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Clinical outcome of biomarker-guided therapies in adult patients with tumors of the nervous system

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Abstract

Background. The clinical utility of molecular profiling and targeted therapies for neuro-oncology patients outside of clinical trials is not established. We aimed at investigating feasibility and clinical utility of molecular profiling and targeted therapy in adult patients with advanced tumors in the nervous system within a prospective observational study.

Methods. molecular tumor board (MTB)@ZPM (NCT03503149) is a prospective observational precision medicine study for patients with advanced tumors. After inclusion of patients, we performed comprehensive molecular profiling, formulated ranked biomarker-guided therapy recommendations based on consensus by the MTB, and collected prospective clinical outcome data.

Results. Here, we present initial data of 661 adult patients with tumors of the nervous system enrolled by December 31, 2021. Of these, 408 patients were presented at the MTB. Molecular-instructed therapy recommendations could be made in 380/408 (93.1%) cases and were prioritized by evidence levels. Therapies were initiated in 86/380 (22.6%) cases until data cutoff. We observed a progression-free survival ratio >1.3 in 31.3% of patients.

Conclusions. Our study supports the clinical utility of biomarker-guided therapies for neuro-oncology patients and indicates clinical benefit in a subset of patients. Our data might inform future clinical trials, translational studies, and even clinical care.

Key Points

- Molecular profiling expands therapeutic options.
- Biomarker-guided treatments result in clinical benefits for a subset of patients.
- We propose neuro-oncology magnitude of clinical benefit scale (Neuro-MCBS), a modified version of European Society for Medical Oncology (ESMO)-MCBS, as a novel assessment parameter.

Importance of the Study

Many tumors of the nervous system remain a therapeutic challenge with a limited availability of established therapies and clinical trial options. Biomarker-based targeted therapies may offer additional therapeutic options. Here we present our real-world experience as part of a prospective observational study using a precision medicine workflow from comprehensive molecular profiling to biomarker-based treatments in adult neuro-oncology patients. This approach expands therapeutic options and results in clinical benefits for a subset of patients. In addition to the intra-patient PFS interval with targeted therapy/PFS interval with the prior systemic therapy ratio, we propose a novel assessment parameter, neuro-oncology magnitude of clinical benefit scale (Neuro-MCBS) based on the ESMO-MCBS to assess the magnitude of clinical benefit in neuro-oncology patients. Our study includes a wide spectrum of tumors in the nervous system including rare alterations and conditions and thus might inform clinical care, future clinical trial designs, and scientific projects.

Molecular-guided tailored treatment strategies hold the promise to improve clinical outcomes and quality of life, particularly for patients for whom no further established therapy or limited clinical trial options are available.[1,2] The rapid advances in next-generation sequencing (NGS) technology have enabled the conduction of clinical trials in which treatments are guided by tumor tissue molecular profiles.[3–5]

Tumors of the nervous system are a heterogeneous group of diseases that include primary and metastatic tumors. The current WHO Classification recognizes >120 primary tumors in the nervous system.[6] Current international treatment guidelines[7–13] often outline a rather limited spectrum of established medical treatment options for various neuro-oncological tumor entities. Innovative clinical trials, for example, the Neuroonkologische Arbeitsgemeinschaft (NOA)-20 umbrella trial in newly diagnosed glioblastoma without hypermethylation of the methylguanine-methyltransferase promoter[14,15] investigate molecular-matched therapeutic strategies. Still, a substantial proportion of neuro-oncology patients cannot participate in clinical trials, either because they are not eligible or simply because there is a lack of clinical trials for their specific tumor entity or tumor stage leading to a substantial unmet clinical need.

Advanced high-throughput molecular diagnostics are readily available at lower costs, and a substantial proportion of molecular diagnostics are currently performed outside clinical trials. The challenge in this "real-world" setting is, however, to connect the complex diagnostic evaluations with the relevant individual clinical data and to formulate rational personalized treatment recommendations. To this end, standardized, quality-controlled, and transparent workflows are necessary for routine clinical care. The Center for Personalized Medicine Tübingen was founded to meet this challenge by establishing systematic interdisciplinary standardized procedures that allow for molecular profiling, informed treatment recommendations, and prospective collection of clinical outcome data. The molecular tumor board (MTB) acts as a linchpin in this setting, consisting of clinicians from all oncology disciplines, as well as pathologists, neuropathologists, pharmacologists, cancer biologists, geneticists, and bioinformatics experts. The prospective observational study MTB@ZPM (NCT03503149) evaluates the impact of this comprehensive workflow on the clinical course and outcome of our patients.

The assessment of clinical utility of a given targeted therapy can be difficult to measure, mainly because molecularly targeted therapies are most commonly used in patients at various stages of their illness. To address this challenge, the use of a progression-free survival (PFS) ratio has been proposed.[16] This PFS2/PFS1 ratio uses the PFS interval with targeted therapy (PFS2) divided by the PFS interval with the prior systemic therapy (PFS1) in a given patient, Thus, PFS2/PFS1 ratio serves as an intra-patient assessment. As successive lines of cancer therapies become less efficient over time due to the accelerating cancer dynamic, a PFS2/PFS1 ratio of ≥1.3 is considered to be indicative of a favorable response to the currently employed targeted therapy.[16]

Recent observational precision medicine trials[17,18] did not include adult patients with tumors of the nervous system. Here we report our results of the first 661 neuro-oncology patients enrolled in the ongoing MTB@ZPM (NCT03503149) observational study.

