Molecular genetics of medulloblastoma in children: diagnostic, therapeutic and prognostic implications

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Medulloblastoma is the most common embryonal tumor in children. The current standard of care comprises surgical resection, radiation and chemotherapy. Patients are stratified into standard and high risk based on the degree of resection, presence of metastatic disease and histopathology. Cure rates dramatically improved during the past decades reaching 70–80% (high and average risk, respectively). Infant medulloblastoma has a worse outcome as the use of radiation therapy is very limited, a group of patients still has dismal outcome despite appropriate therapy, and the unacceptable long-term therapy side effects in survivors. Advanced molecular techniques have allowed scientists to discover four distinct molecular subgroups and correlate them with multiple factors such as histopathology, clinical behavior and possible therapeutic targets.

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Medulloblastoma (MB) is the most common malignant brain tumor (BT) in children. It comprises up to 20% of all pediatric BT and 64% of all embryonal BT [1]. It is commonly diagnosed before 19 years of age and has a male predominance [1,2]. Progress in MB classification, diagnosis, treatment and prognosis reflects a fascinating journey in which survival has improved from near zero to as high as 80% (Figure 1). This has been achieved with better understanding of the disease histology and incorporation of multimodality treatment including surgery, radiation and chemotherapy [3,4]. Patients are currently classified into standard and high risk based on postoperative residual disease (>1.5 and <1.5 cm²), histology and metastatic disease at diagnosis [5,6]. Young age is also prognostic as children <3 –5 years of age do not receive craniospinal irradiation further compromising their outcome [7,8]. This success was not without a cost. Long-term survivors, especially young children (<5 years of age), developed multiple complications, most important of which are the devastating effects on the IQ levels and cognitive function. Other complications included endocrinological, hearing, fertility, cardiopulmonary fitness and physical performance problems, in addition to the risk of secondary malignancies [9–12].

Since the early 1990's, cumulative findings have gradually led to the discovery of molecular characteristics in medulloblastoma and supported the classification into four molecular subgroups (Wingless [WNT], Sonic Hedgehog [SHH], group 3 [G3] and group 4 [G4]). This was reflected in the consensus conference in Boston in 2010 and became internationally accepted following the publication of Taylor and colleagues in 2012 [6,13–21]. In the following years, a massive amount of genomic characterization using advanced molecular techniques has been conducted on hundreds of primary tumors by several independent groups, and each year multiple publications emerge, either introducing new findings in the field or refining earlier ones by discovering substructures within each group reflecting distinct clinicopathological and molecular features [11,20–33]. This has been largely possible due to parallel advances in genetic testing technologies, such as expression profiling, whole-genome sequencing, whole

Figure 1. Historic timeline of medulloblastoma progress.

exome sequencing and methylation profiling, among other technologies [16,34]. In 2016, WHO Classification of Tumors of the Central Nervous System incorporated the MB molecular subgroups into the MB classification [35].

In addition to genetic alterations, epigenetic deregulation occurs in 30–40% of MB, declaring itself as major driver in this disease [33,36,37]. This review will focus on the molecular characteristics of MB in children and their implications on various aspects of the disease (see Table 1 for summary).

WNT-subgroup

The WNT is a family of growth factor receptors that are involved in embryogenesis and in cell–cell control mechanisms [17,38]. WNT/ β -catenin signaling is a highly conserved pathway that regulates key cellular functions including proliferation, differentiation, migration, genetic stability, apoptosis and stem cell renewal. Aberrations in this pathway are implicated in several cancers including colon cancer, breast cancer, adrenocortical tumor, melanoma, high grade glioma and MB [38].

