

# Updates in the Management of Merkel Cell Carcinoma

## Atualizações no manejo do Carcinoma de Células de Merkel

Paulo Henrique do Amor Divino<sup>1</sup>, Gabriel Passos Souza<sup>1</sup>, Maurício Fernando Silva-Almeida Ribeiro<sup>2</sup>, George Horta<sup>1</sup>, Fabíola Ambrosio Silveira-Lima<sup>2</sup>, Alessandra Corte Real-Salgues<sup>2</sup>, Rodrigo Ramella Munhoz<sup>1,2</sup>.

### ABSTRACT

Merkel cell carcinomas are rare cutaneous malignancies with neuroendocrine features that affect elderly individuals with a history of chronic sun exposure and immunosuppression. More recently, a human viral pathogen, the Merkel cell polyomavirus, has been implicated in the carcinogenesis of this disease. Its particularly aggressive biological behavior, the delay in diagnosis related to the lack of awareness, as well as the paucity of effective therapeutic modalities have historically contributed to the high lethality and dismal prognosis. Although surgery and radiation therapy remain the therapeutic pillars for patients with localized disease, the recognition of its immunogenic potential, with the consequent development and successful implementation of immune checkpoint blockade for those with advanced disease has significantly changed the treatment landscape for these patients. In this review, etiopathogenic, diagnostic and therapeutic aspects related to Merkel cell carcinomas are thoroughly addressed.

**Keywords:** neuroendocrine tumors; polyomavirus; immunotherapy; Carcinoma; Merkel Cell

### RESUMO

Carcinomas de células de Merkel são neoplasias malignas cutâneas raras com características neuroendócrinas que afetam indivíduos idosos com história de exposição solar crônica ou imunossupressão. Recentemente, um vírus humano patogênico, o poliomavírus, tem sido relacionado com a carcinogênese da doença. Seu comportamento biológico particularmente agressivo, o atraso no diagnóstico relacionado à falta de informação e a escassez de modalidades terapêuticas eficazes têm contribuído historicamente para a alta letalidade e prognóstico sombrio. Embora a cirurgia e a radioterapia continuem sendo os pilares terapêuticos para pacientes com doença localizada, o reconhecimento de seu potencial imunogênico, com o consequente desenvolvimento e implementação bem-sucedida dos inibidores do checkpoint imune para pessoas com doença avançada mudou significativamente o cenário do tratamento para esses pacientes. Nesta revisão, aspectos etiopatogênicos, diagnósticos e terapêuticos relacionados aos carcinomas de células de Merkel são minuciosamente abordados.

**Descritores:** Merkel cell; immunotherapy; polyomavirus (Células de Merkel, imunoterapia, poliomavírus)

1. Instituto do Câncer do Estado de São Paulo, Medical Oncology Division - São Paulo - São Paulo - Brazil

2. Hospital Sírio-Libanês, Oncology Center - São Paulo - São Paulo - Brazil

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**Correspondence author:** Paulo Henrique do Amor Divino. Instituto do Câncer do Estado de São Paulo.

Avenida Dr. Arnaldo, 251, Cerqueira César, São Paulo-SP, CEP 01246-000.

E-mail: phadmed@hotmail.com.

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## INTRODUCTION AND MECHANISMS OF CARCINOGENESIS

Historically described by Toker in 1972 as a trabecular carcinoma of the skin, Merkel cell carcinomas (MCC) are rare and highly aggressive neuroendocrine cutaneous malignant neoplasms.<sup>1,2</sup> MCC has an estimated annual incidence of 0.4 cases per 100,000 individuals in the American population, reaching 4.85 cases / 100,000 in the population over 80 years of age, with a tendency to increase as demonstrated in the last decades.<sup>3</sup> However, MCC epidemiology exhibits a significant geographic heterogeneity: in Australia, incidence rates four times higher than in the American population have been reported, reaching almost 20.4 cases/100,000, also with a growing number of cases diagnosed over the past decades.<sup>4</sup>

Risk factors traditionally associated with the development of MCC include fair skin, chronic sun exposure, immunosuppression (e.g. solid organ transplantation, presence of HIV infection or concomitant malignant neoplasm) and advanced age, with a marked increase in the incidence rates in the 8<sup>th</sup> and 9<sup>th</sup> decades of life.<sup>2,5</sup> In 2008, the presence of MCC-associated polyomavirus (MCPyV) has been linked to the development of a variable portion of these neoplasms. MCPyV is a human viral pathogen member of the Polyomavirus family, whose infection is common in childhood and typically self-limited. Approximately 60-80% of adults have positive serology for previous infection.<sup>6</sup>

In MCC, MCPyV circular double-strand DNA is capable of integrating into the host genome, resulting in the coding of viral oncoproteins known as Large T (LT) and Small T (ST) antigens.<sup>7,8</sup> Such an event occurs early in the tumorigenesis, as the viral genome can be characterized in different cellular subclones. These antigens capability of directly binding to and negatively interfering with the function of important tumor suppressors such as pRb, p53 and FBXW7, encompasses the basis of carcinogenesis in MCPyV positive tumors.<sup>7,9,10</sup> It is estimated that about 80% of MCC cases in the northern hemisphere are associated with the presence of MCPyV; in Australia, only about 30% of the cases are considered MCPyV positive, being the vast majority attributed to the damage from chronic exposure to ultraviolet (UV) radiation.<sup>1,3,4,7,11,12,13</sup>

MCPyV positive MCCs are amongst the neoplasms with the lowest mutational burden (TMB), presenting on average 0.4-0.75 mutation/Mb and 12.5 somatic single nucleotide variants (SSNV) per exome, without highly recurrent alterations or UV damage signatures. This low TMB observed in MCPyV positive tumors suggests that the disorders caused by the activity of ST and LT oncoproteins are the most important factor in tumorigenesis. Nonetheless, the detection of serum antibodies against T antigens in this subgroup points towards the presence of both recognition and response by the immune system, despite the low TMB, suggesting an intrinsic immunogenic role for MCPyV.<sup>9,14</sup>

MCPyV negative MCCs, on the other hand, exhibit both TMB and median number of neoantigens even higher than cutaneous melanomas, with about 40 mutations/Mb, 1.121 SSNV per exome and 173 neoantigens per sample. Among the genes most frequently affected by pathogenic mutations in this subgroup are *TP53* (70%), *RB1* (45%), *NOTCH1*, *FAT1*, *ATM*, *MSH2*, *BRCA1*, *MAP3K1*, and *TRAF7*. Such profile reinforces the importance of p53 and pRb activity interference in the development of MCC, either by direct protein inactivation or somatic mutations. [9,12,13] It is also important to note that the frequency of C>T transitions, considered signatures of UV-induced damage, is significantly higher in MCPyV negative tumors, being observed in 87% of SSNV, reiterating the importance of chronic sun exposure in the carcinogenesis of this subgroup of neoplasms.<sup>12,14</sup>