Methods

Study Design

The MTB Tübingen at the Center for Personalized Medicine Tübingen (MTB@ZPM) is a prospective single-center observational study for molecular-guided stratification and therapy of patients with advanced tumor diseases, continuously recruiting since the end of March 2018 (NCT03503149) (Figure 1A). The workflow had been first established in a 2-year-pilot phase from April 2016—March 2018 before the initiation of the prospective observational study end of March 2018 (MTB@ZPM and NCT03503149). Furthermore, since January 01, 2021, the whole workflow has been transferred into the healthcare system of the State of Baden-Württemberg (www.zpm-verbund.de). The present study focused on adult patients with tumors of the central nervous system. The ethical board of the University Hospital Tübingen provided an Ethics approval to the present study, that is, retrospective assessments of 132 patients in the pilot phase in 2016–2018 (700/2020BO) and 529 in the ongoing prospective observational study MTB@ZPM until the cutoff date December 31, 2021 (883/2017BO1). Adult patients with tumors in the nervous system (age >18 years) were evaluated for this observational study based on the indication for comprehensive molecular profiling by the neuro-oncology tumor board and after the informed consent process. All steps of the workflow and relevant information were explained to each individual patient by a trained physician.

Main eligibility criteria include (1) advanced tumor disease without further registered and guideline-based treatment options, and (2) rare tumor disease as defined by EURACAN (Supplementary Methods). After processing of tumor and blood samples, quality checks, NGS-based profiling by gene panels and transcriptomic analyses, and subsequent bioinformatic analyses were performed (Supplementary Methods).

Presentation and Discussion in the Molecular Tumor Board

The MTB is a weekly interdisciplinary conference, and external partners can participate. We have presented and discussed 408/661 (61.7%) of patients from the Neuro NGS cohort in the MTB until the cutoff date of December 31, 2021 (Figure 1B, Supplementary Figure S1).

Concordant, Discordant, and Partial Agreement of Immunohistochemistry (IHC)

In general, IHC staining was assessed as consistent with an activation of the signaling pathway if more than one antibody indicated activation of the signaling pathway (*concordant* IHC). If only a single antibody indicated activation of a signaling pathway whereas other antibodies did not indicate downstream activation, a "partial agreement of IHC" was assessed (eg, increased expression of CDK4 but not of p-RB). Whereas "discordant IHC" was assumed when none of the antibodies used indicated activation of the signaling pathway (Supplementary Methods).

Clinical Evaluation of Molecular Profile and Outcome Parameters

We determined the clinical actionability of molecular profiles ("actionable mutations") in weekly interdisciplinary MTB conferences including all clinical oncology disciplines, pathologists, human geneticists, bioinformaticians, pharmacologists, and tumor biologists. All MTB recommendations are centrally documented as outlined in the MTB@ZPM observational study. We performed structured clinical and imaging follow-ups every 8–12 weeks from the time of MTB recommendation.

For the present study, we reviewed and reevaluated all data from patients with tumors in the central nervous system by specialists, residents, and tumor biologists (MR, SCK, JR, BW, HB, HH, PB, DR, LG, LH, MS, DJM, FP, JS, RB, OR, BB, MB, NM, DZ, and GhT).

The MTB recommendations were classified based on levels of evidence in the MTB reports. In addition, we assigned for the present study the respective ESCAT Tiers (Supplementary Table ST5). ESCAT Tier I refers to molecular

targets that are suitable for clinical use, that is, recommendations for therapy with a specific drug when a specific molecular alteration is detected. ESCAT Tier II refers to targets that likely define a patient population that will benefit from targeted therapy, however, additional clinical data are needed. ESCAT Tier III refers to molecules that have previously proven to be beneficial in other tumors or related molecular targets. ESCAT Tier IV refers to targets that call for clinical actionability based on preclinical evidence only. ESCAT Tier V refers to molecular targets that have been demonstrated to have relevant antitumor activity but in clinical studies have not resulted in clinically meaningful benefit. ESCAT Tier X refers to molecular alterations, where a lack of evidence for actionability exists in a given cancer.

The clinical benefit was evaluated for patients who started MTB therapy until the cutoff of the present date December 31, 2021. Clinical assessments were performed per standardized clinical assessments including interval clinical history, physical and neurological examination. Radiological assessments were performed according to radiological assessment in neuro-oncology.[19–22] Clinical outcome parameters included PFS, defined as the time from begin of MTB therapy to the date of clinical or radiographic progression. Overall Survival (OS), is defined as the time from first diagnosis to the date of death. We also assessed a PFS2/PFS1 ratio to compare the PFS associated with the current MTB therapy (PFS2) with the PFS associated with the immediate prior line of treatment (PFS1).

Objective response rate is defined as the proportion of patients with either complete response or partial response and disease control rate is defined as the proportion of patients with either complete response, partial response, or stable disease. In addition, we assessed duration of response, defined as time from radiographic response (partial response + complete response) to time of disease progression, and duration of clinical benefit, defined as time from radiographic response (stable disease + partial response + complete response) to time of disease progression. Furthermore, we assigned our novel Neuro-MCBS (Table 1) to each case.

Statistical Analyses

Data are described by mean and standard deviations for normally distributed continuous variables and by median and range for non-normally distributed continuous variables.

Categorical variables were described in terms of absolute and relative frequencies. Descriptive analyses were stratified by subcohorts.

Data Handling and Availability

All data has been processed through the dedicated MTB data infrastructure. This infrastructure includes an automated generation of pseudonyms at the time of enrollment. These pseudonyms are subsequently used to deliver the NGS raw and processed data to the centralized infrastructure at the Quantitative Biology Center of the University of Tübingen. The established data stores enable a data management concept of patient-derived raw sequencing data separated from their clinical data and metadata. A central web interface functions as a data access gate. The NGS-panel sequencing dataset generated during the current study will thus not be uploaded to a public repository as these are patient samples with potentially identifiable germline information. Data access for researchers beyond the Center for Personalized Medicine Tübingen is possible upon request to the corresponding author. This requires granting by the Data Use and Access Committee of the University Hospital Tübingen (https:// www.medizin.uni-tuebingen.de/dedas-klinikum/ einrichtungen/institute/informationstechnologie-und-medizininformatik/medic/duac). Following the granting of access, accounts are given to researchers and data can be accessed via the web interface and programmatically.

