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BASIC SCIENCE REVIEW

Mechanisms of resistance to T cell-based immunotherapy in head and neck cancer

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Abstract

Background: Most current approved or investigational immunotherapeutic approaches for head and neck squamous cell carcinoma are aimed at activating T cells. The majority of patients receiving such immunotherapy do not demonstrate durable tumor remission.

Methods: Original articles covering tumor heterogeneity, immunoediting, immune escape, and local tumor immunosuppression were reviewed.

Results: In the face of immune pressure, subclones susceptible to T cell killing are eliminated, leaving behind resistant tumor clones in a process known as immunoediting. Such subclones of tumor cells that are resistant to T cell killing may remain sensitive to natural killer (NK) cell detection and elimination, suggesting that patients harboring such tumors may benefit from combination of T and NK cell-based immunotherapy. Even in the setting of optimal immunotherapy, the immunosuppressive tumor microenvironment may arrogate effector immune responses through a number of distinct mechanisms.

Conclusions: Highly effective immunotherapy will likely require multimodality approaches targeting independent mechanisms of immune activation.

KEYWORDS

immune escape, immunoediting, NK cell, T cell, tumor heterogeneity

1 | INTRODUCTION

The immune system is the body's natural defense against pathogens, injury, and malignancy. For a cancer to be established, it must first evade the body's immune response. Recent clinical data suggests that therapeutic enhancement of antitumor immunity can overwhelm an established cancer and induce durable responses or even cure. T cell-based immunotherapies, including immune checkpoint blockade and adoptive T cell transfer, are immune-activating treatments based on the ability of T cells to detect and eliminate cancer cells. Pembrolizumab, a monoclonal antibody targeting the programmed death receptor 1 (PD-1) immune checkpoint, was recently approved by the United States Food and Drug Administration (FDA) as first line treatment in recurrent and metastatic head and neck cancer based on a phase III clinical trial (KEYNOTE 048), which showed an overall survival advantage and favorable adverse event profile compared to the former standard of care regimen containing platinum-based therapy, 5FU, and cetixumab.[1] Another form of immunotherapy not yet FDA approved but with demonstrated clinical efficacy in head and neck carcinoma is adoptive transfer of T cells engineered to target tumor cell antigens.[2,3]

For a T cell to detect a tumor cell, an antigen must be presented on the tumor cell surface via a major histocompatibility complex (MHC) class I molecule. Recognition of an appropriate MHC:antigen complex by a T cell receptor (TCR) activates the T cell, leading to exocytosis of granules containing perforin and granzymes that cause cell death.[4] Head and neck squamous cell carcinomas (HNSCC) have a relatively high somatic mutation rate, such that the formation of neoantigens that can be recognized by T cells is expected to be high.[5,6] However, the great majority of patients receiving immune checkpoint blockade or adoptive T cell transfer do not demonstrate objective responses, let alone cure.[7,8] Why do these T cell-based immunotherapies have such low rates of responses? Is it possible to predict who will respond to T cell-based immunotherapy?

The answers lie in understanding the mechanisms of T cell recognition and killing of tumor cells, and how progressing tumors are shaped in the face of immune pressure in a process called immunoediting. Given that T cells must recognize a processed antigen presented on MHC in order to activate, cells that are weakly antigenic or immunogenic are more likely to evade T cell recognition and survive to populate a progressing tumor. In highly heterogenous tumors, increased numbers of distinct subpopulations escalate the probability that some are resistant to T cells and can adapt in the face of selection pressure. Alarmingly, tumor cells often harbor genetic or epigenetic changes that cause defects in antigen presentation machinery (APM) and interferon-gamma (IFN-γ) signaling, which may render one or more subclones of tumor cells within a carcinoma completely undetectable to T cells.[9] Meanwhile, the microenvironment of a tumor is highly immunosuppressive and represents another barrier of immune suppression.[10] To achieve high rates or treatment response or cure, most patients will require combinatorial immunotherapy to address local immunosuppression and other mechanisms of resistance to T cell immunotherapy. In this article, we review the factors that are predictive of cancer response to T cell-based immunotherapy with a focus on newly emerging patient-correlate data. Understanding the factors that contribute to T cell resistance will inform the study of therapies to combat and circumvent the barriers that limit such treatments to broaden the scope of immunotherapy-sensitive tumors (Figure 1).

