

REVIEW



Immune evasion mechanisms and immune checkpoint inhibition in advanced merkel cell carcinoma

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ABSTRACT

Merkel cell carcinoma (MCC) is a rare skin cancer caused by Merkel cell polyomavirus (MCPyV) infection and/or ultraviolet radiation-induced somatic mutations. The presence of tumor-infiltrating lymphocytes is evidence that an active immune response to MCPyV and tumor-associated neoantigens occurs in some patients. However, inhibitory immune molecules, including programmed death-1 (PD-1) and programmed death-ligand 1 (PD-L1), within the MCC tumor microenvironment aid in tumor evasion of T-cell-mediated clearance. Unlike chemotherapy, treatment with anti-PD-L1 (avelumab) or anti-PD-1 (pembrolizumab) antibodies leads to durable responses in MCC, in both virus-positive and virus-negative tumors. As many tumors are established through the evasion of infiltrating immune-cell clearance, the lessons learned in MCC may be broadly relevant to many cancers.

Abbreviations: AE, adverse event; APC, antigen-presenting cell; B2M, β 2-microglobulin; C/EBP, CCAAT/enhancer-binding protein; CLA, cutaneous lymphocyte antigen; CTLA-4, cytotoxic T-lymphocyte-associated protein 4; DC, dendritic cell; IL, interleukin; INF, interferon; LAG-3, lymphocyte-activation gene 3; MCC, Merkel cell carcinoma; MCPyV, Merkel cell polyomavirus; LT, large T antigen; MHC, major histocompatibility complex; MICA, MHC class I chain-related protein A; MICB, MHC class I chain-related protein B; NK, natural killer; NKG2D, natural killer group 2 D; PD-1, programmed death-1; PD-L1, programmed death-ligand 1; RECIST, Response Evaluation Criteria In Solid Tumors; sT, small T antigen; TCR, T-cell receptor; TGF- β , tumor growth factor β ; TH1, type 1 T helper; TIM-3, T-cell immunoglobulin and mucin-domain containing-3; TLR, Toll-like receptor; TNF- α , tumor necrosis factor α ; Treg, regulatory T cell; UV, ultraviolet

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Introduction

Merkel cell carcinoma (MCC) is an aggressive skin cancer that has etiologic associations with Merkel cell polyomavirus (MCPyV) infection, ultraviolet (UV) radiation exposure, and immunosuppression.¹⁻⁵ As part of the normal immune response against tumors, the host immune system is capable of destroying cells that express tumor-specific antigens; however, tumor-driven dysregulation of immune responses allows tumors to escape immune-mediated elimination.^{6,7} MCC tumors may use multiple strategies for evading the host immune system, many of which modulate CD8⁺ T-cell responses. Recent data from clinical trials with anti-PD-L1/PD-1 monoclonal antibodies, which block negative regulation of activated T-cell responses, demonstrate that the host immune response toward MCC tumor cells can be reactivated, providing durable clinical activity in patients with advanced MCC.^{8,9} Avelumab, a human IgG1 anti-PD-L1 monoclonal antibody, was recently approved in the United States for treatment of patients with metastatic MCC. In this article, we

discuss the scientific rationale for using immunotherapy to treat MCC and summarize progress in achieving sustained benefit for patients with this approach. To develop this non-systematic review, we performed a search of PubMed and ClinicalTrials.gov, including a review of reference lists of articles of interest. Articles were selected for inclusion based on relevance to the planned scope of the article.

Merkel cell carcinoma

Disease characteristics

MCC is a rare and aggressive skin cancer that is associated with a higher mortality rate than melanoma.¹ Approximately 2,000 new cases occur each year in the United States.¹⁰ MCC occurs most frequently in the elderly, on sun-exposed regions of the body, particularly the head and neck, and has a high rate of recurrence and metastatic spread following initial presentation with local disease.¹¹⁻¹³

Risk factors

MCC is considered to be an immunogenic tumor, with tumor cell and immune system interactions being highly relevant to MCC pathogenesis. The link between immunosuppression and tumorigenesis has long been established for other cancer-causing viruses, such as Kaposi’s sarcoma, herpesvirus, Epstein-Barr virus, and human papillomavirus.¹⁴ Immunosuppression and underlying autoimmune diseases are major risk factors for MCC development, with 10% of patients with MCC having overt clinical immunosuppression, defined as patients with HIV/AIDS, certain autoimmune diseases such as chronic lymphocytic leukemia, and iatrogenic suppression for organ transplantation.^{13,15-20} For example, the risk of MCC is increased 66- to 182-fold in organ transplant recipients compared with the general population, which is similar to that reported in patients with cutaneous squamous cell carcinoma, a much more common skin cancer that is also linked to UV exposure.^{21,22} In addition to increased risk of disease, immunosuppressed patients experience poorer MCC-specific survival.^{13,23} As MCC is primarily a disease of the elderly, age-related immune dysfunctions may also be important to MCC development.²⁴ Several causes of immunosuppression are more common in patients with MCC than other tumor types, including chronic lymphocytic leukemia and other hematologic malignancies, HIV/AIDS, and prior solid organ transplant.^{13,15,16,18-20} The increased risk of MCC development in patients with a history of autoimmune disease is thought to be related to the use of corticosteroids and other immunosuppressive medications in this population.^{25,26}

Immunosuppressive effects of UV exposure and advanced age

In addition to chronic disease- and medication-induced immune suppression, development of MCC may be aided by the presence of a weakened immune response from chronic UV exposure and advanced age. Studies in mouse models have shown that UV exposure decreases antigen presentation and

increases the production of immunosuppressive mediators and induction of regulatory T cells (Treg), and the ability of UV exposure to induce tumorigenic mutations is well established.^{27,28} The high rates of MCC in the elderly may be attributable to multiple factors, including chronic UV exposure, increased incidence of immunosuppressive comorbidities, and the gradual decline in immune competence.^{13,25} Immune dysfunction gradually increases with age, leading to increased non-specific inflammatory responses, decreased naive T cells, and impaired T-cell activation.²⁴

