

REVIEW ARTICLE

Merkel cell carcinoma update: the case for two tumours

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Abstract

Merkel cell carcinoma (MCC) is an aggressive tumour with neuroendocrine differentiation. Clinically significant differences within the entity we know as MCC are apparent. This review aims to evaluate the evidence for differences in tumours within Merkel cell carcinoma and to stratify these. A literature search of research pertaining to various characteristics MCC was undertaken from 1972, when Merkel cell carcinoma was first described, to 2018, using PubMed and similar search engines. A total of 41 papers were analysed, including clinical trials, laboratory-based research and reviews. A proportion of MCC has Merkel cell polyomavirus genome integrated (MCPyV+) while others do not (MCPyV-). Both types have a different mutation burden. MCPyV+ tumours are likely true neuroendocrine carcinomas, with a dermal origin, probably from fibroblasts which have been transformed by integration of the viral genome. MCPyV- tumours are likely derived from either keratinocytes or epidermal stem cells, are probably squamous cell carcinomas with neuroendocrine differentiation, and are related to sun damage. Prognostic factors (apart from tumour stage) include the MCPyV status, with MCPyV+ tumours having a better prognosis. P63 expression confers a worse prognosis in most studies. CD8+ lymphocytes play an important role, providing a possible target for PD1/PD-L1 blockade treatment. The incidence of MCC varies from country to country. Countries such as Australia have a high rate and a far greater proportion of MCPyV- tumours than places such as the United Kingdom. MCC doubtlessly encompasses two tumours. The two tumours have demonstrated differences in prognosis and management. One is a neuroendocrine carcinoma related to MCPyV integration likely derived from fibroblasts, and the other is a UV-related squamous cell carcinoma with neuroendocrine differentiation, presumptively derived from either keratinocytes or epidermal stem cells. We propose naming the former Merkel type sarcoma and the latter squamous cell carcinoma, Merkel type.

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Conflicts of interest

The authors declare that they do not have any conflict of interest.

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Introduction

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Materials and methods

A literature search of research pertaining to various characteristics MCC was undertaken from 1972, when Merkel cell carcinoma was first described, to 2018, using PubMed and

similar search engines. A total of 41 papers were analysed, including clinical trials, laboratory-based research and reviews.

Results and discussion

Biology of merkel cell carcinoma: evidence for two tumours

Merkel cell carcinoma is a rare cutaneous tumour with neuroendocrine differentiation. The most common sites on the body are correlated with areas exposed to ultraviolet radiation (UVR),

with the head and neck accounting for 48.8% of cases, followed by the upper limbs and the legs in women.¹

Merkel cell carcinoma was first described by Toker² in 1972 as trabecular carcinoma of the skin in a study of five patients. The tumours were in elderly patients, both male and female. They originated in the dermis and were composed of solid trabecula. Toker postulated origin from cells capable of reproducing sudoriferous structures.

In the early 1980s, electron microscopic studies showed a resemblance of tumour cells to cutaneous Merkel cells and it was then thought trabecular carcinoma arose from them.³ In 1982, three cases of combined small cell carcinoma and squamous cell carcinoma (SCC) were described and thought to be part of the spectrum of trabecular carcinoma⁴ (Fig. 1).

In 2008, Feng *et al.*⁵ demonstrated DNA from a previously unknown polyomavirus integrated into the genome of 8 of 10 MCC, with six having a clonal pattern. Only 8% of various control tissues and 16% of control skin samples demonstrated this viral genome integration. The researchers named the virus Merkel cell polyomavirus (MCPyV) and postulated that it may be a contributing factor to the development of MCC.⁵

Kuwamoto *et al.* in 2011⁶ investigated whether MCC differs in the presence or absence of Merkel cell polyomavirus genome integration (MCPyV). The virus was found only in pure MCC and not in those showing mixed features of MCC and SCC. They concluded that the virus plays an important role in the pathogenesis of MCPyV-positive (MCPyV+) MCC.

In serological studies, Nicol *et al.*⁷ showed that MCPyV has a high prevalence in the community which increases in prevalence throughout early life, with a seroprevalence of 41.7% in children aged 1–4 years, 87.6% in those aged 15–19 years and 79.0%–96.2% in adulthood. Nicol hypothesized that exposure occurs in childhood with reactivation in older age groups as a result of waning immunity. Furthermore, virus-associated malignancies, including MCC, are increased in immunosuppressed people.⁸

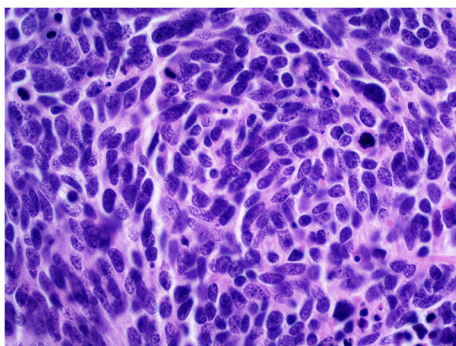


Figure 1 Merkel cell carcinoma. Photo by Dr. Alexander Nirenberg.