2 | TUMOR HETEROGENEITY

Intratumor heterogeneity (ITH) refers to variability among the cancer cells within a single tumor. Cancer likely begins with one tumor cell clone that acquires the ability to proliferate in an uncontrolled manner while evading detection and elimination by the immune system. With tumor progression, genomic instability leads to

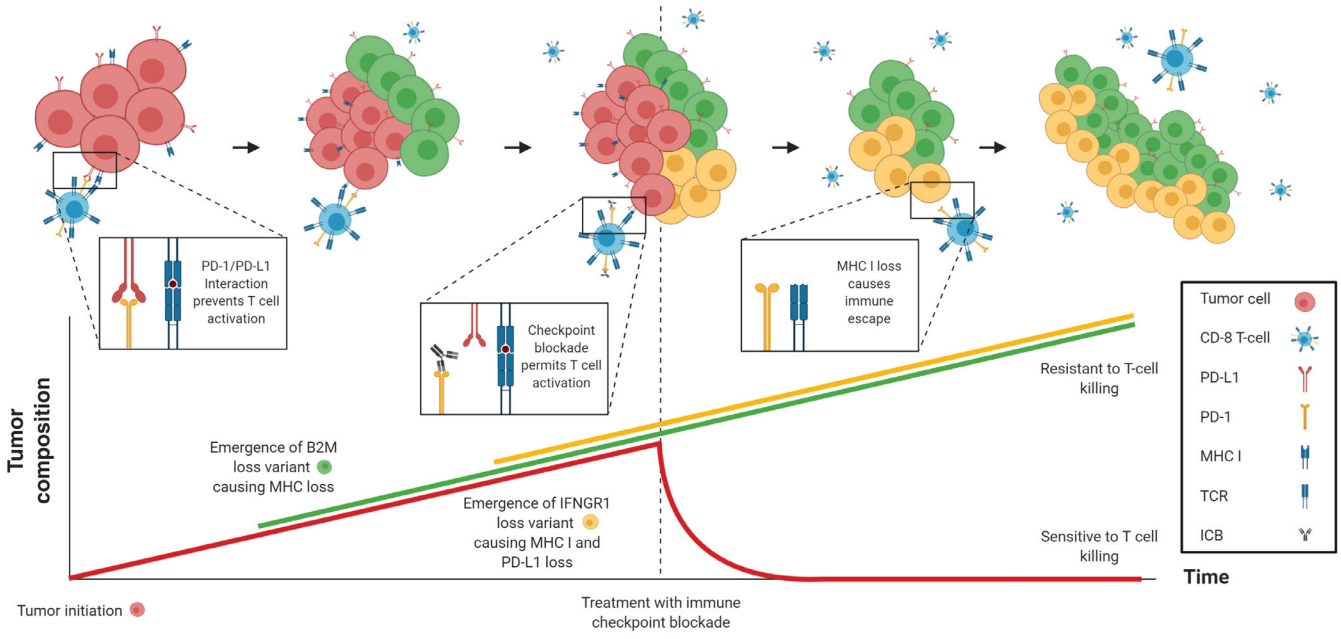

FIGURE 1 Cellular and graphical schema of the development of subclones of tumor cells that harbor genomic alterations that prevent T cell recognition and killing. Early, small homogeneous tumors (red cells) may escape T cell killing through expression of programmed death receptor (PD-1) and ligand (PD-L1) immune checkpoints. As tumors progress, subclones to tumor cells acquire genomic alterations (green and yellow cells) that allow T cell escape. When PD-1/L1 immune checkpoint blockade immunotherapy is administered, tumor cells sensitive to T cell killing are eliminated (red line), whereas resistant tumor clones persist (green and yellow lines) resulting in immunotherapy resistance and tumor progression [Color figure can be viewed at wileyonlinelibrary.com]

the development of subclones of tumor cells that have acquired different genomic alterations.[11] Some of these alterations will provide a selective proliferative or survival advantage. One example is genomic alterations in genes important for immune recognition and elimination by T cells. When this occurs across a tumor composed of millions of tumor cells, dominant subclones arise that co-exist and populate the entire mass of the tumor.

