




Molecular pathological insights reveal a high number of unfavorable risk patients among children treated for medulloblastoma and CNS-PNET in Oslo 2005–2017

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Abstract

Background: An unexplained regional difference in survival was observed in previous publications on outcome for children treated for medulloblastoma and supratentorial primitive neuroectodermal tumor (CNS-PNET) in Norway. We aimed now to reevaluate and perform a retrospective molecular-based risk stratification of all embryonal brain tumors (excluding atypical teratoid rhabdoid tumors [ATRT]) in pediatric patients, who underwent surgery and treatment at Oslo University Hospital between 2005 and 2017.

Procedure: Specimens from all patients <20 years of age with initial diagnosis of medulloblastoma or CNS-PNET were reviewed. Molecular analyses comprised NanoString gene expression, molecular inversion probe profiling, Sanger sequencing, and 850K-methylation analysis. Whole chromosomal aberration signatures were assessed in standard-risk non-WNT/non-SHH medulloblastomas for molecular risk stratification.

Results: We identified 53 non-ATRT embryonal tumors among which 33 were medulloblastomas. Molecular genetic parameters including whole chromosomal aberration signatures allowed classification of 17 medulloblastomas as molecular high risk. These patients had a significantly worse 5-year overall survival than the remaining 16 medulloblastoma patients (52.9% vs. 87.1% $p = 0.036$). Five patients in our cohort had tumors that are considered as new entities in the 2021 classification of tumors of the central nervous system. Five tumors were re-classified as nonembryonal tumors after review.

Conclusion: Molecular-based risk stratification of standard-risk non-WNT/non-SHH medulloblastoma enabled superior identification of medulloblastomas with dismal

Abbreviations: ATRT, atypical teratoid rhabdoid tumor; EFS, event-free survival; FFPE, formalin-fixed, paraffin-embedded; Grp3, Group 3; Grp4, Group 4; MB, medulloblastoma; OS, overall survival; OUH, Oslo University Hospital; PNET, primitive neuroectodermal tumor; WCA, whole chromosomal aberration.

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prognosis. Our cohort demonstrated a significantly increased fraction of standard-risk non-WNT/non-SHH medulloblastoma with molecular high-risk profile compared to other studies, which might have contributed to previously reported unfavorable outcome data.

KEYWORDS

BCOR, CNS neuroblastoma, medulloblastoma, molecular pathology, Norway, PATZ1, risk stratification

1 | INTRODUCTION

Brain tumors are the most common solid neoplasms in childhood and a leading cause of cancer death in young patients.¹ Embryonal tumors account for about 20% of pediatric brain tumors,² and include long-established histological types of medulloblastoma (MB) as well as other entities that were previously collectively referred to as central nervous system primitive neuroectodermal tumors (CNS-PNET). With an increased understanding of the molecular biological background of these neoplasms, it has been shown that CNS-PNET and MB are quite heterogeneous diseases with dramatic prognostic differences associated with genetic subtypes.^{3–5} The revised WHO classification of brain tumors from 2016 considered this heterogeneity and introduced a modular approach for the diagnosis of MB.⁶ In addition to the established histologically defined entities, four molecular entities of MB have been recognized: WNT-activated, SHH-activated and *TP53*-mutant, SHH-activated and *TP53*-wildtype, and non-WNT/non-SHH. The latter encompasses Group 3 (Grp3) and Group 4 (Grp4). Recent studies have identified up to eight molecular subtypes of non-WNT/non-SHH MB, including some with a mixture of Grp3 and Grp4 tumors, indicating overlapping molecular as well as biological features.^{7,8} There is an increasing amount of data confirming the prognostic significance of molecular characteristics in MB. For example, patients younger than 16 years of age with WNT-activated MB have a favorable prognosis with a 5-year overall survival (OS) of >90%,^{9,10} SHH-activated and *TP53*-wildtype MB has a 5-year OS of around 75%, whereas *TP53*-mutant SHH MB is associated with a poor prognosis and an OS of approximately 40% at 5 years.¹¹ Non-WNT/non-SHH MB represent approximately 65% of all MB cases and have heterogeneous clinical characteristics and survival. A recent study showed that the whole chromosomal aberration (WCA) signature strongly correlated with prognosis in patients with standard-risk, non-WNT/non-SHH MB.¹⁰

The basket term CNS-PNET has been discarded and replaced by several newly defined embryonal tumor entities. In this paper, we use the term CNS-PNET because most of our patients were diagnosed and treated before 2016.

