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Clinical, FDG-PET and Molecular Markers of Immune Checkpoint Inhibitor Response in Patients with Advanced Merkel Cell Carcinoma

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Abstract

Recent findings: Merkel cell carcinoma (MCC) is a rare, highly aggressive, neuroendocrine cancer of the skin, associated with immunosuppression, Merkel cell polyoma virus (MCPyV) infection and UV-carcinogenesis. Whilst impressive and durable responses to immune checkpoint inhibitors have revolutionised the treatment of advanced MCC, approximately 50-70% of patients have either primary or acquired resistance to immune checkpoint blockade and robust predictive biomarkers of response are yet to be identified. Exploratory subgroup and biomarker analyses from clinical trials and retrospective studies have evaluated multiple clinical characteristics including age, performance status, immunosuppression, prior therapy, baseline and response imaging assessments, and molecular features including MCPyV status, programmed death ligand-1 (PD-L1) expression, tumour mutational burden (TMB) and tumour infiltrating lymphocytes as potential markers of response.

Summary: Better performance status, earlier line of therapy and early ^{18}FDG-PET response have been consistently associated with favourable response to immune checkpoint inhibitors in MCC. High response rates are seen regardless of viral status, TMB and PD-L1 status.

Purpose of the review: We provide a review of clinical, imaging and molecular markers of response to immune checkpoint inhibitor therapy in MCC to aid patient selection and personalization of therapy.

Keywords: Merkel cell cancer, Immunotherapy, Immune checkpoint inhibitors, Biomarkers, FDG-PET, Merkel Cell Polyoma Virus, Tumour mutational burden, Programmed death ligand-1

Introduction

Merkel cell carcinoma (MCC) is a rare, highly aggressive, neuroendocrine cancer of the skin with an increasing incidence [1,2]. MCC occurs predominantly in older adults with risk factors including high exposure to ultra-violet (UV) light and immunosuppression [3]. The Merkel Cell Polyoma Virus (MCPyV) has been identified as a driver of a subset of MCC, with clonal integration of MCPyV DNA in MCC cells [4]. Abundant tumour infiltrating lymphocytes in a subset (~20%) [5-7] of MCPyV-positive tumours [8] and reactivity of T cells to viral-associated T-antigens [9,10] underpins the immunogenicity of MCPyV-positive MCC. In contrast, MCPyV-negative MCC are associated with UV signature and high tumour mutational burden (TMB), representing a second, distinct subtype with the potential to elicit anti-tumour immunity [11-13], as summarised in Box 1. MCPyV-mediated carcinogenesis predominates in the Northern hemisphere

Box 1: Merkel Cell Cancer Subtypes and Characteristics.	
MCPyV-positive MCC	MCPyV-negative MCC
Low TMB Absence of UV signature Abundant TILs Presence of MCPyV DNA and/or viral-associated antigens Predominates in North America and Europe	High TMB Presence of UV signature Absence of MCPyV DNA and viral associated antigens More common in regions of high UV exposure (e.g. Australia)
MCPyV: Merkel Cell Polyoma Virus; MCC: Merkel Cell Cancer; UV signature: Ultra-Violet mutational signature; TILs: Tumour Infiltrating Lymphocytes	

while UV-associated MCC are more common in regions of high UV exposure, including Australia [2,3,14-16].

Prior to the advent of immunotherapy, prognosis for advanced MCC was dismal with a 5-year overall survival (OS) rate of 14-21% [17]. Although metastatic MCC is noted to be a chemotherapy-sensitive tumour, responses are short-lived with a median progression free survival (PFS) of 3 months [18]. Immune checkpoint inhibitors (ICI) have since revolutionised the treatment of advanced MCC. A number of ICI agents, including the anti-programmed death ligand 1 (anti-PD-L1) monoclonal antibody avelumab and anti-PD-1 monoclonal antibodies pembrolizumab and nivolumab have demonstrated impressive efficacy in advanced MCC, with 3-year survival rates approaching 60% [19]. Nevertheless, a proportion of patients either do not ever respond or develop resistance to ICI after initial response [20,21]. Key questions remain, including treatment strategies for primary non-responders and the optimal duration of ICI therapy in responders. Exploratory subgroup and biomarker analyses from clinical trials and retrospective studies can provide important insights into potential clinical, imaging and molecular markers of response to aid patient selection for ICI and personalization of treatment.

