Update on the Biology and Clinical Management of Merkel Cell Carcinoma

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OVERVIEW

Merkel cell carcinoma (MCC) is a rare but aggressive neuroendocrine cutaneous malignancy, with a predilection for sun-exposed sites in elderly patients. Despite an incidence 30 times less than that of melanoma, its disease-specific mortality is three times higher. Management of MCC remains challenging because of a limited understanding of its molecular biology, lack of prospective clinical trials, and limitations associated with retrospective reviews of therapeutic options. With the recent discovery of an associated human polyomavirus, significant progress has been made in the understanding of the pathogenesis of this malignancy. With this progress, there has been increasing optimism regarding new tools in the therapeutic armamentarium to fight this deadly disease. Here we present an overview on MCC with an emphasis on the most recent biologic discoveries and the rationale for novel targeted and immunotherapies.

Merkel cell carcinoma (MCC) is an aggressive neuroendocrine skin cancer most commonly thought to originate from the Merkel cells of the basal layer of the epidermis. This is supported by its shared cytoplasmic dense-core neuroendocrine granules and keratin filament (cytokeratin-20) expression characteristics, although some have argued that it may arise from an epidermal or dermal pluripotent stem cell. First described histologically in 1972 by Cyril Toker as “trabecular carcinoma of the skin,” detection of MCC markedly improved over the next 20 years with the development of electron microscopy in the late 1970s and immunohistochemistry in the 1990s. The reported MCC incidence has tripled in the last 20 years as a result of improved detection as well as an increased number of patients with relevant risk factors, including age greater than 50, ultraviolet (UV) exposure, and chronic immunosuppression. Despite this increased incidence, it remains an orphan malignancy, with approximately 1,600 new cases per year in the United States.

MCC generally affects elderly individuals, with the average age at diagnosis reported as 76. Clinical features of this cancer have been previously summarized by Heath et al. using the acronym “AEIOU”: asymptomatic/lack of tenderness, expanding rapidly, immune suppression, older than age 50, and UV-exposed site on a person with fair skin. The majority of patients present with skin-limited disease (66%), followed by nodal (27%) and distant metastasis (7%). The lymph node basin is the most common site of metastasis (60%), followed by distant skin (30%), lung (23%), central nervous system (18%), and bone (15%). The 5-year disease-specific survival has been reported to be 56% to 64%, with a reported 0% to 18% 5-year survival for those with metastatic disease at presentation.

BIOLOGIC UPDATES

Merkel Cell Polyomavirus

In 2008, a new human polyomavirus, Merkel cell polyomavirus (MCPyV), was identified by Feng et al and posited to be a contributing factor in the pathogenesis of MCC. MCPyV is a small circular double-stranded DNA virus, belonging to the family Polyomaviridae; it shares genera, Orthopolyomavirus, with the more well-known John Cunningham (JC) and BK polyomaviruses. The 5,387 base pairs of MCPyV encode typical features of polyomaviruses, including large tumor (T) antigen (LT-Ag) and small T antigen (ST-Ag). These are coded within the early region, which promotes cell cycle entry and replication of viral DNA. The T antigens are separated from the late region, which encodes structural capsid proteins VP1, VP2, and VP3 by a noncoding control region. This noncoding region serves as the viral promoter/replication origin. Through genomic sequences, Shuda et al identified selected mutations prematurely truncating the MCPyV LT-Ag helicase in all nine MCC tumors studied, in contrast to none in the four viruses from nontumor sources. These mutations were found not to affect retinoblastoma protein (pRB) binding, but to eliminate viral DNA replication capacity and subsequently cell death. ST-Ag appears to be an independent oncoprotein, acting downstream in the mammalian target of
rapamycin (mTOR) pathway and targeting 4E binding protein 1 regulator, ultimately enhancing cell transformation. The capsid proteins, although likely critical for initial infection, are not thought to promote tumor development.

In Feng et al’s seminal publication, MCPyV sequences were detected in eight of 10 (80%) tumor samples, compared to four of 25 (16%) normal skin control samples and five of 59 (8%) control tissues from various other body sites. Since then, multiple studies have confirmed these findings. In fact, a recent study suggests that all MCC tumors harbor the virus. Using a novel mouse monoclonal antibody, Ab3, with increased sensitivity for a fragment of the MCPyV LT-Ag, Rodig et al found that 56 of 58 (97%) samples stained positive, compared to 46 of 57 (81%) using a previously described murine monoclonal antibody, CM2B4. In addition, using five unique quantitative polymerase chain reaction (PCR) primers developed against both the LT- and ST-Ag, viral DNA was detected in all 60 tumors tested.