Results

Comprehensive Molecular Profiling and Targeted Therapy Recommendations

As of April 2016, we have implemented a standardized workflow to identify biomarker-based therapeutic options for patients with rare and advanced cancers including CNS tumors that lack further registered treatment or clinical trial options (Figure 1A, Supplementary Information). The "Neuro NGS cohort" comprises 661 patients who consented to and were enrolled in this program by December 31, 2021 (Supplementary Table ST1). 408/661 (61.7%) patients from the Neuro NGS cohort underwent molecular profiling and were presented at the MTB, designated as "Neuro MTB presentation" cohort. Targeted therapy recommendations were formulated in 380/408 (93.1%) patients ("Neuro MTB recommendation" cohort). Following confirmed tumor progression, MTB therapy recommended by the multidisciplinary neuro-oncology tumor board and approval of drug coverage by insurance, 86/380 (22,6%) patients of the Neuro MTB recommendation cohort started molecular-guided therapy until December 31, 2021 ("Neuro MTB therapy" cohort, Supplementary Table ST2). Of these 86 patients, 64 (74,4%) ("Neuro MTB PFS2/PFS1 ratio" cohort) were evaluable for an intra-patient progression-free survival ratio (Figure 1B).

Baseline Characteristics of the Neuro NGS Cohort

The majority of patients had a glioblastoma, CNS WHO grade 4 (n = 262, 39.6%) followed by CNS metastases (n = 65, 9.8%), pituitary adenoma (n = 46, 7%), meningioma CNS WHO grade 1 (n = 43, 6.5%), and meningioma CNS WHO grade 2 (n = 43, 6.5%) (Figure 1D, Supplementary Tables ST1 and ST3). We detected around 1600 oncogenic or likely oncogenic somatic mutations and evaluated their frequency within each tumor entity (Table 2, Supplementary Data). Furthermore, we detected 54 pathogenic or likely pathogenic germline variants in the Neuro NGS Cohort (Figure 1D,Table 2) and recommended genetic

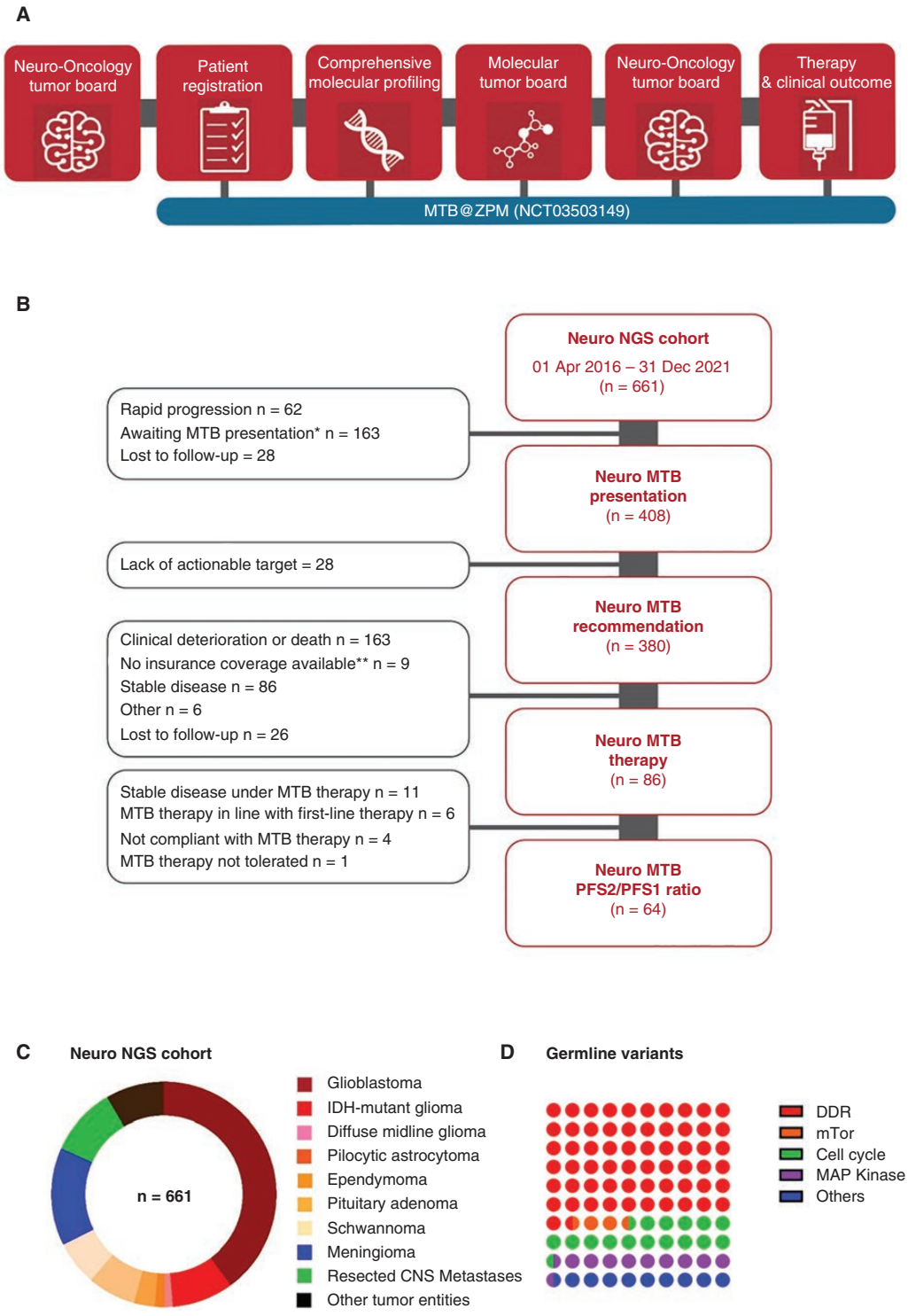

Figure 1. Precision medicine workflow and the Neuro NGS Cohort Tübingen. (**A**) Schematic overview: Clinical indication by the neuro-oncology tumor board, patient consenting and registration, sample processing, molecular diagnostics, assessment and target decision within the molecular tumor board (MTB), approval by the neuro-oncology tumor board, approval of drug coverage, initiation of therapy. (**B**) Neuro-oncology patient cohorts. *163 patients have not yet been presented at the MTB until the data cutoff. **For 9 patients, the insurance companies did not approve a reimbursement. (**C**) The Neuro NGS Cohort (*n* = 661). *CNS*, central nervous system. *IDH*, isocitrate dehydrogenase; *NGS*, next-generation sequencing. (**D**) Relative proportion of germline variants within the Neuro NGS cohort depicted according to their functional pathway affiliation, (1 circle = 1%).

