Merkel cell carcinoma (MCC) is an aggressive neuroendocrine cutaneous cancer that predominantly occurs in patients who are older, and is associated with a high rate of distant failure and mortality. Current management strategies that incorporate surgery and radiotherapy achieve high rates of locoregional control, but distant failure rates remain problematic, highlighting the need for new effective systemic therapies. Chemotherapy can achieve high response rates of limited duration in the metastatic setting, but its role in definitive management remains unproven. Recent developments in our knowledge about the biology of MCC have led to the identification of new potential therapeutic targets and treatments. A key finding has been the discovery that a human polyomavirus may be a causative agent. However, emerging data suggests that MCC may actually be two distinct entities, viral-associated and viral-negative MCC, which is likely to have implications for the management of MCC in the future and for the development of new treatments. In this review, we discuss recent discoveries about the biology of MCC, current approaches to management, and new therapeutic strategies that are being investigated.

Merkel cell carcinoma is an uncommon neuroendocrine carcinoma that mostly arises in sun exposed areas, with the head and neck being the most frequent site. There is geographic variation in incidence with higher rates in Australia than in the United States. It predominantly occurs in patients who are older, with onset occurring at a median age of 75 to 80, and it is more common in males. Recognized risk factors are ultraviolet (UV) sunlight exposure and immunosuppression, and more recently, the Merkel polyomavirus (MCV) has been identified as a causative agent.3

Pathologically, MCC has features of a trabecular neuroendocrine carcinoma arising from Merkel cells, which act as sensory touch receptors in the basal layer of the epidermis.4,5 A diagnosis of MCC from small cell lung cancer is challenging based on morphology alone, but can often be distinguished by the presence of CK20 and absence of TTF-1 with immunohistochemical (IHC) staining.6 Approximately 15% of MCCs are diagnosed at the metastatic stage without evidence of a primary and appear to be associated with a better prognosis.7 Heath et al have recently defined the clinical features that may serve as clues to the diagnosis, summarized by the acronym AEIOU: asymptomatic/lack of tenderness, expanding rapidly, immunosuppression, older than age 50, and UV exposed site on a fair-skinned person.8

BIOLOGY

Merkel Cell Polyoma Virus

A higher risk of developing MCC in patients who are immunosuppressed provided a strong impetus to search for a viral etiology in MCC. The search for viral sequences in tumors was subsequently enabled by the development of deep transcriptome sequencing, which led to the seminal discovery of the MCV in 2008.3 Despite the now clear oncogenic role of the virus, MCV is likely to be part of normal skin flora, as viral DNA can frequently be detected at low levels in the normal skin of healthy individuals.9 This is further supported by serologic studies showing that children can be infected by MCV from a very young age and that up to 80% of adults in North America have been exposed to the virus by age 50.10 MCV is a double-stranded DNA virus that harbors large and small-T antigens (LT; ST) required for modulation of the host cell and viral replication. The oncogenic potential of MCV is thought to only occur on chance sequential events of clonal integration into the host genome and then acquisition of mutations in the 3′ end of the LT. Mutations in the LT truncate the C-terminus of the oncoprotein, disrupting the helicase domain, which renders the virus replication incompetent. This may be important for several reasons, but primarily to prevent cell death through inappropriate DNA replication at integration sites, which would lead to...
replication-fork collisions and DNA-strand breakage. Importantly, the mutation does not affect the functional ability of LT to sequester the retinoblastoma protein (RB), a cell cycle regulator and tumor suppressor that is frequently disrupted in cancer.