Evidence for the presence of subclones of tumor cells harboring different genomic alterations has become abundant with the advent of new technologies such as multiregional and single-cell sequencing.[12,13] A seminal study by Gerlinger et al in 2012 identified that approximately two-thirds of somatic mutations are not detectable across every region within a tumor.[14] Single-cell sequencing of malignant HNSCC cells reveals high variability in expression signatures related to cell cycle, stress, hypoxia, and epithelial to mesenchymal transition.[15] Bioinformatic analysis supports that an individual HNSCC harbors, on average, 2-6 tumor subclones.[16] This can have important implications for how tumor cells and the immune system interface in a process termed cancer immunoediting. Importantly, measures of increased ITH have been shown to be an independent prognostic factor for decreased survival in HNSCC and may actually improve prognostication over traditional variables such as tumor grade and TP53 mutant status.[17]

3 | TUMOR HETEROGENEITY PROMOTES TUMOR ESCAPE

Within a tumor harboring multiple independent antigens, the immune system is only capable of mounting a response against a subset of selected antigens out of the many produced—this phenomenon is termed immunodominance.[18] Immunodominant antigens are established through temporal (first antigen is more likely immunodominant) constraints, spatial (immunodominance occurs at single sites) constraints, or through selective expression of immune checkpoints on individual T cell clones, possibly in response to TCR avidity.[19] T cell response to these immunodominant antigens successively promotes outgrowth of weakly antigenic cells while preventing the development of T cell response to weaker antigens.[18] In special cases, loss of MHC class I antigen presentation on immunodominant tumor cells can spiral the host T cell response into a futile loop of responses against an immunodominant antigen cross-presented on dendritic cells (DCs) but not presented on tumor.[18]

Immunodominance is one contributing factors that leads to the ability of tumors to escape immunity. Immune escape ultimately occurs through cancer immunoediting, a concept elegantly described experimentally by Robert Schreiber and colleagues that proceeds through three phases: elimination, equilibrium, and escape.[20] In elimination, T cell adaptive immunity detects and eliminates transformed cells before a clinically relevant tumor forms. Here, the recognition of tumor-specific antigens (TSA) derived from tumor-specific mutations (neoantigens) by T cells is critical.[21] Within heterogeneous tumors harboring multiple antigens, cells harboring immunodominant TSA are preferentially recognized and killed by T cells while simultaneous attack on immunorecessive antigens on other subclones is suppressed.[18,22] The presence of TSA is associated with an upregulation in antigen presentation genes, which is in turn associated with increased cluster of differentiation 8 (CD8) T cell activation and therefore cancer cell killing.[23] Therefore, the seemingly paradoxical finding that increased mutational burden is independently predictive of positive response to checkpoint blockade in HNSCC can be attributed to increased formation of neoantigens not subject to immune tolerance.[24,25] The killing of sensitive clones by T cells leads to emergence of variants that are either poorly recognized by lymphocytes or insensitive to effector cells. In principle, an equilibrium phase of immunoediting exists where immunity is able to halt progression of a malignancy, but not eliminate it. The exact details of how many tumor subclones need to be sensitive vs resistant to immunity and how long equilibrium can exist (years? decades?) remain unclear. Eventually, one or more populations of tumor cells fully escape immunity leading to escape phase of immunoediting and progressive tumor growth.[20]

4 | KEY GENETIC OR EPIGENETIC ALTERATIONS THAT PROMOTE TUMOR ESCAPE

Loss of antigen presentation itself is a common event in HNSCC that can render tumor cells undetectable by T cells. Loss of expression of even a single gene in the antigen presentation pathway is sufficient to impair antigen presentation.[9,26-31] Genomic alterations in one or more of these genes occurs in 9%-15% of HNSCC.[32,33] Much more common are epigenetic alterations or loss of the IFN signaling pathway that lead to an antigen loss phenotype.[34-38] Through analysis of human tumor specimens, it is estimated that 15%-25% of primary tumor and 40% of metastatic lesions have completely lost MHC class I

expression.[38-40] When taking partial loss into consideration, defects in MHC class I antigen presentation occur in approximately 60% of HNSCC.[40,41] Clinically, downregulation of MHC class I antigen translates into a marker for poor prognosis in HNSCC.[39]