Clear evidence of the importance of the immune response in controlling MCC tumor progression is provided by the unknown primary tumor phenomenon, which is associated with increased MCC-specific survival, believed to occur as a result of immune clearance of primary MCC lesions in patients with nodal disease.²⁹⁻³³ Partial regression of MCC has also been observed following the cessation of immunosuppressive medications.^{34,35}

Etiology

MCC has 2 identified etiologies, one mediated by the actions of oncoproteins encoded by MCPyV and the second as a result of the accumulation of UV-induced mutations^{2-5,36} (Table 1). Early observations that MCC occurred more frequently in immunocompromised patients suggested a potential viral etiology, which was confirmed in 2008 with the discovery of MCPyV.³ MCPyV is found in approximately 80% of MCC tumors and is currently the only human polyomavirus known to cause cancer.^{2,3,37-40} In MCC, tumorigenesis is believed to be mediated by the 2 oncoproteins of MCPyV: large T (LT) and small T (sT) antigens, which disable tumor suppressor pathways by targeting retinoblastoma and p53 proteins^{41,42} (Fig. 1). MCPyV infection is near ubiquitous in the general population and is typically controlled by the immune system without any known sequelae; however, in a small percentage of cases, viral integration occurs.^{3,43,44} In addition, mutations in LT have been found in all MCPyV-positive tumors, such as premature stop codons that produce a truncated LT.^{42,45,46} The truncated LT can no longer support viral replication, but retains the

Table 1. Comparison between MCPyV-positive and MCPyV-negative tumors.

MCPyV-positive Tumors	MCPyV-negative Tumors
<ul style="list-style-type: none">• Viral DNA is integrated into chromosome of tumor cells• Capsid proteins (VP1/VP2) and T-antigen-specific antibodies are typically produced• MCPyV-specific CD8⁺ T cells are often found within tumors and peripheral blood• Somatic/UV mutation rates are very low compared with virus-negative MCCs• Tumor suppressor (<i>TP53</i> and <i>RB1</i>) mutations are absent• Mutations do not follow a UV-associated pattern• MCPyV+ MCC cell lines require ongoing viral protein expression to proliferate• Tumor-infiltrating lymphocytes are often prevalent• PD-L1 is frequently expressed on tumor cells; unknown if viral burden influences PD-L1 levels• PD-1 and TIM-3 are often expressed on virus-specific T cells, indicating that T-cell exhaustion occurs in response to persistent virus infection	<ul style="list-style-type: none">• No viral DNA found in tumor samples• MCPyV capsid protein (anti-VP1/VP2) antibodies are found in some patients• CD8⁺ T cells are generated against tumor neoantigens• Tumors occur more often on sun-exposed sites• Higher mutational burden than MCPyV+ tumors and melanoma tumors• Mutation pattern (UV-induced mutations) similar to those found in other skin cancers (may include <i>TP53</i>, <i>RB1</i>, <i>NOTCH1</i>, <i>FAT1</i>, <i>PRUNE2</i>, and <i>HRAS</i>)• Variable levels of tumor-infiltrating lymphocytes• PD-L1 expression on tumor cells is lower vs virus-positive tumors and correlates with mutational burden

