BRIEF REPORT

Subgroup-specific immune and stromal microenvironment in medulloblastoma

Michael Bockmayr a,b,c, Malte Mohme d, Frederick Klauschen b, Beate Winkler a, Jan Budczies b, Stefan Rutkowski a, and Ulrich Schüller a,c,e

a Department of Pediatric Hematology and Oncology, University Medical Center Hamburg-Eppendorf, Hamburg, Germany; b Charité – Universitätsmedizin Berlin, corporate member of Freie Universität Berlin, Humboldt-Universität zu Berlin, and Berlin Institute of Health, Institute of Pathology, Berlin, Germany; c Research Institute, Children’s Cancer Center Hamburg, Hamburg, Germany; d Department of Neurosurgery, University Medical Center Hamburg-Eppendorf, Hamburg, Germany; e Institute of Neuropathology, University Medical Center Hamburg-Eppendorf, Hamburg, Germany

ABSTRACT
Knowledge on immune and stromal cells in medulloblastoma microenvironment is still limited as previous work was frequently restricted by low sample size and the lack of molecular subgroup information. We characterized 10 microenvironment cell populations as well as PD-L1 from gene expression in 1422 brain tumors and 763 medulloblastomas. All in all, medulloblastomas showed low expression of immune markers. Still, there were substantial differences with a clustering of medulloblastoma subgroups according to their microenvironment profile. Specifically, SHH medulloblastomas displayed strong signatures of fibroblasts, T cells and macrophages, while markers of cytotoxic lymphocytes were enriched in Group 4 tumors. PD-L1 gene expression appeared to be relatively high in single SHH and WNT cases but was undetectable by immunohistochemistry. In addition, two diverse immuno-stromal patterns were identified, indicating distinct types of local tumor immunosuppression, which were primarily controlled by either macrophage and regulatory T cell-mediated mechanisms or immunosuppressive cytokines and checkpoints, respectively. None of the immune cell signatures had an independent prognostic value in the present dataset after multiple testing correction. These results suggest a mild, but subgroup-specific infiltration of immune cells in medulloblastoma.

Introduction
Medulloblastoma is the most frequent malignant brain tumor in children. A comprehensive molecular characterization generating large amounts of genomic, epigenomic, and transcriptomic data has permitted a precise profiling of this entity and the definition of four major molecular subgroups. 1–5 These subgroups and additional genetic alterations as well as clinical factors allow for a precise risk stratification of medulloblastoma patients.6

The tumor microenvironment is of major importance for tumor growth and progression as well as treatment response in many cancers. 7–9 It has further gained interest in the last years with the availability of immune checkpoint inhibitors, which allow for a therapeutic targeting of the interaction between tumor and immune system.10 Tumor-infiltrating immune cells, principally lymphocytes, have been shown to be of prognostic relevance and predictive for response to chemotherapy in various solid tumors. 11,12 In particular, extensive research has been performed on tumor immunology in glioblastoma 13,14 and the potential for immunotherapy has been discussed since many years in medulloblastoma.15 However, despite a very deep molecular characterization of this entity, immunotherapeutic approaches are still rare, which is likely due to limited knowledge about the tumor microenvironment and the prognostic relevance of the cells contributing to it.

Using multicolor FACS analysis in a cohort of 6 medulloblastomas, it was shown that medulloblastoma exhibited a different immune microenvironment compared to 3 other pediatric brain tumor entities and the findings were validated by gene expression.16 Natural killer cell (NK cell) therapy was among the first immunotherapeutic approaches that have been proposed for medulloblastoma (see 17 for review). Further, Margol et al.18 showed that tumor-associated macrophages were more numerous in SHH medulloblastoma than in Group 3 and Group 4 tumors and proposed macrophages as therapeutic targets.