For an antigen to be loaded onto an MHC molecule, proteins must first be cleaved to peptides of 8-10 amino acids through ubiquitination and degradation by a proteasome complex. Specific subunits of the proteasome complex responsible for optimal cleavage of proteins into 8-10 amino acid peptides are inducible by IFN-γ. These peptides are transported into the endoplasmic reticulum via transporter associated with antigen processing complex (TAP) where they are loaded onto a folded MHC-β-2 microglobulin (β2M) complex to form the MHC:antigen complex. Finally, the MHC:antigen complex is transported to the surface of the cell.[28] In HNSCC, TAP1 is a key APM component. Forced expression of TAP1 alone can rescue T cell recognition of HNSCC cells with downregulated APM.[34] TAP1 is downregulated in approximately 50% of HNSCC and among tumors with known downregulation in MHC I, TAP1 is downregulated or lost in 59%.[40,42] TAP2 is downregulated in 70% of HNSCC and tapasin (TAPBP), which plays an important role in stabilization of the MHC-β2M complex, is downregulated in 45% of HNSCC.[40] Defects in the structure of the MHC-β2M complex also allow tumors to evade T cell recognition.[9,43] β2M itself is downregulated in over 50% of HNSCC.[40,44,45] In mouse models, forced expression of β2M with MHC increases the efficacy of MHC gene therapy by virtue of its role in stabilizing cell surface MHC.[46] Analysis of 84 HNSCC specimens detected no genetic mutations in β2M, implying either epigenetic regulation or lack of IFN-γ signaling as the primary mechanism for β2M downregulation.[42] Conversely, loss of heterozygosity in at least one locus of the HLA coding gene at 6P21.3 is present in 36%-49% of HNSCC.[47] In total, these data suggest that downregulation and loss of APM are common events in HNSCC. In patients with tumors harboring such alterations in expression of APM components, T cells are unable to correctly recognize and kill tumor cells, which poses a significant barrier to T cell-based immunotherapy.

In some tumors, IFN-γ produced by immune cells can upregulate APM transcription to rescue MHC I expression in tumor cells where decreased expression is not genetic in origin.[39,48] After binding of IFN-γ to its receptor interferon-gamma receptor (IFNGR1/2), phosphorylation of Janus kinase 1/2 (JAK1/2) and Signal transducer and activator of transcription 1 (STAT1) activates the JAK/STAT signal transduction pathway. STAT1 acts as a transcription factor to increase the expression of interferon regulatory factor 1 (IRF1) and p48 which in turn upregulates MHC I and programmed death-ligand 1 (PD-L1) expression. Many members of the APM including TAP, TAPBP, and immunoproteasome subunits are also upregulated by IFN-γ.[48] Thus, lack of IFN-γ or defects in the IFN-γ response pathway may also lead to downregulation or loss of MHC expression on the cell surface and tumor escape.[48,49] HNSCC harbor somatic mutations in IFNGR1 at a rate of 3% and copy number variations at a rate of 11%.[50] Decreased or absent IFNGR1 expression is common in HNSCC cell lines.[51] When any subunit of the receptor complex is downregulated or mutated, impaired assembly of the full signaling complex leads to deficient IFN-γ responsiveness.[52] Additionally, HNSCC cells express low basal levels of phosphorylated STAT1. STAT1 knockdown significantly reduces both IFN-γ-mediated APM component expression and tumor T cell recognition of IFN-γ-treated HNSCC cells.[53] Alterations in other pathway components including JAK1/2 and IRF1 also impair IFN-γ signaling.[26,54]

Patients with gene expression profiles (GEP) showing high levels of APM in conjunction with high PD-L1, chemokine expression, and other IFN-γ responsive genes are designated "T cell-inflamed". Specifically, an "IFN-γ (6-gene)" GEP which includes STAT1 and IFN-γ was identified from a cohort of melanoma patients as predictive of response to checkpoint inhibitors.[55] This profile was then validated with data from the KEYNOTE-012 (NCT01848834) clinical trial of patients treated with pembrolizumab for HNSCC or gastric cancer. This signature predicted objective response and progression-free survival in HNSCC.[55,56] Further studies of HNSCC KEYNOTE patients confirmed that response to PD-L1 therapy was strongest in patients with T cell-inflamed GEP and high tumor mutational burden (TMB). Notably, GEP and TMB were only modestly correlated with each other, indicating that these factors capture distinct features of T cell activation and neoantigenicity.[25] A GEP indicative of a T cell-inflamed phenotype with intact IFN-γ signaling correlated with progression-free survival in other confirmatory cohorts.[57] Taken together, these data support the crucial role of IFN-γ signaling and expression of APM components in antitumor immunity and supports that patients with defects in IFN-γ response or APM pathway genes are less likely to respond to T cell-based immunotherapies.