An unexplained regional difference in survival was observed in two previous publications on children treated for MB and CNS-PNET in Norway.^{12,13} Solheim and coworkers performed a pure epidemiological study, whereas Stensvold and colleagues reviewed histological specimens according to the WHO 2007 classification of brain tumors.¹² Risk

group allocation was performed in only the most recent study and was based on patient age, extent of residual disease after initial surgery, and evidence of metastatic disease.¹³ However, a major limitation of these studies was the lack of molecular pathological information, including subgroup affiliation. In the light of the substantial advancements in the molecular diagnostic and prognostic factors in MB and the previously limited investigation of these features as part of standard routine at Oslo University Hospital (OUH), we first aimed to perform a retrospective molecularly based risk stratification of all pediatric MB and CNS-PNET patients that underwent surgery at OUH between 2005 and 2017. Second, we wanted to evaluate outcome based on the new molecularly based risk stratification.

2 | MATERIALS AND METHODS

2.1 | Patients

Inclusion criteria were patients younger than 20 years, a histologically confirmed initial diagnosis of MB or CNS-PNET, excluding atypical teratoid/rhabdoid tumor (ATRT), treatment at OUH and date of surgery between January 1, 2005 and December 31, 2017. Limited quality of older tissue specimens hampered retrospective analysis of a longer period. Patients were identified in the archives of the pathological and neurosurgical departments at OUH, as well as from the Cancer Registry of Norway. Medical records were reviewed to register detailed clinical data. September 11, 2020, was defined as the last date of follow-up. See Table S1 for patient characteristics.

2.2 | Histopathological review and molecular analyses

Histopathological specimens from all patients were reviewed by three experienced neuropathologists (authors TP, PN, and GHG). All molecular diagnostic analyses were performed on formalin-fixed, paraffin-embedded (FFPE) samples. Supplementary immunohistochemical analyses were performed in all cases with sufficient biopsy material. The standard panel for MB diagnosis included OTX2, YAP1, p75NGFR, β -catenin, synaptophysin, and NeuN. In addition, silver impregnation (reticulin staining) was used for correct identification of desmoplastic/nodular MB. Supplementary immunohistochemical analyses were

used on a case by case basis depending on relevant differential diagnoses.

Transcription-based molecular stratification was performed using NanoString gene expression profiling, which is known to have a high accuracy using FFPE material, as previously described.¹⁴ We used a molecular inversion probe (MIP) profiling array (335,000 inversion probes; version 2.0; Affymetrix, Santa Clara, CA, USA) to identify aberrant changes in genomic copy number.^{10,15} Raw molecular inversion probe profiles were analyzed using Nexus Copy Number 7.0 Discovery Edition (BioDiscovery; El Segundo, CA, USA). Additional molecular pathological analyses were performed where needed, for example, Sanger sequencing of *CTNNB1* exon 3 if WNT-activated-MB was suspected, *APC* sequencing in *CTNNB1*-wildtype WNT-activated-MB, *TP53* sequencing (exons 4–9) in cases reminiscent of SHH-activated MB, and *H3F3A*-sequencing in cases suspicious for high-grade glioma.^{10,16} DNA methylation profile analysis¹⁷ (MethylationEPIC 850k array platform, Illumina) using the “Heidelberg classifier” version 11b4 and brain classifier prediction version 12.5 was performed for selected cases, particularly if non-WNT/non-SHH MBs could not be classified as Grp3 or Grp4 MBs based on NanoString gene expression profile.

2.3 | Survival analyses and risk stratification

Five-year OS and event-free survival (EFS) were calculated for the entire collective and specific subgroups. Nonembryonal tumors included in the study represent cases, which were initially diagnosed as MB or CNS-PNET, but reclassified as non-embryonal tumor at a later stage or after inclusion in the current study.

2.4 | Clinical high-risk criteria

Clinical high-risk criteria included the presence of metastatic disease, and/or residual tumor $>1.5\text{ cm}^2$ postoperatively, and/or large-cell/anaplastic histology. Additionally, children who had not received radiotherapy due to young age were grouped with high-risk cases in the analyses (see Table S2).

