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Medulloblastoma: From Molecular Subgroups to Molecular Targeted Therapies

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Abstract

Brain tumors are the leading cause of cancer-related death in children, and medulloblastoma (MB) is the most common malignant pediatric brain tumor. Advances in surgery, radiation, and chemotherapy have improved the survival of MB patients. But despite these advances, 25–30% of patients still die from the disease, and survivors suffer severe long-term side effects from the aggressive therapies they receive. Although MB is often considered a single disease, molecular profiling has revealed a significant degree of heterogeneity, and there is a growing consensus that MB consists of multiple subgroups with distinct driver mutations, cells of origin, and prognosis. Here, we review recent progress in MB research, with a focus on the genes and pathways that drive tumorigenesis, the animal models that have been developed to study tumor biology, and the advances in conventional and targeted therapy.

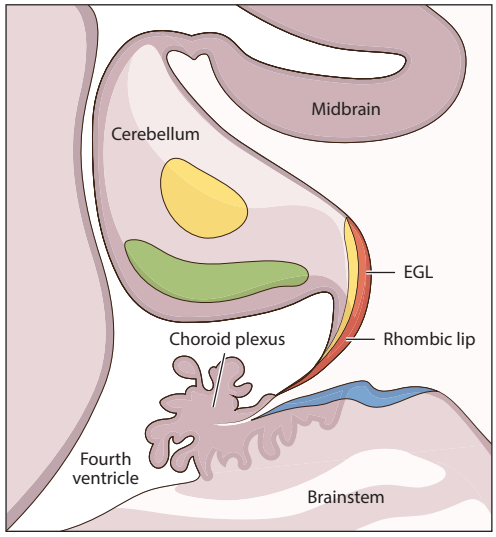
Contents

INTRODUCTION

Medulloblastoma (MB) is the most common malignant brain tumor in children. Despite aggressive therapies, approximately one-third of MB patients die from the disease (Polkinghorn & Tarbell 2007), and those who survive often suffer severe side effects from therapy, including cognitive deficits, endocrine disorders, and secondary tumors. More effective and less toxic therapies are urgently needed to improve outcomes and quality of life for MB patients. In this review, we discuss recent advances in our understanding and treatment of MB.

MOLECULAR CLASSIFICATION OF MEDULLOBLASTOMA

Historically, MB has been classified on the basis of histology into three major forms of the disease: classic, nodular/desmoplastic (ND), and large cell/anaplastic (LCA). Of these forms, LCA tumors have been associated with the worst prognosis, and ND tumors have been considered to have more favorable outcomes. With recent advances in genomics, gene expression profiling, and DNA methylation analysis, MB has been divided into four major subgroups—WNT, Sonic Hedgehog (SHH), Group 3, and Group 4—each with distinct molecular and clinical characteristics (Cho et al. 2011, Kool et al. 2008, Northcott et al. 2011, Thompson et al. 2006) (**Figure 1**). The World Health Organization (WHO) has taken these molecular subgroups into account in its new classification of tumors of the central nervous system (Louis et al. 2016). Because these subgroups exhibit different biologies and are likely to have different responses to therapy, it is helpful to consider them separately. The recent development of advanced algorithms for integrative genomics has provided a deeper understanding of the heterogeneity within these subgroups, subdividing the



	WNT	SHH	Group 3	Group 4
Percentage	10%	30%	25%	35%
Age	Children and adults	Mainly infants and adults	Mainly infants and children	Mainly children and adults
SNVs	*CTNNB1, DDX3, SMARCA4, CREBBP, TP53**	*PTCH1*, SUFU*, SMO, TERT, IDH1, TP53*, KMT2D*	*SMARCA4, CTDNEP1, KMT2D, KBTBD4*	*KDM6A, KMT2C*
Somatic copy number alterations	–	*MYCN, GLI2*	*MYC, PVT1, OTX2, GFI1/1b*	*SNCAIP, MYCN, CDK6, GFI1/1b*
Cytogenetics	Monosomy 6	Gain of 3q, 9p, loss of 9q, 10q, 14q, 17p	i17q, loss of 8, 10q, 11, 16p, 17p, gain of 1q, 7, 17q, 18q	i17q, loss of 8p, 11p, X, gain of 7q, 18q
Prognosis	Very good	Intermediate	Poor	Intermediate
Incidence of metastasis	5–10%	10–15%	40–45%	35–40%
Pattern of relapse	Local and distal	Local	Distal	Distal

Figure 1

Characteristics of the four medulloblastoma (MB) subgroups and their potential origins. (*Bottom*) Each MB subgroup has distinct clinical, histologic, genomic, and epigenomic features, as well as gene expression profiles. The WNT and Sonic Hedgehog (SHH) subgroups derive their names from deregulation of the WNT and SHH pathways, respectively. The molecular drivers for Group 3 and Group 4 are less clear. (*Top*) Different subgroups of MB may originate from different regions of the developing cerebellum, with WNT tumors (*blue*) postulated to arise from the lower rhombic lip, and SHH tumors (*red*) believed to arise from granule neuron precursors in the upper rhombic lip and external granule layer (EGL). Group 3 and Group 4 tumors (*yellow* and *green*, respectively) are thought to arise from primitive progenitors, but the identity of these progenitors remains unclear. Genes associated with germline syndromes, Gorlin syndrome (*PTCH1*, *SUFU*) and Li-Fraumeni syndrome (*TP53*), are denoted with an asterisk. Artwork courtesy of Dr. Christian Smith. Abbreviation: SNV, somatic nucleotide variant.

four major subgroups into 7 to 12 subtypes (Cavalli et al. 2017, Northcott et al. 2017, Schwalbe et al. 2017). Although a detailed discussion of these subtypes is beyond the scope of this review, we refer to them as appropriate in the sections below.

WNT Subgroup

The WNT subgroup represents 10% of all MBs. WNT MBs are rarely metastatic and patients in this subgroup have the most favorable prognosis across all the subgroups (Cho et al. 2011, Northcott et al. 2011, Taylor et al. 2012). Most WNT patients survive after standard therapy, which consists of surgical resection, craniospinal radiation, and chemotherapy. WNT MBs are often located in the fourth ventricle near the brainstem, and recent studies suggest that the cell of origin for this tumor is a progenitor in the lower rhombic lip (Gibson et al. 2010). WNT MBs are characterized by activation of the WNT signaling pathway, often caused by activating mutations in exon 3 of the *CTNNB1* gene, which results in stabilization of the beta-catenin protein (Ellison et al. 2005). Germline mutations in the *APC* gene, associated with Turcot syndrome, are also found in a small number of WNT MB patients. Another common mutation in this subgroup is in the *DDX3X* gene, which encodes a putative RNA helicase that regulates chromosome segregation and cell cycle progression (Jones et al. 2012, Pugh et al. 2012, Robinson et al. 2012). Mutations in chromatin modifier genes such as *SMARCA4* and *CREBBP* are also found in WNT MBs, indicating that dysregulation of the epigenome is likely to be involved in the development of this disease (Robinson et al. 2012). In addition to these focal events, loss of one copy of chromosome 6 (monosomy 6) is a common structural alteration found in WNT MBs (Clifford et al. 2006). Recent studies have suggested that the WNT subgroup consists of at least two subtypes, WNT α (70%) and WNT β (30%). Most WNT α patients are children, and almost all these patients exhibit monosomy 6; WNT β patients are predominantly adults without monosomy 6 (Cavalli et al. 2017).