Garneski *et al.*⁹ compared the rates of MCPyV in MCC in North America and Australia. They found an overall prevalence of 43% in the 37 MCCs tested, with a prevalence of 69% (11 of 16) in tumours from North America and only 24% (five out of 21) tumours from Australia. They also found a prevalence of 13% (two out of 15) in SCC. Garneski raised two possibilities for the difference: firstly, there may be increased sun exposure in Australia, making a possible viral contribution to pathogenesis less frequent. However, they could not exclude the possibility of a different undetectable strain of MCPyV or another virus being present.⁹

Virus+ and virus-negative tumours have been shown to have important biological and clinical differences. Goh *et al.*¹⁰ showed that MCPyV-negative (MCPyV-) MCCs have a high mutation burden (median of 1121 somatic single nucleotide variants per exome), with frequent mutations in genes RB1 and TP53 as well as mutations in JNK (MAP3K1 and TRAF7) and DNA damage pathways (ATM, MSH2 and BRCA1). MCPyV+ tumours had a low mutation burden (median 12.5 somatic single nucleotide variants per exome) with none in the above genes.

González-Vela *et al.*¹¹ noted the types of mutations found in MCPyV- MCCs have a UV signature comparable to melanomas, including enrichment for C to T transitions. They further found shared nuclear accumulation of oncogenic transcription factors (NFAT, P-CREB, P-STAT3).¹¹

In the largest study to date, of 282 cases, Moshiri *et al.*¹² found that MCPyV- tumours had a poorer prognosis than MCPyV+ tumours. MCPyV- tumours were smaller at presentation (1.1 vs. 1.9 cm) but were more likely to present with advanced disease (66.7% vs. 48.3%) and had a 1.8-fold higher risk of progression and a 1.79-fold higher risk of death from MCC. This supports the findings of a previous study by Sihto *et al.*¹³ of 60 cases. However, in a study of 127 cases Schrama *et al.*¹⁴ found no significant survival difference based on viral status.

Using these data, Sunshine *et al.*¹⁵ postulated that the cell of origin for MCC originates in two distinct locations in the skin, one protected from UV light (for MCPyV+ MCC) and the other heavily exposed to UV irradiation (for MCPyV- MCC). Sunshine postulated a compelling argument that Merkel cells, found in the basal layer of the epidermis, are not of the cell origin of MCPyV+ MCC. The vast majority of MCPyV+ MCC are dermal in origin. There are also important immunohistochemical staining differences between the two, and Merkel cells are terminally differentiated cells that arise from differentiated pluripotent epidermal stem cells. The tumour suppressor genes RB1 and TP53 are frequently mutated in MCPyV- MCCs.¹⁰ This may induce epigenetic reprogramming of cancer cells, leading to lineage plasticity and may promote transdifferentiation into a Merkel cell-like neuroendocrine phenotype¹⁵ (Fig. 2).

The evidence points to an epidermal origin for MCPyV- MCC. The burden of mutations in MCPyV-MCC is similar to

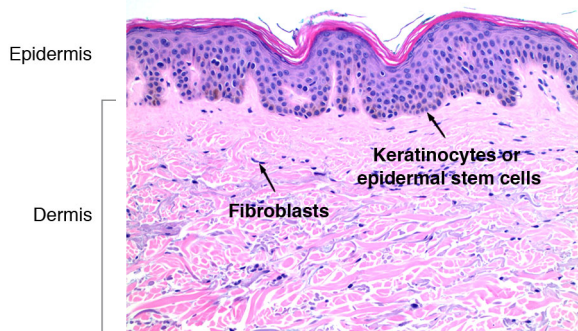


Figure 2 Possible cells of origin. Photo by Dr. Alexander Nirenberg.

those found in UV light-affected, keratinocyte-derived tumours. Furthermore, while a proportion of MCPyV⁻ MCCs are associated with epidermal SCC *in situ* or invasive SCC, no MCPyV⁺ cases associated with these skin cancers have been reported to date.¹⁶