Continuous expression of T-antigens have been shown to be required for maintenance of MCV-positive cell lines. However, unlike the related and intensively studied simian vacuolating virus 40 (SV40), the expression of MCV LT alone does not appear to be sufficient to transform cells, which is in contrast to the MCV ST, which has more potent oncogenic potential. MCV ST has been shown to act downstream of mTOR in the PI3K/AKT/mTOR signaling pathway, preventing dephosphorylation of 4E-BP1, which regulates cap-dependent translation of mRNAs. More recently, the MCV ST has been shown to bind to cellular FBXW7, which is a subunit of the SKP1/CULLIN1/F-box (SCF) protein ubiquitin ligase complex that negatively regulates the LT and other cellular proto-oncogenes such as cMYC and cyclin E. A transgenic mouse harboring MCV ST driven by a bovine keratin 5 promoter has been recently described (K5-ST). Induction of K5-ST in adult mice resulted in epidermal transformation and squamous cell carcinoma in situ. The mice, however, failed to develop MCC, which indicates there is perhaps a requirement of both LT and ST expression or that targeted expression is required in an alternative Merkel cell precursor cell type. Development of an immunocompetent MCC animal model would be an invaluable research tool for the preclinical evaluation of novel therapies.

Viral-Negative MCC

In Europe, the United States, and Japan, the frequency of cases with MCV is variable, but generally thought to be approximately 80% of MCC tumors. This still leaves a substantial fraction of viral-negative cases with a relatively unknown biology. Clues to the underlying origins of viral-negative tumors can be drawn from observations in different geographic regions. In Australia, the association of MCV infection with MCC appears to be much lower. Three independent studies from major Australian cities report a frequency of 18% to 24%, with the exception being a study from Sydney reporting a similar frequency to that found in Germany (80%). Variability in the reported prevalence of viral-associated MCC between studies has been attributed to the choice of the individual antibody used for IHC detection of viral LT expression in tumors. However, the difference between studies in Australia and the Northern hemisphere would appear outside the expected variance between IHC assays. Furthermore, IHC and polymerase chain reaction detection of viral DNA have both been used in Australian studies and these assays have been shown to be mostly concordant.

A higher prevalence of MCC in the Australian population with a predominance of viral-negative tumors suggests that excessive sun damage is likely key to the pathogenesis of the viral-negative subtype. Potential mechanisms explaining increased risk associated with sun damage could involve combined effects of localized immune suppression and the strong mutagenic effects of UV-mediated DNA damage. A telling indicator of UV damage is the DNA mutation signature involving C to T transitions at dipyrimidine bases (i.e., CC to TT) frequently observed in skin cancers such as melanoma. Importantly, these types of mutations have been identified in MCC tumors through sequencing the TP53 tumor-suppressor gene.

Further cumulative evidence to support the notion of two subtypes includes the histologic, clinical, and molecular differences that have been observed between viral and nonviral MCC. MCV-positive and -negative tumors have been reported to have specific morphologic differences. Viral status has also been associated with different growth properties of the respective cell lines. Gene expression profiling can also broadly cluster MCC tumors, which is predominantly driven by an elevated immune signature in the viral-positive group. A poorer prognosis has been associated with viral-negative MCC in some studies, although this has not been consistently demonstrated in different patient cohorts.

Genetic differences observed between MCC tumors based on viral status can be explained in part by the convergent, yet distinct mechanisms for disruption of common pathways. As previously mentioned, the tumor-suppressor protein RB is a key target of the polyoma virus LT. Viral-negative tumors show low to absent protein expression of RB and RB1 copy number loss and loss-of-function mutations account for genetic disruption in a large proportion of viral-negative MCC tumors. Pathogenic mutations in TP53 and overexpression of the p53 protein (commonly associated with TP53 mutation), also appear to be largely restricted to viral-negative tumors. Curiously, unlike SV40, the MCV LT is not thought to directly regulate p53 and therefore may be altered through an indirect mechanism. Oncogenic muta-
tions in PIK3CA predominantly occur in viral-negative tumors, although most MCC cancers regardless of viral status show activated PI3K/AKT signaling and are responsive to PI3K and dual PI3K/mTOR inhibitors. 

