MULTIDISCIPLINARY APPROACH TO THE DIAGNOSIS AND THERAPY OF SKIN NEOPLASMS

EDITED BY: Renato Franco, Giuseppe Argenziano, Aimilios Lallas, Gabriella Brancaccio and Andrea Ronchi

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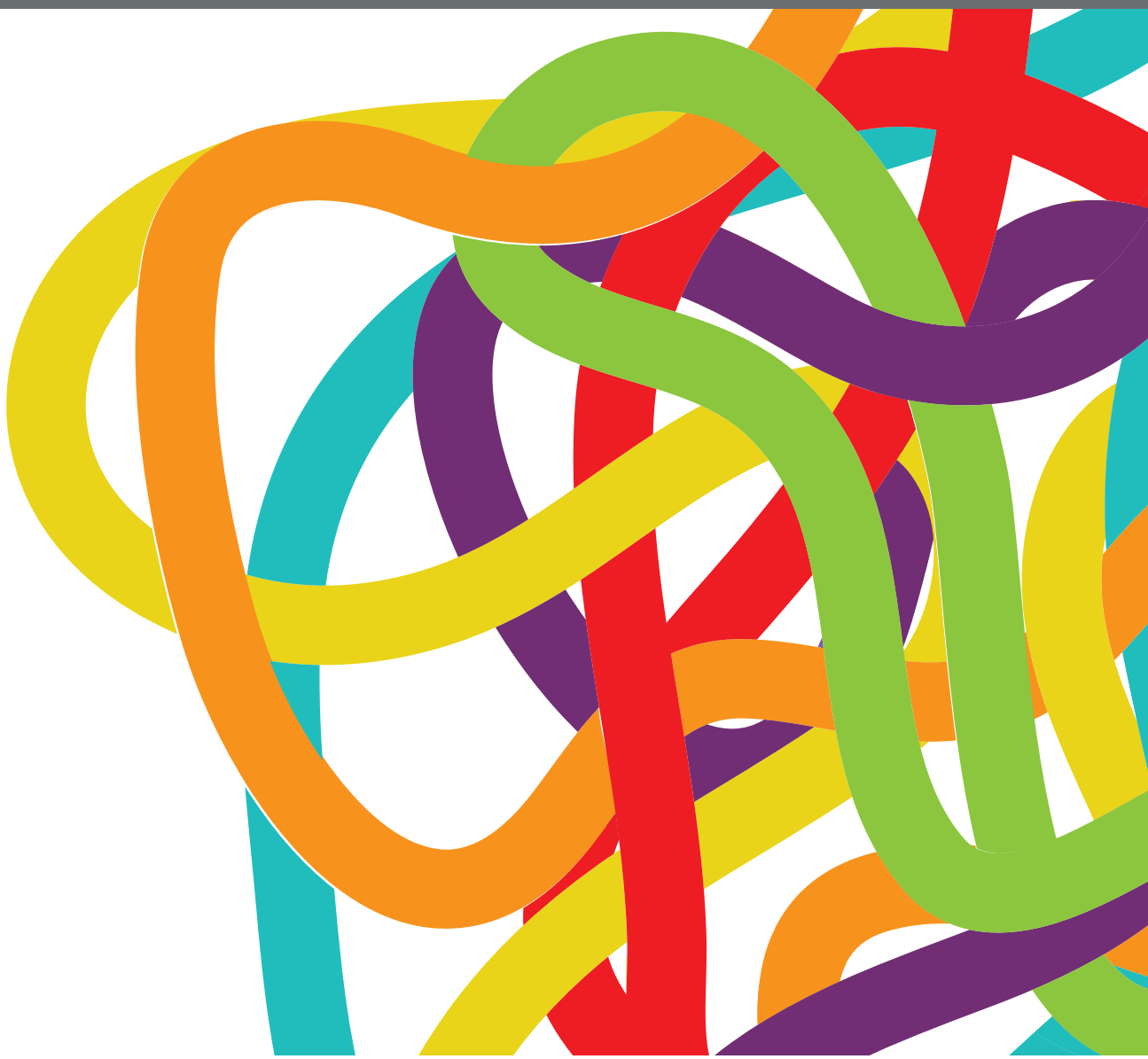

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MULTIDISCIPLINARY APPROACH TO THE DIAGNOSIS AND THERAPY OF SKIN NEOPLASMS

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Table of Contents

05 ***Editorial: Multidisciplinary Approach to the Diagnosis and Therapy of Skin Neoplasms***
Andrea Ronchi, Giuseppe Argenziano, Gabriella Brancaccio, Aimilios Lallas and Renato Franco

07 ***Sonidegib for the Treatment of Advanced Basal Cell Carcinoma***
Gabriella Brancaccio, Federico Pea, Elvira Moscarella and Giuseppe Argenziano

14 ***Immune Checkpoint Inhibitors in Advanced Acral Melanoma: A Systematic Review***
Qingyue Zheng, Jiarui Li, Hanlin Zhang, Yuanzhuo Wang and Shu Zhang

23 ***Research Interest and Public Interest in Melanoma: A Bibliometric and Google Trends Analysis***
Hanlin Zhang, Yuanzhuo Wang, Qingyue Zheng, Keyun Tang, Rouyu Fang, Yuchen Wang and Qiuning Sun

33 ***Clinical and Dermoscopic Factors for the Identification of Aggressive Histologic Subtypes of Basal Cell Carcinoma***
Riccardo Pampena, Gabriele Parisi, Mattia Benati, Stefania Borsari, Michela Lai, Giovanni Paolino, Anna Maria Cesinaro, Silvana Ciardo, Francesca Farnetani, Sara Bassoli, Giuseppe Argenziano, Giovanni Pellacani and Caterina Longo

42 ***The Effectiveness of Different Treatment Modalities of Cutaneous Angiosarcoma: Results From Meta-Analysis and Observational Data From SEER Database***
Siwei Bi, Shanshan Chen, Beiyi Wu, Ying Cen and Junjie Chen

51 ***Case Report: Favorable Response to the Tyrosine Kinase Inhibitor Apatinib in Recurrent Merkel Cell Carcinoma***
Yiyi Feng, Xin Song and Renbing Jia

58 ***Melanoma of Unknown Primary: Evaluation of the Characteristics, Treatment Strategies, Prognostic Factors in a Monocentric Retrospective Study***
Paolo Del Fiore, Marco Rastrelli, Luigi Dall'Olmo, Francesco Cavallin, Rocco Cappellesso, Antonella Vecchiato, Alessandra Buja, Romina Spina, Alessandro Parisi, Renzo Mazzarotto, Beatrice Ferrazzi, Andrea Grego, Alessio Rotondi, Clara Benna, Saveria Tropea, Francesco Russano, Angela Filoni, Franco Bassetto, Angelo Paolo Dei Tos, Mauro Alaibac, Carlo Riccardo Rossi, Jacopo Pigozzo, Vanna Chiarion Sileni and Simone Mocellin

67 ***Correlation Between* In Vivo *Reflectance Confocal Microscopy and Horizontal Histopathology in Skin Cancer: A Review***
Giuseppe Broggi, Anna Elisa Verzì, Rosario Caltabiano, Giuseppe Micali and Francesco Lacarrubba

74 ***Tissue Expression of Carbonic Anhydrase IX Correlates to More Aggressive Phenotype of Basal Cell Carcinoma***
Daniela Russo, Silvia Varricchio, Gennaro Ilardi, Francesco Martino, Rosa Maria Di Crescenzo, Sara Pignatiello, Massimiliano Scalvenzi, Claudia Costa, Massimo Mascolo, Francesco Merolla and Stefania Staibano

81 ***Mucosal Invasion, but Not Incomplete Excision, Has Negative Impact on Long-Term Survival in Patients With Extramammary Paget's Disease***
Hiroki Hashimoto, Yumiko Kaku-Ito, Masutaka Furue and Takamichi Ito

91 ***Malignant Melanoma in Children and Adolescents Treated in Pediatric Oncology Centers: An Australian and New Zealand Children's Oncology Group (ANZCHOG) Study***
Anne L. Ryan, Charlotte Burns, Aditya K. Gupta, Ruvishani Samarasekera, David S. Ziegler, Maria L. Kirby, Frank Alvaro, Peter Downie, Stephen J. Laughton, Siobhan Cross, Timothy Hassall, Geoff B. McCowage, Jordan R. Hansford, Rishi S. Kotecha and Nicholas G. Gottardo

99 ***Are Molecular Alterations Linked to Genetic Instability Worth to Be Included as Biomarkers for Directing or Excluding Melanoma Patients to Immunotherapy?***
Giuseppe Palmieri, Carla Maria Rozzo, Maria Colombino, Milena Casula, Maria Cristina Sini, Antonella Manca, Marina Pisano, Valentina Doneddu, Panagiotis Paliogiannis and Antonio Cossu

110 ***Incidence of Skin Cancer in Patients With Chronic Inflammatory Cutaneous Diseases on Targeted Therapies: A Systematic Review and Meta-Analysis of Observational Studies***
Salvatore Crisafulli, Lucrezia Bertino, Andrea Fontana, Fabrizio Calapai, Ylenia Ingrasciotta, Massimiliano Berretta, Gianluca Trifirò and Claudio Guarneri

121 ***Usefulness of High-Frequency Ultrasonography in the Diagnosis of Melanoma: Mini Review***
Maria Paola Belfiore, Alfonso Reginelli, Anna Russo, Gaetano Maria Russo, Maria Paola Rocco, Elvira Moscarella, Marilina Ferrante, Antonello Sica, Roberto Grassi and Salvatore Cappabianca

128 ***The Challenge of Melanocytic Lesions in Pediatric Patients: Clinical-Pathological Findings and the Diagnostic Value of PRAME***
Giuseppina Rosaria Umano, Maria Elena Errico, Vittoria D'Onofrio, Giulia Delehaye, Letizia Trotta, Claudio Spinelli, Silvia Strambi, Renato Franco, Giuseppe D'Abbronzo, Andrea Ronchi and Alfonso Papparella

135 ***The WHO 2018 Classification of Cutaneous Melanocytic Neoplasms: Suggestions From Routine Practice***
Gerardo Ferrara and Giuseppe Argenziano

151 ***Interference of COVID-19 Vaccination With PET/CT Leads to Unnecessary Additional Imaging in a Patient With Metastatic Cutaneous Melanoma—Case Report***
Rafał Czepczyński, Jolanta Szczurek, Jacek Mackiewicz and Marek Ruchała

156 ***Non-Melanoma Skin Cancer in People Living With HIV: From Epidemiology to Clinical Management***
Emmanuele Venanzi Rullo, Maria Grazia Maimone, Francesco Fiorica, Manuela Ceccarelli, Claudio Guarneri, Massimiliano Berretta and Giuseppe Nunnari

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EDITORIAL
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Editorial: Multidisciplinary Approach to the Diagnosis and Therapy of Skin Neoplasms

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Editorial on the Reseacrh Topic

Multidisciplinary Approach to the Diagnosis and Therapy of Skin Neoplasms

Skin cancer is an extremely heterogenous group of neoplasms, including tumors of the epidermis (basal cell carcinoma, squamous cell carcinoma), tumors of the dermal adnexa (adnexal carcinomas), tumors of melanocytes (cutaneous melanoma), tumors of soft tissue (angiosarcoma and other sarcomas) and tumors of neuroendocrine cells (Merkel cell carcinoma). Despite the better knowledge of its pathological and molecular features, actually skin cancer is a clinical challenge with relevant consequences in terms of morbidity and mortality. Indeed, skin cancer, including cutaneous melanoma and non-melanoma skin cancer, is the most common cancer worldwide. It has been calculated that 1 in 5 subjects will develop skin cancer by the age of 70 in the U.S., and more than 2 subjects die of skin cancer in the U.S. every hour (1, 2). Co-morbidities of patients affected by skin cancer, the challenges of the histological diagnosis, the development of new technologies for the *in-vivo* screening of the patients and the early diagnosis, make the multidisciplinary approach mandatory. In this setting, the aim of this Research Topic is to address the main topics about skin cancer from a multidisciplinary point of view. Cutaneous melanoma is certainly a hot issue, and this Research Topic includes articles about new classification, histological diagnosis, *in-vivo* technologies, new therapies. Umano et al., evaluated the value of Preferentially expressed Antigen in Melanoma (PRAME) for differential diagnosis of spitzoid melanocytic lesions in pediatric patients. Pediatric melanoma is also the topic of a retrospective study by Ryan et al., which provided clinical and prognostic information about this rare neoplasm in a challenging patient group. Ferrara and Argenziano., revised the WHO 2018 classification of cutaneous melanocytic neoplasms, with particular emphasis on intermediate melanocytic tumors and immunohistochemical and molecular algorithms needed to address the morphological ambiguous cases to a correct clinical management.

In the last decades, the development of new technologies has dramatically changed the diagnosis of skin cancer, mainly cutaneous melanoma, and it is to be expected that technologies will acquire an ever-greater role in the next future (3). In the review by Belfiore et al., the Authors examine the role of High frequency Ultrasound (HFUS) in the diagnosis of skin cancer, while Broggi et al., focused on the correlation between *in vivo* reflectance confocal microscopy and horizontal histopathology.

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With regards to the management, immunotherapy is certainly one of the most important novelty in the therapy of skin cancer in the last years, but the effectiveness of immunotherapy in advanced melanoma cannot be predicted (4). In the bibliometric analysis by Zhang et al., immunotherapy is confirmed as a hot issue in cutaneous melanoma research. Palmieri et al., examine the potential role of molecular alterations linked to genetic instability in cutaneous melanoma as predictive biomarkers for response to immunotherapy. Zheng et al. examine current evidence about the effectiveness of immune checkpoint inhibitors therapy in advanced acral melanoma. Melanoma metastases of unknown primary is a relatively common clinical challenge and the recent improvements about clinical management, therapy and prognostic factors are evaluated by the retrospective study by Del Fiore et al.

Although most non-melanoma skin cancer is easily healed with surgery alone, some neoplasms are extremely relevant in terms of morbidity and mortality. This Research Topic includes several articles about the therapy and the prognostic evaluation of these neoplasms. Pampena et al., define the clinical-dermoscopic findings of aggressive subtypes of basal cell carcinoma, while Russo et al., provide evidence that the expression of Carbonic Anhydrase IX may be used as a prognostic marker in basal cell carcinoma and has a potential therapeutic role for target therapy in advanced cases. Data about the use of sonidegib for the treatment of advanced basal cell carcinoma are detailed by Brancaccio et al. Hashimoto et al., analyze the impact of mucosal involvement and surgical treatment on the survival of patients with extramammary Paget's disease. Bi et al., examine the different therapeutic chances for cutaneous angiosarcoma, while Feng et al., describe a case of recurrent Merkel cell carcinoma responding to Tyrosine Kinase Inhibitor Apatinib.

Lastly, the general clinical context always plays an important role in the management of patients affected by skin cancer. A possible misinterpretation of PET/CT images caused by the recent COVID-19 vaccination is described in the case report by Czepczynski et al., Venanzi Rullo et al., describe the peculiar findings of non-melanoma skin cancer in patients affected by HIV. Immunodepression influences not only the epidemiology and the biology of skin cancer, but mainly the clinical management of the patients. Interestingly, Crisafulli et al. systematically review the cutaneous malignancy risk in patients treated for chronic inflammatory cutaneous diseases.

Altogether, the different contributions to this Research Topic offer a comprehensive analysis of improvements in diagnosis and therapy of skin cancer, highlighting the importance of a multidisciplinary approach to this heterogeneous group of neoplasms.

AUTHOR CONTRIBUTIONS

All authors equally contributed to the manuscript. All authors read and approved the final manuscript.

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MINI REVIEW
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Sonidegib for the Treatment of Advanced Basal Cell Carcinoma

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Basal cell carcinoma (BCC) accounts for almost 80% of skin cancers, and its healthcare workload burden is substantial within dermatology departments. Although most BCCs are small, well-defined tumors amenable of surgery or conservative procedures, in a small proportion of patients, BCCs can progress to an advanced stage including locally advanced BCC. The goal of the clinician in the treatment of BCC should be the right therapeutic approach at diagnosis, and different guidelines propose treatment strategies in order to prevent relapses or disease progression. In case of unresectable and untreatable BCC with radiotherapy, the first-choice medical therapy is Hedgehog-GLI (HH) pathway inhibitors. Sonidegib was approved by the U.S. Food and Drug Administration (FDA) and European Medicines Agency (EMA) as a first-line treatment for adult patients with locally advanced BCC, becoming the second HH pathway inhibitor receiving approval after vismodegib. In this review, data on pharmacology, safety, tolerability, and efficacy of sonidegib are summarized and compared to those of vismodegib. Lastly, indications on the management of advanced basal cell carcinoma based on author's clinical experience are provided.

Keywords: basal cell carcinoma, advanced basal cell carcinoma, hedgehog inhibitors, sonidegib, skin cancer

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INTRODUCTION

Basal cell carcinoma (BCC) accounts for almost 80% of skin cancers, and its oncogenesis rely on the interplay between constitutional predisposition (genotypic and phenotypic characteristics) and subsequent exposure to environmental risk factors, with ultraviolet radiation exposure as the principal one (1).

Actual BCC tumor burden is much greater in the population than it is apparent from normal incidence rates. Many reasons make the true BCC incidence difficult to calculate as 1) routine recording of BCC is often not performed by cancer registries; 2) in clinical practice not all the BCCs are histologically confirmed and 3) when recorded, often only the first histologically confirmed BCC per patient is taken into account. These factors translate into a complete absence of BCC rates in the most accounted statistical datasets (2), where it is even excluded from the group of non-melanoma skin cancer. However, the healthcare workload burden and cost of BCC are substantial within dermatology departments (3), and it is even much higher considering the subset of advanced BCC which accounts for the highest morbidity due to cosmetic disfigurement and functional morbidity.

Although most BCCs are small or intermediate-size, well-defined tumors amenable of surgery or conservative procedures, in a small proportion of patients, BCCs can progress to an advanced stage including metastatic BCC (mBCC) or locally advanced BCC (laBCC) (4). Advanced BCC is an entity not yet clearly defined as there is a lack of consensus on the diagnostic criteria which are hardly objectified. Usually, advanced BCCs are extended tumors characterized by destructive growth after multiple relapse, often located on the head and neck areas that have become difficult to treat through standard surgery and radiotherapy. In order to distinguish between BCCs that may progress to mBCC or laBCC, an innovative classification in easy-to-treat and difficult-to-treat BCCs has been recently proposed. It takes into account size, location, definition of borders, previous treatments, and related recurrences and even some patient's characteristics as comorbidities interfering with surgery or reluctance to proposed treatments (5). The distinction between easy- and difficult-to-treat BCC may have practical implication considering the wide availability of therapeutic option for the first group of tumors and the need of an immediate resolutive treatment for the latter one (**Table 1**, **Figure 1**). Different guidelines (5, 6) propose treatment strategies in order to identify the better care pathway and, thus, prevent relapses or persistence of the tumor. The multidisciplinary approach is the mainstay of management of difficult-to-treat BCCs, that should be managed in a tertiary care center (referral center).

Surgery should be always considered as primary therapeutic option, even after neoadjuvant approaches. Mohs surgery should be performed in case of large, high-risk tumors located on the face, in case of surgery after a previous relapse, or in case of BCCs arising on a previous irradiated area, scars or areas of chronic inflammation. However, despite very high cure rate, Mohs

TABLE 1 | Recommended therapeutic approach to easy-to-treat and difficult-to-treat BCCs (5).

	Treatment	Type of recommendation	Grade of recommendation–Level of evidence
Easy-to-treat BCC	Surgery	Highly effective in any type of BCC	A-3
	5% Imiquimod (sBCC)	Effective in sBCC	A-2
		Potential role in nBCC	B-2
	5% 5-Fluoruracil	Effective in sBCC	A-2
	Curettage + electrodedissication and cryoterapy	Potential role in low-risk BCC on the trunk and extremities	B-3
	PDT with MAL or ALA	Effective in sBCC and thin nBCC	A-1
Difficult-to-treat BCC	Surgery	Evaluation of suitability by multidisciplinary team	Expert opinion
	Radiotherapy	Role in elderly patients and patients not candidates for surgery (any BCC)	A-1
	HH inhibitors	To be offered in laBCC and mBCC	B-3

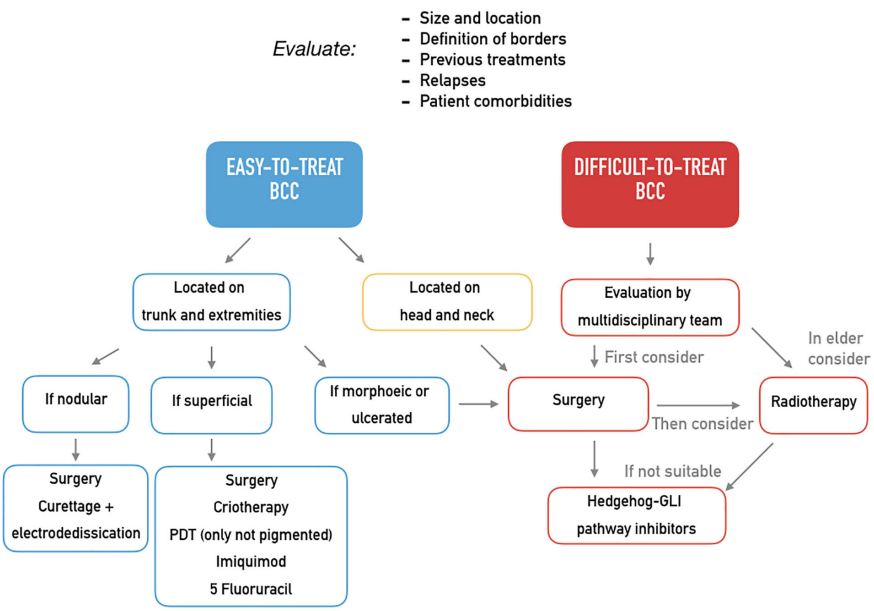

FIGURE 1 | Expert opinion on the treatment of easy-to-treat and difficult-to-treat basal cell carcinomas.

surgery is a costly, time-consuming procedure that requires specialized training and has very little spread in some countries.

Radiotherapy should be taken into account as second-line treatment in elderly patients (>60 years old) suffering from a BCC not amenable of surgery. Radiotherapy is also an option in adjuvant setting in case of positive margins after primary excision. However, due to concerns with long-term sequelae as well as adverse events with intermediate onset, indication to radiotherapy may be questioned by the multidisciplinary team.

Once evolved to laBCC or mBCC, the most appropriate therapeutic option is the target therapy through Hedgehog inhibitors.

HEDGEHOG-GLI PATHWAY AND ITS INHIBITORS

Hedgehog-GLI (HH) signaling plays a major role during the development and is involved in cell proliferation and differentiation (7, 8). The HH pathway is normally silenced in most adult tissues, and it was shown that it may be aberrantly activated in the pathogenesis of various types of tumors (9). This may promote the subsequent activation of transcription factors of the Glioma-associated oncogene (GLI) family, which may favor tumor proliferation (9). Smoothened (SMO) is the main transducer of HH signaling, and in the last few years, it has emerged as a promising therapeutic target for anticancer therapy. Natural and synthetic antagonists have been developed for SMO, and many have undergone clinical trials with varying degrees of success. SMO inhibition was first characterized through binding studies of cyclopamine, a natural steroidal alkaloid derived from *Veratrum californicum*. Derivatives of cyclopamine have been developed with the aim of increasing specificity and pharmacological potency while limiting side effects (10). The first HH pathway inhibitor to be approved by the FDA and EMA was vismodegib, a second-generation cyclopamine derivative. Later, sonidegib was approved by the FDA and EMA as a first-line treatment for adult patients with locally advanced BCC, becoming the second HH pathway inhibitor receiving approval (10). A new SMO inhibitor is also in development for topical administration in patients affected by Gorlin syndrome (11).

SONIDEGIB FOR THE TREATMENT OF ADVANCED BASAL CELL CARCINOMA

Sonidegib is an oral small molecule that acts as a selective antagonist of the SMO receptor, a G protein-coupled receptor-like structure that is fundamental for the correct action of the HH signaling pathway (12).

Sonidegib exhibited dose- and exposure-dependent inhibition of the expression of the GLI homolog 1 in tumor and normal skin biopsies (13) and is currently indicated for the treatment of adults with advanced basal cell carcinomas at the daily dosage of 200 mg (12).

PHARMACOKINETIC PROFILE

Sonidegib pharmacokinetics (PK) was studied in patients with cancer after a single dose ranging between 100 mg and 3000 mg (13). Under fasting condition, absorption resulted quite rapidly with a time to peak concentration (T_{max}) of 2–4 h. Oral bioavailability (F_{OS}) was quite low under fasted state as it was estimated to be around 6–7% after a single 800 mg dose in healthy volunteers (14). F_{OS} increased by 7.8-fold when in the presence of high-fat meal with an almost proportional increase in drug exposure of 7.4-fold in terms of area under the plasma concentration–time curve (AUC) from zero to infinity (15). For this reason, it is recommended that sonidegib is taken under fasting conditions, at least 1–2 h before meal (15).

One of the most interesting pharmacokinetic properties of sonidegib is represented by the wide distribution within tissues (14). A population pharmacokinetic analysis carried out among 351 patients who received sonidegib at a dose ranging between 100 mg and 3,000 mg showed that the volume of distribution (Vd) was of 9,170 L (15). This may explain why sonidegib may either achieve skin concentration sixfold higher than in plasma (15) or effectively cross the blood brain barrier (16). Sonidegib is bound for >97% to plasma protein in a concentration independent mode (15–17).

Sonidegib has a very long-elimination half-life of around 28 days (16, 18). This means that steady-state is reached after more or less 4 months from starting daily dosing treatment (16, 18), with an estimated accumulation of around 19-fold (13, 15). Sonidegib undergoes metabolism mainly *via* oxidation and hydrolysis by the 3A4 isoform of the cytochrome (CYP) P450 (15, 19). All of the metabolites are several-folds less pharmacologically active than the parent compound. Sonidegib is the main circulating moiety in plasma (36%), and both the parent compound and its metabolites are eliminated by the feces (overall 93% of the administered dose) (14).

Overall, the PK profile of sonidegib is quite different from that of the other SMO antagonist vismodegib (**Table 2**). Both drugs are very highly bound to plasma proteins (>97%), but the binding is concentration-independent for sonidegib (16, 17) and concentration-dependent for vismodegib (21, 22). The Vd is much higher for sonidegib than for vismodegib, accordingly to a major grade of lipophilicity. This may reflect in extensive accumulation of sonidegib within tissues, as documented by the finding of concentrations sixfold higher in the skin compared with plasma (15). Conversely, the distribution of vismodegib is mainly limited to the plasma and to the extracellular spaces (23). Theoretically, these differences in the distribution pattern might translate into potential differences in the pharmacodynamic profile of efficacy and toxicity of these two SMO inhibitors (20). Another relevant PK difference is related to the elimination half-life, which is three to fourfold longer for sonidegib (28–30 days) (16, 18) compared with vismodegib (4–12 days) (23, 24). This means that the time needed to achieve steady concentrations during continued treatment (namely the steady-state) is of around 3–4 months for sonidegib (16, 18) and of around 7–21 days for vismodegib (23, 24). The differences in

TABLE 2 | Comparative PK characteristic and efficacy of sonidegib and vismodegib.

PK	Sonidegib 200 mg daily	Vismodegib 150 mg daily
Plasma protein binding	>97% (concentration-independent) (8, 9),	>99% (concentration-dependent) (12, 13),
Vd (L)	9166 (7, 8),	16.4–26.6 (14)
$t_{1/2}$ (days)	28–30 (8, 10),	4–12 (14, 16),
Time to steady-state (days)	90–120 (8, 10),	17–21 (14, 16),
Efficacy	Central review RECIST-like 18-month follow-up (BOLT trial) (20)	Central review RECIST 21-month follow-up (Erivance trial) (20)
Overall response rate n (%); 95% CI	40 (60.6); 47.8–72.4	30 (47.6); 35.5–60.6
Complete response n (%)	14 (21.2%)	14 (22.2%)
Partial response n (%)	26 (39.4%)	16 (25.4%)
Stable disease n (%)	20 (30.3%)	22 (34.9%)
Progressive disease n (%)	1 (1.5%)	8 (12.7%)
Unknown n (%)	5 (7.6%)	3 (4.8%)

RECIST, Response Evaluation Criteria in Solid Tumors. Adapted by Dummer et al. J Eur Acad Dermatol Venereol. 2020.

time to steady state between the two HH inhibitors do not seem to correlate with the time to response, as the median time to response was 3.9 months for sonidegib in BOLT and 5.6 months for vismodegib in ERIVANCE trial (20).

Drug–Drug Interactions

Sonidegib is a substrate of CYP3A4 and it is expected that its pharmacokinetic profile may be altered by modulators of the activity of this metabolizing enzyme (15, 19). Thus, the recommendation on the EMA product label is to avoid co-administration with strong CYP 3A4 inhibitors or to reduce sonidegib dose to 200 mg every other day during co-treatment with strong CYP 3A4 inhibitors in order not to exceed a twofold increase in sonidegib exposure (15, 19). Similarly, co-treatment with strong CYP 3A4 inducers should be avoided (15, 19). However, if co-treatment with inducers is needed, sonidegib dose may be increased to 400–800 mg in order to prevent >80% reduction in sonidegib exposure (15, 19). Concomitant treatment with strong CYP inducers should be avoided in the case of vismodegib as well. The product label does not provide any advice on dose adjustment if co-administration is necessary [Erivedge EMA label].

Pharmacokinetic Profile in Special Patient Populations

The pharmacokinetic behavior of sonidegib was evaluated also in special patient populations. The effect of mild to severe hepatic impairment on the pharmacokinetics of sonidegib was assessed in a phase 1 multicenter, open label, parallel-group study (25) concluding that in patients with any grade of hepatic impairment dose adjustments are unnecessary.

Sonidegib has not been studied in a dedicated pharmacokinetic study in patients with renal impairment. Based on the available data, sonidegib elimination *via* the kidney is negligible. A population pharmacokinetic analysis found that mild or moderate renal impairment did not have a significant effect on the apparent clearance of sonidegib, suggesting that dose adjustment is not necessary in patients with renal impairment. No efficacy and safety data are available in patients with severe renal impairment [Odomzo EMA label].

Additionally, safety and efficacy data in patients aged 65 years and older do not suggest that a dosage adjustment is required in these patients [Odomzo EMA label].

A population pharmacokinetic analysis of sonidegib was carried out among healthy volunteers and patients with advanced solid tumors (18). Covariate analysis showed that age, weight, gender, ethnicity, mild hepatic impairment, mild and moderate renal impairment did not affect sonidegib pharmacokinetics. This means that no sonidegib dose adjustment is indicated in relation to these conditions. Conversely, clinically relevant effects on sonidegib F_{OS} were induced by high-fat meal (fivefold increase), and by co-administration of proton pump inhibitors (30% decrease). In regard to the former effect, it is recommended that sonidegib is assumed under fasted condition for avoiding unpredictable overexposure (15). In regard to the latter effect, a phase 1 study carried out among 42 healthy volunteers showed that co-administration of esomeprazole (40 mg 5-days pretreatment plus combination on day 6) with a single 200 mg dose of sonidegib resulted in a modest reduction of sonidegib absorption under fasted conditions (decreased sonidegib AUC by 32-38%) (26).

TOLERABILITY AND SAFETY

The safety and the tolerability of sonidegib was assessed in the double-blind, phase 2 pivotal trial (BOLT) in which patients with locally advanced or metastatic basal cell carcinoma were randomized to receive 200 or 800 mg oral sonidegib daily (27).

A comprehensive analysis assessed whether an exposure–response relationship would exist for effectiveness and safety of sonidegib among patients with advanced solid tumors (28). For the exposure–efficacy analysis, data from 190 patients receiving sonidegib at 200 or 800 mg daily were included. Logistic regression analysis showed no relationship between sonidegib exposure in terms of trough level (C_{min}) resulting from 200 or 800 mg doses at week 5 and the objective response rate in terms of complete and/or partial response. Exposure–safety analysis was carried out among 336 patients receiving dosages ranging from 100 to 3,000 mg once daily and 250 to 750 mg twice daily. The findings showed that increased exposure was associated with a greater risk of grades 3–4 creatine kinase (CK) elevation, and that the risk was lower in females *vs.* males. Consistently, it is recommended that CK level is monitored periodically throughout the duration of treatment with sonidegib (29).

A pooled analysis of the change in the QT interval was carried out for assessing the eventual prolongation QT caused by sonidegib. Data coming from four patient studies (n = 341) were merged with those coming from four healthy volunteer studies (n = 204) (30). Overall, data showed that sonidegib did not cause QTc prolongation as ΔQTc were always <5 ms both for the 200 and 800 mg dose.

With regard to tolerability, the most frequent adverse events (AEs) resulted in muscle spasms, alopecia, and dysgeusia, mostly of grade 1–2 (17). The most common grade 3–4 AEs occurring in ≥2% of patients receiving the 200 mg daily dose were fatigue, weight decrease, and muscle spasms. Even if data from the two pivotal studies are not directly comparable, sonidegib resulted in being associated with the same AEs of vismodegib but with an approximately 10% lower incidence (4). AEs reported with sonidegib were also slightly less severe and with a slightly longer median time to onset (4). Specifically, the median time to onset of the most frequent AEs with vismodegib 150 mg and sonidegib 200 mg, namely muscle spasms, alopecia and dysgeusia, were 1.89 *vs* 2.07 months, 3.38 *vs* 5.55 months and 1.48 *vs* 3.71 months, respectively.

EFFICACY

The phase 2 trial (BOLT) that led to the approval in both US and Europe compared sonidegib at a dosage of 200 and 800 mg in patients affected by laBCC (n = 194) and mBCC (n = 36). As sonidegib 200 mg demonstrated a better benefit-risk profile than sonidegib 800 mg, we will focus only on the former, which is the approved dose in the setting of laBCC (15).

Primary endpoint of the BOLT trial was overall response rate (ORR) by central review, while secondary endpoints were ORR by investigator review, duration of response (DOR), progression free survival (PFS), overall survival (OS), time to response, safety and quality of life (QoL). Noteworthy, assessment of laBCC in BOLT trial was performed using the BCC-modified RECIST criteria (mRECIST) (27). BCC-mRECIST is a multimodal assessment method integrating magnetic resonance imaging per RECISTv1.1, standard and annotated color photography per WHO guidelines, and histology in multiple biopsy specimens surveying the lesion area. Overall, these criteria for assessing partial and complete response, as well as progression disease, are more stringent compared to the RECISTv1.1 criteria used in vismodegib studies (4). mRECIST is more likely to detect minimal signs of disease and disease progression, thus classifying a given treatment response as partial, whereas the same response may be considered as complete using RECIST. Similarly, mRECIST is more likely to detect signs of slight disease progression that may be classified as stable disease (SD) under RECIST (20). This aspect is crucial when comparing efficacy data from sonidegib and vismodegib trial analyses (17, 27, 31, 32) (**Table 2**). Despite similar baseline patient characteristics, endpoints, and role of central and investigator review, the difference in assessment criteria makes a head-to-head comparison of the two drugs difficult. However, in the 30-month analysis of the BOLT study, a pre-planned analysis adjusted the outcomes from BOLT with RECIST-like criteria. As underlined in a recent expert opinion paper, the most correct match is between adjusted ORR of sonidegib and ORR of vismodegib at the closest follow-up time points across the studies with central review (20). At 21-month follow-up, vismodegib ORR was 47.6%, with 22.2% complete response (CR) and 25.4% partial response (PR). At 18-month follow-up, adjusted ORR of sonidegib was 60.6% with 21.2% CR and 39.4% PR. Adjusting efficacy data using RECIST criteria make just a slight increase in sonidegib overall response rate (ORR) (from 56.1 to 60.6%) while the number of CR increases significantly at the expense of PR. The rate of progressive disease (PD) is higher for vismodegib than for sonidegib (12.7 and 1.5%, respectively) (20), and this data is consistent with reports of acquired resistance during treatment with vismodegib (4). However, it is likely that the responsible genomic mutations affecting SMO confer resistance to different SMO inhibitors. Further studies are needed to find the right therapeutic strategy in constitutionally or acquired resistant laBCC, through drug associations or different molecules. Lastly, the centrally reviewed median duration of response (mDOR) and median progression free survival (mPFS) with sonidegib at 30 months were longer than vismodegib at 21 months (17, 31). The longest (39 months) follow-up report of vismodegib includes only investigator reviewed data, therefore is not appropriate for a comparison (32). However, the investigator reviewed mDOR results are longer with vismodegib.

CLINICAL IMPLICATIONS AND CONCLUSIONS

The goal of the clinician in the treatment of BCC should be the right therapeutic approach at diagnosis, thus preventing the evolution into laBCC or mBCC. Many treatments are available depending on the clinical features of the primitive lesion and on patient characteristics (**Table 1**), and the distinction into easy-to-treat and difficult-to-treat BCCs may be helpful in the clinical practice (**Figure 1**). Easy-to-treat BCCs may be properly managed by the territorial health care or in the private practice, while difficult-to-treat BCCs should be referred to a secondary/tertiary care center in order to be evaluated by a multidisciplinary team. Obviously, the experience of each center differs from one country to another and in the same country and may influence the therapeutic decision, but general recommendations should be followed (5).

For the treatment of difficult-to-treat BCCs, surgery should be the first therapeutic option, but it should be carefully planned, and appropriate imaging to determine the extent of the tumor should be performed when perineural involvement or bone invasion is suspected. When available, Mohs surgery should be preferred. Radiotherapy is an alternative option in elderly patient affected by BCCs not amenable of surgery or in patients who are not candidates to surgery; it is devoted to elderly people because the potential risk of very-long-term trophic disorders is not well addressed (5).

In case of unresectable and untreatable BCC with radiotherapy (laBCC), the first-choice medical therapy is HH pathway inhibitors. Chemotherapy showed a low response rate and a short duration of response in few reports, so it can be considered a last-line treatment, while studies on the efficacy of immunotherapy in BCC are currently ongoing (5).

To date, the choice between the two HH inhibitors available, vismodegib and sonidegib, is based on expert opinion and indirect comparison, as a head-to-head trial is not available. However, a subset of patients who could benefit more from one drug than another has not been clearly identified. Vismodegib, being the first approved HH inhibitor, has been used for longer time and real-world data are available. Although no laboratory tests are required by label (except for pregnancy test), we routinely perform a metabolic panel every 1–2 months, depending on patient comorbidities, with special attention to liver and kidney functionality and creatinine kinase levels. We experienced the efficacy of vismodegib in many laBCC patients, with both complete and partial responses, but also some disease progressions after the onset of resistance, as reported in literature. The main pitfall is the adherence to a long, otherwise chronic, treatment due to the onset of adverse events and their impact on quality of life. The most reported and least tolerated side effect seems to be muscle spasms; it occurs relatively early during the treatment and implementation through magnesium or levocarnitine shows a mild effectiveness in few cases. Dysgeusia and alopecia are of later onset but equally impairing AEs. To overcome this issue, different preventive and management strategies have been proposed, mainly drug holydays. However, since no dose adjustments are present in the vismodegib data sheet, any individual modifications that may be introduced are off-label.

Sonidegib is the latest HH inhibitor to be approved; thus its real-life experience is being built. However, both trial results and clinical experience confirm a similar efficacy profile to vismodegib. Comparing the adjusted results of BOLT trial at 18-month follow-up to the results of ERIVANCE trial at 21-month follow-up points out slightly higher ORR and PR, similar CR and SD, and a lower PD for sonidegib (20). Like vismodegib, also sonidegib is not contraindicated in any specific patient subset, but monitoring of CK levels is indicated. We usually prescribe the same laboratory tests for vismodegib. With regard to tolerability, sonidegib shares the same class-dependent AEs of vismodegib; however, they seem to be less frequent and with a slightly longer time to onset, probably due to a different pharmacokinetic profile. The availability of an alternative administration schedule included in the label (200 mg every other day) is very helpful in managing the entity of specific AEs, such as high CK levels, and thus the rate of treatment discontinuation may be lowered.

To understand which patient could benefit from vismodegib or sonidegib, real-world data on the latter drug are needed. Only one case report described the experience of a laBCC successfully treated with sonidegib with complete response and with no side effects (33). A case series collecting experience in our center is under review. However, making any definitive directives for the choice between the two HHi is premature. Besides real-world data on sonidegib use, a head-to-head trial should be designed in order to produce more reliable comparative data. Also, intermittent trials, sequential trials, or cross-over trials of the two HH inhibitors in laBCC patients who discontinued treatment due to AEs may demonstrate the impact of the pharmacokinetic profile differences and improve the awareness of the clinician on the use of HH inhibitors.

AUTHOR CONTRIBUTIONS

All the authors contributed equally to this work. All authors contributed to the article and approved the submitted version.

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SYSTEMATIC REVIEW
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Immune Checkpoint Inhibitors in Advanced Acral Melanoma: A Systematic Review

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Introduction: Acral melanoma (AM) has different biological characteristics from cutaneous melanoma. Although systemic therapeutic strategies for advanced AM resemble those for advanced cutaneous melanoma, the evidence of the clinical use of immune checkpoint inhibitors (ICIs) for AM is still inadequate. We aimed to systematically analyze the therapeutic effects and safety profile of ICI treatments in advanced AM.

Methods: This systematic review was conducted in line with a previously registered protocol. Three electronic databases, conference abstracts, clinical trial registers, and reference lists of included articles were searched for eligible studies. The primary outcomes were therapeutic effects, and the secondary outcomes were the safety profiles.

Results: This systematic review included six studies investigating anti-CTLA-4 immunotherapy, 12 studies investigating anti-PD-1 immunotherapy, one study investigating the combination therapy of anti-CTLA-4 and anti-PD-1, and one study investigating anti-PD-1 immunotherapy in combination with radiotherapy. In most studies investigating ipilimumab, the anti-CTLA-4 antibody, the objective response rate ranged from 11.4 to 25%, the median progression-free survival ranged from 2.1 to 6.7 months, and the median overall survival was more than 7.16 months. For studies discussing anti-PD-1 immunotherapy with nivolumab, pembrolizumab, or JS001, the objective response rate ranged from 14 to 42.9%, the median progression-free survival ranged from 3.2 to 9.2 months, and the median overall survival was more than 14 months. The combination therapy of anti-CTLA-4 and anti-PD-1 immunotherapy showed better efficacy with an objective response rate of 42.9% than single-agent therapy. The retrospective study investigating the combination therapy of anti-PD-1 immunotherapy and radiation showed no overall response. Few outcomes regarding safety were reported in the included studies.

Conclusions: ICIs, especially anti-CTLA-4 monoclonal antibodies combined with anti-PD-1 antibodies, are effective systematic treatments in advanced AM. However, there remains a lack of high-level evidence to verify their efficacy and safety and support their clinical application.

Keywords: melanoma, immunotherapy, systematic review, ipilimumab, programmed cell death 1 receptor, radiotherapy, combination drug therapy

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INTRODUCTION

Acral melanoma (AM), a relatively uncommon subtype of melanoma, affects palmar, plantar, and subungual surfaces. Although only comprising 2–3% of all melanoma cases, AM tends to be the most common melanoma subtype in Asian, African, and Hispanic patients, who are at lower risk for sun-related melanoma subtypes (1). Compared with other melanoma subtypes, AM is usually diagnosed at a more advanced stage, which has been proved by the study utilizing the Surveillance, Epidemiology and End Reports (SEER) database (2). Nearly two-thirds of AM was diagnosed at stage II or above, while only approximately one-third of cutaneous melanoma was diagnosed at stage II or above. Therefore, most patients have developed distant metastasis when diagnosed with AM, and systemic treatment for advanced AM is of great significance (3).

Unlike cutaneous melanoma, AM is generally not associated with UV-exposure, which partly accounts for its far lower mutational burdens than cutaneous melanoma. An Australian study demonstrated that three of the 35 (9%) acral melanomas were found to be UVR dominant. The three acral melanomas had biological characteristics similar to the cutaneous melanoma, including elevated total mutational burdens and lower levels of structural variations when compared with acral melanomas with a non-UVR signature (4). AM has different oncogenic drivers from the cutaneous melanoma, including fewer BRAF mutations (10–23%), inconstant *KIT* mutation rates (3–29%), *CCND1* and *CDK4* amplification, and deletion or mutations in different genes, such as *CDK2NA*, *PTEN*, *NF1*, and hTERT (2, 5). However, systemic treatment for advanced AM resembles those for advanced cutaneous melanoma, possibly on account of the limited number of clinical trials evaluating optimal interventions in AM. The responses of AM patients to BRAF-inhibitors are modest as AM has lower frequencies of BRAF mutations (6). AM had different kinds of mutations of KIT, such as copy number gains and activating mutations (7), but targeted therapies with inhibitors such as imatinib usually exert poor or non-durable responses (8). There still remains an urgent need for effective systemic treatment for advanced AM.

Recently, immune checkpoint inhibitors (ICIs) have been recommended as first-line treatment for advanced cutaneous melanoma (9). However, given the low incidence of AM worldwide, few clinical trials reported the therapeutic effects and safety profile of ICIs on the AM. To identify whether ICIs are beneficial for the patients of AM, we conducted this systematic review to analyze the therapeutic effects and safety profile of ICIs in advanced AM.

MATERIALS AND METHODS

This systematic review was conducted in line with the protocol registered online in the PROSPERO on May 1, 2020 (ID: CRD42020183476) and was designed in line with the PRISMA guidelines (10).

Literature Search

Considering the rarity of AM worldwide, we identified all randomized controlled trials (RCTs), prospective observational studies, retrospective studies, and expanded access programs of advanced AM treated with ICIs. Single case reports and narrative reviews were not included. Only the articles published in English or Chinese were included.

Three electronic databases: PubMed, Cochrane Central Register of Controlled Trials (CENTRAL), and EMBASE were searched to identify possibly related studies (from January 1, 1990 to July 20, 2020). Besides, clinical trial registers, conference abstracts, and reference lists of the included studies were also checked for additional possibly relevant studies. The search strategies were shown in the **Supplementary Material**.

Data Collection and Analysis

In the screening progress, two authors (ZQ and LJ) independently screened the titles and abstracts of the articles identified from the three electronic databases. The articles considered to be potentially relevant would come to the next step, assessing the eligibility. Two authors (ZQ and LJ) assessed the articles according to their full texts. An additional author (ZS) was consulted and resolved possible disagreements. One author (ZH) searched the clinical trial registers, conference abstracts and references of the included studies, and then assessed the eligibility of the records. The included studies must report the response of the patients with unresectable, metastatic, advanced or stage III or IV AM. Two authors (ZQ and LJ) extracted data independently, and a third author (ZS) reviewed the extracted data and made the decision through discussion whenever discrepancies arose. One author (ZQ) used quality assessment tool for before-after (pre-post) studies with no control group, described by the National Heart, Lung, and Blood Institute (NHLBI) (https://www.nhlbi.nih.gov/health-topics/study-quality-assessment-tools), to evaluate the methodological quality of the included studies and the risk of bias.

The primary and secondary outcome data were extracted. The objective response rate (ORR) counted from the sum of complete response (CR) and partial response (PR), median progression-free survival (PFS), median overall survival (OS), the incidence of one-year progression-free survival and the incidence of one-year overall survival were extracted as the primary outcomes to demonstrate the efficacy of the ICIs. As for the safety of ICIs, immune-related adverse event (irAE) rate of all grades and irAE rate of grade 3 or more were extracted as the secondary outcomes. The irAEs were graded in line with the Common Terminology Criteria for Adverse Events (CTCAE).

RESULTS

We initially identified 247 records in the literature search process. After removing duplicates, 200 of them remained. After screening, 37 potentially relevant studies were selected, and the full texts were obtained for eligibility assessment. Finally,

the primary and secondary outcomes of the 18 records meeting the eligibility criteria were extracted and systemically analyzed (**Figure 1**). The extracted data from the included studies were listed in **Table 1**.

Anti-CTLA-4 Immunotherapy

In the field of anti-CTLA-4 monotherapy, six studies with 177 AM patients treated with ipilimumab were identified (**Table 1**) (11–16). The ORRs for ipilimumab monotherapy ranged from 11.4 to 25%, the median PFS ranged from 2.1 to 6.7 months, and the median OS was more than 7.16 months, demonstrating the therapeutic effects of anti-CTLA-4 immunotherapy in AM. The only study investigating the safety profile of anti-CTLA-4 immunotherapy in AM showed that the frequency of irAEs was 57%, and the frequency of grade 3 or above irAEs was 17%. There remains an unmet need for randomized controlled trials evaluating the anti-CTLA-4 antibodies in AM.

In a prospective, non-interventional, non-controlled, multi-center (146 institutions), observational study, 107 Japanese patients with radically unresectable acral lentiginous melanoma (ALM) receiving ipilimumab had a median OS of 7.16 months (95% CI, 4.99–10.32 months) (11), which was significantly lower than that in other included studies. One possible reason is that the other studies reporting OS all investigated anti-CTLA-4 antibodies as first-line therapy, but this prospective study involved different lines of treatment, in which the patients' overall health condition was worse. In the results of a published expanded access program, five patients with unresectable stage III/IV AM received 3 mg/kg ipilimumab for up to four cycles. None of them was untreated, and two (40%) patients had a PR (12). A retrospective review of 35 AM patients receiving ipilimumab either 3 mg/kg or 10 mg/kg was conducted in America. One patient achieved CR (2.9%), three achieved PR (8.6%), and four achieved stable disease (SD) (11.4%). The ORR was 11.4%, and the clinical benefit rate (CR + PR + SD) was 22.9%. Of note is that all patients with positive responses were in the 3 mg/kg ipilimumab group. The median PFS was 2.5 months (95% CI, 2.3–2.7months). The median OS was 16.7 months (95% CI, 10.9–22.5 months). In this study, 20 patients (57%) had irAEs of any grade, and 17% patients had grade 3 or 4 events, including colitis (n = 2), hypophysitis (n = 2), hepatotoxicity (n = 1), and skin toxicity (n = 1). No patients died of irAEs (13). In a retrospective analysis of 17 patients with metastatic AM treated with ipilimumab as first-line therapy, the ORR was 17.8%. The median PFS was 6.7 months (95% CI, 2.8–17.2 months), and the median OS was 38.7 months (95% CI, 7.8–61.6 months) (14). A single-center retrospective cohort study conducted in Switzerland involved 8 advanced ALM patients with ipilimumab as the first-line treatment. The ORR was 25%. The median PFS and median OS were 2.1 months and 21 months, respectively (15). A retrospective study conducted in Germany evaluated the therapeutic effects of anti-CTLA-4 and

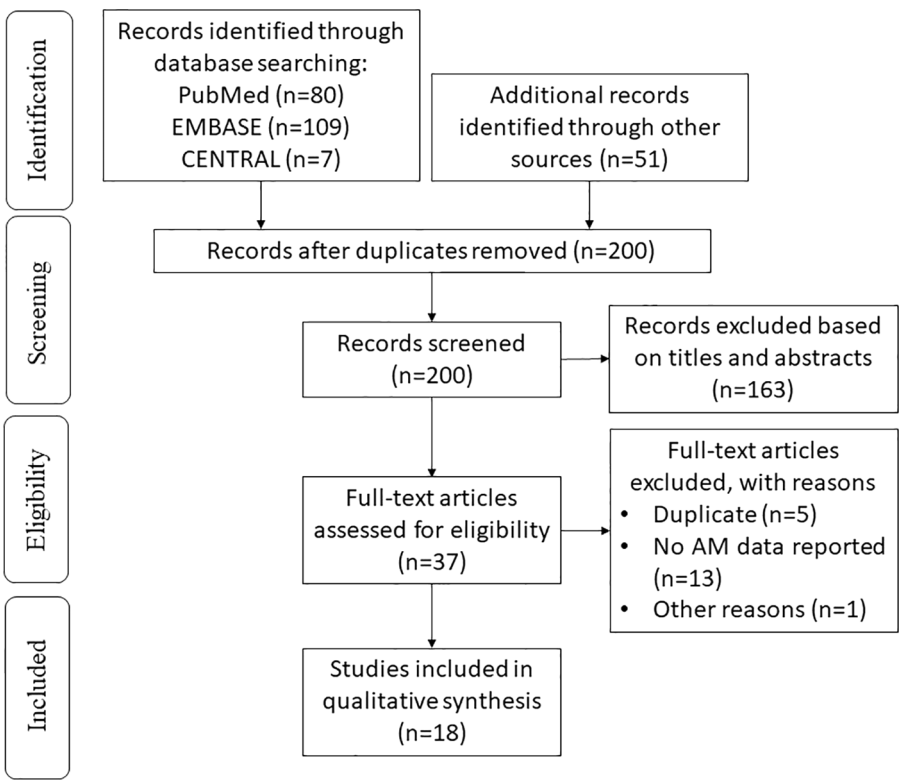

FIGURE 1 | PRISMA 2009 flow diagram of the literature search.

TABLE 1 | Characteristics of the 18 studies included in the qualitative review.

Study characteristics								Primary outcomes							Secondary outcomes		Methodological quality
First author and year	Registration ID	Study design	Population	Location	Intervention (mg/kg)	Line of immunotherapy	Record type	ORR	PR	CR	PFS (median)	OS (median)	1-year PFS	1-year OS	All grades irAEs	Grade 3+ irAEs	
Yamazaki 2020 (11)	NCT02717364	prospective, non-interventional, multi-center, observational study	n = 547 (total), n = 107 (<x>ALM</x>)	Japan	ipilimumab(3)	1+	journal article	NR	NR	NR	NR	7.16 months (95% CI, 4.99–10.32 months)	NR	NR	NR	NR	good
Shaw 2012 (12)	NA	EAP	n = 27 (total), n = 5 (AM)	UK	ipilimumab(3)	2+	conference abstract	NR	2 (40%)	NR	NR	NR	NR	NR	NR	NR	poor
Johnson 2015 (13)	NA	retrospective uncontrolled	n = 35 (AM only)	America	ipilimumab(3 or 10)	NR	journal article	11.40%	3 (8.6%)	1 (2.9%)	2.5 months (95% CI, 2.3–2.7 months)	16.7 months (95% CI, 10.9–22.5 months)	NR	NR	20 (57%)	6 (17%)	good
Saberian 2020 (14)	NA	retrospective uncontrolled	n = 44 (AM only)	America	ipilimumab or pembrolizumab or nivolumab	1	conference abstract	17.8% (anti-CTLA-4, n = 17), 40% (anti-PD-1, n = 15)	NR	NR	6.7 months (95% CI, 2.8–17.2 months, anti-CTLA-4), 9.2 months (95% CI, 2.7–19.7 months, anti-PD-1)	38.7 months (95% CI, 7.8–61.6 months, anti-CTLA-4), 60.1 months (95% CI, 12.4–67.4 months, anti-PD-1)	NR	NR	NR	NR	fair
Hafliger 2018 (15)	NA	retrospective uncontrolled	n = 8 (ALM only)	Switzerland	ipilimumab	1	journal article	25%	NR	NR	2.1 months	21 months	NR	NR	NR	NR	fair
Zaremba 2019 (16)	NA	retrospective uncontrolled	n = 21 (AM)	German	anti-PD-1 and anti-CTLA-4 checkpoint inhibitor, respectively	1	journal article	NR	NR	NR	NR	98 months (anti-PD-1, n=16), 95 months (anti-CTLA-4, n=5)	NR	NR	NR	NR	fair
Nathan 2019 (17)	NCT02156804	open-label, single-arm, multi-center phase II study	n = 1,008 (total), n = 55 (AM)	Europe	nivolumab(3)	2+	journal article	NR	NR	NR	NR	25.8 months (95% CI, 15.1-30.6 months)	NR	35 (63.64%)	42 (76.4%)	14 (25.5%)	fair
Yamazaki 2019 (18)	JapicCTI-142533	open-label, single-arm, multicenter phase II study	n = 23 (total), n = 7 (ALM)	Japan	nivolumab(3)	1	journal article	28.6% (90% CI, 10.0-59.1%)	NR	NR	NR	NR	NR	5 (71.4%)	NR	NR	fair
Maeda 2019 (19)	NA	retrospective uncontrolled	n = 68 (total), n = 16 (ALM)	Japan	nivolumab	NR	research letter	19%	3	0	197 days	421 days	NR	NR	NR	NR	fair
Si 2019 (20)	NCT02821000	open-label, non-randomized, multicenter, phase Ib study	n = 102 (total), n = 38 (AM)	China	Pembrolizumab (2)	2	journal article	15.8% (95% CI, 6.0–31.3%)	6 (15.8%)	0	NR	NR	NR	NR	NR	NR	fair
Tang 2019 (21)	NCT02836795	single-center, phase 1, open-label, 2-part (part A dose-escalation and part B dose-expansion) study	n = 36 (total), n = 13 (AM)	China	JS001(1 or 3 or10)	2+	journal article	23%	2	1	NR	NR	NR	NR	NR	0	good
Tang 2020 (22)	NCT03013101	multi-center, single arm, open-label phase II registration study	n = 128 (total), n = 50 (AM)	China	JS001(3)	2+	journal article	14.00%	NR	NR	3.2 months (95% CI, 1.8–3.6 months)	16.9 months (95% CI, 10.9–not estimable months)	5 (10%)	28 (56%)	NR	NR	good
Nakamura 2020 (23)	NA	retrospective uncontrolled	n = 193 (AM only)	Japan	anti-PD-1 antibody	1+	conference abstract	16.60%	13.50%	3.10%	NR	18.1 months	NR	NR	NR	27 (14.0%)	fair
Betof 2020 (24)	NA	retrospective uncontrolled	n = 396 (total), n = 50 (AM)	America	pembrolizumab or nivolumab	NR	journal article	NR	NR	6 (12%)	NR	NR	NR	NR	NR	NR	good
Shoushtari 2016 (25)	NA	multi-institutional, retrospective cohort analysis	n = 60 (total), n = 25 (AM)	America	nivolumab(0.3 to 10) or pembrolizumab (2 or 10)	1+	journal article	32% (95% CI, 15–54%)	6 (24%)	2 (8%)	4.1 months	31.7 months	5 (20%)	5 (20%)	NR	NR	good
Zhao 2019 (26)	NA	retrospective uncontrolled	n = 51 (total), n = 16 (AM)	China	nivolumab(3) or pembrolizumab (2)	1+	journal article	18.75%	3	0	5.3 months (95% CI, 2.4–8.2 months)	NR	NR	NR	NR	NR	fair
Namikawa 2018 (27)	JapicCTI-152869	open-label, single-arm, multi-center phase II study	n = 30 (total), n = 7 (AM)	Japan	nivolumab(1) and ipilimumab (3)	1	journal article	42.9% (95% CI, 9.9–81.6)	NR	NR	NR	NR	3 (43%)	6 (86%)	NR	NR	good
Kato 2019 (28)	NA	retrospective uncontrolled	n = 10 (total), n = 3 (AM)	Japan	radiotherapy and nivolumab (3 or 2) or pembrolizumab (2)	NR	journal article	0	0	0	NR	NR	NR	NR	NR	0	fair

NA, Not Applicable; NR, Not Reported.

anti-PD-1/PDL1 checkpoint inhibitors, respectively. The five AM patients receiving anti-CTLA-4 monoclonal antibodies as first-line therapy had an OS of 95 months, which was significantly higher in comparison with BRAF inhibitors, MEK inhibitors, and chemotherapy in this study (16).

Anti-PD-1 Immunotherapy

In the field of anti-PD-1 monotherapy, 12 studies with 494 AM patients treated with anti-PD-1 monoclonal antibodies were identified (**Table 1**). The extracted statistics demonstrated that immunotherapy targeting the interaction between PD-L1 and PD-1 had nearly the same effect as the antibodies targeting CTLA-4 in AM. The ORR ranged from 14 to 40.0%, the median PFS ranged from 3.2 to 9.2 months, and the median OS was more than 421 days in these studies. The only two studies assessing the safety profile of the anti-PD-1 monotherapy in AM patients showed that the rate of grade 3 or above irAEs was between 14.0 and 25.5%. One patient died of grade 5 myasthenia gravis, which should not be neglected. IrAEs should be taken into serious consideration in clinical practice. As the two studies exploring the safety of the anti-PD-1 monotherapy in AM patients involved 193 and 55 AM patients, respectively, the results were relatively convincing (17, 29). These outcomes demonstrated that anti-PD-1 monotherapy could extend the lifespan with tolerable toxicities in part of the patients with advanced AM. However, some patients might encounter serious adverse events, such as grade 3 or above irAEs leading to discontinuation of the therapy and even death.

Three studies assessed nivolumab monotherapy (17–19). In an open-label, single-arm, multi-centered phase II study in Europe (CheckMate 172), 55 patients with unresectable AM and disease progression or recurrence after prior treatment including anti-cytotoxic T-lymphocyte antigen 4 (CTLA-4) monoclonal antibodies received nivolumab intravenously 3 mg/kg every 2 weeks for up to 2 years until progressive disease or intolerable adverse events was observed. The median OS was 25.8 months (95% CI, 15.1–30.6), which was similar to that of patients with non-acral cutaneous melanoma [25.3 months (95% CI, 20.9–28.9)]. The 1-year OS rate was 63.64%. The rate of treatment-related AEs was 76.4%, and the rate of grade 3 or 4 treatment-related AEs was 25.5% (17). Another open-label, single-arm, multi-centered phase II study conducted in Japan explored the nivolumab as first-line treatment in unresectable stage III/IV or recurrent AM. The patients received nivolumab via intravenous infusion 3 mg/kg every 2 weeks in a 6-week cycle until disease progression or unacceptable toxicity happened. The ORR was 28.6% (90% CI, 10.0–59.1%) for the seven ALM patients participating in this study. The 1-year OS rate was 71.4% (18). In a retrospective uncontrolled study to explore the efficacy of nivolumab monoclonal antibodies in ALM in Japan, the 16 ALM patients receiving nivolumab monotherapy had an ORR of 19%. Three of the ALM patients achieved a partial response, and none of them achieved a complete response. The estimated median OS and PFS were 421 and 197 days, respectively. Of note is that among the 13 ALM patients with visceral metastasis, only one achieved a partial response. In comparison, two of the three ALM patients without visceral metastasis achieved a partial response. This phenomenon indicated that the efficacy of nivolumab monotherapy for AM patients might differ in different subgroups (19).

Pembrolizumab was independently assessed in one study (20). In an open-label, non-randomized, multi-centered phase Ib study in China, 38 AM patients received pembrolizumab 2 mg/kg *via* intravenous infusion on day 1 of each 3-week cycle for up to 35 cycles as second-line therapy until disease progression, the onset of intolerable toxicity, investigator decision to discontinue treatment, or voluntary withdrawal of informed consent. As none of the AM patients achieved CR, and six of them achieved PR, the ORR was only 15.8% (95% CI, 6.0–31.3%).

JS001, also known as toripalimab, was independently assessed in two studies, both of which were conducted in China (21, 22). One was a single-center, phase 1, open-label, 2-part (part A dose-escalation and part B dose-expansion) study. Among 13 AM patients refractory to standard systemic treatment, one confirmed CR, two confirmed PR, and three confirmed SD were achieved, with an ORR of 23.1% and a disease control rate of 46.2%. No grade 3 or above irAEs were observed in the involved AM patients, which indicated that JS001 was well-tolerated in this study (21). The other study is a multi-centered, single-arm, open-label phase II registration study. Fifty previously treated advanced AM patients received JS001 3 mg/kg once every two weeks intravenously until disease progression, intolerable toxicity, or voluntary withdrawal of informed consent. The median OS was 16.9 months (95% CI, 10.9–not estimable months), and the median PFS was 3.2 months (95% CI, 1.8–3.6 months). The 1-year OS rate was 56%, and the 1-year PFS rate was 10% (22).

Six retrospective studies evaluated nivolumab and pembrolizumab together (14, 16, 23–26). A study involving 21 Japanese institutions evaluated the efficacy of anti-PD-1 antibodies in 193 advanced AM patients. The CR was 3.1%, and the PR was 13.5%. As a consequence, the ORR was 16.6%. The median OS was reported to be 18.1 months, and irAEs of grades 3 to 5 occurred in 27 patients (14.0%). One patient (0.5%) died of grade 5 myasthenia gravis (23). A study conducted in America involved 50 patients with unresectable stage III or stage IV AM. Six patients (12%) achieved CR (24). A multi-institutional, retrospective cohort analysis conducted in America involved 25 AM patients. Eight of them received nivolumab 0.3 mg/kg to 10 mg/kg intravenously every 2 to 3 weeks. Seventeen AM patients received pembrolizumab either 2 mg/kg every 3 weeks or 10 mg/kg every 2 to 3 weeks. As two AM patients had a CR, and six had a PR, the ORR was 32% (95% CI, 15–54%). The median PFS was 4.1 months, and the median OS was 31.7 months. The 1-year PFS rate was 20%, and the 1-year OS rate was also 20% (25). A study involving 16 metastatic AM patients was conducted in China. The patients received nivolumab 3 mg/kg every 2 weeks, or received pembrolizumab 2 mg/kg every 3 weeks by intravenous infusion. None of the patients achieved CR, and three patients achieved PR. The median PFS was 5.3 months (95% CI, 2.4–8.2 months) (26). Another study conducted in Germany evaluated the efficacy of anti-PD-1/PDL1 and anti-CTLA-4 monoclonal antibodies,

respectively. The 16 AM patients receiving anti-PD-1 antibodies as first-line therapy had an OS of 98 months, which was significantly higher in comparison with BRAF inhibitors, MEK inhibitors, and chemotherapy in this study (16). In an analysis of 15 patients with metastatic AM who received pembrolizumab or nivolumab as the first-line treatment, the ORR was 40%. The median PFS of the 15 patients was 9.2 months (95% CI, 2.7–19.7 months), and the median OS was 60.1 months (95% CI, 12.4–67.4 months) (14).

Combination Therapy of Anti-CTLA-4 and Anti-PD-1 Monoclonal Antibodies

One study involving seven AM patients assessed combination therapy of ipilimumab and nivolumab (**Table 1**) (27). An open-label, single-arm, multi-centered phase II study conducted in Japan treated patients with confirmed unresectable stage III/IV or recurrent AM with two doses of nivolumab (1 mg/kg) intravenously plus ipilimumab (3 mg/kg) per cycle for two 3-week cycles, then 6-week cycles with biweekly nivolumab (3 mg/kg) as first-line therapy. The ORR was 42.9% (95% CI, 9.9–81.6), and the number of patients with 1-year PFS and 1-year OS was 3 (43%) and 6 (86%), respectively.

Combination Therapy of Anti-PD-1 Immunotherapy and Radiotherapy

The efficacy and safety of anti-PD-1 immunotherapy and radiotherapy were investigated in one retrospective study conducted in Japan. Three AM patients received one of the following regimens: 3 mg/kg nivolumab every 2 weeks; 2 mg/kg nivolumab every 3 weeks; or 2 mg/kg pembrolizumab every 3 weeks. They were all treated with radiotherapy after the progression of anti-PD-1. None of the patients achieved PR or SD, and two patients achieved SD. There was no grade 3 or above irAEs (28).

DISCUSSION

This systematic review included 16 studies with 542 advanced AM patients and provided a general overview of the efficacy and safety profile of immune checkpoint inhibitors in advanced AM. We conclude that ICIs generally demonstrated remarkable clinical efficacy and acceptable irAEs for most patients.

Anti-CTLA-4 Monotherapy and Anti-PD-1 Monotherapy

High-level evidence of the therapeutic effects and safety profile of anti-CTLA-4 and anti-PD-1 monotherapy in AM is still limited, and its therapeutic effects need to be confirmed *via* high-quality randomized controlled trials. There are three uncompleted clinical trials evaluating anti-PD-1 antibodies for AM patients, which involve different kinds of antibodies from different companies, such as IBI308, IBI310, and pembrolizumab. Two of them were randomized controlled trials. The NCT04277663 will study IBI310 combined with IBI308 in comparison to high-dose interferon in AM removed by surgery. The NCT03698019 will study pembrolizumab in stage III or IV high-risk melanoma before and after surgery. With more clinical trials, the therapeutic effects and safety profile of anti-PD-1 monotherapy will be illustrated more clearly.

Combination Therapy of Anti-CTLA-4 and Anti-PD-1 Immunotherapy

Previous research in cutaneous melanoma showed that the combination of anti-CTLA-4 monoclonal antibodies and anti-PD-1 monoclonal antibodies was more effective but more toxic than single-agent therapy (30, 31). The only study evaluating the therapeutic effects of anti-CTLA-4 (ipilimumab) in combination with anti-PD-1 (nivolumab) in advanced AM showed an ORR of 42.9%, a 1-year PFS rate of 43%, and a 1-year OS rate of 86%, which were all much higher than those of anti-CTLA-4 or anti-PD-1 immunotherapy alone, demonstrating that administering nivolumab plus ipilimumab may provide a more hopeful treatment choice for patients with AM than either agent alone.

However, as the number of patients involved in the study was not enough to exert a convincing conclusion, more clinical trials evaluating the therapeutic effects and safety profile of the combined therapy of anti-CTLA-4 and anti-PD-1 are needed. The NCT02978443 is an uncompleted biomarker study of advanced mucosal melanoma or ALM treated with the combination of ipilimumab and nivolumab.

Combination Therapy of Anti-PD-1 Immunotherapy and Radiotherapy

Radiotherapy is now seldom used due to the remarkable success of targeted therapy and immunotherapy, as well as melanoma's low susceptibility to radiotherapy. Nevertheless, several studies discovered that radiation combined with immune checkpoint inhibitors had a synergistic effect in advanced cutaneous melanoma (32, 33). This systematic review included one retrospective study that assessed the anti-PD-1 immunotherapy combined with radiation (28). The ORR was 0, and the rate of grade 3 or above irAEs was also 0. As only three AM patients were involved in this study, the credibility and convincement of this evidence are poor, calling for more relevant studies to solve this problem. In theory, radiotherapy can enhance the transport of T cells to tumor tissues and enhance the strength of specific anti-tumor immune responses (34), so the combination of ICIs and radiotherapy may be more effective than monotherapy.

Combination Therapy of Tyrosine Kinase Inhibitor and ICIs

As melanomas often overexpress VEGF, which may play a significant role in disease progression, anti-angiogenesis targeting VEGF is a meaningful strategy in treating melanoma (35). Although there is no completed clinical trial investigating the combination of tyrosine kinase inhibitor and ICIs in AM, some clinical trials are recruiting patients, which will fill the gaps in this field. The NCT03955354 investigates the combination of anti-PD-1 monoclonal antibody SHR-1210 and Apatinib as first-

line therapy in advanced AM. The NCT03991975 studies the TQB2450, a kind of PD-L1 antibodies, combined with Anlotinib in patients with advanced AM.

Different Effects of ICIs in AM and Non-Acral Cutaneous Melanoma

Some studies identified in this systematic review compared the therapeutic effects of immune checkpoint inhibitors in AM and other subtypes of melanoma. A retrospective study found that in anti-PD-1 monotherapy, patients with AM (12%) were less likely to have a CR compared to cutaneous melanoma (30.9%) (24). In an open-label, nonrandomized, multi-centered, phase Ib study evaluating the efficacy of pembrolizumab as second-line therapy, the ORR was 15.8% (95% CI, 6.0–31.3%) in AM, 19.5% (95% CI, 8.8–34.9%) in non-acral melanoma (20). An open-label, single-arm, multi-centered phase II study showed that in combination therapy of anti-CTLA-4 and anti-PD-1 immunotherapy, the ORR of patients with AM (42.9%) was much lower than that of patients with non-acral cutaneous melanoma (75.0%) (27). However, a retrospective study found that therapy containing pembrolizumab had the same effect in AM (ORR 26.7%) as in the non-acral cutaneous subtype (ORR 26.7%) (36). Although the quality and size of each one of the studies was not enough to provide strong evidence, the evidence that supports AM has worse efficacy outcomes when treated with ICIs compared with cutaneous melanoma overweighs the few evidence for the same efficacy outcomes. Although the exact reason for the worse efficacy outcomes in AM compared to cutaneous melanoma in most studies was unclear, several studies have revealed unique biological characteristics of AM, which may contribute to uncovering the underlying reason. Unlike cutaneous melanoma, AM is generally not linked to UV-exposure, which results in its far lower mutational burdens than cutaneous melanoma. A study using whole-genome sequencing showed that single-nucleotide variant were 1.02–3.68 per Mb in AM, which is much lower than that in cutaneous melanoma (37). The frequencies of somatic structural variants were more in acral than in cutaneous melanomas, and greater proportions of the acral and mucosal melanoma genomes had copy number variation (38, 39). AM also has different oncogenic drivers from cutaneous melanoma, including inconstant *KIT* mutation rates (3–29%), *CCND1* and *CDK4* amplification, and deletion or mutations in different genes, such as *CDK2NA*, *PTEN*, *NF1*, and hTERT (2). A few studies suggested that the response to immunotherapy is associated with tumor mutational burden, and increased tumor neoantigen load may predict the objective response (40–43). This may partly explain why the efficacy of ICIs for AM is lower than that for the non-acral cutaneous subtype.

A possible reason is that PD-L1 expression is lower in AM than that in the non-acral cutaneous subtype. One study reported the expression of the PD-L1 in different subtypes of melanoma. 33% of AM had PD-L1 expression, compared with 62% of the sun-damaged melanomas (44). As anti-PD-1 antibodies target the interaction between PD-1 and PD-L1, the PD-L1 expression might be a biomarker predictive of the response to ICIs (45, 46).

The tumor microenvironment may also play a role. In a study, grade III TILs were more frequent in cutaneous non-ALM than in ALM (33.3 vs. 22.6%, $p = 0.033$), and lower TIL levels ($p = 0.031$) were significantly associated with shorter OS (47). However, in a study from Korea, there was no significant association between nodular melanoma, superficial spreading melanoma, and ALM with respect to the presence of lymphocytes or LS and DFS and OS (48). So whether there is a difference in TIL in the tumor microenvironment between AM and cutaneous melanomas remains to be determined. As the skin in acral sites is strikingly different from the skin in other anatomical sites, including differences of melanocyte differentiation and the absence of hair follicles and sebaceous glands, the differences between the microenvironment of AM and cutaneous melanoma may suggest a different response rate for ICIs.

Limitations and Prospects

We recognized several limitations in this systematic review. First, the methodological quality of 11 out of 18 studies included in this systematic review was evaluated as poor or fair, and 10 out of 18 studies were retrospective, together with the lack of randomized controlled trials, may result in biases. The number of studies involved in this review was also small due to the limited exploration in this field. Second, the ICIs were applied in mixed lines of therapy in most studies. Nevertheless, ICIs may have variable efficacy and safety outcomes as first-line and further-line treatment of AM. For instance, a prospective study showed that the OS result in treatment-naive AM patients was longer than in those who had received prior treatment when treated with anti-CILA-4 antibodies (11). The conclusion would be more convincing if the studies separated the patients into different subgroups according to the lines of treatment when they received ICIs. Third, most of the studies did not report the primary location of AM, or did not analyze the outcomes of different subgroups of primary sites, but the response to treatment might differ in different primary site of AM. According to a multi-center retrospective study in China, there exist differences in survival in different primary locations in AM. Compared with AM arising from sole, AM arising from palm and nail bed subgroup has a better prognosis (49). AM in different anatomical positions may have variable mutation profiles, which is exemplified by the study result that BRAF mutations were more often found in AM located on the feet. Comparing AM arising from dorsal acral sites with AM on palms and soles, lower frequencies of NRAS (25 *versus* 39.1%) and NF1 (0 *versus* 17.3%) and higher frequencies of BRAF (75 *versus* 21.7%) and TERT promoter (50 *versus* 8.6%) mutations were observed (16). As the variable genetic changes in varying anatomical positions likely influence biological behavior and therapeutic response, it is worthwhile to evaluate the therapeutic effects and safety profile of ICIs in AM arising from specific primary sites. Last, most included studies did not report the outcomes concerning the irAEs of ICIs in AM separately, so the safety of ICIs in AM remains an unsettled question that needs to be further explored.