With the avenue of immune checkpoint inhibitors, the PD-1/PD-L1 axis started being studied in medulloblastoma. Pham et al.19 developed murine SHH and Group 3 models and showed that immune infiltrates were more present in SHH tumors. However, there were more PD-1+ CD8+ T cells in the Group 3 animals. Interestingly, treatment with PD-L1 and CTLA4 inhibitors provided a survival benefit in the Group 3 animals only. In a cohort including 16 tumors, Murata et al. reported that high expression of PD-L1 (seen in 56% of the cases) was associated with low CD8+ T cell infiltration and poor prognosis in medulloblastoma.20 In contrast, Majzner
et al.\textsuperscript{21} (40 cases) and Vermeulen et al.\textsuperscript{22} (29 cases) did not see PD-L1 expression in any of the studied medulloblastomas. None of the last three studies found subgroup-specific patterns of tumor-infiltrating lymphocytes (TILs).

Most of these results are mainly based on preclinical models or on immunohistological profiling of tumors. This yields to limitations in sample size and reduced statistical power as well as small numbers of markers that can be studied simultaneously. Furthermore, some of the immunohistological results on the expression of PD-L1 in medulloblastoma were contradictory. Associations with survival in larger cohorts have not yet been published.

Recently, large gene expression datasets of medulloblastoma have been released, including one dataset of 763 tumors.\textsuperscript{1,5} Also, computational methods have been proposed to characterize the cellular composition of the tumor microenvironment from gene expression data. One of the main methods, CIBERSORT,\textsuperscript{23} uses a deconvolution algorithm that allows for the quantification of 22 immune subpopulations. The algorithm was primarily designed to infer the relative proportion of immune cells and successfully established different immune cell populations as prognostic markers in various cancers.\textsuperscript{24,25} Furthermore, the recently developed Microenvironment Cell Populations-counter\textsuperscript{26} (MCPcounter) uses characteristic gene signatures that are specifically associated with 8 immune cell populations (T cells, CD8+ T cells, cytotoxic lymphocytes, B lineage cells, NK cells, monocytic lineage cells, myeloid dendritic cells, and neutrophils) and 2 stromal cell populations (fibroblasts and endothelial cells) and allows for their absolute quantification.

Hence, we set out to analyze the tumor microenvironment of medulloblastoma using gene expression data. This allows to overcome sample size limitations and to study several immune cell populations simultaneously. We used MCPcounter as it also quantifies two stromal cell populations and yields more consistent results if there are only few infiltrating immune cells, which is frequently the case for pediatric brain tumors. We first compared medulloblastoma to seven other major brain cancers. Further, we used a large published gene expression dataset to infer subgroup-specific differences and associations with survival.

The expression scores for all cell populations and PD-L1 were highly significantly different between entities (all \( p < 5.4e-8 \), see supplement). The overall expression of immune and stromal markers in medulloblastoma was low compared to the other entities (Fig. 1A, B). In the clustering analysis, the expression patterns of medulloblastoma were most similar to the embryonal AT/RT and ependymomas (Fig. 1A). The astrocytic tumors built one cluster and were the only ones to show high expression levels of PD-L1 and markers for cells from the monocytic lineage, e.g. macrophages. There were lower levels of cytotoxic lymphocytes but higher levels of neutrophils in GBM than in the lower grade gliomas. MGM showed a distinct expression pattern to the other tumor entities with large numbers of fibroblasts as well as myeloid dendritic cells and NK cells.

Overall, medulloblastomas had the lowest gene expression level of PD-L1, cytotoxic lymphocytes, and endothelial cells of all studied entities (Fig. 1B). However, there was substantial variation among different medulloblastomas for PD-L1 and the microenvironment cell populations. The expression levels were not uniformly low, indeed, single cases of medulloblastoma showed higher expression than the median of all tumors for all MCPcounter populations and PD-L1.

Hierarchical clustering of all samples revealed a clear-cut aggregation of meningial tumors, but the remaining entities did not form sharply separated groups (Suppl. Fig. 1). In particular, medulloblastomas did not form a distinct cluster but were mixed with the other entities. Hence, although most medulloblastomas showed lower levels of immune infiltration and stromal cells than the remaining brain tumors some tumors exhibited higher expression of certain cell populations similar to brain tumors from other entities.