Both keratinocytes and Merkel cells are derived from the same epidermal progenitor cell,¹⁷ and it is possible that MCPyV⁻ MCC may arise from either keratinocytes or the progenitor cell.¹⁵ However, MCPyV⁺ MCCs have few mutations which are not UV signature mutations. This suggests they may not derive from keratinocytes or epidermal progenitor cells.¹⁵

Liu *et al.*¹⁸ showed that dermal fibroblasts were the host cells for MCPyV and that MCPyV infection is stimulated by matrix metalloproteinase (MMP) genes induction by the WNT/B-catenin signalling pathway and other growth factors. Liu suggested that MCC risk factors such as UV radiation and ageing, which stimulate WNT signalling and MMP expression may promote viral infection and thus drive growth of MCC.¹⁸

Should there be two different mechanisms stimulating MCC from different cell origins, then both pathways must lead to neuroendocrine differentiation. RB1 and TP53 mutations induce epigenetic reprogramming of cancer cells, leading to lineage plasticity and, change in phenotype, including neuroendocrine features.¹⁵ RB1 and TP53 are frequently mutated in MCPyV⁻ MCCs.¹⁰

In a recent study¹⁹ of copy number aberrations and next-generation sequencing detected mutations, Carter *et al.* investigated a cohort of 46 MCCs. Histologically pure tumours consist only of neuroendocrine cells whereas combined tumours contain both neuroendocrine and squamous cell regions. Carter studied 9 histologically pure MCPyV⁺, 9 histologically pure MCPyV⁻ and 10 histologically combined MCPyV-negative tumours for copy number aberrations. The entire cohort of 46 MCCs was studied with next-generation sequencing. They found that pure and combined MCPyV⁻ tumours had more copy number aberrations and a greater fraction of the genome was changed than

the MCPyV⁺ cases. No differences were found between pure and combined MCPyV⁻ tumours. Copy number loss and/or RB1 inactivating mutations was common in combined and pure MCPyV⁻ tumours (80% and 78%, respectively) but not in MCPyV⁺ tumours (11%). Similar results were found for TP53, with copy number losses and/or mutations in 20% of combined and 56% of pure MCPyV⁻ tumours and 0% in MCPyV⁺ tumours.¹⁹ Carter suggests that in combined tumours the original clone may be squamous cell in type, which phenotypically diverges to a neuroendocrine carcinoma in the dermis. The alternative explanation is bidirectional squamous/neuroendocrine tumours.¹⁹

Another hypothesis is that MCC may be derived from early B cells. In 2013, Zur Hausen *et al.* found that 100% of 21 Merkel cell carcinomas tested, expressed PAX5 and 72.8% expressed TdT, markers which are normally co-expressed on pro/pre-B cells and pre-B cells. Most of the MCCs also expressed one or more classes of immunoglobulin. This led the authors to propose that this was the cell of origin in MCC.²⁰

Murakami *et al.*, in a study of 10 MCPyV⁻ and 20 MCPyV⁺ MCCs, found that 60% of MCPyV⁺ but no MCPyV⁻ MCCs expressed at least one immunoglobulin. There was no difference in the expression of PAX5, TdT, Oct-2 and SOX11 between the two groups of MCC. Expression of IgG and PAX5 has been reported in other epithelial carcinomas and may be the result of viral infection. They concluded that expression of these is likely a reflection of cancer-associated dysregulation of protein expression rather than an indication of lymphocytic origin of MCC.²¹ Sauer *et al.*,²² in a recent review paper, however did not exclude this possibility.

Epidemiology

The incidence of MCC varies by country, with a range of 0.10–1.60 per 100 000-person years (100K-py). Schadendorf²³ analysed a total of ten countries: United States, Germany, Denmark, Sweden, Eastern France, Netherlands, Australia, New Zealand, England and Scotland. The lowest rate was in England (0.10), and the highest in Australia (1.60), with the United States in the middle (0.79). The high rate in Australia may be due to its high UV incidence with some papers reporting an increasing incidence.²³

Merkel cell carcinoma is more common in men, with an incidence of 0.4–2.5 per 100 000 for men and 0.18–0.9 per 100 000 for women.²³

Merkel cell carcinoma is a tumour of older Caucasians. In a study in New Zealand by Robertson *et al.*,¹ the average age at diagnosis was 77.2 years, with an overall incidence of 0.88 per 100K-py. The incidence rose from 0.03 per 100K-py in the 35–39 years age group to 17.56 per 100K-py in the 85+ age group.

