different from the outcomes of the NSCLC subgroup.

Pneumonitis/Pneumonia and Colitis as Treatment-Related AEs

In the selected AE analyses, indirect comparisons were conducted on pneumonitis/pneumonia and colitis. The results suggested that nivolumab, durvalumab, two ICIs, and ICI combination chemotherapy would remarkably increase the risk of any-grade pneumonitis/pneumonia compared with chemotherapy. Avelumab was the only ICI that might be ranked higher (lower risk) than chemotherapy. However, the risks did not vary in the NSCLC subgroup among different ICIs.

In the colitis analysis, ipilimumab and two ICIs (nivolumab + ipilimumab) had the highest risk of occurrence. In a sensitive analysis that ignored durvalumab and did not report the risk of colitis, we found that nivolumab combined with ipilimumab could cause more colitis than other ICIs. In general, pembrolizumab and atezolizumab had a lower possibility of leading colitis than other ICIs. All the outcomes are shown in **Figure 4**.

Inconsistency Assessment and Sensitivity Analysis

The node-splitting analysis indicated no significant inconsistencies except for the comparison between nivolumab and two ICIs (**Supplement Figure 5**). Two groups of PWMAs were included, taking chemotherapy and ipilimumab as the control groups (**Table 2**). The direct evidence indicated that atezolizumab, pembrolizumab, and avelumab showed a lower risk of any- or high-grade AEs than other ICIs. Nivolumab was only superior to other ICIs for high-grade AEs. Heterogeneity between groups was found for the comparisons of nivolumab versus chemotherapy and nivolumab versus ipilimumab ($I^2 > 50\%$, $P < 0.05$). For the one direct comparison, obvious inconsistency existed between the network meta-analysis and direct comparison for durvalumab, which presented a drastically higher risk than chemotherapy for any- and high-grade treatment-related AEs in the PWMA.

DISCUSSION

As the number of FDA approvals for ICIs increases, the indications for different ICIs have also expanded. However, different ICIs have distinct immunologic mechanisms and should not be taken as a whole category; even ICIs that belong to the same mechanism might lead to unlikely treatment effects and

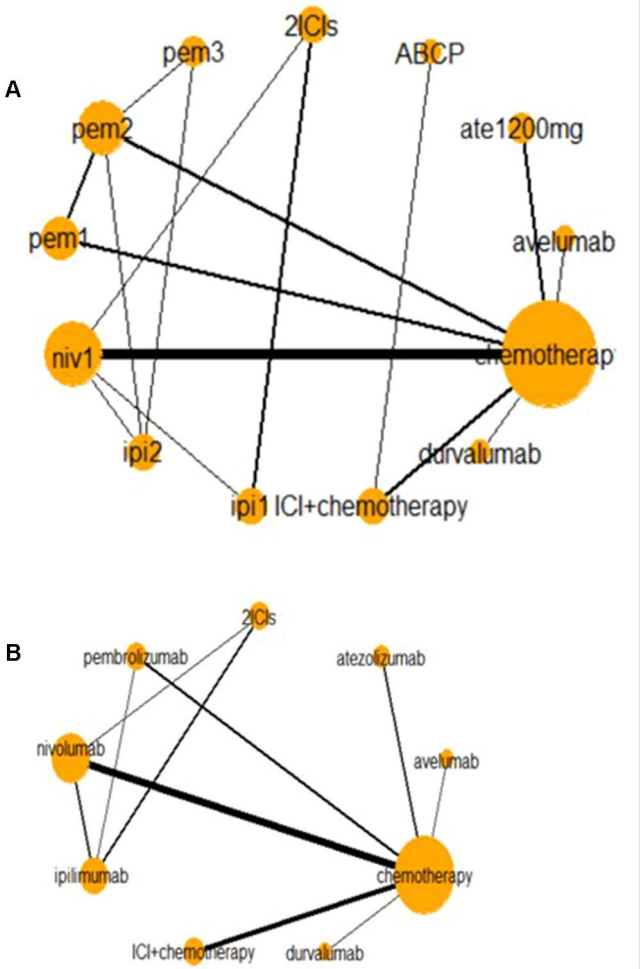


FIGURE 2 | Network of all trials **(A)** and ICIs combined **(B)** for the Bayesian network meta-analysis. Each node presents an invention in the trial. Size of node is proportional to the number of patients. niv1, nivolumab 3 mg/kg, q 2 weeks; pem1, pembrolizumab 2 mg/kg, q 3 weeks; pem2, pembrolizumab 10 mg/kg, q 3 weeks; pem3, pembrolizumab 10 mg/kg, q 2 weeks; ipi1, ipilimumab 3 mg/kg, q 3 weeks; ipi2, ipilimumab 10 mg/kg, q 3 weeks; ABCP, atezolizumab + bevacizumab + carboplatin + paclitaxel. Relative risk (RR) and odds ratio (OR) with 95% confidence interval (CI) in bold means it is statistically significant when comparing these two groups. And values <1 favor the intervention group instead of the control group. For instance, when comparing nivolumab and chemotherapy in high-grades AEs, OR with 95% CI [0.42 (0.20–0.86)] means that fewer AEs happen in intervention group (nivolumab), and it is statistically significant. And when comparing any-grade treatment-related AEs in nivolumab and chemotherapy, RR with 95% CI [1.09 (0.98–1.31)] suggests that fewer AEs happen in the control group (nivolumab), but it is not statistically significant.

tolerability in different diseases (Sukari et al., 2019). This review included 18 phase II/III clinical trials, which involved 11,223 patients suffering from NSCLC and melanoma. In the analysis of all included trials, 10 mg/kg avelumab every 2 weeks was considered the most tolerable, and 1,200 mg atezolizumab was ranked second. When treating NSCLC, nivolumab was ranked as having the lowest risk for both any- and high-grade AEs, followed by avelumab. In the subgroup for melanoma, pembrolizumab was superior to nivolumab, ipilimumab, two combined ICIs, and chemotherapy. Chemotherapy and ICI combined with chemotherapy were ranked low in safety regardless of the dosage or cancer type. It was suggested that nivolumab and avelumab were safe options for NSCLC and pembrolizumab for melanoma regarding any-grade or high-grade AEs. However, due to the failure of avelumab in treating NSCLC (Barlesi et al., 2018), atezolizumab 1,200 mg and nivolumab were favorable choices.

Several meta-analyses and network meta-analyses concerning the safety and tolerability of ICIs have been reported (Nishijima et al., 2017; Baxi et al., 2018; Komaki et al., 2018; Xu et al., 2018; Su et al., 2019; Zoratti et al., 2019). These prior studies focused on simple solid tumors, and select immune-related AEs were also reported. Few of these studies made a comparison among all the inhibitors approved by FDA. In contrast, we comprehensively included all possible ICI regimens for treating NSCLC and melanoma. These two solid tumors were largely potential indications for immunotherapy, so such inhibitors would already be used.

In our analysis, most clinical trials used chemotherapy as a controlled arm, and we performed direct and indirect analyses to compare all types of ICIs, not only head-to-head trials. This process was different from that of a previous meta-analysis, which only contained direct comparisons. More importantly, pneumonia and colitis (two specific AEs related to ICI treatment) were analyzed among different ICIs. This study indicated that ICI leads to more pneumonitis/pneumonia and colitis than chemotherapy. Avelumab has the lowest risk for pneumonitis/pneumonia among all comparators, including chemotherapy. Compared with pembrolizumab and avelumab, the combination of two ICIs (nivolumab + ipilimumab) might lead to a higher risk of any-grade pneumonitis/pneumonia (**Figure 3**, RR > 1). However, no significant differences were observed among the monotherapy ICI regimens. Our findings suggested that there were no notable differences among different ICIs regarding the risk for pneumonitis/pneumonia, which was consistent with the study reported by Nishino et al. (2016). In summary, when treated with ICIs, patients with NSCLC would have a higher risk of pneumonitis/pneumonia than those with melanoma, but this difference was not related to the kind of ICI. In addition, a high correlation was observed between ipilimumab and colitis. Ipilimumab led to a higher risk for colitis than nivolumab, atezolizumab, or pembrolizumab. We also noted that nivolumab, ipilimumab, and the combination of these two ICIs would lead to a higher risk of any-grade colitis than chemotherapy. The combination of nivolumab and ipilimumab led to a higher risk for colitis than even one ICI combined with chemotherapy. In addition, colitis should be given more attention when nivolumab is administered, and pembrolizumab is the much safer option of the two in that aspect. Based on these comprehensive results, this evidence-based analysis might suggest that when nivolumab and ipilimumab are combined, there is concern of colitis. The differences between these two solid tumors might suggest that the specificity of immune-related AEs was closely associated with the mechanism of the ICIs.

A Treatment-related adverse events in different ICIs

nivolumab	1.54 [0.41,5.73]	1.42 [0.44,4.06]	1.61 [0.26,11.46]	0.66 [0.24,1.55]	0.75 [0.49,24.18]	**0.2 [0.06,0.77]**	0.64 [0.15,1.97]	**0.42 [0.20,0.86]**
0.85 [0.52,1.10]	atezolizumab	0.91 [0.22,3.84]	1.11 [0.12,10.49]	0.43 [0.11,1.65]	1.7 [0.26,22.01]	**0.13 [0.03,0.65]**	0.41 [0.07,2.06]	0.28 [0.08,1.02]
0.94 [0.65,1.16]	1.11 [0.70,1.83]	pembrolizumab	1.22 [0.14,7.33]	0.46 [0.12,1.40]	1.91 [0.31,20.77]	**0.14 [0.04,0.50]**	0.44 [0.10,1.70]	**0.29 [0.10,0.90]**
1.06 [0.66,1.30]	1.23 [0.83,1.86]	1.14 [0.76,1.55]	durvalumab	0.39 [0.06,3.17]	1.6 [0.15,24.69]	0.12 [0.02,1.29]	0.38 [0.05,3.07]	0.26 [0.04,1.71]
1.05 [0.88,1.33]	1.23 [0.96,2.10]	1.12 [0.90,1.88]	0.99 [0.79,1.69]	ipilimumab	4.06 [0.72,43.97]	0.3 [0.09,1.16]	0.97 [0.25,3.66]	0.64 [0.26,1.66]
0.82 [0.44,1.19]	0.97 [0.53,1.76]	0.88 [0.46,1.56]	0.78 [0.40,1.41]	0.78 [0.40,1.11]	avelumab	**0.08 [0.01,0.47]**	0.23 [0.02,1.73]	**0.15 [0.02,0.89]**
1.18 [1.02,1.46]	**1.37 [1.10,2.40]**	**1.26 [1.02,2.11]**	1.11 [0.93,1.94]	1.12 [0.99,1.31]	**1.44 [1.06,2.84]**	ICI+ chemotherapy	3.18 [0.47,13.94]	1.99 [0.64,6.77]
1 [0.73,1.34]	1.16 [0.80,2.04]	1.06 [0.73,1.78]	0.95 [0.65,1.67]	0.95 [0.73,1.16]	1.22 [0.81,2.30]	0.85 [0.63,1.01]	2ICIs	0.65 [0.23,2.37]
1.09 [0.98,1.31]	**1.28 [1.05,2.11]**	1.16 [0.97,1.87]	1.03 [0.88,1.73]	1.04 [0.91,1.22]	1.33 [0.98,2.51]	0.92 [0.83,1.04]	1.09 [0.92,1.48]	chemotherapy

B Treatment-related adverse events in different ICIs in NSCLC

nivolumab	0.56[0.24,1.21]	**0.42[0.15,0.99]**	0.11[0.05,1.12]	0.98[0.23,3.32]	**0.12[0.04,0.25]**	**0.16[0.07,0.26]**
1.04[0.80,1.46]	atezolizumab	0.75[0.27,1.80]	0.20[0.08,2.01]	1.74[0.48,6.05]	**0.23[0.07,0.45]**	**0.28[0.14,0.51]**
1.11[0.82,1.54]	1.07[0.79,1.37]	pembrolizumab	0.27[0.10,2.80]	2.36[0.55,8.93]	**0.30[0.09,0.72]**	**0.38[0.15,0.89]**
1.36[0.92,1.77]	1.30[0.85,1.67]	1.22[0.81,1.65]	durvalumab	8.76[0.93,24.81]	1.20[0.08,2.71]	1.44[0.13,3.00]
1.04[0.66,1.42]	0.99[0.59,1.35]	0.93[0.57,1.34]	0.75[0.50,1.21]	avelumab	**0.13[0.03,0.38]**	**0.16[0.05,0.50]**
1.36[1.11,1.98]	**1.30[1.10,1.78]**	**1.22[1.01,1.74]**	0.99[0.87,1.71]	**1.32[1.05,2.12]**	ICI+chemotherapy	1.22[0.84,2.59]
1.31[1.09,1.82]	**1.25[1.07,1.63]**	**1.25[1.07,1.63]**	0.95[0.87,1.55]	**1.27[1.01,2.00]**	0.96[0.87,1.06]	chemotherapy

relative risk(RR) with 95% CI for any grade treatment-related AEs

odds ratio(OR) with 95% CI for high-grades treatment-related AEs

FIGURE 3 | Safety and tolerance of different ICIs in network meta-analysis in consistency model. **A**: treatment-related adverse events in different ICIs; **B**: treatment-related adverse events in different ICIs for NSCLC subgroup.

Any grade pneumonitis/ pneumonia in different ICIs

nivolumab								
0.42 [0.03,2.38]	atezolizumab							
0.34 [0.08,1.46]	0.8 [0.11,16.52]	pembrolizumab						
1.25 [0.16,25.47]	2.87 [0.21,229.40]	3.19 [0.51,98.27]	durvalumab					
0.84 [0.17,2.13]	1.88 [0.27,26.71]	2.38 [0.33,11.46]	0.61 [0.02,6.60]	ipilimumab				
0.06 [0.01,1.09]	0.15 [0.01,10.89]	0.19 [0.01,4.36]	**0.05 [0.01,0.63]**	0.08 [0.01,2.46]	avelumab			
0.62 [0.15,4.21]	1.48 [0.18,54.02]	1.74 [0.37,16.43]	0.55 [0.06,2.53]	0.84 [0.14,7.28]	9.5 [0.81,194.10]	ICI+ chemotherapy		
2.43 [0.59,7.93]	5.64 [0.76,99.26]	7.05 [1.22,38.26]	1.91 [0.07,18.04]	2.95 [0.77,14.90]	**37.21 [1.51,688.80]**	3.91 [0.36,19.10]	2ICIs	
0.23 [0.09,0.69]	0.84 [0.09,9.05]	0.66 [0.19,3.33]	**0.2 [0.01,0.97]**	0.29 [0.08,1.67]	3.56 [0.24,47.08]	**0.38 [0.07,0.99]**	**0.09 [0.03,0.46]**	chemotherapy

Any grade colitis in different ICIs

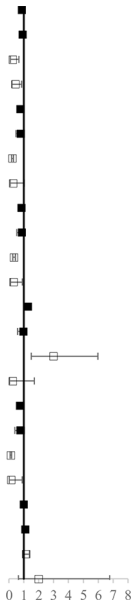
nivolumab								
0.36 [0.01,4.77]	atezolizumab							
0.76 [0.09,4.54]	2.14 [0.09,82.35]	pembrolizumab						
6.36 [1.17,26.43]	**17.65 [1.05,640.30]**	**8.19 [1.21,64.73]**	ipilimumab					
1.46 [0.04,21.60]	4.05 [0.07,267.10]	1.86 [0.06,46.54]	0.23 [0.01,4.45]	avelumab				
2.17 [0.31,11.48]	5.96 [0.30,245.50]	2.77 [0.32,30.86]	0.33 [0.05,2.28]	1.48 [0.08,44.10]	ICI+ chemotherapy			
14.29 [2.83,73.26]	**40.1 [2.33,1722.00]**	**19.31 [2.16,173.30]**	2.23 [0.44,12.37]	10.19 [0.46,329.80]	**6.81 [1.02,44.58]**	2ICIs		
0.15 [0.02,0.80]	0.41 [0.02,15.20]	0.2 [0.02,1.66]	**0.02 [0.01,0.15]**	0.11 [0.01,2.59]	**0.07 [0.01,0.42]**	**0.01 [0.00,0.08]**	chemotherapy	

relative risk (RR) with 95% CI for specific any grade treatment-related AEs

FIGURE 4 | Selected immune-related any-grade AEs in different ICIs.

TABLE 2 | Forest plot of direct and indirect results of head-to-head trials.

Inventions	Study/patients	RR/OR (95% CI)	P	Heterogeneity	
				I^2 (%)	P
Control: chemotherapy					
Nivolumab	5/2,138	0.85 (0.69–1.04)	0.11	97	<0.00001
		0.92 (0.76–1.02)			
		0.25 (0.09–0.67)	0.006	95	<0.00001
		0.42 (0.20–0.86)			
Atezolizumab	2/1,474	0.76 (0.71–0.80)	<0.00001	0	0.46
		0.78 (0.47–0.96)			
		0.23 (0.18–0.30)	<0.00001	0	0.86
		0.28 (0.08–1.02)			
Pembrolizumab	2/1,531	0.84 (0.74–0.96)	0.009	73	0.05
		0.86 (0.54–1.03)			
		0.33 (0.26–0.43)	<0.00001	0	0.41
		0.29 (0.10–0.90)			
Durvalumab	1/447	1.27 (1.11–1.45)	0.0005	NA	NA
		0.97 (0.58–1.13)			
		2.99 (1.50–5.98)	0.002	NA	NA
		0.26 (0.04–1.71)			
Avelumab	1/564	0.74 (0.68–0.81)	<0.00001	NA	NA
		0.75 (0.40–1.03)			
		0.12 (0.08–0.18)	<0.00001	NA	NA
		0.15 (0.02–0.89)			
ICI + chemotherapy	3/1,952	1.00 (0.98–1.01)	0.67	35	0.21
		1.09 (0.96–1.20)			
		1.14 (0.94–1.38)	0.17	0	0.76
		1.99 (0.64–6.77)			
Control: ipilimumab					
2 ICIs	2/276	1.05 (0.93–1.19)	0.44	80	0.03
		0.95 (0.73–1.16)			
		1.36 (0.19–9.52)	0.76	95	<0.00001
		0.97 (0.25–3.66)			
Nivolumab	2/1,529	0.99 (0.80–1.23)	0.94	98	<0.00001
		0.95 (0.75–1.13)			
		0.80 (0.05–12.42)	0.87	99	<0.00001
		0.66 (0.24–1.55)			
Pembrolizumab	1/811	1.08 (0.99–1.17)	0.09	NA	NA
		0.89 (0.53–1.11)			
		0.83 (0.57–1.21)	0.34	NA	NA
		0.46 (0.12–1.40)			



RR in any-grade treatment-related AEs. *OR in high-grade treatment-related AEs.*
Upper is network analysis; below is PWMA. CI, confidence interval; OR, odds ratio; RR, relative risk.

The current analysis has several strengths. By comprehensively including the latest data up to January 2019, we considered all the available evidence on any treatment containing ICIs for NSCLC and melanoma. A detailed assessment of the credibility of the evidence was performed to appraise the results critically. Then, this network meta-analysis was conducted. First, we made a general comparison among all the direct and indirect evidence with different clinical dosages. Thus, a conclusion about the influence of dosage was drawn. Second, we considered any-grade and high-grade AEs from different ICIs to explore the discrepancy among those drugs. A PWMA was also conducted for a head-to-head comparison of the clinical trials of different ICIs. Third, subgroup analyses for NSCLC and melanoma showed different safety and tolerability. Finally, select specific AEs (pneumonitis/pneumonia and colitis) were reported in this review to identify the different immune-related effects.

Limitations also exist in this analysis. Due to the nature of network meta-analyses, missing values always exist in published articles. In the current analysis, we conducted a comprehensive assessment of the evidence we collected and excluded low-quality evidence to improve the quality of this review. Second, some treatments (durvalumab and avelumab) were adopted in only one clinical trial, which might lead to a biased evaluation without enough head-to-head evidence. Third, as the reported AE types were different among the original trials, the specific treatment-related AEs could not be completely evaluated. Thus, we focused on any-grade and high-grade treatment-related AEs as the primary outcome, which could suggest the overall safety and tolerability. Additionally, specific AEs related to ICIs for NSCLC and melanoma were selected to distinguish the differences between tumor types. Third, the incidences of immune-related AEs (including pneumonitis/pneumonia and colitis) were not high, especially those of serious lung toxicities and colitis (Johnson et al., 2018). The low incidence may substantially influence the final results of the indirect comparisons. The influence would be particularly obvious if the specific AE was not reported in the original study. Fourth, this research did not

consider the impact of the different systemic therapies before ICI treatment and the expression level of PD-L1, which might imply inevitable heterogeneity among the included trials.

CONCLUSION

In summary, atezolizumab 1,200 mg and pembrolizumab 2 mg/kg every 3 weeks were generally safer than other ICIs. Nivolumab and pembrolizumab were safer for NSCLC and melanoma than other ICIs, respectively.

AUTHOR CONTRIBUTIONS

Q-QC is the first author of this NMA, and she is responsible for the modification of this paper. J-YD is the second author. JZ is the third author. BW is the corresponding author, and he takes responsibility for the authenticity of the paper and also the modification.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fphar.2019.01260/full#supplementary-material

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Antitumor Activity and Treatment-Related Toxicity Associated With Nivolumab Plus Ipilimumab in Advanced Malignancies: A Systematic Review and Meta-Analysis

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Combining immune checkpoint inhibitors has shown its efficacy compared to monotherapy in advanced malignancies. We conducted this meta-analysis to provide latest evidence on the objective response rate (ORR) and incidence of treatment-related high-grade adverse events (AEs) during nivolumab and ipilimumab combination treatment and further explore from different drug dose level. PubMed and the 2019 American Society of Clinical Oncology (ASCO) annual meeting abstracts were searched for qualified clinical trials up to June 2019. Of the 23 clinical trials (13 from publications and 11 from ASCO abstracts) included, 2,114 and 2,674 patients were eligible for efficacy and safety analysis, respectively. Pooled analysis suggested that the overall ORR was achieved in 34.5% [95% confidence interval (CI), 29.1–40.4%] of patients. There was no significant difference between nivolumab 3 mg/kg plus ipilimumab 1 mg/kg every 3 weeks (N3I1-Q3W) and nivolumab 1 mg/kg plus ipilimumab 3 mg/kg every 3 weeks (N1I3-Q3W) arms in ORR [30.8% vs 41%; odds ratio (OR), 0.72; 95% CI, 0.39–1.30; $P = 0.275$]. Grade 3–4 AEs related to combination therapy occurred in 39.9% (95% CI, 33.5–46.7%) of patients; the most commonly reported grade 3–4 treatment-related AEs were diarrhea (5.28%), colitis (3.96%) and increased alanine aminotransferase (3.51%). Incidence of grade 3–4 AEs were significant lower in N3I1-Q3W arm than in N1I3-Q3W arm (31.3% vs 55.9%; OR 0.52; 95% CI, 0.32–0.87; $P = 0.012$). Treatment-related death was rare and occurred in 2.0% (95% CI, 1.5–2.7%) of patients. Our comprehensive study provides more precise data on the incidence of treatment-related high-grade AEs and ORR among patients receiving nivolumab and ipilimumab combination regimens. Patients on the N3I1-Q3W arm had comparable ORR and significantly occurred less grade 3–4 AEs than patients on the N1I3-Q3W arm. Our finding is of great importance in assisting clinical trial design and clinical medication choice.

Keywords: nivolumab, ipilimumab, combination, dosage, objective response rate, adverse events

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INTRODUCTION

Therapeutic strategies for advanced cancers have dramatically evolved over the past decade. As the traditional chemotherapy gradually couldn't achieve satisfied clinical outcomes in some clinical settings, immune checkpoint inhibitors (ICIs), which specifically target cytotoxic T lymphocyte antigen-4 (CTLA-4) and programmed death-1/ligand-1 (PD-1/PD-L1), have largely altered the treatment predicament in various advanced cancer types (Martins et al., 2019). Compared with monotherapies, combined use of anti-CTLA-4 and anti-PD-1/PD-L1 appears to exert durable response and longer survival benefit in a large proportion of advanced cancer patients (Hodi et al., 2016; Wolchok et al., 2017; Hellmann et al., 2018).

Among all the ICIs, ipilimumab and nivolumab are the most widely used ICI drugs till now, and these two drugs are the earliest and the most frequently used as combination regimens in clinical settings. Ipilimumab is a fully human IgG1 CTLA-4 ICI antibody which block the CTLA-4–B7 interaction and nivolumab is a fully human IgG4 PD-1 ICI antibody which can block the PD-1-PD-L1 interaction between T cells and tumor cells. Both of these two drugs can enhance the T-cell function through different ways in depleting tumor cells and thus might induce clinical response in cancer patients (O'Day et al., 2007; Buchbinder and Desai, 2016).

Accumulating clinical trials has been initiated to evaluate the clinical outcomes of nivolumab plus ipilimumab across various tumor types such as melanoma (Tawbi et al., 2018), lung cancer (Hellmann et al., 2018), renal cell carcinoma (Motzer et al., 2018) and colorectal cancer (Overman et al., 2018). Nevertheless, merely focusing on the response rate and survival benefit brought by the combination use seems insufficient, the treatment-related adverse events (AEs) or immune-related AEs also occur during the ICI treatment. Some AEs were slight and unrecognizable, while other AEs such as grade 3 or more AEs were severe and might lead to treatment discontinuation, hospitalization, and even death (Martins et al., 2019). The frequency and spectrum of high-grade AEs during nivolumab plus ipilimumab combination treatment, however, have not been well investigated. A recent meta-analysis showed that the immunotherapy combination could produce more clinical benefits while with increased high-grade AEs (Wei et al., 2019). Subsequent question was raised that how we clinicians can formulate an optimal combination regimen in reducing the incidence of treatment-related high-grade AEs while not compromising its efficacy at the same time.

Herein, by reviewing the latest evidence in cancer immunotherapy progress, we conducted this meta-analysis trying to exhibit the frequency and spectrum of high-grade/fatal AEs and the objective response rate (ORR) related to nivolumab and ipilimumab combination therapy. We also sought to further explore the outcomes from different drug dose level.

MATERIALS AND METHODS

Search Strategy

We systematically searched the PubMed database to identify the clinical trials that investigated the combined nivolumab and ipilimumab use in cancer patients and report the related results without language restrictions. Besides, the 2019 American Society of Clinical Oncology (ASCO) annual meeting abstracts were also retrieved as potential sources. For PubMed search, the following keywords were used: "Ipilimumab," "Yervoy," "MDX-010," "BMS-734016," "nivolumab," "Opdivo," "BMS-936558," "MDX1106." PubMed search was up to June 1, 2019. We only searched the nivolumab and ipilimumab because they are the most frequently used combined ICIs in clinical trials.

Study Selection

We applied the Population, Intervention, Comparator, Outcome, and Study design (PICOS) approach to identify eligible studies. Clinical trials (S) that investigated nivolumab and ipilimumab combination use (I, C) in advanced cancer patients (P), and provided information on ORR and high-grade AEs (O) were selected. We included all the prospective clinical trials that meet the following items: (1) investigating the combined use of nivolumab and ipilimumab in patients with advanced solid tumors; (2) with results that reported the ORR/the incidence of treatment-related grade 3–4 AEs/the number of treatment-related death; (3) the 2019 ASCO annual meeting abstracts were included if they meet the above two criteria. We excluded trials that: (1) involved combination regimens with other treatment modalities (e.g. nivolumab plus ipilimumab plus radiotherapy); (2) investigated the neo-adjuvant nivolumab combined with ipilimumab in cancer patients; (3) were quality of life analysis or cost-effective assessment of the trials; (4) the results didn't report the specific number or rate of objective response and AEs data. Besides, case reports, editorials, letters and correspondences were excluded. Review and systematic review were screened for potential omitted qualified trials despite they were excluded from our study. In the event of duplicated trials, we selected the most recent trials into our study. Discrepancies regarding the inclusion and exclusion criteria were resolved by consensus (**Figure 1**).

Data Extraction

The data were extracted by 1 reviewer (HX) primarily and were reviewed by another reviewer (PT) following the Preferred Reporting Items for Systematic Reviews and Meta-Analyses guidelines. Efficacy and safety data were separately extracted from trial results. The number of events (objective response, treatment-related grade 3 or more AEs) were recorded. Numbers of objective response were calculated as numbers of partial response + numbers of complete response. In addition, the frequency and spectrum of treatment-related grade 3–4 AEs and fatal AEs (i.e. one specific AE) were also recorded from publications (owing to the limited information on ASCO abstracts, they were not included in this analysis). Besides, information on first author name, ASCO abstracts number, study year, NCT number, phase, cancer type, doses and frequency of nivolumab plus ipilimumab combination, median follow-up duration were also recorded (**Table 1**). For those trials that had multiple arms, we only included the nivolumab plus ipilimumab combination arms and extracted data from each arm. Data were extracted by two reviewers independently and discrepancies were resolved by discussion.

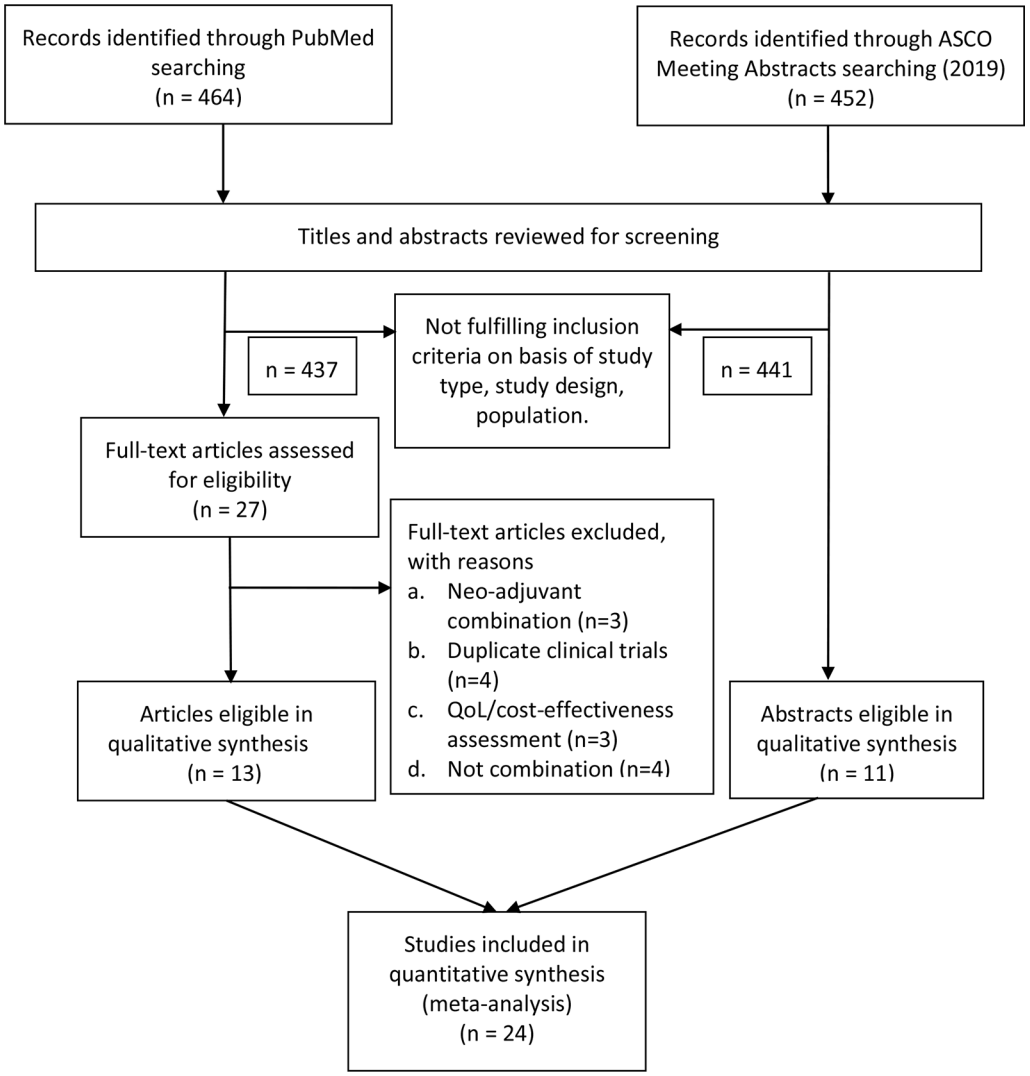

FIGURE 1 | Flow diagram of the eligible trials included in this study. ASCO, American Society of Clinical Oncology; QoL, quality of life.

Statistical Analysis

For efficacy analysis, the number of patients available for efficacy assessment and the number of patients with objective response were recorded from each arm. For safety analysis, the number of patients available for safety assessment and the number of patients with grade 3–4 AEs or fatal AEs were also recorded from each arm. The observed ORR and incidence of treatment-related grade 3–4 or fatal AEs is reported by arm with 95% confidence intervals (CI). Fixed effects models or random effects models were selected according to the heterogeneity. Heterogeneity was assessed according to the I^2 value. The log-odds transformation and restricted maximum likelihood estimation were applied in all models. Besides, the 0.5 adjustment were applied to handle proportions equal to 0 or 1. Meta regression included four variables (sources [publications vs ASCO abstracts], sample size [≥ 100 vs <100], cancer type and different drug dose. Odds ratio [OR] and its corresponding 95% CI were calculated as exponentiate the results from the meta-regression models. Statistical significance was considered as two-side P <0.05. All analyses were conducted using the "meta-for" and "meta" package from R 3.6.0 (R project).

RESULTS

Search Results and Study Characteristics

Four hundred sixty four studies and 452 abstracts were initially retrieved from PubMed search and from 2019 ASCO annual meeting abstracts, respectively. After applying our study selection criteria, 24 clinical trials including 13 trials from PubMed (Wolchok et al., 2013; Antonia et al., 2016b; Hodi et al., 2016; Hammers et al., 2017; Hellmann et al., 2017; Wolchok et al., 2017; D'Angelo et al., 2018; Hellmann et al., 2018; Long et al., 2018; Motzer et al., 2018; Omuro et al., 2018; Overman

TABLE 1 | Baseline characteristics of trials included in this study.

Study	Year	NCT Number	Phase	Cancer Type	Combination Therapy Arms				Median Follow-up	Efficacy, TN	OR, N	Safety, N	Grade 3 or 4, N	FAEs, N
					NIVO		IPI							
					Dosage									
Publications														
Tawbi	2018	02320058	2	Melanoma	1 mg/kg	Q3W	3 mg/kg	Q3W	14	94	48	94	52	1
Omuro	2018	02017717	1	Glioblastoma	1 mg/kg	Q3W	3 mg/kg	Q3W	27.2	10	0	10	9	0
					3 mg/kg	Q3W	1 mg/kg	Q3W		20	2	20	6	0
Motzer[#]	2018	02231749	3	RCC	3 mg/kg	Q3W	1 mg/kg	Q3W	25.2	425	230	547	305	8
Long	2018	02374242	2	Melanoma	1 mg/kg	Q3W	3 mg/kg	Q3W	17	35	16	35	19	0
Hellmann	2018	02477826	3	Lung Cancer	3 mg/kg	Q2W	1 mg/kg	Q6W	11.2*	139	63	576	180	7
D'Angelo	2018	02500797	2	Sarcoma	3 mg/kg	Q3W	1 mg/kg	Q3W	14.2	38	6	42	6	0
Overman	2018	02060188	2	CRC	3 mg/kg	Q3W	1 mg/kg	Q3W	13.4	119	65	119	38	0
Wolchok[#]	2017	01844505	3	Melanoma	1 mg/kg	Q3W	3 mg/kg	Q3W	38	314	183	313	223	2
Hellmann	2017	01454102	1	Lung Cancer	3 mg/kg	Q3W	1 mg/kg	Q12W	12.8	38	18	38	14	0
					3 mg/kg	Q3W	1 mg/kg	Q6W	11.8	39	15	39	13	0
Hammers	2017	01472081	1	RCC	3 mg/kg	Q3W	1 mg/kg	Q3W	22.3	47	19	47	18	0
					1 mg/kg	Q3W	3 mg/kg	Q3W		47	19	47	29	0
					3 mg/kg	Q3W	3 mg/kg	Q3W		NA	NA	6	5	0
Hodi	2016	01927419	2	Melanoma	1 mg/kg	Q3W	3 mg/kg	Q3W	24.5	95	56	94	51	3
Antonia	2016	01928394	1/2	Lung cancer	1 mg/kg	Q3W	3 mg/kg	Q3W	12	61	14	61	18	2
					3 mg/kg	Q3W	1 mg/kg	Q3W	8.7	54	10	54	10	1
Wolchok	2013	01024231	1	Melanoma	0.3mg/kg	Q3W	3 mg/kg	Q3W	NA	14	3	14	6	0
					1 mg/kg	Q3W	3 mg/kg	Q3W		17	9	17	11	0
					3 mg/kg	Q3W	1 mg/kg	Q3W		15	6	16	7	0
					3 mg/kg	Q3W	3 mg/kg	Q3W		6	3	6	4	0
ASCO														
Abstr 2570	2019	02923934	2	Mixed	3 mg/kg	Q3W	1 mg/kg	Q3W	NA	53	17	60	19	0
Abstr 2613	2019	EudraCT 2016-003946-99	2	Mixed	3 mg/kg	Q2W	1 mg/kg	Q6W	4.3	20	1	NA	NA	NA
Abstr 4012	2019	01658878	1/2	HCC	1 mg/kg	Q3W	3 mg/kg	Q3W	24*	50	16	148	55	NA
					3 mg/kg	Q3W	1 mg/kg	Q3W		49	15			
					3 mg/kg	Q2W	1 mg/kg	Q6W		49	15			
Abstr 4517	2019	02982954	3b/4	RCC	3 mg/kg	Q3W	1 mg/kg	Q3W	6.47*	28	8	28	6	0
Abstr 4518	2019	03333616	2	Bladder Cancer	3 mg/kg	Q3W	1 mg/kg	Q3W	3.6	13	4	19	4	0
Abstr 6084	2019	03172624	2	Head and Neck Cancer	3 mg/kg	Q2W	1 mg/kg	Q6W	NA	32	2	32	4	0
Abstr 8563	2019	03083691	2	Lung Cancer	1 mg/kg	Q3W	3 mg/kg	Q3W	NA	18	7	20	NA	2
Abstr 9014	2019	02785952	3	Lung Cancer	3 mg/kg	Q2W	1 mg/kg	Q6W	17.4	123	22	125	48	5
Abstr 9522	2019	01585194	2	Melanoma	1 mg/kg	Q3W	3 mg/kg	Q3W	8.6	30	5	35	14	0
Abstr 11017	2019	02880020	2	GIST	240 mg	Q2W	1 mg/kg	Q6W	NA	12	1	12	4	0
Abstr 11064	2019	03219671	2	Sarcoma	240 mg	Q3W	1 mg/kg	Q6W	3.1	10	5	10	0	0

NIVO, nivolumab; IPI, ipilimumab; OR, Objective Response; TN, total number; FAEs, fatal adverse events; RCC, Renal Cell Carcinoma; HCC, Hepatic Cell Carcinoma; GIST, Gastrointestinal Stromal Tumor; ASCO, American Society of Clinical Oncology; Abstr, abstract; NA, not applicable.