Expression of PD-L1 and microenvironment signatures is highly subgroup-specific

Next, we studied the microenvironment populations (MCP) and PD-L1 expression in a gene expression dataset of 763 medulloblastomas with known molecular subgroup (70 WNT, 223 SHH, 144 Group 3 and 326 Group 4).\textsuperscript{5} The clustering analysis of the 10 MCPs separates most of the SHH and WNT tumors from most of the Group 3 and Group 4 tumors (Fig. 2A). There is one cluster composed mostly of SHH and WNT tumor that shows relatively high PD-L1 expression.

The expression scores were strongly significantly different between subgroups for PD-L1 and all MCPs with the exception of the B lineage and NK cells (all \( p < 3.85e-10 \), Fig. 2B). Median PD-L1 expression was highest in WNT tumors (4.06, log2-scale) followed by SHH tumors (3.67) and lowest in Group 4 tumors (3.54, fold change WNT/Group 4: 1.4). The distribution of PD-L1 expression was heavy-tailed and few tumors mostly from the SHH and the WNT subgroup showed comparably high expression (Fig. 2A). Indeed, 20% of WNT, 6.7% of SHH, 2.7% of Group 3, and 0.3% of Group 4 tumors had expression of PD-L1 higher than 4.5, while 5.7% of WNT, 2.7% of SHH, 1.4% of Group 3 and 0.3% of Group 4 tumors had
expression higher than 5.0. Still, in a series of 10 WNT and 36 SHH medulloblastomas we detected small islets of perivascular PD-L1 positive cells, but none of the samples showed membranous PD-L1 expression in more than 1% of tumors cells by using antibodies against PD-L1 (data not shown).

SHH tumors were associated with the most distinctive pattern of microenvironmental cells (Fig. 2B). In particular, they had larger numbers of T cells, fibroblasts, and cells from the monocytic lineage and lower numbers of neutrophils than the tumors from the other subgroups. Group 3 tumors had low numbers of T cells and macrophages, but the highest number of CD8+ T cells. Group 4 tumors had the lowest number of fibroblasts and largest number of cytotoxic lymphocytes.

**Immuno-stromal classification of medulloblastoma**

To get a more precise overview of the complex relations between molecular subgroups and immuno-stromal context, we computed a two-dimensional embedding of 98 MCP markers using t-SNE to yielding an almost complete separation of the molecular subgroups (Fig. 3A, Suppl. Tables, Suppl. Fig. 2). Using unsupervised k-means clustering, we identified 5 major clusters with distinct immuno-stromal
The unsupervised k-means clustering demonstrates that the two distinct immuno-stromal patterns separate Group 4 tumors into two subgroups (cluster 1 and 4), indicating two potentially diverse mechanisms of immunosuppression within the microenvironment of Group 4 medulloblastoma.

**Associations of microenvironment signatures with survival**

The dataset was split into subgroups prior to survival analysis since molecular subgroups are associated with a significantly different prognosis. The overall association of immune cells with survival was only weak. There were no significant associations with survival in WNT tumors, which might be explained by the good prognosis associated with a low number of events and low statistical power (Fig. 4). The expression score for fibroblasts in SHH tumors in univariate analysis was the only significant finding after multiple testing correction using the Benjamini-Hochberg method.
multivariate analyses adjusted for age, histology, metastatic status, and gender, it narrowly missed significance (p = 0.078). There were no significant associations between MCP-markers or PD-L1 in Group 3 and Group 4 tumors after multiple testing correction. In univariate analysis of Group 3 and Group 4 tumors, myeloid dendritic cells marker expression was associated with better survival (p = 0.028, BH-p = 0.32 and p = 0.05, BH-p = 0.36). In multivariate analysis, this association also lost significance (p = 0.32 and p = 0.41, respectively). There were no significant associations between survival and the expression of the markers for the monocytic lineage (macrophages) or NK cells in any of the subgroups.