Immune Checkpoint Inhibition in MCC

ICI has been adopted as the mainstay of treatment for advanced MCC based on a number of phase II clinical trials. The landmark single-arm JAVELIN Merkel 200 study has established the anti-PD-L1 monoclonal antibody avelumab as the standard of care for the treatment of metastatic MCC. Part A of this study demonstrated an objective response rate (ORR) of 33% with impressive durability (median duration of response 40.5 months) in 88 patients that had progressed following chemotherapy [22,23]. In treatment-naïve patients enrolled in Part B of this study, the response rate was 62.1% [20]. Notably, 83% of responders experienced a duration of response (DOR) of at least 6 months, and 77.8% of responses were ongoing at the time of analysis [20]. A median overall survival of 20.3 months and 12.6 months has been reached for avelumab as first- and second-line treatment respectively [24,25], with a 12-month overall survival rate of 60% in the first-line (median follow up 21.2 months) [25] and a recently reported 5-year overall survival rate of 26% in the second-line setting (median follow up 65.1 months) [26]. In a real-world study of patients receiving first-line avelumab for metastatic MCC, the median overall survival was 20.2 months with an overall survival rate of 66.4% at 12 months [27]. The CITN-09/KEYNOTE-017 trial reported on the use of first-line pembrolizumab for unresectable stage III or metastatic MCC, with an ORR of 58% among 50 patients, of whom 86% had stage IV disease, and 14% had stage IIIB disease. The median DOR was not reached at three years, the median PFS was 16.8 months and 3-year overall survival rate was 59.4% in all comers and 89.5% in responders [28]. In the first-line advanced setting, 14.3 – 32% of patients demonstrate primary resistance to ICI [19,20]. In the KEYNOTE-017 trial, 11 of 29 (37.9%) patients with an initial partial or complete response later relapsed and 8 of these patients received subsequent immunotherapy, with treatment ongoing in 2 patients [19]. In the neoadjuvant setting, 2 doses of pre-operative nivolumab (240 mg, 2 weekly) resulted in a pathological complete response (pCR) rate of 47.2% in patients with resectable stage IIA – IV MCC in the CheckMate 358 trial [29]. The rarity of MCC has precluded large randomised controlled trials comparing ICI to chemotherapy. However, the unprecedented results of these single-arm studies, compared to historically poor outcomes, has cemented single-agent anti-PD-1 (pembrolizumab) and anti-PD-L1 (avelumab) as the front-line standard of care in metastatic MCC.

Clinical Markers of Response to Immune Checkpoint Inhibition in MCC

Clinical trials and retrospective case series have evaluated various clinical factors as potential markers of response. Key findings from these studies are summarised in Table 1. In keeping with the higher ORR observed in Part B (first-line, naïve to chemotherapy) compared to Part A (second-line, post-chemotherapy) of the JAVELIN Merkel 200 study, use of ICI as the first line of therapy has been consistently associated with favourable response, meeting statistical significance in the larger of two retrospective studies [13,30]. The basis of this differential response is poorly understood but may reflect an altered tumour immune environment and impaired adaptive immunity as a result of prior chemotherapy [31]. Better ECOG performance status also correlates with improved response in clinical trials [19] and case series [32]. Younger age has been associated with a trend towards improved response in some retrospective studies [30,32], although this has

Table 1: Summary of key factors associated with response in clinical trials and retrospective analyses.

