



Dendritic Cells and Their Role in Immunotherapy

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Despite significant advances in the field of cancer immunotherapy, the majority of patients still do not benefit from treatment and must rely on traditional therapies. Dendritic cells have long been a focus of cancer immunotherapy due to their role in inducing protective adaptive immunity, but cancer vaccines have shown limited efficacy in the past. With the advent of immune checkpoint blockade and the ability to identify patient-specific neoantigens, new vaccines, and combinatorial therapies are being evaluated in the clinic. Dendritic cells are also emerging as critical regulators of the immune response within tumors. Understanding how to augment the function of these intratumoral dendritic cells could offer new approaches to enhance immunotherapy, in addition to improving the cytotoxic and targeted therapies that are partially dependent upon a robust immune response for their efficacy. Here we will discuss the role of specific dendritic cell subsets in regulating the anti-tumor immune response, as well as the current status of dendritic cell-based immunotherapies, in order to provide an overview for future lines of research and clinical trials.

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INTRODUCTION

Immunotherapy has revolutionized the treatment of many solid and hematological malignancies, with immune checkpoint blockade (ICB), adoptive cell therapy (ACT) using tumor infiltrating leukocytes (TIL), and vaccine strategies targeting different aspects of the immune-oncology cycle to improve the functionality of T lymphocytes. Each of these strategies, however, is necessarily predicated on the initiation of the cycle, namely the presentation of tumor antigens by professional antigen-presenting cells (APCs) (1). APCs can be defined by their ability to capture, process, and present exogenous antigen to T cells, and are usually identified by their constitutive expression of major histocompatibility complex (MHC) II and costimulatory molecules. Thus, dendritic cells (DCs), macrophages, and B cells are normally considered to be the three major populations of APCs. It should be noted that other populations also constitutively express MHCII, including thymic epithelial cells, while still others can acquire exogenous antigen, and express MHCII following activation, including eosinophils and basophils (2, 3). However, in the context of solid tumors, antigen uptake, and presentation are primarily the domain of macrophages and DCs (4). While macrophages are the dominant phagocytic population in tumors, they do not migrate to the lymph nodes and are unable to activate T cells *ex vivo* (4). Instead, macrophages are usually found to blunt T cell responses against tumors via multiple mechanisms and act to suppress therapeutic response to ICB as well as chemotherapy and irradiation (5, 6). DCs thus have a unique ability to transport tumor antigen to the draining lymph nodes to initiate T cell activation, a process that

is required for T cell-dependent immunity and response to ICB (4, 7–10). Tumor-resident DCs also have an emerging role in regulating the T cell response within tumors during therapy (4, 11–14). These functions place DCs at the fulcrum of the anti-tumor T cell response and suggest that regulating the biological activity of these cells is a viable therapeutic approach to indirectly promote a T cell response during therapy.

DENDRITIC CELLS IN CANCER

DCs are the quintessential APCs of the immune system, responsible for bridging the gap between innate and adaptive immunity, including the activation of anti-tumor T cells (4, 7–10). DCs arise from bone marrow progenitors known as common myeloid progenitors (CMPs). From here, two cell subtypes diverge. Expression of the transcription factor Nur77 drives the differentiation of CMPs into monocytes, which can further differentiate into monocyte DCs (moDCs) under inflammatory conditions (15–18). In the absence of Nur77, CMPs differentiate into the common dendritic cell progenitor (CDP), which gives rise both to plasmacytoid DCs (pDCs) and conventional DCs (cDCs) (15). Differentiated cDCs are initially immature, requiring maturation signals (for instance, damage or pathogen associated molecular patterns [DAMPs or PAMPs], or inflammatory cytokines) to fully effect their role in the immune response (15, 18). Upon maturation and activation, DCs downregulate phagocytosis, increase MHC and costimulatory molecule expression, increase cytokine production, and display enhanced migration to lymph nodes, likely driven by higher expression of C-C chemokine receptor 7 (CCR7) (15). As a result of the phenotypic changes that occur during activation, mature DCs are able to prime naïve T cells and initiate the adaptive immune response.

cDCs can be further divided into two subsets, known as type one (cDC1) and type two (cDC2) conventional DCs. cDC1 are defined by reliance on the transcription factors BATF3 and IRF8 for development, and express several common surface markers across species, including XCR1, CLEC9A, CADM1, BTLA, and CD26 (19). However, the cells were originally identified by surface expression of CD8 α (lymphoid organ resident) or CD103 (peripheral tissue resident) in mice (20–22) and CD141 (BDCA-3) in humans (23–25), making these the most commonly used markers. In both organisms, the cDC1 subset displays enhanced ability to cross-present exogenous antigen and activate CD8 $^+$ T cells (15, 18, 26), but this functional demarcation between the cDC1 and cDC2 subset is more pronounced in mice than in humans (19). In both mice and humans cDC1s represent a small percentage of immune cells in circulation. cDC1 accounted for <0.01% of CD45 $^+$ cells in the blood of healthy human donors, as well as <0.1% of CD45 $^+$ cells in surveyed tissue sites (27).

cDC2 are easiest to identify by the absence of cDC1 markers, but higher expression of CD11b, CD1c, and SIRP α (CD172a) is also frequently used to distinguish the population, with IRF4 acting as the key transcription factor (28–31). No specific markers identify migratory from resident cDC2 populations in mice, but differential expression of CD11c and MHCII can be used

as a distinguishing feature (15). In mice, cDC2 are primarily responsible for presentation of endogenous antigen to CD4 $^+$ T cells and shaping the resulting polarization of the cells, with the ability to polarize CD4 $^+$ T cells also observed with human cDC2 (32). As mentioned, however, human cDC2s can cross-present antigen and produce high levels of interleukin (IL)12, properties that are largely restricted to the cDC1 subset in mice (19). Thus, despite the critical role of cDC1s in the development and maintenance of anti-tumor immunity in experimental models (15), it is possible cDC2s have an unidentified role in human cancers. Indeed, a recent study demonstrated a correlation between cDC2 abundance and non-T_{reg} CD4 $^+$ T cell infiltration into head and neck squamous carcinomas. High cDC2 and low T_{reg} infiltration was also associated with longer progression-free survival (33).