Despite evidence for the aberrant expression of common oncogenic and tumor suppressor pathways in MCC, the search for somatic mutations in other known cancer genes has revealed few tangible leads to date. Genome-wide copy number analysis has revealed recurrent large regions of chromosomal gain and loss with more alterations observed in viral-negative tumors. Focal high-level amplification of 1p34 harboring the MYCL1 oncogene is a striking feature in approximately 40% of MCC cases, highlighting an interesting molecular parallel to small cell lung cancer.

A general paucity of new cancer gene discoveries in MCC may reflect the focused interrogation of just a few genes and the small sample sets analyzed to date. Furthermore, given the already potent oncogenic potential of MCC, it is plausible that this disease subtype is genetically quite simple and does not require additional cooperative gene mutations. Focused interrogation of the viral-negative group, which is ostensibly driven by mutagenic effects of sun damage, could be more informative from a genetic perspective and it will be interesting to search for convergence involving mutations in genes targeted by viral LT and ST. A “genomic landscape” interrogation of MCC by massively parallel (next-generation) sequencing is therefore clearly required.

**MANAGEMENT OF MCC**

The main aim of current treatment strategies is to obtain locoregional control without inducing unnecessary toxicity. Cases should be managed in the context of a specialized multidisciplinary team to ensure care can be individualized and all potential treatment modalities considered.

Optimal management of MCC is a therapeutic challenge in part because of its rarity and lack of high-quality evidence to direct treatment. Although we can extrapolate management principles from other skin cancers, such as melanoma and squamous cell carcinoma, MCC differs importantly from these conditions by being extremely sensitive to radiotherapy.

Potential treatment paradigms have been published previously, are readily available, and include the 2015 National Comprehensive Cancer Network (NCCN) guidelines. In this review, we highlight recent developments and some of the differences in approach to the management of MCC.

**Staging**

MCC is associated with high rates of early nodal and distant metastatic spread. Relapse in nodal stations reportedly occurs in up to 76% of cases and is associated with a substantial reduction in survival. Effective staging of nodal and distant metastatic disease at diagnosis is essential. Previously, this has been limited to diagnostic CT scanning, but in recent years other useful staging modalities have emerged.

**PET.** MCC exhibits high fludeoxyglucose (FDG) avidity on PET scanning, permitting the detection of involved subcentimeter nodes that may not be appreciated on the initial CT. Although there are no published multicenter, prospective studies evaluating FDG PET/CT in MCC, there are many retrospective, single-institution studies that have suggested that FDG PET may have a role, as most studies have found that PET changes stage and management in up to 30% of patients. The largest study published to date from the Peter MacCallum Cancer Centre assessed 102 consecutive patients who were staged with PET/CT, which resulted in changed staging in 22% of patients and management in 37% of patients. On multivariable analysis of prognostic factors, PET stage was associated with overall survival. In general, these studies were too small to report the positive and negative predictive value, but a recent systematic review of the literature suggested that PET/CT has a sensitivity of 90% and a specificity of 98% in detecting MCC.

Although there has been no clear evidence to date that PET/CT staging will substantially change clinical outcomes for patients, a change in stage and treatment intent (and/or modality) in up to 30% of patients warrants its inclusion in to the current NCCN guidelines as a potential staging tool. In our department, PET/CT staging is recommended for all patients with T2 or clinically node-positive disease. An ongoing study being conducted by the Trans-Tasman Radiation Oncology Group (TROG) is prospectively evaluating the role of PET/CT in a multicenter trial of patients with MCC (ClinicalTrials.gov identifier NCT01013779).

**Sentinel lymph node biopsy (SLNB).** SLNB has become a standard tool in the nodal staging of MCC in many centers. SLNB detects lymph node spread in up to one-third of patients whose tumors would have otherwise been staged as N0. MCC series data indicate an SLN positivity rate of 20% in T1 tumors, and 45% to 50% in T2 lesions. Primary tumor factors such as size, depth of invasion, and lymphovascular invasion may be prognostic, but this has not been demonstrated conclusively. In a large analysis of prognostic factors in 5,823 patients with MCC, investigators found that pathologic evaluation of node involvement was substantially better at prognosticating with regard to survival compared with clinical/radiologic examination alone. Certainly, SLNB in MCC appears to be able to detect microscopic nodal spread and is prognostic for survival.