	Patients	Clinical features	Imaging features	MCPyV status	PD-L1/ PD-1 status	TMB	Other markers of response
Clinical Trials							
D'Angelo et al, JITC 2020; Kaufman et al, JITC 2018; D'Angelo et al ESMO Open 2021. *JAVELIN 200 Merkel Part A biomarker analysis and extended efficacy update*	N = 88 *1+ prior therapy* *Stage IV*	<2 lines of therapy favours response (ORR 40.4% vs. 22.2%)	NA	MCPyV negative slightly favours response, not significant (ORR 35.5% vs. 28.3%, NS)	PD-L1+ favours response (ORR 36.2% vs 18.8%, NS) Improved mOS in PD-L1+ patients (12.9m vs 7.3m)	TMB ≥ 2 NSSV/ Mb favours response (ORR 45.5% vs 28.0%), 6-month PFS and mOS (NR vs 12.6m)	GSEA: Interferon γ and interferon α and β pathways, Th1, 2 and NK cell pathways enriched in responders
D'Angelo et al, JITC 2021. *JAVELIN 200 Merkel Part B updated overall survival and biomarker analysis*	N = 116 *No prior therapy* *Stage IV*	NA	NA	MCPyV negative slightly favours response, not significant (ORR 48.6% vs 34.2%, NS)	PD-L1 + favour response (ORR 61.9% vs 33.3%, NS) Improved mOS in PD-L1+ patients (NR vs 15.9m)	TMB > 2 NSSV/ Mb slightly favours response (ORR 50.0% vs 41.2%, NS), mOS (NR vs 17.2m)	Median or higher CD8+ T cell density at the invasive margin favours response (ORR 51.2% vs 28.6%, NS) GSEA: interferon Interferon γ and interferon α and β pathways enriched in responders
Nghiem et al, NEJM, 2016. *CITN-09/ KEYNOTE-017*	N = 26 *No prior therapy* *Unresectable stage IIIB or IV*	NA	NA	MCPyV positive favours response (ORR 62% vs. 44%)	No correlation	NA	No correlation with CD8+ T cell infiltration and response
Nghiem et al, JITC 2021. *CITN-09/ KEYNOTE-017 3-year update and correlates*	N = 50 *No prior therapy* *Unresectable stage IIIB or IV (86% stage IV)*	Completion of 2 years of therapy improves OS, HR 0.1 (95% CI 0.01-0.73) ECOG 1 vs 0 reduced survival, HR 2.70 (95%1.10-6.64) No difference for age, gender, baseline tumour burden.	CR/PR improves 3-year OS (89.5% vs. 59.4%)	No difference in OS according to MCPyV status (HR 0.93, 95% CI 0.39-2.17)	No difference in OS (HR 0.48, 95% CI 0.19 -1.20)	NA	

Topalian et al, JCO 2020. *CheckMate 358*	N = 39 *Resectable stage IIA – IV Neoadjuvant*	12-month RFS 100% vs 59.6% for pCR vs non-pCR	Imaging underestimates pCR 5 patients with <30% radiographic reduction had pCR	No difference in response	No difference in response	No difference in TMB for pCR vs non-pCR observed	Increased expression of CCL5, CXCL9, IL16, IL2RB in responders (p < 0.05)
Retrospective Studies							
Weppler et al, JITC 2020.	N = 23	Age < 75 years favours response (ORR 64% vs 50%, NS) irAE favours response (ORR 100% vs 43%, NS)	Low MTV at baseline favours response (p = 0.05) CMR within 12 weeks correlates with improved survival HR for PFS 0.31 (p = 0.38) HR for OS 0.24 (p=0.19)	MCPyV negative favours response (ORR 69% vs 43%, NS)	No correlation with response	No correlation with response	
Knepper et al, ACCR 2019.	N = 317	Line of therapy favours response (ORR 75% 1L, 39% 2L, 18% 3L+, p = 0.006)	NA	No difference in response (ORR 50% vs 41%, p = 0.63)	PD-1+ favours response (ORR 77% vs 21%, p = 0.00598)	No difference in response (ORR 50% vs 41%, p = 0.63)	
Kacew et al, Oncotarget 2020.	N = 45	Higher stage at primary disease diagnosis reduced odds of response (OR 0.06, p = 0.04) Longer time to recurrence reduced odds of response (OR 0.75, p = 0.05)	NA	No difference in response (p = 0.10) No correlation with survival (p = 0.66)	NA	No difference (median TMB 19.7 mut/Mb vs 4.8mut/MB, p = 0.11, for responders vs non-responders)	SNVs in *ARID2* and *NTRK1* correlated with response (p=0.05)
Spassova et al, ACCR 2020.	N = 41	ECOG 0 favours response Absence of immunosuppression favours response Age < 70 years favours response	NA	No correlation with response	No correlation with response	NA	Lower T cell clonality and higher TCR diversity seen in responders