Type 1 Conventional DCs

In mice, cDC1 are responsible for the induction of the “cancer-immune cycle,” as *Batf3*-deficient mice are unable to reject even highly immunogenic tumors or respond to immune-mediated therapies such as checkpoint blockade and adoptive T cell transfer (7–10, 13, 34). This has been traced to the ability of cDC1s to transport antigen from tumors into draining lymph nodes, with migratory cDC1s being the only APC subset capable of causing robust activation and proliferation of CD8 $^+$ T cells *ex vivo* (9, 10). Additionally, migratory cDC1 represented the only cDC subset able to transport antigen to the lymph node in two studies using melanoma models (9, 10). cDC trafficking to the lymph node and generation of a systemic anti-tumor immune response is governed by CCR7 expression (9). Mice lacking CCR7-expressing cDC1 failed to recruit CD8 $^+$ T cells to the tumor, and the T cells that were present in the tumor microenvironment failed to proliferate, leading to an overall lack of immune control (9). Similarly, the inability of tumors to recruit the cDC1 subset prevents an effective CD8 $^+$ T cell response from developing (35, 36), while increasing the number of cDCs in the tumor can restore response to immunotherapy (10, 35). Taken together, these studies strongly support CD103 $^+$ migratory cDC1 as critical for the induction of anti-tumor immunity. In non-tumor models of immunity, lymph node-resident cDC also acquire antigen from migratory cDCs and are needed to initiate an optimum CD8 $^+$ T cell response (37, 38). Whether there are sequential roles for migratory and resident cDC1s during the development of an anti-tumor response is not yet known. However, cross-presentation by cDC1s is critical for the induction of an adaptive immune response by cytotoxic CD8 $^+$ T cells, with mice specifically deficient in cross-presentation-capable cDC1s unable to reject highly immunogenic fibrosarcoma tumors (39). In addition, cross-presentation by cDC1 is enhanced by type I interferon (IFN) signaling (40). The absence of type I IFN in the tumor microenvironment, or the inability of cDC1 to sense type I IFN, are sufficient to impair the development of a CD8 $^+$ T cell response (34, 40). Taken together, these studies emphasize the importance of cross-presentation of tumor antigen to naïve CD8 $^+$ T cells in the lymph node in the induction of a successful anti-tumor immune response.

It is also becoming increasingly clear that cDC1s have a critical role in maintaining CD8⁺ T cell function within tumors. In secondary lymphoid organs and in non-tumor models of immunity, the organization of immune cells is critical for effective signaling (41, 42). The localization of T cells near cDCs, especially, has been shown to be critical to the induction of an adaptive immune response (43, 44). Consistent with this, cytokine production by tumor cDC1s has proven essential for immunotherapy. In the context of adoptive cell therapy (ACT), efficacy required cDCs capable of CXCL9/CXCL10 production in order to drive tumor infiltration by the transferred T cells (13). cDC1 production of CXCL9/CXCL10 and expression of the cognate receptor, CXCR3, on CD8⁺ T cells, has also recently been shown to be critical for response to anti-PD-1 or anti-TIM-3 therapy (11, 14). Surprisingly however, this was not mediated by increased CD8⁺ T cell tumor infiltration, but rather enhanced effector function in endogenous CD8⁺ T cells. How chemokine expression by tumor cDC1s promotes a T cell response is unclear, but may relate to cDC1s being largely responsible for production of IL-12 within tumors (4, 45). In support of this, cDC1 production of IL-12 was found to induce IFN γ production by CD8⁺ T cells following PD-L1 blockade, and the feedback loop between IL-12-producing cDC1s and IFN γ -producing CD8⁺ T cells was necessary for therapeutic efficacy (12). Similarly, IL-12, CXCL9/10, and IFN γ are all required for response to the combination of paclitaxel chemotherapy and TIM-3 blockade (11). Taken together, the data indicate the importance of cDC1 and CD8⁺ T cell crosstalk in the tumor microenvironment and suggest that targeting this interaction is therapeutically viable (**Figure 1**). Interestingly, a recently published study used single cell RNA sequencing (scRNA-seq) to identify a subset of regulatory DCs in lung tumors (46). Although these were shown to arise from both the cDC1 and cDC2 lineage following maturation and uptake of tumor antigen, the authors specifically focused on the regulatory DCs of the cDC1 lineage, and showed that blockade of IL-4 could reestablish IL-12 expression, thus improving CD8⁺ T cell function and tumor control (46).

