Therapeutic targets for malignant peripheral nerve sheath tumors

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Malignant peripheral nerve sheath tumors (MPNSTs) are rare and aggressive soft-tissue sarcomas with dismal prognosis. Complete resection, which is the only known definitive therapy, is not feasible with every tumor, and local recurrence after surgery is another challenge to successful treatment. Treatments used with other sarcoma types have not proven beneficial to MPNST patients. Targeted therapies blocking several signaling pathways known to drive MPNST pathogenesis have also not improved patient outcomes in clinical trials. This review discusses existing therapies and targeted chemotherapeutic options currently being tested clinically, and potential therapeutic avenues identified in preclinical studies that include targeting signaling pathways such as the HIPPO-YAP pathway and epigenetic mechanisms as well as multi-agent strategies.

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Epidemiology & pathology of malignant peripheral nerve sheath tumor

Malignant peripheral nerve sheath tumors (MPNSTs) are highly aggressive peripheral nerve-associated tumors of Schwann cell origin that compose 5–10% of all soft-tissue sarcomas. The tumors occur primarily in adults between 20 and 60 years of age [1]. Approximately 20% of cases are diagnosed in the pediatric population, and MPNSTs are one of the most frequent non-rhabdomyosarcoma tumors in children [2]. The occurrence of MPNST is rare with an estimated incidence of 1.46 per 1,000,000 individuals [3]. MPNSTs either occur independently or in association with neurofibromatosis type 1 (NF1) [4]. Between 8 and 13% of individuals with NF1 mutations develop MPNST, and these patients constitute nearly 50% of all MPNST cases. Approximately 45% of MPNSTs occur sporadically with unidentified genetic anomalies, and the remaining 10% of cases are iatrogenically associated with radiotherapy [5–7]. The prognosis of patients with MPNSTs is dismal with a 5-year survival rate of between 16 and 52%. Disease management poses significant challenges, as surgical resection remains the primary treatment. Surgical resection is often impeded by the large size of tumors, their proximity to complex nerve networks and a low rate of negative resection margins [8]. In addition, MPNSTs usually exhibit strong metastatic proclivity, and patients have a high propensity for relapse following tumor resection and poor responses to cytotoxic chemotherapy and irradiation, resulting in high mortality [4,9] (Figure 1).

MPNSTs arise in nerve roots and bundles in the extremities and pelvis, in particular, large peripheral nerves such as the sciatic nerve, brachial plexus and sacral plexus. Patients present with rapidly enlarging mass, pain and neurological symptoms, and pulmonary metastasis is observed in up to half of the cases. The majority of MPNSTs are classified as intermediate to high grade at diagnosis. Heterologous differentiation to mesenchymal lineages, such as skeletal muscle, bone, cartilage and blood vessels is seen in approximately 15% of tumors and may be predictive of poorer prognosis [4]. The molecular and signaling events responsible for benign neurofibroma-to-MPNST transformation in NF1 patients, and those involved in tumor development and progression of sporadic MPNSTs, are still incompletely understood.

Figure 1. The diagram depicts multiple molecular mechanisms implicated in the pathogenesis of malignant peripheral nerve sheath tumors, which include various receptor tyrosine kinase signaling cascades, HIPPO pathway, Wnt pathway, protein folding mechanism, epigenetic regulation and tumor microenvironment interactions. The mechanisms of action of potential small-molecule inhibitors targeting each of the above-mentioned machinery are denoted.