**Discussion**

Medulloblastoma, an embryonal tumor originating from the posterior fossa, is the most common malignant pediatric brain tumor. With multimodal therapeutic concepts including surgery, radiotherapy, and chemotherapy, about two thirds of the patients can be cured. However, the prognosis of very high-risk patients is still poor. Further, many patients suffer from important treatment associated neurocognitive sequelae...
(reviewed in). Hence, there is a great need for the development of new therapeutic strategies, and immunotherapy, which has been successfully used in the treatment of many adult solid tumors, is a promising treatment option. A precise understanding of the function of the tumor microenvironment appears key to design medulloblastoma specific immunotherapies. However, there is still not much known about the tumor microenvironment of medulloblastoma. Presumably due to the rarity of medulloblastoma compared to major adult cancers, there are no large immunohistochemical studies characterizing the microenvironment of this entity. Previous reports were limited by low sample sizes and/or focused on only one or two cell populations. Hence, to gain a more comprehensive overview, we used a state of the art bioinformatics method to quantify eight immune cell populations and two stromal cell populations as well as PD-L1 in a pancancer dataset of 1422 brain tumors and a medulloblastoma dataset including 763 samples. This allows for the simultaneous characterization of several cell populations and overcomes the described limitations. To our knowledge, this is the largest and most comprehensive study of the microenvironment in medulloblastoma.

The gene signatures proposed by Becht et al. have been carefully selected to be specific for the described immune cell populations and validated in several thousand tumors including a small dataset of medulloblastoma. A study of CD8 and Th17 infiltrates in GBM showed comparable associations with the expression of immunohistological data and data based on gene expression signatures in a similar approach to those proposed by Becht et al. Further, intratumoral heterogeneity of gene expression in medulloblastoma is very low, which also supports our assumption that transcriptome based analysis is suitable for a global description of the tumor microenvironment. However, it cannot be excluded that tumor cells contribute to the expression score, which might significantly confound the analysis. Indeed, the expression of certain genes might be associated with medulloblastoma tumor biology (see supplement and methods). For example, EOMES is highly expressed in cytotoxic lymphocytes but only weakly in CD4+ T cells, making it a suitable marker for this cell population outside of the nervous system. However, it is also associated with embryonal brain development and a specific marker for Group 4 medulloblastoma, which would clearly confound the deconvolution approach. This shows that a careful and tissue-specific selection of the markers is key and should also be performed in further studies.

There have been large numbers of immunohistochemical studies of immune infiltrates particularly in glioma and meningioma including T cells, NK cells and macrophages, with partially contradictory results. This makes it difficult to establish a clear reference level for immune infiltration in these entities, however, for most of the microenvironment populations used in this study, the expression levels are compatible with previously published results based on immunohistochemistry. In the pancancer analysis, PD-L1 expression was highest in GBM in line with results based on immunohistology. The very distinctive profile of meningiomas with a larger number of fibroblasts and a larger number of NK cells might be linked to the different histological origin of these tumors.

Medulloblastoma had the lowest amount of PD-L1 and cytotoxic lymphocytes of all studied entities and overall a very small amount of infiltrating immune cells. This suggests that the tumor either actively modulates the immune response or simply has little immunogenicity as is suggested by the relatively low mutational burden. However, a recent study also demonstrated some form of systemic immune suppression present in medulloblastoma with lower lymphocyte counts and a higher neutrophil-to-lymphocyte count ratio already present at the moment of diagnosis prior to the application of steroids. Our study demonstrated highly significant differences in the pattern of immune and stromal cell signatures as well as PD-L1 between the molecular subgroups of medulloblastoma. Margol
et al. were the first to highlight differences in the microenvironment of medulloblastoma subgroups and showed that there were more macrophages in SHH tumors. They also defined a gene expression signature with 25 tumor genes as well as 6 immune genes able to accurately classify medulloblastoma into subgroups. Here, we showed that these differences apply to all immune and stromal cell populations except NK cells and B cells. Further, our analysis shows that an immune signature alone without genes associated with tumor biology yields a good separation of medulloblastoma subgroups in a clustering analysis (Fig. 3, Suppl. Fig. 2). Recently, it could be shown that WNT medulloblastoma but not the other subgroups lack a functional blood-brain barrier. This might explain differences between WNT tumors and the remaining subgroups, but cannot explain the relatively distinct immune profile of SHH tumors.