Abbreviation: *DDR*, DNA damage repair; *mTOR*, mechanistic target of rapamycin; *MAP*, mitogen-activated protein kinase; NGS, next-generation sequencing.

Table 1. Neuro-Oncology Magnitude of Clinical Benefit Scale (Neuro-MCBS)

Grade	Criteria
1	PFS ≥6 months or PR/ CR (per RANO), ie, reduction in tumor size ≥ 50% or SD (per RANO), ie, ≤ 50% ↓and ≤ 25% ↑ in tumor size AND DoCB ≥ 6 months.
2	PFS 4–6 months or SD (per RANO), ie, ≤ 50% ↓and ≤ 25% ↑ in tumor size or PD (per RANO), ie, ≥ 25% ↑ in tumor size AND DoCB ≥ 3 months.
3	PFS 2–3 months or PD (per RANO), ie, ≥ 25% ↑ in tumor size AND DoCB ≥ 3 months.
0	No clinical benefit.

We developed Neuro-MCBS based on part 1 of the ESMO-MCBS (Supplementary Table ST6) as an additional tool for the assessment of individual clinical outcomes (Methods). Four levels of clinical benefit based on radiological assessment in neuro-oncology criteria and duration of clinical benefit (ie, including clinical and neuro-imaging features); Neuro-MCBS grade 1 is the highest score, followed by grades 2 and 3. Neuro-MCBS grade 0 indicates lack of clinical benefit.
PFS, Progression-Free Survival; *PR*, Partial Response; *CR*, Complete Response; *SD,* stable disease; *DoCB,* Duration of Clinical Benefit (CR + PR + SD).

Table 2. Somatic Mutations and Germline Variants in the Neuro Next-Generation Sequencing Cohort

Tumor Entity	Number of Somatic Mutations	Number of Germline Variants (%)	Genes Where Germline Variants Were Detected (Number of Patients)
Overall	1600/661	54/661 (8.2)	see below per tumor entity
Glioblastoma, IDH-wild type, CNS WHO Grade 4	875/262	14/262 (5.3)	BRCA1 (*n* = 1), BRCA2 (*n* = 1), ERCC3 (*n* = 1), ERCC2 (*n* = 1), FANCA (*n* = 1), MUTYH (*n* = 1), FANCC (*n* = 1), FANCD2 (*n* = 1), FANCM (*n* = 1), MSH2 (*n* = 1), NF1 (*n* = 1), NBN (*n* = 1), SDHD (*n* = 1),TP53 (*n* = 1)
Pilocytic astrocytoma CNS WHO Grade 1	17/9	6/9 (66.7)	BRCA2 (*n* = 1), MUTYH (*n* = 1), FANCG (*n* = 1), NF1 (*n* = 3)
Astrocytoma, IDH-mutant CNS WHO Grade 2	20/12	0/12 (0)	N/A
Astrocytoma, IDH-mutant CNS WHO Grade 3	86/15	3/15 (20.0)	LZTR1 (*n* = 1), PALB2 (*n* = 2)
Astrocytoma, IDH-mutant CNS WHO Grade 4	63/24	4/24 (16.7)	FANCC (*n* = 1), PALB2 (*n* = 1), PMS1 (*n* = 1), SRGAP1 (*n* = 1)
Oligodendroglioma	5/7	2/7 (28.6)	RAD54L (*n* = 1), XPC (*n* = 1)
Diffuse midline glioma, H3 K27-altered, CNS WHO grade 4	67/8	0/8 (0)	N/A
Diffuse hemispheric glioma, H3 G34-mutant, CNS WHO Grade 4	8/2	0/2	N/A
Ependymoma	47/20	0/20 (0)	N/A
Pituitary Adenoma	1/46	0/46 (0)	N/A
Schwannoma CNS WHO Grade 1	1/42	2/42 (4.8)	BRCA2 (*n* = 1), BRCA1 (*n* = 1)
Meningioma CNS WHO Grade 1–3	147/93	2/93 (2.1)	BAP1 (*n* = 1), VHL (*n* = 1)
Resected CNS metastases	136/65	9/65 (13.8)	APC (*n* = 1), BRCA1 (*n* = 1), BRIP1 (*n* = 1), ABRAXAS1 (*n* = 1), PALB2 (*n* = 1), MSH6 (*n* = 1), NF1 (*n* = 2), FANCA (*n* = 1)
Other tumor entities	127/56	12/56 (22.2)	BRCA1 (*n* = 1), BRCA2 (*n* = 2), ERCC3 (*n* = 1), CHEK2 (*n* = 1), ERCC2 (*n* = 1), FANCA (*n* = 1), MUTYH (*n* = 2), MSH6 (*n* = 1), SDHD (*n* = 1), TP53 (*n* = 1)

All genes with oncogenic or likely oncogenic somatic mutations per tumor entity (second column) and likely pathogenic and pathogenic germline variants are outlined per tumor entity (third column). The last column lists all genes in which these germline mutations were detected, followed by the respective number of patients. *N/A*, not applicable.

counseling on the detection of these germline mutations (Supplementary Data). We categorized all affected genes with germline mutations (Table 2) according to functional pathways. Most germline variants occurred within the DNA damage repair pathway (Figure 1D).

