Pathology - Research and Practice 271 (2025) 155869

Contents lists available at ScienceDirect

Pathology - Research and Practice

journal homepage: www.elsevier.com/locate/prp

Review

# Genetic and molecular profiling in Merkel Cell Carcinoma: Focus on MCPyV oncoproteins and emerging diagnostic techniques

Harpreet Singh [a,*], Sourav Mohanto [b], Anil Kumar [c], Arun Kumar Mishra [d], Arvind Kumar [a], Amrita Mishra [e], Mohammed Gulzar Ahmed [b], Mukesh Kr. Singh [a], Amrendra Pratap Yadav [f], Shivani Chopra [g], Hitesh Chopra [h,*]

[a] *School of Pharmaceutical Sciences, IFTM University, Moradabad, Uttar Pradesh 244102, India*
[b] *Department of Pharmaceutics, Yenepoya Pharmacy College & Research Centre, Yenepoya (Deemed to be University), Mangalore, Karnataka 575018, India*
[c] *Moradabad Educational Trust Group of Institutions, Faculty of Pharmacy, Moradabad, Uttar Pradesh 244001, India*
[d] *SOS School of Pharmacy, IFTM University, Moradabad, Uttar Pradesh 244102, India*
[e] *School of Pharmaceutical Sciences, Delhi Pharmaceutical Sciences and Research University, New Delhi 110017, India,*
[f] *Meerut Institute of Technology, Meerut, UP, India*
[g] *Department of Biosciences, Saveetha School of Engineering, Saveetha Institute of Medical and Technical Sciences, Chennai, Tamil Nadu 602105, India*
[h] *Centre for Research Impact & Outcome, Chitkara College of Pharmacy, Chitkara University, Rajpura, Punjab 140401, India*

A R T I C L E I N F O

*Keywords:*
Merkel Cell Carcinoma
Cancer
Immunohistochemistry
MCPyV
Polymerase Chain Reaction
Next-generation sequencing

A B S T R A C T

Merkel Cell Carcinoma (MCC) is an uncommon yet highly malignant form of skin cancer, frequently linked to the Merkel cell polyomavirus (MCPyV). This review comprehensively covers data from year 2000 to 2024, employing keywords such as MCC, MCPyV Oncoproteins, Immunohistochemistry, Southern Blot, Western Blot, Polymerase Chain Reaction (PCR), Digital Droplet PCR (ddPCR), Next-Generation Sequencing (NGS), and *In Situ* Hybridization (ISH). The search engines utilized were Google, PubMed Central, Scopus, and other journal databases like ScienceDirect. This review is essential for researchers and the broader medical community as it consolidates two decades of research on the genetic and molecular profiling of MCC, particularly focusing on MCPyV's role in its pathogenesis. It highlights the diagnostic advancements and therapeutic potential of targeting viral oncoproteins and provides insights into the development of both *in vivo* and *in vitro* models for better understanding MCC. The findings emphasize the significance of early detection, molecular diagnostics, and personalized treatment approaches, aiming to improve outcomes for patients with this malignant malignancy.

## 1. Introduction

Merkel Cell Carcinoma (MCC) is a rare but aggressive form of skin cancer that predominantly arises from the merkel cells, a specialized neuroendocrine cells found at the base of the epidermis layer of the skin [1,2]. These cells play an important role in the sensory motion [3]. MCC is known for its rapid growth and propensity for early metastasis, which makes it a particularly challenging or malignancy to treat [4]. Clinically, MCC tends to present as a painless, firm nodule on the skin, commonly appearing on areas of the body usually exposed to sun [5,6]. Patients at greater risk typically include the elderly, individuals with fair skin, those with a history of extensive sun exposure, or immunocompromised individuals [7,8]. The diagnosis of MCC requires a high index of suspicion and typically involves a combination of physical examination, imaging studies, and confirmatory tissue biopsy [9,10]. Despite its aggressive nature, early-stage MCC can be managed effectively with surgical excision, often followed by radiation therapy [11,12]. These cases may further require systemic treatments such as chemotherapy or, more recently, immune checkpoint inhibitors, which have shown assurance in improving outcomes for patients with metastatic disease [13,14]. Due to its abnormality, research into MCC is ongoing, and clinicians are encouraged to report cases and treatment outcomes to enhance the understanding of this malignancy and enlighten better diagnostic and therapeutic strategies.

The timely identification and therapy are essential for enhancing patient longevity and well-being in MCC [15]. The genetic and molecular profiling of MCC holds significant value for advancing the understanding, diagnosis, and treatment of the disease [16,17]. The molecular

* Corresponding authors.
*E-mail addresses:* harpreetproctor@rediffmail.com (H. Singh), chopraontheride@gmail.com (H. Chopra).

https://doi.org/10.1016/j.prp.2025.155869
Received 9 October 2024; Received in revised form 21 December 2024; Accepted 25 February 2025
Available online 26 February 2025
0344-0338/© 2025 Elsevier GmbH. All rights are reserved, including those for text and data mining, AI training, and similar technologies.

characterization has illuminated the pathogenesis of MCC, dividing it into two main etiological categories, i.e., those caused by the clonal integration of MCPyV and those driven by ultraviolet light-induced mutations [18–20]. MCPyV-positive tumors typically have a better prognosis and are often associated with a different therapeutic response than MCPyV-negative tumors [21,22]. The virus-positive MCC generally exhibits fewer mutations and presents a distinct immuno-biological behavior, suggesting that viral oncogenes play a crucial role in tumorigenesis in these cases [23]. On the other hand, MCPyV-negative MCC harbor a high mutational burden due to ultraviolet radiation-induced DNA damage. This high mutational load may make these tumors more amenable to immunotherapy, as the increased number of mutations could lead to the formation of more neoantigens that are recognizable by the immune system [24]. In addition, the genetic profiling has also facilitated the identification of key mutations in tumor suppressor genes and oncogenes, like p53 and retinoblastoma protein (pRB), which can drive carcinogenesis in MCC [25].

The genetic landscape of MCC is essential for the development of targeted therapies tailored to the molecular characteristics of an individual's tumor. Several molecular diagnosis can further aid in precise staging and decision-making for MCC patients. The presence of certain biomarkers can guide the use of adjuvant therapies following surgical resection or indicate the appropriateness of chemotherapy, radiation therapy, or immunotherapy [26]. As per the literatures, MCC exhibits considerable research gaps and challenges, specifically attributed to its infrequency and aggressive characteristics. The exact molecular pathways that cause MCC to form after MCPyV infection are still not clear, which makes it difficult to identify the effective treatments. A deficiency in thorough understanding of prognostic variables impedes appropriate patient classification and treatment planning. Several literatures further recommended the interventional clinical trials are necessary to determine the effectiveness of new therapeutics or medicines, i.e., PD-1/PD-L1 inhibitors, in treating MCC. The histological diagnosis of MCC is frequently difficult due to its resemblance to other malignancies, resulting in possible misdiagnosis.

The need for enhanced diagnostic indicators and methodologies is essential for early detection and intervention of MCC. Conversely, although the emphasis on the aggressive characteristics of MCC and its treatment modalities is crucial, it is equally important to investigate the psychosocial effects on patients, as these can profoundly influence their quality of life and adherence to therapy. As our molecular understanding of MCC deepens, it holds the promise of more effective and tailored interventions that could significantly improve outcomes for those affected by this aggressive skin cancer.

## 2. Role of MCPyV oncoproteins in MCC pathogenesis

MCPyV is intricately linked to the pathogenesis of MCC, particularly in virus-positive cases of the disease. In addition, approximately 80 % of MCC tumors exhibited this polyomavirus incorporated into their genome, indicating a critical involvement in tumor growth [27], as shown in Fig. 1. The oncogenic mechanism of MCPyV is associated with the virus's ability to express viral oncoproteins, with the two basic proteins being Large T antigen and Small T antigen. These viral oncoproteins interfere with the host cell's mechanism of action, promoting cellular proliferation and survival, which are essential steps in tumorigenesis [28]. Large T antigen of MCPyV possess several functions, most notably, the binding and inactivation of pRB, leading to a loss of cell cycle [29]. In normal cells, pRB regulates the cell cycle by controlling the transition from the G1 phase to the S phase. However, the Large T antigen disrupts this regulation, lead to proliferation [30]. Furthermore, the mutations or deletions in Large T antigen that stabilize its expression are often observed in MCC, implicating its sustained presence as necessary for the maintenance of the cancer [31]. The Small T antigen also contributes to the transformative potential of MCPyV via targeting protein phosphatase 2 A (PP2A), further causing dysregulation of cellular signaling pathways that are normally involved in controlling growth and apoptosis. Additionally, the Small T antigen can modulate gene expression and chromosomal instability, further contributing to cancer development [32]. However, the pathogenic role of MCPyV is not entirely comprehended. The mere presence of the virus is insufficient to cause MCC, as MCPyV is relatively common in the general population and is often asymptomatic [33].

In addition to the direct oncogenic effects of MCPyVLarge T and Small T antigens already discussed, there are other aspects of MCPyV's role in the pathogenesis of MCC. The integration of MCPyV DNA into the host genome is a major event in the pathogenesis of MCC. This viral integration is clonal, indicating that it occurs early in the oncogenic process and before the clonal expansion of the tumor cells. The clonal integration suggests that the virus plays an early and causative role in the development of MCC [34]. Furthermore, MCPyV may contribute to the immune evasion of MCC cells. The virus has developed strategies to

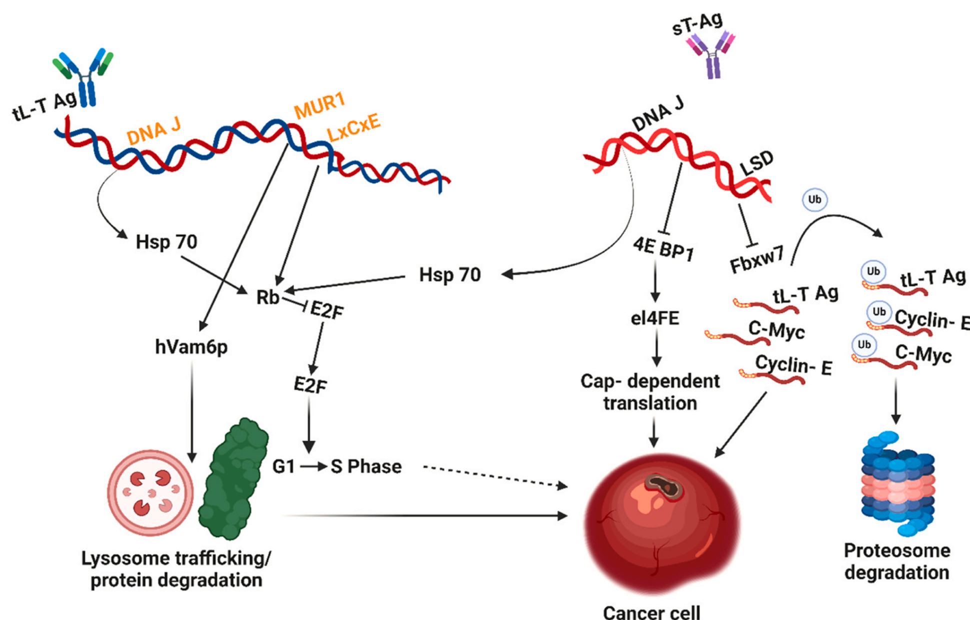


**Fig. 1.** Overview on role of MCPyV oncoproteins and other genetic factors in MCC pathogenesis and progression.

minimize the expression of viral proteins and the production of viral particles, which helps the infected cells to avoid detection and elimination by the host immune system. This immune evasion is aided by the loss of viral capsid protein expression in tumor cells, which lowers the immunogenicity of the virus-infected cells [35]. Simultaneously, MCPyV-positive MCCs possess an immune signal that resembles an "immune-privileged" site, suggesting that these tumors can suppress the local immune response. This suppression might be facilitated by the intracellular oncoproteins, which can inhibit interferon signaling, reducing the ability of the immune system to respond the tumor cells [36,37]. Additionally, the viral oncoproteins may also contribute to genomic instability. For instance, the Large T antigen has been described to bind and inactivate components of the DNA damage response, leading to genomic instability and accumulation of mutations, which can contribute to the carcinogenic process [38], as shown in Fig. 1.

The study of MCPyV's role in MCC pathogenesis also provides insights into potential therapeutic targets. The viral mechanisms enables the development of potential therapeutic strategies such as vaccines targeting MCPyV antigens, antiviral therapies that can disrupt the functions of T antigens, or immunotherapy approaches to boost the immune system's response against virus-infected cancer cells [39]. MCPyV's involvement in the pathogenesis of MCC also includes its contributions to the tumor microenvironment and further impact on cell cycle regulation [28,40]. MCPyV can modulate the microenvironment to create conditions that are corelated to cancer growth and immune evasion. The virus may further influence the expression of various cellular factors, i.e., cytokines and chemokines, that can recruit or suppress various immune cells. This dynamic interaction with the host immune system can be pivotal in allowing the tumor progression [41]. Moreover, the MCPyV's strategy for persistent infection involves the non-lytic shedding of viral particles from the host cell, which may contribute to the maintenance of a chronic inflammatory state in the skin of affected individuals [42,43]. The chronic inflammation has been widely recognized as a potential risk factor for various types of cancer [44,45], may provide additional signals necessary for MCC carcinogenesis [46], as shown in Fig. 1. On the regulatory level, the MCPyV oncoproteins can also have profound effects on the cell cycle. While the virus does not typically cause cell lysis, its impact on cellular controls such as apoptosis and the cell cycle can lead to unchecked cellular division and a reduced capacity for the cells to undergo apoptosis, even in the presence of DNA damage or other cellular stressors [28,47]. The exploration on the role of MCPyV oncoproteins in MCC pathogenesis reveals additional layers of complexity of the virus interacts with host cellular mechanisms and contributes to tumor development. One significant aspect involves the interplay between MCPyV oncoproteins and host cellular metabolic pathways. Several tumor cells, including MCC, often exhibited altered metabolism to support rapid proliferation and survival under stressful conditions [4,48]. MCPyV oncoproteins may re-program cellular metabolism to favor anabolic processes, providing the necessary biomolecules and energy for sustained tumor growth. The viral oncoproteins can influence glycolysis and lipid biosynthesis pathways, ensuring that the cancer cells have a steady supply of energy and structural components for new cell membranes [49,50].

In addition, another intriguing area of research is the epigenetic modifications induced by MCPyV infection. Viral oncoproteins can alter the host cell's epigenetic landscape, leading to changes in gene expression that favor oncogenesis. These modifications may include DNA methylation, histone modifications, and changes in non-coding RNA profiles. Such epigenetic changes can silence tumor suppressor genes or activate oncogenes, contributing to the transformation of normal cells into cancerous [51]. The investigation of these epigenetic alterations can provide insights into the early events of MCC pathogenesis and identify potential biomarkers for early detection and therapeutic targets [52,53]. The interaction between MCPyV oncoproteins and cellular stress response pathways also plays a critical role in MCC development [54]. MCPyV oncoproteins can manipulate the stress-induced cellular pathways to enhance cell survival and proliferation under stress conditions [55]. For instance, the Large T antigen can interfere with the unfolded protein response, preventing apoptosis in response to endoplasmic reticulum stress. Similarly, the Small T antigen can modulate reactive oxygen species levels, allowing cancer cells to survive oxidative stress that would normally induce cell death [56]. Another potential avenue for understanding MCPyV's role in MCC is the study of viral genetic diversity and its impact on tumor biology. Different strains or variants of MCPyV may have varying oncogenic potentials, influenced by mutations in the viral genome that affect the function of the T antigens. The analysis of the MCPyV genetic diversity in MCC patients can reveal correlations between specific viral variants and clinical outcomes, such as tumor aggressiveness, response to therapy, and overall prognosis [57]. However, the development of animal models and organoid systems for MCC research can significantly advance our understanding of MCPyV's role in tumorigenesis. These models allow for the *in vivo* and *in vitro* study of virus-host interactions in a controlled environment, providing valuable insights into the molecular and cellular mechanisms of MCC [28,58].

## 3. Models for studying MCPyV-induced MCC

### 3.1. *In vivo*

Several research on *in vivo* models for studying MCPyV-induced MCC demonstrated significant insights, although not without its critics [28, 59]. In a recent study, Verhaegen et al. characterized the transgenic mice that express the wild-type MCPyV small T antigen under an epidermis-specific promoter. These transgenic mice exhibited striking epithelial dysplasia, underscoring the oncogenic potential of the MCPyV small T antigen [60]. This finding highlights the role of the MCPyV small T antigen in driving carcinogenic processes within epithelial tissues, paving the way for further studies and potential therapeutic approaches. Additionally, Spurgers et al. reported that the expression of the MCPyV large T antigen in transgenic mice led to the development of tumors resembling human MCC, further highlighting the importance of the viral T antigens in MCPyV-driven carcinogenesis [28,60]. Although these transgenic mouse models have provided valuable insights into the oncogenic mechanisms of MCPyV, their utility is limited by the fact that they do not completely replicate the human MCC tumor microenvironment, play a critical role in disease pathogenesis [61]. To address these limitation, several researchers have developed xenograft models in which human MCC cell lines or patient-derived tumor samples are implanted into immunodeficient mice. These models have enabled the study of tumor growth, invasion, and metastasis in the context of a human tumor microenvironment, and have also been assessed to evaluate the efficacy of novel therapeutic agents [62]. The humanized mouse models, which incorporate a functional human immune system, have emerged as a promising platform for studying the interplay between MCPyV and the host immune response in the context of MCC [63]. These models have the potential to provide insights into the immune evasion mechanisms employed by MCPyV and facilitated the development of immunotherapeutic approaches for the management of MCC [63]. In recent years, humanized mouse models have gained significant attention as a powerful tool for studying the complex interplay between tumors, the immune system, and viral infections [64]. The incorporation of the functional human immune system into immunodeficient mice, exhibited numerous advantages for studying the pathogenesis of MCC and MCPyV. These models closely mimic the human immune response, offering a more accurate representation of immune interactions with MCPyV and MCC [65]. In addition, the researchers can also study MCPyV's lifecycle within a human immune milieu, gaining insights into viral infection, replication, immune evasion, and tumor formation [55, 66]. The investigation of the possible mechanisms of MCPyV evades the immune system may enhance treatment strategies, facilitate precision medicine by allowing for patient-specific treatment evaluation via the

engraftment of tumor samples derived from patients [67]. Additionally, the humanized mouse models facilitate the discovery of biomarkers for early detection, prognosis, and treatment response by examining the interactions between human immune cells and MCC tumor cells. Observing the natural disease progression of MCC in the context of a functioning immune system further informs patient care strategies. Thus, these advantages make humanized mouse models essential for modern biomedical research, particularly for diseases like MCC where the human immune system significantly influences disease development and response to treatment [68–70]. Therefore, it can be comprehended that the development of *in vivo* models for studying MCPyV-induced MCC has been critical for advancing our understanding of the underlying biology of this disease and evaluating potential therapeutic approaches [28].