SHH Subgroup

SHH MBs represent approximately 30% of all MB cases and are characterized by aberrant activation of the SHH signaling pathway. Most SHH MBs are located in the cerebellar hemispheres, probably owing to the lateral location of granule neuron precursors (GNPs), which are believed to represent the cells of origin for this type of tumor (Gibson et al. 2010, Perreault et al. 2014, Raybaud et al. 2015). Common alterations in SHH MBs include germline or somatic mutations in components of the SHH pathway, such as *PATCHED1* (*PTCH1*) and *SUPPRESSOR OF FUSED* (*SUFU*), and focal amplifications of *MYCN* and *GLI2* (Jones et al. 2012, Pugh et al. 2012, Robinson et al. 2012). Mutations in the telomerase reverse transcriptase (TERT) promoter are frequently found in adult SHH MBs (Lindsey et al. 2014). *TP53* mutations, frequently germline mutations associated with Li-Fraumeni syndrome, are found in approximately 30% of childhood SHH MBs and are associated with extremely poor outcomes (Louis et al. 2016, Ramaswamy et al. 2016b, Zhukova et al. 2013). Mutations in the isocitrate dehydrogenase 1 (*IDH1*) gene, often seen in gliomas, have also been found in some SHH MB patients, and these lead to a pattern of DNA hypermethylation similar to that found in other *IDH1/2* mutant cancers (Northcott et al. 2017). Other genetic events found in SHH MB include gains of chromosome 3q and losses of 9q, 10q, and 14q. When SHH tumors recur after therapy, recurrence is usually local (near the original tumor) rather than distal, as seen in Group 3 and Group 4 tumors (Ramaswamy et al. 2013).

Recent studies have identified heterogeneity within the SHH subgroup, further dividing it into four subtypes. The SHH α subtype is enriched in *TP53* mutations, as well as *MYCN* and *GLI2* amplification, and is associated with an extremely poor prognosis. SHH β tumors affect mainly

infants and are often metastatic at the time of diagnosis, resulting in poor outcomes. The SHH γ subtype is also found mainly in infants but has a relatively quiet genome and better outcomes. Most MBs with extensive nodularity (MBENs) belong to the SHH γ subtype (Cavalli et al. 2017). The SHH δ subtype is found mainly in adults and frequently contains TERT promoter mutations (Cavalli et al. 2017).

Group 3

Group 3 MBs account for approximately 25% of all MBs and are the most aggressive of the four subgroups. They are characterized by transcriptional signatures resembling photoreceptors and gamma aminobutyric acid–secreting (GABAergic) neurons (Taylor et al. 2012). Group 3 tumors are often located in the fourth ventricle near the brainstem (Raybaud et al. 2015), but nearly 50% of Group 3 MB patients exhibit metastatic dissemination at diagnosis. Although the true cell of origin for Group 3 MB is unknown, several studies have suggested that Prominin1-positive, lineage-negative cerebellar stem cells and GNPs can be transformed into tumors that resemble human Group 3 MB (Kawauchi et al. 2012, Pei et al. 2012). In contrast to WNT and SHH tumors, Group 3 tumors contain few recurrent somatic nucleotide variants and germline mutations (Jones et al. 2012, Northcott et al. 2012, Pugh et al. 2012, Robinson et al. 2012). The most common genetic alteration is amplification of the *MYC* oncogene, found in approximately 20% of Group 3 MB patients. In *MYC*-amplified tumors, amplicons often include PVT1 (plasmacytoma variant translocation 1), a long noncoding RNA thought to stabilize the MYC protein (Northcott et al. 2012, Tseng et al. 2014). Previous studies have also demonstrated that a subset of Group 3 (as well as Group 4) tumors exhibits overexpression of growth factor independent 1 (GFI1) family transcription factors as a result of structural changes in DNA (e.g., duplications, deletions, inversions, translocations) that bring the genes encoding these factors close to superenhancers (a phenomenon called enhancer hijacking) (Northcott et al. 2014). Pathway analysis has indicated that the Notch and transforming growth factor beta (TGF-β) signaling pathways are also activated in Group 3 MB, although the functional significance of these pathways for tumorigenesis remains to be determined (Kool et al. 2008; Northcott et al. 2012, 2017). Recurrent in-frame insertions in the kelch repeat and BTB domain-containing protein 4 (*KBTBD4*) gene were recently identified as hot spot events (Northcott et al. 2017). Finally, Group 3 tumors often have unstable genomes, with multiple chromosomal gains and losses. Among these, one of the most common is coordinate loss of chromosome 17p and gain of chromosome 17q—called isochromosome 17q (i17q). i17q is found in 40% of Group 3 MB patients and is associated with poor outcomes (Shih et al. 2014).

Recent integrative analysis has suggested that there may be three distinct subtypes within Group 3 MB. Group 3α tumors are often found in infants and frequently exhibit metastasis at diagnosis. Group 3β has a high frequency of GFI1 family oncogene activation and orthodenticle homeobox 2 (*OTX2*) amplification. Group 3γ is also associated with a high incidence of metastasis and often exhibits *MYC* amplification; it has the worst prognosis among the three Group 3 subtypes (Cavalli et al. 2017).

Group 4

Group 4 is the most common subgroup, accounting for approximately 35% of all MBs. Group 4 tumors are frequently metastatic at diagnosis and have intermediate outcomes. They are also the least understood form of the disease, due in part to a lack of animal models. Group 4 tumors are characterized by gene expression signatures that resemble glutamatergic neurons (Taylor et al. 2012). Similar to Group 3 tumors, they often invade into the fourth ventricle near the brainstem. The true cell of origin for Group 4 MB remains to be determined, but recent analysis of

superenhancers has suggested that these tumors may originate from eomesodermin (*EOMES*)- and LIM homeobox transcription factor 1 alpha (*LMX1A*)-expressing precursors in the upper rhombic lip (C.Y. Lin et al. 2016). Most Group 4 tumors have i17q, but unlike in Group 3, this lesion does not have prognostic significance in Group 4. Other common alterations in Group 4 include inactivating mutations in the histone lysine demethylase gene *KDM6A*, tandem duplication of the gene encoding synuclein alpha interacting protein (*SNCAIP*, or Synphilin-1), and amplification of the *MYCN* and *CDK6* genes. Recent studies using *cis* expression structural alteration mapping, a method for identifying enhancer-hijacking events, pointed to *PRDM6* as the top-ranked gene affected by enhancer hijacking in Group 4 (Northcott et al. 2017). Interestingly, *PRDM6* is located 600 kb downstream of *SNCAIP* and its expression is markedly elevated in *SNCAIP*-duplicated Group 4 tumors (Northcott et al. 2017).

Similar to Group 3, Group 4 MB also shows intertumoral heterogeneity. Groups 4α and 4γ have focal *CDK6* amplification, chromosome 8p loss, and chromosome 7q gain; however, Group 4α also exhibits *MYCN* amplification, whereas Group 4γ does not. Group 4β is enriched in *SNCAIP* duplication and *PRDM6* overexpression (Cavalli et al. 2017).

ANIMAL MODELS

Animal models are invaluable tools for cancer biology. They can be used to study the mechanisms of tumor initiation and maintenance, to identify oncogenic drivers and therapeutic targets, and to test novel approaches to therapy. The past several years have seen the creation of many models of MB, including genetically engineered mouse (GEM) and patient-derived xenograft (PDX) models. Each of these models has advantages and limitations, but each has helped inform our understanding of the disease. The most widely studied models are discussed below.