There remain several directions of exploration in the application of ICIs in the AM. First, the most suitable clinical setting for the ICIs must be defined to achieve satisfactory outcomes. High-quality clinical trials focusing on ICIs in combination with radiotherapy, chemotherapy, or other immunotherapies in the treatment for AM are in urgent need, especially the randomized controlled trials involving statistically sufficient patients. In addition, the appropriate neoadjuvant and adjuvant therapy also needs to be explored, which could not be accomplished without the efforts and contributions of countries including China where AM is one of the most prevalent melanoma subtypes. Second, there lack laboratory models of AM, which hinders the development of new treatments such as ICIs. Third, prognostic biomarkers that can predict the response of AM to ICIs should be further explored. Tumor neoantigen load and PD-L1 expression level are regarded as promising biomarkers, but the reliability of them in AM needs to be verified, as they might not be applied in the actual situation (50). In a retrospective study, the PD-L1 expression level was not associated with anti-PD-1 ORR ($p = 0.982$) in AM (14). Besides the two markers, lower infiltration of cancer-associated fibroblasts and expression of cancer-associated fibroblast markers are linked to the positive response to anti-PD-1 monoclonal antibodies in AM (51), which is worth further exploring. Finally, possibly effective treatments for AM after the ICI treatment fails also need to be considered. Targeted therapy, or other immunotherapies, even other kinds of ICIs might be effective. In a clinical trial, nivolumab had desirable efficacy and safety results after tumor progression on prior ipilimumab (17), which brought hope to these patients.

CONCLUSIONS

In conclusion, ICIs generally demonstrated remarkable clinical efficacy and acceptable irAEs in patients with advanced AM. ICIs, especially anti-CTLA-4 immunotherapy combined with anti-PD-1 immunotherapy, are promising therapeutic strategy for advanced AM. Nevertheless, there remains a lack of high-level proof to verify their safety and support their clinical application. The effect of ICIs in AM from different primary sites should also be further elucidated in future studies. We hope that this systematic review could benefit physicians and patients, and pave the way for further research on the treatment of advanced AM.

AUTHOR CONTRIBUTIONS

QZ and JL conceived and designed this review. QZ, JL, and HZ conducted the literature search and collected the data. QZ drafted the manuscript and figures. SZ, HZ, JL, and YW reviewed and revised the manuscript. All authors contributed to the article and approved the submitted version.

SUPPLEMENTARY MATERIAL

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Research Interest and Public Interest in Melanoma: A Bibliometric and Google Trends Analysis

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Introduction: Melanoma is a severe skin cancer that metastasizes quickly. Bibliometric analysis can quantify hotspots of research interest. Google Trends can provide information to address public concerns.

Methods: The top 15 most frequently cited articles on melanoma each year from 2015 to 2019, according to annual citations, were retrieved from the Web of Science database. Original articles, reviews, and research letters were included in this research. For the Google Trends analysis, the topic "Melanoma" was selected as the keyword. Online search data from 2004 to 2019 were collected. Four countries (New Zealand, Australia, the United States and the United Kingdom) were selected for seasonal analysis. Annual trends in relative search volume and seasonal variation were analyzed, and the top related topics and rising related topics were also selected and analyzed.

Results: The top 15 most frequently cited articles each year were all original articles that focused on immunotherapy (n=8), omics (n=5), and the microbiome (n=2). The average relative search volume remained relatively stable across the years. The seasonal variation analysis revealed that the peak appeared in summer, and the valley appeared in winter. The diseases associated with or manifestations of melanoma, treatment options, risk factors, diagnostic tools, and prognosis were the topics in which the public was most interested. Most of the topics revealed by bibliometric and Google Trends analyses were consistent, with the exception of issues related to the molecular biology of melanoma.

Conclusion: This study revealed the trends in research interest and public interest in melanoma, which may pave the way for further research.

Keywords: melanoma, bibliometric analysis, Google Trends, research interest, public interest

INTRODUCTION

Melanoma is a severe skin cancer that metastasizes quickly. Cutaneous melanoma causes 55,000 deaths each year, and once the disease spreads, it rapidly becomes life-threatening (1). Cases of cutaneous melanoma account for approximately 1.7% of all newly diagnosed cases of primary malignant cancers (1). The incidence and mortality rate of melanoma vary around the world. Fair-

skinned populations are particularly prone to melanoma, and the incidence of melanoma is the highest in New Zealand and Australia (2). Exposure to ultraviolet radiation, number of atypical moles, and genetic background are common risk factors for melanoma (3).

Bibliometric analysis is a method used to quantify hot topics and research interest in the research community (4–6). Bibliometric analysis can provide physicians and investigators with crucial messages in a specific field. A thorough bibliometric analysis of the most frequently cited articles may facilitate an understanding of disciplinary development and future directions of a research field (7, 8). Google Trends is a commonly used tool for addressing online health issues. Infodemiological methods using Google Trends can estimate the epidemiological characteristics, explore the public interest, and monitor the dynamic variations in infectious diseases (9). Previously, some studies demonstrated positive correlations between the online search frequency of "melanoma" and that of its risk factors (10–12). However, McDonald and Bloom reported negative results on the association between the search index and the incidence of melanoma (13, 14).

Compared to bibliometric analysis, which provides information on research interest, Google Trends analysis provides information on public interest. Physicians and investigators should know not only the hotspots of scientific research on melanoma but also the issues of interest for the general public. This study aimed to update the topics of research interest and public interest in melanoma using bibliometric and Google Trends analyses and compare the similarities and differences, which may pave the way for further research.

METHODS

Bibliometric Analysis

We analyzed the top 15 most frequently cited articles on melanoma each year from 2015 to 2019 according to the bibliometric analysis method. These publications were retrieved from the Web of Science in descending order according to their numbers of annual citations. Two researchers (H. Zhang and Y. Wang) independently screened the abstracts and reached a consensus on the qualifying papers. Articles focusing on multiple diseases, conference articles, patents, comments, or case reports were all excluded. Original articles, reviews, and research letters were all included in this research.

Search Tool and Keyword Selection

Online search data were collected from Google Trends. Google Trends provided an index, namely, the relative search volume (RSV), to facilitate comparisons between terms, times, and locations. The RSV was restricted to a range from 0 to 100. An RSV of 100 represented the highest search count in a given period (weeks, months, or years), and the search counts were proportionally assigned lower numbers in other periods. For example, an RSV of 50 indicates that half as many searches were performed in the selected period compared to the searches indicated by an RSV of 100 (15). An RSV of 0 did not necessarily indicate 0 searches but may represent an extremely low search count compared to other periods (16). Google Trends also automatically adjusted the RSV based on population sizes to allow a comparison between populated areas and underpopulated areas (17).

The keywords were selected under the instruction of a previous guideline (18). Words or short phrases that were specific and not prone to be confused with other words or short phrases were preferable. Google Trends provided two types of query modes. One mode was the "Terms," which could be combined for exhaustive search, but the results would only be shown in the given language. The other type was "Topics," which were defined as groups of terms that shared the same concept in any language. This mode also included related searches in non-English speaking countries and might contain the most associated information (16). The mesh words of PubMed only provided "melanomas" for possible synonyms or homonyms of "melanoma" and allowed us to compare the two types of query modes by inputting different patterns of keywords, including "melanoma" alone as a term or topic, "melanomas" alone as a term, and "melanoma + melanomas" as a combination of terms in Google Trends. Both tests yielded similar fluctuations and patterns, but the topic "melanoma" produced the highest RSV. Therefore, the topic "Melanoma" was selected as the keyword in this study.

Data Query

The "Health" category was chosen to exclude unrelated information. The time range was set from January 2004 to December 2019. On 1 September 2020, the RSV data were exported to Microsoft Excel 2019. Four English-speaking countries with high RSVs were selected for seasonal variation analysis. Two countries (the United Kingdom and the United States) were located in the Northern Hemisphere, and the other two countries (Australia and New Zealand) were located in the Southern Hemisphere.

Google Trends Analysis

Topics related to the search term were also extracted from Google Trends to analyze the public interest. Google Trends provided two types of related topics: "Top related topics" and "Rising related topics." "Top related topics" are defined as the most frequently searched topics within the chosen category, time, or country. "Rising related topics" are topics with high RSV growth and are presented as a percentage of fold changes. We queried the "Top related topics" and "Rising related topics" each year from 2014 to 2019 globally to analyze the variation in the public interest over time. The results were manually examined by two searchers (H. Zhang and Y. Wang) to exclude irrelevant information.

Statistical Analysis

R software (v 3.6.2) was used for statistical analysis and plotting graphs. A diagram was plotted using the "plot" function in R to observe the trend in the annual average RSV. A cosinor model was applied for seasonal analysis according to Barnett's research

(19). Boxplots of the seasonal variation for different countries were plotted by the "season" package in R. A p-value < 0.05 was considered statistically significant.

Ethical Requirements

This study did not involve animal experiments or clinical trials. Thus, permission from the ethical committee was not needed.

RESULTS

Bibliometric Analysis

Table 1 shows the 15 top articles on melanoma with the most annual citations from 2015 to 2019. Seven articles were published in 2015, three were published in 2016, three were published in 2017, and two were published in 2018 (20–34). The annual number of citations of these articles ranged from 167.0 to 485.0, with a median of 212.6 (170.8, 283.5). Seven of the articles were published in the *New England Journal of Medicine*, followed by *Science* (n = 4), *Cell (*n = 2), *Nature* (n = 1), and *Lancet Oncology* (n=1). All of the articles were original articles. These articles were then classified into three different research focuses: immunotherapy (n = 8), omics (n = 5), and microbiome (n = 2).

Annual trends and seasonal variation in Google Trends

The annual trends for the RSV of melanoma in Google Trends are shown in **Figure 1A**. The maximum value appeared in June 2005, and the minimum value was observed in December 2012. The average RSV remained relatively stable across the years. The seasonal variation curve fit with the "cosinor" model for the RSV is shown in **Figure 1B** (p-value < 0.05). The analysis revealed that the peak RSV of melanoma occurred in summer (January for Australia and New Zealand and June for the United States and the United Kingdom) and the valley occurred in winter (July for Australia and New Zealand and December for the United States and the United Kingdom).

Related Topics

Topics related to melanoma from 2004 to 2019 are summarized in **Table 2**. Regarding the top related topics, "Skin" was the most related (RSV = 100), followed by "Skin cancer" (RSV = 70),

TABLE 1 | List of the top 15 most frequently cited articles on melanoma from 2015 to 2019.

Title	Year of publication	Article type	Research focus	Journal of publication	Total citations	Annual citations	Rank by annual citations
Nivolumab in Previously Untreated Melanoma without BRAF Mutation	2015	Original article	Immunotherapy	New England Journal of Medicine	2910	485	1
Pembrolizumab versus Ipilimumab in Advanced Melanoma	2015	Original article	Immunotherapy	New England Journal of Medicine	2783	463.83	2
Gut Microbiome Modulates Response to Anti-PD-1 Immunotherapy in Melanoma Patients	2018	Original article	Microbiome	Science	888	296	3
Overall Survival with Combined Nivolumab and Ipilimumab in Advanced Melanoma	2017	Original article	Immunotherapy	New England Journal of Medicine	1134	283.5	4
Nivolumab and Ipilimumab versus Ipilimumab in Untreated Melanoma	2015	Original article	Immunotherapy	New England Journal of Medicine	1618	269.67	5
Nivolumab versus Chemotherapy in Patients with Advanced Melanoma Who Progressed after Anti-CTLA-4 Treatment (CheckMate 037): a Randomised, Controlled, Open-label, Phase 3 trial	2015	Original article	Immunotherapy	Lancet Oncology	1474	245.67	6
Improved Overall Survival in Melanoma with Combined Dabrafenib and Trametinib	2015	Original article	Immunotherapy	New England Journal of Medicine	1277	212.83	7
Mutations Associated with Acquired Resistance to PD-1 Blockade in Melanoma	2016	Original article	Omics	New England Journal of Medicine	1063	212.6	8
An Immunogenic Personal Neoantigen Vaccine for Patients with Melanoma	2017	Original article	Immunotherapy	Nature	752	188	9
The Commensal Microbiome is Associated with Anti-PD-1 Efficacy in Metastatic Melanoma Patients	2018	Original article	Microbiome	Science	558	186	10
Genomic Classification of Cutaneous Melanoma	2015	Original article	Omics	Cell	1110	185	11
Genomic and Transcriptomic Features of Response to Anti-PD-1 Therapy in Metastatic Melanoma	2016	Original article	Omics	Cell	854	170.8	12
Adjuvant Nivolumab versus Ipilimumab in Resected Stage III or IV Melanoma	2017	Original article	Immunotherapy	New England Journal of Medicine	679	169.75	13
Genomic Correlates of Response to CTLA-4 Blockade in Metastatic Melanoma	2015	Original article	Omics	Science	1005	167.5	14
Dissecting the Multicellular Ecosystem of Metastatic Melanoma by Single-cell RNA-seq	2016	Original article	Omics	Science	835	167	15

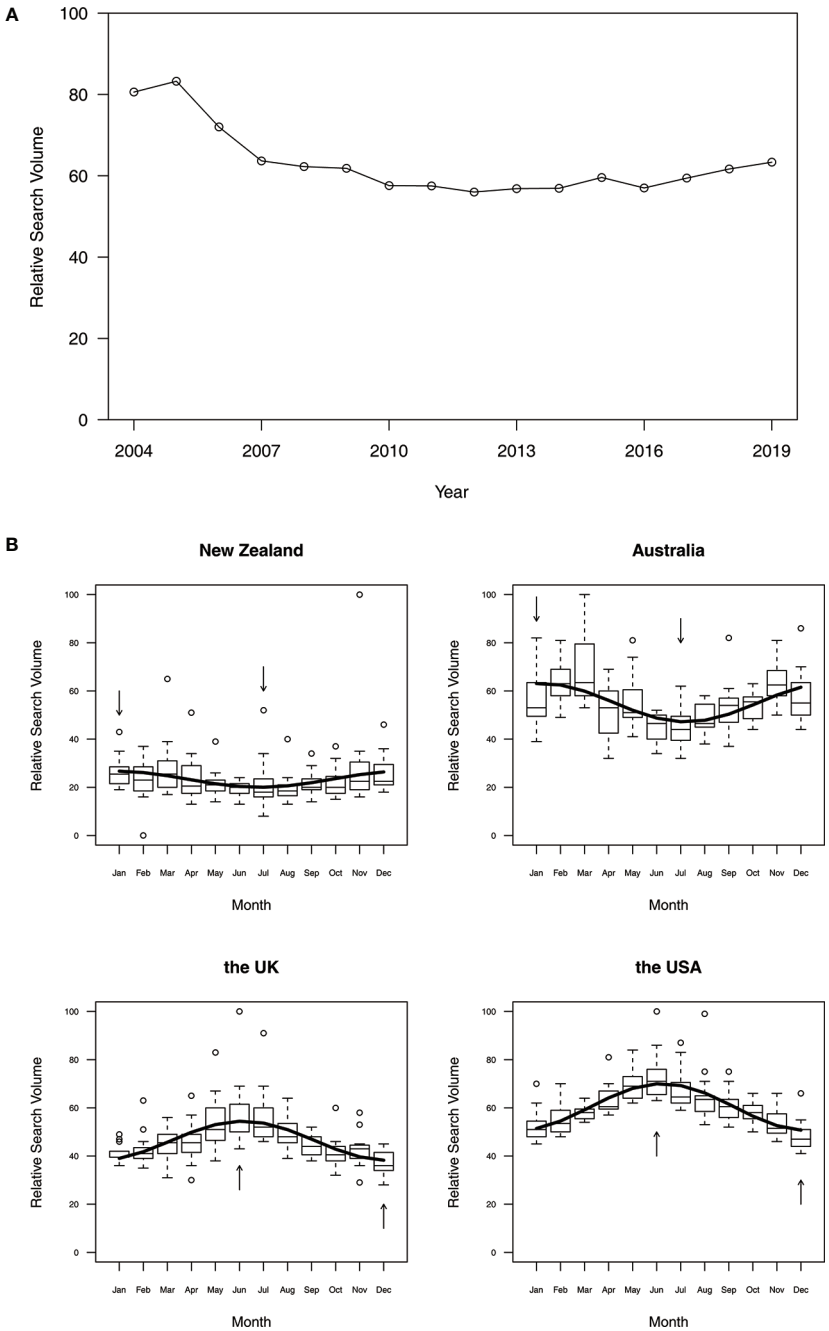

FIGURE 1 | Annual trends **(A)** and seasonal variation **(B)** of the relative search volume on melanoma. a. Annual trends from 2004 to 2019. **(B)** Seasonal variation in New Zealand, Australia, the United Kingdom, and the United States. **(A)** The lines represent the overall trend of RSV variation, and the circles represent the data points of the 12-month average RSV for each year. **(B)** The seasonal analysis was conducted and fit by the cosinor model with a p-value < 0.05. The arrows indicate the extreme value of the 16-year average RSV. (Box: interquartile range (IQR). The horizontal line inside each box: median. Whisker: maximum and minimum within median ± 1.5 × IQR. Circle: outlier outside 1.5 IQR.)

"Metastasis" (RSV = 34), "Melanocytic nevus" (RSV = 32), "Nevus" (RSV = 25), "Basal-cell carcinoma" (RSV = 16), "Prognosis" (RSV = 11), "Squamous cell carcinoma" (RSV = 10), and others. Melanoma mostly originates from the skin and represents a crucial kind of metastatic skin cancer that has a poor prognosis and is difficult to distinguish from benign melanocytic nevus or other metastatic lesions, including basal cell carcinoma and squamous cell carcinoma. Regarding the rising related topics, pathological genes and monoclonal antibodies, including "BRAF," "Ipilimumab," "Nivolumab," "Pembrolizumab," and "Vemurafenib," exhibited an

TABLE 2 | Top related and rising related topics on melanoma from 2004 to 2019.

Top related topics	Relative search volume	Rising related topics	Fold changes
Skin	100	BRAF	Breakout*
Skin cancer	70	Ipilimumab	Breakout*
Metastasis	34	Nivolumab	Breakout*
Melanocytic nevus	32	Pembrolizumab	Breakout*
Nevus	25	Vemurafenib	Breakout*
Basal-cell carcinoma	16	Squamous cell carcinoma	500%
Prognosis	11	Cancer staging	500%
Squamous cell carcinoma	10	Basal-cell carcinoma	400%
Survival rate	8	Melanocytic nevus	350%
Carcinoma	7	Nevus	250%
Malignancy	7	Skin	250%
Cancer staging	7	Carcinoma	200%
Melanin	7	Skin cancer	190%
BRAF	5	Metastasis	170%
Ipilimumab	3	Malignancy	150%
Nivolumab	3	Prognosis	120%
Pembrolizumab	2	Survival rate	110%
Vemurafenib	2		

**Breakout means an increase of over 5000%.*

increase over 5,000%, followed by associated diseases, including the topics "Squamous cell carcinoma" (n = 500%), "Basal-cell carcinoma" (n = 400%), "Melanocytic nevus" (n = 350%), and "Nevus" (n = 250%). Prognosis factors, including "Cancer staging" (n = 500%), "Metastasis" (n = 170%), "Malignancy" (n = 150%) and "Survival rate" (n = 110%), also attracted attention.

Annual Related Topics

The annual related topics are also compared in **Table 3** to identify the trends of the public interest over time. The top related topics each year were consistent with the above results. "Skin," "Skin cancer," "Metastasis," and "Melanocytic nevus" were the only four top related topics during the 16-year interval that had nearly stable ranks, which reflected the search habits of the population. In contrast, 36 rising related topics during this period were identified and showed different emphases across the years. To facilitate comprehension, we summarized the frequency of occurrence and then classified them into several subgroups.

The diseases associated with or manifestations of melanoma appeared most frequently (17/48, 35.4%), including the terms "Freckle," "Liver spot," and "Melanosis" (2/48, 4.2%), followed by "Basal-cell carcinoma," "Birthmark," "Dysplastic nevus," "Eye neoplasm," "Kaposi's sarcoma," "Lentigo," "Melancholia," "Melasma," "Sarcoma," "Subungual hematoma," and "Vulvar cancer" (1/48, 2.1%). Treatment options (13/48, 27.1%) included "Immunotherapy" (3/48, 6.3%), "Nivolumab," "Pembrolizumab" (2/48, 4.2%), "Dacarbazine," "Exeresis," "Ipilimumab," "Lymphadenectomy," "Mohs surgery," and "Vemurafenib" (1/48, 2.1%). Risk factors (5/48, 10.4%), such as the terms "BRAF" (2/48, 4.2%), "Programmed cell death protein 1," "Sun tanning," and "Melanin" (1/48, 2.1%), also attracted attention. Diagnostic tools (5/48, 10.4%) and prognosis (3/48, 6.3%) of melanoma, such as "Dermatoscopy" (4/48, 8.3%), "Cell culture," "Relapse," "Prognosis," and "Survival rate" (1/48, 2.1%), also accounted for small portions of the annual rising related topics. Other topics (5/48, 10.4%) included the "American Joint Committee on Cancer" (3/48, 6.3%); and "Bob Marley" (1/48, 2.1%), who was a celebrity who died of melanoma; and "Stadion" (1/48, 2.1%), which had little relationship with melanoma.

TABLE 3 | Annual topics related to melanoma from 2004 to 2019.

Year	Top related topics	Relative search volume	Rising related topics	Fold Changes
2004	Skin	100	Basal-cell carcinoma	Breakout*
	Skin cancer	73	Melanin	Breakout*
	Metastasis	28	Prognosis	Breakout*
2005	Skin	100	Birthmark	Breakout*
	Skin cancer	79	Kaposi's sarcoma	Breakout*
	Metastasis	30	Lymphadenectomy	Breakout*
2006	Skin	100	Melanosis	Breakout*
	Skin cancer	84	Dacarbazine	160%
	Melanocytic nevus	30	American Joint Committee on Cancer	160%
2007	Skin	100	American Joint Committee on Cancer	Breakout*
	Skin cancer	80	Dermatoscopy	Breakout*
	Metastasis	34	Freckle	250%
2008	Skin	100	Sarcoma	200%
	Skin cancer	71	Immunotherapy	180%
	Metastasis	30	Survival rate	90%
2009	Skin	100	BRAF	300%
	Skin cancer	70	Sun tanning	130%
	Metastasis	37	Dermatoscopy	120%
2010	Skin	100	Ipilimumab	400%
	Skin cancer	74	Freckle	200%
	Metastasis	33	BRAF	180%
2011	Skin	100	Melancholia	Breakout*
	Skin cancer	74	Vemurafenib	170%
	Metastasis	35	Lentigo	90%
2012	Skin	100	Mohs surgery	120%
	Skin cancer	68	Melanosis	60%
	Metastasis	36	Liver spot	60%
2013	Skin	100	Programmed cell death protein 1	300%
	Skin cancer	70	Dermatoscopy	60%
	Metastasis	34	Cell culture	60%
2014	Skin	100	Pembrolizumab	350%
	Skin cancer	70	Nivolumab	180%
	Metastasis	35	Immunotherapy	120%
2015	Skin	100	Bob Marley	150%
	Skin cancer	81	Nivolumab	150%
	Metastasis	34	Pembrolizumab	120%
2016	Skin	100	Immunotherapy	70%
	Skin cancer	75	Liver spot	50%
	Metastasis	35	Dermatoscopy	50%
2017	Skin	100	American Joint Committee on Cancer	100%
	Skin cancer	62	Melasma	90%
	Melanocytic nevus	32	Exeresis	70%
2018	Skin	100	Subungual hematoma	50%
	Skin cancer	66	Relapse	50%
	Metastasis	33	Eye neoplasm	50%
2019	Skin	100	Vulvar cancer	90%
	Skin cancer	62	Stadion	40%
	Metastasis	28	Dysplastic nevus	40%

**Breakout means an increase of over 5000%.*

DISCUSSION

This study updated the topics of research interest and public interest related to melanoma and provided physicians and investigators with a detailed description of the hot issues in which scientists and the public are interested. Google Trends data are a powerful tool to monitor and evaluate public interest in melanoma. The combination of Google Trends and bibliometric analysis may allow researchers to better anticipate research interests to serve melanoma patients.

Using bibliometric analysis, we determined the 15 most frequently cited articles on melanoma with the high numbers of annual citations published from 2015 to 2019. Using annual citations instead of the total citations as bibliometric parameters for ranking yielded benefits because this ranking included newly published articles that can provide emerging insights in the analysis (35). Our analysis indicated that the majority of these articles were published in the *New England Journal of Medicine*, followed by *Science, Cell*, *Nature*, and *Lancet Oncology*, which could be attributed to the high quality of these journals or the inherent bias with which researchers tend to select high impact factor journals for citations (36, 37). All the publications were original articles, reflecting the substantial demand of the community for revolutionary innovation and discoveries related to melanoma. The average numbers of citations of these most frequently cited articles were dramatically higher than those of other bibliometric analysis studies, such as those on rosacea (8), oral lichen planus (38), or psoriatic arthritis (38). This phenomenon reflects a high degree of research interest regarding melanoma. In addition, the articles were all classic with more than 400 citations, even for the articles published in 2018, showing the impact of the literature (8, 39).

Eight of the 15 annual most frequently cited articles were about immunotherapies, such as anti-PD1 therapies (33), nivolumab, or ipilimumab treatment (25), and nivolumab treatment in patients without BRAF mutations (27). The molecular mechanisms and the star genes that the immunotherapeutic drugs targeted, including the "Programmed cell death protein 1" (PD-1) and "B-Raf proto-oncogene" (BRAF), generated research interest (40–42). PD-1 is an immune checkpoint molecule expressed on tumor cells that inhibits CD8+ T cells and induces adaptive immune inhibition (43). PD-1 inhibitors, including "Nivolumab" and "Pembrolizumab," have been demonstrated to show clinical activities in melanoma (44). BRAF mutations were found in approximately 60% of melanomas (45), and the inhibitors "Vemurafenib" and "Dabrafenib" were proven to be efficient in melanoma patients with the mutation (46, 47).

Furthermore, researchers might focus on other topics to provide new insights into melanoma that the public might not know. Examples include omics analysis and microbiome analysis. Genomic studies have identified activating driver mutations that stimulate the development of targeted therapies for patients (48). The overall mutational load, neoantigen load, and expression of cytolytic markers in the immune microenvironment were significantly associated with clinical benefits (29). In addition, the commensal microbiome might have a mechanistic impact on antitumor immunity in melanoma patients (23). The results suggested that patients with a favorable gut microbiome might express enhanced systemic and antitumor immunity (21).

Google Trends was particularly helpful in monitoring health information-seeking behavior and analyzing public interest. The results showed that the global average RSV for melanoma was relatively stable across the years, illustrating the continued attention given by the public to melanoma (49). Regarding seasonal analysis, in Australia and New Zealand, the peak RSV appeared in January (summer). During that time, the incidence of melanoma is predominantly high in those countries (50), and previous research has demonstrated the correlation between the RSV of sun tanning and melanoma (51). Risk factors for melanoma, including exposure to sunshine, lighter clothing, and even sun tanning, might be responsible for this result (52, 53). The health prevention campaign in Australia also promisingly reduced the rates of indoor tanning among young adults and thus helped to decrease the incidence (54). For countries in the Northern Hemisphere, such as the United States and the United Kingdom, the peak RSV appeared in June (summer), and the educational campaign of public awareness month for skin cancers in May might be responsible for increasing the RSV (55).

The related topics illustrated the most concerning themes for the public. The top related topics were defined as the most frequently searched topics within the chosen category, time, or country. As a type of cancer, melanoma mostly originates from the skin; the terms "Skin," "Skin cancer," and "Metastasis" were reasonably ranked in the top 3 related topics. The differential diagnosis of melanoma from other diseases such as "Melanocytic nevus" and "nevus" also attracted attention. Even senior dermatologists had some difficulties in recognizing malignant features to distinguish melanoma from nevus in dermoscopic images (56), and the involvement of artificial intelligence in dermatology liberated dermatologists and made some contributions to solving the problem (57). The terms "Basal cell carcinoma" and "Squamous cell carcinoma" refer to common malignant tumors in the United States and hence have become hot topics (58). "Malignancy," "Prognosis," "Relapse," and "Survival rate" might be the most concerning topics for the patients and appeared in the list.

The rising related topics are of newly emerged public interest. The results marked "Breakout" represent tremendous increases of over 5,000% compared with the previous search, probably representing the rapid development of these topics. Immunotherapies are in the spotlight in this era. The systemic treatment of melanoma has completely changed since the first introduction of ipilimumab in 2011 (59). In less than 10 years, over 10 drugs have been proven or are being proven effective for treating unresectable melanoma and dramatically increase the predicted survival time of patients (60). A review recently summarized the historically published articles and guided clinicians regarding the use of systemic therapy for melanoma (40). The overall success explained the emergence of the public interest in immunotherapies in recent years. "Cancer staging," "Metastasis," "Malignancy," and "Survival rate" also attracted

attention. The complete revolution of melanoma management has invigorated the public interest in the prognoses of patients. The popularization of the concept of personalized medicine caused the public to become more concerned with the outcomes of patients instead of short-term effects. Hence, it was necessary to formulate an individualized systemic medication plan according to the cancer stage and metastasis of the patients to achieve the maximum survival rate.

The annual top related topics were analyzed to reveal the trends in the topics of greatest interest during 2004 to 2019. Most of these topics were consistent with the above discussion, but some interesting terms also emerged. "Basal-cell carcinoma," "Birthmark," "Dysplastic nevus," "Eye neoplasm," "Freckle," "Kaposi's sarcoma," "Liver spot," "Lentigo," "Melancholia," "Melanosis," "Melasma," "Sarcoma," "Subungual hematoma," and "Vulvar cancer" were the diseases associated with or manifestations of melanoma (61–63). Ocular melanoma is the second most common type of melanoma and is often observed as an eye neoplasm. Lentigo maligna might eventually develop into invasive melanoma (64). "Melancholia," "Melanosis," and "Melasma" might have similar spellings as melanoma and hence confuse the searchers.

Treatment methods ranked second among the results. Terms associated with surgical methods including "Exeresis" and "Mohs surgery" refer to effective treatment modalities for early-stage noninvasive melanoma and therefore attract public interest (65, 66). Consistent with the bibliometric analysis, immunotherapies and risk genes attracted attention. In addition to those we discussed above, CTLA-4 was recently the focus of the public and appeared on the list. CTLA-4 is an immune checkpoint molecule that downregulates pathways of T cell activation (67), and "Ipilimumab" can inhibit CTLA-4 to improve survival in patients with metastatic melanoma (68).

Risk factors that had been discussed above, including sun tanning and melanin, illustrated the importance of public educational campaigns (69, 70). The evolution and broad adaption of dermatoscopy in clinical examinations also improved the diagnosis of benign and malignant cutaneous neoplasms compared with diagnosis with unaided eyes. Dermatoscopy also improved the ability of expert readers to make appropriate management decisions (71). Cell cultures can contribute to the diagnosis and development of melanoma management plans and function as an experimental tool to facilitate the development of new drugs (72). Interestingly, American Joint Committee on Cancer and a celebrity, Bob Marley, who died of the disease, also appeared on the list. The former association formulates the guidelines for the cancer staging of melanoma, and the latter reflects the celebrity effect, which can stimulate the recognition of the disease among the public.

Our study revealed the consistency between the research interest and the public interest. Both interests focused on the risk genes of melanoma and their inhibitors or blockers. These included PD-1, BRAF, CTLA-4, ipilimumab, nivolumab, dabrafenib, and trametinib. The use of social media has substantially increased among researchers and the public and could explain this corresponding relationship (73). In Australia, the SunSmart skin cancer prevention program has been demonstrated to contribute to the reduction of melanoma among younger cohorts (74). In addition to Australia, the Euromelanoma campaign also organized a yearly media campaign, which targets the public and focuses on different aspects of melanoma prevention. Euromelanoma Day has been held each year in May, both in university-based and hospital-based outpatient clinics and private dermatology surgeries (75). Patients and even the normal population can enhance their knowledge through these campaigns and become familiar with the latest research interest (76). In addition, the research interest might be influenced by social media, as reported by Pemmaraju (74), and the types of tweets about skin cancer have changed rapidly over time. The number of pharmaceutical companies that is discussed has been increasing, and the topic tags transitioned from "melanoma" to "immunotherapies" from 2011 to 2016 (74).

However, some differences still exist. The public did not show interest in the omics and microbiomes of melanoma that the research community studied. This was comprehensive because the public might not be familiar with these academic terms. More importantly, patients were mostly concerned with the symptoms, differential diagnosis, metastasis, and treatment of melanoma, especially newly emerged targeted drugs, which might improve prognosis and predict survival time. These aspects might become future directions for research and the popularization of science. Mechanisms, pathogenesis, pathophysiology, and epidemiological features were probably less important for patients because the complete elucidation of such factors could not alleviate symptoms, cure the disease, and decrease the high treatment expenses. Although these research fields might not provide patients and their families with hope in this era, they remain valuable for researchers. The development of new techniques and the discovery of key molecules in melanoma are crucial to guide future management. The prognosis of melanoma patients with regional metastases is influenced by the genomic classification, offering insights to further personalize therapeutic decision making (20). In addition, the commensal microbiome might have a mechanistic impact on antitumor immunity in melanoma patients (23). Such research findings might be included in educational campaigns in the future.

There are several limitations to the study. First, the public interest is restricted to Internet users who are conducting Google searches in English. There may be selection bias because the disease might not attract enough attention in underdeveloped areas. Although English remains the most popular official language worldwide, different languages and cultures could have different interests. In addition, other search engines could also be more popular than Google Trends in certain countries. For example, the Baidu engine is the main search engine in China. To compensate for the loss of data, we tried to use "topics" instead of "terms" as keywords, which may include some synonyms of melanoma in other languages. Second, only the Web of Science database was used to search for eligible articles, and some articles may be missed. Notably, fewer citations do not mean that an article is unimportant because it may lack the ability to be accessed by scholars.

CONCLUSION

This study used bibliometric and Google Trends analyses to update the topics and to compare the differences and similarities of research interest and public interest in melanoma. Regarding research interest, the top 15 most frequently cited articles each year focused on immunotherapy (n=8), omics (n=5), and the microbiome (n=2). Regarding public interest, diseases associated with or manifestations of melanoma, treatment options, risk factors, diagnostic tools, and prognosis were of the greatest interest to the public. The results revealed the trends in research interest and public interest in melanoma, which may pave the way for further research.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary material. Further inquiries can be directed to the corresponding author.

AUTHOR CONTRIBUTIONS

HZ and QS conceived and designed the study. HZ and YZW prepared the manuscript, and had equal contribution to the study. QZ and KT prepared the tables and figures. QZ, KT, RF, YCW, and QS reviewed and revised the manuscript. All authors contributed to the article and approved the submitted version.

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Clinical and Dermoscopic Factors for the Identification of Aggressive Histologic Subtypes of Basal Cell Carcinoma

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Background: Infiltrative basal cell carcinoma (BCC) has a higher risk for post-surgical recurrence as compared to the most common low-aggressive superficial and nodular BCC. Independent diagnostic criteria for infiltrative BCC diagnosis have not been still defined. Improving the pre-surgical recognition of infiltrative BCC might significantly reduce the risk of incomplete excision and recurrence.

Objective: The aim of this study is to define clinical and dermoscopic criteria that can differentiate infiltrative BCC from the most common low-aggressive superficial and nodular BCC.

Methods: Clinical and dermoscopic images of infiltrative, superficial, and nodular BCC were retrospectively retrieved from our database and jointly evaluated by two experienced dermoscopists, blinded for the histologic subtype. Pairwise comparisons between the three histologic subtypes were performed and multivariable logistic regression models were constructed in order to define clinical and dermoscopic factors independently associated with each subtype. To validate our findings, two experienced dermoscopists not previously involved in the study were asked to evaluate clinical and dermoscopic images from an external dataset, guessing the proper BCC subtype between infiltrative, nodular and superficial, before and after being provided with the study results.

Result: A total of 481 histopathologically proven BCCs (51.4% nodular, 33.9% superficial, and 14.8% infiltrative) were included. We found that infiltrative BCC mostly appeared on the head and neck as an amelanotic hypopigmented plaque or papule, displaying ulceration on dermoscopic examination, along with arborizing and fine superficial telangiectasia. Shiny white structures were also frequently observed. Multivariate regression analysis allowed us to define a clinical-dermoscopic profile of infiltrative BCC.

Conclusions: We defined the clinical-dermoscopic profile of infiltrative BCC, allowing to differentiate this variant from superficial and nodular BCC. This will improve pre-surgical recognition of infiltrative forms, reducing the risk for post-surgical recurrence.

Keywords: basal cell carcinoma, subtype, infiltrative, superficial, nodular, dermoscopy

INTRODUCTION

Basal cell carcinoma (BCC) is a keratinocyte carcinoma with low aggressive behavior and represents the most common tumor of human being (1). The diagnosis of BCC is generally straightforward integrating clinical and dermoscopic examination, although in a minority of cases BCC may simulate other benign and malignant tumors (2–6). Several histologic classification have been described for BCC being the superficial (sBCC), nodular (nBCC), and infiltrative (iBCC) forms the most commonly referred to. A minority of BCCs belong to a mixed pattern with more than one histotype simultaneously (7, 8). Basically, BCC histotypes can be classified as non-aggressive and aggressive depending on their behavior to deep infiltration, perineural invasion and recurrence after surgical excision (9). Among the three most common BCC histotypes, infiltrative forms are the most aggressive and it has been reported as an independent risk factor for post-surgical recurrence (10). Superficial and nodular BCCs are instead non-aggressive forms, with a very low surgical recurrence (1). Several studies described clinical and dermoscopic criteria associated to different BCC subtypes (11–15), although specific criteria allowing to differentiate the infiltrative subtype from nodular and superficial forms have not been fully elucidated (4, 6, 7, 11–13). The aim of the current study is to define clinical and dermoscopic criteria that can help to differentiate iBCC from the most common low-aggressive sBCC and nBCC.

MATERIALS AND METHODS

Study Population

We retrospectively selected high-quality clinical and dermoscopic images of histopathologically proven BCCs from the digital databases of the Department of Dermatology of the University of Modena and Reggio Emilia (Research Project NET-2011-02347213). BCCs undergoing only partial biopsy or with more than one subtype at histopathological examination were excluded. We focused our analysis on the following histologic subtypes: infiltrative, superficial, nodular. Other subtypes only represented a minority of our case and were therefore excluded. Clinical images were taken *via* conventional clinical photography. Dermoscopic images were taken *via* polarized light contact dermoscopy (DermLite Photo 3Gen, San Juan Capistrano, CA, USA, mounted on a Canon G16 camera). Demographics and clinical data were also retrieved (i.e., skin phototype, maximum diameter and body site). This work was supported in part by Research Project NET-2011-02347213, Italian Ministry of Health. Funding source was not involved in design and conduct of the study, collection, management, analysis and interpretation of data, preparation, review, or approval of the manuscript, or decision to submit the manuscript for publication.

Study Workflow

All clinical and dermoscopic images were jointly evaluated by two of us with different degree of expertise in dermoscopy [GaPa (novice) and RP (expert with 5 years of practice)]. Evaluators were aware of demographics and clinical data, but were blinded for the histological subtype. The following clinical parameter were evaluated: color (white, pink, red, brown, blue, black-gray) and palpability (flat, elevated, nodular) together with 12 BCC-specific dermoscopic criteria: arborizing telangiectasia, superficial fine telangiectasias, blue-gray ovoid nests, blue-gray ovoid globules, ulceration, maple leaf-like, spoke-wheel areas, concentric structures, multiple small erosion, in-focus dots, shiny red-white/structureless areas, short white streaks (chrysalis) (4). Evaluators were finally asked to classify each enrolled lesion, on clinical and dermoscopic basis, as amelanotic, light, normally or heavy pigmented according to the area covered by brown-black colors (0%, <25%, 25–75%, and >75%, respectively). To assess practical implications of our results in improving BCC histotype recognition, we selected 90 BCCs (30 iBCC, 300 nBCC, and 30 sBCC) from the database of the "Centro Oncologico ad Alta Tecnologia Diagnostica" of Reggio Emilia. Clinical and dermoscopic images of this external dataset were evaluated by two experienced Clinicians with more than 10 years training in dermoscopy (GA and GiPa) not previously involved in the study, together with demographics data. They were first blinded for study results and were asked to guess the proper histologic subtype between sBCC, nBCC, and iBCC. After a washout period of 2 weeks, they were provided with study results and repeated the same evaluation.

Statistical Analysis

Quantitative variables were assessed for normal distribution and then compared using the Student's T or the Mann-Whitney U test. For qualitative variables the chi-square or Fisher's exact tests were instead used. Data were descriptively displayed and compared according to the BCC's histologic subtype. Pairwise comparisons between the three histologic subtypes were conducted for demographics, clinical, and dermoscopic variables. Three multivariable logistic regression models were subsequently constructed, one for each pairwise comparison among histologic subtypes, to define which demographics and clinical variables and which dermoscopic features were independently associated with each of the three subtypes. Alpha level was set at 0.05, while an alpha level of 0.10 was used as cut-off for variable inclusion in multivariable models.

TABLE 1 | Demographics, clinical and dermoscopic variables according to the basal cell carcinoma histologic subtype with pairwise comparisons.

Variables		Histologic subtype			Total	p value superf *vs.* infiltrative	p value nodular *vs.* infiltrative	p value superf *vs.* nodular
		Infiltrative	Nodular	Superficial				
Age	**Median (IQR)**	71 (58–79)	67 (52–76)	61 (50–71)	**65 (51–75)**	<0.001	0.034	0.023
Diameter	**Median (IQR)**	7 (5–10)	6 (4–8)	6 (5–10)	**6 (5–10)**	0.267	<0.001	<0.001
Sex	**M**	33	128	80	**241**	0.714	0.427	0.587
		46.50%	51.80%	49.10%	**50.1%**			
	F	38	119	83	**240**			
		53.50%	48.20%	50.90%	**49.9%**			
Phototype	**2**	51	167	111	**329**	0.707	0.629	0.972
		71.80%	67.60%	68.10%	**68.4%**			
	3	20	78	51	**149**			
		28.20%	31.60%	31.30%	**31.0%**			
	4	0	2	1	**3**			
		0.00%	0.80%	0.60%	**0.6%**			
Location	**HN**	56	138	31	**225**	<0.001	<0.001	<0.001
		78.90%	55.90%	19.00%	**46.8%**			
	Trunk	4	79	92	**175**			
		5.60%	32.00%	56.40%	**36.4%**			
	Upper limbs	2	23	16	**41**			
		2.80%	9.30%	9.80%	**8.5%**			
	Lower limbs	9	7	24	**40**			
		12.70%	2.80%	14.70%	**8.3%**			
Palpability	**Macule**	5	7	52	64	<0.001	<0.001	
		7.00%	2.80%	31.90%	13.31%			
	Plaque	55	136	108	299			
		77.50%	55.10%	66.30%	62.16%			
	Papule	11	104	3	118			
		15.50%	42.10%	1.80%	24.53%			
Colors clinical	**White**	36	75	46	**157**	0.001	0.002	0.641
		50.70%	30.40%	28.20%	**32.6%**			
	Pink	66	200	146	**412**	0.414	0.016	0.019
		93.00%	81.00%	89.60%	**85.7%**			
	Red	37	98	28	**163**	<0.001	0.062	<0.001
		52.10%	39.70%	17.20%	**33.9%**			
	Brown	15	44	47	**106**	0.219	0.527	0.009
		21.10%	17.80%	28.80%	**22.0%**			
	Blue	12	62	34	**108**	0.484	0.15	0.321
		16.90%	25.10%	20.90%	**22.5%**			
	Black-gray	18	81	23	**122**	0.038	0.446	<0.001
		25.40%	32.80%	14.10%	**25.4%**			
Degree of clinical pigmentation	**Non-pigmented**	36	93	69	**198**	0.134	0.056	0.016
		50.70%	37.70%	42.30%	**41.2%**			
	Light pigmented	17	64	58	**139**			
		23.90%	25.90%	35.60%	**28.9%**			
	Pigmented	12	37	16	**65**			
		16.90%	15.00%	9.80%	**13.5%**			
	Heavy pigmented	6	53	20	**79**			
		8.50%	21.50%	12.30%	**16.4%**			
Degree of dermatoscopic pigmentation	**Non-pigmented**	31	55	44	**130**	0.084	0.002	0.069
		43.70%	22.30%	27.00%	**27.0%**			
	Light pigmented	20	80	53	**153**			
		28.20%	32.40%	32.50%	**31.8%**			
	Pigmented	11	42	37	**90**			
		15.50%	17.00%	22.70%	**18.7%**			
	Heavy pigmented	9	70	29	**108**			
		12.70%	28.30%	17.80%	**22.5%**			
Dermocopy	**Arborizing (treelike)**	51	202	11	**264**	<0.001	0.067	<0.001
		71.80%	81.80%	6.70%	**54.9%**			
	Short fine superficial telangiectasias	14	8	122	**144**	<0.001	<0.001	<0.001
		19.70%	3.20%	74.80%	**29.9%**			

(Continued)

TABLE 1 | Continued

Variables		Histologic subtype			Total	p value superf *vs.* infiltrative	p value nodular *vs.* infiltrative	p value superf *vs.* nodular
		Infiltrative	Nodular	Superficial				
	Blue-gray ovoid nests	16 22.50%	95 38.50%	10 6.10%	**121** **25.2%**	<0.001	0.013	<0.001
	Multiple blue-gray globules	28 39.40%	141 57.10%	104 63.80%	**273** **56.8%**	0.001	0.009	0.175
	Ulceration	35 49.30%	60 24.30%	9 5.50%	**104** **21.6%**	<0.001	<0.001	<0.001
	Maple leaf-like	6 8.50%	46 18.60%	60 36.80%	**112** **23.3%**	<0.001	0.041	<0.001
	Spoke-wheel areas	1 1.40%	2 0.80%	18 11.00%	**21** **4.4%**	0.013	.533*	<0.001
	Concentric structures	0 0.00%	5 2.00%	16 9.80%	**21** **4.4%**	.004*	.591*	<0.001
	Multiple small erosion	1 1.40%	2 0.80%	30 18.40%	**33** **6.9%**	<0.001	.533*	<0.001
	In-focus dots	4 5.60%	14 5.70%	13 8.00%	**31** **6.4%**	0.526	>0.99*	0.357
	Shiny red-white, structureless areas	49 69.00%	149 60.30%	141 86.50%	**339** **70.5%**	0.002	0.183	<0.001
	Short white streaks (chrysalis)	55 77.50%	153 61.90%	65 39.90%	**273** **56.8%**	<0.001	0.015	<0.001
Total		**71**	**247**	**163**	**481**			

IQR, interquartile range.

Sensitivity, specificity, positive and negative predictive values (PPV and NPV) were calculated to define the diagnostic accuracy of the two evaluators asked to guess the proper BCC histologic subtype before and after being provided with the study results. Statistical analyses were performed using the IBM SPSS 26.0 package (Statistical Package for Social Sciences, IBM SPSS Inc., Chicago, Ill.).

RESULTS

A total of 526 BCCs were initially retrieved. After exclusion of 45 (8.6%) cases with mixed histotypes, 481 BCCs were enrolled belonging to 443 patients [mean age 65 years, interquartile range (IQR): 51–75 years; 218, 49.2% males and 225, 50.8% females]. Three hundred twenty-nine lesions (68.4%) belonged to patients with phototype II, 149 (31.30%) to phototype III, and 3 (0.6%) to phototype IV. Concerning histologic subtype, the majority of the enrolled BCCs were nodular (247/481; 51.4%), followed by superficial (163/481; 33.9%) and infiltrative (71/481; 14.8%) forms. Individual lesions were mainly located on the head/neck (225/481; 46.8%) and trunk (175/481; 36.4%), while only a minority arose on the limbs (upper = 41/481; 8.5%, lower = 40/481; 8.3%). Specific head and neck locations were specified in **Supplementary Table 1**. The iBCC was more frequently located on the temple and the cheek as compared to the other two histotypes. Both iBCC and the nBCC were more frequently seen on the nose than sBCC, with iBCC mainly appearing on the tip and nBCC on the nose wings. The median diameter of the enrolled lesions was 6 mm (IQR: 5–10 mm). Concerning the degree of clinical pigmentation, we found a predominance of amelanotic (198/481) and light pigmented lesions (139/481), with pink as the most widely observed color (412/481; 85.7%), followed by red (163/481; 33.9%), white (157/481; 32.6%), black-gray (122/481; 25.4%), blue (108/481; 22.5%), and brown (106/481; 22%). Dermoscopically, we found a lower number of completely amelanotic lesions (130/481; 27%), while the number of pigmented lesions proportionally increased, as compared to clinical evaluation, with a predominance of light pigmented BCCs (139/481; 28.9%). On dermoscopic examination, the most frequently observed criterion in all cases was shiny red-white structureless areas, in 339/481 (70.5%) BCCs. Multiple blue-gray globules and short white streaks were both detected in 273/481 (56.8%) lesions, arborizing telangiectasia in 264/481 (54.9%) and superficial fine telangiectasias in 144/481 (29.9%) lesions. In all, 121 (25.2%) and 112 (23.3%) out of the 481 BCCs showed blue-gray ovoid nests and maple leaf-like areas, respectively; 104/481 (21.6%) showed ulceration and 33/481 (6.9%) multiple small erosion. Other pigmented criteria, such as in focus dots, spoke-wheel areas, and concentric structures were observed only in a minority of cases. Pairwise comparisons among the three histologic subtypes are reported in **Table 1** according to demographics, clinical, and dermoscopic variables. To evaluate predictors of each BCC histologic subtype, three multivariable logistic regression models were constructed, one for each pairwise comparison. In the models of **Table 2A** demographics and clinical variables were included, together with the degree of dermoscopic pigmentation. In the models of **Table 2B** single dermoscopic criteria were instead included. We found that, as

TABLE 2 | Multivariate logistic regression analysis. Factors associated with each basal cell carcinoma histologic subtypes (infiltrative, nodular, and superficial): pairwise comparisons. Model a) demographic, clinical, and degree of pigmentation; model b) dermoscopic criteria.

A | Histotype comparison	Variables		OR	95% C.I. for OR		p value
				Lower	Upper	
Superficial *vs.* Infiltrative*	**Age**		1.04	1.01	1.07	0.019
	Location	**HN**	ref.			<0.001
		Trunk	0.01	0.00	0.05	<0.001
		Upper limbs	0.03	0.00	0.20	<0.001
		Lower limbs	0.16	0.05	0.49	0.001
	Clinical color	**White color**	3.37	1.34	8.46	0.01
		Red color	7.61	2.66	21.80	<0.001
	Surface	**Flat**	ref.			0.007
		Elevated	3.77	1.12	12.77	0.033
		Nodular	30.05	3.48	259.36	0.002
Nodular *vs.* infiltrative**	**Location**	**HN**	ref.			<0.001
		Trunk	0.137	0.047	0.405	<0.001
		Upper limbs	0.187	0.041	0.853	0.03
		Lower limbs	2.197	0.715	6.748	0.169
	Surface	**Flat**	ref.			0.001
		Elevated	0.605	0.16	2.286	0.459
		Nodular	0.143	0.033	0.618	0.009
Superficial *vs.* nodular***	**Age**		1.021	1.002	1.041	0.029
	Diameter (mm)		0.935	0.889	0.983	0.009
	Location	**HN**	ref.			<0.001
		Trunk	0.193	0.103	0.361	<0.001
		Upper limbs	0.493	0.201	1.208	0.122
		Lower limbs	0.078	0.024	0.254	<0.001
	Clinical color	**Red color**	2.587	1.318	5.077	0.006
		Black-gray color	3.138	1.591	6.189	0.001
	Surface	**Flat**	ref.			<0.001
		Elevated	7.107	2.827	17.866	<0.001
		Nodular	165.1	37.67	723.86	<0.001

B | Histotype comparison	Dermatoscopic variables	OR	95% C.I. for OR		p value
			Lower	Upper	
Superficial *vs.* infiltrative*	**Arborizing (treelike)**	17.60	5.01	61.89	<0.001
	Superficial fine telangiectasias	0.22	0.06	0.78	0.019
	Multiple blue-gray globules	0.25	0.08	0.77	0.015
	Ulceration	10.83	3.33	35.25	<0.001
	Short white streaks (chrysalis)	2.49	0.92	6.78	0.074
	Concentric structures	0.00	0.00	nc	0.998
	Multiple small erosion	0.08	0.01	0.99	0.049
Nodular *vs.* infiltrative**	**Superficial fine telangiectasias**	5.96	2.22	15.97	<0.001
	Multiple blue-gray globules	0.53	0.30	0.96	0.035
	Ulceration	3.36	1.87	6.04	<0.001
	Blue-gray ovoid nests	0.49	0.25	0.95	0.036
Superficial *vs.* nodular***	**Arborizing (treelike)**	15.13	6.01	38.14	<0.001
	Superficial fine telangiectasias	0.07	0.03	0.18	<0.001
	Blue-gray ovoid nests	6.61	2.33	18.74	<0.001
	Ulceration	3.13	0.92	10.73	0.069
	Maple leaf-like	0.32	0.12	0.80	0.015
	Concentric structures	0.20	0.04	1.05	0.057
	Multiple small erosion	0.04	0.00	0.62	0.021

*a) *Variables entered on step 1: age. Location, white color, red color, black-gray color. Degree of dermatoscopic pigmentation. Palpability. **Variables entered on step 1: age. Location, white color, red color, pink color. Degree of dermatoscopic pigmentation. Palpability. ***Variables entered on step 1: age. Diameter (mm). Location, pink color, red color, brown color, black-gray color. Degree of clinical pigmentation. Degree of dermatoscopic pigmentation. Palpability.*

*b) *Variable(s) entered on step 1: arborizing (treelike) telangiectasia. Superficial fine telangiectasias. Ulceration. Maple leaf-like. Short white streaks (chrysalis). Blue-gray ovoid nests. Spoke-wheel areas. Concentric structures. Multiple small erosion. Shiny red-white structureless areas. Multiple blue-gray globules. **Variable(s) entered on step 1: arborizing (treelike) telangiectasia. Superficial fine telangiectasias. Ulceration. Maple leaf-like. Short white streaks (chrysalis). Blue-gray ovoid nests. Multiple blue-gray globules. ***Variable(s) entered on step 1: arborizing (treelike) telangiectasia. Superficial fine telangiectasias. Ulceration. Maple leaf-like. Short white streaks (chrysalis). Blue-gray ovoid nests. Spoke-wheel areas. Concentric structures. Multiple small erosion. Shiny red-white structureless areas.*

compared with sBCC, iBCC had increased odds to be elevated or nodular than flat. Clinically, iBCC also more probably occurred in older individuals, more on the head and neck region than in other body sites and more frequently displayed white and red color. Concerning dermoscopic criteria, iBCC more frequently displayed arborizing telangiectasia and ulceration than sBCCs, which was instead more characterized by superficial fine telangiectasia and multiple blue-gray globules. Comparing iBCC with nBCC, we found higher odds for nBCC to be located on the trunk and upper limbs, while iBCC more frequently appeared on the head and neck. Furthermore, nBCC more frequently appeared as a papule than iBCC. Regarding dermoscopy, superficial fine telangiectasia and ulceration were more associated with iBCC, while multiple blue-gray globules and blue-gray ovoid nests with the nBCC. Finally, we also compared superficial and nodular BCCs, showing higher odds for sBCC to be a macule and to have a larger diameter. The sBCC was also more frequently seen on the trunk and lower limbs and more frequently displayed superficial fine telangiectasia, maple-leaf areas, and multiple small erosion upon dermoscopy. The nBCC, instead, was more frequently characterized by red and black-gray color at clinical examination and by arborizing telangiectasia and blue-gray ovoid nets. The main clinical and dermoscopic differences highlighted among BCC histologic subtypes are illustrated in **Table 3 (Figure 1).** The diagnostic accuracy of the two external readers before and after being instructed for study results is reported in **Table 4**. We registered increased levels of sensitivity and specificity and increased PPV and NPV for each of the three BCC subtypes. Baseline sensitivity for iBCC diagnosis was low for both the evaluators, with only 33.3% of cases correctly identified. After being provided with the study results almost a half of iBCC were instead correctly diagnosed.

DISCUSSION

In this monocentric retrospective observational study, we describe the main clinical and dermoscopic features of the iBCC subtype, as compared to sBCCs and nBCCs. Clinically, we found that iBCC generally appeared as an amelanotic or hypopigmented plaque or papule, located on the head and neck, in particular on the temple, cheek, and tip of the nose. Dermoscopically, iBCC frequently displayed ulceration and a mix of arborizing and superficial fine telangiectasia. Shiny white structures were also frequently observed, such as short white streaks and red-white structureless areas. When compared with

TABLE 3 | Infiltrative. nodular and superficial basal cell carcinoma clinical and dermoscopic profiles. Symbols (+, –, and ≈) were attributed according to the multivariate analysis results.

Variables		Infiltrative BCC *vs.*		Nodular BCC *vs.*
		Superficial	Nodular	Superficial
Age		+	≈	+
Diameter		≈	≈	–
Location	**HN**	++++	++*	+++
	Trunk	–––––	––	––
	Upper limbs	–––––	–	≈
	Lower limbs	––	≈	–––
Color (clinical)	**White**	+	≈	≈
	Pink	≈	≈	≈
	Red	++	≈	+
	Brown	≈	≈	≈
	Black-gray	≈	≈	+
Surface	**Macule**	–––––	++	–––––
	Plaque	+	≈	++
	Papule	++++	––	+++++
Dermoscopic criteria	**Arborizing vessels**	+++	≈	+++
	Superficial fine telangiectasia	-	++	–––
	Ulceration	++	+	+
	Multiple blue-gray globules	–	–	≈
	Blue-gray ovoid nests	≈	–	++
	Maple leaf-like	≈	≈	–
	Short white streaks	≈	≈	≈
	Spoke-wheel areas	≈	≈	≈
	Concentric structures	≈	≈	≈
	Multiple small erosion	≈	≈	–––––
	Shiny red-white structureless areas	≈	≈	≈
	Multiple blue-gray globules	≈	≈	≈
Degree of pigmentation	**Clinical**	≈	≈	≈
	Dermoscopic	≈	≈	≈

**Infiltrative more on the temple. Cheek and tip of the nose; nodular more on the nose wings. Green color highlights the strongest associations, yellow is for intermediate and orange for the weakest.*

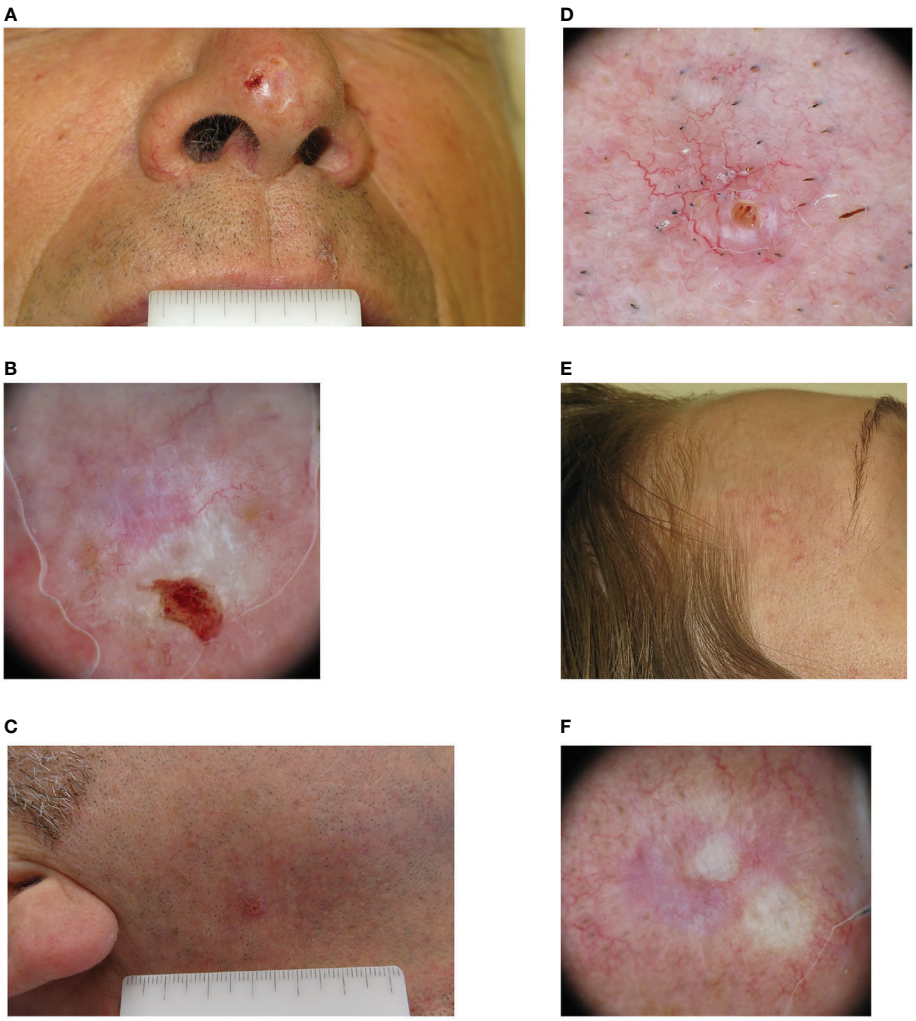

FIGURE 1 | Clinical and dermoscopic images of three cases of infiltrative basal cell carcinoma. **(A)** A man in his 60s with a 7 mm amelanotic plaque located on the tip of his nose. **(B)** Dermoscopically the lesion was ulcerated, with a pinkish-whitish background. Both short white streaks and red-white structureless areas could be seen, together with superficial fine telangiectasia. **(C)** A man in his 50s with a 5 mm pinkish papule located in his right cheek. **(D)** On dermoscopic examination both classic arborizing and more superficial fine telangiectasia are seen on a pinkish background, together with a small ulceration. **(E)** A woman in her 40s with a whitish 8 mm papule located on her right temple. **(F)** Dermoscopy highlights the presence of mixed red and white structureless areas with peripheral white streaks and superficial fine telangiectasia.

the other two histotypes, we found that patients with iBCC were slightly older than those with sBCC, but no age differences were observed with nBCC. Also, the iBCC was more often located on the head and neck and significantly less on the trunk and upper limbs, compared to the other non-aggressive histotypes. Concerning the degree of pigmentation seen on dermoscopy, iBCC was significantly more amelanotic and less heavy pigmented than nBCC in univariate analysis. However, when controlling for age, location, palpability, and clinical color in multivariate analysis no significant differences were observed. As expected, iBCC was more frequently palpable (plaque or papule) than the sBCC and less than nBCC.

Regarding dermoscopic examination, we found a prevalence of arborizing telangiectasia in iBCC, as compared to sBCC, in which superficial fine telangiectasia were instead more frequently seen. No significant differences in arborizing telangiectasia were instead observed between iBCC and nBCC, while in the former superficial fine telangiectasia were more frequently observed. Ulceration was more often reported in iBCC than both sBCC and nBCC, while multiple blue-gray globules and blue-gray ovoid nests were rarely seen among iBCCs. The definition of a specific Clinicians are dermoscopic profile for iBCC, sBCC, and nBCC, allowed external readers to increase their diagnostic accuracy in differentiating these histotypes after being provided with our study results. In particular, they were able to correctly identify a higher number of iBCCs (increased sensitivity). with a reduction of iBCCs misdiagnosed as sBCCs or nBCCs (false negative cases).

TABLE 4 | Diagnostic accuracy of two expert reviewers in diagnosing infiltrative. superficial and nodular basal cell carcinoma (BCC).

BCC histotype		I evaluator		II evaluator		Total	
		Before	After	Before	After	Before	After
Infiltrative	**Sens**	36.7%	50.0%	30.0%	46.7%	33.3%	48.3%
	Spec	80.0%	81.7%	76.7%	81.7%	78.3%	81.7%
	PPV	47.8%	57.7%	39.1%	56.0%	43.5%	56.8%
	NPV	71.6%	76.6%	68.7%	75.4%	70.1%	76.0%
Superficial	**Sens**	66.7%	70.0%	60.0%	63.3%	63.3%	66.7%
	Spec	80.0%	88.3%	80.0%	78.3%	80.0%	83.3%
	PPV	62.5%	75.0%	60.0%	59.4%	61.3%	67.2%
	NPV	82.8%	85.5%	80.0%	81.0%	81.4%	83.3%
Nodular	**Sens**	70.0%	76.7%	66.7%	73.3%	68.3%	75.0%
	Spec	76.7%	78.3%	71.7%	81.7%	74.2%	80.0%
	PPV	60.0%	63.9%	54.1%	66.7%	57.0%	65.3%
	NPV	83.6%	87.0%	81.1%	86.0%	82.4%	86.5%

Before and after being provided with the study results. Evaluation were performed on an external dataset of 90 BCCs (30 infiltrative, 30 nodular, and 30 superficial). Sens, sensitivity; spec, specificity; PPV, positive predictive value; NPV, negative predictive value.

In clinical practice, this would improve pre-surgical recognition of iBCC, allowing the surgeon to keep wider margins and reducing the risk of recurrence. Previous studies mainly defined clinical, demographic and dermoscopic features associated with sBCC (11–13). However, little is known about factors allowing to differentiate sBCC from iBCC. The sBCC has been shown to occur in younger patients than the other BCC histotypes and to be mainly located in non-chronically sun-exposed areas, such as the trunk (16). Concerning dermoscopy, multiple small erosions, superficial fine telangiectasia and structures corresponding to dermo-epidermal pigmentation were shown to predict sBCC subtype. However, the presence of blue-gray ovoid nests seems to exclude the diagnosis of sBCC (12). Dermoscopic criteria more associated with iBCC have been previously reported. However, these findings are mainly based on descriptive analysis and expert opinions, while independent clinical and dermoscopic predictors have not been defined by multivariable analysis so far (4, 6, 11–13, 17). In 2014, Longo and colleagues reported on a study population of 22 iBCCs, 22 nBCC and 44 sBCC, that infiltrative forms were featured by arborizing telangiectasia, superficial fine telangiectasia and shiny white-red structureless areas (11). However, none of these criteria was significantly more observed in iBCC as compared to the other histotypes because of the small number of cases analyzed. Furthermore, multivariable logistic regression analysis was only performed to define confocal criteria predictive of each histotype.

Our study fills this gap by focusing on clinical and dermoscopic criteria independently associated with sBCC, nBCC and iBCC subtypes. In 2020, Conforti and colleagues defined the dermoscopic criteria independently associated with the sclerodermiform BCC subtype as compared to the other subtypes (sBCC + nBCC). They found in multivariate analysis, that ulceration was significantly more frequently seen in sclerodermiform BCC, followed by fine arborizing telangiectasia, pink-white areas and multiple blue-gray dots and globules (14). Recently, a systematic review pointed out that no very specific dermoscopic criteria allow to differentiate different BCC histotypes (7). The authors reported that nBCC was more characterized by arborizing telangiectasia (75%), shiny white structures (43%), and ulceration (31%), while iBCC mainly presented arborizing telangiectasia (76%), ulceration (44%), and short-fine telangiectasia (40%). Only two dermoscopic structures appeared to be relatively unique for one subtype: leaf-like areas and shiny white-red structureless background in sBCC. In our study we failed to find these two criteria as more associated with sBCC, however, we confirmed that sBCC is easier to differentiate from both nBCC and iBCC. Wider differences were indeed observed in multivariable analysis in term of anatomic location, palpability and dermoscopic criteria, when comparing sBCC with nBCC and iBCC. Furthermore, we also reported significant differences between nBCC and iBCC. In particular iBCC was more frequently located on the head and neck as a macule, while nBCC was more frequently seen on the trunk as a papule. Upon dermoscopy, the most important difference regarded the highest occurrence of superficial fine telangiectasia in iBCC. This confirms previous observations, describing the telangiectasia of iBCC as having smaller caliber and less tendency to branch than those of nBCC (6). However, we didn't find significant differences in classic arborizing telangiectasia between iBCC and nBCC. Thus, we can conclude that in iBCC superficial fine and arborizing telangiectasia often coexist in the same lesion.

Some limitations of the current study include the retrospective design, the exclusion of minor BCC histotypes and lack of histopathological specimens' re-assessment. The latter limitation could have influenced the histotype recognition as well as the proportion of lesions showing more than one histotype. We partially controlled for this limitation by asking the pathologist (AMC) for re-assessment in case of doubtful lesions. Another limitation of the current study is the over-representation of patients with photo-type II or III, which is due to the phenotypic characteristics of the Italian population.

To conclude, we defined a clinical-dermoscopic profile of iBCC, allowing to differentiate this variant from sBCC and nBCC when Clinicians are trained on the results of the dermoscopic findings of our study.

DATA AVAILABILITY STATEMENT

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

ETHICS STATEMENT

The study involving human participants was reviewed and approved by Comitato Etico dell'Area Vasta Emilia Nord—Modena, Italy. Protocol number NET-2011-02347213. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

CL, RP, GaPa equally contributed to the study concept and design, data analysis and interpretation, and writing of the report. RP did the statistical analysis. AC did the histopathological reassessment of doubtful cases. SBo, ML, GiPa, AC, SC, FF, SBa, GA, GiPe contributed to the data interpretation and provided expert insight into the writing of the report. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fonc.2020.630458/full#supplementary-material

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SYSTEMATIC REVIEW
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The Effectiveness of Different Treatment Modalities of Cutaneous Angiosarcoma: Results From Meta-Analysis and Observational Data From SEER Database

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Introduction: Cutaneous angiosarcoma (cAS) is an aggressive vascular tumor that originates from vascular or lymphatic epithelial cells. To date, the cAS literature has been limited in a small number with single-center experiences or reports due to its rarity and the optimal treatment strategy is still in dispute. This study aimed to conduct a systematic review and compare the effect of available treatments retrieved from observational studies and Surveillance, Epidemiology, and End Results (SEER) program.

Methods: The authors performed a systematic review in the PubMed, Embase and MEDLINE database identifying the researches assessing the treatment for cAS patients. Clinical and treatment information of patients who had been diagnosed with a primary cAS were also obtained from the SEER program.

Results: Thirty-two studies were eligible but only 5 of which with 276 patients were included in meta-analysis since the unclear or unavailable information. The risk ratio of 5-year death for surgery, surgery with radiotherapy and surgery with chemotherapy were 0.84, 0.96, and 0.69. Meanwhile, in SEER database, there are 291 metastatic and 437 localized patients with cAS. The localized patients receiving surgery showed a significantly worse overall survival result when compared with the surgery combined with RT: hazard ratio: 1.6, 95% confidential interval: 1.05, 2.42, P = 0.03.

Conclusion: In conclusion, our study provided a detailed picture of the effectiveness of present treatments for localized and metastatic cAS patients. The CT could be inappropriate in localized patients. For metastatic patients, the surgery combined RT was recommended compared with surgery alone since its enhanced OS prognosis. Yet, more novel-designed clinical trials with specific targeted populations and rigorous conducting are needed for a solid conclusion on which would be a better treatment strategy.

Keywords: cutaneous angiosarcoma, SEER database, treatment modalities, meta-analysis, clinical efficacy, 5-year death rate, overall survival, cancer-specific survival

INTRODUCTION

Angiosarcomas are a group of vascular malignant tumors that are relatively rare and account for 1-2% of all soft tissue sarcomas (1). With an extremely poor prognosis, patients with angiosarcomas always ending within a year (2). They originate from vascular or lymphatic epithelial cells and can arise in various locations of the body (3, 4). About 60% of angiosarcomas present as cutaneous angiosarcomas (cAS) involving the head and neck predominantly. Others can exist in visceral organs, bones, and other soft tissues (4, 5). Multiple factors are proved to affect the survival rates of cAS, including age, tumor size, tumor site and so on (6).

The prognosis of cAS is relatively poor with a 5-year survival rate ranging from 26% to 51% (6, 7). There are many treatment options for cAS (8, 9), including surgery (10, 11), radiotherapy (RT) (12), chemotherapy (CT) (13), targeted therapy (14, 15) and more recently, immunotherapy (IT) (16). Mainstay therapy remains surgery with adjuvant RT (9). However, with the presence of new effective strategies, the treatment choice for cAS patients could be controversial. Besides, limited literature focused on the possible prognostic significance of treatments on different groups of patients such as metastatic or localized, which would be confusing in clinical practice.

Surveillance, Epidemiology, and End Results (SEER) Program of the National Cancer Institute (17) was initiated in 1973. SEER has now gained enough data that clinical and descriptive characteristics of uncommon tumors can be described at a population level. Based on the clinical characteristics, survival outcomes and corresponding therapy information retrieved from SEER program, we compared the therapeutic effect of different treatments of cAS patients. Moreover, we performed a systematic review and meta-analysis to summarize the previous observational studies evaluating the efficacy of different therapies in treating cAS, through which, independent results of the previous studies could be synthesized.

METHODS

Meta-Analysis: Data Sources and Search Strategy

The following English databases were searched systematically: PubMed, EMBASE and Medline Database with: (cutaneous angiosarcoma [Title/Abstract]) AND (treatment [Title/Abstract]). Only English articles published up to the searching date: 2020.5.17 were included. Reference lists of primary articles were reviewed for more literature.

Meta-Analysis: Inclusion Criteria and Study Selection

Inclusion criteria are as follows: 1) sufficient data including age, tumor size, tumor site, treatments were provided in a full-length article; 2) study design: prospective or retrospective cohort trials; 3) Outcome measurements: survival rate and corresponding follow up duration. Meanwhile, we excluded studies without enough data for effect sizes calculation or any case reports, review articles, letters, or communications. Two reviewers (SWB, SSC) independently went through the titles and abstracts. A senior reviewer (JJC) would be consulted if any differences exist.

Meta-Analysis: Data Extraction and Quality Assessment

By the Cochrane Collaboration for Systematic Reviews guidelines (18), this process was performed separately by two reviewers (SWB, SSC). Relevant data from the eligible studies were extracted including the 1st author's name, the published year, the number of participants, gender proportion, median age, tumor site, tumor size, tumor grade, tumor presentation, average follow-up time, treatment, and outcome measurements. The methodologic quality of each study was evaluated according to the assessment of the Newcastle–Ottawa scale which comprises three categories, including the selection of the study population. comparability of the groups, and ascertainment of the exposure or outcomes. Each parameter consists of a subcategorized questionnaire based on selection, comparability, and outcomes (19, 20). Two of the authors (SWB, SSC) independently scored the questionnaire for each included study following the user manual of the Newcastle–Ottawa scale.

SEER Database: Selection of Population Data and Outcomes

We chose the SEER 18 database which includes cases recorded between 1973 and 2015 spanning 18 different US geographic areas. The clinical data of patients who were diagnosed with cAS were obtained from the SEER Program. cAS was defined by combining the International Classification of Diseases for Oncology, 3rd edition (ICD-O-3) morphological code 9120/3 and 9170/3, which stands for hemangiosarcoma and lymphangiosarcoma, and topographical codes: C44.0-9. The other variables were included such as age at diagnosis, sex, tumor grade, tumor site, tumor size, SEER historic stage, treatment modalities and survival outcomes. For the SEER historic stages, "local," "regional," and "distant" were used as the End Results Group of National Cancer Institute (NCI).

Statistical Analysis

A single group meta-analysis was performed and results were presented with 95% confidence interval (CI). Studies were then pooled together as appropriate with two-sided $P < 0.05$ considered as statistically significant. The authors calculate the Q-statistic (21) for testing heterogeneity among studies, and $P < 0.05$ was considered as significant too. The authors selected the results with the fixed-effects model if the included studies were homogenous with $P > 0.05$; otherwise, the random-effects model results would be picked on. The I^2 statistic (21) was also calculated to efficiently test for the heterogeneity, with $I^2 <$ 25%, 25%–75%, and > 75% to represent a low, moderate and high degree of heterogeneity, respectively. We conducted a subgroup analysis to detect the source of heterogeneity furtherly based on the different treatment strategies.

