**Utilizing capsid protein-specific antibodies and viral T-antigen oncoproteins to elicit active immunity against virus positive Merkel cell carcinoma**

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**This microreview investigates the recent progress made towards a preventative vaccine against Merkel cell polyomavirus’ (MCPyV) positive Merkel cell carcinoma (MCC). MCC is the malignant growth in the skin, usually caused by chronic exposure to UV rays causing mutations in Merkel cells or infection with MCPyV. These different pathways to tumorigenesis result in two forms of MCC, MCPyV-negative and MCPyV-positive, with the ability to protect against only the latter by inciting active immunity against the viral infection that accounts for up to 80% of all MCC cases. It has been shown that when higher levels of antibodies that specifically target VP1 a capsid protein, the survival rate of this aggressive cancer is higher. The objective of clinical research is to upregulate anti-VP1 immune response to prevent or slow MCC growth. Using both VP1-specific CD4+ and CD8+ T cells, it was found that antitumor efficacy was dose dependent, and slowed the progression of tumors in trials using mice with MCPyV-related MCC. Alternative research has focused on utilizing anti-PD-(L)1 checkpoint inhibitors, as PD-(L)1 inhibits T cell function against pathogenic cells. It has been shown that combination therapies to boost immune response while upregulating T cell response have been successful, and that combining these aforementioned therapies with viral T-Antigen (T-Ag) oncoproteins from genomically integrated MCPyV would be more beneficial than monotherapy. T-Ag is responsible for eliciting specific B and T cell responses, and is required to drive virus-positive MCC cell proliferation. This suggests that using T-Ag in a therapeutic vaccination against MCPyV has merit to, when in combination with other immunotherapies, increase survival rate.**

**Introduction**

Merkel cell carcinoma (MCC) is an aggressive form of neuroendocrine carcinoma, with approximately 80% of cases incited and promoted via the continuous expression of a T-antigen (T-Ag) by Merkel cell polyomavirus (MCPyV) cells (Jing et al., 2020) (Pietropaolo et al., 2020). MCC has two primary forms, virus positive MCC due to MCPyV T-Ag expression, and ultraviolet-induced tumor genome damage MCC; this review will focus on the former. It is important to note that both forms of MCC have been the focus of a litany of recent primary research, particularly using deep sequencing techniques. In the past few years, it has been indicated that there are several molecular differences between the two etiologies of cancer, such as; amount and location of point mutations, morphological/structural differences, and significant variation between the copy numbers, but differentiating between virus positive and UV damage MCC during diagnosis is still out of reach (Starrett et al., 2020). With that being said, the two etiologies do share many similarities in the patient such as disease presentation, prognoses, and most importantly, the carcinoma’s response to treatments. This further exacerbates the need for a preventative vaccine effective against both virus positive and UV damage mediated MCC (DeCaprio 2020). MCPyV is an opportunistic oncovirus that is an ubiquitous part of the skin’s microflora, rarely giving rise to oncogenesis. When cancer does proliferate, MCC has a disease-associated mortality rate of 45% as of 2010 and has increased to an estimated 77% from 2011 to 2017 (American Cancer Society, 2022) . This is due, in part, to the staggering occurrence of metastasis, 40-50%, commonly spreading superficial tumors in the bone, liver, or lung tissue (Lemos et al., 2010). From the beginning of the century until 2013, MCC incidence rates increased 95% to 0.7 cases per 100,000 people-years, and that number is only expected to climb as the largest generation in the United States, baby boomers, continue to age past 65 years old, the upper threshold for low-risk (Paulson et al., 2017). It has also been hypothesized that since MCC oncogenesis is also correlated with skin UV radiation damage, there will be a statistically significant increase in incidence rates for other generations as well due to consistent sun and artificial UV exposure for aesthetic purposes.