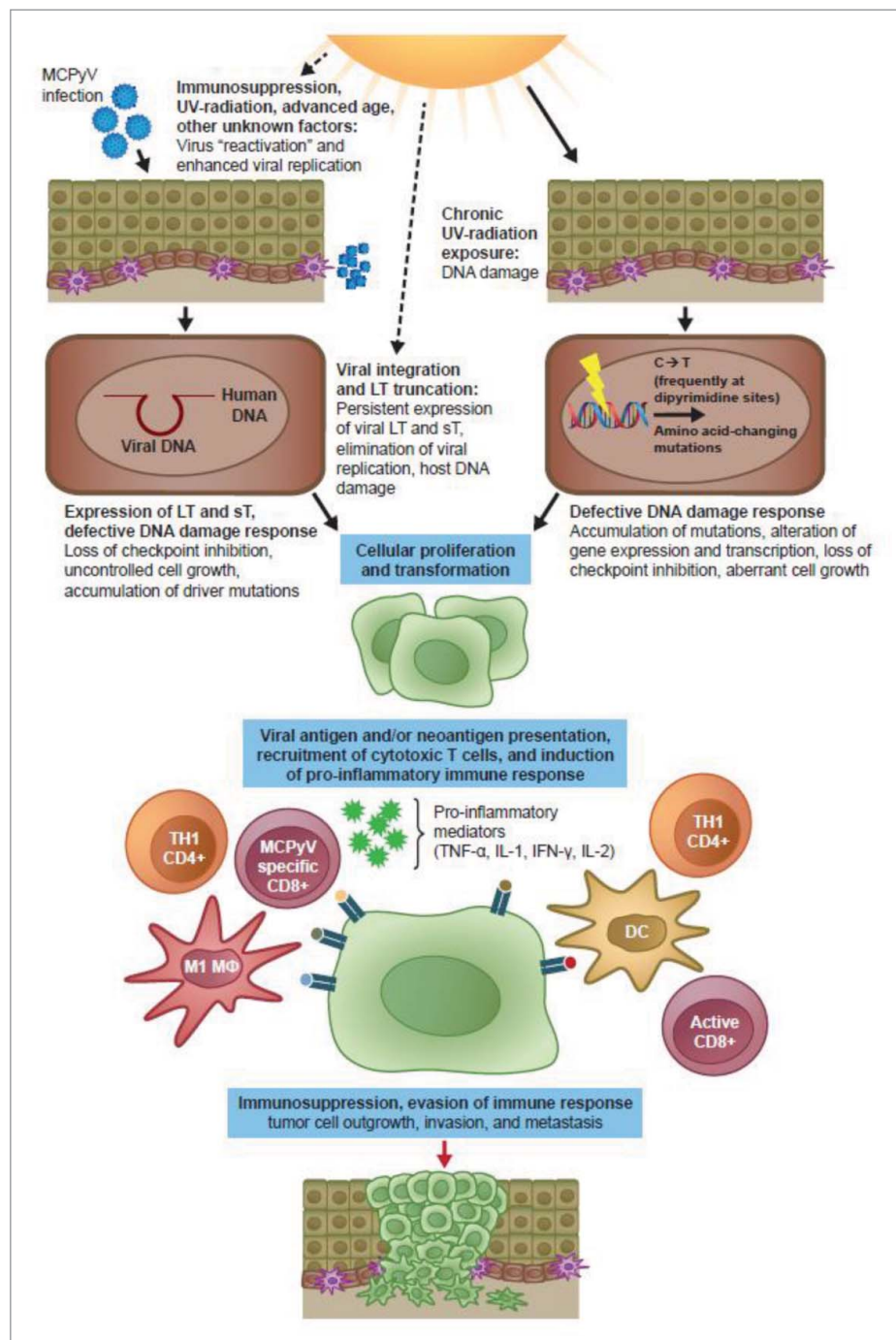


Figure 1. Mechanisms of MCPyV and UV exposure-induced tumorigenesis. Tumorigenesis is a multistep process, and in MCC, this occurs as a result of viral infection of cells within the epithelium or chronic UV radiation exposure of the skin, or both. (C, cytosine; DC, dendritic cell; IFN, interferon; IL, interleukin; LT, large T antigen; M Φ , macrophage; sT, small T antigen; T, thymine; TNF, tumor necrosis factor).

ability to affect gene expression.^{41,42,47} Persistent expression of viral T antigens and a defective DNA damage response further contribute to the dysregulation of host gene expression and loss of checkpoint inhibition, resulting in uncontrolled growth, accumulation of potential driver mutations, and eventually oncogenic transformation.

The lack of detectable tumor-associated MCPyV DNA or oncoproteins in approximately 20% of cases of MCC prompted investigations into other potential etiologies. The mutational burden of virus-negative MCC is higher than that of melanoma, and, as with other skin cancers, MCC is associated with

excessive exposure to UV radiation (Fig. 1).^{12,36,48} UV-induced mutations are found at much higher rates in MCPyV-negative vs MCPyV-positive tumors, suggesting a separate, non-viral mechanism for MCC in which genetic changes accumulate over several decades in the presence of an incomplete DNA damage response (Table 1).^{2,5,36} Over time, mutations in tumor suppressors and oncogenic drivers lead to abnormal cell proliferation and transformation. The role of UV radiation exposure in the development of MCPyV-positive MCC is still being explored, but an MCPyV-positive cell line displayed defective DNA repair and loss of cell-cycle arrest after exposure to UV

radiation, suggesting that these could be mechanisms by which UV synergizes with MCPyV in tumorigenesis.⁴⁹ UV exposure could also potentiate MCPyV-positive tumorigenesis by promoting viral integration.⁴⁵

The immunogenicity of MCC is likely due to the presence of viral antigens and neoantigens, the latter generated as a byproduct of UV radiation-induced mutations. In immunocompetent individuals, the immune response is activated, leading to the production of pro-inflammatory mediators and recruitment of type 1 T-helper (TH1) CD4⁺ and cytotoxic CD8⁺ T cells, M1 macrophages, and dendritic cells into the tumor microenvironment (Fig. 1).^{6,50} Transformed cells that can evade the immune response targeted at virus-infected and malignant cells may progress and eventually invade the surrounding tissues and blood vessels, resulting in distant metastasis.^{7,51}

Immunobiology of MCC

Innate immune response to MCC

Currently, information is limited on the role of the innate immune response in patients with MCC. Gene expression studies in fibroblasts stably expressing MCPyV oncogenes (LT and sT) showed that pro-inflammatory molecules, specifically IL-8, CXCL1, IL-6, IL-1 β , MMP1, and CXCL6, were highly upregulated.⁴⁷ Natural killer (NK) cells, which perform roles in both the innate and adaptive immune response, are found within MCC tumors, and their presence has been shown to correlate with the presence of MCPyV DNA and LT protein expression, although the latter association was not statistically significant.⁵²

Humoral immune response to MCC

MCPyV infection is ubiquitous, with 60–80% of the population possessing MCPyV-specific antibodies that are generally targeted to viral capsid proteins VP1 and VP2.^{43,44,53–55} In one study, MCPyV-capsid antibodies were detected in all patients with MCC and 85% of control subjects.⁵⁶ However, high antibody titers were seen in 65% of patients with MCC but only 7% of controls, and high antibody titers correlated with longer progression-free survival among patients.⁵⁶ Patients with MCC also produce antibodies against MCPyV T antigens, which are rarely detected in the general population.⁵⁷ These results are not surprising, as MCPyV T-antigen oncoproteins are not expressed in the MCPyV virion; however, after viral integration, MCC cells persistently produce these proteins. The T-antigen-specific antibody titer drops following tumor regression in response to successful treatment but starts to rise as the disease progresses, providing evidence that T-antigen-specific antibodies can serve as a measure of tumor burden in patients with MCC.^{57,58} This observation has given rise to a clinically available blood test that can be used to detect MCC recurrence early, potentially improving the likelihood of benefit from immune therapy.⁵⁹

In MCPyV-negative tumors, the humoral immune response to MCC tumors is incompletely understood. Although MCPyV capsid protein antibodies are frequently found in the general population, the capsid protein antibody titer is much higher in

patients with both MCPyV-negative and MCPyV-positive MCC compared with control serum samples obtained from patients without MCC.^{43,56} These findings support the presence of 2 mechanisms of MCC development, although the high titer of antibodies in MCPyV-negative MCC suggests that both etiologies involve MCPyV. More specifically, according to the “hit and run” hypothesis, the virus may be involved in early stages of tumor formation before virus-positive cells are eradicated from tumors by selective pressure from the host immune system, resulting in outgrowth of virus-negative MCC cells.^{60,61}