Transgenic and Knockout Mice

One of the first GEM models of MB was the Ptch-LacZ or *Ptch1*$^{+/-}$ mouse (Goodrich et al. 1997). *Ptch1* encodes the transmembrane protein Patched-1, which suppresses the function of Smoothened (Smo), a key activator of SHH signaling. When SHH ligand binds to Ptch1, Smo is released from inhibition, allowing Gli family transcription factors to enter the nucleus and activate target genes (Briscoe & Thérond 2013). In GNPs, these target genes promote proliferation (Wallace 1999, Wechsler-Reya & Scott 1999). Importantly, loss-of-function mutations in *Ptch1* mimic the effects of SHH ligand, activating the pathway. Although homozygous *Ptch1* knockouts die in utero, *Ptch1* heterozygotes exhibit hyperproliferation of GNPs and a subset of these mice develop cerebellar tumors that resemble human SHH MB. To identify the cells of origin of SHH MB, researchers developed conditional *Ptch1* knockout (*Ptch1*$^{flox/flox}$) mice. When Ptch1 was deleted in GNPs (with *Atoh1-Cre; Ptch1*$^{flox/flox}$ mice), aggressive MBs formed with 100% penetrance (Schüller et al. 2008, Yang et al. 2008). Tumors also developed when Ptch1 was deleted in multipotent stem cells (with *hGFAP-Cre; Ptch1*$^{flox/flox}$ mice); however, these tumors arose only once stem cells had committed to the granule neuron lineage, suggesting that neuronal lineage commitment is a prerequisite for the development of SHH-driven MB. When *Atoh1-Cre* or *hGFAP-Cre* mice were crossed with SmoM2-YFP$^{flox/flox}$ mice, in addition to the cerebellum, tumors also arose from GNPs of the cochlear nuclei of the dorsal brainstem, suggesting that this might represent an alternative cell of origin for SHH MBs (Grammel et al. 2012). Finally, a recent study identified a rare population of Nestin-expressing neuronal progenitors deep in the external granule layer (EGL) of the cerebellum. These cells are particularly susceptible to transformation following inactivation of *Ptch1* (with *Nestin-CreER; Ptch1*$^{flox/flox}$ mice) and might represent an

additional cell of origin for SHH-associated tumors (Li et al. 2013). These studies suggest that several different progenitors may give rise to SHH MB, and provide a variety of animal models that can be used to study the biology and therapeutic responsiveness of this form of the disease.

Although MB is considered a cerebellar tumor, animal models suggest that WNT MBs may originate from progenitors outside the cerebellum, in the lower rhombic lip. *Blbp-Cre; Ctnnb1*$^{lox(ex3)}$ mice, which express a nondegradable form of beta-catenin in brain lipid-binding protein (Blbp)-expressing progenitors, exhibit aberrant accumulations of cells in the lower rhombic lip that persist into adulthood. Although activation of Ctnnb1 alone is not sufficient to transform these cells into tumors, concomitant deletion of the *Trp53* gene (with *Trp53*$^{flox/flox}$ mice) results in MB formation in approximately 15% of mice (Gibson et al. 2010). Introduction of an activating mutation in the phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha (*Pik3ca*) gene further promotes tumor formation: *Blbp-Cre; Ctnnb1*$^{lox(ex3)}$; *Trp53*$^{flox/+}$; *Pik3ca*^{E545K} mice develop MBs with 100% penetrance by 3 months of age. The *Pik3ca*^{E545K} mutation on its own does not cause tumor formation, suggesting that *Pik3ca* mutations promote, rather than initiate, WNT MB (Robinson et al. 2012).

Although Group 3 and Group 4 account for more than half of all MBs, transgenic models of these subgroups have been challenging to develop, partly because of our limited knowledge about the drivers and cells of origin for these subgroups. One model that was thought to resemble Group 4 MB is the GTML mouse, which expresses a MycN transgene under the control of the *Glt1* (glutamate transporter 1) promoter and a tetracycline response element. Glt1 is expressed in the developing hindbrain, and in the presence of doxycycline, MycN is activated and promotes tumor formation in approximately 75% of mice. Tumors in these animals do not express markers of SHH-associated MB, and because a subset of Group 4 MBs exhibit amplification and overexpression of MYCN, it was suggested that GTML tumors might be a model for Group 4 MB (Swartling et al. 2010, 2012). Consistent with this, GTML tumors express elevated levels of KCNA1, a potassium channel described as a marker for human Group 4 MB (Northcott et al. 2011, Swartling et al. 2012). However, recent studies have suggested that the expression profile of GTML tumors more closely resembles that of Group 3 MB (Hill et al. 2015), even though overexpression of MYCN is rare in that subgroup. Additional models of Group 3 and Group 4 MB are clearly needed and are likely to come from a deeper understanding of the cell of origin for these tumors. In this regard, a recent study characterized the enhancer landscape of Group 3 and Group 4 MB and concluded that Group 4 tumors are likely to arise from cells that express the transcription factors LMX1A, EOMES, and LHX2 (C.Y. Lin et al. 2016). Cells with this profile are present early in development in the nuclear transitory zone, which gives rise to the deep cerebellar nuclei. If this is the case, expressing putative Group 4 oncogenes in these cells might result in novel models of Group 4 MB.

Orthotopic Transplantation Models

Although there are no conventional transgenic models of Group 3 MB, several models have been created by orthotopic transplantation of virally infected cells. Pei et al. (2012) isolated Prominin1-positive, lineage-negative cerebellar stem cells, transduced them with a stabilized form of Myc (MycT58A), and transplanted them into the cerebellum of immunodeficient mice. Although cells expressing Myc alone failed to form tumors, coexpression of a dominant-negative form of Trp53 (DNp53) allowed these cells to form tumors that resemble human Group 3 MB at the molecular and histological levels. Similarly, overexpression of Myc in GNPs from *Trp53*$^{-/-}$ mice, followed by orthotopic transplantation of these cells, resulted in aggressive tumors that resemble Group 3 MB (Kawauchi et al. 2012). These models have proved valuable for understanding the biology of Myc-driven MB and for identifying and testing novel approaches

to therapy. In particular, they have been used as platforms for high-throughput drug screening to discover more effective therapies for Group 3 MB (Morfouace et al. 2014, Pei et al. 2016). These studies are discussed in more detail below.

Notably, focal *TP53* alterations are rarely found in human Group 3 MB at diagnosis. Such mutations are seen at relapse (Hill et al. 2015, Morrissy et al. 2016), suggesting that the models described above may be more useful for studying relapsed or recurrent Group 3 MB. However, this raises the question of whether other genes might cooperate with MYC to drive initiation of Group 3 MB. As noted above, *GFI1* and *GFI1B* are activated by enhancer hijacking in a subset of Group 3 and Group 4 MBs. The functional significance of this activation was tested by infecting cerebellar progenitors with viruses encoding Myc and Gfi1 or Gfi1b, and transplanting these cells into the cerebellum of immunodeficient mice. Similar to cells expressing Myc and DNp53, cells expressing Myc and Gfi1 or Gfi1b gave rise to tumors that resemble human Group 3 MB (Northcott et al. 2014, Vo et al. 2017). Whether the mechanisms of transformation by Myc + DNp53 and Myc + Gfi1/1b are related, or whether these models represent distinct forms of MB, remains to be determined.