Genetics & genomics of NF1-associated & sporadic MPNST

In NF1-associated MPNST, malignant transformation of pre-existing benign plexiform neurofibromas, a hallmark of NF1, is believed to contribute to MPNST; MPNSTs are the leading cause of death in neurofibromatosis patients [10]. NF1 is an autosomal-dominant tumor predisposition syndrome caused by a mutation in the *NF1* tumor suppressor gene. *NF1* is located in the chromosome band 17q11 and codes for a 280-kDa protein called neurofibromin that accelerates GTP hydrolysis on Ras proteins and facilitates the conversion of active Ras-GTP to inactive Ras-GDP in various cell types, thereby inhibiting the p21ras signaling pathway [11]. The loss of NF1 protein leads to activation of the Ras signaling pathway. Progression toward MPNST normally requires biallelic loss of the *NF1* gene. NF1 deficiency by itself is insufficient to induce MPNST given that only 8–13% of all NF1 patients develop MPNST. The acquisition of additional genetic aberrations, including the amplification of oncogenes *PDGFRA*, *EGFR* or *MET* or the loss of tumor suppressor genes *CDKN2A*, *RB1*, *TP53* or the genes encoding epigenetic PRC2 complex components *SUZ12* or *EED*, has been associated with MPNST formation [12]. *TP53* point mutations or deletions are observed in approximately 75% of patients with MPNSTs. The loss of *TP53* in one allele is sufficient to drive MPNST [13]. Deletions of the *CDKN2A* tumor suppressor gene, which encodes two cell-cycle inhibitors p16^{INK4A} and p14^{ARF}, occur in about 50% of neoplasms [12]. Moreover, genomic inactivation of *CDKN2A* can be found in a major subset of atypical neurofibromas, which eventually progress into malignancy. Loss of CDKN2A expression contributes to neurofibroma-to-MPNST transformation [14,15].

Sporadic MPNSTs are caused by unidentified genetic or epigenetic anomalies, although nearly 40% also exhibit somatic mutations of *NF1* [16]. *TP53* mutations are more frequently detected in sporadic MPNST, whereas Raf/PI3K/AKT activation and EGFR overexpression are more associated with NF1-associated MPNST [4]. Despite so, recurrent mutations of *NF1*, *TP53*, *CDKN2A*, *SUZ12* and *EED* have been reported across MPNST subtypes from multiple next-generation sequencing studies [17], suggesting that distinct subtypes of MPNST can be driven by similar genomic or epigenetic mechanism.

Standard treatment paradigm for MPNST

The conventional treatment for MPNST involves surgical resection with a wide negative margin, which is defined as the resection margin lacking identifiable tumor within 1 mm from an inked surface of tissue. Surgical resection has significantly prolonged overall survival in patients with NF1. Efficacy of surgery depends on tumor size, location and metastasis. The local recurrence rate after surgical resection is between 32 and 65% [18]. Adjuvant radiation therapy is generally recommended for patients with large high-grade lesions (>5 cm) or with positive resected tumor margins as a post-operative measure to reduce local recurrence and to control symptoms such as pain. The use of radiotherapy, however, has not demonstrated an advantage in terms of overall survival [19].

MPNST has been treated with chemotherapy regimens similar to those used in treatment of soft-tissue sarcomas with agents such as doxorubicin, dactinomycin, cyclophosphamide and vincristine. Chemotherapy is largely reserved for unresectable tumors and metastatic MPNSTs, however. NF1 patients respond less well to chemotherapy than do sporadic MPNST patients [4]. The efficacy of chemotherapy is still not clearly defined as current chemotherapy regimens have provided only marginal survival benefit [4].

Despite current aggressive multimodal therapy, the prognosis of MPNST patients remains dismal. There is an urgent need for improved effective therapeutic modalities for MPNST. Identifying key pathways that are vital to MPNST growth is essential for overcoming this challenge. Suboptimal clinical outcomes with conventional chemotherapeutic agents have prompted interest in the development of rationally targeted therapeutics in MPNST, and recent molecular and genomics studies that have identified signaling networks and epigenetic regulators underlying MPNST pathogenesis have potential to guide this development.

Therapeutics targeting activated signaling pathways in MPNST

Receptor tyrosine kinase signaling

Receptor tyrosine kinases (RTKs) are a family of high-affinity cell-surface receptors for a wide array of growth factors, hormones, cytokines, neurotrophic factors and other extracellular signaling molecules. Multiple soluble or membrane-bound growth factors bind and activate RTKs including NGFR, PDGFR, FGFR, EGFR, VEGFR, MET/HGFR, IR and IGFR, and the proto-oncogene c-KIT. These growth factors are key regulators of normal cellular processes including survival, proliferation, differentiation and migration [20,21].

