

## MINI-SYMPOSIUM

**Modeling pediatric medulloblastoma**

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Group 3, Group 4, medulloblastoma, mouse models, MYC, MYCN, patient-derived xenografts, Sonic hedgehog, WNT.

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**Abstract**

Mouse models of medulloblastoma have proven to be instrumental in understanding disease mechanisms, particularly the role of epigenetic and molecular drivers, and establishing appropriate preclinical pipelines. To date, our research community has developed murine models for all four groups of medulloblastoma, each of which will be critical for the identification and development of new therapeutic approaches. Approaches to modeling medulloblastoma range from genetic engineering with CRISPR/Cas9 or *in utero* electroporation, to orthotopic and patient-derived orthotopic xenograft systems. Each approach or model presents unique advantages that have ultimately contributed to an appreciation of medulloblastoma heterogeneity and the clinical obstacles that exist for this patient population.

**INTRODUCTION**

Medulloblastoma (MB) is the most common pediatric malignant brain tumor and occurs mainly in children aged between 3 and 9 years (48). Medulloblastoma is characterized by four major molecular groups, with different pathologies, outcomes and genetics: two with mutations in developmental pathways, Wingless (WNT) and Sonic Hedgehog (SHH), and two with less well-defined molecular alterations, Group 3 (G3) and Group 4 (G4) (53, 71). Recently, each of the four groups has been further subdivided into subgroups by methylation profiling (14, 52). However, it is not yet clear whether specific subgroups are associated with distinct outcomes.

Since the generation of the first genetically engineered mouse model of Sonic Hedgehog medulloblastoma, in which the Sonic Hedgehog receptor, Patched (Ptch1), was conditionally deleted (26), a large number of mouse models recapitulating each of the four groups has been developed. As medulloblastomas are separated into more subgroups, the development of accurate models recapitulating each of these is warranted if these models are to be used for preclinical studies. Several approaches have been employed over the years, but the discovery of CRISPR-Cas9 enzymes has accelerated the development and the precision with which mouse models are generated. This review will discuss all mouse models developed to date and how they have already been used to inform the development of clinical trials.

**APPROACHES TO MODELING MEDULLOBLASTOMA IN MICE**

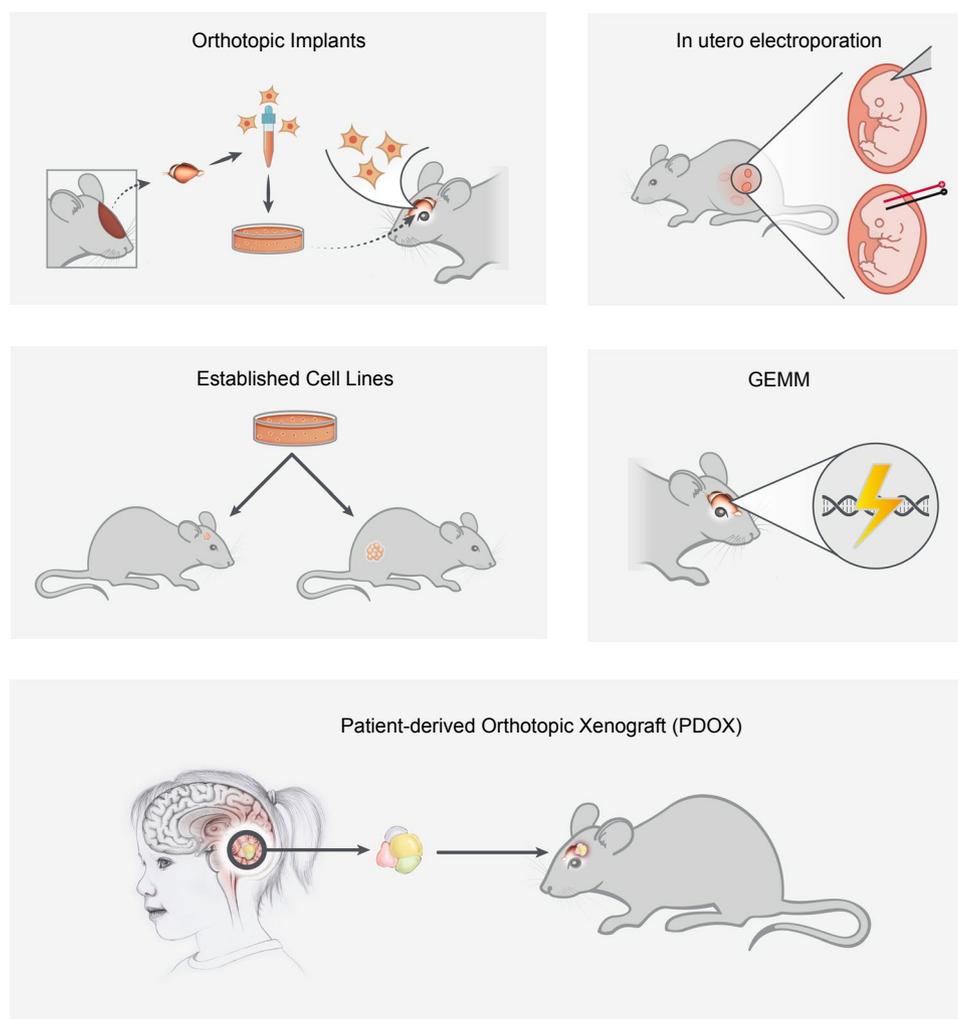
The molecular analysis of human medulloblastoma has been instrumental in the development of mouse models that closely

