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Medulloblastomics revisited: biological and clinical insights from thousands of patients

Volker Hovestadt^{1,2}, Olivier Ayrault^{3,4}, Fredrik J. Swartling⁵, Giles W. Robinson⁶, Stefan M. Pfister^{7,8,9} and Paul A. Northcott^{10*}

Abstract | Medulloblastoma, a malignant brain tumour primarily diagnosed during childhood, has recently been the focus of intensive molecular profiling efforts, profoundly advancing our understanding of biologically and clinically heterogeneous disease subgroups. Genomic, epigenomic, transcriptomic and proteomic landscapes have now been mapped for an unprecedented number of bulk samples from patients with medulloblastoma and, more recently, for single medulloblastoma cells. These efforts have provided pivotal new insights into the diverse molecular mechanisms presumed to drive tumour initiation, maintenance and recurrence across individual subgroups and subtypes. Translational opportunities stemming from this knowledge are continuing to evolve, providing a framework for improved diagnostic and therapeutic interventions. In this Review, we summarize recent advances derived from this continued molecular characterization of medulloblastoma and contextualize this progress towards the deployment of more effective, molecularly informed treatments for affected patients.

Next-generation sequencing (NGS). Technologies enabling massively parallel reading of amplified short nucleotide sequences (typically yielding hundreds of millions of reads, 100–500 bp in length). In contrast, emerging third-generation sequencing technologies read sequences without prior amplification, yielding much longer reads, albeit with reduced accuracy and throughput.

Molecular classification Classification of patient tumour samples based on molecular markers, as opposed to classification based on histomorphological appearance.

Medulloblastoma (MB), a cerebellar tumour, is one of the most common malignant central nervous system (CNS) tumours in children (total of ~440 new diagnoses/year in the USA, >65% diagnosed in children younger than 16 years old¹) and a leading cause of cancer-related death in this age group². The incidence of MB does not appear to be significantly influenced by geographical location or ancestry^{3,4}. During the past two decades, large-scale genomic efforts have helped disentangle the molecular basis of MB, including biologically and clinically relevant intertumoural heterogeneity. Four consensus molecular subgroups — WNT, Sonic hedgehog (SHH), Group 3 and Group 4, each characterized by distinct -omic (that is, genomic, epigenomic, transcriptomic and proteomic) and clinical features — are now widely recognized⁵. As a direct consequence, mechanistic, developmental and preclinical studies are currently being undertaken in a manner that is cognizant of molecular subgroup status. Moreover, clinical protocols have adopted molecular subgrouping strategies into routine diagnosis, treatment stratification and patient selection for molecularly targeted therapies.

In 2012, following the formal recognition of consensus molecular subgroups⁵, the initial wave of next-generation sequencing (NGS) studies conducted on primary MB samples were reported^{6–10}. Since then, additional molecular characterization has ensued on increasingly larger cohorts, resulting in the generation

of a wealth of multidimensional -omics data. As a result, the molecular classification of MB has evolved beyond the four consensus subgroups to include additional subdivisions (also referred to as ‘subtypes’), and new methods for robust and accurate assignment of patients into the relevant subtypes have become mainstream. More recently, spatial and single-cell genomic approaches have been applied, delving into the intratumoural heterogeneity, cellular composition and developmental origins of MB at single-cell resolution. We recently reviewed the epidemiological, biological and therapeutic characteristics of MB in a report that was intended to be all-encompassing of the human disease¹¹. In this Review, we emphasize what has been learned from the -omic analyses of patients with MB since the first NGS studies⁶, highlighting insights into the molecular and biological mechanisms underlying MB heterogeneity and the translational implications emerging from these efforts.

Molecular classification

WHO consensus subgroups and subtypes within subgroups. The first gene expression array study confirmed that MB was molecularly distinct from other embryonal brain tumours, such as primitive neuroectodermal tumour (PNET) and atypical teratoid rhabdoid tumour¹². In this seminal report, published in 2002, a gene expression comparison of MB types with classic histology versus desmoplastic/nodular histology identified specific

*e-mail: paul.northcott@stjude.org
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Intratumoural heterogeneity

The observation that tumours comprise distinct malignant and non-malignant (cells of the microenvironment) cell types. The heterogeneity of malignant cells encompasses genetic heterogeneity (for example, different genetic subclones) and transcriptional heterogeneity (for example, malignant cell states resembling normal development).

Primitive neuroectodermal tumour

(PNET). A class of histologically defined, poorly differentiated childhood brain tumours. More recently, PNET has been reclassified into a number of both novel and previously known brain tumour entities by molecular profiling.

Atypical teratoid rhabdoid tumour

A rare and highly malignant type of childhood brain tumour that is characterized by mutations in the *SMARCB1* gene.

Classic histology

The most common histological variant found in all medulloblastoma subgroups, characterized by densely packed small round or oval cells and a high nuclear:cytoplasmic ratio.

Desmoplastic/nodular histology

A histological variant mostly restricted to Sonic hedgehog medulloblastoma, characterized by a varying number of islands of neurocytic differentiation and internodular desmoplasia.

upregulation of genes involved in Hedgehog signalling, including patched homologue 1 (*PTCH1*), *GLI1* and *MYCN*, in desmoplastic tumours¹². A parallel report comparing the expression profiles of a series of genetically engineered mouse (GEM) models with normal cerebellar controls identified similar activation of Hedgehog pathway-associated gene sets in MB GEM models¹³. Together, these early reports of the gene expression signatures defining MBs in humans and mice paved the way for the molecular era that followed. Multiple independent gene expression array profiling studies conducted on patient cohorts subsequently described distinct molecular subgroups within MB that differed in their demographics, genetic alterations and clinical outcomes^{14–17}, culminating in the definition of consensus subgroups⁵. The WNT and SHH subgroups, which represent approximately 10% and 30%, respectively, of all patients with MB, are associated with activation of the WNT and SHH signalling pathways. The WNT and SHH subgroups are discrete across studies and technologies, providing a basis for their incorporation into the 2016 update of the WHO Classification of Central Nervous System Tumours¹⁸. Further subdivision within the SHH subgroup according to *TP53* mutation status (SHH with wild-type *TP53* and SHH with mutant *TP53*) is also recognized by the WHO. The Group 3 and Group 4 subgroups, which represent the remaining 25% and 35%, respectively, of all patients with MB, exhibit some molecular and biological similarities (detailed below). Consequently, these subgroups are formally recognized by the WHO as non-WNT/non-SHH MB, and the Group 3 and Group 4 designations are listed as provisional entities.

Differential expression analysis between MB subgroups has led to the identification of select biomarkers that enable molecular classification based on immunohistochemistry^{16,19,20}, the NanoString gene expression assay^{21,22} and other panel-based gene expression assays²³. While these assays are widely accessible in clinical practice, DNA methylation arrays, which can measure hundreds of thousands of methylation sites across the genome, have emerged as the platform of choice for MB classification. Owing to the relative stability of DNA, methylation arrays and derivative-targeted DNA methylation-based assays allow for the routine analysis of clinical samples in a diagnostic setting as well as

the profiling of archival tumour material, with limited technical variation between institutions^{24–28}.

In 2017, three independent studies^{29–31} used DNA methylation analysis or combined DNA methylation and gene expression analysis to investigate the additional substructure within subgroups, leading to varying definitions of molecular subtypes across studies (FIG. 1; discussed further below).

WNT medulloblastoma. WNT MB occurs primarily in children from 4 years of age to early adulthood (median age of diagnosis ~11 years) and exhibits a balanced male:female ratio (FIG. 2). WNT-MB tumours are usually of classic histology and are infrequently metastatic at diagnosis. The outcomes for patients with WNT MB are favourable, with 5-year survival of 95% or better^{20,32–35}. Somatic mutations in *CTNNB1* (which encodes β -catenin) are the hallmark genetic event defining this subgroup (~85% of patients)^{17,34}, with most of the remaining patients typified by pathogenic germline variants in adenomatous polyposis coli (*APC*)³⁶. WNT-MB genomes are mostly devoid of somatic copy-number alterations (SCNAs), except for loss of one copy of chromosome 6 (that is, monosomy 6) in most patients^{9,30}.

WNT MB has been described as largely homogeneous between patients, with regard to genome-wide expression and methylation profiles. However, two molecular subtypes, WNT- α and WNT- β , have been suggested that differ in age at diagnosis (median age of 10 versus 20 years, respectively) and frequency of monosomy 6 (REF.²⁹). The survival outcomes of adults with WNT MB (that is, WNT- β ²⁹) have been inconsistent in the literature, with some reports describing outcomes as comparably favourable, relative to those of paediatric patients with WNT MB^{29,37}, and others reporting reduced overall survival³⁵.

SHH medulloblastoma. SHH MB displays an intriguing bimodal age distribution, representing the most common molecular subgroup in both infants (<3 years of age) and adults (>17 years of age), with fewer cases being diagnosed during childhood and adolescence (FIG. 2). Demographically, SHH MB is more common in males than in females (approximately 2:1). Classic and desmoplastic/nodular (including MB with extensive nodularity) histologies occur at similar frequencies (each accounting for ~40% of patients), with a large-cell/anaplastic (LCA) histology making up the remainder^{20,32}. Mutations and focal SCNAs targeting genes in the SHH signalling pathway represent the most common genetic events, including inactivating germline or somatic mutations and deletions of *PTCH1* and of suppressor of fused homologue (*SUFU*), activating mutations in smoothened homologue (*SMO*) and amplifications of *GLI2* (REFS^{30,38}). Frequent chromosomal alterations include loss of chromosomes 9q, 10q, 14q and 17p, as well as gain of chromosomes 2 and 9p^{9,30}.