In a study in Queensland, Australia, which has a high UVR incidence, Youlden *et al.*²⁴ showed similar trends. Between 2006 and 2010, the authors noted an overall incidence of 1.6 per

100 000 population, with 2.5 per 100 000 population for men and 0.9 per 100 000 population for women. The rate peaked at 20.7 per 100 000 population for those 80 years and older. They noted an increase in age-standardized incidence of 2.6% per year from 1993 onwards.

Select prognostic factors

Immune suppression both increases the risk of acquiring MCC and has an adverse effect on prognosis.²⁵ In a study of 195 patients by Heath *et al.*,²⁶ 7.8% of MCC patients had profound immune suppression. Causes included HIV, chronic lymphocytic leukaemia (CLL) and solid organ transplant (SLT). In a larger study of 471 cases by Paulson *et al.*,²⁵ 8.7% of patients had clinically recognized systemic immune suppression, including patients with HIV/AIDS, CLL, other haematological malignancies and long-term immunosuppressive treatment for autoimmune disease or SLT. This study also found a statistically significant reduction in survival in immunosuppressed individuals vs. immune competent patients, with an MCC-specific survival of 40% vs. 73% at 3 years.²⁵

Patients with MCPyV+ tumours have a better prognosis than those with MCPyV– tumours.¹³ P63 is a marker of stratified epithelia, including cutaneous squamous cell epithelium. P63 expression by MCC may be an adverse prognostic factor. In a study of 83 patients, Fleming's group²⁷ showed that staining for P63 correlated with an adverse prognosis, regardless of whether the staining was focal or diffuse. This result was in keeping with several previous studies, although two studies failed to show such a link.^{28,29} P63, as an independent risk factor, was not predicative in multivariate analysis, with age and clinical stage being the only independent parameters.²⁷ This study showed that other significant factors predicting a poorer prognosis were larger tumour size, combined rather than pure tumours and the nature of the lymphocytic infiltrate.²⁷

The role of lymphocytes

In vitro experiments showed that MCPyV+ tumours were dependent on expression of viral T-antigen for survival.³⁰ Paulson *et al.*³¹ found that the circulating level of antibodies against the T-antigen was proportional to disease burden.

In a study of tumour-infiltrating lymphocytes, Sihto *et al.*³² found that MCPyV+ tumours had a statistically significant greater infiltrate of T cells (CD3+), natural killer cells (CD16+) and macrophages (CD68+) compared to MCPyV– tumours. Furthermore, high CD3+ count was associated with a better prognosis in both MCPyV+ and MCPyV– tumours. This was an independent prognostic indicator. Natural killer cells and macrophages' counts were not significant prognostic markers. They further found that of the CD3+ cells, a high Tc cell (CD8+) and regulatory T-cell (FOXP3+) counts were associated with favourable survival, whereas Th (CD4) counts were not. Patients

with a low CD8+/CD4+ or FOXP3+/CD4+ ratio had a poor outcome.³²

Combining viral status with immune cell infiltration levels suggests that patients with MCPyV+ tumours and high CD3+ and CD8+ counts had the best outcome. Sihto *et al.*³³ propose that this may pave the way for immune therapy of MCC with immunostimulatory antibodies such as anticytotoxic T-lymphocyte-associated protein 4 (CTLA-4) and anti-programmed cell death-1 (PD-1).

In a study of 137 MCCs, Paulson *et al.*³⁴ showed that CD8+ infiltration was an independent prognostic factor with denser infiltrates associated with a better prognosis. Three-year MCC-specific survival rates were 56%, 72% and 100% for patients with absent, low and moderate to strong intratumoural lymphocytic infiltrates. A similar trend was observed for overall survival; however, this did not reach statistical significance. The authors hypothesized a high rate of non-MCC-related deaths among older patients.³⁴

Walsh *et al.*³⁵ also found that all inflammatory cells except for B cells and plasmacytoid dendritic cells were present in both intra- and peritumoural locations, with CD8+ cells showing a strong predilection for the intratumoural environment. The CD8+ cells were between and in direct contact with the tumour cells. CD4+ cells and macrophages were located mainly around vessels. This study supported the concept of an anti-tumoural role for CD8+ cells.³⁵

In a larger study of 62 cases, Feldmeyer *et al.*³⁶ showed that increased CD8+ cells at the periphery of the tumour and CD3+ cells at the centre or periphery of the tumour were associated with reduced risk of visceral metastases. Increased density of PD1+ cells at the periphery of the tumour reduced the risk of metastases to any site, including lymph nodes beyond the sentinel node and visceral organs.³⁶ This study confirmed improved survival correlated with increased density of CD8+ cells, especially density at the tumour periphery. Five hundred CD8+ cells/mm², at the tumour periphery, were associated with a 61% reduction in the risk of MCC-specific death. The impact on survival was greater in MCPyV+ tumours. These had greater density of CD8+ cells (median 674.9/mm² vs. 213.3/mm² for MCPyV– tumours). There was no statistically significant difference in PD-L1 positivity between MCPyV+ and MCPyV– tumours.³⁶