resemble their human counterparts. Several characteristics must be met for medulloblastoma mouse models to recapitulate human tumors. They must represent disease heterogeneity, recapitulate developmental origins and mimic molecular, epigenetic and genetic landscapes. Several approaches have been used over the years to develop accurate models, many of which fulfill most, if not all, of these requirements. These models have helped investigators better understand the disease.

Approaches to the development of mouse models or genetically engineered mouse models (GEMMs) include genetic engineering, *in utero* electroporation, viral gene transfer by the replication-competent avian sarcoma leukosis virus and its receptor Tva (RCAS-Tva) system and the Sleeping Beauty transposase, orthotopic transplant models using mouse or human cerebellar and neuronal progenitors, and the development of established mouse and human cell lines and patient-derived orthotopic xenografts (PDOXs) (Figure 1). Each of these methods has advantages and disadvantages but all have facilitated the validation of genetic alterations found in human medulloblastoma groups, with the goal of finding new therapies and improving existing ones.

***In vitro* established cell lines**

Several human medulloblastoma cell lines (44) have been established *in vitro* over the years and have been well described (34). Not surprisingly, most were generated using aggressive Group 3 MBs with *MYC* amplification. Although these established human tumor lines are easy to grow in culture, as monolayers or spheres, and are commonly used to assess the effect of drug treatments in preclinical trials, recent molecular analysis by next generation sequencing (NGS) has revealed that they do not always faithfully mimic



**Figure 1.** Summary of technical methods and approaches to medulloblastoma modeling.

primary tumors and in some cases these lines have acquired additional mutations and/or have lost genetic material. In our own experience, while mouse Group 3 tumors are easily expandable *in vitro* and recapitulate the disease when re-introduced into the cortex or cerebellum from naïve animals (37), we have been unable to generate stable lines from primary patient samples or PDOXs at low passage < 2 (Roussel, personal communication). Mouse or human SHH tumors fail to maintain a SHH signaling pathway signature when grown *in vitro*, and fail to generate tumors when cultured cells are implanted in the brain of mice, as previously reported (67). However, we have successfully grown PDOXs, both SHH tumors with *MYCN* amplification and *TP53* mutations as well as *MYC*-driven Group 3 tumors, transiently for 3–7 days in neural stem cell culture conditions. These transient cultures allow high throughput drug screens and the evaluation of specific compounds for their ability to induce perturbations of cell proliferation, cell death, cell cycle arrest or differentiation. We find that these culture conditions maintain tumor stem-like properties and a tumor's genomic landscape (Roussel, personal communication).

### Genetically engineered mouse models

Some of the most accurate models of medulloblastoma have used conventional knock-out technology to generate genetically engineered mouse models (GEMMs). The Patched 1 model (*Ptch1*<sup>+/-</sup>), the first mouse model of medulloblastoma to be developed, has been extensively used to assess the role of genes that drive tumorigenesis in SHH MBs. This includes mutations in genes of the SHH signaling pathway, from the cell surface to the nucleus, that drive G1 progression and impact DNA repair and apoptotic pathways (Table 1). Realizing that some of the gene deletions were deleterious to embryonic development, many conditional and inducible knock-out models have been developed, enabling precise temporal and spatial gene deletion. This required the development of mouse lines in which Cre recombinase expression is driven in specific cerebellar cell types. In many examples, such transgenic mice express a Cre recombinase fused to the mutated estrogen receptor (ER<sup>TM</sup>) allowing its expression by addition of tamoxifen. When fused to the mutated estrogen receptor, the Cre-ER<sup>TM</sup> fusion protein is sequestered to the cytoplasm, but upon tamoxifen treatment is translocated