The WNT subgroup is the least common among the 4 MB molecular subgroups, comprising around 10–15% of the cases. It can occur at any age; however, it is most commonly encountered in children 6–12 years of age (peak incidence of 10–12 years) with slight female predominance [13,21,30,34,39,40]. Occurrence in children less than 4 years old is extremely uncommon [21,36,41]. The vast majority of WNT tumors are of classic histology; only rare cases are of large cell/anaplastic (LCA) variant and are never nodular desmoplastic (ND). Metastatic disease is encountered in less than 10% of newly diagnosed cases (5–10%) [36,40,42]. The lower rhombic lip progenitor cells of the dorsal midbrain have been proven to be the cells of origin in WNT tumors. This is supported by the fact that anatomically, WNT MB often develop in a central location, frequently abutting the brainstem and protruding through the foramen of Lushcka [28,34,43]. Total of 85–90% of WNT MB cases harbor mutations in *CTNNB1*, the gene encoding for the protein β -catenin. Mutant β -catenin protein is resistant to degradation, leading to its accumulation in the cell nucleus [42]. Whole-genome sequencing has identified recurrent somatic mutations in WNT MB other than *CTNNB1* gene. The most prevalent among those genes are *DDX3X*, *SMARCA4*, *TP53* and *KMT2D*. These gene mutations are found in approximately 50, 26, 15 and 12% respectively, and they are not exclusive to WNT MB. The *DDX3X* mutations are found in 11% of SHH MB and 3% of group 3 MB, *SMARCA4* is a crucial component of the SWI/SNF pathway, which has a major role in the development of multiple cancers including ovarian cancer, melanoma, renal and liver cancer as well as other tumors. The *TP53* mutations are found in 14% of SHH MB, and *KMT2D* is encountered in 13% of SHH MB and 4% of group 3 MB. These genes encode proteins that interact with nuclear β -catenin and remodel chromatin, suggesting that cooperative mutations occur in the development of this tumor subtype [13,21,28,30,40,42,44]. Monosomy 6 is found in 80–85% of WNT MB, which is strongly associated with a WNT pathway immunohistochemical profile [45,46]. It is a hallmark chromosomal aberration of WNT MB and is rarely seen in other subgroups although a minority of tumors retain two copies of the chromosome [36,42,45,47]. The WNT MB subgroup is most accurately diagnosed by sequencing exon 3 of *CTNNB*, DNA methylation profiling or gene expression profiling [48,49]. These methods are expensive

Table 1. Summary of medulloblastoma demographics and molecular characteristics by molecular subgroup.

Subgroup	WNT		SHH				G3			G4

Frequency	10–15%		28–30%				25–28%			40–45%		
Age range (years)	6–12 (peaks at 10–12), >17 (β subgroup)		Bimodal (<4, >16)				Infants and young children			All age groups (median 9)		
Histology	Mostly classic, rarely LCA, never ND		Mostly ND, classic & LCA (less common)				Classic (most common), LCA			Classic and LCA (less common)		
Cell of origin	Lower rhombic lip progenitor cells of the dorsal midbrain		Cerebellar GCP of the EGC				Cerebellar GCP of the EGC (prominin 1+/-lineage–neural stem cells)			Unknown		
Subgroups	α & β		α, β, γ & δ				α, β & γ			α, β & γ		
Anatomic location	Central, frequently abutting brainstem and infiltrating foramen of Lushcka		Hemispheric (rarely midline)				Midline (filling fourth ventricle), hemispheric cases reported			Midline (filling fourth ventricle)		
Metastatic disease at diagnosis	8.6% (α)	21.4% (β)	20%(α)	33%(β)	8.9%(γ)	9.4%(δ)	43.4%(α)	20%(β)	39.4%(γ)	40%(α)	40.7%(β)	38.7%(γ)
IHC	Nuclear β-catenin		Filamin A, YAP1, SFRP1, GAB1				NPR3 (suggested, needs validation)			KCNA1 (suggested, needs validation)		
Chromosomal abnormalities	Monosomy of chromosome 6 (diploid in older patients)		9q deletion, loss of 10q and 17p, gains of 3q and 9p				i17q, 1q gain, loss of 5q and 10q			i17q, loss of 8, 10, 11 and gain of 4, 7 17, and 18		
Genetics	CTNNB1, DDX3X, SMARCA4, TP53 and KMT2D		PTCH1, TP53 (high prognostic impact), KMT2D, DDX3X, MYCN, BCOR, LDB1, GLI				MYC, OTX2, SMARCA4, NOTCH, TGF-β			MYCN, CDKNA, SNCAIP duplications		
Epigenetics	SRFP family (inhibitors of WNT signaling pathway)		MLL2/KMT2D & MLL3/KMT2C (promote H3K4me2/3), SMARCA4				H3K27, H3K4, BRD/BRD			H3K27, H3K4, KDM family		
Diagnosis	Exon 3 sequencing for CTNNB1 or both nuclear β-catenin (IHC) and monosomy 6 (FISH)		Gene expression/methylation profiling and/or IHC for filamin A and YAP1 +/- GAB1				Genome wide methylation, expression array			Genome wide methylation, expression array		
Prognosis (5-year survival)	97% (α)	100% (β)	69.8%(α)	67.3%(β)	88%(γ)	98.5%(δ)	66.2%(α)	55.8%(β)	41.8%(γ)	66.8%(α)	75.4%(β)	82.5%(γ)
Possible targeted therapies	Trichostatin A (HDAC inhibitor), other small molecules to inhibit WNT pathway in preclinical studies		Vismodegib, arsenic trioxide, bromdomain inhibitors, aurora kinase inhibitors				Bromdomain inhibitors, HDAC inhibitors, PI3K inhibitors			None		
Genetic predisposition	APC (germline), most tumors lack CTNNB1 mutation		SUFU, PTCH1, TP53, PALB2, and BRCA2				PALB2 and BRCA2 (rare)			PALB2 and BRCA2 (rare)		
Other	Increased permeability of BBB, excellent outcome even with residual tumors											
BBB: Blood–brain barrier; EGC: External granule cell; G3: Group 3; G4: Group 4; GCP: Granule cell progenitor; HDAC: Histone deacetylases; IHC: Immunohistochemistry; LCA: Large cell/anaplastic; SHH: Sonic Hedgehog; WNT: Wingless.												