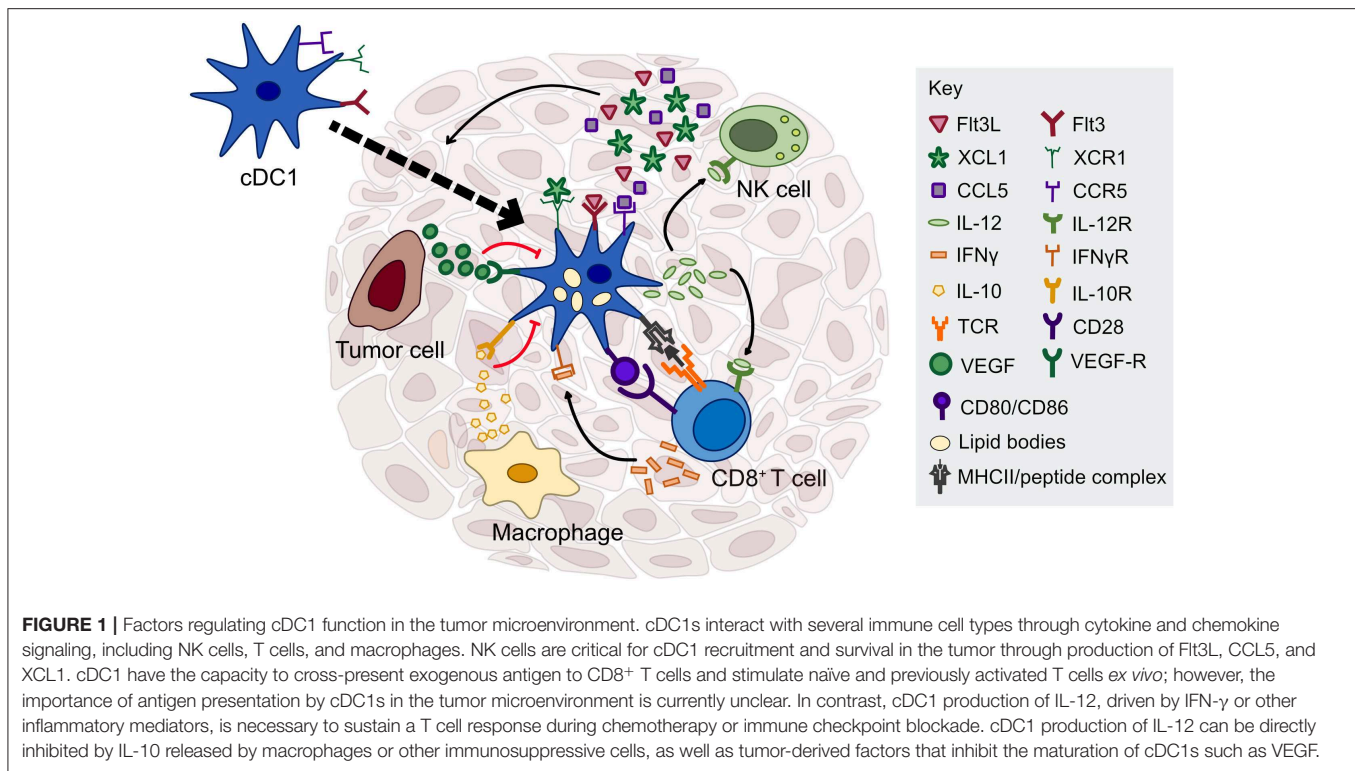
Another recent advancement in the field is the characterization of natural killer (NK) cell and cDC1 interplay within tumors. Two groups independently showed that NK cell production of either FMS-related tyrosine kinase 3 ligand (Flt3L) or CCL5 and XCL1 induces cDC1 recruitment into the tumor microenvironment (36, 47). Analyses of gene signatures in human tumors indicate that the presence of NK cells correlates with the presence of cDC1 in this context as well, suggesting that manipulation of NK cell presence within the tumor could indirectly improve the adaptive immune response (36, 47). Communication in the opposite direction has also been shown to be required, with IL-12 production by cDC1 leading to IFN γ production by NK cells (48). Neutralization of IL-12 or the absence of cDC1 in *Batf3*-deficient mice increased lung colonization following tail-vein injection of multiple tumor cell lines (48). The requirement for cross-talk between cDC1 and multiple immune subtypes is indicative of the complexity of the immune response within the tumor and suggests that the localization of leukocytes within the tumor is a critical regulator

of their function. Improvements in imaging techniques and analysis platforms will help dissect some of this complexity.

At both the genetic and functional level, human cDC1 show similar characteristics to mouse cDC1 (25, 31), suggesting that mouse models to study cDC1 function will be informative in translating the biology to the context of humans. In particular, a recently published study used scRNA-seq to profile myeloid populations in human and mouse lung cancers, and found a high degree of concordance between DC subsets in the two species, including cDC1 (31). The same study assessed the association of the gene signatures most specific to individual cell types and compared them with patient prognosis. cDC1 genes were generally found to be associated with positive prognosis, suggesting that the presence of cDC1 in human lung tumors is associated with better survival (31). Similar findings have been made in hepatocellular carcinoma (49), and the presence of DCs in breast tumors (11), along with the ratio of CD103⁺ cDC1 to CD103⁻ DCs in breast cancer, head and neck squamous cell carcinoma (HNSCC), and lung adenocarcinoma (4), have all been shown to correlate with improved patient prognosis. In addition, the presence of cDC1 within human melanoma tumors correlated with improved response to anti-PD-1 therapy (36) as well as with higher CD8⁺ T cell infiltration into tumors (33), which is associated with a positive prognosis across multiple tumor types (50). Furthermore, genes specific for cDC1 correlate with the presence of CXCL9 expression by human tumors in the TCGA database (11, 13), and cDC1 in human breast tumors exhibit expression of CXCL9 by immunofluorescence (11), further indicating that human cDC1 are likely to produce similar chemokines and play a similar role in the tumor microenvironment as mouse cDC1. As CXCL9 expression also correlates with response to anti-PD-1 (14), there is likely a critical role for cDC1s in the context of patient response to ICB as well, although this has not been directly tested.

Type 2 Conventional DCs

While the aforementioned data suggest that cDC1 may be the only DC subset required for the induction of anti-tumor immunity, this neglects the importance of CD4⁺ T cells, which play a critical role in supporting CD8⁺ T cell activity (suggesting a role for cDC2 antigen presentation to CD4⁺ T cells) (51–54). While cDC2 are dispensable for CD8⁺ T cell activation and proliferation in some tumors (4, 9), this may be due to the specific models and therapies examined. For example, cDC2s were found to be important during response to anthracycline chemotherapy (55), and certain tumor models are responsive to adoptively transferred CD4⁺ T cells (56). There are also several reports describing recognition of tumor antigens by human CD4⁺ T cells (56). As with cDC1, scRNAseq has shown that at the genetic level, mouse and human cDC2 subsets in lung tumors show a high degree of overlap (31). This includes the existence of functionally distinct subsets marked by expression of T-bet and ROR γ t (57). Additionally, it was recently shown that following depletion of regulatory T cells (T_{reg}), a subset of cDC2 can effectively elicit intratumoral CD4⁺ T cell responses and subsequent tumor control in a mouse model of melanoma (33). Upon T_{reg} depletion, cDC2 were able to migrate to the draining



lymph node and effectively induce differentiation of conventional CD4⁺ T cells (33). The observed increase in tumor rejection specifically required CD4⁺ T cell priming in the lymph node, as FTY720 blockade of lymph node egress prevented the anti-tumor immune response (33).