**Table 1.** Models of SHH medulloblastoma

SHH GEMM	Reference
Ptch1 <sup>+/-</sup> , LacZ	(26)
Ptch1 <sup>+/-</sup> , Trp53 <sup>-/-</sup>	(76)
Ptch1 <sup>+/-</sup> , Cdkn2c <sup>-/-</sup>	(73)
Ptch1 <sup>+/-</sup> , Math1-Cre	(68, 78)
Ptch1 <sup>+/-</sup> , hGFAP-Cre	(68, 78)
Ptch1 <sup>+/-</sup> , Math1-CreER	(47)
SmoA2	(5, 16)
NeuroD2-SmoA1 W539L	(27, 30)
Sufu <sup>+/-</sup> , Trp53 <sup>-/-</sup>	(42)
Trp53 <sup>-/-</sup> , H3K27M	(40)
Trp53 <sup>-/-</sup> , XRCC4 <sup>-/-</sup>	(77)
Trp53 <sup>-/-</sup> , LigIV <sup>-/-</sup>	(43)
Nestin-Cre <sup>+/-</sup> , Trp53 <sup>-/-</sup> , Brca2 <sup>fl/fl</sup>	(22)
Ptch1 <sup>+/-</sup> , Cdkn1b <sup>-/-</sup>	(7)
Ptch1 <sup>+/-</sup> , Ptch2 <sup>+/-</sup>	(45)
KU80 <sup>-/-</sup> , Trp53 <sup>-/-</sup>	(43)
Parp <sup>-/-</sup> , Trp53 <sup>-/-</sup>	(72)
PTEN floxed × RCAS-Cre + RCAS-Shh + radiation	(28)
Trp53 <sup>-/-</sup> , PTEN <sup>-/-</sup>	(79)
Trp53 <sup>-/-</sup> , RB <sup>-/-</sup>	(49)
Ink4d <sup>-/-</sup> , Kip1 <sup>-/-</sup> , Trp53 <sup>-/-</sup>	(44)
Ink4d <sup>-/-</sup> , Ink4c <sup>-/-</sup> , Trp53 <sup>-/-</sup>	(44)
Ptch1 <sup>+/-</sup> , Hic <sup>+/-</sup>	(12)
RCAS-Shh + RCAS-Mycn in Ntv-a mice	(13)
RCAS-Shh + RCAS-MycnT48A in Ntv-a mice	(13)
RCAS-Shh + RCAS-IGF2 in Ntv-a mice	(60)
RCAS-Shh + RCAS-BCL2 in Ntv-a mice	(50)
RCAS-Shh, RCAS-MYC, RCAS-BCL2 in Ntv-a mice	(35)
RCAS-Shh + RCAS-WIP1 in Ntv-a mice	(18)
RCAS-Shh + RCAS-HGF in Ntv-a mice	(10)
RCAS-Shh + RCAS-MYC	(60)
Math1-Cre; Nfia <sup>+/+</sup> ; Ptch1 <sup>+/-Lox</sup>	(24)
Nestin-Cre/T2-ONC × PTENFlox/Flox × Rosa26LsLSB11/+	(9)
<i>SHH Orthotopic</i>	
Trp53 <sup>-/-</sup> , MYCN	(31, 37)
Cdk6	(59)
Atoh1 + Gli1	(6)
Trp53 <sup>-/-</sup> , Cdn2c <sup>-/-</sup> , MYCN	(37)
MYCN in human iPSC-derived NES	(33)
<i>SHH In utero electroporation</i>	
CRISPR-Cas9 Ptch1 deletion	(64)

to the nucleus enabling the expression of the Cre recombinase (20, 39). Cre technology permits the deletion of genes at specific times and in specific cell types in the mouse brain during embryonic development and also in adult mice. A number of Cre and Cre-ER<sup>TM</sup> lines have been developed over the years and are now available to investigators. These lines have been well characterized and successfully used to study the role of gene deletion upon Cre excision in specific cerebellar cell types (Table 2).

GEMMs of medulloblastoma have been developed by somatic gene transfer using polyethylenimine (PEI)-mediated transfection (2) and *in utero* electroporation (65) of plasmids carrying the genes of interest in the fourth ventricle of embryos (E) at 13.5 days post coitus. The advantage of these approaches is that they do not suffer the limitations imposed by the size of the retroviral and lentiviral vectors which cannot be much bigger than 10–15 kilobases and

**Table 2.** Models of medulloblastoma

WNT GEMM	Reference
Blbp-Cre, Ctnnb1 <sup>+fl</sup> (ex3), Trp53 <sup>+fl</sup>	(25)
Blbp-Cre, Ctnnb1 <sup>+fl</sup> (ex3), Trp53 <sup>+fl</sup> , PI3CAOE	(25)
Blbp-Cre, DDX3X <sup>fl/fl</sup>	Gilbertson, personal communication
<i>G3 GEMM</i>	
Gt1-tTA: TRE-MYCN-Trp53 <sup>-/-</sup> (GTML)	(70)
Nestin-Cre-MLL4	(17)
GMYC MYC GLT1 Tet-OFF System	Swartling, personal communication
GTML Trp53KI/KI p53ER, TAM	(31)
RCAS-TVA MYC + BCL2 in Ntv-a mice	(35)
RCAS-TVA MYC + Trp53	(35)
Nestin-Cre/ T2- ONC × Trp53LsIR270H × Rosa26LsLSB11/+	(9)
<i>G3 Orthotopic</i>	
Myc OE, Trp53 <sup>-/-</sup> in GNP	(37)
Myc OE, Trp53DN in GNP and NSCs CD133+. Lin-	(57)
Dox inducible MYC, DNTrp53	(57)
MycT58A OE, Trp53DN in GNP and NSCs	(57)
Myc OE, GF1 in CD133 + Lin- NSC	(54)
Myc OE, GF1OE in GNP	(75)
Myc T58A OE, GF1Flox in NSCs from CAG-CreERTM mice	(46)
MycT58A OE, GF1WT in NSCs	(46)
CRISPR-dCas9-MYC	(74)
<i>G3 In utero electroporation</i>	
conditional MYC, DNTrp53 in different neuronal progenitors	(36)
<i>G4 In utero electroporation</i>	
constitutively activated Src, Trp53DN	(21)

could provide tissue and cell-type specificity by combination with CRISPR-Cas9 or with specific Cre-mice. For example, somatic CRISPR-Cas deletion of *Ptch1* by *in utero* electroporation of wild type E13.5 mouse embryos (“CRISPR-Ptch1”) induced SHH medulloblastoma with complete penetrance by 16 weeks of age. Tumor onset is accelerated when these experiments are performed in *Trp53*-null embryos. These tumors have the hallmarks of human SHH medulloblastomas (64) including overexpression of *Gli1* and *Sfrp1*.