Somatic Gene Transfer Models

The avian retrovirus RCAS (replication-competent avian sarcoma-leukosis virus subgroup A long terminal repeat with splice acceptor) has been used to model a variety of cancers in mice (Ahronian & Lewis 2014). Infection with RCAS viruses requires expression of the avian retrovirus receptor TVA (tumor virus A), which is normally not present on mammalian cells. However, transgenic expression of TVA in specific cell types allows these cells to be infected with RCAS viruses encoding complementary DNAs or short hairpin RNAs, making the RCAS-TVA system a powerful tool for modeling human cancer. One of the first uses of the RCAS-TVA system to model brain tumors was in glioma, with the development of glial fibrillary acidic protein (*GFAP*)-*TVA* and *Nestin-TVA* transgenic mice to allow expression of oncogenes in astrocytes and neural progenitor cells, respectively (Holland et al. 1998a,b, 2000). Subsequently, Fults and colleagues (Rao et al. 2003) used a similar approach to model MB: Infection of *Nestin-TVA* mice with RCAS-SHH viruses resulted in MB formation in 9% of recipients. Co-infection with viruses encoding a variety of other genes, including insulin-like growth factor 2 (*Igf2*), *Akt*, *MycN*, *Bcl-2*, or wild-type p53-induced phosphatase 1 (*Wip1*), increased the incidence of MB formation (Browd et al. 2006, Doucette et al. 2012, McCall et al. 2007, Rao et al. 2004). The RCAS/Nestin-TVA system has also been used to validate candidate drivers of leptomeningeal dissemination, such as *Eras*, *Lhx1*, *Ccrk*, *Akt*, *Arnt*, and *Gdi2* (Jenkins et al. 2014, Mumert et al. 2012). Although most tumors generated with the RCAS-TVA system resemble SHH MB, a recent study demonstrated that *Nestin-TVA* mice infected with viruses encoding Myc and Trp53 or Myc and Bcl-2 developed tumors resembling Group 3 MB (Jenkins et al. 2016). Thus, this system can be used to model multiple subgroups of MB.

Whereas the RCAS-TVA system allows delivery of genes to specific cell types, in utero electroporation enables delivery to specific anatomic locations. In a study aimed at testing the tumorigenic potential of multiple embryonic cerebellar cells, in utero electroporation was used to deliver a Cre-inducible Myc expression construct along with DNp53 into the cerebellum of several different Cre driver lines (*Blbp-Cre*, *Atoh1-Cre*, *Atoh1-CreER*, *Gad2-Cre*, *Ptf1a-Cre*, and *Prom1-CreER*). Tumors arose from all Cre lines tested and in all cases resembled human Group 3 MB. These results suggest that Group 3 tumors can arise from multiple cell types and, unlike SHH tumors, do not depend on commitment to a specific lineage for transformation (Kawauchi et al. 2017).

Tumors caused by loss of tumor suppressor genes have traditionally been modeled with cell type-specific knockout mice. However, these approaches are time-consuming and expensive and

cannot be done in a high-throughput manner. But with the advent of CRISPR (clustered regularly interspaced short palindromic repeats)/CRISPR-associated protein 9 (Cas9)-based gene editing, it is now possible to quickly and efficiently modify the mammalian genome, making it relatively easy to validate candidate tumor suppressor genes identified in genomic studies. Zuckermann et al. (2015) used this approach to generate SHH MBs by inducing CRISPR/Cas9-mediated *Ptch1* deletion in mice with a $Trp53^{-/-}$ background, consistent with the results seen in GEM models. CRISPR/Cas9-based gene editing technology holds great promise in testing and generating novel mouse models of MB driven by newly identified loss-of-function mutations in human MBs.

Patient-Derived Xenografts

Although GEM models are valuable for understanding many aspects of MB, they cannot recapitulate all the features of the human disease. Tumors generated in mice are often genetically simpler than human tumors, typically involving mutation of a few genes compared with tens or hundreds of genes altered in human cancer. Moreover, murine models are markedly less diverse than human tumors: A GEM model may resemble one form of MB but cannot represent all the different subtypes of the disease. To compensate for these shortcomings, many investigators have turned to orthotopic PDX models. These models are created by transplanting tumor cells obtained from surgical resection directly into the brain of an immunodeficient mouse and passaging them from mouse to mouse without ever growing them in culture. Whereas cell lines passaged in culture often lose many of the features of the tumors from which they were derived (Daniel et al. 2009), tumors implanted into mice and passaged in vivo maintain many of the molecular and cellular properties of the patient's original tumor (Zhao et al. 2012). By using multiple PDX lines, researchers can capture the heterogeneity of the human disease. In recent years, several labs have begun to generate MB PDX lines and have shared them with one another and with other investigators. This has expanded the use of PDX lines, particularly for testing novel approaches to therapy (Bandopadhayay et al. 2014, Cook Sangar et al. 2017, Morfouace et al. 2014, Pei et al. 2016, Tang et al. 2014, Zhao et al. 2012).

For all their advantages, PDX models have some drawbacks. Not all tumors can be successfully established as xenografts: With some exceptions, more aggressive tumors are more likely to take in mice. As a consequence, PDX lines from Group 3 MB are more prevalent than those from other subgroups, and relatively few PDX lines from WNT MB are available. Moreover, because PDX lines can grow only in immunodeficient mice, they may not be suitable for studying interactions between tumors and the microenvironment or for testing immunotherapy. Through adoptive transfer of human hematopoietic progenitors, it is possible to endow immunodeficient mice with human immune systems (Fujiwara 2017), but ensuring that lymphocytes, monocytes, and natural killer cells are fully competent may require treating mice with human cytokines (because human cells may not recognize the murine versions) (Drake et al. 2012). In addition, human leukocyte antigen (HLA) matching of the tumor, hematopoietic cells, and the microenvironment may be critical to allow for proper recognition of tumors by cells of the immune system. Given these challenges, it may be several years before humanized PDX models of MB become available. In the meantime, the use of both GEM and PDX models may be the most effective approach for understanding the biology and therapeutic responsiveness of the disease.

THERAPY: CURRENT AND FUTURE

Patients with MB usually present with headaches, nausea, vomiting, or difficulties with balance or motor coordination (Dorner et al. 2007). Because these symptoms can be associated with a

multitude of diseases, it can take weeks or even months for the disease to be recognized. As a result, tumors are often relatively large by the time they are diagnosed and treatment is initiated. Moreover, approximately 30% of patients exhibit metastasis at the time of diagnosis (Fouladi et al. 1999). Standard therapy for MB includes surgical resection of the tumor followed by craniospinal radiation and several chemotherapy agents. Despite this multipronged approach to therapy, approximately 30% of patients still die from the disease, and survivors suffer from severe long-term side effects, including neurological deficits, endocrine disorders, and secondary cancers. Most MB patients currently receive similar therapy, with the doses of radiation and chemotherapy modified on the basis of whether a tumor is classified as low, average, or high risk. With the recognition of the molecular subgroups of MB, and a deeper understanding of their prognostic implications, future therapies might be tailored to the characteristics of individual patients and more effective and less damaging therapies might be used for each patient.

Surgery

Surgery is an essential component of MB therapy, and the extent to which a tumor is removed has important implications for prognosis. For many years, total resection of the tumor was considered to be ideal (Albright et al. 1996, Zeltzer et al. 1999), but the long-term consequences of this approach have led some surgeons to question its appropriateness: Complete resection of a tumor may damage normal tissue, resulting in impaired speech, cognition, and motor function. Whether the benefits outweigh these costs varies from patient to patient. In 2016, Thompson et al. (2016) reported that when correcting for molecular subgroup, the relative effect size of extent of resection is significantly reduced, and gross total resection (GTR) does not confer any benefit in terms of overall survival compared with near total resection (NTR) (residual tumor <1.5 cm^2) for most MB patients. The only advantage is seen in Group 4 MB, in which GTR or NTR significantly increases progression-free survival, although this is more pronounced in patients with up-front metastatic disease. Although these results must be validated in other cohorts, they clearly suggest that the benefits of total resection must be balanced with the potential losses in long-term survival and quality of life.