MCPyV is the most recently discovered of the seven known human oncoviruses, which combined account for approximately 12% of cancer cases (Haley et al., 2019) . Human papillomavirus (HPV) and hepatitis B virus (Hep B) are two well-known oncoviruses in this group that promote oncogenesis of several types of cancer, particularly cervical (squamous cell carcinoma) from HPV and liver (hepatocellular carcinoma) from Hep B (Haley et al., 2019). There are preventative vaccines developed and available since 2007 and 1981 respectively (Hakim et al., 2008) (Maugh 1981). The research conducted in the process of developing these vaccines has laid the molecular groundwork for prevention of other forms of oncovirus-mediated cancers, as the molecular characteristics between the oncoviruses in terms of mechanism of pathogenesis, method of diagnosis, and efficacious treatment methods are strikingly similar. Both HPV and Hep B are prevented by inciting a humoral immune response with non-infectious recombinant vaccines containing HPV virus-like particles from its major capsid protein, and Hep B surface antigens (Keating et al., 2003). Unlike its oncovirus counterparts, MCC is not a common form of cancer, meaning that a prophylactic vaccine for mass production and administration would not be profitable for research or pharmaceutical companies to develop. But this may change as the Surveillance, Epidemiology, and End Results (SEER) database has shown a stark increase in reported MCC cases from 2000-2013 with a 95% increase in comparison to melanomas (57%) and all solid tumor cases (15%) (Jacobs et al., 2021). As the largest generational group, Baby Boomers born 1946-1964, continues to age, several factors such as repeated exposure to MCPyV, weakening immune systems, and prolonged exposure to UV rays during the patient's lifetime, will continue to increase the incidence rate of all MCC cases (Paulson et al., 2017). Furthermore, willingly exposing skin to artificial UV rays for aesthetic purposes has been on the rise in recent years, which would over time increase the incidence rate of MCC in younger age groups. In addition to preventing primary malignant growths, MCC has had a high rate of recurrence ranging from 25% to 75% depending on the stage of metastasis at the point of diagnosis (Tabachnick-Cherny et al., 2021)(Xu et al., 2021). These predictions lead researchers to believe that in the foreseeable future, a vaccine protecting against virus positive MCC would not only be financially viable but advantageous as the public becomes increasingly aware of the realities of MCC. In addition to this, a therapeutic vaccine that stimulates antigen-specific CD4+ and CD8+ T cells shows potential against the proliferation of tumor cells for various types of cancers and other diseases (Xu et al., 2021).

**Recent Progress**

Once MCPyV had been isolated in a nodule tumor of MCC, research began into the mechanism of MCPyV mediated oncogenesis. As of 2019, there were two potential pathways of histogenesis, the first step of Merkel cell transformation into malignant cells. The original hypothesis stated that the epithelial cell or MC progenitor suffers UV mutation causing ATOH1 expression and resulting in differentiation in the MC, then MCPyV (and potentially secondary UV mutation) transforms the MC cell into a MCC tumor cell. This would suggest that MC is the cell of origin and T-Ag is capable of transformation. The second, and more complex mechanism, begins with the same physiological differentiation of the MC cell, but this alters the Rb pathway, which is in a family of tumor suppressors, to promote deregulation of cell cycle and proliferation. This hypothesis would suggest that MCC is due to non-MC cells undergoing a mutation mediated oncogenic events, and then later the phenotype of MC cells is taken up by the mutated cell with various possible ancestries: fibroblast/dermal stem cell, pre/pro B cell, MC or MCPyV (Kervarrec et al., 2019). As MCC incidence rates continue to rise nearly ten-fold that of all other solid tumors, researchers began investigating potential therapeutic treatments and preventative measures at the molecular level. Furthermore, MCPyV positive MCC is a prime candidate for vaccination due to several characteristics of the malignant proliferation. The conserved antigenic space of the encoded oncoproteins, they are both easily amenable to vaccine construction, as well as liable to the various immune monitoring tools, tetramers and TCR sequencing. The viral antigens produced by MCPyV are immunogenic, eliciting B and T cell responses while being an immune-sensitive form of cancer (Tabachnick-Cherny et al., 2021). This suggests that vaccinating with MCPyV viral T-Ag would elicit a higher baseline immune status and thus malignant cell death. The challenge arises with non-immunotherapy treatment, that we do not know the precise mechanism of MCPyV positive oncogenesis, or the histogenesis of MCs to MCC, and until these mysteries are solved, starting with uncovering the progenitor cell ancestry, targeted treatments cannot be developed.

 In cases of unknown mechanisms, rather than treat the illness by pharmaceutical targeting of the pathogen, researchers and medical professionals have developed therapies that not only elicit but also augment B and T response to cancer cells. Previous studies of cancer immunotherapy on MCC patients using anti-PD-(L)1 checkpoint inhibitors showed great success initially. PD-1 is a protein found on the surface of immune T cells that binds to PD-(L)1, a protein on the surface of various types of cancer cells. PD-(L)1 is actually a virulence factor for immune evasion, as it binds PD-1 the T cell suffers loss of function, unable to neutralize the cancer cell. Immune checkpoint inhibitors (anti-PD-(L)1 and anti-PD-1) both function by blocking the ability of PD-(L)1 or PD-1 respectively to bind to the other, and restoring T cell function (Liu et al., 2016). As initial clinical trials of these treatments yielded promising results for some patients, nearly 50% of those treated had no response to therapeutic treatment or PD-(L)1-refractory disease can develop and patients do not see a long term benefit (Tabachnick-Cherny et al., 2021). Current clinical research is now focusing on a combination of therapeutic vaccinations to elicit active immunity in a synergistic manner. It was discovered that nearly 80% of MCC cases are reliant on the constant expression of viral T-antigen (T-Ag) oncoproteins from the unknown cell of origin expressing genomically integrated MCPyV, which have been found to elicit B and T cell response in MCPyV positive MCC. It is assumed that if endogenous T-cell response is not sufficient in blocking/clearing MCPyV positive MCC proliferation and tumorigenesis, then the use of T-Ag therapeutic vaccination would need to either be combined with other therapies like PD-(L)1/PD-1 inhibitors or be both potent and synergistic with endogenous T cells to produce an effective and lasting treatment.