### 3.2. *In vitro*

Several researchers have further developed various *in vitro* organoid culture systems that may provide a more physiologically relevant platform for investigating the role of MCPyV in MCC [71]. These organotypic cultures can recapitulate key features of the skin microenvironment, including the interaction of epithelial cells, fibroblasts, and extracellular matrix components [71,72]. A recent study demonstrated that the ability to generate 3D skin grafts comprised of immortalized keratinocytes and fibroblasts, which could then be infected with MCPyV [73]. This system allows for the examination of viral integration events and the effects of MCPyV on cellular differentiation and transformation. However, the detailed investigation is further required to optimize these *in vitro* organoid models to faithfully reproduce the MCC condition [71]. Additionally, the development of patient-derived organoid models may provide opportunities to investigate individual disease heterogeneity and responses to targeted therapeutics. The application of these advanced *in vitro* culture systems represents a promising approach to gain mechanistic insights into the role of MCPyV in MCC that have been difficult to elucidate using traditional cell line and animal models [60]. While *in vitro* organoid culture systems offer a promising platform for studying the role of MCPyV in MCC, there are opposing arguments to consider. Some researchers argue that in *vitro models* may not fully capture the complexity of the tumor microenvironment and the interactions between different cell type [74]. The absence of the dynamic *in vivo* conditions, such as immune responses and tissue structure, could limit the translational relevance of findings from *in vitro* studies. Additionally, there is a concern that the replication of tumor development in *in vitro* organoid models may not fully represent the actual disease process. Several critics further argue that the complexity of tumor-stroma interactions and the influence of systemic factors on tumor growth and progression may not be fully recapitulated in these artificial culture systems [75]. Furthermore, while the inclusion of patient-derived organoid models is considered a potential avenue for studying individual disease heterogeneity, the variability in organoid culture conditions and the challenges associated with maintaining consistency across different patient samples may introduce significant experimental variability and complicate data interpretation [76]. It is important to critically evaluate the limitations of *in vitro* organoid models in studying MCPyV'srole in MCC and consider complementary approaches to ensure a comprehensive understanding of the disease mechanisms [77]. Thus, several *in vitro* organoid systems represent a promising approach for investigating the role of MCPyV in MCC, but their utility and limitations must be carefully considered.

## 4. Molecular diagnostic techniques

The advancements in genetic and molecular diagnostic methods have transformed the field of oncology. These methods have allowed healthcare professionals to gain a better understanding of the mechanisms behind cancer development and progression, leading to improved diagnosis, prognosis, and targeted treatments. This progress has significantly enhanced patient care and outcomes. Furthermore, these advancements have paved the way for personalized medicine tailored to individual patients' unique genetic profiles. By analyzing specific gene mutations and expression patterns in each tumor, clinicians can now prescribe more effective treatments with fewer side effects. This approach holds great promise for improving survival rates and quality of life among cancer patients [78–81]. Fig. 2 further depicted the advanced diagnostic tools or techniques for the genetic and molecular understanding of MCC.

### 4.1. *Immunohistochemistry for viral oncoproteins*

Immunohistochemistry is a powerful tool for the detection and localization of specific proteins, including viral oncoproteins, within tissue samples. This technique utilizes the high specificity of antibodies to identify and visualize the presence of these proteins, which can serve as important biomarkers for various cancer types. By targeting viral oncoproteins, clinicians can gain valuable insights into the role of viral infections in the development of certain cancers, ultimately informing diagnostic and treatment strategies [82]. Immunohistochemistry has emerged as a valuable tool for the detection of MCPyV oncoproteins in MCC. The immunohistochemical analysis has revealed that the small T antigen and truncated large T antigen are consistently expressed in MCPyV-positive MCC; however, these are absent in MCPyV-negative tumors. This differential expression pattern has important implications for diagnosing and understanding MCC pathogenesis [83]. The level of expression of MCPyV oncoproteins has also been correlated with clinical outcomes in MCC patients. Several studies further demonstrated that the higher levels of MCPyV oncoprotein expression are associated with improved prognosis and better overall survival, suggesting that these viral proteins may play a role in the natural history of the disease [84]. This comprehension further raises significant queries about the potential therapeutic implications and the development of targeted interventions aimed at modulating MCPyV oncoprotein expression levels to improve patient outcomes.

In a recent investigation on the high sensitivity and specificity of immunohistochemical detection of MCPyV oncoproteins, provided a robust foundation for the clinical application of this investigative approach. As researchers continue to unravel the complexities of MCPyV-associated MCC, it is evident that the utilization of immunohistochemistry in the study of viral oncoproteins not only offers essential diagnostic value but also serves as a catalyst for advancing our understanding of the molecular mechanisms driving the disease. The identification of MCPyV oncoproteins in MCC *via* immunohistochemistry encounters multiple constraints that impact precision. These obstacles arise from the intricacy of the virus's expression patterns and the technical facets of immunohistochemistry itself. The sensitivity of antibodies employed in immunohistochemistry might fluctuate considerably. A new monoclonal antibody identified MCPyV big T antigen in 97 % of MCC tumors, suggesting that current antibodies may lack universal efficacy [85]. Conversely, other investigations indicated a mere 16.4 % detection rate of big T antigen in keratinocyte carcinomas, implying inconsistency in antibody efficacy among various tumor types [86]. Immunohistochemistry techniques may be affected by variables including tissue fixation, antigen retrieval methods, and the quality of antibodies employed, potentially resulting in inconsistent outcomes [87]. The presence of MCPyV DNA does not consistently correspond with protein expression, confounding the interpretation of immunohistochemistry results [86]. The existence of MCPyV antibodies in the general population hampers the specificity of IHC results, as analogous antibodies may be present in non-cancerous individuals [88]. The fast reduction of T-antigen antibodies in non-recurrent patients may hinder the identification of ongoing disease [88]. Notwithstanding these constraints, continual progress in antibody creation and

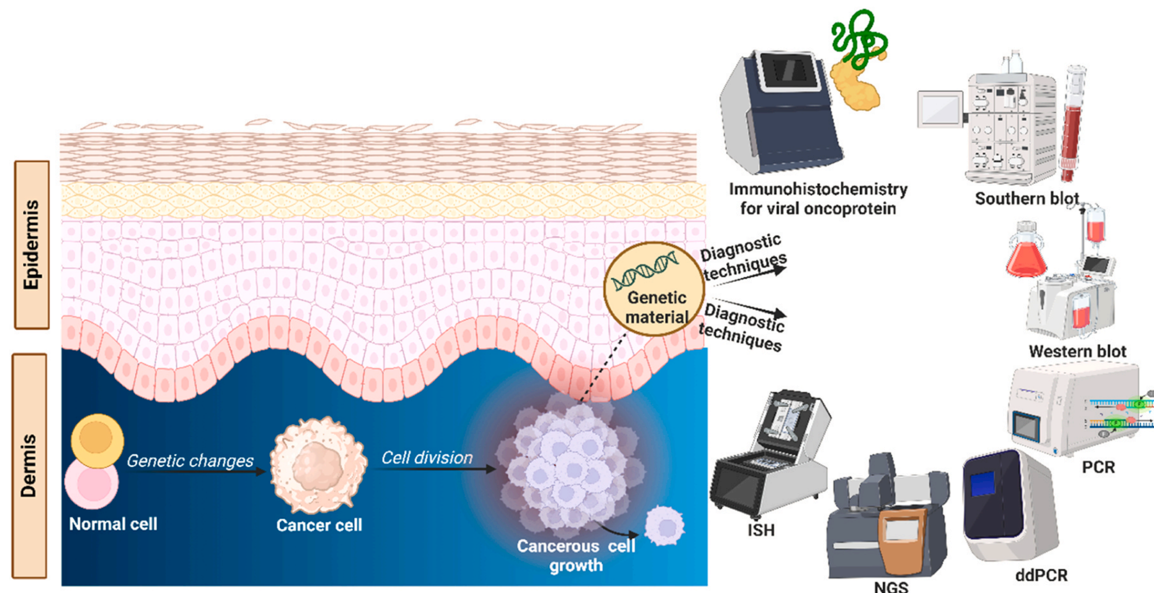


**Fig. 2.** Summarizes the various advanced diagnostic tools for the detection of MCC.

immunohistochemistry techniques may improve the future detection of MCPyV oncoproteins. Nonetheless, the inconsistency in detection rates and the impact of technological variables continue to pose considerable hurdles in the domain. Future explorations into MCPyV oncoproteins through immunohistochemistry holds promise for shaping future therapeutic strategies and refining prognostic assessments in the management of MCC [89].

### 4.2. Southern blot

The Southern blot is a laboratory technique used to detect specific DNA sequences within a complex mixture of DNA. This technique further demonstrate the presence of particular nucleotide sequences and their pattern within the genome, which in the context of MCC, can be used to detect the clonal integration of MCPyV DNA within the host genome [65]. While not a routine clinical test due to its complexity and the advent of more modern techniques, the Southern blot may furnish or provide valuable information about viral integration, which is a hallmark of viral-driven cancers [90]. The Southern blot technique, coined by Edwin Southern, involves transferring DNA fragments from a gel to a membrane and subsequently using probe hybridization to identify certain DNA sequences [91]. The method begins with DNA extraction and fragmentation, in which DNA is isolated from cells and then cleaved into smaller fragments with restriction enzymes. These enzymes cleave DNA at certain sequences, yielding fragments of different lengths. The DNA fragments that have undergone digestion are subsequently sorted based on their size using gel electrophoresis [92]. An electric current is passed through the gel, causing the DNA molecules to move towards the positive electrode. Smaller DNA fragments move quicker than bigger ones. The DNA fragments are subsequently transferred to a nitrocellulose or nylon membrane using a technique known as blotting. This step can be accomplished using several methods, including capillary action or vacuum transfer. A DNA probe specific to the MCPyV genomic sequence is labeled with a radioactive or chemiluminescent tag. This probe can then hybridize to the complementary MCPyV DNA sequence on the membrane. The membrane with transferred DNA is incubated with the labeled probe. The probe will bind, or hybridize, to any DNA fragments that contain the MCPyV sequence. The membrane is then washed to remove excess probes that have not been hybridized, reducing background noise and improving the specificity of the detection [93].

The hybridized probe-DNA complexes are visualized using X-ray film for radioactive probes, or specialized equipment for chemiluminescent tags. The analysis of the Southern blot provides information on the length and quantity of the DNA fragments that have hybridized with the probe. By comparing the pattern of bands on the blot to a ladder of known DNA fragment sizes, researchers can determine the molecular weight of the viral DNA within the sample and its integration pattern within the host genome [94].

In addition, this technique possesses several advantages in detecting MCPyV integration. It can reveal whether MCPyV DNA is present as episomal (non-integrated, circular DNA) or as integrated into the host's genomic DNA, which is indicative of a clonal expansion of the virus-associated cancer cells. It can also provide an estimation of the copy number of the viral genome within the host cells based on the intensity of the bands on the X-ray film or chemiluminescent readout. Additionally, it gives information on the size of the integrated viral DNA fragments, which can suggest the genomic stability and possibly the evolution of the viral integration sites in the tumor cells [27]. However, the Southern Blot technique has its challenges and limitations, i.e., technically complex and time-consuming, making it less suitable for high-throughput or rapid diagnostics [91,95]. It requires a larger amount of DNA compared to PCR-based methods, and detecting low levels of integrated viral DNA can be challenging [96]. The multiple steps involved, from gel electrophoresis to transfer to membrane and exposure, are labor-intensive and require careful handling [92,97]. If using radioactive probes, there are safety considerations for handling and disposing of radioactive materials [98]. In the context of MCC, Southern blot is not commonly used in the routine clinical setting for the reasons mentioned above. Modern molecular techniques such as PCR or NGS have largely replaced it due to their higher sensitivity, specificity, and faster turnaround [99]. The Southern blot technique may be insufficiently sensitive to identify low viral loads or shortened variants of MCPyV big T antigen, which are common in MCC tumors [85,100]. In contrast, several contemporary techniques like quantitative PCR and immunohistochemistry exhibit enhanced sensitivity and specificity for identifying MCPyV in MCC, indicating a transition towards more sophisticated methodology for precise diagnosis and study in this domain [28,85]. However, Southern blot still holds a place in research settings where understanding the detailed pattern of viral integration and the structure of the viral genome within host cells is important [98]. Southern blot can be a valuable tool for detecting clonal integration of MCPyV in MCC, providing insights into the molecular mechanisms of its

oncogenic process [101]. Despite this, the method is surpassed by newer technologies that offer a more practical and efficient approach for clinical diagnosis.

### 4.3. Western Blot

The Western blot, also known as immunoblotting, is a widely used analytical technique for detecting specific proteins in a sample. When discussing MCC and its association with the MCPyV, Western blotting can identify and confirm the expression of viral oncoproteins within cell or tissue samples [20]. The Western blot technique relies on the separation of proteins by gel electrophoresis, followed by the transfer to a membrane and subsequent detection using antibodies specific to the target protein [102]. The process begins with sample preparation. Samples containing proteins, such as tissue lysates from MCC, are prepared by adding a buffer and possibly denaturing agents to ensure proteins are in a linear form. The prepared samples are then loaded onto a polyacrylamide gel. Proteins are separated according to their size by applying an electric current, with smaller proteins traveling faster and further through the gel while larger proteins lag behind [103]. After electrophoresis, the separated proteins are transferred onto a membrane, typically made of nitrocellulose or polyvinylidene difluoride, ensuring that the proteins retain their original positions from the gel. The membrane is then incubated with a protein or other blocking agent that covers all nonspecific sites to prevent non-specific binding of antibodies [104]. Next, the membrane is incubated with a primary antibody that specifically binds to the target protein, for example, MCPyV oncoproteins. After washing away unbound antibodies, the membrane is incubated with a secondary antibody that binds to the primary antibody. This secondary antibody is usually linked to an enzyme or a fluorescent reporter [105]. Furthermore, the detection involves adding a substrate for the enzyme, which in the case of an enzyme-linked secondary antibody, produces a visible signal through a chemical reaction. The intensity of the signal can be quantified to provide an estimate of protein abundance, and the size of the protein can be deduced by its position relative to a molecular weight marker run on the same gel [106]. While Western blot is sensitive, its detection limits may not be as low as those achieved by amplification-based method, i.e., Enzyme Linked Immunosorbent Assay (ELISA). The method requires relatively high-quality protein samples, as degradation can affect results. Additionally, very large or very small proteins can sometimes be difficult to transfer from the gel to the membrane or may not separate well during electrophoresis. The presence of a band must be verified to be the protein of interest through correct controls, and bands can sometimes be non-specific or difficult to interpret, especially if the antibody has cross-reactivity [107]. In the context of MCC, Western blot is not typically used for routine diagnostic purposes in clinical settings due to its time-consuming nature and requirement for specialized equipment. However, it is a powerful tool for research, helping to confirm the role of MCPyV oncoproteins in the pathogenesis of MCC. In research settings, Western blotting is indispensable for studying the expression levels of viral oncoproteins, understanding their role in cancer progression, and developing targeted therapies [108]. The Western blot technique serves as a crucial method for confirming the expression of MCPyV oncoproteins and elucidating their role in the tumorigenesis of MCC. While it is more commonly employed in research due to its intricate procedure and need for analysis by experienced professionals, its contributions to the understanding of MCC on a molecular level are invaluable. However, the Western blot technique may fail to identify all MCPyV variants owing to shortened big T antigens, which may arise from mutations during viral integration [27]. Despite the emergence of more modern protein-detection techniques, Western blotting remains a fundamental tool in the study of MCPyV and its oncogenic mechanisms [109,110].

### 4.4. PCR

The virus, known as MCPyV, is a newly discovered human virus with oncogenic potential, meaning it can contribute to the development of MCC. The detection and study of MCPyV in various clinical samples, including respiratory tract secretions, has become an important area of research. One promising approach for studying MCPyV is through the use of PCR techniques. PCR is a powerful molecular biology technique that allows for the rapid and exponential amplification of specific DNA sequences. This technique has been employed to detect the presence of MCPyV DNA in samples from patients with MCC. PCR detects the presence of MCPyV DNA through several critical steps. Initially, DNA is extracted from clinical samples suspected to contain MCPyV, such as tissue biopsies from MCC tumors or cutaneous swabs [111,112]. The quality and purity of the extracted DNA are crucial for the successful application of PCR. Specific short sequences of DNA, known as primers, are then designed to match unique segments of the MCPyV genome. These primers are essential as they determine the specificity of the PCR, allowing it to target and amplify only MCPyV DNA if present in the sample. The PCR process begins with the denaturation step, wherein the DNA sample is heated to break the hydrogen bonds between the DNA strands, resulting in the separation of the double-stranded DNA into single strands [113]. The temperature is then lowered to allow the primers to bind or anneal to their complementary sequences on the single-stranded viral DNA. Next, a heat-stable DNA polymerase enzyme is used to synthesize new strands of DNA by adding nucleotides to the annealed primers, following the sequence of the template strand. This results in the generation of new copies of the specific DNA region flanked by the primers. These steps are repeated in cycles, typically 25–35 times, leading to the exponential amplification of the target MCPyV DNA segment. With each cycle, the amount of DNA doubles, resulting in over a billion copies of the target sequence after 30 cycles. Then, the amplified DNA can be visualized using gel electrophoresis and confirmed through methods such as sequencing or hybridization with a labeled probe specific to MCPyV [114,115]. Quantitative PCR can also be used to quantify the viral load by measuring the amount of amplified DNA product in real-time during the PCR process. The highly sensitive nature of PCR allows for the detection of low levels of MCPyV DNA in clinical samples, providing insights into the virus's role in the pathology of MCC. The rapid and specific detection capabilities of PCR make it an indispensable tool in the study and diagnosis of MCC [116,117]. On the other hand, some researchers argue that PCR may not always be reliable for the detection of MCPyV in clinical samples. The researchers further raised several potential issues such as PCR can produce false-negative results, especially in instances of low viral load or insufficient extraction of viral DNA from formalin-fixed, paraffin-embedded (FFPE) tissues [118]. The lack of identifiable truncating mutations in the big T antigen sequence in certain tumours indicates that not all MCPyV-associated tumors have identical genetic modifications, which may result in variable PCR outcomes [119]. This further raises concerns about drawing definitive conclusions regarding this virus's biology and clinical implications based solely on PCR-based studies [111].