2.5 | Molecular risk stratification criteria

TP53 mutations in SHH-activated MB as well as *MYC* amplification in non-WNT/non-SHH MB (particularly Grp3) are established molecular high-risk markers in MB patients. In addition, WCA signatures in standard-risk non-WNT/non-SHH MB have been shown to have a prognostic effect and allow separation into favorable-risk and high-risk categories.¹⁰ The presence of at least two of the following features, including chromosome 7 gain, chromosome 8 loss, and chromosome 11 loss, is associated with a favorable outcome. Therefore, we classified *TP53*-mutant SHH-activated MB, *MYC*-amplified non-WNT/non-SHH MB as well as clinical standard-risk non-WNT/non-SHH MB without

the 7/8/11 classifier as molecular high risk. Of note, Goschzik et al. classified patients with residual tumor $>1.5\text{ cm}^2$ lacking other high-risk factors as clinical standard risk.¹⁰

2.6 | Statistical analysis

Time of recurrence was defined as the date of the imaging procedure when the recurrent tumor was confirmed or the date of the first patient notification that included recurrence information. To estimate EFS, time from date of primary surgery to date of death, recurrence, or administrative censoring (September 11, 2020), whichever came first, was used. For OS, follow-up time was defined as time from primary tumor surgery to date of death or administrative censoring, whichever occurred first. For EFS, both recurrence and death were defined as events. Comparisons of OS and EFS were made using Kaplan–Meier curves and corresponding log-rank tests. *p*-Values less than 0.05 were considered statistically significant. Stata 17.0 was used for statistical analysis.

2.7 | Ethics

The Regional Committees for Medical and Health Research Ethics of the South-Eastern Norway Regional Health Authority (#2015/2362) and the Data Protection Officer at OUH approved the study.

3 | RESULTS

We identified 53 children and adolescents <20 years of age diagnosed with and treated for embryonal brain tumors, excluding ATRT, at OUH between 2005 and 2017 (see Table S1 for patient characteristics). Five-year OS and EFS were 65.9% and 56.4%, respectively, for all 53 patients (Tables 1 and 2). Patients under the age of 4 years did not undergo radiotherapy and were treated with surgery and chemotherapy only. For all other patients, multimodal treatment included radiotherapy with cerebrospinal irradiation, with exception of patient ID#40. Five cases (5/53; 9.4%) were excluded from molecular pathological revision due to lack of sufficient tumor material. In five cases (5/53; 9.4%), the initial diagnosis was revised to a nonembryonal tumor entity (four pediatric high-grade gliomas and one neuroepithelial tumor with DNA methylation profile of a neuroepithelial tumor with *PATZ1* fusion). These patients had a 5-year OS and EFS of 40.0%.

Eleven embryonal tumors were located supratentorially and were diagnosed as follows: CNS neuroblastoma, *FOXR2*-activated ($n = 4$, see Figure S1 for a representative example), pineoblastoma ($n = 3$), embryonal tumor with multilayered rosettes ($n = 2$), and CNS tumor with *BCOR* internal tandem duplication ($n = 1$). The classification of the remaining supratentorial tumor was a challenge. Despite the location, the tumor was diagnosed as non-WNT/non-SHH MB, (Grp3) based on the NanoString gene signature (*EGFL11*, *GABRA5*, *IMPG2*, *NPR3*, and *NRL*). In addition, a strong expression of *OTX2* was observed. MIP

TABLE 1 Five-year overall and event-free survival

Group/subgroup	Patients, n	Five-year OS (%)	Five-year EFS (%)
All patients	53	65.9	56.4
Excluded due to lack of tissue	5	60.0	60.0
Nonembryonal tumors	5	40.0	40.0
Embryonal tumors other than MB	10	70.0	37.5
Medulloblastoma	33	69.6	63.5
- WNT-activated	6	83.3 ^a	83.3 ^a
- SHH-activated <i>TP53</i> wild type	4	75.0	75.0
- SHH-activated <i>TP53</i> mutant	1	0.0	0.0
- Non-WNT/non-SHH activated	22	68.2	59.1

Abbreviations: EFS, event-free survival; MB, medulloblastoma; OS, overall survival.

^aDisease-specific OS and disease-specific EFS in the WNT-activated medulloblastoma subgroup was 100%. However, two patients deceased from second malignant neoplasms 4 and 10 years after initial surgery, respectively.

TABLE 2 Five-year overall and event-free survival for different clinical and molecular pathological medulloblastoma subgroups

Medulloblastoma subgroups	Five-year OS (%)	Five-year EFS (%)
MB clinical HR, but not mol. HR (<i>n</i> = 10)	90.0	70.0
MB clinical SR and mol. FR (<i>n</i> = 6)	83.3	83.3
MB mol. FR (<i>n</i> = 12)	82.5	82.5
MB clinical SR (<i>n</i> = 19)	73.7	73.7
All MB (<i>n</i> = 33)	69.6	63.5
MB clinical SR, but mol. HR (<i>n</i> = 13)	69.2	69.2
MB non-WNT/non-SHH (<i>n</i> = 22)	68.2	59.1
MB clinical and/or mol. HR (<i>n</i> = 27)	66.7	59.3
Non-WNT/non-SHH clinical SR, but mol. HR (<i>n</i> = 14)	64.3	64.3
MB clinical HR (<i>n</i> = 14)	64.3	50.0
MB mol HR (<i>n</i> = 17)	52.9	52.9
MB clinical and mol. HR (<i>n</i> = 4)	0	0

Note: See Methods section for definition for clinical and molecular pathological risk stratification.