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and are not readily available in most of the centers. A reliable and more accessible way to diagnose this subgroup is via identification by two markers performed in accredited diagnostic laboratories: nuclear β-catenin accumulation, monosomy 6 (whole chromosome loss) by FISH [48,50,51]. Relying solely on the positivity of immunohistochemistry for beta-catenin may lead to an incorrect diagnosis of a WNT subgroup due to patchy nuclear accumulation in some WNT cases. This applies also to monosomy 6, which can be occasionally observed in other subgroups [48–50].

The WNT-MB was molecularly separated into two subgroups: WNT-α (70%) and WNT-β (30%). The former subgroup occurs mostly in children with ubiquitous monosomy 6, unlike the latter group in which most of the patients are adults and chromosome 6 is diploid [28].

Prognosis in patients with WNT MB is generally excellent even in the presence of somatic *TP53* mutation, incomplete resection and/or metastatic disease at presentation [48,52–54]. There was no significant difference in survival between WNT- α and WNT- β [28]. With current therapy, 5-year survival exceeds 90% in patients below 16 years of age, older patients have worse outcome and are not considered as a low risk group [28,48]. Increase in the permeability of the blood–brain barrier due to the secretion of WNT antagonists allows better penetrance of chemotherapy to the tumor site especially for medications that typically do not cross the blood–brain barrier [23,52,55], which might, in part, explain the outstanding outcome this subgroup.

Although MB patients are currently classified as either standard or high risk [3], nonmetastatic WNT MB has the most favorable outcome among MB subgroups and can be regarded as ‘the low risk group’. Accordingly, studies are being designed to reduce the intensity of therapy in order to minimize long-term toxicities, especially effects on intelligence quotient and cognitive functioning. The ongoing SIOP-PNET5 (NCT02066220) and SJMB12 (NCT01878617) studies are exploring the possibility of reducing craniospinal radiation therapy to 18 and 15 Gray respectively. Surgery is major part of MB treatment and is usually performed upfront prior to radiation therapy. The current practice favors complete tumor resection to attain a better outcome. This is sometimes achieved at a high cost due to the damage caused by the surgery, especially when the tumor is in critical sites like the brainstem and/or infiltrating through foramen of Lushcka. As shown by Thompson *et al.*, WNT MB has an excellent outcome, even when <90% resection is achieved [56]. This permits less aggressive approach, protecting the patient functionality without compromising the outcome.

Targeted therapy for WNT-MB

Small molecules that can inhibit the WNT pathway and are ideally less toxic and more effective for specific patient subgroups seem to be an ideal and promising target to pursue. Direct targeting of WNT signaling has been difficult, largely owing to the lack of pathway-specific targets and the potential redundancy of many pathway components. Nonetheless, with the help of high-throughput screening of WNT-activated luciferase reporter assays, multiple molecules that can inhibit the WNT pathway have been discovered. These molecules are undergoing preclinical testing [46]. The use of histone deacetylases (HDAC) inhibitors, which disrupts chromatin remodeling, has been suggested in WNT MB patients as it can disrupt chromatin remodeling [36].