Clinically Actionable Molecular Alterations and Baskets in the Neuro MTB Presentation Cohort

We determined the clinical utility of molecular alterations in the MTB ("clinically actionable alterations") (Supplementary Methods). Until data cutoff, we discussed molecular profiles of 408 patients (Neuro MTB presentation cohort, Figure 1B, Supplementary Figure S1), comprising clinically actionable molecular targets in 380/408 (93.1%) cases (Neuro MTB recommendation cohort, Figure 1B and 2A), and evaluated their frequency per entity (Supplementary Data).

For each molecular alteration, we determined whether it was associated with a loss of function (LOF) or a gain of function (GOF) regarding the affected cellular pathways or processes. If mutations resulted in an inactivation of protein function, they were defined as LOF, and with activation of protein function as GOF. Clinically actionable LOF alterations included: *PTEN*, *CDKN2A*, *CDKN2B*, *NF2*, and *NF1*. Clinically actionable GOF alterations included the following genes: *EGFR, CDK4, CDK6, MET, MDM2, and PIK3CA*. Overall, we detected 483 GOF and 443 LOF alterations, most of them in glioblastoma (220 GOF, 249 LOF) and in astrocytoma, IDH mutant, CNS WHO grade 3 and 4 (32 GOF, 42 LOF) (Figure 2B, Supplementary data). Composite biomarker profiles (Supplementary information) were detected in 29/380 patients (7.6%) of the Neuro MTB recommendation cohort. High tumor mutational burden (Supplementary Methods) was detected in 14/223 glioblastoma (5.8%), 4/19 (21.1%) IDH-mutant astrocytoma, CNS WHO grade 4; 8/38 (21.1%) CNS metastases (Non-small-cell lung cancer: $n = 6$, CUP: $n = 1$, gastrointestinal cancer: $n = 1$); 2 esthesioneuroblastomas and 1 chondrosarcoma. We categorized the most common molecular alterations in the following biomarker baskets: DNA damage repair basket (*ATM, PALB2, BRCA2, CHEK2, FANCA, MUTYH, POLE, BRCA1, BRIP1, FANCB, MDC1, RAD51C,and STAT5B*), mammalian target of rapamycin (mTOR) basket (*PTEN, NF2, PI3K-family, TSC2, MTOR, AKT1, AKT3, and INPP4B*), cell cycle basket (*CDKN2A, CDKN2B, CDK4, CDK6, CCND1, CCND2, RB1, CCNE1, CCND3, CDKN2C, CDKN1A, and CDKN1B*), mitogen-activated protein kinase pathway (*NF1, BRAF, KRAS, BRAF-KIAA1549, and BRAF-PWWP2A*), tyrosine kinase basket (*EGFR, FGF-family, PDGFRA, KIT, KDR, FGFR-family, MET, FLT-family, PDGFRB, NTRK, ROS1, ERBB2, FRS2, RET, VEGFR3, FGFR3-TACC3, BCR-NTK2, CLIP2-MET, DENND1A-NTRK2, FGFR2-RBFOX2, FSD1L-NTRK2, KANK1-NTRK2, KIF5B-BRET,and PTPRZ1-MET*) (Figure 2B). LOF biomarkers were enriched in the mTOR basket, for example, homozygous *PTEN* deletions. GOF biomarkers were enriched in the cell cycle and tyrosine kinase basket (eg, *CDK4, CDK6,* and *EGFR* amplifications). Gene fusions were enriched in the tyrosine kinase basket (Figure 2B).

Biomarker-Guided Treatment Recommendations

MTB treatment recommendations were based on the molecular profiles and the available clinical evidence. 135/ 380 (35.5%) patients in the Neuro MTB recommendation cohort (Figure 2A) received recommendations for more than one targeted therapy, ranked as priority 1, 2, or 3 recommendations. Criteria for a higher priority included evidence levels and additional support based on immunohistochemistry staining assessments (Figure 3). The majority of recommendations were single agents (priority 1: 375/380, 98.7%; priority 2: 130/135, 96.3%; priority 3 36/37, 97.3%). Combination therapies were recommended for 11/380 patients (Supplementary Data) based on a specific molecular context, for example, BRAFV600E—leading to the recommendation of inhibiting BRAF plus MEK.

Evidence Levels and ESCAT Tiers of MTB Recommendations

All recommendations were categorized according to evidence levels (Supplementary Table ST4) in the final MTB report. Priority 1 recommendations fulfilled criteria for evidence levels m1a (115/380, 30%), m1b (6/380, 1.5%), m1c (128/380, 33.7%), m2a (51/380, 13.4%), m2b (13/380, 3.4%), m2c (33/380, 8.7%), m3 (16/380, 4.2%), and m4 (3/380, 0.8%). Priority 2 recommendations fulfilled evidence levels m1a (15), m1b (2), m1c (17), m2a (43), m2b (6), m2c (36), m3 (5), and m4 (4). Priority 3 recommendations fulfilled evidence levels m1a (5), m1c (4), m2a (12), m2c (12), and m3 (2) (Figure 2C, left graph).

Furthermore, we applied the European Society for Medical Oncology Scale for Clinical Actionability of Molecular Targets (ESCAT),[23] a framework proposed by ESMO to classify and prioritize molecular targets and the respective targeted therapy based on clinical evidence of utility in a given cancer type (Supplementary Table ST5). Most priority 1 recommendations in the Neuro MTB recommendation cohort were constituent to ESCAT tier III-A (156/380, 41%), followed by ESCAT tier V (73/380, 19.2%), (ESCAT tiers I-A (7/380, 1.8%), I-B (37/380, 9.7%), I-C (35/380, 9.2%), II-A (29/380, 7.6%), II-B (26/380, 6.8%), III-B (2/380, 0.5%), IV-A (2/380, 0.5%), IV-B (6/380, 1.6%) (Figure 2C, right graph). Of note, we did not recommend any biomarker-guided therapy without evidence for actionability in neuro-oncology entities (ESCAT tier X).