Abbreviations: EFS, event-free survival; FR, favorable-risk; HR, high-risk; mol, molecular pathological; OS, overall survival.

analysis revealed several gains and losses of whole chromosomes or chromosome arms (gain: 1q, 4p, 5, 6, 7, 10p, 19; loss: 1p, 4q, 10q, 16, 18, 21, 22). DNA methylation profile analysis (MethylationEPIC 850k array platform, Illumina) using the “Heidelberg classifier” revealed a classification score of 0.99 for a medulloblastoma, subclass group 3.¹⁷ Radiologically, the patient had lesions supra- and infratentorially with the former dominating, which led to the interpretation of CNS-PNET with metastases. This tumor was now re-classified as metastatic non-WNT/non-SHH MB based on the results of the molecular and histopathological analyses. This patient showed an EFS with follow-

up >10 years. The other 10 patients (10/53; 18.9%) with supratentorial tumors had 5-year OS of 70.0% and 5-year EFS of 37.5%.

Thirty-two infratentorial tumors were diagnosed as MB with the following subgroup affiliation: WNT-activated MB (*n* = 6), SHH-activated MB *TP53* wild type (*n* = 4), SHH-activated MB *TP53* mutant (*n* = 1), and non-WNT/non-SHH MB (*n* = 21). Five-year OS and EFS of the whole MB-cohort was 69.6% and 63.5%, respectively. The group-specific outcome is summarized in Table 1 and Figure 1.

3.1 | Medulloblastoma types, genetically defined

3.1.1 | WNT-activated MB (ID#5, 25, 31, 38, 45, 49)

The six WNT-MB cases showed histological characteristics of a classic MB-variant. Immunohistochemical analysis revealed nuclear accumulation of beta-catenin in all six cases. Monosomy 6 was observed in five biopsies (not in ID#38). In the tumor without monosomy 6, sequence-analysis showed *CTNNB1* wild type, but somatic *APC* mutations (R283* and R414C). Blood analysis revealed no evidence for a germline mutation. Four of the five cases with monosomy 6 had an activating *CTNNB1* mutation, whereas no *APC* mutation was observed in the patient with *CTNNB1* wild type and monosomy 6. Mean age at surgery was 10.7 years. Two patients had a residual tumor >1.5 cm² and therefore were treated as high-risk patients (ID#38 and ID#45). None of the patients experienced a MB recurrence, but two patients (ID#5 and ID#49) died from cerebellar high-grade gliomas, H3 wild type and IDH wild type, 10 and 4 years after initial surgery, respectively. Notably, one of the high-grade gliomas was initially diagnosed as MB recurrence; the glioma diagnosis was made based on histological and molecular pathological re-evaluation in the context of this study (Figure 2). The recurrent tumor showed a *PTPRZ1-MET* fusion, which has been described previously in a high-grade glioma after radiation therapy.¹⁸ This finding further supported the classification as pediatric high-grade glioma.

3.1.2 | SHH-activated MB (ID#19, 29, 34, 37, 52)

Overall, five SHH-activated MB were identified in our cohort. All five were considered clinically high-risk due to age (ID#29, 34, 37, 52) or metastatic disease (ID#19). One histologically anaplastic MB with metastatic disease at diagnosis and rapid clinical progression (ID#19; OS: 9 months) showed a *TP53* mutation (exon 5, p.A159V). No *TP53* alterations were observed in the remaining four cases. At histological analysis, the *TP53* wild-type SHH-activated MB had desmoplastic/nodular (*n* = 3) or extensive nodular (*n* = 1) morphology. Cytological analysis of a lumbar CSF sample from the patient with extensive nodular subtype (ID#52) revealed intrathecal tumor cells (postoperative day 15). The patient was therefore clinically considered as a high risk. Multiple gene amplifications, including *MYCN*, were observed in the *TP53*-mutant SHH-activated MB. None of the *TP53* wild-type SHH-activated MB had *MYCN* amplification, but increased expression of *MYCN* was seen in patient ID#37, who died 15 months after initial