The cellular origin of MCCs remains controversial. Due to the ultrastructural and immunohistochemical similarities with Merkel cells, it was initially proposed that these tumors would arise from these structures.<sup>15</sup> Nonetheless, characteristics such as (1) very limited proliferative potential (with maintenance of its homeostasis by epidermal progenitor cells), (2) topography divergence between Merkel cells (located in the basal layer of the epidermis) and MCCs arising (dermis or hypodermis), as well as (3) differences in the arrangement of the cytoskeleton have been fostering discussions about its normal counterpart.<sup>9,10</sup> Making this scenario even more complex, in a recent publication, Sunshine et al proposed that MCCs MCPyV positive and MCPyV negative would respectively derive from dermal and epidermal stem-cells, which, in turn, would converge into the process of neoplastic transformation to the neuroendocrine phenotype in the presence of deleterious interference with pRb function. According to the authors, this process of transdifferentiation would be, if corroborated by future studies, the first report of convergent evolution between two distinct embryonic lines (ectodermal and mesodermal) in malignant neoplasms.<sup>9</sup> Finally, a possible origin from pre / pro-B cells has also been suggested, since an expressive percentage of these neoplasms exhibit B-cell line markers (e.g. PAX-5, TdT, bcl-2) and clonal rearrangement of immunoglobulins.<sup>10,16</sup>

Irrespectively of the underlying mechanisms, MCCs present an aggressive clinical course, with lethality rates even higher than paired-stage cutaneous melanomas. Both the presence of MCPyV, initial staging, and the absence of primary lesion at the time of diagnosis appear to be associated with better outcomes; the latter, in turn, highlights the possibility of an existing relationship between the primary lesion regression, higher TMB and a more efficient anti-tumor immune response, which would result in better 5-year overall survival rates favoring those with node-positive disease without an identifiable primary (42% vs. 27%).<sup>2,13,17,18,19,20</sup> According to Harms et al, it is estimated that only about 8% of the patients present with distant metastasis at the time of diagnosis, being the vast majority diagnosed with local (65%) or locally-advanced (26%) disease.

Historically, the 5-year overall survival rates range from 14% to 51% for those with local and metastatic disease.<sup>20</sup>

## DEFINING THE DIAGNOSIS

MCC often presents as a fast-growing violaceous nodule, usually asymptomatic, that appears in sun-exposed skin. Nevertheless, the initial presentation can be non-specific. Multiple cohort studies describe head and neck as the most common anatomic site of the primary tumor.<sup>21</sup> In order to facilitate its early detection, key clinical features were summarized in an acronym: AEIOU - asymptomatic, expanding rapidly (significant growth in  $\leq 3$  months), immune suppression, older than 50 years age, and UV-exposed area in a fair-skinned individual. The presence of at least three of these characteristics increases the suspicion of MCC.<sup>22</sup>

The differential diagnoses encompass benign and malignant cutaneous lesions, including basal cell carcinoma, squamous cell carcinoma, keratoacanthoma, pyogenic granuloma, lipoma and adnexal tumors. Because of their similar microscopic appearance, other possibilities are metastases of small cell carcinoma of the lung, small cell melanoma and Ewing's sarcoma.<sup>23,24</sup>

On histopathologic examination, the tumor presents as a poorly defined, sheet-like mass involving the dermis. Generally, the epidermis is spared and neoplastic infiltrate is confined to the papillary dermis and subcutaneous tissues. Intraepithelial layer impairment may occur. Increased mitotic activity and necrosis are often seen. In hematoxylin-eosin staining (H&E), tumor cells are monotonously small, round, and blue, with a finely dispersed nuclear chromatin pattern and scant cytoplasm, forming peculiar trabeculae.<sup>10,25</sup> Immunohistochemistry demonstrates expression of neuroendocrine markers and particular low-molecular weight cytokeratin immunoreactivity, which is expressed in a characteristic paranuclear punctate or "dot-like" pattern. The main marker of MCC is cytokeratin 20 (CK20), with a sensitivity greater than 90%.<sup>26</sup> Virtually all MCC are positive for neuron-specific enolase (NSE), whereas chromogranin B and A are found in 100% and 72% of the tumors, respectively. Some studies have shown c-kit positivity in up to 95% of cases. S-100 protein, HMB-45, CK7, TTF-1, desmin, and actin are typically negative.<sup>27,28</sup>

MCPyV LT antigen can be detected by immunohistochemistry using mouse monoclonal antibody CM2B4 and the viral DNA by real-time polymerase chain reaction in tumor tissue.<sup>29</sup> Nevertheless, the investigation of MCPyV is not mandatory for the diagnosis of MCC. Antibodies to MCPyV-oncoproteins can also be detected in peripheral blood and are associated with the risk of recurrence. Paulson et al, in a prospective validation study, evaluated 219 patients with newly-diagnosed MCC and the presence of antibody to MCPyV-oncoproteins at diagnosis was an independent predictive factor of decreased recurrence risk (HR 0.58;  $p=0.04$ ). Additionally, among patients with positive antibodies, an increasing titer after definitive therapy was associated with clinically recurrence (positive predictive value of 66%) and it could be useful for ongoing surveillance. Patients with negative antibodies upon initial presentation are at higher risk for recurrence and may benefit from closer follow-up with imaging, in contrast to patients with positive antibodies, for whom alternating serial MCPyV antibody titer assessment with imaging could be an option.<sup>6,30</sup>

Once the diagnosis of MCC is established, initial work-up should include imaging tests. Positron emission tomography with fluorodeoxyglucose (<sup>18</sup>F-FDG-PET/CT), computed tomography (CT) and magnetic resonance imaging (MRI) are viable options. There is no consensus on which method is best for work-up and staging of MCC. Treglia et al. conducted a systematic review and meta-analysis that examined the performance of PET-CT. It showed elevated accuracy and effectiveness in detecting regional lymph node and distant metastatic disease with a sensitivity of 90% and specificity of 98%.<sup>31</sup> The extent of disease at presentation is the most important predictor of survival for MCC and determines management of these patients. According to the American Joint Commission on Cancer (AJCC) 8th Edition, MCC is classified by tumor size, involvement of regional lymph nodes and presence of distant metastases. (Table 1)<sup>32</sup>.

## TREATMENT OF PATIENTS WITH LOCALIZED OR LOCOREGIONAL DISEASE

### Role of surgery

Upon initial diagnosis, patients should be distinguished among those with localized, regional/nodal and metastatic disease. There are no prospective randomized trials interrogating the optimal initial management of patients with MCC, hence most of the treatment definitions are based upon analyses of retrospective data and case series. Patients with localized disease are defined as those with tumor restricted to the skin without nodal disease (AJCC stage I and II).