Aberrant regulation of RTK signaling due to acquisition of genetic and epigenetic alterations is a common cause of cell transformation, cancer development and metastasis, and RTKs play critical roles in regulation of cell

stemness and proliferation [22,23]. Genetic alterations that activate RTKs or components of downstream pathways such as MAPK, PI3K/AKT and JAK/STAT have been identified in a multitude of malignancies [22]. Small-molecule inhibitors and antibodies that target RTKs and the MAP kinase and PI3K/AKT pathways show moderate efficacy against MPNST [24]. Tyrosine kinase inhibitors imatinib and dasatinib, which target the PDGFR pathway and c-KIT, respectively, both upregulated in MPNST [25], have been explored in clinical trials but no significant responses were observed [26,27].

Gene amplification events in the EGFR pathway and dysregulated EGFR expression are frequent in MPNST [28,29]. The EGFR ligands transforming growth factor alpha and EGF are expressed at higher levels in MPNSTs than in neurofibromas [30], and both promote malignant transformation of neurofibroma in a mouse model [31]. Despite this, a Phase II trial of erlotinib, an EGFR inhibitor, in metastatic or unresectable MPNST did not show obvious benefit [32]. Intriguingly, EGFR may mediate its effects through the STAT3 pathway and inhibition of the EGFR-STAT3 axis efficiently impedes MPNST transformation and tumorigenesis in xenograft models [31]. A specific JAK2 inhibitor FLLL32, which blocks JAK2-mediated phosphorylation of STAT3, delays MPNST growth in an MPNST xenograft model, suggesting that combination therapies targeting JAK/STAT3 might be a rational choice for therapeutics. In summary, targeting RTK signaling pathways is a rational but not yet successful approach for treatment of MPNSTs (Table 1).

RAS/RAF/MEK pathway

The RAS/RAF/MEK/ERK cascade integrates inputs from a variety of extracellular cues into intrinsic transcriptional programs that drive a broad spectrum of physiological and pathological cellular processes such as growth, proliferation, differentiation, migration and apoptosis [33]. The tumor suppressor gene *NF1* encodes neurofibromin, a GTPase-activating protein that accelerates GTP hydrolysis on Ras proteins, thus negatively regulating RAS/RAF/MEK signaling. *NF1* loss-of-function mutations result in an increase of the GTP-bound form of Ras [34]. GTP-bound Ras recruits and activates the serine/threonine protein kinase RAF; RAF subsequently activates MEK1/2 and ERK1/2. Activated ERK1 and ERK2 phosphorylate and stimulate downstream effectors and regulate different transcription factors, leading to expression of genes that stimulate proliferation.

Germline or somatic mutations of *NF1* are frequently detected in both NF1-associated and sporadic MPNSTs, suggesting that the RAS-RAF-MEK pathway should be targeted to treat MPNSTs. However, a multicenter Phase II trial exploring responses to sorafenib, a small-molecule orally given RAF kinase and tyrosine kinase inhibitor revealed no significant responses, with overall survival of treated MPNST patients similar to survival of controls [35].

MEK inhibitors show promising antitumor effects in MPNST cells grown in culture [36]. Moreover, a MEK inhibitor, selumetinib, reversed plexiform neurofibroma tumor burden in a recent clinical trial [37]. An ongoing Phase II trial (SARC031; NCT03433183) aims to test the efficiency of selumetinib in combination with the dual mTOR kinase inhibitor vistusertib in patients with unresectable or metastatic NF1-associated or sporadic MPNST.

PI3K/AKT/mTOR pathway

Activation of the mTOR pathway is prevalent in MPNST tumor samples and likely plays a key role in the pathogenesis of MPNST. Alterations in expression or activity of PI3K/mTOR pathway components such as PTEN, p-AKT, p-mTOR and p-S6RP portend a poorer prognosis in MPNST patients [38]. Preclinical studies of mTOR inhibitors *in vitro* and in xenografts have demonstrated tumor growth suppression [38,39]. The combination of mTOR inhibitors with other agents has also been explored in animal models, and combinations have been identified that surpass the cytostatic effects of mTOR inhibition alone. In an MPNST mouse model, dual inhibition of the mTOR pathway and the molecular chaperone hsp90 effectively abrogated the cellular stress response machinery tumor cells employ to survive proteotoxic stress and eventually caused cell death and tumor regression [40]. A multi-institutional Phase I/II trial (SARC023; NCT02008877) of ganetespib, an Hsp90 inhibitor, in combination with the mTOR inhibitor sirolimus in patients with refractory sarcoma including MPNST was recently completed. The investigators hypothesize that these agents that work on separate and potentially synergistic pathways may be useful for treatment of refractory soft-tissue sarcomas. The outcome analysis is yet to be released. Tests of combination therapies involving mTOR inhibitors with agents targeting additional pathways, such as MEK and VEGFR pathways, are ongoing.