On the other hand, for the SEER database analysis, Kaplan-Meier curves were used to illustrate the overall survival (OS) and cancer-specific survival (CSS) probabilities for the selected patients grouped by different therapies. The univariate and multivariate cox proportional hazards regression models were performed using the log-rank test. Predictors for the multivariate model were the factors identified as statistically significant (P value <0.05) in univariate analysis. Moreover, the authors plotted the trends in the management of patients with cAS with linear regression analysis. All the analysis and plots were generated using R 3.6.2 with packages (22–26): "gemtc," "rjags," "dmetar," "survival," "survminer," and "ggplot2".

RESULTS

Meta-Analysis: Eligible Studies Identification

As shown in **Figure 1**, 445 studies were chosen from databases for further screening. We excluded 66 duplicated articles and 347 other articles because of inappropriate topics (n=254), review articles (n=16), lack of full text (n=5), overlapping author (n=59), and not English (n=13). After assessing articles with full text, 32 studies were selected in total. A large number of studies were short of precise data for a specific treatment arm. In the end, five studies with 276 participants were included for the meta-analysis.

Meta-Analysis: Characteristics of Selected Studies

The clinical characteristics of both selected observational studies and SEER population were summarized in **Table 1**. The detailed characteristics of 32 included studies are shown in **Supplementary Files**. The sample size ranged from 5 to 421 with a median of 44 and 1414 participants in total. Participants of 17 studies were divided into two groups by tumor size. Twenty-eight studies involved information about tumor site and 13 studies involved tumor grade. The majority of studies focused on the efficacy of surgery and RT (n=22).

Meta-Analysis: Summary of Prognosis Results in Eligible Studies

The summary of prognosis parameters: 2-, 3-, 5-, 10-year survival rate, disease-free interval (DFI), mean survival time and 3, 5-year regression free survival (RFS) are shown in **Table 2** severally. The 5-year survival rate in patients receiving surgery was 12.5%–46.9%. In patients treated with RT, the 5-year survival rate was 0%–16.7%. Surgery treatment had the highest 3-year survival rate which was close to that of surgery combined with RT (60.2% and 58.4% respectively). Besides, with the follow-up time extending, the survival rate decreased, especially from 3-year to 5-year: for surgery, from 60.2% to 12.5%–46.9%; for RT, from 33.3% to 0%–16.7%; for surgery and RT, from 58.4% to 0%–33.3%.

Meta-Analysis: Results for Death Rate

Similarly, in **Figure 2**, the treatment of RT and CT had the lowest 5-year death rate followed by the treatment of surgery [risk ratio (RR):0.38, 95% confidential interval (CI) = 0.15–0.65; 0.69, 95% CI = 0.51–0.84; respectively]. However, the small number of patients in RT and CT group should be noted. The heterogeneity was in a moderate degree in the pooled effect (I^2 = 70%, P < 0.01) and subgroups of several treatments (**Figure 2**). We also tried to conduct a subgroup analysis to detect the source of heterogeneity furtherly based on other various factors including metastasis condition, age, tumor size, and tumor site, but failed since enrolled articles were lack of appropriate data.

Meta-Analysis: Study Quality of Included Studies

The summary quality assessment of the 32 included studies was illustrated in **Supplementary Files**. We assigned scores of 0–3, 4–6, and 7–9 on the Newcastle-Ottawa scale for the low, moderate and high quality of studies, respectively. The 32 included studies showed the mean quality score was 7 out of 9. In the 5 enrolled studies, three studies reached 8 and two studies were ranked as 7.

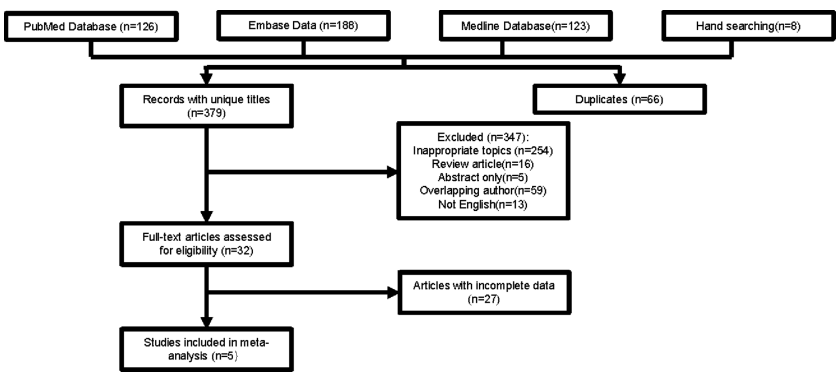

FIGURE 1 | Study selection process.

TABLE 1 | Patient demographics and tumor characteristics for cutaneous angiosarcomas summarized from published literature and SEER database.

	Published literatures	SEER
Sex		
Male	916 (64.8%)	435 (48.4%)
Female	498 (35.2%)	464 (51.6%)
Age		
10–39	72.1 ± 5.15[a]	14 (1.6%)
40–49		31 (3.4%)
50–59		70 (7.8%)
60–69		177 (19.7%)
70–79		280 (31.1%)
80+		327 (36.4%)
Race		
White	–	791 (88.0%)
Black	–	42 (4.6%)
Other	–	49 (5.5%)
Unknown	–	17 (1.9%)
Average follow up (months)[b]	112.9	43.7
Size		
Tumor size ≤5	525 (37.1%)	11 (1.2%)
Tumor size >5	432 (30.6%)	357 (39.7%)
NA/Not reported	457 (32.3%)	531 (59.1%)
Sites		
Scalp/neck/head	721 (51.0%)	345 (39.2%)
Face	367 (26.0%)	211 (21.7%)
Trunk/limb	41 (2.9%)	326 (37.1%)
Unspecific site	152 (10.7%)	17 (1.9%)
Unknown	133 (9.4%)	-
Histologic grade		
Grade I	–	54 (6.0%)
Grade II	–	83 (9.2%)
Grade III	–	138 (15.4%)
Grade IV	–	128 (14.2%)
Unknown	–	496 (55.2%)
SEER historic stage		
Localized	–	437 (51.6%)
Distant	–	291 (34.3%)[c]
Unstaged	–	119 (14.1%)

[a]: *Mean ± Standard deviation.*
[b]: *Mean value of longest follow-up time from each study.*
[c]: *There are 62 distant and 229 regional patients.*
SEER, Surveillance, Epidemiology, and End Results program; NA, not available.

SEER Database: Characteristics of the Population

In **Table 1**, we retrieved 899 cAS patients from the SEER database where 435 patients were male and 464 were female. Interestingly, the ratio of patients with tumor size more than 5 cm versus less than 5cm was exponentially larger than that in published literature data. As for the tumor site, a larger proportion of tumors were documented in the trunk/limb when comparing the SEER data with the published literature data. There are 62 distant and 229 regional patients grouping as distant patients in the following analysis. The number of patients receiving surgery, surgery and RT, surgery and CT, surgery and RT and CT, were 389 (43%), 173 (19%), 61 (7%), and 54 (6%) respectively. There are 108 patients with no treatments recorded (12%).

SEER Database: Factors Influencing the OS and CSS

In the univariate analysis, sites of face (P value < 0.01) and trunk/limb (P value < 0.01) were predictors of both OS and CSS. Ages (P value < 0.01), size (P = 0.03), black race (P value < 0.01), localized stage (P value < 0.01), tumor grades (P value < 0.05) except grade II (P value= 0.54) were all significant predictors of OS. Age (P value < 0.05), sex (P value < 0.01), and SEER historic stage (P value < 0.05) were predictors for CSS (**Supplementary Table 3**). The multivariate models conducted for both OS and CSS included all significant predictors in univariate analysis (**Supplementary Table 4**). We also included the treatment modalities as covariates. All age groups were independently correlated with OS in localized patients. Sites of face and trunk/limb were found to reduce the OS and CSS in localized patients and the OS in metastatic patients when compared with the reference groups (P value < 0.05).

SEER Database: Effectiveness and Trends of Different Treatment Modalities

For a more accurate illustration of the efficacy of different treatment modalities, the multivariate cox regression analysis was performed in which the hazard ratio of OS and CSS were adjusted by the significant factors in the univariate analysis. (Full results were shown in **Supplementary Table 4**). As shown in **Table 3**, the patients were stratified into localized and metastatic groups. Compared with the surgery with RT group, both localized and metastatic patients treated with CT showed significantly worse outcomes in OS and CSS, while the surgery and CT group and surgery and CT and RT group showed significantly worse OS only in localized patients. Particularly, the surgery alone was associated with a higher hazard for OS in metastatic patients compared with the surgery with RT group [hazard ratio (HR): 1.6; 95% CI: (1.05, 2.42); P value: = 0.03]. In **Figure 3**, we plotted the trends of therapies based on the number of patients who received the same therapy each year. Surgery is the most commonly used therapy followed by surgery together with radiotherapy.

DISCUSSION

Given the limited clinical evidence since the rather low incidence of cAS, the discussion for selecting the optimal treatment modality of cAS was in slow progress. Shin et al. (32) conducted a meta-analysis indicating the factors predisposing poor outcomes for angiosarcoma of the scalp and face. In this study, the only treatment-related result was that surgery, compared with no-surgery patients, the 5-year OS rate of angiosarcomas would significantly increase. They also stated the difficulty of comparing different treatment methods since the absence of data. Other studies focusing on the cAS and angiosarcoma patients in SEER database were all short of treatment modalities information (6, 7). To our knowledge, the present study is the first meta-analysis and SEER database research focused on illustrating the prognosis of the cAS patients based on their treatment modalities and extent of the tumor.

Localized cAS Patients

For localized patients, the results from the SEER database suggest that the CT could be inappropriate while the necessity of additional RT to surgery remains uncertain. Because CT alone, surgery and CT, surgery and CT and RT showed worse OS

TABLE 2 | Summary results of prognosis in included studies.

Therapy type	N	2 year-survival rate (%)	3 year-survival rate (%)	5 year-survival rate (%)	10 year-survival rate (%)	3 year-RFS (%)	5 year-RFS (%)	DFI (month)	Mean survival time (month)	First author
Surgery	7–48		60.2	12.5–46.9	14.3	59.8	25–39.9		12.9	Perez, MC (27); Matsumoto, K (28); Holden, CA (29); Zhang, Y (30)
RT	7–45	29	33.3	0–16.7					10.8	Perez, MC (27); Matsumoto, K (28); Holden, CA (29)
CT	2			0					2	Matsumoto, K (28)
Surgery and RT	3–57	66.7	58.4	0–33.3		27.9	0–27.9	42.8	31.3	Perez, MC (27); Matsumoto, K (28); Holden, CA (29); Zhang, Y (30); Barttelbort, SW (31)
Surgery and CT	8–22			0–14			7		11.9	Matsumoto, K (28); Zhang, Y (30)
Surgery, RT and CT	7–22			13.3–15			0		17.1	Matsumoto, K (28); Zhang, Y (30)
RT and CT	13			61.5					9.3	Matsumoto, K (28)

N, Number of patients enrolled in each study; CT, chemotherapy; RT, radiotherapy; RFS, recurrence-free survival; DFI, disease-free interval.

TABLE 3 | Multivariate cox proportions hazards models for overall survival (OS) and cancer-specific survival (CSS) in SEER patients with cAS.

Treatment modality	Localized						Metastatic					
	OS			CSS			OS			CSS		
	HR	95% CI	P value	HR	95% CI	P value	HR	95% CI	P value	HR	95% CI	P value
Surgery and RT	Ref											
CT	**3.6**	**(1.95,6.62)**	**<0.01**	**3.16**	**(1.17,8.53)**	**0.02**	**4.17**	**(2.05,8.46)**	**<0.01**	**3.53**	**(1.24,10.02)**	**0.02**
None	1.72	(1.01,2.91)	0.05	1.83	(0.83,4.05)	0.13	**4.36**	**(2.3,8.25)**	**<0.01**	**6.78**	**(2.77,16.59)**	**<0.01**
RT	1.62	(0.94,2.77)	0.08	0.9	(0.36,2.24)	0.81	1.61	(0.83,3.11)	0.16	1.37	(0.52,3.62)	0.53
RT+CT	1.61	(0.82,3.15)	0.16	1.17	(0.44,3.13)	0.75	1.11	(0.54,2.25)	0.78	1.82	(0.76,4.33)	0.18
Surgery	0.99	(0.71,1.4)	0.98	0.62	(0.36,1.07)	0.08	**1.6**	**(1.05,2.42)**	**0.03**	1.15	(0.62,2.15)	0.66
Surgery and CT	**2.25**	**(1.19,4.24)**	**0.01**	1.71	(0.57,5.12)	0.34	1.18	(0.67,2.06)	0.56	1.04	(0.46,2.39)	0.92
Surgery, RT and CT	**1.79**	**(1.03,3.13)**	**0.04**	1.89	(0.88,4.04)	0.1	1.3	(0.72,2.33)	0.38	1.87	(0.91,3.86)	0.09

cAS, cutaneous angiosarcoma; OS, overall survival; CSS, cancer-specific survival; CT, chemotherapy; RT, radiotherapy; HR, hazard ratio; CI, confidential interval.
The significant results (P<0.05) were showed in bold.

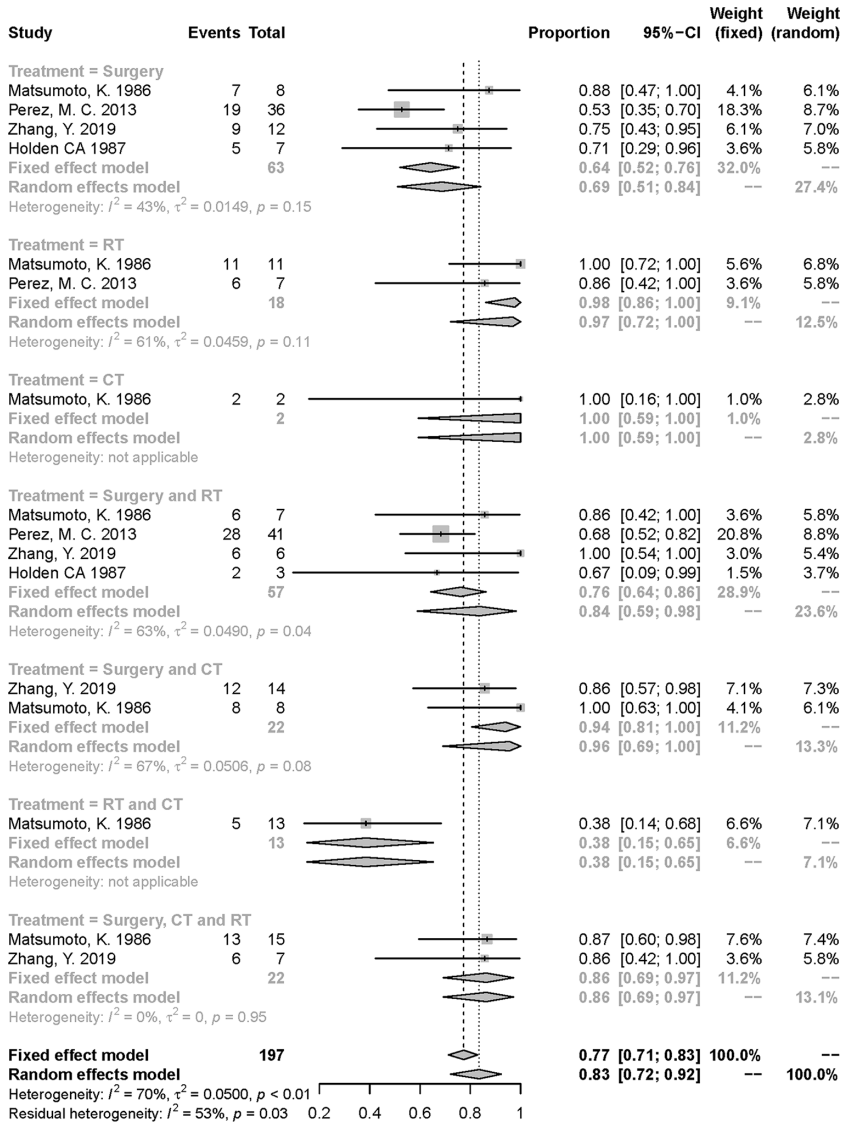

FIGURE 2 | Meta-analysis of 5-year over-all death rate in included studies. RT, radiotherapy; CT, chemotherapy.

results when compared with surgery and RT in the localized patients. The reason could be the intolerance of patients giving a significant proportion of the elderly. What's more, there were no significant results when comparing surgery alone with surgery and RT in the localized patients for both OS and CSS. Several studies (32, 33) have proven that surgery could enhance prognosis in cAS patients with no stratification of patients. Yet, surgery and RT was widely reported for reducing the risk of local recurrence and improving survival rate in localized patients (34, 35). Guadagnolo et al. (36) demonstrated that non-metastatic patients who underwent surgery and RT have statistically greater local control, OS and disease-specific survival compared with those who received surgery or RT alone. Another review (9) stated that surgery followed by RT is the mainstay of the treatment for localized angiosarcoma. Many reasons would cause this ambiguity. Primarily, the assessment of treatment efficacy should be based on the extent of cAS. Localized cAS patients are prone to receive extensive surgery and with a better prognosis since they are in the early stage of cancer while metastatic patients need more systematic treatment and ended up with a poorer outcome. Thus, any comparison of the treatment regardless of the patients' condition should be treated with caution. Secondly, most studies, including ours, are limited by the retrospective nature. The doses, frequency and time of RT (before or after the surgery) can vary a lot. There was another trial demonstrating the efficacy of chemoradiotherapy followed by maintenance CT in localized patients with large tumors that are hard to control with surgery and RT (37). Further clinical trials or guidelines may focus more on systematically conducting and delicately grouping of patients.

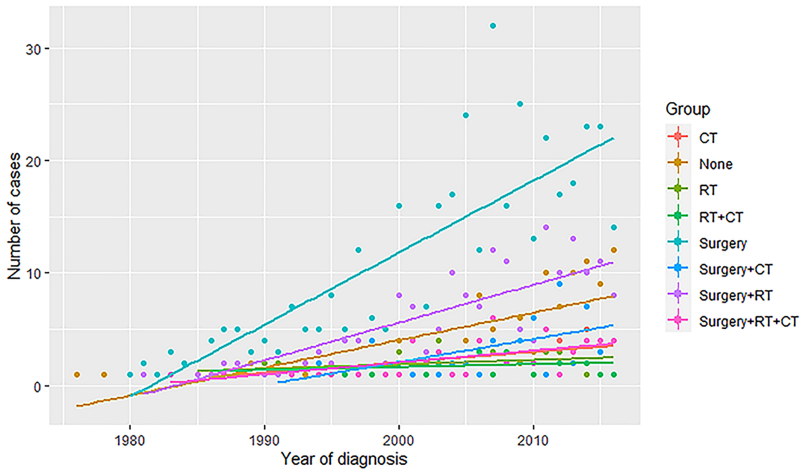

FIGURE 3 | The trends of therapies based on the number of patients who received the same therapy each year. RT, radiotherapy; CT, chemotherapy.

Metastatic cAS Patients

Paclitaxel (taxanes) was recommended as the first-line treatment for metastatic cAS patients in (9), which conflicts with our results: metastatic patients treated with CT alone have worse OS and CSS outcomes than the surgery combined with RT group. This discrepancy could derive from the use of different CT drugs since the quickly evolving process of finding new drugs. Doxorubicin-based drugs have been the preferred choice for advanced soft tissue sarcomas earlier (38, 39), which was replaced by paclitaxel nowadays (9, 38–40). Paclitaxel was rigorously assessed in a phase II trial where 30 metastatic angiosarcoma patients enrolled for a median follow-up of 8 months (40), and the result showed the median time to progression was 4 months and the median overall survival was 8 months. One retrospective study from the same institution including 149 metastatic angiosarcoma patients found there were no statistically significant differences in terms of overall survival between weekly paclitaxel and doxorubicin-based therapy (38).

On the other hand, for metastatic patients, we observed a significantly worse OS outcome receiving surgery alone versus surgery and RT only, which provides evidence for surgery and RT use in metastasis patients except for localized patients. As forementioned, the discussion of the treatment modality for metastatic patients should also consider factors including the patients' tolerance and quality of life and the follow-up duration. Considering the multiple choices of CT drugs, it seems more difficult to reach an agreement. A more systematic treatment modality might be a more reliable choice for metastatic patients based on our findings and current status.

Booming Treatment Options

According to previous results (9, 41), various drugs could be the second-line treatments for advanced cAS including pazopanib (a tyrosine kinase inhibitor), eribulin mesylate (a microtubule-targeting drug), trabectedin (a histone deacetylase inhibitor), bevacizumab (a vascular endothelial growth factor receptor inhibitor), and propranolol (a beta-blocker). Pazopanib, eribulin mesylate, and trabectedin were firstly published to be effective in treating patients with soft tissue sarcomas (42–44). In later times, a Japanese study showed the potential of pazopanib for the treatment of cAS (45). One prospective clinical study evaluating eribulin mesylate in patients with cAS after taxanes showing a promising response rate (46). Another retrospective study found the 3-month PFS rate was 25% with trabectedin in patients with angiosarcoma (47). Bevacizumab was reported to be effective in treating cAS with a PFS of 6.5 months in a phase II study (48). Notably, propranolol was firstly reported to inhibit the progression of infantile hemangioma (49). Following, several case reports described that the propranolol monotherapy or the combination of propranolol with other chemotherapeutic agents had promising responses in advanced angiosarcoma (50–52).

With the field of cancer immunology growing rapidly, there are also studies linking immune therapy, anti-programmed death ligand-1 (anti-PD-L1), to angiosarcoma treatment. A case report showed a remarkable response in a patient with angiosarcoma with the treatment of anti-PD-L1 (16). Nonetheless, for all the second-line treatments and the immunotherapy, there was not enough evidence to make recommendations for patients with advanced cAS and more prospective studies were needed.

Limitations

Our review has some limitations. Firstly, due to the rarity of cAS and the unclear classification of the treatment modalities, the number of enrolled studies and population is pretty small in the meta-analysis, especially for the CT treatment group. There are also no prospective or randomized studies, which would undermine the quality of our study. Secondly, the detailed baseline information is either absent or ununified in a large number of studies, which prevents the more in-depth analysis. It also contributed to the heterogeneity in pooled results. Additionally, although the retrospective study with the

information from SEER was conducted, the treatment details were absent.

CONCLUSION

This study compared the available treatment modalities efficacy of cAS with meta-analysis of observational studies and summarized data from SEER program. The CT could be inappropriate in localized patients. For metastatic patients, the surgery combined RT was recommended compared with surgery alone since its enhanced OS prognosis. Further investigations of long-term and prospective studies are needed for more solid evidence, especially for those newly developed therapies.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**. Further inquiries can be directed to the corresponding authors.

AUTHOR CONTRIBUTIONS

Conception and design: YC, JC, and SB. Administrative support: YC and JC. Collection and assembly of data: SB and SC. Data analysis and interpretation: SB, SC, and BW. Article writing: SB, SC, and BW. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fonc.2021.627113/full#supplementary-material

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CASE REPORT
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Case Report: Favorable Response to the Tyrosine Kinase Inhibitor Apatinib in Recurrent Merkel Cell Carcinoma

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Background: As angiogenesis is an essential step in tumor growth and metastasis, the tyrosine kinase inhibitor (TKI) apatinib has become a revolutionary anticancer therapy across various malignancies. However, its efficiency and safety in Merkel cell carcinoma (MCC) are uncertain.

Case presentation: The current study described the case of a 91-year-old man who presented with a 3.2 × 3.0 × 2.2 cm rapidly growing, solitary tumor of the right lower eyelid. It was diagnosed as MCC pathologically. Twenty-seven days after the surgery, the patient returned to the hospital with recurrent MCC. Apatinib was then administered to this patient. The patient had a complete response (CR) to apatinib after 4.4 months of targeted therapy. Twenty-seven months of progression-free survival (PFS) was achieved with controllable treatment-related adverse events (AEs).

Conclusion: Treatment with apatinib demonstrated clinical benefit in our patient with recurrent MCC, highlighting its potential utility in other MCC patients. Further clinical trials are needed to determine the efficacy and safety of apatinib in MCC patients.

Keywords: Merkel cell carcinoma, tyrosine kinase inhibitor, apatinib, eyelid, targeted therapy

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INTRODUCTION

Merkel cell carcinoma (MCC) is a rare but highly aggressive cutaneous malignancy with neuroendocrine features that has 33–46% mortality (1, 2). Heath M et al. (3) summarized the clinical features of MCC in an acronym: AEIOU—Asymptomatic/lack of tenderness, Expanding rapidly, Immune suppression, Older than age 50, and ultraviolet (UV)-exposed site on a person with fair skin. The incidence rate of MCC varies across the world, with approximately 2,488 cases per year diagnosed in the United States (4). Merkel cell polyomavirus (MCPyV) and UV exposure play a major role in the pathogenesis of MCC (2). The most common primary sites of MCC are head and neck (45%), and eyelid tumors represent only 2.5% of cases (5).

Wide excision of the tumor in combination with adjuvant radiation therapy to the primary site is the first-line strategy (6). Chemotherapy and immunotherapy can be used to treat metastatic or unresectable MCC (6). In immunotherapy, immune checkpoint inhibitors targeting programmed cell death protein 1 (PD-1) or its ligand (PD-L1) are the favored agents. In addition, as tumor angiogenesis is one of the features of cancer, the inhibition of vascular endothelial growth factor (VEGF) signaling pathway has

become a revolutionary anticancer approach across various malignancies (7). However, its efficacy and safety in MCC patients are unknown. Here, we report an elderly male who developed MCC of the eyelid and was treated with apatinib, a small molecule inhibitor of vascular endothelial growth factor receptor-2 (VEGFR-2).

CASE PRESENTATION

The study was carried out according to the principles of the Declaration of Helsinki; informed consent has been obtained from the patient.

A 91-year-old Chinese man presented in the Ninth People's Hospital, Shanghai Jiao Tong University School of Medicine, on May 7, 2018, with a rapidly growing, solitary tumor of the right lower eyelid, which was initially noted in March 2018 without tenderness. Clinically, the tumor was a violet-colored nodule of 3.2 × 3.0 × 2.0 cm with pigmentation and an irregular ulcer in the center (**Figure 1A**). Fine needle aspiration biopsy was performed at Hua Shan Hospital, Fu Dan University, on April 17, 2018, which confirmed the diagnosis of MCC. The patient suffered from prostate cancer, hypertension, coronary heart disease (CHD), chronic cardiac insufficiency (NYHA, II–III) and chronic renal insufficiency and had received treatments of Enantone (3.75 mg, H,

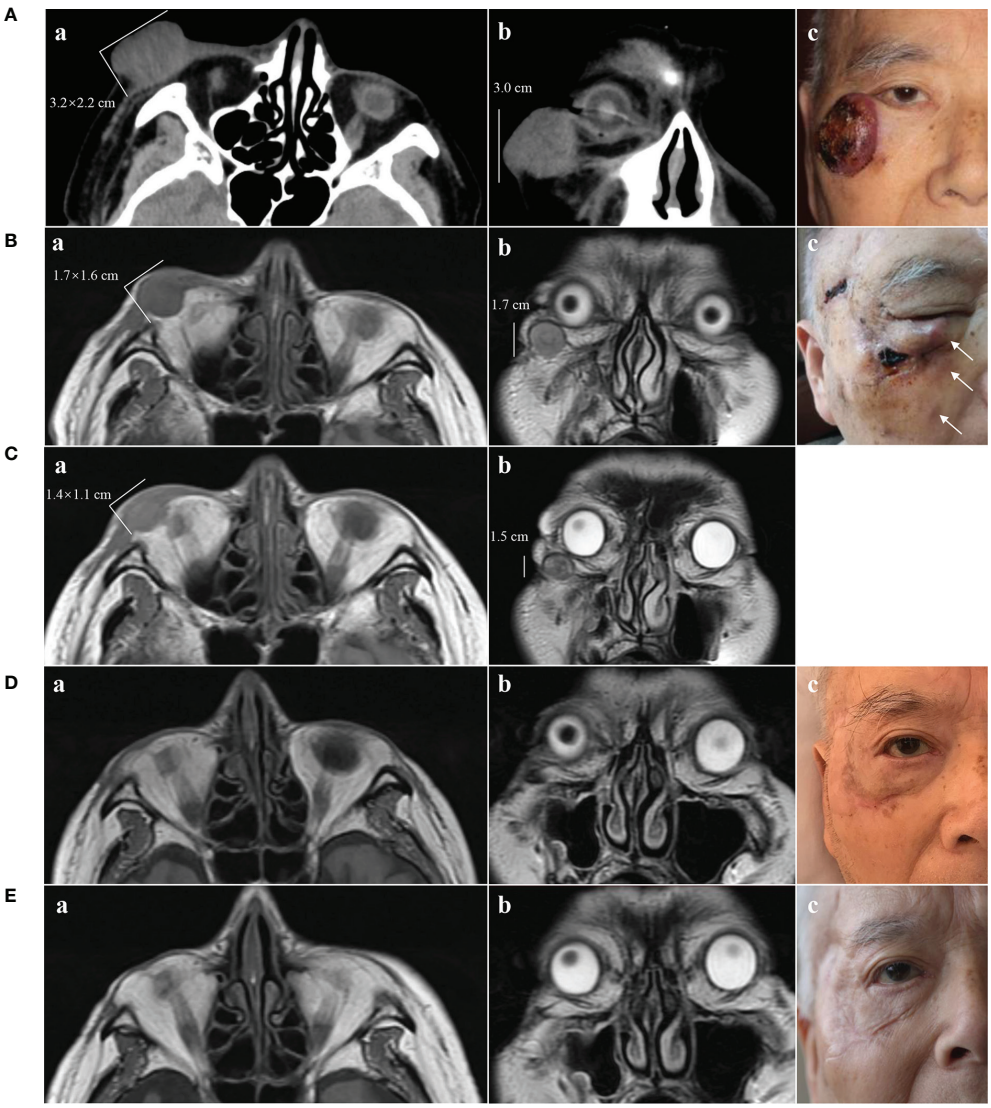

FIGURE 1 | Clinical presentation of MCC and changes on imaging. **(A)** May 2018. **A**(a) and **A**(b) CT scans showing a tumor of 3.2 × 2.2 × 3.0 cm without destruction of bone. **A**(c) Solitary violet-colored nodule of the right lower eyelid with pigmentation and an irregular ulcer in the center. **(B)** June 2018. **B**(a) and **B**(b) MRI showing a tumor of 1.7 × 1.6 × 1.7 cm. **B**(c) Recurrence of the MCC. The white arrows show three hard, subcutaneous nodules. **(C)** July 2018, 2 weeks after treatment with apatinib. **C**(a) and **C**(b) MRI showing regression of the MCC (1.4 × 1.1 × 1.5 cm). **(D)** November 2018, 4 months after treatment with apatinib. **D**(a) and **D**(b) MRI showing MCC disappearance with a favorable response to apatinib. **D**(c) Only a scar with pigmentation was observed. **(E)** November 2019, 17 months after treatment with apatinib. **E**(a) and **E**(b) MRI showing no sign of recurrence. **E**(c) The pigmentation gradually subsided, leaving a pink scar.

q4w), Adalat (30 mg, po, qd), Diovan (80 mg, po, qd), Furosemide (30 mg, po, qd) and spironolactone (20 mg, po, qd). He also had a history of pulmonary tuberculosis when he was young. Ultrasound and computed tomography (CT) were performed to clinically assess the tumor and cervical lymph nodes, and no signs of cervical lymph node metastasis were found. Clinical detection of lymph nodes or metastatic disease was performed *via* inspection and palpation, as the patient could not tolerate the long time required to complete the imaging examination. The physical examination was negative. After a multidisciplinary meeting, we decided to treat this patient with surgery, and sentinel lymph node biopsy (SLNB) was not considered due to the negative results of the imaging examination. Mohs micrographic surgery with a 1 cm excision margin was performed on May 10, 2018. The tumor had infiltrated the periosteum, and all the infiltrated soft tissue was removed together with the tumor. After confirmation of negative margins, reconstruction was performed. A rotation flap was designed to repair skin defects. Histologically small, monomorphic, round-to-oval, low-differentiated cells with a vesicular nucleus and scant cytoplasm were observed, which invaded the muscle, nerve and blood vessels (**Figure 2**). Necrosis was prominent (**Figure 2**). The immunohistochemistry results indicated the following patterns: CK (+), CK20 (+), SYN (+), CAM5.2 (+), CD34 (+), Ki67 (80%+), Vim (−), LCA (−), S100 (−), CD99 (−), DES (−), and CHGA (−) (**Figures 2**, **3**). According to the American Joint Committee on Cancer (AJCC) staging system, the final clinical diagnosis was MCC of the lower right eyelid, IIB. Postoperative radiotherapy was strongly recommended. However, the patient refused.

On June 6, 2018, several subcutaneous, hard nodules were observed at the primary site of surgery (**Figure 1B**). Chest CT, ultrasonography of the liver and kidneys, inspection and palpation of skin and lymph nodes ruled out distant metastasis. Considering the patient's physical condition, surgery was abandoned after a multidisciplinary discussion. Ultimately, apatinib was used to treat MCC in this patient from June 26, 2018 (0.25 g, po, bid). As the treatment was well tolerated by the patient, two days later, we changed the dose of apatinib (0.5 g, po, qd). Blood pressure, routine blood tests, renal function, and liver function were carefully monitored (**Figure 4**). The MCC showed a strong response to apatinib, and the efficacy was significant (**Figure 1C**). However, on July 20, 2018, exacerbated proteinuria and thrombocytopenia led us to reduce the dose of apatinib (0.25 g, po, qd). The patient was treated with leucogen (20 mg, po, tid), and his thrombocytopenia resolved. On September 14, 2018, we stopped the use of apatinib due to a high serum creatinine level (182 μmol/L). Hand–foot syndrome also occurred. However, these treatment-related adverse events (AEs) were well controlled with symptomatic treatment. On October 1, 2018, after another multidisciplinary meeting, we restarted treatment (0.125 g, po, five times a week). After two cycles, we changed the dose of apatinib (0.125 g, po, four times a week) to a low maintenance dose. On November 9, 2018, 4.4 months after the first administration of apatinib, the patient had a complete response (CR) (**Figure 1D**). We continued administering low-dose apatinib for the treatment of MCC. In the following follow-up, the patient's condition was stable (**Figure 1E**). Unfortunately, the patient died on October 4, 2020 due to heart failure and respiratory failure with no sign of recurrence or distant metastasis.

DISCUSSION

To the best of our knowledge, we report the first case of MCC of the eyelid treated with apatinib. As previously described, this case of MCC had a strong response to apatinib with few AEs. In addition, the effect was durable. Finally, the progression-free survival (PFS) of this patient was 27 months.

MCC is an aggressive skin cancer that is associated with exposure to UV radiation and MCPyV, with a median interval to recurrence of 8–9 months (8, 9). Wide excision is the first choice for the treatment of MCC. The National Comprehensive Cancer Network (NCCN) Merkel Cell Carcinoma Panel recommends adjuvant radiotherapy to the primary site for all patients with large primary tumors (≥1 cm) and risk factors such as lymphovascular invasion (LVI) or immunosuppression (6). Whether to apply radiotherapy to the draining nodal basin depends on the result of SLNB (negative or positive). Patients who do not undergo SLNB or LN dissection are also recommended to receive radiotherapy. The dosage of radiotherapy depends on the pathology of the resection margins and the result of SLNB. In this case, SLNB was not performed, and as infiltration of muscle, nerve and blood vessels was observed histologically, radiotherapy was recommended according to the NCCN MCC guidelines. However, the patient refused.

For unresectable MCC and metastatic MCC, systemic therapy is the choice, including chemotherapy and immunotherapy. The effect of chemotherapy varies from study to study. The objective response rate (ORR) for first-line chemotherapy ranged from 29.4 to 55%, and the durability of response (DOR) was 2.8–6.7 months (10–12). In patients who received one or more prior lines of chemotherapy, the ORR was 10.3–28.6%, and the DOR was 1.9–3.4 months (10–12). The PFS was 3.1–4.6 months for those patients receiving first-line chemotherapy and as low as 2–3 months in patients who received one or more prior lines of chemotherapy. In addition to the low response rates and limited durability, chemotherapy may cause toxicity, and it is not a suitable choice for elderly people with many underlying diseases, who have a higher risk of developing AEs. In this case report, the patient was 91 years old and had multiple underlying diseases, and chemotherapy was not chosen to treat the recurrent MCC. Regarding immunotherapy, PD-1 and PD-L1 are immune checkpoint molecules that control tumor growth. Immune checkpoint inhibitors (ICIs), such as avelumab (anti-PD-L1 antibody), nivolumab (anti-PD-1 antibody), and pembrolizumab (anti-PD-1 antibody), are used for the treatment of MCC. Some clinical trials of therapeutic antibodies against PD-1 or PD-L1 have showed high and durable response rates (2, 13, 14). The results of a multicenter, phase II trial of first-line use of pembrolizumab in patients with unresectable advanced MCC

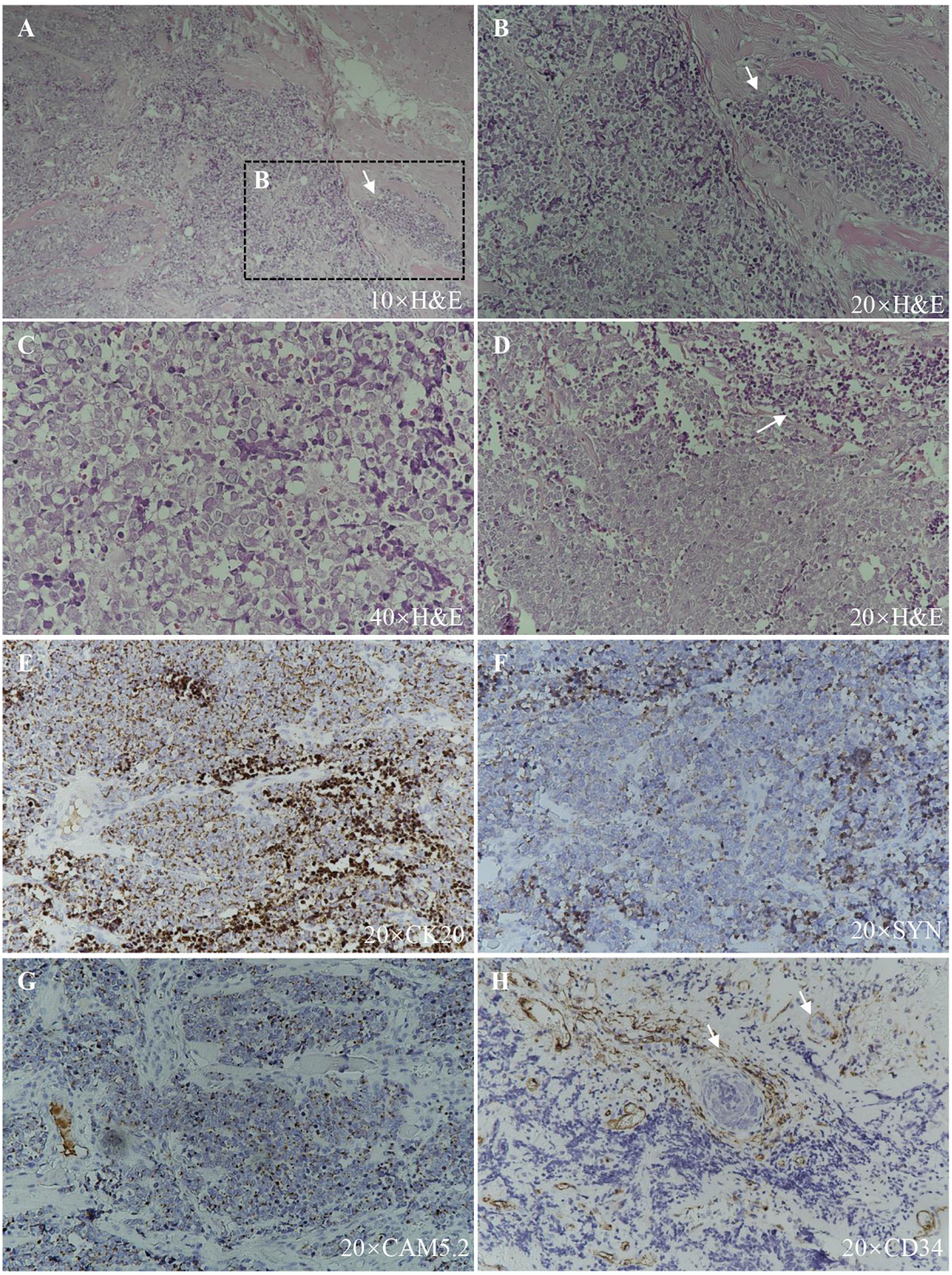

FIGURE 2 | Histopathologic features. **(A)**, **(B)**, and **(C)** Hematoxylin–eosin staining, showing small, monomorphic, round-to-oval, low-differentiated cells with a vesicular nucleus and scant cytoplasm with muscle infiltration (arrow). **(D)** Hematoxylin–eosin staining, showing necrosis (arrow). **(E)** CK20 (+). **(F)** SYN (+). **(G)** CAM5.2 (+). **(H)** CD34 (+), blood vessels and tumor cells within them are indicated with arrows.

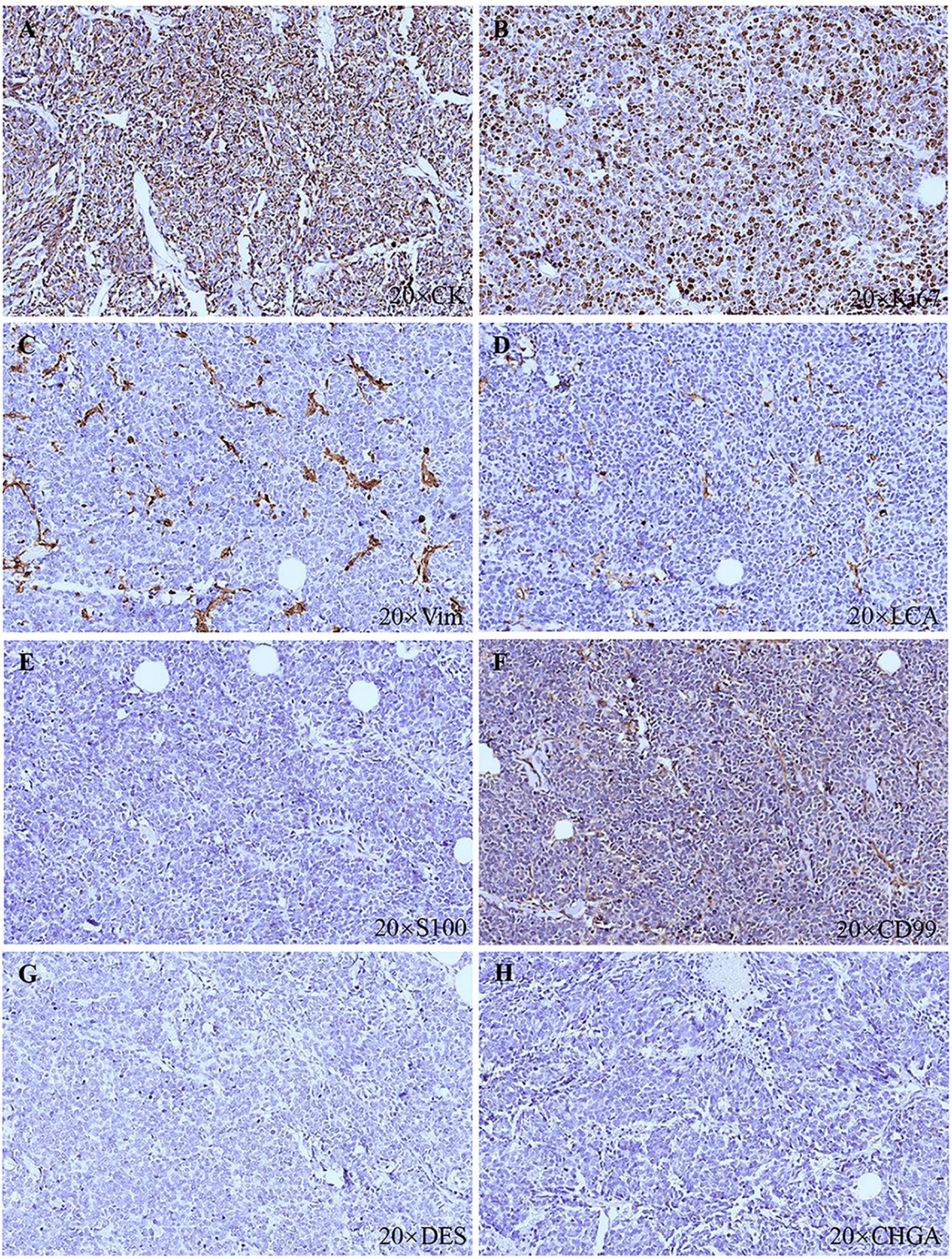

FIGURE 3 | Histopathologic features. **(A)** CK (+). **(B)** Ki67 (80%+). **(C)** Vim (–). **(D)** LCA (–). **(E)** S100 (–). **(F)** CD99 (–). **(G)** DES (–). **(H)** CHGA (–).

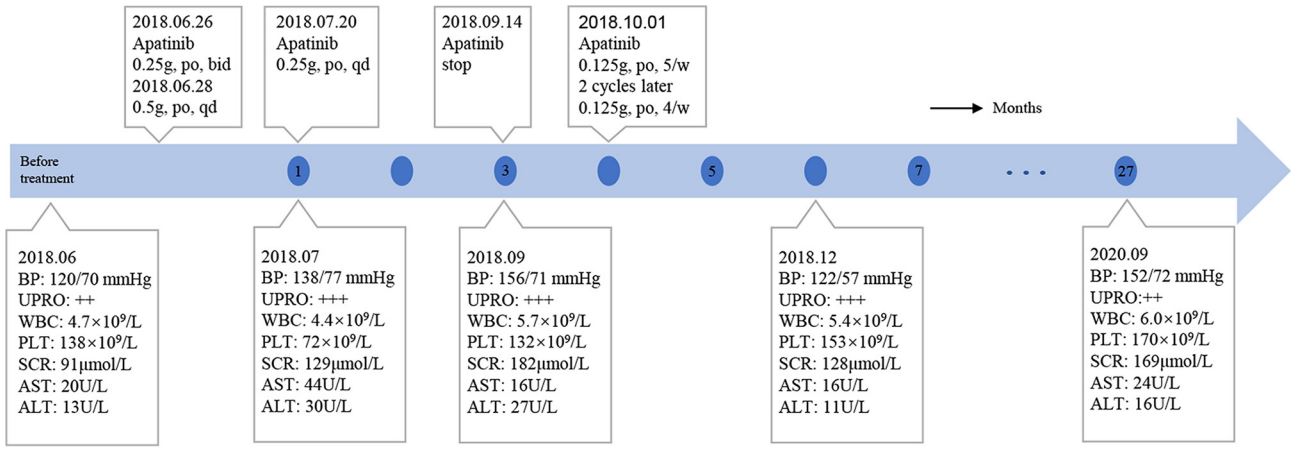

FIGURE 4 | Time Course. Blue dots indicated passing months after the use of apatinib.

demonstrated an ORR of 56% and a DOR of 2.2–9.7 months (NCT02267603) (13). An international, multicenter clinical trial of first-line use of avelumab in metastatic MCC indicated an ORR of 62.1% and a DOR of at least 3 months (93%) (14).

Angiogenesis is a necessary step in tumor growth and metastasis. Among angiogenic factors, VEGF is the most potent. There are three molecular subtypes of the VEGF receptor (VEGFR), including VEGFR-1, VEGFR-2, and VEGFR-3. These receptors are type II transmembrane proteins characterized by tyrosine kinase (TK) activity (7). Among them, VEGFR-2 is the principal subtype of VEGF-induced angiogenic signaling (7). Several studies showed that VEGF and VEGFR-2 were overexpressed in MCC (15–17), and the upregulation of VEGF was associated with aggressive tumor behavior (15). Hence, VEGF and VEGFR can be potential targets for targeted therapy and have attracted increasing attention for the treatment of MCC. One study showed the efficacy of an anti-VEGF antibody (bevacizumab) in MCC in a mouse model (18), however, it has not yet been studied in clinical trials. Tyrosine kinase inhibitors (TKIs) are another potential choice, and their efficacy in other malignancies is impressive. However, little is known about their clinical benefit in MCC. To date, only one clinical trial of TKIs (NCT02036476) has been registered (19); however, due to toxicity and a lack of response, it was closed prematurely. In addition, some case reports have demonstrated the efficiency of TKIs, such as pazopanib and cabozantinib (20, 21). In this case report, we report impressive tumor regression in a patient with recurrent unresectable MCC during treatment with apatinib. Apatinib is a new inhibitor of VEGFR-2 TK activity targeting the intracellular ATP binding site of the receptor (22). The most frequent adverse events of apatinib included hypertension, proteinuria, and hand–foot syndrome, which were also observed in our patient. However, they could be controlled clinically. Our patient had an impressive response to apatinib, and he tolerated the treatment well with controllable AEs.

In conclusion, apatinib had a favorable effect with great durability in this patient, highlighting its potential utility in other MCC patients, especially those who cannot tolerate chemotherapy and those who do not respond to immunotherapy. Further clinical trials are needed to determine the efficacy and safety of apatinib in MCC patients.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary material. Further inquiries can be directed to the corresponding authors.

ETHICS STATEMENT

Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

AUTHOR CONTRIBUTIONS

XS and RJ provided direction and guidance throughout the preparation of this manuscript. XS and YF extracted all data. YF drafted the paper. All authors contributed to the article and approved the submitted version.

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ORIGINAL RESEARCH
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Melanoma of Unknown Primary: Evaluation of the Characteristics, Treatment Strategies, Prognostic Factors in a Monocentric Retrospective Study

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Background: Melanoma of unknown primary (MUP), accounts for up to 3% of all melanomas and consists of a histologically confirmed melanoma metastasis to either lymph nodes, (sub)cutaneous tissue, or visceral sites without any evidence of a primary cutaneous, ocular, or mucosal melanoma. This study aimed to investigate the characteristics, treatment strategies, and prognostic factors of MUP patients, in order to shed some light on the clinical behavior of this malignancy.

Methods: All the consecutive patients with a diagnosis of MUP referring to our institutions between 1985 and 2018 were considered in this retrospective cohort study. The records of 173 patients with a suspected diagnosis of MUP were retrospectively evaluated for inclusion in the study. Patient selection was performed according to the Das Gupta criteria, and a total of 127 MUP patients were finally included in the study, representing 2.7% of the patients diagnosed with melanoma skin cancer at our institutions during the same study period. A second cohort of all consecutive 417 MKP patients with AJCC stages IIIB–IV, referring tions in the period considered (1985–2018), was included in the study to compare survival between MUP and MKP patients. All the diagnoses were based on histopathologic, cytologic and immunohistochemical examination of the metastases.

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All tumors were re-staged according to the 2018 American Joint Committee on Cancer (AJCC) 8th Edition.

Results: Median follow-up was 32 months (IQR: 15–84). 3-year progression-free survival (PFS) was 54%, while 3-year overall survival (OS) was 62%. Worse OS and PFS were associated with older age (P = 0.0001 for OS; P = 0.008 for PFS), stage IV (P < 0.0001 for OS; P = 0.0001 for PFS) and higher Charlson Comorbidity Index (P < 0.0001 for OS and P = 0.01 for PFS). Patients with lymph node disease showed longer PFS (P = 0.001) and OS (P = 0.0008) than those with (sub)cutis disease. Complete lymph node dissection (CLND) was the most common surgical treatment; a worse OS in these patients was associated with the number of positive lymph nodes (P = 0.01), without significant association with the number of retrieved lymph nodes (P = 0.79). Survival rates were lower in patients undergoing chemotherapy (CT) and target therapy (TT), and higher in those receiving immunotherapy (IT). 417 patients with AJCC stages IIIB–IV of Melanoma Known Primary (MKP) were included for the survival comparison with MUP. 3-year PFS rates were 54 and 58% in MUP and MKP, respectively (P = 0.30); 3-year OS rates were 62 and 70% in MUP and MKP, respectively (P = 0.40).

Conclusions: The most common clinical scenario of our series was a male patient around 59 years with lymph node disease. We report that CLND associated with IT was the best treatment in terms of survival outcome. In the current era of IT and TT for melanoma, new studies have to clarify the impact of novel drugs on MUP.

Keywords: melanoma of unknown primary, occult primary melanoma, skin cancer, melanoma, MUP, melanoma treatment, immunotherapy, target therapy

INTRODUCTION

Melanoma of unknown primary (MUP) also known as occult primary melanoma accounts for up to 3% of all melanomas (1) and consists of a histologically confirmed melanoma metastasis to either lymph nodes, (sub)cutaneous tissue, or visceral sites. The diagnosis of MUP is definitive when a primary cutaneous, ocular, or mucosal melanoma is missing after a thorough physical examination and histological revision of previously excised melanocytic lesions. In 1963, Das Gupta and collaborators defined the diagnostic criteria for MUP (2). Such criteria exclude patients who do not receive complete physical examination (including anus/genitalia and ophthalmological visit); those with evidence of previous orbital enucleation, those without histological documentation of prior surgical or non-surgical procedures (*e.g.*, for a mole, birthmark, freckle, chronic paronychia, or skin blemish), and those with nodal involvement and presence of a scar in the skin area drained by the lymphatic basin (2). Of note, according to Kamposioras, only 16% of publications on MUP applied the stringent Das Gupta's exclusion criteria, thus the remaining might have included as MUP some melanoma of known primary (MKP) (3). The peak incidence of MUP occurs between the fourth and fifth decade of age, which is comparable to that of MKP of the skin but earlier than those arising from the mucosa. MUP is also more common in men than women. The management of patients with MUP has been the same to the management of patients with metastatic melanoma and with MKP. Although the survival of patients with stage III–IV MUP as compared to patients with stage III–IV MKP has been richly explained (4–6) including the hypotheses attributable to immune-mediated control of the primary tumor in patients with MUP, a distinct signature of MUP that differentiate the treatment strategies for MUP and MKP has not been defined. To do this, more retrospective cohort studies such as ours are needed to compare outcomes between patients with MUP and stage-matched MKP during novel therapy.

This study aimed to investigate the characteristics, treatment strategies and prognostic factors of MUP patients, in order to shed some light on the clinical behavior of this rare type of melanoma. In addition, survival in MUP patients was compared with survival in MKP patients with the same stage and metastatic sites. The clinical impact of our study is to build a retrospective cohort study for the clinical features and behavior of MUP in the evolving era of immunotherapy, targeted therapies, and their combinations.

MATERIALS AND METHODS

Study Design

All the consecutive patients with a diagnosis of MUP referring to the Melanoma and Sarcoma Clinic of the Veneto Institute of Oncology (IOV) and the Department of Surgery Oncology and Gastroenterology (DISCOG) of the University of Padua (Italy)

between 1985 and 2018 were considered in this retrospective cohort study. IOV and DISCOG are level III referral institutions in Northeastern Italy. Most patients are referred for diagnosis and/or first-line treatment, while some patients are referred for disease progression after being treated in level I–II centers. The study was conducted according to the Helsinki Declaration principles and was approved by the local Ethics Committee (17/04/2020, approval No. 7254). All patients gave their consent for data collection and analysis for scientific purposes.

Patients

The records of 173 patients with a suspected diagnosis of MUP referring to IOV or DISCOG between 1985 and 2018 were retrospectively evaluated for inclusion in the study.

Patient selection was performed according to the Das Gupta criteria (2) (**Table 1**). Forty-six patients were excluded because of unclear information on primary melanoma (14 patients), misdiagnosis of MUP (medical history of previous cutaneous melanoma, 11 patients) or "evidence of previous skin excision or other surgical manipulation of a mole, freckle, birthmark, paronychia or skin blemish", or "evidence of metastatic melanoma in a draining lymph node with a scar in the area of skin supplying the lymph node basin" (21 patients) (1). A total of 127 MUP patients were finally included in the study, representing 2.7% of the patients diagnosed with melanoma skin cancer (127 out of 4,703 patients) at our institutions during the same study period.

A second cohort of all consecutive 417 MKP patients with AJCC stages IIIB–IV, referring to our institutions in the period considered (1985–2018), was included in the study to compare survival between MUP and MKP patients.

Diagnosis and Treatment

All the diagnoses were based on histopathologic, cytologic, and immunohistochemical examination of the metastases. All tumors were re-staged according to the 2018 American Joint Committee on Cancer (AJCC) 8th Edition—TNM staging system (7) was used for tumor staging.

Patients with melanoma metastases in the (sub)cutis, soft tissue, and/or lymph nodes, without a detectable primary tumor were diagnosed s stage III disease, while those with distant metastases including visceral metastases are diagnosed as stage IV.

The surgical treatment included wide resection (WR) in patients with (sub)cutis/soft tissue lesion, complete lymph node dissection (CLND) in those with lymph node metastasis and metastasectomy in those with complete, resectable distant/visceral location.

Radiation therapy (RT) was performed according to location, stage, surgical radicality, and residual disease load. Medical oncology treatments included target therapy (TT), immunotherapy (IT), and classic chemotherapy (CT). In some patients, electrochemotherapy (ECT) and hyperthermic limb perfusion (ILP) were also employed.

IT with high-dose interferon (IFN HD) was used as adjuvant treatment after radical surgery in stage III patients. Since 2012, stage IV patients were treated with targeted therapy (TT) if the melanoma carried the V600E BRAF mutation: in particular, the combination of BRAF and MEK inhibitors (Dabrafenib and Trametinib or Vemurafenib and Cobimetinib, respectively); in case of *BRAF* wild type disease, immune checkpoint blockade with anti-PD1 monoclonal antibodies (Pembrolizumab or Nivolumab) alone or in combination with anti-CTLA4 monoclonal antibodies (Ipilimumab) (8, 9).

Systemic CT (*i.e.* dacarbazine and bio-chemotherapy regimens) was administered before 2012.

Follow-up was performed every three months for the first two years, then every six months up to the 5th year, and once a year thereafter. Disease progression was defined as local disease recurrence, lymph node metastasis and/or distant metastasis.

Data Collection

All data were extracted from a prospectively maintained database. Demographics included age at diagnosis, gender and family history of cancer, while melanoma-related information included clinical presentation, metastasis size, and AJCC TNM stage (7). Tumor stage according to Balch's proposal (which includes stage IV non-visceral tumors in stage III) was also assessed (10). Comorbidity status was summarized using the age-adjusted Charlson Comorbidity Index (11). Neoplastic comorbidity and autoimmune comorbidity were evaluated separately. Information on treatment strategy included surgical therapy (WR, CLND, metastasectomy) and medical therapy (radiotherapy, target therapy, immunotherapy and chemotherapy). Follow-up information was extracted from the reports of scheduled visits. Overall survival was calculated from diagnosis to death (by any cause) or to the last visit, while recurrence/progression-free survival was calculated from diagnosis to recurrence/progression or to the last visit.

Statistical Analysis

Categorical data were summarized as frequency and percentage, while continuous data as median and interquartile range (IQR).

Survival curves were calculated using Kaplan–Meier method. Survival estimates were compared between MUP and MKP patients using the log-rank test.

The association between clinically relevant variables and survival was assessed using Cox regression models. Effects sizes were reported as hazard ratio (HR) with 95 per cent confidence interval (95% CI). Of note, the association between surgical treatments and survival was not evaluated because surgical treatments mirrored the clinical presentation of MUP.

Multivariable analysis of survival was performed with Cox regression models including a set of clinically relevant factors at

TABLE 1 | Das Gupta's exclusion criteria.

Das Gupta's exclusion criteria
Evidence of previous orbital exenteration or enucleation
Evidence of previous skin excision,electrodessication, cauterization or other surgical manipulation of a mole, freckle,birthmark, paronychia, or skin blemish.
Evidence of metastatic melanoma in a draining lymph node with a scar in the area of skin supplying that lymph node basin.
Lack of a nonthorough physical examination, including the absence of an ophthalmologic, anal, and genital exam.

diagnosis (*i.e.* age, Charlson Comorbidity Index, and tumor presentation). Metastasis size was not included in the analysis because this information was available only for lymph node metastases (but not skin metastases). In addition, some potential factors could not be included in the multivariable models due to collinearity with presentation (AJCC stage), rarity of the events (neoplastic and autoimmune comorbidity) or incomplete information (BRAF mutational status).

The association between medical treatments and tumor stage was evaluated using Fisher's exact test.

All tests were two-sided and a p-value less than 0.05 was considered statistically significant. Statistical analysis was performed using R 4.0 (R Foundation for Statistical Computing, Vienna, Austria) (12).

RESULTS

Patients

Of the 173 patients with MUP considered in this study, 46 were excluded, according to the Gupta's criteria. One hundred and twenty-seven patients (78 males and 49 females; median age 59 years) with a diagnosis of MUP between 1985 and 2018 were included in the analysis. Patient and tumor characteristics are shown in **Table 2**. There were 68 AJCC stage III tumors (Balch stage III) and 59 AJCC stage IV tumors, of whom 25 were non-visceral tumors (Balch stage III) and 34 were visceral tumors (Balch stage IV). *BRAF* was mutated in 38 out of 68 evaluable patients (56%).

Treatment

Treatment strategies are shown in **Figure 1**. Ninety-four patients (74%) underwent surgical treatment: 65 CLND, 14 WR, seven metastasectomy, and eight CLND+WR, while 30 patients underwent only medical treatment and three refused the treatment. CLND was performed in axilla (27 patients), groin (eight patients) or neck (12 patients), with a median of 23 retrieved nodes (IQR 18–32) and a median of two positive nodes (IQR 1–5). Such information was not available for six patients.

Medical treatment was administered to 103 patients (81%), with 38 patients receiving more than one treatment, and 65 patients receiving only one treatment. Overall, 34 patients received chemotherapy, which was more frequent among stage IV patients (37 *vs.* 18% in stage III patients, p = 0.02). Seventy-four patients received immunotherapy, which was more frequent among stage III patients (72 *vs.* 42% in IV patients, p = 0.001). Target therapy was administered to 23 patients, with no statistically significant difference between stage III *vs.* IV patients (13 *vs.* 23%, p = 0.19). Twenty-five patients (20%) received radiotherapy, with no statistically significant difference between stage III *vs.* IV patients (23 *vs.* 15%, p = 0.34). Nine patients received chemo-radiotherapy.

Survival

Median follow-up was 32 months (IQR 15–84). At the analysis, seven patients had local recurrence, 39 had recurrence with clinical upstaging, and 19 had disease progression.

3-year recurrence/progression-free survival was 54%, while 3-year overall survival was 62% (**Figure 2**).

Univariate analyses of recurrence/progression-free survival and overall survival are reported in **Table 3**. Impaired recurrence/progression-free survival was associated with older age (HR 1.03, 95% CI 1.01 to 1.04; p = 0.008), stage IV (HR 2.77, 95% CI 1.66 to 4.63; p = 0.0001) and higher Charlson Comorbidity Index (HR 1.16, 95% CI 1.03 to 1.30; p = 0.01). Patients with lymph node metastasis showed longer recurrence/progression-free survival than those with (sub)cutis metastases (HR 0.37, 9%% CI 0.20 to 0.68; p = 0.002). Among patients who underwent RLND, overall survival was associated with the number of positive lymph nodes (HR 1.06, 95% CI 1.01 to 1.11; p = 0.01) but not with the number of retrieved nodes (HR 1.00, 95% CI 0.96 to 1.03; p = 0.79). Impaired overall survival was associated with older age (HR 1.04, 95% CI 1.02 to 1.06; p = 0.0001), stage IV (HR 3.43, 95% CI 2.00 to

TABLE 2 | Patient and tumor characteristics.

Variable			AJCC stage III	AJCC stage IV	
			Patient with lymph node metastases	Patient with (Sub)cutis metastases	Patient with visceral metastases
	N patients:	127	68	25	34
Demographics	Age at diagnosis, year[a]	59 (48–70)	57 (47–67)	60 (48–69)	62 (49–73)
	Sex:				
	Female	49 (39)	24 (35)	12 (48)	13 (38)
	Male	78 (61)	44 (65)	13 (52)	21 (62)
	Family history of cancer[b]	11 (12)	4 (8)	3 (19)	4 (15)
Tumor characteristics	Size of lymph node metastasis, cm[a],[c]	4.0 (2.5–5.0)	4.0 (2.5–5.0)	–	4.0 (3.4–6.0)
	AJCC stage:				
	III	68 (54)	68 (100)	0	0
	IV	59 (46)	0	25 (100)	34 (100)
Comorbidity status	Charlson Comorbidity Index[a]	2 (1–3)	2 (0–3)	2 (1–3)	2 (1–4)
	Neoplastic comorbidity	19 (15)	10 (15)	2 (8)	7 (21)
	Autoimmune comorbidity	22 (17)	9 (13)	3 (12)	10 (29)

Data expressed as n (%) or [a]median (IQR). Data not available in [b]one and [c]29 patients.

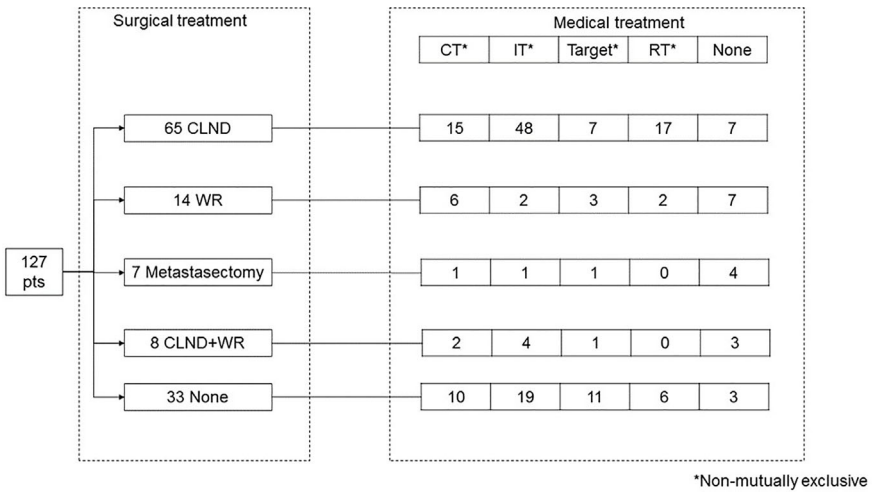

FIGURE 1 | Surgical and medical treatment.

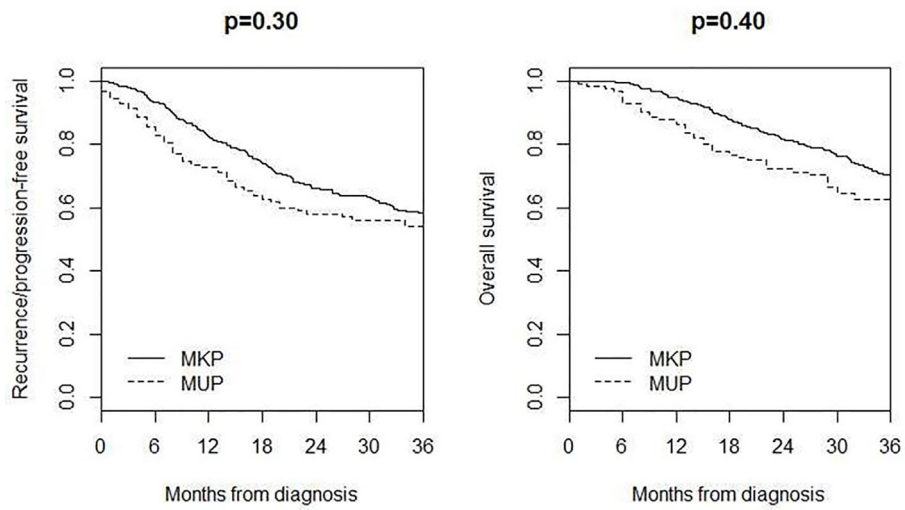

FIGURE 2 | Overall survival and recurrence/progression-free survival in MUP and MKP patients.

5.89; p < 0.0001) and higher Charlson Comorbidity Index (HR 1.25, 95% CI 1.12 to 1.40; p < 0.0001). Patients with lymph node metastasis showed longer overall survival than those with (sub) cutis metastases (HR 0.34, 9%% CI 0.18 to 0.65; p = 0.001). Among patients who underwent CLND, overall survival was associated with the number of positive lymph nodes (HR 1.06, 95% CI 1.01 to 1.11; p = 0.01) but not with the number of retrieved nodes (HR 1.00, 95% CI 0.96 to 1.03; p = 0.79).

Of note, survival was impaired in patients undergoing CT and target therapy and improved in those receiving immune therapy (**Table 3**).

Multivariable analysis identified only stage as independent predictor of survival among clinically relevant factors at diagnosis (**Table 4**). Patients with lymph node metastases had longer recurrence/progression-free survival (HR 0.36, 95% CI 0.19 to 0.67; p = 0.001) and overall survival (HR 0.33, 9%% CI 0.17 to 0.63; p = 0.0008) than those with (sub)cutis metastases.

Comparison of Survival in MUP and MKP Patients

Four hundred and seventeen MKP patients (213 males and 204 females; median age 59 years, IQR 45–70) with AJCC stage IIIB–IV were included in the comparison of survival, 3-year recurrence/progression-free survival was 54% in MUP and 58% in MKP (p = 0.30), and 3-year overall survival was 62% in MUP and 70% in MKP (p = 0.40) (**Figure 3**).

TABLE 3 | Univariate analysis of survival.

Variable	Recurrence/progression-free survival		Overall survival	
	HR (95% CI)	p-value	HR (95% CI)	p-value
Age at diagnosis, years:	1.03 (1.01 to 1.04)	0.008	1.04 (1.02 to 1.06)	0.0001
Sex:				
Female	Reference	–	Reference	–
Male	1.05 (0.63 to 1.75)	0.86	1.37 (0.79 to 2.36)	0.26
Family history of cancer:				
No	Reference	–	Reference	–
Yes	2.10 (0.97 to 4.51)	0.06	1.52 (0.64 to 3.62)	0.34
Size of lymph node metastasis, cm[a]	1.09 (0.97 to 1.22)	0.15	1.09 (0.94 to 1.26)	0.27
AJCC stage:				
III	Reference	–	Reference	–
IV	2.77 (1.66 to 4.63)	0.0001	3.43 (2.00 to 5.89)	<0.0001
Charlson Comorbidity Index	1.16 (1.03 to 1.30)	0.01	1.25 (1.12 to 1.40)	<0.0001
Presentation:				
(Sub)cutis metastases	Reference	–	Reference	–
Lymph node metastases	0.37 (0.20 to 0.68)	0.002	0.34 (0.18 to 0.65)	0.001
Visceral metastases	1.03 (0.54 to 1.96)	0.94	1.36 (0.71 to 2.62)	0.36
Neoplastic comorbidity:				
No	Reference	–	Reference	–
Yes	1.40 (0.71 to 2.74)	0.34	1.73 (0.90 to 3.35)	0.10
Autoimmune comorbidity				
No	Reference	–	Reference	–
Yes	1.23 (0.64 to 2.37)	0.53	1.13 (0.57 to 2.23)	0.73
BRAF:				
Wild Type	Reference	–	Reference	–
Mutation	1.22 (0.65 to 2.29)	0.54	0.71 (0.35 to 1.43)	0.34
CT:				
No	Reference	–	Reference	–
Yes	2.76 (1.66 to 4.57)	<0.0001	2.23 (1.33 to 3.75)	0.002
Immune therapy:				
No	Reference	–	Reference	–
Yes	0.58 (0.35 to 0.95)	0.03	0.53 (0.32 to 0.89)	0.02
Target therapy:				
No	Reference	–	Reference	–
Yes	3.37 (1.94 to 5.87)	<0.0001	1.85 (1.01 to 3.40)	0.04
RT:				
No	Reference	–	Reference	–
Yes	1.35 (0.6 to 2.42)	0.31	1.13 (0.61 to 2.09)	0.71

[a]*Among patients with lymph node metastases or visceral metastases.*

TABLE 4 | Multivariable analysis of overall survival.

Variable	Recurrence/progression-free survival		Overall survival	
	HR (95% CI)	p-value	HR (95% CI)	p-value
Age at diagnosis, years:	1.01 (0.98 to 1.04)	0.42	1.03 (0.99 to 1.06)	0.11
Charlson Comorbidity Index	1.09 (0.89 to 1.32)	0.41	1.10 (0.91 to 1.33)	0.31
Presentation:				
(Sub)cutis metastases:	Reference	–	Reference	–
Lymph node metastases:	0.36 (0.19 to 0.67)	0.001	0.33 (0.17 to 0.63)	0.0008
Visceral metastases:	0.94 (0.49 to 1.80)	0.85	1.12 (0.89 to 2.17)	0.73

DISCUSSION

This study describes patient characteristics, therapeutic approaches, and prognosis of a series of 127 consecutive cases of melanoma of unknown primary (MUP).

The most common clinical scenario in this cohort was a male patient with a median age of 59 years, presenting with a melanoma localized at lymph nodes with neither a detectable primary tumor nor a history of previous melanoma removal, and satisfying all the Das Gupta's exclusion criteria for the definition of MUP.

The median size of lymph node involvement was 4 cm, irrespective of AJCC III or IV stage (*i.e.* with no difference in size between patients with nodal metastases alone, and those with concurrent nodal and visceral metastases). CLND was the most common surgical treatment, and the survival was

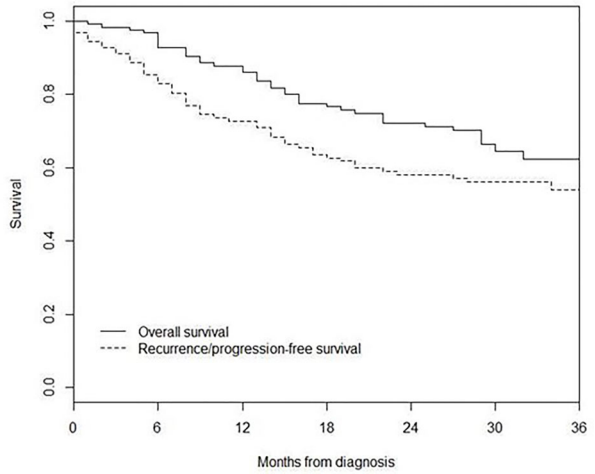

FIGURE 3 | Overall survival and recurrence/progression-free survival.

associated with the number of positive lymph nodes, without significant association with the number of retrieved lymph nodes, in agreement with other studies (13–16). As expected, our results show a worse survival for advanced stage of disease. Considering the staging, our data support AJCC staging system and suggest that the Balch proposal to consider subcutaneous disease as stage III could be not appropriate. In fact, in our series, patients with subcutaneous disease (AJCC stage IV, Balch stage III) had a worse survival than those with lymph nodes metastases (AJCC stage III, Balch stage III), supporting the inclusion of patients with subcutaneous metastases alone in AJCC stage IV.

In addition, the Charlson comorbidity status resulted to be associated with a worse survival in our series.

Considering stage and treatment of MUP, two milestones have been reported. In 2006, the routine use of combined PET/CT at diagnosis in MUP patients increased the shift from stage III to stage IV, and starting from 2011 the introduction of immune and targeted therapy changed the clinical outcome and long-term survival in advanced melanoma. However, in our center, as well in Italy, both therapies were available only in CRTs till 2014; therefore their impact in this series is limited at the last five years. In this historical context, a possible limitation of our study is the long period considered and the imaging and therapeutic changes introduced. Nevertheless, even pooling and considering as "immune-therapy" (IT) the classical interferon option and the novel immune-modulating opportunities (*i.e.* CTLA4 inhibitors and PDL1 inhibitors), IT was the medical treatment associated with the best survival outcome. The lower survival obtained in patients treated with traditional chemotherapy (CT) was in line with the significant superiority of IT compared to CT in all clinical studies. The lower effect of targeted therapy (TT) was due to selection or to more aggressive features in BRAF mutated patients, or could be related to the immune mechanism involved in the initial elimination of melanoma. Indeed a MUP could be considered a recurrence of an immune eliminated melanoma, and IT could restore an effective immune response, and a greater effect of IT in patients with a "fable immunity" was often observed and reported in the literature in old patients and in immune deficient patients. The comparison of IT to TT in this type of melanoma should be tested in large cohorts and prospectively.