Cell-mediated immune responses to MCC

An active, adaptive immune response is critical for clearing virus-infected cells and cancer cells. Following stimulation by CD4⁺ T cells and various cytokines, CD8⁺ T cells can target cells expressing viral or cancer antigens and induce cell death, which eliminates the infected or malignant cells and further activates the immune response. The presence of activated T cells within the tumor microenvironment is a positive prognostic factor in multiple cancers, and in MCC, T-cell infiltration into tumors has been demonstrated in multiple studies and has consistently been associated with a survival benefit.^{52,58,62–66} One group observed that high levels of CD8⁺ T cells at the tumor periphery were associated with lower risk of death.⁶⁵ In addition, gene-expression profiling of MCC tumors revealed an immune response gene signature indicative of high levels of intratumoral CD8⁺ T-cell infiltration that correlates with a better prognosis.⁶² Several reports indicate that there is no association between CD8⁺ T-cell infiltration of MCC tumors and viral status, possibly because both types of MCC can be immunogenic.^{5,62,65,67} In a study of 38 patients with MCC and 30 healthy donors, both populations had T cells recognizing MCPyV VP1.⁶⁷ Similar to humoral immune responses to the viral oncoproteins, however, MCPyV oncoprotein-specific T cells have been found only in patients with MCC.^{66,67}

Overall, these data indicate that both humoral and cell-mediated immune responses, including tumor-specific T cells, are generated in the majority of patients with MCC but are unable to control tumor growth in patients presenting with active disease. Further to this point, there are rare cases in which the infiltrating immune cells undergo reactivation resulting in regression of MCC lesions, which occurs even in patients with metastatic disease. Failure of immune responses despite the presence of tumor-specific T cells has been widely observed in other tumor types, such as colorectal, breast, and ovarian cancers, hepatocellular carcinoma, and head and neck squamous cell carcinoma.^{68–73} This may be related to the functional status of the tumor-infiltrating lymphocytes or the influence of tumor- and stroma-derived suppressive mechanisms. Similar to other tumors, such as malignant melanoma, the presence of infiltrating immune cells in MCC is associated with longer survival.^{63,74}

Immune evasion mechanisms of MCC tumors

MCC immune evasion mechanisms target molecules and pathways of both the innate and adaptive immune response (Fig. 2).