Radiotherapy

Regardless of the extent of resection, surgery almost always leaves behind cells that continue to grow and spread and ultimately mediate tumor recurrence. For this reason, standard therapy for older children with MB includes a course of radiation therapy designed to eliminate tumor cells that remain after surgery. Typically, radiation is delivered to the entire neuraxis (i.e., craniospinal irradiation) to eradicate tumor cells at the primary tumor site as well as cells that have disseminated through the leptomeningeal space in the brain and spinal cord.

Radiation is an extremely effective mode of therapy for MB. The use of 36 Gy of craniospinal irradiation alone, without chemotherapy, can confer survival rates of up to 60% (Paterson & Farr 1953, Thomas et al. 2000). However, this is accompanied by severe long-term side effects, including deficits in cognitive function, endocrine disorders, impaired bone development, ototoxicity, gynecological toxicity, cardiac toxicity, pulmonary toxicity, and an increased incidence of secondary cancers (Cox et al. 2015, Fossati et al. 2009). To mitigate this damage, doctors treat children with average-risk disease with lower doses of craniospinal irradiation and adjuvant chemotherapy. Moreover, because the side effects, particularly long-term cognitive damage, are more severe in infants and young children, most protocols do not use radiation on children less than 3–5 years of age. Instead, these children are treated with intensive chemotherapy with or without autologous stem cell support (Rutkowski et al. 2009). The use of more conformal radiation techniques such as

intensity-modulated radiation therapy (Al-Wassia et al. 2015, Edwards et al. 2010, Schroeder et al. 2008) and proton radiotherapy (Brodin et al. 2011, Ho et al. 2017, Mu et al. 2005, St. Clair et al. 2004) can spare some of the systemic late effects and reduce the dose to the cochlea. Efforts to improve efficacy of radiation and reduce side effects through hyperfractionation (administering small doses every few hours instead of larger doses once a day) were ineffective in a randomized study in Europe [International Society for Pediatric Oncology (SIOPE-PNET4)], and the standard of care worldwide remains daily fractions of 1.8 Gy (Câmara-Costa et al. 2015, Lannering et al. 2012).

In addition to infants, another group of MB patients being considered for reduced radiotherapy are patients with WNT tumors. Because the survival rate for these patients is nearly 100% with standard therapy, trials are under way to test whether reducing or eliminating radiotherapy for these patients would still allow them to survive without the severe long-term side effects. For example, in a Phase II trial led by the Children's Oncology Group (COG) (ClinicalTrials.gov identifier NCT02724579), newly diagnosed WNT MB patients receive a reduced dose of craniospinal radiation (18 Gy instead of 24 Gy) with a boost of 54 Gy to the tumor bed. Another study (NCT02212574) goes even further, testing whether surgery and chemotherapy alone, with no radiation at all, is effective for treatment of WNT MB.

Finally, SHH MB patients with *TP53* mutations (e.g., those with Li-Fraumeni syndrome) have an abysmal prognosis, and it would seem counterintuitive to withhold radiation from these patients. However, because *TP53* mutations render cells hypersensitive to DNA damage, which may increase the risk of mutations that lead to secondary cancers, some investigators have proposed reducing or eliminating radiation for these patients. Given the widespread belief that radiotherapy is one of the most effective forms of therapy for MB, this view is controversial. However, if effective targeted therapies could be developed for these patients, physicians might be willing to consider this approach.

Chemotherapy

Chemotherapy is a broad term that includes the use of drugs to kill cancer cells, to sensitize them to other therapies, or to limit the risk of tumor dissemination and recurrence. As noted above, for patients under 3 years old, intensive chemotherapy is often the only treatment that can be used until the child is old enough to tolerate radiation. Until recently, most of the drugs used for MB chemotherapy have been cytotoxic agents, which interfere with DNA synthesis or replication and thereby kill proliferating cells. The drugs most commonly used for MB treatment are the microtubule-modulating agent vincristine, the platinum-based compounds cisplatin and carboplatin, and the alkylating agent cyclophosphamide (Martin et al. 2014, Packer & Vezina 2008). Like radiation, chemotherapy can cause significant side effects, including nausea, fatigue, alopecia, and increased risk of infection, and long-term risks of kidney disease, neurocognitive deficits, and endocrinopathies (Crawford et al. 2007, Martin et al. 2014). Despite the extensive use of these drugs for MB treatment, multiple clinical trials are under way to increase efficacy and decrease toxicity and resistance (**Table 1**).

Chemotherapy regimens differ according to age and risk factors such as histology (desmoplastic versus anaplastic), extent of resection, and the presence or absence of metastatic disease. Children older than 5 years are treated with risk-adapted radiotherapy followed by adjuvant cytotoxic chemotherapy. Children with average-risk disease, specifically those without metastasis or residual disease, are treated with reduced-dose craniospinal irradiation (23.4 Gy) followed by four to nine cycles of cisplatin-based chemotherapy (Gajjar et al. 2006, Lannering et al. 2012, Packer et al. 2006). Although various combinations of cisplatin and alkylator-based therapy have been used for these patients, survival is consistently 80% as long as radiation is initiated within 4–6 weeks of

Table 1 Ongoing clinical trials for the treatment of medulloblastoma in North America in 2017[a]

Treatment	ClinicalTrials.gov identifier (other ID)	Study title	Phase
Surgery	NCT02462629	Study of BLZ-100 in Pediatric Subjects with CNS Tumors	I
Improvement of standard chemotherapy	NCT02681705 (Sanghaixinhua-003)	Radiation Therapy and Combination Chemotherapy for Medulloblastoma	II
	NCT01878617 (SJMB03)	A Clinical and Molecular Risk-Directed Therapy for Newly Diagnosed Medulloblastoma	II
	NCT01542736 (06–1151)	Concurrent Carboplatin and Reduced Dose Craniospinal Radiation for Medulloblastoma and Primitive Neuroectodermal Tumor (PNET)	II
	NCT00392327 (ACNS0332)	Chemotherapy and Radiation Therapy in Treating Young Patients with Newly Diagnosed, Previously Untreated, High-Risk Medulloblastoma	III
	NCT01356290 (MEMMAT)	Metronomic and Targeted Anti-Angiogenesis Therapy for Children with Recurrent/Progressive Medulloblastoma (MEMMAT)	II
	NCT01331135 (Aflac ST0901)	Aflac ST0901 CHOANOME—Sirolimus in Solid Tumors	I
	NCT02875314 (HeadStart4)	HeadStart4: Newly Diagnosed Children (<10 years/o) with Medulloblastoma and Other CNS Embryonal Tumors	IV
	NCT02025881 (HR MB-5)	Study of Sequential High-Dose Chemotherapy in Children with High-Risk Medulloblastoma	I, II
	NCT01614132 (NOA-07)	Multicenter Pilot-Study for the Therapy of Medulloblastoma of Adults	I, II
	NCT02212574	Study Assessing the Feasibility of a Surgery and Chemotherapy-Only in Children with Wnt Positive Medulloblastoma	NL
Targeted chemotherapy	NCT00867178 (NCI-2012–03167)	Vorinostat Combined with Isotretinoin and Chemotherapy in Treating Younger Patients with Embryonal Tumors of the Central Nervous System	NL
	NCT01708174	A Phase II Study of Oral LDE225 in Patients with Hedgehog (Hh)-Pathway Activated Relapsed Medulloblastoma (MB)	II
	NCT01878617	A Clinical and Molecular Risk-Directed Therapy for Newly Diagnosed Medulloblastoma	II
	NCT02748135 (ONC-403–001)	A Two-Part Study of TB-403 in Pediatric Subjects with Relapsed or Refractory Medulloblastoma	I
	NCT01601184 (MEVITEM)	Study of Vismodegib in Combination with Temozolomide Versus Temozolomide Alone in Patients with Medulloblastomas with an Activation of the Sonic Hedgehog Pathway	II
	NCT02095132 (NCI-2014–00547)	WEE1 Inhibitor MK-1775 and Irinotecan Hydrochloride in Treating Younger Patients with Relapsed or Refractory Solid Tumors	II
	NCT03213678 (NCI-2017–01249)	Pediatric MATCH: PI3K/mTOR Inhibitor LY3023414 in Treating Patients with Relapsed or Refractory Advanced Solid Tumors, Non-Hodgkin Lymphoma, or Histiocytic Disorders with TSC or PI3K/MTOR Mutations	II