Locoregional disease is defined when there is clinical or pathological lymph node involvement (AJCC stage III).<sup>32</sup>

The mainstays of treatment for patients with localized disease have been wide excision followed by tumor bed radiation therapy (RT). It is known that MCC has a high risk of local and locoregional recurrence, which turns mandatory an aggressive strategy for local control.<sup>33-37</sup> In regard to surgery, wide local excision (WLE) with 1-2 cm margins is the most common strategy applied. Historically, many surgical series recommended a wide margin of 2.5 to 3 cm based on high recurrence rates after surgery alone.<sup>38</sup>

**Table I. MCC staging system - AJCC 8<sup>th</sup> Edition**

Stage I	Primary tumors $\leq 2$ cm maximum tumor dimension (T1), without evidence of regional lymph node involvement.
Stage II	IIA. Primary tumors $> 2$ cm (T2 or T3), without evidence of lymph node involvement. IIB. Primary tumor with invasion into fascia, muscle, cartilage or bone (T4), without evidence of lymph node involvement.
Stage III	III. Any primary tumor with regional lymph node disease. IIIA. Clinical occult regional lymph node metastasis identified only by sentinel lymph node biopsy (N1a-sn) or following lymph node dissection (N1a) or clinically and/or radiologically detected regional lymph node metastasis, microscopically confirmed (N1b), without evidence of primary tumor. IIIB. Clinically and/or radiologically detected regional lymph node metastasis, microscopically confirmed or in-transit metastasis (discontinuous from primary tumor; located between primary tumor and draining regional nodal basin, or distal to the primary tumor without (N2) or with (N3) lymph node metastasis.
Stage IV	Metastasis beyond the regional lymph nodes, regardless of the status of the primary tumor and regional nodes

In more recent series and retrospective studies with adjuvant radiotherapy, no additional benefit was observed from wide surgical margins ( $> 2$  cm) compared to margins of 1-1.9 cm.<sup>38-40</sup> Although there is a lack of randomized controlled trials, the National

Comprehensive Cancer Network and European Consensus Guidelines recommend 1-2 cm lateral margins, whereas deep surgical margin is less emphasized.<sup>41,42</sup>

As MCC often presents with extensive vertical growth, Mohs micrographic surgery has been described as an alternative associated with improved local control. This consists on evaluating histologically all major borders, including the deep margins. In a retrospective analysis by O'Connor et al comprising 86 patients submitted to WLE or Mohs

surgery, the latter was associated with a lower local recurrence rate (8.3% vs 31.7%). Additional case series, however, failed to replicate these results. Senchenkov et al published a retrospective series of cases with 38 patients, in which 32 patients were submitted to WLE and six patients to Mohs surgery. There were no differences in local recurrence rates (13,3% vs 16,6%).<sup>43,44</sup>

To this date there are no conclusive data or controlled trials directly comparing surgical strategies and the statistical power of the studies are limited. Thus, the optimal surgical procedure strategy is at discretion of the treating physician, based upon clinical features and feasibility of the procedure, with the aim of achieving at least a 1 to 2 cm excision margin.<sup>45</sup>

## REGIONAL LYMPH NODE EVALUATION

It is known that lymphatic dissemination is frequent and responsible for a considerable amount of regional recurrences. In about 20-30% of patients, nodal involvement is present at initial presentation and, in multiple series, there is a major agreement that lymphatic invasion is an unfavorable prognostic factor.<sup>36,46,47,48</sup> In addition, 30-50% of the patients develop nodal recurrence in the course of the disease.<sup>49</sup> Sentinel lymph node (SLN) mapping and biopsy is a well-established technique in the context of melanoma.

It aims to detect subclinical nodal disease and, therefore, changes treatment decision. Due to the rarity of MCC, the experience of SLN biopsy is mostly extrapolated from the experience with melanoma.<sup>50</sup> Gupta et al. published a single institution series of SLN biopsy in MCC with clinically negative nodes and demonstrated a positive SLN in 32% of the cases; there was a difference in relapse free survival favoring the patients who underwent adjuvant treatment for the involved node bed.<sup>51</sup> Scwhartz et al. examined clinical and pathological features aiming to assess which patients would obtain benefit from SLN biopsy: among 97 tumor

specimens, 45.7% showed SLN involvement. After a multivariate analysis, there was not a subgroup of patients or threshold for precluding the procedure, with a likelihood of at least 15-20% of SLN positivity.<sup>48</sup> Finally, a meta-analysis published by Sadeghi et al. showed that SLN biopsy predicted better disease free survival (DFS) and overall survival (OS) than nodal observation.<sup>52</sup> This led to the recommendation from American and European guidelines to perform SLN mapping and biopsy for all clinically node negative patients.<sup>45,47</sup>

Given that patients with lymph node positive disease are at greater risk of recurrence and have a worse prognosis in terms of DFS and OS<sup>52</sup>, guidelines recommend treatment with regional lymph node dissection and/or RT to the nodal basin for those with clinically involved LN.<sup>45,47</sup> Nonetheless, the available data to support this approach is limited and yet again based on retrospective studies and case series.

## ADJUVANT TREATMENT FOR LOCALIZED DISEASE/PRIMARY SITE

Based upon the well documented radiosensitivity of MCC, tumor bed RT has been used in order to improve local control. Several studies aimed to assess the benefit of adjuvant RT for local and locoregional disease. Veness et al published in 2005 a case series that evaluated the risk of local and nodal recurrence in 86 patients treated with surgical approach followed or not for adjuvant RT. Twenty two percent presented with clinically nodal disease and another 19% presented with lymph node involvement without a primary lesion. There was no difference in rates of local recurrence, but patients treated with surgery plus RT had a lower incidence of nodal recurrence, which translated into an improved DFS (10.5 months vs 4.0 months).<sup>37</sup> In another paper, Lewis et al. performed a database analysis encompassing 1254 patients and found reductions in local and regional recurrences with similar rates of distant metastases, but no statistical difference in terms of overall and MCC specific survival.<sup>53</sup>

In the largest series to date aiming to assess whether adjuvant therapy was associated with better survival that included 6908 cases from the National Cancer Database (NCDB), Bathia et al. showed a reduced risk of death favoring the group receiving adjuvant RT; however, this benefit was limited to patients with negative nodal disease. Patients with node positive disease did not benefit in terms of survival from the addition of adjuvant RT.<sup>36</sup> These findings suggest that the addition of radiotherapy may benefit the local control in localized disease; however, it is reasonable to believe that survival in patients with more advanced disease may be driven by the presence of subclinical distant metastasis.

Several other case series and retrospective studies addressed the risk of recurrence and the use of adjuvant RT.<sup>34,35,46</sup> There are many limitations in these studies, such as selection bias, confounding factors, limited and missing data, lack of randomization, among others. As guideline recommendations from the European and American groups, patients with localized node negative disease are to be offered surgery followed by tumor bed RT.<sup>45,47</sup>

## ADJUVANT TREATMENT FOR LOCOREGIONAL DISEASE

The role of adjuvant RT and chemotherapy (CT) has been assessed by Bathia et al and no difference in terms of survival following the addition of RT or CT to the treatment of stage III disease was observed; nevertheless, limitations to this retrospective study included missing data on CT and RT schemes, as well as status on lymph node dissection.<sup>36</sup>

A prospective trial was designed by the Trans-Tasman Radiation Oncology Group (TROG 96:07), aiming to assess the safety and tolerability of concurrent chemoradiotherapy as adjuvant or definitive treatment for MCC. Candidates were high risk patients, defined by primary tumors larger than 1 cm in size, recurrent disease, gross residual disease following initial surgery, or MCC of unknown primary site with nodal involvement. Fifty-three patients were accrued to receive CT with carboplatin and etoposide days 1-3 in weeks 1, 4, 7 and 10 and RT delivered to the primary site and

regional nodes to a dose of 50 Gy in 25 fractions over 5 weeks. This regimen resulted in a 3-year OS of 76%, locoregional and distant control rates of 75% and 76%, respectively, suggesting that this strategy could provide better outcomes when compared with historical data.<sup>54</sup> Subsequently, Poulsen et al. performed a retrospective comparison between patients with similar baseline characteristics in order to elucidate whether the CT arm would achieve better outcomes when compared to RT alone. In a multivariate analysis, the addition of CT did not provide additional benefit in terms of OS.<sup>55</sup> Furthermore, in line with the aforementioned data, other series have suggested additional morbidity without improving survival for patients receiving adjuvant CT.<sup>56</sup>