[#]Adverse events data were collected from clinicaltrials.gov.

**Minimum follow-up.*

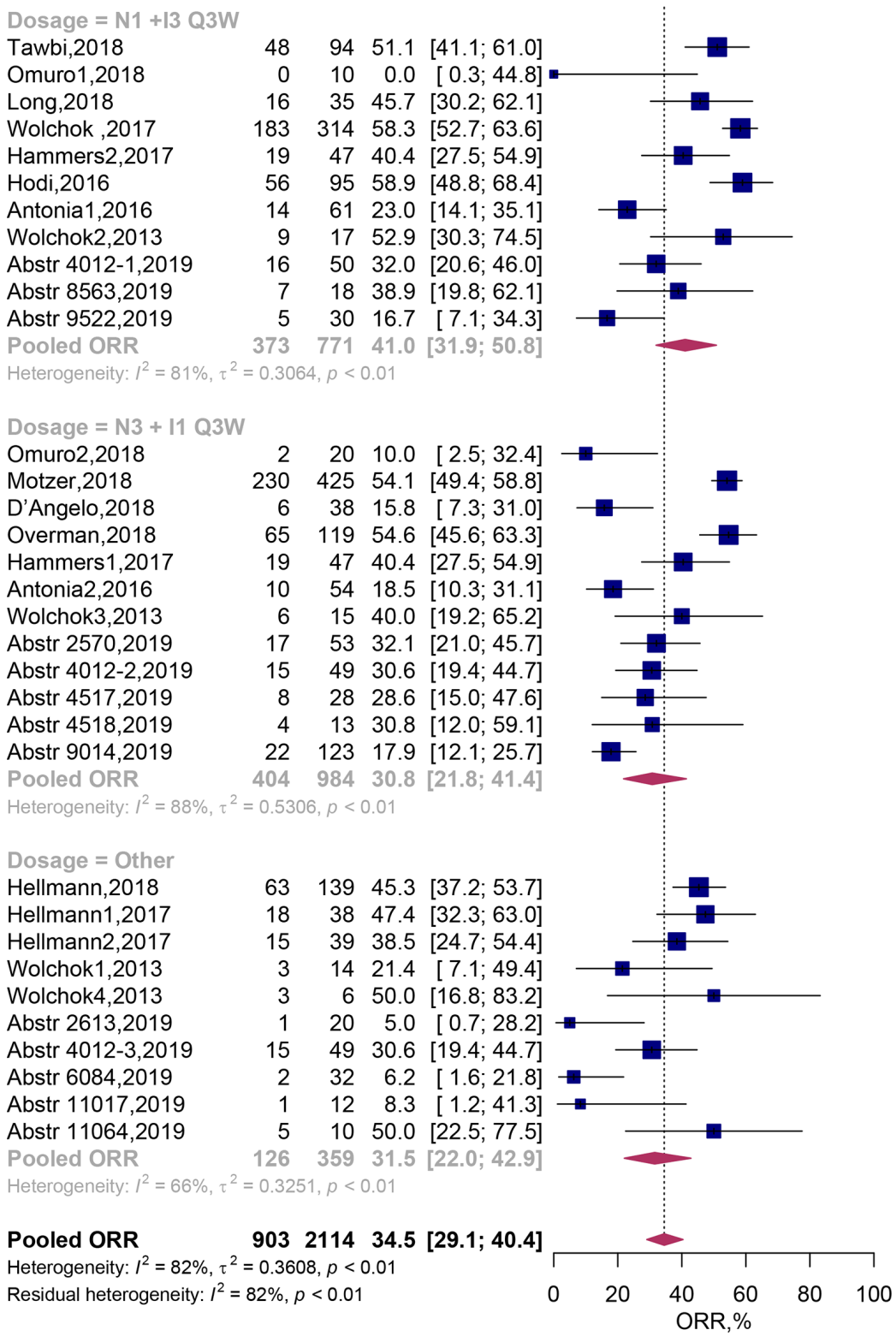

FIGURE 2 | Forest plots of the objective response rate associated with nivolumab and ipilimumab combination treatment. ORR, Objective response rate; N, nivolumab; I, ipilimumab; CI, Confidence interval.

et al., 2018; Tawbi et al., 2018) and 11 trials from ASCO annual meeting (Bazhenova et al., 2019; Emamekhoo et al., 2019; Fischer et al., 2019; Klein et al., 2019; McGregor et al., 2019; Mielgo et al., 2019; Pelster et al., 2019; Singh et al., 2019; Tchekmedyian et al., 2019; Yau et al., 2019; Zer et al., 2019) were finally included in this meta-analysis. The detailed study selection flow diagram can be seen in **Figure 1**.

Of all the trials included, 4, 2, 13, 4 and 1 studies were phase1, phase 1/2, phase 2, phase 3 and phase 3b/4 clinical trial, respectively. For each trial we only included cohorts with nivolumab plus ipilimumab arm, which resulted in 2,114 and 2,674 patients were eligible for efficacy and safety analysis, respectively. The most common cancer types were melanoma (six clinical trials, nine cohorts), lung cancer (five clinical trials, seven cohorts) and renal cell carcinoma (three clinical trials, five cohorts). The most commonly selected dose combination was nivolumab 3 mg/kg plus ipilimumab 1 mg/kg every 3 weeks (N3I1-Q3W, 12 cohorts) and nivolumab 1 mg/kg plus ipilimumab 3 mg/kg every 3 weeks (N1I3-Q3W, 11 cohorts). The median follow-up duration ranged from 3.1 months to 27.2 months. The baseline characteristics of trials included in this study can be seen in **Table 1**.

Objective Response Rate (ORR)

Twenty four clinical trials comprising 33 cohorts (2,114 patients) were available for the ORR analysis. By using random-effects models, the pooled analysis showed the ORR was estimated to be 34.5% (95% CI, 29.1–40.4%; **Figure 2**). Subgroup analysis showed that the predicted ORR was estimated to be 41.0% (95% CI, 31.9–50.8%) in N1I3-Q3W arms and 30.8% (95% CI, 21.8–41.4%) in N3I1-Q3W arms. Multivariate meta-regression analysis showed that there was no significant difference between these two drug doses (N3I1-Q3W vs N1I3-Q3W; OR, 0.72; 95% CI, 0.39–1.30; $P = 0.275$; **Table 2**). The test of residual heterogeneity (after excluding dose level moderator) among studies was statistically significant (Q = 170, $P < 0.0001$, $I^2 = 81.59\%$). While no other study-level factors were found to be associated with ORR (**Supplementary Figure 1A**).

In addition, when we categorized all trial arms according to cancer type, we found that the predicted ORR was achieved in 31.4% (95% CI, 21.7–43.2%) of lung cancer patients, 47.0% (95% CI, 38.2–56.0%) of melanoma patients, 42.8% (95% CI, 31.6–54.8%) of renal cell carcinoma and 24.8% (95% CI, 16.6–35.2%) of patients with other tumor types. Multivariate meta-regression analysis also didn't reveal any significant difference between cancer types (all $P > 0.05$) (**Table 2**).

Treatment-Related Grade 3–4 AEs

Thirty cohorts comprising 2,664 patients were available in assessment of treatment-related grade 3 or 4 AEs. By adopting random-effects models, pooled analysis suggested that grade 3–4 AEs related to the combination therapy occurred in 39.9% (95% CI, 33.5–46.7%) of patients (**Figure 3**). In addition, we recorded the spectrum of these high-grade AEs in our **Table 3**. It exhibited that the most commonly reported grade 3–4 treatment-related AEs were diarrhea [116 (5.28%)], colitis [87 (3.96%)], increased alanine aminotransferase [77 (3.51%)], Increased lipase [66 (3.01%)] and increased aspartate aminotransferase [65 (2.96%)].

TABLE 2 | Meta-regression model results for objective response rate and grade 3–4 adverse events.

Objective Response Rate			
Variable	**Predicted Rate, % (95% CI)**	**Odds Ratio (95% CI)**	***P***
Dosage			
N1 +I3 Q3W	41.0 (31.9–50.8)	Reference	
N3 + I1 Q3W	30.8 (21.8–41.4)	0.72 (0.39–1.30)	0.275
Other	31.5 (22.0–42.9)	0.92 (0.49–1.72)	0.786
Cancer Type			
Lung Cancer	31.4 (21.7–43.2)	Reference	
Melanoma	47.0 (38.2–56.0)	1.74 (0.90–3.35)	0.099
RCC	42.8 (31.6–54.8)	1.76 (0.86–3.62)	0.123
Other	24.8 (16.6–35.2)	1.21 (0.65–2.26)	0.541
Grade 3–4 Adverse Events			
Variable	**Predicted Incidence, (95% CI)**	**Odds Ratio (95% CI)**	***P***
Dosage			
N1 +I3 Q3W	55.9 (44.9–66.3)	Reference	
N3 + I1 Q3W	31.3 (22.7–41.4)	0.52 (0.32-0.87)	0.012
Other	34.1 (27.4–41.5)	0.64 (0.38-1.08)	0.098
Cancer Type			
Lung Cancer	31.9 (27.4–36.8)	Reference	
Melanoma	55.6 (46.4–64.5)	2.23 (1.32–3.75)	0.003
RCC	48.4 (34.4–62.6)	2.31 (1.40–3.80)	0.001
Other	28.4 (20.9–37.2)	1.10 (0.70–1.73)	0.666

RCC, Renal cell carcinoma; CI, Confidence interval.

Subgroup analysis showed that the predicted incidence of treatment-related AEs was 55.9% (95% CI, 44.9–66.3%) in N1I3-Q3W arm and 31.3% (95% CI, 22.7–41.4%) in N3I1-Q3W arm. Multivariate meta-regression analysis showed that patients on the N3I1-Q3W arm were significantly less experience grade 3–4 AEs than patients on the N1I3-Q3W arm (OR 0.52; 95% CI, 0.32–0.87; $P = 0.012$). The test of residual heterogeneity among treatment arms was statistically significant (Q = 144, $P < 0.0001$, $I^2 = 77.42\%$). Still, no other study-level factors were found to be associated with treatment-related grade 3–4 AEs (**Supplementary Figure 1B**).

In addition, when grouping cohorts by cancer type, the predicted incidence of grade 3–4 treatment-related AEs were 31.9% (95% CI, 27.4–36.8%) in lung cancer, 55.6% (46.4–64.5%) in melanoma and 48.4% (95% CI, 34.4–62.6%) in renal cell carcinoma. Incidence of treatment-related grade 3–4 AEs were significant higher in patients with melanoma (OR 2.23; 95% CI, 1.32–3.75; $P = 0.003$) and renal cell carcinoma (OR 2.31; 95% CI, 1.40–3.80; $P = 0.001$), when compared with lung cancer (**Table 2**).

Fatal AEs

Of the 30 combination arms including 2,536 patients, fatal AEs were reported in 31 patients. Pooled meta-analysis using fixed-effects models showed that the incidence of fatal AEs was estimated to be 2.0% (95% CI, 1.5–2.7%; **Figure 4**). Incidence of treatment-related fatal AEs occurred about 2.4% (95% CI, 1.3–4.3%) on the N1I3-Q3W arm and 1.9% (95% CI, 1.2–3.0%)

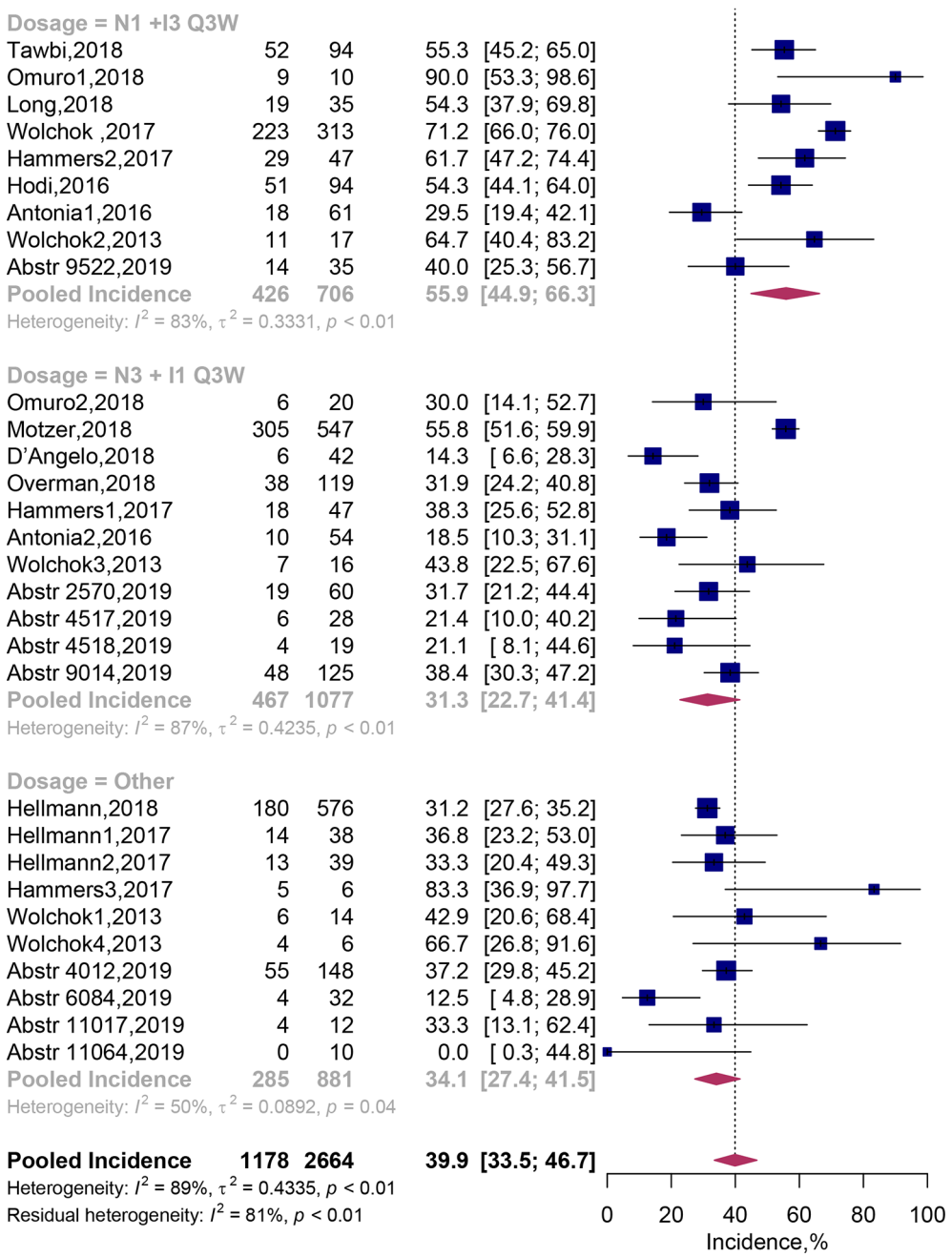

FIGURE 3 | Forest plots of the incidence of grade 3–4 adverse events associated with nivolumab and ipilimumab combination treatment. CI, Confidence interval.

on the N3I1-Q3W arm. In addition, we listed each fatal AE in our **Table 4**. The results showed that incidence of fatal AEs was rare, mostly resulted from respiratory disorders [eight events (0.36%)] and cardiac disorders [seven events (0.32%)]. The most commonly reported fatal AEs was pneumonitis [six events [0.28%)]. The test of residual heterogeneity among treatment arms was not statistically significant (Q = 19, P = 0.8673) (**Supplementary Figure 1**).

DISCUSSION

This meta-analysis investigated the efficacy and safety related to nivolumab and ipilimumab combination therapy in advanced cancer patients. The results showed that roughly 1/3 patients received combined nivolumab and ipilimumab therapy would achieve ORR; meanwhile, nearly 40% of the patients would occur grade 3–4 treatment-related AEs; treatment-related death

TABLE 3 | Incidence of specific grade 3–4 adverse events in included studies (not included ASCO meeting abstracts).

Grade3–4 AEs	Study													Total Events (%)
	Tawbi	Omuro	Motzer	Long	Hellmann	D'Angelo	Overman	Wolchok	Hellmann	Hammers	Hodi	Antonia	Wolchok	
Skin														
Pruritus	0	0	1	0	3	0	2	1	0	0	1	1;0	0	9 (0.41)
Rash	2	0	2	4	9	0	2	0	1;1	0	4	2;0	1;0;1;0	**29 (1.32)**
Maculopapular Rash	7	0	2	0	0	0	0	0	0;1	0	3	2;0	0	15 (0.68)
Gastrointestinal														
Colitis	7	2;1	10	7	3	0	0	31	1;2	0;7;0	12	1;1	1;1;0;0	**87 (3.96)**
Pancreatitis	1	0	3	0	0	0	0	2	1;0	0	2	0	0	9 (0.41)
Gastritis	1	0	1	0	2	0	0	1	0	0	0	0	0	5 (0.23)
Diarrhea	6	7;1	24	7	9	0	2	33	1;0	2;7;1	9	3;1	0;1;2;0	**116 (5.28)**
Vomiting	2	0	5	1	2	0	0	10	0;1	1;0;0	1	1;0	0;1;0;0	**25 (1.14)**
Nausea	2	3;0	8	1	3	0	1	9	0;1	1;0;0	1	1;0	0	**31 (1.41)**
Abdominal Pain	1	0	5	0	0	0	0	5	0	0	0	0	0	11 (0.50)
Hepatic														
Hepatitis	0	0	3	7	5	0	0	4	0	0	2	0	0	21 (0.96)
Acute Hepatitis	1	0	1	0	2	0	0	1	0	0	0	0	0	5 (0.23)
Autoimmune Hepatitis	1	0	0	0	0	0	0	6	0	0	0	0	0	7 (0.32)
Endocrine														
Adrenal Insufficiency	1	0	10	0	9	1	0	7	1;2	0	1	0;1	0	**33 (1.50)**
Hyperthyroidism	3	0	3	0	0	0	2	6	0	0	0	0	0	14 (0.64)
Hypothyroidism	1	0	2	0	2	0	1	2	0	0	0	1;0	0	9 (0.41)
Hypophysitis	5	0	14	1	1	0	0	8	0	0	2	0	0;0;0;1	**32 (1.41)**
Hypopituitarism	0	0	2	1	2	0	0	2	0	0	0	0	0	7 (0.32)
Adrenocortical Insufficiency Acute	0	0	2	0	0	0	0	1	0	0	0	0	0	3 (0.14)
Thyroiditis	0	0	3	0	0	0	0	2	0	0	1	0	0	6 (0.27)
Respiratory														
Pneumonitis	2	0	15	1	13	0	0	6	2;1	0	2	1;1	0	**46 (2.10)**
Dyspnoea	0	0	9	1	0	0	0	6	1;0	0	2	1;2	0	**22(1.00)**
Pulmonary Embolism	0	0;1	4	0	0	0	0	8	1;0	0	0	0	0	14 (0.64)
Respiratory Failure	0	0	2	0	0	0	0	3	0	0	0	0	0	5 (0.23)
Cough	0	0	2	0	0	0	0	1	0	0	0	0	0	3 (0.14)
Musculoskeletal														
Arthritis	0	0	1	0	0	0	0	1	0	0	0	0	0	2 (0.09)
Arthralgia	0	0	4	0	0	0	0	1	0	0	0	0	0	5 (0.23)
Myalgia	0	0	1	0	0	0	0	0	0	0	0	0	0	1 (0.05)
Back Pain	0	0	4	0	0	0	0	2	0	0	0	0	0	6 (0.27)
Pain in Extremity	0	0	1	0	0	0	0	1	0	0	0	0	0	2 (0.09)
Rhabdomyolysis	1	0	1	0	0	0	0	0	0	0	0	0	0	2 (0.09)
Nervous system														
Headache	3	0	3	0	0	0	0	2	0	0;1;2	2	0	0	13 (0.59)
Dizziness	0	0;1	0	0	0	0	0	0	0	0	1	0	0	2 (0.09)
Brain Edema	2	0	1	0	0	0	0	0	0	0	0	0	0	3 (0.14)
Syncope	1	0	2	0	0	0	0	1	0	0	1	0	0	5 (0.23)
Encephalitis	0	0	1	0	0	0	0	1	0	0	0	0	0	2 (0.09)
Meningitis	0	0	2	0	0	0	0	0	0	0	0	0	0	2 (0.09)

(*Continued*)

TABLE 3 | Continued

Grade3–4 AEs	Study													Total Events (%)
	Tawbi	Omuro	Motzer	Long	Hellmann	D'Angelo	Overman	Wolchok	Hellmann	Hammers	Hodi	Antonia	Wolchok	
Renal and Urinary Disorders														
Acute Kidney Injury	1	0	8	0	0	0	0	7	1;0	0	0	0	0	17 (0.77)
Hematuria	0	0	2	0	0	0	0	0	0	0	0	0	0	2 (0.09)
Urinary Tract Infection	0	0	6	0	0	0	0	2	0	0	0	0	0	8 (0.36)
Renal Failure	0	0	0	0	0	0	0	3	0	0	0	0	1;1;1;0	6 (0.27)
Nephritis	0	0	0	1	0	0	0	1	0	0	0	0	0	2 (0.09)
Blood Creatinine Increased	0	0	5	0	0	0	0	0	0	1;1;0	1	0	0	8 (0.36)
Cardiac														
Myocarditis	0	0	1	0	0	0	0	0	0	0	0	0	0	1 (0.05)
Atrial Fibrillation	0	0	2	0	0	0	0	4	0	0	1	0	0	7 (0.32)
Eye														
Diplopia	0	0	2	0	0	0	0	1	0	0	0	0	0	3 (0.14)
Uveitis	1	0	0	0	0	0	0	1	0	0	0	0	0;2;0;0	4 (0.18)
Vascular														
Hypertension	0	0	0	0	0	0	0	1	0	0	0	0	0	1 (0.05)
Hypotension	2	1;0	4	0	0	0	0	2	0	0	2	0	0	11 (0.50)
Hematologic														
Anemia	1	0	6	0	9	1	0	3	0;1	0	0	0;1	0	**22 (1.00)**
Thrombocytopenia	0	0	0	0	0	0	0	1	0	0	0	0;1	0	2 (0.09)
Meabolic														
Hyperglycemia	0	0	0	0	0	0	0	5	0	0	2	1;0	0	8 (0.36)
Diabetes Mellitus	1	0	2	0	0	0	0	1	0;1	0	0	0	0	3 (0.14)
Psychiatric	0	0	0	0	0	0	0	0	0	0	0	0	0	
Confusional State	0	1;0	6	0	0	0	0	3	0	0	0	0	0	10 (0.46)
General														
Decreased Appetite	1	1;0	2	0	3	0	0	2	0	0	0	0	0	9 (0.41)
Fatigue	4	1;3	4	1	8	1	2	5	1;1	0;3;0	5	0	0	**39 (1.78)**
Pyrexia	0	0	18	0	0	0	0	26	0	2;0;1	3	0	0	**50 (2.28)**
Dehydration	2	0	7	0	0	0	0	8	0;1	0;2;0	2	0	0	**22 (1.00)**
Investigations														
Elevated ALT	15	2;2	9	2	4	2	8	3	0;1	2;10;0	10	0;1	2;3;0;1	**77 (3.51)**
Elevated AST	14	1;2	4	2	6	1	9	2	0;1	2;6;0	7	0;1	3;2;1;1	**65 (2.96)**
Increased Lipase Level	8	5;0	1	2	0	2	0	2	3;0	7;13;2	9	5;0	2;1;1;3	**66 (3.01)**
Increased Amylase Level	6	1;0	0	1	1	0	0	0	0	2;3;2	2	1;0	0;2;0;1	**22 (1.00)**
Increased Transaminases	2	0	3	0	0	0	0	8	0;1	0;2;0	1	0;1	0	18 (0.56)
GGT Increased	0	0	0	1	0	0	0	0	0	0	0	0;1	1;0;0;0	3 (0.14)
Hyponatremia	1	0	9	0	0	2	0	2	1;0	0	1	1;0	0	17 (0.77)
Hypokalemia	0	0	0	0	0	0	0	2	1;0	0	0	0	0	3 (0.14)

AEs, adverse events; ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGT, γ-glutamyl transpeptidase.
";" indicates multi-arms in one study by row; Bold values indicate the incidence of a specific adverse event exceeds 1%.

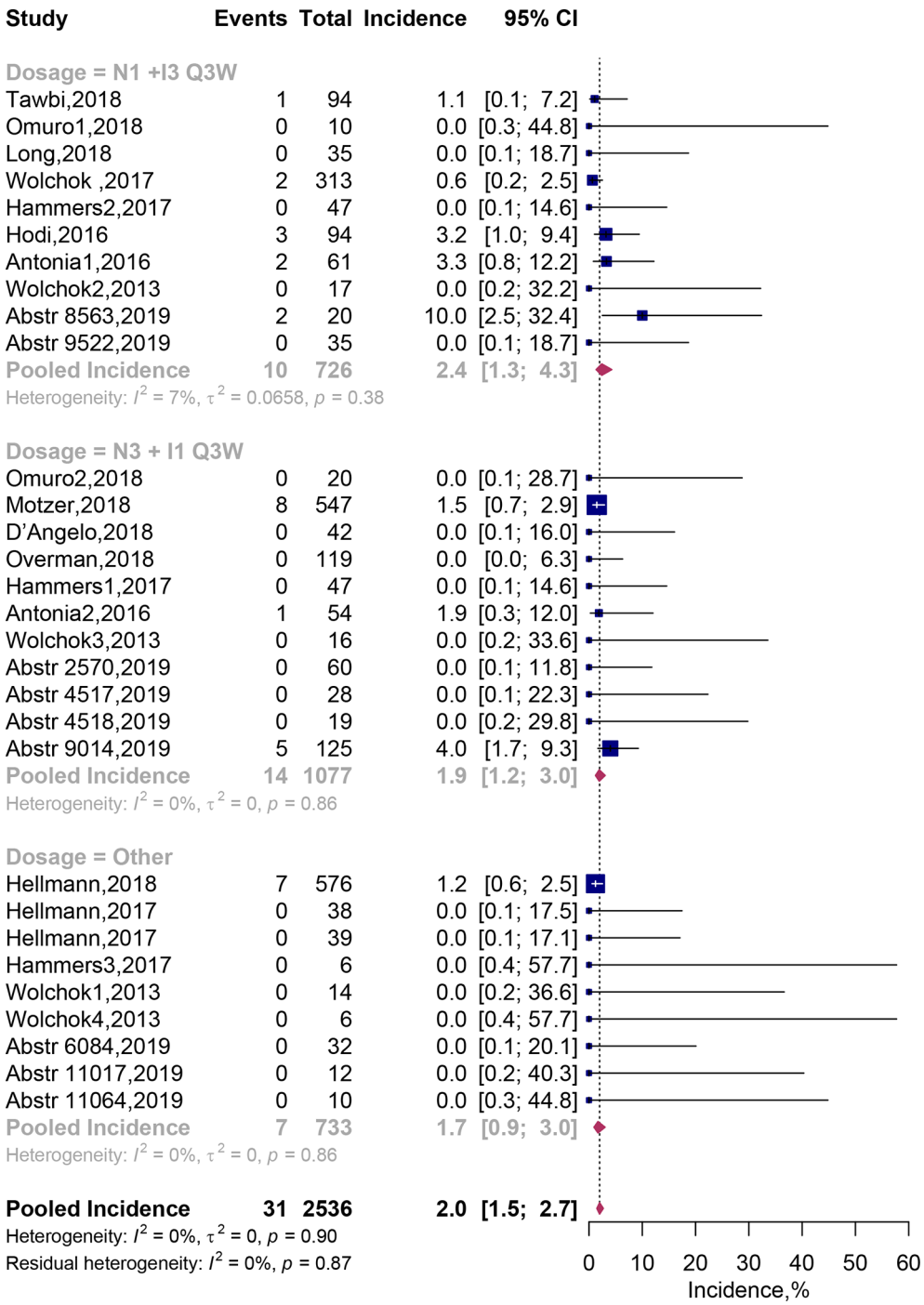

FIGURE 4 | Incidence of fatal adverse events associated with nivolumab and ipilimumab combination treatment. Abstr, Abstract; CI, Confidence interval.

was rare (2%). Moreover, we found that patients on the N3I1-Q3W arm had comparable ORR and significantly experience less grade 3–4 AEs than patients on the N1I3-Q3W arm, suggesting that the N3I1-Q3W regimen might be a better choice when we decided to administrate the combination therapies. By combining the latest clinical trial progress, we were able to draw the spectrum of severe and fatal treatment-related AEs associated with nivolumab plus ipilimumab regimen. Although several previous meta-analyses (Wang et al., 2018; Zhang et al., 2018; Wei et al., 2019) focused on the efficacy and safety of combination ICIs, our study is the first to investigate the estimated ORR and incidence of high-grade treatment-related AEs following the administration of nivolumab plus ipilimumab in solid tumors; moreover, our study is the first that we know of

TABLE 4 | Incidence of specific fatal adverse events (grade 5) in included studies (not included ASCO meeting abstracts).

Fatal adverse events	Study												Total Events (%)
	Tawbi	Omuro	Motzer	Long	Hellmann	D'Angelo	Wolchok	Hellmann	Hammers	Hodi	Antonia	Wolchok	
Cardiac disorders													7 (0.32)
Myocarditis	1				1								2
Autoimmune Myocarditis							1						1
Ventricular Arrhythmia										1			1
Cardiac Insufficiency							1						1
Cardiac Tamponade					1								1
Circulatory Collapse					1								1
Respiratory													8 (0.36)
Pneumonitis			1		3					1	0;1		6
Immune-mediated Bronchitis			1										1
Lung Infection			1										1
Hepatic													2 (0.09)
Liver Toxic Effect			1										1
Liver Necrosis							1						1
Renal													2 (0.09)
Acute Tubular Necrosis					1								1
Renal Failure											1;0		1
Endocrine													2 (0.09)
Panhypopituitarism										1			1
Gastrointestinal													2 (0.09)
Lower Gastrointestinal Hemorrhage			1										1
Hematologic													2 (0.09)
Aplastic Anemia			1										1
The Hemophagocytic Syndrome			1										1
Other													2 (0.09)
Sudden Death			1										1
Myasthenia Gravis											1;0		1

This table only demonstrated the fatal adverse events reported by the included studies.

to compare the efficacy and safety from different drug dose level in combined nivolumab and ipilimumab therapy.

ICIs, including anti-CTL A-4, anti-PD-1 and anti-PD-L1 antibodies, are undoubtedly the most important progress in cancer treatment over the past decade. The indications for these drugs are continuing expanding across many clinical advanced settings, transforming many of the previous standard treatment modalities and bringing new dawn to traditionally "incurable" patients. Clinical evidence has shown the fact that nivolumab combined with ipilimumab could bring more durable responses compared with either agent alone in melanoma or lung cancer patients (Larkin et al., 2015; Antonia et al., 2016b; Hodi et al., 2016; Wolchok et al., 2017). A meta-analysis also concluded that combination ICIs could bring more ORR, progression-free survival (PFS) and overall survival (OS) benefits compared to control arms (Wei et al., 2019). The reason why we select ORR as the main indicator for efficacy of nivolumab and ipilimumab combination rather than PFS or OS is that most of the included studies didn't meet the OS end-point and the definition of PFS is not consistent across various tumor types. The best ORR can be achieved 59% in melanoma in the trial conducted by Hodi et al. (2016). Our study also shows that 47% of melanoma patients receiving nivolumab and ipilimumab combination therapy can achieve complete or partial response. Then comes renal cell carcinoma, in which ORR could be achieved in around 43% of patients. Lung cancer patients only had 31% objective response benefit. Our results might be helpful in patients' selection when the combination ICIs being an option.

Despite combined ICIs therapy showed its efficacy compared to ICI monotherapy in malignancies, however, the treatment-related AEs or immune-related AEs increased accordingly. In a comprehensive network meta-analysis performed by Xu et al., they provided a safety ranking of ICIs in cancer treatment (Xu et al., 2018). Their results demonstrated the pooled incidence of all grade AEs in ICIs combination was 57.7%, while in nivolumab was 14.4% and in ipilimumab was 25.2%. From their study we can know that combined ICIs could increase the AEs incidence, despite this 57.7% associated with ICIs combination might be inappropriate because they only included two trials. Another limitation is that they failed to show treatment-related grade 3–4 AEs associated with ICI combinations. By pooling 30 cohorts comprising 2,664 patients we were able to provide the relatively reliable incidence of grade 3–4 AEs (roughly 40%) related to combination use of nivolumab and ipilimumab, in comparison of 46% in nivolumab and 51% in ipilimumab from Xu's study (Xu et al., 2018). From this point, the combination ICIs therapy might be acceptable and it wouldn't increase the incidence of high-grade AEs compared with monotherapy. In addition, we exhibited the toxicity spectrum of grade 3–5 AEs associated with ICI combination. In a study conducted by Zhao et al. (2018), they demonstrated the most common treatment-related serious AEs were pneumonitis (8.2%), interstitial lung disease (5.6%) and colitis (3.6%) related to nivolumab therapy. While our study demonstrated the most commonly reported grade 3–4 treatment-related AEs were diarrhea (5.28%), colitis (3.96%) and increased alanine aminotransferase (3.51%) in the combination therapy. As for the fatal AEs related to ICI therapy, one meta-analysis found its incidence was 1.23% associated with ICIs combination therapy (Wang et al., 2018), and in our study this index was 2.0%. Regarding the spectrum of the fatal AEs related to ICIs combination, both of us showed the cardiac disorders and pneumonitis were the major cause of treatment-related death, though they rarely happened (< 1%).

In view of the drug doses during ICIs use, previous pooled analyses showed that ipilimumab 10 mg/kg every three weeks had a higher risk of grade 3–4 AEs than 3 mg/kg every three weeks (OR, 3.08; 95% CI, 1.52–6.32) (Xu et al., 2018), and no significant differences were found regarding fatal irAEs across different doses of ipilimumab (3 mg/kg vs 10 mg/kg for ipilimumab monotherapy; 1 mg/kg vs 3 mg/kg for combination ipilimumab therapy) (Wang et al., 2018). These results demonstrated incidence of high-grade AEs ipilimumab might be dose-dependent (Weber et al., 2012; Feng et al., 2013; Eggermont et al., 2016). This might explain our results that incidence of high-grade AEs was significant higher in N1I3-Q3W arm than in N3I1-Q3W arm. N3I1-Q3W is an ideal dose combination which didn't eliminate the efficacy of combination therapy but rather decrease the incidence of grade 3–4 AEs.

Limitations of this study should be stated as well. We performed this meta-analysis from the study level; thus, we were unable to analyze the patient level variables such as patients' sex and previous drug consuming that might affect the outcomes of our results. In addition, a significant proportion of the trials were from ASCO annual meeting abstracts with relatively short follow-up, which might lead to underestimation of their rates and overestimation of drug safety (Saini et al., 2014). Thirdly, published studies only reported the treatment-related AEs with an incidence above ≥1% or ≥5%, and there were only two studies posting their results in clinicaltrials.gov (Wolchok et al., 2017; Motzer et al., 2018); even though we've collected the data from the supplementary materials, some treatment-related grade 3–4 AEs might also be omitted in this study. Fourthly, we only analyzed two typical ICIs combination (nivolumab and ipilimumab) in this study, yet the efficacy or safety profile of other ICI combination [e.g. tremelimumab plus durvalumab (Antonia et al., 2016a; Calabro et al., 2018) and pembrolizumab plus ipilimumab (Long et al., 2017)] still remain unknow.

CONCLUSIONS

In this comprehensive meta-analysis of 23 clinical trials, we provided the efficacy and complete toxicity profile and spectrum of treatment-related grade 3–4 AEs of combining nivolumab and ipilimumab in advanced cancer patients. We found that patients treated with N3I1-Q3W regimen had comparable ORR and experienced significantly less grade 3–4 adverse events than those who treated with N1I3-Q3W regimen. Our finding is of great importance in assisting clinical trial design and clinical medication choice.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this manuscript will be made available by the authors, without undue reservation, to any qualified researcher.

AUTHOR CONTRIBUTIONS

HX and LY conceived and designed the study. HX, PT, JA, SZ, XL, XZ, LY, and QW screened the articles. HX, PT, and JA collated the data. HX, PT, JA, XZ, and SZ interpreted the data. SZ helped in editing the language. All authors drafted and revised critically the manuscript for important intellectual content. All authors gave final approval of the version to be published and have contributed to the manuscript. LY is the guarantor.

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SUPPLEMENTARY MATERIAL

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SUPPLEMENTARY FIGURE 1 | Funnel Plots for studies included in the meta-analysis for objective response rate (ORR), grade 3–4 adverse events (AEs) and fatal adverse events (FAEs).

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REVIEW
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PD-1 Inhibitors in the Advanced Esophageal Cancer

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Esophageal cancer (EC) is a lethal disease, and ranks 7th in incidence and 6th in mortality worldwide. Patients are treated with surgery and/or chemoradiotherapy for a curative intent, but for those with advanced diseases systemic chemotherapy and targeted therapy are the mainstay treatment with poor prognosis. For the patients with squamous cell carcinoma and those progressed after chemotherapy, treatment option is even fewer, and effective treatment modalities are urgently needed. Preclinical and clinical studies have found the PD-1/PD-L1 inhibitors activate T lymphocytes, inhibit cancer growth, and improve survival in cancer patients. Multiple PD-1/PD-L1 inhibitors have been approved for the management of a variety of cancers. Interestingly, a large of proportion of EC patients have tumors with PD-L1 expression and high tumor mutation burden. Trials have been performed to evaluate the efficacy and safety of the PD-1/PD-L1 inhibitors in EC patients. This review will summarize the current progress in this field, especially the toxicities associated with these agents.