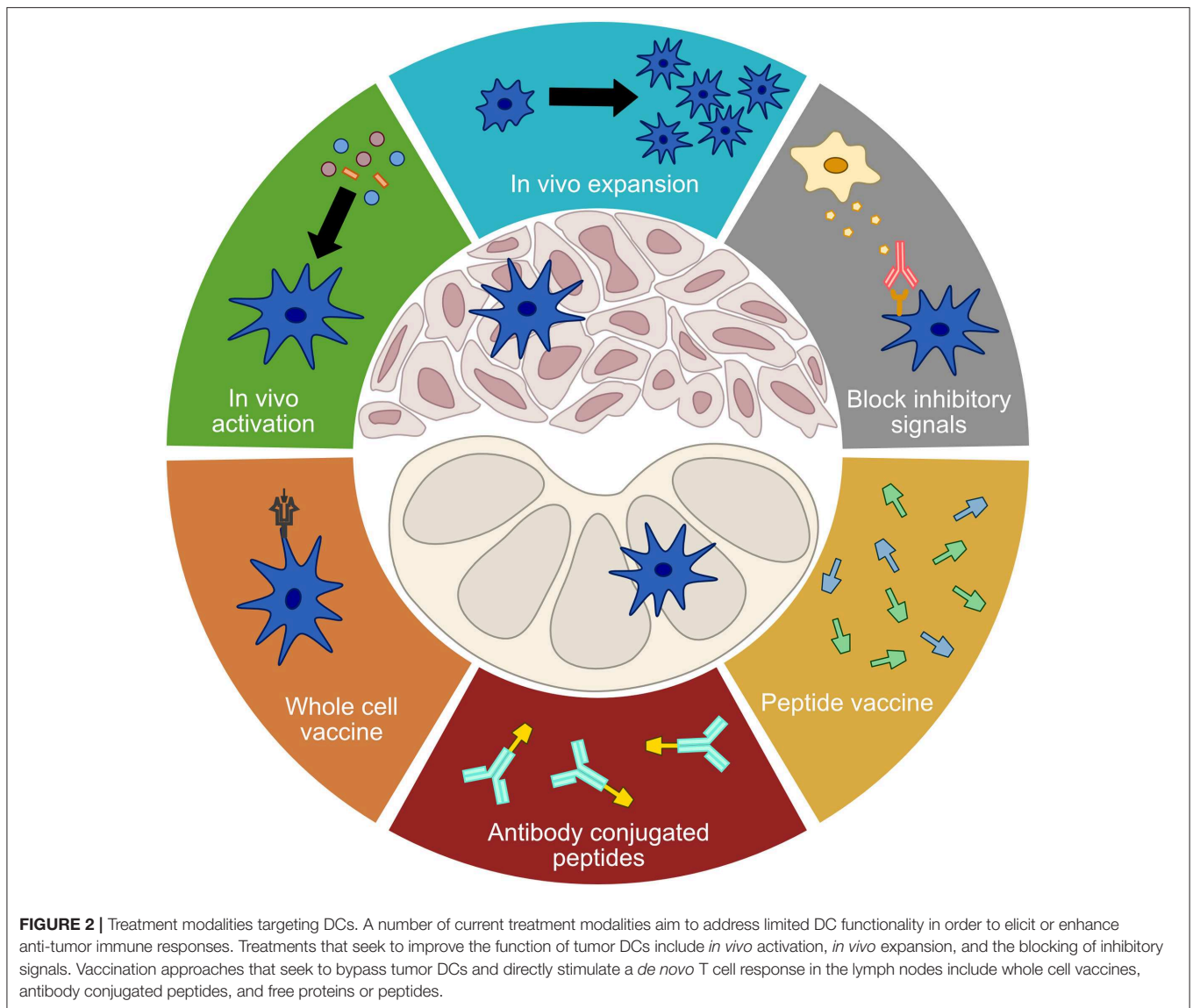
Interestingly, when the cDC2 gene signature was correlated with prognosis for lung adenocarcinoma patients, cDC2 were the DC subset most strongly associated with a positive prognosis (31). Similarly, high levels of cDC2 in HNSCC and melanoma tumors, when combined with low levels of regulatory T cells, correlated with longer progression free survival and higher levels of CD4⁺ T cell infiltration, further suggesting a role for both cDC2 and CD4⁺ T cells in human tumors (33). A substantial degree of heterogeneity in the cDC2 subset isolated from draining lymph nodes of human melanoma patients also correlates with the heterogeneity observed in cDC2 isolated from mouse tumors, with similar characteristics observed in both subsets (33). Given these data, it will be interesting to examine whether T_{reg} are also preventing cDC2 function in contexts other than melanoma, and whether depletion of the T_{reg} may augment the anti-tumor immune response in human tumors via increased cDC2 and CD4⁺ T cell activity.

Plasmacytoid Dendritic Cells

In contrast to cDCs, whose role in anti-tumor immunity is associated with antigen presentation, plasmacytoid DCs (pDCs) are usually associated with response to viral RNA and DNA via production of high levels of type I IFN, along with other inflammatory cytokines such as IL6 and TNF α . However, pDCs

do express MHCII and costimulatory molecules and could therefore potentially act as antigen-presenting cells, although the antigen processing capabilities of the cells are unclear (18, 58). Interestingly, pDCs differentiate from myeloid CDP as well as from IL-7R⁺ lymphoid progenitors (59), resulting in cells that are phenotypically similar but with distinct functional capacities (59). Specifically, only myeloid-derived pDCs were found to process and display antigen (59). The role of pDCs in cancer may therefore depend upon the extent to which they are myeloid derived, in addition to their activation state. At least one study has shown that tumor-associated pDCs are largely inert, but that following intratumoral injection of a TLR7 ligand, pDCs can induce anti-tumor immune responses (60). Whether this response is directly attributable to antigen presentation by myeloid-derived pDCs or is a result of type I IFN activation of cDC function is less clear (61).