Since SHH and WNT tumors had the highest expression levels of PD-L1, we also stained WNT and SHH tumors for PD-L1. Although this is the largest series of medulloblastomas stained for PD-L1 currently reported, we did not find membranous PD-L1 expression in more than 1% of tumor cells in any of the studied samples. This confirms previous studies, which did not see PD-L1 expression in medulloblastoma and contradicts a small study by Murata et al. reporting PD-L1 expression in 9/16 samples. Hence, immunohistological staining of PD-L1 cannot be used as a predictive biomarker for checkpoint inhibition. However, results of PD-L1 immunohistochemistry are variable and patients exist without detectable PD-L1 expression in tumor cells showing a substantial response to PD-L1 inhibitory therapy. In a murine model of medulloblastoma, disruption of CDK5 was associated with lower PD-L1 expression and tumor rejection mediated by CD4 T cells, indicating biological relevance of the PD-L1 pathway in medulloblastoma. High levels of interferon gamma (IFNγ) were present in CDK5 deficient tumors. As we previously reported, TH1 predominance with high expression of IFNγ in the peripheral blood was associated with favorable outcome in children with medulloblastoma treated by high-dose chemotherapy followed by autologous stem cell transplantation. This further corroborates the relevance of the IFNγ/PD-L1 axis in medulloblastoma although its precise functioning in vivo remains to be elucidated.

SHH tumors exhibited larger numbers of fibroblasts, macrophages and T cells than the other subgroups. The fibroblast score is mostly based on the expression of collagens and other elements from the extracellular matrix, and it cannot be excluded that these molecules are produced by tumor cells. Higher numbers of fibroblasts were associated with significantly better prognosis and might be linked to a better differentiation of these tumors. The larger amount of fibroblasts in SHH tumors may be explained by desmoplastic histology, which is known to be associated with better prognosis and the addition of which as a marker reduces the significance of fibroblast expression in multivariate analysis. Nonetheless, the numerical amount of fibroblasts seems to be an important factor.

We identified two distinct immuno-stromal patterns, defined by an either monocytic/macrophage supported pro-tumorigenic microenvironment versus a pattern which suppresses cytotoxic T cell infiltration by immunosuppressive cytokines and checkpoint pathways. Interestingly, as the diverse patterns, with the exception of the divided Group 4 tumors, are accurately distributed to the known medulloblastoma subtypes, a presumable relation to driver pathways has to be considered. Recent studies support such a pathway-specific immunomodulation. For example, an in vivo colitis study showed that a specific activation of the hedgehog pathway resulted in local immunosuppression mediated by stromal and regulatory T cells. A similar immunomodulation of the tumor microenvironment was described in WNT/β-catenin pathway activation. Here, β-catenin activation supported tumor-associated macrophage and regulatory T cell survival (summarized in ). Furthermore, segregation of TCGA datasets according to an inflamed T cell signature set showed that WNT/β-catenin pathway activation inversely correlated with T cell infiltration.

Our analysis showed only weak associations between intratumoral immune infiltration and survival, which all missed significance after multiple testing correction despite a large sample size. This might be explained by the overall very low immune infiltration, with small differences in the absolute levels of immune infiltrating cells. It corroborates findings by Vermeulen et al., who did not see associations between TILs and prognosis. In contrast, peripheral levels of TH1 cells were associated with better prognosis already in a small cohort of medulloblastoma. Hence, a simultaneous investigation of peripheral and intratumoral immune cells seems to be of key relevance in further studies of medulloblastoma tumor immunology.

In summary, we report that medulloblastoma has the lowest level of PD-L1 and cytotoxic lymphocytes of eight studied brain tumors. There are substantial differences in the microenvironment cell populations between molecular subgroups, which are likely to be associated with different interactions between the tumor and the immune system. In particular, two immunostromal patterns (macrophage and regulatory T cell-mediated mechanisms vs. immunosuppressive cytokines and checkpoints) were identified. Both patterns were closely linked to the molecular subgroups. Upcoming immunotherapeutic treatment strategies will have to consider these distinct microenvironmental phenotypes in order to efficiently promote a tumor-specific immune response in the context of different mechanisms of immune escape in medulloblastoma subgroups.