Upon T cell recognition of an MHC:antigen complex and activation, early killing occurs through T cell release of perforin and granzyme and late killing occurs through expression of death receptor ligands such as tumor necrosis factor alpha (TNFa), Fas ligand, and TNF-related apoptosis-inducing ligand.[58,59] Tumor cell insensitivity to these methods of killing can occur via many mechanisms, although the prevalence of these defects has not been clearly elucidated in HNSCC. Other immune effector cells such as natural killer (NK) cells and lymphokine-

activated killer cells depend on similar mechanisms, suggesting that these mechanisms of resistance could be universal to T cell or non-T cell-based immunotherapy. Caspase-8 is a protease enzyme that plays a central role in inflammation and cell death. Activation of caspase-8 via death receptor signaling or granzyme results in apoptosis.[60,61] Mutation in procaspase-8 is present in 9% of primary HNSCC.[33] Mutated procaspase-8 becomes constitutively bound to Fas-associated protein with death domain and impairs the recruitment of death-induced signaling complex components. The presence of a procaspase genomic alteration correlates with poor clinical outcomes.[62] Lastly, many tumor cells bear mutations in cell cycle checkpoint proteins such as TP53, p21, and retinoblastoma (Rb) which dysregulate the G1/S checkpoint. To compensate, cells can pause the cell cycle at the G2/M checkpoint following exposure to granzymes and death receptor signaling to avoid replicating damaged DNA.[63] Several studies have shown that WEE1 kinase inhibition is able to reverse the effect of G2/M cell cycle checkpoint activation and sensitize HNSCC cells to T cell and NK cell-based immunotherapy.[64]

Lastly, recent research has uncovered the role of dysregulated innate immune sensing as mechanism of insufficient activation of antitumor immune responses in head and neck cancer. Production of type I interferon downstream of innate immune receptor signaling on myeloid cells and tumor cells is likely a critical step in activation of adaptive immune responses.[65] Human papilloma virus can induce degradation of stimulator of interferon genes, an important innate immune sensor, in head and neck cancers.[66] Whether genetic loss of innate immune receptors or downstream signaling components also contributes to immune escape highly heterogeneous carcinogen-associated head and neck cancers requires further study.

5 | IMMUNOSUPPRESSIVE MICROENVIRONMENT

Not only do antigen-specific effector immune cells have to contend with tumor heterogeneity and tumor cell-intrinsic mechanisms of resistance to recognition and killing, the immunosuppressive tumor microenvironment (TME) also contributes to immune escape.[10] Physical barriers (high interstitial pressure), nutrient depletion, hypoxia (with low pH), and the recruitment of immunosuppressive immune cells are hallmarks of progressing tumors.[10]

5.1 | Metabolic demands

The metabolic and functional demands of tumor cells lead to competition with immune effector cells for limited resources within the TME.[67] In the majority of solid tumors, rapid tumor development and abnormal angiogenesis create regions of transient or permanent hypoxia.[68,69] Specifically, hypoxia leads to the secretion of the T cell inhibitory compounds adenosine and galectin-1.[70-72] Adenosine triggers accumulation of immunosuppressive levels of intracellular cAMP, while galectin-1 is involved in cellular adhesion, invasion, and angiogenesis and is clinically associated with survival in HNSCC.[70,72,73] Another hallmark of cancer cells is increased use of glycolysis in regions of low glucose and oxygen (Warburg effect).[74-76] Glucose metabolism genes are often overexpressed in cancers, and overexpression of glucose transporter 1 is correlated with decreased T cell infiltration in squamous cell carcinoma.[77,78] Utilization of glucose by tumor cells leaves effector immune cells without the fuel needed for activation and expansion. High rates of anaerobic glycolysis by tumor cells lead to high levels of lactate. Buildup of extracellular lactate inhibits the export of lactate in T and NK cells, leading to decreased production of IFN-γ.[79,80] Another result of extracellular lactate accumulation is a decrease in the extracellular pH to 6.0-6.5.[81] Acidosis leads to loss of T cell function, which can be restored by increasing pH to physiological values.[80,82]