(*Continued*)

Table 1 (*Continued*)

Treatment	ClinicalTrials.gov identifier (other ID)	Study title	Phase
	NCT02255461 (PBTC-042)	Palbociclib Isethionate in Treating Younger Patients with Recurrent, Progressive, or Refractory Central Nervous System Tumors	I
	NCT01857453 (RSMA2010)	Interest of a Dose Decrease for Radiotherapy Associated with Chemotherapy for Treatment of Standard Risk Adult Medulloblastoma	II
Radiotherapy	NCT02724579	Reduced Craniospinal Radiation Therapy and Chemotherapy in Treating Younger Patients with Newly Diagnosed WNT-Driven Medulloblastoma	II
	NCT01063114 (09–361)	Proton Beam Radiotherapy for Medulloblastoma and Pineoblastoma	NL
	NCT00105560 (CDR0000415841)	Proton Beam Radiation Therapy in Treating Young Patients Who Have Undergone Biopsy or Surgery for Medulloblastoma or Pineoblastoma	II
	NCT01682746 (163588–1)	Photodynamic Therapy (PDT) for Recurrent Pediatric Brain Tumors	I
	NCT00058370 (MSKCC-02088)	Intrathecal Radioimmunotherapy, Radiation Therapy, and Chemotherapy after Surgery in Treating Patients with Medulloblastoma	NL
Immunotherapy	NCT02502708 (NLG2105)	Study of the IDO Pathway Inhibitor, Indoximod, and Temozolomide for Pediatric Patients with Progressive Primary Malignant Brain Tumors	I
	NCT02962167	Modified Measles Virus (MV-NIS) for Children and Young Adults with Recurrent Medulloblastoma or Recurrent ATRT	I
	NCT01326104 (Re-MATCH)	Vaccine Immunotherapy for Recurrent Medulloblastoma and Primitive Neuroectodermal Tumor	I
	NCT02100891 (STIR)	Phase 2 STIR Trial: Haploidentical Transplant and Donor Natural Killer Cells for Solid Tumors	II
	NCT02271711	Fourth Ventricle Infusions of Autologous Ex Vivo Expanded NK Cells in Children with Recurrent Posterior Fossa Tumors	I

Abbreviations: ATRT, atypical teratoid rhabdoid tumor; CNS, central nervous system; IDO, indoleamine 2,3-dioxygenase; MB, medulloblastoma; mTOR, mammalian target of rapamycin; NK, natural killer; NL, not listed; PI3K, phosphatidylinositol 3-kinase; TSC, tuberous sclerosis complex.
[a]Information based on **https://clinicaltrials.gov/**.

surgical resection. Children with high-risk disease are treated with higher doses of craniospinal irradiation (36–39 Gy) followed by cisplatin-based chemotherapy. Various approaches, including hyperfractionated radiotherapy, high doses of carboplatin and thiotepa with autologous stem cell transplant, and radiosensitizers such as etoposide and carboplatin, have been used in high-risk patients, and the five-year survival rate for this group using cyclophosphamide-based regimens is consistently 55–65% (Esbenshade et al. 2016, Gajjar et al. 2006, Gandola et al. 2008, Jakacki et al. 2012).

The consideration of molecular subgroups provides significant insight into the efficacy of conventional chemotherapy. Among non-metastatic MB patients, high-risk patients such as those with *TP53*-mutant SHH tumors and *MYC*-amplified Group 3 tumors account for most treatment

failures, even in historical cohorts in whom 36 Gy of radiation is administered (Ramaswamy et al. 2016b). Among standard-risk patients, those with WNT and Group 4 MB (particularly those harboring whole chromosome 11 loss) have excellent outcomes. Among metastatic cases, Group 3 MB patients have the worst outcomes with current therapy, whereas metastatic Group 4 MB patients have survival rates approaching 70%. Interestingly, infants with Group 3 and Group 4 tumors have dramatically worse outcomes than older children do, with Group 4 tumors tending to recur locally with chemotherapy-only approaches, suggesting that these groups are more radiation sensitive (Lafay-Cousin et al. 2016; Ramaswamy et al. 2013, 2016a).

As noted above, infants are currently treated with chemotherapy alone, historically with the goal of deferring radiation but more recently with curative intent (Duffner et al. 1993, Rutkowski et al. 2010). One approach to increasing the efficacy of chemotherapy is to use it more aggressively for shorter periods of time. Since 1990, Finlay and colleagues (Altshuler et al. 2016, Dhall et al. 2008) have led a series of Head Start trials that involve treating children with high-dose chemotherapy without radiation. The initial trials (Head Start I and II) showed that craniospinal radiation could be avoided in 50% of patients under the age of 6, but this therapy still resulted in acute toxicity. Since then, several studies, including CCG99703, ACNS0334, and Head Start III, showed that rescuing patients with autologous hematopoietic stem cells could decrease the toxicity associated with high-dose chemotherapy and significantly increase event-free and overall survival (Altshuler et al. 2016). Whereas Head Start I, II, and III were conducted on patients under the age of 10 with nonmetastatic MB, a new trial, Head Start IV, aims to test the efficacy of this strategy to treat high-risk MB. Another approach has been to add intraventricular methotrexate to intensive induction chemotherapy. Indeed, these trials have shown that intensive chemotherapy without radiation can be effective for some MB patients. Limited follow-up studies have shown that radiotherapy-sparing approaches result in improved neuropsychological, social-emotional, and behavioral functioning, and further cognitive data are urgently warranted as part of these studies (Lafay-Cousin et al. 2016, Rutkowski et al. 2005). Radiation-sparing regimens have been particularly successful in infants with desmoplastic histology, with survival rates as high as 90%. However, in infants with nondesmoplastic tumors, radiation-free survival remains at a dismal 20–40%.

The prognostic value of desmoplastic histology is a function of underlying biology. Infant SHH-activated tumors are highly enriched for desmoplastic histology and have a superior survival as a group, whereas those with Group 3 are composed of nondesmoplastic histology and have a poor survival even with radiation-sparing approaches (Grill et al. 2005, Lafay-Cousin et al. 2016, Rutkowski et al. 2005). This is likely secondary to infant SHH tumors having more favorable biology and the absence of *TP53* mutations, particularly those of the SHH γ subtype (Cavalli et al. 2017). Currently, both SIOPE and COG are planning trials to biologically risk-stratify infants and to intensify therapy for high-risk non-WNT/non-SHH patients.