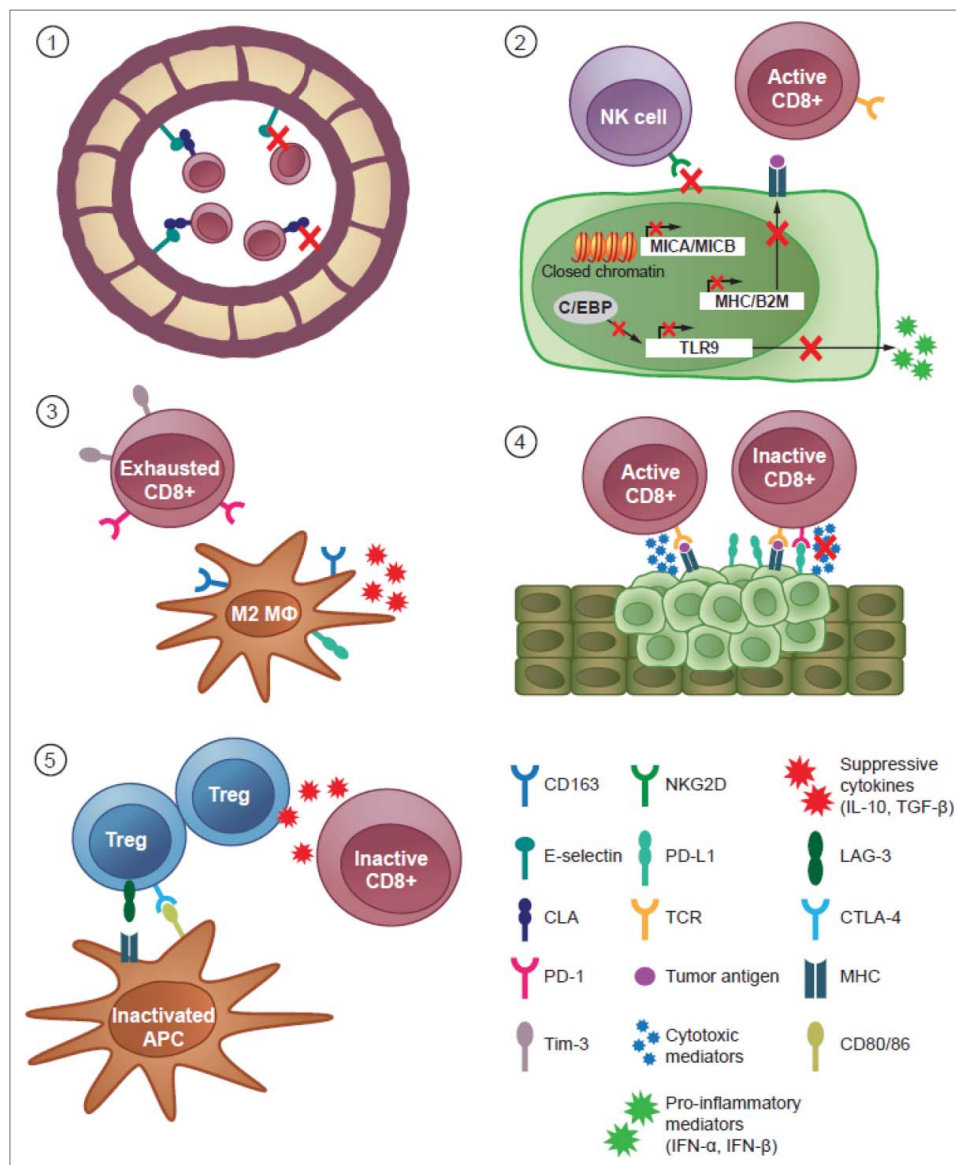


Figure 2. MCC immune evasion strategies. MCC tumors may be targeted by the immune system for eradication. This process may be thwarted by several mechanisms, including 1) inhibition of T-cell migration into areas of inflammation by interrupting interactions between T-cell surface receptors and the tumor endothelium; 2) by alterations in gene expression that reduce the surface expression of MICA/MICB and MHC, molecules that are necessary for NK and T-cell-mediated cytotoxicity of tumor cells; 3) upregulation of inhibitory receptors on effector immune cells in the tumor microenvironment; 4) effector CD8⁺ T cells that have migrated to the tumor site can be inactivated by receptors present on the surface of tumor cells, particularly PD-L1; and 5) the recruitment of Tregs to areas of inflammation, which can suppress immune responses. (APC; antigen-presenting cell; B2M, β 2-microglobulin; C/EBP, CCAAT/enhancer-binding protein; CLA, cutaneous lymphocyte antigen; CTLA-4, cytotoxic T-lymphocyte-associated protein 4; IFN, interferon; IL, interleukin; LAG-3, lymphocyte-activation gene 3; MHC, major histocompatibility complex; MICA, MHC class I chain-related protein A; MICB, MHC class I chain-related protein B; MΦ, macrophage; NK, natural killer; NKG2D, natural killer group 2D; PD-1, programmed death-1; PD-L1, programmed death-ligand 1; TCR, T-cell receptor; TIM-3, T-cell immunoglobulin and mucin-domain containing-3; TLR, Toll-like receptor; TGF, tumor growth factor).

Evasion of innate immunity

The 2 natural killer group 2D (NKG2D) ligands, major histocompatibility complex (MHC) class I chain-related protein A and B (MICA/MICB), are upregulated in cells undergoing a stress response, such as during viral infection or cellular transformation.⁷⁵ The interaction between NKG2D and MICA/MICB stimulates the proliferation and cytotoxic potential of NK cells.^{76,77} In a study of 75 MCC tumors and MCC cell lines, MICA and MICB mRNA levels were low and protein products were rarely observed, indicating that MCC uses mechanisms that affect NK-cell activation (Fig. 2).⁷⁸ In MCC cell lines, MICA and MICB are downregulated through epigenetic

mechanisms, which may be a common pathway of immune evasion for virus-induced cancers.⁷⁸

In MCPyV-positive MCC, T-antigen-mediated inhibition of the CCAAT/enhancer-binding protein (C/EBP) transcription factor, a positive regulator of the Toll-like receptor (TLR) 9 promoter, leads to reduced expression of TLR9, an important mediator of pro-inflammatory immune responses (Fig. 2).⁷⁹ Specifically, TLR9 is activated by binding to non-self DNA containing non-methylated CpG motifs that are found during DNA virus infection.^{80,81} Ligand binding results in activation of NF- κ B-mediated transcription, which produces pro-inflammatory cytokines and type 1 interferons (IFNs) that are important for clearing

virus-infected cells and promoting further immune activation. Downregulation of TLR9 likely contributes to successful viral infection and may also promote tumor growth. However, the involvement of TLR pathway activation is not well understood in non-viral cancers.

Macrophages infiltrate MCC tumors (Fig. 2) and higher levels of infiltrating macrophages are found in MCPyV-positive tumors compared with MCPyV-negative tumors.⁵² Interestingly, a portion of infiltrating macrophages were found to express CD163, a marker of the M2 phenotype that is linked to tumor growth and survival through secretion of suppressive cytokines, rather than the M1 pro-inflammatory phenotype.^{52,82} The number of CD163⁺ macrophages was not associated with the presence or absence of viral DNA.⁵²

Evasion of adaptive immunity

The inability of all activated T cells to properly home to tumor tissues may decrease the effectiveness of immune responses in MCC, even in the presence of a functioning T-cell response. For T cells to migrate to areas of inflammation, interactions must occur between T cells and the endothelium. E-selectin, a receptor present in the endothelium that binds to T cells, is a ligand of cutaneous lymphocyte antigen (CLA), a homing receptor that directs T cells to areas of inflammation in the skin, and enables migration to the tumor microenvironment.⁸³ In a study of 56 MCC samples, E-selectin was downregulated on intratumoral vasculature in 52% of samples.⁸⁴ Indeed, diminished E-selectin expression was associated with both lower CD8⁺ lymphocyte infiltration and poorer outcome.⁸⁴ Dowlatshahi et al found that a subset of MCC tumors contained T cells lacking CLA, which correlated with a lower number of tumor-infiltrating T cells.⁶⁴ These 2 events contribute to the lack of T-cell migration into the sites of MCC tumors (Fig. 2).

Expression of MHC class I receptors on tumor cells is required for identification and eradication by CD8⁺ T cells (Fig. 2). Dysregulation of antigen presentation, including the loss of MHC-1 and β 2-microglobulin (B2M), is a common mechanism of immune escape by various cancers and is observed in both virus- and UV-mediated MCC tumors.⁸⁵ In MCC tumors, MHC-1 cell-surface expression is reduced, and in MCC cell lines decreased gene expression of MHC-1 correlated with that of B2M.⁸⁶ In this study, the negative regulation of MHC-1 was much greater in MCPyV-positive compared with MCPyV-negative tumors.