Keywords: esophageal carcinoma, gastroesophageal junction adenocarcinoma, PD-1 inhibitor, efficacy, safety

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INTRODUCTION

Esophageal cancer (EC) is a dismal disease, with an estimated 5-year survival rate of only 20%. Histologically, this disease entity is categorized to squamous cell carcinoma (SCC) and adenocarcinoma. In 2018, 572,034 new cases and 508,585 deaths were reported worldwide (Bray et al., 2018). Either surgery alone or with peri-operative chemotherapy is a curative treatment modality for locally advanced stage. For those in their late stages, systemic chemotherapy and targeted therapy are the mainstay treatment (Abdo et al., 2017). Platinum-based chemotherapy regimens, commonly combined with fluoropyrimidine or taxane, are the main treatment, with disappointing objective response rate (ORR) of 23.2% to 60.6%, high incidence of adverse event, and a short overall survival (OS) of 7.7 to 15.5 months. And for the SCC patients and those progressed during or after chemotherapy, the treatment options are more limited. Single-agent chemotherapy, such as paclitaxel, docetaxel, and irinotecan, was recommended, resulting in an ORR of 20% and poor OS of approximately 5 months (Shi et al., 2013; Wang et al., 2013; Shirakawa et al, 2014; Prithviraj et al., 2015; Liu et al., 2016; Hiramoto et al., 2018). In summary, the existing treatments for EC have a limited efficacy and severe adverse events. Effective treatment modalities with moderate adverse event are urgently needed (Thallinger et al., 2011).

Lines of direct and indirect evidence show that the interaction between PD-1 and PD-L1 inhibits the function of T lymphocytes to evade persistent inflammatory or autoimmune reaction. However, this protective mechanism is hijacked by the tumors to escape the immune surveillance through upregulating PD-L1 expression on tumor cells (Mcdermott and Atkins, 2013; Araki et al., 2014; Guillebon et al., 2015;

Chen and Han, 2015). Preclinical and clinical studies have found the PD-1/PD-L1 inhibitors activate T lymphocytes. And activated T lymphocytes help to inhibit cancer growth, and improve survival in cancer patients. PD-1/PD-L1 inhibitors have been approved for the management of a variety cancers, such as melanoma, lung cancer, and renal cell cancer etc. (Weber et al., 2015; Chedgy and Black, 2016; Reck et al., 2016). The efficiency of the PD-1/PD-L1 inhibitors is related to the PD-L1 expression, and/or tumor mutation burden (TMB) in tumor cells (Topalian et al., 2012; Rosenberg et al., 2016; Yarchoan et al., 2017; Hellmann et al., 2018a; Hellmann et al., 2018b; Hellmann et al., 2018c; Rizvi et al., 2018; Keenan et al., 2019). Interestingly, a large proportion of EC patients have tumors with PD-L1 expression (14.5–82.8%, in different reports) and high TMB (Lawrence et al., 2013; Hsieh et al., 2018). Not surprisingly, trials have been initiated to evaluate the efficacy and safety of the PD-1/PD-L1 inhibitors in EC patients.

To this end, four antibodies (pembrolizumab, nivolumab, toripalimab, and camrelizumab) were tested in EC patients. Pembrolizumab and nivolumab are authorized globally for a dozen of cancers, including non-small cell lung cancer, head and neck squamous cell carcinoma, urothelial carcinoma, and so on. Toripalimab and camrelizumab are available in China with the indication for melanoma and classical Hodgkin lymphoma, respectively. Pembrolizumab and nivolumab had similar pharmacokinetic parameters. But no published data on that of toripalimab and camrelizumab are available now. Up to now, no clinical trial to directly compare these antibodies regarding safety and tolerability was reported. One report inferred pembrolizumab and nivolumab had similar safety profile (Wang et al., 2019). Data on direct comparison of clinical efficacy for these antibodies are lacking. This review provided a brief summary of current progress of these antibodies in the field of EC treatment, especially the toxicities associated with these agents.

Data Acquisition

The electronic database including PubMed, Clinical trials (https://clinicaltrials.gov/), Embase, Web of science, Cochrane library were retrieved by using the Keywords "esophageal cancer," "esophageal carcinoma," "immunotherapy," "PD-1," "PD-L1," "clinical trial." The literature in abstract form was viewed, and those with only protocol design or preliminary results were excluded. Finally, 12 studies involving PD-1 inhibitor monotherapy with full description of the outcome were selected.

Pembrolizumab

Pembrolizumab is a humanized IgG4 antibody for PD-1. In a pilot phase 1b study, KEYNOTE-012, pembrolizumab was first tested in patients with PD-L1-positive recurrent or metastatic gastroesophageal junction (GEJ) and gastric adenocarcinoma, without limitation on the number of lines of previous therapy (Muro et al., 2016). Thirty-nine patients were enrolled and received pembrolizumab 10 mg/kg every 2 weeks. The ORR was 22%. Median progression-free survival (mPFS), median OS (mOS), and duration of response (DOR) were 1.9 months (mo), 11.4 mo, and 40 weeks. Treatment-related adverse events (TRAEs) occurred in 67% patients, grade 3 or 4 TRAEs in 13% patients (n = 5). Fatigue (18%), decreased appetite (13%), hypothyroidism (13%), pruritus (13%), and arthralgia (10%) were the most common TRAEs. Grade 3–4 TRAEs included grade 3 fatigue (n = 2), grade 3 pemphigoid (n = 1), grade 3 hypothyroidism (n = 1), grade 3 peripheral sensory neuropathy (n = 1), and grade 4 pneumonitis (n = 1). There were no treatment-related death or discontinuation of drugs due to TRAE.

Phase 2 trial KEYNOTE-059 investigated the efficacy and safety of pembrolizumab monotherapy in the late (≥3) lines of therapy (Fuchs et al., 2018). Two hundred fifty-nine patients with similar features as those in KEYNOTE-012 study were enrolled, except for no requirement of PD-L1 expression. Pembrolizumab was given every 3 weeks (at fixed dose of 200 mg). The ORR for the intention-to-treatment (ITT) cohort was 11.6%, and in PD-L1-positive and -negative cohorts, it was 15.5% and 6.4%, respectively. The mDOR for ITT, PD-L1-positive, and PD-L1-negative patients was 8.4 mo, 16.3 mo, and 6.9 mo, respectively. The mPFS and mOS of ITT patients were 2 mo and 5.6 mo. TRAEs of any grade and grade 3–5 occurred in 60.2% and 17.8% patients. Fatigue, pruritus, rash, hypothyroidism, decreased appetite, anemia, nausea, diarrhea, and arthralgia were the most common TRAEs. There were two treatment-related deaths and two cases of treatment-related discontinuation.

The efficacy of pembrolizumab in the second-line therapy was tested in a randomized controlled phase 3 trial KEYNOTE-061 (Shitara et al., 2018). Five hundred ninety-two patients with advanced GEJ or gastric adenocarcinoma who progressed after chemotherapy regimen of fluoropyrimidine and platinum were enrolled. Pembrolizumab (200 mg) every 3 weeks for up to 2 years or paclitaxel 80 mg/m^2 on days 1, 8, 15 in a 4-week cycle was administered. In population with PD-L1 ≥ 1% (PD-L1 CPS ≥ 1), the mOS of pembrolizumab and chemotherapy was 9.1 and 1.5 mo. And mPFS was 8.3 and 4.1 mo, respectively. The ORR of pembrolizumab and chemotherapy was 16% and 14%, and mDOR was 18 and 5.2 mo. In the ITT population, TRAEs occurred in 53% and 84% patients receiving pembrolizumab and chemotherapy, and for grade 3–5 TRAEs, the incidence was 14% and 35%. The most common grade 3–5 TRAE for pembrolizumab were anemia and fatigue. Three percent of the patients in pembrolizumab group discontinued treatment because of TRAEs. The mortality rate was 1% in pembrolizumab group.

KEYNOTE-062 was a phase 3 trial to investigate pembrolizumab with (p+c) or without (p) chemotherapy versus chemotherapy (c, cisplatin, and fluoropyrimidine) for the first-line treatment (Tabernero et al., 2019). This study was also conducted in the GEJ and gastric adenocarcinoma with PD-L1 CPS ≥ 1. Totally, 763 patients were enrolled. Pembrolizumab monotherapy compared with chemotherapy did not show any survival benefit. In patients with PD-L1 CPS ≥ 10, pembrolizumab monotherapy showed improved mOS over chemotherapy (17.4 and 10.8 mo), but inferior mPFS (2.9 and 6.1 mo) and ORR (25% and 36.7%). P+c vs c did not show any benefit in OS and PFS regardless of patients PD-L1 CPS status (CPS ≥ 1 or CPS ≥ 10). Grade 3–5 TRAE rates were 17% (p), 73% (p+c), and 65% (c).

Pembrolizumab was also tested in other histological types, mainly SCC. KEYNOTE-028 was a phase 1b study similar to KEYNOTE-012 study, to explore the efficacy and safety of

pembrolizumab in late-line treatment (87% patients had received ≥2 lines of treatment) for all histological types (including SCC, adenocarcinoma, (Doi et al., 2018). Twenty-three patients with PD-L1-positive tumors were enrolled. The incidence rate of TRAEs was 39%, and the mOS was 7 months.

A phase 2 trial KEYNOTE-180 was similar to KEYNOTE-059 study, investigating pembrolizumab monotherapy in the setting of late (≥3) lines of therapy (Shah et al., 2019). But this study recruited patients of all histological types. PD-L1(+) was mandatory, defined as CPS ≥ 10. One hundred twenty-one patients were enrolled. The ORR was 14.3% among SCC patients, and 5.2% among adenocarcinoma patients. The mPFS and mOS were 2 and 5.8 mo. Subgroup analysis showed mOS was better in patients with SCC. The incidence rates of TRAEs and grade 3–5 TRAEs were 57.9% and 12.4%, respectively. The most commonly TRAEs included fatigue, rash, pruritus, hypothyroidism, and diarrhea. Treatment-related discontinuation (n = 5) and death (n = 1) were reported.

KEYNOTE-181 was a phase 3 trial similar to KEYNOTE-061, where pembrolizumab was used in the second line of therapy, except for recruitment of all histotypes (Kojima et al., 2019). Six hundred twenty-eight patients were enrolled. In the ITT population, pembrolizumab compared with chemotherapy did not show significant benefit in mOS and mPFS. But in subgroup of patients with PD-L1 CPS ≥ 10, pembrolizumab treatment led to longer mOS over chemotherapy (9.3 and 6.7 mo) with statistical significance. The ORR was also improved (21.5% and 6.1%) in this subpopulation. The incidence rate of TRAEs of pembrolizumab and chemotherapy were 64.3% and 86.1%. The incidence rates of grade 3–5 TRAEs were 18.2% and 40.7%, respectively. There was no significant difference in treatment-related discontinuation (6.1% vs 6.4%) and death (1.5% vs 1.7%).

Nivolumab

Nivolumab is another humanized IgG4 monoclonal antibody for PD-1 immune checkpoint. For patients with advanced GEJ and gastric adenocarcinoma, nivolumab was tested in trial ATTRACTION-2 (in Asia) and CheckMate-032 (in Western countries).

ATTRACTION-2 was a randomized, double-blind, placebo-controlled, phase 3 trial to evaluate the efficacy and safety of nivolumab in heavily pretreated adenocarcinoma (Kang et al., 2017). Four hundred ninety-three patients were enrolled and were randomly assigned (2:1) to receive nivolumab 3 mg/kg or placebo every 2 weeks. The mOS of nivolumab and placebo was 5.26 and 4.14 mo, and the mPFS was 1.61 and 1.45 mo. The ORR and mDOR of nivolumab were 11.2% and 9.53 mo. The incidence of TRAEs and grade 3–5 TRAEs of nivolumab was 43% and 10%. The common TRAEs included pruritus, diarrhea, rash, and fatigue. In the nivolumab group, nine cases of treatment discontinuation and five deaths occurred.

Conducted in a cohort with similar demographic features, CheckMate-032 was a phase 1/2 trial where nivolumab monotherapy or nivolumab plus ipilimumab were administered (Janjigian et al., 2018). One hundred sixty patients were enrolled. The treatment consisted three arms: either nivolumab 3 mg/kg every 2 weeks (n = 59), or nivolumab 1 mg/kg plus ipilimumab 3 mg/kg every 3 weeks for four cycles (N1I3, n = 49), or nivolumab 3 mg/kg plus ipilimumab 1 mg/kg every 3 weeks for four cycles (N3I1, n = 52). After four cycles, all patients were maintained on nivolumab 3 mg/kg therapy. For the group of nivolumab monotherapy, N1I3, and N3I1, the ORR was 12%, 24% and 8%. The mOS was 6.2, 6.9, 4.8 mo, and the mPFS was 1.4, 1.4, and 1.6 mo. The incidence rates of TRAEs and grade 3–4 TRAEs of these groups were 69%, 17%, 84%, and 47%, 75%, 27%, respectively. The most frequently occurred TRAEs were fatigue, pruritus, rash, diarrhea, decreased appetite, and increased transaminase. The incidence rates of discontinuation of drug related to TRAEs were 3%, 20%, and 13% in three groups.

Another phase 2 study ATTRACTION-1 was conducted in Japan, where patients with EC were enrolled (Kudo et al., 2017). Sixty-five patients with heavily treated SCC were enrolled and received nivolumab 3 mg/kg every 2 weeks. The ORR was 17%, mOS was 10.8 months, and mPFS was 1.5 months. The incidence rates of TRAEs and grade 3 or worse TRAEs were 60% and 17%, respectively. The most common adverse events were diarrhea, decreased appetite, constipation, rash, and fatigue. Seven patients discontinued therapy due to TRAEs, and no death related to TRAE occurred.

Toripalimab (JS001) and Camrelizumab (SHR1210)

Toripalimab and camrelizumab are two of Chinese domestic me-too antibodies in this class. A phase 1b/2 trial (Clinicaltrial identifier: NCT02915432) evaluated the efficacy and safety of toripalimab in refractory/metastatic esophageal SCC (Xu et al., 2018). Fifty-six patients were enrolled and received toripalimab at the dose of 3 mg/kg every 2 weeks. Till September 2017, 34 patients were evaluated, and 8 patients achieved partial response with an ORR of 23.5%. TRAEs were mostly grade 1 or 2. Another trial (NCT02742935) was a dose-escalating phase 1 study investigating the efficacy and safety of camrelizumab in ≥2 line treatment of esophageal SCC (Huang et al., 2018). The dose was given at 60, 200, and 400 mg every 2 weeks. The ORR was 33.3% and the mPFS was 3.6 months. The incidences of TRAEs and grade 3 TRAEs were 83.3% and 10%, respectively. The most common TRAEs included reactive capillary hemangiomas, pruritus, hypothyroidism, and fever. There was no treatment-related discontinuation due to toxicity.

DISCUSSION

EC is a lethal disease affecting millions of people worldwide. Histologically, it is composed of two main subtypes, i.e., SCC and adenocarcinoma. They differ to a large extent in their genetic aberrations, epidemiology, etiology, and clinical manifestations. Thus, the two subtypes should have distinct strategy of therapy. Previously radio- and chemo-therapy remain the mainstay of the therapy for those unsuitable for surgery. Targeted therapy including anti-angiogenesis agents and epidermal growth factor receptor inhibitors

obtains authorization for the treatment of adenocarcinoma, but not SCC. Therefore, there is a large unmet need for the improvement of SCC treatment. It is in high expectance that the immune checkpoint inhibitors help to advancing the progress in this field. Our summary showed that most of the studies were performed in adenocarcinoma till now, but the trends toward SCC became obvious (**Figure 1**).

The current review summarized 12 trials on PD-1 inhibitor monotherapy for the treatment of advanced EC, including phase 3 (n = 4) and phase 1/2 trials (n = 8). Among them, KEYNOTE-062 is the only one investigating pembrolizumab monotherapy in the first-line treatment. Both KEYNOTE-061 and KEYNOTE-181 investigated the efficacy of pembrolizumab monotherapy in the second-line treatment. The rest nine trials investigated efficacy and safety of PD-1 inhibitors in late lines. The immune checkpoint inhibitors showed promising results, with minimal to mild toxicities (**Figure 2**). TRAEs in EC were similar to those reported in other solid tumors, and no unexpected TRAEs occurred (Topalian et al., 2012; Garon et al., 2015; Ferris et al., 2016; Tomita et al., 2017).

It was interesting to compare the PD-1/PD-L1 inhibitors and traditional chemotherapy for EC patients. The two modalities were compared in a head-to-head fashion in three of the trials (**Table 1**). Although relative a small sample, a clear trend could be easily found favoring the former, with elevated ORRs, prolonged PFS and OS, and less frequency of any-grade or grade 3–5 AE in immunotherapy.

Pembrolizumab, nivolumab, and toripalimab had similar incidence of TRAE (**Figure 2**, 39%–67%, 43%–60%, 62.7%, respectively), lower than that of camrelizumab (83.3%). Also, grade 3–5 TRAEs seemed less likely in pembrolizumab

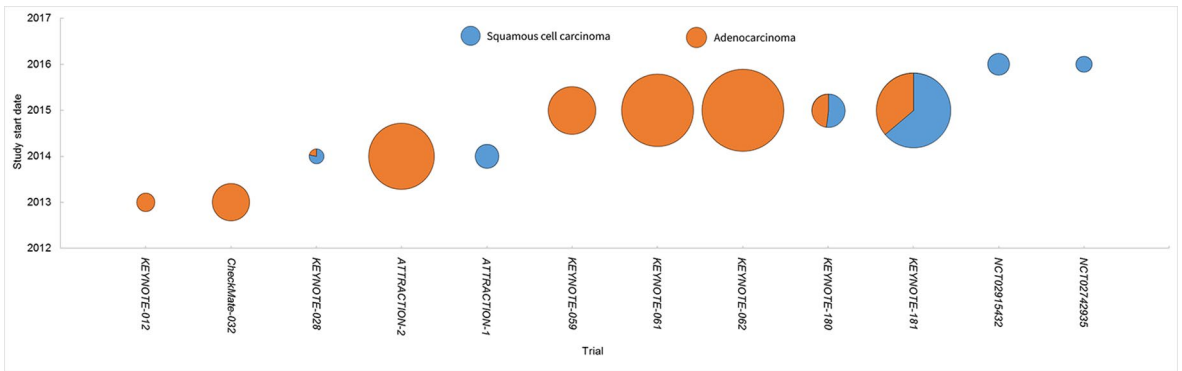

FIGURE 1 | Clinical studies of PD-1 inhibitors in EC. Each trial was plotted against the year of the initiation. The circle area denoted the sample size, and SCC and adenocarcinoma were depicted in different colors.

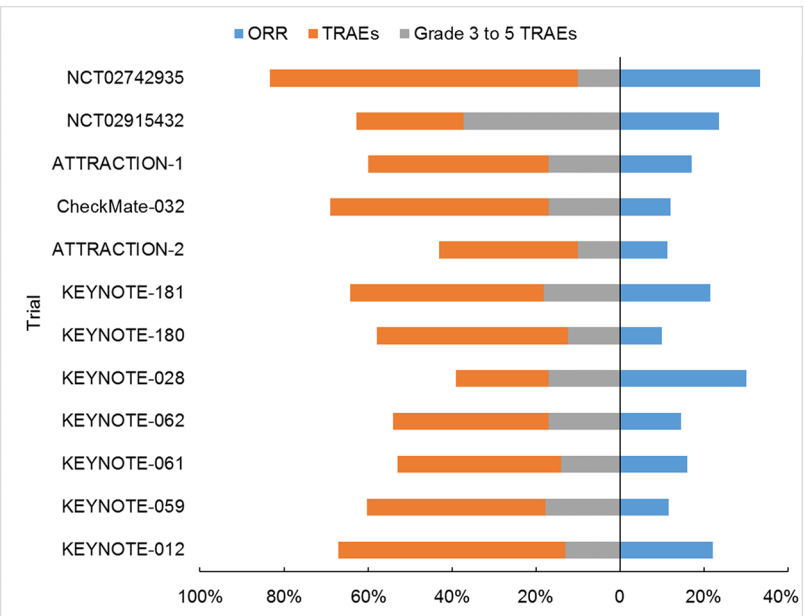

FIGURE 2 | Summary of toxicities and ORRs in each study.

TABLE 1 | The comparison of PD-1 inhibitors and chemotherapy.

Trial	Drug	PD-L1 CPS	ORR (%)	DOR (mo)	PFS (mo)	OS (mo)	AEs (%)	AE ≥Grade 3 (%)
		<1	2 vs 10.4	NA	NA	4.8 vs 8.2		
KEYNOTE-061	Pembro vs Chemo	≥1	16 vs 14	8 vs 5.2	1.5 vs 4.1	9.1 vs 8.3	53 vs 84	14 vs 35
		≥10	24.5 vs 9.1	NA	NA	10.4 vs 8		
KEYNOTE-062	Pembro vs Chemo	≥1	14.5 vs 36.8	NA	2 vs 6.4	10.6 vs 11.1	54.3 vs 91.8	17 vs 69
		≥10	25 vs 36.7	NA	2.9 vs 6.1	17.4 vs 10.8		
KEYNOTE-181	Pembro vs Chemo	All comer	13.1 vs 6.7	8.5 vs 10.7	2.1 vs 3.4	7.1 vs 7.1	64.3 vs 86.1	18.2 vs 40.9
		≥10	21.5 vs 6.1	9.3 vs 7.7	2.6 vs 3	9.3 vs 6.7		

TABLE 2 | Ongoing phase 2/3 trial with PD-1 inhibitor combined with chemotherapy in first-line treatment of esophageal carcinoma.

Trial	Phase	Status	Drug	Tumor	Treatment
KEYNOTE-590—China Extension Study	3	Recruiting	Pembrolizumab+Cisplatin+5-FU/Placebo+Cisplatin+5-FU	Esophageal Carcinoma	First-line
KEYNOTE-590	3	Active	Pembrolizumab+Cisplatin+5-FU/Placebo+Cisplatin+5-FU	Esophageal Carcinoma	First-line
NCT02954536	2	Recruiting	Pembrolizumab +Trastuzumab+ Chemotherapy (Capecitabine/5-Fluorouracil+Cisplatin/Oxaliplatin)	Esophagogastric Carcinoma	First-line
NCT03342937	2	Recruiting	Pembrolizumab + Oxaliplatin +Capecitabine	Esophagogastric Carcinoma	First-line
NCT03615326	3	Recruiting	Pembrolizumab+Trastuzumab+Chemotherapy/Placebo+Trastuzumab+Chemotherapy (Capecitabine/5-Fluorouracil/S-1+Cisplatin/Oxaliplatin)	Gastroesophageal junction and gastric adenocarcinoma	First-line
Checkmate 648	3	Recruiting	Nivolumab + Ipilimumab/Nivolumab + Cisplatin + Fluorouracil/Cisplatin + Fluorouracil	Esophageal Carcinoma	First-line
NCT03409848	3	Recruiting	Nivolumab and Trastuzumab +Ipilimumab/FOLFOX	Esophagogastric Carcinoma	First-line
NCT03829969	3	Recruiting	JS001 +paclitaxel +cisplatin/placebo +paclitaxel +cisplatin	Esophageal Squamous Cell Carcinoma	First-line
NCT03691090	3	Recruiting	SHR-1210 + paclitaxel + cisplatin/placebo +paclitaxel +cisplatin	Esophageal squamous cell carcinoma	First-line
NCT03603756	2	Recruiting	SHR-1210 + Apatinib+ Chemotherapy (irinotecan/paclitaxel+ nedaplatin)	Esophageal Squamous Cell Carcinoma	First-line

(12.4%–18.2%), nivolumab (10%–17%), camrelizumab (10%) than toripalimab (37.3%). The incidence of immune-related adverse events (irAEs) was 18% to 26% in pembrolizumab, 10.2% in toripalimab, and 83.3% in camrelizumab. It should be noted the toxicities of toripalimab and camrelizumab were both extracted from small-sized, phase 1 studies, and might be over-estimated.

Next, specific AE was analyzed. Because the information was lacking for toripalimab and camrelizumab, only pembrolizumab and nivolumab were compared. A consistent higher incidence was observed in hyperthyroidism (3.5%–7.7% and 1%), hypothyroidism (7.4%–12.8% and 0), pneumonitis (1.9%–4.9% and 0.3%), colitis (1%–2.6% and 1%), and hepatitis (0.4%–2.6% and 0) for pembrolizumab than nivolumab. For the severe (grade 3–5) irAE, pembrolizumab also had worse record in hypothyroidism (0.4%–2.5% and 0), pneumonitis (0.3%–2.6% and 0.3%), colitis (0.3%–1.2% and 0.3%), and hepatitis (0.4%–1% and 0) than nivolumab. But it was imprudent to make direct comparison of data from different trials. For EC treatment, these four agents had comparable safety and efficiency, based on the direct comparison of their reported outcomes (**Figure 2**). This conclusion also got supports from the biochemical features of these drugs. They are monoclonal antibodies blocking PD-1, and they have the same, if any difference, of action mechanism.

From these trials, one reasons that PD-1 inhibitors would play a role in the treatment of advanced EC. But the question is when and how to apply these agents appropriately. At this time point, Food and Drug Administration (FDA) authorized pembrolizumab for the late (≥2) line treatment for the cancer patients whose tumors harbor high TMB, irrespective of tissue origin, also including those with EC. Additionally, FDA approved pembrolizumab for the 2-line treatment for the patients with SCC with CPS ≥ 10 and for the 3-line treatment for the patients of with GEJ and gastric adenocarcinoma with PD-L1 CPS ≥ 1. Based on the encouraging results, PD-1 inhibitor combined with chemotherapy for the first-line therapy for EC is in underway (**Table 2**).

CONCLUSION

In general, PD-L1 inhibitor monotherapy in the treatment of pretreated EC has a promising antitumor activity and manageable toxicity.

AUTHOR CONTRIBUTIONS

Z-YD and YH contributed conception and overall idea of the study. YH wrote the first draft of the manuscript. Z-YD wrote sections of the manuscript. Both authors contributed to manuscript revision, read and approved the submitted version.

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REVIEW
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Immune Checkpoint Inhibitor-Associated Cardiotoxicity: Current Understanding on Its Mechanism, Diagnosis and Management

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Immune checkpoint inhibitors (ICIs) that target cytotoxic T lymphocyte antigen 4, programmed cell death-1, and PD-ligand 1 have revolutionized cancer treatment, achieving unprecedented efficacy in multiple malignancies. ICIs are increasingly being used in early cancer settings and in combination with various other types of therapies, including targeted therapy, radiotherapy, and chemotherapy. However, despite the excellent therapeutic effect of ICIs, these medications typically result in a broad spectrum of toxicity reactions, termed immune-related adverse events (irAEs). Of all irAEs, cardiotoxicity, uncommon but with high mortality, has not been well recognized. Herein, based on previous published reports and current evidence, we summarize the incidence, diagnosis, clinical manifestations, underlying mechanisms, treatments, and outcomes of ICI-associated cardiotoxicity and discuss possible management strategies. A better understanding of these characteristics is critical to managing patients with ICI-associated cardiotoxicity.

Keywords: immune checkpoint inhibitors, cardiotoxicity, myocarditis, pericarditis, cytotoxic T lymphocyte-associated antigen-4, programmed cell death protein 1, programmed cell death-ligand 1

INTRODUCTION

The immune system employs several suppressive molecules and pathways to maintain T lymphocyte cell tolerance and prevent autoimmunity (Boussiotis, 2016). Cytotoxic T lymphocyte-associated antigen-4 (CTLA-4), a coinhibitory molecule, is expressed on stimulated $CD4^+$/$CD8^+$ T cells to attenuate T cell activation. Of note, CTLA-4 also constitutively resides on $Foxp3^+$ regulatory $CD4^+$ T cells and directly facilitates the inhibitory function of regulatory T cells (Peggs et al., 2009; Walker and Sansom, 2011). Moreover, the programmed cell death protein 1 (PD-1)/programmed cell death-ligand 1 (PD-L1) pathway plays a predominant role in regulating T-cell-driven immune response. PD-1 exists inherently on the surface of T cells and is expressed on antigen-presenting cells (APCs), such as on macrophages and dendritic cells. The binding of these two molecules (PD1/PD/L1) can inhibit the immune response by reducing cytokine production and suppressing T-cell proliferation (Swaika et al., 2015). Intriguingly, numerous cancer cells overexpress PD-L1 on their surface, which

contributes to their immune evasion by enhancing immune escape ability, resulting in a poor prognosis (Hino et al., 2010).

Based on the inhibitory roles of these checkpoint molecules or pathways, several immune checkpoint inhibitors (ICIs), including PD-1 inhibitors (nivolumab and pembrolizumab), PD-L1 inhibitors (atezolizumab, avelumab, and durvalumab), and CTLA-4 inhibitors (ipilimumab and tremelimumab) have been developed to restore the T cell-mediated immune response and improve the efficacy of anti-tumor treatments (Wolchok, 2015; Ribas and Wolchok, 2018). Encouragingly, these agents have revolutionized the treatment of various hematological and solid tumors (Powles et al., 2014; Tumeh et al., 2014; Pi et al., 2016).

The combination of ICIs, either use of multiple ICIs or ICIs combined with other therapies, such as chemotherapy, radiation, and anti-angiogenic drugs, has been associated with a significantly better prognosis than monotherapy (Larkin et al., 2015a). However, these reagents, both alone and in combination, also produce a wide spectrum of immune-related adverse events (irAEs), mainly due to aberrant autoreactive T cell activation (Weber et al., 2012; Nishino et al., 2015; Postow et al., 2018). Immune-mediated toxicities can affect any organ or tissue involving the skin, gastrointestinal system, endocrine system, lung, or liver (Champiat et al., 2016; Michot et al., 2016) and can largely be controlled by glucocorticoid therapy (Friedman et al., 2016). Among these toxicities, cardiotoxicity, a potentially fatal irAE, has rarely been reported in early clinical trials of ICI therapy because of its low incidence and nonspecific symptomatology (Mahmood et al., 2018a). Over the years, although increasing cases and case series of ICI-associated cardiotoxicity have been reported, it has not been fully recognized (Varricchi et al., 2017). Herein, to strengthen understanding of cardiotoxicity induced by ICIs and reduce deaths, we elaborate on the incidence, clinical manifestations, diagnosis, mechanisms, and outcomes of cardiotoxicity associated with ICIs. We will also discuss prophylactic strategies, potential treatments, and management of ICI-associated cardiotoxicity based on relevant literatures and current knowledge.

INCIDENCE AND CLINICAL MANIFESTATIONS OF IMMUNE CHECKPOINT INHIBITOR-ASSOCIATED CARDIOTOXICITY

Since the first specific case of ICI-associated cardiotoxicity was reported in 2014 (Heery et al., 2014), cardiotoxicity during ICI treatment has been reported in a gradually increasing number of patients (Geisler et al., 2015; Laubli et al., 2015; Heinzerling et al., 2016; Johnson et al., 2016a; Koelzer et al., 2016; Tadokoro et al., 2016; Behling et al., 2017; Norwood et al., 2017; Reuben et al., 2017; Tajmir-Riahi et al., 2018). Cardiotoxicity attributed to ICIs spreads to almost all parts of the heart (**Figure 1**) and involves both inflammatory cardiotoxicity and non-inflammation-mediated cardiotoxicity. The former includes myocarditis, perimyocarditis, pericarditis, left ventricular dysfunction without myocarditis, and others (Lyon et al., 2018). The latter includes asymptomatic noninflammatory left ventricular dysfunction (Roth et al., 2016), Takotsubo-like syndrome with both basal (Ederhy et al., 2018) and apical (Geisler et al., 2015; Anderson and Brooks, 2016) variants, coronary vasospasm (Nykl et al., 2017), arrhythmias (Salem et al., 2018), and myocardial infarction (Weinstock et al., 2017). Of all the cardiotoxicity-associated ICIs, myocarditis is the most common cardiotoxic reaction. Pericardial diseases and conduction diseases were reported in 15 and 12% of patients with ICI-related cardiotoxicity, respectively (Mir et al., 2018). One published study (Mahmood et al., 2018a) on 964 patients between 2013 and 2017 indicated that 1.14% of patients developed myocarditis and 0.52% developed a major adverse cardiovascular event (MACE), such as complete heart block, cardiogenic shock, and cardiac arrest, during treatment with ICIs. As for the presentation of myocarditis, it is highly variable and nonspecific (**Table 1**). Based on previously published studies, the manifestations of cardiotoxicity range from subclinical disease with asymptomatic cardiac biomarker elevation, fatigue, and general malaise to chest pain, dyspnea, palpitations, multiorgan failure, cardiogenic shock, and cardiac arrest (Laubli et al., 2015; Yun et al., 2015; Gibson et al., 2016; Escudier et al., 2017; Reuben et al., 2017; Mahmood et al., 2018a; Neilan et al., 2018; Wang et al., 2018; Samara et al., 2019).

As for the incidence and severity of cardiotoxicity, it is still not well recognized due to limited small-sample retrospective analyses and case reports regarding cardiotoxicity induced by ICIs. A multicenter retrospective study of 752 patients with melanoma treated with ipilimumab had shown that only one case of myocardial fibrosis had occurred (Voskens et al., 2013). Despite the lack of prospective randomized controlled trials to assess myocarditis, retrospective evaluation literature has estimated that the incidence of ICI-related myocarditis ranges from 0.09 (Johnson et al., 2016a) to 1.14% (Mahmood et al., 2018a). Although cardiotoxicity is rare, a high case fatality rate (35%) of myocarditis from ICIs had been reported in a systematic review by Hassan Mir (Mir et al., 2018). A systematic review by Wang et al. of fatal toxic effects associated with ICIs had found that ICI-related myocarditis appeared to present the highest (39.7%) death rate, with 52 deaths among 131 cases (Wang et al., 2018).

In patients treated with the combination of two ICIs, the incidence and death rate of cardiotoxicity is higher than with immunotherapy alone (Larkin et al., 2015b). The largest study of ICI-associated cardiotoxicity to date, using the global database (VigiBase) of the World Health Organization, has revealed that the incidence of myocarditis in patients treated with ICIs is 11 times greater than those without ICI treatment (Salem et al., 2018). A significantly higher case fatality rate (46%) in combination therapy was reported in this study. In addition, myocarditis was found to be more frequent (0.27 *vs.* 0.06%) and severe (60 *vs.* 10%) in patients prescribed a combination of nivolumab and ipilimumab than in those prescribed nivolumab alone (Johnson et al., 2016a). For the adverse events induced by the combination of nivolumab and ipilimumab, the rates of 1.3% for tachycardia, 1.1% for hypertension, 0.4% for arrhythmias, and 0.2% for atrial fibrillation were reported by the European Medicines Agency's European public assessment report, Opdivo (Hassel et al., 2017).

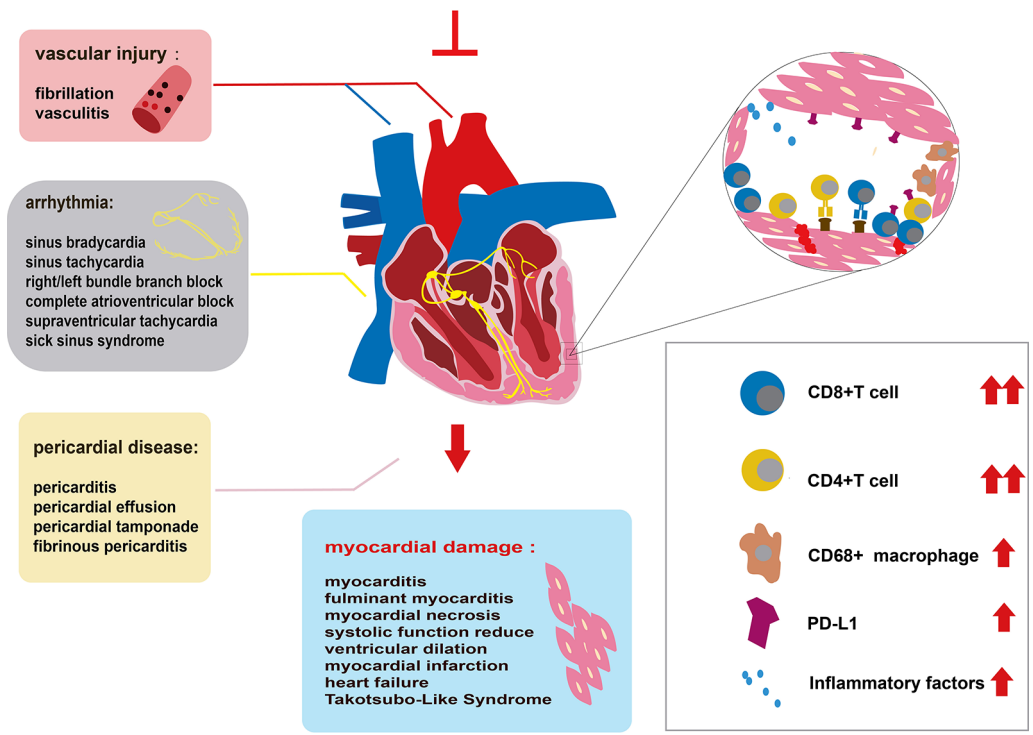

FIGURE 1 | Immune checkpoint inhibitor (ICI)-related cardiac toxicity and its underlying mechanism. Anatomically, ICI-related cardiac toxicity involves almost all parts of the heart. The myocardium is most sensitive to ICI toxicity, showing impaired heart function. The cardiac conduction system, vascular system, and pericardium are also influenced by ICIs. Many infiltrating cells including hyperactivated CD4+/CD8+ T lymphocytes and a few macrophages (CD68+ cells) can microscopically be found in the heart tissue of patients with ICI-related cardiac toxicity. The infiltrating T cells are regarded as the main cause of ICI-related cardiac toxicity. The production of inflammatory factors promotes T cell activity. Elevated PD-L1 expression on cardiac muscle cells and T cells also contributes to the ICI-related cardiac toxicity.

Nevertheless, when ICIs are combined with other non-ICI therapies, it remains unknown whether ICI-related myocarditis is more frequent. In a phase 1b trial of 55 patients treated with avelumab (anti-PD-L1 monoclonal antibody) plus axitinib (a vascular endothelial growth factor [VEGF] inhibitor), only one (1.8%) case developed lethal myocarditis (Choueiri et al., 2018).