Most series indicate that patients with SLNB-positive disease receive treatment to the nodal region. Regional relapse rates following treatment in this setting are very low. However, in the setting of a negative SLNB result, it is less clear whether prophylactic treatment can be omitted. The false-negative rate of SLNB has been re-
ported to be 10% to 20%. This may be particularly problematic in the head and neck region where SLNB may be less accurate as a result of the complex and variable lymphatic drainage, postoperative tissue changes, and the presence of more than one sentinel lymph node.

SLNB has been recommended as a standard procedure for the staging of patients with MCC in the NCCN guidelines. This is not unreasonable, but patients should be carefully selected for the procedure in light of the limited evidence of efficacy in some settings (postreconstructive surgery, immunosuppressed patients, or high-risk lesions). In our department, patients with lesions larger than 2 cm are considered sufficiently high risk to warrant prophylactic nodal irradiation, regardless of a SLNB result and so it is not recommended. For patients with tumors smaller than 2 cm, we will recommend SLNB in patients with tumors larger than 1 cm and is planned as part of their initial surgery. In patients with lesions smaller than 1 cm, we make an individual assessment based on risk factors. Further studies are required to better define the benefit and utility of SLNB.

**Definitive Management**

**Stage I and II disease (node-negative).** Treatment in stage I and II MCC generally involves surgical resection of the primary tumor with clear margins followed by adjuvant radiotherapy. However, it is not necessary to obtain wide or even clear surgical margins if this would compromise cosmesis or function, or delay planned adjuvant radiotherapy. Adjuvant radiotherapy provides superior locoregional control rates compared with surgery alone. Patients with very small primary tumors (< 1 cm), or negative SLNB with clear margins and no adverse features, such as lymphovascular invasion and immunosuppression, may be able to avoid adjuvant radiotherapy. If surgery is not feasible or refused, definitive radiotherapy can achieve high rates of locoregional control. Veness et al reported a 3-year locoregional control rate of 75% with radiation alone in a population with poor prognosis, but overall 60% of patients relapsed, most commonly outside the radiation field.

**Stage III disease (node-positive).** Stage IIIA disease (microscopic nodal) is usually detected via SLNB, and treatment of the nodal basin is recommended with radiotherapy or lymphadenectomy. Both modalities achieve excellent results with radiotherapy permitting concurrent adjuvant treatment to the primary site. In IIIB disease, clinically evident node disease can be treated with lymphadenectomy and radiotherapy, or definitive radiotherapy. The NCCN guidelines recommend initial surgery as the standard therapy in this setting, although a different approach may be adopted, particularly for MCC localized to the head and neck. Radiotherapy alone has been shown to provide good regional control of gross node disease, with isolated regional recurrence being uncommon. The MD Anderson Cancer Center has recently reported its experience with radiotherapy in MCC localized in the head and neck with 96% local and regional control rates, and notably no regional recurrences in 22 patients with gross nodal disease who were treated with radiation alone.

If IIIB disease is treated surgically it is likely that the majority of patients would be recommended for postoperative radiotherapy based on multiple nodes or extracapsular extension. Decisions about the optimal approach must take into account the lack of evidence that bimodality treatment is more effective in achieving regional control than radiotherapy alone, as well as the predominant distant pattern of failure, and the additional toxicity and effect on quality of life associated with bimodality treatment.