### 4.5. ddPCR

The ddPCR is a highly sensitive variation of traditional PCR that enables the absolute quantification of target DNA sequences. It is particularly useful in applications requiring high precision, such as measuring viral load in infections or detecting rare genetic events [120]. ddPCR works by partitioning a sample of DNA into thousands or even millions of individuals, nanoliter-sized droplets, with each droplet acting as an independent micro-PCR reactor. This partitioning allows for the segregation of DNA molecules, with ideally one or zero target DNA molecules in each droplet [121]. Sample preparation for ddPCR involves extracting DNA from biological samples, such as tissues or cells from patients with MCC. This step ensures that the DNA is suitable for

downstream applications. In the reaction mix, the extracted DNA is combined with PCR reagents. These reagents include primers specific to the target sequences, which may be MCPyV DNA, as well as fluorescent probes, nucleotides, and DNA polymerase [122]. Droplet creation is achieved by emulsifying the reaction mix with oil using a specialized droplet generator instrument. This process creates thousands of droplets that function as individual reaction chambers. PCR amplification occurs within these droplets. When the target DNA sequence is present, it generates fluorescent signals within the droplets during amplification. Following PCR amplification, a droplet reader analyses each droplet. The reader detects and counts droplets that contain amplified DNA (positive) compared to those without any amplification (negative). The final step, data analysis, uses the number of positive droplets to calculate the absolute concentration of the target DNA in the original sample [123,124]. The calculation of the absolute concentration of target DNA in a sample using ddPCR relies on poison statistical analysis to account for the distribution of DNA copies across droplets. After PCR amplification, the process begins with counting the number of positive droplets, which fluoresce due to the presence of amplified target DNA, and negative droplets, which do not fluoresce, indicating the absence of target DNA [125]. The next step involves calculating the fraction of negative droplets by dividing the number of negative droplets by the total number of droplets. This fraction is crucial for applying Poisson statistics. According to the Poisson distribution, if λ represents the mean number of target DNA molecules per droplet, then the fraction of negative droplets is given by e^(-λ) [126]. Solving for λ requires taking the natural logarithm of the fraction of negative droplets, expressed as-

$$\lambda = -\ln\left(\frac{Negative\ Droplets}{Total\ Droplets}\right)$$

To determine the absolute concentration of target DNA molecules in the reaction, λ is multiplied by the total volume of the reaction. This calculation yields the total number of target DNA molecules. To find the concentration of target DNA in the original sample, this number is divided by the volume of sample DNA added to the reaction [127]. If the original sample was subjected to dilution before the reaction, the final concentration must be adjusted by multiplying by the dilution factor. The resulting concentration is typically reported in copies per μL or copies per mL of the original sample. It is important to note that ddPCR provides absolute quantification without the need for standard curves. The accuracy of ddPCR is highly dependent on precise droplet counting and the proper application of poisson statistics [128]. ddPCR offers several key advantages for detecting MCPyV in MCC. Due to its partitioning capability, ddPCR can detect very low amounts of viral DNA, making it incredibly sensitive and ideal for detecting MCPyV even when the virus is present at low levels [129]. One of the primary benefits of ddPCR is its ability to provide absolute quantification of target DNA molecules. Unlike quantitative PCR, ddPCR does not require a standard curve for quantification, which allows for more precise viral load measurements. Additionally, ddPCR reduces variability via utilizing individual droplets as separate reaction chambers, thereby minimizing the impact of potential PCR inhibitors and yielding highly reproducible results [130]. The clinical applications of ddPCR in MCC are significant due to its capacity for absolute quantification of MCPyV DNA. This can aid in several aspects of patient care. For diagnosis, ddPCR can detect MCPyV in tissue samples of MCC with high sensitivity, confirming the viral association with the carcinoma. In terms of prognosis, quantifying the viral load may correlate with disease outcomes, as patients with higher MCPyV loads might have different prognoses compared to those with lower levels of the virus. For treatment monitoring, ddPCR allows for the assessment of viral load over time, helping to determine the effectiveness of therapy. A decrease in MCPyV DNA levels could indicate a positive response to treatment, while stable or increasing levels may suggest resistance to treatment or disease progression [131]. Additionally, ddPCR can detect minimal residual disease after treatment, identifying very low levels of MCPyV DNA that may indicate the presence of remaining cancer cells that have evaded treatment. Despite these advantages, there are challenges and limitations associated with the use of ddPCR. The technical complexity of setting up and interpreting ddPCR assays requires specialized equipment and expertise, which may not be available in all laboratory settings. The cost of ddPCR is higher than standard PCR methods due to the need for specialized equipment and consumables. Standardization across different laboratories is crucial to ensure consistency in measurements, necessitating uniform protocols for droplet generation and reading [132,133]. Moreover, although ddPCR is sensitive, it still requires a minimum quality and quantity of DNA, which can be difficult to obtain from certain types of samples. ddPCR represents a powerful advancement in the molecular diagnostics of MCC, particularly for the quantification and study of MCPyV. Its high sensitivity and precise quantification potential make it an invaluable tool for research and clinical management of MCC. However, considerations regarding its accessibility, cost, and the need for specialized technical expertise must be addressed when implementing ddPCR as a routine diagnostic assay [120,129]. ddPCR markedly improves the precision of MCPyV identification in MCC relative to conventional PCR techniques. This enhancement is chiefly because to ddPCR's superior sensitivity and quantification abilities, facilitating more dependable detection of viral DNA in clinical specimens. Comparative research of quantitative Real-Time PCR (qPCR) and ddPCR revealed that qPCR discovered MCPyV in 35 % of MCC samples, whereas ddPCR recognised 65 % of the samples as positive. ddPCR exhibited an elevated detection rate in tumours situated at UV-exposed locations, underscoring its efficacy in identifying MCPyV across diverse tumour habitats. Moreover, ddPCR facilitates accurate measurement of MCPyV DNA, which is essential for comprehending the viral load in tumours and its possible association with disease progression [129].

### 4.6. NGS

NGS represents a transformative advancement in the field of genomic studies, allowing for the comprehensive examination of genetic sequences at an unprecedented scale and speed. In the context of MCC, NGS has become an invaluable tool for assessing the mutational landscape, particularly in relation to the MCPyV, which is implicated in a significant proportion of MCC cases. As authors have discussed earlier also that, MCC is a rare but highly aggressive skin cancer that has been increasingly associated with MCPyV [18,53,134]. This virus contributes to oncogenesis through the integration of its DNA into the host genome, whereby it continually expresses its oncoproteins, including the large T antigen and small T antigen. These viral oncoproteins are capable of manipulating the host cell's machinery, leading to unchecked cellular proliferation and tumorigenesis [135]. NGS technology has enabled researchers to explore the genetic underpinnings of MCC, providing insights into the role MCPyV plays in the MCC development. Through high-throughput sequencing, NGS offers a granular view of the genetic alterations and allows for the identification of MCPyV integration sites within the host genome. By sequencing both tumor and matched normal tissues, mutational patterns unique to the cancer can be isolated, revealing potential drivers of MCC [136]. Furthermore, the use of NGS facilitates the detection and characterization of oncogenic pathways modulated by the viral oncoproteins. For instance, the Large T antigen is known to inactivate tumor suppressor proteins such as pRb, which is crucial for cell cycle regulation. Detailed genomic analyses via NGS can illuminate the broader impacts of such viral-host interactions, contributing to the molecular profile of MCC [28,137]. NGS also plays a pivotal role in uncovering the mutational burden and heterogeneity of MCC tumors, which are linked to patient prognosis and response to therapies. This information can be critical in guiding personalized treatment strategies, such as the use of immune checkpoint inhibitors, which have shown effectiveness against high mutational burden cancers. So, it can be said that the application of NGS in the study of MCC allows for a

comprehensive analysis of the genetic aberrations and the influence of MCPyV oncoproteins [138]. This approach not only accelerates the discovery of potential therapeutic targets but also refines the prognostic and diagnostic frameworks of MCC. NGS stands as a cornerstone technology in the pursuit of tailoring personalized and more effective therapeutic regimes for patients with MCC [139]. However, some critics argue that the cost of NGS is prohibitive and may not be accessible to all patients. Additionally, they point out that the technology has limitations in accurately predicting treatment response in certain cases [140–142]. Although NGS can detect MCPyV in a considerable proportion of MCC patients, its capacity to differentiate between pathogenic and non-pathogenic viral presence is constrained.The lack of identifiable truncating mutations in the big T antigen of MCPyV in certain tumours prompts enquiries on its oncogenic function, suggesting that NGS may not discover all pertinent changes linked to tumorigenesis [119]. Next-generation sequencing may encounter difficulties in distinguishing between MCPyV-positive and MCPyV-negative MCC [143]. The existence of mutations in additional genes (e.g., NF1, PIK3CA) in MCPyV-negative MCCs complicates the scenario, indicating that NGS results require careful interpretation. Despite its limitations, NGS is a potent instrument for detecting genetic variations in MCC, and continuous developments may improve its specificity and sensitivity in finding MCPyV-related modifications.

### *4.7. ISH*

ISH is a laboratory technique used to detect specific nucleic acid sequences within fixed tissues and cells in their natural location [144]. For MCC, ISH can be particularly useful for identifying the presence of MCPyV DNA directly within the tumor tissues [145]. ISH is based on the complementary nature of DNA or RNA strands [146]. A labelled nucleic acid probe is designed to be complementary to the sequence of interest—in this case, MCPyV DNA. When the probe is applied to the tissue sample, it can hybridize (bind) specifically with the target sequence if it is present. The process begins with tissue preparation, where MCC tissue samples are fixed, typically embedded in paraffin, and sectioned thinly onto microscope slides. These preparations preserve morphology and immobilize the DNA or RNA targets [65]. A probe, either RNA or DNA, that has a sequence complementary to a segment of the MCPyV genome is synthesized. This probe is labelled with either a fluorescent tag or an enzyme that produces a chromogenic substrate. During the prehybridization treatment, tissue sections are treated to make the DNA or RNA in the cells accessible to the probe. This may include deparaffinization, rehydration, and application of enzymes or chemicals to permeabilize cells [147]. The probe is then applied to the tissue sections and incubated under conditions that allow for specific binding between the probe and its target MCPyV DNA within the tissue. After incubation, slides are washed to remove unbound and non-specifically bound probes, leaving only the specifically bound probes attached to their target MCPyV DNA. Detection methods vary: for fluorescent in situ hybridization, the slides are examined under a fluorescence microscope; for chromogenic in situ hybridization, an enzyme reaction results in a colorimetric change that can be seen under a regular light microscope [148,149]. The pattern and intensity of the signal provide qualitative and quantitative data on MCPyV DNA presence and abundance. Experienced pathologists then interpret the signal, determining the presence and distribution of MCPyV within the tumor tissue. Signal intensity may also provide quantitative information regarding viral load. ISH offers several advantages in MCC [147]. It allows for the detection of MCPyV within the actual context of the tissue architecture, showing exactly which cells contain the virus, which is important in confirming the diagnosis and understanding the biology of MCC [150]. Additionally, ISH provides both quantitative and qualitative information. Apart from revealing the presence of the virus, ISH can also give an idea of the viral load within the tumor cells. Moreover, the pattern of viral DNA distribution can provide insights into the biology of the infection and the tumor [151]. Modern ISH techniques, such as fluorescence in situ hybridization, can be highly sensitive and specific when carefully designed probes are used, making them well-suited to detect the low-abundance MCPyV DNA within MCC [152]. Furthermore, ISH can complement other molecular assays, like PCR and immunohistochemistry, by providing spatial information that these other techniques cannot offer. However, there are challenges associated with ISH. The technique requires careful optimization of probe design and hybridization conditions, as well as controlled processing of tissue samples to preserve nucleic acid integrity and tissue morphology [153]. The interpretation of ISH results can depend on the experience and skill of the pathologist, particularly with chromogenic substrates, where background staining and signal specificity can sometimes be challenging to discern. Standardization across different laboratories is crucial for reproducible results, necessitating standardized probe design, hybridization protocols, and detection methodology [154]. While ISH is sensitive, it may not detect very low levels of MCPyV DNA that fall below the threshold of the utilized probes and detection methods. Additionally, the ISH process from tissue preparation to result interpretation can be lengthy compared to other techniques like PCR, potentially impacting its utility in clinical settings requiring rapid diagnostic turnaround [155]. ISH offers a unique approach to visualize and quantify MCPyV DNA in the context of MCC tissue sections. It is an essential tool for the diagnosis of MCC and contributes valuable information on the distribution and load of MCPyV within tumors. Despite its advantages, the application of ISH requires optimized protocols, experienced personnel, and may not always be suitable for the rapid diagnosis required in clinical practice [28,145]. Although ISH can detect viral presence, it may not consistently align with the true viral load or integration status, resulting in some misinterpretations. ISH may be ineffective in identifying MCPyV in tumors with low viral levels or in instances where the virus is integrated into the host genome, as observed in roughly 80 % of MCC cases. Notwithstanding these constraints, alternative techniques as quantitative PCR and CRISPR-based diagnostics exhibit the potential for more dependable detection of MCPyV in MCC, possibly addressing the deficiencies of ISH [156].

## 5. Integrative approaches in MCC research

Integrative approaches in MCC research leverage the complexity of biological systems and the interplay between various factors contributing to the disease. By utilizing various scientific and computational methods, researchers aim to gain a more comprehensive understanding of MCC development, progression, and treatment response[157].

### *5.1. Combined analysis of viral oncoproteins and tumor suppressor mutations*

Combined analysis of viral oncoproteins and tumor suppressor mutations in MCC research involves examining the role of both these factors in the pathogenesis and development of the cancer. This comprehensive approach is essential because MCC can result from the interplay between the integration and expression of MCPyV oncoproteins, such as large T antigen and small T antigen, and the mutation or inactivation of cellular tumor suppressor genes [158,159]. The implications of combined analysis in MCC are significant. This examination underscores how the presence of the virus and mutations in the host genome can together push cells towards malignancy. Understanding these mechanisms can highlight specific proteins or pathways that might be intercepted with targeted therapies. By recognizing the combined molecular signatures of viral oncogenesis and cellular mutations, unique biomarkers can be identified, potentially leading to more accurate diagnosis and prognosis of MCC [160]. Identifying specific viral oncoproteins interacting with genetic mutations can aid in designing personalized treatment regimens that address the unique characteristics of an individual's tumor. Creating predictive models based on the interaction between MCPyV

oncoproteins and host tumor suppressor mutations may also help predict disease course and treatment response [110]. Research perspectives highlight the importance of cell culture and animal models in studying the interaction between MCPyV oncoproteins and tumor suppressor gene mutations. Researchers use these models to observe the effects of these interactions on cellular behavior and tumorigenesis [71]. Functional studies involving the introduction or knockdown of oncoproteins and tumor suppressor genes in cell lines can provide direct evidence of their roles in MCC and determine the potential impact of therapeutic interventions. Advanced bioinformatics tools allow for the analysis of pathways affected by MCPyV oncoproteins and mutated tumor suppressors, offering insights into the network of interactions that could contribute to carcinogenesis and potential points of intervention [52]. Correlating the findings from combined molecular analyses with clinical data, such as patient outcomes and treatment responses, can validate the relevance of identified targets and biomarkers [161]. Challenges and future directions in this field include the complexity of integrating large-scale genomic and proteomic data sets to analyze both viral and host factors in MCC, which requires sophisticated computational resources and expertise. MCC tumors can be heterogeneous, meaning that the interplay between viral oncoproteins and tumor suppressor mutations might vary across different tumor regions and between patients, complicating the development of universal treatments [162]. Understanding the mechanisms of resistance to current therapies is crucial, and combined analysis can shed light on whether changes in oncoprotein expression or the emergence of new mutations contribute to treatment failure [108]. The combined analysis of viral oncoproteins and tumor suppressor mutations also offers a holistic view of the molecular dynamics at play in MCC [163]. It provides a pathway to novel therapeutic interventions, enhances our understanding of the disease's etiology, and potentially leads to improvements in the clinical management of MCC through more personalized approaches to treatment and care. These integrative efforts suggest a promising direction for ongoing and future MCC research [164].

### 5.2. Systems biology approaches to understanding MCC complexity

Systems biology is a comprehensive approach that aims to understand complex biological systems through the study of the interactions between their components. In the context of MCC, systems biology approaches can provide an integrated view of how various cellular and molecular constituents interact and contribute to the disease's complexity. This can include the influence of viral oncoproteins from the MCPyV, host genetic mutations, epigenetic changes, and the tumor microenvironment [89]. A holistic perspective in systems biology does not focus on individual genes, proteins, or pathways in isolation but instead looks at how they function collectively within the network that makes up the biological system [165]. In MCC research, this could include studying how viral oncoproteins perturb cellular signaling networks and interact with host immune responses [135]. Integration of multi-omics data often involves combining data from genomics, transcriptomics, proteomics, and metabolomics. By combining these data types, researchers gain a multidimensional view of the biological processes involved in MCC. Genomics provides insights into DNA alterations, including mutations in tumor suppressor genes and the integration of viral DNA [166]. Transcriptomics reveals changes in RNA expression levels, indicating the genes that are upregulated or downregulated due to MCPyV oncoproteins [167]. Proteomics offers a closer look at the functional proteins, how their expression is altered in MCC, and any post-translational modifications [16]. Metabolomics shows the metabolic changes in tumor cells, influenced by genetic mutations and viral interference with cellular pathways [168]. Advanced computational methods are employed in systems biology to model the networks and systems identified through omics studies. This can help predict the behavior of the system under various conditions and understand disease progression. Simulations can also be used to test potential interventions in a virtual environment prior to experimental validation [169,170]. By constructing and analyzing interaction networks, such as protein-protein interaction networks, systems biology helps identify key regulatory nodes and pathways that could be potential targets for therapy. Network analysis can also shed light on robustness and vulnerability within the biological system, possibly uncovering points where the system can be manipulated to treat or prevent MCC [171]. Systems biology acknowledges that biological systems are dynamic. Positive and negative feedback loops, as well as regulatory mechanisms within the system, can be pivotal in understanding how MCC cells respond to environmental stresses or therapeutic interventions [172]. Changes in the tumor microenvironment, for instance, can lead to adjustments in the behavior of cancer cells and may also influence the immune system's ability to recognize and destroy tumor cells [173]. Signal pathway mapping involves the detailed mapping of signaling cascades affected by both the presence of MCPyV and the mutations within host cellular pathways. It includes understanding which pathways are activated or inhibited and how these cascades interact with each other to promote carcinogenesis or tumor maintenance [174]. Through the global analysis of cellular systems, systems biology approaches can help identify biomarkers that predict disease outcomes or response to therapy. This could profoundly impact patient stratification for different treatments and clinical trial design. Understanding the systematic changes in MCC due to interactions of viral oncoproteins and genetic changes can uncover unexpected drug targets. It can also reveal why certain drugs might work or fail, potentially guiding drug repositioning. Furthermore, it can inform the development of combination therapies that target multiple aspects of the cancer's biology. By identifying specific pathways or network nodes altered in individual patients' tumors, systems biology may eventually lead to personalized medicine approaches tailored to the unique molecular profile of each patient's cancer. It could also help define therapeutic windows—optimal times to administer treatment based on the biology of the tumor and its microenvironment. Systems biology models can help track how MCC progresses over time, including the processes that lead to metastasis. By modeling the evolutionary pathways of cancer cells, researchers can better understand how to interrupt these processes [175]. The comprehensive nature of systems biology presents challenges, primarily in handling and interpreting vast amounts of complex data. These challenges necessitate the development of new bioinformatics tools and cross-disciplinary collaboration. Moreover, there is a need for high-quality and standardized data to fuel reliable systems biology models. As technology advances and more data become available, systems biology is expected to provide deeper insights into the complexity of MCC. Integrating this understanding into clinical practice could lead to more effective, personalized, and dynamic treatment strategies, improving care for patients with this challenging disease. Fig. 3 further explain the various signaling molecules and their role in MCC.