Additionally, the origin of MUP is still an open question, and future studies elucidate whether MUP has to be considered and treated as a melanoma with a known primary (MKP) or represents a different entity. As for survival, we could not demonstrate a difference among MUP and MKP as already reported by other groups. However many authors showed a significant improved survival of MUP compared with MKP (3–5, 17–22).

This was originally explained by Smith and Stehlin in 1965 with a phenomenon of immunological *spontaneous regression* of the primitive tumor (T of TNM). Of note, in contrast to this interpretation, a partial regression of the primary tumor at dermatoscopy has traditionally been recognized as a negative prognostic sign. Therefore linking *regression* to better survival seems at least in part a contradiction, as for melanoma. However the explanation by Smith and Stehlin has been re-proposed by many authors afterwards and is cited also by Anbari and coworkers in 1997 alongside with other criteria of exclusion of MUP (*i.e. a concurrent, unrecognized melanoma or a previously excised, misdiagnosed melanoma*). Indeed, the original contribution of the latter report at the end of last century was the proposal of a new explanation for the origin of MUP: it could represent a primary tumor (T of TNM) within a node rather than a metastatic process to the regional basin (N of TNM). This could explain the better prognosis of MUP patients when compared to MKP, but this does not explain subcutaneous metastases without nodes or visceral metastasis only.

Whatever the origin, it should be considered that the absence of cutaneous/mucosal malignancy in MUP patients could explain by itself their better prognosis for the lesser tumor load (*i.e.* lower amount of cancer stem cells able to metastasize and/or give rise to recurrent disease).

Recently, new reports tried to assess the existence of any correlation between mutations in the main genes (BRAF/NRAS) involved in melanoma initiation and progression (23); they have proposed a distinct molecular classification for MUP to explain the differences in patient outcomes. MUP patients presents consistently BRAF and TERT promoter mutations, suggesting a cutaneous origin. BRAF mutations rate in MUPs appears similar to MKPs; however, for MUPs the rate for V600K seems higher than the rate for MKPs (24). Melanomas with the V600K mutation are characterized by a lower dependence on the activation of the ERK pathway and greater use of alternative pathways; against these melanomas they have a higher mutational load and respond better to immunotherapy; this would concretely explain the better response to immunotherapy and the worse response to BRAFi/MEKi of the MUPs (25–27). The strengths of our study include the diagnosis of MUP based on Das Gupta's criteria (2), the sample size (one of the largest in MUP literature), the evaluation of Balch's staging proposal, and the evaluation of systemic treatments.

The present study has also some limitations. First, it is a single-center study, thus the generalizability of the findings is limited. Second, the retrospective nature of the study limited the

availability of data (*e.g.* mutational status). Third, the included patients were treated with heterogeneous modalities because of the long period of inclusion. Fourth, the new medical options now available both in in the adjuvant as in the metastatic setting for all patients could make the distinction between MUP and MKP clinically needless.

DATA AVAILABILITY STATEMENT

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found below: Del Fiore, Paolo (2020), "Melanoma of Unknown Primary (MUP) Monocentric Retrospectivr Study", Mendeley Data, V1, doi: 10.17632/xj636ftgff.1.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Ethics Committee of Veneto Institute of Oncology CESC-IOV. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

Study concepts: PF, MR, SM. MA, VC, JP. Study design: PF, FC, SM, MA, CR. Data acquisition: PF, RS, FC, GA, AP, BF, AS. Quality control of data and algorithms: PF, FC. Data analysis and interpretation: PF, FC, SM, DL, GA. Statistical analysis: FC. Manuscript preparation: PF, FC, SM, AB, RC, GA. Manuscript editing: PF, FC, SM, DL, MR. Manuscript review: SM, AB, AF, FB, MA, RM, CR. All authors contributed to the article and approved the submitted version.

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REVIEW
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Correlation Between *In Vivo* Reflectance Confocal Microscopy and Horizontal Histopathology in Skin Cancer: A Review

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In dermatopathological daily practice, vertical histopathology sections are classically used to analyze skin biopsies. Conversely, horizontal histopathological sections are currently used for the diagnosis of some types of alopecia. In the last years the morphological findings obtained by horizontal histopathology have been correlated to those obtained by *in vivo* reflectance confocal microscopy which provides the same "point of view" of the skin. This review paper emphasizes the strong matching and correlation between reflectance confocal microscopy images and horizontal histopathology in cutaneous neoplasms, further demonstrating the strong reliability of this innovative, non-invasive technique in the management of skin tumors.

Keywords: horizontal histopathology, reflectance confocal microscopy, skin cancer, correlation, horizontal histopathological sections

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INTRODUCTION

One of the major application fields of dermatological research has always been the identification of new diagnostic tools capable of improving the diagnostic precocity and accuracy of skin neoplasms (1, 2). In the last decade, *in vivo* reflectance confocal microscopy (RCM) is gradually establishing itself as a non-invasive diagnostic technique for several skin diseases, being able to provide a horizontal high-resolution "point of view" of the skin, from the stratum corneum to the papillary dermis; horizontal skin images up to a 250 µm of maximum depth may be studied through this technique (3–6). The use of RCM in the diagnostic approach to many inflammatory and neoplastic skin diseases is still increasing, representing one of the major diagnostic aids in the dermatological clinical practice (7). However, the horizontal "point of view" provided by RCM does not allow an optimal correlation with classical histopathology that, as known, produces a full-thickness vertical overview of the skin (8, 9). Instead, horizontal histological sections (HHSs) allow a better correlation as they reflect the same skin plane observed by RCM (10).

The possibility of optimally comparing horizontal histopathology and RCM images represents a relatively new trend, and quite a few papers have been published in this field regarding both inflammatory and neoplastic disorders (11–17). The purpose of this review paper is to establish the

"state of the art" on RCM and HHS findings in skin tumors, emphasizing how well horizontal histopathology reflects the images provided by RCM.

SQUAMOUS CELL CARCINOMA *IN SITU* (BOWEN'S DISEASE)

Squamous cell carcinoma *in situ* (SCCis) represents the earliest and non-invasive form of squamous cell carcinoma, in which, by definition, the neoplastic cells do not infiltrate the basement membrane and therefore lack distant metastatic potential (14). SCCis mainly affects photoexposed skin of elderly, and the head and neck are the most commonly affected sites (14). Clinically, SCCis arises in the form of flat/raised, reddish/brownish in color, often scaly, papules or plaques; due to the low specificity of the clinical presentation, further non-invasive diagnostic tools, such as dermoscopy and RCM, are often required to enhance the diagnostic accuracy of SCCis (14, 18). The detection of "red dots", representing glomerular vessels in the superficial dermis, is the most typical dermoscopic finding of SCCis (18). In addition, RCM has been also validated as useful diagnostic tool and its application in the dermatological practice has been supported by the perfect matching with HHS found by our research group (14). SCCis shows the following RCM features (14) (**Figures 1A, C**): i) at the level of stratum corneum, highly refractive amorphous structures and sporadically polygonal, nucleated cells; ii) at the level of the stratum granulosum/spinosum, marked architectural disarray, consisting of keratinocytes highly variable in size, shape, and nuclear morphology; scattered bright dendritic cells may also be found; iii) at the level of the dermoepidermal junction, large rounded dark areas, corresponding to enlarged dermal papillae. Horizontal histopathology perfectly matches with the previous reported RCM findings (14) (**Figures 1B, D**): hyperkeratosis and parakeratosis are the histopathological causes of the refractive amorphous structures and the nucleated cells observed in the stratum corneum at RCM; the loss of architectural array visible in the stratum granulosum/spinosum at RCM reflects the presence of atypical keratinocytes with nuclei of variable size and shape along the entire thickness of epidermis; some S-100 positive, CD1a negative and Melan-A negative dendritic cells may be occasionally found scattered among the neoplastic cells; lastly, at the dermoepidermal junction, HHSs show enlarged dermal papillae containing glomeruloid capillary vessels, corresponding both to the rounded dark areas and to the "red

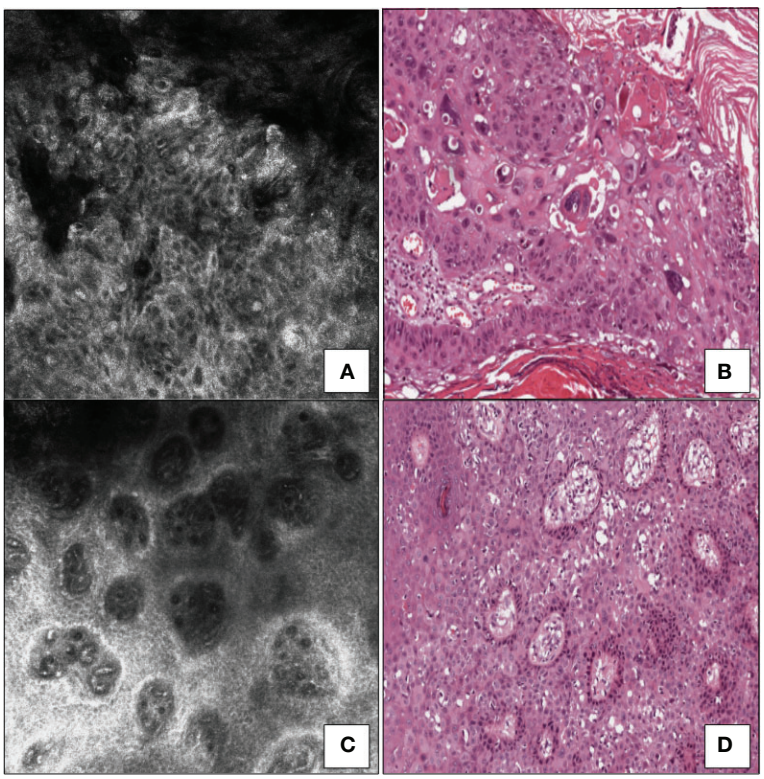

FIGURE 1 | Squamous cell carcinoma *in situ*. **(A)** RCM image at the stratum spinosum showing a marked loss of the normal honeycomb pattern (architectural disarray) due to the presence of markedly variable size, shape, and nuclear morphology keratinocytes. **(B)** Horizontal histopathology at the same level revealing neoplastic keratinocytes with high-grade nuclear atypia (hematoxylin and eosin; original magnification 400×). **(C)** RCM image at the dermoepidermal junction showing dilated blood vessels within enlarged edged dermal papillae. **(D)** Horizontal histopathology at the same level confirming the RCM finding (hematoxylin and eosin; original magnification 100×).

dots" observed at RCM and dermoscopy, respectively. Since the horizontal histopathology does not allow to evaluate the possible presence of dermal invasion, the concept that its use is only for the purpose of comparing it with the RCM findings, in order to further validate the diagnostic use of RCM, must be emphasized.

MYCOSIS FUNGOIDES WITH PATCH LESIONS

Mycosis fungoides (MF) is the most frequent T-cell lymphoma of the skin and seems pathogenetically related to a monoclonal T-cell receptor (TCR) gene rearrangement, leading to a monoclonal proliferation of cutaneous CD4-positive T lymphocytes (19, 20). Clinically, MF exhibits a higher predilection for dark skin (2:1) males (2:1) and, in its classical form, presents a slow-growing clinical course with a progressive shift from patches to plaques and, in final stages, tumors (19, 20). A variable combination of patches, plaques and tumors is frequently observed in MF with tumor lesions (20). Both clinical presentation and histopathology of MF are often non-specific, especially when it occurs in the form of patchy lesions, to such an extent that multiple biopsies are often necessary to obtain a definitive diagnosis (19, 21). RCM may improve the diagnostic accuracy of MF (13, 22, 23). In the upper portion of epidermis, epidermal disarray with disruption of the normal "honeycomb" appearance and sometimes hyporefractive areas, combined to the detection of small sized bright cells interspersed within epidermal layers are usually identifiable with RCM (13) (**Figure 2A**); the same bright cells are found at the dermoepidermal junction both inside and around dermal papillae, visible as round darker areas (13). RCM features of MF perfectly match with HHS (13): the presence of spongiosis, epidermotropic CD4-positive lymphocytes (**Figures 2B, C**) forming Pautrier's microabscesses and band-like distributed CD4-positive lymphocytes at dermoepidermal junction are the histopathological "mirror" of what is detectable with RCM. In addition, the differential diagnosis with eczematous disorders can become more straightforward using RCM (13), that shows in the stratum spinosum widespread round, deeply dark areas, intercellular spaces and few mildly bright cells: these findings are confirmed by horizontal histopathology, displaying marked spongiotic features combined to a less conspicuous lymphocytic exocytosis than MF (13).

ECCRINE POROMA

Eccrine poroma (EP) is a sweat gland derived adnexal tumor, first described by Pinkus in 1956 (24), that clinically arises as a slow-growing, sometimes ulcerated, reddish, and firm in consistency nodule, mostly located to the acral regions (25, 26). Usually, EP has a benign clinical course, even if a malignant counterpart, called "porocarcinoma" and characterized by low distant metastatic potential, has been also described (27). EP usually occurs on photodamaged skin, mimicking cutaneous malignancies, such as basal cell carcinoma (BCC), squamous cell carcinoma (SCC) or malignant melanoma (MM) (25, 26). Although the definitive diagnosis of EP is still based on conventional histopathology, non-invasive techniques, including dermoscopy and RCM, allow ruling out malignant conditions, and to suspect a benign adnexal neoplasm (28, 29). Dermoscopically, EP usually presents milky red areas at the periphery of the lesion and a polymorphous vascular pattern in the center, including glomerular, flower-like and dotted vessels (30). RCM shows a uniformly well-circumscribed neoplasm, consisting of hyper-reflective clusters surrounded by a darker stromal component (28, 30). Neoplastic cells are bright and homogeneous in size and shape, with round and dark nuclei, and may be arranged around non-reflective rounded areas (28, 30). Deeper sections show a richly vascularized stroma intermingled with tumor nests (28, 30). RCM images of EP correspond well with HHS (28, 30): neoplastic cells are monomorphic, cuboid-shaped, arranged in

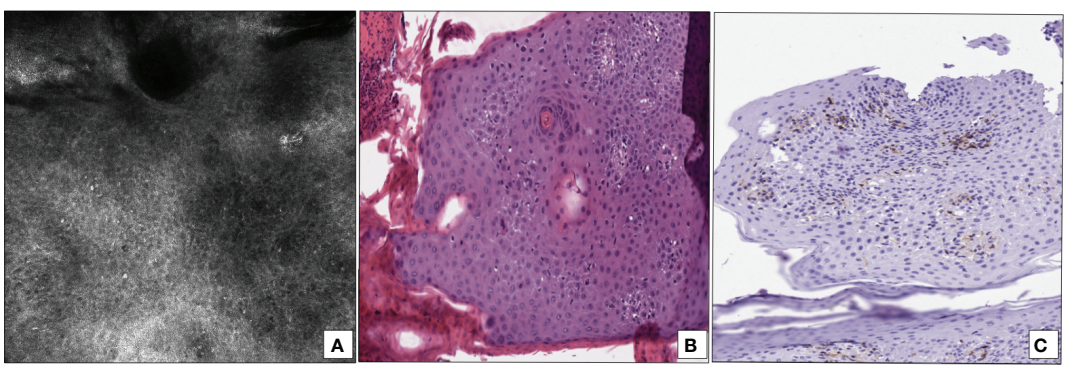

FIGURE 2 | Mycosis fungoides with patch lesions. **(A)** RCM at the stratum spinosum revealing a diffuse epidermal disarray with scattered small hyperreflective cells (epidermotropic lymphocytes). **(B)** Horizontal histopathology at the same level showing the presence of lymphocyte epidermotropism (hematoxylin and eosin; original magnification 400×). **(C)** Immunohistochemical staining for CD4 revealing the CD4-positive phenotype of epidermotropic T-lymphocytes (immunoperoxidase staining; original magnification 350×).

basaloid nests and occasionally forming round/slit-like ducts with eosinophilic material inside; these ducts strongly match with the non-reflective round dark areas visible with RCM and represent foci of ductal differentiation of EP. Bright uniformly shaped and sized cells interspersed within the tumor island or scattered in the upper dermis are often present at RCM in the pigmented variant of EP (28); these cells histologically correspond to melanocytes and melanophages, respectively. The presence of melanocytes in pigmented EP makes the differential diagnosis with MM mandatory: neoplastic melanocytes in MM are usually more irregularly shaped/dendritic or fusiform than those observed in pigmented EP (31, 32).

DISSEMINATED SUPERFICIAL ACTINIC POROKERATOSIS

Disseminated superficial actinic porokeratosis (DSAP) represents the most frequent variant of porokeratosis. It clinically presents as multiple scaly macules with a whitish central area surrounded by a slightly raised rim that mainly occurs on photoexposed regions (33). Dermoscopy frequently shows a double free edged scaly rim, whitish in color, representing the dermoscopic equivalent of the cornoid lamella, that is the histopathological hallmark of porokeratosis (34, 35). RCM may be useful in the diagnostic approach to DSAP, and its finding has been validated on the basis of the correlation with HHS (36). At RCM, architectural disarray with loss of the normal "honeycomb" pattern is observed in the center of the lesion (36); proceeding towards the periphery, a less refractile destructured area, containing more refractile amorphous substance (cornoid lamella) and surrounded by normal skin with regular "honeycomb" array, is found (36). HHS strongly matches with these RCM features and shows columns of parakeratosis (cornoid lamella) combined with moderately atypical keratinocytes (36).

SOLITARY MASTOCYTOMA

The term "mastocytosis" includes a wide spectrum of diseases caused by a clonal proliferation of mast cell and affecting simultaneously or at different times several organs, including the skin, bone marrow, liver, spleen, and lymphatic system (37). Based on the involved organs, the World Health Organization identifies two different variants of mastocytosis: cutaneous mastocytosis, if the disease exclusively affects the skin, and systemic mastocytosis, if there are other organs affected, regardless of the skin. Furthermore, cutaneous mastocytosis may be clinically further subdivided into maculo-papular cutaneous mastocytosis, diffuse cutaneous mastocytosis, and cutaneous mastocytoma (38). The latter includes not only the cases when there is a single cutaneous lesion (solitary mastocytoma; SM), but also those in which up to three skin lesions are seen (38). Clinical presentation of SM is variable and ranges from brownish/reddish macules to papules, plaques and nodules, showing swelling spontaneously or after rubbing (Darier's sign). Zhang et al. (39) first described RCM findings of mastocytosis in a huge group of 200 patients, including all different clinical presentation; regardless of the specific variant examined; all cases showed similar RCM features: the absence of aggregates of bright element in the context of finely granular and edematous papillary dermis was a constant finding. Following these results, our group first described more specific RCM features of SM and correlated them with HSS for validation (15): in particular, the presence of enlarged dermal papillae, containing tortuous vessels and large, uniformly round-shaped, bright cells at the level of dermoepidermal junction (**Figure 3A**) perfectly matched with the finding of aggregates of round, CD117-positive mastocytes with granular cytoplasm located to dermal papillae on HHS (**Figure 3B**).

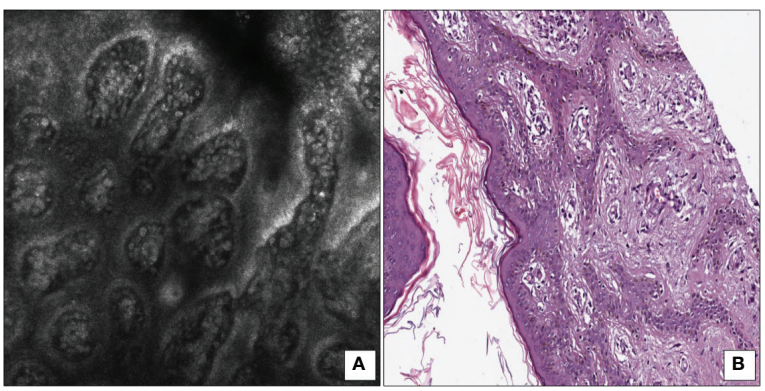

FIGURE 3 | Solitary mastocytoma. **(A)** RCM at the level of dermoepidermal junction showing multiple, large and rounded bright cells within dilated dermal papillae. **(B)** Horizontal histopathology at the same level revealing the presence of round mastocytes with pale and granular cytoplasm within dermal papillae (hematoxylin and eosin; original magnification 150×).

TABLE 1 | Correlation between reflectance confocal microscopy and horizontal histopathology in skin tumors: summary.

	Depth	RCM	HHS
SCCis (14)	Stratum Corneum	- Hyperrefractive amorphous structures - Polygonal, nucleated cells	- Hyperkeratosis - Parakeratosis
	Stratum granulosum/ spinosum	- Architectural disarray - Bright dendritic cells	- Large atypical keratinocytes - Langerhans cells (S-100 +, CD1a+, Melan-A -)
	Dermoepidermal junction	- Enlarged edged papillae with widened dermal papillae - Tortuouscapillary vessels	- Enlarged papillae with widened dermal papillae - Tortuouscapillary vessels
MF (13)	Upper epidermis	- Darker spots compared to the surrounding epidermis. - Epidermal disarray and presence of small bright cells	- Spongiosis - CD4-positive T-cellepidermotropism
	Dermoepidermal junction	- Small bright cells scattered within and among roundish hyporefractive areas (dermal papillae)	- CD4-positive lymphocytes infiltrating dermal papillae
EP (28, 30)	Epidermis	- Clusters of small, hyperrefractive and uniformly shaped cells with round dark nuclei surrounded by keratin - Parakeratosis	- Monomorphic basophilic neoplastic cells with large and round nuclei surrounded by amorphic keratin - Parakeratosis
	Dermis	- Larger and confluent cell clusters embedded in a denser and highly vascularized stroma- Neoplastic clusters arranged around darker hyporefractive rounded areas- Presence of bright, uniformly shaped and sized cells interspersed within tumor island or scattered in the upper dermis (pigmented variant)	- Increased tumor volume and denser and more vascularized stromal compartment - Intratumoral round or slit-like areas filled with eosinophilic substance (spots of ductal differentiation) - Intratumoral melanocytes or melanophages (pigmented variant)
DSAP (36)	Epidermis	- Architectural disarray with loss of the normal "honeycomb" pattern (central zone) - Hyperrefractive amorphous material (cornoid lamella) within hyporefractivedestructured areas, surrounded by skin with regular "honeycomb" pattern (peripheral zone)	- Columns of parakeratosis (cornoid lamella) combined with moderately atypical keratinocytes
SM (15)	Dermoepidermal junction	- Tortuous vessels and large, uniformly round-shaped, bright cells within enlarged dermal papillae	- Dermal papillae containing aggregates of round, CD117-positive mastocytes with granular cytoplasm
MTs (16, 17)	Dermoepidermal junction	- Atypical pigment network: proliferation of bright dendritic cells, forming "bridge" from epidermis to the superficial dermis (*in situ* melanoma) - Atypical pigment network: atypical nests of rounded and spindled hyperreflective melanocytes combined to an architectural disarray of dermal papillae and some bright cells or small dots within dermal papillae (*in situ* melanoma) - Hair follicles surrounded by multiple dendritic bright melanocytes and layers of keratinocytes filled at the periphery with rounded/ elongated hyperreflective melanocytes (lentigo maligna).	- Presence of atypical Melan-A-positive melanocytes surrounding dermal papillae and bulging into dermis (*in situ* melanoma) - Atypical melanocytes arranged in nests and presence of lymphocytes within dermal papillae (*in situ* melanoma) - Heavily pigmented keratinocytes of the basal layer of the epidermis combined with an increased number of junctional melanocytes (lentigo maligna).
	Upperdermis	- Dermoscopic globules: small nests of monomorphous non-atypical bright melanocytes non connected with epithelium in nevi and larger nests of pleomorphic neoplastic melanocytes in melanomas - Non-atypical peripheral pseudopods	- Small nests of non-atypical melanocytes in nevi and larger clusters of atypical neoplastic melanocytes in melanomas - peripheral confluent clusters of pigmented neoplastic melanocytes

RCM, reflectance confocal microscopy; HHS, horizontal histopathological section; SCCis, squamous cell carcinoma in situ; MF, mycosis fungoides; EP, eccrine poroma; DSAP, disseminated superficial actinic porokeratosis; SM, solitary mastocytoma; MTs, melanocytic tumours.

MELANOCYTIC TUMORS

While the introduction of dermoscopy has definitely represented a turning point in the diagnostic accuracy of melanocytic tumors, allowing the detection of some architectural patterns corresponding to specific histopathological features, in recent years RCM has emerged as a valid tool capable of providing architectural and morphological information at the cellular level (40–42); in particular, the combined use of dermoscopy and RCM proved to increase the accuracy for facial tumor detection, compared with RCM alone (43).

Braga et al. (17) compared RCM findings of melanocytic tumors and HHS. They selected four MMs and two benign nevi and compared specific dermoscopic patterns of cutaneous MM such as pigment network, irregular globules and pseudopods, and their benign counterparts, detectable in nevi, to RCM findings and both vertical and horizontal histopathology. Regarding the pigment network, two melanomas showed two different types of atypical network: the first MM presented on RCM a proliferation of bright dendritic cells at the level of dermoepidermal junction, some of them protruding from the epidermis to the superficial dermis to form "bridges"; conventional vertical histopathology revealed an *in situ* melanoma, and HHS showed the same features observed on RCM, confirming the presence of many atypical Melan-A-positive melanocytes surrounding dermal papillae and bulging into dermis. RCM of the second MM with an atypical pigmented network showed at dermoepidermal junction atypical nests of both rounded and elongated hyperreflective melanocytes combined to an architectural disarray of dermal papillae and some bright cells or small dots within dermal papillae; vertical histopathology revealed an *in situ* melanoma, and RCM findings were confirmed by HHS showing pleomorphic

melanocytes arranged in nests and presence of lymphocytes within dermal papillae. Based of RCM, Braga et al. (17) were also able to discriminate dermoscopic globules in nevi and melanomas on the basis of morphological atypia: both RCM and HHS showed small nests of monomorphous non-atypical bright melanocytes non-connected with epithelium in nevi and larger nests of pleomorphic neoplastic melanocytes in MMs. Lastly, pseudopods were not characterized by morphological atypia on RCM, corresponding to peripherally visible, confluent clusters of pigmented neoplastic melanocytes on horizontal histopathology. Navarrete-Dechent et al. (16) also matched the dermoscopic sign "circle within a circle" of lentigo maligna (presence of pigmentation within and around hair follicles) with its RCM and HHS: RCM revealed the presence of hair follicles surrounded by numerous dendritic bright melanocytes and layers of keratinocytes filled at the periphery with rounded/elongated hyperreflective melanocytes. HHS strongly overlapped with RCM, showing a high pigmentation of the keratinocytes of the basal layer of the epidermis combined with an increased number of junctional melanocytes.

As previously mentioned regarding SCCis, also for melanocytic tumors, the use of horizontal histopathology has only the purpose of validating the RCM application in clinical practice without replacing conventional histopathology as diagnostic gold standard.

DISCUSSION

In dermatology, the majority of skin specimens from biopsy or surgical procedures is analyzed using classical vertical histopathological sections, which represents the diagnostic gold standard. Horizontal histopathology is currently used for the diagnosis of some types of alopecia allowing a more correct visualization of follicular and perifollicular features (44).

More recently, HHS has been used to correlate with the morphological features obtained by RCM which provides the same transversal "point of view" of the skin. In particular, the strong matching and correlation between RCM images and HHS in skin tumors (**Table 1**), as shown in this review, further demonstrates the reliability of this innovative, non-invasive technique in the management of skin tumors. Based on such correlations, some considerations can be made: in SCCis and melanoma RCM may confirm the clinical suspect addressing the correct therapeutic approach; in clinically atypical SM, RCM evaluation may avoid biopsy or excision as it is generally self-resolving; in MF and DSAP, RCM is particularly useful for the selection of the best site for biopsy thus avoiding multiple biopsies often quite bothersome for the patient; a further application of RCM in skin tumors may consist in the early recognition of local recurrences after medical or surgical treatments of the disease (14).

AUTHOR CONTRIBUTIONS

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

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Tissue Expression of Carbonic Anhydrase IX Correlates to More Aggressive Phenotype of Basal Cell Carcinoma

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Basal cell carcinoma (BCC) is the most common cancer in the white-skinned population accounting for about 15% of all neoplasms. Its incidence is increasing worldwide, at a rate of about 10% per year. BCC, although infrequently metastasizing, very often causes extensive tissue losses, due to the high propensity toward stromal infiltration, particularly in its dedifferentiated forms, with disfiguring and debilitating results. To date, there still is limited availability of therapeutic treatments alternative to surgery. We evaluated the immunohistochemical expression of the carbonic anhydrase IX (CAIX), one of the main markers of tissue hypoxia, in a set of 85 archived FFPE BCC tissues, including the main subtypes, with different clinical outcomes, to demonstrate a possible relationship between hypoxic phenotype and biological aggressiveness of these neoplasms. Our results showed that the expression level of the CAIX protein contributes to the stratification of BCC in the different risk classes for recurrence. We hypothesize for CAIX a potential therapeutic role as a target therapy in the treatment of more aggressive BCCs, thus providing an alternative to surgical and pharmacological therapy with Hedgehog inhibitors, a promising example of target therapy in BCCs.

Keywords: basal cell carcinoma, carbonic anhydrase IX, IHC, skin cancer, prognosis, risk stratification

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INTRODUCTION

Basal cell carcinoma (BCC) is defined by the World Health Organization (WHO) as a locally invasive, slow-growing tumor that originates from the basal layer cells of the epidermis, placed peripherally to the hair bulbs, and that rarely hesitates in metastasis. The main risk associated to BCC are multiple relapses, an event more frequently occurring in case of incomplete excision or multiple primitive tumors, Relapsing BCC can produce, over time, serious anatomic, functional and aesthetic damage, with serious problems of co-morbidity, severely affecting the quality of life (1). Accounting for about 15% of all solid tumors, BCC is the most common malignant neoplasm in the world, with more than 2.8 million new cases diagnosed each year in the

United States of America (2). In the context of non-melanoma skin cancer (NMSC), BCC accounts for about 80% of the cases (3), with a global incidence increase of 3% to 7%/year over last decades (4), BCC represents a serious public health problem. In Italy, the incidence is approximately 100 cases per 100,000 inhabitants (5). These figures could be underestimated because of the diagnostic-therapeutic management for this neoplasia. BCC treatment, in fact, does not usually include hospitalization, and BCC generally does not cause patients' death. BCC develops predominantly in the mature-elderly population (>40 years), prevalently males, with average age at diagnosis of 68 years, in regions of the body chronically exposed to the sun (particularly face and neck, 70% to 85% of cases; 25% to 30% being represented by the nose alone, to follow the trunk and less frequently the limbs). Recently, an epidemiological shift has been reported, with increased incidence in young female population, probably due to the varied habits of exposure of the population (not adequately protected) in the Sun (6). BCC recognizes as the main risk factor exposure to sunlight, especially UVA and UVB ultraviolet rays. Different BCC variants have been described, based on clinical behavior, morphology, growth pattern, architecture, and differentiation (7). Hypoxia is a pathological condition determined by a lack of oxygen in the whole organism (generalized hypoxia) or in one tissue (tissue hypoxia). Hypoxia has emerged as an important feature of tumor microenvironment of neoplasms with more aggressive biological behavior. The uncontrolled growth of tumors is, in fact, accompanied by the induction of insufficient vascularization which results in the formation in most of the malignant solid tumors of heterogeneously distributed hypoxia regions (8, 9). Hypoxia generates a passage to the glycolic metabolism that allows the production of energy in low or absent oxygen conditions and is crucial for the survival of hypoxic cancer cells. Among the molecules most expressed in hypoxia condition are HIF-1 α and carbonic anhydrase IX (CAIX). These molecules are responsible for the process of adapting cells to oxygen deficiency with the formation of new blood vessels, a mechanism that is exploited by tumor tissues to grow and metastasize (10). CAIX belongs to the family of Carbonic Anhydrases (CA), a group of metal zinc-containing enzymes that catalyze the reversible hydration of CO2 in HCO3 and H + ions and has recently emerged as the most promising endogenous marker of cellular hypoxia (10, 11). This reaction is fundamental at the level of cells, tissues, and organs in a wide range of biochemical and physiological processes such as acid-base equilibrium, gas exchange, ionic transport, and carbon dioxide fixation. To date, 15 human isoforms of CA have been characterized that differ in catalytic activity, subcellular localization, and tissue distribution (11). Carbonic Anhydrase IX is encoded by a gene located on chromosome 9 and is a transmembrane isoform with a catalytic site in the extracellular portion and has the highest efficiency for the transport of H + between CAs. It consists of a proteoglycan-like domain at the N-terminal end (involved in adhesion and intercellular communication), an extracellular catalytic domain, a transmembrane hydrophobic portion and a C-terminal cytoplasmic tail (essential for correct localization on the plasma membrane and proper functioning of the enzyme) (12). It is a tumor-associated protein, as it is expressed in limited quantities in normal tissue, such as the stomach or intestine, and the expression is however limited to the basolateral membrane of epithelial cells endowed with increased proliferative activity, while it is hyper expressed in solid tumor cells linked to a hypoxic phenotype (13). The overexpression of CAIX on the cell membrane of many solid tumors is mediated by the HIF-1 transcription factor and is often associated with poor reactivity to classical radio and chemotherapy. In a recent work, a close association between overexpression of CAIX and the markers of staminality CD44 and Nestin, has been demonstrated in several aggressive and metastasizing neoplasms, with relevance in a series of squamous carcinomas of the tongue (14). This indicates that CAIX action in hypoxic tumors goes beyond intra-tumoral PH control. The clear majority of existing data, in fact, indicates that CAIX has multiple functions in solid tumors, in particular, it plays a key role in encouraging the establishment of chemo-and radio-resistance in the most advanced cases and opens new therapeutic perspectives (14). In the present study, we deepened the role of the Carbonic Anhydrase IX as a possible leading actor and marker of hypoxia in BCC, by evaluating the immunohistochemistry expression of the CAIX protein in a series of archived FFPE BCC tissue samples.

MATERIALS AND METHODS

Patients and Tissue Samples

Formalin-fixed, paraffin-embedded tissue blocks of 85 BCCs, diagnosed and excised with healthy surgical margins from February 2002 to November 2017, were retrieved from the archives of the Pathology Section of the Department of Advanced Biomedical Sciences, "Federico II" University of Naples. Out of 85 cases, 55 males and 30 females, the age at diagnosis ranged between 38 and 88 years (mean age, 67 years). **Table 1** summarizes the histological groups of the study population, together with the associated risk. The clinical data and pathological features of the tumors are reported in **Table 2**. The study design and procedures involving tissue samples collection and handling were performed according to the

TABLE 1 | Study population summary grouped by histological types.

Risk of recurrence	Histotype	Count
Higher risk	Basosquamous carcinoma	14
	Infiltrating BCC	34
	Micronodular BCC	4
	Sclerosing/morphoeic BCC	10
Higher risk, total		62
Lower risk	BCC with adnexal differentiation	2
	Nodular BCC	16
	Superficial BCC	5
Lower risk, total		23
Total		85

Declaration of Helsinki, in agreement with the current Italian law, and to the Institutional Ethical Committee guidelines.

TMAs Construction and Immunohistochemistry

Two pathologists (SS and DR) reviewed the whole routine hematoxylin-eosin (H&E) sections to confirm the original diagnosis and to mark the most representative tumor areas useful for the TMA construction. Tissue cores with a diameter of 3 mm were punched from morphologically representative tissue areas of each "donor" tissue block and brought into one recipient paraffin block using a manual tissue arrayer. The filled recipient blocks were then placed on a metal base mold. The paraffin-embedding was then carried-out, by heating the blocks at 42°C, for 10 min, and flattening their surface by pressing a clean glass slide on them. As a result, four TMAs were built. 4-μm sections were cut from each TMA using an ordinary microtome (15, 16). The first section was stained with H&E to confirm the presence of the tumor and the integrity of tissues. The other section was mounted on a super frost slide (Microm, Walldorf, Germany) for the immunohistochemical evaluation of CAIX. For CAIX IHC assay the sections were deparaffinized routinely in xylene and rehydrated through a series of graded ethanol. CAIX antigen retrieval was performed in EDTA buffer (pH 8) in a hot water bath (94°C) for 20 min and in CITRATE buffer (pH 6) by microwave oven (3 min × 3 times); the backdrop (for blocking non-specific background staining) was removed using the universal blocking serum (Dako Diagnostics, Glostrup, Denmark) for 15 min at room temperature. Endogenous alkaline phosphatase activity was quenched adding Levamisole to buffer AP (Substrate Buffer); the slides were rinsed with TRIS+Tween20 pH 7.4 buffer and incubated in a humidified chamber with the primary rabbit polyclonal antibody anti-CAIX (sc-25599, Santa Cruz Biotechnology, diluted 1:200 overnight at 4°C). Then used a biotinylated secondary antibody and streptavidin conjugated with alkaline phosphatase. The reaction has been highlighted with the chromogen Fast Red, which showed the presence of antigen that we sought in red (Dako REAL Detection System, Alkaline Phosphatase/RED, Rabbit/Mouse). Again, after a weak nuclear counterstain with hematoxylin, the sections were then mounted with a synthetic medium (Entellan, Merck, Darmstadt, Germany). Positivity for CAIX was visualized as red membranous and cytoplasmic staining. The CAIX expression was defined as high or low depending on whether the percentage of neoplastic cells stained was respectively >/= or <5%.

TABLE 2 | Clinical-pathologic characteristics of the study population.

		n	%
Patients	Total	85	100%
Age	Mean	67	
	Range (Min-Max)	38–88	
Sex	Male	55	65%
	Female	30	35%
Tumor site	Area H	46	54%
	Area M	10	12%
	Area L	27	32%
	ND	2	2%
Histologic subtype	BCC with indolent growth	23	27%
	BCC with aggressive growth	62	73%
Follow-up	Recurrence	30	35%
	No recurrence	55	65%
Follow-up time (months)	Mean	39	
	Median	42	
	Min	2	
	Max	153	
Tumor size	>2 cm	25	29%
	<2 cm	59	70%
	N.D.	1	1%

Area H: "mask areas" of face (central face, eyelids, eyebrows, periorbital, nose, lips [cutaneous and vermilion], chin, mandible, preauricular, and postauricular skin/sulci, temple, ear), genitalia, hands, and feet; Area M: cheeks, forehead, scalp, neck, and pretibial; Area L: trunk and extremities; BCC with indolent or ordinary growth: BCC with solid nest, superficial, adenoid, keratotic; BCC with aggressive or aggressive growth: BCC morphoeic, basosquamous, micronodular, dedifferentiated.

Statistical Analysis

Correlation between CAIX immunohistochemical expression and BCC clinical-pathologic characteristics was asses through contingency analysis with Fisher exact test. Statistical analysis has been performed using SPSS software (IBM Corp. Released 2013. IBM SPSS Statistics for Windows, Version 22.0. Armonk, NY: IBM Corp.).

RESULTS

Our case series included 85 tumor samples (**Table 2**), out of which, 7 (8%) were not evaluable for CAIX tissue expression due to loss of core integrity. The CAIX protein showed LOW expression score in 35 (45%) out of 78 cases, and a HIGH score in the residual 43 (55%) (**Table 3**). The study population was subdivided, according to the histotype, into two groups: aggressive BCCs (i.e., BCCs with higher risk of recurrence; including basosquamous, morphoeic, infiltrating and micronodular) consisting of 60 cases (60/78, 77%) and the group of ordinary BCCs (i.e., BCCs with lower risk of recurrence; including nodular, superficial, and with adnexal differentiation) consisting of 18 cases (18/78, 23%) (**Table 1**). Among aggressive BCC, 41 out of 60 evaluable cases (68.3%) showed a HIGH CAIX expression score, while in the group of ordinary BCCs only 2 out of 18 cases (11.1%) showed a high score (**Table 4**; **Figure 1A**). **Table 5** shows the distribution of CAIX expression scores per histologic subtypes. The follow-up data for recurrence, detailed per tumor variants, are shown in **Table 6**.

TABLE 3 | CAIX IHC tissue expression score frequency distribution in the studied population.

CAIX expression score frequency distribution

CAIX		Frequency	Percentage (Total)	Percentage (Valid)
Valid	Low	35	41%	45%
	High	43	51%	55%
	Tot. Valid	78	92%	100%
Missing		7	8%	
Total		85	100%	

Correlation between CAIX immunohistochemical expression and BCC histotype was assessed through contingency analysis with Fisher exact test, that proved to be statistical significant, with a P value <0.0001. A survival analysis, taking recurrence as endpoint, was carried out and Kaplan-Meier curves are shown in **Figure 1B**: difference between CAIX HIGH and LOW curves is significant as resulted from Log-Rank test (p = 0.05). Taken together our results show that the higher CAIX expression significantly correlates with BCC aggressive behavior. Representative images of CAIX IHC staining in low-risk BCCs are shown in **Figure 2**; representative high-risk BCCs immunostained with anti-CAIX antibody are shown in **Figure 3**.

DISCUSSION

Basal carcinoma (BCC) represents 15% of all neoplasms and constitutes a serious public health problem, being the most common cancer in the white-skinned population. Its incidence is increasing worldwide, with an increase of ≥ 10%/year [Lomas et al. (2)]. BCC is a tumor that, despite its low frequency of distant metastasis, frequently causes extensive tissue losses, due to a marked tendency to stromal infiltration, particularly in its dedifferentiated forms, with disfiguring and debilitating results. The maximum expression of this aggressive behavior is the so-called Ulcus Rodens with destructive consequences for the cartilage and bone tissues. To date, there is still limited availability of alternative therapeutic treatments to surgery.

TABLE 4 | Contingency table of BCC histologic classification by CAIX score.

Contingency Table Classification * CAIX score

		CAIX score		
		High	**Low**	**Total**
Classification	Aggressive	41 (68.3%)	19 (31.7%)	60 (100%)
	Ordinary	2 (11.1%)	16(88.9%)	18 (100%)
Total		43(55.1%)	35 (44.9%)	78 (100%)

TABLE 5 | Crosstab of CAIX expression by histologic subtypes.

Histologic type	**CAIX score**		
	High	**Low**	**Total**
Basosquamous carcinoma	9 (64.3%)	5 (35.7%)	14 (100%)
BCC with adnexal differentiation	0	2 (100%)	2(100%)
Infiltrating BCC	22 (66.7%)	11 (33.3%)	33 (100%)
Micronodular BCC	2 (50%)	2 (50%)	4 (100%)
Nodular BCC	1 (8.3%)	11 (91.7%)	12 (100%)
Sclerosing/morphoeic BCC	8 (88.9%)	1 (11.1%)	9 (100%)
Superficial BCC	1 (25%)	3 (75%)	4 (100)

TABLE 6 | Crosstab of recurrence follow-up data by tumor variants.

Histologic type	**Follow-Up**		
	Not recurrent	**Recurrent**	**Total**
Basosquamous carcinoma	10	4	14
BCC with adnexal differentiation	1	1	2
Infiltrating BCC	15	18	33
Micronodular BCC	1	3	4
Nodular BCC	11	1	12
Sclerosing/morphoeic BCC	5	4	9
Superficial BCC	3	1	4
Total	46	32	78

Recently, promising results seem to emerge from the early follow-up of patients treated with molecular anti-Sonic Hedgehog therapy, a pathway associated with the BCC carcinogenesis process. It is compelling to unravel the pathogenetic mechanisms underlying the aggressiveness potential of each BCC subtypes, in order to achieve an effective personalized therapy for these tumors. The need of greater understanding of BCC biology appears even more urgent given that this neoplasia preferentially affects the adult and elderly population and that prevention and early diagnosis are still unattained goals, especially in emerging areas of the World and in Western countries peripheral areas. In recent years, a large body of data has highlighted the importance of the interaction of cancer cells with the tumor microenvironment,

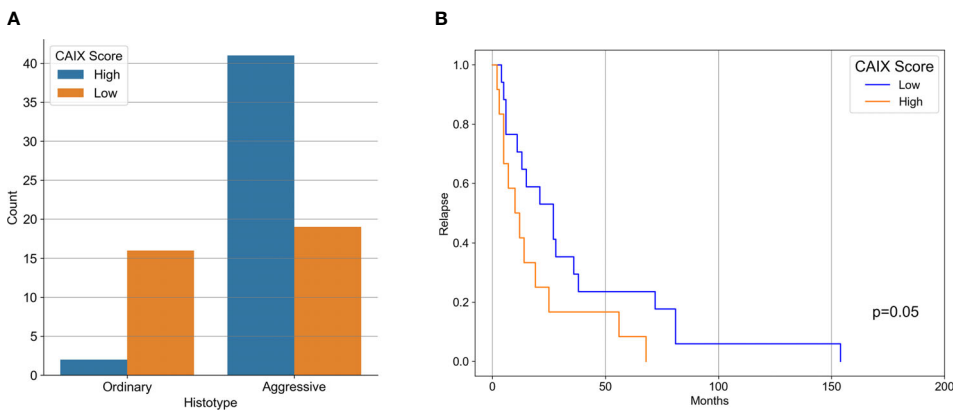

FIGURE 1 | **(A)** Bar-Graph representation of CAIX immunohistochemical expression in aggressive and ordinary BCC. **(B)** Kaplan-Meier curves of recurrence survival. Difference between CAIX HIGH and CAIX LOW curved proved statistically significant (p = 0.05) as tested by Log-Rank.

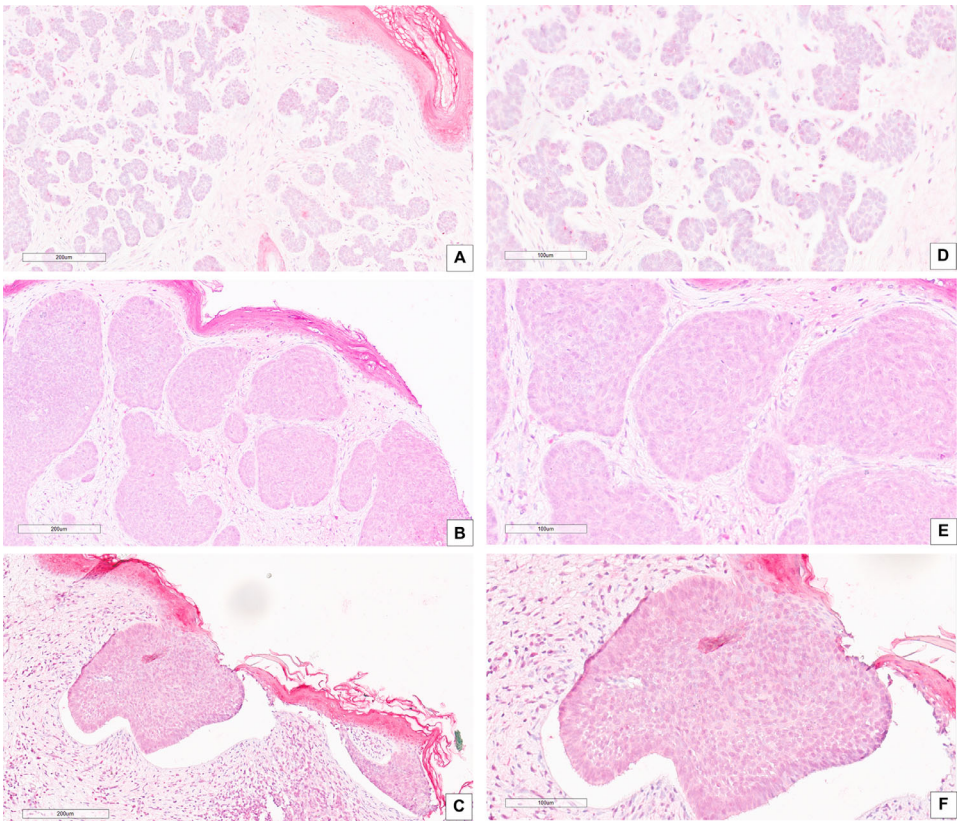

FIGURE 2 | IHC stain with an anti-CAIX antibody in low risk BCC histological variants: **(A–D)** BCC with adnexal differentiation (magnification, 20× and 40×, respectively); **(B–E)** Nodular BCC (magnification, 20× and 40×, respectively); **(C–F)** Superficial BCC (magnification, 20× and 40×, respectively). Scale bars are shown.

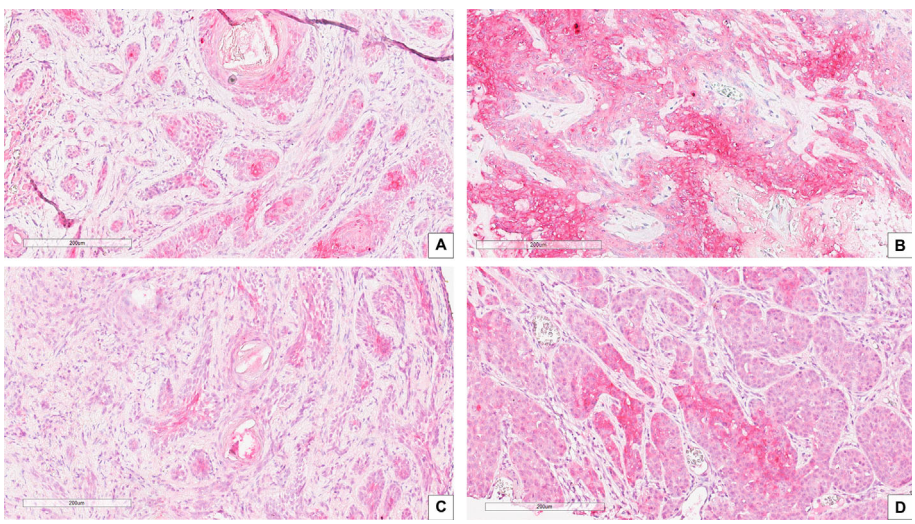

FIGURE 3 | IHC stain with an anti-CAIX antibody in high risk BCC histological variants: **(A)** Infiltrating BCC; **(B)** Morpheaform BCC; **(C)** Micronodular BCC; **(D)** Basosquamous BCC. Scale bars are shown, magnification is 20×.

which provides support for the growth and development of neoplasia. This assumption brought to light new study hypotheses, in order to characterize the heterotypic interaction between the tumor and its microenvironment. Among the alterations of the tumor microenvironment, much attention has been paid, in recent decades, to hypoxia, a pathophysiological feature of locally advanced tumors, resulting from genetic instability, diminished apoptotic potential, and angiogenesis. The hypoxic state also plays an important role in relapsing, metastasis and poor response to treatments, including radiotherapy, chemotherapy and angiogenic treatment. Among the molecules most expressed in hypoxic condition, the carbonic anhydrase IX (CAIX) is considered a marker of hypoxia *in vivo* (17), whose overexpression has been correlated with increased tumor aggression in different types of cancer (18–22). To date, a series of CAIX inhibitors have been synthesized, both in the form of small inhibitory molecules and as monoclonal antibodies, used as antitumor agents in different models of neoplasms (23–31). In a previous work we tested the expression of CAIX in several human solid tumors, extending the CAIX expression information to the expression of the stem cells markers CD44 and nestin in solid cancers, to explore their relationship with the biological behavior of tumors. We found that CAIX is strongly expressed in advanced tumors, including squamous cell invasive cancer of the tongue (14). The role of CAIX as a prognostic biomarker in oral cancer has been recently reviewed by (32), whose systematic review and meta-analysis showed that that immunohistochemical CAIX assessment is a useful OSCC prognostic biomarker. In the present work, the immunohistochemistry expression of the CAIX protein was evaluated in a selected series of patients with BCC divided into two groups based on the histological subtype. The highest levels of protein were found in the aggressive BCC group consisting mainly of morphoeic BCC and Basosquamous BCC. 68.3% of cases showed a high level of expression, and the remaining 31.7% a low level. CAIX expression frequency distribution has been reported in all the histotypes described in our case series, and the statistics of CAIX expression correlation with BCC subtypes have been carried out grouping BCC samples into two categories, aggressive and ordinary one, according to clinical behavior of each subtype, in order to overcome the relative small number of subjects for some subtypes. In the ordinary group, 92% of cases expressed low levels of CAIX and only 8% show a high score. The Fisher Exact Test confirmed that the difference in CAIX immunostain observed between the two BCC groups was statistically significant. The most significant result was obtained by comparing the averages of expression between the aggressive and ordinary groups, and the difference showed a value of $P<0.0001$. In conclusion, these results suggest that the expression levels of the CAIX protein can help to stratify BCCs in different risk classes; moreover, our results let envisage a role for CAIX as a therapeutic target to counteract the most aggressive BCC, providing a viable alternative to the surgical approach, and to the inhibitors of Hedgehog Pathway, a promising tool for target therapy in BCCs, often associated with various degrees of toxicity (muscle spasms, alopecia, dysgeusia, weight loss, fatigue, nausea, decreased appetite and diarrhea) that in the most severe forms (hypovolemic shock, myocardial infarction, meningeal disease, Ischemic stroke) determine the interruption of treatment, with no resolution of the pathology in place.

DATA AVAILABILITY STATEMENT

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

ETHICS STATEMENT

The study was performed according to the Italian law, and according to the Declaration of Helsinki for studies based only on retrospective analyses on routine archival FFPE-tissue.

AUTHOR CONTRIBUTIONS

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication. All authors contributed to the article and approved the submitted version.

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Mucosal Invasion, but Not Incomplete Excision, Has Negative Impact on Long-Term Survival in Patients With Extramammary Paget's Disease

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Background: Extramammary Paget's disease (EMPD) sometimes spreads from the skin to mucosal areas, and curative surgical excision of these areas is challenging. The aim of this study is to analyze the impact of mucosal involvement and surgical treatment on the survival of patients with EMPD.

Methods: We conducted a retrospective review of 217 patients with EMPD. We also assessed the associations between tumor involvement in boundary areas (anal canal, external urethral meatus, vaginal introitus), prognostic factors, and survival in 198 patients treated with curative surgery.

Results: Of 217 patients, 75 (34.6%) had mucosal boundary area involvement. Lesions in these areas were associated with frequent lymphovascular invasion ($p = 0.042$), lymph node metastasis ($p = 0.0002$), incomplete excision ($p < 0.0001$), and locoregional recurrence ($p < 0.0001$). Boundary area involvement was an independent prognostic factor associated with disease-specific survival, per multivariate analysis (HR: 11.87, $p = 0.027$). Incomplete excision was not significantly correlated with disease-specific survival (HR: 1.05, $p = 0.96$).

Conclusion: Boundary area tumor involvement was a major risk factor for incomplete excision, local recurrence, and poor survival outcomes. However, incomplete removal of primary tumors was not significantly associated with poor prognosis. A less invasive surgical approach for preserving anogenital and urinary functions may be acceptable as the first-line treatment for resectable EMPD.

Keywords: extramammary Paget's disease, mucosal invasion, surgery, prognostic factor, invasive surgery, radical surgery

INTRODUCTION

Extramammary Paget's disease (EMPD) is a rare neoplastic condition (1). It commonly affects areas rich in apocrine sweat glands, including the vulva, perineal area, perianal area, scrotal area, and penile skin (1, 2). EMPD typically affects Caucasian females and Asian males older than 60 years (3–7). Most EMPD tumors are restricted to the epidermis as *in situ* lesions, and they are associated with good prognosis because of their slow-growing nature (1, 8). However, approximately 15–40% of EMPD lesions display dermal invasion, which is known as invasive EMPD, and this increases the risk of lymph node and distant metastasis (2, 4). Management is notoriously complicated, and the recurrence rate is high (15–61%) despite aggressive surgeries (9–12).

Several prognostic factors regarding primary tumors have been reported, including tumor thickness (13, 14), level of tumor invasion (15–18), lymphovascular invasion (8, 17, 19), and perianal location (13, 20–22). Ohara et al. (8) recently conducted a multicenter analysis of 301 invasive EMPD cases, and they proposed a new tumor, node, and metastasis (TNM) classification and staging system in which the T category was determined based on tumor thickness and lymphovascular invasion. The Japanese Skin Cancer Society is currently proposing the use of this EMPD-specific TNM classification and staging system. However, the classification is still tentative.

EMPD lesions sometimes spread from the skin to mucosal areas *via* boundary areas (anal canal, external urethral meatus, vaginal introitus) and deep toward internal organs (rectum, uterus, urinary bladder). Curative surgical excision of lesions in boundary areas is challenging since radical excision impairs organ functions and requires additional functional reconstruction (colostomy, etc.). To preserve organ function, surgical margins are determined at specific sites (e.g., dentate line) regardless of tumor spread, but it can be difficult to maintain sufficient surgical margins at these sites. Perianal lesions indicate poor prognosis partly due to difficult total excision (20). A recent report suggested frequent incomplete excision in cases of EMPD with mucosal involvement (23). However, the prognostic impact of mucosal involvement has not been elucidated.

In this study, we reviewed the data of 217 EMPD patients in our institution over a 23-year period. We showed that lesions involving boundary areas were associated with high risk for poor survival outcomes, regardless of whether complete surgical removal was achieved, and that incomplete excision of EMPD did not affect patient outcomes. We also aimed to verify the newly proposed EMPD-specific TNM staging system (8).

MATERIALS AND METHODS

Patients

This retrospective review was conducted according to the guidelines of the Declaration of Helsinki. This study was approved by the Ethics Committee of Kyushu University Hospital (30–363; November 27, 2018). We retrieved the data of 217 patients with primary EMPD lesions. These patients were treated at the Department of Dermatology of Kyushu University in Fukuoka, Japan, between January 1997 and October 2020. At least three experienced dermatopathologists confirmed the diagnosis. Patients with secondary EMPD, which involved direct invasion from visceral organs, were carefully excluded.

The following data on all patients were retrieved from our prospectively maintained databank and then analyzed: demographic data (sex, age at initial presentation), clinical data (tumor site, primary lesion size), and histopathological data obtained *via* hematoxylin and eosin staining (tumor thickness [measured to the second decimal place, as per the latest melanoma classification guidelines of the American Joint Committee on Cancer] (24), lymphovascular invasion). For patients with two or more primary lesions, we recorded the greatest tumor thickness and the total tumor size. Tumor thickness was measured from the total excised specimen. For cases without total excision, tumor thickness was calculated from biopsy specimens. In situ lesions on biopsy were further confirmed by clinical findings (lack of erosions, ulcerations, formation of nodules). Involvement of mucosal boundary areas (anal canal, external urethral meatus, vaginal introitus) was recorded from clinicopathological data. Lymph node metastasis was primarily determined by histopathology. Patients who had lymphadenopathy detected by physical examination or imaging studies (ultrasonography, computed tomography [CT], and/or positron emission tomography with computed tomography [PET/CT]) were also considered to have metastasis. The N category was defined according to the classification system proposed by Ohara et al. (8): N0, no lymph node metastasis; N1, metastasis involving one lymph node; and N2, metastasis involving two or more lymph nodes. Distant metastasis was determined by using imaging studies (ultrasonography, chest X-ray, CT, and/or PET/CT). Lymph node metastasis beyond the regional lymphatic basin was also classified as distant metastasis. For the M category, M0 indicated no distant metastasis, and M1 indicated distant metastasis (8).

Mucosal Boundary Area Involvement and Surgical Outcomes

Next, the data of patients treated with curative surgery were collected. Patients were divided into two groups, that is, with or without involvement of mucosal boundary areas, as involvement of these areas influences surgical strategies. In addition to the data mentioned above, we compared data pertaining to surgical treatments and outcomes, including surgical margin, margin status after surgery (complete or incomplete excision), local recurrence, and new regional lymph node metastasis after initial treatment, between these two groups. Complete excision was defined as complete removal of the primary tumor with histopathologically negative margins and complete dissection of regional lymph nodes (if lymph node metastases were present). Patients with distant metastases at surgery were excluded when comparing surgical outcomes. Reconstruction of skin/mucosal

Abbreviations: EMPD, extramammary Paget's disease; TNM, tumor, node, and metastasis; DSS, disease-specific survival; SLNB, sentinel lymph node biopsy; CLND, completion lymph node dissection.

defects was performed by using simple sutures, skin grafting, or musculocutaneous flaps, as appropriate.

Follow-Up

The patients were monitored by physical examination every 3–6 months and imaging studies (ultrasonography, chest X-ray, and/or CT). Survival data, including time of locoregional and distant recurrence, survival length, and cause of death, were recorded. The median follow-up period was 61.4 months (range: 2.0–264.7 months). By the last follow-up, 164 patients were alive, 20 died of EMPD, and 33 died of other causes.

Statistical Analysis

All statistical analyses were performed by using JMP version 14.2 (SAS Institute, Cary, NC, USA). The χ^2 test or Fisher's exact test and Mann-Whitney U test were used for analysis of categorical variables and continuous variables, respectively. We used the Kaplan-Meier method to evaluate disease-specific survival (DSS), and we compared survival curves by using the log-rank test. DSS was calculated from the date of the first histological examination to the date of death due to EMPD or the last follow-up prior to October 31, 2020. Data on patients who did not die were censored on October 31, 2020. Data on patients who died of other causes were censored at the time of death. The associations between clinical and histopathological factors and DSS were determined by using a multivariate Cox proportional hazards regression model. Probability values less than 0.05 were regarded as statistically significant.

RESULTS

Clinicopathological Data of the Study Cohort

The demographic and clinical data of the 217 patients with primary EMPD are shown in **Table 1**. All patients were Japanese, with a mean age of 72.9 years (range: 34–95 years). There were 130 male patients (59.9%) and 87 female patients (40.1%). Tumors were predominantly localized in the genital area (83.9%), followed by the perianal area (4.1%), then the axillary area (2.3%). Multiple lesions or tumors spreading over two areas were seen in 21 patients (9.7%). There were 95 patients (44.4%) with small primary lesions (< 25 cm^2) and 119 (55.6%) with large lesions (≥ 25 cm^2). A total of 109 patients (50.2%) had tumors in situ. Tumor thickness was stratified as ≤ 1 mm, 1–4 mm, or > 4 mm for invasive tumors. There were 38 patients (17.5%) with tumors ≤ 1 mm, 45 (20.7%) with tumors 1–4 mm, and 19 (8.8%) with tumors > 4 mm. Lymphovascular invasion was observed in 14 patients (6.5%); lymphovascular invasion was not evident in 203 patients (93.5%). A total of 75 patients (34.6%) exhibited boundary area involvement. Regional lymph node metastasis was found in 27 patients (12.4%). Seven patients (3.2%) had one metastatic lymph node, and 20 (9.2%) had two or more. Distant metastasis was observed in six patients (2.8%). Data on primary lesion size and tumor thickness were unavailable for three and six patients, respectively.

TABLE 1 | Demographics and clinical data of all 217 patients.

Parameter	n (%)
Sex	
Male	130 (59.9)
Female	87 (40.1)
Age (years)	
Mean ± SD	72.9 ± 10.0
Median (range)	73 (34-95)
Tumor site	
Genital area only	182 (83.9)
Perianal area only	9 (4.1)
Axillary area only	5 (2.3)
Genital + perianal areas	13 (6.0)
Genital + axillary areas	5 (2.3)
Other areas	3 (1.4)
Primary lesion size (cm^2)	
<25	95 (44.4)
≥25	119 (55.6)
Unknown	3 (0.4)
Tumor thickness (mm)	
In situ	109 (50.2)
≤1	38 (17.5)
1-4	45 (20.7)
>4	19 (8.8)
Unknown	6 (2.8)
Lymphovascular invasion	
Present	14 (6.5)
Absent	203 (93.5)
Boundary area involvement	
Present	75 (34.6)
Absent	142 (65.4)
Metastasis	
Regional lymph node metastasis	
N0	190 (87.6)
N1	7 (3.2)
N2	20 (9.2)
Distant metastasis	
M0	211 (97.2)
M1	6 (2.8)

SD, standard deviation.

Treatment, Locoregional Recurrence, and Distant Metastasis

A total of 204 patients (94.0%) underwent surgical excision for primary lesions. Of these patients, 200 underwent curative excision with wide margins (0.5–5.0 cm), typically after mapping biopsy, and four underwent palliative surgery. Surgical margins were positive in 46 of these 204 patients (22.5%). Additional excision was performed in seven of these 46 patients. A total of 13 patients (6.0%) with disseminated metastasis or complications or who were unable to give consent for surgical excision received the following alternative treatments, alone or in combination: topical imiquimod cream (n = 3), topical 5-fluorouracil ointment (n = 3), cryotherapy (n = 2), photodynamic therapy (n = 1), radiation therapy (n = 5), or systemic chemotherapy (n = 4). Only two patients received palliative care as the primary treatment. There were 33 patients without lymphadenopathy who underwent sentinel lymph node biopsy (SLNB); eight of them (24.2%) were positive. There were 19 patients with lymphadenopathy who underwent swollen lymph node biopsy; nine of them (47.4%) had confirmed metastasis. Completion lymph node dissection (CLND) was

performed in 18 patients (8.3%). Systemic chemotherapy/targeted therapy was performed in six patients (2.8%). Radiation therapy was performed in seven patients (3.2%). A summary of the initial treatments is available in **Supplementary Table 1**.

Of 200 patients who underwent curative excision with wide margins, 13 patients had local recurrence during the follow-up period. They underwent wide surgical excision (n = 9), radiation therapy (n = 2), or treatment with topical imiquimod cream (n = 2). The details of the 13 patients with local recurrence are shown in **Supplementary Table 2**. Regional lymph node metastasis or distant metastasis (distant lymph node, lung, liver, brain, or bone metastasis) occurred for the first time in 18 patients during the follow-up period, and 13 of these patients underwent CLND, systemic chemotherapy/targeted therapy, or radiation therapy (alone or in combination).

Stage Classification and Disease-Specific Survival: Corroboration of the Newly Proposed TNM Staging System

Most patients were stage 0 (T0N0M0) (n = 109, 50.2%), followed by stage I (T1N0M0) (n = 70, 32.3%), stage II (T2N0M0) (n = 9, 4.1%), stage IIIa (TanyN1M0) (n = 7, 3.2%), stage IIIb (TanyN2M0) (n = 16, 7.4%), and stage IV (TanyNanyM1) (n = 6, 2.8%). The 5-year DSS of each stage was 100.0%, 97.4%, 42.9%, 80.0%, 23.3%, and 0.0%, respectively. The prognosis between stages I and II, classified by tumor thickness of invasive EMPD without remote regional lymph node or distant metastasis, showed a significant difference ($p < 0.0001$). All patients with distant metastasis (stage IV) died within 5 years, and the survival rate was significantly different from that of all other stages (0 vs. IV, $p < 0.0001$; I vs. IV, $p < 0.0001$; II vs. IV, $p = 0.0027$; IIIa vs. IV, $p = 0.0003$; IIIb vs. IV, $p < 0.0001$). No significant difference was found between stages IIIa and IIIb, classified by the number of lymph node metastases ($p = 0.066$). There were significant differences in survival between stages I and IIIa ($p = 0.034$) and stages I and IIIb ($p < 0.0001$). The survival rate of stages II was opposite that of patients in stage IIIa, although there was no significant difference ($p = 0.47$). The Kaplan-Meier DSS curves of patients stratified by TNM stage are shown in **Figure 1**.

Characteristics of Patients Treated With Curative Surgery

Next, the data of 198 patients treated with curative surgery were analyzed to assess the associations between mucosal boundary area involvement and prognostic factors. Patients with distant metastasis (stage IV) were excluded from this analysis. There were 65 patients (32.8%) with boundary area involvement and 133 (67.2%) without.

The demographic and clinicopathological data of each group are listed in **Table 2**. Patients with involvement of boundary areas were mostly female ($p < 0.0001$), and the location was most frequently the perianal area ($p = 0.0018$). Tumor size showed no significant difference between the two groups ($p = 0.29$). Histopathologically, patients with boundary area involvement

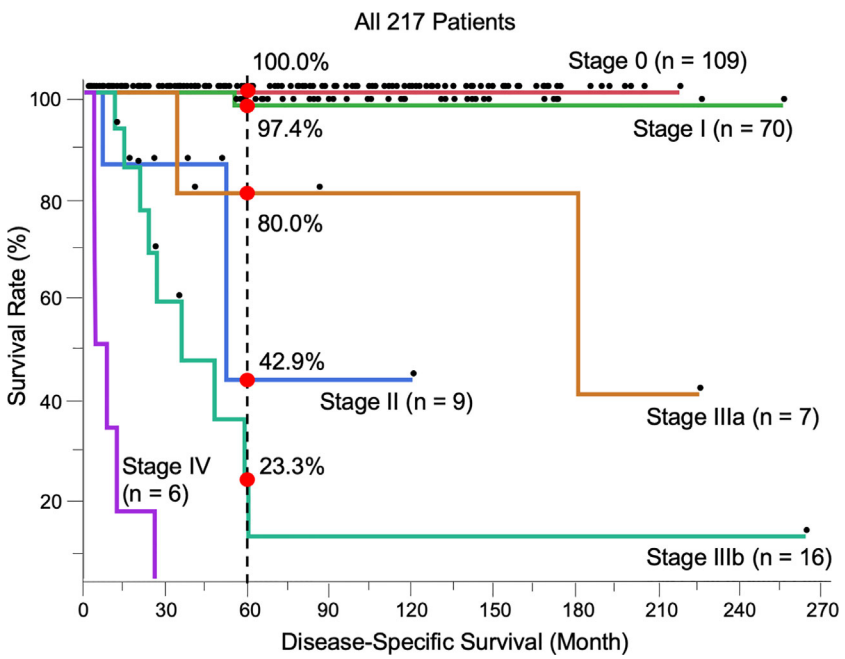

FIGURE 1 | Kaplan-Meier disease-specific survival curves of all 217 patients stratified by TNM stage. The 5-year survival was 100.0% (Stage 0, n = 109), 97.4% (I, n = 70), 42.9% (II, n = 9), 80.0% (IIIa, n = 7), 23.3% (IIIb, n = 16), and 0.0% (IV, n = 6). The log-rank test showed the results of survival as follows; 0 vs I, $p = 0.17$; I vs II, $p < 0.0001$; I vs IIIa, $p = 0.034$; I vs IIIb, $p < 0.0001$; II vs IIIa, $p = 0.47$; II vs IIIb, $p = 0.24$; IIIa vs IIIb, $p = 0.066$; 0 vs. IV, $p < 0.0001$; I vs. IV, $p < 0.0001$; II vs. IV, $p = 0.0027$; IIIa vs. IV, $p = 0.0003$; IIIb vs. IV, $p < 0.0001$.

TABLE 2 | Demographics and clinical data of the 198 patients treated with curative surgery.

Parameter	Involvement of mucosal boundary areas		*P*-value*
	Present (n = 65)	Absent (n = 133)	
Sex			
Male	16 (24.6%)	105 (78.9%)	**<0.0001**
Female	49 (75.4%)	28 (21.1%)	
Age (year)			
Mean ± SD	69.7 ± 10.3	73.5 ± 9.12	**0.0091**
Tumor site			
Perianal area	12 (18.5%)	5 (3.8%)	**0.0018**
Other areas	53 (81.5%)	128 (96.2%)	
Primary lesion size (cm^2)			
<25	26 (40.0%)	64 (48.1%)	0.29
≥25	39 (60.0%)	69 (51.9%)	
Tumor thickness (mm)			
In situ	30 (46.2%)	72 (54.1%)	0.12†
≤4	26 (40.0%)	54 (40.6%)	
>4	9 (13.8%)	7 (5.3%)	
Lymphovascular invasion			
Present	7 (10.8%)	4 (3.0%)	**0.042**
Absent	58 (89.2%)	129 (97.0%)	
Regional LN metastasis			
Present	13 (20.0%)	4 (3.0%)	**0.0002**
Absent	52 (80.0%)	129 (97.0%)	
Number of regional LN metastases			
1	4 (30.8%)	3 (75.0%)	0.25
2 or more	9 (69.2%)	1 (25.0%)	
TNM stage			
0	30 (46.2%)	72 (54.1%)	**0.0014**
I	20 (30.8%)	50 (37.6%)	
II	2 (3.1%)	7 (5.3%)	
IIIa	4 (6.2%)	3 (2.3%)	
IIIb	9 (13.9%)	1 (0.8%)	
Local recurrence			
Present	12 (18.5%)	0 (0.0%)	**<0.0001**
Absent	53 (71.5%)	133 (100.0%)	
Follow-up period (month)			
Mean ± SD	82.8 ± 64.0	83.7 ± 57.4	0.73
Median (range)	58.2 (7.2–256.5)	78.9 (2.0–264.7)	

Significant values are shown in boldface.

**Mann-Whitney U tests were used for continuous variables, and χ^2 or Fisher's exact tests were used for categorical variables.*

†In situ vs. ≤ 4 mm, p = 0.65; in situ vs. > 4 mm, p = 0.040; ≤ 4 mm vs. > 4 mm, p = 0.077. SD, standard deviation; LN, lymph node; TNM, tumor, node, metastasis.

tended to have thicker tumors in invasive EMPD (*in situ* vs. ≤ 4 mm, $p = 0.65$; *in situ* vs. > 4 mm, $p = 0.040$; ≤ 4 mm vs. > 4 mm, $p = 0.077$). Lymphovascular invasion was more frequently observed in patients with involvement of boundary areas ($p = 0.042$). Patients with boundary area involvement had more advanced primary tumors. The rate of regional lymph node metastasis in patients with boundary area involvement was statistically higher than in patients without boundary area involvement ($p = 0.0002$). In each group, patients were classified in accordance with the TNM staging system. Patients with involvement of boundary areas tended to be classified with advanced TNM stages.

Twelve patients had local recurrence during the follow-up period, and all of them had involvement of boundary areas. They underwent wide surgical excision (n = 9), radiation therapy (n = 1), or treatment with topical imiquimod cream (n = 2). The details of the patients with local recurrence are shown in **Supplementary Table 2**.

Initial Treatment of Patients Treated With Curative Surgery: Boundary Area Involvement as a Risk Factor for Incomplete Excision

The initial treatment patterns of these 198 patients, who were divided into two groups based on boundary area involvement, are summarized in **Table 3**.

For primary tumor excision, the distance of the surgical margin showed no significant difference in the two groups (mean: 1.56 cm vs. 1.72 cm, $p = 0.18$). Surgical margins were positive in 42 of the 198 patients (21.2%). The positive site was predominantly at the mucosal side (n = 30), followed by the skin side (n = 8), and then both the mucosal and skin sides (n = 4). The positive surgical margin rate was significantly higher in patients with boundary area involvement than in patients without boundary area involvement ($p < 0.0001$). Additional excision was performed in seven of the 42 patients with positive surgical margins (six patients with additional mucosal excision and one with additional skin excision), and all seven of these patients were confirmed to have negative surgical margins. Only three patients underwent colostomy or urinary diversion. There was no significant difference in the rate of SLNB performed ($p = 0.41$). However, the rate of metastasis in SLNB cases was significantly different between the two groups ($p = 0.0048$). The rate of metastasis in lymphadenopathy cases was not significantly different between the two groups ($p = 0.12$). CLND was performed in 13 patients with boundary area involvement and four patients without boundary area involvement ($p = 0.0002$). Curative excision was completed in 37 patients with boundary area involvement (56.9%) and 126 patients without boundary area involvement (94.7%) ($p < 0.0001$). All incomplete excisions were for primary tumors. There were no patients with incomplete removal of regional lymph nodes. Five patients among 35 patients with incomplete excision (14.3%) experienced local recurrence (**Supplementary Table 2**).