Immunohistochemistry as an Additional Molecular Assessment Tool

In 192/380 patients of the Neuro MTB recommendation cohort (50.5%), we performed additional immunohistochemistry (IHC), particularly in the presence of several actionable mutations. In total, 294 IHC staining panels were performed (Figure 3A, first row) and classified as concordant, discordant, or partial agreement (Supplementary Methods) with the NGS results. Among 192 patients and 294 IHC staining panels that we evaluated for the present study, the actionable molecular target was confirmed by IHC (concordant) in 154 cases and was discordant in 93 cases (Figure 3A, upper circle). Partial agreements

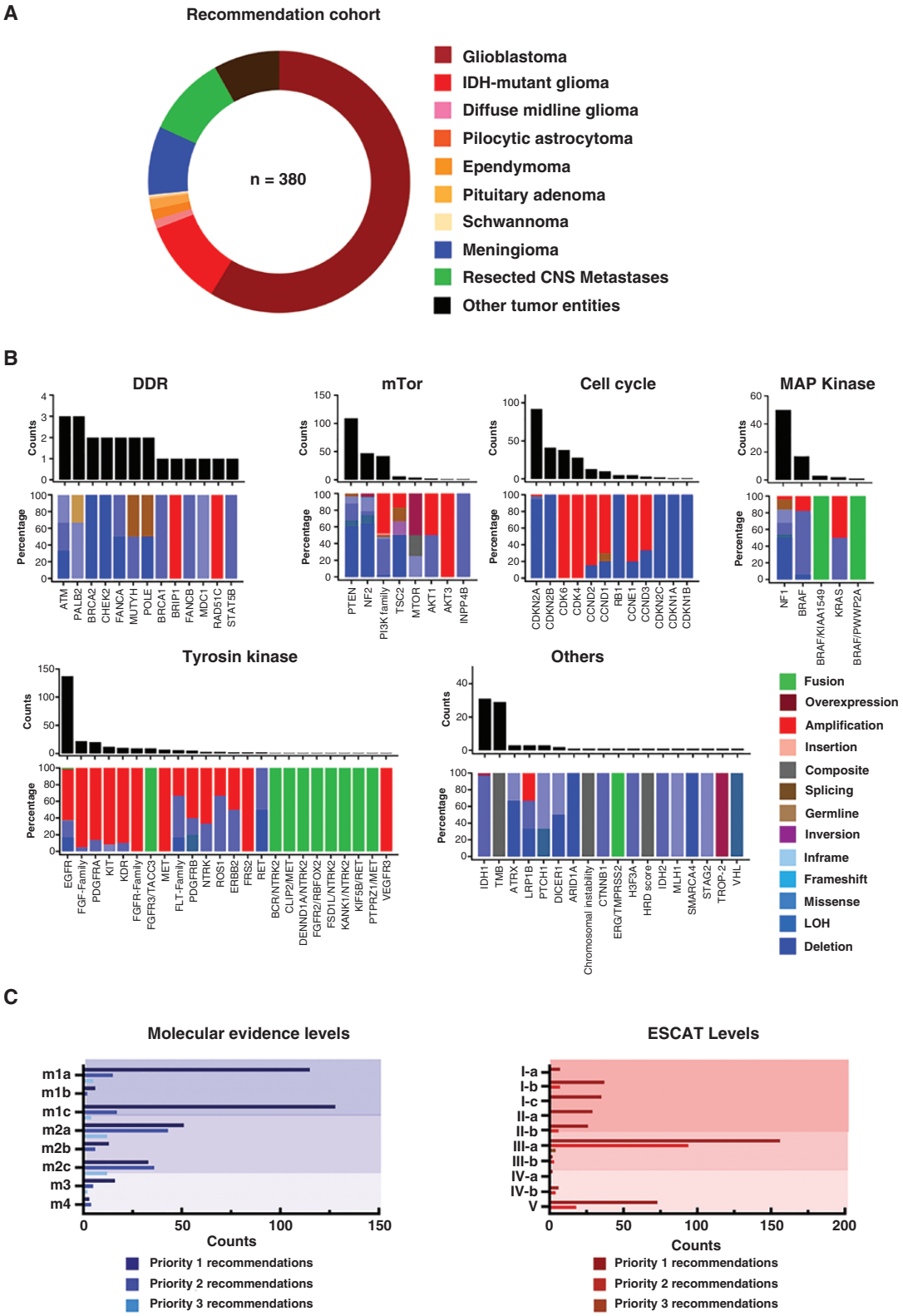

Figure 2. Biomarkers and molecular profiles for MTB discussions. (**A**) Neuro MTB recommendation cohort. (**B**) Incidence and relative proportions of actionable genetic alterations sorted by molecular baskets. *DDR*, DNA damage repair; *mTOR*, mechanistic target of rapamycin; *MAP*, mitogen-activated protein. (**C**) Level of evidence of MTB recommendations (left) and ESCAT tiers (right).

Abbreviation: *ESCAT*, ESMO Scale for Clinical Actionability of molecular Targets; *ESMO*, European Society for Medical Oncology, MTB, molecular tumor board.