The time to onset of cardiotoxicity presentation varies depending on the medical history, type of medication, duration of usage, and double or single medication (**Table 1**). Approximately 80% of ICI-associated myocarditis occurs within the first 3 months of starting ICI therapy (Larkin et al., 2015a; Postow et al., 2015). Approximately 62–64% of patients received only one or two doses of ICIs before the onset of myocarditis (Moslehi et al., 2018; Atallah-Yunes et al., 2019). Cardiac disorders, including myocarditis, pericarditis, and cardiomyopathy are reported to occur between 2 and 17 weeks after ICI treatment onset (Wang et al., 2017; Oristrell et al., 2018). An analysis of an eight-center institutional registry indicated the median time for myocarditis was 34–65 days after initiation of treatment (Mahmood et al., 2018a). In contrast, a patient with melanoma had been reported to develop pericarditis 3 months after four cycles of ipilimumab (Yun et al., 2015). Interestingly, patients without any obvious symptoms had been found to have fulminant myocarditis after 1 year of ICI treatment (Yamaguchi et al., 2018). We reviewed previously published cases of adverse cardiac reactions and found that the onset time of cardiotoxicity was earlier in the combination of two ICIs. In the combination of two ICIs, in more than half (53%) of patients, cardiac toxicity occurred within 4 weeks after ICI initiation, whereas in ICIs alone, cardiac toxicity occurred in 17% of patients around the first ICI dose, and it occurred in 34% of patients 4 months later (**Figure 2**).

POTENTIAL MECHANISM OF IMMUNE CHECKPOINT INHIBITOR-RELATED CARDIAC TOXICITY

The mechanism of ICI-related cardiac toxicity is not yet fully understood. Histological analyses of patients and monkey models with ICI-associated myocarditis have revealed that the infiltration of predominant $CD4^+/CD8^+$ T lymphocytes and a few macrophages ($CD68^+$ cells) are the main cause of ICI-associated myocarditis (Johnson et al., 2016a; Ganatra and Neilan, 2018; Ji et al., 2019) (**Figure 1**). In addition, the expression change of multiple chemokine receptors further proves the enhancement of T cells. CXCR3–CXCL9/CXCL10 and CCR5/CCL5 are required for T cell

TABLE 1 | Published case reports and case series of immune checkpoint inhibitor-associated cardiotoxicity.

References	Patient	Medical history	Cancer type	Drug	Time of onset	Symptoms	Cardiotoxicity	Withdraw the drug	Treatment	Outcome
(Khoury et al., 2019)	68/M	Hypertension, Prostate cancer	Melanoma	Ipilimumab Nivolumab	2 weeks after second dose	Dyspnea, irregular heartbeats, achycardia	Myocarditis	YES	Solumedrol 1 g/day divided into four doses for 3 days. Prednisone 2 mg/kg and decreasing the dose daily by 7.5%.	Death
(Salem et al., 2019)	66/F	NR	Lung cancer	Nivolumab	Three doses	Chest pain	Myocarditis	NR	Methylprednisolone (50 mg/day) iv for 3 days, plasmapheresis, abatacept (500 mg q2w for five doses)	NR
(Esfahani et al., 2019)	71/F	NR	Melanoma	Pembrolizumab	Second cycle of treatment	Dyspnea	Myocarditis, Cardiac arrhythmia	NR	Methylprednisolone (1 g/day) iv for 3 days, then (2 mg/kg/day) mycophenolate mofetil (2 g/day), plasmapheresis, rituximab (375 mg/m^2), alemtuzumab (30 mg)	NR
(Dhenin et al., 2019)	79/F	Asthma, Hypertension	Lung cancer	Pembrolizumab	After the third infusion	Chest pain	Pericarditis	Yes (Drug was reintroduced)	Pyridostigmine (30 mg, five times daily), methylprednisolone (80 mg/day).	Clinical recovery
(Altan et al., 2019)	72/M	Hypertension, CAD, smoking	Lung cancer	Anti-PD-L1	78 days	Dyspnea, hypotension hypoxia	Pericarditis	Yes	NR	Death
(Altan et al., 2019)	65/F	II-DM Hypertension, smoking	Lung cancer	Anti-CTLA-4, anti-PD-1	131 days	Loss of consciousness hypotension	Arrhythmias	Yes	Pacemaker	Death
(Altan et al., 2019)	57/M	Smoking	Lung cancer	anti-PDL1	98 days	Dyspnea, orthopnea, bilateral lower edema	Cardiac tamponade	Yes	NR	No additional toxicity after reintroduction
(Martin Huertas et al., 2019)	80/M	None	Kidney cancer	Nivolumab	After four cycles	Severe asthenia	Myocarditis, AF	Yes	Methylprednisolone (2 mg/kg/day IV)	Death
(Lindner et al., 2019)	73/M	PVD	UC	Pembrolizumab	After 22 cycles	Sweats, fatigue, fever, severe pain in the right limb	Vasculitis	Yes	NR	NR
(Fazel and Jedlowski, 2019)	78/F	Hypertension, Intermittent Asthma, PE, Depression	Melanoma	Nivolumab	5 days after the first cycle	Muscle weakness, dyspnea	Myocarditis	Yes	Methylprednisolone (1–1.5 mg/kg/day IV) to pulse steroid 1,000 mg/day IV, IGI (2 g/kg/day IV)	Deterioration
(Liu et al., 2019)	61/F	NR	Lung cancer	Atezolizumab	3 days after first dose of atezolizumab	Dyspnea, fatigue	Myocarditis	NR	Methylprednisolone 5 mg/kg/day IV, mycophenolate mofetil 1000 mg/day orally	Deterioration

(Continued)

TABLE 1 | Continued

References	Patient	Medical history	Cancer type	Drug	Time of onset	Symptoms	Cardiotoxicity	Withdraw the drug	Treatment	Outcome
(So et al., 2019)	55/F	A thymectomy for thymoma	Melanoma	Nivolumab	After the second infusion	Dysphagia, dyspnea, limb weakness	Myocarditis	Yes	IGI for four cycles, steroid pulse plus two cycles of plasma exchange	Symptoms improved
(Sakai et al., 2019)	74/M	NR	Lung cancer	Nivolumab	After the second infusion	General malaise, appetite decrease, dyspnea	MN	Yes	Large amount of catecholamine	Death
(Sharma et al., 2019)	76/F	Psoriatic arthritis	T-cell lymphoma	Brentuximab and Nivolumab	After the first infusion	Fatigue, dyspnea, orthopnea	AHF	Yes	Solumedrol 1 mg/kg for 3 days, Impella implantation.	Deterioration
(Charles et al., 2019)	33/M	NR	HL	Nivolumab	After the eight infusion	NR	CHB, Myocarditis	Yes	Mycophenolate mofetil, steroids (1 to 2 mg/kg), IGI	Death
(Agrawal et al., 2019)	73/M	NR	Malignant Mesothelioma;	Pembrolizumab;	32 days later	Progressive dyspnea, fatigue	Myocarditis	Yes	Prednisolone 60 mg/day orally, permanent pacemaker, IGI, plasmapheresis	Death
(Agrawal et al., 2019)	89/M	II-DM, Hypertension, Dyslipidemia, AF	Melanoma	Pembrolizumab	After the first dose	Weakness, myalgias, and dyspnea	Myocarditis	Yes	Methylprednisolone 1 g/day IV was started, then oral prednisone 60 mg twice daily, ATG	Death
(Agrawal et al., 2019)	65/F	Hypertension, MR	Lung cancer	Nivolumab	6 days later	Dyspnea, edema, bradycardia	ACS, ADHF	Yes	Methylprednisolone 1 g/day for 3 days, prednisone, furosemide, ATG	Deterioration
(Agrawal et al., 2019)	67/M	CAD	Melanoma	Nivolumab	Three cycles later	Chest pain, palpitations	Myocarditis	Yes	Prednisone 80 mg BID for 5 days then tapering, infliximab, oral corticosteroids	Symptoms improved
(Monge et al., 2018)	79/M	AF	Prostate cancer	Nivolumab	After 8 weeks	Blurred vision, pain, stiffness in the upper back	Myocarditis	Yes	Methylprednisolone 1 mg/kg/day and oral prednisone taper	Clinical recovery
(Hsu et al., 2018)	42/M	HBV carrier	HCC	Pembrolizumab	After six circles	Fatigue, dizziness and anorexia	Bradycardia	Yes	Cortisone 12.5 mg/day orally	Symptoms improved
(Gallegos et al., 2019)	47/F	CAD	Melanoma	Ipilimumab and Nivolumab, then Nivolumab	4 months	Dyspnea, achycardic, pulmonary edema	HF, ASVT	Yes	Methylprednisolone 500 mg intravenous BID for 5 days), infliximab (10 mg/kg/day for 2 days)	Death
(Thibault et al., 2018)	52/M	None	RCC	Nivolumab and Ipilimumab	Three circles later	None	Myocarditis	Yes (Nivolumab reintroduce)	Beta-blocker therapy	No subsequent clinical event
(Berner et al., 2018)	69/M	None	RCC	Avelumab and Axitinib	4 days after second dose	Fatigue, constipation	Hypertension, Cardiac arrest	Yes	Reduction of axitinib, amlodipine	Death
(Jain et al., 2018)	67/M	NR	Melanoma	Nivolumab and Ipilimumab	16 days after the first dose	Dyspnea, cough, dyspnea on exertion	ADHF, Arrhythmia, CHB	Yes	Methylprednisolone 500 mg twice daily, ATG and permanent pacemaker implanted	Deterioration

(Continued)

TABLE 1 | Continued

References	Patient	Medical history	Cancer type	Drug	Time of onset	Symptoms	Cardiotoxicity	Withdraw the drug	Treatment	Outcome
(De Almeida et al., 2018)	69/M	NR	Lung cancer	Nivolumab	5 days after the 24th cycle	Dyspnea, tachycardia, fever	Pericarditis, PT	Yes	Prednisone (1 mg/kg) for 2 weeks, gradually tapered for 8 weeks	Clinical recovery
(Ederhy et al., 2018)	45/F	NR	Melanoma	Nivolumab and Ipilimumab	5 days after the first infusion	NR	AHF, TLS	NR	Methylprednisolone, 1 g/day IV	Complete recovery
(Ederhy et al., 2018)	77/M	NR	Melanoma	Ipilimumab Nivolumab.	After 3 perfusions	NR	TLS	NR	Methylprednisolone 1 g/day IV for 3 days	NR
(Ganatra and Neilan, 2018)	41/F	Hashimoto's thyroiditis	Melanoma	Ipilimumab and Nivolumab	6 days after four cycles	Dyspnea	Myocarditis	Yes	Methylprednisolone 1g/day for 3 days	Symptoms improved
(Yamaguchi et al., 2018)	60/M	None	Melanoma	Nivolumab	13 cycles later	Fatigue, fever	Fulminant Myocarditis	Yes	Prednisolone pulse therapy was initiated at 1000 mg/d for 3 days, IGI at 50 g/d for 2 days	Symptoms improved
(Mahmood et al., 2018b)	75/F	NR	EMC	Durvalumab and Tremelimumab	3 weeks after the first dose	Difficulty ambulating, dyspnea	Myocarditis, HF,CHB	Yes	Methylprednisolone 1 mg/kg, mycophenolate mofetil 1,000 mg oral twice daily	Symptoms improved
(Oristrell et al., 2018)	55/F	NR	Breast cancer	Pembrolizumab	Five cycles later	Pericardial chest pain	PT	Yes	Anterior pericardiectomy, corticosteroids 2 mg/kg/day IV and keep low doses	Symptoms improved
(Frigeri et al., 2018)	76/F	CD	Lung cancer	Nivolumab	After seven biweekly administrations of Nivolumab	Rapidly progressive dyspnea	Myocarditis, CAB	Yes	Methylprednisolone 5 mg/kg/d and three doses of infliximab 5 mg/kg	Deterioration
(Tajmir-Riahi et al., 2018)	72/M	NR	Melanoma	Nivolumab and Ipilimumab	After the 10th therapy	Dyspnea, edema of the legs	Myocarditis	Yes (Pembrolizumab reintroduce)	Prednisolone 1 mg/kg/day	Cardiacarrest
(Katsume et al., 2018)	73/M	Smoking	Lung cancer	Pembrolizumab	16 days after first dose	Faintness	CAB, Myocarditis	NR	Methylprednisolone, 1g/day IV for 3 days and temporary pacemaker implantation	NR
(Chen et al., 2018)	43/M	NR	Thymoma	Nivolumab	10 days later	Chest discomfort, fatigue, myalgias of lower limbs	Myocarditis	Yes	IGI 300 mg/kg IV for 4 days, methylprednisolone 1 g/day for 3 days followed by 500 mg/day for 4 days then 60 mg/day	Death
(Matson et al., 2018)	55/M	Hypertension, COPD	Lung cancer	Nivolumab	3 days after the second dose	Lethargy, dyspnea	ADRHF, cardiogenic shock	Yes	NR	Death
(Norwood et al., 2017)	49/F	Hyperlipidemia	Melanoma	Nivolumab and Ipilimumab	2 weeks after the first dose	Atypical chest discomfort at the cardiac apex	Myocarditis	Yes (following the Ipilimumab)	Methylprednisolone was initiated at 125 mg/day IV, IGI 400 mg/kg/day IV for 2 days	Clinical recovery

(Continued)

TABLE 1 | Continued

References	Patient	Medical history	Cancer type	Drug	Time of onset	Symptoms	Cardiotoxicity	Withdraw the drug	Treatment	Outcome
(Arangalage et al., 2017)	35/F	NR	Melanoma	Ipilimumab	15 days after the first infusion	Progressive dyspnea	Fulminant Myocarditis	Yes	Methylprednisolone, 1 g/day IV, and IGI IV, plasma exchanges	Completely recovered
(Penel et al., 2017)	61/M	Dyslipidemia, Smoking	Lung caner	Nivolumab	After the 11th dose	NR	ACS	Yes	Corticosteroids	Recovered
(Kimura et al., 2017)	54/M	NR	Lung cancer	Nivolumab	4 weeks after PD-1 therapy	Dizziness, nausea, loss of consciousness, general paralysis	HF	Yes	High-dose steroid, pacemaker	Death
(Behling et al., 2017)	63/M	Hypertension, Hyperlipoproteinemia, II-DM, COPD	Melanoma	Nivolumab	3 days after the second dose	Dyspnea, dysphagia, worsened muscle pain	AB, MI	Yes	Prednisone1.5 mg/kg IV and an antibiotic therapy with sultamicillin 3 g IV TID 500 mg aspirin and 5,000 IU unfractionated heparin	Death
(Johnson et al., 2016a)	65/F	NR	Melanoma	Nivolumab and Ipilimumab	12 days after the first doses	Atypical chest pain, dyspnea, fatigue	Fulminant myocarditis	Yes	Methylprednisolone 1 mg/kg/day IV	Death
(Johnson et al., 2016a)	63/M	NR	Melanoma	Nivolumab and Ipilimumab	15 days after the first doses	Fatigue, myalgias	Fulminant myocarditis	Yes	Methylprednisolone 1 g/kg/day IV for 4 days and infliximab	Death
(Roth et al., 2016)	60/M	Hypertension, anxiety, RS	Melanoma	Ipilimumab	2 years after the first dose.	None	AF	Yes	Lisinopril 5 mg/day, metoprolol was changed to carvedilol 6.25 mg twice daily	NR
(Tadokoro et al., 2016)	69/F	NR	Melanoma	Nivolumab	2 months	General malaise, palpitation	Myocarditis	Yes	Oral prednisolone (2 mg/kg) was initiated	Symptoms improved
(Heinzerling et al., 2016)	72/M	MI, II-DM, Hypertension, PVD, Hyperuricemia	Melanoma	Ipilimumab	After three infusions	Dyspnea, anasarca	Myocarditis	Yes	Corticosteroids were initiated at 1 mg/kg orally	Symptoms improved
(Heinzerling et al., 2016)	68/M	ADC, Alcohol abuse	Melanoma	Ipilimumab	After four doses	Dyspnea, lower extremity edema	Cardiomyopathy	Yes	Diuresis, coronary catheterization	Resolved
(Heinzerling et al., 2016)	71/M	None	Melanoma	Ipilimumab	After the second infusion	No obvious cardiac symptoms	MF	Yes	High dose steroids (2 mg/kg)	Death
(Heinzerling et al., 2016)	81/M	AF, CAD	Melanoma	Ipilimumab	11 weeks following the third dose	Progressive subacute dyspnea	HF, Myocarditis	Yes	Diuretics	Symptoms improved
(Heinzerling et al., 2016)	23/M	NR	Melanoma	Ipilimumab	7 months after initiating Ipilimumab	Chest pain and cough	Myocarditis/HF	Yes	Methylprednisolone (2 mg/kg/day) converted to 80 mg prednisone/day with taper over 1 month,	Resolved to baseline
(Heinzerling et al., 2016)	64/M	PVD	Melanoma	Ipilimumab	After the second dose	Fatigue, seizures, abdominal pain (Yun et al., 2015)	Myocarditis	Yes	Dopamine and fentanyl	Death
(Heinzerling et al., 2016)	88/M	CAD	Melanoma	Pembrolizumab	After the eight infusion	Myalgia, pain in the shoulder	Cardiac arrest	Yes	Corticosteroids 125 mg IV for 4 days	Resolved

(Continued)

TABLE 1 | Continued

References	Patient	Medical history	Cancer type	Drug	Time of onset	Symptoms	Cardiotoxicity	Withdraw the drug	Treatment	Outcome
(Heinzerling et al., 2016)	80/M	Melanoma	NHL	Ipilimumab	2 weeks after two doses	Dyspnea, edema, arrhythmias	Fatal myocarditis	Yes	Methylprednisolone (1 mg/kg) IV then prednisone 60 mg by mouth daily	Death
(Laubli et al., 2015)	73/F	NR	Melanoma	Pembrolizumab	Five cycles later	Progressive dyspnea	AHF	Yes	AT2-receptor blocker, a beta-blocker, spironolactone, diuretics	Symptomatic recovery
(Yun et al., 2015)	59/M	None	Melanoma	Ipilimumab	12 weeks after four cycles	Chest pain and dyspnea	AFP	Yes	Methylprednisolone 125 mg/day, prednisone 40mg/day, budesonide 9 mg/day on the third day, and tapered down over a month	Symptoms improved

NR, no report; HL, Hodgkin lymphoma; NHL, non-Hodgkin's lymphoma; UC, urothelial carcinoma; HCC, hepatocellular carcinoma; RCC, renal cell carcinoma; EMC, endometrial cancer; AF, atrial fibrillation; MN, myocardial necrosis; HF, heart failure; AHF, acute heart failure; CHB, complete heart block; ACS, acute coronary syndrome; ADHF, acute decompensated heart failure; ASVT, asymptomatic supraventricular tachycardia; PT, pericardial tamponade; TLS, Takotsubo-like syndrome; CAB, complete atrioventricular block; ADRHF, acute decompensated right-sided heart failure; AB, atrioventricular block; MI, myocardial infarction; MF, myocardial fibrosis; AFP, acute fibrinous pericarditis; IV, intravenous; ATG, anti-thymocyte globulin; QD, once daily; BID, twice daily; TID, three times per day; II-DM, diabetes mellitus type II; RS, Raynaud syndrome; COPD, chronic obstructive pulmonary disease; CAD, coronary artery disease; PVD, peripheral vascular disease; AF, atrial flutter; MR, mitral regurgitation; ADC, asymptomatic dilated cardiomyopathy; CD, cardiovascular disease; PE, pulmonary embolism; mAb, monoclonal antibody; IGI, immunoglobulin.

activities to upregulate (Tokunaga et al., 2018; Ji et al., 2019). Tumor necrosis factor-α, granzyme B, and interferon-γ are produced by activated T cells, inducing cell death. These inflammatory molecules are overexpressed, which might contribute to cardiac injury (Varricchi et al., 2017; Tocchetti et al., 2018).

The most likely explanation is the "shared antigen" between the tumor and cardiac muscle, with muscle-specific antigens (desmin and troponin) detected in the tumor. Moreover, similar clonal T cell populations have been found infiltrating tumors and cardiac muscle. In this case, hyperproliferative T lymphocytes and macrophages aberrantly infiltrate the cardiac muscle after treatment with ICIs, thereby inducing fatal myocarditis (Johnson et al., 2016a). This theory is also supported by the shared epitope between myeloma cells and cardiomyocytes (Martinez-Calle et al., 2018).

Like tumor cells, cardiomyocytes might also employ the PD-1/PD-L1 and CTLA-4 pathways to prevent T cells from hyperactivation in physiological condition. ICIs, a promising anticancer agent, liberate the T-cell inhibition by tumor cells, may also relieve the same type of suppression by cardiomyocytes, which leads to T-cell hyperactivation in the heart. Subsequently, T-cell hyperactivation may result in ICI associated cardiotoxicity. CD28 binds to CD80 (B7-2)/CD86 (B7-2) on antigen-presenting cells (APCs) as a second activation signal to stimulate TCR signaling. CTLA-4 can complete with CD28 for binding with CD80/86 to inhibit the immune response. In contrast, silencing the genes that encode CTLA-4 promotes the proliferation and infiltration of $CD8^+$ T cells in the heart, contributing to the development of myocarditis (Love et al., 2007). In many preclinical models, PD-1 has also been demonstrated to limit the T cell response in the heart as a negative immunoregulatory receptor. The disruption of PD-1 induces $CD8^+$ T cell-mediated autoimmune dilated cardiomyopathy and myocarditis (Nishimura, 2001; Wang et al., 2010), suggesting PD-1 is protective against inflammation and cardiac damage (Tarrio et al., 2012).Similarly, the knockdown of PD-L1 also leads to mortal autoimmune myocarditis in a preclinical model of Murphy Roths Large mice (Lucas et al., 2008). Moreover, in patients with injured myocardium, PD-L1 is overexpressed on injured cardiomyocytes and infiltrating $CD8^+$ T cells (Johnson et al., 2016a). Consistently, in preclinical studies of T cell-mediated myocarditis, the expression of PD-L1 is also upregulated (Grabie et al., 2007).The upregulation of PD-L1 might protect the myocardium from damage; however, this upregulation can be neutralized by ICIs (Grabie et al., 2007). In summary, these limited findings indicate that PD-1/PD-L1 and CTLA-4 play crucial roles in the emergence and development of ICI-related cardiac toxicity. Further studies are needed to elucidate the underlying mechanisms of these effects.

THE DIAGNOSIS OF IMMUNE CHECKPOINT INHIBITOR-ASSOCIATED CARDIOTOXICITY

The detection of ICI-associated cardiotoxicity is currently challenging due to the lack of consistency of its clinical manifestation (**Table 1**). If ICI-related cardiotoxicity is observed

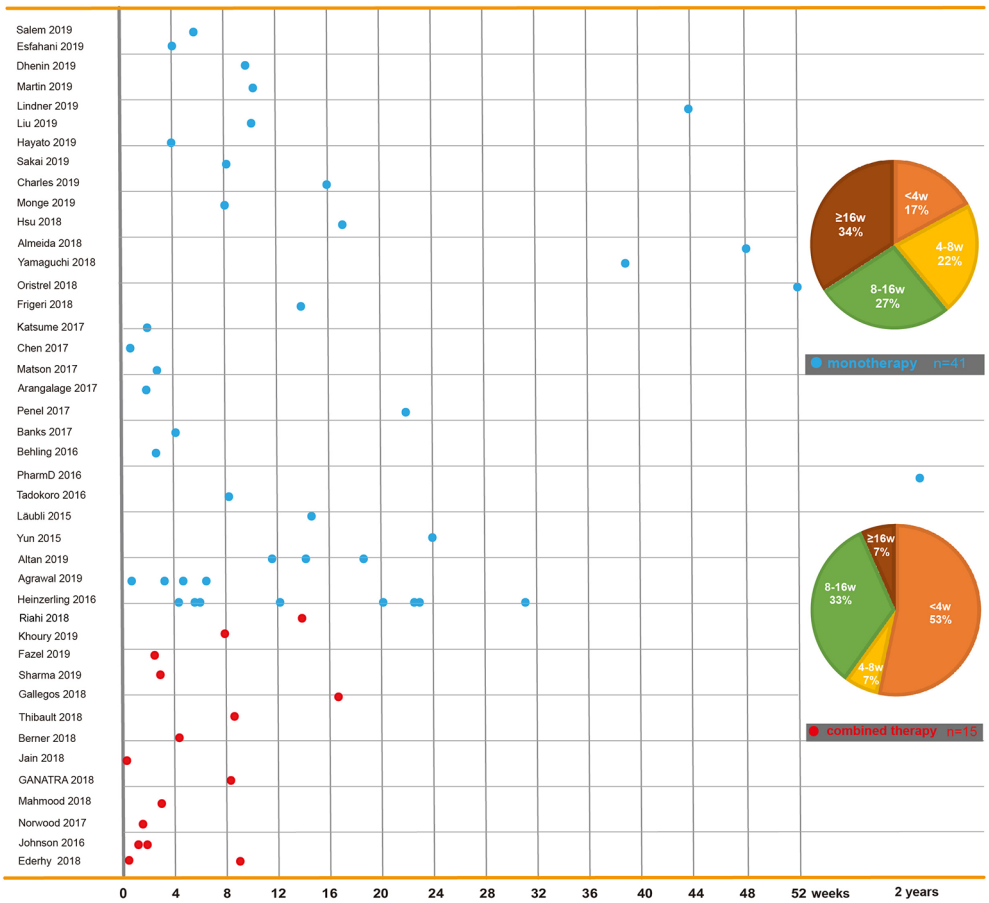

FIGURE 2 | Time to onset of immune checkpoint inhibitor-related cardiac toxicity. In reported cases or case series, reagents including PD-1 inhibitors (nivolumab and pembrolizumab), PD-L1 inhibitors (atezolizumab, avelumab, and durvalumab), and CTLA-4 inhibitors (ipilimumab and tremelimumab) are used solely or in combination. This figure is built based on the cases' therapy types (monotherapy and combined therapy). The time when the ICI-related cardiac toxicity occurred since the first dose of each case is recorded as a dot. The time to onset of monotherapy (the blue dot) and combined therapy (the red dot) are illustrated. The onset time trend of ICI-related cardiac toxicity in each group is shown. On the right, two pie charts reveal case percentages of different onset time periods in each group.

in a patient, a detailed history and physical exams are required to exclude alternative cardiomyopathy etiologies, such as viral and autoimmune cardiac disease, infectious myocarditis or myocardial infarction. This comprehensive diagnostic information is helpful to correct circulation in a timely manner, to provide patients with specific treatment, and to help improve their symptoms (Catena et al., 2009).

For early diagnosis of subclinical myocarditis, serial laboratory tests, electrocardiograms (ECGs) and transthoracic echocardiograms (TTEs) can be beneficial for patients treated with ICIs. Laboratory tests typically include troponin (cardiac troponin I [cTnI] or troponin T [cTnT]), creatine phosphokinase (CPK), creatine kinase (CK), creatine kinase-myocardial band (CK-MB), brain natriuretic peptide (BNP), and N-terminal pro-brain natriuretic peptide (NT-proBNP) (**Table 2**). Of all the markers, troponin is generally the most sensitive marker for confirming or excluding the diagnosis of myocarditis (Asnani, 2018). Mahmood et al. have indicated that almost all (94%) myocarditis cases had elevated troponin at the time of manifestation (Mahmood et al., 2018a). We reviewed the previous cases/case series and found that 84 and 89% of patients with ICI-associated cardiotoxicity had elevated cTnI and abnormal ECG, respectively (**Figure 3**). Even very low serum concentrations of troponin can provide important diagnostic and/or prognostic information for oncologists (Jaffe, 2011; Mahajan and Jarolim, 2011; Eggers and Lindahl, 2017). Notably, in patients with myositis, cTnT and CPK can also be elevated; thus, cTnI is the preferred marker for cardiac injury (Hughes et al., 2015). Interestingly, Mahmood et al. have also indicated that higher levels of serum cTnT might be associated with a greater risk of MACE (Mahmood et al., 2018a). BNP or NT-proBNP, the sensitive indicators (**Figure 3**), were reported to increase in most patients (Lyon et al., 2018); these are also helpful for the diagnosis of ICI-associated cardiotoxicity (Norwood et al., 2017). In cynomolgus monkeys with moderate mononuclear cell infiltration or with myocardial degeneration, an NT-proBNP or cTnI increase was observed at multiple time points after the first dose of ICIs; thus, they might serve as valuable biomarkers for ICI-induced myocarditis (Ji et al., 2019). However, BNP is a poorly specific marker for the diagnosis of

TABLE 2 | Laboratorial, radiological, and histopathological features of immune checkpoint inhibitor-associated cardiotoxicity.

Laboratory tests	ECG	TTE	CMR	Histopathological features
cTnI mildly elevated (a* = 9)	Sinus bradycardia (k* = 2)	LVSF diminished (Martin Huertas et al., 2019)	T2 intramyocardial intensity consistent with edema (Agrawal et al., 2019)	Inflammatoryinfiltrate beneath the thick fibrinous layeron the epicardium ($CD4^+$, $CD8^+$ T cells, some $CD68^+$ macrophages, scattered $CD20^+$ B cells (Altan et al., 2019)
cTnI moderately elevated (b* = 7)	Sinus tachycardia with ST-segment elevation in V1-6 (Sakai et al., 2019)	Reduced LVEF of 9% and akinesis of anteroseptal wall and apex (Sakai et al., 2019)	An elevated regional T2 ratio and EGE (Agrawal et al., 2019)	Myocardial necrosis (Martin Huertas et al., 2019)
cTnI massively elevated (c* = 8)	Sinus tachycardia with no ST-T changes (Jain et al., 2018; Sharma et al., 2019)	Severely reduced LVEF, moderate PE, moderate MR, severe TR and mildly RVD (Sharma et al., 2019)	A non-ischemic pattern of LGE, four-chamber dilation with severe biventricular dysfunction (Gallegos et al., 2019)	Intense inflammatory infiltrate: $CD3^+$, $CD8^+$, $CD4^+$, 40% of all lymphocytes were PD-1 positive, some $CD68^+$ macrophages (Martin Huertas et al., 2019)
cTnT elevated (Laubli et al., 2015; Yamaguchi et al., 2018)	Atrial rate was faster than ventricular rate (Charles et al., 2019)	Mild concentric LVH, mild RAE, moderate LAE, and mild AR, MR, TR (Agrawal et al., 2019)	Patches of LGE were seen in the basal and mid inferior wall showing an epicardial pattern compatible with myocarditis (Monge et al., 2018)	Myocardial necrosis with few inflammatory cells scattered in both ventricles (Sakai et al., 2019)
cTnI decreased (Liu et al., 2019)	Alternating RBBB and LBBB, episodes of asystole, third-degree block with a junctional escape rhythm (Agrawal et al., 2019)	RVD (Agrawal et al., 2019)	Diffuse myocardial edema (Ederhy et al., 2018)	Intense inflammatory infiltrate: $CD4^+$, $CD8^+$ T cells. PD-L1 stain showed focal membrane positivity in the areas of LGE (Gallegos et al., 2019)
CK mildly elevated (Katsume et al., 2018)	RBBB (Agrawal et al., 2019)	EF was severely decreased to 25–30% (Agrawal et al., 2019)		Lymphocytic infiltrate: $CD3^+$, $CD4^+$, $CD8^+$ T cells, $CD68^+$ macrophage within the myocardium, cardiac sinus and atrioventricular nodes (Johnson et al., 2016a)
CK moderately elevated (d* = 7)	Sinus rhythm with new lateral ST segment depressions (Agrawal et al., 2019)	Mild BVD with reduced RVSF, BAD (Agrawal et al., 2019)		T-cell and macrophage infiltrates in the myocardium, cardiac conduction system and skeletal muscle (Johnson et al., 2016a)
CK massively elevated (Chen et al., 2018; Agrawal et al., 2019)	Atrial tachycardia (Gallegos et al., 2019)	GBF with LVEF of 26%, severe LVD (Fazel and Jedlowski, 2019), and a trivial PE (Gallegos et al., 2019)		Heavy infiltration of $CD68^+$ and $CD3^+$, $CD20^-$ T-lymphocytes (Berner et al., 2018)
CPK mildly elevated (Sakai et al., 2019)	PR prolongation with normal QRS complexes (Johnson et al., 2016a)	LVEF of 65% with LAE, RVD and increased PAP (Johnson et al., 2016a)		Diffuse cardiomyocyte necrosis with lymphocytic infiltration and predominance of $CD3^+$ and $CD20^-$ T cells (Jain et al., 2018)
CPK moderately elevated (e* = 3)	Profound ST segment depression (Johnson et al., 2016a)	Severe LV hypokinesis and LVEF decline to 20% (Jain et al., 2018).		Nonspecific chronic inflammation with extensive fibrosis and lymphocyte infiltration (De Almeida et al., 2018)
CPK massively elevated (Johnson et al., 2016a; Katsume et al., 2018)	AF with QT prolongation and LAFB (Monge et al., 2018)	Restrictive PE (De Almeida et al., 2018)		Diffuse infiltration with inflammatory cells (histocytes, lymphocytes, macrophages, and giant cells) with cardiac myocyte necrosis (Mir et al., 2018)
CK-MB mildly elevated (f* = 5)	Complete atrioventricular block with wide QRS complexes (Katsume et al., 2018)	Reduced LVEF (40%) with apical and mid-ventricular akinesia (Ederhy et al., 2018)		Lymphocytic infiltration: $CD3^+$, $CD4^+$, $CD8^+$ $CD20^-$, strong expression of PD-L1 (Chen et al., 2018)
CK-MB moderately elevated (g* = 3)	Intraventricular conduction delay progressed into episodes of ventricular tachycardia (Jain et al., 2018)	GlobalLV systolic dysfunction with an EF of 15% (Ganatra and Neilan, 2018)		Extensive lymphocytic infiltration, interstitial edema, and myocardial necrosis and with predominant $CD4^+$, $CD8^+$, $CD20^-$, PD-L1 strongly expressed on myocardium (Yamaguchi et al., 2018)
CK-MB massively elevated (Johnson et al., 2016a)	Sinus tachycardia with T-wave inversion in the anteroseptal leads (Ederhy et al., 2018)	Thickened interventricular septum (12 mm), regular ventricular motion with LVEF of 49% (Chen et al., 2018)		Lymphocytic infiltration with occasional eosinophils (Mahmood et al., 2018b)

(Continued)

TABLE 2 | Continued

Laboratory tests	ECG	TTE	CMR	Histopathological features
BNP mildly elevated (h = 5*)	T-wave inversion on leads V2, V3, and V4 (Ederhy et al., 2018)	Diffuse hypokinesis and reduced LVEF (15%) with myocardial edema (Yamaguchi et al., 2018)		Diffuse lymphoplasmacytic infiltrates ($CD3^+$, $CD4^+$, $CD8^+$, $CD20^-$ cells) with foci of active myocyte injury and necrosis throughout the atria, ventricles, and interventricular septum, (Matson et al., 2018)
BNP moderately elevated (i = 4*)	Low QRS voltage and T wave inversion on V1–V4 leads (Yun et al., 2015)	54% LVEF with regional areas of hypokinesis (Mahmood et al., 2018b)		$CD3^+$ infiltrated in the pericardium; huge infiltration in pericardium with predominance of neutrophils (Oristrell et al., 2018)
BNP massively elevated (Agrawal et al., 2019)	Sinus rhythm with prolongation of the PR interval and RBBB (Chen et al., 2018)	RVD with reflux into the hepatic veins, suggestive of RHF (Matson et al., 2018).		Interstitial fibrosis with inflammation, fiber necrosis, signs of hypertrophy (Tajmir-Riahi et al., 2018)
NT-pro BNP elevated (j = 5*)	ST segment Elevation in V4–V6, leads II, III, and aVF (Yamaguchi et al., 2018)	Moderate PE and right atrial systolic collapse (Oristrell et al., 2018)		Early collagen deposition admixed with inflammatory cells; the majority of $CD3^+$, $CD4^+$, $CD68^+$; the rarity of $CD20^+$, CD138 (Norwood et al., 2017)
CRP mildly elevated (Katsume et al., 2018; Fazel and Jedlowski, 2019)	CAB (Kimura et al., 2017; Mahmood et al., 2018b)	A severely reduced LVEF, MAB (Frigeri et al., 2018)		Lymphocytic infiltration: $CD3^+$, $CD8^+$ cells with the myocardium (Johnson et al., 2016a)
CRP massively elevated (Martin Huertas et al., 2019)	Sinus tachycardia (Oristrell et al., 2018)	Diffuse hypokinesis of the LVEF (30.2%) (Katano et al., 2011)		Lymphocytic infiltration with a predominance of CD8+ T cells (Katano et al., 2011)
AChR Ab mildly elevated (Martin Huertas et al., 2019)	Sinus tachycardia with a RBBB and ST-segment elevation in the anteroseptal and inferolateral leads (Arangalage et al., 2017)	A severely impaired LVEF of 30% with marked ventricular desynchrony (Laubli et al., 2015)		Patchy lymphocytic infiltration: $CD3^+$, $CD8^+$, $CD68^+$ cells (Heinzerling et al., 2016)
AChR Ab massively elevated (So et al., 2019)	ST-segment elevation in leads II, III, and aVF (Katano et al., 2011) A suspected non-ST segment elevation MI (Behling et al., 2017) PR interval prolongation with normal QRS complexes; rapid progression to CHB (Johnson et al., 2016a) Tachycardiac sinus rhythm with ventricular bigamy (Laubli et al., 2015)			Lymphocytic infiltration with a predominance of $CD8^+$ T cells (Laubli et al., 2015) Mixed inflammatory infiltrates in the pericardial wall, accompanied by abundant surface fibrin (Yun et al., 2015)

Compared with the normal value of laboratory tests: mildly elevated: < 10 times; moderately elevated: ≥10 and <100 times; massively elevated: ≥100 times. cTnI, cardiac troponin I; cTnT, cardiac troponin T; BNP, brain natriuretic peptide; TTE, transthoracic echocardiogram; ECG, electrocardiogram; CMR, cardiovascular magnetic resonance; CK-MB, creatine kinase-myocardial band; NT-pro BNP, N-terminal pro-brain natriuretic peptide; CPK, creatine phosphokinase; CK, creatine kinase; CRP, C-reactive protein; AChR Ab, acetylcholine receptor antibody; EF, ejection fraction; LV, left ventricular; LVEF, left ventricular ejection fraction; PE, pericardial effusion; MR, mitral regurgitation; AR, aortic regurgitation; BVD, bi-ventricular dilatation; BAD, bi-atrial dilatation; LVD, left ventricle dilatation; RVD, right ventricle dilatation; TR, tricuspid regurgitation; LAFB, left anterior fascicular block; LBBB, left bundle branch block; RBBB, right bundle branch block; LVH, left ventricular hypertrophy; RAE, right atrial enlargement; LAE, left atrial enlargement; GBF, global biventricular failure; LGE, late gadolinium enhancement; EGE, early gadolinium enhancement AF, atrial fibrillation; PAP, pulmonary artery pressure; LVSF, left ventricular systolic function; RVSF, right ventricular systolic function; RHF, right heart failure; CHB, complete heart block; MI, myocardial infarction; MAB, multiple apical thrombi; CAB, complete atrioventricular block