SHH-subgroup

The SHH-MB constitutes 28–30% of all MBs and has a bimodal age distribution (infants < 4 years and adults > 16 years old). Metastatic disease at diagnosis occurs in 15–25% of cases. Histologically, ND variant is the most common (~30%) and is almost exclusive for SHH-MB, followed by classic and large cell anaplastic subtypes) [26,34,41,42]. Immunoreactivity for filamin A and YAP1 in SHH tumors are usually strong and widespread. Cytoplasmic GAB1 immunoreactivity ranges from weak to absent [40]. Hence, immunostaining is a good option to identify SHH subgroup if gene expression and methylation profiling are not available or unaffordable.

This MB subgroup was named after the SHH signaling pathway, which is one of the major signaling networks that regulates the key events during developmental processes [21,57]. Deregulations in the SHH signaling pathway may lead to birth defects, tissue regeneration, stem cell renewal and cancer growth [57]. In the brain, granule cell (GC) progenitors migrate from the upper rhombic lip to the external granule layer, where they initiate proliferation in the outer external granule layer. The SHH, secreted by Purkinje neurons starting from embryonal week 18, is a critical mitogen regulating GC progenitor proliferation. Hence, SHH tumors mainly arise in the hemispheric areas, although cases are reported in the midline [58,59]. Germline mutations in SHH receptor PTCH was first linked with MB as a result of the finding that patients with Gorlin syndrome were found to have a strong predisposition to the disease [60]. This opened the door to the discovery of multiple somatic mutations and driver genes in SHH-MB through transcriptional profiling. The most prominent driver genes are *PTCH1* (28%); *TP53* (13.6–21%, almost exclusively in patients 5–18 years old); *KMT2D* (12.9%); *DDX3X* (11.7%); *MYCN* amplification (8.2%); *BCOR* (8%); *LDB1* (6.9%); *TCF4* (5.5%) and *GLI2* amplification (5.2%) [13,15,17,21,47,52,61,62]. The *MYCN* is almost exclusively associated with deletions of chromosome 9q, which is also the location of the *PTCH1* gene (9q22). Less frequently than chromosome 9 loss, SHH-MB might have 10q and 17p losses and gains of 3q and 9p [13].

Since the discovery of the four core MB subtypes in 2010, molecular subgrouping has remarkably revolutionized, ending up with 12 molecular subtypes. Cavalli *et al.* analyzed 763 primary MB samples collected from the Medulloblastoma Advanced Genomics International Consortium and proved that SHH-MB can now be divided into four subgroups (SHH- α , β , γ and δ) reflecting distinct somatic copy-number aberrations, activated pathways

and clinical outcomes [28]. The SHH α affect children aged 3–18 years and have the worst prognosis in this subgroup. Histologically, it can be of LCA or ND subtypes. They are enriched for *MYCN*, *GLI2*, *YAP1* amplifications, *TP53* mutations and copy number alterations (9q, 10q, 17p losses). When compared with other SHH subgroups and WNT-MB, *TP53* mutation was prognostically significant in SHH α subgroup. Metastatic disease occurred in around 70% of cases at diagnosis.

Infant SHH-MBs (age <3 years) mainly belong to β and γ subgroups, although with dissimilar molecular profiles and outcomes. Total of 25% of SHH β harbor focal *PTEN* deletions and have multiple focal amplifications. A third of SHH β are metastatic at diagnosis, which explains the dismal outcome (67% 5-year survival) in this subgroup. Unlike SHH β , SHH γ have a better outcome (88% 5-year survival), balanced genome and no recurrent amplifications. The ND and MBEN are the main subtypes (all MBEN MB are SHH γ). The SJYC07 (NCT00602667) Phase II study showed consistent results with the Cavalli group where infant SHH-MB (iSHH) was separated into two subgroups; iSHH-I and iSHH-II based on the methylation subgroup analysis in relation to the EFS. The iSHH-I was enriched for *SUFU* aberrations and chromosome 2 gain. The 5-year progression free survival (PFS) was 75 versus 28% in iSHH-II versus iSHH-I respectively. This is very important to note as iSHH-II group can be cured with less aggressive therapy (craniospinal radiotherapy, myeloablative chemotherapy or intraventricular therapy), while iSHH-I require aggressive treatment approaches [63].

Adults dominate the SHH- δ subgroup with favorable outcome (88.5%, 5-year survival) and less than 10% cases with metastatic disease at presentation. This subgroup is enriched with ND subtype and *TERT* promotor mutations [64].