5.2 | Tumor-associated macrophages

In tumors, lactic acid also enhances polarization of macrophages into immunosuppressive M2 macrophages.[83(p132)] Tumor-associated macrophages differentiate along a spectrum of M1 tumoricidal macrophages to M2 tumor-promoting macrophages.[84] M1 macrophages exhibit expression of proinflammatory cytokines while M2 macrophages are identified by expression of arginase-1 and mannose and scavenger receptors.[84] M2 polarized macrophages induce production of regulatory cytokines such as transforming growth factor beta (TGF-β) and interleukin-10 (IL-10) and suppress T cell proliferation. In oral SCC (OSCC), M2 macrophages are the predominant phenotype and are seen at even higher levels in metastatic tumor deposits.[85] Importantly, patients with tumors harboring high levels of M2 macrophages have poorer outcomes compared to patients with low M2 macrophages, indicating that M2 macrophage-induced immunosuppression may contribute to tumor escape and progression.[85]

5.3 | Myeloid-derived suppressor cells

Myeloid-derived suppressor cells (MDSC) are a population of immature myeloid cells that play an immunosuppressive role in cancer.[86] Classically, MDSC are recruited to the tumor environment by myeloid cytokines including

granulocyte-macrophage colony-stimulating factor, monocute chemoattractant protein 1, C-X-C motif chemokine ligand 1 (CXCL1), IL-8, and colony-stimulating factor 1.[10,87,88] MDSC are elevated in the peripheral blood and tumors of HNSCC patients and have several immunosuppressive mechanisms.[89,90] Production of arginase 1 (Arg-1) and nitric oxide synthase (iNOS) deplete L-arginine from the TME, shunting T cells away from oxidative phosphorylation, which decreases survival capacity and antitumor activity.[91-93] Arg-1 is regulated by pSTAT3, which binds to multiple sites in the Arg-1 promoter. Functionally, inhibition of STAT3 negates MDSC suppressive activity and decreases expression of Arg-1.[93] Similarly, MDSC express indoleamine-pyrrole 2,3-dioxygenase (IDO) which converts tryptophan into immunosuppressive metabolites.[94] MDSC from HNSCC patients suppress T cell activity through expression of arginase, CD86, PD-L1, and TGF-β. In the context of a hypoxic TME, PD-L1 is upregulated on MDSC and other immune cells by HIF-1α to inhibit T cell activation.[95] Lastly, MDSC can present peptides to T cells, resulting in nitration of T cell surface molecules and TCR dysfunction. Through this mechanism, MDSC can induce antigen-specific T cell tolerance.[96]

The levels of circulating MDSC are associated with clinical tumor burden and recruitment of MDSC to the tumor site is associated with tumor progression in animal models of HNSCC.[97] A subset of granulocytic polymorphonuclear (PMN) MDSC is the most clinically important subset in HNSCC for its ability to suppress T cell proliferation greater than monocytic or early stage MDSC. In vitro, PMN-MDSC reduced T cell proliferation by approximately 75% and IFN-γ release by more than 80%. Inhibition of either arginase or iNOS restored T cell activity, but T cell proliferation was better restored by iNOS inhibition while IFN-γ release was better restored by arginase inhibition. Accordingly, a high frequency of PMN-MDSC was strongly correlated with poor overall survival with a subset of CD11b+/CD16+ PMN-MDSC being most strongly associated with poor survival in HNSCC.[89]

To address this immunosuppressive capability, several clinical trials have attempted to target MDSC in HNSCC. Epacadostat is an IDO inhibitor that was used in combination with pembrolizumab in a phase I/II trial (ECHO-202/KEYNOTE-037) of solid tumors. The combination drug was generally well tolerated and showed encouraging antitumor activity across several tumor types with 2 of 3 HNSCC patients demonstrating objective response.[98] Tadalafil is a phosphodiesterase 5 (PDE5) inhibitor that can suppress the production of Arg-1 and iNOS, thereby inhibiting MDSC-mediated immunosuppression.[99] Several clinical trials have shown that Tadalafil lowers MDSC and Treg in the blood and tumor, and increases activated intratumoral CD8 T cells in HNSCC.[100,101] However, intratumoral PD-L1 was upregulated in one trial of Tadalafil plus antitumor vaccine. This presents a possible mechanism for tumor escape and supports a future trial of combination checkpoint and PDE5 inhibitors, which is currently underway (NCT03993353).[101] Given the evidence that modulating MDSC activity can alter tumor immunological balance and the promising oncological responses seen in these trials, MDSC are clearly a key component of tumor immune evasion and warrant further study.