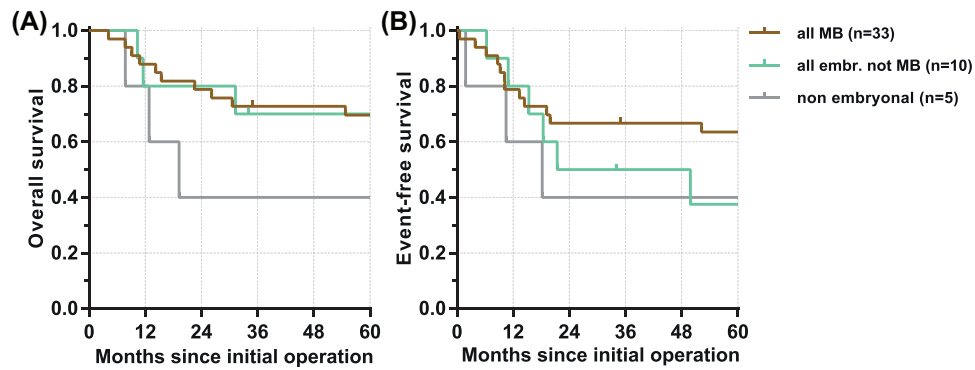


FIGURE 1 Overall survival (A) and event-free survival (B) according to diagnosis

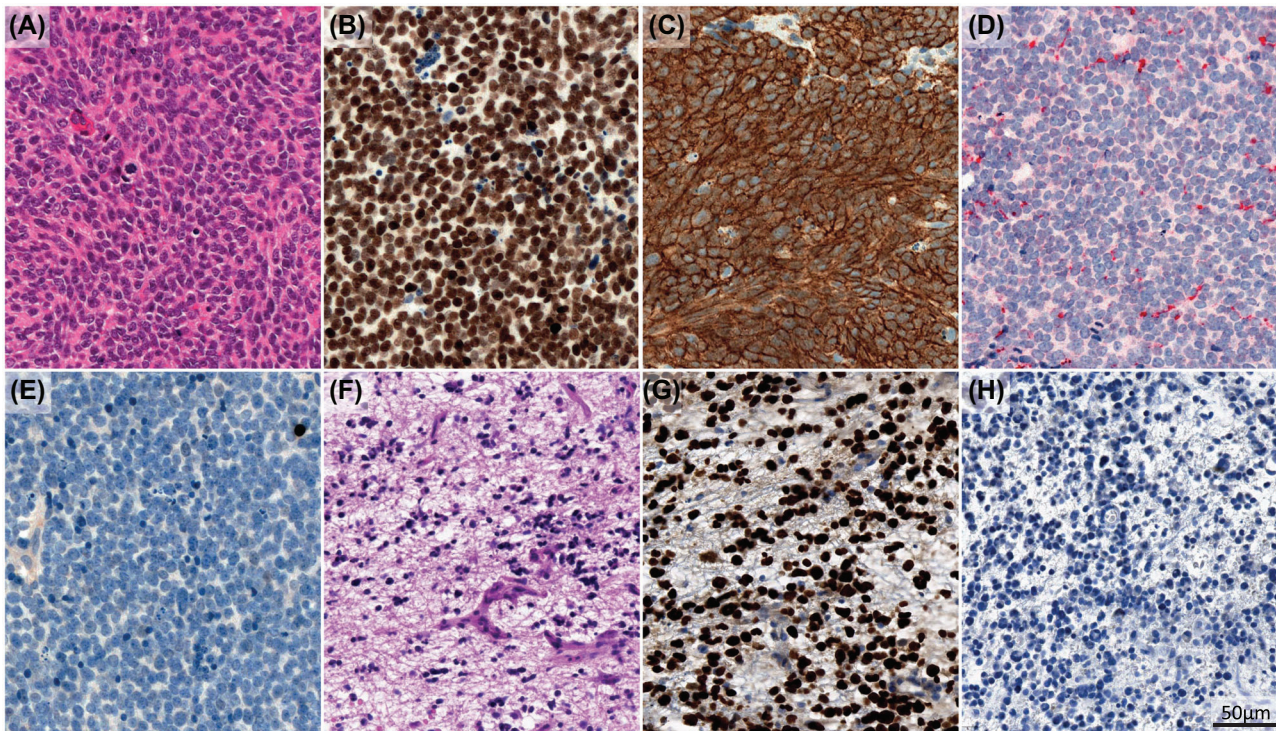


FIGURE 2 WNT-activated medulloblastoma with secondary diffuse high-grade glioma. The first tumor resection (fossa posterior) in patient ID#49 showed a small blue round cell tumor with increased mitotic activity (A, H&E staining). Immunohistochemical analysis revealed nuclear immunoreactivity for OTX2 (B) and β -catenin (C). Substantial expression of p75-NGFR (D) or Olig2 (E) was not observed. Molecular pathological analysis revealed a *CTNNB1* mutation (exon 3, S33F; not shown). The tumor was classified as classic medulloblastoma, WNT-activated. A new cerebellar lesion was resected 4 years after initial surgery (F, H&E staining). The tumor showed strong expression of Olig2 (G), but no expression of OTX2 (H). Molecular pathological analysis revealed a *PTPRZ1-MET* fusion (exon1/exon2). The tumor was classified as radiation-associated diffuse high-grade glioma

diagnosis due to tumor progression. The remaining three patients were alive without recurrence at date of last follow-up (3, 8, and 10 years from diagnosis, respectively).

3.1.3 | Non-WNT/non-SHH MB

Twenty-two non-WNT/non-SHH MBs were identified, including the above-mentioned supratentorial case clinically diagnosed as CNS-

PNET and herein reclassified as MB. Five-year OS and EFS were 68.2% and 59.1%, respectively. Seven patients were initially clinically considered as high-risk patients due to at least one of the following risk factors: metastatic disease ($n = 3$; ID# 21, 33, 41), tumor cells in the cerebrospinal fluid ($n = 1$; ID#32), young age ($n = 1$, ID# 20), *MYC* amplification ($n = 2$, ID# 39, ID #41) or residual tumor $>1.5 \text{ cm}^2$ ($n = 1$; ID# 18). Two cases showed a large-cell/anaplastic histomorphology (ID#39, ID#41), whereas the remaining 20 non-WNT/non-SHH MBs were histologically classified as classic MB. Using NanoString gene