Schwalbe *et al.* published the results of their study based on the analysis of 428 centrally reviewed MB cases from patients aged 0–16 years from multiple European centers. They categorized SHH patients into two groups (SHH infant and SHH child). Both groups were enriched in *SUFU* and *PTCH1* mutations and had a 10-year overall survival (OS) of 58 and 48% respectively. The SHH child group showed mutations in *TP53*, *TP53* *GLI* and *TERT*, in addition to *SUFU* and *PTCH1* as well as 9p gain, 1p and 9q deletions [27].

Targeted therapy for SHH MB

The PBTC-025 trial showed promising responses with vismodegib (GDC-0449, Genetech Corporation, MA, USA), a smoothened protein inhibitor, in a subset of adult patients with recurrent SHH-MB. However, responses were shortlived as patients developed resistance to the drug after prolonged exposure. Additionally, patients with immature bony skeletons develop premature fusions of the epiphyses; hence, SMO inhibitors are only indicated with mature skeletons. Patients with downstream *SUFU* and *GLI2* mutations will not benefit from such therapy [31]. The currently ongoing St Jude SJMB12 (NCT01878617) incorporates vismodegib in the treatment of SHH-MB patients. Pending the results of the SJMB12 study, subsets of SHH-MB with poor prognosis (*TP53* [germline and somatic], *SUFU*, *GLI2* mutations), pose a great challenge and mandate newer therapeutic options to improve their prognosis. Arsenic trioxide (*GLI2* inhibitor) and bromodomain (BRD) inhibitors, targeting SHH transcriptional output, seem to have a promising potential [65,66]. Aurora-kinase inhibitors have been suggested as possible therapeutic option for tumors with *MYCN* mutation [67]. All these drugs are still in the preclinical testing phase and require validation in Phase I trials in patients with recurrent disease.

G3-Subgroup

The G3-MB accounts for 25–28% of all MBs [34,42]. It occurs predominantly in males (2:1 male to female ratio). Only two histologic variants are encountered in G3-MB, classic (most common) and LCA. Metastatic disease is very common at diagnosis affecting up to 45% of cases [17,21,42]. Most of the patients recur with metastatic dissemination while tumor bed is free of disease [68]. The proposed cells of origin for this subgroup are cerebellar granule-neuron precursor cells of the external granule-cell layer and are usually located in the midline filling the fourth ventricle [42,43,69].

They display cytoplasmic immunoreactivity for β -catenin and do not stain for GAB1 and YAP1 [40]. Immunohistochemistry for natriuretic peptide receptor-3 has been proposed by several studies [13,70].

Unlike previous data showing that less than a third of G3-MB had at least one probable driver gene [54,70,71], recent evidence based on a large dataset has confirmed that 76% of G3-MB can be related to a potential driver gene [30].

The most common recurrent somatic genetic mutations occurring in G3-MB are the mutually exclusive *MYC* and *OTX2* (16–20 and 7.7% respectively) [42,72]. The *MYCN* amplification is detected in 5% of G3-MB [30,34].

Structural variants, including deletions, duplications and inversions, place the growth factor independent family of proto-oncogenes *GFI1* and *GFI1B* next to active enhancer elements, resulting in their aberrant activation in 41% of the cases [30,34,73]. The most frequently mutated gene in G3-MB is *SMARCA4* (11%). Significant overrepresentation of genes involved in the notch and tumor growth factor- β (TGF- β) signaling pathways are currently well documented in G3-MB [30,74,75].

G3-MB has a remarkably low number of recurring single nucleotide variants and is represented by a series of recurrent DNA copy number gains or losses of chromosomal arms or of whole chromosomes [36,48]. Isochromosome 17 (iq17) is detected in about 25% of the cases. Gain of chromosome 1q and/or loss of chromosome 5q and 10q is also associated with this subgroup [21,34,58,74].

Identification of this subgroup is best performed using either genome-wide methylation, expression array methods or limited panels (22 NanoString signature) [76].