**Material and methods**

All data analyses were performed using the statistical programming language R including the packages *survival*, *gplots*, *bee-swarm* and *Rtsne*.
Datasets and preprocessing

Raw gene expression data (.CEL files) were downloaded from Gene Expression Omnibus (GEO). For the pancancer analysis, 18 datasets covering 8 different tumor entities measured on the Affymetrix Human Genome U133 Plus 2.0 Array were used (see supplement). To allow for comparisons between different series, fRMA normalization, which includes a batch effect correction, was used as implemented in the R-package fRMA using the input vectors from the R-package hgu133plus2frmavecs. Duplicated samples (defined by a Pearson correlation > 0.995) were removed. This yielded a final dataset of 1422 unique tumor samples (421 glioblastomas (GBM), 282 medulloblastomas (MB), 272 ependymomas (EPN), 99 meningiomas (MGM), 159 pilocytic astrocytomas (PA), 61 anaplastic oligodendrogliomas (AO), 69 anaplastic astrocytomas (AA) and 59 atypical teratoid/rhabdoid tumors (AT/RT). To exclude major remaining batch effects after preprocessing, we compared samples from entities present in multiple gene expression series. Clustering analysis showed that MCPcounter profiles for the same entity were similar with substantial variance between entities (data not shown).

The data published by Cavalli et al. was used for medulloblastoma specific analyses. Raw data were normalized using the RMA method as implemented in the R-package affy with the custom chip definition file hguene11sth-sensgcdf (v22.0.0). The data published by Doucette et al. was used as the mean of the two corresponding probes (see supplement). MCPcounter scores were defined as the average of the log2-scaled values of the corresponding probes or genes (see supplement) for the U133 Plus 2.0 Array and the 1.1 ST array respectively. Expression of PD-L1 was defined as the mean of the two corresponding probes (223834_at and 227458_at) for the U133 Plus 2.0 Array. The resulting scores are expressed in arbitrary units. Immunosuppressive signatures were obtained from Doucette et al. and computed analogously.

Computation of immune and stromal signatures

Published signatures of mRNA markers from 10 cell populations (MCPcounter) were used to estimate the content of T cells, CD8+ T cells, cytotoxic lymphocytes, B lineage cells, NK cells, monocyctic lineage cells, myeloid dendritic cells, neutrophils, fibroblasts and endothelial cells in tumor tissue. All markers were manually reviewed and those associated with medulloblastoma tumor biology were removed to avoid bias (see supplement). MCPcounter scores were defined as the average of the log2-scaled values of the corresponding probes or genes (see supplement) for the U133 Plus 2.0 Array and the 1.1 ST array respectively. Expression of PD-L1 was defined as the mean of the two corresponding probes (223834_at and 227458_at) for the U133 Plus 2.0 Array. The resulting scores are expressed in arbitrary units. Immunosuppressive signatures were obtained from Doucette et al. and computed analogously.

Heatmap, differential expression, k-means clustering, t-SNE and survival analysis

Medulloblastoma data was trimmed at Z-scores ± 4 prior to heatmap analysis. Heatmaps were generated using the average linkage method and Pearson correlations as similarity measure. Significance of differential expression between subgroups of medulloblastoma was assessed using the Kruskal-Wallis test (two-sided).

Immuno-stromal clusters were defined using k-means clustering of the MCPcounter marker genes. The number of clusters (5) was selected using the elbow method. For visualization, t-SNE was used with perplexity 100.

Survival analysis was performed using the proportional hazards model as implemented by the coxph function from the R package survival. For multivariate analysis, age, metastatic status, histology and gender were included. Samples with missing clinical data were excluded. Expression values for the different signatures were transformed to 3 quantiles (tertiles) and included as numerical values. Multiple testing correction for the cell populations and PD-L1 was performed using the Benjamini-Hochberg (BH) method where appropriated.

Immunohistochemistry

Immunohistochemical stains were performed on formalin-fixed, paraffin-embedded tumor material according to standard protocols on an automated Ventana HX IHC system (Ventana-Roche Medical systems, Tucson, AZ, USA) following the manufacturer’s instructions. Diaminobenzidine (Sigma) was used as Chromogen and sections were counterstained with hematoxylin. Antibodies against PD-L1 (E1L3N, Cell Signaling) were used in a dilution of 1:200.