Age-associated molecular differences discriminating infant and adult SHH MB have been observed through both gene expression and DNA methylation array profiling^{31,38,39}. Compared with their paediatric counterparts, adult SHH MBs are characterized by a higher overall

Author addresses

¹Department of Pathology and Center for Cancer Research, Massachusetts General Hospital and Harvard Medical School, Boston, MA, USA.

²Broad Institute of Harvard and MIT, Cambridge, MA, USA.

³Institut Curie, PSL Research University, CNRS UMR, INSERM, Orsay, France.

⁴Université Paris Sud, Université Paris-Saclay, CNRS UMR 3347, INSERM U1021, Orsay, France.

⁵Department of Immunology, Genetics and Pathology, Science for Life Laboratory, Rudbeck Laboratory, Uppsala University, Uppsala, Sweden.

⁶Department of Oncology, St Jude Children's Research Hospital, Memphis, TN, USA.

⁷Hopp Children's Cancer Center Heidelberg (KiTZ), Heidelberg, Germany.

⁸Division of Paediatric Neurooncology, German Cancer Research Center (DKFZ) and German Cancer Consortium (DKTK), Heidelberg, Germany.

⁹Department of Paediatric Haematology and Oncology, Heidelberg University Hospital, Heidelberg, Germany.

¹⁰Department of Developmental Neurobiology, St Jude Children's Research Hospital, Memphis, TN, USA.

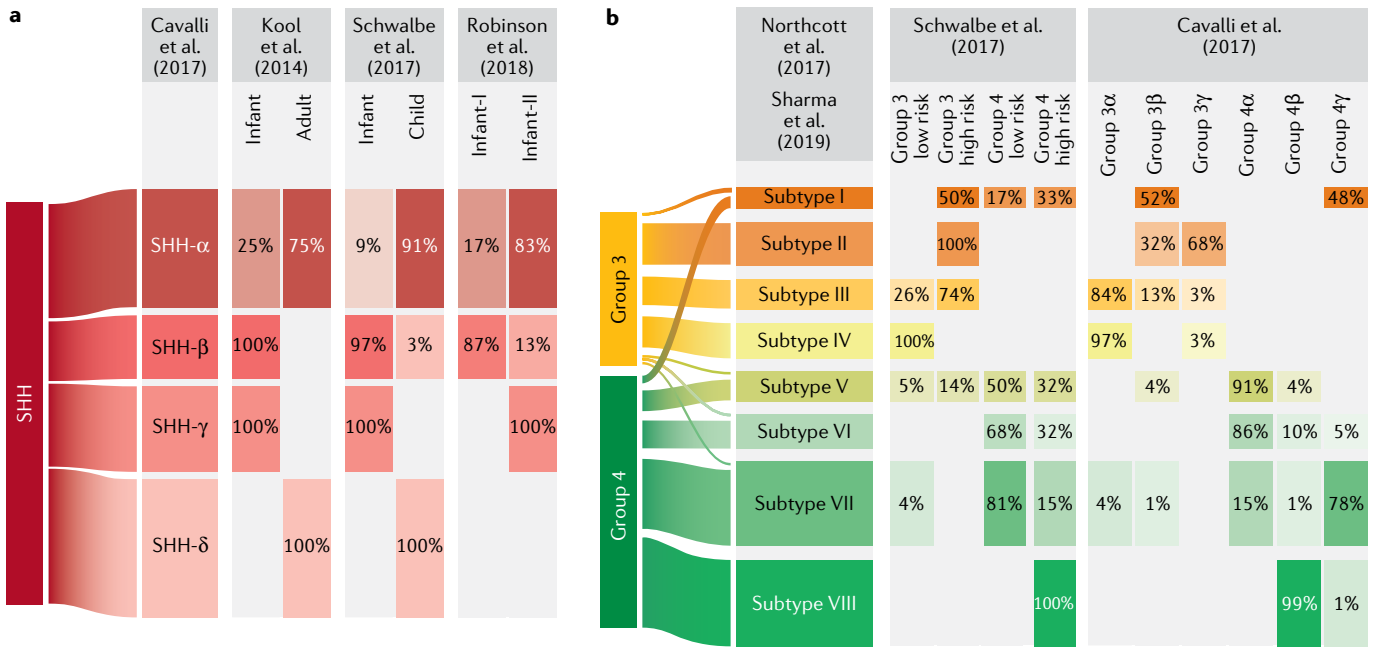


Fig. 1 | Comparison of MB DNA methylation-derived subtypes described across recent studies. a | Correspondence between the four molecular subtypes of the Sonic hedgehog medulloblastoma (SHH-MB) subgroup, described by Cavalli et al.²⁹ (n = 223 samples) and the subtypes described in three additional studies (Kool et al.³⁸, n = 129 samples; Schwalbe et al.³¹, n = 103 samples; Robinson et al.⁴⁴, n = 82 samples). DNA methylation profiles from all samples of each additional study were used to classify patients into the four molecular subtypes using a machine-learning approach. The height of each row corresponds to the fraction of samples per subtype in the Cavalli et al. study²⁹ (SHH-α = 29%; SHH-β = 16%; SHH-γ = 21%; SHH-δ = 34%). Percentages indicate overlap of the predicted subtypes with the original subtype annotations in each additional study. The samples from the study by Robinson et al.⁴⁴ were not predicted as SHH-δ, consistent with the study including only patients under the age of 6 years. **b** | A similar comparison of the eight molecular subtypes of Group 3 and Group 4 MB described by Northcott et al.³⁰ and Sharma et al.¹³⁴ (n = 1,370 samples; subtype: I = 4%, II = 13%, III = 9%, IV = 10%, V = 8%, VI = 9%, VII = 22%, VIII = 25%) and the subtypes described in two additional studies (Schwalbe et al.³¹, n = 273 samples; Cavalli et al.²⁹, n = 470 samples). The line widths between the two consensus subgroups (Group 3 and Group 4) and the eight DNA methylation subtypes indicate the fraction of samples per subtype that were originally classified as Group 3 or Group 4 MB.

mutational burden, a higher prevalence of SHH pathway-associated mutations (including higher incidence of *PTCH1* and *SMO* alterations) and a more expansive list of chromatin modifier mutations (that is, in bromodomain and PHD finger containing 1 (*BRPF1*) and CREB-binding protein (*CREBBP*)); virtually all such MBs also harbour telomerase reverse transcriptase (*TERT*) promoter mutations^{38,40–42}. Intriguingly, transcriptional comparison of tumours between available SHH-MB mouse models and patients has suggested that, despite the mouse tumours being primarily driven by inactivation of *Ptch1* or activation of *Smo* — genetic events that occur in both paediatric and adult tumours — the current models are more molecularly similar to adult SHH MB⁴³.

More recently, four molecular subtypes of SHH MB have been reported²⁹ (FIGS 1a,2): SHH-β and SHH-γ correspond to infant subtypes (median ages of diagnosis 1.9 and 1.3 years, respectively), whereas SHH-α and SHH-δ correspond to childhood/adolescent and adult subtypes, respectively (median ages of diagnosis 8 and 26 years, respectively). Subtype SHH-α is enriched for patients harbouring *TP53* mutations (~1/3 of patients with SHH-α) and is associated with inferior outcomes, compared with patients with SHH-δ. Infant subtype SHH-β showed a lower 5-year survival than SHH-γ. Similar observations were made in an independent study

reporting on a clinical trial in infants and young children (under 6 years of age) that identified two molecular subtypes, iSHH-I (equivalent to SHH-β) and iSHH-II (equivalent to SHH-γ and SHH-α)⁴⁴.

Group 3 and Group 4 medulloblastoma. Group 3 MB occurs during infancy and childhood and is rarely seen in patients older than 18 years of age, whereas Group 4 MB occurs across all age groups (FIG. 2). The male:female ratios are 2:1 or higher for both subgroups. LCA histology is more prevalent in Group 3 than in Group 4, and 30–40% of patients are metastatic at diagnosis in both subgroups³². *MYC* amplification is a common genetic feature of Group 3 MB and is associated with a particularly poor clinical outcome^{14,16}. *MYCN* and cyclin-dependent kinase 6 (*CDK6*) amplifications are notable genetic alterations seen in Group 4 MB^{9,30}. Isochromosome 17q (that is, i17q) is a hallmark cytogenetic event in both subgroups, found in >50% of patients from either subgroup^{9,30}.

The definitions and substructures of Group 3 and Group 4 MBs have been a topic of debate since their initial discovery^{14–17}. Indeed, early nomenclature did not always define Group 3 and Group 4 into distinct subgroups and in some cases described them as a single ‘mixed’ subgroup of patients designated as

Large-cell/anaplastic (LCA) histology

A histological variant associated with both Group 3 medulloblastoma and a more aggressive phenotype, characterized by the co-occurrence of groups of large cells with round nuclei and cells exhibiting cytological pleomorphism (anaplasia).

Isochromosome

An abnormal chromosome in which both chromosome arms are identical. Isochromosome 17q is one of the most frequent somatic copy-number alterations in Group 3 and Group 4 medulloblastoma. In most cases a second q-arm is fused to the p-arm proximal to the centromere.