Cavalli *et al.* recently subcategorized 144 G3-MB into G3- α (46.5%), G3- β (25.7%) and G3- γ (27.8%) subgroups with distinct copy-number aberrations, activated pathways and clinical outcomes. The G3- α mostly affects infants and young children up to 10 years of age, with 60% of infants in G3-MB belonging to this subgroup. Metastatic disease is present in 43.4% of cases at diagnosis, and the 5 year OS is 66.2%. Chromosomal abnormalities frequently encountered in this subgroup are chromosomes 8, 10 and 11 loss, chromosome 7 gain, and i17q. *MYC* amplification occurs in 7.1% of the cases. The G3- β commonly occurs in older children and is less frequently metastatic at diagnosis (20%). It is enriched with *OTX1* gain and *DDX31* loss on chromosome 9 and a high expression of *GFI1/1B*. A 5-year OS is 55.8% [28].

The G3- γ has the worst outcome in this group with a 5-year survival of 41.9%, and almost 40% of patients are metastatic at diagnosis. This group has a similar age range as G3- α . It is enriched for iq17 (25%) and frequently harbor *MYC* amplification (20.8%) [28]. Interestingly, G3- γ has a poor prognosis regardless of the *MYC* status, amplifying the role of other factors related to bad prognosis and questioning the previous observations that *MYC* nonamplified G3 MB has a better prognosis [15]. Prognosis of G3 patients in the SJYC07 was poor in both intermediate and high risk groups (5-year PFS 15.4 vs 0.0% respectively, p-value = 0.22) [63].

Therapeutic options for G3-MB

Finding new effective therapies for G3-MB is of high importance given the overall dismal prognosis. High-throughput screening has identified pemetrexed and gemcitabine as possibly effective against G3-MB [32] and is currently being tested by the ongoing St Jude trial (SJMB12, NCT01878617). The BRD inhibitors, interfering with *MYC*-associated transcriptional activity, seem to be a promising option [77]. Targeting the histone methylases EZH2 and KDM6A represents possible future targets [24]. The HDAC inhibitors potentially inhibit *MYC*-driven MB cell growth *in vitro* and synergize with PI3K inhibitors to inhibit tumor growth *in vivo* [78,79].

G4-subgroup

The G4-MB is the most common subtype of MBs. It rarely arises in infants and occurs much more frequently in males than in females (3:1 ratio) across all age groups (median age = 9 years). It accounts for 40–45% of childhood MBs [21,42,52,72]. Classic histology is the commonest finding in G4-MB with LCA less frequently encountered [72]. They are usually located in the midline filling the fourth ventricle [59]. The *KCNA1* has been suggested as a possible immunohistochemical marker for the diagnosis of G4-MB; however, validation of this test is required [13,15]. Similar to G3, G4 has been recently subdivided into three subgroups, based on the analysis of 326 G4-MB, into G4- α (30%), G4- β (33.4%) and G4- γ (36.6%). The G4- α is enriched for *MYCN* (16.7%) and *CDKNA* amplifications, as well as 8p loss and 7q gain. The *MYCN* amplification is not associated with an inferior outcome as in SHH group [34]. The G4- β is strongly enriched for synuclein, α interacting protein (*SNCAIP*) duplications (15%). Similar to G4- α , G4- γ is enriched for with 8p loss, 7q gain and *CDKNA* amplifications. The i17q is encountered in all G4 subgroups, with the biggest share for G4- β (80 vs 41% in G4- α and 26% in G4- γ) [28]. Metastatic disease at diagnosis is almost identical in the three subgroups (~40%). Analysis of outcome (5-year OS) did not show significant difference between the three groups (68.8, 75.4 and 82.5% respectively). In general, G4-MB patients with metastatic disease at diagnosis have a higher risk of relapse, except in the presence of either whole chromosome 11 loss or chromosome 17 gain [34]. Infant G4 seems to experience poor survival as compared with children and adults [80]. In the SJYC07 trial, G4 group was divided into intermediate and high risk subgroups. The 5-year PFS was 16.7 and 0.0% respectively. When compared with the SHH group, the results were inferior (13.3 vs 51.1% respectively); however, not statistically significant (p-value = 0.095) [63].

Therapeutic options for G4-MB

Unfortunately, unlike with other MB subgroups, there are no currently active clinical or preclinical studies for G4-MB. The molecular overlap between G3 and G4-MB might be of use in extrapolating future therapies for this subgroup [34,42].

The good news about breaking down the currently overlapping G3 and G4 MB into several subgroups with distinct molecular characteristics, clinical behavior and prognosis is that it opens the door to reclassifying them into more distinct subgroups. This should eventually reflect on improving our therapeutic strategies and survival rates.