Several GEMMs of SHH or Group 3 medulloblastoma made use of the RCAS-TVA system (4). This technique relies on the use of an avian retroviral vector, RCAS, to target gene expression to neuronal progenitors in transgenic mice in which the Nestin gene promoter drives expression of the viral receptor, tva (3, 23, 41, 55). Several SHH models were developed by infection with RCAS virus expressing Shh, alone or in combination with genes that include *MYCN*, activated Akt, HGF, WIP1, BCL2 all of which accelerate the onset of SHH medulloblastoma (Table 1). GEM models of Group 3 MB were also generated in which the chicken retrovirus expressed the oncogenes *Myc* and *Bcl2* in addition to RCAS-Shh which when transferred into the brain of mice induces Group 3 medulloblastoma (35) with large cell/anaplastic (LCA) features.

Another approach involves insertional mutagenesis with the transposase Sleeping Beauty that when conditionally

expressed in Nestin-Cre mice together with a *Trp53* mutation (Nestin-Cre/T2-ONC × *Trp53*LsIR270H × *Rosa26*LsSB11/+) or PTEN knock-out (Nestin-Cre/T2-ONC × PTEN Flox/Flox × *Rosa26*LsSB11/+) induces SHH or Group 3/Group 4 medulloblastoma (9).

### Orthotopic transplant of genetically modified precursors

Whereas GEMMs were the only mouse models of medulloblastoma initially developed for the SHH and WNT groups, for which mutations of *Ptch1* and  $\beta$ -catenin had been identified in human tumors, a lack of obvious genetic drivers was initially responsible for the delay in developing GEMMs for the other two MB groups. As a result, we and other investigators initially decided to use purified granule neuronal progenitors (GNPs) (37) or neural stem cells (NSCs/CD133<sup>+</sup>/Prominin<sup>+</sup>, Lin<sup>-</sup>) (56), to assess the role of potential drivers of medulloblastoma, including *Myc* and *Gfi1* (53, 56). This evaluation made use of retroviral or lentiviral vectors that could conditionally express or repress genes of interest to modify mouse neuronal progenitors or human induced pluripotent stem cells (iPSC)-derived neuroepithelial stem cells (NSC) (33). Marked progenitors are then implanted into the cortices or cerebella of naïve immune-compromised NSG or CD1-nude mice or of naïve syngeneic animals, giving rise to tumors consistent with Group 3 based on histopathological and molecular analyses.

### Patient-derived xenograft models

GEMMs of MB provide valuable tools for *in vitro* and *in vivo* testing but fall short of capturing the heterogeneity or microenvironment of human tumors. Patient-derived orthotopic xenograft (PDOX) models address these limitations and have become increasingly prevalent in MB preclinical research. PDOXs are generated by implanting tissue from a patient's tumor into immune-compromised mice. After initial processing of the tumor sample, there are no intermediate *in vitro* steps, eliminating the risk of artifact or genetic drift that can arise during cell culture. Most groups working with MB PDOXs utilize orthotopic models, amplifying tumors intracranially *in vivo*. The presence of stromal environmental components and the heterogeneity of the tumor cell population provide a significant advantage, particularly with regard to preclinical evaluation of small molecules or other interventions (11, 66, 69). Research from St. Jude Children's Research Hospital, the German Cancer Research Center and the Fred Hutchinson Cancer Research Center suggests that the genetic, epigenetic and molecular signatures of MB tumors are maintained from patient sample to PDOX models ((11, 32, 66), Roussel, personal communication). Unlike the artificially homogenous cell lines generated from genetic manipulation of progenitor cells, PDOX models retain intra- and inter-tumoral heterogeneity that facilitates more accurate interrogation of disease mechanism and therapeutic response. Currently, PDOX models exist for each of the major MB groups, some from primary tumor biopsy and others from disease relapse or metastasis. Single cell RNA sequencing of PDOX models continues to provide

insights into spatial and temporal tumor evolution, while analysis of the genetic and epigenetic landscape reveals new insights into tumorigenesis and progression (32).