Factors Associated With Disease-Specific Survival of Patients Treated With Curative Surgery: Negative Impact of Boundary Area Involvement on Long-Term Survival

We evaluated the possible clinical and histopathological factors associated with DSS in the 198 patients treated with curative surgery by using a multivariate Cox proportional hazards regression model. The following factors were included as explanatory variables: sex, age, tumor site, tumor thickness, boundary area involvement, complete excision, and regional lymph node metastasis. The results are listed in **Table 4**. Univariate analysis results revealed that tumor thickness > 4 mm, boundary area involvement, and regional lymph node metastasis were statistically significant factors for poor survival. Multivariate analysis results showed that tumor thickness > 4 mm (HR: 7.23, $p = 0.0037$), boundary area involvement

TABLE 3 | Initial treatment of the 198 patients treated with curative surgery.

Treatment		Involvement of boundary areas		P-value*
		Present (n = 65)	Absent (n = 133)	
For primary lesions	Surgical margin (cm)			
	Mean ± SD	1.56 ± 0.84	1.72 ± 0.84	0.18
	Surgical margin status			
	Positive	34 (52.3%)	8 (6.0%)	**<0.0001**
	Negative	31 (47.7%)	125 (94.0%)	
	Additional excision			
	Done	6 (17.7%)	1 (12.5%)	1.00
	Not done	28 (82.3%)	7 (87.5%)	
For regional LNs	SLNB			
	Done	8 (12.3%)	24 (18.1%)	0.41
	Not done	57 (87.7%)	109 (81.9%)	
	SLNB			
	LN metastasis present	5 (62.5%)	2 (8.3%)	**0.0048**
	No LN metastasis	3 (37.5%)	22 (91.7%)	
	Biopsy of lymphadenopathy			
	Done	8 (12.3%)	8 (6.0%)	0.16
	Not done	57 (87.7%)	125 (94.0%)	
	Biopsy of lymphadenopathy			
	LN metastasis present	5 (62.5%)	1 (12.5%)	0.12
	No LN metastasis	3 (37.5%)	7 (87.5%)	
	CLND			
	Done	13 (20.0%)	4 (3.0%)	**0.0002**
	Not done	52 (80.0%)	129 (97.0%)	
Overall	Complete excision†			
	Complete	37 (56.9%)	126 (94.7%)	**<0.0001**
	Incomplete	28 (43.1%)	7 (5.3%)	
Adjuvant therapy	Chemotherapy	0 (0.0%)	1 (0.75%)	1.00
	Radiation therapy	1 (0.75%)	0 (0.0%)	1.00

Significant values are shown in boldface.
**Mann-Whitney U tests were used for continuous variables, and Fisher's exact tests were used for categorical variables.*
†Complete excision was defined as complete removal of the primary tumor with histopathologically negative margins and complete dissection of regional lymph nodes (if lymph node metastases were present).
SD, standard deviation; LN, lymph node; SLNB, sentinel lymph node biopsy; CLND, completion lymph node dissection.

(HR: 11.87, $p = 0.027$), and regional lymph node metastasis (HR: 27.91, $p = 0.031$) were also statistically independent factors associated with DSS. Incomplete excision was not significantly correlated with survival (HR: 1.05, $p = 0.96$). The Kaplan-Meier curves of patients stratified by boundary area involvement and achievement of complete excision are shown in **Figures 2**, **3**.

As an additional analysis, these possible prognostic factors were evaluated in the 65 patients with boundary area involvement by using a multivariate analysis for DSS. The results revealed that incomplete excision was not significantly correlated with survival (HR: 3.11, $p = 0.34$). The detailed data are available in **Supplementary Table 3**.

DISCUSSION

Complete surgical tumor removal is the treatment of choice for resectable EMPD. Due to the slow-growing nature of this kind of tumor, nearly 90% of the patients at our hospital show no lymph node or distant metastasis. Treatment strategies for primary lesions are therefore key for curing this disease in these patients. EMPD lesions are most likely to arise in the anogenital area, sometimes extending toward visceral organs *via* boundary areas (anal canal, external urethral meatus, vaginal introitus). When tumors involve these boundary areas, surgeons are forced to choose whether radical surgical excision with extensive reconstruction should be performed or whether less invasive surgery should be performed to preserve defecation and urination functions. This choice is challenging, as most EMPD patients are elderly, and radical surgery impairs patients' quality of life. The latter choice is often chosen in our institute after deep discussion with patients and their families, unless the tumors are invasive (with nodule formation, etc.) in boundary areas. Reconstruction of skin/mucosal defects is typically accomplished by using simple sutures or split-skin grafting. One of the aims of this study was to evaluate the reasonability of this kind of surgery. We retrospectively summarized 23 years of experience treating 217 patients with EMPD and assessed their outcomes. This is one of the largest studies conducted at a single institute, and we identified several important findings.

We showed for the first time that patients with EMPD lesions in boundary areas had significantly shortened DSS compared to other patients ($p < 0.0001$, **Figure 2**). This was corroborated by the results of multivariate analyses, which were adjusted by some known prognostic factors (HR: 11.87, 95% CI: 1.32–106.73, $p = 0.027$). Representative prognostic factors of primary tumors

TABLE 4 | Multivariate Cox proportional hazard analyses for disease-specific survival.

Variable	Univariate analysis			Multivariate analysis		
	HR	95% CI	*P*-value	HR	95% CI	*P*-value
Sex, male	1.78	0.47-6.72	0.39	0.26	0.012-5.42	0.38
Age (year)†	1.01	0.92-1.05	0.49	1.05	0.97-1.14	0.24
Perianal lesion	1.11	0.14-8.72	0.92	1.53	0.13-16.90	0.73
Tumor thickness > 4 mm	30.56	8.73-109.94	**<0.0001**	7.23	1.13-46.19	**0.037**
Boundary area involvement	21.13	2.70-165.60	**0.0037**	11.87	1.32-106.73	**0.027**
Incomplete excision	0.94	0.20-4.38	0.94	1.05	0.16-6.74	0.96
Regional LN metastasis	36.60	9.51-140.92	**<0.0001**	27.91	1.35-576.63	**0.031**

Significant values are shown in boldface.
†Continuous variable.
HR, hazard ratio; CI, confidence interval; LN, lymph node.

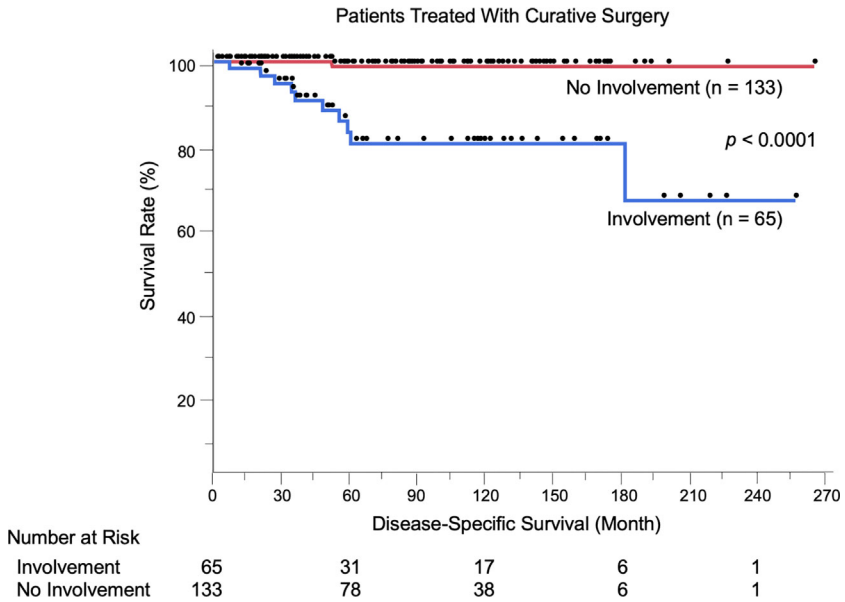

FIGURE 2 | Kaplan-Meier disease-specific survival curves of the 198 patients treated with curative surgery stratified by boundary area involvement. Patients with EMPD lesions in boundary areas had significantly shortened their survival ($p < 0.0001$). The number at risk is also shown.

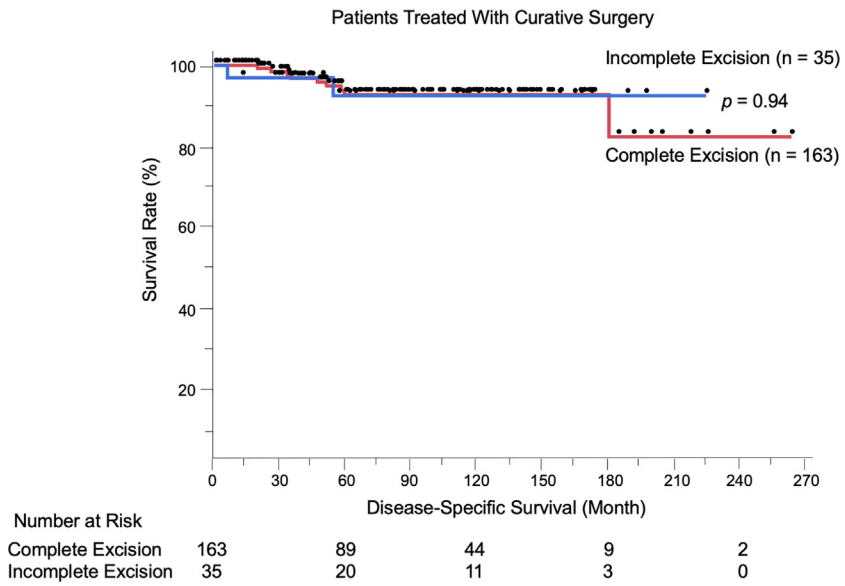

FIGURE 3 | Kaplan-Meier disease-specific survival curves of the 198 patients treated with curative surgery stratified by achievement of complete excision. Incomplete excision was not correlated with worse survival compared to complete excision ($p = 0.94$).The number at risk is also shown.

include nodule formation (14, 25), tumor thickness (8, 13, 14), level of tumor invasion (15–18), lymphovascular invasion (8, 17, 19), perianal location (13, 20–22), and vaginal location (26). Human epidermal growth factor receptor 2/neu (27–29) and nectin cell adhesion molecule 4 (30) expression are other factors associated with tumor recurrence and DSS, respectively. We previously evaluated the efficacy of mapping biopsy and surgical treatment of EMPD, and we found a high tumor-positive rate of surgical margins in EMPD lesions with mucosal boundary area involvement (19/36, 52.8%) (23). This high positive rate may be

due to difficulty both in delineating tumor borders and in setting sufficient surgical margins in these areas. In the current study, the positive rate was similar to our previous one (34/65, 52.3%). Some factors were associated with the presence of boundary area involvement. Female patients more frequently had boundary and perianal lesions compared to male patients (data not shown) since female anogenital areas are close to boundary areas. Other factors included thicker tumors, the presence of lymphovascular invasion, and lymph node metastasis, suggesting that advanced EMPD lesions are likely to extend to boundary areas. In this study, 12 patients experienced local recurrence of primary lesions, and all had boundary lesions.

Of note, among the 198 patients treated with curative surgery, incomplete excision of primary tumors was not correlated with worse DSS compared to complete removal (p = 0.94). Similarly, when analyzing the patients with boundary area involvement (n = 65), incomplete excision was not a poor prognostic factor (p = 0.34 per Cox multivariate analysis). Furthermore, only five patients among 35 patients with incomplete excision (14.3%) experienced local recurrence. Most of the patients with the disease were elderly (mean age: 72.9 years), and among the 53 patients who died during the follow-up period, EMPD was the direct cause only in 20 patients (37.7%); the other 33 patients (62.3%) died of other causes. These results raise an important question: is it always necessary to pursue negative margins in primary EMPD? Previous studies have reported no correlation between positive surgical margins and local recurrence in vulvar EMPD (9–11, 31, 32). Nasioudis et al. (6) conducted a large database study and reported that the presence of positive surgical margins was not associated with overall survival. Correlations between surgical margins and patient survival have been controversial, and the current study offered new insights into this issue. Furthermore, some radical surgical procedures (proctectomy, urethrectomy, total cystectomy) are accompanied by simultaneous creation of colostomy and urinary diversions, which can lead to troublesome complications (33–36). Formijne Jonkers et al. (37) reported that 82% of patients who underwent creation of an intestinal stoma experienced one or more stoma-related complications within 1 year. Radical surgeries with creation of colostomy or urinary diversions deteriorate patients' organ functions, as well as patients' quality of life (33, 38–40). In our cohort, only three of 75 patients (4.0%) with boundary area involvement underwent colostomy or urinary diversion. Whereas lesions in boundary areas had increased risks of incomplete excision and local recurrence, these lesions were also associated with advanced tumor status (thicker tumors, frequent lymphovascular invasion, and lymph node metastasis). Most localized EMPD lesions were unaggressive, with high 5-year survival rates (100% in stage 0 and 97.4% in stage I). Collectively, the less invasive approach we performed (preserving anorectal and urinary functions) may be a reasonable treatment choice for patients with EMPD.

Another interesting finding was that patient survival in this study fit well with the newly proposed TNM staging system (8). Although TNM staging is crucial in cancer treatment, no widely accepted staging system specific for EMPD has been established due to the rarity of the disease. In this study, we classified patients in accordance with the newly proposed, EMPD-specific TNM staging system (8) and assessed its validity. The T category (classified by tumor thickness and lymphovascular invasion), N category (classified by lymph node metastasis), and M category (classified by distant metastasis) were significantly associated with worse survival, and their survival curves were consistent with previous reports. Interestingly, the survival of patients in stage II (localized invasive tumors) was worse than that of patients in stage IIIa (one regional lymph node metastasis), although the difference did not reach statistical significance (p = 0.47). These inverse survival results were also observed in the original report of the TNM staging system for EMPD (8). The exact mechanisms of this inversion is still unclear but this is also noted in malignant melanoma (41, 42). EMPD and melanoma exhibit a similar invasion process (first arising in the epidermis, horizontally spreading, and later invading vertically into the dermis with the destruction of basal membrane). One possible explanation is the hematogenous metastasis, however, more data is required to test this hypothesis.

CONCLUSION

We retrospectively reviewed 23 years of data of 217 patients with EMPD. Most patients (n = 198, 91.2%) were candidates for curative surgery. Tumor involvement in boundary areas was a major risk factor for incomplete excision, local recurrence, and poor survival outcomes. However, incomplete removal of primary tumors was not significantly associated with poor prognosis. A less invasive surgical approach for preserving anogenital and urinary functions may be acceptable as the first-line treatment for resectable EMPD.

DATA AVAILABILITY STATEMENT

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

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Kyushu University Hospital. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

HH, TI, and YK-I participated in manuscript preparation. TI designed the methodology. HH participated in data analysis and figure preparation. HH and YK-I collected the detailed information of the patients. TI and MF reviewed and revised the manuscript. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fonc.2021.642919/full#supplementary-material

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Malignant Melanoma in Children and Adolescents Treated in Pediatric Oncology Centers: An Australian and New Zealand Children's Oncology Group (ANZCHOG) Study

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Objectives: Unlike adults, malignant melanoma in children and adolescents is rare. In adult melanoma, significant progress in understanding tumor biology and new treatments, including targeted therapies and immunotherapy have markedly improved overall survival. In sharp contrast, there is a paucity of data on the biology and clinical behavior of pediatric melanoma. We report a national case series of all pediatric and adolescent malignant melanoma presenting to ANZCHOG Childhood Cancer Centers in Australia and New Zealand.

Methods: A retrospective, descriptive, multi-center study was undertaken to identify patients less than 18 years of age treated for cutaneous malignant melanoma over a twenty-year period (1994 to 2014). Data on clinical characteristics, histopathology, and extent of disease, treatment and follow-up are described.

Results: A total of 37 cases of malignant melanoma were identified from all of the Australasian tertiary Childhood Cancer Centers. The median age was 10 years (range 1 month – 17 years). Clinically, the most common type of lesion was pigmented, occurring in sixteen (57%) patients, whilst amelanotic was seen in 7 patients (25%). In 11 (27.9%) the Breslow thickness was greater than 4mm. A total of 11 (29.7%) patients relapsed and 90% of these died of disease. Five-year event free survival (EFS) and overall survival were 63.2 (95% CI: 40.6 – 79.1) and 67.7% (95% CI: 45.1 – 82.6) respectively.

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Conclusion: Our data confirms that melanoma is a rare presentation of cancer to tertiary Australasian Childhood Cancer Centers with only 37 cases identified over two decades. Notably, melanoma managed in Childhood Cancer Centers is frequently at an advanced stage, with a high percentage of patients relapsing and the majority of these patients who relapsed died of disease. This study confirms previous clinical and prognostic information to support the early multidisciplinary management in Childhood Cancer Centers, in conjunction with expert adult melanoma centers, of this rare and challenging patient group.

Keywords: cutaneous melanoma, childhood, dermatology, outcome, rare tumors

INTRODUCTION

Cutaneous melanoma in children and adolescents is rare, with an incidence ranging between 0.3 and 1 per 100 000 children a year, and only a small percentage occurring before puberty (1–4). Pediatric melanoma has not been studied as extensively as adult melanoma and our current understanding of the outcomes for melanoma presenting in children and adolescents is limited to mainly single-institution review series and a recent prospective European rare pediatric cancer consortium registry study (3–7). In Australia <2% of all cases of cutaneous melanoma occur before the age of 25 years (1, 7–9). Given that certain geographical areas of Australia and New Zealand have been reported to have the highest rates of adult melanoma in the world, it is important to review pediatric data and evaluate specific factors that influence prognosis and overall survival (7–9).

The rarity of pediatric melanoma combined with differences in the clinical presentation compared to adults (10), especially in young children can make diagnosis challenging. Moreover, histopathological diagnosis is complicated due to the similar histological appearance of malignant melanoma with more benign lesions in childhood, such as spitz nevi, atypical spitz nevi and the concept of melanocytic tumors of uncertain prognosis (MELTUMP). Molecular diagnostic tools, such as fluorescent *in situ* hybridization and genomic testing, are now assisting pathologists to distinguish between these different entities (11, 12).

Over the past decade, significant advances have been made in elucidating the molecular pathogenesis of adult melanoma. Approximately 60% of adult melanoma patients have identifiable oncogenic mutations in the *BRAF* gene, whilst another 20% have oncogenic *NRAS* mutations (13). These genetic discoveries have been translated into the clinic, with mitogen-activated protein kinase (MAPK) pathway inhibitors such as BRAF and mitogen-activated protein kinase/extracellular signal-regulated kinase (MEK) inhibitors inducing dramatic responses and significantly improving survival (14, 15). In addition, the highly immunogenic nature of melanoma has successfully been exploited using immunotherapy, with major improvements in patient outcomes (16).

Despite these advances, melanoma that has spread to distant sites remains incurable in the majority of patients. Clinical trials are ongoing to develop novel and more effective targeted therapies and immunotherapies to treat metastatic melanoma (16–19). The management of pediatric melanoma patients has been extrapolated from the treatment of adults with melanoma. However, the limited understanding surrounding the diagnosis and prognosis of childhood melanoma initially led to the almost uniform exclusion of these patients from clinical trials offered to adult patients; a strategy that has hampered research efforts and access to treatment in this population (20). The increased use of precision medicine to molecularly characterize tumors in children has further guided specific treatments including molecular target therapies and immunotherapy. In Australia this is being undertaken through the Precision Medicine in Children with Cancer (PRISM) clinical trial (https://clinicaltrials.gov/ct2/show/NCT03336931).

For these reasons, we evaluated the clinical characteristics and outcomes of all pediatric and adolescent malignant melanoma patients managed at pediatric oncology centers in Australia and New Zealand over the past two decades.

METHODS

We undertook a retrospective, descriptive, multicenter study of children and adolescents with malignant melanoma. All ten pediatric oncology centers in the Australia and New Zealand Children's Oncology Group (ANZCHOG) participated in the study. Patients aged less than 18 years and treated for malignant melanoma between 1994 and 2014 were included. A detailed review of each patient chart was undertaken and data collected for each case included age, gender, ethnicity, site of disease, staging, extent of disease (including Breslow thickness), ulceration and *BRAF* status. Treatment outcomes, mode of follow-up, relapse and cause of death were also recorded. All data were all collected in accordance with the approval of institutional research ethics boards.

The number of cases of pediatric and adolescent melanoma patients was compared to the number of cases published in the national cancer registry, for both Australia and New Zealand, over the same timeframe.

Data has been presented as medians and ranges and as percentages. The overall survival (OS) has been calculated according to the Kaplan-Meier method: from the date of

diagnosis to the date of death or latest follow-up for patients still alive. The event free survival (EFS) has been calculated from the date of diagnosis to the date of disease recurrence, death or latest follow-up for patients still alive and in complete remission.

RESULTS

Thirty-seven patients with malignant melanoma were identified over the twenty-year period timeframe. The ratio of males to females was 1:1 and the median age at diagnosis was 10 years age (range 1 month to 17 years). A total of 16 (43%) patients were less than 10 years old. The majority of patients were of Caucasian ethnicity (83.7%) with only five New Zealand Maori (n=3), African (n=1) and Australian Aboriginal (n=1) patients. Tumors were located on the head and neck (n = 14, 37.8%), trunk (n = 10, 27%), upper limb (n = 5, 13.5%) and lower limb (n = 5 cases, 13.5%). The primary location was unknown in two patients (**Table 1**).

The Australia and New Zealand cancer registries reported 1,778 children and adolescents with melanoma over the same twenty-year time period (**Table 2**).

Melanoma arose from congenital nevi in six patients (16.3%) and two patients had a history of malignancy with one patient being treated for acute leukemia, including total body radiation conditioning for an allogeneic bone marrow transplant and another patient with previous anaplastic astrocytoma and leukemia and known Li Fraumeni Syndrome.

A description of lesions at clinical presentation was available in 28 patients. The majority (16 cases, 55%) had a pigmented lesion reported, whilst seven (25%) had amelanotic lesions which were described as scaly, warty or friable in appearance. Two (7%) patients presented with subungal nodular lesions on the toe and index finger and three (11%) patients had nodal enlargement as the presenting clinical feature.

Histologically the most common melanoma subtypes were nodular and Spitzoid, with eight cases (21.6%) reported for each group respectively. Breslow thickness was reported in 25 cases and nearly 30% (11 cases) had thick lesions with a measurement greater than 4mm at presentation.

Based on the American Joint Committee on Cancer (AJCC) classification, our study found that eight patients (21.6%) were stage I, nine patients (24.3%) were stage II, four patients were stage III (10.8%) and the remaining were stage IV (11 cases, 29.7%) at diagnosis. For five patients, no exact staging classification was possible. *BRAFV600E* testing was conducted in seven (18%) patients and was positive for one patient. There was also one patient who was tested for and found positive for an *NRAS* exon 3 mutations.

TABLE 1 | Patient characteristics and Clinical Features of the 37 patients with malignant melanoma in Australia and New Zealand 1st January 1995 – 31st December 2014.

	N (%)
Gender: Male/Female	18/19 (48.6/51.4)
Age:	
0 – 4 years	5 (13.6)
5 – 9 years	11 (29.7)
10 – 14 years	13 (35.1)
15 – 18 years	8 (21.6)
Ethnicity:	
Caucasian	31 (83.7)
African	1 (2.7)
Aboriginal	1 (2.7)
Maori	3 (8.1)
Unknown	1 (2.7)
Site of Disease:	
Trunk	10 (27)
Head and Neck	14 (37.8)
Extremity - Upper	5 (13.5)
Extremity - Lower	5 (13.5)
Other	1 (2.7)
Unknown	2 (5.6)
Histology:	
Superficial Spreading	4 (10.8)
Nodular	8 (21.6)
On congenital naevus	6 (16.3)
Spitzoid	8 (21.6)
Not classified	11 (29.7)
Breslow Thickness:	
≤ 1.00mm	4 (10.8)
1.01 – 2.00mm	4 (10.8)
2.01 – 4.00mm	6 (16.3)
> 4.00mm	11 (29.7)
Unknown	12 (32.4)
AJCC Stage at diagnosis:	
Stage I	8 (21.6)
Stage II	9 (24.3)
Stage III	4 (10.8)
Stage IV	11 (29.7)
Unknown	5 (13.6)

Initial Treatment

All but three patients underwent initial surgical resection of their tumor. Of the three patients who did not receive surgical resection, one had an unknown primary lesion; one initially had a shave biopsy before proceeding to further surgery and one had a fine needle aspirate of an enlarged lymph node before subsequent nodal excision. Primary re-excision, in order to obtain adequate margins, was performed in seven (19%) of patients. Lymph node biopsy was undertaken in 13 (35%) patients and lymphoscintigraphy was performed in 1 patient. Lymph nodes were positive following biopsy in five (38%) patients. Among the cases with positive lymph node biopsies, three had nodular histology and two were associated with congenital nevi. All but one of these patients relapsed and subsequently died of the disease.

Chemotherapy was used in five patients, following initial surgical resection, and included interferon in four patients (3 patients stage III and 1 stage IV) and a combination of cisplatin, dacarbazine and fotemustine in another patient (stage IV).

Relapses and Treatment

Eleven patients (29.7%) relapsed, with a median time from diagnosis to first relapse of 22 months (range 2 months – 9 years). All but one of the 11 patients who relapsed died from malignant melanoma. Among the patients who relapsed, 3 (27%) had melanoma arising from a congenital nevus, four (37%) had

TABLE 2 | Incidence Count from 1st Jan 1994 – 31st Dec 2013, based on Australia and New Zealand national cancer registry data.

0 – 4 years	19
5 – 9 years	43
10 – 14 years	198
15 – 19 years	1289
Number of Deaths: Australia 1st Jan 1994 – 31st Dec 2013	
0 – 4 years	1
5 – 9 years	2
10 – 14 years	3
15 – 19 years	26
Incident Count: New Zealand 1st Jan 1994 – 31st December 2013	
0 – 4 years	3
5 – 9 years	3
10 – 14 years	30
15 – 19 years	193
Number of Deaths: New Zealand 1st Jan 2007 – 31st December 2012	
0 – 4 years	1
5 – 9 years	0
10 – 14 years	1
15 – 19 years	8

Mean population of children 0 - 19 years in Australia and New Zealand over the duration of the study = 3, 069, 745 per annum (http://www.stats.govt.nz/topics/population, https://abs.gov.au/statistics/people/population.

nodular histology, one (9%) had superficial spreading histology and in three (27%) patients the histology was unknown. The site of relapse was in regional lymph nodes for five patients, local cutaneous for two patients and metastatic in four patients. A summary of relapsed treatment can be found in **Table 3**. Relapsed treatments were varied and included surgery, when feasible (five cases), but more predominately chemotherapy (seven cases) and palliative radiotherapy (five cases). Targeted therapy was used in two patients and included immunotherapy with ipilimumab and pembrolizomab in one patient and the combination of targeted therapy with the *BRAF* inhibitor dabrafinab followed by ipilimumab in the other patient.

Survival Outcomes

At the end of the follow-up period 10 patients (27%) had died of disease (**Table 3**). A total of 26 patients were still in first Complete Response (CR) and one in second CR. **Figures 1** and **2** show Kaplan-Meier curves for EFS and OS. After a median follow-up of 5.8 years (2 months – 16.5 years) the 5 year EFS and OS were 63.2 (95% CI: 40.6 – 79.1) and 67.7% (95% CI: 45.1 – 82.6) respectively.

DISCUSSION

We report here a retrospective case series of all malignant melanomas occurring in children and adolescents in Australia and New Zealand from the ANZCHOG, who were managed at tertiary pediatric oncology centers over two decades. Patients were referred from various other health professionals, including dermatologists, primary health care physicians general and plastic surgeons, often after initial surgery.

Our finding that only 37 pediatric patients were treated at tertiary pediatric oncology centers over a twenty-year period is in stark contrast with national registry data, where the total incidence of malignant melanoma among children and adolescents in Australia and New Zealand over the same time period was 1,778 across both registries. These data reveal that malignant melanoma in children and adolescents is rarely treated at pediatric oncology centers in Australia and New Zealand. In keeping with our data, a recent Italian study estimated that only one in three children and one in ten adolescents with melanoma are treated in tertiary pediatric referral centers (3). However, there is a paucity of similar data for other countries.

Another important aspect of our findings concerns the stage of the melanoma in referred patients. Our data shows that a large percentage (29.7%) of patients referred to tertiary oncology centers had stage IV disease at presentation. Our findings strongly suggest that patients are usually referred to tertiary oncology centers only when harboring advanced stages of the disease, which likely also explain the relatively high death rate of 27% that was observed. Consistent with our hypothesis, Réguerre et al., analyzed 52 cases of malignant melanoma in children and adolescents and suggested that the relatively poor prognosis noted in their cohort could be explained by having selected patients referred to expert oncology hospitals (21). Compared to other published series, patients reported by Réguerre et al. had more advanced stages or the worst initial presentations, such as metastatic relapse after the excision of a supposedly benign lesion.

For a cancer like melanoma, early diagnosis is a crucial factor determining the outcome of a patient. For both adults and children, the successful management of melanoma is stage dependent and surgical treatment alone, with adequate margins, is curative for both adult and pediatric patients who present with early-stage localized disease (22, 23). In addition, early diagnosis has also been shown to significantly improve the quality of life for patients with melanoma. For example, a study of 395 melanoma patients evaluated with the EQ-5D-5L questionnaire showed that postoperative stage I–II melanoma patients experienced better health outcomes >2 years after treatment, compared to patients with stage III melanoma (24, 25).

The morphological appearance of lesions is also of paramount significance. The presence of thick lesions (>4mm) is associated with a higher risk of disease spread, and these patients may benefit from additional chemotherapy (26). In a large pediatric series of melanoma, Brecht et al., reported that the presence of histological ulceration, nodular histology, Breslow thickness of more than 2mm and AJCC classification of III or IV, were indicative of a poor prognosis (27). Our data, although contained a relatively small number of cases, is consistent with these findings, with tumor thickness, nodular histology and advanced stage having worst survival outcomes (All six patients with known nodular histology and Breslow thickness of >2mm died of disease). However, these histological factors need to be evaluated further in larger cohorts of children and adolescents with melanoma before their true prognostic value can be evaluated. The presence of

TABLE 3 | Relapse characteristics.

Site of Primary Disease	Site of Relapse	Age at Diagnosis	Time to relapse	Histology	Breslow Thickness	AJCC Stage at Diagnosis	Therapy for Relapse	Outcome
Subungal	Metastatic (Scalp, lymph nodes)	7 years, 3 months	21 months	Nodular	3.5mm	II	Chemotherapy Radiotherapy	DOD
Trunk	Trunk	20 months	2 months	On congenital naevus	8mm	IV	Chemotherapy Radiotherapy	DOD
Ear	Metastatic (lymph nodes, Lung)	11 years, 7 months	18 months	Nodular	10mm	IV	Surgery Chemotherapy Immunotherapy	DOD
Trunk	Trunk	5 years, 7 months	10 months	On congenital naevus	7.4mm	IV	Surgery Radiotherapy	DOD
Scalp	Metastatic (lymph nodes, bone, liver)	15 years, 4 months	8 months	Superficial Spreading	3.2mm	IV	Surgery, Chemotherapy, Immunotherapy Radiotherapy	DOD
Lymph node	Lymph nodes	17 years, 3 months	2 years, 4 months	Unknown	Unknown	IV	Chemotherapy	DOD
Scalp	Lymph nodes	13 years, 6 months	1 year, 2 months	Nodular	7.5mm	II	Chemotherapy	DOD
Trunk	Lymph nodes	16 years, 2 months	9 months	Nodular	6.4mm	II	Chemotherapy Immunotherapy	DOD
Meninges	Lymph nodes	3 years, 9 months	1 year, 5 months	Unknown	Unknown	Unknown	Surgery	DOD
Trunk	Metastatic (lymph nodes, liver, bone)	1 year, 6 months	9 months	On congenital naevus	11.4mm	II	Radiotherapy	DOD

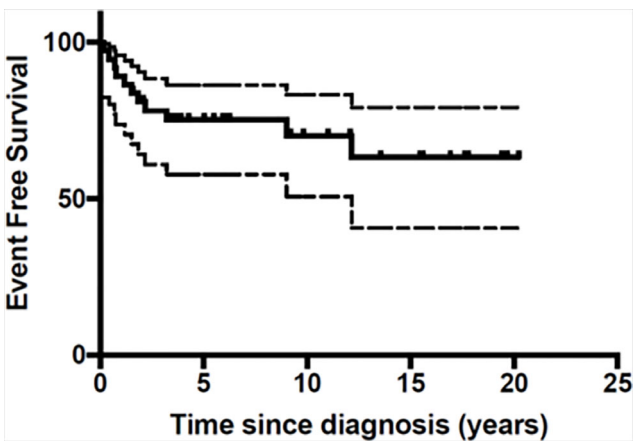

FIGURE 1 | Event Free Survival (EFS) of the 37 patients with malignant melanoma.

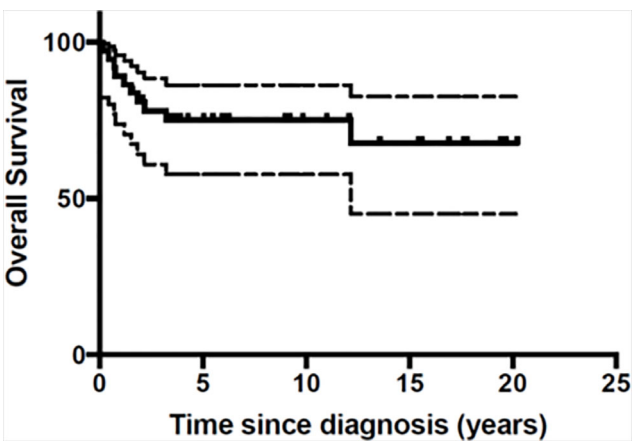

FIGURE 2 | Overall Survival (OS) of the 37 patients with malignant melanoma.

ulceration was rarely documented and its prognostic significance should be stressed in the histopathological work-up of such cases in the future. Lymph node evaluation with the use of sentinel node biopsy was not routinely documented in this cohort but would offer additional important prognostic information for childhood melanoma patients. In addition, other missing information relating to comorbidities, family history and further details of treatment such as surgical techniques and margins would have been valuable to evaluate in relation to survival outcomes. Another important aspect concerns treatment location. It is difficult to ascertain whether the location of diagnosis and treatment for children and adolescents with malignant melanoma ultimately influences outcome. It is well recognized that children and adolescents diagnosed with cancer benefit from access to a specialized multidisciplinary team with ongoing systematic clinical reviews and surveillance imaging (21, 28, 29). A recent Italian study that analyzed nationwide hospital discharge of adolescents with melanoma found that patients were dispersed across a large number of hospitals, not always in a pediatric oncology center. The study identified 418 adolescents diagnosed with cutaneous melanoma between 2007 and 2014. These patients were referred to 137 different hospitals, where they were treated in various units, such as pediatric and adult oncology, adult general surgery and dermatology. These findings highlight the need to develop better ways to manage

melanoma patients, to ensure that they are referred to an appropriate specialized clinic (28).

Given the association between childhood and adolescent malignant melanoma and the presence of an underlying cancer predisposition syndrome, any such case should be considered for referral to clinical genetics and for genetic counseling (30–32).

As in adults, changes in the appearance of a pigmented lesion should alert to the possibility of melanoma. However, the ABCDE clinical rule (asymmetry, border, irregularity, color variability and diameter >6mm and evolving), often used to identify concerning skin lesions in adults, may be difficult to apply to children (33). Common benign lesions such as Spitz nevi and benign nevi that grow as the child grows often have these clinical features. A study by Cordoro et al. showed that 60% of children aged 0 to10 years and 40% of children aged 11 to 19 years with melanoma did not present with the conventional ABCDE criteria, but rather with amelanosis, bleeding, uniform color and *de novo* development were the most common clinical presentations (33). In our cohort, while the majority of patients had pigmented lesions, a large number were described as amelanotic and associated with non-specific skin changes or bleeding. The low index of clinical suspicion for malignant melanoma in such lesions has been reported as the cause of delays or misdiagnosis in 50 to 60% of patients (21, 26).

Despite the advances in targeted therapies of adult melanoma, the genomic landscape of pediatric melanoma has only recently been explored. Only 18% of our cohort underwent analysis of *BRAF* V600E mutation, which was present in only one case. In addition, a patient in which melanoma arose from a congenital nevus was positive for an *NRAS* mutation. The limited molecular information in this study reflects the era over which many of the patients were treated; molecular analyses, especially for tumors such as melanoma, were still in their infancy and not widely available. Such molecular information is now essential and should be collected in future prospective clinical studies to fully characterize this rare childhood malignancy and to potentially guide treatment with targeted therapies a. Indeed, a study by Lu et al. provides the most comprehensive genomic analysis of pediatric melanoma to date (34) and shows that there are three distinct groups of childhood melanoma, each with a unique clinical behavior and molecular profile. The first group is the conventional melanoma that shares the histopathological and clinical features of adult melanoma, where 50 – 60% of patients harbor the *BRAF* V600E mutation and the condition rarely develops before puberty. The second group arises in association with congenital nevi, where approximately 5 – 10% of all patients with large or giant congenital nevi develop melanoma. The condition arises most often in the first decade of life and harbors *NRAS* mutations. Finally, the third group is Spitzoid melanoma, where *NRAS* and *BRAF* mutations are absent and the lesions often have a less aggressive clinical course (34).

Collectively, the data from these initial genomic studies suggest that the therapeutic targets for genotype specific melanoma in adults might be applicable to some cases of melanoma in children. What remains to be determined is the safety and efficacy of targeted therapies currently used in adults in children and adolescents with malignant melanoma. Consequently, it is critical that the molecular pathogenesis of future cohorts of pediatric melanoma lesions be evaluated to continue to resolve these important clinical issues.

Due to the rarity of malignant melanoma in young people, it has been difficult to conduct prospective clinical trials tailored to children. In addition, most adult treatment protocols are generally not accessible to children. Recent approval and early phase trials with immune checkpoint inhibitors, such as ipilimumab and nivolumab, *BRAF* inhibitors (e.g. Dabrafanib) and MEK inhibitors (e.g. Binimetinib) has begun for adolescents with advanced malignant melanoma at selected pediatric centers (35). In this study, only 2 out of the 11 children with relapsed disease were treated with immunotherapy. This was due to them being treated in an era prior to immunotherapy being an established treatment for metastatic melanoma and not due to contraindications to the use of immunotherapy.

CONCLUSION

Whilst the limited number of cases identified in this study precludes any definitive conclusions on the clinical behavior of melanoma in children and adolescents, some important observations can be made. Consistent with previous reports, the diagnosis of malignant melanoma is challenging, especially in young children as their clinical and histopathological features are poorly characterized. The cases we identified have been compared to published national cancer registry data and build on previous international studies revealing that only a small proportion of children and adolescents with malignant melanoma are managed in tertiary oncology centers (3, 21, 28). Malignant melanoma patients treated in these centers often have more advanced disease and subsequent poor prognosis.

As with many rare pediatric cancers, the diagnosis and subsequent treatment of malignant melanoma is challenging. This study confirms previous clinical and prognostic information in pediatric melanoma to support the early multidisciplinary management in Childhood Cancer Centers, in conjunction with expert adult melanoma centers, of this rare and challenging patient group (21, 25, 28). Scientific advancement together with growing collaborative efforts provide opportunities to advance understanding and treatment (34–36). Further progress involves taking advantage of sophisticated molecular analysis and application of this knowledge in the clinical setting, such that a therapeutic multi-center prospective trial, which includes the collection of tumor samples, be considered in the near future.

DATA AVAILABILITY STATEMENT

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

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Child and Adolescent Health Service- Western Australia GEKO - Governance Evidence Knowledge Outcomes. Written informed consent from the participants' legal guardian/next of kin was not required to participate in this study in accordance with the national legislation and the institutional requirements.

AUTHOR CONTRIBUTIONS

AR: conception design, data acquisition, analysis and interpretation, and manuscript drafting. CB: data acquisition. AG: data acquisition. DZ: conception designs and data acquisition. MK: data acquisition and manuscript drafting. FA: data acquisition. PD: data acquisition. SL: data acquisition. SC: data acquisition, and analysis and interpretation. TH: data acquisition, and analysis and interpretation. GM: data acquisition, and analysis and interpretation. JH: conception design, data acquisition, analysis and interpretation, and manuscript drafting. RSK: conception design and manuscript drafting. NG: conception design, data acquisition, analysis and interpretation, and manuscript drafting. All authors contributed to the article and approved the submitted version.

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REVIEW
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Are Molecular Alterations Linked to Genetic Instability Worth to Be Included as Biomarkers for Directing or Excluding Melanoma Patients to Immunotherapy?

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The improvement of the immunotherapeutic potential in most human cancers, including melanoma, requires the identification of increasingly detailed molecular features underlying the tumor immune responsiveness and acting as disease-associated biomarkers. In recent past years, the complexity of the immune landscape in cancer tissues is being steadily unveiled with a progressive better understanding of the plethora of actors playing in such a scenario, resulting in histopathology diversification, distinct molecular subtypes, and biological heterogeneity. Actually, it is widely recognized that the intracellular patterns of alterations in driver genes and loci may also concur to interfere with the homeostasis of the tumor microenvironment components, deeply affecting the immune response against the tumor. Among others, the different events linked to genetic instability—aneuploidy/somatic copy number alteration (SCNA) or microsatellite instability (MSI)—may exhibit opposite behaviors in terms of immune exclusion or responsiveness. In this review, we focused on both prevalence and impact of such different types of genetic instability in melanoma in order to evaluate whether their use as biomarkers in an integrated analysis of the molecular profile of such a malignancy may allow defining any potential predictive value for response/resistance to immunotherapy.

Keywords: melanoma, microsatellite instability, aneuploidy, tumor mutation burden, immunotherapy response

INTRODUCTION

The increasing efficacy of immunotherapy with immune checkpoint inhibitors (ICIs) has deeply changed life expectancy for different types of fatal cancer: melanoma, lung cancer, renal carcinoma, advanced squamous cell carcinoma of the head and neck or skin districts, some colorectal cancers, and refractory lymphomas (1–5). At the same time, it is widely recognized that the therapeutic indication of ICI cannot be extended to all subtypes of tumor histology since it has been observed

that majority of patients are not responsive (6). Therefore, the identification of biomarkers able to accurately predict either response or resistance to the treatment represents a crucial need in cancer immunotherapy.

Although the introduction into clinical practice of validated immuno-oncological biomarkers is currently limited by the heterogeneity of the types of specimens analyzed, because of the diversity of the used methodologies and the absence of a real sharing of the produced data, it is necessary to continue to support the efforts in conducting biomarker-driven trials (7). In recent years, multidisciplinary approaches have significantly increased the quest for an even more accurate molecular classification through the assessment of the mutational status in multiple oncogenes and tumor suppressor genes; in the immuno-oncological field, such efforts have already produced some approved tests (PD-L1 expression and microsatellite instability rates) and other advanced tests yet to be fully proven for efficacy (tumor mutation load, neoantigen pattern, intratumor T-cell infiltration rate) (5, 8–10).

Toward a holistic approach aimed at implementing precision oncology for treatment of "difficult" human cancers, should evaluation of genetic instability be included into the patients' molecular classification, probably even for the cancer types—like cutaneous melanoma—with a recognized low prevalence of such an alteration? In supporting a positive answer to this question, it has been recently demonstrated that a detailed tumor molecular profiling with identification of all low-frequency actionable alterations in pancreatic cancer—a definitely difficult-to-treat tumor—may produce a significant benefit from receiving a matched therapy (11). Before moving in this sense, we retain to firstly go through the features bringing to the classification of an unstable genome.

GENETIC INSTABILITY

The accumulation and fixation of mutations into the genome, both in the transcribed or regulatory sequences and in those apparently inactive, is one of the most important ways through which evolution is carried out (12). Excluding mutations having deleterious effects with functional consequences, the great majority of sequence variants often display an undefined role (neither harmful nor beneficial) in disease pathogenesis (13). These apparently neutral genetic variants can spread and become fixed in a population, making a large contribution to the evolutionary change in genomes. Focusing on single individuals, the establishment of germinal mutations or the accumulation of somatic mutations can lead to serious cell dysfunctions. **Figure 1** represents the main mechanisms inducing the increase of the mutations' content in cancer cells.

An accurate and articulated system of control and repair of genomic DNA integrity has evolved into the cells (14, 15). The DNA damage can be caused by genetic instability that may exist at two distinct mechanistic levels. In most cases, genomic instability is observed at the chromosomal level as whole chromosome or segmental/focal aneuploidy; in a more limited fraction of tumors, instability is observed at the nucleotide level and is revealed by the presence of alterations in particular highly repeated DNA sequences with a uniform nucleotide composition, the satellite DNA loci (16, 17). Such satellite

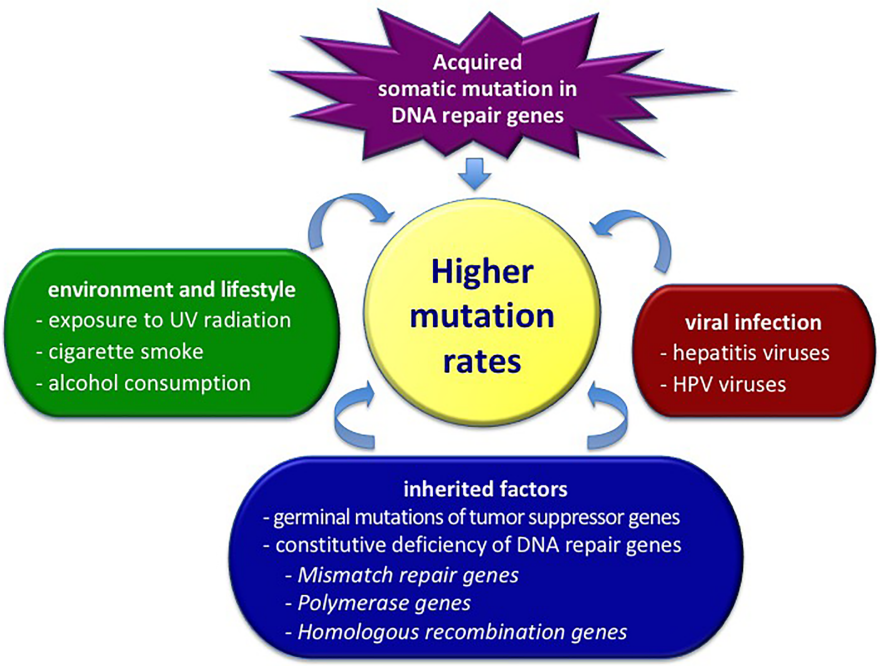

FIGURE 1 | Factors determining the total level of mutations in cancer cells. HPV, human papilloma virus; UV, ultraviolet radiation.

DNA regions are classified as minisatellite or microsatellite DNA, depending on the length of the repeated sequences (18–21). Minisatellites consist of repetitive motifs that range in length from 10 to over 100 base pairs. They are located mainly at the centromeres and at the sub-telomeric and telomeric chromosome regions (telomeres itself are constituted by tandem repeats). Minisatellites may play a role in modifying levels of transcription, alternative splicing, or imprinting changes; therefore, they can participate in cell functioning as regulators of gene expression (18, 19, 22). Microsatellites consist of tandem repeats of 1 to 6 base pairs, often organized in long strings, which are subject to mutational events such as insertions and deletions (18, 19, 21).

Aneuploidy—which is due to a genomic imbalance in terms of gain or loss of chromatid or chromosome regions—can be actually classified as a somatic copy number alteration (SCNA), being demonstrated to play a critical role during the process of tumorigenesis and prognosis (23). Occurrence of aneuploidy/SCNA seems to contribute to immune evasion through the reduction of a cytotoxic immune infiltrate into the tumor microenvironment (TME); on this regard, TME can be immunosuppressive *per se*, facilitating tumor progression through mobilization of cytokines, chemokines, and inhibitory factors (24). Moreover, the TME can also recruit immunosuppressive immune cells including regulatory T cells (TREGs), myeloid-derived suppressor cells (MDSCs), and tumor-associated macrophages (TAMs) to evade immune clearance (25). The aneuploid status may potentiate the immunosuppressive TME activity by also negatively interfering with the presentation of the antigens of the major histocompatibility complex (MHC), which represents a fundamental moment into the recognition of the tumor by the immune system (26). The content of peptide neoantigens seems to vary based on the levels of tumor SCNAs, with a relative concentration that is significantly lower in aneuploid tumors than diploid ones acting in an opposite way from the increased overall mutation load and correspondent tumor neoantigen expression levels, which are both positively correlated with the induction of cytotoxic immune infiltrates (27).

Microsatellite instability (MSI) seems to be usually due to deficient DNA damage repair; it has been associated with promotion of a higher load of tumor mutations (28, 29). The MSI occurrence (MSI+) is subsequent to impairment of at least one main gene regulating the different DNA repair mechanisms: homologous recombination (involving *BLM*, *BRCA1/2*, *BRIP1*, *PALB2*, *RAD50/51*, Fanconi Anemia genes), mismatch repair (*MLH1*, *MSH2*, *MSH6*, *PMS2*), cell cycle checkpoints (*ATM*, *CHEK1/2*), base excision repair (*POLE*) (30, 31). A high tumor mutation burden (TMB-high) is generally defined as the >10–20 mutations per megabase of genomic area (threshold is deeply varying according to the cancer type) and can somehow act as a surrogate marker of the neoantigen load (32–34). Tumor specific peptide epitopes, which are usually absent in the normal human genome, can be recognized and targeted by the immune system (33–35). Both MSI+ and TMB-high have been both associated with favorable outcome to ICI therapy in some cancer types (33, 34, 36), but their role in predicting overall survival is still controversial. Vast majority of MSI+ samples present with TMB-high (83%), but the converse is not true, since only 16% of samples with TMB-high are classified as MSI+ (37).

Overall, next-generation sequencing (NGS) analysis through a whole genome or exome screening is being used for detecting the high-level SCNAs, the MSI+ status, and the TMB-high in tumor tissues. The MSI+ and TMB-high conditions have been associated with the long-term response to ICI treatment in different human malignancies—including melanoma, lung and renal/bladder cancer, head and neck squamous cell carcinoma (38–45). Conversely, occurrence of aneuploidy/SCNA negatively correlates with the presence of a favorable immune signature, conferring resistance to ICI treatment (26). **Figure 2** summarizes the effects exerted by the different conditions on the activity of the immune system.

Although additional factors are involved in augmenting the adaptive immunity under ICI therapy—such as the histocompatibility leukocyte antigen (HLA) evolution pattern and tumor-infiltrating lymphocyte (TIL) reactivity (27), the simultaneous assessment of the SCNA burden and the rates of TMB and MSI in tumor tissue sections might be strongly useful for classifying patients who are more or less likely to respond to immunotherapies (46). Despite such recognized predictive values, the NGS-based test was not yet routinely included in

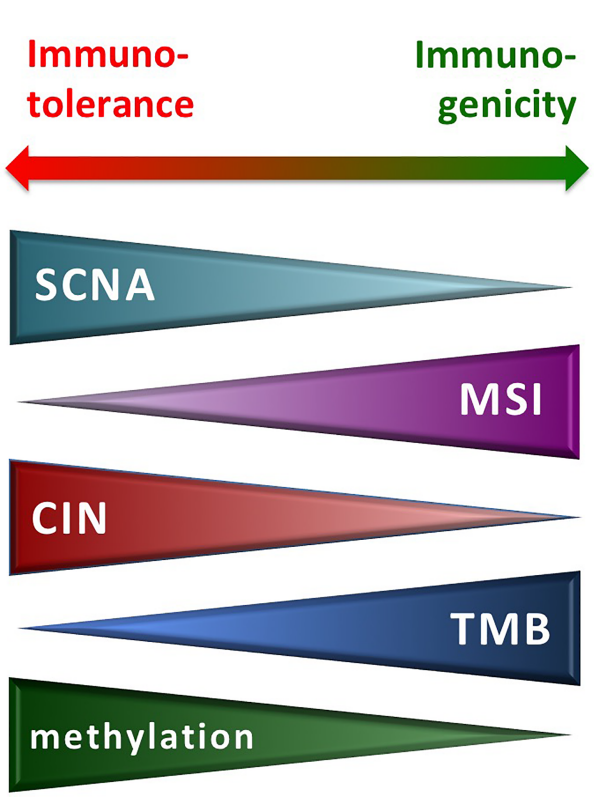

FIGURE 2 | Molecular alterations from genetic instability and immune reactivity. CIN, chromosomal instability; MSI, microsatellite instability; SCNA, somatic copy number alteration; TMB, tumor mutation burden.

clinics due to the required high level of technical expertise, the lack of standardization, the high cost, and the pretty-long time required to perform an extensive genomic screening (47, 48). Recently, the combination of reducing the costs of NGS technologies and developing large but manageable multi-gene panels has contributed to facilitate continuous implementations for the use of NGS-based assays in daily clinical practice (49). In other words, the aim of simplifying the sequencing of multiple genes per tumor sample, in order to detect targetable genomic alterations, is becoming a reality and NGS is presenting a really good analytical validity, with an increasingly favorable cost–benefit ratio. To achieve the most currently accurate molecular classification for guiding treatment decisions among cancer patients, recommendations on how multi-gene NGS assays should be used to profile human tumors for improving patients' management are being provided by scientific societies (50).

Aneuploidy: Mechanism and Effects

Aneuploidy can be mostly considered as the result of the impairment of the cell cycle checkpoints, which consist of mechanisms that verify DNA replication accuracy and control the cell cycle progression, detecting errors in DNA repair, DNA synthesis, and chromosome segregation (51). Occurrence of structural alterations significantly affecting the genome integrity constitutes a signal sent to the replication/segregation machinery in order to repair the damage (52).

Several cyclin-dependent kinases (CDKs) physiologically drive cell division and regulate the different phases of the cell cycle through phosphorylation of a complex network of substrates and activation of cascades of transduction signals (53). In case of genomic DNA damage, the cell cycle checkpoints arrest the G1/G2 and G2/M transitions by repressing the CDK activity. Hyperactive CDKs, caused by mutations in genes controlling the DNA damage response pathway, lead to the progression into the cell cycle and cell survival (52). On this regard, inactivating mutations in TP53 gene have a permissive role, strongly contributing to the propagation of genetic errors in descendant daughter cells (54). As consequence, deregulation of the TP53-driven pathway—also including impairment of the activity of its downstream effectors (*i.e.*, RB1)—contributes to aneuploidy (55). A number of cancers with mutated TP53 are chromosomal stable and show MSI+, whereas TP53 loss-of-function is predominant in non-hypermutated tumors (54–56). Indeed, the TP53 inactivation is mostly dependent on whether or not mutations in this gene affect the function of p53 on repressing the activity of the Cyclin D1–CDK2 system controlling centrosome duplication and preventing aneuploidy (53).

Activating mutations in oncogenes (such as *CCND1*, *EGFR*, *PIK3CA*, *KRAS*, *BRAF*) and inactivating changes in tumor suppressor genes—like *RB1*, *APC*, and WNT signaling pathway components (*CHK1* and *CHK2-BRCA1*)—can dramatically enhance cell proliferation and increase the replication stress levels, causing double-strand breaks in the DNA, with consequent genomic instability that affects tumor progression (57). This seems due to the fact that the unbalanced activity of the driver genes involved in promotion of cell proliferation and survival leads to a sort of oncogene-induced mitotic stress status (58). The enormous variation of segregation errors among different malignancies is indeed a strong indicator that mitotic events act as important players in aneuploidy occurrence (59). Deregulation of the centrosome duplication may indeed promote the formation of multiple centrosomes, which in turn leads to multipolar spindles and aneuploidy (58, 59). Molecular alterations favoring instability of centromeres can thus lead to chromosome segregation defects.

Actually, assessment of aneuploidy is mostly based on measuring SCNA rates in malignancies through bioinformatics approach, the allele-specific copy number analysis of tumors (ASCAT), using data generated by whole-genome/exome sequencing strategies (60). The rates of intratumor karyotype heterogeneity can accurately be determined by simultaneous estimation of the allele-specific total copy number after adjusting for both tumor ploidy—including gains, losses, copy number-neutral events, and loss of heterozygosity (61).

Individual chromosome arm-level alterations were found to be related to expression changes in immune and cell-cycle markers, independent of aneuploidy level; however, increased arm- and chromosome-level SCNA burdens were associated with proliferation signatures and immune evasion profiles (62). Moreover, tumor aneuploidy is likely to increase intratumor heterogeneity, which may inhibit tumor immunity (63). Many solid cancers presenting with a high somatic copy alteration burden exhibit features of immune exclusion, whereas tumors displaying low rates of aneuploidy present an immune active profile (26, 27, 64). High-level SCNAs are classified through bioinformatic approaches as events where focal copy number gain (or loss) are higher (or lower) than the maximum (or minimum) median arm-level copy number gain (or loss), hence avoiding artifacts or false positives after comparison with low-level SCNAs linked to the ploidy of tumor samples and thus obtaining more reliable thresholds (65, 66). High-level SCNA profile in activating beta-catenin signaling pathway elements including *CTNNB1*, *APC*, and *AXIN1-2* genes has been reported in metastatic melanoma but not in primary melanoma (67). A significantly higher concordance between mutated SCNA profiles in beta-catenin signaling pathway activated samples with a low level of T-cell tumor inflammation has been demonstrated, thus suggesting that SCNA signature may act as a progression marker in advanced melanoma (67). For its prediction of the T-cell-inflamed gene expression signature, the SCNA score is worth to be included in molecular tests aimed at somehow anticipating probabilities of resistance to immunotherapies. Further supporting this, the SCNA level has been found lower in lung cancer patients with a responsive disease than those with stable or progressive disease under ICI treatment (68).

Finally, SCNAs can be intrinsically linked to complex structural variants (CSVs) in affecting the efficacy of ICI treatment in melanoma. In particular, CSVs—which are represented by deletions, duplications, translocations, or

inversions and arise through the breakage and fusion of one or two genomic locations—are particularly reported in acral melanoma (69). In bioinformatic analysis of NGS-generated data, SCNAs and CSVs are detected as changes in sequencing read depth and in junction-spanning read pairs across the candidate genomic loci (70).

Microsatellite Instability

MSI is characterized by small insertions or deletions within short tandem repeats in tumor DNA when compared with the corresponding normal DNA. In other words, regions that contain sequences of repeated nucleotides are intrinsically unstable and the insertion of inappropriate nucleotide(s) or the slippage events during DNA replication give rise to the insertion or deletion of single bases or small tandem DNA sequences (56). These alterations, which are normally recognized and repaired, in the absence of an efficient MMR function, are maintained giving origin to alleles of different sizes during the successive replication cycles. The accumulation of unpaired alleles is at the basis of such a genome-wide genetic instability, which is recognized as MSI+ phenotype and observed at higher prevalence in gastrointestinal and endometrial cancers (37, 44, 56, 71). **Table 1** report frequencies of MSI+ in different tumor types, as inferred taking into the consideration the main published studies (72–76).

In colorectal carcinoma (CRC), the MSI+ phenotype has been long evaluated for its impacts on disease pathogenesis and behavior as well as for correlations with prognostic effects. While some distinct clinical and pathological features (proximal location, poor differentiation, mucinous histology) have been consistently associated with the occurrence of MSI, more controversial data have been produced on the prognostic role of this alteration (77). In early stage CRC, the MSI+ phenotype has been described in patients with a better prognosis; conversely, detection of unstable microsatellites seems to confer a negative prognosis in patients with metastatic disease (77–79).

MSI reflects a defect in genes involved in DNA replication fidelity and mostly, is due to inactivation of the mismatch repair (MMR) genes (29, 31). The MMR genes may be impaired by inactivating or down-regulating genetic mutations as well as by gene-silencing epigenetic changes (80). The result of such alterations is the expression of normal levels of functionally deficient MMR proteins or lack of the MMR protein expression, both conditions progressively inducing genetic instability and somehow providing a selective advantage during neoplastic transformation and progression (80). The important components of the DNA mismatch repair system are represented by seven specific ATP-binding proteins that work coordinately in sequential steps to initiate repair of DNA mismatches in genomic DNA: MLH1, MSH2, MLH3, MSH3, MSH6, PMS2, and PMS1 (81). Inactivation of MLH1 and MSH2 was detected in more than 85% of the MSI+ tumors (80, 81). Nearly all MMR genes contain a mononucleotide repeat and thus represent the first target of inactivating mutations when the MSI+ phenotype coexists (71).

The real breakthrough in defining a more impacting role of the MSI in the clinic practice for the management of neoplastic patients has been registered in 2017, when the U.S. Food and Drug Administration (FDA) granted approval of an immune checkpoint inhibitor (the anti-PD-1 pembrolizumab) for treatment of patients with cancers carrying MSI or deficient-MMR (82). The approval by FDA of the anti-PD-1 treatment for all advanced MSI+ solid tumors still represents the first regulatory authorization based exclusively on the use of a specific biomarker, regardless of the anatomic location in the body where the tumor originated ("tumor agnostic") (83). The MSI and the mutation load underlie the response to PD-1 blockade immunotherapy in deficient-MMR human tumors; the extent of response seems to be particularly associated with the accumulation of insertion-deletion (indel) mutational load (84). In a recent meta-analysis of patients with MSI+ cancer, the ICI treatment was significantly confirmed to be associated with high activity independent of tumor type and drug used and MSI status assessment may have a predictive value for the selection of patients to be addressed to immunotherapy (85).

Epigenomic studies have shown that tumors with MSI exhibit hypermethylation of key genes implicated in tumor development (75, 86). The hypermethylated promoters were identified in some genes that regulate some main molecular signaling cascades (75, 76, 87): WNT (in the absence of WNT-signals, β-catenin—a key downstream effector of this pathway—is targeted for degradation through phosphorylation; the WNT signals thus stabilize the intracellular levels of β-catenin and subsequently increase transcription of downstream target genes in many human cancers), hedgehog (essential for embryonic and postnatal development, this pathway remains in the quiescent state in adult tissues but gets activated upon inflammation and injuries), and PTEN (its inactivation through mixed genetic/epigenetic mechanisms results in persistent activation of PI3K effectors, with an important impact on cell proliferation, apoptosis resistance, angiogenesis, metabolism regulation, genomic

TABLE 1 | MSI+ frequency in different tumor types.

Cancer	Number	MSI+	%
Endometrial carcinoma	1426	401	28.1
Gastric adenocarcinoma	573	117	20.4
Colorectal adenocarcinoma	1,456	196	13.5
Thyroid carcinoma	584	18	3.1
Hepatocellular carcinoma	375	11	2.9
Kidney renal clear cell carcinoma	278	6	2.2
Cutaneous melanoma	359	7	1.9
Ovarian carcinoma	63	1	1.6
Prostate adenocarcinoma	463	3	0.6
Lung nonsquamous cell adenocarcinoma	480	3	0.6
Head and neck squamous cell carcinoma	506	3	0.6
Lung squamous cell carcinoma	443	2	0.5
Urothelial carcinoma	253	1	0.4
Glioblastoma	262	1	0.4
Glioma	513	1	0.2
Kidney papillary cell carcinoma	207	0	0.0
Breast carcinoma	266	0	0.0
TOTAL	**8,507**	**771**	**9.1**

Total numbers and percentages were obtained summing data from literature (see text for references).

instability, cellular senescence, and cell migration). The hypermethylated status is also tightly correlated with the occurrence of somatic mutations in *BRAF* oncogene, overall causing a strong inhibition of the senescence mechanisms and a consequent promotion of an uncontrolled cell proliferation and survival (88, 89). Hypermethylation has also been related to the facilitation of tumor escape by repressing transcriptional expression of interferon (IFN) regulatory factors (90). Indeed, demethylating agents and histone deacetylases are being combined with ICI treatments in numerous clinical trials and types of malignancies (91, 92).

Several additional factors, other than those mainly underlying MSI, have been shown to be involved in determining a hypermutated status, such as inactivating mutations in the DNA polymerases as well as exposure to external (cigarette smoke, UV radiation, chemicals) and endogenous (reactive oxygen species) mutagens (93, 94). The hypermutated condition may be related to driver mutations in the DNA *polymerase* ϵ (*POLE*) and $\delta 1$ (*POLD1*) genes among different tumor types, including colorectal, endometrial, and other cancers such as melanoma and lung cancer (95, 96). Deleterious mutations in *POLE*/*POLD1* genes compromise proofreading of genomic DNA during cell replication and the timing of their onset may vary, with constitutional defective MMR followed by acquired secondary *POLE*/*POLD1* defects or *vice versa* (97). It has been shown that the presence of mutations in *POLE* may promote a high level of non-synonymous single-nucleotide variations (ns-SNVs), not tightly associated with the presence of a MSI+ phenotype (the highest mutation rates were observed in MSS tumors) (71). The *POLD1* gene has been found silenced in several cancer types—mostly, in conjunction with a defective *POLE* gene—with increased genome instability and DNA damage effects (98–100). POLD1 is involved in different forms of DNA repair induced by exposure to mutagens, including nucleotide excision repair, double strand break repair, base excision repair, and mismatch repair (101). The coexistence of MSI+ and mutated *POLE* may be associated with higher densities of CD8+ TILs, PD-1-expressing CD8+ TILs, and tumor-infiltrating immune cells with a Th1 phenotype in the TME, strongly predicting response to checkpoint inhibitors (102).

As mentioned above, tumors with the hypermutated status present similar sensitivity to ICI. Indeed, a strong correlation was found between increased load of non-synonymous mutations and clinical benefits to PD-1 inhibition in non-small cell lung cancer (39) or to cytotoxic T-lymphocyte antigen T 4 (CTLA-4) blockade in melanoma (103). Considering such reported outcomes, one can speculate that increased production of neoepitopes predicting response to ICI might be even generated in cohorts of patients with low (<10% of case) or very low (<1%) prevalence of MSI (**Table 1**).

The hypermutated status can be actually defined with more extensively detailed approaches such as NGS or mass spectrometry assays (104). Among strategies not requiring to match normal DNA material, the single-molecule molecular inversion probe (smMIP) assay is able to detect the existence of an impaired intracellular capability of correcting smMIP-induced errors (105). All these screening strategies are useful in a research context, but technically difficult to translate into clinical practice for routine diagnostic application, since either requiring an extensive bioinformatics analysis of the obtained results either remaining still expensive methods (48—50). Conversely, a simple method to directly detect MSI on formalin-fixed paraffin embedded tumor tissue sections is represented by the Idylla™ test, a fully automated PCR-based assay including a high-resolution melting curve analysis. The Idylla™ MSI test is able to detect mutations in seven tumor-specific MSI loci (ACVR2A, BTBD7, DIDO1, MRE11, RYR3, SEC31A, and SULF2), not requiring the analysis of paired normal tissue samples. For more extensive and detailed information about the methodologies aimed at investigating the MSI status, one can refer to the recent report from our group (106, 107).

The contextual assessment of the MSI+ phenotype and the hypermutated status may be strongly indicative for the existence of a higher tumor immunogenicity, though none of the alterations described as immediate biological effects of the MSI+ phenotype and the hypermutated status—the mutation load, the neoantigen prediction, and the intratumor immune cell infiltration rate—may be considered as a reliable predictor of response to anti-PD-1 treatment (108). Several additional molecular factors are suggested to be involved in immune response. Occurrence of mutations inactivating *JAK1*—within the JAK-STAT pathway that regulates different cellular processes—has been reported to confer resistance to the anti-PD-1 treatment by reducing both the PD-L1 expression and the ability to promote the IFN-γ driven response (109, 110). The relationship between such *JAK1* mutations and MSI status is however complex. In patients with tumors characterized by a low prevalence of MSI—including cutaneous melanoma, invasive breast cancer, and prostate adenocarcinoma—deleterious *JAK1* mutations are associated with unfavorable prognosis (109, 110). In MSI+ tumors, *JAK1* silencing seems to instead impair the tumor growth, playing a positive prognostic role (109, 110). This further confirms that often the same molecular alterations occurring in different tumor types have a distinct impact on biological behavior according to the different genetic backgrounds.

Classification of Melanoma Patients for Genetic Instability

According to their mutational status inferred by NGS analysis at somatic level, one could classify melanoma patients using:

- "qualitative" parameters, aimed at discriminating all classes of sequence changes or structural alterations (non-synonymous single-nucleotide variants/ns-SNVs, indels, copy number variations/CNVs, fusions, and splice variants) in tumor suppressor genes and/or oncogenes. These alterations occur at high frequency in melanoma samples. Research efforts should be aimed at defining the clinical role of the distinct mutational patterns of driver ns-SNVs as well as whether the increased load may rather represent the consequence of the sequential accumulation of "passenger" mutations in specific pathways during disease progression;
- "quantitative" parameters, aimed at defining the above described threshold-depending parameters representing the

main immuno-oncology content (SCNA, MSI, and TMB). These alterations occur at low frequency in melanoma samples (**Figure 3**).

Most of such key features are actually achieved using large NGS-based panels, which usually include over 400 unique driver genes in correspondent genomic loci for the achievement of a comprehensive and simultaneous genomic profiling (**Table 2**).

MSI Detection on Liquid Biopsies

In cancer patients, the assessment of PD-L1 status in circulating tumor cells (CTC) and the determination of specific somatic mutations in circulating tumor DNA (ctDNA) represent non-invasive tools acting as predictive markers of the efficacy of the therapeutic response to ICI. The technology for CTC isolation is not widely available, whereas genomic analyzes on ctDNA are methodologically feasible. In NSCLC, undetectable ctDNA levels

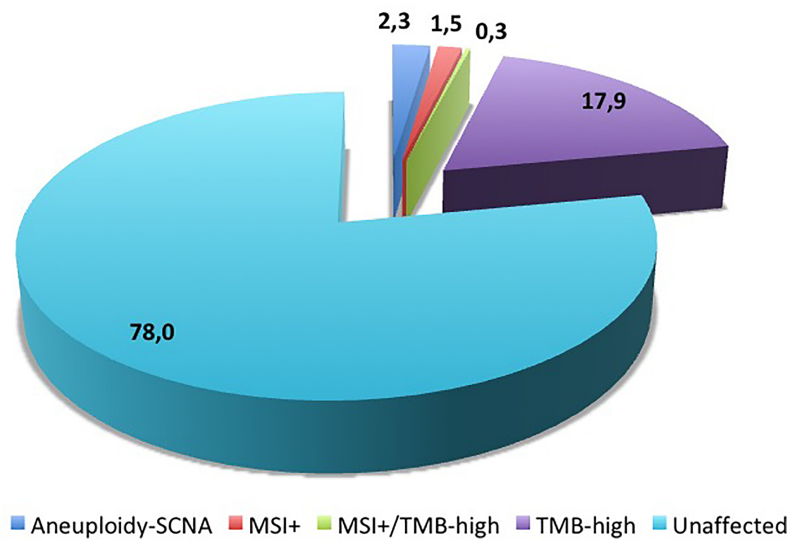

FIGURE 3 | Distribution of molecular alterations linked to genetic instability in melanoma samples. Numbers indicate the percentages of cases reported in literature (see text for references).

TABLE 2 | Molecular alterations underlying genetic instability useful in cancer patients' stratification for immunotherapy.

Type	Detection method	Identified alteration
SCNA	whole genome sequencing (WGS)	gene/locus gain or loss
	whole exome sequencing (WES)	copy number variation
	targeted multiple-gene NGS assays (panels)	complex structural variants
		loss of heterozygosity (LOH)
MSI	Bethesda panel assay (5 microsatellite loci)	genome-wide instability
	≥ 2 unstable markers (different microsatellite lengths between tumor and normal samples)	
	extended Bethesda panel (8 microsatellite loci and 2 homo-polymer markers: BAT25, BAT26, BAT40, D5S346, D17S250, D2S123, TGFB, D18S58, D17S787, D18S69 or BAT25, BAT26, BAT40, D2s123, D10s197, D13s153, D17s250, D18s58, D5s346, Mycl)	genome-wide instability
	≥30% unstable markers	mutations in seven MSI loci (ACVR2A, BTBD7, DIDO1, RYR3, MRE11, SEC31A, and SULF2)
	real-time PCR by Idylla™ MSI Test	
	≥ 1 mutated locus	
dMMR	protein expression by immunohistochemistry	lack of MMR protein(s)
	targeted multiple-gene NGS assays	mutations inactivating MMR genes (MLH1, MSH2, MLH3, MSH3, MSH6, PMS2, PMS1)
CIN	comparative genomic hybridization (CGH) fluorescence in-situ hybridization (FISH)	whole chromosome or segmental/focal aneuploidy
	gene fusion (mRNA) microarrays	
TMB	whole exome sequencing	mutations per megabase of genomic area
	targeted multiple-gene NGS assays	mutations inactivating DNA polymerases (POLE, POLD1)
Methylation	whole genome methylation	genome-wide DNA methylation with RRBS
	gene promoter methylation	methylation levels of candidate gene promoters

SCNA, somatic copy number alteration; MSI, microsatellite instability; dMMR, deficient mismatch repair; CIN, chromosomal instability; TMB, tumor mutation burden; NGS, next-generation sequencing; RRBS, reduced representation bisulfite sequencing.

after two months of ICI were demonstrated to be associated with a marked and lasting response to therapy, while an increase in ctDNA load after initiation of ICI was associated with poorer survival (111, 112). In melanoma, detectable ctDNA at baseline and post-surgical tumour removal may predict a shorter median disease-specific survival among stage III melanoma patients (113, 114) as well as detection of persistent or increasing ctDNA levels during follow-up was shown to predict worse prognosis when compared to patients with undetectable or falling ctDNA levels (115, 116). Currently, plasma-based commercially available assays ("liquid biopsies") can be used to assess the MSI or the mismatch repair deficiency (dMMR) through genomic analysis by realt-time PCR or DNA sequencing assays in a large variety of cancer types (117–119). From the practical point of view, the real-time PCR is mainly based on the Idylla™ MSI assay (Biocartis, Bruxelles, Belgium; catalog n. A0101/6), which includes a set of seven MSI biomarkers consisting of short homo-polymers located in the above mentioned genes. The NGS tests on ctDNA are performed using complex multigene panels (*i.e.* the Oncomine Comprehensive Assay Plus panel, which provides highly multiplexed target selection of >400 genes implicated in cancer pathogenesis, carried out on the Ion GeneStudio S5 System) (120). These NGS-based tests are now feasible in clinical practice and they have very high concordance, sensitivity and specificity and a detection limit of 0.1% tumor content for MSI-H status. Moreover, such panels allow identification of further genomic alterations (*i.e.* the tumor mutation burden or TMB) with potential implications for predicting response to immunotherapy.

CONCLUSIVE REMARKS

Considering the steadily increasing advances in the knowledge of the molecular mechanisms underlying the genetic instability at the chromosomal and nucleotide levels as well as the recognized ascertainment of their clinical impact on cancer management, selection of the subgroups of patients according to the type of instability (SCNA+ *vs.* SCNA–, MSI+ *vs.* MSI–) or mutational composition (TMB-high *vs.* TMB-low; neoantigen-high *vs.* neoantigen-low) present is becoming mandatory. Further advancements will be however achieved by increasing correlations between such molecular features—through a continuous dissemination of the methodologies to be used for their assessment into the clinical practice—and all disease-related and therapy-dependent parameters. These efforts should facilitate the development of innovative diagnostic, predictive, and/or prognostic tools for a better molecular classification of cancer patients, even in a malignancy like melanoma with lower rates of such alterations. Nevertheless, more extensive applications of the NGS technologies could improve the assessment of all driver alterations putatively acting as disease markers to be transferred into the daily clinical practice.

AUTHOR CONTRIBUTIONS

All authors contributed to the conception, design, and writing of the manuscript. All authors contributed to the article and approved the submitted version.

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SYSTEMATIC REVIEW
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Incidence of Skin Cancer in Patients With Chronic Inflammatory Cutaneous Diseases on Targeted Therapies: A Systematic Review and Meta-Analysis of Observational Studies

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Cancer is one of the several comorbidities that have been linked with chronic cutaneous inflammatory diseases namely psoriasis/psoriatic arthritis and hidradenitis suppurativa. Although the chronic inflammatory state, typical of the diseases, may induce pro-tumorigenic effects, the debate whether or not the drugs currently used in clinical practice do in facts increase a patient's risk of malignancy remains largely unsolved. The therapeutic armamentarium has been greatly enhanced at least in the last two decades with the advent of biologics, a heterogeneous group of laboratory-engineered agents with more in the pipeline, and other targeted small molecules. Among the organ systems, skin results as one of the most commonly affected, non-melanoma skin cancers being the main drug-induced manifestations as side effect in course of these treatments. The objective of the study is to systematically review the cutaneous malignancy risk of the newer therapies through an overview of meta-analyses and observational studies on the topic.