(a = 9 (Norwood et al., 2017; Ganatra and Neilan, 2018; Katsume et al., 2018; Monge et al., 2018; Thibault et al., 2018; Agrawal et al., 2019; Charles et al., 2019; Sakai et al., 2019; Sharma et al., 2019).*

(b = 7) (Ederhy et al., 2018; Frigeri et al., 2018; Matson et al., 2018; Agrawal et al., 2019; Charles et al., 2019; Fazel and Jedlowski, 2019; Khoury et al., 2019).*

(c = 8) (Johnson et al., 2016a; Arangalage et al., 2017; Chen et al., 2018; Martin Huertas et al., 2019; Salem et al., 2019).*

(d = 7) (Arangalage et al., 2017; Monge et al., 2018; Yamaguchi et al., 2018; Agrawal et al., 2019; Martin Huertas et al., 2019; Salem et al., 2019; So et al., 2019).*

(e = 3) (Behling et al., 2017; Kimura et al., 2017; Khoury et al., 2019).*

(f = 5) (Norwood et al., 2017; Chen et al., 2018; Katsume et al., 2018; Liu et al., 2019; Sakai et al., 2019).*

(g = 3) (Johnson et al., 2016a; Monge et al., 2018; Agrawal et al., 2019).*

(h = 5) (Laubli et al., 2015; Monge et al., 2018; Agrawal et al., 2019; Fazel and Jedlowski, 2019; Sharma et al., 2019).*

(i = 4) (Gallegos et al., 2019; Liu et al., 2019; Martin Huertas et al., 2019; Sakai et al., 2019).*

(j = 5) (Chen et al., 2018; Frigeri et al., 2018; Ganatra and Neilan, 2018; Liu et al., 2019; Salem et al., 2019).*

(k = 2) (Hsu et al., 2018; Liu et al., 2019).*

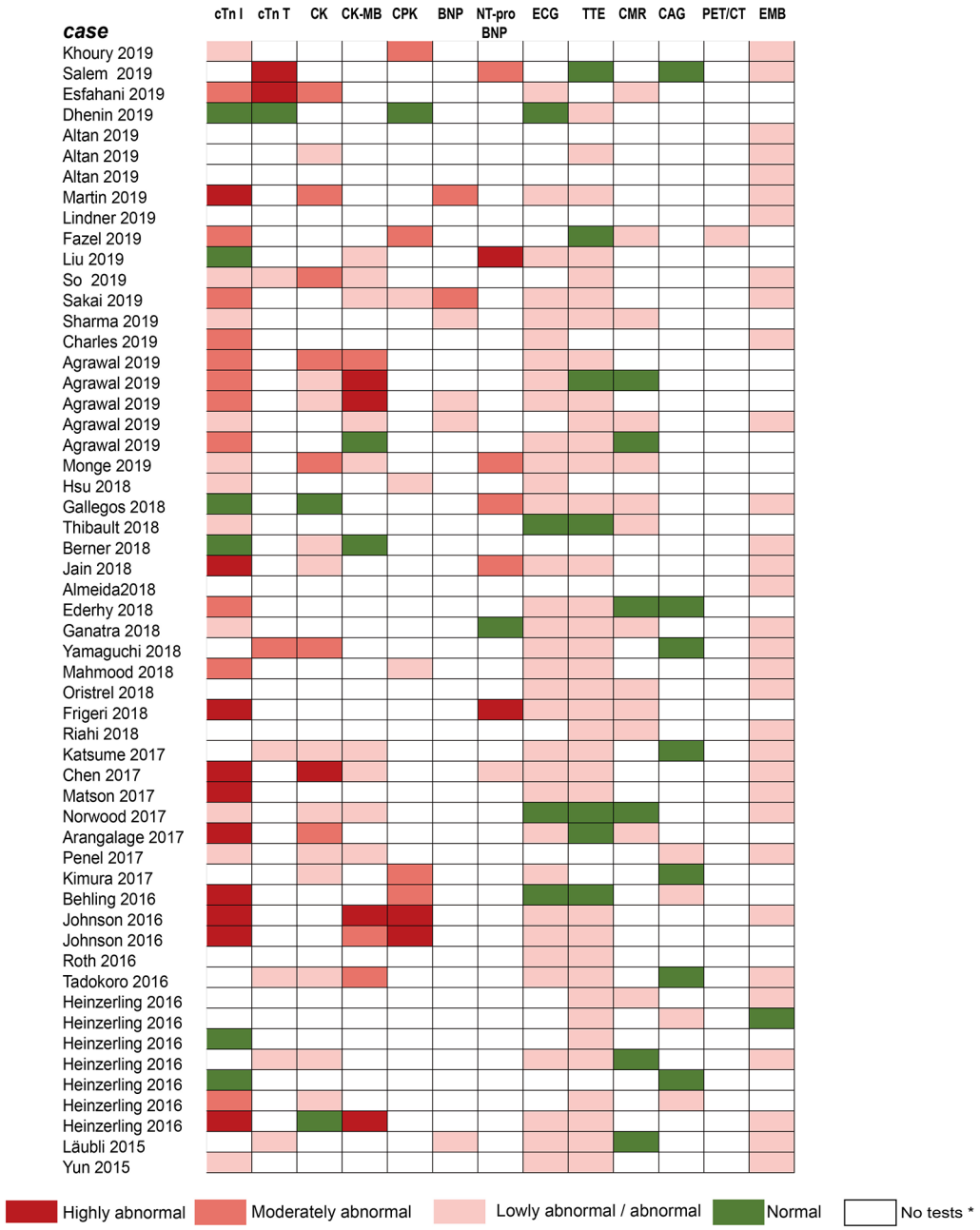

FIGURE 3 | Summarized results of laboratory tests and other examinations for diagnosis in published cases reports/case series. The laboratory tests include cTnI, cTnT, CK, CK-MB, CPK, BNP, NT-pro-BNP. Other examinations include ECG, TTE, CMR, CAG, PET/CT, and EMB. No tests* means no laboratory testing or examination is performed in cases. Compared with the normal value of laboratory tests: Lowly abnormal: 10 times; Moderately abnormal: ≥10 and 100 times; Highly abnormal: ≥100 times. cTnI, cardiac troponin I; cTnT, cardiac troponin T; CPK, creatine phosphokinase; CK, creatine kinase; CK-MB, creatine kinase-myocardial band; BNP, brain natriuretic peptide; NT pro BNP, N-terminal pro-brain natriuretic peptide; ECG, electrocardiograph; TTE, transthoracic echocardiogram; CMR, cardiovascular magnetic resonance; CAG, coronary angiography; PET/CT, positron emission tomography/computed tomography; EMB, endomyocardial biopsy.

ICI-associated cardiotoxicity, because it not only can be elevated in noninflammatory left ventricular dysfunction or other causes of acute cardiac injury, but also in many patients with cancer who have cardiotoxicity (Bando et al., 2017). The elevation of CK can also be observed in ICI-induced myocarditis (**Figure 3**) (Chen et al., 2018; Agrawal et al., 2019; So et al., 2019). Moreover, several reports have indicated that a complete atrioventricular block is usually associated with ICI-related myocarditis, with a considerably elevated CK level (Heinzerling et al., 2016; Johnson et al., 2016a). Of note, mild to massive elevation of serum CK was found in patients diagnosed with ICI-related myositis or myasthenia gravis (Kimura et al., 2016; Chang et al., 2017; Suzuki et al., 2017; March et al., 2018); therefore, the specificity of elevated CK was relatively poor.

Given ECG has widespread availability and is easy to perform, it is considered a first-line test to identify patients with suspected ICI-associated myocarditis (Ganatra and Neilan, 2018). In our review of previous case reports, most patients had had an ECG performed (**Figure 3**). Abnormal ECGs have been reported in 40–89% of patients with ICI-related cardiotoxicity; however, these changes are often nonspecific (**Table 2**) (Escudier et al., 2017; Mahmood et al., 2018a). TTE has also been applied to provide further insight into left ventricular ejection fraction (LVEF) impairment, pericardial effusions, and wall motion abnormalities. However, a normal TTE report does not rule out ICI-associated myocarditis (Neilan et al., 2018; Tocchetti et al., 2018). Patients presenting with cardiac marker elevation, ST elevation, and ischemic symptoms should receive emergency coronary angiography to eliminate acute coronary syndrome (Tajiri et al., 2018).

To accurately diagnose myocarditis, further diagnostic technologies, such as cardiac magnetic resonance (CMR) imaging or an endomyocardial biopsy (EMB) are also necessary (Guglin and Nallamshetty, 2012; Bami et al., 2016). Gadolinium contrast-enhanced CMR imaging, a meaningful noninvasive diagnostic tool superior to echocardiography, can identify tissue characterization and offer accurate diagnosis of fibrosis and inflammation for hemodynamically stable patients in the early course of the disease (Friedrich et al., 2009; Aquaro et al., 2017). The CMR features of myocarditis, including edema, necrosis, and scar formation, were previously defined as the Lake Louise Criteria (**Table 2**) (Friedrich et al., 2009). For instance, an enhanced T2 signal on CMR could be indicative of underlying myocardial edema or myocarditis (Sharma et al., 2019). However, for patients who require invasive hemodynamic or respiratory and/or circulatory support, CMR imaging might not be feasible. Furthermore, although CMR imaging to detect myocardial edema and late gadolinium enhancement is accurate, its sensitivity is relatively poor (Laubli et al., 2015; Escudier et al., 2017; Norwood et al., 2017; Mahmood et al., 2018a) and an absence of positive findings on CMR does not rule out myocarditis (Abdel-Aty et al., 2005).

EMB, a gold standard for the diagnosis of myocarditis (Kindermann et al., 2012; Leone et al., 2012; Caforio et al., 2013), should be conducted when the treatment course is affected by suspected cardiotoxicity. This is especially true in unclear situations in which the oncologist does not know whether to continue or terminate ICI treatment. An EMB can reveal various features of interstitial inflammation suggested by interstitial fibrosis and lymphocyte infiltration (Sharma et al., 2019). Previous reports on ICI-related myocarditis have shown that significant T cells ($CD4^+$, $CD8^+$) and macrophage infiltration were observed in the myocardium (Laubli et al., 2015; Heinzerling et al., 2016; Johnson et al., 2016a; Koelzer et al., 2016; Tadokoro et al., 2016), cardiac conduction system (Johnson et al., 2016a), interventricular septum (Matson et al., 2018) and pericardium (Oristrell et al., 2018) (**Table 2**). Similar T cell populations were also observed in cardiomyocytes, according to a postmortem report of a patient who died from ICI-induced myocarditis (Johnson et al., 2016a), suggesting hyperactivated cytotoxic T cells directly injuring themyocardium as the probable mechanism of myocarditis induced by ICIs. B lymphocytes and/or plasma cells are usually rare or absent (Altan et al., 2019). Inflammatory infiltration can be transient and focal, however, and can sometimes be inaccessible to pathological puncture. Therefore, a biopsy sampling error from patients with myocarditis can result in a false negative diagnosis (Leone et al., 2012). In this case, it is suggested that EMB should be reattempted in cases with unexplained progressive heart failure (Caforio et al., 2013).

TREATMENT AND OUTCOME OF IMMUNE CHECKPOINT INHIBITOR-ASSOCIATED CARDIOTOXICITY

The treatment regimens for ICI-associated cardiotoxicity vary depending on the case (**Table 1**); however, the principal strategy concentrates on targeting the hyperactive T-cell response. High-dose steroids have constituted the first-line treatment for ICI-related myocarditis (Heinzerling et al., 2016; Johnson et al., 2016a; Kimura et al., 2016; Semper et al., 2016; Tadokoro et al., 2016; Haanen et al., 2017; Brahmer et al., 2018). Prompt initiation of high-dose intravenous methylprednisolone and immediate ICI discontinuation are associated with improved symptoms (Escudier et al., 2017; Mahmood et al., 2018a). A higher starting dose of steroids (intravenous methylprednisolone 1 g) was related to a lower rate of MACE according to a recent study by Mahmood et al. (Mahmood et al., 2018a). Although data regarding treatment for irAEs from rigorous studies are lacking, rapidly initiating intravenous or oral prednisone (1–2 mg/kg) for most patients and intravenous methylprednisolone (0.5–1.0g) for refractory cases with progressive tapering are recommended according to consensus guidelines (Brahmer et al., 2018). However, multiple studies have indicated that corticosteroids alone might not be sufficient to improve immune-mediated cardiac adverse reactions, and patients with ICI-associated cardiac events might even progress to malignant arrhythmias and severe heart failure symptoms during steroid treatment (Heinzerling et al., 2016; Johnson et al., 2016a). Many patients had received corticosteroids early in their cardiotoxicity management, tapering them over 1 month; however, no significant effect was observed (Norwood et al., 2017; Agrawal et al., 2019; Martin Huertas et al., 2019).

For patients with a poor response to corticosteroids, other immunosuppressive drugs should be administered, including immunoglobulin (Caforio et al., 2013), plasmapheresis, mycophenolate mofetil, tacrolimus, and infliximab (Haanen et al., 2017; Jain et al., 2017; Norwood et al., 2017; Reddy et al., 2017; Brahmer et al., 2018; Frigeri et al., 2018; Lyon et al., 2018). Infliximab, a chimeric immunoglobulin G1 monoclonal antibody blocking tumor necrosis factor-α, is used to treat patients with steroid-refractory ICI-associated colitis (Pages et al., 2013). The use of infliximab has been documented in the context of severe steroid-refractory myocarditis (Heinzerling et al., 2016; Johnson et al., 2016a; Tay et al., 2017; Frigeri et al., 2018) and has demonstrated significant clinical recovery and biochemical normalization (Agrawal et al., 2019). It is cautioned that infliximab could be potentially associated with deteriorating heart failure and is prohibited for patients with

moderate to severe heart failure (Kwon et al., 2003). Considering the histological similarity between ICI-associated myocarditis and cardiac transplantation rejection, anti-transplant rejection medications (e.g., anti-thymocyte globulin [ATG]) have also been used for treating patients with ICI-related myocarditis (Tay et al., 2017). One case series has indicated that two patients treated with ATG after their clinical course worsened during steroid treatment responded well to ATG therapy, with remission of cardiogenic shock and malignant arrhythmias (Agrawal et al., 2019). The underlying mechanism could be associated with ATG leading to a rapid reduction in lymphocyte infiltration and T cell superactivation, thereby resulting in myocardial conduction improvement (Tay et al., 2017).

Recently, two reports (Esfahani et al., 2019; Salem et al., 2019) have shown that the new therapeutic agents alemtuzumab and CTLA-4 agonists (abatacept and belatacept), could be associated with significant relief of symptoms of cardiotoxic reactions caused by ICIs. Alemtuzumab, a monoclonal antibody that binds to CD52, can result in destruction of complement-mediated peripheral immune cells (monocytes, lymphocytes, macrophages, natural killer cells, and dendritic cells). Although the use of alemtuzumab in the context of cardiac allograft rejection has previously been evaluated, data on its use in patients with irAEs is limited (Cahoon et al., 2012). A recent report has indicated that 30 mg of alemtuzumab led to rapid T-cell depletion and was associated with the resolution of cardiac immunotoxic effects (Esfahani et al., 2019). CTLA-4 agonists, either abatacept or belatacept, can inhibit T cell costimulation mediated by CD28/B7 at the dendritic cell level, thereby abrogating the costimulation of T cells upstream of PD-1/PD-L1 and the CTLA-4 pathways. Abatacept can rapidly cause global T cell anergy (the inactivation of normal immune response) with specific reverse pathways activated by ICIs (Ingelfinger and Schwartz, 2005). When high-dose methylprednisolone injection and sustained plasmapheresis did not work, abatacept resulted in a rapid reduction in cTnI levels and recovery of LVEF (Salem et al., 2019). However, given the potential risks of infectious complications and tumor growth, it is necessary to further evaluate of the risk–benefit balance of abatacept in ICI-induced myocarditis (Ingelfinger and Schwartz, 2005).

Apart from the immunosuppressive therapies above, when necessary, beta-blockers, angiotensin converting enzyme inhibitors (ACEIs), and high-dose aspirin could be required as auxiliary therapies for patients with heart failure and in the context of raised troponin and the indication of cardiac ischemia (Berner et al., 2018). Extracorporeal membrane oxygenation is also required for a patient with severe myocarditis induced by the combination therapy of nivolumab and ipilimumab (Arangalage et al., 2017).

POSSIBLE MANAGEMENT OF IMMUNE CHECKPOINT INHIBITOR-ASSOCIATED CARDIOTOXICITY AND FUTURE DIRECTIONS

Cardiotoxicity, a rare but fatal irAE, is particularly difficult to supervise and manage (Puzanov et al., 2017; Rapoport et al., 2017; Brahmer et al., 2018). Currently, no standard management guidelines on ICI-related cardiotoxicity have been established, due to its low incidence and the limited data on its manifestation, diagnosis, therapy, and outcomes. Based on previous studies and evidence, we summarized the possible management of ICI-related cardiotoxic reactions (**Figure 4**) as follows.

Before initiating ICI therapy, a comprehensive assessment of cardiovascular risk factors and a detailed cardiac history should be obtained for all patients, given the risk factors for ICI-associated cardiotoxicity remain unclear to date. According to previous reports, potential risk factors such as autoimmune disease, pre-existing cardiac disease, the combination of ICIs, and age should be evaluated before ICI treatment. In 50% of patients treated with ipilimumab, pre-existing autoimmune diseases worsened (Bowyer et al., 2016). Several studies have also revealed that patients with underlying autoimmune disease might be at high risk of irAEs, including cardiotoxicity (Johnson et al., 2016b; Johnson et al., 2017; Postow et al., 2018). For instance, patients with systemic autoimmune disorders are more likely to have subclinical myocarditis than those without autoimmune diseases. These results show that clinical and subclinical autoimmune diseases are an important consideration before initiation of ICI therapy (Varricchi et al., 2017). Physicians should be aware of potentially cardiotoxic events during ICI treatment, especially for those with pre-existing cardiac conditions (Hsu et al., 2018). In Heinzerling's report, pre-existing cardiac disease or peripheral arterial disease had been present in most patients (5 of 8) who developed autoimmune cardiotoxicity (Heinzerling et al., 2016). The combination therapy of ICIs might also be a risk factor for ICI-associated myocarditis (Varricchi et al., 2017). Some VEGF inhibitors can increase the risk of thrombosis and coronary ischemia (Jain et al., 2018). They are also known to be related to cardiotoxicity and left ventricular dysfunction (Shah and Morganroth, 2015). Therefore, when ICIs are combined with anti-VEGF therapies, we should be vigilant for cardiac adverse effects. Given that the common adverse reaction to anti-VEGF therapies, such as axitinib, is hypertension (Hamnvik et al., 2015), it is not a likely causal agent in case of myocarditis; however, it can result in poor heart function in reaction to the physiological challenge. In addition, age can be associated with the incidence of ICI-associated cardiotoxicity. In Wang's retrospective cohort, fatal irAEs involving cardiovascular toxicities were more common in the elderly than in the young (median,70 *vs* 62 years, $P = .009$) (Wang et al., 2018). Other risk factors, such as diabetes mellitus, a history of smoking, and dyslipidemia, might be included (Behling et al., 2017; Norwood et al., 2017; Tomita et al., 2017; Katsume et al., 2018; Agrawal et al., 2019; Altan et al., 2019), however, large sample studies are needed for confirmation.

To accurately diagnose ICI cardiotoxicity, several appropriate steps can be taken into consideration: First, serum troponin tests, baseline ECG, and serial surveillance are necessary. TTE and other laboratory tests, such as CK, CK-MB, BNP, or NT-proBNP can also be performed. Notably, for some special cases with a high risk of cardiotoxicity, TTE is strongly recommended to record the patient's baseline cardiac function before the initiation of ICIs, and other laboratory inspections should be performed to record their baseline cardiac status. When a cardiotoxic reaction is

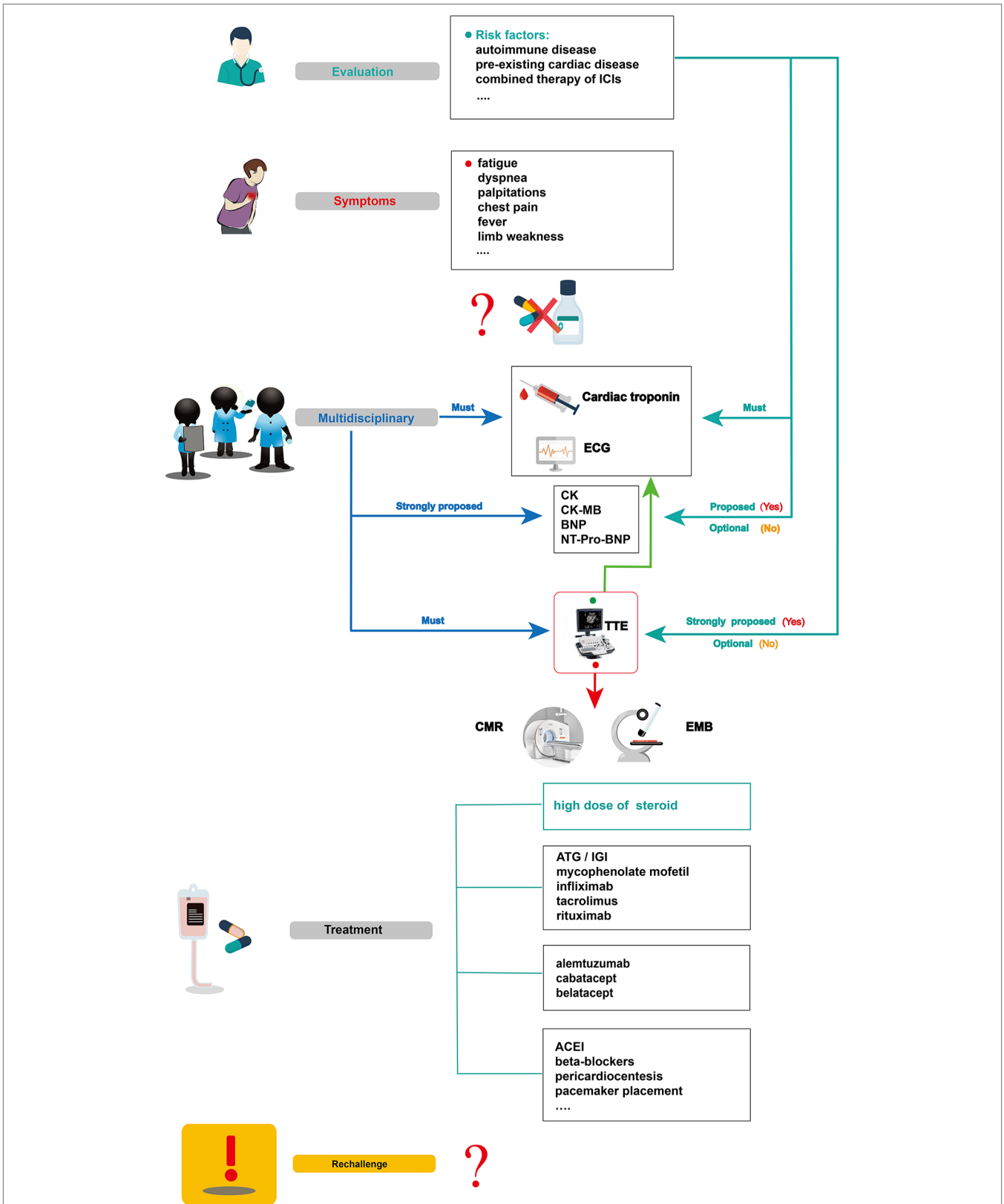

FIGURE 4 | Summarized overview on potential monitoring and management of ICI-related cardiotoxicity. CK, creatine kinase; CK-MB, creatine kinase-myocardial band; BNP, brain natriuretic peptide; NT pro BNP, N-terminal pro-brain natriuretic peptide; TTE, transthoracic echocardiogram; CMR, cardiovascular magnetic resonance; EMB, endomyocardial biopsy; ATG, anti-thymocyte globulin; IGI, immunoglobulin; ACEI, angiotensin converting enzyme inhibitor.

suspected during treatment with ICIs alone and in combination—for example, the patient's symptoms might include chest pain, dyspnea, fatigue, palpitations, fever, and limb weakness—one major challenge for oncologists is to identify whether these symptoms are clinical manifestations of cardiovascular irAEs. In a highly suspected ICI-associated cardiotoxic event, temporary discontinuation of ICIs is suggested; it is then necessary for the oncologists, cardiologists, and immunologists to together discuss further diagnosis and treatment. Second, apart from cardiac troponin and ECG, other laboratory biomarker tests, such as serum CK, CK-MB, BNP, and NT-proBNP tests, are strongly proposed. Although a TTE must be performed to record the heart function, normal cardiac function does not rule out ICI-induced myocarditis. CMR is also advisable to further evaluate abnormal cardiac structure. When CMR is not available or contraindicated, cardiac positron emission tomography/computed tomography is beneficial to diagnose myocardial inflammation. Third, in uncertain cases, EMB should be performed and samples from multiple sites should be collected to optimize the diagnostic accuracy of focal myocarditis and reduce sampling errors (Leone et al., 2012). After a comprehensive diagnosis, if there is no evidence of cardiac dysfunction, myocarditis, or other cardiotoxic events, ICI therapy can be slowly reintroduced under close troponin and ECG monitoring.

For patients with confirmed ICI-associated myocarditis, permanent discontinuation of ICIs after cardiac adverse events (G1) has been recommended by the American Society of Clinical Oncology guidelines, though the recommendation is based on anecdotal evidence (Brahmer et al., 2018). Then, prompt administration of oral prednisone (1–2 mg/kg/day) (Jain et al., 2017) or intravenous methylprednisolone (1–2 mg/kg/day) (Puzanov et al., 2017; Wang et al., 2017; Brahmer et al., 2018; De Almeida et al., 2018) should be initiated. If improved signs are observed, a slow tapering dose of glucocorticoid over at least 4 weeks has been recommended (Hu et al., 2019). In patients with pericarditis, despite the resolution of pericardial effusion *via* pericardial window, prednisone (1 mg/kg) initiation approximately 2 weeks later can prevent constrictive pericarditis (De Almeida et al., 2018). For patients with sick sinus syndrome, a low dose of cortisone (12.5 mg/day) taken orally might help relieve symptoms (Hsu et al., 2018). Nonetheless, if a patient shows a poor response to glucocorticoids, secondary drugs, including ATG, immunoglobulin, infliximab, tacrolimus, mycophenolate mofetil, rituximab, CTLA-4 agonists, and alemtuzumab can be considered (Jain et al., 2017; Norwood et al., 2017; Reddy et al., 2017; Frigeri et al., 2018; Esfahani et al., 2019; Salem et al., 2019). What should be emphasized is that infliximab is generally contraindicated because it can induce congestive heart failure (Kwon et al., 2003). Although the safety and efficacy of these immunosuppressive agents (*e.g.*, ATG, alemtuzumab, abatacept, belatacept) need further confirmation, these drugs could be a viable choice, especially for a critically ill patient with rapidly deteriorating cardiovascular function when high-dose glucocorticoid therapy is not possible.

In parallel with the immunosuppressive agents above, guideline-based therapy and supportive care is recommended for patients with ICI-associated cardiotoxicity. Patients with congestive heart failure should be treated with tolerable medications, including renin-angiotensin system inhibitors and beta-blockers (Yancy et al., 2017). Those with progressive life-threatening arrhythmias should be treated with appropriate antiarrhythmic drugs, or a patient with advanced conduction disease should be considered for temporary/permanent pacemaker placement. When necessary, invasive therapies such as pericardial window placement or pericardiocentesis might also be needed (Yang and Asnani, 2018).

It is controversial whether immunotherapy should be reintroduced after recovery from cardiac toxicity (Brahmer et al., 2018). Although cardiac dysfunction can be significantly improved by high-dose glucocorticoid therapy, an anti-PD1 antibody rechallenge might aggravate immune-related toxicity (Tajmir-Riahi et al., 2018). Given the potential for fulminant or fatal ICI-related myocarditis, ICIs are not recommended for reintroduction in patients (Champiat et al., 2016). However, in a retrospective analysis of 30 patients who were diagnosed with cardiotoxic irAEs, four patients resumed ICIs safely, without cardiotoxic event recurrence (Escudier et al., 2017).Therefore, the clinical oncologist, cardiologist, and immunologist collaboration should give discreet consideration to patients according to their manifestations, outcome, and alternative cancer treatment options to determine the safety of reintroducing ICI therapy.

In summary, the detection and management of ICI-associated cardiotoxic reactions are challenging, and more efforts are needed in future. First and foremost, one of the most important challenges is to improve preventive measures and increase early detection of cardiac toxicity *via* monitoring of cardiac damage. Second, a multidisciplinary team constituting of oncologists, cardiologists, radiologists, immunologists, and pathologists should be organized to achieve optimal management of ICI-induced cardiotoxicity and to decrease its lethal capacity. Further, developing cardiac protectants that can be used in conjunction with ICIs will be critical in preventing ICI cardiotoxicity. Last but not least, research into new immunotherapeutic agents with unknown cardiotoxicity incidence, such as anti-T cell Ig or anti-lymphocyte-activated gene-3 and mucin-containing protein 3, as well as V-domain Ig suppressor of T cell activation or B and T lymphocyte attenuator blockade, would be a new challenge for physicians. In addition, more clinical trials should focus on the effects of T cell costimulation blockers on cardiovascular disease. For example, CD40–TRAF6 inhibitors have already been well examined. Blocking OX40 and anti-4-1BB costimulation could be a promising strategy in the future (Simons et al., 2019).

CONCLUSION

Immune checkpoint inhibitors, either alone or in combination, can result in cardiotoxic adverse reactions, such as myocarditis, pericarditis, conduction abnormalities, cardiomyopathy, acute coronary syndrome, and others. Of all ICI-related cardiotoxic events, myocarditis is the most common cardiotoxic reaction. Though the incidence of ICI-associated cardiotoxicity remains relatively low, clinicians must be aware of these adverse events due to their high fatality rate. It mainly occurs in the early stage

after ICI initiation, with nonspecific symptoms ranging from asymptomatic cardiac biomarker elevation, fatigue, and general malaise to chest pain, dyspnea, palpitations, multiorgan failure, cardiogenic shock, and cardiac arrest. A high level of clinical suspicion and early diagnosis indicators are required due to the rapid progress and fulminant course of the disease. The assessment of clinical features in combination with laboratory examinations (cTnI, cTnT, CK, CK-MB, BNP, and NT-proBNP), ECG, TTE, CMR, and EMB contribute to the diagnosis of ICI-associated cardiotoxicity. Among these diagnostic methods, troponin is generally the most sensitive marker, ECG has widespread availability and is easily performed, and EMB is a gold standard diagnosis. Before initiating ICIs, a comprehensive assessment of cardiovascular risk factors and a detailed cardiac history should be obtained, especially for patients with autoimmune disease or pre-existing cardiac disease, and when ICIs are combined with other treatments. For patients with confirmed cardiotoxic events, prompt high-dose steroids and other immunosuppressors, such as ATG, immunoglobulin, infliximab, tacrolimus, mycophenolate mofetil, rituximab, CTLA-4 agonists, and alemtuzumab can result in clinical recovery and increased survival. Auxiliary therapies, such as ACEIs, beta-blockers, aspirin, diuretics, antiarrhythmic drugs, pacemaker placement, and pericardiocentesis can also help. In addition, cardiac function assessment and frequent monitoring are necessary. To better understand the pathogenesis of this disease and provide effective treatment strategies, larger studies are needed.

AUTHOR CONTRIBUTIONS

YZhou collected and reviewed the literature and wrote the manuscript. YZhu wrote and revised the manuscript. MW and YX rechecked the manuscript and put forward meaningful comments on it. CC, TZ, and FX assisted in drawing. JL and ZD contributed equally to writing the design and revised the manuscript. All authors read and approved the final manuscript.

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Comprehensive Survey of Clinical Trials Registration for Melanoma Immunotherapy in the ClinicalTrials.gov

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Objective: Comprehensively evaluate the immunotherapeutic clinical trials and provide reference for melanoma treatment and research.

Methods: The website of *ClinicalTrials.gov* was searched to retrieve and download all registered clinical trials for melanoma immunotherapy on August 1 (updated on August 25), 2019. All registration trials met the inclusion criteria were collected regardless of the type of study, the status of recruitment, and the results of the study. The general characteristics, methodological characteristics, and the types of immunotherapeutic drugs included of these trials were analyzed.

Results: Finally, 242 eligible trials were included and evaluated. Of them, 30.6% were completed, 16.9% were terminated, and two were withdrawn; 77.7% recruited less than 100 participants; 30.5% were randomized; 45.5% was single group assignment; 88.8% were not masked; the primary purpose was treatment; 44.2% had data on monitoring committees; 27.7% used US FDA-regulated immunization drugs; 78.5% without results posted; 43.0% were sponsored by the industry. Immunological checkpoint inhibitors were most often studied, with 53.6% of the trials involving PD-1, the most commonly studied was Nivolumab.

Conclusions: Currently, most of the registered clinical trials for melanoma immunotherapy were interventional open-label trials. Most immunotherapy research hotspots were in the FDA-regulated drug product, and a few trials reported available test results. It is necessary to strengthen the supervision of results and explore and disseminate more effective and safe immunotherapy methods.

Keywords: melanoma, immunotherapy, *ClinicalTrials.gov*, trial registration, vaccines

INTRODUCTION

Melanoma is a malignant tumor originating from melanocytes and often occurs in the skin, uvea, oral cavity, intracranial, etc. (Smith et al., 2016; Tang et al., 2017; Yang et al., 2018). According to the global cancer statistics in 2018, there are more than 280,000 new cases of melanoma of skin worldwide, accounting for 1.6% of new cancer cases; and more than 60,000 cases of melanoma deaths worldwide, accounting for 0.6% of total cancer deaths (Bray et al., 2018). Although the morbidity and mortality of melanoma are not as high as other malignancies, the global burden of disease (GBD) of melanoma is increasing year by year. It is reported that the GBD of melanoma increased by 51% in 2016 compared with 2015, and the incidence of melanoma increased by 39% (Collaboration, 2018). In addition, the 5-year survival rate of advanced melanoma patients is only about 20% (Maio et al., 2015; Hamid et al., 2019). For early melanoma, it can be cured by surgical treatment; moreover, immunotherapy, radiation therapy, chemotherapy, targeted therapy, and other treatments are used for the supplementary treatment of surgery or the treatment of patients with unresectable or metastatic melanoma (Wang et al., 2018).

As a form of biological therapy, immunotherapy is widely used to treat tumors. Some researchers believe that tumor cells develop and proliferate in the tumor microenvironment, while evading the identification and clearance of the immune system in a variety of ways (Ude et al., 2018). Due to the inhibition of the production and activity of immune effector cells in the body, many signals and factors were released to the tumor microenvironment to help tumor cells spread and metastasis. Immunotherapy can stimulate immune system, activate recognition surface antigens of tumor cells by immune cells, and induce immune cells to remove tumor cells, so as to achieve the purpose of cancer treatment (Muenst et al., 2016). Up to now, many trials have been designed for immunotherapy of melanoma, including vaccines, immunomodulators, adoptive cell transfer therapy (ACT), immune checkpoint inhibitors (ICI), etc. (Koller et al., 2016). Vaccine induces immune response of immune system by active immunity, stimulates immune cells to recognize tumor specific antigens, and then destroys tumor cells. Common vaccines include dendritic cell (DC) vaccine, peptide vaccine, DNA vaccine, autologous tumor cell vaccine, etc. (Ott et al., 2014; Sarbu et al., 2017). Cytokines such as interleukin (IL)-2, IL-12, IL-15, and interferon-α (INF-α) can promote the immune recognition of melanoma and thus have function of regulating immunity (Nicholas and Lesinski, 2011). Adoptive cell immunotherapy separates lymphocytes from blood or tumor-infiltrating lymphocytes from tumors that have been surgically removed, and then transfuse them to patients after activation and proliferation *in vitro*, that to kill tumors or stimulate the anti-tumor immune effect of the body (Maus et al., 2014; Rosenberg and Restifo, 2015). In addition, immune checkpoint inhibitors (ICI), such as pembrolizumab and nivolumab, play a role in regulating the immune response of T lymphocytes in tumor microenvironment, and has made some progress in previous clinical trials (Pulluri et al., 2017).

Clinical trials of immunotherapy for melanoma continue to increase. In 1970s, the concept of "clinical trial registration" was proposed in the United States. Simes RJ (Simes, 1986) found that clinical trial with positive or promising outcomes was preferred to publish and the clinical trial registration helps to reduce this publication bias. Currently, the International Committee of Medical Journal Editors (ICMJE) requires all prospective clinical trials be registered before the first subject were included (De Angelis et al., 2004). In 2000, the *ClinicalTrials.gov* (https://clinicaltrials.gov/) was open to the public. As one of the most widely used clinical trial registration platform, its high weekly growth rates for new entries, high transparency and accessibility, and detailed information on past and present clinical trials (Ma et al., 2019), making *ClinicalTrials.gov* a representative of 16 clinical trial registry centers around the world (Zarin et al., 2017). *ClinicalTrials.gov* has received more than 300,000 clinical trials registration so far, including a number of trials on immunotherapy for melanoma. Hence, more details could be obtained from trials than those reported in final peer-reviewed publications (Cihoric et al., 2017). Moreover, harnessing the immune system for therapeutic benefit in cancer becomes an aim of immunologists and oncologists in recent years. With the development of immunotherapy for melanoma, great progress has been made, but immune-related adverse events (irAE) have also observed. Therefore, we searched and analyzed all of these trials on immunotherapy for melanoma registered in *ClinicalTrials.gov* to assess the characteristics of them and the current status of immunotherapy.

METHODS

Data Source

We retrieved and downloaded all registered clinical trials for melanoma immunotherapy in the *ClinicalTrials.gov* website. We used its search function to search the term "melanoma" for "Condition or disease" and "Immunotherapy" for "Other terms" on August 1 (updated on August 25), 2019. Intervention (clinical trials), observation, and expanded studies were all included. Trials of open (not yet recruited, recruited) and closed (by invitation to register; active, unrecruited; suspended; terminated; completed; withdrawn; unknown) status were considered to include. There are no restrictions on the results of the study or the age of the patients enrolled. All finally included clinical trials must have a definitive record of established immunotherapy.

Statistical Analysis

The selected records were imported into the Microsoft Excel 2007 software and all of the following information was extracted: the NCT number, status, conditions, groups or arms, experimental and control medications, sponsor, collaborators, gender, age, study phases, enrollment, funder type, study types (allocation, intervention model, masking, primary purpose, and time perspective), start date, completion date, locations, data

monitoring committee (DMC), US Food and Drug Administration (FDA)-regulated product, IPD sharing statement, study documents, and study result.