‘non-WNT/non-SHH’ MB²⁰. However, the recognition of Group 3 and Group 4 MB has continued to evolve in recent years, with the majority of the neuro-oncology community, and the WHO¹⁸, acknowledging their definition as mostly discrete molecular entities and supporting the utility of their distinction, as is supported in the literature (see^{11,45–47} for recent reviews).

More recently, three independent studies have identified a varying number of subtypes within Group 3 and Group 4 (FIG. 1b). Schwalbe and colleagues identified four molecular subtypes in Group 3 and Group 4 MB that split each subgroup into high- and low-risk subtypes³¹. Cavalli and colleagues identified three molecular subtypes in each subgroup: Group 3 α , β and γ , and Group 4 α , β and γ ²⁹. In a combined analysis of Group 3 and Group 4 cases, we identified eight molecular subtypes, designated I to VIII³⁰. To harmonize these subtype definitions, a joint analysis of 1,501 Group 3 and Group 4 MBs from all three studies was recently undertaken⁴⁸. In this analysis, we showed that separation into largely the same eight subtypes best unified the substructures observed in the aforementioned studies (FIG. 1b).

Subtype I represents the least common subtype and comprises a mix of Group 3 and Group 4 tumours (FIG. 2). Subtype I tumour genomes are generally balanced and enriched for amplification of the orthodenticle homeobox 2 (*OTX2*) oncogene and activation of growth factor independent 1 (*GFI1*) or *GFI1B*, which encode transcriptional repressors (described in detail below). Subtypes II, III and IV are bona fide Group 3 subtypes. Subtypes II and III are characterized by amplification of the *MYC* oncogene and are associated with poor outcomes. In comparison to other subtypes, Subtype IV is enriched for younger patients (median age of diagnosis 3 years) and is associated with a favourable outcome in non-infants; the low progression-free survival observed in infant patients with Subtype IV suggests that survival is dependent on treatment with craniospinal axis irradiation (CSI)⁴⁴. Subtypes V, VI and VII consist mostly of Group 4 MBs but also include some Group 3 tumours. Subtype V genomes are characterized by amplification of both *MYC* and *MYCN* and are associated with modest outcomes. Subtype VIII is the most common and only pure Group 4 subtype, mostly occurring in older children (median age of diagnosis 10 years). Subtype VIII tumours display a balanced genome, except for the presence of i17q in most cases. Subtype VIII is associated with favourable 5-year survival; however, many patients are affected by late relapse and death, a feature that is unique to this subtype⁴⁸.

Medulloblastoma genomic analyses

Gene mutations. MB NGS studies have primarily detailed somatic non-synonymous mutations affecting protein-coding genes in relatively modest patient cohorts^{7,8,10,49}. New recurrently mutated genes emerged from these analyses, including DEAD-box helicase 3X-linked (*DDX3X*), BCL-6 co-repressor (*BCOR*), CTD nuclear envelope phosphatase 1 (*CTDNEP1*), and T-box brain transcription factor 1 (*TBR1*), among others. In addition, genes encoding chromatin modifiers such as the lysine methyltransferases *KMT2D* (also known

as *MLL2*) and *KMT2C* (also known as *MLL3*), the lysine demethylase *KDM6A*, and SWI/SNF-related matrix-associated actin-dependent regulator of chromatin subfamily A member 4 (*SMARCA4*), previously identified by large-scale exome resequencing⁵⁰, were also confirmed, and their mutational frequencies and distributions were contextualized by subgroup. Despite these advances, the initial standalone studies were underpowered to adequately detail the broader scope of the low-frequency driver gene alterations that contribute to MB, especially in Groups 3 and 4, which were heterogeneous and devoid of highly recurrent protein-coding gene mutations⁶.

Recently, an international collaborative effort, co-led by authors of this Review, towards comprehensive characterization of the MB genomic landscape summarized putative driver gene alterations across a series of 491 primary MB samples³⁰. As expected, the WNT and SHH MB subgroups were largely characterized by mutations and SCNAs affecting known genes. Of interest, functional annotation of recurrently altered genes identified somatic deregulation of SWI/SNF family chromatin-remodelling genes (namely, *SMARCA4*, *ARID1A* and *ARID2*) in one-third of WNT-MB cases, and recurrent targeting of histone acetyltransferase genes (namely, *CREBBP*, *KANSL1*, *BRPF1* and others) in nearly 20% of SHH MBs. Detailed mechanistic studies will be required in order to determine how somatic targeting of these chromatin-associated complexes potentiates MB pathogenesis in the affected subgroups.

In Group 3 and Group 4 MBs, the multidimensional molecular analysis performed on this broader set of patients ($n = 324$ total) confirmed that recurrent protein-coding gene mutations remained relatively rare. *SMARCA4* mutations occurred in 9% of Group 3 and only 2% of patients from Group 4. *KDM6A* (7%), zinc finger MYM-type containing 3 (*ZMYM3*) (6%) and *KMT2C* (6%) represented the most commonly mutated genes in Group 4. Previously unknown somatic in-frame insertions that affected Kelch repeat and BTB domain containing 4 (*KBTBD4*) were evenly distributed between Group 3 and Group 4 MBs (6% of patients from either subgroup). Although it is poorly characterized to date, *KBTBD4* is a member of the BTB–Kelch protein family and is predicted to recruit protein substrates to cullin–RING ligases for targeted ubiquitylation and protein degradation⁵¹. MB-associated *KBTBD4* insertions were confined to the Kelch domain and were deemed unlikely to disrupt the overall domain structure but instead converged on the known substrate-binding interface described for other BTB–Kelch protein family members. Recently, somatic in-frame insertions identical to those seen in MB have been reported in pineal parenchymal tumours of intermediate differentiation (PPTID)⁵², suggesting that common oncogenic mechanisms may be shared between patients affected by MB and PPTID.

Structural alterations and enhancer hijacking. The Group 3 and Group 4 MB genomes are characterized by a preponderance of SCNAs and structural variants (SVs)⁹, suggesting that these alterations play an integral role

Craniospinal axis irradiation (CSI). Standard therapy for patients with medulloblastoma following surgery, to reduce risk of tumour regrowth and metastatic dissemination. The application of CSI is also associated with neurological impairments and secondary malignancies.

Non-synonymous mutations
Genetic alterations that alter the amino acid sequence of an affected protein, possibly altering protein function. Most described recurrent mutations in medulloblastoma are non-synonymous mutations.

BTB–Kelch protein family
Family of proteins characterized by the presence of an N-terminal BTB domain and C-terminal Kelch motifs. The BTB domain facilitates protein binding, and the Kelch motifs associate to form a β -propeller facilitating protein–protein interactions. This family function as adaptor proteins that link cullin–RING ligases to substrates for ubiquitylation.

Pineal parenchymal tumours of intermediate differentiation (PPTID). A very rare tumour type of intermediate grade arising from the pineal parenchyma.

| Subgroup | | WNT | SHH | | | |
|--------------------|--|-----------------------------------|--|--|--|--|
| Subtype | | | α | β | γ | δ |
| Demographics | Frequency (%) | 100 | 29 | 16 | 21 | 34 |
| | Age (bar height corresponds with percentage) | | | | | |
| | Gender (%) | 45 ♂ 55 ♀ | 63 ♂ 37 ♀ | 47 ♂ 53 ♀ | 55 ♂ 45 ♀ | 69 ♂ 31 ♀ |
| Clinical features | Histology | Classic | Classic > desmoplastic > LCA | Desmoplastic > classic | Desmoplastic > MBEN classic | Classic > desmoplastic |
| | Metastasis (%) | 12 | 20 | 33 | 9 | 9 |
| | 5-year OS (%) | 98 | 70 | 67 | 88 | 89 |
| Molecular features | Cytogenetics | | | | | |
| | Driver events | CTNNB1, DDX3X or SMARCA4 mutation | <ul style="list-style-type: none"> MYCN or GLI2 amplification TP53 mutation PTCH1 mutation (less) | <ul style="list-style-type: none"> PTCH1 or KMT2D mutation SUFU mutation/deletion PTEN deletion | <ul style="list-style-type: none"> PTCH1, SMO or BCOR mutation PTEN deletion | <ul style="list-style-type: none"> PTCH1 mutation TERT promoter mutation |

| Subgroup | | Group 3 | | | | | | Group 4 | |
|--------------------|--|---|--|--------------------------|-------------------------|---------------------------|---|-----------------|--|
| Subtype | | I | II | III | IV | V | VI | VII | VIII |
| Demographics | Frequency (%) | 4 | 13 | 9 | 10 | 8 | 9 | 22 | 25 |
| | Age (bar height corresponds with percentage) | | | | | | | | |
| | Gender (%) | 60 ♂ 40 ♀ | 77 ♂ 23 ♀ | 78 ♂ 22 ♀ | 68 ♂ 32 ♀ | 71 ♂ 29 ♀ | 67 ♂ 33 ♀ | 66 ♂ 34 ♀ | 75 ♂ 25 ♀ |
| Clinical features | Histology | Classic > desmoplastic | LCA, classic | Classic > LCA | Classic | Classic | Classic | Classic | Classic |
| | Metastasis (%) | 35 | 57 | 56 | 58 | 62 | 45 | 45 | 50 |
| | 5-year OS (%) | 77 | 50 | 43 | 80 | 59 | 81 | 85 | 81 |
| Molecular features | Cytogenetics | | | | | | | | |
| | Driver events | <ul style="list-style-type: none"> GFI1 and GFI1B activation OTX2 amplification | <ul style="list-style-type: none"> MYC amplification GFI1 and GFI1B activation KBTBD4, SMARCA4, CTDNEP1 or KMT2D mutation | MYC amplification (less) | No common driver events | MYC or MYCN amplification | <ul style="list-style-type: none"> PRDM6 activation MYCN amplification (less) | KBTBD4 mutation | <ul style="list-style-type: none"> PRDM6 activation KDM6A, ZMYM3 or KMT2C mutation |

in disease pathogenesis. Analysis of the genomes of 137 Group 3 and Group 4 MB samples identified a series of atypical SVs (that is, deletions, duplications, inversions and more complex genomic alterations) mapping to chromosome 9q34 that were specific to these

subgroups⁵³. Integration with sample-matched gene expression data uncovered pronounced, SV-associated upregulation of GFI1B in affected samples. GFI1B is a transcriptional repressor that is primarily known for its role in T cell and B cell development, as well as in