In addition to the downregulation of cellular receptors necessary for immune cell recognition, inhibitory receptors are upregulated on tumor-targeted immune cells (Fig. 2). Negative regulatory pathways provide the immune system with a mechanism for successfully clearing pathogens and malignant cells, while limiting immunopathology.⁸⁷ One such receptor, PD-1, involved in the attenuation of the immune response to infections or tumorigenesis is also essential for the induction and maintenance of self-tolerance, as evidenced by the development of autoimmune disorders in PD-1 null mice.⁸⁸ However, chronic antigen stimulation from viral infections and tumor cells upregulates inhibitory receptors on active T cells, causing T cells to lose functionality over time.⁸⁹ Increased expression of

the T-cell receptors PD-1 and TIM-3 occurs in response to persistent viral or tumor antigen exposure and is referred to as T-cell exhaustion.^{90,91} T-cell exhaustion correlates with immune dysfunction and prevents CD8⁺ T-cell-mediated clearance of virus-infected and malignant cells.^{92,93} MCPyV-specific T cells found in the blood and within MCC tumors show simultaneous expression of PD-1 and TIM-3.⁶⁶ Furthermore, one study has shown that effector T cells isolated from virus-positive MCC tumors have lower levels of activation markers (CD25 and CD69) and higher levels of PD-1 compared with normal skin T cells.⁶⁴ In this study, MCC tumor-infiltrating lymphocytes that displayed markers of exhaustion could be isolated from primary tumors or metastases, expanded, and rescued through cytokine stimulation, resulting in in vitro anti-tumor activity. Because tumor-specific epitopes necessary for the isolation of virus-negative MCC-specific T cells have not been identified, it is not clear whether markers of T-cell exhaustion are present on tumor-specific effector T cells present in the tumor microenvironment of UV exposure-mediated MCC. PD-1 expression on tumor-infiltrating T cells could be considered a surrogate marker for tumor-specific T cells, as upregulation requires exposure to cognate antigen; however, PD-1 expression in MCPyV-negative MCC has not been assessed.⁹⁴ In melanoma, tumor-specific CD8⁺ T cells also upregulate PD-1 and TIM-3, indicating that somatic mutations induced by UV exposure contribute to T-cell dysfunction; this may also occur in UV exposure-mediated MCC.⁹⁵

Active CD8⁺ T cells that reach the tumor site may also be inactivated through receptors present on the surface of tumor cells (Fig. 2).⁹⁶ The best-studied example of this is PD-L1, which is upregulated in many tumor types, including MCC.⁹⁷ PD-L1 expression is induced by type II interferons, which can be produced in the tumor microenvironment by activated T cells and NK cells.^{96,98} Following the activation of the adaptive immune response to foreign antigens, the expression of PD-L1 serves to reinstate immune homeostasis and protect tissue from cytotoxic immune cell damage.⁹⁹ PD-L1 inactivates T cells through engagement of PD-1 and B7.1, resulting in a loss of ability to induce tumor cell death.⁹⁹ Additionally, PD-L1 can be upregulated on antigen-presenting cells (APCs) in the tumor microenvironment, which may result in the induction of T-cell tolerance during presentation of tumor antigens within tumor-draining lymph nodes.¹⁰⁰ A study analyzing MCC tumor specimens from 49 patients demonstrated PD-L1 expression on tumor cells and infiltrating lymphocytes.⁹⁷ The presence of PD-L1 on tumor cells was strongly associated with the presence of MCPyV DNA, with 50% of virus-positive samples expressing PD-L1 compared with 0% of virus-negative samples. An absence of PD-L1 expression was associated with shorter overall survival, suggesting that an initially robust immune response that results in upregulated PD-L1 may lead to a survival benefit. However, a different study found that virus-negative MCC tumors have upregulated PD-L1 levels that correlate with increasing mutational burden.⁵ The role of other B7 family members, such as PD-L2, in the development and progression of MCC is currently unknown. PD-L2 is capable of inhibiting T-cell activation through the engagement of PD-1 and has been observed on a subset of APCs infiltrating MCC tumors.^{64,101}

The recruitment of Tregs to areas of inflammation is a mechanism to suppress an overactive immune response and help reestablish peripheral immune homeostasis (Fig. 2). Tregs can inactivate CD8⁺ T cells and APCs, and may contribute to disease progression in response to UV radiation exposure and viral infection.¹⁰² Levels of Tregs are higher in MCC tissues than in normal skin.⁶⁴ In contrast to reports in other cancer types, one study of 116 patients with MCC found that the presence of Tregs was associated with longer survival, possibly indicating that the association between MCC and virus infection results in a distinct profile of T-cell responses.⁵² In another study, CD4⁺ and CD8⁺ Tregs were found within MCC tumors, but their presence was not associated with overall survival.⁶⁴ Thus, the role of Tregs in the establishment and progression of MCC, in addition to the association of Tregs with MCPyV infection, is unclear.

Improving treatment of MCC: Immunotherapy

Standard treatment of local or regional MCC consists of surgical removal with or without adjuvant radiotherapy.^{103,104} No treatment in current clinical practice, including radiotherapy or chemotherapy, has been shown to increase survival in advanced MCC. Advanced-stage MCC can be responsive to chemotherapy initially, but progression usually occurs within weeks.^{105,106} Responses to second-line chemotherapy in patients with metastatic MCC are rarely durable, as shown by real-world data.¹⁰⁷ High levels of MCPyV-specific antibodies and T cells in MCPyV-positive tumors, and the infiltration of CD8⁺ T cells into MCC tumors irrespective of MCPyV status, indicate that patients develop a functional immune response initially, which may be evaded through the various mechanisms outlined previously.

Early-phase trials and case reports of immunotherapy approaches, including anti-PD-L1/PD-1 antibodies,^{8,9} intratumoral IL-12 injection,¹⁰⁸ intratumoral injection of the TLR4 agonist G100,¹⁰⁹ and adoptive T-cell therapy,¹¹⁰ provided preliminary evidence of the potential efficacy of immune-based agents in MCC.