The virus appears to be common in the environment with human exposure likely occurring early in life. Results of a large (828 patients) Italian study revealed 41.7% of children age 1 to 4 and 87.6% of those age 15 to 19 were seropositive for MCPyV.

**Immune Response**

It has long been recognized that patients with immunosuppression are not only at higher risk of developing MCC, but have a worse prognosis. However, the majority (90%) of patients with this disease are immunocompetent, and mortality is also high in this population, suggesting that the tumor demonstrates mechanisms of immune evasion.

The effect of UV radiation in the pathogenesis of MCC has been suggested to be more likely a result of immune modulation than direct effects on DNA itself. UVB can decrease dendritic epidermal T cells and increase immunosuppressive cytokines. UVA inhibits Langerhans cells and encourages hapten tolerance, presumably resulting in increased viral susceptibility. In addition, ST-Ag has a UV-inducible promoter region.

Further strengthening the hypothesis of an immune-mediated process is the description of a dense lymphocytic infiltrate of T cells seen in cases of spontaneous regression of MCC after biopsy. A recent study described the tumor microenvironment of MCC, demonstrating markedly reduced expression of CD69 (a marker of early T-cell activation) in tumor-infiltrating lymphocytes (TIL). An increased number of CD25+ (a marker of late T-cell activation) regulatory T cells (Treg) was also seen, when compared to CD25+ FOXP3+ activated effector T cells. On immune stimulation with interleukin (IL)-2 and IL-15 in vitro, increased expression of both CD69+ and CD25+ effector T cells was seen; additionally, there was T-cell proliferation, particularly of CD8+ T cells. These results suggest that decreased effector T-cell activation is at least in part a result of increased Treg expression and activation. Moreover, the same authors identified expression of programmed death-1 (PD-1), a T-cell inhibitory receptor, in 50% of nonactivated T cells. Following treatment with IL-2 and IL-15, PD-1 levels decreased to that of normal skin cells and there was a subsequent loss of viable tumor cells.

Further, Afanasiev et al demonstrated that circulating MCPyV-specific CD8+ T cells and MCC TIL express higher levels of PD-1 receptor in comparison to T cells specific to other human viruses, and higher expression of its ligand PD-L1 correlated with CD8+ TIL. The above studies suggest that therapies that increase T-cell activity, either through inhibition of Treg or blockade of the PD-1 signaling pathway, may have therapeutic activity in MCC.

**Potential Clinical Targets**

In addition to potential immunologic targets, recent years have expanded our understanding of the molecular pathways that are deranged in MCC. This has led to increasing excitement and optimism surrounding novel targeted therapies. MCC has been found to express high levels of survivin, c-KIT, somatostatin receptor type 2, CD56, and vascular endothelial growth factor receptor-2 (VEGFR2). Phosphoinositide 3-kinase (PI3K)/mTOR and hedgehog signaling pathways have also been shown to be activated in MCC. Clinical trials testing some of these concepts are underway and results are eagerly expected.

**CLINICAL MANAGEMENT**

Because of the rarity of this malignancy, clinicians and patients are limited by a lack of robust data regarding natural history, prognostic predictors, and preferred treatment strategies. General recommendations for work-up, treatment, and follow-up of MCC are outlined in the National Comprehensive Cancer Network (NCCN) Clinical Practice Guidelines in Oncology website (www.nccn.org). With each
iteration of the NCCN guidelines, we continue to move toward a consensus on management of MCC, however, there continues to be a high degree of institutional variation.

Staging
Before 2010, the literature and subsequent data analysis was muddled by the lack of a consensus staging system, with both 3-stage and 4-stage systems in frequent use, derived from populations between 77 and 250 patients. In 2010, Lemos et al proposed a 4-stage system, based on the analysis of 5,823 cases from the National Cancer Data Base tumor registry, and subsequently adopted by the American Joint Committee on Cancer. The current system divides stages I and II (skin-limited disease \( \leq 2 \text{ cm} \) and \( > 2 \text{ cm} \), respectively) from stage III (regional lymphatic) and stage IV (distant metastatic) disease. Local disease is further subdivided based on whether nodes were pathologically staged (Ia, IIA) with either sentinel lymph node biopsy (SLNB) or dissection, or clinically staged with physical exam or imaging (Ib, IIb), and further by extension into bone/muscle/fascia/cartilage (IIC). Regional disease is subdivided by micrometastasis (IIIA) and macrometastasis (IIIB, clinically detectable node or in-transit metastasis).

Prognostic Indicators
Multiple factors have been implicated as predictors of prognosis in MCC. Lymphovascular invasion identified within the primary tumor, advanced disease stage, and immunosuppression have all been shown to correlate with poor prognosis. In contrast, TIL and high titers of MCPyV antibody have been associated with better outcomes. In addition, overexpression of VEGFR2 and survivin are associated with more aggressive clinical behavior. Taken together, these factors may prove to be tools for predicting outcomes for individual patients.