## MOUSE MODELS OF THE FOUR MAJOR MEDULLOBLASTOMA GROUPS

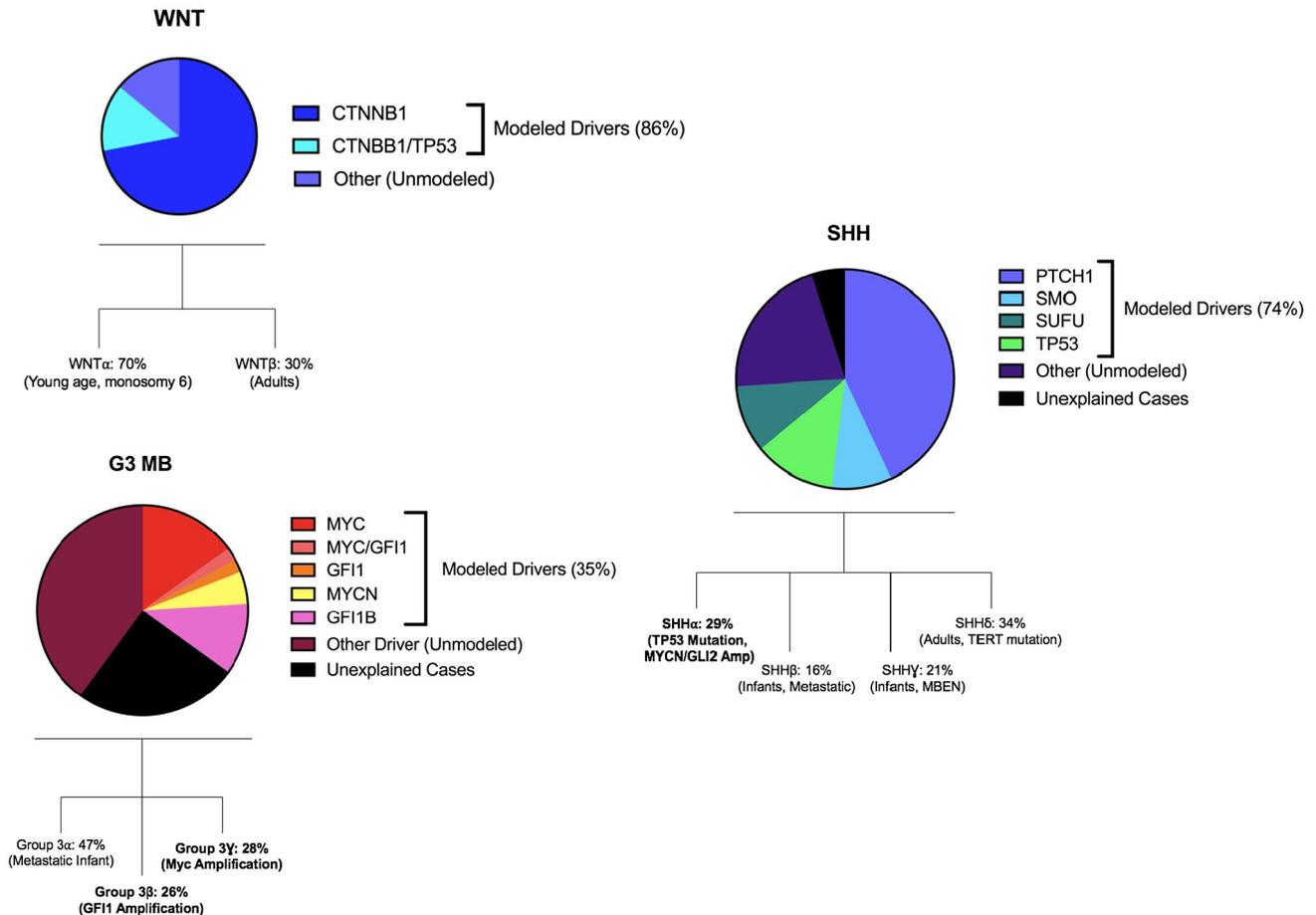
The wealth of data on human medulloblastomas from multiple “omics” approaches has facilitated the development of mouse models for each medulloblastoma group and some of the recently described subgroups (Figure 2).

### WNT

The molecular landscape of Wingless (WNT) group medulloblastomas is relatively well understood, and as such the genetically engineered mouse model (GEMM) developed by Gibson and collaborators has proved ideal to study this disease group (25). The majority (~85%) of patients with WNT MB harbor mutations in  $\beta$ -catenin (*CTNNB1*) leading to over-activation of the WNT signaling pathway, the nuclear localization of  $\beta$ -catenin, and accelerated cell division and proliferation (19). Initial experiments targeted overexpression of a mutated *CTNNB1* S37F in mouse *Atoh1*-positive granule neural progenitors with or without *Trp53* loss. Whereas  $\beta$ -catenin protein was clearly overexpressed, no tumors ever formed, suggesting that GNPs were not the cell of origin of WNT MBs. It was only by mapping genes expressed in WNT MBs during embryonic cerebellar development that Gibson and collaborators discovered the cell of origin of WNT MBs in the floor of the fourth ventricle. Crossing the *Blbp*-Cre line into *Ctnnb1*<sup>lox(ex3)</sup> × *Trp53*<sup>flx/flx</sup> mice drove the initiation of tumors closely resembling WNT MB in anatomic features, chromosomal changes and transcriptomic landscape. The penetrance of the disease was further increased by enforced expression of *PI3KCA* to activate the PI3K pathway. Importantly, analysis of this WNT model revealed an aberrant tumor blood–brain barrier, consistent with increased propensity for hemorrhage at surgery and improved response to chemotherapy (58). Because patients with WNT MBs respond well to current therapy and recognizing that intensive craniospinal irradiation causes long-term neurological sequelae, this model has facilitated the evaluation of CSI dose reduction (ongoing SJ clinical trial with lower radiation doses—NCT02724579). Recently, a new model of WNT MB has been created based on recurrent *DDX3X* mutation found in a subset of WNT MB tumors (52) (Richard Gilbertson, personal communication).