Informed consent was obtained from all patients prior to the analysis.

Abbreviations

| Abbreviation | Description |
|--------------|-------------|
| AA           | anaplastic astrocytoma |
| AO           | anaplastic oligodendroglioma |
| AT/RT        | atypical teratoid rhabdoid tumor |
| BH           | Benjamini-Hochberg |
| EPN          | ependymoma |
| GBM          | glioblastoma |
| IFNγ         | interferon gamma |
| MB           | medulloblastoma |
| MCP          | microenvironment cell population |
| MGM          | meningioma |
| PA           | pilocytic astrocytoma |
| PD-1         | programmed death 1 |
| PD-L1        | programmed death-ligand 1 |
| TIL          | tumor-infiltrating lymphocyte |
| Treg         | regulatory T cell |

Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

Authors’ contributions (CRediT)

Conceptualization: MB and US, Methodology: MB, MM, FK, JB and US, Formal Analysis: MB, Investigation: MB, MM, FK, BW, JB, SR and US, Resources: FK and US, Data Curation: MB, Writing – Original Draft: MB and US, Writing – Review & Editing: MB, MM, FK, BW, JB, SR and US, Visualization: MB, Supervision: US, Funding: US.

Funding

This research was supported by the Fördergemeinschaft Kinderkrebs-Zentrum Hamburg.
References

1. Northcott PA, Jones DTW, Kool M, Robinson GW, Gilbertson RJ, Cho Y-J, Pomeroy SL, Korshunov A, Lichter P, Taylor MD, et al. Medulloblastomas: the end of the beginning. Nat Rev Cancer. 2012;12:818–34. doi:10.1038/nrc3410. PMID:22317512.

2. Taylor MD, Northcott PA, Korshunov A, Remke M, Cho Y-J, Clifford SC, Eberhart CG, Parsons DW, Rutkowski S, Gajjar A, et al. Molecular subgroups of medulloblastoma: the current consensus. Acta Neuropathol (Berl). 2012;123:465–72. doi:10.1007/s00401-011-0922-z. PMID:22134537.

3. Northcott PA, Shih DHJ, Peacock J, Garzia L, Morrisry AS, Zichner T, Stütz AM, Korshunov A, Reimand J, Schumacher SE, et al. Subgroup-specific structural variation across 1,000 medulloblastoma genomes. Nature. 2012;488:49–56. doi:10.1038/nature11327. PMID:22832581.

4. Northcott PA, Buchhalter I, Morrisry AS, Hovestadt V, Weisenfeld J, Ehrenberger T, Gröbner S, Segura-Wang M, Zichner T, Rudneva VA, et al. The whole-genome landscape of medulloblastoma subtypes. Nature. 2017;547:311–7. doi:10.1038/nature22973. PMID:28726821.

5. Cavalli FMG, Remke M, Rampasek L, Peacock J, Doz F, Kool M, Dufour C, Vassal G, Milde T, et al. Risk strata for childhood medulloblastoma in the molecular era: the current consensus. Acta Neuropathol (Berl). 2016;131:821–31. doi:10.1007/s00401-016-1569-6. PMID:27040285.

6. Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. Cell. 2011;144:646–74. doi:10.1016/j.cell.2011.02.013. PMID:21376230.

7. Chen F, Zhuang X, Lin L, Yu P, Wang Y, Shi Y, Hu G, Sun Y. New horizons in tumor microenvironment biology: challenges and opportunities. BMC Med. 2015;13:45. doi:10.1186/s12916-015-0278-7. PMID:25857315.

8. Quail DF, Joyce JA. The Microenvironmental Landscape of Brain Tumors. Cancer Cell. 2017;31:326–41. doi:10.1016/j.ccell.2017.02.009. PMID:28292436.

9. Pardoll DM. The blockade of immune checkpoints in cancer immunotherapy. Nat Rev Cancer. 2012;12:252–64. doi:10.1038/nrc3239. PMID:22437870.