Metronomic chemotherapy, an alternative approach that has recently gained attention, is the frequent administration of chemotherapy at doses below the maximum tolerated dose and with no prolonged drug-free break. The underlying idea is that more frequent administration of a low-dose standard chemotherapy agent would be more effective with less toxicity and a rare chance of developing acquired drug resistance, as has been shown in a variety of studies of pediatric brain tumors (Andre et al. 2011, Choi et al. 2008, Peyrl et al. 2012). In one study (Andre et al. 2011), investigators tested daily or twice-daily combinations of five drugs for 3 weeks or more; the doses used were 10 times less than the normal doses used in the clinic. Another trial (Peyrl et al. 2012) tested the combination of cyclophosphamide and etoposide with antiangiogenic drugs on patients with recurrent MB. Initial results of these trials in terms of treatment efficacy and tolerability have been favorable, with no unacceptable toxicities due to therapy (Choi et al. 2008),

and are being evaluated prospectively in combination with intrathecal therapies in the ongoing MEMMAT study (NCT01356290).

Molecular Targeted Therapy

As we gain a deeper understanding of the molecular basis of MB, we will be able to identify drivers of the disease and then deliver therapies that target these drivers without killing normal cells. Because different forms of MB are likely to have different drivers, distinct targeted therapies may be needed for each MB subgroup. Indeed, there are already some targeted agents in clinical trials, and in many cases, these are likely to work on only a subset of MB patients. To date, targeted therapies for SHH and Group 3 tumors have been identified.

Therapies for SHH MB. The identification of mutations in *PTCH1* and *SMO* as drivers of SHH MB led investigators to test SMO inhibitors initially in preclinical studies (Berman et al. 2002, Kool et al. 2014, Romer et al. 2004) and more recently in clinical trials (Gajjar et al. 2013, Lee et al. 2012, Robinson et al. 2015). However, SMO inhibitors (GDC-0449/vismodegib, LDE225/erismodegib, IPI-926/saridegib) are effective only on the subset of SHH MBs that harbors mutations upstream of SMO (Kool et al. 2014). Vismodegib was tested in a Phase II trial on recurrent or refractory MB and was effective in 41% of SHH MBs but not in other subgroups of MB (Robinson et al. 2015). Clinical trials for erismodegib in the treatment of MB are still under way. As with many targeted agents, prolonged exposure leads to resistance and relapse. Resistance has been attributed to three mechanisms (Buonamici et al. 2010, Dijkgraaf et al. 2011). In some cases, mutations in *SMO* cause conformational changes that prevent the first-generation inhibitors from binding. Notably, a new SMO inhibitor, MK-4101, is structurally distinct from most other SMO inhibitors and has shown efficacy even in vismodegib-resistant SHH MB (Filocamo et al. 2016). A second mechanism of resistance to SMO inhibitors involves alterations that activate the SHH pathway downstream of SMO; these alterations (such as amplification of *GLI* genes) circumvent the effects of SMO inhibitors and render them ineffective. Finally, alterations in other pathways, such as the phosphatidylinositol 3-kinase (PI3K) pathway, can allow tumor cells to grow in a manner that is independent of SHH signaling. Importantly, concomitant treatment with SMO inhibitors and PI3K inhibitors can delay the onset of resistance that is seen with SMO inhibitors alone (Buonamici et al. 2010), suggesting that combination therapies such as these might increase the utility of SMO inhibitors in the clinic.

GLI transcription factors are the terminal effectors of the SHH-SMO signaling pathway. For SHH MBs that are driven by downstream mutations (e.g., *SUFU* or *GLI2*), or that acquire such mutations after therapy with SMO inhibitors, antagonists of GLI might be effective drugs. Agents that target GLI proteins include the GLI antagonist (GANT) and arsenic trioxide (ATO). GANT molecules were found in a screen on HEK293 cells expressing a GLI-dependent luciferase reporter. GANT61 and GANT58 exhibit specificity for SHH signaling and can induce regression in human prostate tumor xenografts (Lauth et al. 2007). More recently, GANT61 tested on MB cell lines enhances apoptosis on its own and in combination with cisplatin (Z. Lin et al. 2016). ATO inhibits the activity of GLI proteins as well as decreases their expression and stability. In vivo, daily treatment with ATO can inhibit the growth of murine SHH-driven MB (Kim et al. 2010). Phase I clinical trials with brain tumor patients have demonstrated the safety of ATO, and Phase II trials are under way (Cohen et al. 2013). Another strategy for targeting GLI proteins are bromodomain and extraterminal (BET) protein inhibitors, which in addition to targeting MYC (see below) modulate GLI expression (Tang et al. 2014). Combining these agents with SMO antagonists could prevent or delay the development of resistance.

In addition to drugs that target the SHH pathway itself, inhibitors of Aurora kinase A (AURKA) and Polo-like kinase (PLK) are effective against SHH MB (Markant et al. 2013, Triscott et al. 2013). In both GEM and PDX models of SHH MB, AURKA and PLK inhibitors can each block tumor growth and synergize with conventional chemotherapy (Markant et al. 2013). Triscott et al. (2013) demonstrated that PLK1 is a marker for poor prognosis in MB and that PLK inhibitors can impede in vitro proliferation and self-renewal, and in vivo growth of SHH-activated MBs with an efficacy similar to that seen with standard chemotherapy (Triscott et al. 2013). These studies suggest that SHH-associated MBs may be susceptible to multiple classes of targeted agents.

Therapies for Group 3 MB. Group 3 MB is characterized by overexpression or amplification of the *MYC* oncogene. *MYC* expression can be regulated by epigenetic factors such as the BET protein BRD4, which binds acetylated lysines and allows the recruitment of transcriptional regulators (Henssen et al. 2013). Treatment of MB cells with BET inhibitors such as JQ1 inhibits *MYC* expression and decreases viability of Group 3 MB cells (Bandopadhayay et al. 2014). Although not all clinical bromodomain inhibitors cross the blood-brain barrier (BBB) (Pastori et al. 2014), brain-penetrant bromodomain inhibitors may have significant benefits for patients with Group 3 MB.

Beyond MYC, several studies have demonstrated other vulnerabilities of Group 3 MB cells. In 2014, Morfouace et al. (2014) screened libraries of FDA-approved drugs on GEM and PDX models of Group 3 MB. They found that MYC-overexpressing tumor cells are sensitive to folate pathway inhibitors. They combined one of these, pemetrexed, with the DNA/RNA synthesis inhibitor gemcitabine and demonstrated that the combination decreased tumor growth and increased survival of MB tumor–bearing mice. These two drugs are already used as chemotherapy agents for other types of cancer and, on the basis of these studies, are now in clinical trials for the treatment of MB (NCT01878617).

A high-throughput screen carried out by our group (Pei et al. 2016) identified histone deacetylase (HDAC) inhibitors as potent therapeutic agents for Group 3 MB. HDAC inhibitors act in part by increasing expression of the tumor suppressor gene *FOXO1*. Notably, FOXO1 nuclear localization is increased by PI3K inhibitors, which impede the growth of Group 3 tumors (Pei et al. 2012). Importantly, HDAC inhibitors synergize with PI3K inhibitors to inhibit tumor growth and prolong survival of Group 3 MB–bearing mice. Because many HDAC and PI3K inhibitors are in trials for other cancers, the efficacy of these drugs—or of the dual HDAC/PI3K inhibitor CUDC-907—may be worth testing in the clinic (Younes et al. 2016, Sun K et al. 2017).