Checkpoint inhibition

Suppression of the cell-mediated immune response can occur as a result of upregulated inhibitory receptors, such as PD-1 and PD-L1, on tumor and immune cells. PD-L1 is expressed on tumor and immune cells in both virus-positive and virus-negative MCC tumors, providing a rationale for investigating checkpoint inhibitors targeting PD-1 or PD-L1 in MCC. The presence of PD-1 and PD-L1 in MCC is likely the result of chronic antigen presentation of processed viral proteins and neoantigens, the latter being generated as a result of UV-induced somatic mutations. Checkpoint blockade has shown efficacy and tolerability in patients with head and neck squamous cell carcinoma, another tumor with a viral etiology.¹¹¹ Anti-PD-1 and anti-PD-L1 antibodies have been investigated as first-line and as second-line or later therapy in patients with advanced-stage MCC (Table 2).

Pembrolizumab is a humanized IgG4 anti-PD-1 monoclonal antibody (Table 2).⁸ Pembrolizumab has been investigated

as first-line treatment of immunocompetent patients with advanced MCC in a phase 2 clinical trial (NCT02267603). Of 25 patients with stage IIIB or stage IV MCC and no prior systemic therapy who received pembrolizumab, 16% (n = 4) had a complete response and 40% (n = 10) had a partial response, resulting in an objective response rate of 56%.⁸ Response to pembrolizumab did not correlate with PD-L1 expression or MCPyV positivity. Twenty-six patients were included in the safety analysis and treatment was generally well tolerated, with 77% (n = 20) of patients reporting an adverse event (AE) of any grade, of which 15% (n = 4) were grade 3 or 4. Grade 3 or 4 events that occurred in more than one patient included increased aspartate aminotransferase (n = 3, 12%), increased alanine aminotransferase (n = 2, 7.7%), and hyponatremia (n = 2, 7.7%). These events were managed through the discontinuation of pembrolizumab and, if necessary, glucocorticoid treatment was provided.

Avelumab is a human IgG1 anti-PD-L1 monoclonal antibody.⁹ Avelumab has a wild-type IgG1 Fc region that may further activate the immune response via NK cell-mediated antibody-dependent cell-mediated cytotoxicity, as shown in pre-clinical studies.¹¹² In a phase 2 study (NCT02155647), immunocompetent patients with distant metastatic (stage IV) disease that had progressed after chemotherapy received avelumab as second-line or later therapy. Of 88 patients treated, 9% (n = 8) had a complete response and 23% (n = 20) had a partial response, resulting in an objective response rate of 32%. By Kaplan-Meier estimates, the proportion of responses with a duration of at least 6 months was 92%.⁹ Responses to avelumab occurred irrespective of PD-L1 levels or MCPyV status. Avelumab was well tolerated, with 70% (n = 62) of patients reporting an AE, but only 5% (n = 4) were grade 3, and there were no grade 4 events. Grade 3 events consisted of lymphopenia (n = 2, 2%), and increased blood creatine phosphokinase, increased blood cholesterol, and increased aminotransferase (n = 1, 1% each). Only fatigue (n = 21, 24%) and infusion-related reaction (n = 15, 17%) occurred in more than 10% of patients. Potential immune-mediated treatment-related AEs, derived by both the search-term method and manual medical review, occurred in 11% (n = 10) of patients. Based on the findings from this phase 2 study, avelumab received approval by the US Food and Drug Administration for treatment of metastatic MCC, including in chemotherapy-naïve patients, and is currently the first and only approved therapy for metastatic MCC.

The impressive results from the previously mentioned 2 trials offer powerful new tools for managing advanced MCC. Both checkpoint inhibitor therapies displayed manageable safety profiles, with no treatment-related deaths. No grade 4 treatment-related AEs for avelumab were reported in patients who had received prior chemotherapy, a population that has shown high rates of serious toxicities during systemic treatment with chemotherapy.¹¹³ The durable responses to anti-PD-1/PD-L1 confirm the importance of immune mechanisms in MCC pathogenesis. However, not all patients respond and a key question remains as to what tumor characteristics might be used to predict response. MCPyV-positive tumors have a low mutation rate, lack mutations in typical tumor suppressor genes, have high levels of tumor-infiltrating lymphocytes, and a higher frequency of tumor PD-L1 expression, whereas MCPyV-negative tumors have a high burden

Table 2. Summary of data from trials of immunotherapy for the treatment of patients with advanced MCC.

Parameter	Pembrolizumab Study ⁷	Avelumab Study ⁸
Patient population	Treatment naïve (first-line treatment)	Chemotherapy refractory (second-line or later treatment)
N	26 ^a	88
Primary end point	Objective response rate by RECIST v1.1	Confirmed best overall response by independent review committee per RECIST v1.1
Patient and disease characteristics		
Median age (range), years	68 (57–91)	73 (33–88)
Stage IIIB MCC, n (%)	2 (8)	0
Stage IV MCC, n (%)	24 (92)	88 (100)
Prior lines of systemic therapy, n (%)		
0	26 (100)	0
1	0	52 (59)
≥ 2	0	36 (41)
Median baseline extent of disease (range), mm	69 (13–182)	79 (16–404)
MCPyV-positive, n (%)	17 (65)	46 (52) ^b
Median duration of follow-up (range), months	7.6 (1.6–12.2)	10.4 (6–19)
Minimum duration of follow-up, months	1.6	6
Objective response rate		
Overall, % (95% CI)	56 (35–76)	32 (22–43) ^c
MCPyV-positive, % (n/N1) ^d	62 (10/16)	26 (12/46)
MCPyV-negative, % (n/N1)	44 (4/9)	36 (11/31)
Response durability		
Number of patients with ongoing response at data cutoff, % (n/N1)	86 (12/14)	82 (23/28)
Median duration of response (range), months ^e	Not reached (2+ to 10+)	Not reached (3+ to 18+)
Kaplan-Meier estimate of proportion of responses with ≥ 6 months' duration, % (95% CI)	Not reported	92 (70–98)
Durable response rate, % (95% CI) ^f	Not reported	29 (20–39) ^e
Progression-free survival		
Median, months (95% CI)	9 (5–not reached)	2.7 (1.4–6.9)
6-month rate, % (95% CI)	67 (49–86)	40 (29–50)
Overall survival		
Median, months (95% CI)	Not reported	11.3 (7.5–14.0)
6-month rate, % (95% CI)	Not reported	69 (58–78)
Treatment-related AE, n (%)		
Any grade	20 (77)	62 (70)
grade 3	2 (8)	4 (5)
grade 4	2 (8)	0 (0)