◀ Fig. 2 | **Summary of demographic, clinical and molecular features of novel MB subtypes.** Values for age and gender distribution, frequency of metastasis, and 5-year overall survival (OS) for the WNT and Sonic hedgehog (SHH) subgroups are derived from the Cavalli et al. study²⁹. Driver events were additionally derived from the Kool et al.³⁸ and Robinson et al.⁴⁴ studies. Similarly, values for the Group 3 and Group 4 subgroups of medulloblastoma (MB) were derived from the Sharma et al. study¹³⁴. *BCOR*, BCL-6 co-repressor; *CTDNEP1*, CTD nuclear envelope phosphatase 1; *CTNNB1*, β -catenin; *DDX3X*, DEAD-box helicase 3X-linked; *GFI1*, growth factor independent 1 transcriptional repressor; i17q, isochromosome 17q; *KBTBD4*, Kelch repeat and BTB domain containing 4; *KDM6A*, lysine demethylase 6A; *KMT2C*, lysine methyltransferase 2C; LCA, large-cell/anaplastic; MBEN, MB with extensive nodularity; *OTX2*, orthodenticle homeobox 2; *PRDM6*, PR/SET domain 6; *PTCH1*, patched homologue 1; *SMARCA4*, SWI/SNF-related matrix-associated actin-dependent regulator of chromatin subfamily A member 4; *SMO*, smoothened homologue; *SUFU*, suppressor of fused homologue; *TERT*, telomerase reverse transcriptase; *ZMYM3*, zinc finger MYM-type containing 3.

haematopoietic malignancies in which it functions as an oncogene⁵⁴. The related family member *GFI1* was also determined to be aberrantly expressed in a mutually exclusive set of Group 3 and Group 4 MBs harbouring SV breakpoints proximal to the *GFI1* locus. Using an orthotopic transplantation approach, *GFI1B* and *GFI1* were validated as novel MB oncogenes capable of cooperating with *MYC* to promote highly aggressive Group 3-like MB in mice⁵³. Histone chromatin immunoprecipitation followed by sequencing (ChIP-seq) data for histone H3 lysine 27 acetylation (H3K27ac) that marked active enhancers suggested that the activation of *GFI1B* and *GFI1* expression in MB was accomplished via SV-dependent misappropriation of distal, highly active enhancers or super-enhancers to their normally repressed gene promoters. Similar to classical translocations that lead to overexpression of established oncogenes, such as *IgG-MYC* in Burkitt lymphoma^{55,56}, this mechanism of SV-dependent gene activation was designated ‘enhancer hijacking’ and has since been explored and documented in numerous follow-up studies in other cancer types^{57–61}. Overall, enhancer hijacking-associated *GFI1* and *GFI1B* activation is estimated to account for ~12–15% of patients with Group 3 and Group 4 MB, with clear enrichment of these events in Subtype I, and to a lesser extent in Subtype II³⁰.

Motivated by the discovery of enhancer hijacking, a novel computational pipeline, termed *cis*-expression structural alteration mapping (CESAM)⁶⁰, was developed to systematically identify additional enhancer-hijacking events through the integration of SV breakpoints and gene expression data. By applying CESAM to sample-matched genomic datasets derived from 164 patients with MB, we discovered *PRDM6* as a novel target of enhancer hijacking in 17% of patients with a Group 4 diagnosis³⁰. *PRDM6* maps to chromosome 5q23, approximately 600 kb downstream of *SNCAIP* — a locus known to be targeted by highly recurrent, stereotypical tandem duplications exclusively in Group 4 MB⁹. Through multi-omic data integration for a series of Group 4 MBs, a putative model of enhancer hijacking-mediated activation of *PRDM6* was proposed. *PRDM6* is described as a transcriptional repressor, mediating gene silencing through intrinsic histone H4 lysine 20 (H4K20) methyltransferase activity, in concert with known chromatin-associated, repressive complexes^{62,63}. To date, *PRDM6* represents the most frequent somatically altered gene in Group 4

MB, which, together with mutations targeting other chromatin-modifying genes, further implicates deregulation of physiological transcriptional control as an essential mechanism underlying Group 4 MB pathogenesis.

Transcriptomic and epigenomic analyses

Compared with childhood leukaemias⁶⁴ and other paediatric brain cancers (for example, supratentorial ependymoma⁶⁵ and pilocytic astrocytoma⁶⁶), recurrent gene fusions are rare in MB. Early transcriptome sequencing discovered recurrent *PVT1* gene fusions in Group 3 MB that were linked to chromothripsis and *MYC* amplification on chromosome 8q24 (REF.⁹). Additional *PVT1*-associated fusion events have since been reported in Group 3, including *PVT1-NDRG1*, *PVT1-LINC00964*, *PVT1-ZCH3* and others³⁰. *PVT1* encodes a long intergenic non-coding RNA (lincRNA) harbouring a cluster of six annotated microRNAs (miRNAs; namely, miR-1204, miR-1205, miR-1206, miR-1207-5p, miR-1207-3p, and miR-1208). Several reports have linked *PVT1* overexpression and activity with pro-tumorigenic phenotypes^{67–73}. In contrast, a recent study suggested that the *PVT1* gene promoter is a tumour suppressor DNA element that inhibits *MYC* expression through enhancer-promoter competition in *cis*⁷⁴. Future mechanistic and phenotypic studies in relevant model systems will be necessary to decipher the role of the *PVT1* fusions, as well as other fusions, seen in MB.

Paired DNA methylation and transcriptome analyses of patient samples with MB have also led to the identification of alternate promoters, indicating that transcriptional initiation of specific genes might reside outside previously annotated promoter regions. Analysis of high-coverage RNA sequencing (RNA-seq) data in 43 MB samples revealed 262 novel first exons that were spliced to internal exons in excess of 15 kb upstream of the previously annotated transcriptional start sites (TSSs), with some being located more than 500 kb away⁷⁵. Many of these alternate transcripts were expressed in a subgroup-specific manner, which often coincided with patterns of differential DNA methylation in the region of the novel TSS. One notable example is the pluripotency factor *LIN28B*, which has been described as regulating multiple oncogenic processes, including downregulation of the tumour-suppressive let-7 miRNA family⁷⁶. In MB, *LIN28B* is expressed specifically in Group 3 and Group 4 MBs. In about half of Group 3 cases and nearly all Group 4 cases, the annotated promoter region is fully hypermethylated, and transcription is initiated at a novel first exon that is spliced to the second annotated exon.

Systematic miRNA profiling of patient samples resulted in the identification of a number of differentially expressed miRNAs, both between MB subgroups and relative to normal controls^{14,77,78}. Among the best studied is the oncogenic miR-17–92 cluster, which was described as being genetically amplified and overexpressed specifically in SHH MB^{77,79} and as being required for the formation of SHH MB in the *Ptch1*^{+/-} mouse MB model⁸⁰. Furthermore, locked nucleic acid-mediated silencing of miR-17–92 prolonged the survival of mice with intracranial SHH-MB allografts⁸¹. Another well-studied example is the miR-183–96–182 cluster,

Chromothripsis

Clustered occurrence of a large number of structural variants restricted to a single chromosome or chromosomal arm, emerging through a single catastrophic event.

Ptch1^{+/-} mouse MB model

Transgenic mouse model that is heterozygous for the *Ptch1* gene. Sporadic Sonic hedgehog medulloblastoma develops in ~15% of *Ptch1*^{+/-} mice.

which is highly expressed in WNT, Group 3 and Group 4 MB, targeting the AKT–PI3K–mTOR pathway and regulating cell proliferation and migration both in vitro and in vivo^{82–84}. Other miRNAs are downregulated in MB and have been described as tumour suppressors, including miR-125b, miR-326 and miR-324-5p, which have been described to regulate the SHH signalling pathway⁸⁵, and miR-124a and miR-9, which regulate both the RE1-silencing transcription factor (REST) complex and cell proliferation^{86–89}. An extensive review of the role of miRNAs in MB has recently been published⁹⁰.