5.4 | Regulatory T cells

Regulatory T cells (Tregs) are a subset of FoxP3 expressing CD4+ T cells that are highly enriched in HNSCC and other tumors. The enrichment of Tregs at tumor sites likely contributes to suppressing antitumor immunity. Tregs are recruited to the TME as either natural Tregs, which have matured in the thymus or naïve CD4 T cells, which are then polarized into T cells at the tumor site.[102,103] In the TME, Tregs proliferate through recognition of TSA and the presence of IL-2.[104,105] Tregs suppress proliferation of immune effector cells through IL-10 and TGF-β secretion.[106,107] In addition, Tregs express increased levels of immune modulatory proteins including OX40, PD-1, cytotoxic T-lymphocyte-associated protein 4 (CTLA4), and T cell immunoglobulin and mucin domain-containing protein 3 (TIM3).[108]

Neuropilin-1 (Nrp1) is a transmembrane glycoprotein required to maintain intratumoral Treg stability; remarkably, patients with HNSCC show relatively high intratumoral NRP1+ Treg levels (approximately 60%).[109] Studies in animal models uncovered a reciprocal role for fragile (NRP1−) and stable (NRP1+) Tregs where fragile Tregs block stable Treg function and promote antitumor immunity through production of IFN-γ, which in turn drives increased Treg fragility. In fact, IFN-γ-induced Treg fragility is required for response to anti-PD1 therapy, supporting the case that an essential component of effective immunotherapy is the induction of sufficient IFN-γ to promote Treg fragility.[109] This distinction in the Treg population may be the reason why studies show conflicting evidence over the prognostic value of Tregs in HNSCC. CTLA4+ Tregs in cetuximab-treated HNSCC were found to be correlated with poor prognosis, but a meta-analysis of 167 HNSCC found that increased tumor infiltrating Tregs were actually associated with better prognosis.[110,111] An increased percentage of NRP1+ Tregs was correlated with poor prognosis in HNSCC indicating that NRP1+ Tregs may be an essential population with regards to immune evasion and cancer prognosis.[109]

More research is warranted on the immunosuppressive impacts of Tregs with a specific focus on stable subpopulations that may be the primary culprit.

Therapeutic options targeting Tregs largely focus on Treg depletion through markers such as CD25, C-C chemokine receptor type 4 (CCR4), and OX40. However, few trials have been completed in patients with HNSCC and the results have been less promising than those of MDSC-targeted therapies. CCR4 is preferentially expressed on Tregs and can be targeted to deplete Tregs in humans.[112] In a clinical trial of PF-05082566 (utomilumab, stimulates CD-137 signaling) in combination with Mogamulizumab (anti-CCR4 antibody) in patients with advanced solid tumors including HNSCC, only 1 out of 24 patients demonstrated objective or immune-related objective response (NCT02444793). One other trial aims to study OX40, a costimulatory molecule of the TNF receptor family that plays an inhibitory role in Treg functioning.[113] This currently ongoing trial studies anti-OX40 antibody in HNSCC patients as neoadjuvant therapy (NCT02274155). Further preclinical and clinical study, especially of specific Treg subsets, is warranted to uncover the role and scope of Tregs in cancer immunity.