Keywords: skin cancer, non-melanoma skin cancer, melanoma, biologics, psoriasis

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INTRODUCTION

Psoriasis, psoriatic arthritis and hidradenitis suppurativa are three common inflammatory and immune-mediated skin diseases characterized by increased levels of pro-inflammatory cytokines and chemokines such as tumor necrosis factor (TNF)-α, interleukin (IL)-17 and IL-23 (1–7). Chemical inflammatory mediators involved in the pathogenesis of these diseases may increase the

risk of malignancies through the induction of pro-cancerous mutations, adaptive responses, resistance to apoptosis and environmental changes such as the stimulation of angiogenesis (8, 9). A number of observational studies suggested that patients affected by these diseases are at increased risk of developing cancer (10–13). In particular, increased rates of cancer, especially keratinocyte skin cancer and lymphomas were reported in patients with psoriasis or psoriatic arthritis (14). A significantly increased risk of overall cancer was observed also among patients affected by hidradenitis suppurativa in a recently published population-based cohort study (15).

The recent marketing of systemic biological (i.e. the TNF-α inhibitors etanercept, infliximab and adalimumab, the anti-IL-12/23 ustekinumab, the IL-17/IL-17 receptor antagonists secukinumab, ixekizumab and brodalumab and the anti-IL-23 agents tildrakizumab, guselkumab and risankizumab) and chemically synthetized drugs (e.g. apremilast and tofacitinib) as targeted therapies has improved the management of these diseases (16–18). However, since these drugs target molecules that may be relevant to cancer immunosurveillance mechanisms, some concerns were raised about their association with an increased risk of cancer occurrence (19–23). A recent meta-analysis of randomized clinical trials (RCTs) and open-label extension (OLE) studies reported that TNF inhibitors are associated with an increased risk of non-melanoma skin cancers (NMSC) in people with psoriasis. However, the authors of this study found that no real-world evidence was available and acknowledged the significant limitations associated with the study design of the articles included, that make it difficult to extrapolate to real-world practice (24). Evidence on the risk of skin cancer in patients with chronic inflammatory cutaneous diseases on targeted therapies is still sparse controversial. Therefore, the aim of this systematic review and meta-analysis was to assess the risk of cutaneous malignancies in patients with psoriasis, psoriatic arthritis or hidradenitis suppurativa treated with targeted therapies.

METHODS

Search Strategy and Study Selection Criteria

This systematic review and meta-analysis was conducted in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement, following an *a priori*-established protocol registered on the International Prospective Register of Systematic Reviews (PROSPERO: CRD42020212137). The completed PRISMA checklist is provided in **Supplementary Figure 1**. Two authors (SC, FC) independently searched the bibliographic databases PubMed and EMBASE for literature related to the risk of skin cancer in patients affected by inflammatory cutaneous diseases and treated with targeted therapies. Literature was searched from databases inception until 15th September 2020. The search strategy concerned terms related to inflammatory cutaneous diseases (i.e. psoriasis, psoriatic arthritis and hidradenitis suppurativa), skin cancers (e.g. squamous cell carcinoma, basal cell carcinoma and melanoma) and targeted therapies (i.e. etanercept, infliximab, adalimumab, ustekinumab, secukinumab, ixekizumab, brodalumab, tildrakizumab, guselkumab, risankizumab, apremilast and tofacitinib). Citations, titles and abstracts were exported into Endnote X9. The detailed literature search strategy for different databases is provided in **Supplementary Table 1**. Original observational studies were included if they (a) included patients affected by psoriasis, psoriatic arthritis or hidradenitis suppurativa; (b) clearly reported a well-defined measure of skin malignancies incidence; (c) included patients treated with biological drugs and/or the small molecules, apremilast and tofacitinib; (d) were written in English. To reduce the risk of publication bias, conference abstracts were also eligible for inclusion. Narrative or systematic reviews, meta-analyses, book chapters, editorials and pooled analyses were not included, but the reference lists in reviews and meta-analyses were screened to potentially identify further studies to include.

After duplicate studies were removed, two authors (SC and FC) individually reviewed titles and abstracts to remove clearly irrelevant articles and, subsequently, full text of the articles that both reviewers considered potentially eligible. Any inconsistencies were resolved at this stage through discussion or the intervention of a third independent assessor (GT or CG).

Data Extraction

For eligible studies, information on the following items was independently collected by the same two authors and stratified by skin cancer type: study authors, year of publication, catchment area, data source, study population, study years, study design and risk estimate. Any disagreements were resolved by consensus with a third author (GT or CG).

Assessment of Risk of Bias and Overall Quality of the Evidence

The risk of bias of the observational studies included in this systematic review was independently assessed by two authors (SC and FC) using the Newcastle-Ottawa quality assessment scale (25). This instrument consists of eight different domains for cohort studies (representativeness of the exposed cohort, selection of the non-exposed cohort, ascertainment of exposure, demonstration that outcome of interest was not present at start of study, comparability of cohorts on the basis of the design or analysis, assessment of outcome, follow-up long enough for outcomes to occur, adequacy of follow up) and case-control studies (adequate case definition, representativeness of the cases, selection of controls, definition of controls, comparability of cases and controls on the basis of the design or analysis, ascertainment of exposure, same method of ascertainment for cases and controls, non-response rate). The included studies were categorized as "low risk of bias" if at least six of the eight domains were judged to be at low risk of bias.

Statistical Analysis

For each included study, skin cancer incidence rates (IR) per 10,000 person-years (PY) were considered as the primary

outcome for the meta-analysis. Meta-analysis of IRs was performed assuming that the logarithm of each study-specific rate was normally distributed and the corresponding standard error, used to perform the inverse-variance weighting, was computed from the 95% CI (or p-value) reported in the original IRs. Between-study heterogeneity of the estimates was assessed using the Cochran's Q-test (26) along with its derived measure of inconsistency (I^2), and was considered to be present when Cochran's Q-test p-value was < 0.10 or I^2 > 40% (27). Estimates were summarized by fixed-effects or random-effects models, according to the absence or the presence of heterogeneity, respectively. It is generally accepted that when there are fewer than ten studies in a meta-analysis, both meta-regression (27) and test for publication bias (28) should not be considered. Both the study specific as well as the pooled epidemiological estimates, were graphically depicted, with their 95% CI, on a forest plot. Analyses were stratified for specific skin cancer types, i.e. NMSCs and melanoma. If a study presented more than one estimate, the most recent one was used. Two-sided p-values<0.05 were considered for statistical significance. All calculations were carried out using R Foundation for Statistical Computing (version 4.0, package: metafor).

RESULTS

Characteristics of the Studies Included

The original electronic search yielded 1762 (1549 after removing duplicates) papers potentially relevant for this review (**Figure 1**). After removing duplicates, 1549 were initially screened. Of these, 1467 were excluded after the screening of study titles and abstracts. The remaining 82 studies were retrieved for more detailed evaluation and 10 of them met the review inclusion criteria. The main characteristics of the included studies are reported in **Table 1**. Most of the included studies were prospective cohort studies (N= 5; 50.0%) (33–36, 38), three (30.0%) (29, 31, 32) were retrospective cohort studies, one was

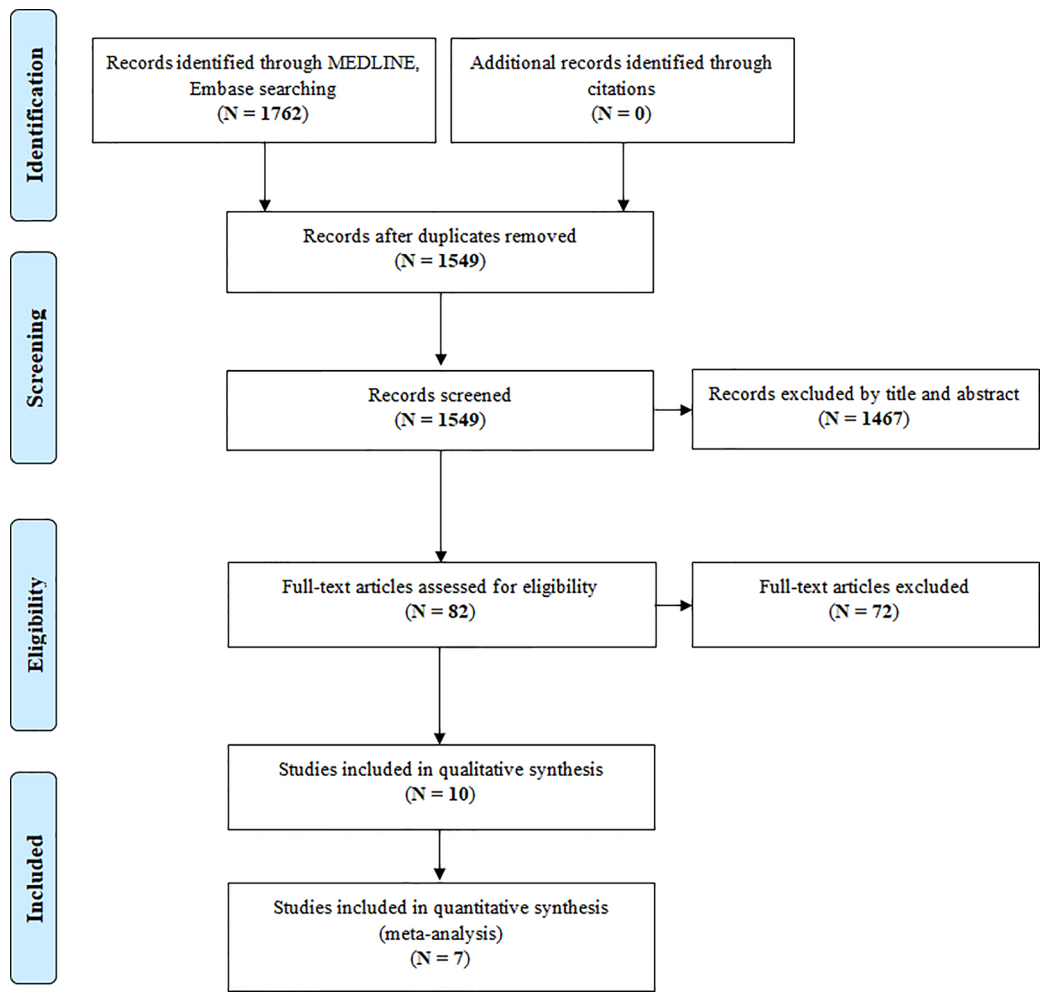

FIGURE 1 | PRISMA flow-chart showing the process of literature search and study selection.

TABLE 1 | Characteristics of the studies included in the systematic review.

Reference	Catchment area	Data source	Study population	Study drugs	Study years	Study design	IR per 10,000 PYs [95%CI]
Non-melanoma skin cancer							
29	California (USA)	Kaiser Permanente Northern California (KPNC)	All KPNC members aged ≥ 18 years, diagnosed with psoriasis between 1998 and 2011 and treated with a systemic antipsoriatic agent	Adalimumab, etanercept, infliximab, ustekinumab	1998-2011	Retrospective cohort study	120 [98-143]
30	USA	US Truven MarketScan database	Patients with moderate to severe PsA, defined by ≥1 inpatient or ≥2 outpatient 696.0 diagnosis codes on 2 unique calendar days	Adalimumab, etanercept, infliximab, apremilast	2010-2015	Clinical trial and real-world data comparison	149.3 [116.5-182.0]
31	United Kingdom	British Society for Rheumatology Biologics Register + National cancer and death registers	All patients diagnosed with PsA starting a TNF-inhibitor and registered in the British Society for Rheumatology Biologics Register	Etanercept, adalimumab, infliximab	2002-2012	Retrospective cohort study	N.A.
32	USA	Market-Scan® database and Medicare	Patients with a diagnosis of psoriasis, with the first outpatient qualifying ICD-9 CM code	Etanercept Adalimumab Infiximab	2005-2009	Retrospective cohort study	185.8 [160.2-211.42]
33	USA, Canada, Germany, France, Czech Republic, Greece, Netherlands, Spain, UK, Austria, Denmark, Ireland, Sweden	ESPRIT Registry	Patients aged ≥ 18 years of age with chronic plaque psoriasis who had been prescribed adalimumab	Adalimumab	2008-2015	Prospective cohort study	62 [52-72]
34	Canada	OBSERVE-5 surveillance registry	Adult patients with moderate to severe psoriasis initiating etanercept	Etanercept	2006-2012	Prospective cohort study	125 [60-240]
34	USA	OBSERVE-5 surveillance registry	Adult patients with moderate to severe psoriasis initiating etanercept	Etanercept	2006-2012	Prospective cohort study	262 [220-310]
35	Germany	The German Psoriasis Registry PsoBest	Adult patients with moderate-to-severe psoriasis at the time point of a new drug to be started	TNF-α inhibitors	2008-2012	Prospective cohort study	38 [12-90]
35	Germany	The German Psoriasis Registry PsoBest	Adult patients with moderate-to-severe psoriasis at the time point of a new drug to be started	Ustekinumab	2008-2012	Prospective cohort study	24 [10-136]
36	The Netherlands	Radboud University Nijmegen Medical Centre pharmacovigilance registry	Patients starting biological treatment for psoriasis in the Dermatology outpatient clinic of the Radboud University Nijmegen Medical Centre	Etanercept, adalimumab, infliximab, ustekinumab	2005-2010	Prospective cohort study	N.A.
Melanoma							
29	California (USA)	Kaiser Permanente Northern California (KPNC)	All KPNC members aged ≥ 18 years old, diagnosed with psoriasis between 1998 and 2011 and treated with a systemic antipsoriatic agent	Adalimumab, etanercept, infliximab, ustekinumab	1998-2011	Retrospective cohort study	8 [3-14]
31	United Kingdom	British Society for Rheumatology Biologics Register + National cancer and death registers	All patients diagnosed with PsA starting a TNF-inhibitor and registered in the British Society for Rheumatology Biologics Register	Etanercept, adalimumab, infliximab	2002-2012	Retrospective cohort study	NA
37	America and Europe	Psoriasis Longitudinal Assessment and Registry (PSOLAR)	Patients aged ≥ 18 years with moderate-to-severe psoriasis who were receiving, or were candidates to receive, systemic therapy	TNF-α inhibitors	2007-2015	Nested case-control study	NA
37	America and Europe	Psoriasis Longitudinal Assessment and Registry (PSOLAR)	Patients aged ≥ 18 years with moderate-to-severe psoriasis who were receiving, or were candidates to receive, systemic therapy	Ustekinumab	2007-2015	Nested case-control study	NA

(Continued)

TABLE 1 | Continued

Reference	Catchment area	Data source	Study population	Study drugs	Study years	Study design	IR per 10,000 PYs [95%CI]
38	USA, Canada, Germany, France, Czech Republic, Greece, Netherlands, Spain, UK, Austria, Denmark, Ireland, Sweden	ESPRIT Registry	Patients aged ≥ 18 years of age with chronic plaque psoriasis who had been prescribed adalimumab	Adalimumab	2008-2013	Prospective cohort study	5 [3-10]
35	Germany	PsoBest Registry	Adult patients with moderate-to-severe psoriasis at the time point of a new drug to be started	Adalimumab, etanercept, infliximab	2008-2012	Prospective cohort study	8 [0-43]

ICD-9 CM: international classification of diseases, 9th revision, clinical modification; IR, incidence rate; NA, not available; PsA, psoriatic arthritis; PYs, person-years; SIR, standardized incidence ratio; TNF, tumor necrosis factor; UK, United Kingdom; USA, United States of America.

a nested case-control study (10.0%) (37) and one was a study comparing clinical trials data and real-world data (10.0%) (30).

All included studies focused on the incidence of skin malignancies in patients treated with TNF-α inhibitors, three of them included also patients treated with ustekinumab (29, 35, 36) and only one study reported NMSC IRs also for apremilast and tofacitinib (30). No observational studies assessing the incidence of skin cancer in patients with inflammatory cutaneous diseases and treated with secukinumab, ixekizumab, brodalumab, tildrakizumab or risankizumab were found. All the included studies used real-world data sources, such as drug or disease registries and claims databases.

Of the 10 studies included in this systematic review, 7 provided data suitable for meta-analysis.

Risk of Bias in Individual Studies

Figure 2 summarizes the risk of bias assessment of individual studies. The overall risk of bias was rated as low for 7 (29, 30, 32–34, 35, 38) of the 10 included studies, while 3 (31, 36, 37) studies

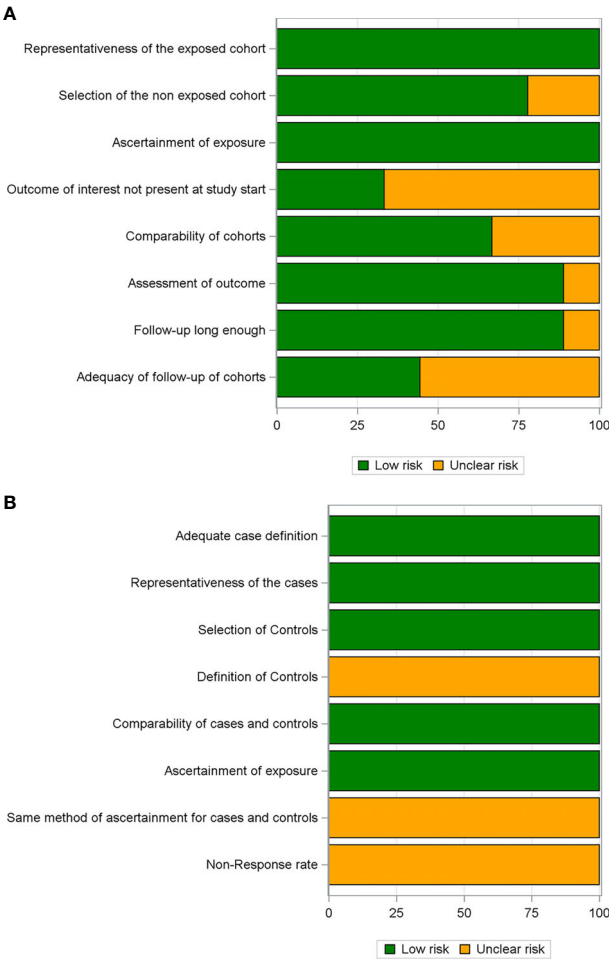

FIGURE 2 | Risk of bias assessment through the Newcastle-Ottawa Scale presented as percentages across all included cohort studies **(A)** and case–control studies **(B)**.

proved to have an unclear risk of bias. Limitations mainly concerned the assessment of the presence or absence of prognostic factors and the adequacy of follow-up.

Targeted Therapies and Skin Cancer Incidence Rates

IRs of NMSC and melanoma reported in the articles included in this systematic review are summarized in **Figure 3**.

Overall, the IR of NMSC in the included studies ranged from 38 (95% CI: 12-90) (35) to 262 (95% CI: 220-310) (34) cases per 10,000 PYs. The pooled IR for the overall risk of NMSC was 124.5 (95% CI 83.4 – 185.8) per 10,000 PYs. A considerable heterogeneity was found among these studies (Cochrane's Q = 173.0; I^2 = 96.5%).

A comparison of the incidence ratio for the overall risk of NMSC in patients exposed to biologics and small molecules versus non-biologic drugs users could be obtained only in two studies (29, 36). In one case (36), the hazard ratio (HR) was 1.42 (95% CI:1.12-1.80), while in the other one the Incidence Rate Ratio (IRR) was 0.74 (95% CI:0.60-0.91) (29).

The IR of melanoma in the included studies ranged from 5 (95% CI: 3-10) (38) to 8 (95% CI: 0-43) (35) cases per 10,000 PYs. The pooled IR for the overall risk of melanoma was 6.1 (95% CI 3.9 – 9.6) per 10,000 PYs. No heterogeneity among studies reporting melanoma IRs was found (Cochrane's Q= 1.0; I^2 = 0.0%). The only study reporting an HR for melanoma between users of biologic drugs and small molecules versus non-biologic users (36) showed no statistically significant difference (HR:1.57, 95% CI: 0.61-4.09).

It was not possible to investigate both the source of heterogeneity and the presence of publication bias, as fewer than ten studies were included in the meta-analysis (28).

DISCUSSION

In recent years, we have witnessed a revolution in the treatment of many skin diseases, ranging from bullous diseases, urticaria, atopic dermatitis, to hidradenitis suppurativa and psoriasis (39). In particular, psoriasis is a chronic cutaneous inflammatory disease affecting an estimated 125 million people worldwide, that is often associated with systemic manifestations such as major adverse cardiovascular event, obesity, inflammatory bowel disease and arthropathic psoriasis (40, 41). The decision to use one therapy over another is significantly influenced by these comorbidities and the severity of the disease. Moreover, a better understanding of the pathogenesis of this systemic disease had led to identification of new therapeutic targets (42). Whereas the older treatment options, such as phototherapy, methotrexate and cyclosporine A, are still effective, biotechnological drugs are substantially improving the therapeutic arsenal. The success of these new therapies lies in their great selectivity of action which allows to obtain, in most cases, a significant therapeutic efficacy in a short time with a reduction in side effects compared to traditional therapies. Through these therapies, even the severest symptoms of psoriasis and psoriatic arthritis can be excellently treated (43, 44). The biological drugs produced so far are

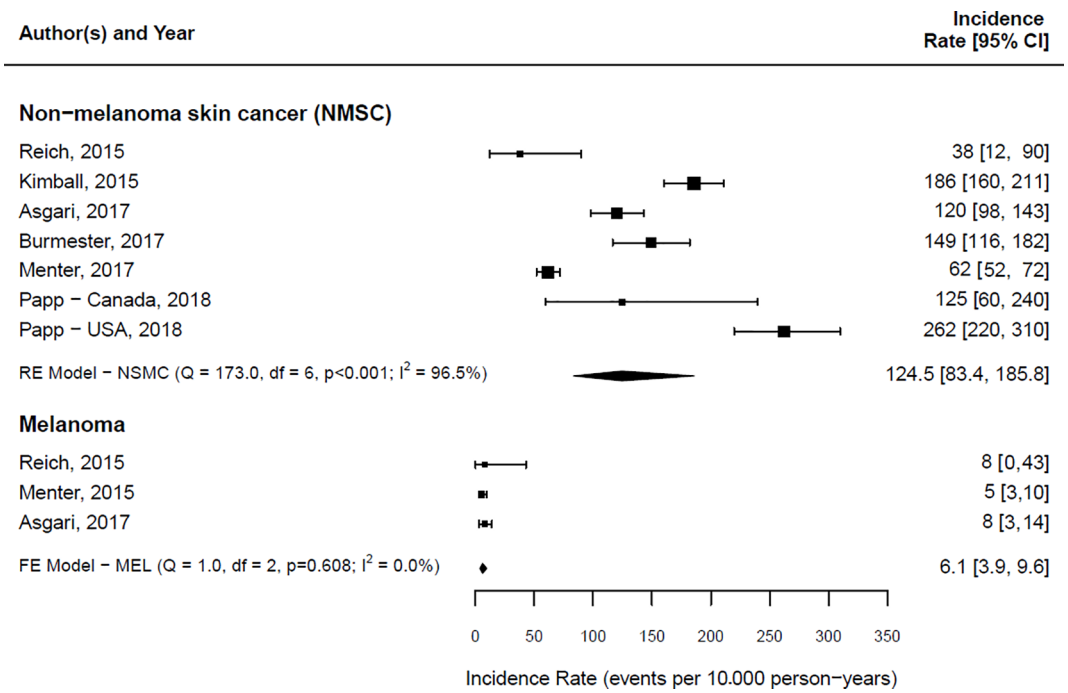

FIGURE 3 | Forest plot of the estimated skin cancer incidence per 10,000 person-years along with 95% confidence intervals, stratified by skin cancer type. RE, Random-Effects model; FE, Fixed Effect model.

monoclonal antibodies and fusion proteins. These products have the enormous advantage of being able to selectively interfere, at various levels and with different modes of action, in the immunological processes that trigger and sustain psoriasis (45). To date they are divided into five classes: TNF-α inhibitors, IL-12/23 inhibitors, IL-17 inhibitors, IL-23 inhibitors and phosphodiesterase type 4 (PDE4) inhibitors (40).

According with the above-mentioned results, our review found no observational studies assessing the incidence of skin cancer in patients with inflammatory cutaneous diseases and treated with biologics targeting selectively IL-17 or IL-23, thus obtaining mainly data on patients under anti-TNF-α therapy and, to a more limited degree, under ustekinumab, apremilast and tofacitinib.

TNF-α inhibitors infliximab, etanercept, adalimumab and certolizumab pegol are the oldest class of currently approved biotechnological drugs for the treatment of both psoriasis and psoriatic arthritis and, limited to adalimumab, of hidradenitis suppurativa. TNF-α exerts several effects. It could promote the progression of cancer (46), but also blocking TNF-α could result in arresting antitumor immune response and in promoting the growth of immunogenic tumors (47–49).

Some of the studies analyzed in this systematic review also included patients receiving ustekinumab, apremilast and tofacitinib (29, 30, 35, 36). Ustekinumab belongs to the class of biologics targeting the IL-12/23 pathway, whereas apremilast is an anti-PDE4 small molecule and tofacitinib a janus kinase inhibitor. The inhibition of these pathways causes a downregulation of the inflammatory response by modulating the expression of TNF-α, IL-23, IL-17 and other inflammatory cytokines, all involved at least in part in the tumorigenesis.

Consequently, whereas these drugs have shown dramatically excellent efficacy, concerns have been raised about the risks related to this class of agents.

Undoubtedly, patients with psoriasis are at an increased risk of cancer. Assessing the baseline risk of cutaneous malignancies in psoriasis patients is challenging due to most studies including both treated and untreated patients, and due to confounding factors like phototherapy and immunosuppressive therapy (50). Moreover NMSC and melanoma are known to arise with increased incidence among patients that have undergone medical radiation procedures or immunosuppressive therapy (51–53), such as those immunosuppressed in an iatrogenic way after a solid organ transplantation (54–56). According to the World Health Organization, age standardized world incidence of melanoma and NMSC are respectively 3,4 and 11 per 100.000 PYs. On the other hand, recent data emerging from literature show that skin cancers have a higher incidence in psoriasis patients than general population with a standardized incidence ratio of 3.37 (95% CI 1.84-5.66) (57). More in detail, Pouplard et al. in a meta-analysis reported a standardized incidence ratio of 5.3 for squamous cell carcinoma (SCC) (95% CI 2.63–10.71) and of 2.00 for basal cell carcinoma (BCC) (95% CI 1.83–2.20), whereas the authors reported a similar risk of melanoma in psoriatic patients compared to the general populations.

When considering the risk of skin cancer in psoriatic patients under treatment, many aspects should be analyzed: predisposing factors, duration and timing of exposure, the cumulative dose, the interaction with other carcinogens and, also, the latency. Despite all these data to be considered, enough evidence confirmed the relation between skin cancer and specific treatment for psoriasis and it has emerged that the risk increases even more respect untreated patients (58).

In particular, oral psoralen and ultraviolet A (PUVA) is associated with an increased risk for skin cancer in a dose dependent fashion: risk of NMSC is greatest with >350 treatments, while melanoma risk is increased with >250 treatments (59, 60). However, the carcinogenic mechanism of PUVA has not been elucidated: it maybe acts in a mutagenic and immunologic way (61). Instead, even if UVB phototherapy may increase photoaging acting with multiple mechanisms (inhibition of DNA synthesis, epidermal keratinocyte hyperproliferation, induction of T-cell apoptosis and of anti-inflammatory cytokines), no increase in skin cancer has been observed, especially with <100 treatments. Only when patients have been treated previously with PUVA and, in a second time, with broadband UVB (>300 treatments), it has been noted a modest increase in SCC (incidence rate ratio 1.37, 95% CI 1.03–1.83) and BCC (incidence rate ratio 1.45, 95% CI 1.07–1.96) (62).

Also systemic non biologic therapies are associated with an increased risk of skin cancers (63), acting primarily as immunosuppressants. Treatment with methotrexate results in higher risk for NMSC, but no association with risk for melanoma was observed (64). In detail, it has been shown that patients in treatment with methotrexate seem to have a doubled risk of SCC compared with people who receive PUVA therapy (65). Cyclosporine is associated with an elevated risk of SCC, which could increase even more in relation to treatment duration (>2 years) and previous therapy (PUVA) (66, 67), as already seen in transplant patients treated with high doses of cyclosporine and for long periods (68–70).

In our systematic review, we also considered studies evaluating the risk of skin cancers in patients with hidradenitis suppurativa in treatment with adalimumab, the only approved biologic agent for moderate-to-severe hidradenitis (71, 72). No articles were found that met the inclusion criteria. Nevertheless, data from literature point to a higher risk of developing NMSC in patients with hidradenitis than general population (15). Compared with psoriatic patients who underwent biologic treatment, patients with hidradenitis start treatment with TNF-α inhibitors after fewer months/years from the diagnosis of the disease and the guidelines do not provide obligatory treatment with first line systemic immunosuppressive drug, such as cyclosporine or methotrexate, before approaching the biologic therapy.

Considering all together the studies included in the metanalysis, the IR emerging from our systematic review shows an incidence of skin cancer in biologic treated patients, 124.5 per 10000 PYs for NMSC and 6.1 per 10000 PYs for melanoma. With regard to NMSC, IRs in literature presented large variability, from 24 in a psoriatic cohort of a German registry to 262 coming from a USA surveillance registry on patients treated with etanercept. The IR has been established on 8 out of 10 studies (**Table 1**). Concerning melanoma, 3 out of 6 studies reported an IR, ranging from 5 to 8 (**Table 1**). As a

comparison, these IRs are significantly lower than post-transplant skin cancer IR, that is 1355 per 100.000 PYs for SCC and 125 per 100.000 PYs for melanoma (73).

Our figures substantially agree with those reported in a recent systematic review and metanalysis by Vaengebjerg et al. (14) who reviewed 112 observational studies and more than 2 million persons, thus assessing them for prevalence, incidence and overall risk of cancer in patients with psoriasis and psoriatic arthritis. The reported IR per 1000 PY for overall cancer was 11.75 (95% CI, 8.66-15.31) and 4.35 (95% CI, 3.18-5.70) for keratinocyte cancer, whereas the IR for melanoma was 0.37 per 1000 PYs.

A study by Esse and collaborators was focused on melanoma risk in patients treated with biologics for common inflammatory diseases, such as inflammatory bowel diseases, rheumatoid arthritis and psoriasis (68). In detail, they considered a total of 7 studies, consisting of patients treated with TNF-α inhibitors, one of which regarding patients with psoriasis and, moreover, included in our review (74). According with their findings, the risk of melanoma in biologic-treated patients with IBD and psoriasis compared with their biologic-naïve counterparts receiving conventional systemic therapy showed no statistically significant increases. Esse et al. included in their paper only one study (36) concerning psoriatic patients; this study is currently the only one reporting an HR for melanoma in patients treated with TNF-α inhibitors compared with non-biologic users and shows no significant difference between the two groups.

With regard to NMSC, the paper by Asgari (36) explicitly reported an HR for the same comparison. Our review considered an additional study in which we were able to calculate IRR from the reported data (29). While Asgari et al. (36) reported an increased HR for NMSC in patients treated with TNF-α inhibitors compared with non-biologic users, data coming from the other study (29) showed no statistically significant differences.

The main strengths of our analysis included the use of a well-defined protocol with strict inclusion and exclusion criteria. Complying with the protocol, our search addressed a clearly focused question with standardized data extraction and quality assessment to minimize errors. In addition, the real-world setting of the studies, the inclusion of biologic agents and of patients treated exclusively for common cutaneous inflammatory diseases represent distinctive features of our review and metanalysis.

The main limitation was the small number of eligible studies. The studies were also heterogeneous, which makes comparison difficult. In addition, a major weakness of the analysis was the absence of adjustment for established risk factors for NMSC and melanoma.

Furthermore, in previous studies performed only on patients with PSO it was found that there were no univocal data on the higher or lower incidence of tumors in patients with PSO. In particular, they were studies that analyzed both patients treated with systemic drugs and patients treated with biological drugs (50, 75). In our systematic review and meta-analysis, we considered only patients treated with target therapies suffering from psoriasis, PSA and/or HS.

In common with previous studies, on the other hand, there is the fact that the risk of skin tumors itself cannot be excluded because patients had to undergo immunosuppressive therapy (systemic or not) before being able to carry out treatment with a target therapy.

Another limit that emerges from our systematic review, in common with other articles already present in the literature, is the follow-up time. As demonstrated by many studies, the development and growth times of skin tumors are long and may exceed the observation periods of the clinical trials in the literature.

SUMMARY AND PERSPECTIVES

Although with some limitations, the metanalysis of currently available real-world data seems to suggest that treatment of psoriasis, psoriatic arthritis and/or hidradenitis suppurativa with TNF-α inhibitors, ustekinumab, apremilast or tofacitinib does not increase the risk of NMSC or melanoma compared to "non-biologic" systemic treatments. The cumulative sample size of the studies in literature is certainly conspicuous, but, in the light of the worldwide diffusion and frequency of the aforementioned diseases as well as their multifactorial nature and response to treatment, including undesired effects, further data are desirable.

Additionally, the ending years of the periods analyzed in the available studies range from 2009 to 2015. Similar evaluations of real-world evidence concerning molecules marketed in the last 10-15 years, such as secukinumab, ixekizumab, brodalumab, tildrakizumab or risankizumab, would be of great interest, particularly when considering that these molecules are widely used in current clinical practice. Consequently, to conduct future trials it is necessary to consider the above data and the fact that the number of studies comparing newer molecules and conventional drugs are small. A greater number of new trials will have to be conducted, considering longer follow-up times and, above all, common methods will have to be applied to allow a comparison between the various studies.

In summary, this updated systematic review and meta-analysis seems to suggest that no differences exist between treatment of chronic cutaneous diseases with biotechnological drugs/small molecules and conventional DMARDs in terms of HR/IRR for melanoma, while data on NMSC are more controversial. Nevertheless, periodic dermatologic screening should be ensured for all patients undergoing these therapies.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**. Further inquiries can be directed to the corresponding author.

AUTHOR CONTRIBUTIONS

Conceptualization: CG and SC. Methodology: SC, FC, GT, LB, and CG. Validation: SC, LB, and CG. Resources: SC, AF, FC, GT, YI, LB, MB, and CG. Data curation: SC, AF, FC, YI, GT, MB, and CG. Writing-original draft preparation: SC, LB, and CG. Writing-review and editing: SC, LB, and CG. Supervision: SC, LB, and CG. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fonc.2021.687432/full#supplementary-material

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MINI REVIEW
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Usefulness of High-Frequency Ultrasonography in the Diagnosis of Melanoma: Mini Review

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High-frequency equipment is characterized by ultrasound probes with frequencies of over 10 MHz. At higher frequencies, the wavelength decreases, which determines a lower penetration of the ultrasound beam so as to offer a better evaluation of the surface structures. This explains the growing interest in ultrasound in dermatology. This review examines the state of the art of high-frequency ultrasound (HFUS) in the assessment of skin cancer to ensure the high clinical approach and provide the best standard of evidence on which to base clinical and policy decisions.

Keywords: Melanoma, high frequency ultrasound, oncology research and diseases, MDT, Dermatology

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INTRODUCTION

Cutaneous melanoma (CM) has a high incidence rate, even among young people; it has steadily increased over the last several decades (1, 2). Moreover this incidence is 1.5 times higher in males (3). However, this data is related to the age of onset; it has been seen that melanoma affects young women and older men. The main risky factors implicated in melanoma development are exposure to ultraviolet (UV) for their genotoxic effect, the number of melanocytic nevi, familiar history, and genetic susceptibility (3). It has been noted that patients with a previous history of melanoma have a 1% to 8% risk of developing other primary melanomas (4). These numbers highlight the health and socio-economic implications of this skin cancer. Melanoma is related to a poor prognosis in the general population. The main important prognostic factors for survival are the Breslow's index and the presence of ulceration. In the eighth edition, the AJCC melanoma expert panel described the impact of the tumor thickness subcategorizing T1 melanomas (5). The main prognostic factors for survival are still primary tumor (Breslow) thickness and ulceration. They are also useful to define T-category strata in cutaneous melanoma. As in prior editions, also in the eighth edition, tumor thickness has to be measured to the nearest 0.1 mm, not 0.01 mm. In this edition, melanoma thickness threshold of 1.0, 2.0, and 4.0 mm continues to define the T category. Consequently, those tumors that measure from 0.95 to 1.04 would be rounded to 1.0 mm. While in the seventh edition, a subset of these melanomas measuring 1.01 to 1.04 would have been staged as T2 (a: w/o ulceration, b: with ulceration). The clinical implication, if any, of this small group of patients who are mentioned in the eighth edition, has not yet been formally explored. Previous studies have detected a clinically significant treshold in the region of 0.7 to 0.8 mm in patients with T1 melanoma. In the eighth edition AJCC the analysis of the T1 melanoma patient cohort, multivariable analysis of

factors that predict melanoma-specific survival (MSS) [i.e. tumor thickness, ulceration, mitotic rate as a dichotomous variable (<1 mitosis/mm^2 vs ≥1 mitosis/mm^2)] revealed that tumor thickness dichotomized as < 0.8 mm and 0.8 to 1.0 mm and ulceration could predict MSS more efficiently than mitotic rate (as a dichotomous variable).

The subcategorization of T1 melanomas (0.8 threshold) is important for the role of Sentinel Lymph nodes biopsy(SNLB) considering that SLN metastases are very infrequent (< 5%) in patients whose melanoma is < 0.8 mm in thickness and nonulcerated (i.e., AJCC eighth edition T1a) but it occurs in approximately 5% to 12% of patients with primary melanomas 0.8 to 1.0 mm in thickness. The SLN biopsy can be performed in the patients with a primary tumor thickness 0.8–1.0 mm and also in patients with thinner ulcerated tumors (i.e., all patients with AJCC eighth edition T1b melanomas). The SLN biopsy had to be performed for patients with T2 and thicker melanomas, and when performed in patients with a T1 melanoma, the status of the SLN was used (5).

The thickness of the melanoma also determines an increased risk of lymph node involvement. Patients with melanoma spread to the nearby lymph nodes have a survival rates at 5 years of 65% (6). For all patients with primary melanoma with Breslow's index > 0.8 mm is indicated the Sentinel lymph nodes. This procedure allows the detection of metastatic involvement of the lymph nodes and the detection of nodal disease with no clinical or radiographic evidence. The outcome of SNLB may change future therapeutic management, including the choice of performing a complete lymph nodes dissection, or an adjuvant therapy, but also set up different program of clinical and imaging follow-up. For whole-body staging are used advanced imaging techniques, such as computed tomography (CT), magnetic resonance (MR), and positron emission tomography-CT (PET-CT) (7). There is no single consensus regarding surveillance imaging in melanoma patients, in fact, according to National Comprehensive Cancer Network (NCCN), the CT or PET scan is recommended every 3 to 12 months for patients with stage IIB-IV asymptomatic melanoma. While, The European Society of Medical Oncology recommends only physical examination every three months (8). However, ultrasound is the first diagnostic approach used to monitor regional lymph node basins for recurrence. It has been demonstrated that ultrasound has the highest sensitivity and specificity, 96% and 99% respectively, for lymph node surveillance (9–11), as well as for the evaluation of nodal disease. Thanks to the use of high-frequency probes, it has proved useful for the determination of ultrasound Breslow index, which means evaluating the depth of tumor invasion (**Figure 1**). Moreover, Color Doppler is an additional tool that can improve diagnostic accuracy through the identification of intra-tumor vessels and characterizations of their distributions (12) (**Figure 2**).

High accurate pre-treatment evaluation of the melanoma is useful tool for taking a correct therapeutic approach and improving the survival rate and follow-up (13).

The HFUS, and even more the ultra-HFUS, provide important information, previously obtained only thanks to biopsy samples.

Further information can be obtained thanks to the use of strain elastography (SE). This technique estimates the tissues elasticity according to assumption that tissues affected by tumor invasion are less deformable than normal tissues (14). An evaluation is then achieved by comparing the elasticity of the target lesion with the surrounding tissues. The data obtained on the relative stiffness is converted into a color-coded image that overlaps the two-dimensional images (15–17) (**Figure 3**).

This review examines the state of the art of HFUS in the assessment of melanoma to ensure the best clinical evaluation for the correct therapeutic strategies.

METHODS

Using the Medline, Embase, and ISI web of Science (Science Citation Index Expanded) databases, we searched different articles with these keywords: "melanoma", "melanoma ultrasound", "skin cancer melanoma diagnosis" (18).

The reference lists of all retrieved studies were used as additional sources of pertinent documents (18). We evaluated the title and abstract of these selected articles. If the abstract was eligible, the article was downloaded and read by two of the authors

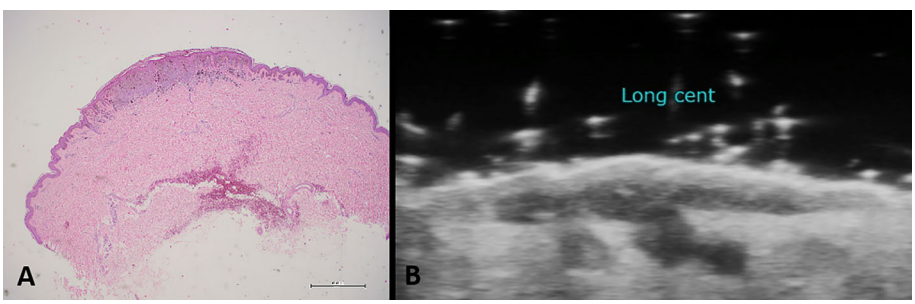

FIGURE 1 | Histological specimen **(A)** and ultrasound examination **(B)** in case of cutaneous melanoma. High-frequency probes are useful for the determination of the ultrasound Breslow index, which means evaluating the depth of tumor invasion.

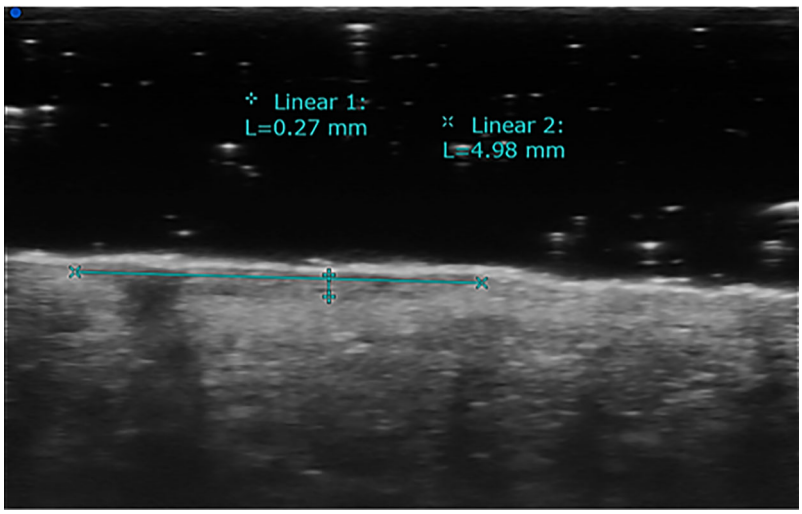

FIGURE 2 | High-frequency transducers allow the determination of ultrasound Breslow index, which means evaluating the depth of tumor invasion. This example shows skin melanoma considered with HFUS (70 MHz).

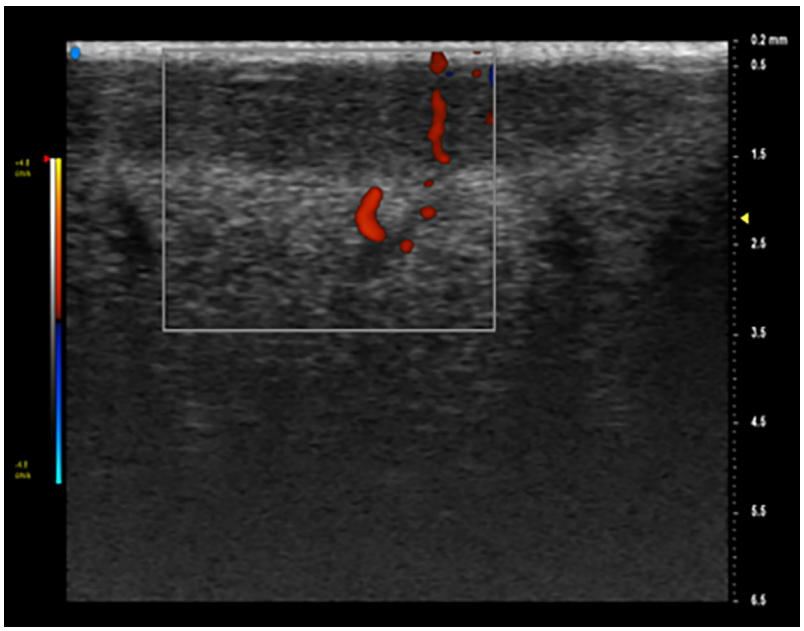

FIGURE 3 | Doppler is an additional tool that can identify intra-tumor vessels and characterize their distribution, improving diagnostic accuracy. On Color Doppler examination, it is possible to see a hypoechoic lesion with an increased vascular signal.

(MB and AR). We included human observational studies published from 1997 to 2020. These studies reported melanoma thickness with ultrasound (US). Furthermore, the ability to identify with HFUS the skip lesions and lymph nodes using 95% confidence intervals or other measures of statistical uncertainty. The studies included in the meta-analysis consider different epidemiological data. Many of these studies relied on specific reference incidence rates based on gender, age, and provided a relative standardized incidence ratio as risky measures (**Table 1**).

We excluded case reports, editorials, non-independent studies, and cohort or case-control studies.

Between two articles with overlapping numbers of melanoma cases, we chose the study with the highest number of total patients (18) (**Figure 4**).

TABLE 1 | List of the main studies related to the use of the HFUS in melanoma.

Author	Year	Frequency Probes	Results
Lassau et al.	1997	20 MHz	Proved that in 12 cases of melanoma the difference between histologic and US measurement was ≤ 0.2 mm.
Harland et al.	2000	20 MHz	US is a non-invasive aid for evaluating the acoustic differences between common pigmented lesions.
Clement et al.	2001	20 MHz	US is useful for differential diagnosis of skin lesions.
Bessoud et al.	2003	20 MHz	Sonographic and histologic measurement of melanoma thickness are strongly related, and US coupled with Color Doppler is a simple and useful tool for pigmented skin lesions management.
Pellacani et al.	2003	20 MHz	US measurements were slightly overestimated compared to the histological size but US has a strength correlation with melanoma thickness.
Rallan et al.	2007	20 MHz	Demonstration of quantitative differences between benign and malign skin lesions.
Gambichler et al.	2007	20 MHz	US measurements were slightly overestimated compared to the histological size but US has a strength correlation with melanoma thickness.
Machet et al.	2009	20 MHz	US measurements were slightly overestimated compared to the histological size but US has a strength correlation with melanoma thickness.
Kaikaris et al.	2011	14 MHz	They found a low US correlation between the Breslow index for thin melanomas (1-2 mm) and a significant correlation for thicker melanomas (> 2 mm).
Solivetti et al.	2014	18MHz or 22MHz (in case of very small and superficial lesions)	All of 52 lesions (in-transit metastases) were detected with HFUS.
Botar et al.	2015	40 MHz	There is not substantial difference between Breslow index and US thickness.
Reginelli et al.	2019	50-70 MHz	There is a favorable agreement between HFUS and Breslow thickness in 7 lesions examined.

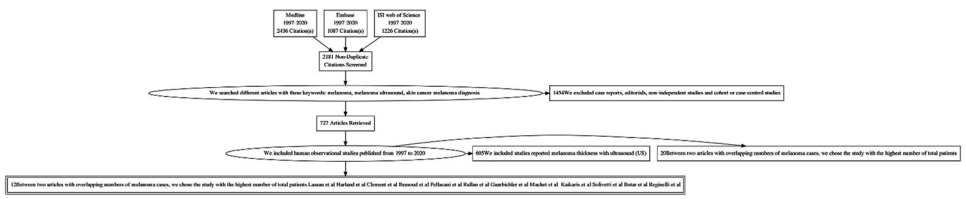

FIGURE 4 | PRISMA flow diagram.

DATA EXTRACTION

Only one co-author (MB) pulled the data into a predefined database.

The following information was considered valid for the analysis: study's year, country, type of melanoma, number of patients, the average age, gender, and lastly, median person years accumulated by patients (18).

DISCUSSION

The application of new imaging techniques has also changed the staging work-up of patients with cutaneous melanoma. Chest and abdominal computed tomography (CT) scanning should be restricted to patients with high-risk melanoma (stage IIIA with a macroscopic lymph node, IIIB, IIIC) and used to evaluate the potential metastatic sites. Magnetic resonance imaging (MRI) of the brain is used in patients with stage IV, optional in stage III and not used in patients with stage I and II disease. The diagnosis of metastases is evaluated by Positron emission tomography (PET)/CT. This technique complements conventional CT/MRI imaging in the staging of patients who have solitary or oligometastatic disease where surgical resection is most relevant. The lesions suspected of cutaneous melanoma are subjected to dermoscopic examination and if dermatologist deems it necessary, evaluated with excisional biopsy. The histological examination allows to decide whether to perform a further surgical excision and an SNLB; after a correct melanoma staging to decide the subsequent treatment (19, 20). Therefore after the excision of the lesion and histologic evaluation it is mandatory to perform a correct staging to decide whether a further surgical excision should be performed. Ultrasonography is widely used in medicine (21–23). In recent years, US and especially HFUS have become popular among dermatologists. Skin US offers essential information for the diagnosis, therapeutical management, and follow-up of tumoral and non-tumoral cutaneous pathology. It seems that HFUS examination may be useful in pre-operative evaluation of CM, and it may correlate with histology (24). Modern HFUS equipment allows highly accurate visualization of the skin layers and appendages up to histological details (25–28). Probes ranged from 15 to 22 MHz allowed visualization of the epidermis and dermis, including adjacent tissues 1 to 2 cm deep from the basal dermal layer (16).

Moreover ultra-HFUS has ultrasound frequencies higher than 30 MHz, which allow to obtain submillimeter resolution of superficial anatomical structures (29).

The image quality is influenced by the resolution, the key element in measuring the thickness and depth of skin changes (30). The typical ultrasound image of healthy skin is composed of three elements: epidermis, also known as epidermal echo, dermis and subcutaneous tissue (30).

HFUS cannot detect pigments such as melanin but allows a non-invasive evaluation of the primary tumor. It is already able to calculate a Breslow index in a large number of patients with CM (1).

Many literature studies provide US information on primary skin melanoma lesions (30–32). The first US evaluations were performed with 14 MHz probes. The 20-MHz probe was used in five studies, it has an axial resolution that goes from 50 to 80 μm and lateral resolutions to 100 μm in Bessoud et al., 2003, Clement et al., 2001, Lassau et al., 1997 and Rallan et al., 2007 at 300 μm in Harland et al., 2000 (12, 33–36).

As far as these studies are concerned, it remains unclear how the authors obtained the resolution values. Some parameters such as dynamic signal range and signal-to-noise ratio were not reported in the studies, and more often the diagnostic information provided on the lesions appeared to be poorly detailed (37).

Bessoud et al., 2003 evaluated with HFUS 130 pigmented lesions and added a Color Doppler study in 107 lesions. Ultrasound features were linked with anatomo-pathological specimen. Of these lesions: 57% invasive melanoma, 29% benign nevi, 4% basal cell carcinoma (BCC), 4% seborrheic keratosis and other benign lesions (32, 34).

Lassau et al., 1997 evaluated 70 skin lesions, clinically suspected of CM (35) and of BCC (32). HFUS and color Doppler were performed for each lesion, only eight lesions of these were not visualized and therefore excluded. Of these lesions 19 (27%) were invasive melanoma, 31 (44%) BCC, one neurosarcoma, and 12 (17%)were benign nevi (3 of the seven lesions not visualized on HFUS were melanomas) (12). In both studies, the sensitivity of the combined characteristics of HFUS was 100% with a specificity of 33% (95% CI 20% to 48%) in Bessoud et al, 2003 (130 lesions; 65 melanomas) and 73% (95% CI 57% to 85%) in Lassau et al., 1997 (62 lesions; 19 melanomas) (the lower limits of the 95% CIs for sensitivity were 94% and 82%, respectively).

Lassau et al., 1997 determined a specificity of 8% (95% CI 0% to 36%) on 32 lesions, 19 of which were melanomas. Both studies have not visualized five melanomas in the US (38).

Lassau et al., 1997 who evaluated the hypoechoic, homogeneous, well-defined and vascularized lesions, saw that there is no difference in the sensitivity and specificity achieved using HFUS alone for the discrimination of invasive melanoma (n = 19) from all other included lesions (n = 44) (39).

The HFUS and Doppler features can be combined according to both Bessoud et al., 2003 and Lassau et al., 1997, sensitivities were 34% (95% CI 22% to 47%; n = 65 melanomas) and 16% (95% CI 3% to 40%; n = 19 melanomas) with 100% specificity (95% CI 92% to 100%) respectively for both studies (n = 45 and n = 44).

Harland et al., 2000 and Rallan et al., 2007 reported quantitative assessments of the US image evaluating the acoustic differences between common pigmented lesions.

Both studies included only melanoma, melanoma in situ, benign naevi, or seborrheic keratosis (n = 19, 6, 15, 29 in Harland et al., 2000; and n =14, 11, 38, 24 in Rallan et al., 2007).

Harland et al., 2000 compared melanoma and seborrheic keratosis (benign naevi excluded) (35, 36).

Rallan et al., 2007's work on a prototype 3D HFUS C-scan with "reflex transmission" imaging found significant differences in the mean values between melanoma and seborrheic keratosis and between melanoma and benign naevi (39).

Kaikaris et al., 2011 described the use of HFUS (14 MHz) and the association between US and morphological findings in measuring melanoma thickness.

They found a low US correlation between the Breslow index for thin melanomas (1–2 mm) and a significant correlation for thicker melanomas (> 2 mm). Measurements made with ultra-HFUS (20 MHz) were found to be well correlated with the depth of thick melanomas but were not accurate enough for thinner melanomas.

Evidence suggests that HFUS (20 MHz) may be the best tool for the estimations of tumor volume more than 2D-US (40). The first significant US reports of melanoma were performed using fixed HF probes ranging from 20 to 100 MHz.

Solivetti et al., 2014, define the HFUS as a useful technique for the detection of melanoma in-transit metastases (41). This study was performed on 600 patients with melanoma (thickness> 1 mm) resulted negative to objective examination at clinical follow-up; the US detected in-transit metastases in 63 patients with a total of 95 lesions (41). All these lesions have not reported false positive or false negative (41).

Botar et al., 2015 document the positive correlation between the Breslow index with the involvement of the lymph nodes and risk of distant metastasis. This study performed the characterization of the lesion with elastography but used the 40-MHz probe for the semiquantitative analysis. The information obtained with HFUS showed a good correlation between sonometry and histometry (r = 0.88), with an average difference of 0.39 mm (relative difference 28%) (35, 42). Tumors with a thickness between 0.55 and 0.95 mm were found to be incorrectly classified according to histology in 34%, and tumors with a thickness between 1.30 and 1.70 mm were classified incorrectly in 50% of cases. These last results are due to the low penetration of ultrasound with fixed frequency equipment (about 6 mm at 20 MHz, 3 mm at 75 MHz, and 1 mm at 100 MHz).

On the other hand, probes with variable frequency from 10 to 15 MHz and multi-channeled color Doppler evaluation allow differentiating melanomas measuring < o > 1 mm in thickness (43). This evaluation is essential in choosing to perform an SNL biopsy, which is indicated in melanomas measuring more than 1 mm in thickness (42).

Gambichler et al., found an almost similar relationship to histology, with a correlation coefficient of 0.99 with both 20- and 100-MHz transducers (44).The use of 100 MHz was more accurate than the 20 MHz. They included only lesions ≤ 1 mm thick, limiting the evaluation of lesions> 1 mm thick. Machet et al., Gambichler et al., and Pellacani et al., found that the US measurements were slightly overestimated compared to the histological size but concluded that US has a strength correlation with melanoma thickness (10, 45, 46).

For the first time, Reginelli et al., described the HFUS analysis of the CM using probes ranged from 50 to 70 MHz. In this study 14 CM have been analyzed. They present oval aspects and a fusiform shape, inhomogeneous, hypoechoic, smooth edges, and variable vascularization (1, 47, 48).

After several studies on small animals, the first HFUS for clinical use could be introduced for clinical use. The availability to use HF between 50 and 70 MHz is much higher than the conventional US systems, providing a resolution up to 30 microns and a penetration of about 15 mm (1). They considered the US performed with HF probes more accurate because the result corresponds to *in vivo* tissue without dehydration or fixation. The thickness obtained from US evaluation was compared to that obtained on the biopsy piece, and a favorable agreement was seen with the Breslow thickness (39, 49–51).

CONCLUSIONS

The application of ultrasound to dermatology is becoming more and more frequent. The ultrasound examination offers significant advantages and being it minimally invasive it is easily repeatable. In particular, the use of equipment with high-frequency probes provides important information, especially in the pre-operative, thus allowing a broader diagnostic-therapeutic evaluation, as well as later follow-up.

AUTHOR CONTRIBUTIONS

All the authors contributed equally to this work. All authors contributed to the article and approved the submitted version.

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The Challenge of Melanocytic Lesions in Pediatric Patients: Clinical-Pathological Findings and the Diagnostic Value of PRAME

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Pediatric melanoma is a rare disease especially in children aged younger than 10 years old. Recent estimates report a rise of disease incidence in both adults and children. Diagnostic work-up is challenging in pediatric melanoma, as it displays a wide range of clinical presentations. Immunohistochemical biomarkers have been reported as predictors of malignancy in melanoma, however data specific to pediatric melanoma are poor. Our study aims to contribute to provide evidence of pediatric melanoma clinical features and differential diagnosis in this patient population. We describe our experience with a retrospective case series of pigmented skin lesions including malignant melanoma, atypical spitzoid tumor, and benign nevi in children and adolescents aged less than 16 years. We described the clinical and demographic characteristics of the cohort and evaluated the immunohistochemical expression of the PReferentially expressed Antigen in MElanoma (PRAME) for differential diagnosis of melanoma in children. The series displayed a similar distribution of melanoma between males and females, and the most common site of melanoma onset were the upper and lower limbs. In our cohort, PRAME was negative in most cases. Focal and slight positivity (from 1 to 5% of the neoplastic cells) was observed in four cases (two Spitz nevi and two atypical Spitz tumors). A moderate positivity in 25% of the neoplastic cells was observed in one case of atypical Spitz tumor. Immunohistochemical expression of PRAME might be useful in the differential diagnosis of malignant melanoma.

Keywords: melanoma, children, atypical spitzoid tumor, PRAME, immunohistochemistry

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INTRODUCTION

Malignant melanoma (MM) affects mainly the adult population, and about 14% of patients aged >18 years-old develop MM during their life according to recent studies (1). Although MM is rare in pediatric age, it is the most common form of skin cancer in children. The incidence increases with age: it is a rare neoplasm in children aged less than 10 years (annual incidence of 0.7–0.8 per million). However, this cancer cannot be considered a rare disease in teenagers, as its incidence is above two cases per million (2). Teenagers aged 15–19 years represent about 73% of pediatric MM cases; patients aged 10–14 years of age represent about 17%, while those aged 5–9 years and 1–4 old represent 6 and 4%, respectively (3). Overall, MM incidence in pediatric patients ranges from 1.1 per million in children younger than 5 years to 10.4 per million in those aged 15–19 years in the United States (4). However, data about trends in subjects aged less than 20 years are poor and contrasting (5, 6). In 2011, a literature review reported an incidence increase of 1–4% per year in the pediatric population (5). Conversely, Campbell et al. observed a decreased incidence in teenagers from 2004 to 2010 in the United States (6).

These data highlights how our understanding of pediatric MM is limited because clinical studies rarely involve children and adolescents. In addition, MM diagnosis in children is challenging, as it exhibits a wide range of clinical presentations (7). Clinical surveys have reported that MM in younger children might be amelanotic, uniformly pigmented, bleeding, thicker, and more frequently associated with lymph node metastasis compared to MM in adult patients, and thus displays a different biological behavior (8, 9).

A correct diagnosis is mandatory, as the patient's management and the correct therapy are directly dependent on diagnosis. Indeed, the therapeutic options for MM include not only surgery, but also targeted therapy using BRAF and MET inhibitors and immunotherapy. Moreover, a better understanding of the MM molecular landscape has led to the identification of new prognostic biomarkers (ALK, NTRK, MYC, C-KIT, and others) and will allow new targets for therapy in the near future (10). The diagnosis of melanocytic lesions is one of the most difficult aspects of dermatology and pathology. The development of dermoscopy in the last decades has improved the recognition of atypical lesions that need to be excised. However, the diagnosis still relies on histological examination, and the differential diagnosis in pediatric patients mainly includes Spitz nevus, atypical Spitz tumors, and Spitz melanoma. Histological diagnosis of melanocytic proliferations is certainly a challenge, as it mainly relies on morphological findings, which are almost partially subjective and requires trained pathologists with specific expertise (11). Recently, immunohistochemical and molecular biomarkers have been applied to the differential diagnosis, and have improved the diagnostic specificity.

Immunohistochemistry (IHC) is one of the most used techniques in pathology laboratories, as it is inexpensive, automatized, and can precisely evaluate the cellular population expressing a specific protein. Several immunohistochemical markers are tested on melanocytic neoplasms in everyday practice, mainly including HMB45, p16, and Ki67. Nevertheless, IHC plays an ancillary role in the diagnosis of melanocytic neoplasms in pediatric patients, and no immunohistochemical marker is entirely specific in differentiating benign from malignant neoplasms.

PRAME (PReferentially expressed Antigen in MElanoma) is a tumor-associated antigen recently identified in some neoplasms, including myxoid liposarcoma, synovial sarcoma, and MM (12). Current data suggest that PRAME is expressed by MM cells, but not by benign melanocytic neoplasms, and consequently it may be applied in the differential diagnosis of challenging melanocytic lesions. However, data about the expression of PRAME by melanocytic lesions in pediatric patients are limited. To fill the gap in this field, our study aims were twofold: first, to provide a description of cases presenting with suspected pigmented skin lesions and clinical findings of atypical melanocytic neoplasms including MM based on the experience of three hospital centers, and second, to evaluate the expression of PRAME in the subset of atypical spitzoid neoplasms in children.

MATERIALS AND METHODS

Patient Cohort

We retrospectively included clinical and histopathological data of children and adolescents referred to participating institutions for pigmented skin lesions suspected of melanoma. Three centers participated in the study: Santobono Hospital (Naples, Italy), the Pediatric Surgery Unit of University of Campania Luigi Vanvitelli (Naples, Italy), and the Pediatric, Adolescents and Young Adults Surgery Division of University of Pisa (Pisa, Italy). From databases containing data of patients subjected to excisional biopsy at these three centers, we selected patients satisfying the following criteria for inclusion in the study: 1) subjects referred to the participating centers from 2006 to 2020; 2) age ≤16 years; and 3) availability of demographic, clinical, surgical, and histopathological results. Data regarding benign pigmented skin lesions were obtained as a control group.

The present study was retrospectively conducted using archival biological samples. The diagnoses had already been rendered in all included cases. Approval by the participating institutions ethical review boards was collected.

At diagnosis, each patient received a baseline evaluation, which included medical history assessment and physical examination. Demographic and clinic characteristics included: sex, age, anatomical site of onset, signs of bleeding, itching, growth speed, and shape/color changes. Surgical characteristics recorded were removal of sentinel lymph node and sentinel lymph node state.

Through a telephone history we also obtained data about the presence of possible risk factors, such as clear skin phenotype, UV exposure levels, familiarity for skin melanoma in first degree relatives, number and presence of congenital nevi, dysplastic

nevus syndrome, immunodeficiency status, and residence in polluted areas in patients that were diagnosed with either *in situ* or invasive MM.

This study was reviewed and approved by the ethics committee of University of Campania Luigi Vanvitelli (Naples, Italy).

Morphological Evaluation

Histological slides of all cases were reviewed by two experienced pathologists trained in melanocytic pathology. Histological studies were performed when necessary for diagnostic purposes. The histological review included immunohistochemical slides, when available. In some cases, further immunohistochemical markers were tested for diagnostic purposes, including HMB45 and p16. We applied diagnostic criteria defined in the most recent WHO classification of skin tumors (13).

PRAME Immunohistochemistry

Inclusion criteria for PRAME IHC included: 1) spitzoid morphology; and 2) availability of archived residual biomaterial in paraffin blocks. Immunocytochemistry was performed on 5-micron thick sections cut from formalin-fixed and paraffin-embedded (FFPE) tissue blocks. A commercially available anti-PRAME monoclonal antibody (dilution 1:200; EPR20330, Abcam, Cambridge, United Kingdom) was used on the Ventana Bench Mark Ultra System, (Ventana, Oro Valley, USA) autostainer platform, according to the manufacturer's instructions. The staining of PRAME IHC was recorded as the percentage of immunoreactive tumor cells with nuclear labeling per total number of tumor cells. A positive control was added to each slide, consisting in a PRAME-positive MM. The non-melanocytic tissue in the slide was considered the negative control.

The immunohistochemical slides were interpreted by two experienced dermatopathologists, evaluating both the intensity of the staining and the percentage of stained neoplastic cells on the total number of neoplastic cells. In cases where a consensus was not obtained, it was achieved through review by a third experienced pathologist. Intensity of the staining was graded as follows: score 1+: slight positivity; score 2+: moderate positivity; score 3+: intense positivity. The percentage of the positive cells was recorded, as well as the location of positive cells in the setting of the lesion (junctional *versus* intradermal).

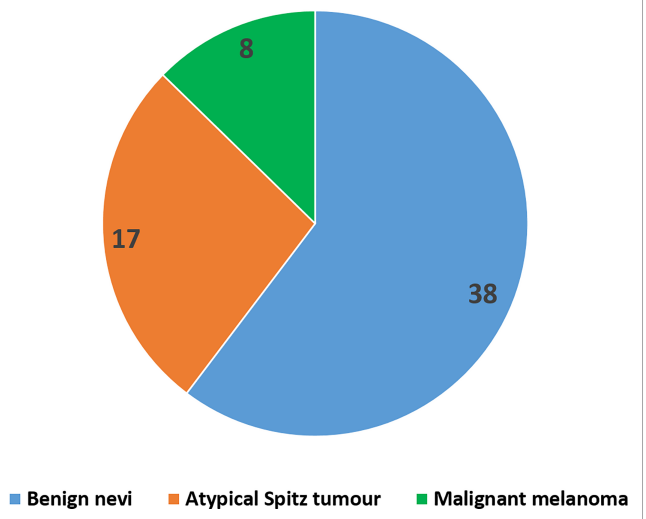

FIGURE 1 | Number of patients included in the study according to skin lesion type.

TABLE 1 | Clinical and demographic characteristics of the pediatric cohort according to lesion type.

Feature	Melanoma (n = 8)	Atypical Spitz Tumor (n = 17)	Benign Pigmented Skin Lesions (n = 38)
Sex			
Male	4	9	20
Signs/Symptoms			
Fast growth	5	17	28
Color changes	3	0	10
Asymmetry	1	0	1
Bleeding	0	0	0
Itching	0	0	0
Site of onset			
Trunk	2	2	12
Upper limb	4	4	7
Lower limb	3	11	12
Head/Neck	0	0	7

RESULTS

Clinical and Pathological Findings

We evaluated a total of 63 lesions in 63 subjects. Eight of 63 lesions were diagnosed as MM, 17 as atypical Spitz tumor (AST), and 38 as benign nevi (**Figure 1**). Overall, 52% of subjects were males, and the mean age was 6.1 ± 3.3 years. MM lesions were more frequently located in the lower and upper limbs, whereas benign lesions were equally distributed between lower limbs and trunk (see **Table 1**). With regards to clinical characteristics, none of the benign lesions were associated with signs of bleeding and/or itching. One patient exhibited recent shape, dimensions, and color changes of the pre-existing lesion with asymmetry. Moreover, two children presented an increased in size of the lesion. All lesions were diagnosed with cellular dysplasia on histopathologic examination. The remaining benign pigmented lesions underwent surgical excision because of recent fast growth and/or color changes on dermatologic consultation.

With regards to MM lesions, the majority did not arise from a pre-existing nevus and in four cases a rapid growth was reported. No cases of familial melanoma syndrome were observed. One melanoma developed from a congenital nevus and it presented with a rapid change in shape and color. No signs of itching and bleeding were reported.

In this group, a 5-year follow-up was carried out with a survival rate of 100% and neither relapses nor the appearance of

metastases occurred. Only one patient underwent an additional surgical excision of a benign skin lesion.

PRAME Immunohistochemistry

PRAME immunohistochemistry was performed on 38 melanocytic neoplasms with spitzoid features, including 19 Spitz nevi, 17 ASTs, and 2 MMs. Six cases diagnosed as MM were not included in the immunohistochemical evaluation, as no residual bioptic material was available in paraffin blocks after the histological and molecular evaluations performed for diagnostic purposes.

Overall, the mean age of the tested population was 7 years, ranging from 1 to 16 years. For 20 of the 38 (52.6%) cases, lesions were located on the lower limbs, while in 7 (18.4%) cases lesions were located on the trunk, in 6 (15.8%) cases on the upper limbs, and 5 (13.2%) cases on the head and neck region.

Concerning the 19 cases diagnosed as Spitz nevi, the patients ranged in age from 1 to 10 years (mean age: 5.1 years). The lesions were located on the lower limbs in 7 patients (36.8%), on head and neck in 5 (26.3%), on the trunk in 4 (22.2%), and on the upper limbs in 3 (15.8%) patients. One of these patients was diagnosed with a desmoplastic Spitz nevus, with the lesion located on the dorsal trunk of the 8-year-old child (**Table 2**).

Regarding the 17 patients diagnosed as ASTs, ages ranged from 2 to 13 years (mean age: 7.2 years). Twelve of 17 (70.6%) patients presented lesions on the lower limbs, while in 3 (17.6%) and 2 (11.8%) lesions were located on the upper limbs and the trunk, respectively. The two cases diagnosed as MM presented lesions on the right foot of a 4-year-old child and on the dorsal trunk of a 10-year-old child (**Table 2**).

Overall, PRAME immunohistochemistry was negative in 33 of 38 (86.8%) cases. Two cases diagnosed with MM tested negative. Five of 38 (13.2%) cases showed some PRAME positivity. PRAME immunohistochemistry testing was positive in 25% of the neoplastic cells in a case of AST arising at the lower limb of an 8-year-old child. The intensity of the staining resulted in a score 2+, and the positive cells included both junctional and intradermal cells. The remaining four positive cases included two ASTs and two SN. In these cases, the percentage of positive cells ranged from 1 to 5%, and the intensity of the staining yielded a score of 1+. The positive cells were junctional in three cases and intradermal in one case (an AST located at the lower limb of a 2-year-old child) (**Figure 2**). The clinical and pathological features of the positive cases are listed in **Table 3**.

TABLE 2 | Clinical features of Spitz nevus and atypical Spitz tumor lesions subjected to PRAME immunohistochemistry testing.

Lesion	SN	AST
Cases	19	17
Age (mean age, range)	5.1; 1–10	7.; 2–13
Location (N, %)		
Head and neck	5 (26.3%)	0 (0%)
Trunk	4 (22.2%)	2 (11.8%)
Upper limbs	3 (15.8%)	3 (17.6%)
Lower limbs	7 (36.8%)	12 (70.6%)

SN, spitz nevi; AST, atypical Spitz tumors.

DISCUSSION

Although MM is relatively rare, it is the most common skin cancer in pediatric age. The estimated incidence in children under 10 years of age is 1.8 cases for 1 million in the United States (14). MM incidence increases during puberty, with a rate of 14 and 23 cases per million in adolescent males and females, respectively (15). Consequently, MM may be considered a rare tumor in pediatric patients, but the same cannot be said for adolescents. Although MM is a significant problem even in this population, clinical data are insufficient. Moreover, the differential diagnosis of melanocytic neoplasms remains a challenge, mainly in the setting of spitzoid lesions.

Herein, we analyzed a series of melanocytic lesions, and tested the expression of PRAME in a subset of cases. The first part of our study assessed the demographic and clinical presentation of suspected pigmented skin lesions. The ratio of pigmented lesions was equivalent between sexes and the most frequent site of onset was the limbs. The sex distribution of lesions was similar to that reported in previous case series including subjects of similar ages as our study (16). Conversely, in adolescents and youths, females were more frequently diagnosed with MM (17).

In our cohort, we more frequently observed malignant lesions in the lower and upper limbs. This finding is consistent with the data described by Dean et al. who reported the same body distribution for melanoma (16). This trend could be explained, as indicated by Strouse et al. (18), by the greater exposure of the upper and lower limbs to environmental disruptors and/or sunbathing. The latter is considered a risk factor also in adults, as well as phenotypic traits including red hair, blue eyes, and poor tanning ability (19). In addition, if we consider body surface distribution in children compared to adults, in pediatric subjects there is a relative higher prevalence on the upper and lower extremities over trunk surfaces. The common risk factors reported for pediatric melanoma, as well as giant melanocytic nevi, xeroderma pigmentosum, and neurocutaneous melanosis (19) were not detected in our cohort. Moreover, it has been reported in scientific literature that germline variants, such as *MC1R*, *CDKN2A*, and *p16* gene variants are also associated with increased risk of melanoma (19).