The general characteristics of clinical trials were shown in descriptive statistics. The categorical data was expressed by calculating the frequency and percentage. All analyses were performed using the Microsoft Excel 2007 software.

RESULTS

General Characteristics of Included Clinical Trials

A total of 395 records were identified on the *ClinicalTrials.gov*. After excluded repeated records, non-immunotherapy, and melanoma with other organ diseases trials, we finally include 242 trials. Among them, the vast majority of trials (n = 241, 99.6%) did not restrict gender of participants. Most trials (n = 224, 92.6%) were solely focused on adults, and a small number of trials (n = 18, 7.4%) were focused on both children and adults. The number of registered trials had increased significantly since 2008 (**Figure 1**), and most trials (n = 154, 63.6%) began in 2011 and beyond. The majority of trials (n = 192, 79.3%) spanned more than 24 months, and more than one-third of the trials (n = 79, 32.6%) were over 60 months. Of the eligible trials, 233 (96.3%) were interventional, eight (3.3%) were observational, and one was expanded access trial. Most of them (n = 188, 77.7%) recruited less than 100 participants, only 4.6% recruited more than 400 participants. 74 trials (30.6%) were in the completed state, followed by the recruiting state (n = 65, 26.9%); 41 trials (16.9%) were terminated (lacking funds or statistical power, business reasons, expired commitment) and two were withdrawn (no patients were enrolled). **Table 1** presented the detailed information.

Methodological Quality of Included Clinical Trials

Among the 233 interventional trials, 95.5% were in Phase I to III. 71 (30.5%) were randomized, while 68 (29.2%) were non-randomized. The most common intervention model was single group assignment (n = 106, 45.5%), followed by parallel assignment (n = 101, 43.4%). Most of them (n = 207, 88.8%) were not masked, only eight (3.4%) were double masked, three (1.3%) were triple masked, and four (1.7%) were quadruple masked. Most (n = 227, 97.4%) commonly adopted primary aim were treatment. In addition, eight observational trials were cohort design, including five (62.5%) prospective design and three (37.5%) retrospective design. **Table 2** presented the detailed information.

Detailed Characteristics of Included Clinical Trials

In 242 trials, less than half (n = 107, 44.2%) had DMCs, 27.7% used immunization drugs were the US FDA-regulated products, eight trials (3.3%) had IPD sharing statement, seven trials (2.9%) had results submitted, 45 (18.6%) posted results on *ClinicalTrials.gov*, and 78.5% without any results posted. Nearly half of the trials (n = 114, 47.1%) had collaborations. 104 trials (43.0%) were sponsored by the industry, less than one-third (n = 66, 27.3%) were funded by the NIH, and 16.1% were funded only by NIH (**Table 3**). 148 trials (61.2%) were conducted in North America, then in the Europe (n = 47, 19.4%), and 9.9% were based on international cooperation (**Figure 2**).

Description of Immunotherapies in Included Clinical Trials

All included trials involved four categories of immunotherapy: ACT, ICI, immunomodulators, and vaccine (**Table 4**). Among them, ICI was the most frequently (n = 155, 42.8%), followed by

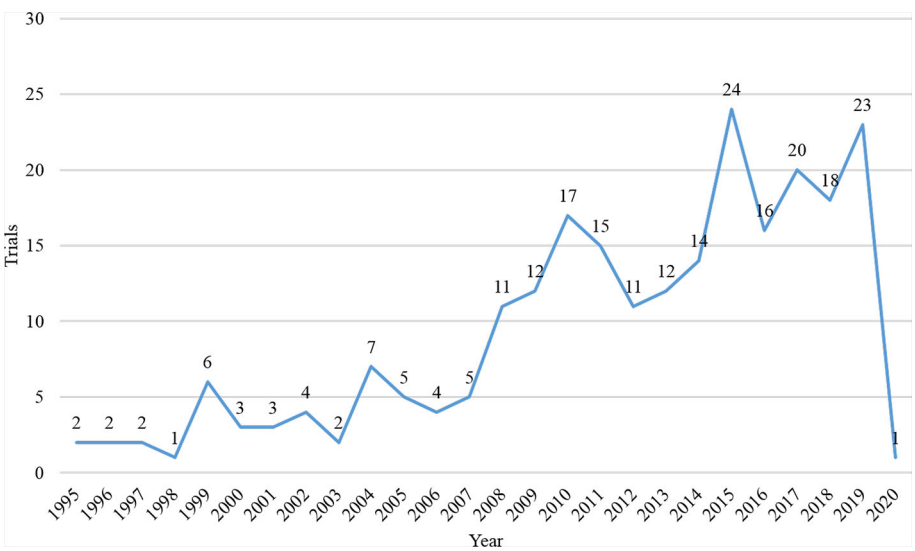

FIGURE 1 | Quantity trend of registered trials per year.

TABLE 1 | General characteristic of included trials (n = 242).

Name	Detail	Number	Percent
Gender			
	All	241	99.6%
	Not provided	1	0.4%
Age			
	Adult	224	92.6%
	Child and adult	18	7.4%
Start Date			
	Prior to 2000	16	6.6%
	2001–2010	70	28.9%
	2011–2020	154	63.6%
	Not provided	2	0.8%
During Date			
	0–12 months	7	2.9%
	13–24 months	27	11.2%
	25–36 months	36	14.9%
	37–48 months	38	15.7%
	49–60 months	39	16.1%
	60-	79	32.6%
	Not provided	16	6.6%
Study Type			
	Interventional	233	96.3%
	Observational	8	3.3%
	Expanded access	1	0.4%
Enrollment			
	0–100	188	77.7%
	101–400	35	14.5%
	401~	11	4.6%
	NP	8	3.3%
Status			
	Active, not recruiting	39	16.1%
	Completed	74	30.6%
	No longer available	1	0.4%
	Not yet recruiting	9	3.7%
	Recruiting	65	26.9%
	Suspended	3	1.2%
	Terminated	41	16.9%
	Unknown status	8	3.3%
	Withdrawn	2	0.8%

TABLE 2 | Design data of included trials (n = 242).

Study Type	Study Design	Number	Percent
Interventional		233	96.3%
	Phases		
	·Phases 1–3	231	95.5%
	·Phase 4	1	0.4%
	·Not applicable	1	0.4%
	·Not provided	9	3.7%
	Allocation		
	·Randomized	71	30.5%
	·Non-randomized	68	29.2%
	·Not provided	94	40.3%
	Intervention Model		
	·Crossover assignment	4	1.7%
	·Factorial assignment	1	0.4%
	·Parallel assignment	101	43.4%
	·Sequential assignment	7	3.0%
	·Single group assignment	106	45.5%
	·Not provided	14	6.0%
	Masking		
	·None	207	88.8%
	·Double	8	3.4%
	·Triple	3	1.3%
	·Quadruple	4	1.7%
	·Not provided	11	4.7%
	Primary Purpose		
	·Diagnostic	1	0.4%
	·Prevention	1	0.4%
	·Treatment	227	97.4%
	·Other	3	1.3%
	·Not provided	1	0.4%
Observational			
	Cohort	8	3.3%
	·Prospective	5	62.5%
	·Retrospective	3	37.5%
Expanded Access		1	0.4%

vaccine (n = 83, 22.9%). Among the ICI, the most studied were PD-1 (n = 83, 53.6%), followed by CTLA-4 (n = 64, 41.3%). The most studied single drug in PD-1 was nivolumab, followed by pembrolizumab. The most studied single drug in CTLA-4 was ipilimumab, and one study was tremelimumab. Among the vaccines, peptide vaccine was the most frequently studied vaccine, followed by DC vaccine, and then Autologous Tumor Cell vaccine. Among the immunomodulators, cytokines were the most widely studied, especially IL-2 (n = 50, 76.9%).

DISCUSSION

This study comprehensively analyzed drug trials registered on *ClinicalTrials.gov*, all of which explored immunotherapy and common adverse reactions to melanoma. Through analysis, we found most of the trials were interventional trials, and one third trials had been completed. Most interventional trials were phase 1–3, small sample size, and single group assignment, not blinded, for therapeutic purposes. At the same time, nearly half of the trials included the data monitoring committee, and one fifth submitted and published results. ICI and vaccine were the most widely studied immunotherapies, of which ipilimumab, nivolumab, and IL-2 were the most single drug widely studied.

Almost all subjects in these trials were gender-neutral, and more than 92% of the trials included only adults. Even though women diagnosed with cutaneous melanoma have a survival advantage due to the effects of sex hormones, there is no difference in overall survival rates between men and women (Enninga et al., 2017). Although melanoma often occurs in adults, it is also the most common skin cancer in children (Dunn et al., 2018). According to these registrations, only a few trials have been included children; obviously, there is still a great shortage of research on melanoma in children. Hence, we recommend researchers expand scope of the population in future clinical trials to get more clinical data for children with melanoma. Since 2008, more than 10 trials have been conducted each year, and 63% of trials have been carried out after 2010. The registration of clinical trials helps to increase the sharing of information of clinical trials, increase the openness of the research process, and reduce publication bias (Aslam et al., 2013). In 2004, the ICMJE issued a statement requesting that

TABLE 3 | Detailed characteristics of included trials (n = 242).

Name	Detail	Number	Percent
Data Monitoring Committee			
	Yes	107	44.2%
	No	93	38.4%
	Not provided	42	17.4%
U.S. FDA-regulated Product			
	Yes	67	27.7%
	No	26	10.7%
	Not provided	149	61.6%
IPD Sharing Statement			
	Yes	8	3.3%
	No	41	16.9%
	Undecided	19	7.9%
	Not provided	174	71.9%
Results			
	Results submitted	7	2.9%
	Posted on ClinicalTrials.gov	45	18.6%
	No results posted	190	78.5%
Collaborators			
	Yes	114	47.1%
	No	128	52.9%
Funder type			
	NIH	39	16.1%
	Industry	49	20.3%
	Industry and (NIH +Other)	55	22.7%
	Other	72	29.8%
	NIH and other	27	11.2%

NIH, the National Institution of Health.

prospective clinical trials need to be registered prior to inclusion in patients (De Angelis et al., 2004). From this study, we found the number of clinical trial registrations for melanoma immunotherapy has increased significantly compared to before after 2004, this may be related to this publication policy.

More than 96.3% were interventional studies, and among them only 30.5% clearly indicated random allocation was used, 43.4% used parallel assignment models, and only 6.4% used double-masking, triple-masking, or quadruple-masking. Randomization is a very powerful method that can largely prevent confusion and reduce selection bias in treatment comparisons (Sessler and Imrey, 2015). The implementation of masking can bring many benefits to participant, care provider, investigator, and outcomes assessor (Schulz and Grimes, 2002). However, due to the different toxicity profiles of the comparators, the trials were difficult to perform blindly, so most of them were open label design. More than 64% of the research continued for three years or more, and 79 trials were conducted for more than five years. Because melanoma is invasive, patients with stage IV melanoma have an average survival of about eight months and a low five-year survival rate (Grob et al., 2017). Most clinical trials were still exploring the long-term survival of immunotherapy for melanoma (Faries et al., 2017; Schachter et al., 2017). According to result of enrollment, the sample size of most studies was still small, and 77% included fewer than 100 patients. The sample size affects population mean, variance, statistical power, and effect size (del Rio et al., 2014), which is directly related to the credibility of the results (Ruberg and Akacha, 2017). Therefore, this suggested that the minimum sample size should be estimated in advance in the design stage of clinical trials to meet the accuracy and reliability of statistics and ensure the reliability of results.

Most of the selected trials were conducted in North America, and 9.8% were conducted on more than two continents. The *ClinicalTrials.gov* is a database of privately and publicly funded clinical studies conducted around the world, which currently contains registration information for nearly 300,000 studies in more than 200 countries (Tse et al., 2018). 47.1% of trials had collaborators, 50% were conducted by NIH participate in sponsorship. Rare adverse events are unlikely to be found in small sample clinical studies, because the effect size may be too small to be evaluated. One way to increase the sample size is to conduct multi-center collaborative research to increase the external validity of the study (Yusuf et al., 1984). At the same time, the support of funds from sources such as the NIH provides a strong guarantee for the smooth development of multi-center research (Allareddy et al., 2014). 44.2% of the trials had DMCs, and DMC is critical to maintaining the scientific integrity of the trial, the accuracy and authenticity of the trial data, and the safety of the study participants (Filippatos et al., 2017). 21.5% of the trials submitted or posted their results, although the reporting rate had improved, but still need to adhere to the principle to provide accurate, complete, and timely information for all studies (Zarin et al., 2017). 27.7% of the trials reported FDA-regulated product, and federal law requires sponsors to submit summary results for applicable clinical trials, including those following the first phase of the FDA new drug approvals to *ClinicalTrials.gov* for public releasing (Schwartz et al., 2016).

The included trials were classified according to the type of immunotherapy. The results showed that most studies explored ICI and vaccines, among the ICI, the most studied were PD-1, followed by CTLA-4. The most studied single drug in PD-1 was nivolumab, followed by pembrolizumab. The most studied single drug in CTLA-4 was ipilimumab. Immunological checkpoint inhibitors (ICI) have greatly changed the treatment of advanced skin melanoma and gradually replaced traditional chemotherapy, showing great potential for the treatment of melanoma. Common ICIs include cytotoxic T-lymphocyte-associated antigen 4 (CTLA-4) antibodies (O'Day et al., 2007), programmed cell death protein 1 (PD-1) antibodies (Topalian et al., 2012), PD-L1 antibodies (Sullivan et al., 2019), and lymphocyte-activation gene-3 (LAG-3) antibodies (O'Day et al., 2007). CTLA-4 and PD-1 downregulate T cell response in lymphoid tissues and tumor microenvironments. And their monoclonal antibodies can interfere with this pathway and promote the activation of anti-cancer T cells (Levine et al., 2017). PD-L1 binds to PD-1 on T cells, which down-regulates T cell activity, and PD-L1 antibodies achieve anti-tumor effects by interfering with this pathway (Zou et al., 2016). LAG-3 is

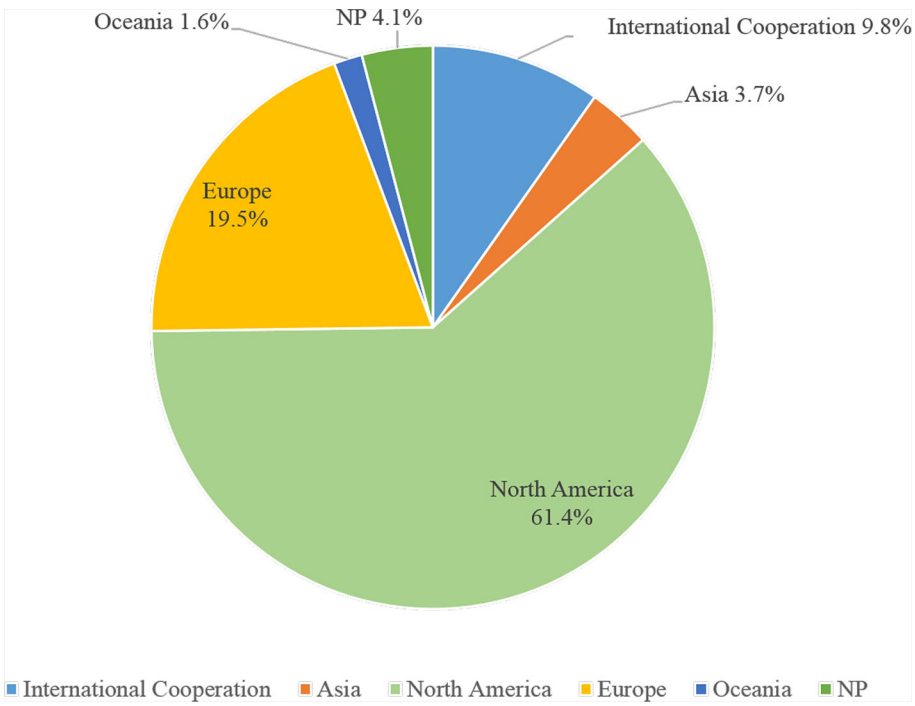

FIGURE 2 | Regional pie chart for included clinical trials.

another important immunological checkpoint, and co-expression of PD-1 is associated with T cells exhaustion (Grosso et al., 2009; Park and Cheung, 2017). Common monoclonal antibodies corresponding to these four types of ICI are ipilimumab (NCT00324155), nivolumab (NCT01585194), atezolizumab (NCT03175432), and relatlimab (NCT03743766).

Vaccine is a hot spot drug and made some progress in the immunotherapy of melanoma. Among the vaccines, peptide vaccine was the most frequently studied vaccine, followed by DC vaccine, and then autologous tumor cell vaccine. Most cancer vaccines are designed to activate tumor-specific $CD8^+$ cytotoxic T cells, so the most common peptide vaccination strategy is based on MHC class I-restricted peptide epitopes on TAA (Butterfield, 2015). DCs are the most effective antigen presenting cell in the immune system and have the unique ability to induce the differentiation of naive T lymphocytes into effector T cells, which have specific cytotoxic activity against a variety of antigens, including antigens expressed by tumor cells (Anguille et al., 2017). The principle of DC vaccine preparation is to collect lymphocytes from peripheral blood, induce them into DC *in vitro*, and present tumor antigens to DCs, thereby providing a large number of these cells for active immunotherapy (Dannull et al., 2013). Tumor antigen presentation to DC can be accomplished in a variety of ways (Osada et al., 2015; Wei et al., 2016). A new method was developed to present autologous tumor antigens to the cytoplasm of DCs. This method is more effective than conventional foreign aid loading. In the mouse melanoma model, the new method produces DC vaccines that show more excellent effect (Hardin et al., 2018). Geskin *et al.* conducted the efficacy of three MODC vaccines for the treatment of metastatic melanoma, differing only in the antigen loading method of autologous tumors: co-culture, fusion, or lysate pulse, and found vaccines to be safe with few side effects (Geskin et al., 2018). Other common vaccines such as recombinant vaccinia virus, plasmid DNA vaccine, autologous tumor cell vaccine, and dinitrophenyl (DNP)-modified melanoma vaccine are still in the stage of clinical trials. Although monotherapy with these vaccines is unlikely to produce substantial complete remission or cure rates in metastatic melanoma, the use of these vaccines to promote anti-tumor immunity may be an important method of future combination therapy (Wolchok et al., 2013).

Immunomodulators are an important part of melanoma immunotherapy (Nicholas and Lesinski, 2011). Common immunomodulators are cytokines such as IL-2, IL-12, IL-15, and IFN. These cytokines can help lymphocytes to recognize melanoma and achieve the purpose of treating tumors (Marabondo and Kaufman, 2017; Mirjacic Martinovic et al., 2017). IFN-α and IL-2 have been used in the immunotherapy of melanoma for decades (Buchbinder and McDermott, 2014). High-dose IL-2 is one of the first immunotherapeutic drugs to demonstrate initial clinical efficacy in advanced cancer patients (Atkins, 2006). The US-FDA approved it in 1996 for the treatment of metastatic malignant melanoma (MM). However,

TABLE 4 | Descriptions of immunotherapies in clinical trials.

Immunotherapies	Type of Drugs	Name of Drugs	Number	Percent
ICI			155	42.8%
	PD-1		83	53.6%
		Nivolumab*	50	
		Pembrolizumab*	30	
		Spartalizumab*	2	
		Camrelizumab	1	
	CTLA-4		64	41.3%
		Ipilimumab*	63	
		Tremelimumab	1	
	PD-L1	Atezolizumab*	5	3.2%
	LAG-3	Relatlimab	3	1.9%
Immunomodulators			66	18.2%
	Cytokine		58	
		IL-2*	50	
		IFN-α-2b	3	
		IFN-γ	1	
		IL-12	1	
		IL-15	1	
		rIL-21	2	
	TLRA	TLRA	1	
	Oncolytic viral	Oncolytic viral*	6	
	Not provided	Not provided	1	
Vaccine	Vaccine		83	22.9%
		ATC	12	
		BCG	2	
		DNA	5	
		DC*	21	
		Dinitrophenyl	2	
		RNA	4	
		Viral	3	
		Peptide	34	
ACT			58	16.0%

**U.S. FDA-regulated drug product; TLRA, toll-like receptor agonist; ATC, autologous tumor cell.*

due to the high toxicity of HD IL-2, it is rarely used in clinical trials to treat MM patients (Ye et al., 2014). Davar *et al.* (Davar et al., 2017) retrospectively analyzed data from 237 patients receiving high-dose (HD) IL-2 from 1992 to 2015. The results showed that the overall response (OR) was 18.1% and complete response (CR) was 8.0%. The median overall survival (OS) was 64.9 months. In addition, this study found that pre-treatment level of lactate dehydrogenase (LDL) and sites of metastatic disease may be useful markers for patients who benefit from HD IL-2 therapy. The anti-tumor effect of IFN-α is expected to be induced by CD8+ T cell-mediated autologous tumor cell lysis. High-dose IFN is currently the standard adjuvant therapy, despite the high incidence of adverse events (Espinosa et al., 2016). The significant clinical efficacy of oncolytic virus (Andtbacka et al., 2015) and toll-like receptor agonist (Mauldin et al., 2015) had opened up a new path for melanoma immunotherapy, and follow-up clinical research is underway. It is expected that it will have better clinical research results and be used in clinical practice as soon as possible.

ACT is another hot spot in immunotherapy for melanoma. Common adoptive cells include tumor infiltrating lymphocytes (TIL) (Lee et al., 2016), chimeric antigen receptor modified T cells (CAR-T) (Wiesinger et al., 2019), and T cell receptor (TCR) gene modified T cells (Lagisetty and Morgan, 2012). TIL is a lymphocyte isolated from tumor tissue. After induction by interleukin-2 *in vitro*, TIL can be amplified in large quantities (Itoh et al., 1986). A clinical trial of TIL treatment for melanoma was followed up to 17 years. The results showed that the major adverse events experienced during treatment were transient and reversible, with no grade 3/4 toxicity or drug-related death observed. The recurrence-free survival of the TIL group was 14 months and nearly 4 months longer than the control group (Khammari et al., 2014). CAR-T cells are a promising approach in adoptive cell therapy for melanoma. The technique requires screening a monoclonal antibody that specifically recognizes certain tumor antigens, and then coupling the binding region of the antibody to certain peptide chains on the T cell surface membrane molecule to construct a chimeric antigen receptor; then, it is introduced into the patient's T cells for expression, and its ability to specifically recognize the antigen is activated to exert an anti-tumor effect (Firor et al., 2015; Ogba et al., 2018). One study has shown a way to stabilize the production of CAR-T cells (Wiesinger et al., 2019). In addition, genetic modification of T cells by altering the specificity of TCR is another strategy of ACT. The antigen specificity of T cells can be manipulated by genetic modification and targeted to antigens expressed by tumors. The production of tumor-specific TCR requires identification of target sequences in advance, then tumor-specific T cells are isolated from patients with tumor remission, and the reactive TCR sequences are transferred to T cells from another patient (de Witte et al., 2006). The tumor killing activity can be enhanced by altering the sequence of TCR to T cells *in vitro* to increase the strength of interaction of TCR with antigen (Robbins et al., 2008; Sharpe and Mount, 2015).

Immunotherapy has revolutionized the treatment of cancer. At the same time, given this growing success, treatment response rates, duration of treatment, why patients respond or not, and if combined with different immunotherapy will overcome this lack of response, delay acquired resistance and increase (Cooper et al., 2014). There are major limitations and unresolved issues in terms of opportunities for success. Given the complexity of immune activation and the considerable variability of tumor biology in patients and tumor types, it is necessary to understand the body's immune pathways, the molecular and immune basis of the disease, and develop interventions and combinatorial strategies that are more suitable for the treatment of cancer patients. Explore patient choices and biomarkers (Ingles Garces et al., 2019). Although immunotherapy has shown promising success, further and ongoing research is needed to determine safety, efficacy, optimal combination, dosage, and timing. Our study also has some limitations. This study only retrieves trials in the *ClinicalTrials.gov*, although approximately two-thirds of total global registrations, we might miss some trials registered in other 15 registration centers (Zarin et al., 2017) that were not fully evaluated. All information is obtained from the *ClinicalTrials.gov*, and some information of registration trials that has not been submitted to the website, therefore, some studies cannot be fully evaluated.

In conclusion, up to now, most clinical trials related to melanoma immunotherapy registered in the *ClinicalTrials.gov* were interventional trials; and although the number of registered

studies increases gradually every year, the number of registered trials was still small. At the same time, it is encouraged to register on the clinical trial registration platform. In addition, we noticed that the results of some clinical trials were not uploaded to registration platform after the end of the trial. It is suggested that the researchers of clinical trials update the latest results of the trial regularly, which will help disseminate information in this field and help doctors get the research frontier as soon as possible. Although some adverse reactions may occur in the course of immunotherapy for melanoma, as an effective treatment for melanoma and even other malignant tumors, we should increase our energy and financial investment in the exploration of immunotherapy.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation, to any qualified researcher.

AUTHOR CONTRIBUTIONS

Y-MY designed this study. Y-BW and GL performed search and collected data. F-HX re-checked data. L-LM and GL performed analysis. Y-BW wrote the manuscript, Y-MY reviewed the manuscript.

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frontiers in Pharmacology

SYSTEMATIC REVIEW
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Comparative Efficacy and Safety of Nivolumab and Nivolumab Plus Ipilimumab in Advanced Cancer: A Systematic Review and Meta-Analysis

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Background: Combination therapy with immune checkpoint inhibitors (ICIs) has been applied in the clinic to achieve synergistic effects and to improve clinical efficacy. Compared with monotherapy, combination therapy has promising efficacy against various advanced cancers. To further verify the effectiveness of combination therapy, we conducted a meta-analysis of the efficacy and safety of nivolumab (NIVO) and NIVO plus ipilimumab (IPI) in advanced cancer.

Methods: Electronic databases (PubMed, EMbase, and The Cochrane Library) were systematically searched for applicable studies published in English between January 1990 and June 2019. Relevant outcomes included objective response rate (ORR), disease control rate (DCR), median progression-free survival (mPFS), median overall survival (mOS), and grade 3–4 adverse events (AEs).

Results: A total of 1,297 patients from six studies were included. Compared with NIVO alone, NIVO + IPI was more efficacious for advanced tumors. Pooled outcome values were: ORR, 1.73 (95% CI: 1.34–2.23); DCR, 1.80 (95% CI: 1.21–2.69); mPFS, 0.22 (95% CI: 0.03–0.41); mOS, 0.03 (95% CI: –0.20–0.26); and grade 3–4 AEs, 3.64 (95% CI: 2.86–4.62).

Conclusion: NIVO + IPI is more effective than NIVO alone for the treatment of advanced cancer and can significantly improve ORR and DCR and prolong mPFS. Due to the limited quality and quantity of the included studies, more high-quality studies are needed to validate the above conclusions.

Keywords: nivolumab, ipilimumab, advanced cancer, combination immunotherapy, efficacy, safety

INTRODUCTION

Cancers remain difficult to cure because the inherent intrinsic genomic instability of tumors facilitates their escape from cytotoxicity and targeted therapy (Miller and Sadelain, 2015). However, the discovery of cancer immune checkpoints and the success of immune checkpoint inhibitors (ICIs) may improve patient survival.

Cytotoxic T-lymphocyte-associated antigen-4 (CTLA-4) (Pardoll, 2012), programmed cell death-1 and its ligands (PD-1/PD-L1/2) (Topalian et al., 2012), and lymphocyte activation gene-3 (Yu et al., 2019) inhibit the T cell immune response. CTLA-4 signaling limits the initiation of the T cell response in the lymph nodes early in the immune response, whereas PD-1 restricts T cell activity later in the process in the tumor microenvironment (Fife and Bluestone, 2008). The CTLA-4 and PD-1–PD-L1/PD-L2 checkpoints are commonly exploited by tumors to evade and/or suppress the immune system. Therefore, many monoclonal antibodies have been developed to block proteins that are involved in the downregulation of immune responses (Meng et al., 2015; Ngiow et al., 2015) by stimulating T cell-dependent cytotoxicity against tumor cells through abrogating peripheral tolerance (Cuende et al., 2015). Therefore, the use of monoclonal antibodies to block immune checkpoints has become a promising cancer treatment strategy (Anagnostou et al., 2017) and can lead to long-lasting antitumor activity, improving survival rates for various malignancies compared with other systemic therapies (Bang et al., 2017). Anti-CTLA-4 antibody (ipilimumab [IPI]), anti-PD-1 antibodies (nivolumab [NIVO] and pembrolizumab), and anti-PD-L1 antibodies (atezolizumab, avelumab, and durvalumab) have been approved for clinical use in various advanced solid tumors, such as melanoma (Hodi et al., 2016), nonsmall cell lung cancer (NSCLC) (Dal Bello et al., 2017), renal cell cancer (Atkins et al., 2017), small cell lung cancer (Schneider and Kalemkerian, 2016), gastro-esophageal cancer, and liver cancer (Gong et al., 2018).

NIVO is a human IgG4 PD-1 ICI antibody that selectively blocks the PD-1 receptor on the surface of cytotoxic T cells to prevent downregulation of the immune response in malignant tumor cells induced by PD-L1 (Minguet et al., 2016). Because it has been shown to significantly improve overall survival (OS) and safety in selected patients, NIVO has been approved by the United States (US) and the European Union (EU) for the treatment of locally advanced or metastatic NSCLC (Minguet et al., 2016; Vokes et al., 2018), advanced renal cell carcinoma, and advanced melanoma (Larkin et al., 2015; Raedler, 2015; Wolchok et al., 2017; Schuyler, 2018). In addition, NIVO can treat recurrent or refractory Hodgkin's lymphoma with good efficacy and safety (Ansell et al., 2015). Ipilimumab (IPI) is a human monoclonal IgG4 that acts as an antineoplastic ICI by selectively binding to cytotoxic T lymphocyte-associated antigen 4, a molecule located on the surface of cytotoxic T cells, suppressing the immune response (Hodi et al., 2010). IPI blocks CTLA-4, leading to a continuously active immune response in malignant cells. The US and EU have approved IPI monotherapy to treat melanoma (Lipson and Drake, 2011).

Although significant progress has been made, the effect of immunotherapy is not completely satisfactory. Despite some durable responses, most patients did not respond to their initial treatment (primary resistance) and some responders later relapsed (acquired resistance). Insufficient infiltration of cytotoxic T lymphocytes, lack of tumor-associated antigens, or activation of other immunosuppressive pathways are significant causes of resistance to immunotherapy (Sharpe and Pauken, 2018).

Compared with monotherapy, ICI-combined therapy can provide a significant OS benefit. Combination therapy has been shown to be efficacious against different malignancies; clinical data show that chemotherapy can induce the expression of PD-L1 in tumor cells and regulate their immune function (Wei et al., 2019). The combination of anti-CTLA4 and anti-PD1 leads to significantly better response rates and progression-free survival than anti-PD1 agents alone. In patients with metastatic melanoma, NIVO monotherapy and NIVO + IPI treatment resulted in significantly longer median progression-free survival (PFS) than chemotherapy or IPI treatment (Hodi et al., 2016; Hao et al., 2017). The mechanism might involve enhanced simultaneous blockade of the CTLA-4 and PD-1 pathways, cell infiltration, and/or activated expression of markers and inflammatory cytokines (Curran et al., 2010). Additionally, a greater ratio of $CD8^+$ T cells to regulatory T cells and myeloid-derived suppressor cells in the tumor may contribute to multiple coinhibitory blockades. However, combination therapy might increase the incidence of adverse events (AEs). The vast majority of these are grade 3–4 AEs that appear in the first few weeks to months after treatment initiation, and the most common ones include pruritus, nausea, rash, diarrhea, and atony. There are also some serious grade 5 AEs, such as pneumonia, neurotoxic effects, myocarditis, and hepatitis, some of which may be fatal (Omuro et al., 2018). The efficacy and safety of combination immunotherapy is still controversial, thus we undertook the current meta-analysis.

MATERIALS AND METHODS

The current systematic review and meta-analysis conformed with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement.

Search Strategy

We searched PubMed, Embase, and The Cochrane Library databases for relevant English-language articles that had been published by 1 June 2019. The following terms were used: (nivolumab or Opdivo) AND (ipilimumab or Yervoy) AND (neoplasm* OR tumor* OR cancer* OR malignant* OR malignant neoplasm*). We also performed a manual search to find applicable studies in the references and related citations.

Eligibility Criteria

We included studies that fulfilled the following criteria: (a) population, patients with stage III–IV malignancies; (b) intervention, NIVO + IPI; (c) control, NIVO monotherapy; (d) prospective study, phase II or III clinical trials; and (e) inclusion

of any of the outcome measures. Where multiple articles had analyzed the same trial, the most recent study was used.

Outcome Measures

The primary outcomes were objective response rate (ORR, percentage of patients who achieved an objective response as defined by the Response Evaluation Criteria in Solid Tumors), disease control rate (DCR), mPFS, median OS (mOS), and AEs. The severity of AEs was graded according to the National Cancer Institute Common Terminology Criteria for Adverse Events, version 4.0.

Data Extraction

Two reviewers independently screened the titles and abstracts of retrieved citations. Discrepancies were resolved by discussion. A standardized extraction form was prepared using Microsoft Excel (Microsoft, Redmond, Washington). The extracted data included first author, study design, population, information for assessment for risk of bias (ROB), treatments, and measured outcomes (ORR, DCR, mPFS, mOS, grade 3–4 AEs).

ROB Assessment

ROB was assessed by two independent reviewers using the Cochrane Collaboration's tool for ROB assessment (Lundh and Gotzsche, 2008).

Statistical Analysis

For the meta-analysis, we estimated the standard mean difference for continuous outcomes. Odds ratio (OR) was used to compare dichotomous variables, and Peto odds ratio was used to compare rare AEs. All the results were reported with 95% confidence intervals (CI). Pooled OR and 95% CIs for dichotomous data were estimated using the Mantel–Haenszel method. The *I*-square (I^2) test was performed to assess the impact of study heterogeneity. If severe heterogeneity was present at $I^2 > 50\%$, the random effect model was chosen; otherwise, the fixed-effect model was used. In the case of a missing SD of the mean change from baseline, it was calculated from the SE or the 95% CI. We used Review Manager (RevMan, version 5.3; Copenhagen: The Nordic Cochrane Centre, The Cochrane Collaboration, 2014).

RESULTS

Search Results and Studied Characteristics

The initial search identified 1,052 publications. After excluding duplicates, 699 publications remained. Of these, 682 studies were discarded after reading the titles and abstracts. After assessing the full texts, 11 reports were further excluded and six studies were included for data analysis. Details regarding the selection of studies are outlined in the flow diagram in **Figure 1**. The included studies were published between 2018 and 2019. The six studies were all randomized controlled trials (D'Angelo et al., 2018; Hodi et al., 2018; Janjigian et al., 2018; Long et al., 2018; Scherpereel et al., 2019; Sharma et al., 2019) and included 1,189 patients with advanced-stage cancers. There were five phase II studies and one phase III study. The intervention group received intravenous NIVO (3 mg/kg) + IPI (1 mg/kg) or intravenous NIVO (1 mg/kg) + IPI (3 mg/kg), while the control group received intravenous NIVO (3 mg/kg) (**Table 1**).

Quality Assessment

The results of the quality assessment are shown in **Figure 2**. Most studies had a low risk of bias. Random sequence generation was not found in two studies (Janjigian et al., 2018; Scherpereel et al., 2019), and some studies did not clearly report concealment (Janjigian et al., 2018; Hodi et al., 2018; Scherpereel et al., 2019; Sharma et al., 2019). The blinding of participants was explicitly reported in only one study (Hodi et al., 2018). Furthermore, some studies did not clearly report selective reporting (Long et al., 2018; Sharma et al., 2019; Janjigian et al., 2018) or other bias (Janjigian et al., 2018).

Efficacy

The efficacy of NIVO + IPI or NIVO for advanced tumors was evaluated by combining ORR, DCR, mPFS, and mOS. We included all six studies to analyze ORR, DCR, and mPFS, and four studies to evaluate mOS. The combined results revealed an ORR of 1.73 (95% CI: 1.34–2.23, $I^2 = 0\%$, $P = 0.46$), suggesting that compared with NIVO monotherapy, patients were more likely to respond to NIVO + IPI therapy, thus improving the ORR. The DCR was 1.80 (95% CI: 1.21–2.69, $I^2 = 53\%$, $P = 0.06$), showing that the PFS of the NIVO + IPI group could control the progression of cancer better than the NIVO group. There was heterogeneity between these two studies and the random effect model was used. PFS was 0.22 (95% CI: 0.03–0.41, $I^2 = 51\%$, $P = 0.07$), indicating that the PFS of the NIVO + IPI group was significantly improved when compared with the NIVO group. There was slight heterogeneity among the studies and the random effect model was used. OS was 0.03 (95% CI: ¯0.20–0.26, $I^2 = 39\%$, P = 0.18), and there was no statistical difference between the NIVO + IPI group and the NIVO group. Significant differences in ORR, DCR, and mPFS were found. These results are shown in **Figures 3**–**6**.

Safety

The combined incidence of grade 3–4 AEs in the six included studies was 3.64 (95% CI: 2.86–4.62; $I^2 = 70\%$; $P = 0.005$); the results showed that the incidence of AEs in the NIVO + IPI group was higher than that in the NIVO group. The total risk of AEs significantly differed between the combination and monotherapy arms (**Figure 7**). The most common AEs in the combined treatment group (n = 606) were hepatotoxicity (n = 71, 11.71%), diarrhea (n = 49, 8.08%), increased lipase (n = 44, 7.26%), rash (n = 27, 4.45%), and fatigue (n = 24, 3.96%). The most common AEs in the monotherapy group (n = 583) were increased lipase (n = 26, 4.45%), hepatotoxicity (n = 13, 2.22%), diarrhea (n = 11, 1.88%), rash (n = 10, 1.71%), and fatigue (n = 9, 1.54%).