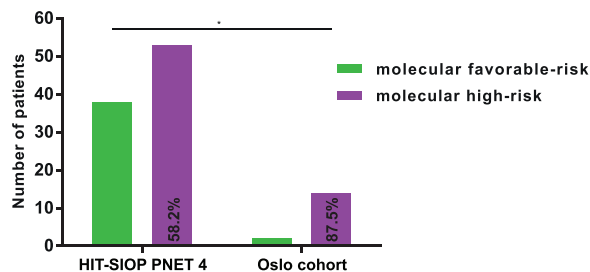


FIGURE 3 Statistical analysis revealed a significantly increased number of molecular high-risk patients among clinical standard-risk non-WNT/non-SHH medulloblastomas in the Oslo cohort compared to the HIT-SIOP PNET 4 series, as published by Goschzik et al. ($p = 0.0273$)

expression profiling and DNA methylation analysis, these 22 non-WNT/non-SHH MBs could be divided in 11 Grp3 MBs and 10 Grp4 MBs. Group allocation failed in one clinical standard-risk non-WNT/non-SHH MB (ID#11). Both large-cell/anaplastic MBs with MYC amplification belong to the Grp3.

3.2 | Molecular risk stratification of clinical standard-risk non-WNT/non-SHH MBs based on whole chromosomal aberration signatures

Identification of WCA signatures as a biomarker of molecular risk in non-WNT/non-SHH MBs was restricted to clinical standard-risk MBs, due to inclusion criteria in the HIT-International Society of Paediatric Oncology (SIOP) PNET 4 trial. To follow the criteria by Goschzik et al., we allowed for one exception: ID# 18 this case had a clinical HR criterium with residual tumor $>1.5 \text{ cm}^2$ but this HR-criterium was not used by Goschzik et al. As alluded to above, we identified 15 clinical standard-risk non-WNT/non-SHH MBs in our series, and with the one clinical high-risk included (ID# 18) we ended up with 16 cases to be analyzed for WCA signatures.

Stratification of these 16 non-WNT/non-SHH MBs based on the 7/8/11 classifier revealed 14 (87.5%) cases with a WCA signature associated with high risk, whereas only two tumors (12.5%) showed a WCA pattern associated with favorable outcome. None of the patients in the latter group had a recurrence (5-year OS and EFS 100%), whereas the 5-year OS and EFS was 64.3% in the high-risk group. Strikingly, the Oslo-series had a significantly increased percentage of patients with WCA signatures indicating poor outcome compared to the HIT-SIOP PNET 4 series had ($p = 0.0273$; see Figure 3).