Only one trial was able to show a survival benefit with the use of CT in the adjuvant setting. Chen et al. evaluated the use of adjuvant RT, adjuvant CRT and surgery alone. It showed a survival benefit for the use of adjuvant RT and adjuvant CRT over surgery alone and a survival benefit for CRT over adjuvant RT for a

subset of patients with tumors > 3 cm, positive surgical margins or male sex.<sup>57</sup> The data regarding the use of adjuvant CT are conflicting (with controversy over the real benefit on survival) and this modality is not routinely recommended by neither American nor European guidelines.<sup>45,42</sup>

## MANAGEMENT OF PATIENTS WITH UNRESECTABLE, RECURRENT OR METASTATIC DISEASE

CT has historically been the standard approach for patients with advanced (unresectable or metastatic) MCC, either at presentation or after prior definitive therapy. However, given the rarity of this tumor, there have been no definitive prospective clinical trials of CT for patients with this tumor, and most of the evidence and rationale has been extrapolated from regimens applied to neuroendocrine tumors of distinct primary sites. Treatment options are based in classical schemes used to treat small-cell lung cancer, and platinum doublets (including irinotecan or etoposide) have been long regarded as the historical standard approaches for those with advanced MCC. Topotecan and cyclophosphamide/doxorubicin/vincristine (CAV) have also been used in distinct series.<sup>59</sup> Despite overall response rates (ORR) of up to 60%, the benefit from these strategies has been usually short-lived, with most patients developing disease progression in less than 6 months. As an example, Voog et al analyzed 107 published cases between 1980 and 1995. The median age at diagnosis was 66 years old, 70% of the patients underwent CT due to distant metastasis and 42 different regimens were employed. The most prescribed regimens were: cyclophosphamide/ifosfamide-based (56%), anthracyclin-based (49%) and platinum-based ones (25%); ORR were 57%, 45% and 20% in the first, second and third-line, respectively. The median OS was 9 months for patients with metastatic disease and no specific regimen was associated with significantly superior survival. Myelotoxicity was the most commonly reported toxicity and nine treatment associated deaths were reported (five due to febrile neutropenia and septic shock).<sup>60</sup> Another case series published by Tai et al analyzed 204 cases, in which the most commonly used chemotherapy regimens were CAV, cyclophosphamide/epirubicin/vincristine (CEV) or etoposide/cisplatin (EP). CAV or CEV had an overall response rate of 75.7% and etoposide-platinum regimens led to an ORR of 60%. The median OS for the 204 patients was 21.5 months (including local, locoregional and advanced disease in the analysis). There were 6 deaths certainly related to treatment toxicity. [61] Becker et al analyzed retrospectively refractory patients beyond second-line therapies; CT achieved an ORR of 8.8%, median duration of response (DOR) of 1.9 months, median PFS of 3.0 months and median OS of 5.3 months, in line with previous reports of the ineffectiveness of second-line or subsequent treatments in patients with previously-treated metastatic MCC.<sup>62</sup>

To date, there is no defined role for adjuvant immunotherapy on MCC. Ongoing clinical trials are currently enrolling patients with completely resected tumors to evaluate the efficacy of nivolumab and ipilimumab as potential adjuvant therapies.<sup>58</sup>

The limitations to those studies are particularly the lack of standardized chemotherapy regimens, heterogeneous patient population, the limited number of patients, and their retrospective nature.<sup>45,63,64</sup> Until the approval of immune checkpoint blockade, some guidelines would recommend the inclusion in clinical trials or best support of care as the standard options for patients with advanced MCC.<sup>45,59</sup>

The role of immune system in the development of MCC has been long suggested. Higher rates of MCC in immunosuppressed patients and better outcomes in patients with unknown primary lesion, suggesting immunological response and regression, were the first evidence of the association between the immune system and MCC. More recently, the discovery of MCPyV positive tumors associated with antibodies production as well as high TMB and neoantigens in MCPyV negative tumors confirmed the immunogenic potential of MCC and the rationale for the use of immunotherapy with PD1/PDL1 inhibitors. Indeed, half of MCC express PD-1 on tumor infiltrating lymphocytes and express PD-L1 on tumor cells.<sup>65</sup> The performance of different treatment regimens in unresectable or metastatic MCC is shown in table 2.

Nghiem et al evaluated the efficacy of first-line pembrolizumab, in a phase II trial that included 26 patients with stage IIIB or IV MCC. The median age of the patients was 68 years, 35% of patients were classified as MCPyV negative, and 65% were MCPyV positive tumors. The median TMB was 1121 muts/Mb e 12.5 muts/Mb in MCPyV negative and MCPyV positive MCC, respectively. Pembrolizumab was administered intravenously at a dose of 2 mg per kilogram of body weight every 3 weeks for a maximum of 2 years or until a complete response, dose-limiting toxic effects, or progressive disease occurred. The median follow-up was 33 weeks. The ORR was 56% and complete response (CR) rate was of 15%. The ORR was 62% among MCPyV positive tumors and 44% in MCPyV negative. Responses with pembrolizumab were durable, and 14 of 26 patients were still on treatment by the time of the final analysis. The estimated progression-free survival rate at 6 months was 67% and 77% of the patients experienced any grade adverse events (AE) during treatment (15% of grade 3 or 4). The most common AE were fatigue and laboratory abnormalities.<sup>66</sup> Recently, updates reported on the complete cohort of 50 patients have confirmed the benefit of pembrolizumab in treatment-naïve patients with advanced disease. After a median follow-up of 14.9 months, a 56% ORR was observed, regardless of MCPyV status, with 24% achieving a CR and a median PFS of 16.8 months for the entire cohort.<sup>67</sup>

**Table II. Performance of different treatment regimens in stage IIIB or IV MCC**

Author	N	Design	Intervention	ORR (%)	12 months PFS (%)	12 months OS (%)
Voog et al.	107	Historical cohort	DOX, CDDP, DTIC, CPM, MTX, 5-FU	57 (1L), 45 (2L) and 20 (3L)	ND	9.1 months
Kaufman et al	88	Phase 2 single arm – Cohort A	Avelumab 10 mg/Kg every 2 weeks	33 (≥ 2L) [11% CR]	30	52
D'Angelo et al	29	Phase 2 single arm – Cohort B	Avelumab 10 mg/Kg every 2 weeks	62.1 (1L) [13% CR]	ND	ND
Ngheim et al	50	Phase 2 single arm	Pembrolizumab 2 mg/Kg every 3 weeks	56 [24% CR]	ND	72
Topalian SL	25	Phase 1/2 trial	Nivolumab 240 mg/Kg every 2 weeks	71 (1L) and 63 (2L or 3L) [14% CR]	ND	ND