Excisional biopsy is mandatory in cases of melanocytic lesions with atypical features in pediatric patients, and the diagnosis relies on histological examination. In this setting, the histological diagnosis of spitzoid neoplasms is one of the most difficult issues in dermatopathology. Despite a better understanding of the molecular biology underlying these neoplasms, the differential diagnosis between benign lesions and malignant lesions is still difficult, and largely based on qualitative and albeit, partially subjective findings (11). PRAME has recently emerged as a novel immunohistochemical marker able to distinguish benign from malignant melanocytic proliferations (20). However, the value of PRAME in the setting of differential diagnosis of spitzoid melanocytic neoplasms in pediatric patients is not well defined. We performed PRAME immunohistochemistry on a series of 38 spitzoid melanocytic neoplasms, including 19 Spitz nevi, 17 ASTs, and 2 MMs. Overall, PRAME was negative in 33 of 38 (86.8%) cases, including three ASTs and two SN. Notably, the

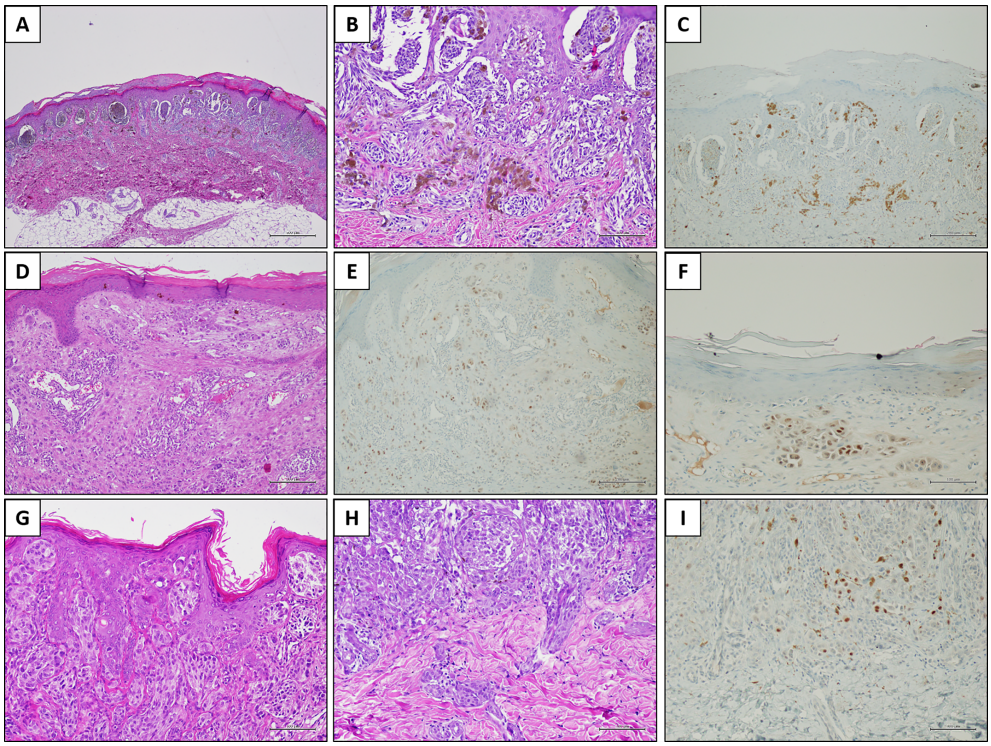

FIGURE 2 | PRAME immunostaining in three explicative lesions. Case 1 (Spitz nevus): a melanocytic lesion located on the right foot of an 8-year-old child. Histologically, the neoplasm was characterized by large junctional nests with peripheral clefting [**(A)** H&E, original magnification 40×]. Some junctional nests are confluent; smaller nests are present in the dermis, in addition to melanophages [**(B)** H&E, original magnification 200×]. PRAME immunostaining was negative [**(C)** immunostaining, original magnification 100×]. Case 2 (Atypical Spitz tumor): a melanocytic lesion located on the leg of an 8-year-old child. In this field, the melanocytic population is arranged in single epithelioid cells and small nests, located in the dermis [**(D)** H&E, original magnification 100×]. Overall, PRAME immunostaining was positive in about 25% of the melanocytic population [**(E)** immunostaining, original magnification 100×] with a moderate (score 2+) intensity [**(F)** immunostaining, original magnification 200×]. Case 3 (Spitz nevus): a melanocytic lesion located on the face of a 3-year-old child. Histologically, the junctional component was organized in confluent nests and constituted by epithelioid and spindle cells, in the context of a hyperplastic epidermis [**(G)** H&E, original magnification 200×]. The dermal component was organized in smaller nests, and peri-adnexal spread was present [**(H)** H&E, original magnification 200×]. PRAME immunostaining showed slight positivity (score 1+) in a few cells, corresponding to the 2% of the melanocytic population [**(I)** immunostaining, original magnification 200×].

TABLE 3 | Clinical and pathological features of PRAME-positive cases.

N.	Diagnosis	Location	Age (y)	% Positivity	Score	Location
1	AST	Lower limb	8	25	2+	Junctional and dermal
2	SN	H/N	3	2	1+	Junctional
3	AST	Upper limb	7	5	1+	Junctional
4	SN	Lower limb	2	1	1+	Junctional
5	AST	Lower limb	2	1	1+	Dermal

two cases diagnosed as MM tested negative as well. In five cases, including three ASTs and two SN, some PRAME positivity was observed. In particular, 25% of both junctional and intradermal neoplastic cells showed a score 2+ staining in an AST located at the lower limb of an 8-year-old child. In the remaining four cases, only a few cells resulted slightly positive (score 1+), ranging between 1 and 5% of the melanocytic population. To more decisively evaluate these results, it is mandatory to define a cut-off value to be applied for positive cases. PRAME stains mainly in the nucleus and consequently the results are of high quality and are easily interpretable in all cases, despite the amount of melanin pigment. In our experience, PRAME staining is diffusely positive, in most neoplastic cells, and in both junctional and intradermal cells, in cases morphologically diagnosed as MM. Nonetheless, we are accustomed to defining negative cases with only few positive cells. Our experience matches observations reported by other studies. Lezcano et al. recently examined the immunohistochemical expression of PRAME in a heterogenous series of 400 melanocytic lesions. The Authors considered PRAME positivity significant when observed in ≥76% of neoplastic cells (20). Similarly, Raghavan et al. defined positive cases showing PRAME staining in at least 60% of the cells (21). Based on these data, in our series the positivity observed in the five cases does not appear to be significant, and we considered all cases tested as negative. Nevertheless, we might speculate that the data reported in literature relied on a higher cut-off value of cellular staining to define PRAME positivity.

Differential diagnosis in the setting of spitzoid melanocytic lesions is challenging, and ancillary tests may be useful. In this

setting, PRAME immunohistochemistry is emerging as a novel immunohistochemical test that has been recently introduced in the routine diagnostic work-up of dermatopathologists. When faced with the diagnosis of a melanocytic lesion, a basic immunohistochemistry panel may include HMB45, p16, Ki67, and PRAME expression. However, data regarding the diagnostic value of PRAME in the setting of the spitzoid melanocytic lesions in pediatric patients are missing. In the single paper available in the literature, Raghavan et al. evaluated the expression of PRAME in a series of atypical melanocytic lesions, including 35 spitzoid neoplasms (20 SN, 13 ASTs, and 2 MMs). The authors found that PRAME was expressed in 7.7% of ASTs and in 4% of SNs (21). The study evaluated only two cases of MM, and PRAME expression was in one case (21). However, the series did not consider pediatric patients. In this study, we evaluated PRAME expression specifically focusing on spitzoid lesions in pediatric patients. In our series, PRAME tested negative in all cases. Although some cells showed PRAME expression in five lesions, its expression was focal (25% of the cells in one case and ≤5% in the remaining four cases) and did not reach the cut-off value for positivity. We can conclude that PRAME is not expressed in SN and ASTs in pediatric patients, and therefore it is not useful for the differential diagnosis of SN and AST in this clinical setting. Conversely, we tested only two MMs, and therefore no significant information could be obtained from our series relative to the expression of PRAME in MM lesions in pediatric patients.

In conclusion, in our case series we observed that pediatric MM equally affects young boys and girls, and that the limbs are the most common site of onset. These findings highlight the different clinical behavior of MM in children compared to adults. In addition, we tested PRAME expression in a series of 38 spitzoid melanocytic lesions in pediatric patients. Although PRAME is an emerging IHC marker for the characterization of melanocytic lesions in adults, data regarding its utility in the diagnosis of spitzoid lesions in pediatric patients are lacking. Herein, we demonstrated that PRAME is not expressed in either SN or ASTs in this clinical setting; thus PRAME positivity may be considered an element useful for the differential diagnosis of MM. However, there are insufficient data in pediatric populations about PRAME expression in MM with spitzoid morphology, as only two cases have been reported by a previous study, of which only one case resulted positive. Considering the paucity of clinical and histopathological data in pediatric cohorts, additional studies should be conducted in this field with the aim of identifying predictors of malignant forms.

DATA AVAILABILITY STATEMENT

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

AUTHOR CONTRIBUTIONS

AR, RF, and GD'A revised the histological and immunohistochemical slides and contributed to design the study. ME and GU contributed to design the study, contributed to write the manuscript and data analysis. VD'O, GD, LT, CS, and SS provided the clinical data and biological material, contributed to the analysis of the data. AP contributed to design the study and reviewed the manuscript. All authors contributed to the article and approved the submitted version.

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REVIEW
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The WHO 2018 Classification of Cutaneous Melanocytic Neoplasms: Suggestions From Routine Practice

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The "multidimensional" World Health Organization (WHO) classification 2018 of melanocytic tumors encompasses nine melanoma pathways (seven of which for cutaneous melanoma) according to a progression model in which morphologically intermediate melanocytic tumors are cosidered as simulators and/or precursors to melanoma. These "intermediates" can be subclassified into: i) a "classical" subgroup (superficial/thin compound: dysplastic nevus), which is placed within the morphologic and molecular progression spectrum of classical (Clark's and McGovern's) melanoma subtypes (superficial spreading and, possibly, nodular); and ii) a "non-classical" subgroup (thick compound/dermal: "melanocytomas") whose genetic pathways diverge from classical melanoma subtypes. Such a progression model is aimed at giving a conceptual framework for a histopathological classification; however, routine clinicopathological practice strongly suggests that most melanomas arise *de novo* and that the vast majority of nevi are clinically stable or even involuting over time. Clinicopathological correlation can help identify some severely atypical but benign tumors (*e.g.*: sclerosing nevus with pseudomelanomatous features) as well as some deceptively bland melanomas (*e.g.*: lentiginous melanoma; nested melanoma), thereby addressing some ambiguous cases to a correct clinical management. The recently available adjuvant therapy regimens for melanoma raise the problem of a careful distinction between severely atypical (high grade) melanocytoma and "classical" melanoma: conventional morphology can guide an algorithmic approach based on an antibody panel (anti-mutated BRAF, BAP1, PRAME, ALK, TRKA, MET, HRAS-WT, ROS; beta catenin; R1alpha; p16; HMB45; Ki67), a first-line molecular study (identification of hot spot mutations of *BRAF* and *NRAS*) and an advanced molecular study (sequencing of *NF1, KIT, BRAF, MAP2K1, GNAQ, GNA11, PLCB4, CYSLTR2, HRAS*; fusions studies of *BRAF, RET, MAP3K8, PRKCA*); as a final step, next-generation sequencing can identify melanocytic tumors with rare genetic signatures and melanocytic tumors with a high tumor mutation burden which should be definitely ascribed to the category of classical melanoma with the respective therapeutic options.

Keywords: melanoma, melanocytoma, dysplastic nevus, clinicopathological correlation, histopathology, immunohistochemistry, molecular biology

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INTRODUCTION

The histopathological diagnosis and classification of melanocytic skin tumors is probably the greatest conceptual and practical challenge in modern dermatopathology and is expected to rapidly evolve in the next future, with the WHO 2018 classification being the basis for the forthcoming studies (1). One major problem, however, is that the histopathological diagnosis itself is not based upon the search of a single (or a few), objective, and easily reproducible morphological diagnostic feature(s) but rather, it is born by a constellation of diagnostic criteria whose implementation, meaning, and relative weight considerably vary case by case and is responsible for a worrisome list of diagnostic pitfalls (**Table 1**). Thus, the histopathological diagnosis of melanocytic skin neoplasms, being based upon the simultaneous evaluation of several criteria, is no more than an *assessment of probability* and, as such, is often a matter of a sizable disagreement and inter-observer variability (2). In addition, and even more importantly, the time-honored "unifying concept of melanoma" (melanoma as a single entity evolving with a well-defined and repetitive "sequence of events") (3) has been questioned, because both clinicopathological (4) and molecular studies (5) point toward the existence of melanocytic neoplasms of low malignant potential (putative low-grade melanocytic malignancies different from "classical" melanoma).

In order to face with these problems in routine histopathological practice, the WHO Working Group supports the use of descriptive and provisional terminology, *i.e:* i) "intraepidermal atypical melanocytic proliferation of uncertain significance (IAMPUS)": a melanocytic neoplasms raising the differential diagnosis with melanoma *in situ*; ii) "superficial atypical melanocytic proliferation of uncertain significance (SAMPUS)": a thin compound melanocytic neoplasm whose differential diagnosis is with early invasive, radial growth phase (thin non-mitogenic and non-tumorigenic) melanoma; iii) "melanocytic tumor of uncertain malignant potential (MELTUMP)": a compound or dermal-based neoplasm whose differential diagnosis includes melanoma in vertical growth phase (typified by dermal mitotic figures and/or by dermal nests/sheets which are larger than the larger junctional nest) (6). Based on the these definitions, such a descriptive terminology applies to simulators (morphologically atypical nevi and deceptively bland melanomas) (2) as well as to biological "intermediates" (melanocytic neoplasms of low malignant potential) (4); and a strong suggestion is made that several neoplasms belonging to both categories may be in fact precursors to melanoma. The present review is aimed at giving some suggestions in the multidisciplinary approach based on the WHO 2018 classification.

TABLE 1 | Main settings of diagnostic difficulties in melanocytic skin neoplasms.

1. Unrecognized melanoma on partial (shave/punch) biopsies
2. Nevoid melanoma *vs*. "common" or "congenital" compound/dermal nevus
3. Desmoplastic melanoma *vs*. desmoplastic nevus *vs*. scar
4. Recurrent/persistent nevus *vs*. (recurrent) melanoma
5. Spindle cell melanoma *vs*. spindle cell nevus
6. Spitz/spitzoid melanoma *vs*. atypical Sptz nevus/tumor *vs*. Spitz nevus
7. Superficial spreading melanoma *vs*. dysplastic nevus
8. Superficial spreading melanoma *vs*. haloed nevus
9. Melanoma (in special site) *vs*. nevus with site-related atypia
10. Melanoma with regression *vs*. compound nevus with regression-like fibrosis
11. Melanoma with regression *vs*. melanosis
12. Melanoma *in situ* in chronic sun-damaged skin *vs*. melanocytic hyperplasia/photoactivation
13. Dermal melanoma over congenital nevus *vs*. proliferative nodule in congenital nevus
14. Cellular blue nevus *vs*. animal-type melanoma *vs*. blue nevus-like metastatic melanoma
15. Deep penetrating nevus *vs*. deep penetrating nevus-like melanoma
16. Pigmented epithelioid melanocytoma *vs*. animal-type melanoma

THE PATHWAYS TO MELANOMA

The WHO 2018 classification of melanocytic tumors sets forth nine pathways to melanoma (6), seven of which being primary cutaneous (**Table 2**), by largely transposing a previously proposed "multidimensional" pathogenetic scheme based on: i) the role of ultraviolet (UV) radiation; ii) the cell (or tissue) of origin; iii) driving and/or recurrent genomic changes (7).

The most common melanomas in Whites arise from epithelium-associated melanocytes in cutaneous sites with some degree of cumulative sun damage (CSD); these neoplasms are characterized by a high number of point mutations, mostly consisting in the so-called "UV signature" (cytosine to thymidine transitions at dipyrimidine sites); as a rule, the higher the degree of CSD the higher the tumor mutation burden (TMB) (on average: 30 mutations/megabase in high-CSD melanoma; 15 mutations/megabase in low-CSD melanoma) (10). Desmoplastic melanoma is a subtype of high-CSD characterized by a particularly high TMB (on average: 62 mutations/megabase) (11). The degree of CSD is related with the histopathological evidence of dermal solar elastosis, graded according to a three-tiered scale (grade 1: single elastic fibers; grade 2: bunches of fibers; grade 3 basophilic masses) (6).

The other subtypes of melanoma are UV-unrelated. The most common melanomas in non-White population arise from epithelium-asssociated melanocytes on acral skin (palms, soles, nail apparatus) or mucous membranes and are characterized by an early onset of major chromoscomal rearrangements, such as chromotripsis, with gene copy number changes, including multiple high-level amplifications (8). Spitz melanoma and melanomas arising from non-epithelium associated melanocytes (uveal melanoma, melanoma arising in blue nevus and in congenital nevus) also have a very low TMB, but lack the highly rearranged genomes of acral and mucosal melanomas (7, 20). The separation among melanomas with different TMBs is clinically relevant because the TMB may be predictive of response to immune checkpoint inhibitors (21, 22); parenthetically, the assessment of the TMB may be even proposed as a tool for the management of some cases of severely atypical MELTUMP (see below).

Next generation sequencing (NGS) studies have identified many recurrently mutated genes in melanoma, incuding well known genes (*PTEN*, *MAP2K1-2*, *RB1*) and recently identified genes (*ARID2*, *PPP6C*, *RAC1*, *DDX3X*, *IDH1*) (23, 24); however,

TABLE 2 | The WHO 2018 classification of melanoma according to pathways.

Relationship with sun exposure/sun damage	Pathway n.	Subtype	Genetic hallmarks
Melanomas arising in sun-exposed skin	1	*Low-CSD melanoma/superficial spreading melanoma*	High frequency of *BRAF* p.V600 mutations (7–9)
	2	*High-CSD melanoma (including lentigo maligna melanoma and high-CSD nodular melanoma)*	Predominating mutually exclusive *NF1*, *NRAS*, other *BRAF* (non-p.V600E), and perhaps *KIT* mutations (7–9)
	3	*Desmoplastic melanoma*	Recurrent inactivating *NF1* mutations, *NFKBIE* promoter mutations, and several different activating mutations in the MAPK pathway (*e.g.*: *MAP2K1*) (9–11)
Melanomas arising at sun-shielded sites or without known etiological associations with UV radiation exposure	4	*Malignant Spitz tumor (Spitz melanoma)*	Mutations in *HRAS* and kinase fusions in *ROS1*, *NTRK1*, *NTRK3*, *ALK*, *BRAF*, *MET*, and *RET*; *CDKN2A* homozygous deletion, *TERT* promoter mutations and *MAP3K8* fusions/truncating mutations only in aggressive or lethal variants (7, 12–15)
	5	*Acral melanoma (including nodular melanoma in acral skin)*	Multiple amplifications of *CCND1*, *KIT*, and *TERT*; mutations of *BRAF*, *NRAS*, and *KIT*; kinase fusions of *ALK* or *RET* in a few cases (7, 8)
	6	*Mucosal melanoma*	Numerous copy number and structural variations; uncommonly, *KIT* and *NRAS* mutations (16)
	7	*Melanoma arising in congenital nevus*	In large to giant congenital nevi: *NRAS* mutation; in small to medium-sized congenital nevi, *BRAF* mutations (17, 18)
	8	*Melanoma arising in blue nevus*	Initiating mutations in the Gαq signalling pathway (*GNAQ*, *GNA11, CYSLTR2, PLCB4*); monosomy 3 (associated with loss of *BAP1*) and chromosome 8q gains in aggressive cases; additional secondary copy number aberrations in *SF3B1* and *EIF1AX* (7, 19)
	9	*Uveal melanoma*	Mutually exclusive mutations in the Gαq pathway (*GNAQ*, *GNA11*, *PLCB4*, *CYSLTR2*); *BAP1*, *SF3B1*, and *EIF1AX* mutations during progression (16)

most of these genes are involved in melanoma progression, rather than in melanoma initiation. Based on the presence of specific driver mutations, The Cancer Genome Atlas (TCGA) classified melanomas into four molecular subtypes: *BRAF*-mutated, *RAS*-mutated, *NF1*-mutated, and triple wild-type (lack of mutations in all three genes); among the latter were cases characterized by *KIT* mutations and by early onset of somatic copy number variations in terms of both gene amplifications in *KIT*, *CCND1*, *CDK4*, *MITF*, and *TERT* and gene deletion/loss-of-function of *TP53* and *CDKN2A* (9).

TCGA molecular subtypes correspond to most cases of the classical (Clark's and McGovern's) (25, 26) types of melanoma and roughly identify melanoma pathways 1–3 of the WHO 2018 classification; melanoma arising in congenital nevus may be also genetically related to classical melanoma because they harbor multiple DNA copy number changes (17) superimposed to *NRAS* mutation. By contrast, the genetic profiles of Spitz melanoma (mutations in *HRAS* and kinase fusions in *ROS1*, *NTRK1*, *NTRK3*, *ALK*, *BRAF*, *MET*, and *RET*) (12, 13) as well as of melanoma arising in blue nevus (mutations in the Gαq signalling pathway) (19, 27) are not encompassed within the TCGA classification. Such cases will unlikely harbor numerous DNA copy number changes or a high TMB; thus they may be genetically considered as "non-classical" subtypes of melanoma.

NEVI AS POTENTIAL PRECURSORS TO MELANOMA

As a rule, all nevi may be virtually simulators of melanoma (and *vice versa*). In addition, the recent identification of the presence of shared genomic abnormalities between some melanomas and associated nevi has provided support for a potential role of some nevi (28) as both simulators and precursors. However, only some of the WHO 2018 pathways to melanoma may have their putative startpoint in nevi harboring the same mutation:

- Pathway 1: the vast majority of acquired nevi possess single driver mutations of either *BRAF* V600E or *NRAS* Q61R/L (29);
- Pathway 4: some Spitz nevi harbor *HRAS* mutation or translocations with kinase gene fusions involving *ALK*, *ROS*, *RET*, *MET*, and *NTRK* (12, 13).
- Pathway 7: *NRAS* mutation is most frequently observed in congenital melanocytic nevi (18);
- Pathway 8: some blue nevi harbor the *GNAQ* or *GNA11* mutation (19, 27).

In contrast to melanomas, which acquire additional driver mutations, nevi usually enter a suppressive state of replicative senescence which is regulated by the tumor suppressor gene *CDKN2A via* its proteins, p14 and p16, and various transcriptional controls of the cell cycle (30, 31). Therefore, the above-listed mutations, as a single event, appear to be insufficient for melanomagenesis, but bear partially transformed melanocytes which may have an increased susceptibility to additional pathogenic mutation(s) (16). Such a progression model also encompasses neoplasms that have an intermediate number of pathogenetic mutations between nevi and melanomas: within this category, the WHO Working Group lists atypical junctional/thin compound neoplasms (dysplastic nevus and melanoma *in situ*) as well as papulonodular tumorigenic dermal proliferations ("melanocytomas"), and

both categories are subclassified into low-grade and high-grade (16). Like Pathway 1 to melanoma, dysplastic nevi are associated with activating mutations of *BRAF* or *NRAS* (18, 29); additional mutation of the *TERT* promoter and, sometimes, hemizygous loss of *CDKN2A* are involved in the morphological progression to a "classical" (superficial spreading) melanoma *in situ* (32).

Many melanocytomas are instead dermal-based, thick, "combined" melanocytic tumors in which an activating mutation of *BRAF* (or, much less commonly, *NRAS*) is followed by a second genetic hit with expansion of a morphologically peculiar ("non-classical") clone of melanocytes. Morphology of this secondary clone strictly depends on the type of second genetic hit: inactivation of the *BAP1* (*BRCA1*-associated protein) gene is the hallmark of *BAP1*-inactivated nevus (BIN) (33, 34); gain-of-function mutations of *CTNNB1* or loss of *APC* is found in deep penetrating nevus (DPN) (35, 36); loss-of-function of *PRKAR1A* is typical of pigmented epithelioid melanocytoma (PEM) (37, 38). However, several melanocytomas arise *de novo* (without a pre-exsisting common nevus): for example, cases of "pure" (non-combined) PEM are also genetically peculiar because often they harbor kinase (most commonly *PRKA*, but also *NTRK1* and *NTRK3*) (38) fusions as the initiating event. Most of these dermal-based tumors are clinically stable; however, they can display various degrees of histopathological atypia (39–42). Increasing atypical histopathological features may correlate with increased risk of disease progression (43), but available data are too weak because of the relative rarity of these tumors and the need of long-term follow-up data. Since the initiating genetic change of such neoplasms is often an activating mutation of *BRAF* or *NRAS*, the three above-mentioned types of melanocytomas are placed within Pathway 1 of melanomagenesis, whose endpoint is superficial spreading melanoma; however, cases of superficial spreading melanoma dysplaying the genetic signature of the above-listed melanocytomas are exceedingly rare. Therefore, in real life such melanocytomas are probably unrelated to the vast majority of classical (Clark's and McGovern's) (25, 26) types of melanoma. **Figure 1** shows a case of early superficial spreading melanoma over a combined BIN, with the malignant component being BAP1-positive, and being thus unrelated with the dermal melanocytoma.

According to Table 2.06 of the WHO classification (16), even the other pathways to melanoma starting from the respective nevi have their own "melanocytomas", namely: atypical Spitz tumor (Pathway 4), (atypical proliferative) nodule in congenital nevus (Pathway 7), and (atypical) cellular blue nevus (Pathway 8). It has been suggested that these entities share with BIN, DPN, and PEM the existence of a "spectrum within the spectrum" (43), namely: a set of atypical histopathological features which can be variously combined with each other, thereby bearing a "spectrum" of lesions with increasing risk of disease progression up to overtly malignant neoplasms. However, the WHO Working Group underlines that regarding Pathway 7, there is no convincing evidence that *bona fide* proliferative nodules in congenital nevi evolve into melanoma (44); and that regarding Pathway 8, a histopathological diagnosis of malignancy is straightforward for melanoma arising in blue nevus (45). Instead, regarding atypical Spitz tumor, it is acknowledged that there is the need of a "risk stratification" (46), evidently because neoplasms belonging to the Spitz lineage distribute along a spectrum of increasing histopathological atypia, with their malignant end being Spitz melanoma (14, 15).

Interestingly, atypical Spitz tumor shares at least with PEM a peculiar biological behavior, featuring a high incidence of nodal metastases with a very low incidence of distant metastases (41, 47): such as unique biological property that strongly favors ultrasonograpy monitoring over sentinel node biopsy in the clinical management of such cases (47, 48). Based on these data, PEM and atypical Spitz tumor might represent melanocytic tumors of low-grade (mostly lymphotropic) malignancy different from "classical" melanoma: it seems thus reasonable to include atypical Spitz tumor into the "melanocytoma" rubric, as suggested since the beginning (49). Interestingly enough, the list of putative low-grade melanocytic malignancies with a peculiar genetic and morphologic profile has been growing for the last years and has thus been increasingly supporting the concept itself (50–53). An example of CRTC1-TRIM11 (50) fused melanocytoma is provided in **Figure 2**; like several other melanocytomas, such a putatively low-grade malignant melanocytic tumor does not likely progress from a common nevus.

For the above, intermediate melanocytic tumors may be subclassified into: i) a "classical" subgroup (dysplastic nevus and melanoma *in situ*), which is placed within the morphologic and molecular progression spectrum of "classical" melanoma subtypes (superficial spreading and, possibly, nodular; WHO 2018 Pathway 1); and ii) a "non-classical" subgroup ("melanocytomas") whose genetic pathways diverge from "classical" melanoma subtypes. Among the latter are probably low-grade melanocytic malignancies whose list has been increasing for the last years and whose risk stratification needs a careful and systematic approach (48).

Not surprisingly, neoplasms belonging to the WHO 2018 intermediate category are prone to a lower interobserver agreement and are classified as ambiguous by multiple pathologists. Thus, the intermediate rubric also encompasses the provisional categories IAMPUS, SAMPUS, and MELTUMP (6), whose definitions (see above) imply a "subjective" diagnostic uncertainty, rather than a morphologic subset of melanocytic neoplasms. Immunohistochemical and genetic investigations may help classify the WHO 2018 provisional entities into the proper subgroup of melanoytic tumors: this goal is of paramount importance because the "provisional" terminology should be adopted as less as possible (48).

THE WHO 2018 PROGRESSION MODEL: WHAT MATTERS IN ROUTINE PRACTICE

The WHO 2018 progression model is aimed at giving a framework for a histopathological classification; it is therefore a relatively simplifed linear scheme which must be accepted with the awareness that not only are there multiple pathways to

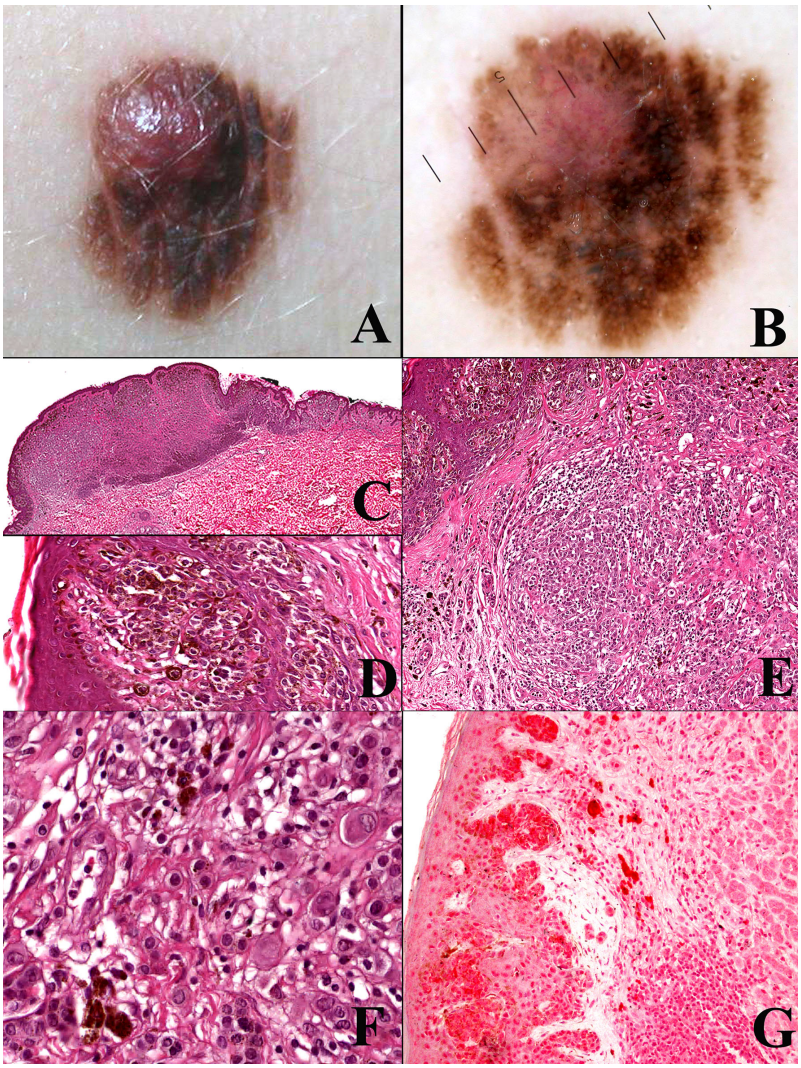

FIGURE 1 | Man, 54 years; a severely atypical melanocytic tumor of the abdomen characterized by a flat pigmented area with an eccentric nodule **(A)**. On dermoscopy, the flat area is typified by a prominent and focally irregular pigment network, whereas the nodular area is characterized by an atypical vascular pattern **(B)**. Histopathologically, the tumor is strikingly asymmetric (**C**; hematoxylin–eosin, ×25), with a broad highly cellular "shoulder" composed by junctional melanocytes arranged in irregular nests and in single unit (**D**; hematoxylin–eosin, ×400); the severely atypical junctional component spans above the dermal nodule, the latter being characterized by a lymphoid cell infiltrate (**E**; hematoxylin–eosin, ×250) and nests of nevocytes intermingled with moderately pleomorphic epithelioid melanocytes with "inclusion-like" cytoplasms (**F**; hematoxylin–eosin, ×400); all the melanocytic components of this tumor were BRAFv600e mutated protein positive (not shown) and only the dermal epithelioid cell component disclosed loss of the nuclear expression of BAP1 (**G**; ×250). The tumor was interpreted as an early melanoma developing as a neoplastic progression of a common nevus and not as a progression of a BIN.

melanomagenesis but also that some of the intermediate steps may be bypassed and that other non-linear pathways exist. The most frequent and most important non-linear pattern is by far melanoma *de novo* of the "classical" type. In a meta-analysis carried out by Pampena et al. on 38 observational cohort and case–control studies, only 29.1% of melanomas likely arose from a preexisting nevus and 70.9% arose *de novo* (54). Studies on nevus-associated melanoma based on histopathology alone may have several biases: a benign component may be absent in the tissue levels examined or, else, it may be completely destroyed by the malignant growth; on the contrary, peripheral or deep areas of melanoma may have a deceptive "nevus-like" appearance ("pseudomaturation"). Dermoscopy and dermoscopic digital monitoring can help differentiate between melanoma characterized by a homogeneous remodeling of the tumor (likely melanoma *de novo;* **Figures 3A–D**) and melanoma characterized by focal changes ("dermoscopic island"; likely nevus-associated melanoma) (55) (**Figures 3E–H**). An early melanoma may be missed if grossing of the specimen is carried out blind to the clinicodermoscopic features of a given melanocytic lesion (56). Dermoscopic digital monitoring also shows that the overwhelming majority of nevi are stable and are

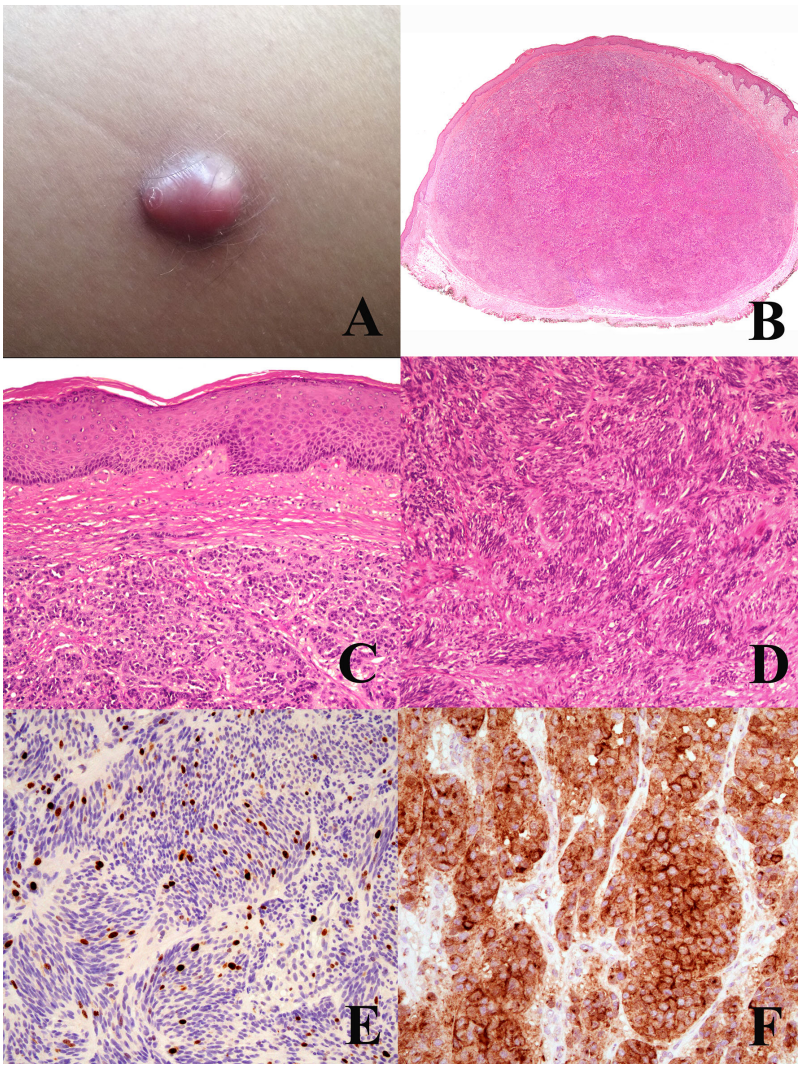

FIGURE 2 | Woman, 44 years; a reddish nodule of the thigh **(A)**. Histopathology shows an expansile dermal nodule (**B** hematoxylin–eosin, ×25) composed by nests of epithelioid cells (**C** hematoxylin–eosin, ×250) and fascicles of spingle cells separated by thin fibrotic bands (**D** hematoxylin–eosin, ×250); the proliferation rate (Ki67-positive cells) is 5%, with no clusters of proliferating cells (**E**; ×250); the tumor cells are diffusely positive for TRKA (**F**; ×400). Molecular studies allowed to exclude the possibility of a dermal clear cell sarcoma and to establish a diagnosis of CRTC1-TRIM1 fused melanocytoma. Courtesy of Dr. Arnaud de la Fouchardière, Lyon, F.

more likely to involute according to one of the following: i) a fading pattern (progressive replacement of the nevus by normal skin); ii) a haloed pattern (progressive replacement of the nevus by centripetal extension of a peripheral white vitiligo-like ring); iii) a regression-like pattern (replacement of the nevus by dermoscopic regression structures (peppering, white scarlike ares) (57). The regression-like pattern is seldom documented with dermoscopic monitoring, but is peculiar enough to allow a clinicopathological differential diagnosis between melanoma with regression and its main benign simulator, the so-called "sclerosing nevus with pseudomelanomatous features" or "compound nevus with regression-like fibrosis" (58, 59). The latter is a kind of "chronically recurrent nevus" following chronic unnoticed trauma, and has been described mainly, albeit not exclusively, in the convex area of the back of young to middle aged patients. Histopathologically, this neoplasm is usually large and asymmetric with a typical "trizonal" pattern featuring: i) an irregular junctional component with irregular epidermal hyperplasia and areas of prevailing single cell proliferation; ii) a significant area of dermal sclerosis with architecturally atypical melanocytic nests; iii) a residual, bland-appearing nevus tissue (very often with congenital nevus-like features) around and deep into the cicatricial tissue (**Figure 4**). The presence of a clear-cut benign dermal component is the main clue to the diagnosis, because regressing melanoma is usually not associated with a nevus. Such a severely atypical melanocytic tumor, in our experience often cautiously diagnosed as MELTUMP, can be indeed diagnosed with confidence when considering the proper

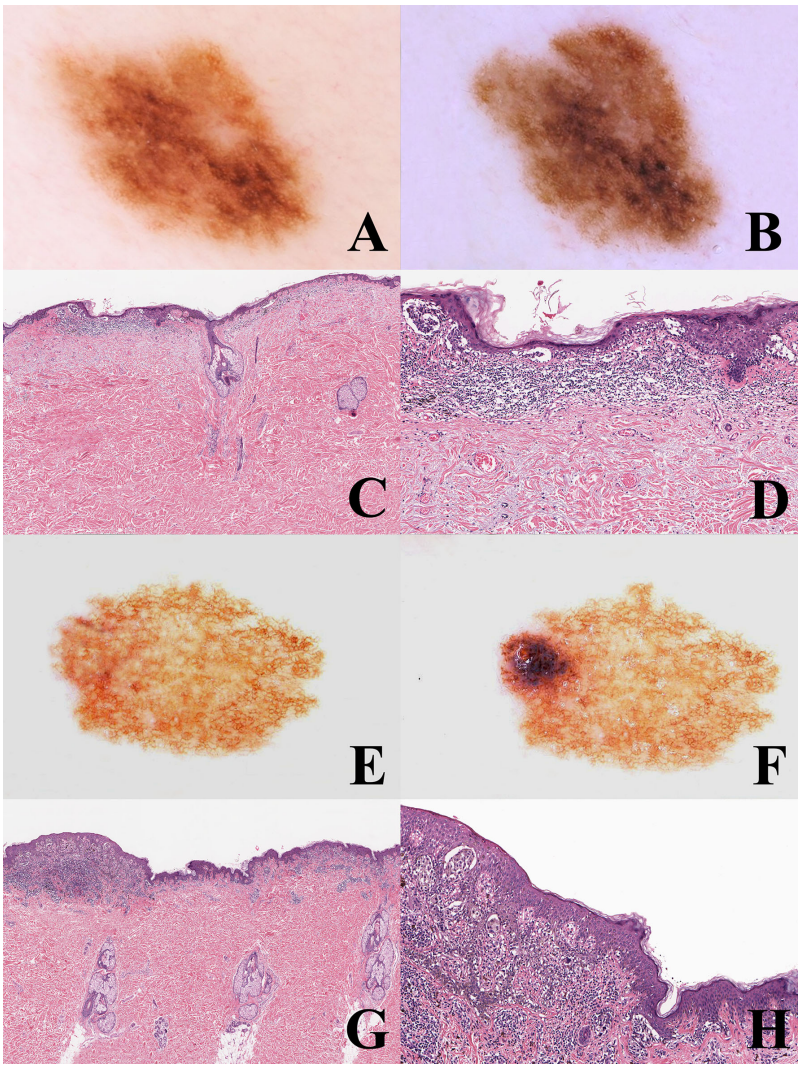

FIGURE 3 | **(A–D)** man, 53 years; a pigmented lesion of the back with a slightly irregular pigment network **(A)**; after six months, the tumor appears as uniformly enlarged, with increasingly irregular pigment network **(B)**. Histopathologically, the tumor is strikingly asymmetric (**C**; hematoxylin–eosin, ×25), with a lichenoid infiltrate at the base of its more severely atypical half (**D**; hematoxylin–eosin, ×100). Even if the histopathological picture might be interpreted as a melanoma *in situ* developing in the background of a dysplastic nevus, the homogeneous remodeling of the tumor documented with dermoscopic digital monitoring favored the diagnosis of melanoma *de novo*. E-H: Woman, 35 years; a pigmented lesion of the back with a thin and regular pigment network at the baseline **(E)**; after eight months, a raised bluish areas is evident at the periphery ("dermoscopic island") **(F)**. Histopathologically the tumor shares with the previous case the striking asymmetry **(G)** hematoxylin-eosin, ×25) and the presence of a lichenoid infiltrate at the base of its more severely atypical half **(H)** hematoxylin-eosin, ×100). However, dermoscopic digital follow up data clarify that this case likely represents an early melanoma *in situ* over a junctional dysplastic nevus.

clinicopathological setting; together with the many nevi in special sites (nevi with site-related atypia), it is an example of histopathological atypia probably unrelated with a signficantly higher risk of progression toward melanoma. This entity also underlines the role of clinically identifiable "environmental modifiers" (trauma, epilation, acute sun exposure) which may increase the histopathological features of atypia in nevi (2, 34) presumably without any impact in melanomagenesis.

As also underlined by the WHO Working Group in a paper published shortly after the 2018 Classification, the risk of an individual nevus progressing to melanoma has been estimated to be in the order of one in 33,000 or less per year (60). Therefore, from a practical point of view, we can conclude that:

1. the vast majority of nevi are, at worse, clinicopathological simulators and not precursors to melanoma;
2. besides esthetic reasons, indication to their excision is solely related to the impossibility to rule out melanoma on clinical grounds alone;
3. with the possible (but not universally accepted) exception of medium (1.5–20 cm) and large/giant (>20 cm) congenital nevi, which carry a definite size-related melanoma risk [up to

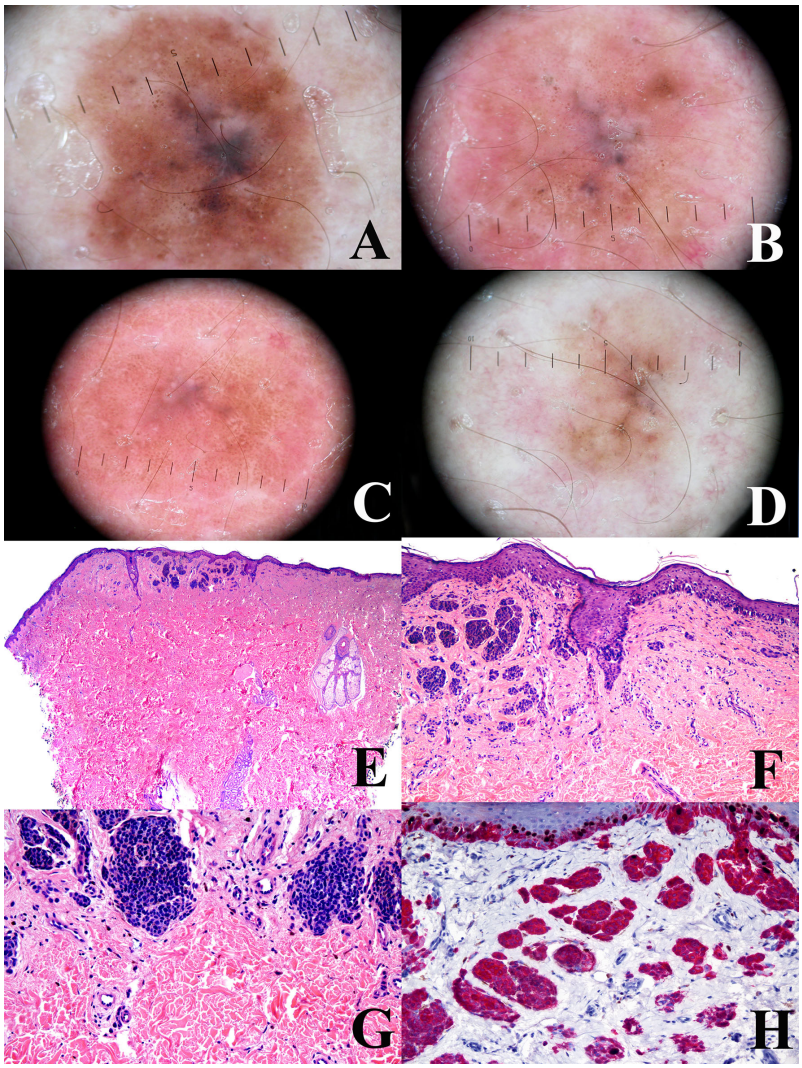

FIGURE 4 | Man, 38 years at the time of the surgical excision of a pigmented lesion of the scapular area; at the baseline, the tumor shows a a relatively regular peripheral pigment network associated with slightly eccentric globules and a central bluish area **(A)** the tumor shows a progressive and relatively symmetric fading after 1 year **(B)**, four years **(C)**, and 6 years **(D)**. The tumor discloses a "trizonal" histopathological pattern (**E**; hematoxylin–eosin, ×25), with an atypical junctional component, a scar-like dermal thickening (**F**; hematoxylin–eosin, ×100) and a very bland-appearing deep dermal component (**G**; hematoxylin–eosin, ×100); the proliferation rate (Ki67-positive dermal melanocytes, evaluated with a KI67/MART1 double stain) is very low (**H**; ×250). These histopathological features are consistent with the so-called "sclerosing nevus with pseudomelanomatus features". Such a histopathological diagnosis is in keeping with the slowly progressive and relatively symmetrical involution of the tumor, as documented with dermoscopic digital monitoring. Clinical images provided by Dr. Luigi Ligrone, Salerno, I.

15% (61)], by no means the excision of a nevus must be viewed as a tool of primary prevention ("prophylactic excision").

These statements also apply to dysplastic nevus and dysplastic nevus syndrome. The WHO Working Group defines dysplastic nevus as a clinically atypical, histopathologically benign junctional or compound melanocytic tumor, >4 mm in breadth on fixed sections (>5 mm clinically), with architectural disorder plus cytological atypia (62). The former is typified by irregular (horizontally oriented, bridging adjacent rete, and/or varying in shape and size) and/or dyscohesive nests of intraepidermal melanocytes plus increased density of non-nested junctional melanocytes (*e.g.* more melanocytes than keratinocytes in an area ≥1 mm^2); the latter is evaluated on the basis of the highest degree of cytological atypia present in more than a few melanocytes as low grade (nuclei ≤1.5× larger than basilar keratinocytes, with small or absent nucleoli and uniformly hyperchromatic or dispersed chromatin, and with "random" variation in size and shape) or high grade (nuclei ≥ larger than basilar keratinocytes, with prominent nucleoli and coarse or peripherally condensed chromatin, and with slightly

confluent variation in size and shape) (62). It is stated that nevi with high-grade dysplasia and/or with additional genetic alterations such as TERT promoter mutation should be considered for complete excision (62); this implies that a nevus with high-grade displasia needs no re-excision if already excised with clear margins.

Some studies are reported in which the degree of dysplasia is related with an increased melanoma risk (63–66); however, with the sole exception of a retrosective review considering the personal history of melanoma (66), these studies were histopathologically based, *i.e.*: they did not take into account the clinical features of risk of the individual patients (familial history of melanoma, skin type, personal history of sunburns, number of nevi, number of clinically atypical nevi). Thus, from a practical point of view, a histopathological diagnosis of dysplastic nevus must be evaluated in the clinical context in order to assess the risk of the individual patient to develop a melanoma; and, since genetic findings are relatively inconsistent to date (62), the diagnosis of dysplastic nevus syndrome (aka: Familial Atypical Multiple Mole and Melanoma, FAMMM; OMIN #155600) is largely based on clinical criteria, *i.e.*: number of nevi, number of clinically atypical and/or large nevi, personal/famlial history of melanoma (64, 66).

Excluded from the rubric of dysplastic nevus is lentiginous nevus, because being very common, unassociated with a relevant risk of progression to melanoma, and prone to poor diagnostic riproducibility (67). Lentiginous nevus is defined as a benign, junctional, or compound melanocytic tumor, <4 mm in width (on fixed sections), usually symmetrical but with poorly defined borders, with increased density of regularly spaced, non-nested junctional melanocytes around the tips and sides of the rete ridges, with no to mild cytological atypia and minor/variable features also seen in dysplastic nevi (67). These definitional features must be kept in mind because not uncommon in clinical practice are broad and irregular lentiginous melanocytic proliferations of the trunk and the proximal limbs, mostly found in elderly patients, which are probably the clinicopathological counterpart of lentigo maligna on non-chronically sun-exposed skin and are called lentiginous melanoma (68, 69). Dermoscopic digital monitoring of some of these lesions has demonstrated a homogeneous remodelling over many years, thereby suggesting that these are very slow-growing melanomas *de novo* and not the evolution to melanoma from lentiginous nevi (**Figures 5A–E**). In our experience on lentiginous melanoma, histopathological criteria alone are often weak and may result in a provisional diagnosis of IAMPUS or SAMPUS; the clinical picture of these cases is, however, very often unequivocal for melanoma and must be therefore incorporated into the decision-making process regarding their management.

Nested melanoma (of the elderly) is another example of deceptively bland melanoma (70) whose recognition often depends on a thorough clinicopathological correlation. Like lentiginous melanoma, it is often removed from the trunk and limbs in elderly patients as being large, growing and dermoscopically atypical flat pigmented tumor (71); histopathology features a junctional nesting which is not invariably irregular enough to allow a confident histopathological diagnosis; thus, the result is often a provisionla diagnosis of high-grade dysplasia, IAMPUS, or SAMPUS which, however, is not consistent with the clinical picture. Dermoscopic features of nested melanoma (70) suggest that it conceivably a slow growing melanoma *de novo*, rather than a melanoma evolving from a nevus (**Figures 5F–I**).

A MANAGEMENT-BASED APPROACH: THE MPATH-DX SYSTEM AND BEYOND

A histopathological diagnosis is aimed at giving a Mutidisciplinary Team the main (albeit not the sole) information for the clinical management. However, such an approach centered on histopathology having some major limitations, more or less explicitly underlined by the WHO Working Group, namely:

1. the diagnostic terminology varies depending on the individual cultural background and on local giudelines (72);
2. the diagnostic interobserver reproducibility is poor even among experts (73);
3. all the available evidence-based clinical guidelines are set upon a dichotomic diagnostic approach (all melanocytic tumors are either nevi or melanomas) and upon a unifying concept of melanoma (all melanocytic malignancies have the same biological behavior which can be predicted on the basis of a universally applicable set of histopathological parameters) (3).

In 2014, the Melanocytic Pathology Assessment Tool and Hierarchy for Diagnosis (MPATH-Dx) schema was proposed in an effort to reduce uncertainty and offer guidelines, mostly for melanocytic tumors different from melanoma (the "classical" melanocytic malignancy with its own evidence-based guidelines) (74): notably, the original schema excluded some melanocytic tumors (pigmented spindle cell; Spitz; epithelioid blue; cellular blue; deep penetrating/plexiform spindle cell) from Class 1 (no apparent risk), thereby anticipating the WHO 2018 concept of intermediate melanocytic tumors. The MPAT-Dx system stratified melanocytomas into four classes (Classes 2 to 5) of melanocytic tumors, with the first two being discriminated on the basis of the degree of histopathological atypia, and the last two discriminated on the basis of Breslow's thickness. The latter criterion, however, should not be applied to melanocytomas, because they are morphologically, genetically, and biologically different from "classical" melanoma with its "classical" prognostic parameters.

In order to specifically address the clinical management of dermal-based tumorigenic "intermediate" melanocytic tumors, practical recommendations have been delivered by the ESP, the EORTC, and the EURACAN (48). Morphological evaluation of these tumors is based on the evaluation of a list of general criteria, both architectural (diameter >6 mm; asymmetry; epidermal effacement; ulceration; high dermal cellularity; tumor clones; loss of grenz zone; absence of vertical "maturation"; expansile nodule formation; destriucive growth pattern; deep subcutaneous extension; pagetoid spread) and cytological (cellular pleomorphism; macro-eosinophilic

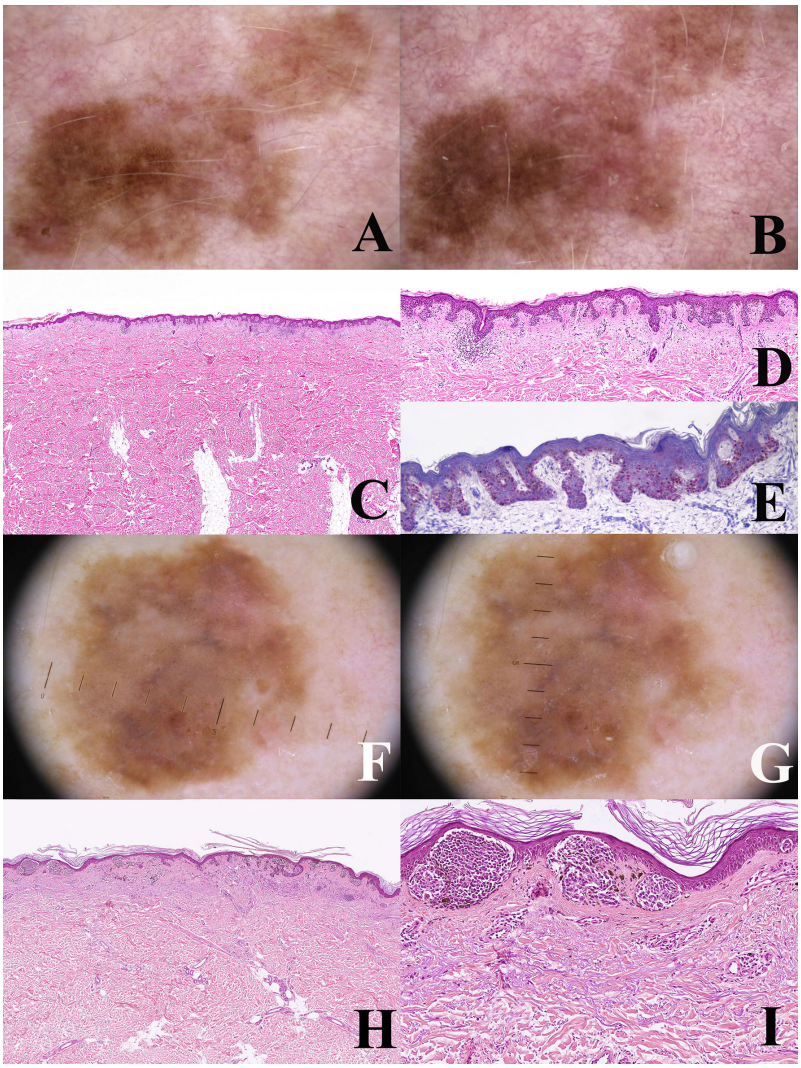

FIGURE 5 | **(A–E)** Man 52 years. Dermoscopy of a large pigmented lesion of the back with an irregular pigment network at the baseline **(A)** after one year, the lesion shows an increase in size with a homogeneous remodeling and a more prominent pigment network **(B)** such a slow clinical evolution is akin to a lentigo maligna of chronically sun-exposed skin and virtually excludes a diagnosis of nevus. Histopathologically, the tumor has a dysplastic nevus-like silhouette (**C**; hematoxylin–eosin, ×25) but is severely atypical because of the striking predominance of tightly packed single melanocytes at the junction (**D**; hematoxylin–eosin, ×100). PRAME immunostain shows a strong and diffuse nuclear positivity in intraepidermal melanocytes **(E)** ×250), as expected in melanoma. Clinicopathological features of the lesion are diagnostic for lentiginous melanoma *in situ*. **(F–I)** Man, 59 years. A large pigmented lesion of the abdomen, dermoscopically characterized by tiny eccentically grouped globules and structureless peripheral areas **(F)** after seven months the peripheral strucureless areas show a clear-cut increase in size **(G)**. Histopathologically there are some areas with a dysplastic nevus-like silhouette, but the epidermis is largely atrophic (**H**; hematoxylin–eosin, ×25) and junctional nests are very large and irregular (**I**; hematoxylin–eosin, ×250). These features suggest a diagnosis of melanoma *in situ* with a focally "nested" architecture.

nucleoli; variable density of nuclear chromatin; irregular nuclear membrane; >1 mitosis/mm^2; overlapping nuclei; tumor necrosis). Melanocytomas are then stratified into "low-grade" (few criteria present) and "high grade" (roughly up to half of them present), with excision margins estimated as adequate at 2 mm for the former and at 5–10 mm for the latter. Since a 2-mm excision margin is recommended for every melanocytic tumor, no further excision is required for low-grade melanocytomas. Pigmented epithelioid melanocytoma is by definition an intermediate-high-grade tumor; sentinel node staging is recommended only for "unclassified atypical dermal tumors" and for cases in which a Spitz melanoma cannot be ruled out; cases labeled as MELTUMP should be managed as per melanoma of the same thickness.

The ESP-EORTC-EURACAN recommendations concerning Spitz melanoma should be applied also on the basis of the recent observation that a "spitzoid" morphology is not invariably associated with a "Spitz" genetic signature (14, 15); in other words, malignant Spitz tumor (Spitz melanoma) is different from "spitzoid" melanoma, which can be regarded as a melanocytic

malignancy with "Spitz-like" morphology but genetically ascribed to a "classical" melanoma subtype because of the presence of a specific driver mutation, or numerous DNA copy number changes, or a high TMB. **Figure 6** illustrates the clinicopathological features of an ulcerated melanocytic malignancy histopathologically composed of large epithelioid cells with Spitz-like features, but immunohstichemically typified as a "classical" melanoma because of its immunohistochemical positivity to the anti-BRAF mutated protein VE1 antibody. Parenthetically, PEM-like (75, 76) and DPN-like melanomas (77, 78) might be differentiated from their "melanocytoma counterpart" based on immunohistochemical and/or genetic findings akin to "classical" melanoma.

Based on the above, a new problem is thus rising in dermatopathology, *i.e.*: the differential diagnosis between severely atypical melanocytoma and melanocytoma-like "classical" melanoma. This is not merely a speculative problem, because both a severely atypical melanocytoma and a melanocytoma-like "classical" melanoma will likely spread to the regional nodes, but only the latter will be candidates to sentinel node biopsy and, possibly, to an adjuvant therapy with *BRAF*-inhibitors or with immune checkpoint inhibitors (79, 80). This means that underdiagnosing a "classical" melanoma as a severely atypical melanocytoma may address the patient to an improper wait-and-watch strategy. Many melanocytomas (comprising Spitz tumors) currently lack an identifiable genetic

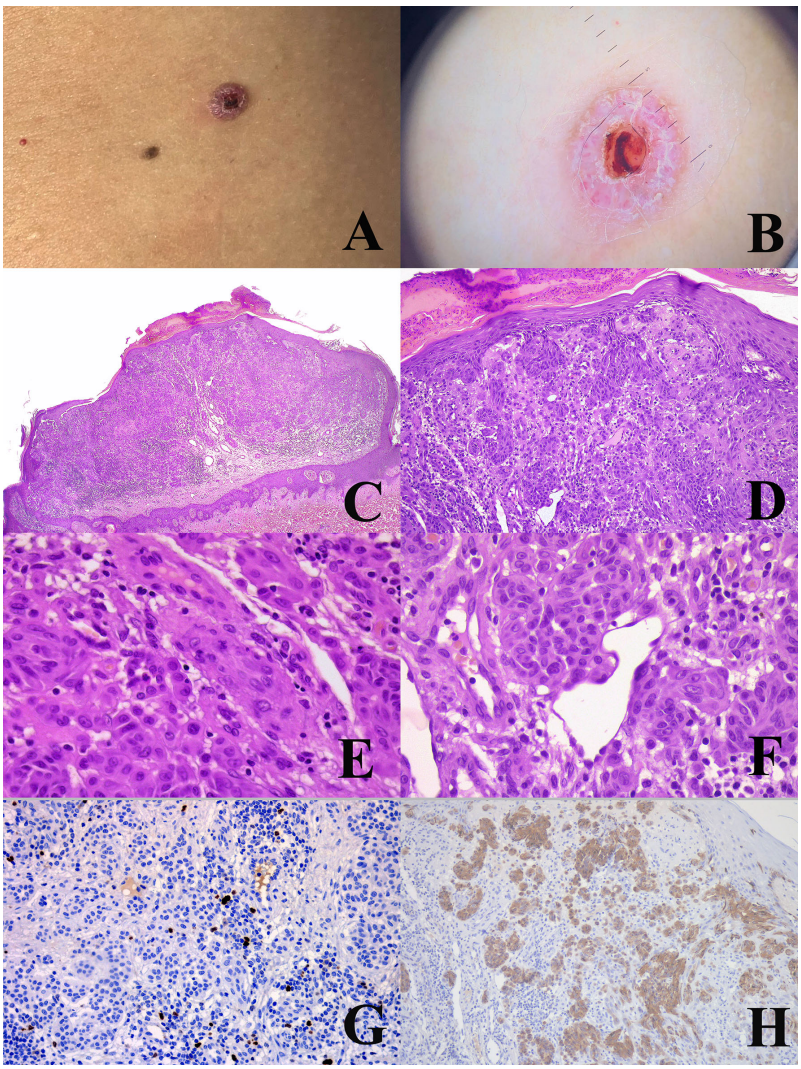

FIGURE 6 | Woman, 22 years. An ulcerated nodule of the right flank **(A)** dermoscopically characterized by keratoacanthoma-like features with vessels surrounded by a white halo **(B)**. Histopathologically, the tumor has an irregularly nodular, exophytic silhouette with an epidermal "collarette", a superficial crust, and a "brisk" inflammatory infiltrate in the dermis (**C**; hematoxylin–eosin, ×25); the superficial nests are very irregularly confluent with no sharp circumscription from the overlying epidermis (**D**; hematoxylin–eosin, ×250); dermal melanocytes show a "spitzoid" morphology, with spindle (**E**; hematoxylin–eosin, ×400) and epthelioid (**F**; hematoxylin–eosin, ×400) cells, both with reatively abundant and eosinophilic cytoplasms. In spite of the severe architectural atypia, the proliferation rate of the tumor (Ki67-positive dermal melanocytes) is low **(G)** ×250); however, the tumor is not an atypical Spitz tumor, but a classical nodular melanoma because it is positive to the antibody anti-BRAFv600e-mutated protein **(H)** ×250).

"signature"; by definition, however, they lack *BRAF*-mutation and a high TMB which are predictive parameters for neoadjuvant therapy (79, 80). Thus, the differential diagnosis between a severely atypical melanocytoma with no known genetic signature and a classical "melanocytoma-like" melanoma may be approached by looking for predictive (rather than diagnostic) paramenters; the same might apply for cases provisionally labeled as MELTUMP or as unclassified atypical dermal lesion (48).

A THERAPY-ORIENTED DIAGNOSTIC APPROACH

When dealing with an atypical melanocytic tumor of the skin, the first step can be the differential diagnosis between a "classical" type of melanocytic tumor and a "melanocytoma" (comprising Spitz tumor). Immunohistochemistry can assist such a differential diagnosis as follows:

- The anti BRAF-mutated protein VE1 antibody identifies the subset of melanocytic tumors of the "classical" type harboring the $BRAF^{v600e}$ mutation (or a "combined" melanocytoma) (48, 81);
- The immunostain for BAP1 can document loss of the consitutive nuclear immunoreactivity in BAP1-inactivated melanocytic tumors (33, 34);
- The anti PRAME immunostain can assist the differential diagnosis between benign and malignant "traditional" melanocytic tumors (82); in our experience, particularly for lentiginous neoplasms and for the differential diagnosis between congenital nevus and nevoid melanoma;
- The anti-ALK, anti-TRKA, anti-MET, anti-HRAS-WT, and anti-ROS1 antibodies identify the subset of melanocytic tumors of the Spitz lineage with the respective kinase gene changes (48, 83, 84);

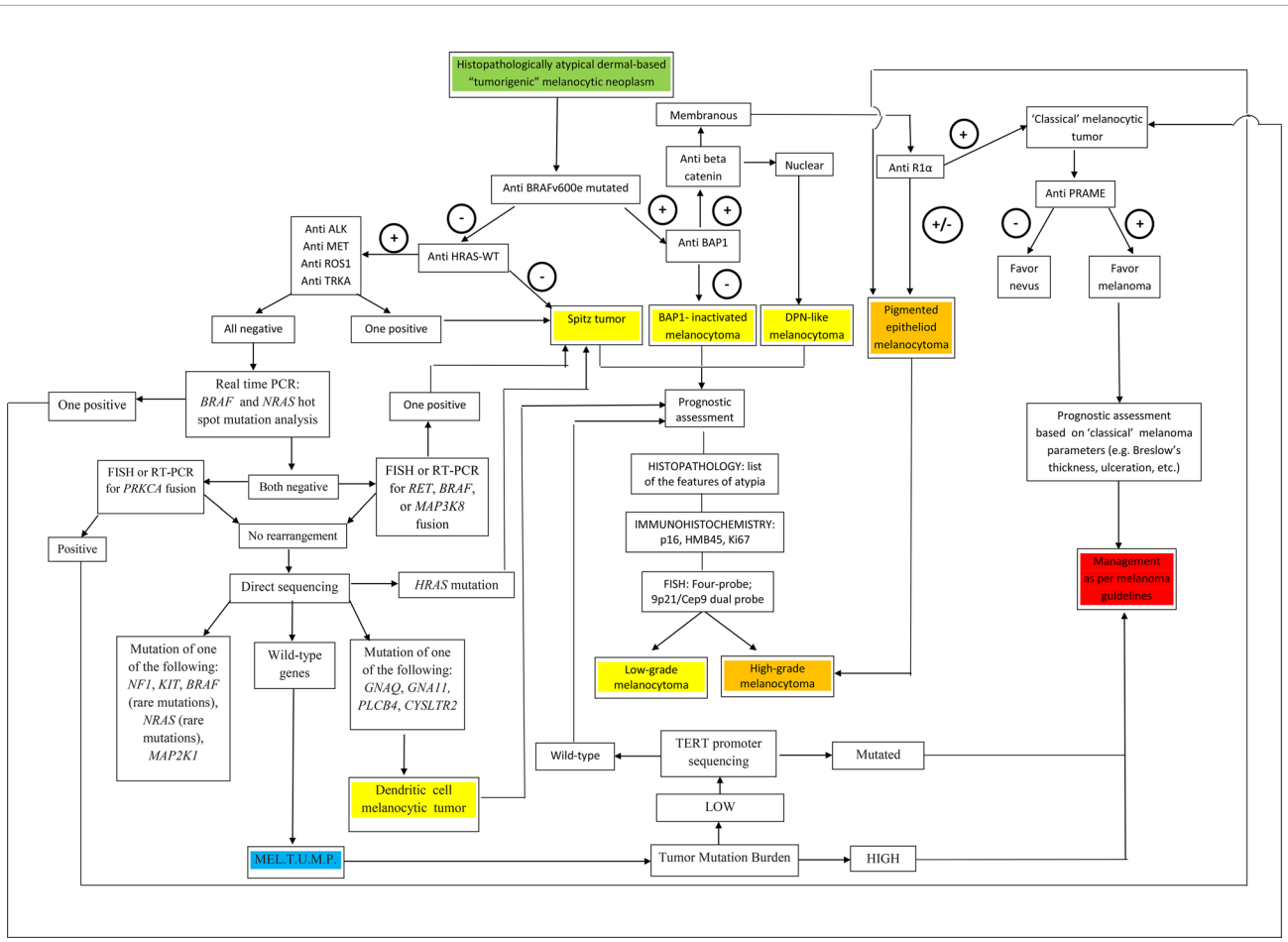

FIGURE 7 | A flow chart illustrating a therapy-oriented morphomolecular approach to atypical dermal-based tumorigenic melanocytic neoplasms. Of paramount importance are: i) the distinction between melanocytomas (recognized as such by specific genetic signatures) and melanocytic tumors of uncertain malignant potential (MEL.T.U.M.P.; provisionally defined as tumors with unknown driver mutations); ii) among melanocytomas, the distinction between low-grade and high-grade tumors; iii) among MELTUMP, the distinction between tumors with a low tumor mutation burden and tumors with a a high tumor mutation burden, the latter being best managed as per "classical" melanoma.

- The anti-beta catenin immunostain identifies the aberrant nuclear positivity definitional for DPN and related tumors (36);
- Tha anti-R1alpha can document loss of constitutive nuclear immunoreactivity in PEM with inactivating mutation or epigenetic inactivation of *PRKAR1A* (85).

An immunohistochemical panel aimed at a risk stratification can encompass:

- p16, which may disclose uneven immunoreactivity or "clonal" loss as an atypical feature (2, 48);
- HMB45, which may be unevenly distributed, with loss of the "gradient" pattern seen in benign tumors (2);
- Cell cycle-related protein Ki67, which may show a high rate of expression and/or "proliferative clusters" in atypical lesions (2).

The traditional four-probe (targeting *MYB*, *RREB*, *Cep6*, and *CCND11*) plus the anti-*CDKN2A*/*Cep9* dual probe FISH examination may help refine the risk stratification of melanocytic tumors as recently proposed (86).

If morphology and immunohistochemistry are not contributory in assigning the melanocytic tumor to a given lineage, molecular analysis guided by morphology may be implemented as follows:

- Identification of hotspot mutations of *BRAF* (codon 600) and *NRAS* [exon 2 (odons 12, 13), exon 3 (codons 59, 61), and of exon 4 (codons 117, 146)];
- Sequencing techniques for the following: *NF1*, *KIT* (exons 11, 13, 17, and 18), *BRAF* (rare mutations), *NRAS* (rare mutations), and *MAP2K1* (exons 2 and 3; in-frame deletion) for "classical" melanocytic tumors; *GNAQ* (exons 4 and 5), *GNA11* (exons 4 and 5), *PLCB4*, and *CYSLTR2* for dendritic melanocytic tumors (WHO 2018 Pathways 8 and 9); *HRAS* (exons 2 and 3) for a subset of Sptz tumors; TERT promoter for a subset of aggressive malignencies (some characterized by a 'Spitz-like' morphology);
- Fluorescence *in situ* hybridization (FISH) or reverse transcriptase polymerase chain reaction (RT-PCR) examination for fusions involving: *BRAF* and *RET* for Spitz tumors; *MAP3K8* for morphologically malignant epithelioid cell Spitz neoplasms (87, 88); *PRKCA* for PEM.

As per ESP-EORTC-EURACAN guidelines, if the immunohistochemical screening implies additional procedures, immuno-positive cases (of Spitz neoplasms) should be confirmed for the respective genomic aberration by molecular examinations (48); this is, however, a theroretically uncommon scenario.

As a final step for an approach akin to tumor-agnostic therapy, NGS analysis can help identify melanocytic tumors with "rare" genetic signatures, and—even more important—melanocytic tumors with a high TMB which should be definitely ascribed to the category of classical melanoma with the relative therapeutic options. Specialized referral centers must be involved for sequencing, fusion studies, and NGS examination (48).

A visual summary of the above-proposed algorithmic diagnostic approach is given in **Figure 7**.

TAKE-HOME MESSAGE

The traditional "dichotomic" (benign *vs* malignant) view of melanocytic tumors and the concept of melanoma as a "unique" clinicopathological entity no longer fit with the routine diagnostic approach. Along with "classical" (Clark's and McGovern's) subtypes of melanoma, other melanocytic malignancies, each charcaterized by peculiar biological behavior probably exist, must be distinguished from "classical" melanoma subypes and require specific clinical guidelines. Clinicopathological correlation can allow both reducing the histopathological diagnostic uncertainty and addressing patients to a proper management.

AUTHOR CONTRIBUTIONS

Both authors listed have made a substantial, direct, and intellectual contribution to the work and approved it for publication.

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CASE REPORT
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Interference of COVID-19 Vaccination With PET/CT Leads to Unnecessary Additional Imaging in a Patient With Metastatic Cutaneous Melanoma—Case Report

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The COVID-19 pandemic has widely influenced oncological imaging mainly by presenting unexpected pulmonary and mediastinal lesions. The ongoing global program of vaccination has led to incidental diagnosis of axillary lymphadenopathy. We present a case of increased accumulation of ^{18}F-FDG in an axillary lymph node in a PET/CT scan performed in a 43-year-old female patient with metastatic melanoma. The scan was performed 4 days after the AZD1222 vaccination. The occurrence of lymphadenopathy was verified with another PET/CT scan scheduled one month later. This case report presents a possible misinterpretation of PET/CT images caused by the recent COVID-19 vaccination. To avoid distress of the patient and unnecessary oncological diagnostics to verify the findings, we recommend avoiding scheduling PET/CT shortly after vaccination.

Keywords: malignant melanoma, PET/CT, COVID-19, vaccination, metastases

INTRODUCTION

Positron emission tomography with computed tomography (PET/CT) using ^{18}F-fluoro-deoxyglucose (^{18}F-FDG) is a valuable tool used to monitor treatment of melanoma, especially its metastatic forms subjected to immunotherapy (1). In stage III cutaneous melanoma, sensitivity in detecting distant metastases during follow-up ranges between 82 and 100%, and the specificity ranges between 45 and 100% (2). With regard to lymph node metastases, PET/CT shows sensitivity of 91% for nodes >10 mm and 69% for smaller nodes (with a similar specificity of 71%) (3). The inflammatory reaction of the lymph nodes is one of the main causes of the false positive PET/CT findings in oncological patients. A non-specific nodal ^{18}F-FDG uptake may lead to a false diagnosis of metastases and to the initiation of an unnecessary treatment.

The widespread COVID-19 vaccination has raised a lot of questions with regard to its potential complications and side-effects. Many patients experience local pain in the injection site; some of them suffer from generalized inflammatory reactions, including fever and fatigue (4). As it has been recently shown, local inflammatory reaction in the lymphatic system may have potential

implications for imaging. The vaccine-induced lymphadenopathy may also pose a challenge in the PET/CT interpretation (5). In this paper, we report a patient with stage IV melanoma who had a PET/CT performed incidentally few days after COVID-19 vaccination that resulted in a false positive finding in an axillary lymph node.

CASE PRESENTATION

A 43-year-old female with the diagnosis of metastatic melanoma treated with nivolumab was reported for a ^{18}F-FDG PET/CT scan to exclude disease progression shortly after COVID-19 vaccination.

The timeline of the history of the patient is presented in **Figure 1**. She was diagnosed of a primary cutaneous melanoma of the right thigh in June 2015. The patient was previously healthy, with no history of other malignancies, surgery, or medication. There was no personal or family history of melanoma. The lesion was removed, and the final diagnosis was: cutaneous melanoma, *BRAF* wild-type, pT2aN0M0. In January 2019, a recurrence of the disease in form of the subcutaneous and brain metastases was diagnosed with the use of CT. A single cerebral metastasis was confirmed by MRI. After two weeks, nivolumab treatment was initiated, with a dose of 480 mg every 4 weeks. After the first dose of nivolumab, the cyberknife radiotherapy of the brain metastasis was performed. After two months of systemic treatment, all subcutaneous metastases disappeared; however six new brain metastases were detected in another MRI. All these new lesions were subsequently treated with cyberknife. The patient continued the nivolumab therapy beyond progression. Thereafter, during nivolumab treatment, she developed a further disease progression in the brain (04.2019, 05.2019, 01.2020, 12.2020). With each progression, one or two new brain metastases were found in the MRI. These lesions did not exceed 1 cm and were asymptomatic. After each occurrence, the cerebral metastases were treated with cyberknife. All extracerebral metastases were still in regression until December 2020 when some metabolically active lymph nodes in the right iliac region were detected in a PET/CT scan (PET1). In January 2021, a robot-assisted right iliac lymphadenectomy was performed, and the metastatic character of the iliac lymph nodes was histologically confirmed.

In February 2021, the patient underwent AZD1222 COVID-19 vaccination (first dose injected into her left arm). Four days later, another PET/CT was performed to exclude new extracerebral metastases (PET2). No sign of melanoma recurrence was found in the iliac lymph nodes or central nervous system. However, a metabolically active lymph node in the left axillary region was noted (**Figure 2**). The lymph node had the dimension of 9 × 7 mm, and the maximal standardized uptake value was 5.2. Additionally, an area of increased ^{18}F-FDG accumulation was found in the left deltoid muscle that corresponded to the site of the recent vaccination. An inflammatory reaction to the injection was suspected to be responsible for the ^{18}F-FDG accumulation in the axillary lymph node. However, in order to rule out a melanoma metastasis in the axillary lymph node, a follow-up PET/CT (PET3) was recommended 28 days later (March 2021, 32 days after vaccination). This scan did not present any ^{18}F-FDG accumulation in the reported lymph node. The diameter of the node did not change. No other finding was reported, except for a focus of slightly increased ^{18}F-FDG accumulation (diameter of 10 mm) in the right cerebellar lobe that had not been present in the PET2 scan. Fortunately, the subsequent MRI did not confirm any lesion in the cerebellum and did not show any other intracranial recurrence. However, further MRI monitoring of the central nervous system has been recommended.