In a similar vein, the role of pDCs in human tumors is less established than that of the cDC subsets. As with cDC1 and cDC2, scRNAseq indicates that the human pDCs mirror mouse pDCs (31). The human pDC gene signature also correlates with a positive prognosis in lung adenocarcinoma, although to a lesser degree than either cDC1 or cDC2 (31). In contrast, the presence of pDC in breast tumors, as assessed by immunohistochemical staining, strongly correlated with a poor overall prognosis (62). Additionally, pDCs found in the ascites of patients with ovarian carcinoma induced IL-10-producing CD8⁺ regulatory T cells and inhibited T cell proliferation (63). High-dimensional analysis has recently been employed by several groups to identify heterogeneity within the classically defined pDC population

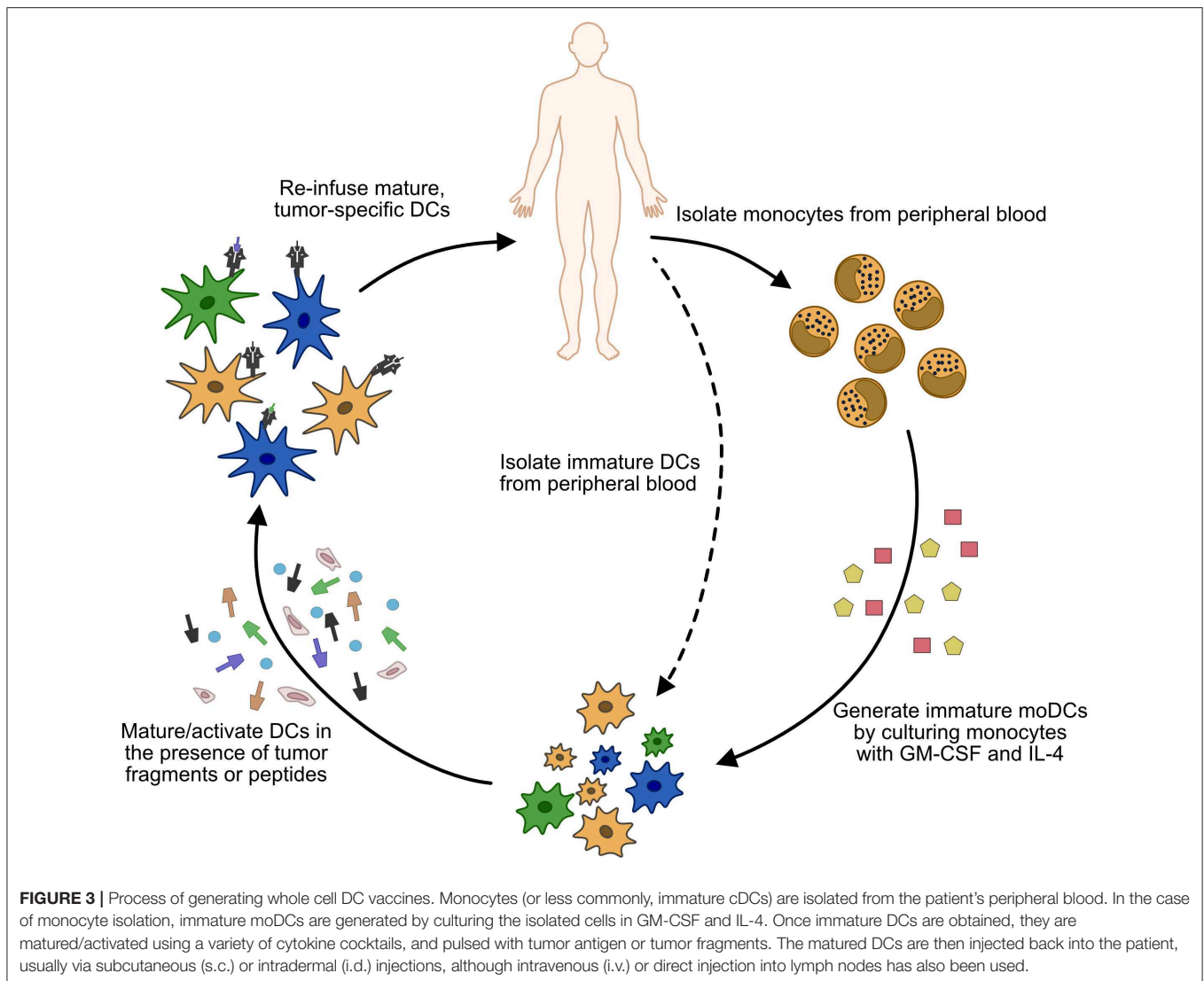


in human samples (64–66), raising the possibility that the conflicting roles of pDCs in human tumors could be attributed to the conflation of multiple subsets.

Monocyte Dendritic Cells

Monocyte-derived DCs (moDCs) differentiate from Ly6C⁺ or CD14^{hi} monocytes in mice and humans, respectively, generally under inflammatory conditions (19). Identification of moDCs has historically been difficult, as the markers used for identification overlap substantially with those expressed by macrophages and CD11b⁺ DCs in mice. Recently, however, expression of the Fc receptors FcγRI and FcεRI were used to distinguish the subset (67). In contrast to the ability of cDCs to present antigen to T cells, moDCs have not been shown to transport antigen to the lymph nodes and activate T cells. As a result, it is unclear what role moDCs can have in inducing a

de novo T cell response. However, the recruitment of moDCs is enhanced under inflammatory conditions, which can lead to the induction of “TipDCs” (tumor necrosis factor (TNF) and NOS2-producing inflammatory dendritic cells) from moDCs. It was also recently shown that for mice given adjuvant therapy with polyinosinic:polycytidilic acid (Poly [I:C]), moDCs were required for the anti-tumor response, whereas cDC1 were dispensable (68). moDCs have also been shown to enhance the survival of adoptively transferred T cells (69) and may further regulate T cell activity within tumors through production of TNFα and NOS2 (18). Activation of p53 in myeloid precursors can even promote the formation of CD103⁺ moDCs with the capacity to cross-present antigen and produce high amounts of IL-12 (70). moDCs also appear to play a critical role in the regulation of graft-vs.-leukemia (GVL) responses following therapeutic bone marrow transplants, with inhibition of XBP-1 splicing



helping to prevent graft-vs.-host disease while maintaining a GVL response in both murine and human xenograft models (71, 72). Thus, while the role of moDCs in the development of spontaneous anti-tumor immunity is unclear, they appear critical in sustaining an immune response during certain inflammatory conditions.