The efficacy of nivolumab has also been evaluated in metastatic MCC. In the phase I/II CheckMate-358 trial, patients with 5 subtypes of virus-associated advanced cancers (including MCC), who had received ≤2 prior therapies, were treated with nivolumab 240 mg every 2 weeks until disease progression or unacceptable toxicity. Among 25 treated patients, 60% were treatment naïve; 22 patients were available for radiological response assessment, with ORR of 68%. The ORR were 71% in treatment-naïve patients and 63% in the pretreated subgroup (1-2 prior systemic therapies), with similar activity in both MCPyV positive and MCPyV negative tumors. At 3 months, PFS and OS rates were 82% and 92%, respectively. Treatment-related AE of any grade occurred in 68% of patients and grade 3/4 occurred in 20%.<sup>68</sup> In the cohort of 25 patients with stage IIA to IV resectable MCC, neoadjuvant nivolumab was administered intravenously at a dose of 240 mg on D1 and D15 and surgery was planned on D29. Radiological response was observed in 80% of the 20 evaluable patients. Surgery occurred without significant delay in 88% of the patients and, among 17 surgical specimens evaluated for pathological response, 47% presented CR.<sup>69</sup>

In March of 2017, the Food and Drug Administration approved avelumab to treatment of metastatic MCC based on data published by Kaufman et al, part A of JAVELIN Merkel 200 trial.<sup>70</sup> This phase II trial enrolled 88 patients with stage IV MCC chemotherapy-refractory disease. The median age of the patients was 72.5 years, 59% received one previous systemic anticancer treatment, 66% had tumor PD-L1 expression and 52% of tumors were MCPyV positive. Avelumab was given intravenously at a dose of 10 mg/kg every 2 weeks. After median follow up of 16.4 months the ORR was

33.0% with 11.4% reaching CR. Responses to avelumab were observed irrespective of PD-L1 expression or MCPyV status, but ORR was higher in PD-L1 positive (36.2% vs 18.8%) and in MCPyV negative tumors (35.5% vs 28.3%). The 12 month PFS and OS rate were 30% and 52%, respectively. 72.4% of were ongoing at data cutoff, and a median duration of response (DOR) was not yet reached.<sup>71</sup> The treatment was well tolerated, with an incidence of grade 3 or higher treatment-related AE of 5%. The most common AE were fatigue and infusion-related reactions.<sup>72</sup> Preliminary results from part B of Javelin Merkel 200 trial were presented at the American Society of Clinical Oncology Annual Meeting in 2017.<sup>73</sup> This separate cohort included chemotherapy-naïve patients that received first-line avelumab 10 mg/Kg every 2 weeks. In this prespecified interim analysis, cut off on March 2017, 39 of 112 patients were enrolled in safety analysis and 29 of 112 patients in efficacy analysis. The median of follow-up was 5.1 months, the ORR and CR were 62.1% and 13.8%, respectively. Responses were ongoing in 21/29 patients (72.4%) at last report. Among the 14 patients with at least 6 months of follow-up, the confirmed ORR was 71.4% and CR was 28.6%. Among 39 patients evaluable for safety analysis, 71.8% had a treatment-related AE, with 20.5% of grade 3. No grade 4 or treatment-related deaths occurred.<sup>74</sup>

Unlike other tumors, for which high PD-L1 expression and TMB are well-known predictive biomarkers of response, no biomarker has been proven useful in predicting response to checkpoint inhibitors (CPI) in advanced MCC so far. Georges et al evaluated the association of TMB, PD-L1 expression, MCPyV status and CD8+ tumor-infiltrating T-cell density with ORR and survival. No biomarker alone was predictive of response.<sup>75</sup>

Therefore, CPI are becoming the new standard-of-care for treating patients with metastatic or unresectable MCC. The efficacy of anti-PD-1 and anti-PD-L1 is higher and duration of response is longer

than with chemotherapy and new dates of immunotherapy safety have been reported not only in the treatment of MCC but also in the management of other tumors.<sup>76,77,78</sup>

## PERSPECTIVES

In the metastatic setting, there are newly designed approaches that are currently in progress. These include the open label phase II study NCT01758458 [79] that tests the use of viral oncoprotein targeted laboratory treated autologous T-cell therapy together with aldesleukin (IL-2). In this trial, these T-cells comprehend a polyclonal autologous CD8+ cells that recognize MCPyV T-antigen. Combined modality therapies are also under investigation in NCT03071406 [80]; in this regard, the open label, randomized, phase 2 study of nivolumab + ipilimumab +/- stereotactic body radiation therapy (SBRT) at the start of week 2 of treatment (in both arms, nivolumab is administered every 2 weeks and ipilimumab every 6 weeks until progression or unacceptable toxicity) is

underway. We eagerly await for the results, given the bad prognosis intrinsically associated to this disease.

In the adjuvant scenario, efforts have been made to address the potential role of immunotherapy. An ongoing phase 2, open-label, randomized trial (NCT02196961) of adjuvant nivolumab or ipilimumab as monotherapy in complete resected MCC, with PFS at 12 months as the primary endpoint<sup>81</sup>. To date, despite immature data, in this study, ipilimumab arm is already closed due to worst outcomes. There is also high expectancy on an ongoing phase 3, randomized, double blind, placebo controlled study that investigates the role of avelumab in the setting of clinically positive nodal disease after definitive therapy (surgery with/without adjuvant radiation therapy). The results of both trials are expected after 2021.<sup>82</sup>

## CONCLUSION

Merkel cell carcinoma remains a challenging disease marked by low awareness and numerous diagnostic challenges. Despite optimal upfront therapies that include surgery and radiation therapy, a significant proportion of patients will develop disease recurrence. In this setting, the characterization of the immunogenic potential of MCC has paved the way for the development of immune checkpoint blockade with anti-PD-1

or anti-PD-L1 agents, that now represent the standard of care for patients with irresectable or metastatic disease, leaving the use of CT to those who are not candidates or are refractory to immunotherapy. Ongoing studies addressing the role of these strategies at earlier stages of MCC are under development, and may lead to additional changes in the management of this disease in the near future, translating into even better outcomes for these patients.

## CONFLICTS OF INTEREST

RRM - Research involvement: BMS, Lilly, Merck, MSD, Novartis, Roche. Honoraria: BMS, Merck, MSD, No-

vartis, Roche, Sanofi. Travel grants: BMS, Novartis, Sanofi.

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## AUTHOR'S CONTRIBUTION

**Paulo Henrique do Amor-Divino:** Collection and assembly of data, Conception and design, Data analysis and interpretation, Final approval of manuscript, Manuscript writing.

**Gabriel Passos Souza:** Collection and assembly of data, Manuscript writing.

**Maurício Fernando Silva-Almeida Ribeiro:** Collection and assembly of data, Data analysis and interpretation, Manuscript writing.