Refining these studies, Walsh *et al.*³⁵ evaluated 22 patients and developed a morphological and immunotypic map of the immune response in MCC, providing a global semi-quantitative and topographic appreciation of the relevant agonists and antagonists involved in this process.³⁵ They also found that in three of four cases with available biopsy tissue, there was no significant difference in tumour-infiltrating lymphocyte (TIL) density between the original biopsy and the subsequent excisional specimen. There was a significant correlation between TILs & MCPyV+ status and a non-statistically significant trend with

histologically pure MCC. The TIL infiltrates were concentrated at the stromal-tumour interface, with piecemeal penetration of the tumour periphery and perivascular tracking of lymphocytes into the centre of the tumour. T cells predominated with a median CD8:CD4 ratio of 30% : 20%. Remaining cells were B cells (20%) and macrophages (30%).³⁵

Lipson *et al.*³⁷ investigated the potential of MCC being a target for PD-1/PD-L1 blockade. PD-1 expressed by T cells is the receptor for PDL-1, which is expressed by other immune and stromal cells. Binding of PD-L1 to PD-1 inhibits T-cell function, thus dampening the immune response. They found that PD-L1 expression was co-located with immune infiltrates. They also saw an association between the presence of MCPyV DNA, a brisk inflammatory response and PD-L1 expression. This suggested that a local tumour-specific and potentially MCPyV-specific immune response drives tumour PD-L1 expression. In multivariate analysis, PD-L1 tumours were independently associated with worse overall survival.³⁷

Current conventional therapies

The mainstay of MCC treatment remains surgical excision to investing muscle fascia or periosteum with 1–2 cm clinical margins. In sites where margin tissue sparing is a factor, Mohs surgery (MS), modified MS, or complete circumferential peripheral and deep margin assessment with permanent sections may be considered. If these alternatives are utilized, a debulking specimen of the central tumour should be sent for microstaging. These alternative excisional techniques should not interfere with sentinel lymph node biopsy (SNLB), when indicated.³⁸

To identify metastatic spread, all patients should be considered for a whole body PET scan or neck/chest/abdomen/pelvis CT with contrast, with or without brain MRI. MCPyV antibody status may be evaluated as part of the initial workup. Additional workup and treatment recommendations include clinical and ultrasound examination of local lymph nodes, with SLNB for clinical N0 disease. SLN+ patients should undergo complete node dissection or radiation therapy (RT) to the nodal basin. For SLN- patients, clinical observation or adjuvant RT may be considered to the primary tumour site and in the case of high-risk patients, to the nodal basin. For disseminated disease, immune checkpoint inhibition is recommended (refer to section Immunotherapy) unless inappropriate, in which case conventional chemotherapy may be given. Close follow-up is maintained.³⁸

Immunotherapy

The discovery of the role of CD8+ lymphocytes and the PD1-PD-L1 immune inhibitory pathway in MCC laid the groundwork for new therapies. Tumours may upregulate PDL1 as adaptive immune resistance, dampening the immune response against the tumour. Tumeh *et al.*³⁹ showed that pre-existing

CD8 T cells distinctly located at the invasive tumour margin are associated with expression of the PD-1/PD-L1 immune inhibitory axis.

In view of these findings, two recent trials were undertaken to test the effectiveness of PD-1 immune inhibitory pathway blockade in the management of MCC. Nghiem *et al.*⁴⁰ treated 26 patients with the PD-1 blocker pembrolizumab. This was a multicentre phase 2, non-controlled trial of patients with advanced MCC who had not received previous systemic therapy. The objective response rate among the 25 patients who had at least one evaluation was 56%, with four patients having a complete response and 10 having a partial response. The median follow-up of 33 weeks (range 7–52 weeks) and the response duration was 2.2–9.7 months. Relapse occurred in two of 14 patients (14%) who experienced an initial response. Seventeen of the 26 patients (65%) had MCPyV+ tumours. Response rates were 62% for patients with MCPyV+ tumours and 44% for those MCPyV-. Fifteen percent of patients had drug-related grade 3 or 4 adverse effects.⁴⁰

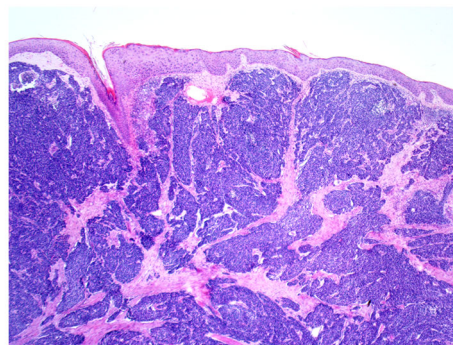


Figure 3 Pure neuroendocrine carcinoma. Photo by Dr. Alexander Nirenberg.