### SHH

Since the development of the first *Ptch1*<sup>+/-</sup> GEM model in 1997 (26), a plethora of SHH medulloblastoma mouse models has been developed using multiple approaches (Table 1). Many investigators generated these models by initially testing the role of mutations and overexpressed genes found in human SHH medulloblastomas. Initial GEMMs all led to tumors mimicking the SHH group (16, 42, 68). This



**Figure 2.** For each subgroup of medulloblastoma, the proportions of driver events as determined by Next-Generation Sequencing (NGS) are displayed; Copy Number Variants (CNV) and mutation analysis. *Modeled Drivers*: of all driver events identified by NGS for this subgroup, the proportion represented by established *in vivo* models. *Other*: Driver events identified by NGS which have not been modeled *in vivo*.

*Unexplained Cases*: cases of this MB group for which no events have been identified through NGS approaches. Recently, defined subdivisions of groups into subgroups are also displayed (14), with those in bold having corresponding *in vivo* models.

was the first set of data pointing to GNPs as the cell of origin of SHH medulloblastoma. Subsequent experiments confirmed that SHH medulloblastomas arose from GNPs that were blocked in their exit from the cell cycle and differentiation to post-mitotic granule cells, thus remaining in the external granule layer (6). Recognizing that GNPs are highly susceptible to DNA damage, the McKinnon laboratory and others tested the role of DNA repair enzymes responsible for homologous recombination (HR) and non-homologous end joining (NHEJ) (43, 77). These investigators found that loss of enzymes, including Ligase IV, Xrcc4 and Brca2 led to embryonic lethality, which was rescued by the loss of Trp53. While mice developed into adulthood, they all succumbed to SHH medulloblastomas. These studies emphasized the importance of intact p53 and DNA repair pathways in maintaining a healthy cerebellum during development. Whereas SHH MB models recapitulate the human tumors, they rely on the modification of mouse cells. Recent humanized models have been developed using human

neuroepithelial stem cells (NSC) from iPSCs that are representative of cerebellar progenitors. Transduction of *MYCN* in human NSC induced tumors of the SHH medulloblastoma subgroup (33). Interestingly, these tumors did not lose TP53 function nor the *PTCH1* gene. The same group showed that orthotopic transplantation of NSC cells from a Gorlin patient carrying a germline *PTCH1* mutation also induced SHH medulloblastoma. It is likely that the use of human NSC will be important to model mutations and gene fusions found in human medulloblastoma.

### Group 3

Overexpression of the *MYC* oncogene typifies Group 3 medulloblastomas. *MYC* is amplified in about 17% of cases and correlates with frequent metastasis at diagnosis, an aggressive clinical behavior and poor prognosis. While *MYC* overexpression is seen in most human Group 3 MBs, rare tumors in this group display *MYCN* amplification.

The first mouse model of G3 MB was developed in a GEMM in which *MYCN* expression was driven by the glutamate transporter 1 (*Glt1*) promoter expressed in hind-brain progenitors (GTML) (70). In this model, most tumors exhibit a classic or large cell/anaplastic (LCA) morphology and a G3 MB molecular profile, although some more closely resemble other groups. Crossing the *Glt1-tTA*; TRE-*MYCN*/luciferase model to mice lacking *Trp53* accelerates tumorigenesis. Because many recurrent cases of human G3 MB have deletion of one copy of *TP53* as part of an isochromosome 17q, this transgenic model might represent a more efficient tool to examine MB relapse (31). Despite the histological relevance of the GTML model, *MYCN* amplification is far less common than *MYC* amplification in G3 MB. To overcome this criticism, the Swartling group recently developed a derivative model (GMYC), in which *MYC* is driven from the hindbrain-specific glutamate transporter 1 (*Glt1*) promoter. Demonstrating differential expression of key features between *MYC*- and *MYCN*-driven tumors, this new GMYC model highlights a role for *CDKN2A* in MB pathogenesis (Swartling, Personal Communication).