Giraldo et al, JITC 2018.	N = 26	NA	NA	NA	No correlation with response for PD-L1 status using 1% as threshold Higher PD-L1 correlated with response when analysed as a continuous variable (p = 0.02)	NA	Higher density of PD-1+ cells in responders vs non-responders (p = 0.03). Density of PD-1+ cells adjacent to PD-L1+ correlates with response (p<0.05)

N: Number of patients; NA: Not Assessed; NS: Not Significant; NR: Not Reached; ORR: Objective Response Rate; Mos: Median Overall Survival; RFS: Recurrence Free Survival; OR: Odds Ratio; HR: Hazard Ratio; Pcr: Complete Pathological Response; CMR: Complete Response; PR: Partial Response; CMR: Completed Metabolic Response On FDG-PET; MTV: Metabolic Tumour Volume; 0L: Treatment naïve; 1L: 1 prior line of therapy; 2L: 2 prior lines of therapy; 3L+: 3 or more prior lines of therapy; SNVs: Single Nucleotide Variants; ECOG: Eastern Co-operative Oncology Group Performance Status (0-5); TCR: T cell Receptor; irAE: Immune-related Adverse Event; GSEA: Gene Set Enrichment Analysis

not been replicated in the clinical trial population [19]. In a retrospective series of 23 patients by Weppler et al., 6 of 6 patients who experienced an immune-related adverse event (irAE) responded (overall response 100% compared to 47% without irAE) [30]. These numbers are too small to meet statistical significance, however are supported by similar observations in patients with melanoma and NSCLC [33-35]. Conversely, immunosuppression was identified as a negative predictor of response in two retrospective studies [32,36]. Depth and duration of response appear to be prognostic: in the CheckMate-358 trial recurrence-free survival (RFS) significantly correlated with pCR with no relapses observed at 12 months of follow up in patients who achieved pCR [29]. In KEYNOTE-017, greater percentage of target lesion reduction and completion of 2 years of pembrolizumab were associated with improved overall survival at 30 months [19].

It is unclear how prior or concurrent radiation impacts response to ICI. Radiation can induce immunogenic cell death and upregulate PD-L1 expression, potentially synergising with ICI [37,38]. One retrospective study of 45 patients found that patients with a longer disease-free interval between definitive radiation therapy and subsequent use of ICI for metastatic disease were less likely to respond (odds ratio 0.75, p =0.05). The authors hypothesised that more recent radiation in patients with shorter-disease free interval may improve response to ICI [36]. Weppler et al. described an overall response rate of 75% and complete response (CR) rate of 50% in four patients who received concurrent palliative radiation with commencement of ICI. Salvage radiation resulted in response in all 3 patients with isolated ICI-resistant lesions. However, all 3 patients who received salvage radiation in the setting of multisite progressive disease continued to experience disease progression at sites outside the radiation field [30].