In addition to their effects on SHH MB, Aurora kinase inhibitors have been suggested to be valuable for Group 3 MB. Tumor cells expressing MYC are sensitive to mitosis inhibition (Yang et al. 2010), and Aurora kinase B expression is regulated by MYC (den Hollander et al. 2010). Thus, MYC-expressing MB cells are extremely sensitive to Aurora kinase B inhibitors (Diaz et al. 2015). AURKA is also a key regulator of mitosis but in addition functions as a scaffold that binds to MYC proteins (MYC and MYCN) and stabilizes them (Brockmann et al. 2013). Some inhibitors of AURKA, such as MLN8237/alisertib, alter its conformation and decrease its affinity for MYC, enhancing its degradation (Richards et al. 2016). Indeed, Hill et al. (2015) have shown that alisertib can inhibit the growth of MycN-driven GTML tumors. Building on these observations, Gustafson et al. (2014) developed a novel AURKA inhibitor, CD532, that is even more effective at disrupting interactions with MYC. Compounds such as these are likely to become important tools for therapy of MYC-driven MB.

A novel computational approach called DiSCoVER (disease-model signature versus compound-variety enriched response) identifies new therapeutic targets on the basis of gene expression profiles (Hanaford et al. 2016). Using this method, the investigators predicted that the

CDK4/6 inhibitor palbociclib would be effective against Group 3 MB. They showed that this agent could inhibit growth of a model of Group 3 MB generated by transducing neural stem cells with *MYC*, dominant-negative *TP53*, constitutively active *AKT*, and human telomerase reverse transcriptase (*hTERT*). Strikingly, their findings were validated in separate studies by Cook Sangar et al. (2017) that showed dramatic inhibition of tumor growth in PDXs derived from Group 3 MB. Thus, targeting CDK4/6 might be a valuable approach to therapy for Group 3 (and perhaps CDK6-amplified Group 4) MB.

CHALLENGES FOR THE FUTURE

Personalizing Therapy for Medulloblastoma

MB is a heterogeneous disease, and the current strategy of treating all patients with standard chemotherapy is clearly outmoded. Although there may be treatments that work on multiple forms of MB, the diversity of genetic and epigenetic events even within a particular subgroup (Cavalli et al. 2017, Northcott et al. 2017, Schwalbe et al. 2017) makes it more likely that each patient will be responsive to distinct therapies and combinations thereof. Identifying appropriate therapies for each patient may require detailed molecular and cellular analysis of tumor tissues. Analysis of DNA—whole genome sequencing, whole exome sequencing, or targeted sequencing of known oncogenes and tumor suppressors—is an important component of this analysis, and several studies have shown that this approach is feasible for pediatric cancers (Janeway 2017, Ramkissoon et al. 2017, Seibel et al. 2017). Indeed, the COG and the National Cancer Institute have initiated a pediatric version of their Molecular Analysis for Therapeutic Choice (MATCH) study that directs patients with particular mutations into treatment groups with appropriate targeted therapies (Allen et al. 2017). For example, patients whose tumors exhibit phosphatase and tensin homolog (*PTEN*) loss or mammalian target of rapamycin (*mTOR*) or *PIK3CA*-activating mutations receive PI3K or mTOR inhibitors, those with *BRAF V600E* mutations receive BRAF inhibitors, and those who have fibroblast growth factor receptor (*FGFR*)-activating mutations or fusions receive FGFR inhibitors.

Although prioritizing therapies on the basis of mutations, amplifications, and deletions certainly has value, many pediatric cancers lack such alterations. In fact, recent studies suggest that DNA sequencing identifies actionable genetic events in only 9% of pediatric cancer patients (Allen et al. 2017). In the context of MB, Group 3 and Group 4 exhibit relatively few somatic mutations (Jones et al. 2012, Northcott et al. 2012, Pugh et al. 2012). Thus, assessment of parameters beyond DNA may be warranted. As noted above, gene expression profiles can be used to identify candidate therapies that can treat cancer; in addition to the DiSCoVER approach described above, Califano and colleagues (Alvarez et al. 2016) have developed a method called virtual inference of protein activity by enriched regulon analysis (VIPER), which infers protein activity from gene expression data and then uses the inferences to predict drug responses. In vitro assays showed that VIPER-inferred protein activity outperformed mutational analysis in predicting sensitivity to targeted inhibitors (Alvarez et al. 2016). DNA methylation analysis can also point to possible therapies. For example, tumors that exhibit a CpG island methylator phenotype may be responsive to drugs that inhibit DNA methylation (Mack et al. 2014). Finally, directly screening drugs on patients' tumor cells—or on low-passage cell lines or PDXs derived from them—may also inform the prioritization of therapies that are effective for a particular patient (Pauli et al. 2017). Ultimately, a combination of these approaches may be necessary to give clinicians the information they need to choose most effective treatment plans for patients, particularly those with high-risk or recurrent disease.

Breaching the Blood-Brain Barrier

As our understanding of MB biology increases, new targets will be identified and new drugs will become available for therapy. But many therapeutic agents that can inhibit the growth of tumor cells in culture are not effective for treating brain tumors in patients. One major reason for this is the inability of most small molecules to cross the BBB. Although most brain tumors exhibit some disruption of the BBB (as evidenced by contrast enhancement on MRI imaging), this disruption is often localized to particular regions of the tumor (Hong et al. 2016, Phoenix et al. 2016). Tumor cells that are distant from these disruptions, and especially cells that have infiltrated into normal tissue, are likely to be behind an intact BBB and therefore inaccessible to poorly brain-penetrant drugs. The BBB is a major challenge for neuro-oncology, and circumventing it is the subject of extensive research. Strategies for increasing the penetration of drugs into tumors include modifying the physicochemical properties of existing drugs so that they are more brain penetrant (Pajouhesh & Lenz 2005); using physical, genetic, or pharmacological approaches to transiently open the BBB so that drugs can get to the brain (Campbell et al. 2008, Cosolo et al. 1989, Etame et al. 2012, Kim & Bynoe 2015, Parrish et al. 2015); using convection-enhanced delivery to introduce drugs directly into the tumor (Debinski & Tatter 2009); and linking drugs to carriers or transporters that can deliver the drugs across the BBB (Georgieva et al. 2014, Oller-Salvia et al. 2016, Pardridge 2002, Vieira & Gamarra 2016, Wohlfart et al. 2012). Each of these approaches holds promise for enhancing the delivery of drugs to MB, and it will be exciting to see how they develop and become integrated into the clinic.

Integrating Therapeutic Modalities

Beyond pharmacological agents, several new therapeutic modalities have emerged as candidates for treating brain tumors. Immunotherapies, including immune checkpoint inhibitors (anti-PD-1, anti-PD-L1, IDO), dendritic cell vaccines (NCT01326104), adoptive T cell therapies, activators of macrophage-induced phagocytosis (e.g., anti-CD47) (Gholamin et al. 2017), and natural killer cell–based therapies (NCT02100891, NCT02271711) (Pérez-Martínez et al. 2016), are being evaluated for their efficacy in treating pediatric brain tumors. In addition, oncolytic viruses, which have been studied for many years, have begun to make their way into clinical trials. Therapies based on measles virus (Studebaker et al. 2012, Studebaker et al. 2010) are already in clinical trials for the treatment of MB (Clinical Trial: NCT02962167), and therapies involving modified poliovirus (e.g., PVSRIPO), herpesviruses (e.g., G207) (Brown & Gromeier 2015, Brown et al. 2017, Friedman et al. 2016, Studebaker et al. 2017), and murine leukemia virus–based retroviruses (Toca-511) (Cloughesy et al. 2016, Perez et al. 2012) are all in trials for other brain tumors. These viruses were originally designed to kill tumor cells directly, but their efficacy appears to depend on their ability to elicit an immune response (Alvarez-Breckenridge et al. 2015, Dunn-Pirio & Vlahovic 2017, Hardcastle et al. 2017, Saha et al. 2017). Learning which tumors are most responsive to these therapies, when to administer them, and how best to combine them with standard therapies will be critical for improving patient care in the future.

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