6 | BEYOND T CELL THERAPY

Immune checkpoint blockade unleashes existing T cell responses being held back by the expression of immune checkpoints. Although immune checkpoint blockade is FDA-approved for the first-line treatment of recurrent/metastatic head and neck cancer, less than half of all treated patients demonstrate an objective response and complete responses are very rarely achieved. A possible theory is that tumor antigen-specific T cells are terminally exhausted and cannot be rescued with immune checkpoint blockade. This could potentially be addressed by replacing T cell immunity with adoptive cell transfer of expanded tumor infiltrating lymphocytes or autologous T cell engineered to express a therapeutic TCR or chimeric antigen receptor.[114,115] Importantly, both immune checkpoint blockade and T cell transfer immunotherapies are dependent upon the ability of T cells to detect and eliminate cancer cells. Based on ITH and the presence of genetic and epigenetic defects in antigen processing and presentation described above, the sobering truth is that there are likely a significant subset of patients harboring progressing carcinomas that cannot be cured with T cell-based immunotherapy alone. This is in stark contrast to hematologic malignancies that are often composed of highly clonal malignant cells and demonstrate high response rates to chimeric antigen receptor-engineered cellular therapies.[116,117]

Some tumors that harbor subclones of tumors cells with alterations in genes critical for any immune response, such as caspase 8 alterations, may not benefit from any form of immunotherapy. For these patients, standard anticancer therapies are likely to remain the most effective treatment options for the foreseeable future. However, such alterations are relatively rare. Much more common are the presence of alterations that render tumor cells undetectable by T cells.[9] For these patients, NK cell-based immunotherapy, alone or as an adjunct to T cell-based immunotherapy, may be a promising approach.[118-120] NK cells do not require specific tumor antigen presented on MHC molecules for the detection of malignant cells. NK cells are defined as a set of CD3−/CD56+ cells, which represent a small proportion of circulating lymphocytes in humans. They are governed by a set of excitatory and inhibitory receptors that in summation determine activation status. Notably, NK cell killer-cell immunoglobulin-like receptors interact with MHC class I in an inhibitory fashion, leading to potent activation in the absence of MHC class I expression. When activated, NK cells are cytotoxic lymphocytes that can kill cancer cells through direct and indirect mechanisms. In addition to release of perforin and granzyme, NK cells also express CD16 (Fc receptor) which mediates antibody-dependent cellular cytotoxicity.[121] Furthermore, recent research has uncovered a novel NK-DC axis where stimulatory DCs are recruited to tumors through chemokines and cytokines released by NK cells. Tumor cell killing by NK and T cells causes DCs to uptake and present tumor debris at secondary lymphoid organs, promoting increased T cell migration and activity. As such, the presence of NK and DCs improves the efficacy of T cell-based therapy, specifically in melanoma.[122,123] Newly engineered NK cell therapies have demonstrated potent antitumor activity in numerous preclinical studies.[124,125] In humans, the use of anti-CD19 chimeric antigen receptor (CAR)-transduced NK cells in CD19-positive lymphoid malignancies demonstrated response in eight of 11 treated patients (and complete remission in seven) without the development of major toxicities, establishing the potential for NK cells as safe and efficacious therapies.[126] Additionally, there is an ongoing phase I clinical trial testing PD-L1 CAR-transduced NK cells (PD-L1t-haNK) in subjects with solid tumors (NCT04050709). Whether NK cell therapies will demonstrate antitumor activity as a monotherapy, or are best used as an adjuvant to T cell immunotherapy, requires further study. Unfortunately, the immunosuppressive microenvironment remains a significant barrier to both T cell- and NK cell-based immunotherapy. In patients with hostile TMEs, even combination therapies may not be effective; it is possible that any immunotherapeutic approach will require manipulation of the TME in order to be maximally effective. M2 macrophages, Tregs, and MDSCs are the main mediators of the immunosuppressive microenvironment, and manipulation of these cells individually has shown therapeutic effect in preclinical and clinical studies.[10,89,100]

7 | CONCLUSIONS

Rapid advances in the field of tumor immunology have ushered immunotherapy to first line status for recurrent and metastatic HNSCC. Checkpoint inhibitors and T cell-based cell therapies hinge on the activity of T cells to recognize and kill tumor cells, but many tumors have developed strategies to evade recognition or killing. Many genomic and epigenetic alterations within tumor cells that lead to immune escape may be specific for T cell recognition, and there is a solid foundation of evidence that supports combination therapy with NK cell-based immunotherapy warrants further study. On the other hand, the immunosuppressive microenvironment presents a challenge to all effector cells and may need to be addressed in the context of any immunotherapeutic approach. With the development of sequencing technologies that allow rapid assessment of both intrinsic tumor genomic alterations and expression profiles and the TME, we may soon be able to tailor specific T cell and/or NK cell-based immunotherapies to individual patients.

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