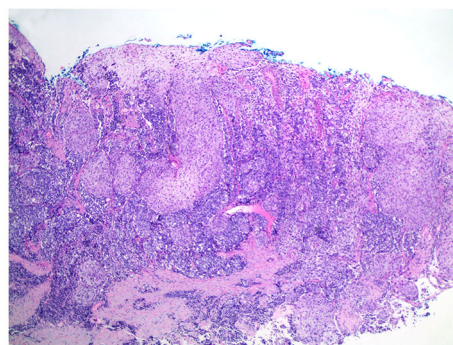


Figure 4 Combined squamous cell and neuroendocrine carcinoma. Photo by Dr. Alexander Nirenberg.

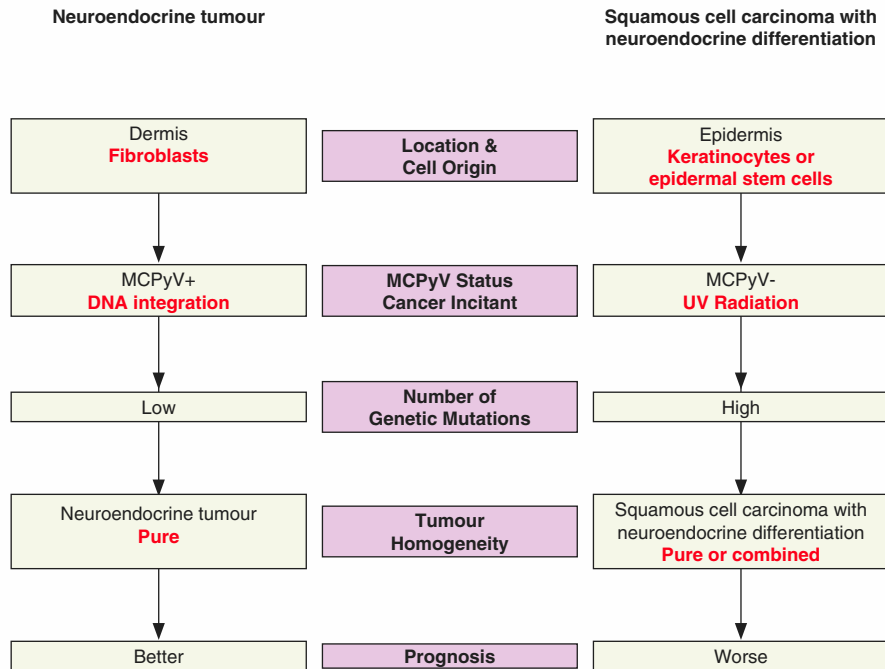


Figure 5 Summary.

Kaufman *et al.*⁴¹ conducted a phase-2, single-group open-label trial of the 88 patients with stage IV MCC which were refractory to chemotherapy, using the anti-PD-L1 monoclonal antibody avelumab. Median follow-up was for a median 10.4 months (range 8.6–13.1). An objective response was seen in 31.8% of cases (28 patients), with eight complete and 20 partial responses. Responses were ongoing in 23 of the 28 patients (82%) at the time of analysis.

Conclusion

Merkel cell carcinoma is an aggressive tumour with neuroendocrine differentiation. Evidence supports the hypothesis that there are two subtypes of MCC, with distinct pathophysiology and prognosis.

The first is a pure neuroendocrine carcinoma, possibly arising from dermal fibroblasts, related to MCPyV DNA integration. This tumour has a low number of genetic mutations and has a high expression of RB1. It has a better prognosis (Fig. 3).

The second is probably a subtype of SCC with neuroendocrine differentiation, forming pure or combined tumours. It is related to UV radiation and is likely derived from either keratinocytes or an epidermal stem cell for keratinocytes (and Merkel cells) (Fig. 4). It is MCPyV– and has a high mutation load and high P63 expression. This subtype has a worse prognosis (Fig. 5).

Given the two very different patterns and behaviours, nomenclature should be adjusted to reflect different tumours. We propose naming the former Merkel type sarcoma and the latter squamous cell carcinoma, Merkel type.