Surgical resection by molecular subgroup

Analysis of degree of resection of 787 MB patients by molecular subgroup showed a benefit of in progression free survival (PFS) of GTR over STR but no significant PFS or EFS benefit between GTR and NTR and no survival benefit to greater extent of resection for patients with WNT, SHH and G3 subgroups. Group 4 patients had increased risk of progression with STR as compared with GTR and NTR, especially in metastatic patients [56]. This study evaluated the largest cohort of patients for the role of extent of resection in the treatment and outcome of MB subgroups. Although it was limited by the absence of central radiology review, it highlights the importance of individualizing the surgical approach in MB patients to avoid surgical morbidity and maintain adequate cure rates.

Epigenetics in MB

Epigenetics involves changes in the gene function that are mitotically and/or meiotically heritable and that do not involve changes in the individual's DNA sequence. This is achieved through DNA methylation, histone modifications, chromatin remodeling, microRNAs and lncRNAs [33,81]. As mentioned in this paper, childhood MBs harbor multiple genetic mutations leading to tumorigenesis. However, there are subtypes (mainly G3 and G4) that have no reported mutations, suggesting the role of epigenetic dysregulation [33]. Hypermethylation of 5'-C-phosphate-G-3' (CpG) dense promoters (CDKN2A, H1C1 and RASSF1) leads to the silencing of tumor suppressor genes, including *PTCH1*, *SFRP* family and *ZIC2* [33,37,82]. The DNA methylation inhibitor like 5-azacytidine has been suggested as a therapeutic option [33,83]. Somatic mutations and copy number aberrations in HDACs, demethylases, histone lysine methyltransferases, as well as members of the polycomb transcriptional repressor complex (PRC2 and PRC1) have been identified across all four MB subgroups. Aberrant histone methylation at H3K27 and H3K4 are found in G3 and G4 MB. Mutations in KDM family members are among the most common recurrent events in G4 MB. The BRD and extraterminal (BET)-containing proteins control MYC levels, a common driver in G3 MB. Small molecule BET inhibitors are currently being studied in preclinical settings [33,77,84,85]. The HDAC inhibitors such as valproic acid and trichostatin might have a role in MB treatment [33]. miRNAs and long noncoding RNAs (lncRNAs) are reported to have a role in MB. miRNAs can either be suppressed (miR-124) or overexpressed (miR-17~192). The lncRNAs regulate the nuclear structure, gene expression and post-transcriptional processing [33,86].

Genetic predisposition in MB

Even prior to the discovery of the four MB subgroups, MB has been observed in conjunction with several rare disorders, including Gorlin syndrome (associated with mutations in *SUFU* and *PTCH1*), Li-Fraumeni syndrome (*TP53*), Fanconi anemia (*BRCA2*) and *APC*-associated polyposis conditions [14,87–89]. In a very recent study, Waszak *et al.* analyzed blood samples for germline mutations from 1022 patients with MB (673 from previous retrospective cohorts and 349 from prospective studies) and matched them with 800 available tumor samples for 110 cancer predisposition genes. Damaging germline mutations were identified in 11% of the retrospective cohort and results were replicated in the prospective study. The most relevant genes identified were *APC*, *BRCA2*, *PALB2*, *PTCH1*, *SUFU* and *TP53* [25]. The prevalence of the above six genes was 6% overall and as high as 20% in SHH-MB. Three important findings were documented in this study: G3 and G4 subgroups were rarely associated with germline mutations and the absence of biallelic germline mutations in mismatch repair genes (only one patient harbored heterozygous germline mutation in *MSH6* gene) [25]. The authors conclude that patients with WNT and SHH MBs are most commonly associated with a genetic predisposition, and half of the identified patients lacked relevant family history. This mandates genetic counseling for those patients for earlier detection and management of associated malignancies.