Genome-wide analysis of differential DNA methylation between MB subgroups and control tissues showed that the classical notion of gene silencing through promoter hypermethylation was not a prominent feature in MB⁷⁵. The most abundant pattern of differential methylation was identified in regions extending several kilobases downstream of the promoter into the gene body, in which hypomethylation correlated with elevated gene expression (in promoter downstream correlated regions, pdCRs). About 20% of genes that exhibited MB subgroup-specific expression contained a pdCR, which suggests that this pattern plays an important regulatory role in distinct tumour-specific transcriptomes. Large, megabase-scale blocks of reduced DNA methylation (partially methylated domains, PMDs) represented another pattern of differential methylation in MB⁷⁵. PMDs are a prominent feature in many cancer types and coincide with nuclear lamina-associated domains and other heterochromatic regions^{91–93}. In MB, PMDs are primarily detected in the WNT and Group 3 subgroups and can cover up to one-third of the genome, often in a subgroup-specific manner.

The integration of MB RNA-seq and enhancer ChIP-seq (namely, H3K27ac) datasets has further illuminated the mechanisms of gene regulation⁹⁴. Enhancer ChIP-seq data generated for 28 primary MBs and 3 MB cell lines enabled annotation of the active *cis*-regulatory landscape across MB subgroups. In total, nearly 80,000 enhancers were inferred, 25% of which had not previously been annotated by the Encyclopedia of DNA Elements (ENCODE)^{95,96} or the Roadmap Epigenomics Consortium⁹⁷. Computationally linking highly active, subgroup-specific enhancers, or ‘super-enhancers’, to putative target genes revealed new insights into the gene regulatory networks underlying MB subgroup biology and identity. Known cancer-associated genes were identified as prominent super-enhancer targets, including *ALK* (encoding the ALK receptor tyrosine kinase, RTK) in WNT MB; *GLI2*, *SMO* and neurotrophic RTK 3 (*NTRK3*) in SHH MB; the transcriptional regulators *LMO1* and *LMO2*, as well as *MYC* in Group 3 MB; and the transcription factors *ETV4* and *PAX5* in Group 4 MB, among others. Differential analysis of enhancers and their inferred gene targets identified aberrant transforming growth factor- β (TGF β) signalling activity that was specific to Group 3, substantiating prior genomic-based evidence implicating oncogenic TGF β signalling in a subset of tumours⁹.

Proteomic analyses

During the past decade, tremendous technical progress has been made towards sensitive detection and accurate quantification of the proteome⁹⁸, and recent studies

have begun to explore the proteomic landscape of MB. Quantitative mass spectrometry (MS)-based proteomics of primary human MBs has predominantly confirmed the classification of MB into consensus subgroups in three independent cohorts^{99–101}. The proteome also revealed notable substructure within both the SHH (designated as SHH-a and SHH-b) and Group 3 (designated as Groups 3a and 3b) subgroups. These observations were not immediately supported by companion DNA methylation or transcriptomic datasets, indicating a potential role for post-transcriptional mechanisms in the pathobiology of SHH MB.

Concordance between mRNA transcripts and protein abundance is known to vary between species and cell types, with only ~40% of protein variance explained by variance in mRNA abundance¹⁰². Group 3 and Group 4 MBs exhibit the lowest correlation between mRNA and protein expression, emphasizing the potential role of post-transcriptional mechanisms in their underlying biology^{100,101}. At the proteomic level, Group 3 MB exhibited elevated expression of subunits of eukaryotic initiation factor (eIF: EIF2s, EIF3s, EIF4Gs and EIF4As)¹⁰⁰, a complex implicated in the initiation of protein synthesis in eukaryotes. Supporting this finding, pharmacological inhibition of formation of the eIF4F complex reduced the viability of cell lines derived from Group 3 MBs in vitro¹⁰¹. Interestingly, discrepancies between mRNA and protein expression revealed activation of RTK signalling in Group 4 through aberrant expression of ERBB4 and the phosphorylated form of the tyrosine-protein kinase SRC (FIG. 3). In utero (embryonic day 13.5) electroporation-mediated overexpression of activated SRC in combination with dominant-negative p53 in the developing mouse cerebellum induced Group 4-like MB¹⁰⁰, functionally substantiating observations gleaned through proteomics. While the mechanisms responsible for the mRNA- and protein-level discrepancies seen in Group 3 and Group 4 MBs are likely due to a multitude of factors, translational effects mediated through MYC or MYCN are suspected to play a role^{100,103,104}.

Phosphoproteomics can indicate protein kinase activity and potential opportunities for therapeutic intervention through the administration of pharmacological inhibitors. Bioinformatic analyses of MB phosphoproteomic data predicted the activation of several prominent kinases, including glycogen synthase kinase 3 β (GSK3 β ; Group 4 and SHH-b), PRKDC (also known as DNA-PKcs; Group 3 and WNT), cyclin-dependent kinase 5 (CDK5; Group 4) and CDC-like kinase 1 (CLK1; Group 3) and casein kinase 2 (CK2; Group 3). Also, kinome analyses using high-throughput peptide phosphorylation profiling revealed two distinct protein-signalling signatures in MB: MYC-like protein signalling, observed in the majority of SHH and Group 3 MBs, and protein-signalling profile 2, characterized by DNA damage response and apoptotic and neuronal signalling in the majority of Group 4 MBs (REF.¹⁰⁴). Hence, these findings reinforce the idea that protein activity might reveal unifying and/or distinct tumour biologies among MB subgroups. Also, several post-translational modifications (PTMs) of key players in MB have been

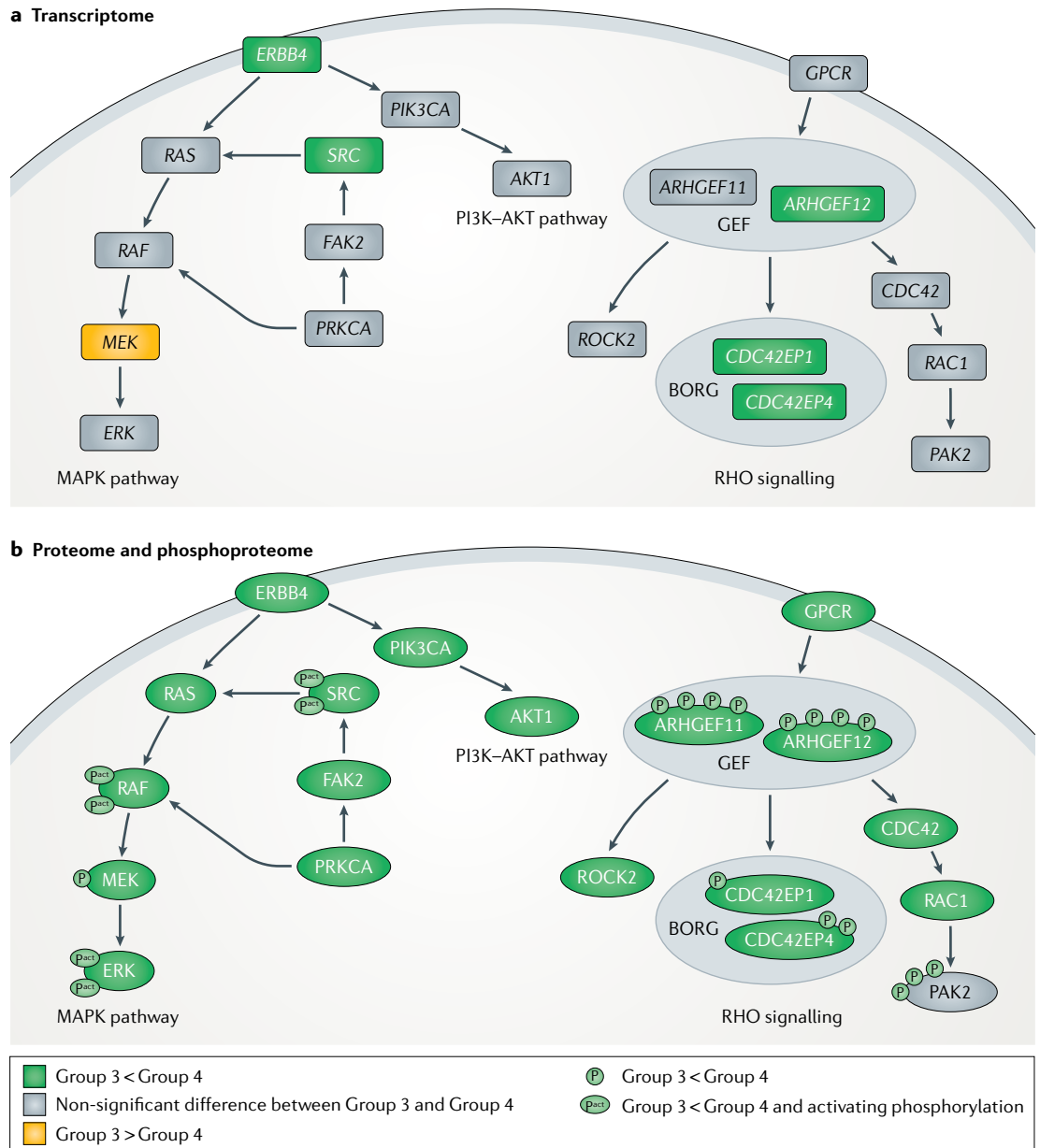


Fig. 3 | Aberrant signalling pathways implicated in Group 3 and Group 4 MB by proteomics. Colours indicate differential expression of mRNA (part **a**; $P < 0.05$, *t*-test) or protein and phosphoprotein abundances (part **b**) between Group 3 and Group 4 medulloblastomas (MBs), as reported in the Forget et al. study¹⁰⁰. Phosphorylation is indicated as a circled P, and P^{act} indicates that the phosphorylation event has previously been shown to activate the protein. ARHGEF11, RHO guanine nucleotide exchange factor 11; BORG, binder of RHO GTPases family; CDC42EP1, CDC42 effector protein 1; FAK2, focal adhesion kinase 2; GEF, guanine nucleotide exchange factor; GPCR, G protein-coupled receptor; PAK2, p21 activated kinase 2; PIK3CA, PI3K catalytic subunit α ; PRKCA, protein kinase C α ; ROCK2, RHO-associated protein kinase 2.

identified. For instance, in SHH MB, deacetylation of GLI1 and GLI2 proteins induces their transcriptional activity¹⁰⁵, while phosphorylation of the transcription factor ATOH1 by Janus kinase 2 (JAK2) controls tumour growth¹⁰⁶. Besides phosphorylation and acetylation, a myriad of other PTMs, including ubiquitylation, methylation and others, have yet to be investigated in MB. The field of proteomics is rapidly advancing, and, along with further technological and bioinformatic breakthroughs, a deeper characterization of MB subgroup biology will undoubtedly follow.