Imaging Features of Response

The role for 2-[18F]fluoro-2-deoxy-D-glucose ([^{18}F]FDG, ^{18}FDG) positron emission tomography (PET) in assessing response and directing management in MCC was established in the pre-immunotherapy era [39,40]. A clear association between complete metabolic response (CMR) and improved overall survival in MCC has been demonstrated [41]. More recently, ^{18}FDG-PET has been evaluated as a potential marker of response in patients treated with ICI. Patients who achieved CMR had a significantly lower metabolic tumour volume (MTV) at baseline [30], consistent with findings from clinical studies in MCC and melanoma that lower burden of disease is associated with favourable response to ICI [22,42]. These data support the role of routine surveillance ^{18}FDG-PET to detect asymptomatic, smaller volume recurrence following definitive therapy for MCC [41]. Patients treated with ICI who achieved CMR on early ^{18}FDG-PET (within 12 weeks of treatment initiation) demonstrated a trend towards improved progression free and overall survival [30]. These findings support the ongoing use of ^{18}FDG-PET in response assessments and identify a need for specific research into additional treatment strategies for patients with high MTV.

Like other neuroendocrine tumours, approximately 70% of MCC express somatostatin receptors [43], presenting an opportunity for disease assessment with Gallium68 (^{68}Ga)-labelled octreotide derivates using PET, the most widely used tracer being [^{68}Ga]Ga-DOTA-octreotate (^{68}Ga-DOTATATE). Several retrospective case series and retrospective analyses have demonstrated high sensitivity, specificity and diagnostic accuracy for ^{68}Ga-DOTATATE PET in MCC [44]. Interestingly, when FDG-PET was directly compared to ^{68}Ga-DOTATATE PET in MCC, similar avidity was seen with both tracers [45]. This is an unusual finding in poorly differentiated neuroendocrine tumours which are generally associated with lower SSTR-expression and higher FDG-avidity compared to their well-differentiated counterparts, with prognostic implications [45]. There are no data on ^{68}Ga-DOTATATE PET specifically assessing response to ICI, however this is being explored as the companion diagnostic for a potential theranostic strategy in metastatic MCC. The GOTHAM trial (NCT04261855) utilising ^{68}Ga-DOTATATE PET to select patients to receive upfront ^{177}Lu-DOTATATE (LuTate) peptide receptor radionuclide therapy in combination with avelumab.

Molecular Markers of Response

Despite the distinct molecular features of MCPyV-positive and MCPyV-negative MCC, both subtypes respond to immune checkpoint inhibition [13,21,23,29]. A clear predictive biomarker for response to ICI is yet to be elucidated. In addition to MCPyV status, tumour mutational burden (TMB) and PD-1 or PD-L1 status have been investigated as potential biomarkers in clinical trials. Changes in peripheral and intra-tumoral T cell populations and immune-related gene signatures have also been explored in retrospective studies.

MCPyV status

Traditionally, MCPyV-positive MCC has been associated with a more favourable prognosis [46], attributed to the increased immune-infiltrates and PD-L1 expression seen in this subtype [8,47]. Although responses to ICI have been observed in both MCPyV-positive and -negative cases of MCC, some differences in response rates have been observed. A recent real world retrospective study identified a non-significant trend towards improved ORR in MCPyV-negative tumours compared to MCPyV-positive tumours [30]. This is supported by a similar but non-significant finding in the JAVELIN Merkel 200 biomarker analysis with an ORR of 35.5% for MCPyV-negative MCC compared to 28.3% for MCPyV-positive MCC [23]. In contrast, a numerically higher ORR was seen in MCPyV-positive tumours in the initial analysis of the single-arm phase 2 trial of pembrolizumab (62% vs 44%) [21] although this finding was not reproduced in the subsequent 3-year update of this trial [19]. No association between MCPyV status and pathological response rate was seen in the neoadjuvant nivolumab trial [29], and a retrospective review of 57 patients with MCC by Knepper et al. did not find any difference in response according

to MCPyV status [13]. Overall, no clearly consistent trend or statistically significant association between viral status and response to ICI has been observed.