3.3 | Summarized molecular risk stratification

Within the 33 MB cases, we identified 17 patients with either established molecular high-risk factors (MYC-amplification, $n = 2$; TP53-mutant SHH $n = 1$) or WCA signatures associated with poor outcome ($n = 14$). These 17 cases had a significant worse 5-year OS (52.9%)

than the remaining 16 MBs (87.1%; $p = 0.036$, Figure 4A,B). Interestingly, application of molecular high-risk factors separated the 14 clinical high-risk MB into a group with molecular high-risk factors and very poor outcome ($n = 4$, 5-year OS and PFS = 0%) and a group without molecular high-risk factors and significantly better outcome ($n = 10$, 5-year OS = 90.0%, EFS = 70.0%; $p > 0.001$ and $p = 0.002$; Figure 4C,D, Table 2).

4 | DISCUSSION

The comprehensive molecular analysis conducted in this study allowed for re-examination of embryonal tumor diagnoses and further molecular-based risk stratification of MB cases. Of the 53 identified cases, five could not be included in this series due to lack of sufficient material. Another five patients turned out to suffer from nonembryonal tumors with an unfavorable outcome (5-year OS and EFS 40%). The 43 verified embryonal tumors had a 5-year OS and EFS of 69.6% and 57.8%, respectively. Interestingly, our cohort included five patients with embryonal tumors that are now considered as novel entities in the 2021 classification of tumors of the central nervous system.¹⁹

Our molecularly analyzed series included 33 MB. Five-year OS and EFS of the whole MB cohort was 69.6% and 63.5%, respectively. The 2016 classification of tumors of the central nervous system included for the first time a genetically defined medulloblastoma classification in addition to the established histologically defined classification.⁶ As a consequence, the ongoing HIT-SIOP PNET 5 study includes biological testing alongside clinical and histopathological parameters in order to separate favorable- and high-risk biological profiles from standard-risk and low-risk groups. The current molecular benchmark challenges previously accepted risk stratification criteria. For instance, the prognostic benefit of gross total resection (GTR) for patients with MB is controversial when molecular subgrouping is taken into account.²⁰ In our series, we had two WNT-activated MB patients with residual tumor $>1.5 \text{ cm}^2$. None showed progression or recurrence after initial treatment. In contrast, two patients with WNT-activated MB died from most probably radiation-induced secondary malignancies (cerebellar diffuse high-grade gliomas).

One aim of our study was further elucidation of the previously observed regional difference in survival of children treated for MB and CNS-PNET in Norway.^{12,13} Stensvold et al. analyzed the outcome of all patients <21 years at age diagnosed with MB or CNS-PNET in Norway between January 1, 1974 and December 31, 2013.¹³ All patients were treated at one of the following four university hospitals in Norway: University Hospital of North Norway, St. Olavs Hospital Trondheim University Hospital, Haukeland University Hospital, and OUH. There were no differences in inclusion and histopathological classification between these hospitals. The authors have not succeeded in their efforts to identify possible explanations for the survival differences, in spite of a thorough review of diagnostic work-up, surgery, radiotherapy, and chemotherapy given to all patients.¹³ Five patients had a congenital genetic condition, confirmed by genetic testing. Only one of these patients is included in the current study (ID#26, Nijmegen