Intratumoural heterogeneity

Phenotypic heterogeneity of individual cells within tumours has been a longstanding interest in MB research. Following similar discoveries in other solid tumours¹⁰⁷ and in leukaemia^{108,109}, Peter Dirks and colleagues first reported the discovery of a stem-like tumour cell population in samples from patients with MB characterized by the neural stem cell surface marker CD133 (positive for 6–21% of cells)^{110,111}. These cells show a marked capacity for proliferation, self-renewal and neuronal differentiation, both in vitro by neurosphere

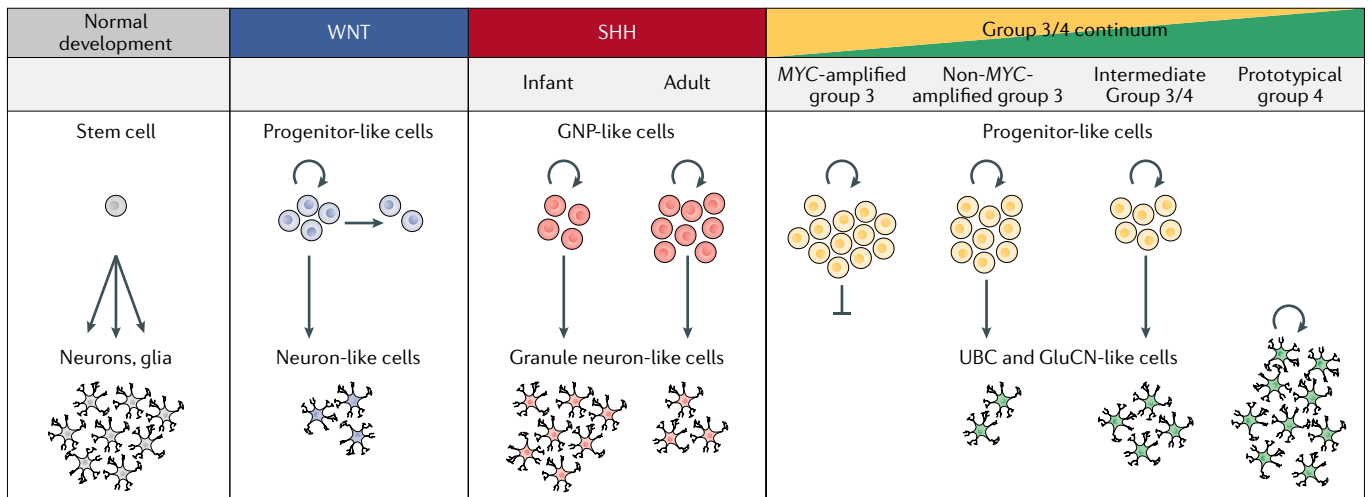


Fig. 4 | MB subgroup cellular hierarchies deduced from single-cell RNA sequencing. Illustration of malignant cell types inferred in medulloblastoma (MB) subgroups^{121,122}. WNT MB comprises undifferentiated progenitor-like populations and more differentiated neuron-like cells. The undifferentiated, cycling cells in Sonic hedgehog (SHH) MB resemble granule neuron progenitor (GNP) cells, whereas differentiated cells resemble granule neurons. The frequency of these cell populations varies with age-associated subtypes, with more differentiated cells in infant patients and more GNP-like cells in adults. Group 3 and Group 4 MBs comprise undifferentiated progenitor-like cells and more differentiated types of neurons that share expression of marker genes with glutamatergic cerebellar nuclei (GluCN) and unipolar brush cells (UBCs). A continuum of frequencies of these cell types is observed, with Group 3 tumours being fully or mostly undifferentiated, Group 4 tumours being predominantly differentiated and some intermediate tumours being located in between. These transcriptional states are superimposed on genetic alterations that are associated with molecular subtypes.

Single-cell RNA sequencing

Emerging technology that enables unsupervised characterization of transcriptional profiles in individual cells of healthy and diseased tissues. The throughput of this technology has steadily increased over recent years, now enabling profiling of tens of thousands of individual cells in a single experiment.

Granule neuron progenitor (GNP).

A progenitor cell type that gives rise to granule cells, the most common type of neuron in the mature cerebellum; the presumed developmental origin of Sonic hedgehog medulloblastoma.

Unipolar brush cells (UBCs).

Rare glutamatergic interneurons found in the cerebellar cortex and in the dorsal cochlear nucleus. Recent studies have identified transcriptional similarities between UBCs in mouse and human Group 4 medulloblastoma.

Glutamatergic cerebellar nuclei (GluCN).

Also referred to as deep cerebellar nuclei. Cells that function (along with GABAergic interneurons) as the main output centres of the cerebellum.

culture and in vivo by mouse xenotransplantation. In mice, as few as 1,000 CD133⁺ cells were sufficient to initiate tumours that phenotypically resembled the original tumour, whereas 50,000 CD133⁻ cells failed to establish tumours. CD133 is most highly expressed in Group 3 MB¹¹², but it does not mark tumour-propagating cells in SHH-MB GEM models^{113,114}. Instead, the neural stem cell surface antigen CD15 was found to enrich for tumour-propagating cells in this subgroup. Further analysis of the *Ptch1*^{+/-} SHH-MB GEM model identified a rare, quiescent SOX2⁺ cell population that gave rise to rapidly cycling progenitors¹¹⁵.

In recent years, single-cell transcriptome sequencing (that is, single-cell RNA sequencing; scRNA-seq) has emerged as a powerful method to decipher cellular states in healthy and diseased tissues in an unbiased way, as exemplified in adult and paediatric gliomas¹¹⁶⁻¹²⁰. Recently, two independent studies applied scRNA-seq to cohorts of primary MBs, showing that MB displays subgroup-specific transcriptional heterogeneity at the single-cell level^{121,122}. Analysing eight patients from either the SHH, Group 3 or Group 4 subgroup, Vladoiu and colleagues demonstrated mixed populations of cells with divergent differentiation along cerebellar neuronal lineages¹²¹. Analysing 25 patients across all molecular subgroups, we identified subgroup-specific undifferentiated and differentiated neuronal-like malignant populations¹²² (FIG. 4). Both studies confirmed the resemblance of SHH tumours to cerebellar granule neuron progenitor (GNP) cells, in agreement with earlier experimental evidence^{13,123} (FIG. 4; BOX 1). Interestingly, adult SHH-MB tumours showed a higher fraction of undifferentiated GNPs than did infant tumours, which might be linked to the divergent biologies of these groups¹²² (FIG. 4).

Combined analysis of Group 3 and Group 4 tumours revealed a related developmental trajectory, from primitive and progenitor-like to more mature, neuronal-like cells, the relative proportions of which distinguished the subgroups¹²² (FIG. 4). MYC-amplified Group 3 MBs only comprised undifferentiated progenitor-like cells and did not show any capacity to differentiate¹²². Most other Group 3 MBs showed a small degree of neuronal differentiation (<10% of cells), indicating that tumour cells maintained the capacity to differentiate. Interestingly, a subset of tumours characterized as ‘intermediate’ cases by DNA methylation-based classification exhibited both undifferentiated and differentiated populations in varying proportions¹²², providing an explanation for the known challenges associated with confident classification of some Group 3 and Group 4 MB cases to either subgroup by bulk molecular profiling (FIG. 4). Prototypic Group 4 MBs almost exclusively comprised more differentiated neuronal-like cells resembling unipolar brush cells (UBCs)^{121,122} and glutamatergic cerebellar nuclei (GluCN)¹²² (FIG. 4; see BOX 1).

Recent parallel gene expression profiling and exome sequencing of 47 multiregional biopsies from eight patients demonstrated that MB is characterized by spatially homogeneous transcriptomes, in which biopsies from the same patient were more similar to each other than to those of other patients¹²⁴. This was contrary to findings in glioblastoma, in which different biopsies from the same patient were classified as different transcriptional subtypes¹²⁴. However, the same study reported high levels of genetic heterogeneity in MB at the level of SCNAs and somatic mutations. As the affected genes included therapeutic targets, these observations put into question whether single biopsies

are truly representative of the entire tumour and whether monotherapies can effectively treat MB.