Publication Bias Test and Sensitivity Analysis

Publication bias analysis was not performed because our analysis included fewer than 10 studies. Sensitivity analysis was

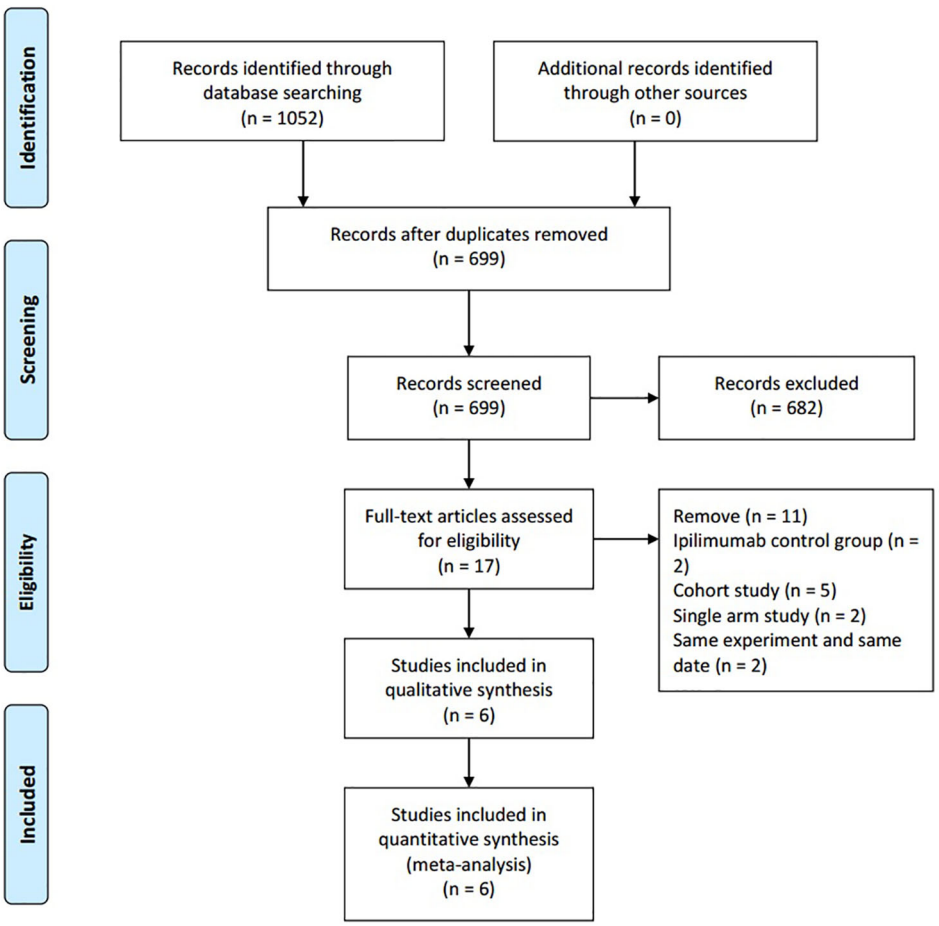

FIGURE 1 | PRISMA chart.

performed on the results, but no significant change was observed after the fixed effect model was adopted, indicating that the results of this study were stable (**Table 2**).

DISCUSSION

Our meta-analysis showed that NIVO + IPI combined immunotherapy significantly improved antitumor efficacy and led to better ORR and DCR compared with NIVO monotherapy. Combined treatment was also associated with longer PFS, but OS did not significantly differ between the two groups. Adverse events ≥ grade 3 were more frequent but controllable in the combined treatment arm.

In the meta-analysis, we found that combination therapy was superior to monotherapy. This may be because: (a) the efficacy of monotherapy is limited by low response rates, with only a small proportion of patients responding to treatment (Rotte et al., 2018; Hellman et al., 2018) (b) combining anti-CTLA-4 and anti-PD-1 therapies was suggested to activate the antitumor immune response synergistically, thus increasing response rates (Curran et al., 2010); (c) combining anti-CTLA-4 and anti-PD-1 therapies significantly increases the ratios of both $CD8^+$/regulatory T cells and $CD4^+$ effector/regulatory T cells within the tumor, so that $CD8^+$ and $CD4^+$ T cells to continue to survive, proliferate, and perform effector functions in the tumor (Duraiswamy et al., 2013; Beavis et al., 2018); (d) combining anti-CTLA-4 and anti-PD-1 therapies allows the accumulation of active T cells that express CTLA-4 and PD-1 and would otherwise be energized (Curran et al., 2010); and (e) combining anti-CTLA-4 and anti-PD-1 therapies increases the production of inflammatory cytokines (such as IFN-γ and TNF-α) in the tumor itself and in its draining lymph nodes (Shi et al., 2016). Some clinical trials support this idea. Combined immunological checkpoint blockade synergistically inhibited tumor immune escape, and thus improved the efficacy of single-agent anti-PD-1 therapy in esophagogastric cancer; however, the clinical effect was not related to the expression of tumor PD-L1 (Janjigian et al., 2018). A previous study (Hodi et al., 2018) reported that NIVO + IPI or NIVO monotherapy could achieve lasting and sustained clinical efficacy in patients with advanced melanoma regardless of *BRAF* mutation status. Although the efficacy of NIVO

TABLE 1 | Characteristics of included studies.

No. Study	Trial phase	Study design	Disease	Participants	Intervention	Comparator	No. of patients (I/C)	Ages(years)		ECOG	
								I	C	I	C
Scherpereel et al., 2019	II	Multicenter open-label, randomized noncomparative,	relapsed malignant pleural mesothelioma	Patients were aged 18 years or older, histologically proven malignant pleural mesothelioma progressing after first-line or second-line pemetrexed and platinum based treatments, measurable disease by CT,	NIVO (3 mg/kg every 2 weeks) + IPI (1 mg/kg every 6 weeks)	NIVO (3 mg/kg) every 2 weeks	125 (62/63)	71.2 (48.1–88.1)	72.3 (32.5–87.2)	0:25 1:36 2:1	0:19 1:42 2:0
Sharma et al., 2019	I/II	Multicenter open-label multiarm randomly assigned	Metastatic Urothelial Carcinoma	Patients in the locally advanced or metastatic platinum pretreated urothelial carcinoma	NIVO 3 mg/kg + IPI 1 mg/kg every 3 weeks for four doses	NIVO (3 mg/kg) every 2 weeks	182 (104/78)	63.0 (39–83)	65.5 (31–85)	0:40 1:64	0:42 1:36
D'Angelo et al., 2018	II	two open-label, noncomparative, randomized,	metastatic sarcoma	patients aged 18 years or older and had central pathology confirmation of sarcoma with at least one measurable lesion, evidence of metastatic, locally advanced or unresectable disease,	NIVO 1 mg/kg + IPI 3 mg/kg every 3 weeks for four doses	NIVO (3 mg/kg) every 2 weeks	85 (42/43)	57.0 (27.0–81.0)	56.0 (21.0–76.0)	0:24 1:18	0:28 1:15
Hodi et al., 2018	III	multicenter, randomized	advanced melanoma	Patients were aged 18 years or older with previously untreated, unresectable, stage III or stage IV melanoma, known BRAFV600 mutation status.	NIVO 1 mg/kg + IPI 3 mg/kg every 3 weeks for four doses	NIVO (3 mg/kg) every 2 weeks	630 (314/316)	–	–	–	–
Janjigian et al., 2018	III	open-label two-stage randomized	Metastatic Esophagogastric	Patients with locally advanced or metastatic chemotherapy–refractory gastric, esophageal, or gastroesophageal junction cancer from centers in the United States and Europe	NIVO 1 mg/kg + IPI 3 mg/kg every 3 weeks for four doses	NIVO (3 mg/kg) every 2 weeks	108 (49/59)	53 (27–77)	60 (29–80)	0:27 1:22	0:29 1:30
Long et al., 2018	II	multicenter randomized	melanoma brain metastases	Immunotherapy-naive patients aged 18 years or older with melanoma brain metastases.	NIVO 1 mg/kg + IPI 3 mg/kg every 3 weeks for four doses	NIVO (3 mg/kg) every 2 weeks	63 (35/25)	59 (53–68)	63 (52–74)	0 +1:34 2:1	0 +1:25

monotherapy was better supported, combination therapy was more likely to prolong survival than NIVO monotherapy. However, PD-L1 levels did not predict the efficacy of combination therapy. Similar to Hodi's research, NIVO + IPI was a suitable first-line treatment for asymptomatic brain metastases, and patients whose baseline biopsy PD-L1 expression was ≥1% had a numerically higher overall mPFS than did patients whose tumor PD-L1 expression was <1% (Long et al., 2018). Other studies (Scherpereel et al., 2019) have pointed out that the combined regimen was most effective in patients with PD-L1^{+} malignant pleural mesothelioma, especially in patients whose tumors had high PD-L1 expression (≥25% positive cells). This view was also supported by a single-arm experiment (Disselhorst et al., 2019). A recent study (D'Angelo et al., 2018) reported that patients with locally advanced, unresectable, or metastatic soft-tissue sarcomas who received combination immunotherapy achieved significant therapeutic effects compared with patients who received monotherapy, but this study did not mention biomarkers that could predict prognosis. Identifying highly sensitive and specific immunotherapeutic biomarkers is an important topic in oncology. In contrast, monotherapy has been shown to be superior to combination therapy for glioblastoma (Omuro et al., 2018). The lesser efficacy in the combination group

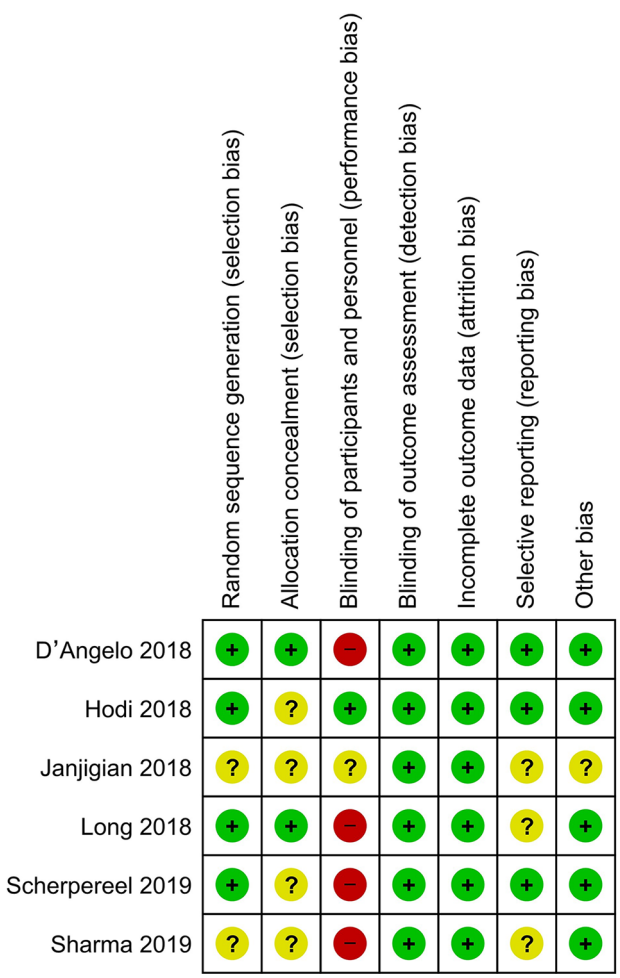

FIGURE 2 | Risk of bias of included studies.

might reflect ICI-enhanced inflammatory infiltration in some patients with central nervous system tumors. Of note, based on previous research, the survival benefit for patients whose tumors have >1% PD-L1+ cells is greater than for patients whose tumors have <1% PD-L1+ cells (Brahmer et al., 2012). However, some of our included studies found that the therapeutic effect was unrelated to PD-L1 expression. Tumor mutation burden (TMB) has shown some clinical predictive value in clinical trials[33].

A recent study also found that the effects in the combined group were influenced by the doses of both drugs (Rozeman et al., 2019); they identified a tolerable combination dose plan (two cycles of NIVO 1 mg/kg + IPI 3 mg/kg) with a high response rate. Another study compared different doses (Sharma et al., 2019) and found that the effective rates of NIVO (3 mg/kg) + IPI (1 mg/kg) *versus* NIVO (1 mg/kg) + IPI (3 mg/kg) were 26.9% and 38.0%, respectively, and mPFS was 2.6 months (95% CI: 1.4–3.9) *versus* 4.9 months (95% CI: 2.7–6.6). Administration cycles also affected outcomes: mPFS was 8.1 months (95% CI: 5.6–13.6) or 3.9 months (95% CI: 2.6–13.2) using NIVO (3 mg/kg) + IPI (1 mg/kg) every 12 or 6 weeks, respectively. Twelve-week cycles appear to be safe.

Of note, NIVO + IPI combination immunotherapy was shown to be effective in many clinical trials that did not meet our study inclusion criteria. NIVO + IPI showed significant advantages over sunitinib in advanced renal cell carcinoma (Motzer et al., 2018), which led to FDA approval of NIVO + IPI for the treatment of advanced renal cell carcinoma (Schuyler, 2018; Cella et al., 2019). Another study (Reck et al., 2019) showed that first-line NIVO + IPI led to continuous early improvement in patients with advanced NSCLC and high TMB compared with chemotherapy. Japan's single-arm experiment (Namikawa et al., 2018) also highlighted the advantages of NIVO + IPI.

This meta-analysis also evaluated grade 3–4 AEs. The combined treatment groups in our study had a higher overall incidence of AEs than the monotherapy groups. The most common AEs associated with combined immunotherapy were hepatotoxicity, diarrhea, increased lipase, fatigue, and rash. Therefore, preventing or treating these AEs among patients who receive these combinations should be considered. Four deaths that might have been associated with combination therapy were reported, including one each from tumor lysis syndrome (Sharma et al., 2019), fulminant hepatitis, encephalitis, and acute kidney failure (Scherpereel et al., 2019).

Other studies analyzed the potential causes of toxicity (Sharma et al., 2019). The NIVO (1 mg/kg) + IPI (3 mg/kg)

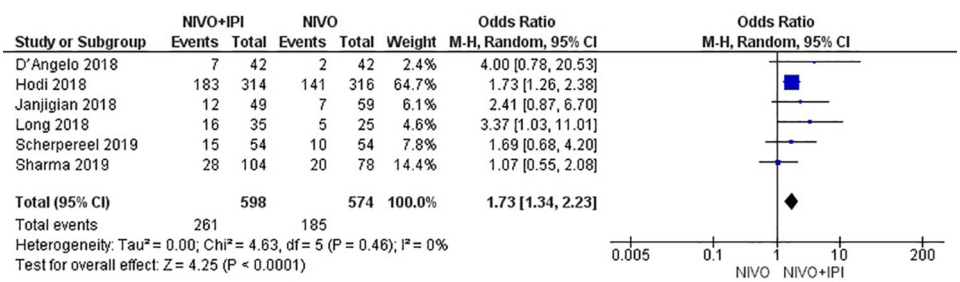

FIGURE 3 | Forest plot of nivolumab plus ipilimumab *vs*. nivolumab for improving ORR.

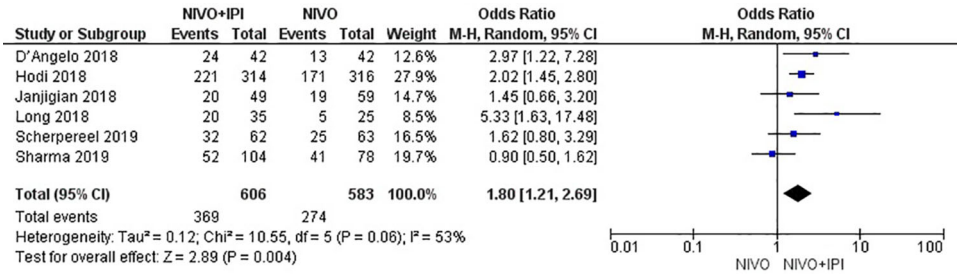

FIGURE 4 | Forest plot of nivolumab plus ipilimumab *vs*. nivolumab for improving DCR.

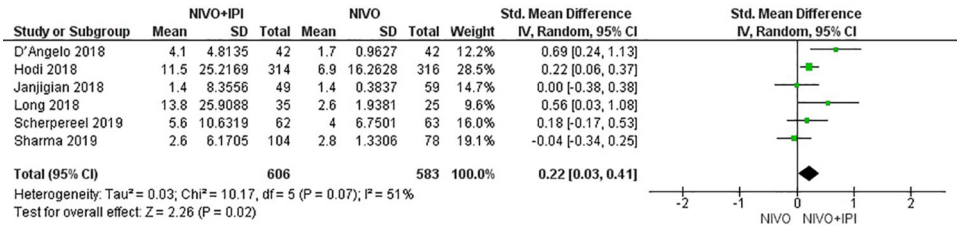

FIGURE 5 | Forest plot of nivolumab plus ipilimumab *vs*. nivolumab for prolonging mPFS.

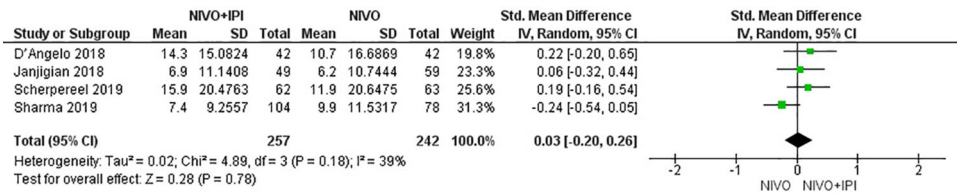

FIGURE 6 | Forest plot of nivolumab plus ipilimumab *vs*. nivolumab for prolonging mOS.

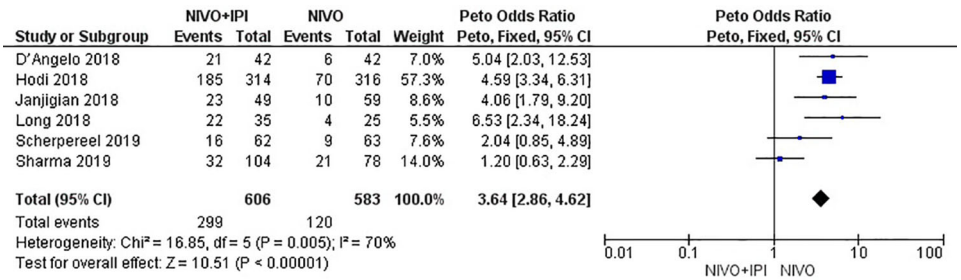

FIGURE 7 | Forest plot of nivolumab plus ipilimumab *vs*. nivolumab for increasing grade 3–4 AEs.

TABLE 2 | Sensitivity analysis.

	objective response rate (ORR)	disease control rate (DCR)	median progression-free survival (mPFS)	median overall survival (mOS)
Random Effect Model	1.73 (95% CI: 1.34–2.22)	1.79 (95% CI: 1.18–2.72)	0.22 (95% CI: 0.03–0.41)	0.03 (95% CI: –0.20–0.26)
Fixed Effect Model	1.73 (95% CI: 1.35, 2.23)	1.78 (95% CI: 1.41, 2.25)	0.20 (95% CI: 0.09, 0.32)	0.01 (95% CI: –0.16, 0.19)

group had the highest incidence of high-grade AEs, possibly due to the dose-related toxicity of IPI. One study (D'Angelo et al., 2018) supported the finding that a lower dose of IPI (1 mg/kg *vs.* 3 mg/kg) might reduce AE incidence and make this combination therapy safer. Notably, another report (Scherpereel et al., 2019) demonstrated that the safety of NIVO alone or combined with IPI compared favorably with what had been proposed for platinum-based chemotherapy. As the AEs observed in our studies were similar to those reported for immunotherapy drugs used in other settings and in previous trials, we hypothesize that the safety of combination therapy was correlated with drug dose and pretreatment. However, further trials with larger study cohorts are required to validate this hypothesis.

Because the included studies were from different tumors, and because of the dose and sequence of the combination, heterogeneity may also result. However, in clinical practice, advanced tumor progression and outcome vary, but the primary therapeutic goal is to control symptoms and prolong survival, consistent with the results of various studies, and thus heterogeneity may not affect the outcome.

This study had some limitations. First, differences in tumor types may lead to heterogeneity between studies. Second, because of the varying designs of the studies, we could not analyze differences in dosages. Third, we only included phase I/II studies; ongoing studies were not included due to incomplete data.

CONCLUSION

In patients with advanced tumors, NIVO + IPI therapy significantly improved ORR, DCR, and mPFS. AEs ≥ grade 3 were more common but were controllable. Due to the limited quality and quantity of the included studies, additional high-quality studies are needed to validate the above conclusions.

AUTHOR CONTRIBUTIONS

YY collected and analyzed the data and wrote the article. GJ and YP collected data. PW, YH, and WW prepared the pictures and tables. ZW, HZ, and GT modified the article. ZZ provided the idea. All authors read and approved the final manuscript.

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REVIEW
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PD-1/PD-L1 Based Combinational Cancer Therapy: Icing on the Cake

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Cancer has been a major global health problem due to its high morbidity and mortality. While many chemotherapy agents have been studied and applied in clinical trials or in clinic, their application is limited due to its toxic side effects and poor tolerability. Monoclonal antibodies specific to the PD-1 and PD-L1 immune checkpoints have been approved for the treatment of various tumors. However, the application of PD-1/PD-L1 inhibitors remains suboptimal and thus another strategy comes in to our sight involving the combination of checkpoint inhibitors with other agents, enhancing the therapeutic efficacy. Various novel promising approaches are now in clinical trials, just as icing on the cake. This review summarizes relevant investigations on combinatorial therapeutics based on PD-1/PD-L1 inhibition.

Keywords: cancer, PD-1, PD-L1, immunotherapy, combinational therapy

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INTRODUCTION

Cancer has become one of the major problems threatening human health based on its high rates of morbidity and mortality (Huang and Fu, 2015; Zhang et al., 2016; Zhang et al., 2017; Huang et al., 2020). Chemotherapeutic drugs play a major role in cancer treatment (Shi et al., 2011; Lin et al., 2017a; Lin et al., 2017b; Jiang et al., 2019). It is undeniable that these treatments are effective at present, but they also destroy the physiological state of normal cells while killing tumor cells, resulting in irreversible damage and therefore poor patient tolerability (Shi et al., 2007; Kathawala et al., 2015; Siegel et al., 2018; Liu et al., 2019). Recently, cancer immunotherapy has been on the rise. It has been shown that immunotherapy has achieved excellent therapeutic efficacy in a variety of tumors, including melanoma, non-small cell lung cancer, renal cell carcinoma, colorectal cancer, as well as breast cancer (Hanahan and Weinberg, 2011; Siegel et al., 2017; Sanmamed and Chen, 2018; Yu et al., 2019). Antibodies specifically against programmed death-1 (PD-1), programmed death-ligand 1 (PD-L1), and cytotoxic T lymphocyte antigen 4 (CTLA-4) (e.g., ipilimumab, tremelimumab) are regarded as recent breakthroughs in cancer immunotherapy (Quezada and Peggs, 2013; Herbst et al., 2014; Turajlic et al., 2018; Rahimi Kalateh Shah Mohammad et al., 2020).

PD-1/PD-L1 OVERVIEW

PD-1 pertains to a suppressive T-cell receptor that is generally expressed by activated T cells, and antigen-specific T cells, which are chronically exposed to various antigens (Day et al., 2006;

Tian et al., 2019; Wang and Wei, 2019). PD-1 is highly selective for immune-inhibitory signals that are mediated by programmed death-ligand 1 (PD-L1, B7-H1), which is generated by malignant cells, myeloid-derived suppressor cells (MDSCs), and leukocytes (Iwai et al., 2002; Blank et al., 2004; Von Knethen and Brüne, 2019). Cancer cells escape immune responses by overexpressing PD-L1 (**Figure 1**) (Okazaki and Honjo, 2007; Markham, 2016; Cao et al., 2019). The immune system is activated by diseases, whereas PD-L1 inhibits the immune system by preventing foreign antigen-specific T cells from accumulating and reducing antigen-specific $CD8^+$ T cell proliferation (Trautmann et al., 2006; Sanmamed and Chen, 2018). The inhibitory effect of therapeutic antibodies on PD-1/PD-L1 is expected to be highly specific to tumor antigen-specific T cells and exhibits lower specificity for auto-reactive T cells (Sznol and Chen, 2013; Homet Moreno et al., 2015). It has been recently confirmed that PD-1/PD-L1 treatment can regulate T-cell activation, including the disruption of suppression of T cell receptor (TCR) activation that is caused by PI3K/Akt/Ras-MEK/ERK, as well as the negative feedback loop involving the cell cycle, thereby leading to apoptosis (Day et al., 2006; Butte et al., 2007; Quigley et al., 2010; Markham, 2016; Kamta et al., 2017; Li X. et al., 2019).

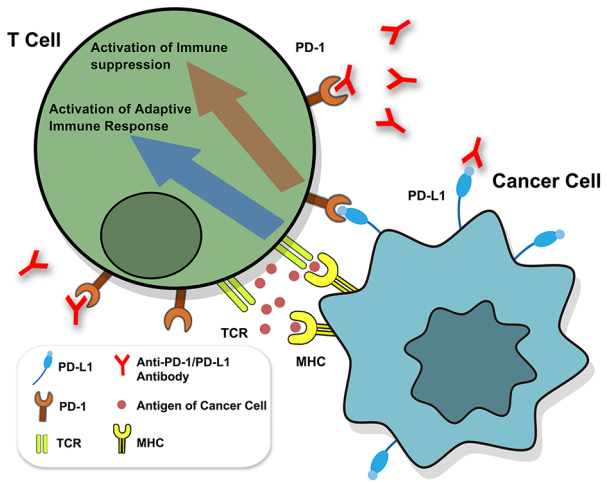

FIGURE 1 | Identification of PD-1/PD-L1. The binding of TCR and MHC activates adaptive immune response. The binding of PD1 and PD-L1 can prevent the signaling transduction of T cells to inhibit the immune response, while anti-PD-1/PD-L1 antibody can reverse the inhibition. TCR, T cell receptor.

DRUGS TARGETING PD-1/PD-L1

Until now, six PD-1/PD-L1 targeted drugs have been listed in dozens of countries in Europe and United States, which are made up of three PD-1 antibodies and three PD-L1 antibodies (Sanmamed and Chen, 2018). See **Table 1** for details. In addition, four innovative anti-PD-1/PD-L1 mAbs have been on the Chinese market, including toripalimab, sintilimab, camrelizumab, and tislelizumab.

ANTI-PD-1/PD-L1 DRUGS BASED COMBINATIONAL THERAPY

Nivolumab Based Combinational Therapy

Preclinical Study

Synergistic antitumor activity in mouse MC38 and CT26 colorectal tumor models was observed with concurrent, but not sequential CTLA-4 and PD-1 blockade. Significant antitumor activity was maintained using a fixed dose of anti-CTLA-4 antibody with decreasing doses of anti-PD-1 antibody

TABLE 1 | Six PD-1/PD-L1 targeted drugs.

Abbreviation	O drug	K drug	T drug	I drug	B drug	L drug
Trade name	Opdivo	Keytruda	Tecentriq	Imfinzi	Bavencio	Libtayo
Common name	Nivolumab	Pembrolizumab	Atezolizumab	Durvalumab	Avelumab	Cemiplimab
Manufacturer	Bristol-Myers Squibb, USA	Merck, USA	Genentech, USA	AstraZeneca, UK	Merck, USA	Regeneron Pharmaceuticals Inc
Target	PD-1	PD-1	PD-L1	PD-L1	PD-L1	PD-1
Indication	Melanoma, metastatic squamous NSCLC, etc.	Melanoma, NSCLC, renal cell carcinoma, head and neck squamous cell carcinoma, etc.	Urothelial carcinoma	NSCLC, urothelial carcinoma	Merkel cell carcinoma, urothelium carcinoma	metastatic cutaneous squamous cell carcinoma (CSCC) or locally advanced CSCC who are not candidates for curative surgery or curative radiation.
Approval year	2014	2014	2016	2017	2017	2018
Time to market	2018	2018	–	–	–	

in the MC38 model. Immunohistochemical and flow cytometric analyses confirmed that $CD3^+$ T cells accumulated at the tumor margin and infiltrated the tumor mass in response to the combination therapy, resulting in favorable effector and regulatory T-cell ratios, increased pro-inflammatory cytokine secretion, and activation of tumor-specific T cells. Similarly, *in vitro* studies with combined ipilimumab and nivolumab showed enhanced cytokine secretion in superantigen stimulation of human peripheral blood lymphocytes and in mixed lymphocyte response assays. In a cynomolgus macaque toxicology study, dose-dependent immune-related gastrointestinal inflammation was observed with the combination therapy, which had not been observed in previous single agent cynomolgus studies. Together, these *in vitro* assays and *in vivo* models comprise a preclinical strategy for the identification and development of highly effective antitumor combination immunotherapies (Selby et al., 2016).

Melanoma

The first clinical trial of combinational treatment of PD-1 plus CTLA-4 inhibitors was reported in 2013 (Wolchok et al., 2013). Here, 53 melanoma patients were treated with nivolumab + ipilimumab, whereas 33 patients received nivolumab alone. Results showed that the efficacy of the combinatorial treatment was superior to ipilimumab or nivolumab alone as earlier reported. In the combinatorial treatment group, the 2-year survival was 79%, and the objective response rate (ORR) was 42%. Responding patients showed an 80% tumor reduction, and 17% of the patients had a complete response (Pico De Coaña et al., 2015). Nivolumab monotherapy and combination with ipilimumab increase proportions of patients achieving a response and survival, versus ipilimumab in patients with metastatic melanoma. In 2015, the United States Food and Drug Administration (USFDA) approved ipilimumab + nivolumab for the treatment of metastatic or unresectable melanoma (Swart et al., 2016).

In a double-blind study involving 142 patients with metastatic melanoma who had not previously received treatment, the ORR and the progression-free survival (PFS) were significantly greater with nivolumab combined with ipilimumab, than that with ipilimumab monotherapy. Combination therapy had an acceptable safety profile (Postow et al., 2015). In a phase 1 dose-escalation study, combined inhibition of T-cell checkpoint pathways by nivolumab and ipilimumab was associated with a high ORR, including complete responses, among patients with advanced melanoma. In the advanced melanoma (CheckMate 067), the phase 2 trial (at 2 years of follow-up) revealed that the combination of first-line nivolumab plus ipilimumab might lead to improved outcomes, compared with first-line ipilimumab alone (Hodi et al., 2016). Nivolumab combined with ipilimumab resulted in longer progression-free survival and a higher ORR than ipilimumab alone in a phase 3 trial involving patients with advanced melanoma. In the advanced melanoma patients, significantly longer overall survival (OS) occurred with combination therapy of nivolumab plus ipilimumab or nivolumab alone, than with ipilimumab alone (Wolchok et al., 2017). The following phase 3 trial (at 4 years of follow-up) showed that a durable, sustained survival benefit can be achieved with first-line nivolumab plus ipilimumab or nivolumab alone in the advanced melanoma patients (Hodi et al., 2018). Among patients with advanced melanoma, sustained long-term OS at 5 years was observed in a greater percentage of patients who received nivolumab plus ipilimumab or nivolumab alone, than monotherapy of ipilimumab. In addition, no patients who received regimens containing nivolumab got apparent loss of quality of life. These results suggest encouraging survival outcomes with immunotherapy in this population of patients (Larkin et al., 2019).

In addition, a multicenter open-label randomized phase 2 trial (NCT02374242) was done and revealed nivolumab combined with ipilimumab and nivolumab monotherapy were active in melanoma brain metastases. A high proportion of patients achieved an intracranial response with the combination. Thus, nivolumab combined with ipilimumab should be considered as a first-line therapy for patients with asymptomatic untreated brain metastases (Long et al., 2018).

The above are some evidence that PD-1 and CTLA-4 are efficacious *via* dependent immune pathways. The simultaneous inhibition of both pathways can induce synergistic effects.

NSCLC and SCLC

A single-center phase Ib study investigated the tolerability, safety, and pharmacokinetics of nivolumab combined with standard chemotherapy in patients with advanced non-small-cell lung cancer (NSCLC). Results indicated that combination of nivolumab 10 mg/kg and chemotherapy showed an acceptable toxicity profile and encouraging antitumor activity in patients with advanced NSCLC (Kanda et al., 2016). In three academic hospitals in the USA, an open-label, non-randomized, phase Ib clinical trial was conducted with patients with ages ≥18 years. These individuals were previously treated histologically or confirmed cytologically to be at stage IIIB or IV NSCLC. From January 2016 to June 2017, 21 patients received ALT-803 (an IL-15 superagonist) plus nivolumab at four dose levels. The results showed that the ALT-803 + nivolumab is safe in the outpatient setting, using a dose of ALT-803 at 20 μg/kg that was administered subcutaneously once per week plus nivolumab administered intravenously at 240 mg every 2 weeks. This is the first report on using IL-15 in the treatment of patients with NSCLC, the potential of ALT-803 + nivolumab (Wrangle et al., 2018). In addition, Oshima Y, et al. found a higher proportion of reports about Interstitial Pneumonitis (IP) for nivolumab in combination with EGFR-TKI, than treatment with either drug alone, including concomitant and sequential use, and careful monitoring for IP is recommended (Oshima et al., 2018; Li D. et al., 2019).

Hellmann MD, et al. indicated that in SCLC patients, nivolumab plus ipilimumab appeared to provide a greater clinical benefit than nivolumab monotherapy in the high tumor mutational burden tertile (Hellmann et al., 2018).

Metastatic Sarcoma

Patients with metastatic sarcoma have limited treatment options. In the two open-label, non-comparative, randomized, phase 2 trials (NCT02500797), the activity and safety of nivolumab alone or in combination with ipilimumab in patients with locally advanced, unresectable, or metastatic sarcoma were investigated. The results indicated nivolumab combined with ipilimumab demonstrated promising efficacy in certain sarcoma subtypes, with a manageable safety profile comparable to current available treatment options. The combination therapy met its predefined primary study endpoint; further evaluation of nivolumab plus ipilimumab in a randomized study is warranted (D'angelo et al., 2018).

Renal-Cell Carcinoma

Purpose combination treatment with immune checkpoint inhibitors has shown enhanced antitumor activity. The open-label, parallel-cohort, dose-escalation, phase I CheckMate 016 study evaluated the efficacy and safety of nivolumab plus ipilimumab, and nivolumab plus a tyrosine kinase inhibitor in metastatic renal cell carcinoma (mRCC). This investigation showed that nivolumab plus ipilimumab therapy demonstrated manageable safety, notable antitumor activity, and durable responses with promising OS in patients with mRCC (Hammers et al., 2017).

OS and ORR were significantly higher with nivolumab plus ipilimumab than with sunitinib among intermediate- and poor-risk patients with previously untreated advanced renal-cell carcinoma. Further study showed that treatment-related adverse events, grade 3 or 4 events, and treatment-related adverse events leading to discontinuation were lower in the nivolumab-plus-ipilimumab group than in the sunitinib group (Motzer et al., 2018).

Lymphoma

In the phase 1/2 study, brentuximab vedotin (BV) and nivolumab administered in combination was an active and well-tolerated first salvage regimen, potentially providing patients with R/R HL an alternative to traditional chemotherapy (Clinical Trials: NCT02572167) (Herrera et al., 2018).

Combining local irradiation with anti-PD-1 checkpoint blockade treatment is feasible and synergistic in refractory Hodgkin's lymphoma. Correlative studies also suggest that the expression of PD-L1, DNA damage response, and mutational tumor burden can be used as potential biomarkers for treatment response (Qin et al., 2018).

The combination of ibrutinib and nivolumab had an acceptable safety profile and preliminary activity was similar to that reported with single-agent ibrutinib in chronic lymphocytic leukemia or small lymphocytic lymphoma, follicular lymphoma, and diffuse large B-cell lymphoma (Clinical Trials: NCT02329847) (Younes et al., 2019).

Colorectal Cancer

The clinical trial CheckMate-142 evaluated the efficacy and safety of nivolumab + ipilimumab in 119 patients with microsatellite instability-high (MSI-H)/DNA mismatch repair-deficient (dMMR) metastatic colorectal cancer (mCRC). The patients received a combination of 3 mg/kg nivolumab and 1 mg/kg ipilimumab at 3-week intervals (for a total of four doses), followed by nivolumab 3 mg/kg at 2-week intervals (Gourd, 2018; Overman et al., 2018). Approximately 76% of patients earlier received two or more systemic treatments. The nivolumab + ipilimumab regimen showed acceptable tolerability, high response rate, and significantly higher PFS and OS at 12-month follow-up. Nivolumab + ipilimumab was thus considered as a potential novel treatment option for patients with dMMR/MSI-H mCRC (Sznol, 2014; Gourd, 2018).

The details for clinical trials of nivolumab based combinational therapy were summarized in **Table 2**.

Pembrolizumab Based Combinational Therapy

Melanoma

Standard-dose pembrolizumab given in combination with four doses of reduced-dose ipilimumab followed by standard-dose pembrolizumab has a manageable toxicity profile and provides robust anti-tumor activity in patients with advanced melanoma. These data suggest that standard-dose pembrolizumab plus reduced-dose ipilimumab might be a tolerable, efficacious treatment option for patients with advanced melanoma (Clinical Trials: NCT02089685) (Long et al., 2017).

For melanoma brain metastases patients, Radiosurgery/stereotactic radiotherapy in combination with immunotherapy and targeted agents has been shown to be feasible and well tolerable (Trino et al., 2017).

A phase Ib trial evaluated intratumoral SD-101, a synthetic CpG oligonucleotide that stimulates Toll-like receptor 9 (TLR9), in combination with pembrolizumab in patients with unresectable or metastatic malignant melanoma. Results indicated that the combination of pembrolizumab with intratumoral SD-101 is well tolerated and can induce immune activation at the tumor site. Combining an intratumoral TLR9 innate immune stimulant with PD-1 blockade can potentially increase clinical efficacy with minimal additional toxicity relative to PD-1 blockade alone (Clinical Trials: NCT02521870) (Ribas et al., 2018).

NSCLC

Pembrolizumab is a humanized monoclonal antibody against programmed death 1 (PD-1) that has antitumor activity in advanced non-small-cell lung cancer (NSCLC), with increased activity in tumors that express programmed death ligand 1 (PD-L1). In patients with advanced NSCLC and PD-L1 expression on at least 50% of tumor cells, pembrolizumab was associated with significantly longer progression-free and OS and with fewer adverse events than was platinum-based chemotherapy (Clinical Trials: NCT02142738) (Reck et al., 2016).

More recently, pembrolizumab plus chemotherapy was shown to be an effective and tolerable first-line treatment option for patients with advanced non-squamous NSCLC.

TABLE 2 | Nivolumab based combinational therapy.