Work-Up
There is no consensus on the role of imaging for staging MCC; it is recommended “as clinically indicated” in the NCCN guidelines. The most recent version, however, does suggest PET/CT as first-line imaging over CT or MRI. Recent studies indicate that PET/CT may change the stage and subsequent treatment strategy in as many as 16% to 22% of patients, and may be more effective at detecting bone and bone marrow metastases. Important to note, however, is the finding that PET/CT does not replace SLNB for detection of micrometastatic disease. In a subset of patients without palpable adenopathy before a positive SLNB, regional nodal involvement was detected in only 14% of patients by PET/CT. Multiple studies have shown that relative survival is worse in patients who only have clinical nodal exams, as the risk of upstaging following SLNB is approximately 30%.

Treatment
A multidisciplinary approach is recommended for optimal management of this rare and aggressive cancer.

Treatment of local disease (stages I and II). Treatment of primary MCC has traditionally consisted of surgical resection with the goal of clear margins, with or without adjuvant radiation therapy. Surgical margins of 1 to 2 cm remain the standard technique, but in keeping with updated NCCN guidelines, clinical margins “should not be pursued to the degree of significantly delaying any planned adjuvant radiation therapy” (www.nccn.org). SLNB should be performed in all patients who are medically able, at the same time as excision of the primary tumor, ideally before resection to maximize the test’s accuracy.

Radiation therapy to the tumor bed has been commonly used in the adjuvant setting, based on retrospective data demonstrating improvement in both locoregional control and survival when compared to series managed with surgery alone. However, adjuvant radiation has not uniformly been shown to confer a benefit, and thus institutions vary in their practice. A large single-institution prospective study demonstrated no difference in rates of local recurrence with adjuvant radiation to the surgical bed. It is still unclear which patients can safely avoid radiation. Miller et al have suggested that adjuvant radiation therapy may be omitted for those with small primary tumors (<1 cm), clear margins, negative SLNB, and absence of poor prognostic factors like lymphovascular invasion and immunodeficiency.

The only prospective randomized trial evaluating the benefit of prophylactic radiation therapy to the draining nodal basin in early-stage disease closed prematurely because of a rapid decrease in recruitment following the introduction of SLNB. Patients with stage I disease were randomly assigned to radiation therapy to the draining lymph node basin versus observation, after resection of stage I disease and radiotherapy of the tumor bed. A significant decrease in probability of regional relapse was seen with nodal basin radiation therapy (p = 0.007), but there was no difference in overall survival. Given this finding, if a patient is not a candidate for SLNB, then radiation may be considered as adjuvant therapy for the draining nodal basin.

Treatment of regional nodal disease (stage III). If the SLNB is positive, completion lymphadenectomy (CLND) and/or radiation therapy is recommended. Whether nodal radiation therapy is required after a negative SLNB is unclear. Although some series suggest high failure rates after negative SLNB, others report no survival difference for those with or without adjuvant nodal therapy.

In 2010, Fang et al published a cohort study analyzing treatment of patients with stage IIIa and IIIb disease with definitive radiation to the lymph node basin, compared to CLND ± radiation. Regional control was 100% for microscopic lymph node disease, regardless of modality. For patients with palpable node disease, there was no statistically significant difference in 2-year regional recurrence-free survival for the radiation group (78%, 9 patients) and the CLND ± radiation group (73%, 15 patients). Additionally, no difference was noted in overall survival, suggesting that radiation alone was a reasonable modality for obtaining re-
gional control, although the study was limited by its size and lack of randomization.

Two prospective trials were conducted evaluating adjuvant chemoradiotherapy in MCC. In the Trans-Tasman Radiation Oncology Group (TROG) 96:07, 53 patients with high-risk disease (primary tumor size > 1 cm, involved lymph nodes, recurrence after initial surgery or gross disease after surgery) underwent concurrent chemoradiotherapy with carboplatin and etoposide. Grade 3 or higher neutropenia and febrile neutropenia rates were 57% and 35%, respectively. There was no treatment-related death. The 3-year overall and relapse-free survival rates were 76% and 65%, respectively. To decrease the toxicity seen in TROG 96:07, another study was done using weekly carboplatin during the 5 weeks of radiation therapy, followed by three cycles of carboplatin and etoposide every 3 weeks, starting 3 weeks post-radiation therapy. Eighteen patients with high-risk MCC entered the study. Only one did not complete the weekly carboplatin and three did not complete all three cycles of adjuvant chemotherapy. Again, no treatment-related death was seen. As predicted, grade 3 skin toxicity and grade 3 and 4 neutropenia were much lower in comparison to TROG 96:07. Given the excellent results seen in TROG 96:07, an attempt was made to identify a survival benefit with the addition of chemotherapy to the treatment regimen of high-risk patients. Poulsen et al compared the outcome of the patients on TROG 96:07 to a single-institution matched set of historic controls that would have fulfilled the eligibility criteria of TROG 96:07. No survival benefit was seen with the addition of chemotherapy on multivariate analysis. While some studies have shown a benefit of chemotherapy on multivariate analysis, a randomized trial is needed to confirm these findings.