Table 1. Targeted therapies of past and ongoing clinical trials for malignant peripheral nerve sheath tumor and potential therapeutics tested in preclinical studies.

Drug/therapeutics	Target	Phase/models	Year	Results	Clinical trial identifier	Study (year)	Ref.
Erlotinib	EGFR	II		No objective responses; median progression-free survival = 2 months; median overall survival = 4 months		Albritton <i>et al.</i> (2006)	[32]
Sorafenib	RAF/VEGFR/c-KIT/PDGFR	II		No responses; median progression-free survival = 1.7 months		Maki <i>et al.</i> (2009)	[35]
Imatinib	VEGFR/c-KIT/PDGFR	II		No responses; 1 stable disease		Chugh <i>et al.</i> (2009)	[26]
Dasatinib	C-KIT/SRC	II		No responses or stable disease		Schuetze <i>et al.</i> (2010)	[27]
Bevacizumab everolimus	Angiogenesis/mTOR	II	2012–2017	Clinical benefit rate of 12%	NCT01661283	Widemann <i>et al.</i> (2016)	[43]
Ganetespib sirolimus	Hsp90 mTOR	I/II	2013–2018	Complete, awaiting analysis	NCT02008877		
Selumetinib sirolimus	MEK mTOR	II	2018–2021	Currently ongoing	NCT03433183		
CPI-0610	BET family proteins	II	2017–2020	Currently ongoing	NCT02986919		
PLX3397 sirolimus	CSF1R/c-KIT mTOR	I/II	2015–2021	Currently ongoing	NCT02584647		
Pembrolizumab (MK-3475)	PD-1 (T cells) and PD-L1 (MPNST cells) interaction	II	2016–2025	Currently ongoing	NCT02691026		
Oncolytic virus vaccine	RAS overexpressing tumor cells	I	2017–2021	Currently ongoing	NCT02700230		
IWR-1 or XAV-939 RAD001	Wnt- β -catenin mTOR	MPNST cell lines		Drug synergistic effects; induction of MPNST cell apoptosis		Watson <i>et al.</i> (2013)	[47]
Verteporfin sorafenib	HIPPO-TAZ/YAP PDGFR/RAF	Mouse MPNST cells and allografts; MPNST cell lines		Combination therapy inhibits tumor proliferation and decelerates growth		Wu <i>et al.</i> (2018)	[60]
JQ1 PD-901	BET family proteins MEK	Mouse MPNST model; MPNST lines		Drug combination triggered significant tumor regression		De Raedt <i>et al.</i> (2014)	[40]

BET: Bromodomain and extra terminal domain; MPNST: Malignant peripheral nerve sheath tumor.

VEGF pathway

VEGF signaling promotes vasculogenesis and angiogenesis and is critical to tumor-induced new vasculature formation, which is essential for tumor growth and metastasis [41]. Anti-angiogenic therapy has been a vital strategy for cancer therapy; however, pharmacological inhibition of the VEGF pathway in Phase II studies did not benefit MPNST patients [42]. Therefore, rationally designed combination therapies are currently being explored. A Phase II trial testing the combination of anti-angiogenic VEGF inhibitor bevacizumab and mTOR inhibitor everolimus in 25 patients with NF1-associated and sporadic MPNSTs has been completed (NCT01661283). The therapy outcome was dismal, with a clinical benefit rate of 12% [43].

Emerging potential therapeutic options

Despite a prevalent association with *NF1* genetic aberrations in most MPNST cases, the genomic complexity of the disease renders therapy development challenging. Moreover, the focus of most clinical trials aimed at treating MPNSTs has been on reducing RAS signaling or downstream effector expression using inhibitors of RTK, RAS, MEK, AKT and mTOR signaling pathways. Unfortunately, none of these trials have yet shown efficacy against MPNSTs. Therefore, it is imperative to elucidate additional key molecular drivers of MPNST.