Tumour mutational burden

High TMB is classically associated with high response rates to ICI across various tumour types [48]. Increased neoantigen expression is the putative mechanism of immunogenicity. Several studies have confirmed the bimodal distribution of TMB in MCC according to viral status, characteristically high in MCPyV-negative tumours and low in MCPyV-positive tumours, with very few (8%) intermediate cases [13,49]. In one large study of 317 patients, 37% were TMB high with a median TMB of 53.6 mutations per megabase (mut/Mb) and 55% were TMB-low with a median TMB 1.2 mut/Mb [13]. MCPyV DNA was not identified in any of the TMB high patients in this study [13], and was only detected at very low levels in another smaller study (median viral copy number 0.0037) [49]. Furthermore, 94% of patients with high TMB had a detectable UV mutational signature [13]. UV mutational signature and MCPyV DNA were mutually exclusive in intermediate TMB cases [13]. A clear molecular and oncogenic distinction between TMB-high, UV-associated and TMB-low, viral-driven MCC is thus drawn.

In the biomarker analyses of JAVELIN Merkel 200, patients with a TMB of 2 or more non-synonymous mutations per megabase had numerically but not significantly higher ORR compared to those with a lower TMB [23,25]. Despite this, no difference in the median TMB was seen between responders and non-responders was identified [25]. A non-significant trend towards improved PFS and median overall survival is noted in both first- and second-line settings (Table 1) [23,25]. None of these results met statistical significance and are limited by small population of only 36 evaluable patients. Interestingly, a very high ORR of 83.3% was seen in patients who exhibited high TMB in combination with another factor, such as high $CD8^+$ T cell density at the invasive margin [23]. Contrary to these signals, no difference in TMB was observed between pathological responders and non-responders in the neoadjuvant nivolumab study, although only 14 patients were evaluable for TMB [29]. Multiple retrospective cohort studies have also failed to identify a clear correlation between TMB and response [13,30,36]. The absence of a definite association between high TMB and response in MCC differs from other tumour types and suggests an alternative mechanism of immunogenicity in MCPyV-positive tumours, presumably related to the presence of viral antigens and their ability to elicit an immune-response.

PD1/PDL1 status

PD-L1 status has been identified as a useful biomarker for response to ICI in some tumour types but not others. The JAVELIN 200 Merkel, KEYNOTE-017 and CheckMate 358 trials all included analyses of PD-L1 status. In the neoadjuvant nivolumab study, 27 patients had a quantifiable PD-L1 status: 7 had positive staining with greater than or equal to 1% of tumour cells staining for PD-L1 and 20 were deemed negative with staining less than 1% of tumour cells. No trends for radiographic or pathologic response or recurrence free survival according to PD-L1 status were observed [29]. Similarly, no correlation between PD-L1 status and response to CPI was observed in KEYNOTE-017 [21]. Although PD-L1 positive patients demonstrated a numerically higher ORR than PD-L1 negative patients in both first- and second-line settings in the JAVELIN Merkel 200 trial, this was not statistically significant [23,25] (Table 1). Long-term responses were observed in patients with both PD-L1 positive and negative tumours, although the majority (81.8%) of long-term survivors were PD-L1 positive [23]. A non-significant improvement in overall survival for PD-L1 positive patients is also seen in both cohorts (chemotherapy pretreated and chemotherapy naive) in JAVELIN Merkel 200 (Table 1) [25,26]. No significant correlation between PD-L1 status and response has been observed in retrospective studies [13,30].

Interestingly, one retrospective study also assessed PD-1 status on peritumoral lymphocytes and found a stark difference in response rate at 77% for PD-1 positive compared to 21% for PD-1 negative patients [13]. In an extended biomarker analysis of PD-1 and PD-L1 status from KEYNOTE-017, quantitative analysis showed higher densities of PD-1 and PD-L1 expression in responders compared to non-responders [50]. The geographic distribution of PD-1 and PD-L1 expressing cells also appeared to be meaningful, with higher responses seen in patients with a high number of PD-1 expressing immune cells adjacent to PD-L1 positive cells, indicative of adaptive rather than constitutive PD-L1 expression [50]. These findings suggest that categorical PD-L1/PD-1 positivity or negativity may be too simplistic as an approach to this biomarker. It is important to note that many of the studies evaluating PD-L1 status used different clones to assess PD-L1 expression, and whilst all considered tumours with 1% or greater staining as positive, some assessed tumour cells alone (tumour proportional score or TPS) [23,29,32] whereas others also included assessment of immune cells (IC score) [19,30], potentially contributing to the observed inconsistencies between studies.