Insights into relapse and metastasis

Since MB relapse and metastases remain the most significant morbidity factors influencing patient outcomes¹²⁵, an improved understanding of the molecular events driving treatment resistance and recurrence represents a major priority for the field. To date, comparative

studies of primary versus relapsed disease have been exceedingly limited and restricted to relatively modest cohorts. Application of the NanoString-based molecular classification assay confirmed that MB subgroup status is conserved between primary and relapsed disease, reinforcing the concept that the individual MB subgroups constitute distinct diseases¹²⁶. A complementary subgrouping approach later independently confirmed this observation¹²⁷. The specific emergence of *MYC* or *MYCN* amplifications and p53 pathway defects (namely *TP53* mutation and *CDKN2A* deletion) at relapse was also reported¹²⁷. An elegant SHH-MB GEM model made using the *Sleeping Beauty* transposon system (*Ptch1*^{+/-}, *Math1-SB11*, *T2Onc* or *T2Onc2*) functionally substantiated the genetic divergence of primary versus recurrent tumours¹²⁸. In the same study, genomic characterization of a cohort of 36 primary and relapsed tumours from patients with MB indicated that recurrent tumours harboured a profound increase in mutational burden and SVs. More extensive efforts are ongoing that are sufficiently powered to build on these studies and to provide further understanding of the molecular basis of MB treatment failure and relapse.

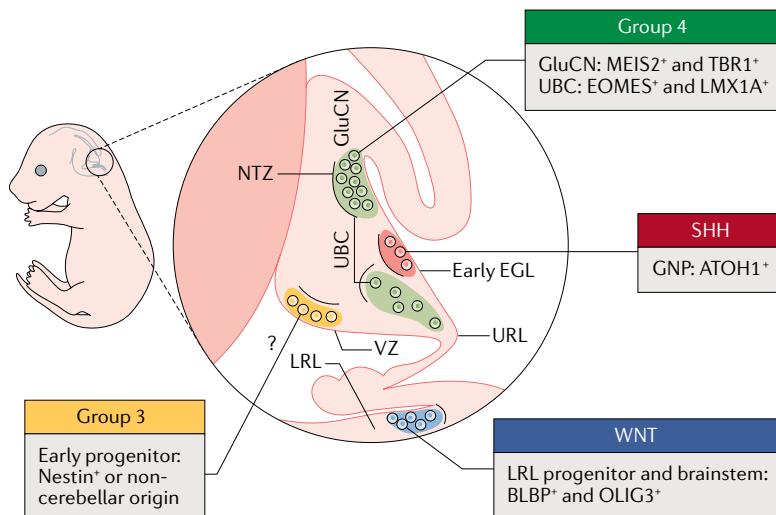
MB metastasis usually occurs via leptomeningeal dissemination (LMD) that remains confined to the CNS and spinal cord¹²⁵. Approximately one-third of all patients with MB have metastatic disease at diagnosis, with patterns and frequencies that vary considerably according to molecular subgroup¹²⁹. Patients who fail conventional therapy and/or who relapse with metastatic disease share a universally dismal prognosis, with nearly all patients succumbing to their refractory disease. Garzia and colleagues inferred the presence of circulating tumour cells (CTCs) on the basis of careful analysis of specific mutations in simultaneously collected patient-matched primary MB, leptomeningeal metastatic MB and peripheral blood¹³⁰. These mutations were clonal in the metastasis, clonal or subclonal in the primary tumour, and present at very low fractions in the peripheral blood. In three of six peripheral blood samples, exceedingly rare CD56⁺/CD45⁻ morphologically abnormal cells were identified, further supporting the presence of rare CTCs in the blood of some patients with MB. These molecular and cellular observations derived from patient samples were strengthened by a series of innovative mouse-modelling experiments, including the implementation of a parabiosis model that established the haematogenous spread of implanted MB cells and recipient (sibling mouse) animals. Comparison of gene expression profiles for a limited set of patient-matched primary and metastatic MB samples in the same study identified overexpression of the chemokine *CCL2* in the metastatic compartment, which was further substantiated through additional molecular and functional analyses, suggesting that aberrant *CCL2* signalling may be an important mediator of LMD in MB.

Emerging clinical implications

Risk stratification. There has been a cautious yet steady transition of discoveries made in the research arena into the clinic. Indeed, clinical protocols for patients with

Box 1 | Cellular origins of MB subgroups

Because medulloblastoma (MB) subgroups are enriched for specific genetic alterations and exhibit different epigenetic and transcriptional profiles, it has long been suspected that they arise from distinct cellular populations or developmental lineages¹⁴³. The figure depicts an embryonic mouse cerebellum (estimated development at ~13.5 days) highlighting cellular populations proposed to be developmentally linked to specific MB subgroups. Mouse models of the WNT and Sonic hedgehog (SHH) subgroups have substantiated lower rhombic lip (LRL) progenitors (BLBP⁺) and granule neuron progenitor (GNP) populations (ATOH1⁺), respectively, as probable cells of origin for these subgroups¹⁴⁴. Multiple orthotopic, transgenic and somatic gene transfer mouse models have demonstrated that a variety of stem/progenitor cell populations can be effectively transformed in order to replicate the molecular and phenotypic features of MYCN-driven SHH MB¹⁴⁵ or MYCN- or MYC-driven Group 3 MB¹⁴⁶⁻¹⁴⁹. Mapping the MB enhancer landscape enabled inferences as to the master transcription factors (TFs) governing subgroup-specific tumour biology. LMX1A, EOMES and LHX2 were predicted to function as master regulators of Group 4 MB⁹⁴. Developmentally, these master TFs regulate lineage specification for restricted glutamatergic progenitor cell populations born out of the cerebellar upper rhombic lip (URL) during cerebellar morphogenesis^{150,151}, including glutamatergic cerebellar nuclei (GluCN; also known as deep cerebellar nuclei) and unipolar brush cells (UBCs). Since LMX1A, EOMES and LHX2 were inferred to be highly specific super-enhancer-regulated candidate master TFs in Group 4 MB, the aforementioned glutamatergic populations expressing these markers were proposed as the putative lineages of origin for this MB subgroup⁹⁴. More recently, comparative cross-species transcriptomic analyses have complemented these observations, using single-cell transcriptional profiles of the developing mouse cerebellum as a reference for mapping MB subgroup origins^{121,122}. Granule neuron lineage populations were highly correlated with SHH MBs, reinforcing the expansive literature implicating GNPs as their developmental origin. Interestingly, GluCN and UBCs were both shown to be highly transcriptionally similar to Group 4 MBs, suggesting that these populations could represent bona fide cells of origin for this subgroup of patients. Early embryonic Nestin⁺ progenitor cells were suggested as being highly correlated with Group 3 MB¹²¹; however, these results were not supported in the companion study¹²². Deeper molecular analyses of these novel candidate populations and functional studies that mimic the relevant candidate driver alterations in the correct lineage at the correct developmental stage are ongoing and will be required in order to confirm these initial findings.



EGL, external granule layer; NTZ, nuclear transitory zone; VZ, ventricular zone.

MB have begun to implement molecular subgroup-informed strategies for treatment stratification. SJMB12 (NCT01878617 (REF.¹³¹)), an active trial for newly diagnosed patients with MB, includes separate treatment arms for patients in the WNT, SHH and non-WNT/non-SHH (that is, Group 3 and Group 4) subgroups. According to this protocol, patients with clinically standard-risk WNT MB (non-metastatic with near total surgical tumour resection) receive reduced CSI (15 Gy, as opposed to the standard dose of 23.4 Gy administered to patients with standard-risk MB), owing to the highly favourable outcomes that have been consistently reported for WNT MB since 2005 (REFS^{33,34}). Skeletally mature (that is, females with a bone age ≥ 15 years or males with a bone age ≥ 17 years) patients with SHH MB who are enrolled in SJMB12 receive the SMO inhibitor vismodegib on top of standard-of-care chemotherapy and CSI. In contrast, patients with non-WNT/non-SHH forms of MB are further stratified into standard- and high-risk treatment arms based on a combination of clinical- and molecular-risk factors, including the extent of resection, metastatic status and *MYC* amplification status. A trial for newly diagnosed patients that is ongoing in Europe, International Society of Paediatric Oncology PNET 5 (NCT02066220 (REF.¹³²)), is likewise stratifying patients with WNT MB in a low-risk treatment arm and administering reduced-dose CSI (18 Gy) compared with the patients in other MB subgroups, who are treated according to standard of care; a similar trial for patients with WNT MB by the Children's Oncology Group (ACNS1422; NCT02724579 (REF.¹³³)) is ongoing in North America. Despite these examples of molecularly informed risk stratification in current MB trials, the clinical arena still lags behind the pace of retrospective research studies. Recent molecular studies conducted on both large retrospective and trial cohorts have provided an increased rationale for further molecularly driven stratification in future protocols, especially within specific MB subgroups^{29,31,35,44,134}. However, due to the reasonably high cure rates for all patients with MB (~70–75%), the way forward must continue to be methodical. With children's lives at stake, rather than leaping into untested waters, trials need to simultaneously build on past successes, chip away at lessening the morbidities of current therapy and improve cure rates for the patient population who consistently fail to benefit from current therapies. The answer lies in using the new molecular understandings to improve risk stratification, such that judicious dose reductions can be applied to the lowest-risk groups while promising experimental therapy can be given to the highest-risk patients. In this way, reductions in long-term treatment-related morbidities are prioritized for patients who are surviving, and the chance of survival is increased for patients who are currently dying of the disease.