In 2012, our group and Wechsler-Reya's laboratory independently developed mouse models of G3 MB that mimicked the histology and gene expression signatures of human tumors. Both groups leveraged enforced *MYC* overexpression by retroviral gene transfer and the loss of *Trp53* function to reprogram granule cell neuronal progenitors (GNPs) or Proliferating Cell Nuclear Antigen (PCNA)/CD133-positive neural stem cells (37, 57). These two models relied on orthotopic transplants of modified neuronal progenitors in the cerebellum or cortices of naïve recipient mice. To better define the cell of origin of Group 3 medulloblastomas, Kawauchi and colleagues conditionally enforced *MYC* co-expression with luciferase and a dominant form of *Trp53* (DNp53) by *in utero* electroporation in embryonic cerebellar progenitor cells using several specific Cre lines. Irrespective of the cerebellar lineage targeted, all tumors were Group 3 suggesting that the combination of the two genetic insults, rather than a specific cell of origin, drive this MB group. This conclusion was recently confirmed by single cell sequencing of primary tumor samples from patients with human Group 3 MBs (32). Because these G3 MB mouse models grow *in vitro* as spheres in stem cell media conditions, they have been instrumental for conducting high-throughput drug screens and identifying novel therapies, some of which are currently in clinical trials (SJMB12-NCT01878612, SJDAWN-NCT03434262 and SJELiOT-NCT04023669).

Most existing models of G3 MB rely on predetermined genetic perturbation and complex preimplantation protocols. In order to mimic the sporadic genetic changes that occur during tumor initiation in somatic cells, Jenkins *et al* developed a retroviral transfer system whereby *MYC* is delivered to and ectopically expressed in Nestin-positive neural progenitor cells in postnatal mice (35). Similar to previous models, this RCAS/tv-a model of G3 MB requires *Trp53* loss or overexpression of *Bcl-2* to escape *Myc*-induced apoptosis. Uniquely, this model develops within an intact host

immune system which enables interrogation of the native microenvironment and immunotherapeutic testing.

In most of the murine models of G3 MB developed to date, *MYC* overexpression is driven by strong retroviral promoter elements. While this approach is effective at generating G3 MB tumors, it presents a limitation in investigating the signals that regulate *MYC* transcription. To circumvent this challenge, Vo and collaborators developed CRISPR/dCas9 gene activation in which endogenous *MYC* is activated by dCas9-VP160 in *Trp53*-null neural progenitors (74). Many of these tumors recapitulate G3 MB by histology and microarray analysis and importantly provide a useful model to interrogate endogenous *Myc* regulation and signaling.

Since the development of GEMM and orthotopic mouse models of G3 MB that all rely on the loss of *Trp53*, recent studies have demonstrated that enforced *MYC* expression can collaborate with *GFI1* or *GFI1B* overexpression in neural progenitors, bypassing the requirement for *Trp53* loss (54, 75). Prevalent genomic structural variants have been identified in human G3 and G4 MBs that result in the activation of the transcription factor repressors *GFI1* and *GFI1B* by rearranging coding sequences to juxtapose them to active enhancer elements. These novel mouse models recapitulate one of the recently described subgroups of *MYC*-driven Group3 medulloblastoma (14). Because medulloblastomas have a paucity of mutations but many chromosomal anomalies, it is likely that currently undiscovered rearrangements may lead to new targets and mouse models.

Recent sequencing studies have revealed mutations in epigenetic regulators in all medulloblastoma groups, including Group 3, but it is unclear whether these mutations drive tumor development. One epigenetic regulator *Mll4* mutated in Group 3 MB was recently found to be a driver mutation (17). Whether other genetic alterations in chromatin regulators are driving medulloblastoma development will require further studies including CRISPR or/and shRNA screens of libraries of epigenetic regulators.

#### Group 4

Phosphoproteomic studies of human medulloblastomas have provided a better understanding of potential drivers in G4 tumors and facilitated the development of a G4 medulloblastoma model that has eluded investigators up to now. Investigators showed that G4 tumors harbor a phosphoproteomic landscape distinct from their RNA signature and characterized by activation of a tyrosine kinase program highlighting a unique receptor tyrosine signaling network (*ERBB4-SRC*) specific to this group (21). Using these insights, the first mouse model of G4 MB was created using *in utero* electroporation to deliver a dominant negative form of *Trp53* (DNp53) and a constitutively active form of *SRC*, with a truncated C-terminal domain (*SRC-CA*), into the fourth ventricle of E13.5 mouse embryos, an age selected to target the nuclear transitory zone (NTZ) progenitors of the developing cerebellum. Comparative analysis by cross-species comparison confirmed that gene expression most closely resembled G4 MB. This model for the first time recapitulates

the landscape integrity of G4 MBs and suggests that G4 MBs could be susceptible to kinase inhibitors, many of which are widely available and used in treating adult cancers.

## PRECLINICAL USE OF MOUSE MODELS OF MB

A key impetus for the development of accurate and representative mouse models of medulloblastoma was to create preclinical testing tools. The requisite characteristics of preclinical models are now represented across the spectrum of mouse models. Tumors driven by specific genetic perturbations are useful for assessing response to targeted therapies (SMO inhibitors, BET inhibitors, etc), while syngeneic orthotopic models will be invaluable to explore the immune landscape in medulloblastoma. PDOXs are increasingly becoming the gold standard for preclinical testing, consistent with a trend away from established cell lines that fail to recapitulate the primary tumors from which they are derived. The heterogeneity of MB warrants testing interventional approaches in multiple models, particularly to define relevant patient populations for trial stratification. Most of the preclinical work in MB has focused on SHH and Group 3, due in part to the development of multiple representative models and the need for improved therapies for the high-risk subsets of these groups. However, major challenges are the blood–brain and blood–tumor barriers. These have limited the number of drugs that can be used to treat MB patients. While several drugs and small molecules have been tested in mouse models and found to be efficacious in suppressing medulloblastoma proliferation, most used established cell lines and flank tumors. Thus, ideally preclinical trials should be performed in multiple mouse models *in vitro* and *in vivo* and should require that all drugs be investigated for their brain and tumor penetration.