DENDRITIC CELL-BASED THERAPIES

Immunotherapy continues to represent a promising avenue for new cancer therapies, especially since many patients who respond exhibit durable responses. However, response rates for many tumor types are still low, underscoring the need for continued improvement in our understanding of anti-tumor immunity and approaches to enhance it. As expanded upon in the first section, cDCs are central inducers of the immune response, and targeting them may provide a method of improving immune responses in cases where targeting T cells alone is ineffective. As DCs,

especially cDC1, tend to correlate with a positive prognosis when they are present in tumors, therapies targeting DCs focus on enhancing DC function, increasing their numbers, or bypassing the tumor microenvironment to promote systemic *de novo* anti-tumor immunity (Figure 2).

In vivo Activation

One of the earliest approaches to immunotherapy was the attempt to revert suppression of cDCs in the tumor microenvironment by providing exogenous activation signals. Toll-like receptors (TLRs) are one of the major pathogen- and damage-sensing pathways, with 13 different TLRs present in mice (TLR1-TLR13) and the first 10 also present in humans. DCs subsets display differential TLR expression patterns in both species (73, 74). For example, in humans, pDCs preferentially express TLR7 and 9, cDC1 preferentially express TLR3 and 8, and cDC2 preferentially express TLR1 and 6 (73). This means DCs preferentially recognize different pathogenic/danger-associated

signals and can be targeted with specific agonists, potentially with the goal of optimally shaping the anti-tumor immune response. However, the identification and therapeutic use of TLR agonists predates the classification of the cDC subsets, and limited work has been done in this area.

In humans, TLR7 and TLR9 are among the more widely explored targets given their capacity for inducing a type I IFN response. Topical TLR7 agonists including imiquimod and R848 have been shown to induce an immune response as well as promote some level of tumor control in a variety of cancer types, including melanoma and breast cancer (75–77). Indeed, a number of clinical trials are currently ongoing to test TLR agonists in breast cancer patients, with one having observed immune-mediated rejection of skin metastases following treatment with imiquimod (75). Topical application carries a clear drawback, in that it can only reasonably be used in situations where either the induction of a systemic immune response will be able to induce tumor control, or where tumors are close enough to the body's surface that a local immune response can be induced. As a result, TLR7 agonists with non-topical application methods are also under development. One such agonist is 852A, which has been shown to induce CXCL10 and IL-1RA production, although minimal tumor control was observed in initial clinical trials (78, 79). In addition to TLR7 activation, DCs can be targeted via TLR9 agonists (73), with activation of TLR9 using CpG oligodeoxynucleotides (ODNs) causing pDC maturation and cytokine production. The classes of CpG ODNs have different routes of administration and produce unique downstream effects (73). In addition to CpG ODNs, a novel TLR9 agonist, IMO-2125, has also been shown to engage TLR9 leading to downstream immune signaling and suppression of A20 lymphoma and CT26 colon carcinoma tumor models in mice (80).

TLR3 and TLR8 are preferentially expressed by cDC1, which, owing to their established role in anti-tumor immunity, makes them attractive therapeutic targets (73). Polyinosinic:polycytidylic acid (Poly[I:C]) is one of the most well-known TLR3 agonists and administration of poly(I:C) is effective in inducing cDC1 maturation as well as production of IL-12, type I IFNs, and chemokines. However, as it is not well-tolerated clinically (81), variants have been developed that aim to reduce the toxicity of poly(I:C) administration. One such variant is poly-ICLC, an RNase resistant form of poly(I:C) that leads to immune activation and some tumor responses, either alone or as an adjuvant to conventional therapies (82, 83). Poly(I:C12U), another poly(I:C) variant, introduces unpaired bases in order to increase the degradation rate of the drug in an effort to reduce adverse effects (84, 85). In addition to TLR3, cDC1 also express TLR8, which can be targeted with the TLR7/8 agonist mentioned previously, R848. Agonists of TLR8 alone are also in development. For example, VTX-2337 was shown to activate cDC1 and monocytes (86) and was well-tolerated in phase I clinical trials, although progression free survival was unchanged in a phase II trial conducted in squamous cell head and neck cancer (73, 87).

STING (stimulator of interferon genes) mediates type I IFN responses following recognition of cytosolic DNA by cGAS

(cyclic GMP-AMP synthase) and production of 2'3'-cGAMP (88). Host STING is required for the induction of anti-tumor immunity, as STING-deficient mice fail to develop spontaneous immunity against immunogenic tumor lines and show reduced responses to radiation therapy (89, 90). STING knockout mice also exhibit increased susceptibility to inflammation-associated carcinogenesis following administration of AOM/DSS to induce colitis (91, 92). It is currently unclear whether STING expression by cDCs or other host cells is important for promoting an immune response, and the specifics of the tumor model and therapy being evaluated will likely impact the underlying biology. For example, blockade of CD47 promotes uptake of tumor cells by SIRPα⁺ cDC2, leading to activation of the cGAS-STING pathway (93), whereas in other tumor models it is production of 2'3'-cGAMP by tumor cells that is responsible for activation of host STING (94). Regardless, the intratumoral injection of STING agonists such as 2'3'-cGAMP and DMXAA can induce tumor rejection, both alone and in combination with other therapeutic modalities (95, 96).

Despite the pre-clinical efficacy of intratumoral injection of STING or TLR agonists, single agent efficacy in the clinic has remained elusive. This has hampered development of TLR agonists in the past, but in the age of cancer immunotherapy these are now being reevaluated as part of combinatorial therapies. For instance, a recent pre-clinical study showed that treatment with the TLR9 agonist CpG led to increased OX40 expression on CD4⁺ T cells (97). Accordingly, while intratumoral injection of CpG alone led to rejection of the directly treated tumor, the addition of an OX40 agonist antibody led to clearance of contralateral tumors (97), and a phase I study testing this combination in non-Hodgkin lymphoma is currently underway (NCT03410901). As STING agonists have been developed more recently, these trials are already incorporating anti-PD-1 into their phase I treatment arms (e.g., NCT03010176). That said, it remains to be seen if this approach will be successful, and the development of systemic therapies targeting these pathways will be important to expand treatments beyond accessible tumors (98).