**George Horta:** Collection and assembly of data, Manuscript writing.

**Fabíola Ambrosio Silveira-Lima:** Data analysis and interpretation, Manuscript writing.

**Alessandra Corte Real-Salgues:** Data analysis and interpretation, Manuscript writing.

**Rodrigo Ramella Munhoz:** Collection and assembly of data, Conception and design, Data analysis and interpretation, Final approval of manuscript, Manuscript writing

## REFERENCES

1. Toker C. Trabecular carcinoma of the skin. *Arch Dermatol.* 1972; 105:107-10. doi:10.1001/archderm.1972.01620040075020.
2. Uchi H. Merkel cell carcinoma: An update and immunotherapy. *Fron. Oncol.* 2018; 8:48. doi 10.3389/fonc.2018.00048.
3. Hodgson N. Merkel cell Carcinoma: changing incidence trends. *J. Surg. Oncol.* 2005; 89:1-4.
4. Youlden DR, Soyer HP, Youl PH, Fritschi L, Baade PD. Incidence and survival for Merkel cell carcinoma in Queensland, Australia, 1993-2010. *JAMA Dermatol.* 2014; 150(8): 864-72. doi:10.1001/jamadermatol.2014.124.
5. Lanoy E, Costagliola D, Engels EA. Skin cancers associated with HIV infection and solid organ transplant among elderly adults. *Int J Cancer.* 2010; 126(7): 1724. doi: 10.1002/ijc.24931.
6. Paulson KG, Carter JJ, Jonhson LG et al. Antibodies to Merkel Cell Polyomavirus T Antigen Oncoproteins Reflect Tumor Burden in Merkel Cell Carcinoma Patients. *Cancer Research.* 2010; 70(21)8388-8397
7. Feng H, Shuda M, Chang Y, Moore PS. Clonal integration of a Polyomavirus in human Merkel cell carcinoma. *Science.* 2018; 319(5866): 1096-1100. doi: doi:10.1126/science.1152586.
8. Shuda M, Feng H, Kwun HJ, Rosen ST, Gjoerup O, Moore PS et al. T antigens mutations are a human tumor-specific signature for Merkel cell polyomavirus. *Proc Natl Acad Sci USA.* 2008;42(105):16272-277. doi: 10.1073/pnas.0806526105.
9. Sunshine JC, Jahchan NS, Sage J, Choi J. Are there multiple cells of origin of Merkel cell carcinoma?. *Oncogene.* 2018;37:1409-16. doi: 10.1038/s41388-017- 0073-3.
10. Tetzlaff MT, Nagarajan P. Update on Merkel cell carcinoma. *Head and Neck Pathology.* 2018;12:31-43. doi: 10.1007/s12105-018-0898-2.
11. Garneski KM, Warcola AH, Feng Q, Kiviat NB, Leonard JH, Nghiem P. Merkel cell polyomavirus is more frequently present in North American than Australian Merkel cell carcinoma tumors. *Journal of Investigative Dermatology.* 2009;129: 246-48. doi: 10.1038/jid.2008.229.
12. Wong SQ, Waldeck K, Vergara IA, Schröder J, Madore J, Wilmott JS et al. UV- associated mutations underlie the etiology of MCV-negative Merkel cell carcinomas. *Cancer Res.* 2015;75(24):5228-34. doi: 10.1158/0008-5472.CAN-15- 1877.
13. Moshiri AS, Doumani R, Yelistratova L, Blom A, Lachance K, Shinohara MM et al. Polyomavirus-negative Merkel cell carcinoma: a more aggressive subtype based on analysis of 282 cases using multimodal tumor virus detection. *Journal of Investigative Dermatology.* 2017;137:819-27. doi:10.1016/j.jid.2016.10.028.
14. Goh G, Walradt T, Markarov V, Blom A, Riaz N, Doumani R et al. Mutational landscape of MCPyV-positive and MCPyV-negative Merkel cell carcinomas with implications for immunotherapy. *Oncotarget.* 2015;3(7):3403-15.
15. Tang CK, Toker C. Trabecular carcinoma of the skin: an ultrastructural study. *Cancer.* 1978;42:2311-21. doi:10.1002/1097-0142(197811)42:5<2311::AID-CNCR2820420531>3.0.CO;2-L.
16. Sauer CM, Haugg AM, Chteinberg E, Rennspiess D, Winnepenninckx V, Speel E-J et al. Reviewing the current evidence supporting early B-cells as the cellular origin of Merkel cell carcinoma. *Critical Reviews in Oncology/Hematology.* 2017; 116:99-105. doi: 10.1016/j.critrevonc.2017.05.009.
17. Kuromi T, Matsushita M, Iwasaki T, Nonaka D, Kuwamoto S, Nagata K. Association of expression of the hedgehog signal with Merkel cell polyomavirus infection and prognosis of Merkel cell carcinoma. *Human Pathology.* 2017; 69:8-14. doi: 10.1016/j.humpath.2017.05.011.
18. Sihto H, Kukko H, Koljonen V, Sankila R, Böhling T, Joensuu H. Merkel cell polyomavirus infection, large T antigen, retinoblastoma protein and outcome in Merkel cell carcinoma. *Clin Cancer Res.* 2011;17(14):4806-13. doi: 10.1158/1078-0432.CCR-10-3363.
19. Vandeven N, Lewis CW, Makarov V, Riaz N, Paulson KG, Hippe D et al. Merkel cell carcinoma patients presenting without a primary lesion have elevated markers of immunity, higher tumor mutation burden and improved survival. *Clin Cancer Res.* 2017;24(4):963-71. doi: 10.1158/1078-0432.CCR-17-1678.
20. Harms KL, Healy MA, Nghiem P, Sober AJ, Johnson TM, Bichakjian CK. Analysis of prognostic factors from 9387 Merkel cell carcinoma cases forms the basis for the new 8th edition AJCC staging system. *Ann Surg Oncol.* 2016;23: 3364-3571. doi:10.1245/s10434-016-5266-4.
21. Smiths VA, Camp ER, Lentsch EJ. Merkel cell carcinoma: identification of prognostic factors unique to tumors located in the head and neck based on analysis of SEER data. *Laryngoscope.* 2012;122:1283-90. doi:10.1002/lary.23222.
22. Heath M, Jaimes N, Lemos B, Mostaghimi A, Wang LC, Peñas PF et al. Clinical characteristics of Merkel cell carcinoma at diagnosis in 195 patients: the AEIOU features. *J Am Acad Dermatol.* 2008;58(3):375-81.
23. Cheuk W, Kwan MY, Suster S, Chan JK. Immunostaining for thyroid transcription factor 1 and