## 6. Future directions

Genetic and molecular profiling in MCC, with a focus on the MCPyV oncogenes, is critical for advancing the diagnosis and treatment of this aggressive skin cancer. These profiles extensively detail genomic alterations, expression of viral oncoproteins, and molecular changes within tumor cells, promising significant benefits to human health [176]. The diagnostic accuracy can be achieved through advanced molecular profiling techniques, leading to the development of highly sensitive and specific diagnostic tests for MCC. The detection of MCPyV oncoproteins and other genetic abnormalities unique to MCC can result in earlier and more accurate diagnoses, essential for better prognosis and personalized treatment plans [177]. The early detection and screening may become feasible as molecular profiling techniques become more refined and accessible. This could include analyzing specific biomarkers or applying liquid biopsy techniques to detect traces of MCPyV DNA or mRNA in blood samples, particularly in at-risk populations [178]. Several

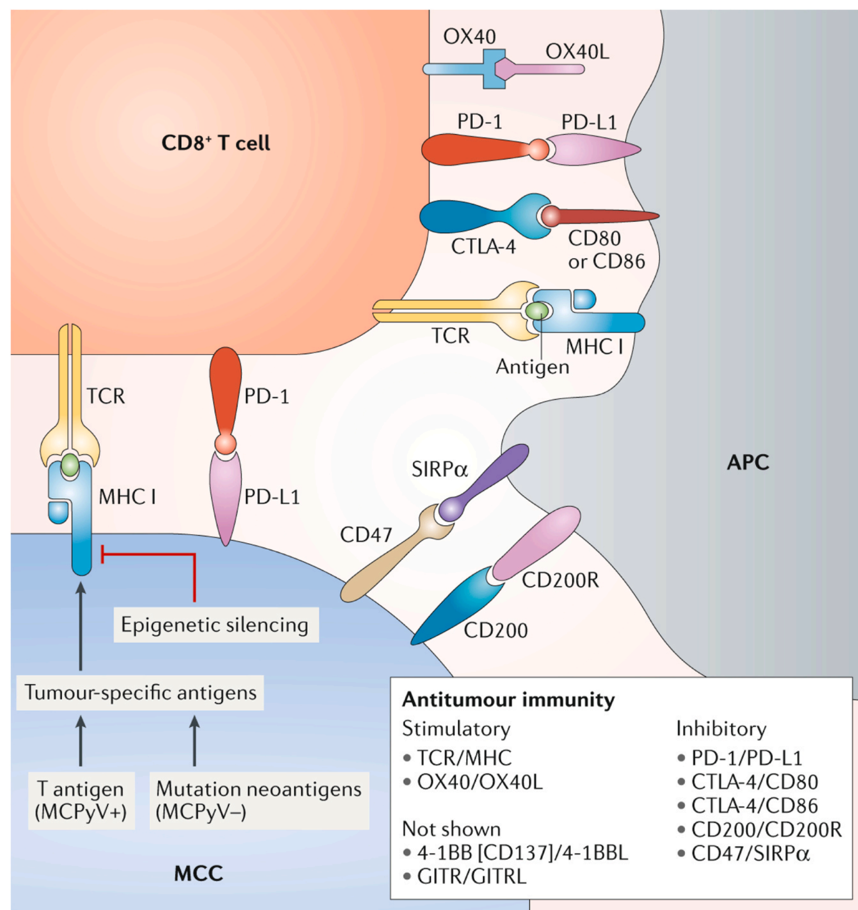


**Fig. 3.** Summarized the immune response against tumors is influenced by signaling molecules on immune cells like T cells, antigen-presenting cells (APCs), and MCC cells. The body's adaptive antitumor immunity relies on the presentation of tumor antigens on the MHCs of either tumor cells or APCs. In the case of MCC, tumor-associated antigens can be viral protein products in Merkel cell polyomavirus (MCPyV)-positive MCC or, in MCPyV-negative tumors, neoantigens resulting from somatic mutations. In virus-positive MCC tumor cells, the presentation of antigens by MHC complexes is often suppressed through epigenetic silencing. Several signaling pathways have the potential to enhance either (e.g., OX40–OX40L) or inhibit antitumor immunity (e.g., programmed cell death protein 1 (PD-1)– programmed cell death protein 1 ligand 1 (PD-L1)). These different immune signaling pathways could serve as potential targets for therapy, i.e., CTLA-4, cytotoxic T antigen 4; GITR, glucocorticoid-induced TNFR-related protein; SIRP α, signal-regulatory protein α; TCR, T cell receptor [110].

targeted therapeutics can be designed by understanding the role of MCPyV oncoproteins in MCC pathogenesis. These targeted therapies, aimed at viral proteins such as the large T and small T antigens, could lead to more effective treatments with fewer side effects by inhibiting their functions or downstream signaling pathways [179]. Upon administration of a nanovaccine, the body's innate immune system will recognize oncoproteins as exogenous entities, triggering the production of antibodies in response to their distinct physiochemical characteristics [180]. Vaccine development is a promising future direction, potentially including preventive vaccines against MCPyV and therapeutic vaccines designed to elicit an immune response against MCC tumors expressing MCPyV antigens [181]. In this context, the personalized medicine can be guided by molecular profiling, tailoring treatment to the unique characteristics of each patient's tumor. By understanding the mutational landscape and the presence of viral oncoproteins, clinicians can choose the most effective treatments and adjust them as the tumor evolves [182]. Predictive modeling, based on detailed genetic and molecular information, can forecast tumor progression and response to treatment, allowing for more informed decision-making and proactive disease management [183]. Companion diagnostics can identify patients most likely to benefit from new treatments targeting specific molecules or pathways. Genetic and molecular profiling, including MCPyV status, can also help clinicians better stratify patients according to their risk of recurrence or aggressive disease, guiding clinical decisions and potentially reducing overtreatment [177]. Understanding the tumor microenvironment and its influence on MCC progression, particularly how it is affected by MCPyV oncoproteins, might open new therapeutic strategies. The interactions between tumor cells and their microenvironment could disrupt immune evasion mechanisms employed by MCC. Advanced immunotherapies can be optimized using genetic and molecular profiling to identify new immune-related targets [184]. This approach could include personalized cancer vaccines or engineered T-cell therapies tailored to individuals' MCPyV status [185]. Technology advancements in sequencing and bioinformatics analysis will probably reduce costs and increase the accessibility of comprehensive genetic and molecular profiling, making precision medicine a more standard part of care for MCC and other cancer types [186,187]. Molecular profiling can significantly influence the design of clinical trials by identifying patient subgroups with specific molecular characteristics who are most likely to respond to new drugs or therapies, enhancing trial efficiency and the likelihood of regulatory approval [188]. Functional genomics research will focus on understanding the effects of identified mutations on cellular behavior, leading to the discovery of new therapeutic targets and drugs that correct dysfunctional molecular pathways. Profiling the genetic evolution of MCC during treatment could uncover mechanisms behind treatment resistance, guiding the development of combination therapies that prevent or overcome resistance. The interdisciplinary research approaches, combining the expertise of clinicians, molecular

biologists, and computer scientists, will be crucial for translating genetic and molecular findings into clinical practice [189,190]. Investigating the epigenetic landscape of MCC, including DNA methylation patterns and histone modifications, can provide a fuller picture of regulatory changes due to MCPyV oncoproteins, leading to therapies that reverse aberrant epigenetic modifications [191]. Targeting epigenetic regulators for cancer therapy: mechanisms and advances in clinical trials. Enhanced risk management can be achieved through molecular profiling, allowing clinicians to determine which patients are at greater risk of metastasis and allocate aggressive treatments accordingly. This approach can minimize the burden on patients with less aggressive disease [187]. Molecularly informed surgery and radiotherapy can be guided by the genetic and molecular characteristics of MCC, optimizing surgical margins and the precision of radiotherapy to effectively target tumor cells while minimizing damage to healthy tissues [12,192]. Metabolic targeting, based on a better understanding of altered metabolism in MCC, could lead to novel therapies, particularly if viral oncoproteins influence metabolic pathways. Bioinformatics and data sharing will accelerate discovery and the translation of genetic and molecular knowledge into clinical practice through the development of sophisticated tools and publicly available databases [193]. Neoadjuvant and adjuvant therapies can be optimized using profiling to identify the most effective treatments for individual patients, improving overall survival rates by shrinking tumors before surgery or preventing recurrence afterward [194,195]. Healthcare policy and reimbursement must evolve to ensure patients have access to diagnostic technologies and that profiling costs are covered by insurance providers as molecular profiling becomes integral to cancer care [196]. International collaboration and standardization are key to ensuring consistent and replicable research findings that can be universally applied to benefit patients worldwide [197,198]. Educational initiatives will be necessary to keep healthcare professionals updated on the latest scientific advancements and their practical applications in patient care [199,200]. Ethical, legal, and social implications of genetic and molecular profiling must be addressed, including genetic privacy and discrimination. Initiatives focused on ethical management, genetic counseling, and safeguarding patient rights are essential for responsible data handling and patient care practices [201]. Quality of life studies can lead to improvements in symptom management and patient well-being by developing targeted therapies with fewer side effects than conventional chemotherapy [202–204]. Health disparities research may explain differences in MCC incidence and outcomes among different populations, leading to targeted public health interventions and equitable healthcare delivery [205]. Combining molecular profiling with imaging can enhance tumor detection, monitoring, and treatment planning, leading to more personalized and timely interventions [206]. AI and machine learning can analyze complex molecular datasets to uncover patterns and predictive markers, leading to breakthroughs in predicting disease trajectories and treatment responses [207]. Genetic therapy advances could develop strategies to correct or suppress defective genetic components of carcinoma cells or enhance the immune response against tumor cells, guided by MCPyV's impact on genetic stability and function [108,208]. Understanding the links between molecular profiles, patient lifestyle, and environmental exposures could yield insights into MCC etiology and progression, identifying modifiable risk factors for disease prevention [15].These prospective directions represent a convergence of pioneering research, clinical practice, and technology poised to significantly enhance our understanding of MCC and improve patient outcomes. As cancer research evolves, integrating novel diagnostic techniques and discovering biomarkers and therapeutic targets hold promise for making MCC a more treatable and potentially preventable disease.

## 7. Discussion

Skin cancers of the aggressive MCC variety have a greater chance of metastasis, recurrence, and death. Over the past twenty years, the prevalence of MCC has almost tripled. For a given stage of MCC, the overall survival rate for people with MCC is 70 %. The MCC risk factors are immunosuppression, old age, and exposure of UV. Most patients have radiation therapy first, then extensive surgical excision; only chemotherapy is administered if there is metastasis and positive lymph nodes [209]. Patients with MCC have a longer survival percentage if they receive an early diagnosis. Gene expression profiling-based molecular subtyping of cancers has influenced the development of subtype-specific targeted therapies as well as subtype-specific diagnosis and prognosis [210]. In order to have a thorough understanding of the genes and pathways involved in MCC, it is necessary to analyze the molecular heterogeneity of MCC. Additionally, it will open up new possibilities for targeting MCC patients who fit particular subtypes.

We have discovered some MCC causing elements in our investigation. Differentiated gene signatures in the development of MCC were found using MCPyV oncoproteins and gene set enrichment analysis. MCCs showed that overexpression of BCL-2 and CD56 genes was accompanied by overexpression of genes related to cell cycle, spliceosome, replication of nucleotides, and mismatch DNA repair, among other processes. On the other hand, TILs, SSTR2, CD56, and BCL-2 were identified as the overexpressed pathways by gene ontology and gene set enrichment analysis (Table 1). It has been discovered that in a number of cancer tissues, including bladder cancer and hepatocellular carcinoma, among others, the cycle of expression, repression, and repression of genes i.e. PD-1/PD-L and CTLA-4 is a significant carcinogenic component [211].

**Table 1**
MCC causing elements and therapeutic agents.

| Factors involved in MCC | Indication | Examples of potential therapeutic agents | Reference |
|---|---|---|---|
| Intratumoral CD8+ T-cells (TILs) | Ligand CTLA−4 and PD-L1/PD−1that bring back the activity of cytotoxic T cells | Anti CTLA−4 (Ipilimumab) and anti PD−1 (Pembrolizumab) | [223,224] |
| CD8+ T-cells specific to viral antigens | Exhibit elevated levels of ligand PD-L1 tumor-specific expression that vary in response to therapy. | Ipilimumab and Pembrolizumab | [225] |
| Adoptive immunotherapy | Autologous T-cell therapy | Aldesleukin | [226] |
| Tyrosine kinases, including PDGFR, VEGFR2, and cKIT | Focusing on pathways for aberrant cell growth and proliferation | Imatinib; pazopanib; Cabozantinib | [227,228] |
| Overexpression of BCL−2 | Inducing apoptotic death | Oblimersen sodium; ABT−263 | [229,230] |
| MCC Survivin expression | Cytostatic response in MCC xenografts | Xenografts with YM155 | [231] |
| Somatostatin receptor type 2 (SSTR2) | Bind to SSTR2 in both functional and nonfunctional neuroendocrine tumors to produce antiangiogenic, antisecretory, and antiproliferative effects. | Octreotide | [232] |
| CD56 (NCAM) expression on the cell surface | Antibody-drug conjugates and immunocytokines | Lorvotuzamab mertansine (IMGN901) | [233] |
| Tenascin-C and interleukin (IL)−2 | Angiogenesis IL−2 is a strong immune stimulant, and tenascin-C is a marker that is expressed in the reactive stroma of many solid tumors. | F16-(IL)−2 immunocytokine; F16-IL2 in conjunction with paclitaxel | [234] |

Additionally, we discovered several known target genes and possible therapeutic drugs associated with them for the treatment of MCCs. PTCH1, CDKN2A, AURKA, BRCA1, and MCL1 are among these genes in subtype 1, and MCL1 in subtype II [212]. In order for MCC patients to be resistant to PD-L1/ PD-1 suppression and to have low infiltration of CD8+ T cells, HDACis functions to accelerate stunted HLA class-I expression. This aids the tumors' ability to elude the host's immune system [212]. Thus, PTCH1 and HDACi inhibitors may be beneficial for patients with subtype I MCC. Further information regarding chemotherapy resistance in different types of cancer has been described regarding the expansion of BCL2-class antiapoptotic proteins such as MCL1, BCL2, and BCL-xL [212]. ABT-263 effectively reduced various pro-survival BCL-2 proteins in 10 out of 11 MCC xenografts in one investigation, providing significant evidence for the curative effect of these compounds on targeting of MCC [213]. It was discovered that Sabutoclax, another pan-active BCL2 inhibitor, may target each of the five anti-apoptotic proteins. Following up on the potential that MCL1 inhibition appears to offer as specific inhibitors of MCL1 [214].

In this work, we examined two approaches for identifying MCPyV in melanoma and MCC samples. The efficacy of the MCPyV monoclonal antibody, which targets the big T-antigen (clone CM2B4), varied from 39.0 % to 90.0 % [215]. The preanalytic changing parameters, such as tissue fascination and viral replica levels in the tumor cells, also have an impact on the sensitivity of IHC [216]. Research that compares MCPyV detection by PCR and IHC typically show appropriate concordance. Based on our findings and those of other research, we concluded that PCR was more responsive than IHC [216–218]. Additionally, we identified MCPyV-associated melanoma samples exclusively by PCR. Molecular profiling on MCCs has been the subject of few, if any, controversial studies. Patients with lower levels of malignant cells's KIT, had longer survival times than those with higher expression of the KIT receptor tyrosine kinase in primary MCCs; nevertheless, activating mutations in KIT have not been found in MCCs [219,220].

In a different study, the molecular genetics of MCPyV-positive and -negative tumors were examined. It was found that MCPyV-positive MCCs have comparatively few mutations and lack a clear UV signature, supporting the oncogenic significance of T-antigens as the primary drivers of these cancers [221]. The immune checkpoint inhibitor response's molecular landscape was described in a sizable genomics investigation conducted in MCC. MCPyV sequences were found exclusively in subjects with a low tumor load. A response was seen in 50.0 % of high VTB tumors and 41.0 % of low VTB and MCPyV-positive tumors [17]. In contrast, VTB was higher in positive samples of MCPyV because fewer MCPyV-negative cases were evaluated.

Our investigation of gene expression revealed that MCL1 inhibitors greatly aided MCC when BCL-2 family proteins were present. In a similar vein, imatinib, another possible blocker of the intended MCC, was also discovered. It is believed that patients with metastatic MCC and inoperability issues can benefit from imatinib inhibitor therapy [222]. These findings suggested that these inhibitors and diagnostic methods would be helpful for MCC patients.

## 8. Conclusion

This review has highlighted significant advancements in the understanding and treatment of MCC, particularly emphasizing the role of MCPyV. The integration of molecular profiling, innovative diagnostic techniques, and personalized medicine approaches has shown promise in improving patient outcomes and guiding treatment strategies. This article serves as a comprehensive resource for researchers worldwide by providing a holistic view. It integrates multi-omics data and systems biology approaches, offering a detailed perspective on the molecular dynamics of MCC. This can guide future research directions and the development of novel therapeutic interventions. The discussion on advanced diagnostic techniques, such as immunohistochemistry and molecular profiling, can result in the development of more accurate and efficient diagnostic tools. Additionally, emphasizing the need for interdisciplinary and international collaboration encourages the pooling of resources and expertise to overcome current research challenges. For the general public, the advancements discussed in this article translate to improved patient care. Personalized medicine approaches tailored to the unique genetic profiles of patients can lead to more effective treatments with fewer side effects, enhancing overall survival rates and quality of life. The development of biomarkers for early detection and prognosis can lead to timely interventions, potentially reducing the burden of advanced MCC. Furthermore, insights into the tumor microenvironment and immune evasion mechanisms pave the way for novel immunotherapies, offering new hope for patients with MCC.

## CRediT authorship contribution statement

**Harpreet Singh:** Writing – original draft, Conceptualization, Formal analysis. **Sourav mohanto:** Writing – original draft, Writing – review & editing, Formal analysis, Visualization, Software. **Anil Kumar:** Writing – original draft, Project administration, Formal analysis. **Arun Kumar Mishra:** Writing – original draft, Project administration. **Arvind Kumar:** Writing – original draft, Writing – review & editing, Visualization, Conceptualization, Formal analysis. **Amrita Mishra:** Writing – original draft, Project administration, Methodology, Formal analysis. **Mohammed Gulzar Ahmed:** Writing – original draft, Writing – review & editing, Supervision, Data curation. **Mukesh Kr. Singh:** Writing – original draft, Visualization, Resources, Formal analysis. **Amrendra Pratap Yadav:** Writing – original draft, Formal analysis, Validation, Supervision. **Shivani Chopra:** Writing – original draft, Formal analysis, Data curation. **Hitesh Chopra:** Writing – review & editing, Supervision, Writing – original draft, Formal analysis.

## Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## References

[1] J.C. Becker, A. Stang, J.A. DeCaprio, L. Cerroni, C. Lebbé, M. Veness, P. Nghiem, Merkel cell carcinoma, Nat. Rev. Dis. Prim. 3 (2017) 17077, https://doi.org/10.1038/nrdp.2017.77.
[2] P.B. Munde, S.P. Khandekar, A.M. Dive, A. Sharma, Pathophysiology of Merkel cell, J. Oral Maxillofac. Pathol. 17 (2013) 408–412, https://doi.org/10.4103/0973-029X.125208.
[3] J.H. Leonard, A.L. Cook, M. Van Gele, G.M. Boyle, K.J. Inglis, F. Speleman, R. A. Sturm, Proneural and proneuroendocrine transcription factor expression in cutaneous mechanoreceptor (Merkel) cells and Merkel cell carcinoma, Int. J. Cancer 101 (2002) 103–110, https://doi.org/10.1002/ijc.10554.
[4] E.M. Zwijnenburg, S.F.K. Lubeek, J.E.M. Werner, A.L. Amir, W.L.J. Weijs, R. P. Takes, S.A.H. Pegge, C.M.L. van Herpen, G.J. Adema, J.H.A.M. Kaanders, Merkel cell carcinoma: new trends, Cancers 13 (2021), https://doi.org/10.3390/cancers13071614.
[5] S.E. Broida, X.T. Chen, C.L. Baum, J.D. Brewer, M.S. Block, J.W. Jakub, B. A. Pockaj, R.L. Foote, S.N. Markovic, T.J. Hieken, M.T. Houdek, Merkel cell carcinoma of unknown primary: clinical presentation and outcomes, J. Surg. Oncol. 126 (2022) 1080–1086, https://doi.org/10.1002/jso.27010.
[6] P.U. Farooq Baba, Z. Rasool, I. Younas Khan, C.J. Cockerell, R. Wang, M. Kassir, H. Stege, S. Grabbe, M. Goldust, Merkel cell CArcinoma: from Pathobiology to Clinical Management, Biology 10 (2021), https://doi.org/10.3390/biology10121293.
[7] K. Ouyang, D.X. Zheng, G.W. Agak, T-cell mediated immunity in Merkel cell carcinoma, Cancers 14 (2022), https://doi.org/10.3390/cancers14246058.
[8] K. Coggshall, T.L. Tello, J.P. North, S.S. Yu, Merkel cell carcinoma: an update and review: pathogenesis, diagnosis, and staging, J. Am. Acad. Dermatol. 78 (2018) 433–442, https://doi.org/10.1016/j.jaad.2017.12.001.
[9] C. Lebbe, J.C. Becker, J.-J. Grob, J. Malvehy, V. Del Marmol, H. Pehamberger, K. Peris, P. Saiag, M.R. Middleton, L. Bastholt, A. Testori, A. Stratigos, C. Garbe, Diagnosis and treatment of Merkel Cell Carcinoma. European consensus-based interdisciplinary guideline, Eur. J. Cancer 51 (2015) 2396–2403, https://doi.org/10.1016/j.ejca.2015.06.131.
[10] S. Ashique, N. Mishra, A. Garg, N. Kumar, Z. Khan, S. Mohanto, D.K. Chellappan, A. Farid, F. Taghizadeh-Hesary, A critical review on the role of probiotics in lung

cancer biology and prognosis, Arch. Bronconeumol. (2024), https://doi.org/10.1016/j.arbres.2024.04.030.
[11] J. Jabbour, R. Cumming, R.A. Scolyer, G. Hruby, J.F. Thompson, S. Lee, Merkel cell carcinoma: assessing the effect of wide local excision, lymph node dissection, and radiotherapy on recurrence and survival in early-stage disease—results from a review of 82 consecutive cases diagnosed between 1992 and 2004, Ann. Surg. Oncol. 14 (2007) 1943–1952, https://doi.org/10.1245/s10434-006-9327-y.
[12] S. Naseri, T. Steiniche, M. Ladekarl, M.L. Bønnelykke-Behrndtz, L.R. Hölmich, S. W. Langer, A. Venzo, E. Tabaksblat, S. Klausen, M. Skaarup Larsen, N. Junker, A. H. Chakera, Management recommendations for Merkel cell carcinoma-a Danish perspective, Cancers 12 (2020), https://doi.org/10.3390/cancers12030554.
[13] C.V. Angeles, M.S. Sabel, Immunotherapy for Merkel cell carcinoma, J. Surg. Oncol. 123 (2021) 775–781, https://doi.org/10.1002/jso.26319.
[14] I.S. Chan, S. Bhatia, H.L. Kaufman, E.J. Lipson, Immunotherapy for Merkel cell carcinoma: a turning point in patient care, J. Immunother. Cancer 6 (2018), https://doi.org/10.1186/s40425-018-0335-9.
[15] D. Schadendorf, C. Lebbé, A. Zur Hausen, M.-F. Avril, S. Hariharan, M. Bharmal, J.C. Becker, Merkel cell carcinoma: epidemiology, prognosis, therapy and unmet medical needs, Eur. J. Cancer 71 (2017) 53–69, https://doi.org/10.1016/j.ejca.2016.10.022.
[16] B.Z. Sundqvist, S.K. Kilpinen, T.O. Böhling, V.S.K. Koljonen, H.J. Sihto, Transcriptomic analyses reveal three distinct molecular subgroups of Merkel cell carcinoma with differing prognoses, Int. J. Cancer 152 (2023) 2099–2108, https://doi.org/10.1002/ijc.34425.
[17] T.C. Knepper, M. Montesion, J.S. Russell, E.S. Sokol, G.M. Frampton, V.A. Miller, L.A. Albacker, H.L. McLeod, Z. Eroglu, N.I. Khushalani, V.K. Sondak, J.L. Messina, M.J. Schell, J.A. DeCaprio, K.Y. Tsai, A.S. Brohl, The genomic landscape of Merkel cell carcinoma and clinicogenomic biomarkers of response to immune checkpoint inhibitor therapy, Clin. Cancer Res. Off. J. Am. Assoc. Cancer Res. 25 (2019) 5961–5971, https://doi.org/10.1158/1078-0432.CCR-18-4159.
[18] K.L. Harms, L. Lazo de la Vega, D.H. Hovelson, S. Rahrig, A.K. Cani, C.-J. Liu, D. R. Fullen, M. Wang, A.A. Andea, C.K. Bichakjian, T.M. Johnson, S.A. Tomlins, P. W. Harms, Molecular profiling of multiple primary Merkel cell carcinoma to distinguish genetically distinct tumors from clonally related metastases, JAMA Dermatol. 153 (2017) 505–512, https://doi.org/10.1001/jamadermatol.2017.0507.
[19] P.W. Harms, P. Vats, M.E. Verhaegen, D.R. Robinson, Y.-M. Wu, S. M. Dhanasekaran, N. Palanisamy, J. Siddiqui, X. Cao, F. Su, R. Wang, H. Xiao, L. P. Kunju, R. Mehra, S.A. Tomlins, D.R. Fullen, C.K. Bichakjian, T.M. Johnson, A. A. Dlugosz, A.M. Chinnaiyan, The distinctive mutational spectra of polyomavirus-negative Merkel cell carcinoma, Cancer Res. 75 (2015) 3720–3727, https://doi.org/10.1158/0008-5472.CAN-15-0702.
[20] H. Singh, H.B. Choudhary, D.S. Mandlik, M.S. Magre, S. Mohanto, M.G. Ahmed, B.K. Singh, A.K. Mishra, A. Kumar, A. Mishra, T. Venkatachalam, H. Chopra, Molecular pathways and therapeutic strategies in dermatofibrosarcoma protuberans (DFSP): unravelling the tumor's genetic landscape, EXCLI J. 23 (2024) 727–762, https://doi.org/10.17179/excli2024-7164.
[21] P.T. Nghiem, S. Bhatia, E.J. Lipson, R.R. Kudchadkar, N.J. Miller, L. Annamalai, S. Berry, E.K. Chartash, A. Daud, S.P. Fling, P.A. Friedlander, H.M. Kluger, H. E. Kohrt, L. Lundgren, K. Margolin, A. Mitchell, T. Olencki, D.M. Pardoll, S. A. Reddy, E.M. Shantha, W.H. Sharfman, E. Sharon, L.R. Shemanski, M. M. Shinohara, J.C. Sunshine, J.M. Taube, J.A. Thompson, S.M. Townson, J. H. Yearley, S.L. Topalian, M.A. Cheever, PD-1 blockade with pembrolizumab in advanced Merkel-cell carcinoma, N. Engl. J. Med. 374 (2016) 2542–2552, https://doi.org/10.1056/NEJMoa1603702.
[22] V. Laniosz, O. Onajin, S. Sominidi-Damodaran, A. Meves, L.E. Gibson, C.L. Baum, Natural killer cell response is a predictor of good outcome in MCPyV(+) Merkel cell carcinoma: A case series of 23 patients, J. Am. Acad. Dermatol. 77 (2017) 31–32, https://doi.org/10.1016/j.jaad.2017.02.013.
[23] K. Gomez, G. Schiavoni, Y. Nam, J.-B. Reynier, C. Khamnei, M. Aitken, G. Palmieri, A. Cossu, A. Levine, C. van Noesel, B. Falini, L. Pasqualucci, E. Tiacci, R. Rabadan, Genomic landscape of virus-associated cancers, MedRxiv Prepr. Serv. Heal. Sci. (2023), https://doi.org/10.1101/2023.02.14.23285775.
[24] G. Goh, T. Walradt, V. Markarov, A. Blom, N. Riaz, R. Doumani, K. Stafstrom, A. Moshiri, L. Yelistratova, J. Levinsohn, T.A. Chan, P. Nghiem, R.P. Lifton, J. Choi, Mutational landscape of MCPyV-positive and MCPyV-negative Merkel cell carcinomas with implications for immunotherapy, Oncotarget 7 (2016) 3403–3415, https://doi.org/10.18632/oncotarget.6494.
[25] D. Brazel, P. Kumar, H. Doan, T. Pan, W. Shen, L. Gao, J.T. Moyers, Genomic alterations and tumor mutation burden in Merkel cell carcinoma, JAMA Netw. Open. 6 (2023) e2249674, https://doi.org/10.1001/jamanetworkopen.2022.49674.
[26] N. Singh, I.A. Jaiyesimi, N. Ismaila, N.B. Leighl, H. Mamdani, T. Phillips, D. H. Owen, Therapy for stage IV non-small-cell lung cancer without driver alterations: ASCO Living Guideline, Version 2023.1, J. Clin. Oncol. Off. J. Am. Soc. Clin. Oncol. 41 (2023) e51–e62, https://doi.org/10.1200/JCO.23.00282.
[27] S. Passerini, C. Prezioso, G. Babini, A. Ferlosio, T. Cosio, E. Campione, U. Moens, M. Ciotti, V. Pietropaolo, Detection of Merkel Cell Polyomavirus (MCPyV) DNA and transcripts in Merkel Cell Carcinoma (MCC), Pathogens 12 (2023), https://doi.org/10.3390/pathogens12070894.
[28] A.S.W. Loke, P.F. Lambert, M.E. Spurgeon, Current In vitro and in vivo models to study MCPyV-associated MCC, Viruses 14 (2022), https://doi.org/10.3390/v14102204.
[29] O. Gjoerup, H. Chao, J.A. DeCaprio, T.M. Roberts, pRB-dependent, J domain-independent function of simian virus 40 Large T antigen in override of p53 growth suppression, J. Virol. 74 (2000) 864–874, https://doi.org/10.1128/jvi.74.2.864-874.2000.
[30] M.E. Spurgeon, J. Cheng, E. Ward-Shaw, F.A. Dick, J.A. DeCaprio, P.F. Lambert, Merkel cell polyomavirus large T antigen binding to pRb promotes skin hyperplasia and tumor development, PLoS Pathog. 18 (2022) e1010551, https://doi.org/10.1371/journal.ppat.1010551.
[31] J. Cheng, O. Rozenblatt-Rosen, K.G. Paulson, P. Nghiem, J.A. DeCaprio, Merkel cell polyomavirus large T antigen has growth-promoting and inhibitory activities, J. Virol. 87 (2013) 6118–6126, https://doi.org/10.1128/JVI.00385-13.
[32] J.D. Arroyo, W.C. Hahn, Involvement of PP2A in viral and cellular transformation, Oncogene 24 (2005) 7746–7755, https://doi.org/10.1038/sj.onc.1209038.
[33] C. Mazziotta, C. Lanzillotti, M. Govoni, G. Pellielo, E. Mazzoni, M. Tognon, F. Martini, J.C. Rotondo, Decreased IgG antibody response to viral protein mimotopes of oncogenic Merkel cell polyomavirus in sera from healthy elderly subjects, Front. Immunol. 12 (2021), https://doi.org/10.3389/fimmu.2021.738486.
[34] A. Kassem, A. Schöpflin, C. Diaz, W. Weyers, E. Stickeler, M. Werner, A. Zur Hausen, Frequent detection of Merkel cell polyomavirus in human Merkel cell carcinomas and identification of a unique deletion in the VP1 gene, Cancer Res. 68 (2008) 5009–5013, https://doi.org/10.1158/0008-5472.CAN-08-0949.
[35] J.C. Becker, S.R. Hadrup, D. Schrama, C. Ritter, 31 MCPyV and the immune system: target and modulator, Oral. Oncol. 51 (2015) e36–e37, https://doi.org/10.1016/j.oraloncology.2015.02.032.
[36] P.L. Triozzi, A.P. Fernandez, The role of the immune response in Merkel cell carcinoma, Cancers 5 (2013) 234–254, https://doi.org/10.3390/cancers5010234.
[37] M.G. Dimitraki, G. Sourvinos, Merkel cell polyomavirus (MCPyV) and cancers: emergency bell or false alarm? Cancers 14 (2022) https://doi.org/10.3390/cancers14225548.
[38] M.E. Spurgeon, A. Liem, D. Buehler, J. Cheng, J.A. DeCaprio, P.F. Lambert, The Merkel cell polyomavirus T antigens function as tumor promoters in murine skin, Cancers 13 (2021), https://doi.org/10.3390/cancers13020222.
[39] L. Cappabianca, S. Guadagni, R. Maccarone, M. Sebastiano, A. Chiominto, A. R. Farina, A.R. Mackay, A pilot study of alternative TrkAIII splicing in Merkel cell carcinoma: a potential oncogenic mechanism and novel therapeutic target, J. Exp. Clin. Cancer Res. 38 (2019) 424, https://doi.org/10.1186/s13046-019-1425-3.
[40] I. Erovic, B.M. Erovic, Merkel cell carcinoma: the past, the present, and the future, J. Ski. Cancer 2013 (2013) 929364, https://doi.org/10.1155/2013/929364.
[41] S. Jani, C.D. Church, P. Nghiem, Insights into anti-tumor immunity via the polyomavirus shared across human Merkel cell carcinomas, Front. Immunol. 14 (2023) 1172913, https://doi.org/10.3389/fimmu.2023.1172913.
[42] K.D. Mertz, T. Junt, M. Schmid, M. Pfaltz, W. Kempf, Inflammatory monocytes are a reservoir for Merkel cell polyomavirus, J. Invest. Dermatol. 130 (2010) 1146–1151, https://doi.org/10.1038/jid.2009.392.
[43] M.Y. Alshahrani, A.G. Alkhathami, M.A.A. Almoyad, M.Z. Ahmad, S. Mohanto, W. Ahmad, S. Wahab, Phytochemicals as potential inhibitors of interleukin-8 for anticancer therapy: in silico evaluation and molecular dynamics analysis, J. Biomol. Struct. Dyn. 0 (2023) 1–12, https://doi.org/10.1080/07391102.2023.2294387.
[44] S. Das, T. Mukherjee, S. Mohanty, N. Nayak, P. Mal, S. Ashique, R. Pal, S. Mohanto, H. Sharma, Impact of NF-κB signaling and sirtuin-1 protein for targeted inflammatory intervention, Curr. Pharm. Biotechnol. (2024), https://doi.org/10.2174/0113892010301469240409082212.
[45] A.D. Gholap, H.S. Kapare, S. Pagar, P. Kamandar, D. Bhowmik, N. Vishwakarma, S. Raikwar, A. Garkal, T.A. Mehta, S. Rojekar, N. Hatvate, S. Mohanto, Exploring modified chitosan-based gene delivery technologies for therapeutic advancements, Int. J. Biol. Macromol. 260 (2024) 129581, https://doi.org/10.1016/j.ijbiomac.2024.129581.
[46] H. Sahi, H. Sihto, M. Artama, V. Koljonen, T. Böhling, E. Pukkala, History of chronic inflammatory disorders increases the risk of Merkel cell carcinoma, but does not correlate with Merkel cell polyomavirus infection, Br. J. Cancer 116 (2017) 260–264, https://doi.org/10.1038/bjc.2016.391.
[47] V. Pietropaolo, C. Prezioso, U. Moens, Merkel cell polyomavirus and Merkel cell carcinoma, Cancers 12 (2020), https://doi.org/10.3390/cancers12071774.
[48] U. Hani, B.H.J. Gowda, N. Haider, K. Ramesh, K. Paul, S. Ashique, M.G. Ahmed, S. Narayana, S. Mohanto, P. Kesharwani, Nanoparticle-based approaches for treatment of hematological malignancies: a comprehensive review, AAPS PharmSciTech 24 (2023) 233, https://doi.org/10.1208/s12249-023-02670-0.
[49] K.G. Paulson, C.W. Lewis, M.W. Redman, W.T. Simonson, A. Lisberg, D. Ritter, C. Morishima, K. Hutchinson, L. Mudgistratova, A. Blom, J. Iyer, A.S. Moshiri, E. S. Tarabadkar, J.J. Carter, S. Bhatia, M. Kawasumi, D.A. Galloway, M.H. Wener, P. Nghiem, Viral oncoprotein antibodies as a marker for recurrence of Merkel cell carcinoma: a prospective validation study, Cancer 123 (2017) 1464–1474, https://doi.org/10.1002/cncr.30475.
[50] S. Nag, O. Mitra, G. Tripathi, I. Adur, S. Mohanto, M. Nama, S. Samanta, B.H. J. Gowda, V. Subramaniyan, V. Sundararajan, V. Kumarasamy, Nanomaterials-assisted photothermal therapy for breast cancer: state-of-the-art advances and future perspectives, Photodiagnosis Photodyn. Ther. 45 (2024) 103959, https://doi.org/10.1016/j.pdpdt.2023.103959.
[51] S. Nag, O. Mitra, G. Tripathi, S. Samanta, B. Bhattacharya, P. Chandane, S. Mohanto, V. Sundararajan, S. Malik, S. Rustagi, S. Adhikari, A. Mohanty, D. A. León-Figueroa, A.J. Rodriguez-Morales, J.J. Barboza, R. Sah, Exploring the theranostic potentials of miRNA and epigenetic networks in autoimmune diseases: a comprehensive review, Immun. Inflamm. Dis. 11 (2023) e1121, https://doi.org/10.1002/iid3.1121.

[52] V. Pietropaolo, C. Prezioso, U. Moens, Role of virus-induced host cell epigenetic changes in cancer, Int. J. Mol. Sci. 22 (2021), https://doi.org/10.3390/ijms22158346.

[53] J.C. Rotondo, C. Mazziotta, C. Lanzillotti, M. Tognon, F. Martini, Epigenetic dysregulations in Merkel cell polyomavirus-driven Merkel cell carcinoma, Int. J. Mol. Sci. 22 (2021), https://doi.org/10.3390/ijms222111464.

[54] A.M. Pham, L.E. Ortiz, A.E. Lukacher, H.J. Kwun, Merkel Cell polyomavirus large T antigen induces cellular senescence for host growth arrest and viral genome persistence through its unique domain, Cells 12 (2023), https://doi.org/10.3390/cells12030380.

[55] R. Wang, J.F. Yang, T.E. Senay, W. Liu, J. You, Characterization of the impact of Merkel cell polyomavirus-induced interferon signaling on viral infection, J. Virol. 97 (2023) e0190722, https://doi.org/10.1128/jvi.01907-22.

[56] X.-H. Tang, X. Li, Y. Zhou, Y.-T. He, Z.-Y. Wang, X. Yang, W. Wang, K. Guo, W. Zhang, Y. Sun, H.-Q. Li, X.-F. Li, Golgi anti-apoptotic proteins redundantly counteract cell death by inhibiting production of reactive oxygen species under endoplasmic reticulum stress, J. Exp. Bot. 73 (2022) 2601–2617, https://doi.org/10.1093/jxb/erac011.

[57] Z.-Z. Lin, D.-S. Ming, Y.-B. Chen, J.-M. Zhang, H.-H. Chen, J.-J. Jiang, Z.-S. Zhang, KMT5A promotes metastasis of clear cell renal cell carcinoma through reducing cadherin-1 expression, Oncol. Lett. 17 (2019) 4907–4913, https://doi.org/10.3892/ol.2019.10163.

[58] M. Bleijs, M. van de Wetering, H. Clevers, J. Drost, Xenograft and organoid model systems in cancer research, EMBO J. 38 (2019) e101654, https://doi.org/10.15252/embj.2019101654.

[59] U. Bokova, M. Tretyakova, A. Korobeynikova, E. Denisov, In vivo models in cancer research, Adv. Mol. Oncol. 10 (2023) 8–16, https://doi.org/10.17650/2313-805X-2023-10-2-8-16.