An important prognostic factor is the presence of CD8+ lymphocytes. There is involvement of the PD1-PDL1 immune inhibitory pathway. This has paved the way for immune therapy, which is a promising addition to treatment options.

References

- Robertson JP, Liang ES, Martin RC. Epidemiology of Merkel cell carcinoma in New Zealand: a population-based study. *Br J Dermatol* 2015; **173**: 835–837.
- Toker C. Trabecular carcinoma of the skin. *Arch Dermatol* 1972; **105**: 107–110.
- Sibley RK, Rosai J, Foucar E, Dehner LP, Bosl G. Neuroendocrine (Merkel cell) carcinoma of the skin. A histologic and ultrastructural study of two cases. *Am J Surg Pathol* 1980; **4**: 211–221.
- Tang CK, Toker C, Nedwich A, Zaman AN. Unusual cutaneous carcinoma with features of small cell (oat cell-like) and squamous cell carcinomas. A variant of malignant Merkel cell neoplasm. *Am J Dermatopathol* 1982; **4**: 537–548.
- Feng H, Shuda M, Chang Y, Moore PS. Clonal integration of a polyomavirus in human Merkel cell carcinoma. *Science* 2008; **319**: 1096–1100.
- Kuwamoto S, Higaki H, Kanai K *et al.* Association of Merkel cell polyomavirus infection with morphologic differences in Merkel cell carcinoma. *Hum Pathol* 2011; **42**: 632–640.
- Nicol JT, Robinot R, Carpentier A *et al.* Age-specific seroprevalences of merkel cell polyomavirus, human polyomaviruses 6, 7, and 9, and