Wnt signaling

Largaespada and colleagues have developed an unbiased *in vivo* forward genetic screen to identify pathways critical for MPNST tumorigenesis. Using their *Sleeping Beauty* transposon-based somatic mutagenesis system in mice, in which a mutagenic transposable element tags insertional mutagenesis events that drive cancer in the Schwann cell lineage, they identified Wnt signaling pathway genes as potential drivers of benign neurofibromas and MPNSTs [44]. Evidence suggests that the canonical WNT signaling pathway crosstalks with other pathways to drive MPNST pathogenesis [45,46]. Targeting the Wnt pathway in conjunction with mTOR inhibition synergistically induced cell death in MPNST cell lines [47]. The same *Sleeping Beauty* screening platform also enabled identification of new proto-oncogenes key to MPNST maintenance such as *FOXR2*, further validating the potential use of this tool for discovery of cancer-driving genes and therapeutic design [44].

Epigenetic mechanisms

Epigenetic-based therapies have gained tremendous attention in cancer research in recent years. In 70–90% of all MPNST subtypes, loss-of-function mutations in *SUZ12* or *EED*, which encode polycomb repressive complex 2 (PRC2) constituents, have been identified [48]. These mutations are associated with malignant progression from benign plexiform neurofibroma to MPNST [49,50]. *SUZ12* or *EED* mutations cause loss of the repressive mark, trimethylation of histone 3 on K27 (H3K27), resulting in de-repression of genes that drive cell growth. Upon inactivation of PRC2 and loss of H3K27 methylation, H3K27 may become acetylated. The bromodomain and extra terminal domain (BET) family proteins (e.g., BRD protein family) bind to these acetylated loci and recruit transcription factor complexes thereby modulating transcription elongation of specific genes, such as oncogenic Ras signaling genes [51]. A potential strategy is to reverse the loss of PRC2 activity with bromodomain inhibitors such as JQ1. JQ1 potently inhibits bromodomain protein activities, thus suppressing the genes normally repressed by PRC2. Furthermore, JQ1 in conjunction with a MEK inhibitor promotes cell death and tumor regression in a mouse model [49].

Another study finds that the chromatin regulator, BRD4, a member of the BET family, is upregulated in a mouse MPNST model. Inhibition of BRD4 robustly suppresses MPNST growth and tumorigenesis via induction of apoptosis through proapoptotic Bim activation [52]. A Phase II clinical trial is ongoing with the use of CPI-0610, a selective inhibitor of BET family proteins (NCT02986919).

Additionally, tumors with elevated Ras signaling, including MPNSTs, are sensitive to histone deacetylase inhibitors, which induce autophagy in MPNSTs and might be a useful therapeutic option [53].

HIPPO signaling pathway

Despite the rarity of genetic mutations of HIPPO core components, persistent activation of HIPPO signaling effectors YAP and TAZ has been reported in a multitude of cancer types. A few inactivating mutations of the HIPPO pathway components have also been identified in cancers [54], including mutations in *NF2*, encoding an upstream HIPPO component Merlin, in benign schwannoma [55] and occasionally high-grade tumors [56], as well as *LATS2* and *SAV1* in malignant mesothelioma [57]. Constitutive activation of YAP/TAZ oncoproteins is observed in a wide variety of sarcoma types including MPNST [58]. YAP/TAZ hyperactivity bestows tumor cells with stem cell properties and resistance to chemotherapeutic agents [59]. Importantly, a recent study shows that the HIPPO/YAP gene signature is activated in both NF1-associated and sporadic MPNSTs regardless of *NF1* mutation status [60]. Pharmacological manipulation of the HIPPO pathway may be a potential approach to treat tumors with high YAP and/or TAZ activity.