- cytokeratin 20 aids the distinction of small cell carcinoma from Merkel cell carcinoma, but not pulmonary from extrapulmonary small cell carcinomas. *Arch Pathol Lab Med.* 2001;125(2):228-31. doi: 10.1043/0003-9985(2001)125<0228:IFTFA>2.0.CO;2.
24. Chen AM, Fu JM, Grekin RC, Margolis L. *Cancer of the Skin, Leibel and Phillips Textbook of Radiation Oncology.* 3rd ed. 2010.
  25. Andea AA, Coit DG, Amin B, Busam KJ. Merkel cell carcinoma: histologic features and prognosis. *Cancer.* 2008;113(9):2549-58. doi: 10.1002/cncr.23874.
  26. Tello TL, Coggs K, Yom SS, Yu SS. Merkel cell carcinoma: an update review: current and future therapy. *J Am Acad Dermatol.* 2018;78(3):445-54. doi:10.1016/j.jaad.2017.12.004.
  27. Wong HH, Wang J. Merkel cell carcinoma. *Arch Pathol Lab Med.* 2010; 134(11):1711-1716. doi:10.1043/2009-0165-RSR2.1.
  28. Barksdale SK. Advances in Merkel cell carcinoma from a pathologist's perspective. *Pathology.* 2017;49(6):568-74. doi: 10.1016/j.pathol.2017.07.003.
  29. Martin B, Poblet E, Rios JJ, Kazakov D, Kutzner H, Brenn T et al. Merkel cell carcinoma with divergent differentiation: histopathological and immunohistochemical study of 15 cases with PCR analysis for Merkel cell polyomavirus. *Histopathology.* 2013; 62(5): 711. doi: 10.1111/his.12091.
  30. Paulson KG, Lewis CW, Redman MW et al. Viral Oncoprotein antibodies as marker for recurrence of Merkel cell carcinoma: A prospective validation study. *Cancer,* 2017; 123(8) 1464-1474
  31. Treglia G, Annunziata S, Sobic-Saranovic D, Bertagna F, Caldarella C, Giovanella L. The role of 18F-FDG-PET and PET-CT in patients with sarcoidosis: an updated evidence-based review. *Acad Radiol.* 2014; 21(5):675-84. doi: 10.1016/j.acra.2014.01.008.
  32. Amin M, Edge S, Greene F, et al, eds. *AJCC Cancer Staging Manual.* 8th ed. New York, NY: Springer, 2017.
  33. Fields RC, Busam KJ, Chou JF, Panageas KS, Pulitzer MP, Kraus DH et al. Recurrence after complete surgical resection and selective use of adjuvant therapy for stage I through III Merkel cell carcinoma. *Cancer.* 2012;118(13): 3311-20.
  34. Fields RC, Busam KJ, Chou JF, Panageas KS, Pulitzer MP, Kraus DH et al. Recurrence and survival in patients undergoing sentinel lymph node biopsy for Merkel cell carcinoma: analysis of 153 patients from a single institution. *Ann Surg Oncol.* 2011;18(9):2529-37. doi: 10.1245/s10434-011-1662-y.
  35. Senchenkov A, Barnes SA, Moran SL. Predictors of survival and recurrence in the surgical treatment of merkel cell carcinoma of the extremities. *J Surg Oncol.* 2007;95(3):229-34.
  36. Bhatia S, Storer BE, Iyer JG, Moshiri A, Parvathaneeni U, Byrd D et al. Adjuvant radiation therapy and chemotherapy in Merkel cell carcinoma: survival analyses of 6908 cases from the national cancer data base. *J Natl Cancer Inst.* 2016; 108 (9): djw042. doi: 10.1093/jnci/djw042.
  37. Veness MJ, Perera L, McCourt J, Shannon J, Hughes TM, Morgan GJ et al. Merkel cell carcinoma: improved outcome with adjuvant radiotherapy. *ANZ J Surg.* 2005;75(5):275-81. doi: 10.1111/j.1445-2197.2005.03353.x
  38. Poulsen M. Merkel-cell carcinoma of the skin. *Lancet Oncology,* 2004; 5(10)593- 599
  39. Gillenwater AM, Hessel AC, Morrison WH et al. Merkel cell carcinoma of the Head and Neck Effect of Surgical Excision and Radiation on Recurrence and Survival. *Arch Otolaryngol Head Neck Surg,* 2001; 127(2):149-154
  40. Hukko H, Bohling T, Koljonen V, et al. Merkel cell carcinoma – a population- based epidemiological study in Finland with a clinical series of 181 cases. *Eur J of Cancer,* 2012; 48: 737-742
  41. Lebbe C, Becker JC, Grob JJ, et al. Diagnosis and treatment of Merkel Cell Carcinoma. European consensus-based interdisciplinary guideline. *Eur J Cancer,* 2015; 51(16) 2396-2403
  42. Miller SJ, Alam M, Andersen J, Berg D, Bichakjian CK, Bowen G et al. Merkel cell carcinoma: Clinical practice guidelines in oncology™. *JNCCN Journal of the National Comprehensive Cancer Network.* 2006;4(7):704-12.
  43. Allen PJ, Zhang ZF, Coit DG. Surgical management of Merkel cell carcinoma. *Ann Surg.* 1999;229(1): 97-105.
  44. O'Connor WJ, Roenigk RK, Brodland DG. Merkel Cell Carcinoma: Comparison of Mohs micrographic surgery and wide excision in eighty-six patients. *Dermatol Surg.* 1997;23(10):929-33.
  45. Lebbe C, Becker JC, Grob J-J, Malvehy J, del Mar mol V, Pehamberger H et al. Diagnosis and treatment of Merkel cell carcinoma. European consensus-based interdisciplinary guideline. *Eur J Cancer.* 2015;51(16):2396-2403.
  46. Medina-Franco H, Urist MM, Fiveash J, Heslin MJ, Bland KI, Beenken SW. Multimodality treatment of Merkel cell carcinoma: case series and literature review of 1024 cases. *Ann Surg Oncol.* 2001;8(3):204-8.
  47. Miller SJ, Alam M, Andersen J, Berg D, Bichakjian CK, Bowen G et al. Merkel cell carcinoma: Clinical practice guidelines in oncology™. *JNCCN Journal of the National Comprehensive Cancer Network.* 2006;4(7):704-12.
  48. Schwartz JL, Griffith KA, Lowe L, Wong SL, McLean SA, Fullen DR, et al. Features predicting sentinel lymph node positivity in Merkel cell carcinoma. *J Clin Oncol.* 2011; 29(8):1036-41.