Blocking Inhibitory Signals

Extracting murine cDCs from tumors allows them to activate and restimulate CD8⁺ T cells (4), implicating the suppressive microenvironment as a key regulator of cDC function. An alternative approach to enhance the activation state of tumor cDCs is therefore to block inhibitory pathways that reduce cDC functionality. One advantage of this approach is that it allows for systemic administration of inhibitors, as opposed to the local administration required for many immune agonists. One of the first examples of this is targeting vascular endothelial growth factor (VEGF), as VEGF inhibits DC maturation and prevents an effective anti-tumor immune response (99). VEGF inhibitors are already in clinical use to inhibit increased angiogenesis, and evidence indicates that antibodies against VEGF enhance the anti-tumor immune response by counteracting DC inhibition (100, 101). This is supported by several pre-clinical studies showing that inhibitors of VEGF increase immune function and decrease the rate of tumor growth (101–103). VEGF

inhibition has also been shown to enhance DC maturation in human patients (104), suggesting that this may contribute to the efficacy of VEGF inhibitors in the clinical setting. However, it should be noted that the impact of VEGF on the vasculature and other immune populations may be more relevant to the immune impact of VEGF pathway inhibitors (105).

Another potent immunosuppressive signal in the tumor microenvironment is IL-10, which can be produced by tumor cells, macrophages, regulatory T cells, as well as other components of the stroma. Using isolated human DCs in co-culture with human melanoma cell lines, researchers have shown that IL-10 prevents DC maturation and induces a tolerogenic phenotype (106). Blockade of IL-10 in pre-clinical models, either directly or via depletion of macrophages has been shown to improve CD8⁺ T cell mediated anti-tumor immune responses in both murine and human systems (45, 106–108). At least in a mammary tumor model, this has been directly linked to the ability of IL-10 to suppress IL-12 production by cDC1s, reducing the percentage of CD8⁺ T cells that display a cytotoxic effector phenotype (45). TIM-3 expression by cDCs has also been shown to prevent response to chemotherapy in several tumor models (11, 109). How this occurs is unclear, but may relate to TIM-3 binding to high mobility box 1 protein (HMGB1) and limiting response to nucleic acids (109). Thus, while anti-TIM-3 antibodies can promote response to PD-1/L1 blocking by reducing T cell exhaustion (110, 111), TIM-3 blockade might prove efficacious even in patients with tumors that do not display substantial T cell infiltration.

Regulation of immunometabolism to increase anti-tumor immunity has been an increasing focus of cancer research. Although our understanding of basic immunometabolism is still evolving, several key insights have been made that are of relevance to tumor-associated DCs. As this has been expertly reviewed previously (99, 112), we will here highlight only two key metabolic aspects of tumor-associated DCs, and the therapeutic approaches being taken to counteract this metabolic inhibition. First, DC expression of indoleamine 2,3-dioxygenase 1 (IDO1) is thought to reduce L-tryptophan availability by converting it to L-kynurenine, leading to an increase in the suppressive capacity of regulatory T cells (113, 114). That said, IDO1 can be highly expressed by tumor cells themselves, and evidence that IDO1 expression by tumor DCs is a major mechanism of immune suppression is lacking. Several IDO1 inhibitors have also failed to demonstrate efficacy over the past few years, raising questions about the validity of this approach. Second, lipid accumulation in DCs has been shown to limit the function of DCs via interference in antigen processing and subsequent antigen presentation (115, 116). Accumulation of lipids in tumor-associated DCs is promoted by DC-specific activation of the endoplasmic reticulum (ER) stress sensor XBP1 (117). DC-specific siRNA silencing of XBP1 led to decreased lipid accumulation by DCs and enhanced immune-mediated tumor control in mouse models of ovarian cancer (117). Although further research will be required before ER stress can be effectively targeted to treat cancer, it is an active area of investigation.

In vivo Expansion

Tumor cDCs are relatively infrequent in human and murine epithelial malignancies (4, 11, 33). Thus, increasing the number of intratumoral cDCs represents an alternative approach to increasing the cumulative function of the population. Rather than the injection of exogenously expanded and activated cDCs (DC vaccination; described below), it has been shown in pre-clinical studies that systemic injection of Flt3L leads to systemic expansion of the cDC1 population, increasing the number of these cells within B16 melanomas and significantly delaying tumor growth (10). This approach also showed promise in increasing both the number of cDCs in pancreatic tumors and overall control of pancreatic tumor lesions in an autochthonous disease model, highlighting the importance of DC infiltration, and expansion even in cancer types with typically low immune infiltration (118). Combined administration of Flt3L with TLR agonists, STING agonists, radiation, and/or checkpoint blockade results in additional tumor control, even in advanced tumors (7, 10, 118, 119). This approach is being tested clinically in several tumor types, including metastatic breast cancer and non-Hodgkin's lymphoma (NCT03789097, NCT01976585). The key advantage of this therapy is the potential for targeting a wider range of antigens, rather than those selected for vaccination, bypassing the need for patient-specific vaccine development. In addition, both systemic T cell activation and local T cell infiltration are enhanced by this combination, increasing the potential for synergy with other immunotherapies.

Dendritic Cell Vaccines

In contrast to *in vivo* expansion, whole-cell DC vaccines rely on exogenous maturation and/or expansion of monocyte-derived DCs or cDC precursors (**Figure 3**), although most trials utilize moDC due to the rarity of cDCs or pre-DCs (27). These cells are isolated from a patient's peripheral blood, loaded with tumor lysate or tumor antigens, and matured using various cytokine cocktails (120, 121). Whole cell DC vaccines are associated with limited toxicities, are therefore considered a relatively safe therapeutic approach, and are being extensively evaluated in the clinic (121, 122). Multiple vaccine formulations can lead to increased antigen-specific T cell responses. There have even been trials in AML involving the fusion of cancer cells with autologous moDCs (123). However, the presence of an immune response has not correlated with clinical efficacy (124), with response rates in general between 8 and 15% in single arm trials (122). The only whole cell DC vaccine approved by the FDA to date is sipuleucel-T, which consists of isolated PBMCs cultured with a GM-CSF/prostatic acid phosphatase fusion protein (125). This approval to treat metastatic prostate cancer was based upon a 4.1 month improvement in overall survival without an accompanying delay in disease progression (125).