Our recent study showed that deficiencies in HIPPO signaling kinases *LATS1/2* in Schwann cells induces transformation to malignant nerve sheath tumors with full penetrance, suggesting that HIPPO signaling dysregulation is critical for MPNST pathogenesis. Tumors that arise are highly invasive and differentiated, resembling the characteristics of human MPNST. Oncogenic activities of YAP and TAZ are dependent on their association

with the TEADs transcription factors. Analysis of TEAD1 genome occupancy and transcript levels demonstrated that YAP/TAZ directly regulate multiple oncogenic pathways including those involved in cell cycle progression, regulation of cell migration, extracellular matrix organization and, in particular, PDGFR signaling. Pharmacologically targeting HIPPO/YAP/TAZ and PDGFR signaling was explored in allograft models as well as in human MPNST cell lines *in vitro*. Combined attenuation of YAP/TAZ/TEAD1 and PDGFR/RAF1 signaling decelerates tumor cell proliferation in LATS1/2-deficient mice and human MPNST cells. Although single-agent therapy with PDGFR/RAF1 inhibitors alone (imatinib or sorafenib) was not effective in treatment of MPNST patients in a Phase I/II trial [26,35], the combination treatment paradigm has potential.

The YAP/TAZ inhibitor verteporfin is an FDA-approved porphyrin-family drug clinically used as a photosensitizer in photodynamic therapy that has been shown to disrupt the interaction of YAP/TAZ with TEAD [61]. Verteporfin effectively inhibits YAP expression and sensitizes esophageal cancer cells [62] and lung cancer cells [63] in culture to cytotoxic drugs. Although a Phase I/II trial of verteporfin photodynamic therapy in locally advanced pancreatic cancer indicates therapy feasibility [64], its toxicity to non-malignant cells and its low aqueous solubility pose limitations to clinical use.

The interactions between TEAD and YAP/TAZ can also be inhibited by peptides and small molecules. Super-TDU, designed to mimic the tandem TDU domain of VGLL4, which directly competes with YAP for binding to TEADs, robustly inhibits gastric cancer growth *in vitro* and in xenografts [65]. Intriguingly, its anti-tumor activity appears to be specific for tumors with an elevated ratio of YAP to VGLL4. Another peptide engineered to target the YAP-TEAD interaction is the cyclic peptide 17; the peptide specifically targets the TEAD-binding interface on YAP. *In vitro* assays and xenograft models show that it potently impairs tumor growth. CA3, a small-molecule inhibitor of TEAD/YAP1 transcriptional activity, inhibits proliferation and tumor sphere formation, induces apoptosis and reduces the proportion of cancer stem cells in esophageal adenocarcinoma xenografts without apparent toxicity [66]. In summary, targeting oncogenic proteins YAP/TAZ is promising for cancer therapeutics [59]. The animal model of MPNST with LATS deficiency will facilitate identification and development of combinatorial strategies coupling inhibition of the HIPPO pathway and other oncogenic pathways.

Targeting cancer-associated cells in the tumor microenvironment

One of the hallmarks of cancer is the evolution of a tumor microenvironment whereby malignant cells recruit, co-exist and communicate with nonmalignant cells such as fibroblasts, macrophages and other immune infiltrates to establish a growth-permissive milieu. The myriads of cellular interactions between cancer cells and cancer-associated cells in the tumor microenvironment can be growth-promoting and growth-inhibitory, which determines whether a given host environment favors tumor proliferation, invasiveness, metastatic potential and stemness maintenance and confer drug resistance [67]. A complete understanding of these cellular interactions may therefore shed light on novel anti-tumor therapies aimed at increasing the resistance of the host environment.

MPNST is composed of a mixture of Schwann cells, fibroblasts and perineurial cells, all of which contribute to the establishment of a tumor niche. Indeed, mouse model studies reveal that *Nf1* heterozygosity in the tumor environment promotes neurofibroma formation [68]. In a conditional *Nf1* mouse model, in which a floxed *Nf1* allele is deleted by a Cre transgene under control of the Schwann cell-specific promoter, Krox-20 (*Nf1^{flax/-};Krox20-cre*; containing *NFI^{-/-}* Schwann cells and *NFI^{+/-}* mast cells), the *Nf1^{flax/-};Krox20-cre* mice develop plexiform neurofibromas and massive mast cell infiltration, whereas *Nf1^{flax/flax};Krox20-cre* mice (containing *NFI^{-/-}* Schwann cells and normal mast cells) only developed small, infrequent hyperplastic lesions in the cranial nerves with reduced mast cell infiltration. This suggests an important role of the heterozygous environment in neurofibroma development. However, subsequent studies by other groups suggests that ablation of *Nf1* or codeletion of *Nf1* and *Pten* solely in Schwann cell precursors give rise to neurofibroma and MPNST, respectively [47,69].