49. Hitchcock CL, Bland KI, Laney RG, Franzini D, Harris B, Copeland EM. Neuroendocrine (Merkel cell) carcinoma of the skin. Its natural history, diagnosis, and treatment. *Ann Surg.* 1988;207(2):201-7.
50. Eng TY, Boersma MG, Fuller CD, Goytia V, Jones WE, Joyner M, et al. A comprehensive review of the treatment of Merkel cell carcinoma. *Am J Clin Oncol.* 2007;30(6):624-36.
51. Gupta SG, Wang LC, Peñas PF, Gellenthin M, Lee SJ, Nghiem P. Sentinel lymph node biopsy for evaluation and treatment of patients with Merkel cell carcinoma: the Dana-Farber experience and meta-analysis of the literature. *Arch Dermatol.* 2006;142(6):685-690.
52. Sadeghi R, Adinehpour Z, Maleki M, Fallahi B, Giovanella L, Treglia G. Prognostic significance of sentinel lymph node mapping in Merkel cell carcinoma: systematic review and meta-analysis of prognostic studies. *BioMed Res Int.* 2014;2014. doi: 10.1155/2014/489536.
53. Lewis KG, Weinstock MA, Weaver AL, Otley CC. Adjuvant local irradiation for Merkel cell carcinoma. *Arch Dermatol.* 2006;142(6):693-700.
54. Poulsen M, Rischin D, Walpole E, Harvey J, Mackintosh J, Ainslie J et al. High-risk Merkel cell carcinoma of the skin treated with synchronous carboplatin/etoposide and radiation: a Trans-Tasman Radiation Oncology Group Study-TROG 96:07. *J Clin Oncol.* 2003;21(23):4371-6.
55. Poulsen MG, Rischin D, Porter I, Walpole E, Harvey J, Hamilton C et al. Does chemotherapy improve survival in high-risk stage I and II Merkel cell carcinoma of the skin? *Int J Radiat Oncol Biol Phys.* 2006;64(1):114-9.
56. Fields RC, Busam KJ, Chou JF, et al. Recurrence after complete resection and selective use of adjuvant therapy for stage I through III Merkel Cell Carcinoma. *Cancer,* 2012; 3311-3320
57. Chen MM, Roman SA, Sosa JA, Judson BL. The role of adjuvant therapy in the management of head and neck Merkel cell carcinoma: an analysis of 4815 patients. *JAMA Otolaryngol Head Neck Surg.* 2015;141(2):137-41.
58. Adjuvant therapy of completely resected Merkel cell carcinoma with immune checkpoint blocking antibodies versus observation [Internet]. Available from: <https://clinicaltrials.gov/ct2/show/NCT02196961>.
59. NCCN Guidelines Version 1.2018 Panel Members. Merkel cell carcinoma. 2018. 2018. p. [https://www.nccn.org/professionals/physician\\_gls/P](https://www.nccn.org/professionals/physician_gls/P).
60. Voog E, Biron P, Martin J-P, Blay J-Y. Chemotherapy for patients with locally advanced or metastatic Merkel cell carcinoma. *Cancer.* 1999;85(12):2589-95.
61. Tai PTH, Yu E, Winquist E, Hammond A, Stitt L, Tonita J et al. Chemotherapy in neuroendocrine/Merkel cell carcinoma of the skin: case series and review of 204 Cases. *J Clin Oncol.* 2000;18:2493-99. doi: 10.1200/JCO.2000.18.12.2493.
62. Becker JC, Lorenz E, Ugurel S, Eigentler TK, Kiechler F, Pföhler C et al. Evaluation of real-world treatment outcomes in patients with distant metastatic Merkel cell carcinoma following second-line chemotherapy in Europe. *Oncotarget.* 2017;8(45):79731-41.
63. Nghiem P, Kaufman HL, Bharmal M, Mahnke L, Phatak H, Becker JC. Systematic literature review of efficacy, safety and tolerability outcomes of chemotherapy regimens in patients with metastatic Merkel cell carcinoma. *Future Oncol.* 2017;13(14):1263-79. doi:10.2217/fon-2017-0072.
64. Desch L, Kunstfeld R. Merkel cell carcinoma: chemotherapy and emerging new therapeutic options. *J Skin Cancer.* 2013; 2013:327150. doi: 10.1155/2013/327150
65. Chan IS, Bhatia S, Lipson EJ, et al. Immunotherapy for Merkel cell carcinoma: a turning point in patient care. *Journal of Immunotherapy of Cancer,* 2018; 6: 23
66. Nghiem PT, Bhatia S, Lipson EJ, Kudchadkar RR, Miller NJ, Annamalai L et al. PD-1 blockade with pembrolizumab in advanced Merkel cell carcinoma. *N Engl J Med.* 2016;374(26):2542-52. doi: 10.1056/NEJMoa1603702.
67. Nghiem PT, Bhatia S, Lipson EJ, et al. Durable Tumor Regression and Overall Survival in Patients with Advanced Merkel Cell Carcinoma Receiving Pembrolizumab as First-Line Therapy. *Journal of Clinical Oncology,* 2019; 37(9) 693-702
68. Topalian SL, Bhatia S, Hollebecque A, Awada A, Boer JPD, Kudchadkar RR et al. Abstract CT074: non-comparative, open-label, multiple cohort, phase 1/2 study to evaluate nivolumab (NIVO) in patients with virus-associated tumors (Check-Mate 358): efficacy and safety in Merkel cell carcinoma (MCC). *Cancer Res.* 2017; 77: CT074-CT074.
69. Topalian SL, Bhatia S, Kudchadkar RR, et al. Nivolumab as neoadjuvant therapy in patients with resectable Merkel Cell Carcinoma in Check-Mate 358. 2018 ASCO Annual Meeting. Abstract 9505. Presented June 4, 2018
70. Kaufman HL, Russell J, Hamid O, Bhatia S, Terheyden P, D'Angelo SP et al. Avelumab in patients with chemotherapy-refractory metastatic Merkel cell carcinoma: a multicentre, single-group, open-label, phase 2 trial. *Lancet Oncol.* 2016;17(10):1374-85. doi: 10.1016/S1470-2045(16)30364-3.

71. Kaufman HL, Russell JS, Hamid O, Bhatia S, Terheyden P, D'Angelo SP et al. Updated efficacy of avelumab in patients with previously treated metastatic Merkel cell carcinoma after  $\geq 1$  year of follow-up: JAVELIN Merkel 200, a phase 2 clinical trial. *J Immunother Cancer*. 2018;6(1):1-7. doi: 10.1186/s40425-017-0310-x.
72. Joseph J, Zobniw C, Davis J, Anderson J, Trinh VA. Avelumab: A review of its application in metastatic Merkel cell carcinoma. *Ann Pharmacother*. 2018; 00(0):1-8. doi: <https://doi.org/10.1177/1060028018768809>.
73. D'Angelo SP, Russell J, Hassel JC, Lebbe C, Chmielowski B, Rabinowits G et al. First-line (1L) avelumab treatment in patients (pts) with metastatic Merkel cell carcinoma (mMCC): preliminary data from an ongoing study. *J Clin Oncol*. 2017; 35(15\_suppl (May 20 2017)): 9530-30.
74. D'Angelo SP, Russell J, Lebbé C, Chmielowski B, Gambicher T, Grob J-J et al. Efficacy and safety of first-line avelumab treatment in patients with stage IV metastatic Merkel cell carcinoma. *JAMA Oncol*. 2018;10065:1-5. doi:10.1001/jamaoncol.2018.0077.
75. Georges S, Shah PK, Shapiro I, et al. Integrative molecular analysis of metastatic Merkel cell carcinoma to identify predictive biomarkers of response to avelumab. 2019 ASCO Annual Meeting. Abstract 9569
76. Chan IS, Bhatia S, Kaufman HL, Lipson EJ. Immunotherapy for Merkel cell carcinoma: a turning point in patient care. *J Immunother Cancer*. 2018; 6:23. doi:10.1186/s40425-018-0335-9.
77. Wang M, Ma X, Guo L, Xia F. Safety and efficacy profile of pembrolizumab in solid cancer: pooled reanalysis based on randomized controlled trials. *DrugDesDevelTher*.2017;11:2851-60.doi:10.2147/DDDT.S146286.
78. Kelly K, Infante JR, Taylor MH, Patel MR, Wong DJ, Iannotti N et al. Safety profile of avelumab in patients with advanced solid tumors: a pooled analysis of data from the phase 1 JAVELIN solid tumor and phase 2 JAVELIN Merkel 200 clinical trials. *Cancer*. 2018;124(9):2010-17. doi: 10.1002/cncr.31293.
79. [www.clinicaltrials.gov/ct2/show/NCT01758458](http://www.clinicaltrials.gov/ct2/show/NCT01758458). Consulted in April 22, 2018.
80. [www.clinicaltrials.gov/ct2/show/NCT03071406](http://www.clinicaltrials.gov/ct2/show/NCT03071406). Consulted in April 22, 2018.
81. [www.clinicaltrials.gov/ct2/show/NCT02196961](http://www.clinicaltrials.gov/ct2/show/NCT02196961). Consulted in April 22, 2018.
82. [www.clinicaltrials.gov/ct2/show/NCT03271372](http://www.clinicaltrials.gov/ct2/show/NCT03271372). Consulted in April 22, 2018.