To date (June, 2021), the patient does not present any active metastases (NED—no evidence of disease). Moreover, her performance status remains WHO 0 from the initial diagnosis until now. The treatment beyond disease progression was beneficial to the patient. In addition, at each disease progression, the patient was offered a second line treatment

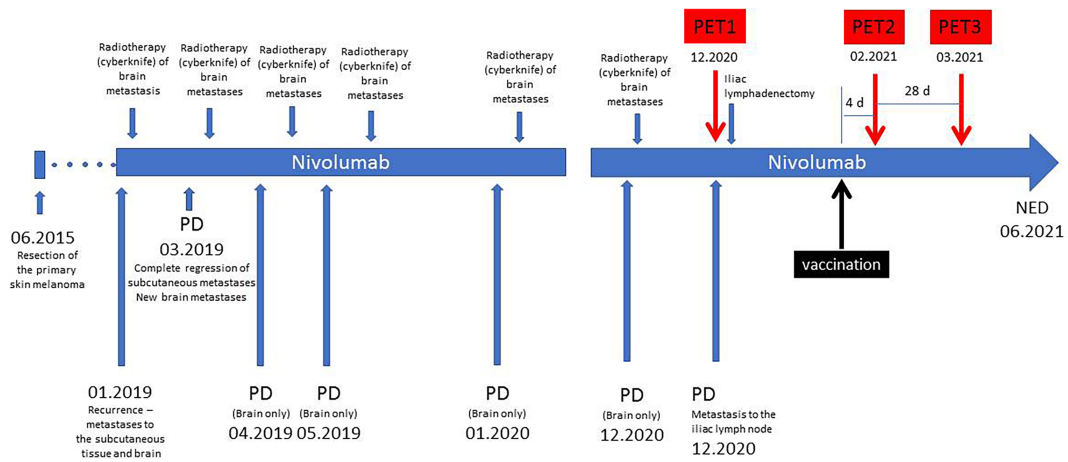

FIGURE 1 | Timeline of the treatment. PD, progression of the disease; NED, no evidence of the disease.

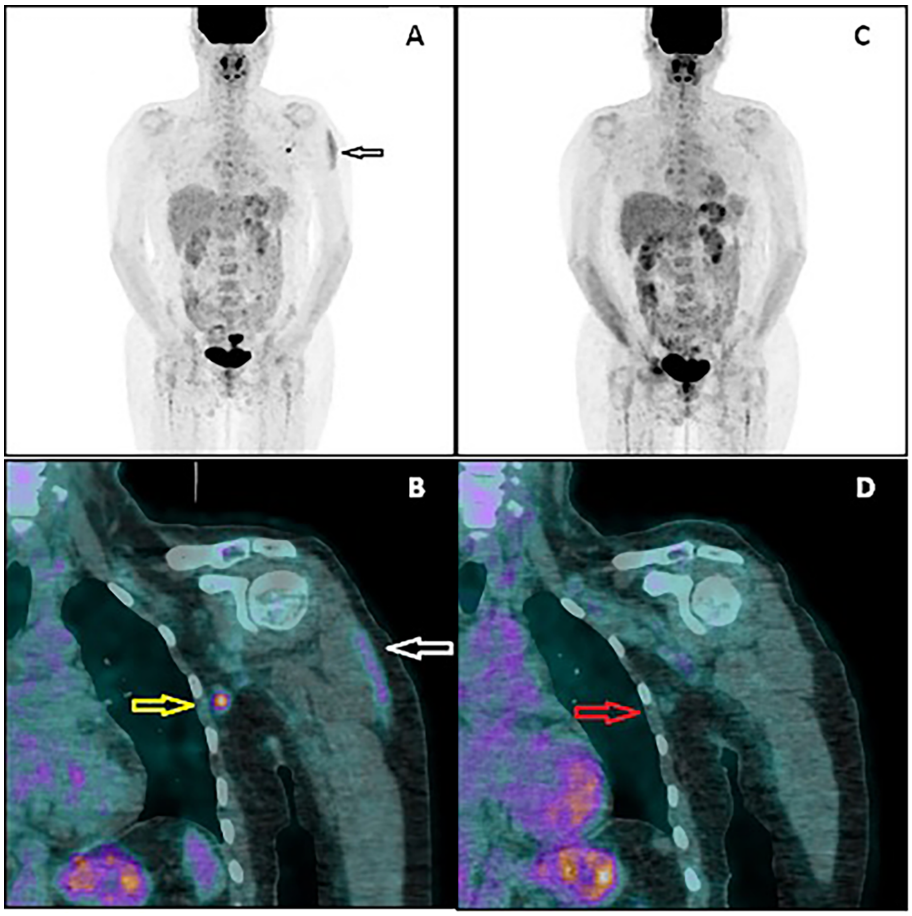

FIGURE 2 | PET/CT image performed 4 days after vaccination **(A, B)**. The multiple-intensity projection image **(A)** showing increased ^{18}F-FDG uptake in the left deltoid muscle (black arrow) and in the left axillary region. Fused coronal image **(B)** showing the uptake in the muscle (white arrow) and in the axillary lymph node (yellow arrow). PET/CT image performed 32 days after vaccination **(C, D)**. Both the muscular uptake and nodal uptake have disappeared. The referred axillary lymph node (red arrow) shows similar morphology but no ^{18}F-FDG accumulation.

with ipilimumab, to which the patient did not consent due to its high toxicity and low efficacy (6). The patient was informed that there was insufficient evidence for the treatment with nivolumab beyond confirmed progression (7). Due to the fact that the patient did not consent to the ipilimumab treatment, as well as to the lack of a clinical trial, the continuation of nivolumab therapy was the only reasonable treatment option. In conclusion, the continued treatment beyond progression was decided due to the low tumor burden, the motivation of the patient and good performance status and, the lack of other treatment options.

The patient gave consent for the publication of her case.

DISCUSSION

PET/CT is an established imaging modality used in oncology on an every-day basis. It is well-known that foci of non-oncological pathology can accumulate ^{18}F-FDG similarly to the malignant tumors, nodal and distant metastases. The examples of such benign, metabolically active lesions include pulmonary tuberculosis, benign thyroid nodules, diverticulitis, *etc.* (8–10). Also, reactive lymph nodes can present as metabolically active, mimicking nodal metastases (11). Common situations like that include cervical lymphadenopathy after infection of the upper respiratory tract or tonsillitis, mediastinal lymphadenopathy in case of pneumonia or sarcoidosis, and inguinal lymph node metabolic stimulation due to a lower extremity injury. Careful anamnesis prior to the scan, not excluding apparently irrelevant conditions, like tooth pain or transient fever, may prevent a misinterpretation of the images.

In the every-day practice of a PET/CT department, the occurrence of vaccination-induced lymphadenopathy is a new phenomenon. Several authors have already reported the unexpected findings of increased ^{18}F-FDG accumulation in the axillary lymph nodes (5, 12–14). It may cause serious doubts regarding the character of lymphadenopathy in cases of melanoma and other malignancies with an aggressive dissemination pattern. The presented patient had a history of

lymph node metastases in the inguinal region that was obviously correlated with the primary location in the ipsilateral lower extremity. However, the metastatic behavior of melanoma can be unpredictable, and a metastasis in the contralateral axillary fossa could not be excluded, especially when knowing that the progression of the disease with new brain metastases during systemic treatment had occurred several times. The coexistence of all these risk factors has led to the recommendation of an early follow-up scan (PET3). Although the axillary metastasis has been excluded by the negative PET/CT, an increased caution of the reporting physician, who was aware of the history of the patient, led to another false positive finding—the cerebellar focus suspected of being another recurrence in the central nervous system. The reporting attention of the physician to possible intracranial foci was alerted because of the well-known low sensitivity of ^{18}F-FDG PET/CT in the detection of brain metastases due to the physiological radionuclide uptake in the gray matter. The rapid application of MR has led to the exclusion of relapse.

Worldwide COVID-19 vaccination is an unprecedented program of the medical interventions performed on an enormous global population in a relatively short time (4). What is more, commonly, the intramuscular vaccine injection is performed twice in each subject. Also oncological patients, referred to a PET/CT scan as a part of their routine management, are independently vaccinated and the schedules of the vaccination and imaging are not always coordinated, as they are being organized by separate institutions. This may lead to a situation of equivocal PET/CT findings as presented in this case report.

Interestingly, the sign of elevated ^{18}F-FDG accumulation does not occur in all vaccinated patients. In a recent study by Schroeder et al., the ^{18}F-FDG-positive axillary lymph nodes were found in four out of 54 patients subjected to COVID-19 vaccination performed at the median time of 10–13 days earlier (15). This observation may cause even more uncertainty of the PET/CT image interpretation. Recently, authors from Israel have reported a much higher incidence of the vaccination-related axillary lymphadenopathy: 36.4% after the first vaccine dose and as much as 53.9% after the booster dose (16). It must be emphasized, however, that these results refer to the mRNA vaccine (Pfizer BNT162b2), not the viral vector vaccine, as in the presented case. If the vaccination and the PET/CT are to be performed in a short interval, we recommend to schedule the PET/CT before the COVID-19 vaccination. This may not always be feasible, especially if the patient undergoes an oncological treatment with a strict protocol. In such a situation, a delayed PET/CT scan would be a preferable solution. Considering the optimal time of PET/CT after vaccination, no firm data are available. From our experience, a great majority of patients who present the sign of increased axillary ^{18}F-FDG accumulation received their vaccine in the recent 10 days. In rare cases, however, we have seen this sign even more than 4 weeks after the injection and this observation is supported by other authors (5). It is noteworthy that a prolonged nodal hypermetabolism is more likely to be found after the booster dose of a mRNA vaccine (16). Therefore, we recommend performing the PET/CT imaging *ca*. 4 weeks after the vaccination if the treatment protocol allows that. In any case, the physician responsible for reporting the PET/CT scan must be aware of the vaccination date.

CONCLUSION

This case report presents possible misinterpretation of PET/CT images caused by a recent COVID-19 vaccination. To avoid distress of the patient and unnecessary oncological diagnostics to verify the findings, we recommend avoiding scheduling PET/CT shortly after vaccination.

DATA AVAILABILITY STATEMENT

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

AUTHOR CONTRIBUTIONS

RC wrote the manuscript. JS performed image acquisition and prepared images. JM prepared clinical data and the time-line and reviewed the manuscript. MR reviewed the manuscript. All authors contributed to the article and approved the submitted version.

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REVIEW
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Non-Melanoma Skin Cancer in People Living With HIV: From Epidemiology to Clinical Management

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Skin cancers represent the most common human tumors with a worldwide increasing incidence. They can be divided into melanoma and non-melanoma skin cancers (NMSCs). NMSCs include mainly squamous cell (SCC) and basal cell carcinoma (BCC) with the latest representing the 80% of the diagnosed NMSCs. The pathogenesis of NMSCs is clearly multifactorial. A growing body of literature underlies a crucial correlation between skin cancer, chronic inflammation and immunodeficiency. Intensity and duration of immunodeficiency plays an important role. In immunocompromised patients the incidence of more malignant forms or the development of multiple tumors seems to be higher than among immunocompetent patients. With regards to people living with HIV (PLWH), since the advent of combined antiretroviral therapy (cART), the incidence of non-AIDS-defining cancers (NADCs), such as NMSCs, have been increasing and now these neoplasms represent a leading cause of illness in this particular population. PLWH with NMSCs tend to be younger, to have a higher risk of local recurrence and to have an overall poorer outcome. NMSCs show an indolent clinical course if diagnosed and treated in an early stage. BCC rarely metastasizes, while SCC presents a 4% annual incidence of metastasis. Nevertheless, metastatic forms lead to poor patient outcome. NMSCs are often treated with full thickness treatments (surgical excision, Mohs micro-graphic surgery and radiotherapy) or superficial ablative techniques (such as cryotherapy, electrodesiccation and curettage). Advances in genetic landscape understanding of NMSCs have favored the establishment of novel therapeutic strategies. Concerning the therapeutic evaluation of PLWH, it's mandatory to evaluate the risk of interactions between cART and other treatments, particularly antiblastic chemotherapy, targeted therapy and immunotherapy. Development of further treatment options for NMSCs in PLWH seems needed. We reviewed the literature after searching for clinical trials, case series, clinical cases and available databases in Embase and Pubmed. We review the incidence of NMSCs among PLWH, focusing our attention on any differences in

clinicopathological features of BCC and SCC between PLWH and HIV negative persons, as well as on any differences in efficacy and safety of treatments and response to immunomodulators and finally on any differences in rates of metastatic disease and outcomes.

Keywords: human immunodeficiency virus, non-melanoma skin cancer, basal cell cancer, squamous cell cancer, immunedeficiency, review (article)

INTRODUCTION

The natural history of HIV has been significantly modified by the advent of combined antiretroviral therapy (cART) that has prolonged life expectancy and reduced mortality and morbidity of people living with HIV (PLWH). Even if highly active, cART cannot cure HIV and so it is a lifelong therapy because of a hidden, even though active, reservoir (1, 2) that is able to escape the treatment. Over the past twenty years, many important factors, as increased age of PLWH and (3) coinfection with oncogenic viruses have promoted the emergence of other malignant neoplasms that collectively are classified as non-AIDS-defining cancers (NADCs) and that, over the years, overtook the incidence of AIDS-defining cancers in PLWH (4–14).

Non-melanoma skin cancers (NMSCs) include primarily basal cell (BCC) and squamous cell carcinoma (SCC). They represent the most frequent malignant neoplasms in the white population, with a worldwide increasing incidence (15). NMSCs develop from epidermal cells and their incidence increases in older age. The pathogenesis is multifactorial: chronic sun exposure is the main environmental risk factor. Other risk factors include increased longevity, genetic mutations, immunodeficiency, concurrent disease and dedicated therapy (i.e., psoriasis) (16). In immunocompromised patients, such as HIV positive patients, the incidence of more malignant form or the development of multiple tumors seems to be higher than among immunocompetent people. In PLWH these malignancies are often more aggressive compared with the general population and they need multidisciplinary assistance (17–26).

The purpose of this review is to describe the incidence of NMSCs among PLWH, focusing on any difference in clinicopathologic features of BCC and SCC between PLWH and HIV negative persons, as well as on any difference in efficacy and safety of treatments and response to immunomodulators, and finally any differences in rates of metastatic disease and outcomes.

MATERIALS AND METHODS

A systematic search of the EMBASE and Medline databases was performed to identify potentially relevant papers reporting original research on NMSCs in PLWH. This research was performed from inception to 3 March 2021, and it was restricted to humans. Clinical trials, prospective and retrospective studies, case series, case control studies and metanalysis concerning the topic of NMSCs in PLWH published in English, Spanish and Italian with available abstracts, were selected if they addressed one or more of the following topics: BCC, SCC, basal cell carcinoma, squamous cell carcinoma, HIV. The following search strings were used: "BCC OR basal cell carcinoma AND HIV", "SCC OR squamous cell carcinoma AND HIV". Reviews, expert opinions, book chapters and articles lacking original data were excluded. The title and abstract of all articles retrieved were check by two reviews (EVR and MGM) who selected relevant articles for full text evaluation according to predetermined criteria. Discrepancies were resulted by a third reviewer (MB). Studies were compared by title and abstracts to eliminate duplicates. A Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) flow diagram (Downloaded 03 March 2021, http://prisma-statement.org/PRISMAStatement/FlowDiagram.aspx.) was set to illustrate the review process (**Supplementary Materials**). We summarized the review according to PRISMA guidelines, represented below).

EPIDEMIOLOGICAL PROFILE OF NMSCs

Non-melanoma skin cancers (NMSCs) are the most frequent neoplasms in Caucasians and their incidence is increasing worldwide, with 80% diagnosed as BCC followed by SCC being both more common than melanomas (27). They are much common in white population than in skin color people. Their incidence results 18-20 times higher than that of melanoma (28). Epidemiologic studies highlight that the worldwide incidence varies widely. In fact, BCC has higher incidence in equatorial latitudes and lower in polar latitudes. Australia is the country with highest incidence of BCC, followed by the US and Europe, although the real incidence is globally underestimated (29). In Australia the rate for BCC is more than 1,000 per 100,000 person-years (2,448/100.000), followed by Europe (91 in women and 129 in men per 100,000 person-years) and the US (450 per 100,000 person-years). Cutaneous SCC is the most common skin cancer, behind BCC, and it represents approximately 20 percent of NMSCs (30). Its incidence increases more quickly with age than BCC. In PLWH cancer is becoming a growing problem representing now the first cause of death. It is clear that cancer risk is higher in PLWH in comparison with the general population (31), less clear are the reasons behind it. The advent of cART has improved the morbidity and mortality of PLWH, prolonging their life expectancy (32). A large body of literature has highlighted that HIV infection is associated to an increased risk of several

different type of cancers besides NMSCs, such as lung cancer, cancer of the colon and rectum, Hodgkin disease, hepato-cellular carcinoma, head and neck SCC (HNSCC), conjunctival SCC and anogenital SCC (10, 17, 19, 20, 33, 34). BCC in PLWH show a 1.8-fold increased risk in comparison with HIV negative people (35), but it could be better in patients that have a good control of the infection. The occurrence of multiple BCC in PLWH without additional risk factors is uncommon. In HIV positive patients BCC is essentially more frequent than SCC (36) and ratios around 4:1 of BCC versus SCC have been found, similar to the general population (4:1) (33). In a retrospective cohort that studied 36821 HIV negative and 6560 HIV positive patients it has been shown an increased risk for BCC among PLWH. In fact, in this Californian cohort, the risk of developing a BCC was about twice as likely in non-Hispanic white PLWH than in the same HIV negative population. So that, it has been denoted that patients with HIV showed a meaningful tendency to develop BCC as HIV negative persons (37). Regarding HNSCCs, they are a heterogeneous group of cancers occurring in various anatomic sites, including scalp, oral cavity, lips, oropharynx, nasopharynx and larynx.

Focus on SCC of the Scalp

SCC of the scalp represents approximately 16% of scalp cancers (38), with a mean age of 65 years at diagnosis. It has a positive correlation with advanced age.

Known risk factors for developing SCC of the scalp are older age, history of ionizing radiation chronic scarring, androgenetic alopecia, ultraviolet light exposure, actinic damage.

Immunosuppression is a crucial risk factor for all SCCs (39) that represent the most common cancer in immunosuppressed patients, with greater potential for tumor growth, cell differentiation, and aggressiveness. Furthermore, SCC may show a higher risk of metastatic disease and death in immunocompromised patients compared with immunocompetent individuals (40).

A retrospective study showed that twenty out of fifty-tree immunocompromised patients affected by cutaneous SCC of the scalp had bone invasion, that is associated with poor prognosis (41).

The aggressive behavior of SCC on the scalp in immunosuppressed patients has been described by Lang et al. (42). It is recommendable to manage scalp tumors aggressively and appropriately because they are associated with important morbidity and mortality. So that, it is essential to monitor for bone invasion, recurrence, perineural invasion and metastasis. A better knowledge of the mechanisms of recurrency could be helpful to prevent morbidity and mortality in this specific group of patients. Concerning the clinical presentation of SCC of the scalp in HIV positive patients, Ferreira CP et al. have described a case report of a sixty years-old male, white, and HIV positive in use of zidovudine, lamivudine and efavirenz, presenting tumor located in scalp, progressing with rapid growth for one year. The histopathological examination revealed a diagnosis of well differentiated SCC. Immuno-virological profile revealed CD4: 62 cells/mm^3; CD8: 1,654 cells/mm^3; viral load: 91,000 copies. CT brain scan revealed cerebral foci of calcification in the suprasellar region as well as in basal ganglia on the left, with a diameter of 15 mm and invasion to the skull along the interparietal suture. The patient had subsequent pneumonia that was the final cause of death. Fortunately, SCC is often diagnosed before the invasion to the skull because of its slow progression. Rarely, SCC can extend to the brain and invade, in late stages, the skull and the dura mater. When this event occurs, patients may present neurological symptoms (43). Because of the anatomical profile of the scalp region, margin excision is not always possible. Preoperative imaging is essential to define the proper extent of invasion and choose the correct treatment strategy. The treatment of SCC in advanced stages is challenging starting from the multidisciplinary surgical approach needed for a proper excision. Further studied are required for advanced disease.

RISK FACTORS AND PATHOGENESIS

Among immunocompetent light-skin color people, the development of NMSCs is favored mainly by chronic sun exposure and increasing age. There are important phenotypic characteristics, such as fair skin type, light-colored eyes, red hair, northern European origin and childhood freckling (44) that influence vulnerability to solar radiation. The frequency and intensity of sun exposure are also important.

Other environmental risk factors that contribute an increased risk for NMSCs include older age, family history of skin cancer, immunodeficiency (45), previous radiotherapy, long-term immunosuppressive treatment, genetic syndromes and chronic, mostly occupational, exposure to arsenic (46).Moreover, several observational studies have documented a correlation between use of photosensitizing molecules and increased risk for BCC (47).

The Genetic Landscape of NMSCs

Mutations of numerous tumor suppressor genes and proto-oncogenes play a key role as drivers in BCC formation (48). In almost 90% of cases, mutations that activate the Hedgehog pathway (HH) play an established role in the development of BCC (48), while SCC is characterized by a high neoantigen burden (37). In about 50% of BCC cases, TP53 tumor-suppressor gene mutations are caused by UV radiation. TP53 encodes the P53 protein involved in maintain genomic stability by regulating the cell cycle, inducing apoptosis and activating DNA repair. Furthermore, mutations identified in PTCH1 and TP53 are so-called UV signature mutations, because in most cases they are consistent with ultraviolet radiation-induced mutagenesis.

Among genetic syndromes that may increase the risk for the development of BCC, we should keep on mind Gorlin-Goltz syndrome, also called Nevoid BCC syndrome, an autosomal dominant disease with multiple lesions of the skin, pits of the palm and developmental defects (49).

Moreover, oculocutaneous albinisms and xeroderma pigmentosum, which are known as genetic diseases with deficiencies of the protective mechanisms against UVR, are characterized by multiple and early BCCs (50).

Concerning the genetic landscape of SCC, multiple studies have shown that genes altered by UVR exposition are TP53, CDKN2A, NOTCH1, NOTCH2 and p16 suppressor gene. Moreover, mutations in DNA repair pathways include missense mutations in ATR, PIK3CA, ERRB4 and NF1 (51). In addition, association between SCC and genetic syndromes as oculocutaneous albinism, xeroderma pigmentosum, Fanconi anemia, epidermolysis bullosa and Lynch syndrome has been found (52).

A Brief Focus on Possible Links Between the Innate Immune System and NMSCs

A large body of studies highlights that innate immunity play a key role in NMSCs development and progression. Their role has attracted increasing attention recently. As well known, the innate immune system cells can recognize numerous exogenous ligands, such as infectious agents, through various mechanisms. The most important genetic pathway networks involve a crucial group of receptors, called toll-like receptors (TLRs) (53). They are a family of ten transmembrane glycoproteins that directly recognize a wide spectrum of pathogen-associated molecular patterns (PAMPs) and damage-associated molecular patterns (DAMPs), against which they activate the innate immune response and initiate the adaptive immune response (54).

TLRs play a crucial role in the activation of innate immunity, promoting cancer progression; therefore, their activation induces genes that encode for numerous inflammatory cytokines, such as tumor necrosis factor-α (TNF- α), INF-1, IL-6, IL-1, granulocyte-colony stimulating factor and different chemokines, including CCL2 and CXCL10 (54, 55).

It has been observed that some TLRs are involved in the pathogenesis of numerous inflammatory and autoimmune skin disorders. Particularly, there is evidence that Imiquimod, a synthetic agonist of TLR-7, presents high efficacy for treatment of superficial BCC, with a cure rate ranging from 43-94% (56).

The high efficacy of this TLR-7 agonist against superficial BCC, suggests a possible role of this receptor in the pathogenesis of BCC. As a possible consequence, polymorphisms of this receptor could change host immune responses, determining a different susceptibility to BCC and others cancers and autoimmune diseases (57).

A recent case control study performed by Russo et al. (58) highlights the possible association between the susceptibility to BCC and a functional single-nucleotide polymorphism within the promoter of TLR-7 gene (SNP rs 179008/Gln11Leu).

Further genetic research of this receptor and its ligands are needed to improve the knowledge of the pathogenesis of BCC and other UV-related skin cancers.

An increasing body of evidence shows that BCC is an immunogenic tumor (59). Several immune-related markers have been implicated in BCC pathogenesis. IL-23/Th17 related cytokines, as 17, 23, 22, play a significant role in cutaneous inflammatory diseases, but their involvement in skin carcinogenesis is controversial and is poorly investigated in BCC. A recent study of Pellegrini C et al. has highlighted the role of INF-γ in BCC pathogenesis, supporting the involvement of IL-23/Th 17 related cytokines. Particularly, it has observed that BCC is characterized by higher levels of IFN-γ, IL-17, IL-22 and IL-23. Their expression could be correlated to the severity of the inflammatory infiltrate.

Concerning cSCCs, as well known, they are characterized by high mutational burden and cellular heterogeneity (60).

The role of immunosuppression in cSCC risk is supported by higher incidence among recipients of solid organ transplants and PLWH (37, 61), suggesting that this tumor type has enhanced many elements of innate immune response compared to normal skin. The immune system plays complex roles over the entire process of cancer initiation, promotion and progression.

Presentation of tumor antigens to CD8+ cytotoxic T cells and CD4+ helper T cells by HLA class I and class II molecules, respectively, is a key component of this process. The immune response is modulated by human leukocyte antigens (HLAs), which are encoded by a cluster of highly polymorphic genes located on chromosome 6. At the same time, inflammation can facilitate cell transformation by providing pro-tumorigenic cytokines and growth factors to tumor cells and forming an immune suppressive microenvironment within the tumor, which ultimately lead to immune escape and clinical manifestation of the tumors (62). A growing body of literature shows that variation in the expression pattern of these proteins, involved in the presentation of tumor antigens to T lymphocytes, has been implicated in multiple cancers by influencing host defenses against tumorigenesis. The exact mechanisms underlying these associations need to be elucidated. The strongest association between amino acid changes and cSCC risk was found for codon 26 of HLA-DRB1. However, the true functional impact of the phenylalanine to leucine change remains to be elucidated. The identification of specific amino acid changes in the HLA class II genes, if confirmed, helps provide mechanistic clues to the relationship between HLA-mediated immune response and cSCC tumorigenesis. Future studies that examine the mechanism underlying the association between HLA class II and cSCC risk need to be performed. The immune system impacts cSCC susceptibility and pathogenesis, as evidenced by the substantially higher incidence of cSCC in immunocompromised patients. Furthermore, susceptibility to the effects of UVR is known to be genetically determined (63). Variations in immunological makeup of human hosts may influence their ability to recruit immune responses needed to prevent cSCC development. Particular HLA genetic variants are associated with cSCC in immunocompetent and immunosuppressed patients, with more evidence for class I HLA-cSCC associations in immunosuppressed patients than in immunocompetent patients. Class I HLA could play a more important role in cSCC in immunosuppressed patients because HPV may be a co-factor in tumorigenesis- class I HLA proteins present intracellular peptide antigens, including viral proteins degraded into peptides. Further researches of tumor antigens involved in cSCC pathogenesis are needed, to better understand cSCC pathogenesis from an immunological point of view, and try to provide an effective prevention and treatment of cSCC (64).

Skin Cancer, Chronic Inflammation, and Immunodeficiency: A Mènage A Tròis

Cutaneous manifestations often may reveal themselves important clinical clues of many diseases in general, including neoplastic skin diseases, that brings the patient to the physician.

The cutaneous immune system is usually linked to defense against pathogens and external agents; it can also promote the neoplastic process and tumor progression through inflammation.

As known, inflammation plays a key role in oncogenesis (**Figure 1**). Different kinds of cancers arise from infections or chronic inflammation that represent the main promoters of chronic activation of immune system. This prolonged immune activation triggers various stages of carcinogenesis.

As known, immunodepression HIV-related determines an increased risk of tumors (65).

Moreover, HIV shows a tropism for cells of the human immune system, such as macrophages, dendritic cells and T-lymphocytes. HIV infection, through different processes, leads to the reduction of CD4 T-cells to a critical level. Below this level, cell-mediated immunity is lost, and this event allows the rise of opportunistic infections and AIDS development.

Regarding the mechanisms by which HIV virus induced lytic activities, Pope et al. (66) suggested that direct contact between CD4 T cells and HIV pulsed dendritic antigen-presenting cells triggers replication of the virus, leading to a death to both cell types. Furthermore, delayed-type hypersensitivity tests usually have been used as monitors for the progression of the infection, because of the compromise of cutaneous immune system is crucial (67). When CD4 and antigen-presenting cells count decrease meaningfully, skin becomes susceptible to numerous opportunistic infections and neoplastic diseases. In addition, HIV virus seems to activate proto-oncogenes (68), cause alterations in cell cycle regulation and inhibit tumor suppressor genes including p53 (69). Moreover, HIV could determine microsatellite gene instability and genetic alterations, promoting formation of different cancers, including NMSCs (70) (**Figure 2**).

Finally, HIV infection may booster pro-angiogenesis signaling that could lead to endothelial abnormalities. These alterations could promote tumor growth and metastasis (71).

Cutaneous malignancies are the majority of cancers among HIV positive patients (72) and NMSCs are now the most frequent cutaneous malignancies among PLWH. The main risk factors for NMSCs are similar to HIV negative people. Accumulate worldwide studies have shown that NMSCs are usually more aggressive in immunocompromised patients, as evidenced by an increased risk of metastatic disease and mortality in comparison with immunocompetent individuals (73). Frequent opportunistic infections represent also important risk factors for NMSCs (74).

In a study by BURGI et al. (72), cART therapy was associated with lower rates of NMSCs, whereas the standardized incidence ratio (SIR) for NMSCs was reported not to be decreased in the post-cART era among patients recorded in the Swiss cohort study (36). Moreover, a study by Silverberg et al. has suggested that the cART use is associated with decreased risk. Generally, PLWH with BCC and SCC tend to be younger, to have an increased rate of recurrence and they seem to have an overall poorer outcome (75). They often present with more advanced stages of the disease, with a greater degree of infiltrative disease and poorer outcomes (76). In PLWH possible etiologies of NMSCs include the HIV virus, coinfection with oncogenic viruses, such us hepatitis B virus (HBV) (77), hepatitis C virus (HCV) (78), human papilloma virus (HPV) and Epstein Barr virus (EBV) (79), cART agents and tobacco exposure. HPV skin infections are common but the exact correlation between HPV infection and the developing of cutaneous SCC remains still less clear (80). Multiple studies have reported indirect evidence supporting an etiologic relationship (81).

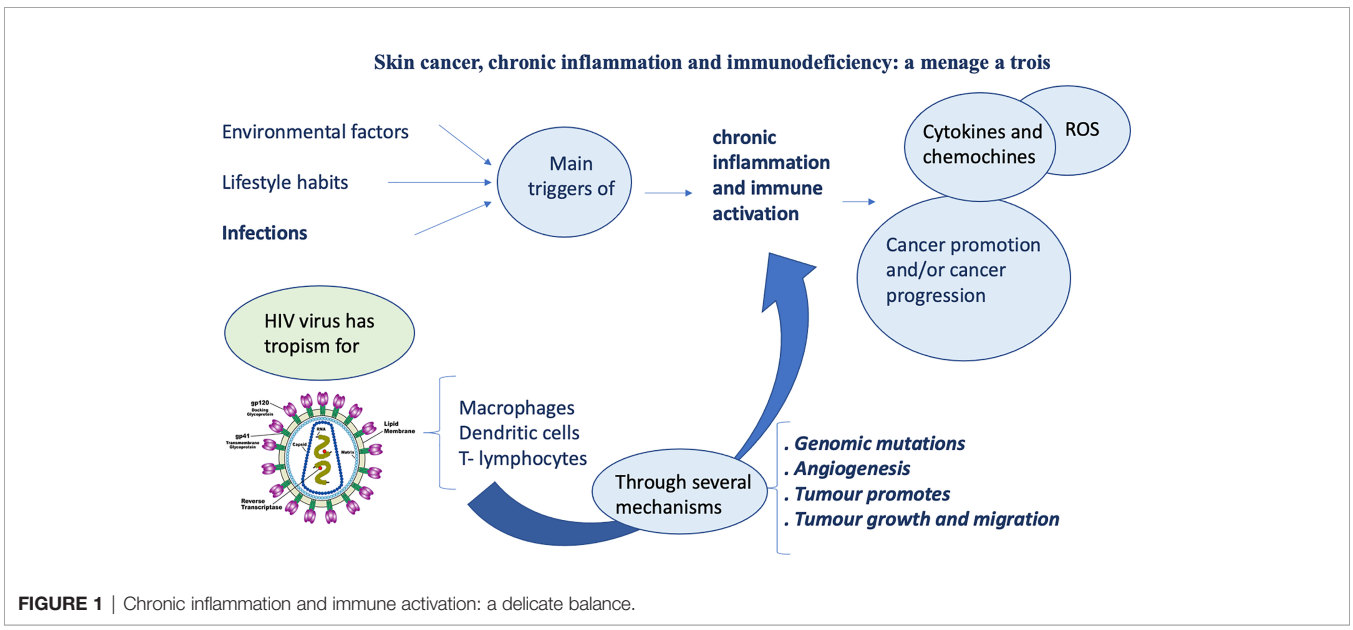

FIGURE 1 | Chronic inflammation and immune activation: a delicate balance.

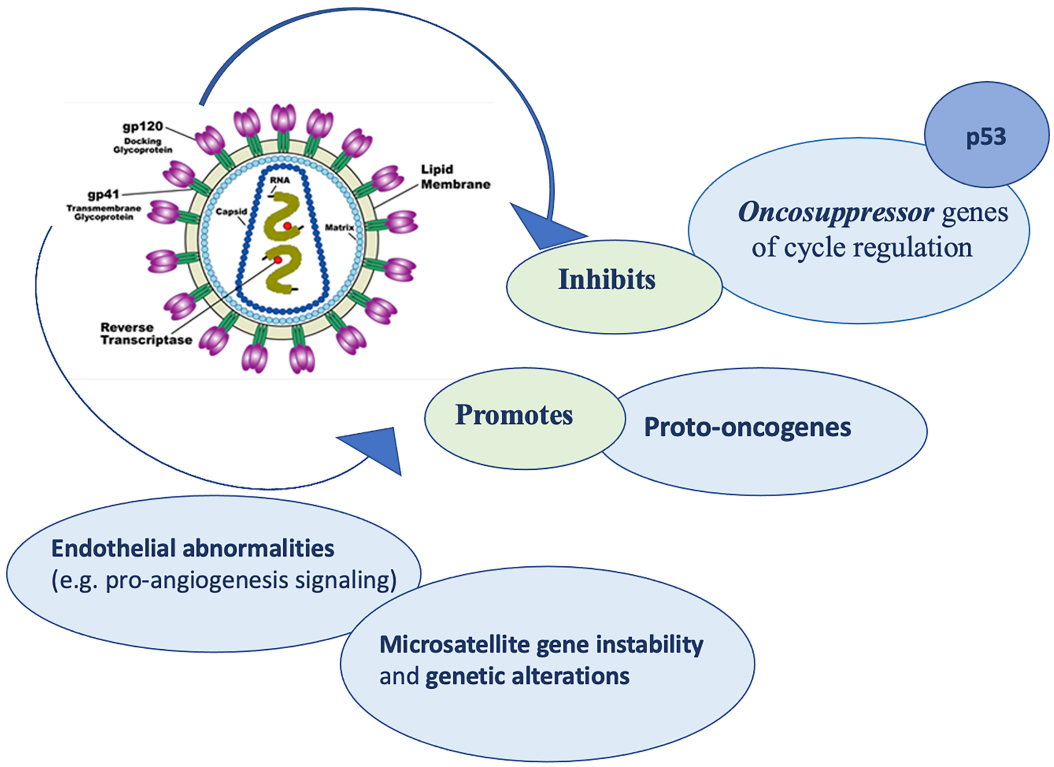

FIGURE 2 | The pathogenetic role of HIV.

Exploring the Link Between Viral-Immunologic Profile of HIV Positive Patients and Risk of NMSCs

Current knowledge of the correlation between viral-immunologic profile of HIV positive patients and NMSCs is evolving. A peculiar correlation between decreased immune-surveillance and carcinogenic virus co-infections might favor oncogenesis, increasing the risk of developing tumors in these subjects (see **Figure 3**).

CD4 cell count is one of the main investigations in the clinical evaluation and management of HIV-infected patients and the skin is richly endowed with these cells. Immunocompetent and PLWH seemed to share the same genetic and environmental factors that lead to the formation of NMSC. Immunosuppression can increase risk to develop NMSCs, mostly SCC (82). An increased rate of neoplasms could be likely to explained by the progressive decline and dysfunction of T cells associated with HIV infection.

HIV infection causes reduced activation of both CD4 and CD8 cells and an increased synthesis of TH2 cytokine subsets. This event leads to cell-mediated immunity deficiency and accumulation of genetic mutations. HIV produces specific proteins, such as nef and tat, that alter MHC signaling and chemokine production (83).

How HIV infection could be the cause of oncogenesis it is complicated to demonstrate, especially because it seems not to be correlated with the overall immune status (CD4 counts and viral load) (84). A meta-analysis of Grulich et al. (85) have showed that immune deficiency caused an increased risk of cancer. HIV positive patients, with CD4 counts <200/microL and high viral loads > 10,000 copies/mL, have a twofold increased risk of developing a primary SCC. The association between level of immunodeficiency and risk of NMSCs is less clear, with a correlation with only SCC having been observed (37). Recently, it has been demonstrated an increased rate of NMSCs among PLWH (37). In 2017, Asgari et al. reported that non-Hispanic white PLWH had a greater risk of developing a new subsequent SCC and that this risk is correlated with lower CD4 counts and higher viral loads. The study failed to demonstrate the same for BCC. In PLWH a 15% increased risk of NMSC has been demonstrated. In particular, the possibility of a subsequent NMSC seemed to be correlated with profound immune-compromission (CD4 <200) (86).

These findings suggest that HIV-related immunodeficiency can determine an increased risk of NMSC overall and SCC in particular. In addition, the HIV viral load, often influenced by antiretroviral therapy adherence, was associated with subsequent primary SCC (hazard ratio of 2.28 with a VL above 10,000 copies/mL) but not for BCC (86). However, this study presents some limitations. The confidence intervals surrounding their HRs are not wide, suggesting that their findings were sufficiently powered. PLWH, especially those with poor immune control,

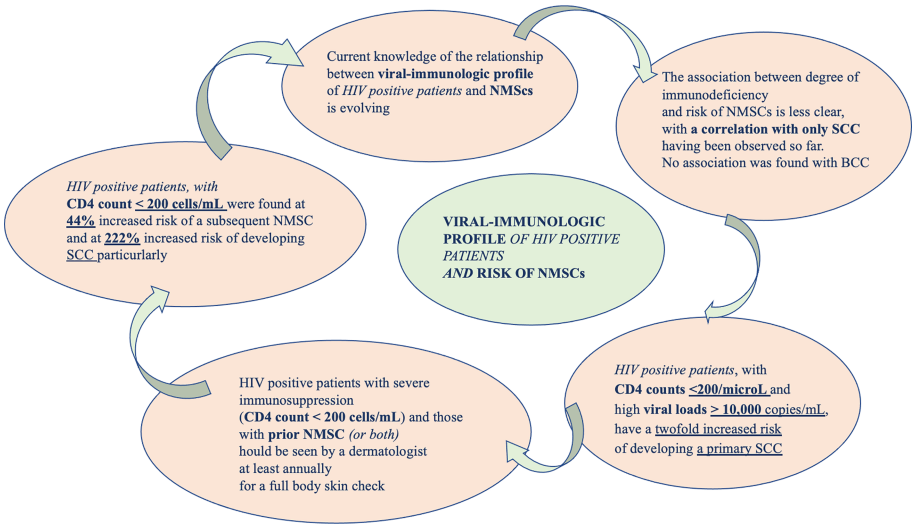

FIGURE 3 | The exploration of a bond between NMSCs and immuno-viral profile of PLWH.

could potentially benefit from targeted monitoring for SCC. In these cases, Sarah J Coates et al. recommended that patients with prior NMSC should undergo a careful dermatologic evaluation at least every year (87).

CLINICAL PRESENTATION AND DIAGNOSIS

BCC derives from the deepest cell layer of the epidermis, the basal layer of keratinocytes. Its clinical presentation is notably heterogeneous. It usually appears as a waxy, translucent, or pearly lesion that often shows a central ulceration and a raised pale border. Telangiectasias are frequent and they often bleed. Moreover, they lead to friability and poor healing. The lesion can appear atrophic and the borders can be indistinct (88). Approximately, in 9 cases out of 10, BCC arises on the head and in 7 cases out of 10 on the trunk and extremities (89). Although BCC shows minimal metastatic potential (<0,1%), local tissue effects can be destructive and disfiguring (88). Diagnosis is primarily histologically. The main histologic patterns are: nodular, superficial, morpheaform/infiltrative, basosquamous, micronodular and pigmented. Morpheaform/infiltrative, micronodular and basosquamous are considered more "aggressive growth" subtypes of BCC. Moreover, some lesions present a mixed histology.

SCC arises from atypical proliferation of keratinizing cells of the epidermis or its appendages. It often develops from actinic keratosis and Bowen's disease (SCC *in situ*) which are considered precancerous lesions. It can also grow *de novo* or on irradiated skin regions, or on chronic inflammatory skin disorders. In contrast to BCC which rarely metastasizes, SCC can metastasize initially to regional lymph-nodes and subsequently to distant regions (90). Typical clinical aspect of SCC is a raised pink papule or plaque, sometimes with scaling or an ulcerated center. The borders often are irregular and bleed easily. During the first years of follow-up, it seems to be less frequent that AKs turns into invasive SCC. When SCC arises from actinic keratosis, it appears scaly, but it tends to grow thicker, and a pink macular area develops into an erythematous raised base. Because SCC may seem quite similar to actinic keratosis, only skin biopsy accurately identifies significant cytologic atypia and invasion of SCC (89). Clinical appearance of SCC is extremely heterogeneous, and it depends also on the anatomical region and subtype. The diagnosis of SCC is primarily histologically. In all clinically suspicious lesions, a skin incisional biopsy or excision, need for a histologic confirmation, should be performed initially, depending on the size of the cancer and treatment approach (see **Figure 4**). It is possible to perform an incisional (punch or shave biopsy) or an excisional biopsy of the whole lesion. Moreover, in rare cases of uncertain diagnosis, immunohistochemical markers of differentiation, such as cytokeratin or molecular biological markers can be applied (50).

Generally, PLWH with SCC and BCC present identically to immunocompetent individuals (91). BCC generally appears on the trunk, while SCC on the head and neck regions. Superficial type BCC is the most typical clinical and histologic presentation, which tends to be multiple, involving the trunk. Generally, in PLWH malignant cancers show a more aggressive phenotype and poorer survival rates in comparison with immunocompetent persons. NADCs show often earlier age at onset, higher tumor degree, more aggressive clinical course and/or more advanced stage at presentation, highlighting the need for prompt and aggressive treatment. More aggressive clinical course has been correlated with multiple factors, such as anatomic site, size at onset, growth rate, histologic features and recurrence after treatment (92). A substantial body of evidence on metastatic

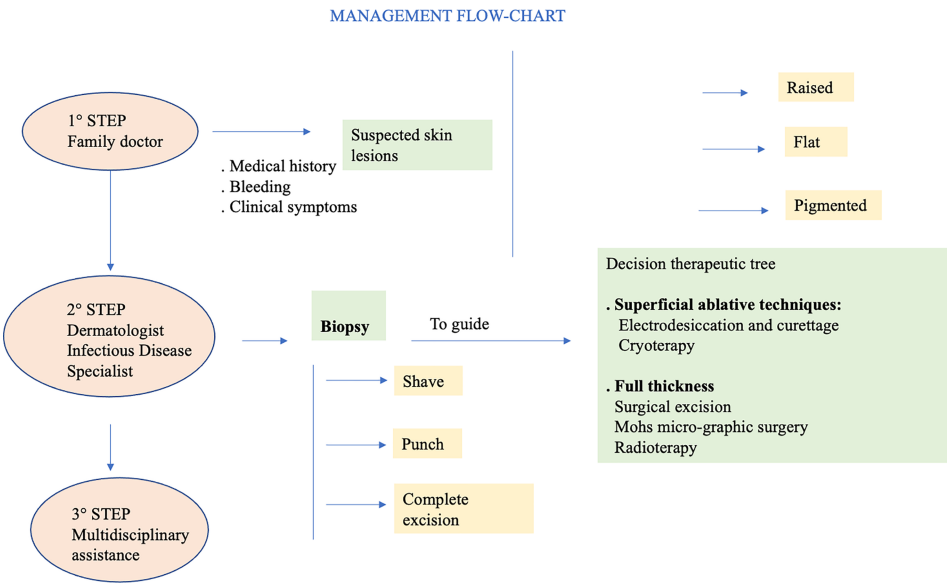

FIGURE 4 | Diagnostic Management flow chart of NMSCs.

SCC highlights that head and neck are primary sites; particularly the temporal and zygomatic regions seem to have a clear tendency for metastasis, maybe because of rich and direct lymphatic drain-age to the parotid gland (93).

Nguyen et all. have demonstrated that PLWH can develop rapidly growing SCC at a young age, with a high risk of local recurrence and metastasis. Management of high-risk SCC should be aggressive and not palliative in PLWH (92). However, cART has certain improved the life quality of PLWH and their outcome that appears more similar as in the general population. Several worldwide studies have highlight that in PLWH, NMSCs are usually characterized by a more aggressive clinical course, higher cancer grade, advanced stages at cancer diagnosis and shorter survival compared with HIV negative individuals (74). SCC seem to be more dangerous in the context of HIV disease. R. N. Motta et al. have described the case of a 59-year-old male patient with advanced HIV infection who presented with a highly aggressive SCC lesion scalp area with destruction of the underlying parietal bone and fulminant clinical progression (94).

Nguyen et al. (92) have described ten cases of aggressive SCC. They recorded 41 different SCC lesions: 75% in head and neck, 7% in the trunk and 8% in extremities.

based on rapid growth rate, a diameter of over 1.5 cm, a history of recurrence and/or evidence of metastasis. A total of 41 SCC lesions were recorded from 10 patients. The head and neck were the most frequently involved regions (31 lesions), followed by the trunk (7 lesions) and extremities (1 lesion). The article stated that those patients initially treated with radiation therapy and surgery combined as well as those treated with radical neck dissection had the best outcomes (92). This paper suggests that high-risk SCC should be treated aggressively and not palliatively in patients infected with HIV.

FROM PREVENTION TO THERAPY

Skin cancer can be avoided by following simple prevention rules (95). Primary prevention is of utmost importance. In particular, sun exposure should be reduced and totally avoided when at its peak during the day, and intensive tanning discouraged. Secondary prevention should be aimed to reduce morbidity and mortality, mainly through early detection of skin cancer, as close clinical evaluation of the arms, face and upper chest can uncover many lesions. In PLWH it is vital a careful evaluation with early biopsy of suspicious lesions. Precancerous lesions should be undergone an early diagnosis to prevent the development of invasive SCC. When cycle of therapy is concluded, patients should undergo a regular follow-up with evaluation of local recurrence or nodal metastasis, particularly for SCC. Other important prevention strategies include smoking cessation and prevention and/or treatment of oncogenic viruses' coinfections, such as HPV, HBV, HCV (13, 26). HPV plays an important etiologic role in genital SCC, so that the quadrivalent HPV vaccination has been strongly suggested (96). Generally, cancer therapy is chosen on the basis of location of primary disease, extension and spread and host comorbidities. Moreover, it depends on histology, lesion aspect, size and location, as well as patient compliance. BCC and SCC should be primarily treated with complete surgical excision (97) (see **Figure 5**).

Management of BCC remains primarily surgical (98), as in immunocompetent people. Management of SCC is influenced by clinical presentation (i.e., palpable lymph nodes) and histopathologic features. Generally, a full skin examination should be performed in all patients, followed by lymph node examination and by surgical and medical management involving a team of experts. It is important to extend the excision at least

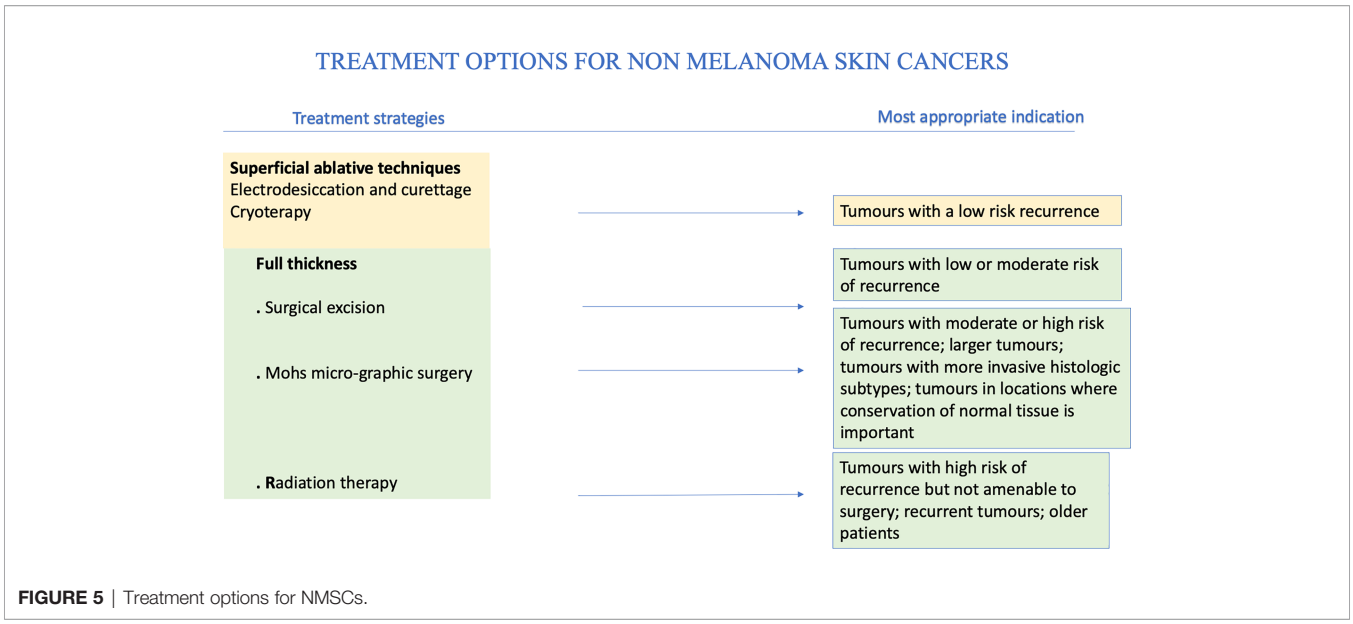

FIGURE 5 | Treatment options for NMSCs.

6 mm from the margins independently from the site whenever it is possible (98). Standard treatment should be applied to all PLWH with a newly diagnosed NMSCs (99); however, when combining cART with chemotherapy, potential drug-drug interactions and overlapping toxicities such as nausea and diarrhea, myelosuppression, neuropathies may occur (99). In case of overlapping toxicity occurs, it is recommendable to change cART or the chemotherapy agent rather than stopping the antiretroviral therapy or decreasing the dosage of chemotherapy (99). Many studies suggested that outcomes can be similar in PLWH with a good control of their infection and HIV negative people (100). However, PLWH with advanced disease show a poor tolerance of therapy and they more likely have worse outcomes compared with HIV negative individuals (101). In immunocompromised individual oral retinoids could be effective to reduce cancer load and to partially prevent the occurrence of new lesions. Unfortunately oral retinoids are teratogenic and that represent a limitation in their use (102). Morbidity and mortality of aggressive SCC in PLWH depend on the control of the disease in the early stages (92). People with higher risk cancers should receive loco regional adjunctive radiotherapy or chemotherapy or both and sentinel node procedures. These recommendations apply regardless of CD4 counts. There is no evidence that BCC in PLWH need more aggressive therapy. For example, Wilkins et al. recommend the use of the same treatment protocols for treatment of BCC even if there is no evidence for imiquimod for BCC in PLWH (91). Among factors influencing prognosis, any kind of immunocompromised patient present more rapid growth, an higher risk of local recurrence and metastasis, even 10 times higher (82). Intensity and duration of immunodeficiency plays a great role (103). Immunocompromised patients should be followed-up closely, at least twice a year (50). PLWH can die of a metastatic SCC, so the treatment of SCC in PLWH should never be less aggressive or prompt than the treatment of HIV negative individuals. Concerning metastatic SCC, it is important to keep in mind that late treatment of high-risk SCC could lead to metastatic diseases especially in immunocompromised people. Moreover, perineural invasion is clearly linked to recurrence and higher risk of metastasis. Generally, the most chosen surgical option in these high-risk cases is Mohs surgery. But the presence of perineural invasion requires additional adjuvant therapy (104). Similarly, high-risk SCC in HIV infected patients should be treated initially by ablative therapy with histologic control and, if necessary, adjuvant therapy. A retrospective study of Nguyen has illustrated the potential for rapid growth of SCC in HIV infected people. An initial less aggressive therapeutical approach in PLWH is linked to higher rates of recurrence, metastasis and death. For this reason PLWH with SCC should receive a combination of surgery and radiotherapy or of surgery and radical neck dissection (92). NMSCs are a striking example of immunodeficiency-related neoplasm, and they offer further opportunities for therapeutic and pathogenetic insights. In fact, multiple clinical phenomena highlight the close correlation between immunity and skin cancers.

The main therapeutic techniques, superficial ablative and full thickness, for NMSCs will be broadly reviewed above (see **Table 1**).

Surgical Excision

Surgery is the treatment of choice. Depending on the affected area, it can be followed by plastic reconstruction. Moreover, histological examination of the excised tissues allows diagnosis, prognosis and treatment tailoring.

In SCC, surgical excision is immediately followed by histopathological examination of excision margins, which allows to confirm the cancer type and assess the absence of cancer cells from the resection margins. Another procedure to obtain the same result is micrographically controlled surgery

TABLE 1 | Classification of BCC according to risk for recurrence (105, 106).

LOW RISK	INTERMEDIATE RISK	HIGH RISK
Superficial primary BCC	Superficial recurrent BCC	Clinical forms: Morpheaform or ill-defined
Nodular primary BCC when:	Nodular primary BCC when:	Nodular primary BCC when:
<1 cm in intermediate risk area	<1 cm in high-risk area	>1 cm in high-risk area
<2 cm in low-risk area	>1 cm in intermediate risk area	
	>2 cm in low-risk area	
Pinkus tumor BCC		Histological forms:
		Aggressive
		Recurrent forms

(MCS). For low-risk NMSCs limited to dermis, traditional excision preferred (89). Aesthetically, excision offers better results than ablative techniques. Moreover, it offers the advantage of obtaining specimens for histologic examination. With surgery, cure rates are higher than 90%. It is neither recommended, nor cost-effective, storing frozen sections of tumor margins of every suspected NMSC. MMS is applied for recurrent tumors, tumors in high-risk areas, tumors ≥ 2 cm, recurrent tumors, tumors which margins are not clear and tumors in cosmetically sensitive areas (107). Wide removals should be done when margins are smaller than the recommended safety margins due to the tissue shrinkage, while re-excision should be done for operable cases in the event of positive margins (108). In the context of high-risk SCC, usefulness of a sentinel lymph node biopsy is still not clear (109). In fact, SCC does not invade deeper tissues as quickly as cutaneous malignant melanoma. The reason consists in absence of lymphatic drainage in superficial dermis and epidermis. Therefore, SCC is less likely to spread *via* lymphatics. There are still no guidelines about how to approach regional nodal disease in patients with SCC. Moreover, available directions are based on studies concerning head and neck mucosal SCC (110). Patients affected by metastases from SCC spread to lymph nodes should be treated surgically, as well as patients with melanoma or Merkel cell carcinoma. When surgery is not indicated, e.g., for patient-related factors, a nonsurgical approach by a multidisciplinary group should be evaluated.

Radiotherapy

Radiotherapy (RT) may be applied in an adjuvant setting, after surgical resection, in patients with high-risk features. A host factors as immunosuppression is considered by the American Joint Committee of Cancer (AJCC) as a risk for having a poorer outcome when diagnosed with NMSC. Obviously, the presence of other risk factors such as location, particularly ears and lips, poor differentiation and perineural invasion (PNI) can worsen outcomes. American Society for Radiation Oncology (ASTRO) guidelines recommends postoperative radiotherapy (PORT) in the setting of chronic immunosuppression (111).

Bimodality therapy (surgery and PORT) is used in the context of immune suppression, especially with head and neck cutaneous SCC. As a matter of fact, it frequently presents a lower outcome than immunocompetent patients, with a significantly lower progression-free survival at 2 years ($p = 0.002$) (112). When necessary, adjuvant RT should not be delayed. It is demonstrated that exceeding a time of 6 weeks after the excision may worsen the prognosis (113). Irradiation volume must consider cancer location and risk factors, such as PNI, lymphatic and vascular invasion, to decide whether to include the first lymph node. The results of phase III TROG 05.01 trial (114) suggest no benefit in overall survival, disease free survival and locoregional relapse with the addition of weekly carboplatin to RT as adjuvant therapy.

RT is recommended as the only treatment modality in patients with NMSCs who cannot benefit from surgical resection. In fact, NMSCs can obtain an optimal local control because they are radio responsive carcinomas. Marconi et al. (115), using definitive RT, demonstrated that BCC had a 5- and 10-year local control of 96% and 94%, while for SCC 5- and 10-year control were 92% and 87%, respectively. It is important to keep in mind that in case of underlying genetic syndromes RT is discouraged because of higher radio-sensitivity in patients affected by Li-Fraumeni or Gorlin syndrome, ataxia telangiectasia. Furthermore, connective tissue disorders represent a contraindication to treatment whenever not under control (111).

Cryotherapy

Cryotherapy represents a therapeutic option for BCC, although tissue destruction is not perfectly targeted. It is based on two consecutive 30-second freeze-thaw cycles and is particularly effective on facial lesions, with a 95% cure rate (116).

Electrodesiccation and Curettage

Generally, these therapeutic options are considered only when assessing low-risk lesions. These techniques have a worse cosmetic yield than surgical excision, often ending in a round, hypopigmented and possibly hypertrophic scar (89). National Comprehensive cancer Network (NCCN) guidelines reported that curettage and electrodessication may be considered for small and low-risk primary SCC (117).

Chemotherapy

Systemic chemotherapy has a meaningful role in the management of local advanced and/or metastatic NMSC. Aggressive management with polychemotherapy should be considered for difficult to treat cases. Usually, mono-chemotherapy should be considered as a first-line treatment (50). Metastatic SCCs are notably difficult to treat, representing a challenge for clinicians. Platinum based chemotherapeutic

agents, such as cisplatin or carboplatin, can be considered for local advanced and metastatic SCCs not amenable for surgical excision or radiotherapy. Other chemotherapeutic drugs, such as cyclophosphamide, bleomycin, doxorubicin, methotrexate and 5-FU, may also be used alone or in combination (118). However, guidelines for the use of classic chemotherapy in NMSC are based on low-level evidence, as the trials had several limitations, such as lack of randomization and heterogeneous patient populations. Recently, it has been highlighted that patients with stage I and II lip SCC can be successfully treated with monotherapy *via* superficial temporal artery administration of bleomycin, in order to obtain a cure in 70.8% of patients (119). Currently, chemotherapy is recommended in NCCN guidelines in a combination with radiotherapy, especially in localized, high-risk SCCs for patients who cannot undergo surgery (117). Before the advent of molecular target therapies, metastatic BCC had been treated with various conventional chemotherapeutic agents. However, metastatic BCC is rare, and the available literature about the effectiveness of these treatments is mostly episodic. In a short review collecting twelve elsewhere published cases treated with platinum, five showed complete response and four showed partial response (120).

Immunotherapy and Target Therapy of NMSCs: New Promising Neoadjuvant Therapy

Given actual evidence, targeted therapy and immunotherapy represent the frontiers in neoadjuvant therapy of NMSCs, being much more selective than traditional chemotherapy. Emerging clinical data (see **Table 2**) show that immunotherapy, particularly checkpoint inhibition, is a useful therapy option for advanced cSCC, while targeted therapy with sonic hedgehog pathway inhibitors results an effective treatment option for locally advanced or multiple BCC (121). The role of immune system has been linked to the occurrence of NMSCs by epidemiologic evidence that led to several studies about the immunology of NMSCs (122). These studies demonstrated the elevated number of neoantigens expressed by NMSCs' cells that could represent the right target for a successful immune therapy. These kinds of observations have led to ongoing clinical trials based on novel immunotherapies of NMSCs as a neoadjuvant approach (123). By definition, a neoadjuvant approach aims to reduce the size of the tumor, before the subsequent potentially curative techniques. Immunotherapy acts by inhibiting immune checkpoints, eventually improving the activity of the immune system against the tumoral cells and reducing regulatory T cell-mediated immunosuppression. Unfortunately, these new treatment options appear quite expensive; moreover, immunotherapy can cause important and irreversible adverse effects (121). A thorough knowledge of SCC carcinogenesis is needed to develop new treatment approaches. The main immune checkpoints include CTLA-4, PD-1 and PD-L1, while sonic hedgehog pathway inhibitors include Vismodegib and Sonidegib, that we briefly describe above.

Anti-Programmed Cell Death Receptor-1 Immune Checkpoint Inhibitor

Cemiplimab

It is indicated for advanced or metastatic SCC in patients who are not amenable for surgery or radiotherapy. The phase I/II study (EMPOWER-CSCC-1) of patients with locally advanced or metastatic SCC has been the first trial that led to drug approval, producing a response rate of 47% in a cohort of 59 patients (124). Recently, this study led to the U.S. Food and Drug Administration approval of cemiplimab for locally advanced or metastatic SCC on September 28, 2018. The phase II clinical study of Cemiplimab in patients with advanced cutaneous SCC is ongoing and it is currently recruiting participants. (NCT02760498).

Another study (NCT03969004) is currently recruiting participants to study cemiplimab use in the adjuvant setting after surgery and radiation in patients with high risk of recurrence. Numerous ongoing clinical trials are studying the use of cemiplimab in patients with advanced BCC with a progression of disease while on Hedgehog pathway inhibitor therapy (125). Between them, the study (NCT03132636) is

TABLE 2 | Immune Checkpoint inhibitors.

Stage of disease	SCC	BCC
I	Nivolumab ± Ipilimumab(II)	N/A
II	Nivolumab ± Ipilimumab (II) Cemiplimab (II)	N/A
III	Nivolumab ± Ipilimumab (II) Cemiplimab (II)	N/A
IVA	Nivolumab ± Ipilimumab (II) Avelumab (II) Cemiplimab (II) Pembrolizumab (II)	Nivolumab ± Ipilimumab (II)
IVB	Nivolumab (II) Avelumab (II) Cemiplimab (II) Pembrolizumab (II)	Nivolumab ± Ipilimumab (II)

Immune Checkpoint inhibitors currently under investigation for the treatment of squamous cell carcinoma and basal cell carcinoma. Between brackets the phase of the study. SCC, squamous cell carcinoma; BCC, basal cell carcinoma; N/A, not applicable. Data extracted from https://clinicaltrials.gov/.

active, not recruiting. Another ongoing clinical trial is studying CTLA-4/PD-1 combinations, such as ipilimumab/nivolumab for treatment of advanced BCC. This study (NCT03521830) is currently recruiting participants with locally advanced or metastatic BCC.

Pembrolizumab

There are currently ongoing studies that are investigating the treatment of recurrent or metastatic cSCC (126). Between them, (NCT02964559) is an active study, not recruiting participants. It is also being evaluated in advanced SCC (NCT03284424), an active study, not recruiting for treatment of recurrent or metastatic cSCC.

Nivolumab

It is also being evaluated in advanced SCC: (NCT04204837) is an active study, not recruiting.

Anti-Programmed Cell Death Ligand-1

Avelumab

Several ongoing studies for advanced SCC are investigating avelumab with or without cetuximab (121). The study (NCT03944941) is currently open to enrollment. Another study, (NCT03737721) is currently recruiting participants with unresectable SCC treated with avelumab and radical radiotherapy. This study is called UNSCARRed study.

Atezolizumab

The study (NCT03108131) studies how cobimetinib/atezolizumab association works in treating participants with rare tumors that have spread to other places in the body (advanced) or that does not respond to treatment (refractory). This study is currently recruiting participants. Cobimetinib may block some of the enzymes involved in cell growth. So that, immunotherapy with monoclonal antibodies, such as atezolizumab, could interfere with the capability of tumor cells to grow and spread.

Cosibelimab

Cosibelimab is a fully human monoclonal antibody of IgG1 subtype that directly blocks its interactions with the Programmed Death-1 (PD-1) and B7.1 receptors (121). The study (NCT03212404), based on cosibelimab/atezolizumab association, is currently recruiting participants. The aim of this study is to assess the safety, tolerability and efficacy of CK-301 when administered intravenously as a single agent to subjects with recurrent or metastatic cancers.

Anti-Cytotoxic T-Lymphocyte-Associated Protein 4 Immune Checkpoint Inhibition

Ipilimumab

Emerging data showing ipilimumab use in SCC are limited to case reports. A patient with metastatic cSCC had a durable remission of both malignancies. Concerning BCC, there is an ongoing study regarding locally-advanced unresectable or metastatic BCC which investigates ipilimumab in association with nivolumab in one of the arms (NCT03521830) (127). This study is currently open to enrollment.

Hedgehog Pathway Inhibitors: Vismodegib and Sonidegib

Genetic and molecular studies have highlighted genetic mutations in the hedgehog signaling pathway characterize almost all BCCs. These alterations result in excessive activation leading to uncontrolled proliferation of basal cells. In addition, they determine loss of function of patched homologue 1 (PTCH1). PTCH1 blocks the signaling activity of smoothened homologue (SMO), a seven-transmembrane protein.

Vismodegib and sonidegib are two anti-tumor drugs targeting the HH pathway, called hedgehog pathway inhibitors (HPIs). Currently, there are no recommendations about when to prefer one molecule rather than the other. Moreover, these molecules have similar efficacy and tolerability, although they differ under a pharmacokinetic aspect (128). As a matter of fact, both are metabolized through cytochrome P450. Vismodegib is prevalently metabolized by CYP2C9, while sonidegib passes through CY3A4. Therefore, CYP3A4 inhibitors increases the blood concentration of sonidegib. Among them, ritonavir e saquinavir, two antiretroviral drugs. Whenever it is not possible to avoid the simultaneous use of sonidegib and strong inhibitors of CYP3A4, a dose reduction to sonidegib 200 mg every second day is recommended (129). Muscle spasms, alopecia, dysgeusia and weight loss are the most frequent side effects described in the literature. Of interest, many cases of SCC have been observed in patients treated with vismodegib for BCC therapy or single agent (BRAF) inhibitors, such as vemurafenib, for melanoma therapy (130).

All the current conventional treatments and ongoing trials are summarized in **Table 3**. Further studies are required to better understand the correct management of the drug, alternative dosing regimens and differences with the other HPIs.

Target Therapy in PLWH

Immunotherapy has paved new paths for treatment of HIV-related cancers and, thanks to monoclonal antibodies and immunomodulatory drugs, have shown to be effective in HIV-related cancers. In particular, the effectiveness of checkpoint inhibitors targeting the PD-1/PD-L1 pathway in the treatment of many malignancies in PLWH it has been suggested by recent data, hopefully stronger evidence on this matter will follow with the inclusion of PLWH in immune-oncology studies. Recently, ASCO and the Food and Drug Association (FDA) have provided guidance to include PLWH in clinical trials on neoplastic diseases.

A recent FDA-approved sonic hedgehog (SHH) signaling pathway inhibitor, Vismodegib, can be used to treat locally advanced, metastatic and recurrent BCCs that are inoperable and cannot be treated with radiotherapy, showing promising results (131). Although this molecule seems to be a safe option for those patients that cannot undergo surgery for advanced and metastatic BCC, in high-risk patients the optimal treatment protocol is unknown. The safety of Vismodegib in PLWH and its interactions with cART are not well known. Recently, Scalvenzi et al. have described a case-report of a HIV positive patient with an inoperable ulcerative BCC of the ear.

TABLE 3 | Conventional and New promising neoadjuvant therapies.

CONVENTIONAL THERAPY	
SURGICAL EXCISION	Generally adopted as **first step** for most NMSCs and it is considered a **potentially curative treatment**
RADIOTHERAPY	Effective non-surgical option and used in the **definitive, adjuvant and palliative settings**
CRYOTHERAPY	Reserved only for **low-risk lesions**
ELECTRODESICCATION AND CURETTAGE	Reserved only for **low-risk lesions**
CHEMIOTHERAPY	Topic mono-chemotherapy, e.g. with **5-fluorouracil** or **Imiquimod**, can be considered for **superficial lesions**
NEW PROMISING NEOADJUVANT THERAPY	
TARGETED THERAPY -Sonic hedgehog pathway inhibitors: Vismodegib and Sonidegib	Targeted therapy with sonic hedgehog pathway inhibitors is very effective in **locally advanced or multiple BCC**.
IMMUNOTHERAPY - Anti-programmed cell death receptor-1 checkpoint inhibitor **(Anti PD-1)** - Anti-cytotoxic T-lymphocyte-associated protein 4 immune checkpoint inhibition **(Anti CTLA-4)** - Anti-programmed cell death ligand-1 **(Anti PD-L1)**	Immunotherapy with immune checkpoint inhibitors appears to be promising for **advanced cutaneous SCC**. Several **ongoing clinical trials** are investigating their use. **Cemiplimab** is the only checkpoint inhibitor approved for locally advanced or metastatic cSCC.

Conventional and New promising neoadjuvant therapies. SCC, squamous cell carcinoma; cSCC, cutaneous squamous cell carcinoma; BCC, basal cell carcinoma.

After a specialistic evaluation also of the immune status (high CD4 T cell count) the patient started oral Vismodegib 150 mg daily. In about 6 months of therapy the patient obtained a complete resolution, after which Vismodegib was discontinued. The article reports good tolerance and no interactions between Vismodegib and the previous cART (132). Reports on SHH inhibitors in immunocompromised patients witch locally advanced or metastasizing BCC are rare (133). Effectual use of Vismodegib and the lack of drug to drug interaction with cART (tenofovir/emtricitabine/rilpivirine) has been described in a single case (132). Recently, Hoffmann V. et al. have described a successful case of treatment with Sonidegib in a patient on cART. However, it's mandatory to evaluate the risk of interactions between cART and antiblastic chemotherapy, target therapy and immunotherapy (134). In fact, it is true that the new antiretroviral drugs (135) are less toxic but they still have long-term side effects that need to be careful evaluated (136).

CONCLUSIONS AND FUTURE PERSPECTIVES

PLWH have an elevated propensity to develop cancers compared to the general population. It has been clearly shown that in this population immunosuppression and concomitant infection with oncogenic viruses play an important role. NMSCs are the most frequent cause of cutaneous malignancy in PLWH, and they represent a new oncologic challenge due increasing age of HIV-infected patients. In this paper, we tried to review the incidence of NMSCs among PLWH, any different clinical presentations of squamous cell and basal cell carcinoma between PLWH and HIV negative persons and any differences in efficacy and safety of treatments and response to immunomodulators (see **Table 4**). According to several authors ratios of BCC and SCC are similar between PLWH and HIV negative persons (4:1) (140), with BCC essentially more frequent than SCC. PLWH with NMSCs tend to be younger, to have a higher risk of local recurrence and to have an overall poorer outcome. The main risk factors for NMSCs are similar to HIV negative individuals. Superficial BCC is the most frequent variant and is more often found on the trunk and in multiple lesions. SCC tends to be more aggressive in HIV infected people and it presents at significantly younger age, with higher risk of local recurrence and metastasis (141). The treatment of SCC in people with HIV should be at least aggressive as the treatment in HIV seronegative individuals. There is no strong evidence of how the depth of the immune compromission (CD4 counts) directly influence the risk of NMSCs, and this evidence supports mainly the risk of SCC rather than BCC. It is mandatory to suggest to PLWH a proper screening of all precancerous lesions besides a careful prevention with sun avoidance and use of sunscreen. Notably, there is a lack of official recommendation and guidelines on these subjects. Recently, Vismodegib and Sonidegib, two hedgehog signaling pathway inhibitors, have been approved to treat unresectable BCCs that are not amenable for surgery and radiotherapy. It is difficult to compare the efficacy of Sonidegib and Vismodegib due to the absence of trials designed to prove it and also because the first is only approved for locally advanced BCC while the last is also used for metastasizing BCC (128). It is time to answer to this lack of knowledge with appropriate trials that study the role of targeted therapy for BCC, in PLWH that result to be inoperable. The effectiveness of checkpoint inhibitors targeting the PD-1/PD-L1 pathway in the treatment of many malignancies in PLWH it has been suggested by recent data, hopefully stronger evidence on this matter will follow. In order to improve knowledge, PLWH must be included in immune-oncology studies. In conclusion, the treatment of advanced NMSC represents still an important challenge for clinician, mainly because of the lack of high-quality evidence and randomized trials. Further studies are required to focus on the best therapeutic approaches to NMSCs and mostly on the impact of cancer screening interventions among HIV-infected patients, in order to improve cancer diagnosis at an earlier stage. Further

TABLE 4 | Synoptic picture with differences between PLWH and immunocompetent.

	GENERAL POPULATION	HIV POSITIVE PATIENTS
RATIOS BCC/SCC	4:1 (30)	4:1 (33)
MORE FREQUENT SUBTYPE OF BCC	Nodular (75)	Superficial (137)
PREVALENCE M/W	Slight male predilection (after age of 45 years) (138)	Increased risk of NMSC in both male and female patients (139)
AGE AT DIAGNOSIS	60 -70 years	Tendency to earlier age (~ 45 years) (83)
RISK FACTORS FOR DEVELOPMENT OF NMSCs	Chronic sun-exposure, fair phenotypic features, family history, older age, genetic mutations	The same as for general population plus immunodeficiency and coinfection with oncogenic viruses (83)
ANATOMIC DISTRIBUTION	Sun-exposed areas (more frequent on head and neck regions)	Trunk and extremities (generally lesions are multiple) (83)
CLINICAL COURSE	Usually indolent; nevertheless, metastatic forms lead to poor patient outcome	Generally more aggressive compared to the general population (83)
RISK METASTATIC DISEASE	Generally BCC shows minimal metastatic potential (88); SCC has a 4% annual incidence of metastatic disease (90)	Higher compared to the general population (83)
TREATMENT	**Full thickness Treatment** (93) Surgical excision Mohs micro-graphic surgery Radiotherapy **Superficial ablative techniques** **Targeted Therapy**	The same as standard of care for general population. However, it's mandatory to evaluate the risk of drug interactions (between cART and other treatments) (134)

studies are needed to learn and apply pathogenetic insights to obtain new therapeutic options and correlate the degree of HIV-related immunodeficiency with disease outcome.

AUTHOR CONTRIBUTIONS

ER, MB, and GN designed the study. ER, MM, MB, and MC performed the screening of articles following the inclusion criteria. ER, MM, and FF wrote the article. CG, MB, and GN supervised the part of diagnosis and treatment. All authors contributed to the article and approved the submitted version.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fonc.2021.689789/full#supplementary-material

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