 Alternative current research is investigating an immunotherapy option that targets the MCPyV  capsid protein VP1. This protein is used in serological diagnosis, and showed an increased survival rate as well as a decreased recurrence rate in patients with more anti-VP1 antibodies at the site of primary metastasis. MCC’s aggressive nature is due to the high mutation burden, which interestingly is higher in MCPyV negative MCC cases, as well as the accumulation of tumor neoantigens is higher in MCPyV MCCs than in both melanomas and small-cell lung cancers. This supports the justification for research further in that immune checkpoint blockades are potentially highly effective at producing clinical responses in MCPyV negative MCC cases, unlike some immunotherapies that are only effective against virus positive MCC (Goh et al. 2015). It is relevant to note that while VP1 is the serological evidence from MCPyV positive MCC cases, 23% of MCPyV negative MCC patients had positive antibody results against the MCPyV capsid protein, suggesting that VP1 is not expressed by MCC tumors as originally assumed. Expression of this antigen is lost after malignant growth has begun, as is also seen in other oncoviruses such as HPV-mediated cervical cancers, Kaposi’s sarcoma incited by chronic human herpesvirus 8, and liver carcinomas that arise from HBV-related chronic hepatitis. In the same study it was found that VP1 mutations have the potential to alter viral DNA integration from MCPyV into host cells and the proliferation of initial tumor growth. This is thought to be done by targeting multiple vital functions such as membrane binding, various stages of protein folding/unfolding, and antibody escape. The most important discovery of this investigation was that, like T-Ag, VP1-derived epitopes have the capability to elicit CD8+ T-cells, and in healthy patients with both seropositive and seronegative results, VP1-specific CD4+ T-helper function was uninhibited, while it was found to be absent in patients with MCC (Xu et al., 2021). While clinical trials to test the efficacy of this individual treatment have not yet been conducted, it is assumed that VP1, in combination with other immunotherapies such as PD-1/PD-(L)1 inhibitors and T-Antigen vaccination would bolster immune response by increasing elicitation of B and T cells, blocking malignant cell immune evasion strategies to increase T cell function, and altering viral DNA integration to decrease proliferation.

**Discussion**

As MCPyV positive MCC was identified as a unique mechanism of oncogenesis in 2008, much of the research into treatment is still in the developmental stages. There is still a litany of research that needs to be conducted to conclusively determine the best targeted pharmaceutical treatment or therapy. Many of the gaps in knowledge that exist are due to time constraints and the limited amount of long-term trials that could be conducted. In regards to upregulating endogenous T-cell response via T-Ag vaccination, initial trials have produced promising results, but previous studies have suggested that over time there is a low immunological response to MCPyV. As of right now, it is unclear if T-Ag therapies’ efficacy would wane over time requiring recurrent administration, which opens new avenues for other complications. Further research will need to first identify the half-life of efficacy independently, as well as with varying numbers and frequencies of re-administration. If T-Ag therapies were to become ineffective overtime, researchers could also attempt to find a balance between current cytotoxic therapies, such as chemotherapy and radiation treatments, at lower doses in combination with immunotherapies. This would potentially reduce healthy tissue damage by decreasing current therapy dosage, as well as increase immune response systematically rather than exclusively at the site of tumorigenesis resulting in a decrease in micrometastases-mediated recurrence.

 While it is not currently financially advantageous for pharmaceutical companies to invest time, money, and resources into an MCC vaccine that prevents initial asymptomatic MCPyV infection before mutation into MCC, the increasing incidence rate of MCPyV positive MCC has motivated researchers to continue investigating the molecular mechanisms of MCPyV in case development begins. Further research could produce a dual treatment including a T-Ag immunotherapeutic approach for current patients vastly increasing immune response immediately, and a VP1-specific cellular target to incite a longer-lasting immune response. One potential result from this would be a stark contrast between consistent B and T cell responses due to therapies and the natural transitory responses of the pathogen, allowing for differentiation between the two. This is supported by data showing decreased T and B cell response once MCPyV positive MCC tumor removal. While at times, it feels as if there is more about MCC we do not know than we do, but the information we currently have suggests that MCPyV positive MCC is a prime candidate for unprecedented therapeutic vaccination against immunogenic cancers.

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