Treatment of unresectable disease (stages I to III). For those with unresectable disease or in whom surgery is otherwise contraindicated, radiation therapy alone or in combination with chemotherapy can be considered.

Treatment of distant metastatic disease (stage IV). Cytotoxic chemotherapy remains the most commonly used modality for patients with metastatic disease. Given the histologic and biologic similarities of MCC to small cell carcinoma of the lung, commonly used regimens include platinum-based therapies, anthracyclines, etoposide, cyclophosphamide, vincristine, bleomycin, and 5-fluorouracil, either alone or in combination. No randomized studies have compared different chemotherapy regimens. Further, most of the studies evaluating chemotherapy treated a mixture of patients with metastatic and locally advanced disease, where chemotherapy was used in the adjuvant setting. Despite 60% to 75% response rates seen with platinum/etoposide combination and anthracycline-based regimens, median duration of response is only approximately 8 months and toxicity can be prohibitive for this patient population. Less toxic and more effective agents are clearly needed. The recent and significant progress made in the understanding of the biology of this disease has identified a new armamentarium of potential targeted and immunotherapies with potential activity against MCC; several are currently under investigation, including somatostatin analogs, VEGFR inhibitors, and immunotherapies including anti-PD-1 and PD-L1. Providers and patients are eagerly awaiting the results of these trials.

Surveillance: New Horizons

Current recommendations suggest close clinical monitoring of the skin and lymph nodes every 3 to 6 months for 2 years after diagnosis, and every 6 to 12 months thereafter. New to the guidelines is the suggestion to “consider routine imaging for high-risk patients.” In Hawryluk et al., PET/CT scans performed for subsequent management strategy revealed 45% of scans identifying disease in 42% of patients, and 35% of their cohort had at least one recurrence. The mean time to recurrence was 15.3 months and, consistent with prior reports, 91% of recurrences occurred within 2 years of diagnosis. In their study, disease recurred in all stages, from 13% in stage I to 35% in stage III. They observed a higher incidence of bone/bone-marrow metastases (33%) than in previously published reports, which they attributed to improved detection by PET/CT.

Currently, our standard methods of monitoring patients following treatment have significant drawbacks. These include the inadequate detection of recurrence/metastasis by clinical evaluation and the cost, radiation exposure, and false-negative and -positive rates associated with imaging studies. There is a great need for novel biomarkers that are both cost-effective and reliable in this disease.

To this end, antibodies to a shared portion of the LT-Ag and ST-Ag have been found in 40.5% of patients with MCC, as compared to 0.9% of controls. Antibodies correlated with disease progression, rising rapidly in those with progressive disease and falling precipitously in patients whose disease did not recur; increase in titers preceded clinical detection of disease.

Recently, Blom et al evaluated circulating tumor cells (CTCs) in a cross-sectional study of patients with MCC. Fifty-two blood samples from 34 patients were analyzed and 40% of samples (and 41% of patients) had detectable CTCs. Patients included those with no evidence of disease, as well as local, regional, and metastatic disease, representing both new diagnoses and follow-up patients. Presence of CTCs was strongly correlated with subsequent disease progression and death (MCC-specific survival was 25% of CTC-positive cases compared to 72% of CTC-negative cases). In individual patients, serial CTC counts helped assess treatment response. It was not, however, highly sensitive for presence of disease (15 of 27 patients with active disease had negative CTC results). Both predictive antibodies and CTC appear to be promising new mechanisms of surveillance that may help improve our current methods of post-treatment surveillance.
CONCLUSION
MCC is an aggressive cutaneous malignancy that is increasing in incidence. Although this orphan disease has been historically associated with unclear management recommendations and limited systemic options, recent advances in our understanding of the pathophysiology and molecular mechanisms involved in this disease have led to potential novel targeted and immunotherapies which are currently under investigation. Uniform staging criteria and widespread use of pathologic staging through SLNB will assist our prognostic accuracy, giving our patients a clearer view of the road ahead. Progress in both management and surveillance of MCC through the use of biomarkers has brought great excitement and optimism to the field. It is our hope that this progress will bring improvements in survival for patients faced with this grave diagnosis.

Disclosures of Potential Conflicts of Interest
The author(s) indicated no potential conflicts of interest.

References

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