In view of the high risk of distant metastases, and the similarities to small cell carcinoma of the lung, there has been interest in incorporating chemotherapy into the definitive management of patients at high risk. The TROG 96.07 trial evaluated the treatment of 53 patients with high-risk local and nodal MCC with radiotherapy and four cycles of carboplatin and etoposide. Radiotherapy doses were moderate (50 Gy/25 fractions), and the bulk of disease treated ranged from microscopic to lesions larger than 5 cm. Gross node disease was present in 62% of patients. The 3-year overall survival, locoregional control, and distant control was 76%, 75%, and 76%, respectively. However, a high febrile neutropenia rate was observed that predominantly occurred during the peak of the radiation skin reaction. A subsequent trial demonstrated that giving weekly carboplatin during radiation followed by adjuvant carboplatin and etoposide was much better tolerated. However, the single arm design of these trials does not permit any definitive conclusions about efficacy. Retrospective comparisons to patients treated with radiation alone have yielded mixed results with some studies finding no evidence of benefit, whereas a recent analysis restricted to head and neck primaries has suggested improved overall survival with chemoradiation. The role of chemotherapy in this setting remains unproven, and could only be established by a randomized trial.

**Stage IV disease (distant metastases).** Distant metastases develop in 20% to 30% of patients with MCC. The mainstay of anticancer management of metastatic MCC has been chemotherapy, which achieves high response rates (60% to 75%), but of limited duration. Based on apparent similarities to small cell carcinoma, regimens such as platinum and etoposide or cyclophosphamide, doxorubicin, and vincristine are most commonly used for first-line chemotherapy. However, there is limited evidence to guide decision making, with no randomized trials evaluating different regimens, and limited data about the effect of chemotherapy on survival, symptom benefit, or quality of life. Bearing in mind that patients are frequently older with comorbidities, many patients are not good candidates for chemotherapy and are best managed by supportive care alone.

**NEW TREATMENTS**

The viral etiology and an epidemiologic link to immunosuppression suggest that immunotherapies may be effective in
treating MCC tumors. Many MCC tumors elicit a strong immune response with brisk infiltrates of intratumoral CD8+ T-cells (TILs), which is an independent indicator of better survival.22-24 Viral-antigen specific CD8+ T-cells can also be detected in the peripheral blood of patients with MCC and fluctuate in response to treatment.25 Antibody blockade of immune checkpoint receptors and ligands, such as CTLA-4 and PD-1/PD-L1 that reactivate cytotoxic T-cell activity, have demonstrated durable responses in the treatment of refractory solid tumors.76,77 Importantly, viral specific T-cells in MCC express PD-1 and high tumor specific expression of the ligand PD-L1 has been observed in viral-positive, but not viral-negative tumors.75,78 Phase II trials using anti-PD-L1 (MSB0010718C; NCT02155647), anti-PD-1 (pembrolizumab; NCT02267603), and anti-CTLA-4 (ipilimumab; NCT02196961) are currently open. An alternative or complementary immune strategy is adoptive immunotherapy, which involves the isolation of tumor-specific autologous T-cells from a patient, which are then cultured in vivo and infused back into the patient. This strategy has proven effective for treating melanoma.79 Methods have been described for the isolation of MCV-specific cytotoxic T-cells from patients with MCC,80 and a phase I/II clinical trial using autologous T-cell therapy with aldesleukin is currently underway (NCT01758458).

Targeting dysregulated cell growth and proliferation pathways within MCC tumors presents another potential therapeutic avenue. MCC tumors overexpress receptor tyrosine kinases such as cKIT, PDGFR, and VEGFR2.81-83 Despite promising early preclinical evaluation of imatinib (targeting KIT and PDGF), a low response rate was observed in a clinical trial, although a complete response has been reported elsewhere.84 The multikinase inhibitor pazopanib targets receptor tyrosine kinases, including cKIT, FGFR, PDGFR, and VEGFR, and a complete response to this drug has been observed in MCC resistant to cytotoxic therapy.85 A phase II trial of pazopanib in patients with neuroendocrine tumors including MCC is currently open (NCT01841736). Cabozantinib, which targets VEGFR2/cMET, is being investigated in a phase II MCC trial (NCT02036476). As previously mentioned, MCC tumors may be responsive to inhibition of the PI3K/AKT/mTOR axis, and a number of clinical trials are currently active for treatment of solid tumors using PI3K inhibitors.