[60] C.D. Church, P. Nghiem, How does the Merkel polyomavirus lead to a lethal cancer? Many answers, many questions, and a new mouse model, J. Invest. Dermatol. 135 (2015) 1221–1224, https://doi.org/10.1038/jid.2015.4.

[61] D.S. Chulpanova, K.V. Kitaeva, C.S. Rutland, A.A. Rizvanov, V.V. Solovyeva, Mouse tumor models for advanced cancer immunotherapy, Int. J. Mol. Sci. 21 (2020), https://doi.org/10.3390/ijms21114118.

[62] I. Oliveira, G. Silva, F. Cordeiro, E. Pinheiro Junior, isabela gobbo, F. Cerni, umberto zottich, M. Pucca, Research models in biomedical sciences: advantages and limitations, Open Access J. Biomed. Sci. 2 (2020), https://doi.org/10.38125/OAJBS.000197.

[63] A. Chen, I. Neuwirth, D. Herndler-Brandstetter, Modeling the tumor microenvironment and cancer immunotherapy in next-generation humanized mice, Cancers 15 (2023), https://doi.org/10.3390/cancers15112989.

[64] M. Kitsera, J.E. Brunetti, E. Rodríguez, Recent developments in NSG and NRG Humanized Mouse Models for Their Use in Viral and Immune Research, Viruses 15 (2023), https://doi.org/10.3390/v15020478.

[65] M. Czech-Sioli, T. Günther, M. Therre, M. Spohn, D. Indenbirken, J. Theiss, S. Riethdorf, M. Qi, M. Alawi, C. Wülbeck, I. Fernandez-Cuesta, F. Esmek, J. C. Becker, A. Grundhoff, N. Fischer, High-resolution analysis of Merkel Cell Polyomavirus in Merkel Cell Carcinoma reveals distinct integration patterns and suggests NHEJ and MMBIR as underlying mechanisms, PLoS Pathog. 16 (2020) e1008562, https://doi.org/10.1371/journal.ppat.1008562.

[66] J.C.M. Prado, T.A. Monezi, A.T. Amorim, V. Lino, A. Paladino, E. Boccardo, Human polyomaviruses and cancer: an overview, Clinics 73 (2018) e558s, https://doi.org/10.6061/clinics/2018/e558s.

[67] A.R. Mazzocchi, S.A.P. Rajan, K.I. Votanopoulos, A.R. Hall, A. Skardal, In vitro patient-derived 3D mesothelioma tumor organoids facilitate patient-centric therapeutic screening, Sci. Rep. 8 (2018) 2886, https://doi.org/10.1038/s41598-018-21200-8.

[68] D.-J. Cheon, S. Orsulic, Mouse models of cancer, Annu. Rev. Pathol. Mech. Dis. 6 (2011) 95–119, https://doi.org/10.1146/annurev.pathol.3.121806.154244.

[69] M.R. Gaiser, K. Daily, J. Hoffmann, M. Brune, A. Enk, I. Brownell, Evaluating blood levels of neuron specific enolase, chromogranin A, and circulating tumor cells as Merkel cell carcinoma biomarkers, Oncotarget 6 (2015) 26472–26482, https://doi.org/10.18632/oncotarget.4500.

[70] A. Buqué, L. Galluzzi, Modeling tumor immunology and immunotherapy in mice, Trends Cancer 4 (2018) 599–601, https://doi.org/10.1016/j.trecan.2018.07.003.

[71] A.S.W. Loke, B.J. Longley, P.F. Lambert, M.E. Spurgeon, A novel in vitro culture model system to study Merkel Cell polyomavirus–associated MCC using three-dimensional organotypic raft equivalents of human skin, Viruses 13 (2021), https://doi.org/10.3390/v13010138.

[72] S. Mohanto, A. Biswas, A.D. Gholap, S. Wahab, A. Bhunia, S. Nag, M.G. Ahmed, Potential biomedical applications of terbium-based nanoparticles (TbNPs): a review on recent advancement, ACS Biomater. Sci. Eng. 10 (2024) 2703–2724, https://doi.org/10.1021/acsbiomaterials.3c01969.

[73] J. Ahn, K. Ohk, J. Won, D.-H. Choi, Y.H. Jung, J.H. Yang, Y. Jun, J.-A. Kim, S. Chung, S.-H. Lee, Modeling of three-dimensional innervated epidermal like-layer in a microfluidic chip-based coculture system, Nat. Commun. 14 (2023) 1488, https://doi.org/10.1038/s41467-023-37187-4.

[74] N.T. Elliott, F. Yuan, A review of three-dimensional in vitro tissue models for drug discovery and transport studies, J. Pharm. Sci. 100 (2011) 59–74, https://doi.org/10.1002/jps.22257.

[75] H. Clevers, D.A. Tuveson, Organoid models for cancer research, Annu. Rev. Cancer Biol. 3 (2019) 223–234, https://doi.org/10.1146/annurev-cancerbio-030518-055702.

[76] H. Xu, Y. Jiao, S. Qin, W. Zhao, Q. Chu, K. Wu, Organoid technology in disease modelling, drug development, personalized treatment and regeneration medicine, Exp. Hematol. Oncol. 7 (2018) 30, https://doi.org/10.1186/s40164-018-0122-9.

[77] J.A. Hickman, R. Graeser, R. de Hoogt, S. Vidic, C. Brito, M. Gutekunst, H. van der Kuip, I.M.I.P. consortium, Three-dimensional models of cancer for pharmacology and cancer cell biology: capturing tumor complexity in vitro/ex vivo, Biotechnol. J. 9 (2014) 1115–1128, https://doi.org/10.1002/biot.201300492.

[78] M.F. Berger, E.R. Mardis, The emerging clinical relevance of genomics in cancer medicine, Nat. Rev. Clin. Oncol. 15 (2018) 353–365, https://doi.org/10.1038/s41571-018-0002-6.

[79] A.P. Sokolenko, E.N. Imyanitov, Molecular diagnostics in clinical oncology, Front. Mol. Biosci. 5 (2018), https://doi.org/10.3389/fmolb.2018.00076.

[80] V. Gambardella, N. Tarazona, J.M. Cejalvo, P. Lombardi, M. Huerta, S. Roselló, T. Fleitas, D. Roda, A. Cervantes, Personalized medicine: recent progress in cancer therapy, Cancers 12 (2020), https://doi.org/10.3390/cancers12041009.

[81] M. Kalia, Biomarkers for personalized oncology: recent advances and future challenges, Metab. - Clin. Exp. 64 (2015) S16–S21, https://doi.org/10.1016/j.metabol.2014.10.027.

[82] N. Sukswai, J.D. Khoury, Immunohistochemistry innovations for diagnosis and tissue-based biomarker detection, Curr. Hematol. Malig. Rep. 14 (2019) 368–375, https://doi.org/10.1007/s11899-019-00533-9.

[83] A. Grundhoff, N. Fischer, Merkel cell polyomavirus, a highly prevalent virus with tumorigenic potential, Curr. Opin. Virol. 14 (2015) 129–137, https://doi.org/10.1016/j.coviro.2015.08.010.

[84] A. Yang, W.A. Wijaya, L. Yang, Y. He, Y. Cen, J. Chen, The impact of merkel cell polyomavirus positivity on prognosis of merkel cell carcinoma: a systematic review and meta-analysis, Front. Oncol. 12 (2022), https://doi.org/10.3389/fonc.2022.1020805.

[85] S.J. Rodig, J. Cheng, J. Wardzala, A. DoRosario, J.J. Scanlon, A.C. Laga, A. Martinez-Fernandez, J.A. Barletta, A.M. Bellizzi, S. Sadasivam, D.T. Holloway, D.J. Cooper, T.S. Kupper, L.C. Wang, J.A. DeCaprio, Improved detection suggests all Merkel cell carcinomas harbor Merkel polyomavirus, J. Clin. Invest. 122 (2012) 4645–4653, https://doi.org/10.1172/JCI64116.

[86] T.R.B. Bellott Nascimento, F.B. Luz, A.K. Fausto da Silva, R.B. Varella, M. C. Rochael, R.E. Rozza-de-Menezes, L. Pantaleão, Presence of Merkel cell polyomavirus DNA and large-T antigen in keratinocyte carcinomas and its correlation with immunohistochemical markers p16, p53 and ki67, An. Bras. Dermatol. 99 (2024) 688–695, https://doi.org/10.1016/j.abd.2023.12.002.

[87] T.R. Bellott, F.B. Luz, A.K. Fausto, R.B. Varella, M.A.A.M. Guimarães, M. T. Venceslau, M.C. Rochael, L. Pantaleão, Detection of Merkel cell polyomavirus in tumor cells and peritumoral lymphocytes of non-melanoma skin cancer by Immunohistochemistry, GSC Adv. Res. Rev. 13 (2022) 162–168, https://doi.org/10.30574/gscarr.2022.13.1.0282.

[88] K.G. Paulson, J.J. Carter, L.G. Johnson, K.W. Cahill, J.G. Iyer, D. Schrama, J.C. Becker, M.M. Madeleine, P. Nghiem, D.A. Galloway, Data from Antibodies to Merkel Cell Polyomavirus T Antigen Oncoproteins Reflect Tumor Burden in Merkel Cell Carcinoma Patients, 2023. https://doi.org/10.1158/0008-5472.c.6501566..

[89] P. Donizy, J.P. Wróblewska, D. Dias-Santagata, K. Woznica, P. Biecek, M. C. Mochel, C.-L. Wu, J. Kopczynski, M. Pieniazek, J. Ryś, A. Marszalek, M. P. Hoang, Merkel cell carcinoma of unknown primary: immunohistochemical and molecular analyses reveal distinct UV-signature/MCPyV-negative and high immunogenicity/MCPyV-Positive Profiles, Cancers 13 (2021), https://doi.org/10.3390/cancers13071621.

[90] M.E. Verhaegen, P.W. Harms, J.J. Van Goor, J. Arche, M.T. Patrick, D. Wilbert, H. Zabawa, M. Grachtchouk, C.-J. Liu, K. Hu, M.C. Kelly, P. Chen, T.L. Saunders, S. Weidinger, L.-J. Syu, J.S. Runge, J.E. Gudjonsson, S.Y. Wong, I. Brownell, M. Cieslik, A.M. Udager, A.M. Chinnaiyan, L.C. Tsoi, A.A. Dlugosz, Direct cellular reprogramming enables development of viral T antigen-driven Merkel cell carcinoma in mice, J. Clin. Invest. 132 (2022), https://doi.org/10.1172/JCI152069.

[91] M.-M. Chang, Plasmid-to-plasmid Southern blot analysis validates the presence of nucleotide binding site (nbs) sequences in cloned plasmids, Biochem. Mol. Biol. Educ. A Bimon. Publ. Int. Union Biochem. Mol. Biol. 50 (2022) 373–380, https://doi.org/10.1002/bmb.21642.

[92] M.R. Green, J. Sambrook, Analysis of DNA by southern blotting, Cold Spring Harb. Protoc. 2021 (2021), https://doi.org/10.1101/pdb.top100396.

[93] W. Liu, N.A. Krump, C.B. Buck, J. You, Merkel cell polyomavirus infection and detection, J. Vis. Exp. (2019), https://doi.org/10.3791/58950.

[94] L.F. Lincz, F.E. Scorgie, M.B. Garg, J. Gilbert, J.A. Sakoff, A simplified method to calculate telomere length from Southern blot images of terminal restriction fragment lengths, Biotechniques 68 (2020) 28–34, https://doi.org/10.2144/btn-2019-0082.

[95] T. Brown, Southern blotting, Curr. Protoc. Protein Sci. 13 (1998), https://doi.org/10.1002/0471140864.psa04gs13. A.4G.1-A.4G.8.

[96] N.K. Mohd-Ismail, Z. Lim, J. Gunaratne, Y.-J. Tan, Mapping the Interactions of HBV cccDNA with Host Factors, Int. J. Mol. Sci. 20 (2019), https://doi.org/10.3390/ijms20174276.

[97] T.A. Brown, Southern blotting and related DNA detection techniques, in: ELS, John Wiley & Sons, Ltd, 2001, https://doi.org/10.1038/npg.els.0000996.

[98] G.F. Codner, V. Erbs, J. Loeffler, L. Chessum, A. Caulder, N. Jullien, S. Wells, M.-C. Birling, L. Teboul, Universal Southern blot protocol with cold or radioactive probes for the validation of alleles obtained by homologous recombination, Methods 191 (2021) 59–67, https://doi.org/10.1016/j.ymeth.2020.06.011.

[99] G. Mellars, K. Gomez, Mutation detection by Southern blotting, Methods Mol. Biol. 688 (2011) 281–291, https://doi.org/10.1007/978-1-60761-947-5_19.