T cells

Tumour infiltrating lymphocytes (TILs) have been shown to be prognostic in breast cancer and melanoma [51,52]. In MCC, T cell infiltration itself does not appear to be associated with response in several studies [19,23,30], although the updated biomarker analysis from the first-line cohort in JAVELIN indicates a trend towards improved ORR for patients with a median or higher density of $CD8^+$ T cells at the invasive margin [25]. Additionally, some differences in T cell characteristics have been noted between responders and non-responders. Spassova et al. demonstrated lower clonality and more diverse T cell repertoire characterised the TILs of responders compared to non-responders [32]. Central memory T cells were the predominant TIL subtype in responders, a feature also associated with ICI efficacy in other

tumour types including melanoma [32,53]. In another study evaluating peripheral mononuclear blood cells, the frequency of a highly activated $CD8^+$ T cell subtype expressing both PD-1 and T cell immunoreceptor with Ig and ITIM domains (TIGIT) at baseline and following treatment with pembrolizumab was predictive of response [54]. These so-called "double-positive" cells in peripheral blood samples are compelling as a potential biomarker and requires validation in larger patient populations.

Genes and gene sets

Several studies have identified enrichment of pathways involved in inflammation and immune response in responders to ICI [23,25,32]. Interferon gamma signatures were most common in responders, MCPyV-negative, PD-L1 positive tumours, and tumours with median or higher $CD8^+$ T cell infiltration at the invasive margin [23,25]. Whilst MHC class I gene expression did not correlate with response or overall survival in the JAVELIN Merkel Part B biomarker analysis, increased MHC class I expression was associated with upregulation of inflammatory gene sets and was higher in patients with median or higher $CD8^+$ T cell density [25]. Differential expression of genes involved in T cell attraction and activation has been noted in tumours of responders compared to non-responders [32]. While most emphasis has been placed on detection of conventional T cells, Gherardin et al. identified dominant infiltration of gamma delta T cells in some MCC and a gene-expression signature derived from these unconventional T cells was found elevated in bulk-tissue RNA from a subset of ICI-responsive cases [55]. Mutations in *TP53* and *RB1* are the most common genetic aberrations in both subtypes but are seen at higher frequencies in TMB-high population [13]. In a study of 45 patients, Kacew et al. found there were significantly more *ARID2* and *NTRK1* mutations in responders, a finding that requires further validation given small patient numbers [36].

Future Directions

Despite recent advances in the treatment of MCC, approximately 50 to 70 percent of patients demonstrate either primary or acquired resistance to immunotherapy within one year [20,22,28]. Definative predictive biomarkers of response to ICI are yet to be identified in MCC, with current research efforts limited by small patient numbers. Beyond further translational research to identify potential immune-related biomarkers and identify mechanisms of resistance, other approaches to be considered in the subset that exhibit primary and acquired resistance include treatment intensification with combined immunotherapy agents, targeted agents in conjunction with immunotherapy, or utilising multi-modality treatments, including radiation, to modulate the tumour microenvironment.

Combination ipilimumab and nivolumab has been explored in limited case series of patients resistant to anti-PD-L1 therapy, with response seen in 3 of the 5 treated patients [56,57]. This is a strong positive signal in a difficult to treat, refractory population, but requires validation with higher patient numbers. A randomised phase II trial of combination ipilimumab and nivolumab with or without radiation is ongoing, recruiting approximately 50 patients (NCT03071406). Novel immuno-oncology agents including toll-like receptor agonists [58] and T cell receptor therapy are being explored in MCC [59]. T cell receptor therapy is a focus of some early phase trials (NCT03747484) and involves the collection of T cells and programming high-affinity anti-MCPyV T-cell receptors into immature T cells for expansion.