- trichodysplasia spinulosa-associated polyomavirus. *Clin Vaccine Immunol* 2013; **20**: 363–368.
- 8 Schulz TF. Cancer and viral infections in immunocompromised individuals. *Int J Cancer* 2009; **125**: 1755–1763.
 - 9 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. *J Invest Dermatol* 2009; **129**: 246–248.
 - 10 Goh G, Walradt T, Markarov V *et al*. Mutational landscape of MCPyV-positive and MCPyV-negative Merkel cell carcinomas with implications for immunotherapy. *Oncotarget* 2016; **7**: 3403–3415.
 - 11 González-Vela MDC, Curiel-Olmo S, Derdak S *et al*. Shared oncogenic pathways implicated in both virus-positive and UV-induced Merkel cell carcinomas. *J Invest Dermatol* 2017; **137**: 197–206.
 - 12 Moshiri AS, Doumani R, Yelistratova L *et al*. Polyomavirus-negative merkel cell carcinoma: a more aggressive subtype based on analysis of 282 cases using multimodal tumor virus detection. *J Invest Dermatol* 2017; **137**: 819–827.
 - 13 Sihto H, Kukko H, Koljonen V, Sankila R, Böhlting T, Joensuu H. Clinical factors associated with Merkel cell polyomavirus infection in Merkel cell carcinoma. *J Natl Cancer Inst* 2009; **101**: 938–945.
 - 14 Schrama D, Peitsch WK, Zapatka M *et al*. Merkel cell polyomavirus status is not associated with clinical course of Merkel cell carcinoma. *J Invest Dermatol* 2011; **131**: 1631–1638.
 - 15 Sunshine JC, Jahchan NS, Sage J, Choi J. Are there multiple cells of origin of Merkel cell carcinoma? *Oncogene* 2018; **37**: 1409–1416.
 - 16 Martin B, Poblet E, Rios JJ *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**: 711–722.
 - 17 Woo SH, Stumpfova M, Jensen UB, Lumpkin EA, Owens DM. Identification of epidermal progenitors for the Merkel cell lineage. *Development* 2010; **137**: 3965–3971.
 - 18 Liu W, Yang R, Payne AS *et al*. Identifying the target cells and mechanisms of Merkel cell polyomavirus infection. *Cell Host Microbe* 2016; **19**: 775–787.
 - 19 Carter MD, Gaston D, Huang WY *et al*. Genetic profiles of different subsets of Merkel cell carcinoma show links between combined and pure MCPyV-negative tumors. *Hum Pathol* 2018; **71**: 117–125.
 - 20 Zur Hausen A, Rennspiess D, Winpenninckx V, Speel EJ, Kurz AK. Early B-cell differentiation in Merkel cell carcinomas: clues to cellular ancestry. *Cancer Res* 2013; **73**: 4982–4987.
 - 21 Murakami I, Takata K, Matsushita M *et al*. Immunoglobulin expressions are only associated with MCPyV-positive Merkel cell carcinomas but not with MCPyV-negative ones: comparison of prognosis. *Am J Surg Pathol* 2014; **38**: 1627–1635.
 - 22 Sauer CM, Haugg AM, Chteinberg E *et al*. Reviewing the current evidence supporting early B-cells as the cellular origin of Merkel cell carcinoma. *Crit Rev Oncol Hematol* 2017; **116**: 99–105.
 - 23 Schadendorf D, Lebbé C, Zur Hausen A *et al*. Merkel cell carcinoma: epidemiology, prognosis, therapy and unmet medical needs. *Eur J Cancer* 2017; **71**: 53–69.
 - 24 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**: 864–872.
 - 25 Paulson KG, Iyer JG, Blom A *et al*. Systemic immune suppression predicts diminished Merkel cell carcinoma-specific survival independent of stage. *J Invest Dermatol* 2013; **133**: 642–646.
 - 26 Heath M, Jaimes N, Lemos B *et al*. Clinical characteristics of Merkel cell carcinoma at diagnosis in 195 patients: the AEIOU features. *J Am Acad Dermatol* 2008; **58**: 375–381.
 - 27 Fleming KE, Ly TY, Pasternak S, Godlewski M, Doucette S, Walsh NM. Support for p63 expression as an adverse prognostic marker in Merkel cell carcinoma: report on a Canadian cohort. *Hum Pathol* 2014; **45**: 952–960.
 - 28 Lim CS, Whalley D, Haydu LE *et al*. Increasing tumor thickness is associated with recurrence and poorer survival in patients with Merkel cell carcinoma. *Ann Surg Oncol* 2012; **19**: 3325–3334.
 - 29 Higaki-Mori H, Kuwamoto S, Iwasaki T *et al*. Association of Merkel cell polyomavirus infection with clinicopathological differences in Merkel cell carcinoma. *Hum Pathol* 2012; **43**: 2282–2291.
 - 30 Houben R, Shuda M, Weinkam R *et al*. Merkel cell polyomavirus-infected Merkel cell carcinoma cells require expression of viral T antigens. *J Virol* 2010; **84**: 7064–7072.
 - 31 Paulson KG, Carter JJ, Johnson LG *et al*. Antibodies to merkel cell polyomavirus T antigen oncoproteins reflect tumor burden in merkel cell carcinoma patients. *Cancer Res* 2010; **70**: 8388–8397.
 - 32 Sihto H, Böhlting T, Kavola H *et al*. Tumor infiltrating immune cells and outcome of Merkel cell carcinoma: a population-based study. *Clin Cancer Res* 2012; **18**: 2872–2881.
 - 33 Sihto H, Joensuu H. Tumor-infiltrating lymphocytes and outcome in Merkel cell carcinoma, a virus-associated cancer. *Oncimmunology* 2012; **1**: 1420–1421.
 - 34 Paulson KG, Iyer JG, Simonson WT *et al*. CD8+ lymphocyte intratumoral infiltration as a stage-independent predictor of Merkel cell carcinoma survival: a population-based study. *Am J Clin Pathol* 2014; **142**: 452–458.
 - 35 Walsh NM, Fleming KE, Hanly JG *et al*. A morphological and immunophenotypic map of the immune response in Merkel cell carcinoma. *Hum Pathol* 2016; **52**: 190–196.
 - 36 Feldmeyer L, Hudgens CW, Ray-Lyons G *et al*. Density, distribution, and composition of immune infiltrates correlate with survival in Merkel cell carcinoma. *Clin Cancer Res* 2016; **22**: 5553–5563.
 - 37 Lipson EJ, Vincent JG, Loyo M *et al*. PD-L1 expression in the Merkel cell carcinoma microenvironment: association with inflammation, Merkel cell polyomavirus and overall survival. *Cancer Immunol Res* 2013; **1**: 54–63.
 - 38 Bichakjian CK, Olencki T, Aasi SZ *et al*. Merkel cell carcinoma, version 1.2018, NCCN clinical practice guidelines in oncology. *J Natl Compr Canc Netw* 2018; **16**: 742–774.
 - 39 Tumei PC, Harview CL, Yearley JH *et al*. PD-1 blockade induces responses by inhibiting adaptive immune resistance. *Nature* 2014; **515**: 568–571.
 - 40 Nghiem PT, Bhatia S, Lipson EJ *et al*. PD-1 Blockade with pembrolizumab in advanced Merkel-cell carcinoma. *N Engl J Med* 2016; **374**: 2542–2552.
 - 41 Kaufman HL, Russell J, Hamid O *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**: 1374–1385.