10. Fridman WH, Pagès F, Sautès-Fridman C, Galon J. The immune contexture in human tumours: impact on clinical outcome. Nat Rev Cancer. 2012;12:298–306. doi:10.1038/nrc3425. PMID:22419253.

11. Gooden MJM, de Bock GH, Leffers N, Daemen T, Nijman HW. The prognostic influence of tumour-infiltrating lymphocytes in cancer: a systematic review with meta-analysis. Br J Cancer. 2011;105:93–103. doi:10.1038/bjc.2011.189. PMID:21629244.

12. Pernet FD, Bousset V, Hainsworth G. Immunotherapies for malignant glioma. Oncogene. 2017;36:818–29. doi:10.1038/onc.2016.178. PMID:28022800.

13. Gentles AJ, Newman AM, Liu CL, Bramat SV, Feng W, Kim D, Nair VS, Xu Y, Khuong A, Hoang CD, et al. The prognostic landscape of genes and infiltrating immune cells across human cancers. Nat Med. 2015;21:938–45. doi:10.1038/nm.3909. PMID:26193342.

14. Ali HR, Chlon L, Pharoah PDP, Markowitz F, Calsdas P. Patterns of Immune Infiltration in Breast Cancer and Their Clinical Implications: A Gene-Expression-Based Retrospective Study. PLOS Med. 2016;13:e1002194. doi:10.1371/journal.pmed.1002194. PMID:27995923.

15. Becht E, Giraudon NA, Lacroux L, Buttard B, Elarouci N, Petitprez J, Selves J, Laurent-Puig P, Sautès-Fridman C, Fridman WH, et al. Estimating the population abundance of tissue-infiltrating immune and stromal cell populations using gene expression. Genome Biol. 2016;17:218.

16. Maaten L van der, Hinton G. Visualizing Data using t-SNE. J Mach Learn Res. 2008;9:2579–605. doi:10.1162/jmlr.2017.15-0713. PMID:21875497.

17. Doucette T, Rao G, Rao A, Shen L, Aldape K, Wei J, Dzurzynski K, Gilbert M, Heimberman AB. Immune Heterogeneity of Glioblastoma Subtypes: Extrapolation from the Cancer Genome Atlas. Cancer Immunol Res. 2013;1:112–22. doi:10.1158/2326-6066.CIR-13-0028. PMID:24409449.

18. Palmer SL, Reddick WE, Gajjar A. Understanding the Cognitive Impact on Children Who Are Treated for Medulloblastoma. J Pediatr Psychol. 2007;32:1040–9. doi:10.1093/jpepsy/jsi056. PMID:17329318.

19. Majzner RG, Simon JS, Grosso JF, Martinez D, Pawel BR, Santi M, Merchant MS, Goegeer B, Hezam I, Marty V, et al. Assessment of programmed death-ligand 1 expression and tumor-associated immune cells in pediatric cancer tissues. Cancer. 2017;123:3807–15. doi:10.1002/cncr.30724. PMID:28608950.

20. Vermeulen JP, Hecke WV, Adrianaeen EJM, Esmen MK, Bouma RG, Hidalgo JV, Fisch P, Broekhuizen R, Splet WGM, Kool M, et al. Prognostic relevance of tumor-infiltrating lymphocytes and immune checkpoints in pediatric medulloblastoma. Oncoimmunology. 2017;6:e139877. doi:10.1080/2162402X.2017.1293940.

21. Newman AM, Liu CL, Chatelain A, Feng W, Xu Y, Hoang CD, Dinh E, Alizadeh AA. Robust enumeration of cell subsets from tissue expression profiles. Nat Methods. 2015;12:453–7. doi:10.1038/nmeth.3337. PMID:25822800.

22. Delaunay M, Junginger C, Otremba A, Friedel D, Brunner J, Eichenberger F, Moosmann C, Castresana-Rubertes A, et al. The anti-tumor activities of natural killer cells to target medulloblastoma. Expert Rev Anticancer Ther. 2012;12:298–306. doi:10.1586/14737596.12.1.298. PMID:22832583.