A low level of apoptosis is a feature of MCC. BCL-2, a prosurvival member of the intrinsic apoptosis pathway, is overexpressed in approximately 80% of MCC tumors.86 Oblimersen sodium (G3139), a phosphorothioate antisense oligonucleotide that targets BCL-2, demonstrated good efficacy in a preclinical assessment,87 but proved ineffective in patients with MCC.88 The orally available drug ABT-263, which targets multiple BCL-2 family members, has also demonstrated preclinical activity against MCC cell lines by inducing apoptotic death.89 Theoretically, ABT-263 may be more effective than G3139 in patients, given that it targets multiple BCL-2-family members. Survivin is a member of the inhibitor of apoptosis family and is upregulated by MCV LT.90 High survivin expression corresponds with an aggressive clinical course and poor prognosis in patients with MCC.91 Survivin expression can be attenuated using sepantronium bromide, also called YM155.92 Treatment of MCC xenografts with YM155 demonstrated cytostatic response in MCC xenografts; however, this has yet to be tested in patients with MCC.93

The somatostatin receptor type 2 (SSTR2) is expressed in 90% of MCC tumors.94 Somatostatin analogs such as octreotide bind to SSTR2 and elicit antiangiogenic, antitumor, and antiproliferative responses in functional and nonfunctional neuroendocrine tumors.95 Long-term response to octreotide has been reported in MCC,96 and a French phase II trial testing the efficacy of the drug lanreotide is about to begin (NCT02351128). Peptide receptor radionuclide therapy (PRRT) involves covalent attachment of radioactive isotopes to somatostatin peptide analogs (e.g., 177lutetium octreotide). Given the exquisite radiosensitivity of MCC cells, there is a strong rationale for using PRRT in MCC. There have been several case reports demonstrating responses to PRRT used alone or with chemotherapy.97,99

Other biologically-targeted therapeutic strategies involve the use of antibody-drug conjugates and immunocytokines. Most MCC tumors demonstrate cell surface expression of CD56 (NCAM).100 Lorvotuzamab mertansine (IMGN901) is a conjugate of a humanized anti-CD56 antibody with maytansinoids, such as DM1 (a microtubule targeting agent). The drug IMGN901 has showed efficacy against MCC in early phase trials and has been granted orphan status by the U.S. Food and Drug Administration (FDA). The immunocytokine F16-interleukin (IL)-2 is the fusion of the monoclonal antibody fragment F16 specific to tenascin-C fused to IL2. Tenascin-C is an angiogenesis marker and expressed in the reactive stroma of many solid tumors, whereas IL-2 is a potent immune stimulator. Preclinical studies have shown efficacy in human xenograft models of breast carcinoma and glioblastoma,101,102 and phase IB and II trials in solid tumors and breast cancer have shown the drug is well tolerated and efficacious in some patients. A phase II trial using F16-IL2 in combination with paclitaxel for metastatic MCC is currently underway (NCT02054884).

CONCLUSION
Current management strategies achieve high rates of locoregional control, but distant failure rates remain problematic. Management of metastatic disease is challenging; chemotherapy can achieve high response rates of limited duration and is often associated with toxicity in this older population. The recent upsurge in our understanding of the biology of MCC is opening up new potential therapeutic targets and treatments. Finally, the recognition that MCC may be two distinct entities, viral-associated and viral-negative MCC, is likely to have implications for the management of MCC in the future and for the development of new treatments, somewhat analogous to oropharyngeal cancer following the identification of the HPV as a causative agent.
Disclosures of Potential Conflicts of Interest

Relationships are considered self-held and compensated unless otherwise noted. Relationships marked “L” indicate leadership positions. Relationships marked “I” are those held by an immediate family member; those marked “B” are held by the author and an immediate family member. Institutional relationships are marked “Inst.” Relationships marked “U” are uncompensated.


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