^a25/26 patients had ≥ 1 tumor assessment during treatment.^b77/88 patients were evaluable for MCPyV status.^cA repeated CI for the ORR in the modified intent-to-treat analysis set (95.9% CI for the primary analysis) was calculated to account for the group sequential testing approach.^dN1, number evaluable.^e+ denotes a censored observation for durability of response.^fDurable response rate defined as the proportion of patients with a response of at least 6 months' duration and was estimated as the product of the objective response and the Kaplan-Meier estimate of 6 months' durability of response.

of UV-induced mutations, variable levels of tumor-infiltrating lymphocytes, and lower PD-L1 expression (Table 1). Clinical evidence indicates that checkpoint inhibitors can be effective treatments for MCC of either etiology. In both the pembrolizumab and avelumab studies,^{8,9} treatment responses occurred in patients with virus-positive and virus-negative tumors. Additionally, patients with PD-L1-positive and PD-L1-negative tumors responded. These results suggest that viral status and PD-L1 expression may not be useful biomarkers for determining which patients with MCC would most likely not respond to anti-PD-L1/PD-1 therapy. Additionally, these studies suggest that in both MCPyV-positive and MCPyV-negative tumors, a proportion of patients have MCC-specific T cells that can be reactivated to provide clinically beneficial anti-tumor activity. While current clinical studies have shown that PD-L1 expression is not necessary for patients to respond to checkpoint inhibitor therapy, PD-1 upregulation on tumor-infiltrating lymphocytes may be a potential biomarker of interest for future study. PD-1 expression is an indicator of the presence of tumor-specific T cells and other pro-inflammatory activities within the tumor microenvironment and

therefore may better predict patients who will respond to checkpoint inhibition.

The early successes with checkpoint inhibitors have increased interest in other clinical studies using other agents from this class. Currently, clinical trials testing the safety and efficacy of checkpoint inhibitors are limited to otherwise immunocompetent patients with MCC. Ipilimumab (an anti-CTLA-4 antibody) is being investigated as an adjuvant therapy for completely resected MCC (NCT02196961), and nivolumab (an anti-PD-1 antibody) is being combined with ipilimumab in virus-associated tumors, including MCC (NCT02488759). Similarly, a triple-combination study of tremelimumab (an anti-CTLA-4 antibody), durvalumab (an anti-PD-L1 antibody), and TLR3 agonist poly-ICLC in advanced MCC (NCT02643303) is testing the hypothesis that the TLR3 agonist will influence the tumor microenvironment and potentiate the activity of the checkpoint inhibitors. A study to investigate localized radiation or IFN- β with avelumab with or without adoptive immunotherapy (MCPyV T-antigen-specific T cells) is also recruiting patients (NCT02584829); IFN- β has

been shown to increase the expression of MHC molecules in vitro and in patients with MCC.⁸⁶

Emerging approaches

Immunotherapeutic approaches other than checkpoint inhibitors are also being investigated in clinical studies. In one study, paclitaxel is combined with F16-IL2 (a fusion protein targeting-tenascin-C –expressing tumor cells with simultaneous stimulation of NK cells, macrophages, and T cells) in metastatic MCC (NCT02054884). In the QUILT-3.009 study, infusions of activated NK-92 (an NK cell line developed from a large granular lymphoma patient sample) are administered to patients with advanced and metastatic MCC (NCT02465957), based on the hypothesis that administration of activated NK cells will promote tumor-cell lysis in the absence of co-stimulatory molecules. In a small study (NCT01440816), patients with MCC are treated with an IL-12 gene therapy (pro-inflammatory cytokine) and plasmid DNA vaccine therapy. Early-phase studies are investigating synthetic TLR4 agonist GLA-SE (NCT02035657) and TTI-621, a recombinant fusion protein targeting CD47 (NCT02890368) in patients with MCC. A trial to evaluate the oncolytic virus talimogene laherparepvec, approved for melanoma treatment with or without anti-PD-1 (nivolumab) treatment in patients with advanced MCC, is expected to begin enrollment in mid-2017 (NCT02978625). Additionally, clinical trials assessing the treatment of MCC with immunotherapy in combination with radiotherapy are being considered. The clearance of non-irradiated lesions through systemic immune activation following radiotherapy, referred to as the abscopal effect, may enhance the effect of checkpoint inhibitors and combination treatments of immunotherapy and radiotherapy are being investigated in melanoma.^{114,115} Currently, a phase 2 trial is evaluating talimogene laherparepvec with or without radiotherapy in patients with advanced MCC (NCT02819843).

Concluding remarks

Recent research, which has uncovered the role that MCPyV and UV-induced mutations play in the etiology of MCC, has led to the testing of new therapeutic approaches for MCC—a disease that has seen few advances in recent years. Two recent trials with pembrolizumab and avelumab have explored the role of checkpoint inhibitors in metastatic MCC. Because of this research and the subsequent approval of avelumab in the United States, patients now have a new treatment option with the potential to provide durable responses. Future research will focus on enabling the full potential of immunotherapeutic approaches by applying combination therapies and elucidating biomarkers with the potential to predict which patients will have long-lasting benefit.

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