Genetic predisposition. Hereditary genetic predisposition to MB remains an underappreciated clinical challenge. Recent data collected on large retrospective MB cohorts suggest that, especially in the case of SHH MB, the proportion of patients with an underlying cancer predisposition might approach 25% or even higher³⁶. In patients with WNT MB, the proportion appears to

be in the range of 5–10%. At this stage, these estimates only account for known cancer predisposition genes, and efforts to systematically identify other pathogenic events in the germ line of patients with MB are currently ongoing. American Association for Cancer Research (AACR) guidelines recommend genetic testing and counselling for paediatric tumour types that frequently occur in the context of genetic predisposition (where a threshold of $\geq 10\%$ of a given cancer's incidence is attributed to pathogenic germline mutations)¹³⁵. The prevalence of pathogenic germline events in known cancer predisposition genes qualifies SHH MB, and potentially WNT MB, for a general recommendation to offer genetic testing and counselling prior to adjuvant therapy. This infrastructure, however, is currently not in place in the majority of treatment centres across the world. The largest MB predisposition study to date³⁶ identified *APC* germline mutations in patients with *CTNNB1* mutation-negative WNT MB, as well as significant enrichment of *TP53* (Li–Fraumeni syndrome), *SUFU*, *PTCH1* (Gorlin syndrome), *BRCA2* and *PALB2* pathogenic germline variants in SHH MB. These genes can be tested together by clinical-grade whole-exome or panel sequencing, but also successively as single-gene tests based on their age associations and, in some cases, a family history. AACR surveillance guidelines are available for *APC*, *TP53*, *SUFU* and *PTCH1*, whereas it remains to be determined how to appropriately manage patients with damaging heterozygous germline MB variants in *BRCA2* and *PALB2*. As this considerable proportion of patients with hereditary MB disease and their families require special clinical attention, including potential treatment modifications, family testing and surveillance, this challenge has to be tackled systematically but expeditiously.

Therapeutic targets. Arguably, it was the discovery of small molecule Hedgehog pathway antagonists (that is, SMO inhibitors) and their preclinical potency against *Ptch1*^{+/−} GEM models¹³⁶ that sparked the need to molecularly identify patients with MB who would benefit from these inhibitors. However, what emerged was molecularly far more complex than had first been envisioned. SHH pathway gene alterations in SHH MB differ according to patient age at diagnosis and subtype^{29,30,38,44}, collectively accounting for the variable responses to SMO inhibitors. *PTCH1* mutations (both germline and somatic) are the most common ones but are present in less than half of all patients with SHH MB, and mostly within infants and adults^{30,38,44}. Germline and somatic *SUFU* mutations are largely restricted to infant SHH MBs, whereas activating somatic *SMO* mutations are enriched in adult patients with SHH MB^{30,38,44}. Similarly, germline and somatic *TP53* mutations, predominantly coincident with *GLI2* and *MYCN* amplifications, are found exclusively in children between the ages of 8 and 17 years^{29,30,38}. These observations are of direct clinical relevance when considering the treatment of SHH MB with SMO inhibitors. The *Ptch1*^{+/−} GEM model¹³⁶ and a patient-derived xenograft (PDX) model from a patient with SHH MB³⁸ harbouring a *PTCH1* mutation were sensitive to SMO inhibitors (HhAntag, vismodegib or sonidegib), whereas a GEM model lacking *Sufu*¹³⁷ or

Sleeping Beauty transposon system

A synthetic DNA transposon system used for random mutagenesis screening and in a recent Sonic hedgehog-medulloblastoma mouse model. Genes affected by genomic insertion of the transposon can be identified through sequencing.

Bone age

Degree of skeletal maturity, an important parameter for determining the clinical use of SMO inhibitors in patients with Sonic hedgehog medulloblastoma. Prolonged exposure to the targeted inhibitor vismodegib has been associated with growth defects in children that have not reached skeletal maturity.

Patient-derived xenograft (PDX)

A model of cancer in which tumour cells from a patient are implanted and maintained in a non-human carrier, most commonly immunodeficient or humanized laboratory mice. PDX models are thought to resemble patient tumours more closely than cell cultures do.

Blood–brain barrier

A semipermeable border formed by endothelial cells lining the cerebral microvasculature that separates the brain from the circulating blood and protects the brain from fluctuations in plasma composition and from circulating agents such as neurotransmitters and pathogens. The blood–brain barrier also presents a challenge for drug delivery when treating brain tumours.

Blood–tumour barrier

Tumour-associated compromise of the blood–brain barrier, resulting in a highly heterogeneous vasculature characterized by non-uniform permeability to small and large molecules.

PDX harbouring *TP53* mutation and *MYCN* amplification exhibited primary resistance to these inhibitors³⁸. These preclinical findings were corroborated in an early-phase clinical trial on patients with relapsed SHH MB who were treated with vismodegib¹³⁸. As was molecularly predicted, responders to SMO inhibition were more likely to have tumours that harboured a *PTCH1* mutation, whereas no beneficial clinical responses were observed in patients harbouring *TP53* or *SUFU* mutations and concurrent *MYCN* and *GLI2* amplifications. Moreover, another significant concern that has restricted the clinical use of SMO inhibitors is the emergence of permanent growth plate fusions in the long bones of small children¹³⁹, a morbidity that was predicted in early preclinical studies of young mice¹⁴⁰. Given this, the current clinical strategy has shifted to skeletally mature patients with upstream (that is, *PTCH1* or *SMO*) SHH pathway mutations. While this is an admittedly small population, this approach attempts to first define the benefit of SMO inhibitors in a targeted population, prior to exposing a more vulnerable population to the inhibitors and their morbidities. Collectively, these findings demonstrate the importance of performing thorough preclinical and molecular testing to identify the appropriate patient populations as candidates for molecularly targeted therapy.

Additional translational implications relevant to other MB subgroups have also emerged. For instance, the oncogenic activity of GFI1 in MB is promoted through direct interaction with KDM1A (also known as LSD1)¹⁴¹, a histone lysine demethylase associated with transcriptional repression. Treatment of MBs driven by MYC and GFI1 with LSD1 inhibitors attenuated the malignant phenotype *in vitro* and *in vivo* in a flank PDX model. No treatment effect was observed when treating the same tumours in an orthotopic setting, demonstrating that this model might serve as a preclinical testing vehicle for blood–brain barrier or blood–tumour barrier penetration. Taken together, these results provide preclinical support for treating patients with GFI1 (or GFI1B)-driven MB with LSD1 inhibitors, if a brain-penetrant LSD1 inhibitor becomes available.

With the identification of aberrant TGFβ signalling in Group 3 MB, and RTK signalling in Group 4 MB, new opportunities for preclinical testing of inhibitors of these pathways have likewise emerged^{100,142}. Additional studies evaluating the efficacy of such agents in treating accurate preclinical MB models will be necessary before their translation to the clinic.

Conclusion and future outlook

Through continued multi-omic analyses conducted on unprecedented patient cohorts, MB now represents one of the most extensively characterized cancer entities. Deep understanding of its molecular substructure

and the assignment of known and novel driver gene alterations to specific disease subtypes have created a more refined understanding of MB tumour biology. This knowledge will enable the development of better models that more accurately recapitulate human disease. Moreover, these advances pave the way for the critical functional studies that will be required in order to determine the mechanistic contribution of newly discovered genes and molecular complexes to MB pathogenesis, in the appropriate cellular context. A continued transition from bulk tumour profiling to more detailed analyses of intratumoural heterogeneity in single cells is expected, especially as methods for analysing genetic alterations and the epigenome, transcriptome and proteome at single-cell resolution continue to evolve and become attainable on archival clinical samples. These studies should help further resolve the recently described molecular subtypes within subgroups and elucidate the molecular and cellular basis of MB recurrence and metastasis.

Clinically, the separation of MB along molecular lines into subgroups, and now into subtypes within subgroups, generates concern among many treating physicians that the number of divisions far outpaces our ability to give subtype-specific care. However, while this argument against a complicated classification system is understandable, given that the majority of subtype-specific findings are not immediately targetable by individual medicines, it ignores the clinical benefit of improved risk stratification. Despite reasonably high cure rates, in the range of 70–75% for all patients with MB, the cost of current therapy that combines surgery, CSI and chemotherapy remains unacceptably high. Molecular subgrouping has already demonstrated pronounced differences in survival, and this has afforded the opportunity to trial judicious reductions in therapy to the lower-risk WNT subgroup, while maintaining and optimizing intensive therapy for the high-risk patients with Group 3 MB. Moreover, the more precise and more well defined the subtypes become, the better the opportunity to hone this risk stratification approach. For example, the new subtyping of very young children with MB is anticipated to rapidly lead to new trials, as the toxicities of CSI and chemotherapy remain unacceptably high in this most vulnerable population⁴⁴. Furthermore, if promising targeted therapies, such as SMO inhibitors, LSD1 inhibitors and RTK inhibitors, are to be successful, then it is imperative that these agents be given to the appropriate populations. Even though the end of further evolution of MB molecular sub-classification may be in sight, the impact of these molecularly informed advances on therapy is only just beginning.

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Author contributions

V.H. and P.A.N. researched data for the article, contributed substantially to discussion of the content, wrote the article and reviewed/edited the manuscript before submission. O.A., F.J.S., G.W.R. and S.M.P. researched data for the article,

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Competing interests

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