Macrophage depletion

Macrophage infiltration is prevalent in neurofibromas and MPNSTs, accounting for nearly 50% of cells within a tumor [70]. While evaluating the efficacy of PLX3397, a selective RTK/c-KIT and colony stimulating factor 1 receptor (CSF1R) inhibitor against MPNST cells, Schwartz and colleagues demonstrated that PLX3397 efficiently blocks c-KIT, PDGFR- β and CSF1R activity in MPNST cells *in vitro* and in xenografts, impairing tumor growth and inducing macrophage depletion. Combining PLX3397 and an mTOR inhibitor, rapamycin further enhances macrophage depletion and tumor growth inhibition [71]. This strategy is currently being explored in a Phase I/II trial (NCT02584647) of the orally dosed combination of PLX3397 and an mTOR inhibitor, sirolimus.

Immune checkpoint blockade

Tumor cells evade immune surveillance and T-cell mediated clearance by expression of inhibitory immune molecules, including programmed death-ligand 1 (PD-L1). Manipulation of immune checkpoints such as CTLA4 or PD-1 with targeted antibodies has emerged as an effective anticancer strategy in various malignancies. Since PD-L1 expression is more prevalent in MPNST than normal nerves, benign neurofibromas and schwannomas, immune checkpoint blockade immunotherapy may be a potential therapy for MPNST [72,73]. A Phase II clinical trial is ongoing using a checkpoint inhibitor to PD-1, pembrolizumab (MK-3475) in the patients with metastatic or locally advanced/unresectable MPNST (NCT02691026). Pembrolizumab is a humanized antibody that binds to PD-1, expressed on the surface of activated T cells, and blocks the interaction and activation of PD-1 by its ligand PD-L1 on tumor cells, which results in the activation of T-cell-mediated killing of tumor cells.

Oncolytic virus vaccine therapy

There has been growing interest in using oncolytic viruses as therapy due to their ability to kill cancer cells, via direct lytic activity of the virus life cycle or activation of the innate immune response against tumor cells. Oncolytic herpes simplex viruses selectively infect MPNST cells expressing high levels of RAS in culture and inhibit the growth of MPNST cells in immunodeficient mice [74,75]. A Phase I trial for investigating the side effects and the suitable dose of vaccine therapy in the patients with recurrent or unresectable MPNST is ongoing (NCT02700230).

Conclusion & future perspective

The poor prognosis, generally dismal drug response rate, and high rates of drug resistance of patients with MPNST present a pressing need for a better understanding of MPNST biology and development of effective treatment paradigms. The rarity of MPNST poses a challenge for patient recruitment and testing of potential therapies. In the past two decades, the outcome of MPNST has not improved significantly. However, a more thorough understanding of the genomic, epigenetic, signaling pathway and microenvironment changes during plexiform neurofibroma-to-MPNST malignant transformation has offered hope for the development of more effective diagnostic and therapeutic strategies for MPNST. In the next years, developing novel animal models that recapitulate human MPNST transformation from atypical plexiform neurofibroma is necessary to enable dissection of the molecular mechanisms underlying MPNST initiation and progression. Identification of the biomarkers of MPNST and their validation in preclinical models are critically important to the success of clinical trials. In addition, targeting individual steps in a druggable pathway may induce negative feedback responses resulting in treatment failure. Understanding all steps in multiple signaling pathways activated in MPNST and how different pathways crosstalk within tumor cells or with tumor microenvironment will facilitate design of rational combinations of targeted therapies to increase treatment efficacy and reduce resistance mechanisms.

Furthermore, a comprehensive database including genomic, transcriptomic, phenotypic, metabolomic data, drug treatment responses and tumor microenvironment niches is requisite for accelerating our understanding of the molecular basis of this challenging disease. These important resources must be shared to inform therapeutic strategies in MPNST. Coordinated efforts between preclinical and clinical research teams will help establish criteria for rational selection of combination therapies for clinical trial evaluation in MPNST patients.