Combining DNA Damage Repair (DDR) inhibitors, such as ATR (ataxia telangiectasia and Rad3-related) inhibitors, with ICI is another attractive strategy for MCC [60]. These agents target the cell cycle dysregulation that occurs as a result of *TP53* and *RB1* loss in MCC, and also induce immunogenic cell death, potentially augmenting the effect of ICI [60]. A number of ATR inhibitors have demonstrated tolerability in early-phase clinical trials [61-63] and several trials combining ATR inhibitors and ICI in other solid tumours are planned or ongoing (NCT05061134, NCT04216316, NCT04095273).

Trials to formally evaluate combining ICI with radiation with ICI in MCC are underway. In addition to the GOTHAM trial, NCT04261855 (evaluating avelumab with external beam radiotherapy and avelumab with LuTate in highly somatostatin receptor-expressing MCC in the metastatic setting), the CARTA trial (NCT04792073) is evaluating the role of comprehensive ablative radiation therapy with or without avelumab in patients with advanced disease progressing after anti-PD-1 therapy. In the adjuvant setting, the Immunotherapy Merkel Adjuvant Trial (I-MAT, NCT04291885) is randomising patients to receive concurrent, adjuvant avelumab or placebo following surgery and /or radiation for stage I-III MCC. Other ongoing adjuvant studies include the STAMP study (NCT03271372) and ADEMEC-O study (NCT02196961), randomising patients to observation or anti-PD-1 therapy (pembrolizumab and nivolumab respectively) after complete surgical resection of MCC, and the ADAM trial evaluating adjuvant avelumab following completion of surgery and/or radiotherapy (NCT03271372).

For patients who do respond to ICI, questions remain regarding the optimal duration of therapy. A retrospective study of 40 patients evaluated rates of progression following treatment cessation for response or otherwise and found relatively high rates of progression on treatment cessation, even among patients who initially achieved a CR [64]. Thirty (75%) patients were in CR at the time of treatment cessation and 26% of these patients and 57% of patients in partial response (PR) developed progression with a median time to progression of 5.5 months. Receiving fewer cycles of ICI was significantly associated with increased risk of progression. Ultimately, robust biomarkers are still required to identify those patients who will need intensified therapy, those who can be successfully treated with anti-PD-1/PD-L1 alone and those who can safely discontinue therapy after achieving a response.

Conclusion

Immune checkpoint inhibition has led to dramatic improvements for patients with advanced MCC. However, resistance remains a challenge and as yet there is no clear biomarker to aid patient selection or personalization of treatment. Performance status, line of therapy and early ^{18}FDG-PET response have all been associated with favourable outcomes. Despite two clearly molecularly distinct subgroups of MCC, high ICI responses are seen in both subgroups, regardless of viral status, TMB and PD-L1 status. However, a significant subset of these patients who initially respond to ICI subsequently develop disease progression. More work is needed to identify and understand mechanisms of response and resistance to ICI, to select patients for standard or emerging therapies.

Disclosures

JDD, LSM, GK and AC have nothing to disclose. RWT has served on an advisory board for Merck Serono. SS has served on advisory board for Bristol Myer Squibb, Merck Sharp and Dohme, AstraZeneca, Janssen and has received grant funding to the institute from Pfizer, Merck Sharp and Dohme, AstraZeneca, Amgen, and Advanced Accelerators Applications (AAA), a Novartis Company (outside the submitted work). RJH holds shares in Telix Pharmaceuticals.

Author Contribution Statement

JDD, LM and SS devised the proof outline. All authors contributed to the final manuscript, with particular attention to respective areas of expertise from RJH and GK (nuclear medicine and imaging), and RWT and AC (immunology and molecular studies). SS provided oversight and supervision.

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