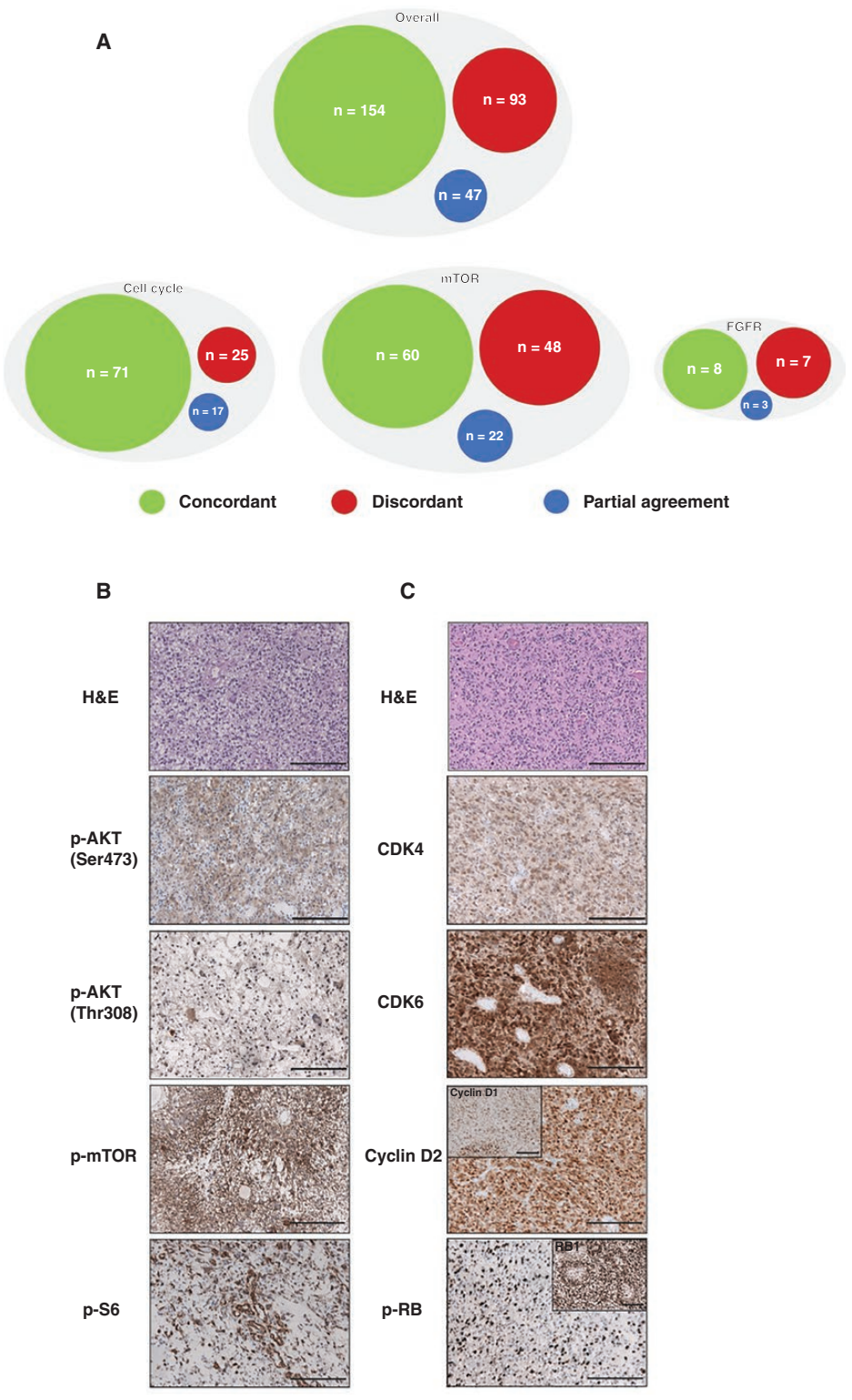

Figure 3. Immunohistochemistry antibody panels for molecular tumor board recommendations. (**A**) Matching assessments of next-generation sequencing diagnostics and additional selected immunohistochemistry (IHC). First line: concordance (green), discordance (red), and partial agreements (blue); second line: Matching assessment per selected IHC panels (cell cycle, mTOR, and FGFR, the composition of the respective IHC panel is outlined in the text). (**B**) Example of activated "cell cycle panel" (in a glioblastoma, CNS WHO grade 4, scale bar: 200 µm). (**C**) Example of activated "mTOR panel" (in a glioblastoma, CNS WHO grade 4, scale bar: 200 µm). H&E, hematoxylin and eosin; *CDK4,* cell division protein kinase 4; *CDK6,* cell division protein kinase 6; *p-AKT,* phosphorylated protein kinase B, *p-mTOR,* phosphorylated mammalian target of rapamycin; *p-S6,* phospho-S6 ribosomal protein, *p-RB,* phosphorylated retinoblastoma protein.

occurred in 47 cases (Figure 3A, upper circle).The frequency of concordance varied between different pathways (Figure 3A, circles in the second row). The most frequent IHC staining panels in our cohort were the "cell cycle" (n = 115) and the "mTOR" (n = 131) panel (Supplementary Methods), as outlined in selected staining examples (Figure 3B–C).The IHC assessments supported the prioritization of treatment recommendations, particularly in cases with multiple molecular targets based on NGS data.

Clinical Outcome and Benefit of Biomarker-Guided Treatments

At the time of data cutoff, 86/380 (22.6%) patients of the Neuro MTB recommendation cohort had started MTB therapy (Neuro MTB therapy cohort, Figure 1B, Supplementary data, Supplementary Table ST2). We observed tumor progression under MTB therapy in 70/86 patients and evaluated the clinical outcome parameter of these patients (Supplementary Table ST6, Figure 4A). Median PFS was 3.3 (range 0.04–30.9) months with differences within the entities: mPFS was 2.5 months (range 0.3–25.8) in 36 patients with glioblastoma; 1.7 months (range 0.8–3.4) in 10 patients with other glioma subtypes; 12.8 months (range 2.1–20.6) in 8 patients with meningioma (one patient with meningioma CNS WHO grade 1, 4 patients with meningioma CNS WHO grade 2 and three patients with meningioma CNS WHO grade 3). In 10 patients with brain metastases from melanoma, breast cancer, NSCLC and CUP, mPFS was 8.2 months (range 2.5; 30.9). We further determined radiological outcome parameters, is that, disease control rates, and best radiographic response (Supplementary Data). Median overall survival (mOS) was 33 months (range 8– 271) (Supplementary Data). As the design of MTB@ZPM leads to the enrollment of a highly heterogeneous group, we next performed intra-patient assessments.