[100] A.S.W. Loke, P.F. Lambert, M.E. Spurgeon, Current In vitro and in vivo models to study MCPyV-associated MCC, Viruses 14 (2022) 2204, https://doi.org/10.3390/v14102204.
[101] N. Fischer, J. Brandner, F. Fuchs, I. Moll, A. Grundhoff, Detection of Merkel cell polyomavirus (MCPyV) in Merkel cell carcinoma cell lines: cell morphology and growth phenotype do not reflect presence of the virus, Int. J. Cancer 126 (2010) 2133–2142, https://doi.org/10.1002/ijc.24877.
[102] T. Mahmood, P.-C. Yang, Western blot: technique, theory, and trouble shooting, N. Am. J. Med. Sci. 4 (2012) 429–434, https://doi.org/10.4103/1947-2714.100998.
[103] R. Sule, G. Rivera, A.V. Gomes, Western blotting (immunoblotting): history, theory, uses, protocol and problems, Biotechniques 75 (2023) 99–114, https://doi.org/10.2144/btn-2022-0034.
[104] B.T. Kurien, R.H. Scofield, Common artifacts and mistakes made in electrophoresis, Methods Mol. Biol. 869 (2012) 633–640, https://doi.org/10.1007/978-1-61779-821-4_58.
[105] D.A. Griffiths, H. Abdul-Sada, L.M. Knight, B.R. Jackson, K. Richards, E. L. Prescott, A.H.S. Peach, G.E. Blair, A. Macdonald, A. Whitehouse, Merkel cell polyomavirus small T antigen targets the NEMO adaptor protein to disrupt inflammatory signaling, J. Virol. 87 (2013) 13853–13867, https://doi.org/10.1128/JVI.02159-13.
[106] L. Pala, T. Sirec, U. Spitz, Modified enzyme substrates for the detection of bacteria: a review, Molecules 25 (2020), https://doi.org/10.3390/molecules25163690.
[107] J.J. Bass, D.J. Wilkinson, D. Rankin, B.E. Phillips, N.J. Szewczyk, K. Smith, P. J. Atherton, An overview of technical considerations for Western blotting applications to physiological research, Scand. J. Med. Sci. Sports 27 (2017) 4–25, https://doi.org/10.1111/sms.12702.
[108] K. Stachyra, M. Dudzisz-Śledź, E. Bylina, A. Szumera-Ciećkiewicz, M.J. Spałek, E. Bartnik, P. Rutkowski, A.M. Czarnecka, Merkel cell carcinoma from molecular pathology to novel therapies, Int. J. Mol. Sci. 22 (2021), https://doi.org/10.3390/ijms22126305.
[109] V. Leroux-Kozal, N. Lévêque, V. Brodard, C. Lesage, O. Dudez, M. Makeieff, L. Kanagaratnam, M.-D. Diebold, Merkel cell carcinoma: histopathologic and prognostic features according to the immunohistochemical expression of Merkel cell polyomavirus large T antigen correlated with viral load, Hum. Pathol. 46 (2015) 443–453, https://doi.org/10.1016/j.humpath.2014.12.001.
[110] P.W. Harms, K.L. Harms, P.S. Moore, J.A. DeCaprio, P. Nghiem, M.K.K. Wong, I. Brownell, The biology and treatment of Merkel cell carcinoma: current understanding and research priorities, Nat. Rev. Clin. Oncol. 15 (2018) 763–776, https://doi.org/10.1038/s41571-018-0103-2.
[111] L. Garibyan, N. Avashia, Polymerase chain reaction, J. Invest. Dermatol. 133 (2013) 1–4, https://doi.org/10.1038/jid.2013.1.
[112] M.A.A. Valones, R.L. Guimarães, L.A.C. Brandão, P.R.E. de Souza, A. de Albuquerque Tavares Carvalho, S. Crovela, Principles and applications of polymerase chain reaction in medical diagnostic fields: a review, Braz. J. Microbiol. [Publ. Braz. Soc. Microbiol. 40 (2009) 1–11, https://doi.org/10.1590/S1517-83822009000100001.
[113] N. Gupta, DNA extraction and polymerase chain reaction, J. Cytol. 36 (2019) 116–117, https://doi.org/10.4103/JOC.JOC_110_18.
[114] C.M. Li, H. Dong, Q. Zhou, K.H. Goh, CHAPTER 11 - Biochips – fundamentals and applications, in: X. Zhang, H. Ju, J. Wang (Eds.), Electrochem. Sensors, Biosens. Their Biomed. Appl., Academic Press, San Diego, 2008, pp. 307–383, https://doi.org/10.1016/B978-012373738-0.50013-1.
[115] T.C. Lorenz, Polymerase chain reaction: basic protocol plus troubleshooting and optimization strategies, J. Vis. Exp. (2012) e3998, https://doi.org/10.3791/3998.
[116] F. Watzinger, K. Ebner, T. Lion, Detection and monitoring of virus infections by real-time PCR, Mol. Asp. Med. 27 (2006) 254–298, https://doi.org/10.1016/j.mam.2005.12.001.
[117] I.M. Artika, Y.P. Dewi, I.M. Nainggolan, J.E. Siregar, U. Antonjaya, Real-time polymerase chain reaction: current techniques, applications, and role in COVID-19 diagnosis, Genes 13 (2022), https://doi.org/10.3390/genes13122387.
[118] M. Barchitta, A. Maugeri, E. Campisi, R. Magnano San Lio, G. Favara, H.J. Soto Parra, L. Salvatorelli, G. Magro, G. Basile, A. Agodi, Comparison of quantitative real-time PCR and digital PCR to detect the polyomavirus in Merkel Cell Carcinoma, Viruses 14 (2022) 2195, https://doi.org/10.3390/v14102195.
[119] R. Arvia, M. Sollai, D. Massi, P. Asensio-Calavia, C. Urso, K. Zakrzewska, No detectable truncating mutations in large T antigen (LT-Ag) sequence of Merkel cell polyomavirus (MCPyV) DNA obtained from porocarcinomas, Infect. Agent. Cancer 19 (2024) 9, https://doi.org/10.1186/s13027-024-00568-5.
[120] H. Li, R. Bai, Z. Zhao, L. Tao, M. Ma, Z. Ji, M. Jian, Z. Ding, X. Dai, F. Bao, A. Liu, Application of droplet digital PCR to detect the pathogens of infectious diseases, Biosci. Rep. 38 (2018), https://doi.org/10.1042/BSR20181170.
[121] P.-L. Quan, M. Sauzade, E. Brouzes, dPCR: a technology review, Sensors 18 (2018), https://doi.org/10.3390/s18041271.
[122] X.J.D. Lu, K.Y.P. Liu, Y.S. Zhu, C. Cui, C.F. Poh, Using ddPCR to assess the DNA yield of FFPE samples, Biomol. Detect. Quantif. 16 (2018) 5–11, https://doi.org/10.1016/j.bdq.2018.10.001.
[123] H.R. Shehata, J. Li, S. Chen, H. Redda, S. Cheng, N. Tabujara, H. Li, K. Warriner, R. Hanner, Droplet digital polymerase chain reaction (ddPCR) assays integrated with an internal control for quantification of bovine, porcine, chicken and turkey species in food and feed, PLoS One 12 (2017) e0182872, https://doi.org/10.1371/journal.pone.0182872.
[124] V. Kokkoris, E. Vukicevich, A. Richards, C. Thomsen, M.M. Hart, Challenges using droplet digital PCR for environmental samples, Appl. Microbiol. 1 (2021) 74–88, https://doi.org/10.3390/applmicrobiol1010007.
[125] C.M. Hindson, J.R. Chevillet, H.A. Briggs, E.N. Gallichotte, I.K. Ruf, B.J. Hindson, R.L. Vessella, M. Tewari, Absolute quantification by droplet digital PCR versus analog real-time PCR, Nat. Methods 10 (2013) 1003–1005, https://doi.org/10.1038/nmeth.2633.
[126] A. Lievens, S. Jacchia, D. Kagkli, C. Savini, M. Querci, Measuring digital PCR quality: performance parameters and their optimization, PLoS One 11 (2016) e0153317, https://doi.org/10.1371/journal.pone.0153317.
[127] A.S. Basu, Digital Assays Part I: partitioning statistics and digital PCR, SLAS Technol. 22 (2017) 369–386, https://doi.org/10.1177/2472630317705680.
[128] N. Coccaro, G. Tota, L. Anelli, A. Zagaria, G. Specchia, F. Albano, Digital PCR: a reliable tool for analyzing and monitoring hematologic malignancies, Int. J. Mol. Sci. 21 (2020), https://doi.org/10.3390/ijms21093141.
[129] M. Barchitta, A. Maugeri, E. Campisi, R. Magnano San Lio, G. Favara, H.J. Soto Parra, L. Salvatorelli, G. Magro, G. Basile, A. Agodi, Comparison of quantitative real-time PCR and digital PCR to detect the polyomavirus in Merkel Cell Carcinoma, Viruses 14 (2022), https://doi.org/10.3390/v14102195.
[130] R. Arvia, M. Sollai, F. Pierucci, C. Urso, D. Massi, K. Zakrzewska, Droplet digital PCR (ddPCR) vs quantitative real-time PCR (qPCR) approach for detection and quantification of Merkel cell polyomavirus (MCPyV) DNA in formalin fixed paraffin embedded (FFPE) cutaneous biopsies, J. Virol. Methods 246 (2017) 15–20, https://doi.org/10.1016/j.jviromet.2017.04.003.
[131] B. Woodhouse, T.J. Robb, J.I. Hearn, P.S. Houseman, G. Hayward, R. Miller, A. P. Restall, M. Findlay, B. Lawrence, C.G. Print, K. Parker, C. Blenkiron, Merkel cell polyomavirus is uncommon in New Zealand Merkel cell carcinomas, Br. J. Dermatol. 179 (2018) 1197–1198, https://doi.org/10.1111/bjd.16903.
[132] D. Cilloni, J. Petiti, V. Rosso, G. Andreani, M. Dragani, C. Fava, G. Saglio, Digital PCR in myeloid malignancies: ready to replace quantitative PCR? Int. J. Mol. Sci. 20 (2019) https://doi.org/10.3390/ijms20092249.
[133] R. Shirai, T. Osumi, D. Keino, K. Nakabayashi, T. Uchiyama, M. Sekiguchi, M. Hiwatari, M. Yoshida, K. Yoshida, Y. Yamada, D. Tomizawa, S. Takae, N. Kiyokawa, K. Matsumoto, T. Yoshioka, K. Hata, T. Hori, N. Suzuki, M. Kato, Minimal residual disease detection by mutation-specific droplet digital PCR for leukemia/lymphoma, Int. J. Hematol. 117 (2023) 910–918, https://doi.org/10.1007/s12185-023-03566-2.
[134] A.D. King, H. Deirawan, P.A. Klein, B. Dasgeb, C.I. Dumur, D.R. Mehregan, Next-generation sequencing in dermatology, Front. Med. 10 (2023) 1218404, https://doi.org/10.3389/fmed.2023.1218404.
[135] N.A. Krump, J. You, Molecular mechanisms of viral oncogenesis in humans, Nat. Rev. Microbiol. 16 (2018) 684–698, https://doi.org/10.1038/s41579-018-0064-6.
[136] S. Chowdhary, R. Deka, K. Panda, R. Kumar, A.D. Solomon, J. Das, S. Kanoujiya, A.K. Gupta, S. Sinha, J. Ruokolainen, K.K. Kesari, P.K. Gupta, Recent updates on viral oncogenesis: available preventive and therapeutic entities, Mol. Pharm. 20 (2023) 3698–3740, https://doi.org/10.1021/acs.molpharmaceut.2c01080.
[137] D. Shyr, Q. Liu, Next generation sequencing in cancer research and clinical application, Biol. Proced. Online 15 (2013) 4, https://doi.org/10.1186/1480-9222-15-4.
[138] R. Groisberg, V. Subbiah, Immunotherapy and next-generation sequencing guided therapy for precision oncology: what have we learnt and what does the future hold? Expert Rev. Precis. Med. Drug Dev. 3 (2018) 205–213, https://doi.org/10.1080/23808993.2018.1480898.
[139] W. Lydolph, S.P. Desikan, S. Wang, J. Allgood, C. 3rd McClains, J. Mclaughlin, R. Desikan, Advances in immunology: a cornerstone in diagnosis and therapy of Merkel cell carcinoma, J. Investig. Med. High Impact Case Rep. 10 (2022) 23247096221089492, https://doi.org/10.1177/23247096221089492.
[140] K.A. Johansen Taber, B.D. Dickinson, M. Wilson, The promise and challenges of next-generation genome sequencing for clinical care, JAMA Intern. Med. 174 (2014) 275, https://doi.org/10.1001/jamainternmed.2013.12048.
[141] O. Tan, R. Shrestha, M. Cunich, D.J. Schofield, Application of next-generation sequencing to improve cancer management: a review of the clinical effectiveness and cost-effectiveness, Clin. Genet. 93 (2018) 533–544, https://doi.org/10.1111/cge.13199.
[142] A. Tafazoli, H.-J. Guchelaar, W. Miltyk, A.J. Kretowski, J.J. Swen, Applying next-generation sequencing platforms for pharmacogenomic testing in clinical practice, Front. Pharmacol. 12 (2021), https://doi.org/10.3389/fphar.2021.693453.
[143] E. Hartsough, M. Mino-Kenudson, J.K. Lennerz, D. Dias-Santagata, M.P. Hoang, Clinical next-generation sequencing panels reveal molecular differences between Merkel Cell polyomavirus–negative merkel cell carcinomas and neuroendocrine carcinomas, Am. J. Clin. Pathol. 159 (2023) 395–406, https://doi.org/10.1093/ajcp/aqac176.
[144] M.J.E.M.F. Mabruk, In situ hybridization: detecting viral nucleic acid in formalin-fixed, paraffin-embedded tissue samples, Expert Rev. Mol. Diagn. 4 (2004) 653–661, https://doi.org/10.1586/14737159.4.5.653.
[145] L. Wang, P.W. Harms, N. Palanisamy, S. Carskadon, X. Cao, J. Siddiqui, R. M. Patel, S. Zelenka-Wang, A.B. Durham, D.R. Fullen, K.L. Harms, F. Su, S. Shukla, R. Mehra, A.M. Chinnaiyan, Age and gender associations of virus positivity in merkel cell carcinoma characterized using a novel RNA In situ hybridization assay, Clin. Cancer Res. Off. J. Am. Assoc. Cancer Res. 23 (2017) 5622–5630, https://doi.org/10.1158/1078-0432.CCR-17-0299.
[146] M. Jaouannet, C.-N. Nguyen, M. Quentin, S. Jaubert-Possamai, M.-N. Rosso, B. Favery, In situ Hybridization (ISH) in preparasitic and parasitic stages of the plant-parasitic nematode Meloidogyne spp, Bio-Protoc. 8 (2018) e2766, https://doi.org/10.21769/BioProtoc.2766.

[147] M.E. Spurgeon, P.F. Lambert, Merkel cell polyomavirus: a newly discovered human virus with oncogenic potential, Virology 435 (2013) 118–130, https://doi.org/10.1016/j.virol.2012.09.029.
[148] C.J. Burrell, C.R. Howard, F.A. Murphy, Laboratory diagnosis of virus diseases, Fenner White'S. Med. Virol. (2017) 135–154, https://doi.org/10.1016/B978-0-12-375156-0.00010-2.
[149] S. Bialasiewicz, S.B. Lambert, D.M. Whiley, M.D. Nissen, T.P. Sloots, Merkel cell polyomavirus DNA in respiratory specimens from children and adults, Emerg. Infect. Dis. 15 (2009) 492–494, https://doi.org/10.3201/eid1503.081067.
[150] A.S. Moshiri, R. Doumani, L. Yelistratova, A. Blom, K. Lachance, M.M. Shinohara, M. Delaney, O. Chang, S. McArdle, H. Thomas, M.M. Asgari, M.-L. Huang, S. M. Schwartz, P. Nghiem, Polyomavirus-negative merkel cell carcinoma: a more aggressive subtype based on analysis of 282 cases using multimodal tumor virus detection, J. Invest. Dermatol. 137 (2017) 819–827, https://doi.org/10.1016/j.jid.2016.10.028.
[151] G. Bevilacqua, The viral origin of human breast cancer: from the Mouse Mammary Tumor Virus (MMTV) to the Human Betaretrovirus (HBRV), Viruses 14 (2022), https://doi.org/10.3390/v14081704.
[152] N.M. Chrzanowska, J. Kowalewski, M.A. Lewandowska, Use of fluorescence in situ hybridization (FISH) in diagnosis and tailored therapies in solid tumors, Molecules 25 (2020), https://doi.org/10.3390/molecules25081864.
[153] C. Cosi, M. Millar, M. Beltran, L. Sherry, S. Gatti-McArthur, Quantitative analysis of RNAscope staining for c-fos expression in mouse brain tissue as a measure of Neuronal Activation, MethodsX 8 (2021) 101348, https://doi.org/10.1016/j.mex.2021.101348.
[154] J.M. Monné Rodríguez, A.-L. Frisk, R. Kreutzer, T. Lemarchand, S. Lezmi, C. Saravanan, B. Stierstorfer, C. Thuilliez, E. Vezzali, G. Wieczorek, S.-W. Yun, D. Schaudien, European society of toxicologic pathology (Pathology 2.0 Molecular Pathology Special Interest Group): review of in situ hybridization techniques for drug research and development, Toxicol. Pathol. 51 (2023) 92–111, https://doi.org/10.1177/01926233231178282.
[155] M. Matsushita, D. Nonaka, T. Iwasaki, S. Kuwamoto, I. Murakami, M. Kato, K. Nagata, Y. Kitamura, K. Hayashi, A new in situ hybridization and immunohistochemistry with a novel antibody to detect small T-antigen expressions of Merkel cell polyomavirus (MCPyV), Diagn. Pathol. 9 (2014) 65, https://doi.org/10.1186/1746-1596-9-65.
[156] R. Arora, K. Gupta, A. Vijaykumar, S. Krishna, DETECTing Merkel cell polyomavirus in Merkel tumors, Front. Mol. Biosci. 7 (2020), https://doi.org/10.3389/fmolb.2020.00010.
[157] M.R. Karimi, A.H. Karimi, S. Abolmaali, M. Sadeghi, U. Schmitz, Prospects and challenges of cancer systems medicine: from genes to disease networks, Brief. Bioinform. 23 (2022), https://doi.org/10.1093/bib/bbab343.
[158] M.M. Ahmed, C.H. Cushman, J.A. DeCaprio, Merkel Cell polyomavirus: oncogenesis in a stable genome, Viruses 14 (2021), https://doi.org/10.3390/v14010058.
[159] W.-S. Ryu, Chapter 6 - polyomaviruses: SV40, in: W.-S. Ryu (Ed.), Mol. Virol. Hum. Pathog. Viruses, Academic Press, Boston, 2017, pp. 85–95, https://doi.org/10.1016/B978-0-12-800838-6.00006-0.
[160] A. Faghihkhorasani, A. Dalvand, E. Derafsh, F. Tavakoli, N.K. Younis, S. Yasamineh, O. Gholizadeh, P. Shokri, The role of oncolytic virotherapy and viral oncogenes in the cancer stem cells: a review of virus in cancer stem cells, Cancer Cell Int. 23 (2023) 250, https://doi.org/10.1186/s12935-023-03099-y.
[161] P. Gupta, N. Shahzad, A. Harold, M. Shuda, A. Venuti, M.C. Romero-Medina, L. Pacini, L. Brault, A. Robitaille, V. Taverniti, H. Hernandez-Vargas, G. Durand, F. Le Calvez-Kelm, T. Gheit, R. Accardi, M. Tommasino, Merkel cell polyomavirus downregulates N-myc downstream-regulated Gene 1, leading to cellular proliferation and migration, J. Virol. 94 (2020), https://doi.org/10.1128/JVI.00899-19.
[162] K.L. Harms, L. Zhao, B. Johnson, X. Wang, S. Carskadon, N. Palanisamy, D. R. Rhodes, R. Mannan, J.N. Vo, J.E. Choi, M.P. Chan, D.R. Fullen, R.M. Patel, J. Siddiqui, V.T. Ma, S. Hrycaj, S.A. McLean, T.M. Hughes, C.K. Bichakjian, S. A. Tomlins, P.W. Harms, Virus-positive Merkel cell carcinoma is an independent prognostic group with distinct predictive biomarkers, Clin. Cancer Res. Off. J. Am. Assoc. Cancer Res. 27 (2021) 2494–2504, https://doi.org/10.1158/1078-0432.CCR-20-0864.
[163] U.N. Mui, C.T. Haley, S.K. Tyring, Viral oncology: molecular biology and pathogenesis, J. Clin. Med. 6 (2017), https://doi.org/10.3390/jcm6120111.
[164] A.M.E. Elkhalifa, S.U. Nabi, O.S. Shah, S.M. Bashir, U. Muzaffer, S.I. Ali, I. A. Wani, N.A.N. Alzerwi, A.Y. Elderdery, A. Alanazi, F.O. Alenazy, A.H.A. Alharbi, Insight into oncogenic viral pathways as drivers of viral cancers: implication for effective therapy, Curr. Oncol. 30 (2023) 1924–1944, https://doi.org/10.3390/curroncol30020150.
[165] J. Bard, Systems biology - the broader perspective, Cells 2 (2013) 414–431, https://doi.org/10.3390/cells2020414.
[166] T. Ivanisevic, R.N. Sewduth, Multi-omics integration for the design of novel therapies and the identification of novel biomarkers, Proteomes 11 (2023), https://doi.org/10.3390/proteomes11040034.
[167] T. Schlemeyer, D. Ohnezeit, S. Virdi, C. Körner, S. Weißelberg, S. Starzonek, U. Schumacher, A. Grundhoff, D. Indenbirken, S. Albertini, N. Fischer, Merkel cell carcinoma and Immune Evasion: Merkel Cell polyomavirus small T-antigen-induced surface changes can be reverted by therapeutic intervention, J. Invest. Dermatol. 142 (2022) 3071–3081, https://doi.org/10.1016/j.jid.2022.04.029, e13.
[168] G.S. Suri, G. Kaur, G.M. Carbone, D. Shinde, Metabolomics in oncology, Cancer Rep. 6 (2023) e1795, https://doi.org/10.1002/cnr2.1795.
[169] J. Zou, M.-W. Zheng, G. Li, Z.-G. Su, Advanced systems biology methods in drug discovery and translational biomedicine, Biomed. Res. Int. 2013 (2013) 742835, https://doi.org/10.1155/2013/742835.
[170] A. Dasgupta, R.K. De, Chapter 6 - Artificial intelligence in systems biology, in: S. G. Krantz, A.S.R. Srinivasa Rao, C.R. Rao (Eds.), Artif. Intell., Elsevier, 2023, pp. 153–201, https://doi.org/10.1016/bs.host.2023.06.004.
[171] A. Badkas, S. De Landtsheer, T. Sauter, Construction and contextualization approaches for protein-protein interaction networks, Comput. Struct. Biotechnol. J. 20 (2022) 3280–3290, https://doi.org/10.1016/j.csbj.2022.06.040.
[172] N. Rhind, P. Russell, Signaling pathways that regulate cell division, Cold Spring Harb. Perspect. Biol. 4 (2012), https://doi.org/10.1101/cshperspect.a005942.
[173] T.L. Whiteside, The tumor microenvironment and its role in promoting tumor growth, Oncogene 27 (2008) 5904–5912, https://doi.org/10.1038/onc.2008.271.
[174] U. Moens, A. Macdonald, Effect of the large and small T-antigens of human polyomaviruses on signaling pathways, Int. J. Mol. Sci. 20 (2019), https://doi.org/10.3390/ijms20163914.
[175] A.M. Gonzalez-Angulo, B.T.J. Hennessy, G.B. Mills, Future of personalized medicine in oncology: a systems biology approach, J. Clin. Oncol. Off. J. Am. Soc. Clin. Oncol. 28 (2010) 2777–2783, https://doi.org/10.1200/JCO.2009.27.0777.
[176] A. Mokánszki, G. Méhes, S.L. Csoma, S. Kollár, Y.-C. Chang Chien, Molecular profiling of merkel cell polyomavirus-associated Merkel cell carcinoma and cutaneous melanoma, Diagnostics 11 (2021), https://doi.org/10.3390/diagnostics11020212.
[177] G.J. Starrett, M. Thakuria, T. Chen, C. Marcelus, J. Cheng, J. Nomburg, A. R. Thorner, M.K. Slevin, W. Powers, R.T. Burns, C. Perry, A. Piris, F.C. Kuo, G. Rabinowits, A. Giobbie-Hurder, L.E. MacConaill, J.A. DeCaprio, Clinical and molecular characterization of virus-positive and virus-negative Merkel cell carcinoma, Genome Med. 12 (2020) 30, https://doi.org/10.1186/s13073-020-00727-4.
[178] V. Mishra, A. Singh, X. Chen, A.J. Rosenberg, A.T. Pearson, A. Zhavoronkov, P. A. Savage, M.W. Lingen, N. Agrawal, E. Izumchenko, Application of liquid biopsy as multi-functional biomarkers in head and neck cancer, Br. J. Cancer 126 (2022) 361–370, https://doi.org/10.1038/s41416-021-01626-0.
[179] N.A. Krump, J. You, From Merkel cell polyomavirus infection to Merkel Cell Carcinoma oncogenesis, Front. Microbiol. 12 (2021), https://doi.org/10.3389/fmicb.2021.739695.
[180] M.A.H. Priyanka, H. Abusalah, A. Chopra, S.A. Sharma, O.P. Mustafa, M. Choudhary, M. Sharma, R. Dhawan, A. Khosla, A. Loshali, J. Sundriyal, Saini, Nanovaccines: a game changing approach in the fight against infectious diseases, Biomed. Pharmacother. 167 (2023) 115597, https://doi.org/10.1016/j.biopha.2023.115597.
[181] T. Gambichler, D. Schrama, R. Käpynen, S.S. Weyer-Fahlbusch, J.C. Becker, L. Susok, F. Kreppel, N. Abu Rached, Current progress in vaccines against Merkel cell carcinoma: a narrative review and update, Vaccines 12 (2024), https://doi.org/10.3390/vaccines12050533.
[182] B.K. Das, A. Kannan, G.J. Velasco, M.D. Kunika, N. Lambrecht, Q. Nguyen, H. Zhao, J. Wu, L. Gao, Single-cell dissection of Merkel cell carcinoma heterogeneity unveils transcriptomic plasticity and therapeutic vulnerabilities, Cell Rep. Med. 4 (2023) 101101, https://doi.org/10.1016/j.xcrm.2023.101101.
[183] V. Prakash, L. Gao, S.J. Park, Evolving applications of circulating tumor DNA in Merkel cell carcinoma, Cancers 15 (2023), https://doi.org/10.3390/cancers15030609.
[184] S. Bhatia, O. Afanasiev, P. Nghiem, Immunobiology of Merkel cell carcinoma: implications for immunotherapy of a polyomavirus-associated cancer, Curr. Oncol. Rep. 13 (2011) 488–497, https://doi.org/10.1007/s11912-011-0197-5.
[185] V. Leko, S.A. Rosenberg, Identifying and targeting human tumor antigens for T cell-based immunotherapy of solid tumors, Cancer Cell 38 (2020) 454–472, https://doi.org/10.1016/j.ccell.2020.07.013.
[186] C. Yam, B.B.Y. Ma, T.A. Yap, Global implementation of precision oncology, JCO Precis. Oncol. 5 (2021), https://doi.org/10.1200/PO.21.00001.
[187] E.R. Malone, M. Oliva, P.J.B. Sabatini, T.L. Stockley, L.L. Siu, Molecular profiling for precision cancer therapies, Genome Med. 12 (2020) 8, https://doi.org/10.1186/s13073-019-0703-1.
[188] O. Kuznetsova, M. Ivanov, A. Tryakin, A. Lebedeva, E. Ignatova, M. Fedyanin, Comprehensive multigene profiling impact on clinical decisions in patients with advanced cancers: a multicenter, retrospective analysis, J. Clin. Oncol. 41 (2023) e15163, https://doi.org/10.1200/JCO.2023.41.16_suppl.e15163.
[189] M. Ikegami, Prognostic benefit of comprehensive genomic profiling in clinical practice remains uncertain, Cancer Sci. 114 (2023) 3053–3055, https://doi.org/10.1111/cas.15826.
[190] A. Melguizo-Garín, M.D. Benítez-Márquez, I. Hombrados-Mendieta, M.J. Martos-Méndez, Importance of social support of parents of children with cancer: a multicomponent model using partial least squares-path modelling, Int. J. Environ. Res. Public Health 20 (2023), https://doi.org/10.3390/ijerph20031757.
[191] Y. Cheng, C. He, M. Wang, X. Ma, F. Mo, S. Yang, J. Han, X. Wei, Targeting epigenetic regulators for cancer therapy: mechanisms and advances in clinical trials, Signal Transduct. Target. Ther. 4 (2019) 62, https://doi.org/10.1038/s41392-019-0095-0.
[192] P. Ghadjar, J.H. Kaanders, P. Poortmans, R. Zaucha, M. Krengli, J.L. Lagrange, O. Özsoy, T.D. Nguyen, R. Miralbell, A. Baize, N. Boujelbene, T. Collen, L. Scandolaro, M. Untereiner, H. Goldberg, G.A. Pesce, Y. Anacak, E.E. Friedrich, D.M. Aebersold, K.T. Beer, The essential role of radiotherapy in the treatment of Merkel cell carcinoma: a study from the rare cancer network, Int. J. Radiat. Oncol. Biol. Phys. 81 (2011) e583–e591, https://doi.org/10.1016/j.ijrobp.2011.05.028.