### SHH

Some of the earliest preclinical studies for MB capitalized on PTCH and SMO mutations in SHH tumors and explored the potential of SMO inhibitors like Vismodegib and Sonidegib (63). The most common model to evaluate efficacy of SHH pathway inhibitors has been the *Ptch1<sup>-/-</sup>; Trp53<sup>-/-</sup>* mouse model, followed closely by SHH PDOXs. Our understanding of the signaling pathways involved in SHH MB, and the availability of a GEMM model that so closely recapitulates the genetic signature of these tumors has been immensely valuable in translating preclinical testing into Phase II clinical trials PBTC-025B and PBTC-032 (Vismodegib for recurrent or refractory MB with a 41% response in SHH patients) (62). However, as suggested by mouse studies, treatment of children with Vismodegib experienced growth plate fusion, and this has stimulated the identification of alternative therapeutics for this patient population (38, 61).

Molecular analysis of tumor propagating cells in the Math-GFP/*Ptch1<sup>+/-</sup>* mouse model revealed increased expression of G2/M regulators and led to the evaluation of Aurora

Kinase (Aurka) and Polo-like kinase 1 (Plk1) inhibitors as targeted therapies (29). Whether these small molecules effectively cross the blood–brain barrier is unknown. Preliminary studies from collaborative teams at St. Jude Children's Research Hospital, Telethon Kids Institute in Perth, Australia and the German Cancer Research Center (DKFZ) in Heidelberg, Germany are utilizing high-risk SHH PDOX models of MB to test the preclinical efficacy of checkpoint inhibitors in combination with traditional chemotherapy and the DNA damage agent cyclophosphamide. The clinical trial based on this work (SJELiOT–NCT04023669) was recently approved by the FDA and began enrolling in 2019.

### Group 3

Despite the relatively quiet mutational landscape of Group 3 MBs, early mouse models faithfully recapitulated the most common perturbation of MYC overexpression. Many groups use the Myc-driven, *Trp53<sup>-/-</sup>* mouse model to investigate the utility of small molecule inhibitors. Because this model is amenable to *in vitro* culture, high-throughput screening can be conducted to identify novel small molecules. Pei and collaborators identified a cooperative effect between HDAC inhibitors and PI3K inhibitors in this, and other PDOX models, while Morfouace and collaborators demonstrated a new combination approach of pemetrexed and gemcitabine (1, 51, 56). From these, preclinical efforts have emerged early stage clinical trials including SJMB12–NCT0187861 (Pemetrexed and Gemcitabine for Newly diagnosed patients with Non-WNT, Non-SHH, MB). A more recent St. Jude clinical trial, SJDAWN–NCT03434262 include the use of a CDK4/6 inhibitor (ribociclib) in combination with gemcitabine for recurrent G3 MB. Inhibition of CDK4/6 with Palbociclib (IBRANCE) was found to significantly extend the survival of mice harboring a MYC-amplified Group3 medulloblastoma patient-derived orthotopic xenograft, Med-311FH (15). These studies stimulated a phase I study with Palbociclib in children with recurrent, progressive or refractory CNS tumors including medulloblastoma (PBTC-042). More recently, inhibitors of DNA repair enzymes Check 1 and Check 2 were found to efficiently suppress the proliferation of mouse and human Group 3 medulloblastomas, with or without TP53 mutation, in combination with the DNA-damaging agents, gemcitabine or cyclophosphamide (Roussel and Gottardo, personal communication). The clinical trial designed based on this work (SJELiOT, NCT04023669) was recently approved by the FDA and began enrolling in July 2019.

Several groups have used the Myc-amplified GEMM model and various PDOX models to demonstrate the efficacy of epigenetic regulation perturbation via BET bromodomain inhibitors (8, 74). Early G3 models were created using exogenous plasmids with foreign promoters to drive overexpression of Myc. Although they have provided preclinical value, Vo and collaborators recently developed a CRISPR-Myc-driven system that leveraged the endogenous Myc promoter and created a better tool to interrogate inhibitors that impact epigenetic regulation at the MYC promoter (74).

## CONCLUSION

Since the development of the SHH medulloblastoma *Ptch1*<sup>+/-</sup> mouse model, all four medulloblastoma groups have been modeled in mice, and many have been used in preclinical studies that have led to clinical trials. It is likely that in the near future new mouse models will be developed that will accurately recapitulate MB subgroups. This should strengthen the preclinical pipeline and contribute to finding novel therapies for the most aggressive forms of medulloblastoma.

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## CONFLICT OF INTEREST

The authors have no conflict of interest to declare.

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