Given the ability of most vaccines to induce an immune response against a specific antigen, it is unclear why vaccines have shown limited efficacy to date. One possibility is that the immunosuppressive microenvironment of the tumor blocks T cell infiltration, survival, or effector function. Several

pre-clinical studies have shown that PD-1 and/or CTLA-4 blockade can improve tumor control in combination with tumor cell vaccines (126, 127). Similarly, in a mouse mammary tumor model, the efficacy of a HER2-loaded BMDC vaccine was improved by sequential anti-PD-1 therapy (128). Treatment with DC vaccines have also been shown to augment responses to standard-of-care therapy (129). Clinical trials have begun to evaluate the efficacy of combining DC vaccines with standard-of-care therapies and of vaccination with different DC subsets. In glioblastoma, a phase III clinical trial to assess the efficacy of a whole cell DC vaccine administered in combination with tumor resection, temozolomide, and radiotherapy (NCT00045968) exhibited safety and potential efficacy based on interpretation of early results (130). In contrast, a phase III trial of tumor-RNA loaded whole cell vaccines in combination with sunitinib following surgical debulking for the treatment of renal cell carcinoma (NCT01582672) was terminated early due to a lack of efficacy.

The limited efficacy of DC vaccines could also be a result of protocols that do not produce the optimal T cell response. GM-CSF maturation of PBMCs produces moDCs that are limited in their capacity to migrate to lymph nodes (131, 132), and several studies have shown endogenous DCs are actually required for T cell priming following administration of moDC vaccines (133–135). Murine cDC1s have been used in a vaccine in at least one study (136), but whether this is a viable approach in the clinic remains to be determined, particularly given the paucity of circulating, mature cDC1 in human peripheral blood (27, 137). Instead, studies have largely focused on improving baseline efficacy by assessing activation with different maturation cocktails. For many years, the “gold standard” maturation cocktail consisted of TNF α , IL-1 β , IL-6, and PGE₂ (120). However, PGE₂ induces T regulatory cells and lowers IL-12 production, so methods of maturation which omit it are being explored. For example, an interferon cocktail along with TLR3, TLR7, and TLR8 agonists produced superior T cell mediated cytotoxicity against a breast cancer cell line (138), while the combination of TNF α , IL-1 β , IFN γ , and a TLR7/8 agonist induced higher levels of the T cell chemoattractants CXCL9/10 (139). At the same time, the “gold standard” cocktail induces the highest level of DC migratory capacity (120). Given that increased DC migration to the lymph node following vaccination has been associated with increased overall survival in a small cohort of patients (140), it is unclear which approach would be better at promoting tumor control. DC migration to the lymph node can also be directly enhanced by pre-treating the injection point with DC activating agents such as tetanus toxoid and CCL3, or TLR agonists such as imiquimod or poly-ICLC (140, 141). The number of DCs injected also plays a role in achieving optimal responses, with 10⁶–10⁷ DCs per injection representing the optimal range for efficacy (142, 143). Given the range of approaches, it remains to be seen which, if any, will produce anti-tumor responses that can induce tumor regression, either alone or in combination with other therapeutic modalities.

Peptide/Protein Vaccines

Another possible reason for the failure of many DC vaccines may be the reliance on overexpressed or tissue-specific antigens (e.g., NY-ESO-1, MUC1, MAGEA3, MART1, HER2). In addition to their use in DC vaccines, these antigens have been fused to DC-targeting antibodies against Clec9a, DEC205, or DC-SIGN to enhance their ability to induce an immune response (122). DEC205-fused tumor-associated antigens demonstrate improved ability to induce T cell responses over administration of free antigen (144, 145). Additionally, partial clinical responses were observed following administration of DEC205-fused NY-ESO-1 and TLR agonist adjuvants in a phase I clinical trial (146). While targeting Clec9a generally induces tolerance, different adjuvants can be added in order to drive immune responses (124). For example, when combined with poly(I:C) and other adjuvants, Clec9a-fused antigens induce CD4- and CD8-mediated anti-tumor immunity (147, 148), while fusion of human IFN α to Clec9a led to an anti-tumor response that was improved by treatment with checkpoint blockade in the murine 4T1 mammary tumor model (149). Peptide fusions to antibodies against several other DC surface proteins are also in pre-clinical and clinical development (122). Given that different DC subsets can be targeted using antibodies against specifically expressed surface proteins, this represents another mechanism by which the anti-tumor immune response could be optimally shaped to induce the best outcomes for a given patient. However, one of the most recent advances in the development of cancer vaccines has been the ability to generate vaccines with patient-specific neoantigens. Although expensive and technically challenging, neoantigen vaccines are safe and able to induce strong systemic T cell responses (150, 151). More importantly, complete and durable responses have been observed in patients receiving neoantigen vaccines in combination with anti-PD-1 therapy in early phase clinical trials. Dozens of studies are now underway testing neoantigen vaccines either alone or in combination with ICB (e.g., NCT02950766, NCT03639714, NCT03953235, NCT04161755, NCT03359239).

CONCLUDING REMARKS

Poor responses to current immunotherapies are frequently associated with tumors that have low mutational burdens or low T cell infiltration. For these patients, alternate approaches are likely necessary to elicit favorable responses on par with those observed in disease contexts such as melanoma and lung adenocarcinoma. Increasingly, the role of tumor DCs in the anti-tumor immune response is being recognized as targetable. Although single-agent therapies targeting DCs have been minimally successful, combination with standard-of-care therapies with novel immunotherapies is a promising avenue of investigation. Further research to fully understand the role of the tumor immune microenvironment as a whole is certainly warranted given the complex nature of the interactions between the tumor and immune system.

A more complete understanding will hopefully lead to the development of effective therapeutic strategies that improve patient outcomes.

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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