[193] J. Singer, A. Irmisch, H.-J. Ruscheweyh, F. Singer, N.C. Toussaint, M.P. Levesque, D.J. Stekhoven, N. Beerenwinkel, Bioinformatics for precision oncology, Brief. Bioinform. 20 (2017) 778–788, https://doi.org/10.1093/bib/bbx143.
[194] T. Jouary, N. Lalanne, F. Siberchicot, A.-S. Ricard, J. Versapuech, S. Lepreux, M. Delaunay, A. Taieb, Neoadjuvant polychemotherapy in locally advanced Merkel cell carcinoma, Nat. Rev. Clin. Oncol. 6 (2009) 544–548, https://doi.org/10.1038/nrclinonc.2009.109.
[195] F. Petrelli, A. Ghidini, M. Torchio, N. Prinzi, F. Trevisan, P. Dallera, A. De Stefani, A. Russo, E. Vitali, L. Bruschieri, A. Costanzo, S. Seghezzi, M. Ghidini, A. Varricchio, M. Cabiddu, S. Barni, F. de Braud, S. Pusceddu, Adjuvant radiotherapy for Merkel cell carcinoma: a systematic review and meta-analysis, Radiother. Oncol. 134 (2019) 211–219, https://doi.org/10.1016/j.radonc.2019.02.015.
[196] C.Y. Lu, S. Loomer, R. Ceccarelli, K.M. Mazor, J. Sabin, E.W. Clayton, G. S. Ginsburg, A.C. Wu, Insurance coverage policies for pharmacogenomic and multi-gene testing for cancer, J. Pers. Med. 8 (2018), https://doi.org/10.3390/jpm8020019.
[197] E.L. Trimble, J.S. Abrams, R.M. Meyer, F. Calvo, E. Cazap, J. Deye, E. Eisenhauer, T.J. Fitzgerald, D. Lacombe, M. Parmar, N. Seibel, L. Shankar, A.M. Swart, P. Therasse, B. Vikram, R. von Frenckell, M. Friedlander, K. Fujiwara, R.S. Kaplan, F. Meunier, Improving cancer outcomes through international collaboration in academic cancer treatment trials, J. Clin. Oncol. 27 (2009) 5109–5114, https://doi.org/10.1200/JCO.2009.22.5771.
[198] I.A. Cree, B.I. Indave Ruiz, J. Zavadil, J. McKay, M. Olivier, Z. Kozlakidis, A. J. Lazar, C. Hyde, S. Holdenrieder, R. Hastings, N. Rajpoot, A. de la Fouchardiere, B. Rous, J.C. Zenklusen, N. Normanno, R.L. Schilsky, for the IC3R participants, the international collaboration for cancer classification and research, Int. J. Cancer 148 (2021) 560–571, https://doi.org/10.1002/ijc.33260.
[199] A.P. Abernethy, L.M. Etheredge, P.A. Ganz, P. Wallace, R.R. German, C. Neti, P. B. Bach, S.B. Murphy, Rapid-learning system for cancer care, J. Clin. Oncol. Off. J. Am. Soc. Clin. Oncol. 28 (2010) 4268–4274, https://doi.org/10.1200/JCO.2010.28.5478.
[200] L. Johnson, A. Ousley, J. Swarz, R.J. Bingham, J.B. Erickson, S. Ellis, T. Moody, The art and science of cancer education and evaluation: toward facilitating improved patient outcomes, J. Cancer Educ. 26 (2011) 27–35, https://doi.org/10.1007/s13187-010-0147-1.
[201] K. Offit, P. Thom, Ethical and legal aspects of cancer genetic testing, Semin. Oncol. 34 (2007) 435–443, https://doi.org/10.1053/j.seminoncol.2007.07.007.
[202] P. Nghiem, S. Bhatia, E.J. Lipson, W.H. Sharfman, R.R. Kudchadkar, A.S. Brohl, P. A. Friedlander, A. Daud, H.M. Kluger, S.A. Reddy, B.C. Boulmay, A. Riker, M. A. Burgess, B.A. Hanks, T. Olencki, K. Kendra, C. Church, T. Akaike, N. Ramchurren, M.M. Shinohara, B. Salim, J.M. Taube, E. Jensen, M. Kalabis, S. P. Fling, B. Homet Moreno, E. Sharon, M.A. Cheever, S.L. Topalian, Three-year survival, correlates and salvage therapies in patients receiving first-line pembrolizumab for advanced Merkel cell carcinoma, J. Immunother. Cancer 9 (2021), https://doi.org/10.1136/jitc-2021-002478.
[203] A. Bi, S. Yang, Y. Ding, Y. Yu, W. Zhan, T. Song, Prognostic value of radiotherapy and chemotherapy in stage I–III Merkel cell carcinoma, Front. Med. 9 (2022), https://doi.org/10.3389/fmed.2022.845905.
[204] P. Mojica, D. Smith, J.D.I. Ellenhorn, Adjuvant radiation therapy is associated with improved survival in merkel cell carcinoma of the skin, J. Clin. Oncol. 25 (2007) 1043–1047, https://doi.org/10.1200/JCO.2006.07.9319.
[205] N. Mohsin, M.R. Martin, D.J. Reed, S.M. Vilasi, L. Miao, N.T. Hill, I. Brownell, Differences in merkel cell carcinoma presentation and outcomes among racial and ethnic groups, JAMA Dermatol. 159 (2023) 536–540, https://doi.org/10.1001/jamadermatol.2023.0061.
[206] V. Beylergil, J.A. Carrasquillo, Molecular imaging and therapy of merkel cell carcinoma, Cancers 6 (2014) 1020–1030, https://doi.org/10.3390/cancers6021020.
[207] K. Kourou, T.P. Exarchos, K.P. Exarchos, M.V. Karamouzis, D.I. Fotiadis, Machine learning applications in cancer prognosis and prediction, Comput. Struct. Biotechnol. J. 13 (2015) 8–17, https://doi.org/10.1016/j.csbj.2014.11.005.
[208] M.D. Carter, D. Gaston, W.-Y. Huang, W.L. Greer, S. Pasternak, T.Y. Ly, N. M. Walsh, Genetic profiles of different subsets of Merkel cell carcinoma show links between combined and pure MCPyV-negative tumors, Hum. Pathol. 71 (2018) 117–125, https://doi.org/10.1016/j.humpath.2017.10.014.
[209] U.A.K. Saddozai, F. Wang, Y. Cheng, Z. Lu, M.U. Akbar, W. Zhu, Y. Li, X. Ji, X. Guo, Gene expression profile identifies distinct molecular subtypes and potential therapeutic genes in Merkel cell carcinoma, Transl. Oncol. 13 (2020) 100816, https://doi.org/10.1016/j.tranon.2020.100816.
[210] M.D. Wilkerson, D.N. Hayes, ConsensusClusterPlus: a class discovery tool with confidence assessments and item tracking, Bioinformatics 26 (2010) 1572–1573, https://doi.org/10.1093/bioinformatics/btq170.
[211] R. Tothill, V. Estall, D. Rischin, Merkel cell carcinoma: emerging biology, current approaches, and future directions, Am. Soc. Clin. Oncol. Educ. B (2015) e519–e526, https://doi.org/10.14694/EdBook_AM.2015.35.e519.
[212] S. Ugurel, I. Spassova, J. Wohlfarth, C. Drusio, A. Cherouny, A. Melior, A. Sucker, L. Zimmer, C. Ritter, D. Schadendorf, J.C. Becker, MHC class-I downregulation in PD-1/PD-L1 inhibitor refractory Merkel cell carcinoma and its potential reversal by histone deacetylase inhibition: a case series, Cancer Immunol. Immunother. 68 (2019) 983–990, https://doi.org/10.1007/s00262-019-02341-9.
[213] M.E. Verhaegen, D. Mangelberger, J.W. Weick, T.D. Vozheiko, P.W. Harms, K. T. Nash, E. Quintana, P. Baciu, T.M. Johnson, C.K. Bichakjian, A.A. Dlugosz, Merkel cell carcinoma dependence on bcl-2 family members for survival, J. Invest. Dermatol. 134 (2014) 2241–2250, https://doi.org/10.1038/jid.2014.138.
[214] R.H. Whitaker, W.J. Placzek, Regulating the BCL2 family to improve sensitivity to microtubule targeting agents, Cells 8 (2019), https://doi.org/10.3390/cells8040346.
[215] M.T. Tetzlaff, P.W. Harms, Danger is only skin deep: aggressive epidermal carcinomas. An overview of the diagnosis, demographics, molecular-genetics, staging, prognostic biomarkers, and therapeutic advances in Merkel cell carcinoma, Mod. Pathol. Off. J. U. S. Can. Acad. Pathol. Inc. 33 (2020) 42–55, https://doi.org/10.1038/s41379-019-0394-6.
[216] S. Sirikanjanapong, J. Melamed, R.R. Patel, Intraepidermal and dermal Merkel cell carcinoma with squamous cell carcinoma in situ: a case report with review of literature, J. Cutan. Pathol. 37 (2010) 881–885, https://doi.org/10.1111/j.1600-0560.2009.01407.x.
[217] H.S. Jung, Y.-L. Choi, J.-S. Choi, J.H. Roh, J.K. Pyon, K.-J. Woo, E.H. Lee, K.-T. Jang, J. Han, C.-S. Park, Y.S. Park, Y.K. Shin, Detection of Merkel cell polyomavirus in Merkel cell carcinomas and small cell carcinomas by PCR and immunohistochemistry, Histol. Histopathol. 26 (2011) 1231–1241, https://doi.org/10.14670/HH-26.1231.
[218] M. Leitz, K. Stieler, A. Grundhoff, I. Moll, J.M. Brandner, N. Fischer, Merkel cell polyomavirus detection in Merkel cell cancer tumors in Northern Germany using PCR and protein expression, J. Med. Virol. 86 (2014) 1813–1819, https://doi.org/10.1002/jmv.23808.
[219] A.A. Andea, R. Patel, S. Ponnazhagan, S. Kumar, P. DeVilliers, D. Jhala, I. E. Eltoum, G.P. Siegal, Merkel cell carcinoma: correlation of KIT expression with survival and evaluation of KIT gene mutational status, Hum. Pathol. 41 (2010) 1405–1412, https://doi.org/10.1016/j.humpath.2010.02.010.
[220] B.L. Swick, R. Srikantha, K.N. Messingham, Specific analysis of KIT and PDGFR-alpha expression and mutational status in Merkel cell carcinoma, J. Cutan. Pathol. 40 (2013) 623–630, https://doi.org/10.1111/cup.12160.
[221] D. Schrama, W.K. Peitsch, M. Zapatka, H. Kneitz, R. Houben, S. Eib, S. Haferkamp, P.S. Moore, M. Shuda, J.F. Thompson, U. Trefzer, C. Pföhler, R. A. Scolyer, J.C. Becker, Merkel cell polyomavirus status is not associated with clinical course of Merkel cell carcinoma, J. Invest. Dermatol. 131 (2011) 1631–1638, https://doi.org/10.1038/jid.2011.115.
[222] C. Frenard, L. Peuvrel, A. Brocard, M. Saint-Jean, A. Moreau, B. Dreno, G. Quéreux, Dramatic response of an inoperable Merkel cell carcinoma with imatinib, JAAD Case Rep. 2 (2016) 16–18, https://doi.org/10.1016/j.jdcr.2015.10.007.
[223] K.G. Paulson, J.G. Iyer, A.R. Tegeder, R. Thibodeau, J. Schelter, S. Koba, D. Schrama, W.T. Simonson, B.D. Lemos, D.R. Byrd, D.M. Koelle, D.A. Galloway, J.H. Leonard, M.M. Madeleine, Z.B. Argenyi, M.L. Disis, J.C. Becker, M.A. Cleary, P. Nghiem, Transcriptome-wide studies of Merkel cell carcinoma and validation of intratumor lymphocyte invasion as an independent predictor of survival, J. Clin. Oncol. 29 (2011) 1539–1546, https://doi.org/10.1200/JCO.2010.30.6308.
[224] H. Sihto, T. Böhling, H. Kavola, V. Koljonen, M. Salmi, S. Jalkanen, H. Joensuu, Tumor infiltrating immune cells and outcome of Merkel cell carcinoma: a population-based study, Clin. Cancer Res. Off. J. Am. Assoc. Cancer Res. 18 (2012) 2872–2881, https://doi.org/10.1158/1078-0432.CCR-11-3020.
[225] O.K. Afanasiev, L. Yelistratova, N. Miller, K. Nagase, K. Paulson, J.G. Iyer, D. Ibrani, D.M. Koelle, P. Nghiem, Merkel polyomavirus-specific t cells fluctuate with Merkel cell carcinoma burden and express therapeutically targetable PD-1 and Tim-3 exhaustion markers, Clin. Cancer Res. 19 (2013) 5351–5360, https://doi.org/10.1158/1078-0432.CCR-13-0035.
[226] E.J. Lipson, J.G. Vincent, M. Loyo, L.T. Kagohara, B.S. Luber, H. Wang, H. Xu, S. K. Nayar, T.S. Wang, D. Sidransky, R.A. Anders, S.L. Topalian, J.M. Taube, PD-L1 expression in the Merkel cell carcinoma microenvironment: association with inflammation, Merkel cell polyomavirus, and overall survival, Cancer Immunol. Res. 1 (2013) 54–63, https://doi.org/10.1158/2326-6066.CIR-13-0034.
[227] D.E. Loader, R. Feldmann, M. Baumgartner, F. Breier, D. Schrama, J.C. Becker, A. Steiner, Clinical remission of Merkel cell carcinoma after treatment with imatinib, J. Am. Acad. Dermatol. 69 (2013) e181–e183, https://doi.org/10.1016/j.jaad.2013.03.042.
[228] M. Davids, A. Charlton, S.-S. Ng, M.-L. Chong, K. Laubscher, M. Dar, J. Hodge, R. Soong, B.C. Goh, Response to a novel multitargeted tyrosine kinase inhibitor pazopanib in metastatic merkel cell carcinoma, J. Clin. Oncol. 27 (2009) e97–e100, https://doi.org/10.1200/JCO.2009.21.8149.
[229] H. Schlagbauer-Wadl, G. Klosner, E. Heere-Ress, S. Waltering, I. Moll, K. Wolff, H. Pehamberger, B. Jansen, Bcl-2 antisense oligonucleotides (G3139) inhibit merkel cell carcinoma growth in SCID mice, J. Invest. Dermatol. 114 (2000) 725–730, https://doi.org/10.1046/j.1523-1747.2000.00937.x.
[230] H. Sahi, V. Koljonen, H. Kavola, C. Haglund, E. Tukiainen, H. Sihto, T. Böhling, Bcl-2 expression indicates better prognosis of Merkel cell carcinoma regardless of the presence of Merkel cell polyomavirus, Virchows Arch. 461 (2012) 553–559, https://doi.org/10.1007/s00428-012-1310-3.
[231] L.R. Dresang, A. Guastafierro, R. Arora, D. Normolle, Y. Chang, P.S. Moore, Response of Merkel cell polyomavirus-positive merkel cell carcinoma xenografts to a survivin inhibitor, PLoS One 8 (2013), https://doi.org/10.1371/journal.pone.0080543.

[232] R.J. Hicks, Use of molecular targeted agents for the diagnosis, staging and therapy of neuroendocrine malignancy, Cancer Imaging Off. Publ. Int. Cancer Imaging Soc. 10 (2010) S83-91, https://doi.org/10.1102/1470-7330.2010.9007.
[233] M. Kurokawa, K. Nabeshima, Y. Akiyama, S. Maeda, T. Nishida, F. Nakayama, M. Amano, K. Ogata, M. Setoyama, CD56: a useful marker for diagnosing Merkel cell carcinoma, J. Dermatol. Sci. 31 (2003) 219–224, https://doi.org/10.1016/S0923-1811(03)00029-X.
[234] M. Pedretti, C. Verpelli, J. Mårlind, G. Bertani, C. Sala, D. Neri, L. Bello, Combination of temozolomide with immunocytokine F16–IL2 for the treatment of glioblastoma, Br. J. Cancer 103 (2010) 827–836, https://doi.org/10.1038/sj.bjc.6605832.