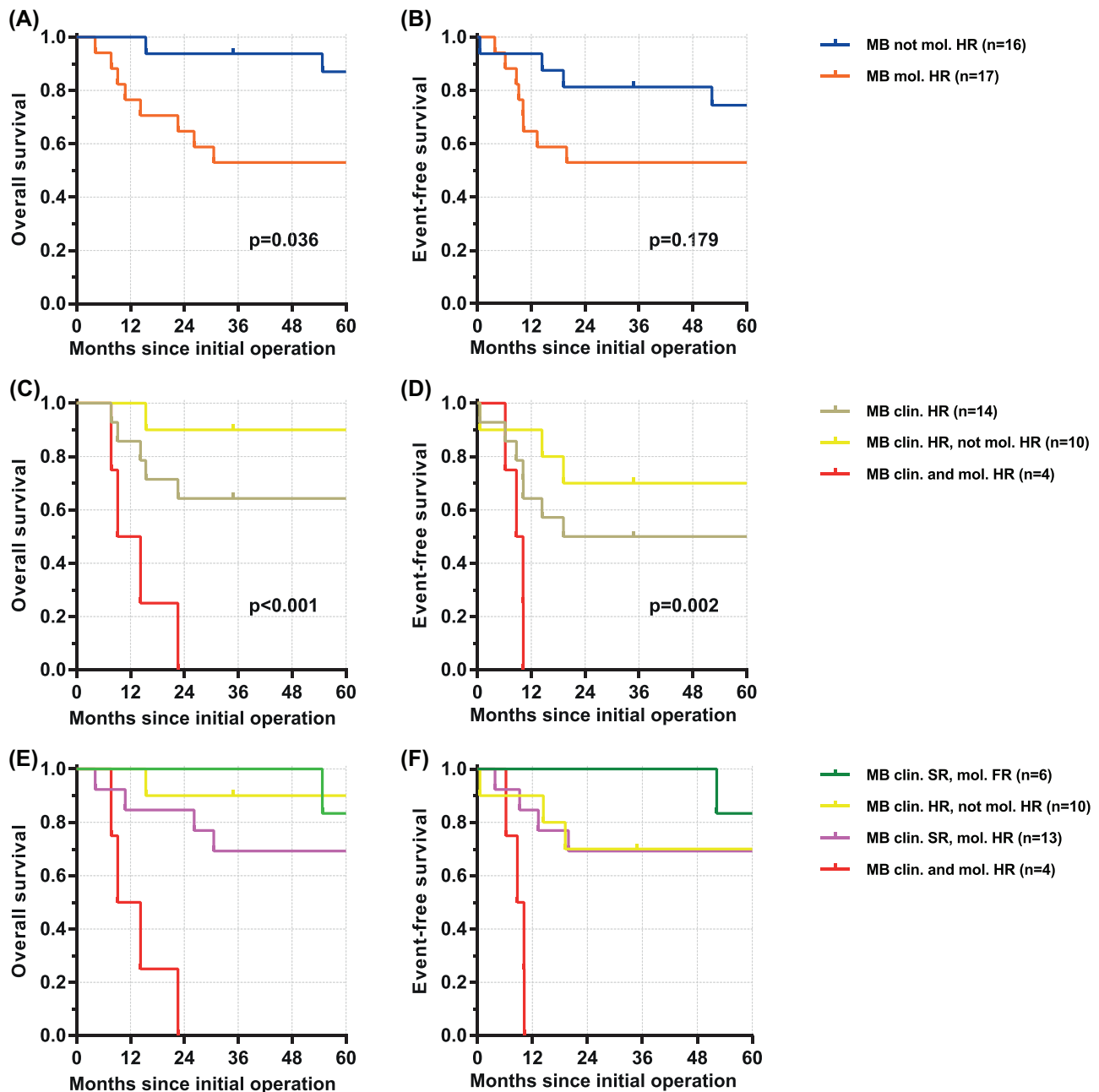


FIGURE 4 Molecular-based risk stratification identifies significant survival differences in the entire medulloblastoma cohort (A and B) and medulloblastomas with clinical high-risk features (C and D). Combined clinical and molecular pathological risk parameters for overall survival and event-free survival are shown in (E) and (F), respectively

breakage syndrome). Lack of suitable FFPE tissue from 2004 and before hampered analysis of the entire cohort described by Stensvold and co-workers. Selected clinical high-risk criteria in the aforementioned studies^{12,13} are no longer appropriate, since SHH-activated desmoplastic MB and MB with extensive nodularity in young patients are currently considered prognostically favorable without irradiation. We adopted these older criteria in order to leverage the comparison between the studies. However, in contrast to these studies, we had substantial molecular pathological data for risk stratification. WCA signatures as a biomarker of molecular risk in non-WNT/non-SHH MBs

enabled identification of several molecular high-risk MB. Indeed, MBs classified as molecular favorable risk showed a significant better outcome than the remaining MBs, supporting the WCA-signature criteria published by Goschzik et al.¹⁰ We performed molecular inversion probe profiling to identify changes in genomic copy number, but other methods such as DNA methylation analysis could be used equally.¹⁷ Statistical analysis on further stratified groups should be interpreted with caution due to low numbers in most groups. However, 5-year OS and EFS of 83.3% in the clinical standard risk and molecular favorable risk, on the one hand ($n = 6$), and 0% in the clinical HR and molecular

HR group, on the other hand ($n = 4$), argues for superior prediction by combined risk allocation. Interestingly, our cohort demonstrated a significantly increased number of standard-risk non-WNT/non-SHH medulloblastoma with molecular high-risk profile compared to previously published series.¹⁰ We did not succeed to identify a explanation for this observation. Our results support the usefulness of an integrated risk stratification based on both clinical as well as molecular parameters.

The difference in outcome between molecular high-risk and favorable-risk patients indicates that a comparison of different published patient series with unknown molecular profile bears considerable limitations. Thus, the previously observed unexplained regional difference in survival of children treated for MB and CNS-PNET in Norway has to be interpreted with caution as long as substantial molecular pathological parameters of most patients are unknown.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.

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