Cancer type	Treatment	Dose schedule	Efficacy	Adverse rate	Notes	References
Melanoma	Nivolumab ± ipilimumab	N + I q3w × 4 doses, followed by N q3w × 4 doses, continued q12w for up to 8 doses Escalating doses of N: 0.3, 1, 3, 10 mg/kg; of I: 1, 3, 10 mg/kg N q2w for up to 48 doses (previously treated with ipilimumab) Escalating doses of N: 1, 3 mg/kg	All: 40% ORR Acceptable level of AEs (1 mg/kg N + 3 mg/kg I): 53% ORR 20% ORR 73.4% OS	53% Grade 3/4 AEs	NCT01024231 Patients with a diagnosis of measurable, unresectable, stage III or IV melanoma;	(Wolchok et al., 2013)
		1 mg/kg N + 3 mg/kg I q3w for 4 doses, followed by 3 mg/kg N q2w	59% ORR 73.4% OS	92% AEs	NCT01927419 CheckMate 069	(Hodi et al., 2016)
		3 mg/kg I q3w × 4 doses	11% ORR 63.8% OS	94% AEs	Patients with unresectable stage III or IV melanoma	
		1 mg/kg N + 3 mg/kg I q3w × 4 doses, followed by 3 mg/kg N q2w	58% ORR 58% OS	59% Grade 3/4 AEs	NCT01844505 CheckMate 067	(Wolchok et al., 2017)
		3 mg/kg N q2w × 4 doses	44% ORR 52% OS	21% Grade 3/4 AEs	Patients with stage III (unresectable) or stage IV melanoma	
		3 mg/kg I q3w × 4 doses	19% ORR 34% OS	28% Grade 3/4 AEs		
		1 mg/kg N + 3 mg/kg I q3w × 4 doses, followed by 3 mg/kg N q2w	58% ORR	59% Grade 3/4 AEs	NCT01844505 Patients with unresectable or stage III or stage IV melanoma,	(Hodi et al., 2018)
		3 mg/kg N q2w ×4 doses	45% ORR	22% Grade 3/4 AEs		
		3 mg/kg I q3w × 4 doses	19% ORR	28% Grade 3/4 AEs		
		1 mg/kg N + 3 mg/kg I q3w × 4 doses, followed by 3 mg/kg N q2w	58% ORR 22% CR	59% Grade 3/4 AEs	NCT01844505 CheckMate 067	(Larkin et al., 2019)
		3 mg/kg N q2w	45% ORR 19% CR	23% Grade 3/4 AEs		
		3 mg/kg I every 3 weeks × 4 doses	19% ORR 6% CR	28% Grade 3/4 AEs		
		1 mg/kg N + 3 mg/kg I q3w × 4 doses, then 3 mg/kg N q2w	46% ORR 78% OS	97% TRAEs 54% Grade 3/4 AEs	NCT02374242 Patients with melanoma brain metastases	(Long et al., 2018)
		3 mg/kg N q2w	20% ORR 68% OS	68% TRAEs 16% Grade 3/4 AEs		
		3 mg/kg N q2w (local therapy failed, neurological symptoms, or leptomeningeal disease)	6% ORR 44% OS	50% TRAEs 13% Grade 3/4 AEs		
Lung cancer	Nivoluma + standard chemotherapy	10 mg/kg N (D1) + 1250 mg/m^2 gemcitabine (D1 and 8) + 80 mg/m^2 cisplatin (D1), q3w for up to four cycles, followed by 10 mg/kg N (D1) q3w	50% ORR 6.28 months mPFS	66.7% Grade 3/4 AEs	JapicCTI-132071 Patients with stage IIIB (without indication for definitive radiotherapy)	(Kanda et al., 2016)
		10 mg/kg N (D1) + 500 mg/m^2 pemetrexed (D1) + 75 mg/m^2 cisplatin (D1) q3w for up to four cycles, followed by 10 mg/kg N (D1) + 500 mg/m^2 pemetrexed (D1) q3w	50% ORR 9.63 months mPFS	66.7% Grade 3/4 AEs	stage IV, or recurrent NSCLC	
		10 mg/kg N (D1) + 200 mg/m^2 paclitaxel (D1) + 6 mg/ml/min (AUC) carboplatin (D1) + 15 mg/kg bevacizumab (D1) q3w for up to six cycles, followed by 10 mg/kg N (D1) + 15 mg/kg bevacizumab (D1) q3w	100% ORR None mPFS	100% Grade 3/4 AEs		
		10 mg/kg N (D1) + 75 mg/m^2 docetaxel (D1)	16.7% ORR 3.15 months mPFS	100% Grade 3/4 AEs		
	ALT-803 + nivolumab	3 mg/kg N q2w + ALT-803 q1w × four cycles Escalating dose of ALT-803: 6, 10, 15, or 20 μg/kg	ORR 29% 17.4 months mPFS	–	NCT02523469 Patients with IIIB or IV NSCLC (or recurrent disease following previous radiotherapy or surgical resection)	(Wrangle et al., 2018)
	Nivolumab ± ipilimumab	1 mg/kg N + 3 mg/kg I q3w for four cycles, followed by 3 mg/kg N q2w	46.2% ORR	–	NCT01928394 CheckMate 032	(Hellmann et al., 2018)

(Continued)

TABLE 2 | Continued

Cancer type	Treatment	Dose schedule	Efficacy	Adverse rate	Notes	References
		3 mg/kg N q2w	21.3% ORR	–	Patients with limited- or extensive-stage SCLC with progression after at least one platinum-based chemotherapy regimen	
Metastatic sarcoma	Nivolumab ± ipilimumab	3 mg/kg N + 1 mg/kg I q3w for 4 doses, followed by 3 mg/kg N q2w for up to 2 years	16% ORR 4.1 months mPFS	26% Serious TRAEs	NCT02500797 Patients with bone or soft tissue sarcoma,	(D'angelo et al., 2018)
		3 mg/kg N q2w, followed by 3 mg/kg N q2w for up to 2 years	5% ORR 1.7 months mPFS	19% Serious TRAEs	locally advanced, unresectable, or metastatic sarcoma	
Renal-cell carcinoma	Nivolumab + ipilimumab	3 mg/kg N + 1mg/kg I q3w × 4 doses, followed by 3 mg/kg N q2w	40.4% ORR 67.3% OS	38.3% Grade 3/4 TRAEs	CheckMate 016 Patients with advanced	(Hammers et al., 2017)
		1 mg/kg N + 3 mg/kg I q3w × 4 doses, followed by 3 mg/kg N q2w	40.4% ORR 69.6% OS	61.7% Grade 3/4 TRAEs	RCC or mRCC with a clear-cell component	
		3 mg/kg N + 1 mg/kg I q3w × 4 doses, followed by 3 mg/kg N q2w	55.2% ORR 80% OS	93% TRAEs 46% Grade 3/4 AEs	NCT02231749 Patients with advanced	(Motzer et al., 2018)
		50 mg sunitinib q1d for 4 weeks	25.5% ORR 72% OS	97% TRAEs 63% Grade 3/4 AEs	renal-cell carcinoma with a clear-cell component	
Colorectal cancer	Nivolumab + ipilimumab	3 mg/kg N + 1 mg/kg I q3w × 4 doses, followed by 3 mg/kg N q2w	55% ORR 85% OS	–	CheckMate-142 Patients recurrent CRC or mCRC assessed as dMMR and/or MSI-H per local guidelines	(Overman et al., 2018)

Cohort G of KEYNOTE-021 (NCT02039674) evaluated the efficacy and safety of pembrolizumab plus pemetrexed-carboplatin (PC) versus PC alone as first-line therapy for advanced nonsquamous NSCLC. At the primary analysis (median follow-up time 10.6 months), pembrolizumab significantly improved ORR and PFS; the hazard ratio (HR) for OS was 0.90 (95% confidence interval [CI]: 0.42–1.91) (Langer et al., 2016).

The updated analysis indicated that significant improvements in PFS and ORR with pembrolizumab plus PC versus PC alone observed in the primary analysis were maintained, and the HR for OS with a 24-month median follow-up was 0.56, favoring pembrolizumab plus PC (Borghaei et al., 2019).

In patients with previously untreated metastatic nonsquamous NSCLC without EGFR or ALK mutations, the addition of pembrolizumab to standard chemotherapy of pemetrexed and a platinum-based drug resulted in significantly longer OS and PFS than chemotherapy alone (Clinical Trials: NCT02578680) (Gandhi et al., 2018).

In patients with previously untreated metastatic, squamous NSCLC (Clinical Trials: NCT02775435), the addition of pembrolizumab to chemotherapy with carboplatin plus paclitaxel or nab-paclitaxel resulted in significantly longer OS and PFS than chemotherapy alone (Paz-Ares et al., 2018).

Insinga RP et al. describe cost-effectiveness of pembrolizumab plus platinum and pemetrexed chemotherapy in metastatic, non-squamous, NSCLC patients in the US. As a result, the addition of pembrolizumab to chemotherapy is projected to extend life expectancy to a point not previously seen in previously untreated metastatic non-squamous NSCLC. Although ICERs vary by sub-group and comparator, results suggest pembrolizumab + chemotherapy yields ICERs near, or in most cases, well below a 3-times US per capita GDP threshold of $180,000/QALY, and may be a cost-effective first-line treatment for metastatic non-squamous NSCLC patients (Insinga et al., 2018).

Renal-Cell Carcinoma

The treatment combination of axitinib plus pembrolizumab is tolerable and shows promising antitumour activity in patients with treatment-naive advanced renal cell carcinoma (Clinical Trials: NCT02133742) (Atkins et al., 2018). In addition, among patients with previously untreated advanced renal-cell carcinoma, treatment with pembrolizumab plus axitinib resulted in significantly longer OS and PFS, as well as a higher ORR, than treatment with sunitinib (Clinical Trials: NCT02853331) (Rini et al., 2019).

Advanced Solid Tumors

Purpose Stereotactic body radiotherapy (SBRT) may stimulate innate and adaptive immunity to augment immunotherapy response. Multisite SBRT is an emerging paradigm for treating metastatic disease. Anti-PD-1-treatment outcomes may be improved with lower disease burden. A phase I study to evaluate the safety of pembrolizumab with multisite SBRT in patients with metastatic solid tumors and indicated that multisite SBRT followed by pembrolizumab was well tolerated with acceptable toxicity. Additional studies exploring the clinical benefit and predictive biomarkers of combined multisite SBRT

and PD-1-directed immunotherapy are ongoing (Luke et al., 2018).

The phase Ib study (NCT02179918) evaluated the safety, antitumor activity, pharmacokinetics, and pharmacodynamics of utomilumab, a fully human IgG2 mAb agonist of the T-cell costimulatory receptor 4-1BB/CD137 in combination with the humanized, PD-1-blocking IgG4 mAb pembrolizumab in patients with advanced solid tumors. Results showed that patients received combination treatment with no dose-limiting toxicities. Treatment-emergent adverse events were mostly grades 1 to 2, without any treatment-related discontinuations. 26.1% patients had confirmed complete or partial responses (Tolcher et al., 2017).

Gastric/Gastroesophageal Junction Cancer

The multicohort, phase II, nonrandomized KEYNOTE-059 study evaluated pembrolizumab ± chemotherapy in advanced gastric/gastroesophageal junction cancer. In detail, in the combination therapy and monotherapy cohorts, 25 and 31 patients were enrolled; median follow-up was 13.8 months (range 1.8–24.1) and 17.5 months (range 1.7–20.7), respectively. In the combination therapy cohort, grade 3/4 treatment-related adverse events occurred in 19 patients (76.0%); none were fatal. In the monotherapy cohort, grade 3–5 treatment-related adverse events occurred in seven patients (22.6%); one death was attributed to a treatment-related adverse event (pneumonitis). The ORR was 60.0% [95% confidence interval (CI), 38.7–78.9] (combination therapy) and 25.8% (95% CI 11.9–44.6) (monotherapy). This study indicated that pembrolizumab demonstrated antitumor activity and was well tolerated as monotherapy and in combination with chemotherapy in patients with previously untreated advanced gastric/gastroesophageal junction adenocarcinoma (Bang et al., 2019).

The details for clinical trials of pembrolizumab based combinational therapy were summarized in **Table 3**.

Atezolizumab Based Combinational Therapy

NSCLC and SCLC

Atezolizumab, which restores anticancer immunity, improved OS in patients with previously treated NSCLC and also showed clinical benefit when combined with chemotherapy as first-line treatment of NSCLC. To assess the efficacy and safety of atezolizumab plus chemotherapy versus chemotherapy alone as first-line therapy for non-squamous NSCLC, IMpower130 showed a significant and clinically meaningful improvement in OS and a significant improvement in PFS with atezolizumab plus chemotherapy, than chemotherapy as first-line treatment of patients with stage IV non-squamous NSCLC and no ALK or EGFR mutations. No new safety signals were identified. This study supports the benefit of atezolizumab, in combination with platinum-based chemotherapy, as first-line treatment of metastatic non-small-cell lung cancer (Clinical Trials: NCT02367781) (West et al., 2019).

The phase Ib clinical trial NCT01633970 involved patients with metastatic or locally advanced NSCLC (n = 30) who received 15 mg/kg atezolizumab at 3-week intervals combined with standard chemotherapy (carboplatin + paclitaxel, pemetrexed, or nab-paclitaxel for a total of 4–6 cycles and then maintained with atezolizumab until progression). The ORR was 67% (18 partial responses; two complete responses) (Markham, 2016; Liu et al., 2018).

The addition of atezolizumab to chemotherapy in the first-line treatment of extensive-stage small-cell lung cancer resulted in significantly longer OS and PFS than chemotherapy alone. (Clinical Trials: NCT02763579) (Horn et al., 2018).

Breast Cancer

Atezolizumab plus nab-paclitaxel prolonged PFS among patients with metastatic triple-negative breast cancer in both the intention-to-treat population and the PD-L1-positive subgroup. Adverse events were consistent with the known safety profiles of each agent (Clinical Trials: NCT02425891) (Schmid et al., 2018).

In the phase Ib clinical trial NCT01633970, patients diagnosed with triple-negative breast cancer received atezolizumab (800 mg at 2-week intervals) plus nab-paclitaxel (125 mg/m^2, once a week for 3 weeks in a 4-week treatment course), and five patients were evaluated for efficacy at three-month follow up (four partial responses and one complete response) (Markham, 2016; Liu et al., 2018).

Renal-Cell Carcinoma

In the phase Ib clinical trial NCT01633970, patients (n = 12) diagnosed with metastatic renal cell carcinoma received atezolizumab (20 mg/kg) plus bevacizumab (15 mg/kg, at 3-week intervals). At a minimum follow up of 2.1 months, a total of 10 evaluable patients exhibited an ORR of 40%. This study indicated that atezolizumab in combination with bevacizumab enhances antigen-specific T-cell migration in metastatic renal cell carcinoma (Wallin et al., 2016).

The details for clinical trials of atezolizumab based combinational therapy were summarized in **Table 4**.

Durvalumab Based Combinational Therapy

NSCLC

Clinical Trials NCT02000947 assess durvalumab plus tremelimumab in patients with advanced squamous or non-squamous NSCLC. Durvalumab 20 mg/kg every 4 weeks plus tremelimumab 1 mg/kg showed a manageable tolerability profile, with antitumor activity irrespective of PD-L1 status (Antonia et al., 2016).

Clinical trial NCT02088112 evaluated the combinational therapy of durvalumab (10 mg/kg intravenously Q2W) plus gefitinib (250 mg once daily) in TKI-naive patients harboring sensitizing EGFR mutations associated with advanced NSCLC (Gibbons et al., 2016). Approximately 10 patients were assigned to group 1 and given durvalumab + gefitinib, whereas the other 10 patients of group 2 were administered gefitinib monotherapy for the first 4 weeks, followed by gefitinib plus durvalumab (Gibbons et al., 2016). The results observed grade 3–4 adverse effects, and the treatment was

TABLE 3 | Pembrolizumab based combinational therapy.

Cancer type	Treatment	Dose schedule	Efficacy	Adverse rate	Notes	References
Melanoma	Pembrolizumab + ipilimumab	2 mg/kg P + 1 mg/kg I q3w × 4 doses, followed by 2 mg/kg P q3w for up to 2 years	61% ORR 89% OS	45% Grade 3/ 4 TRAEs	NCT02089685 Patients with advanced melanoma	(Long et al., 2017)
	SD-101 + pembrolizumab	1, 2, 4, or 8 mg SD-101 (Naive to prior anti-PD-1/PD-L1 therapy)	ORR 78%	–	NCT0252189 Patients with unresectable or metastatic malignant melanoma	(Ribas et al., 2018)
		1, 2, 4, or 8 mg SD-101 (Received prior anti-PD-1/PD-L1 therapy)	ORR 15%	–		
	Pembrolizumab ± chemotherapy	200 mg P for four cycles + 5 mg/ml/min (AUC) carboplatin + 500 mg/m^2 pemetrexed q3w, followed by P for 24 months + pemetrexed maintenance	55% ORR	93% TRAEs	NCT02039674 Patients with chemotherapy-naive, stage IIIB, or IV, non-squamous NSCLC	(Langer et al., 2016)
		Carboplatin + pemetrexed for four cycles, followed by pemetrexed maintenance	29% ORR	90% TRAEs		
	Pembrolizumab ± PC	500 mg/m^2 pemetrexed + 5 mg/ml/min (AUC) carboplatin q3w for four cycles + 200 mg P q3w for 2 years	56.7% ORR	16.9% TRAEs	NCT02039674 MK-3475-021/KEYNOTE-021 Patients with stage IIIB/IV nonsquamous NSCLC	(Borghaei et al., 2019)
		500 mg/m^2 pemetrexed + 5 mg/ml/min (AUC) carboplatin q3w for four cycles	30.2% ORR	12.9% TRAEs		
	Pembrolizumab ± Pemetrexed + platinum-based drug	Pemetrexed + platinum-based drug+ 200 mg P q3w for four cycles, followed by P for up to 35 cycles + pemetrexed maintenance	69.2% OS	–	NCT02578680 KEYNOTE-189 Patients with metastatic non-squamous NSCLC	(Gandhi et al., 2018)
		Pemetrexed + platinum-based drug q3w for four cycles, followed by pemetrexed maintenance	49.4% OS			
	Pembrolizumab ± carboplatin + [nab]-paclitaxel	200 mg P (D1) for up to 35 cycles + 6 mg/ml/min (AUC) carboplatin (D1) + 200 mg/m^2 paclitaxel (D1) or 100 mg/m^2 nab-paclitaxel (D1, 8, and 15) for the first four cycles	15.9 months mOS	98.2% AEs 69.8% Grade > 3 AEs	NCT02775435 KEYNOTE-407 Patients with untreated metastatic, squamous NSCLC	(Paz-Ares et al., 2018)
		200 mg P (D1) for up to 35 cycles	13.2 months mOS	97.9% AEs 68.2% Grade ≥ 3 AEs		
Renal-cell carcinoma	Pembrolizumab + axitinib	5 mg axitinib q2d + 2 mg/kg P q3w	73% ORR 20.4 months mOS	65% Grade ≥ 3 AEs 54% TRAEs	NCT02133742 Patients with advanced renal cell carcinoma (predominantly clear cell subtype)	(Atkins et al., 2018)
		200 mg P q3w + 5 mg axitinib q2d	59.3% ORR 15.1 months mPFS	75.8% Grade ≥ 3 AEs	NCT02853331 KEYNOTE-426 Patients with untreated advanced clear-cell renal-cell carcinoma	(Rini et al., 2019)
		50 mg sunitinib q1d for the first 4 weeks of each 6-week cycle	35.7% ORR 11.1 months mPFS	70.6% Grade ≥ 3 AEs		
Advanced solid tumors	SBRT + pembrolizumab	SBRT + 200 mg P q3w (within 7 days)	ORR 13.2% 9.6 months mOS 3.1 months mPFS	–	NCT02608385 Patients with metastatic solid tumor previously treated with standard-of-care therapy	(Luke et al., 2018)
	Pembrolizumab + utomilumab	2 mg/kg P q3w + 0.45–5.0 mg/kg utomilumab	26.1% ORR	–	NCT02179918 Patients with advanced/ metastatic solid tumor malignancy	(Tolcher et al., 2017)
Gastric/ gastroesophageal junction cancer	Pembrolizumab ± chemotherapy	200 mg P for over 30 min infusion (D1) + 80 mg/m^2 cisplatin (D1) for up to six cycles + 800 mg/m^2 5-fluorouracil (D1–5 of each 21-day cycle) for continuous infusion	60.0% ORR	100% TRAEs	NCT02335411 KEYNOTE-059 Patients with recurrent or metastatic G/GEJ adenocarcinoma	(Bang et al., 2019)
		200 mg pembrolizumab for over 30 min infusion (D1 of each 21-day cycle)	25.8% ORR	77.4% TRAEs		

TABLE 4 | Atezolizumab based combinational therapy.

Cancer type	Treatment	Dose schedule	Efficacy	Adverse rate	Notes	References
NSCLC and SCLC	Atezolizumab + Chemotherapy	1200 mg A q3w + 6 mg/ml/min (AUC) carboplatin q3w + 100 mg/m² nab-paclitaxel q1w	18.6 months mOS	24% Serious TRAEs	NCT02367781 Patients with stage IV non-squamous NSCLC	(West et al., 2019)
		6 mg/ml/min (AUC) carboplatin q3w + 100 mg/m² nab-paclitaxel q1w for 4 or 6 21-day cycles, followed by maintenance therapy	13.9 months mOS	13% Serious TRAEs		
	Atezolizumab + platinum-based doublet chemotherapy	15 mg/kg A + 6 mg/ml (AUC) carboplatin q3w + 200 mg/m^2 paclitaxel q3w	36% ORR 12.9 months mOS	76% Grade≥3 TRAEs	NCT01633970 Patients with stage IIIB/IV NSCLC	(Markham, 2016; Liu et al., 2018)
		15 mg/kg A + 6 mg/ml (AUC) carboplatin q3w + 500 mg/m^2 pemetrexed q3w	68% ORR 18.9 months mOS	52% Grade≥3 TRAEs		
		15 mg/kg A + 6 mg/ml (AUC) carboplatin q3w + 100 mg/m^2 nab-paclitaxel q1w	46% ORR 17 months mOS	89% Grade≥3 TRAEs		
	Atezolizumab + Carboplatin and Etoposide	5 mg/ml/min (AUC) carboplatin for four 21-day cycles + 100 mg/m^2 etoposide (D1-3 of each cycle) + 1200 mg A (D1 of each cycle)	60.2% ORR 12.3 months mOS	56.6% Grade 3/4 AEs	NCT02763579 Patients with extensive-stage SCLC	(Horn et al., 2018)
		5 mg/ml/min (AUC) carboplatin for four 21-day cycles + 100 mg/m^2 etoposide (D1-3 of each cycle)	64.4% ORR 10.3 months mOS	56.1% Grade 3/4 AEs		
Breast cancer	Atezolizumab ± nab-paclitaxel	840 mg A (D1 and 15) + 100 mg/m^2 nab-paclitaxel (D1, 8, and 15) for 28-day cycle	56.0% ORR 21.3 months mOS	48.7% Grade 3/4 AEs	NCT02425891 Patients with metastatic TNBC	(Schmid et al., 2018)
		100 mg/m^2 nab-paclitaxel (D1, 8, and 15) for 28-day cycle	45.9% ORR 17.6 months mOS	42.2% Grade 3/4 AEs		

discontinued in four patients (all included in arm 2). Observed partial response (PR) or complete response (CR) was 77.8% or 80%, respectively, in patients belonging to group 1 and 2 (Gibbons et al., 2016).

Women's Cancers

A study of the PD-L1 inhibitor, durvalumab, in combination with a PARP inhibitor, olaparib, and a VEGFR1-3 inhibitor, cediranib, in recurrent women's cancers with biomarker analyses were conducted and results showed that the recommended phase 2 dose (RP2D) is tolerable and has preliminary activity in recurrent women's cancers (Lee J.M. et al., 2017; Zimmer et al., 2019).

A pilot study of durvalumab and tremelimumab and immunogenomic dynamics in metastatic breast cancer showed that responses are low in unselected metastatic breast cancer, however, higher rates of clinical benefit were observed in triple negative breast cancer (TNBC). This study suggested that immunogenomic dynamics may help identify phenotypes most likely to respond to immunotherapy (Santa-Maria et al., 2018).

In the NCT02484404 phase I trial, durvalumab plus olaparib resulted in higher clinical activity in patients diagnosed with triple-negative breast cancer or ovarian cancer in the absence of germline BRCA mutations (Lee J.M. et al., 2017).

In NCT02291055 phase I/II trial, the combinatorial treatment of durvalumab and axalimogene filolisbac were determined to be efficacious in previously treated patients who were diagnosed with HPV-associated cervical cancer (recurrent/metastatic) (Syed, 2017).

Prostate Cancer

In metastatic castration-resistant prostate cancer, durvalumab plus olaparib has acceptable toxicity, and the combination demonstrates efficacy, particularly in men with DNA damage repair (DDR) abnormalities (Karzai et al., 2018).

Lymphoma

The phase 1b/2, multicenter, open-label study evaluated ibrutinib plus durvalumab in relapsed/refractory follicular lymphoma (FL) or diffuse large B-cell lymphoma (DLBCL). In FL, GCB DLBCL, and non-GCB DLBCL, ibrutinib plus durvalumab demonstrated similar activity to single-agent ibrutinib with the added toxicity of the PD-L1 blockade; the combination resulted in a safety profile generally consistent with those known for each individual agent (Herrera et al., 2020).

Melanoma

In the NCT02027961 phase I/II trial, durvalumab + darafenib + trametinib was administered to unresectable patients with wild-type metastatic or BRAF-mutant melanoma (Syed, 2017).

Solid Tumors

In the NCT02141347 phase I trial, the combination durvalumab plus tremelimumab resulted in early effects in Japanese patients diagnosed with advanced solid tumors (Syed, 2017).

The details for clinical trials of durvalumab based combinational therapy were summarized in **Table 5**.

Avelumab Based Combinational Therapy

Preclinical Study

NHS-muIL12 and avelumab combination therapy enhanced antitumor efficacy relative to either monotherapy in two tumor models-BALB/c mice bearing orthotopic EMT-6 mammary tumors and μMt-mice bearing subcutaneous MC38 tumors. Most EMT-6 tumor-bearing mice treated with combination therapy had complete tumor regression. Combination therapy

TABLE 5 | Durvalumab based combinational therapy.

Cancer type	Treatment	Dose schedule	Efficacy	Adverse rate	Notes	References
NSCLC	Durvalumab + tremelimumab	D q4w × 13 doses + T q4w for 6 doses, followed by T q12w × 3 doses Escalation dose of D: 3, 10, 15, 20 mg/kg Escalation dose of T: 1, 3, 10 mg/kg	17% ORR	36% TRAEs	NCT02000947 Patients with locally advanced or metastatic NSCLC, immunotherapy-naïve	(Antonia et al., 2016)
	Durvalumab + gefitinib	10 mg/kg D q2w + 250 mg gefitinib q1d	ORR 77.8%	100% TRAEs	NCT02088112 Patients harboring sensitizing EGFR mutations associated with advanced NSCLC, TKI-naive	(Gibbons et al., 2016)
		250 mg gefitinib q1d for 4 weeks, followed by 10 mg/kg D q2w + 250 mg gefitinib q1d	ORR 80%	100% TRAEs		
Women's cancers	Durvalumab + olaparib	10 mg/kg D q2w or 1,500 mg D q4w + olaparib Escalation dose of olaparib: 200, 300 mg	17% ORR	–	NCT02484404 Patients with TNBC or ovarian cancer	(Lee J.M. et al., 2017)
		10 mg/kg D q2w or 1,500 mg D q4w + cediranib Escalations dose of cediranib: 20, 30 mg	50% ORR	–		
Lymphoma	Durvalumab + ibrutinib	560 mg ibrutinib q1d + 10 mg/kg D q2w for 28-day cycles	25% ORR	20% TRAEs	NCT02401048 Patients with relapsed/refractory DLBCL or FL	(Herrera et al., 2020)

also induced the generation of tumor-specific immune memory, as demonstrated by protection against tumor rechallenge and induction of effector and memory T cells. Combination therapy enhanced cytotoxic NK and $CD8^+$ T-cell proliferation and T-bet expression, whereas NHS-muIL12 monotherapy induced $CD8^+$ T-cell infiltration into the tumor. Combination therapy also enhanced plasma cytokine levels and stimulated expression of a greater number of innate and adaptive immune genes, compared with either monotherapy. These data indicate that combination therapy with NHS-muIL12 and avelumab increased antitumor efficacy in preclinical models, and suggest that combining NHS-IL12 and avelumab may be a promising approach to treating patients with solid tumors (Xu et al., 2017).

Renal-Cell Carcinoma

In a single-group, phase 1b trial, avelumab plus axitinib resulted in objective responses in patients with advanced renal-cell carcinoma (Choueiri et al., 2018).

The next phase 3 trial involving previously untreated patients with advanced renal-cell carcinoma compared avelumab plus axitinib with the standard-of-care sunitinib. PFS was significantly longer with avelumab plus axitinib than with sunitinib among patients who received these agents as first-line treatment for advanced renal-cell carcinoma (Clinical Trials: NCT02684006) (Motzer et al., 2019).

Head and Neck Cancer

The JAVELIN Head and Neck 100 study is a multinational, Phase III, double-blind, placebo-controlled, randomized clinical trial assessing the efficacy of avelumab, a PD-L1 inhibitor, in combination with CRT compared with placebo in combination with CRT for high-risk HNSCC (Trial registration: Javelin Head and Neck 100; NCT 02952586) (Yu and Lee, 2019).

Cemiplimab Based Combinational Therapy

Preclinical Study

In an engineered T cell/antigen-presenting cell (APC) bioassay, REGN3767 alone, or in combination with cemiplimab (REGN2810, human anti-PD-1 Ab), blocked inhibitory signaling to T cells mediated by hLAG-3/MHCII in the presence of PD-1/PD-L1. To test the *in vivo* activity of REGN3767 alone or in combination with cemiplimab, human PD-1×LAG-3 knock-in mice were generated, in which the extracellular domains of mouse Pdcd1 and Lag3 were replaced with their human counterparts. In these humanized mice, treatment with cemiplimab and REGN3767 showed increased efficacy in a mouse tumor model and enhanced the secretion of proinflammatory cytokines by tumor-specific T cells. The favorable pharmacokinetics and toxicology of REGN3767 in non-human primates, together with enhancement of antitumor efficacy of anti-PD-1 Ab in preclinical tumor models, supports its clinical development (Burova et al., 2019).

Toripalimab Based Combinational Therapy

A single-center, phase IB trial (NCT03086174) evaluated the safety and preliminary efficacy of toripalimab combined with the VEGF receptor inhibitor axitinib in patients with advanced melanoma, including chemotherapy-naive mucosal melanomas). 33 patients were enrolled to receive 1 or 3 mg/kg toripalimab every 2 weeks, in combination with 5 mg axitinib twice a day, in a dose-escalation and cohort-expansion study. The results showed no dose-limiting toxicities observed, while 97% patients experienced treatment-related adverse events (TRAEs). The most common TRAEs were mild, while grade 3 or greater TRAEs occurred in 39.4% of patients. Among patients with chemotherapy-naive mucosal melanoma, 48.3% patients achieved objective response, and the median PFS was 7.5 months. Although the combination therapy was tolerable and showed promising antitumor activity, due to patients enrolled in this study were all Asian, these results must be validated in a randomized phase III trial that includes a non-Asian population (Sheng et al., 2019).

Camrelizumab Based Combinational Therapy

The first-line standard of care for patients with recurrent or metastatic nasopharyngeal carcinoma are platinum-based doublet chemotherapy regimens, specially gemcitabine combined with cisplatin. Two single-arm, phase 1 trials (NCT02721589 and

NCT03121716) were designed to evaluate the safety and preliminary anti-tumor activity of camrelizumab in combination with gemcitabine plus cisplatin for patients with recurrent or metastatic nasopharyngeal carcinoma. Camrelizumab combined with first-line standard therapy exhibited a manageable toxicity profile and promising preliminary anti-tumor activity for this disease in treatment-naive patients (Fang et al., 2018).

Tislelizumab Based Combinational Therapy

A multicentre, open-label, phase 1a/b study (NCT02660034) was designed to investigate the safety and anti-tumor effects of pamiparib, PARP 1/2 inhibitor, in combination with tislelizumab. Forty-nine patients with advanced solid tumors were enrolled to determine the optimum doses for further evaluation. The recommended phase 2 dose was determined as tislelizumab 200 mg every 3 weeks in combination with pamiparib 40 mg twice daily. Pamiparib plus tislelizumab exhibited generally well tolerance and were associated with anti-tumor responses and clinical benefit in patients with advanced solid tumors, supporting further investigation of the combined therapy (Friedlander et al., 2019).

TOXICITY AND SIDE EFFECTS CAUSED BY PD-1/PD-L1-BASED MONOTHERAPY OR COMBINATION THERAPY

Similar to any other drug, checkpoint inhibitors provide benefits as well as risks. Generally speaking, side effects of PD-1 inhibitors are less common than those of CTLA-4 inhibitors. The spectrum of side effects caused by PD-1/PD-L1 inhibitors includes gastrointestinal, hepatic, dermatologic, and endocrine events (Naidoo et al., 2016; Davis et al., 2017). It is usually recommended that patients with grade 2 toxicity should refrain from receiving checkpoint inhibitors transiently. For patients exhibiting grade 3 or higher adverse effects, treatment should be terminated and systemic corticosteroids should be given (1 to 2 mg/kg or equivalent) daily (Naidoo et al., 2016; Davis et al., 2017).

Data from mouse gene knockout studies indicated that blocking the PD-1/PD-L1 pathway results in relatively low incidence of autoimmune reactions that can be managed with immune suppression or supportive care. Toxicological studies involving monkeys indicated gastrointestinal toxicity may reach grades 3 to 4 after application of nivolumab and ipilimumab (Sznol, 2014). Toxicities due to combinational treatment of nivolumab + ipilimumab are similar to that generated using ipilimumab alone. In return for high rates of activity and efficacy, high rates of reversible autoimmune adverse events of grade 3 to 4 caused by combination regimens could be tolerated if toxicities are reversible with acceptable morbidity (Sznol, 2014). Combining anti-PD-1/PD-L1 inhibitors with chemotherapeutic agents was reported in quite a few clinical trials. There was a single-center phase Ib study investigating the tolerability and safety of nivolumab combined with standard chemotherapy in patients with NSCLC. Skin toxicities and hepatic toxicities were more frequently than chemotherapy or nivolumab alone, they were mild and intervention with systemic corticosteroids was not needed. Only two patients with interstitial lung disease were resolved by systemic corticosteroids, which happened in two patients several months after the start of treatment. It suggests that combination therapy with nivolumab and standard chemotherapy strengthens the anti-tumor activity of each monotherapy (Kanda et al., 2016).

Thyroid disorders are one of the most common adverse events caused by anti-PD-1 monotherapy or combinatorial therapy of anti-CTLA-4 plus anti-PD-1 (Lee H. et al., 2017). Studies comparing the prevalence of drug-related thyroid disorders due to monotherapy or combination therapy have been performed. The dynamic evolution of thyroid disorders has also been assessed in 45 patients who received anti-PD-1 monotherapy or anti-CTLA-4/anti-PD-1 combinatorial therapy. Results indicate that thyrotoxicosis or hypothyroidism are the initial form of thyroid disorders (Lee H. et al., 2017). Thyrotoxicosis occurs in most of the treated patients, with a prevalence of 93% for combination therapy and 56% for monotherapy. Additionally, the onset pattern of the thyroid disorder differs significantly between these two groups ($p = 0.01$). Subsequently, 76% and 90% of thyrotoxicosis shifted into hypothyroidism in patients of combination and monotherapy groups, respectively (Lee H. et al., 2017). The median time for onset of thyrotoxicosis and hypothyroidism was 31 and 68 days after first treatment, and 21 and 63 days for monotherapy groups and combination therapy, respectively. The median time was 42 days for the transition from thyrotoxicosis to hypothyroidism in both groups (Lee et al., 2017).

The most common side effects include immune-related and were observed in about 60% of patients enrolled in phase II and III studies. These side effects were mainly low grade and the majority involved skin conditions such as pruritus and rash or GI conditions, including diarrhea and colitis (Weinstock et al., 2017).

PROSPECTS

Immunotherapy based on PD-1/PD-L1 has revealed its efficacy in melanoma, NSCLC, gastric cancer, as well as head and neck cancer. The frequency of side effects of PD-1/PD-L1 therapy due to immune suppression is relatively lower than using traditional cancer therapy and are better tolerated. However, due to the immunomodulating nature of the mAbs, the measurement of the biological activities (release or stability test) made a great problem in quality control laboratories (Wang et al., 2017). As therapeutic antibodies, the limited half-life and multiple-dosages-caused immunogenicity, which might induce over-activity of immune system, were inevitably emerged, some small-molecule immune checkpoint inhibitors to avoid these shortcomings are under developing (Lee et al., 2016; Magiera-Mularz et al., 2017; Li and Tian, 2019). The above factors made these drugs a high cost for biopharmaceutical industrials, which is not conducive to benefit more patients (Kandolf Sekulovic et al., 2017; Ward et al., 2017).

Despite some disadvantages, checkpoint inhibitors possess a great prospect. The recent findings suggest that PD-1/PD-L1 inhibitors may be combined with other immunotherapies or traditional treatments to enhance efficacy relative to that using PD-1/PD-L1 therapy alone, which always exhibit higher response rates, reducing adverse reaction and drug resistance (Li J. et al., 2019; Zhang et al., 2019; Li et al., 2020; Shao et al., 2020; Sonpavde et al., 2020; Wan et al., 2020; Weiss et al., 2020; Zhang et al., 2020). Some researchers have shown the prospects of anti-PD-L1 and anti-CTLA-4 combination therapy, which revealed PD-L1:CD80 (CTLA-4 ligand) cis-heterodimerization inhibited both PD-L1: PD-1 and CD80:CTLA-4 interactions. Therefore, exploration of the efficacy and mechanism of co-blockade of PD-L1 and CTLA-4 is promising (Sugiura et al., 2019; Zhao et al., 2019). The emerging nanovaccine was reported to profoundly potentiate the immunogenicity of the neoantigen, enhancing responsiveness (Ni et al., 2020). Furthermore, some studies reveal that angiotensin-converting enzyme 2 (ACE2) expression is increased after interleukin (IL)-1β treatment (Clarke et al., 2014), blockade of IL-1β synergized with blockade of PD-1 can inhibit tumor growth (Tian et al., 2020). This correlation can provide new ideas for anti-PD-1/PD-L1 therapy (Sui et al., 2014). Above all, the combination therapy using PD-1/PD-L1 may pave the way for a new era for cancer immunotherapy.

AUTHOR CONTRIBUTIONS

L-WF conceived the review. J-YZ and Y-YY searched the literature and drafted the manuscript. J-JL revised literature. RA edited the manuscript. All authors approved the final version of the manuscript.

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