Executive summary

Epidemiology & pathology of malignant peripheral nerve sheath tumor

- The major subtypes of malignant peripheral nerve sheath tumors (MPNSTs) include NF1-associated, sporadic and radiotherapy-associated MPNST. These highly aggressive peripheral nerve-associated tumors of Schwann cell origin compose 5–10% of all soft-tissue sarcomas.
- The clinical outcome of MPNSTs is poor with a dismal 5-year survival rate. Surgical resection remains the primary treatment. In addition, MPNSTs are strongly metastatic and resistant to cytotoxic chemotherapy.

Genetics & genomics of NF1-associated & sporadic MPNST

- Biallelic loss of the *NF1* gene alone is insufficient to induce MPNST.
- The acquisition of additional genetic aberrations, including the amplification of oncogenes *PDGFRA*, *EGFR* or *MET* or the loss of tumor suppressor genes *CDKN2A*, *RB1*, *TP53* or the genes encoding epigenetic PRC2 complex components *SUZ12* or *EED*, has been associated with MPNST formation.

Standard treatment paradigm for MPNST

- The conventional treatment for MPNST involves surgical resection.

- Adjuvant radiation therapy is generally recommended for patients with large high-grade lesions (>5 cm) or with positive resected tumor margins.
- Chemotherapy is largely reserved for unresectable tumors and metastatic MPNSTs, and is similar to treatment paradigms for other soft tissue sarcomas. However, these regimen are considered ineffective.

Therapeutics targeting activated signaling pathways in MPNST

- **Receptor tyrosine kinase signaling**
- **RAS/RAF/MEK pathway**
 - *NF1* loss-of-function mutations result in Ras activation.
 - MEK inhibitors show promising anti-tumor effects in MPNST cells *in vitro*.
 - A clinical trial is ongoing using a MEK inhibitor in combination with other oncogenic pathway inhibition.
- **PI3K/AKT/mTOR pathway**
 - Preclinical studies of mTOR inhibitors *in vitro* and in xenografts have demonstrated tumor growth suppression.
 - Several ongoing clinical trials use combination of mTOR inhibitors and other agents.
- **VEGF pathway**
 - Pharmacological inhibition of the VEGF pathway in Phase II studies did not benefit MPNST patients.

Emerging potential therapeutic options

- **Wnt signaling**
 - *In vivo* forward genetic screen identified Wnt signaling pathway genes as potential drivers of benign neurofibromas and MPNST.
 - Targeting the Wnt pathway in conjunction with mTOR inhibition synergistically induced cell death in MPNST cell lines.
- **Epigenetic mechanisms**
 - Loss-of-function mutations of PRC2 components are associated with malignant progression from benign plexiform neurofibroma to MPNST.
 - BRD4 inhibition suppresses MPNST tumor growth.
 - HDAC inhibition induces autophagy in MPNST.
- **HIPPO signaling pathway**
 - HIPPO/YAP gene signature is activated in both *NF1*-associated and sporadic MPNSTs regardless of *NF1* mutation status.
 - Pharmacological manipulation of the HIPPO pathway may be a potential approach to treat tumors with high YAP and/or TAZ activity.
- **Targeting cancer-associated cells in the tumor microenvironment**
 - **Macrophage depletion**
 - Macrophage infiltration is prevalent in neurofibromas and MPNSTs, accounting for nearly 50% of cells within a tumor.
 - PLX3397 efficiently blocks c-KIT, PDGFR β and CSF1R activity in MPNST cells *in vitro* and in xenografts.
 - **Immune checkpoint blockade**
 - PD-L1 expression is more prevalent in MPNST than normal nerves, benign neurofibromas and schwannomas.
 - A phase II clinical trial is ongoing using a checkpoint inhibitor to PD-1 in MPNST.
 - **Oncolytic virus vaccine therapy**

Future perspective & conclusion

- Identification of the biomarkers of MPNST and their validation in preclinical models are critically important to the success of clinical trials.
- A comprehensive database including genomic, transcriptomic, phenotypic, metabolomic data, drug treatment responses and tumor microenvironment niches is requisite for accelerating our understanding of the molecular basis of this challenging disease.

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