Molecular genetics at recurrence

Despite the advances in MB therapy [3,5,90], a third of patients suffer from relapse. Outcome in relapsed MB (rMB) is dismal with less than 10% survival rates at best in patients who have received prior irradiation. Therapy in rMB is nonuniform, ranging from palliative therapy in most of the cases, to high dose chemotherapy [81,91,92]. The results of the COG trial for rMB ACNS0821 (NCT01217437) were disappointing with a median event-free survival (EFS) and OS of 6 and 19 months respectively [93]. Ramaswamy *et al.* showed in a group of 34 matched samples of newly diagnosed and rMB patients that MB does not change subgroup at recurrence. Subgroup analysis in this study demonstrated that SHH-MB mostly recurs locally in the tumor bed, while G3 and G4-MB mostly recur at distant sites with de novo tumor bed [68]. Similarly, Hill *et al.*, in a cohort of 29 recurrent tumors with matched samples at diagnosis, demonstrated that MB does not change subgroup at relapse. However, they showed that relapsed tumors commonly acquire combined MYC family amplifications and P53 pathway defects (32%). This combination was encountered across all subgroups, although most commonly seen in SHH (60%) and least encountered in G4 (11%) subgroups [67]. These findings are of great essence to the future trials which can be directed based on tumor biology and patterns of recurrence.

Conclusion

The MB is not a single disease anymore, it is a group of four diseases characterized by genomic, biologic and clinical diversity even within the same group. As discussed earlier, group or subgroup targeted therapies are yet to be investigated; nonetheless, treatment decisions based on specific subgroup characteristics can be made and can readily affect patient survival and long-term side effects.

Future perspective

It is amazing how much MB diagnosis and treatment have advanced since the early years of the 20th century. The discovery of the four robust molecular subgroups, and the recent refinements has transformed our understanding of this disease. Unfortunately, treatment-related morbidity and mortality are still a significant burden. Additionally, outcomes are still suboptimal when it comes to high risk, metastatic and relapsed patients. The immense amount of genetic and epigenetic findings and their correlation with multiple factors including age, anatomic location, risk stratification, surgical approach and patterns of relapse and possible therapeutic targets should be the foundation of future trials that will hopefully deliver a perfectly crafted individualized therapy, providing best outcomes and minimizing treatment side effects. In clinical practice, subgrouping should soon become a standard of care and should be strictly performed in certified laboratories following international guidelines to avoid inaccurate grouping leading to catastrophic consequences. Validating immunohistochemical tests to replace the more advanced expensive genomic testing will ensure accurate subgrouping in centers with high load and low budgets (low and middle income countries).

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Executive summary

- Until recently, medulloblastoma (MB) was classified according to histologic characteristics.
- Current standard of care for MB incorporates maximum safe surgical resection, irradiation and chemotherapy.
- In 2010, four molecular subgroups (Wingless [WNT], Sonic Hedgehog [SHH], group 3 and group 4) were identified and validated by all major research groups.
- WHO CNS classification included the four groups for the first time in 2016.
- Groups were further refined into multiple subgroups reflecting genomic, biologic and clinical heterogeneity.
- Epigenetic dysregulation plays a role in MB.
- Recurrent tumors retain their original molecular characteristics.
- Genetic counseling is important especially for WNT and SHH-MB.

WNT subgroup

- A total of 10–15% of MB, peak age 10–12 years, classic histology most common, lower rhombic lip progenitor cell of the dorsal midbrain are the cells of origin, usually located centrally and infiltrating foramen of Lushcka.
- Presence of nuclear β -catenin and monosomy of chromosome 6 is diagnostic.
- Less aggressive surgery and lower doses of radiation are required.
- Excellent prognosis even with metastatic disease and somatic *TP53* mutation.

SHH subgroup

- Total of 28–30% of MB, bimodal age distribution, nodular desmoplastic histology most common. Cerebellar GCP of the EGC are the cells of origin. Usually located in the cerebellar hemispheres.
- Immunohistochemistry for filamin A and YAP 1 are diagnostic.
- *PTCH1* and *TP53* mutations have high prognostic impact.
- Prognosis is variable by subgroup and dependent on multiple factors (intermediate to very poor).
- Infant SHH-MB may have good or poor outcome based on methylation profiling.
- Targeted therapies are under investigation.

Group 3

- A total 25% of MB, mostly affects infants and young children. Classic histology is most common.
- Usually located in the midline filling the fourth ventricle.
- High rate of metastatic disease at diagnosis.
- High rate of i17q and *MYC* amplification.
- Diagnosis made by genome wide methylation and expression array.
- Prognosis generally poor.
- Suggested targeted therapies, no current clinical trials.

Group 4

- The most common group (40–45%), can occur at any age. Classic histology is most common. Cell of origin is unknown.
- Usually located in the midline filling the fourth ventricle.
- High rate of metastatic disease at diagnosis.
- Intermediate to good prognosis depending on molecular subgroup.
- No possible targeted therapies identified.

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