Assessment of Intra-patient PFS2/PFS1 Ratio

We reevaluated all 70 patients who had experienced tumor progression during MTB therapy (Figure 4A). We excluded

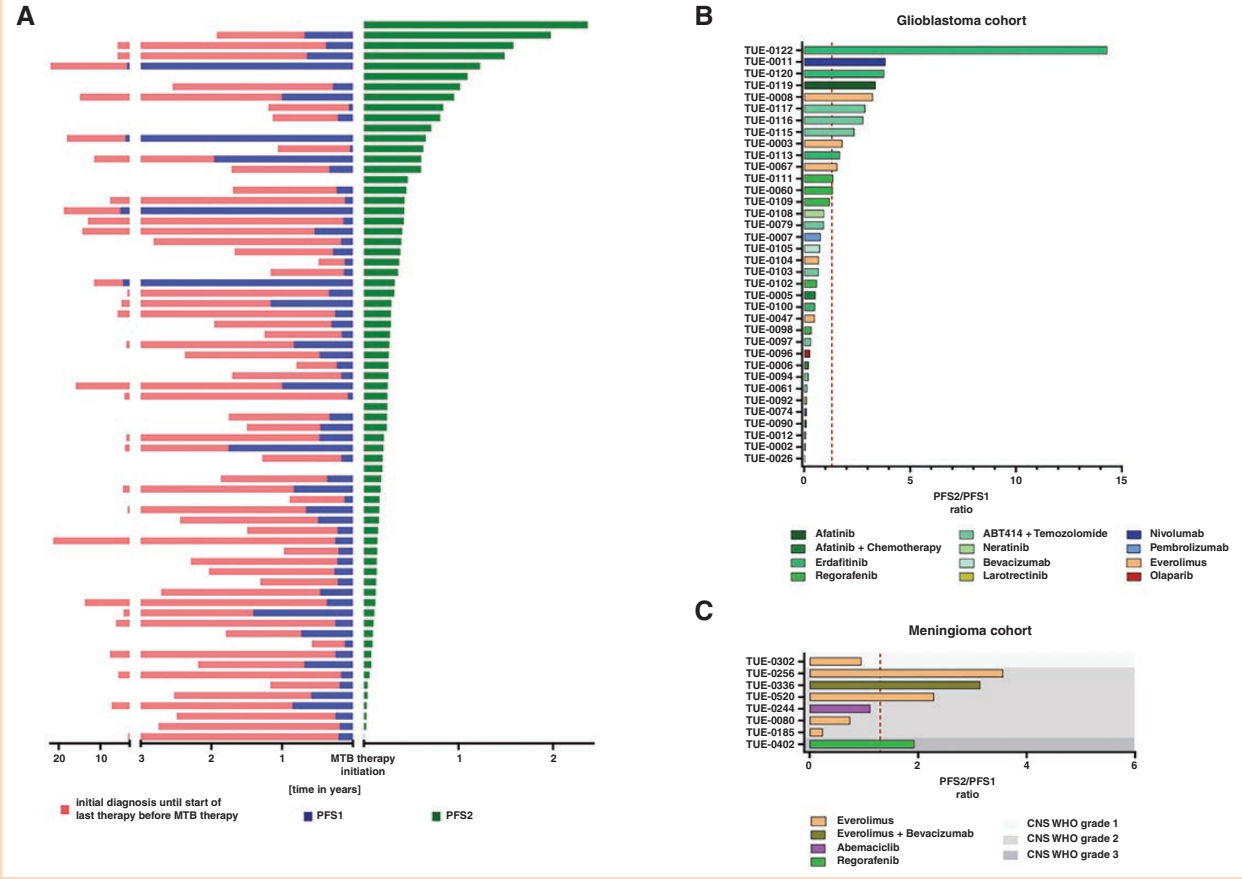

Figure 4. Clinical outcome: Neuro MTB Therapy Cohort and PFS2/PFS1 of selected entities. (**A**) Clinical outcome of n = 70 patients is indicated with documented tumor progression under MTB therapy. PFS2 (green) indicates progression-free survival of each patient since start of the biomarker-based therapy (MTB therapy); PFS1 (blue) indicates progression-free survival during the last treatment before initiation of MTB therapy; the time frame of the initial diagnosis until the last treatment before MTB therapy initiation is outlined in pink. The x-axis indicates time frame in years. (**B**, **C**): Selected entities of the PFS2/PFS1 cohort (B: glioblastoma, C, meningioma). Dashed line indicated PFS2/PFS1 ratio of 1.3.

Abbreviation: *MTB*, Molecular Tumor Board; *PFS*, progression-free survival. *CNS*, central nervous system; *WHO*, world health organization.

6 patients with resected CNS metastases within the MTB therapy cohort, because their molecular-based therapy was the first systemic treatment, thus a PFS2/PFS1 ratio was not feasible (Supplementary Table ST6). In the remaining 64 evaluable patients, we identified a PFS ratio >1.3 in 20/64 (31.3%) patients (Supplementary Figure S2). Glioblastoma (36/64) (Figure 4B) and meningioma (CNS grades 1–3) (8/64) (Figure 4C) were the main entities within the Neuro MTB PFS2/PFS1 cohort, and we observed PFS >1.3 in 36% (13/36) of glioblastoma and in 62.5% (5/8) of meningioma (Figure 4B–C, Supplementary Table ST6). The frequency of PFS ratio >1.3 was similar for patients with or without additional glucocorticoid use (Supplementary data). The proportion of therapies associated with PFS2/PFS1 >1.3 was highest for MTB recommendations based on evidence level m1A ($n = 7$), followed by evidence level m1C ($n = 5$) (not shown). We identified 13 patients within the MTB PFS2/PFS1 ratio cohort who had an IHC assessment, 6 concordant and 7 discordant/partial agreement (Figure 3A, Supplementary Figure S3). Patients with concordant IHC had a PFS2/PFS1 ratio = 1.3 (median, range 0.1–6.6), whereas, patients with discordant/partial agreement had PFS2/PFS1 ratio = 0.68 (median, range 0.4–4.1, Supplementary Figure S3).

Assessment of Clinical Benefit in Individual Patients by Neuro-Oncology Magnitude of Clinical Benefit Scale (Neuro-MCBS)

We reasoned that all neuro-oncology patients within MTB@ZPM could also be viewed as a series of single arms of a clinical study. For clinical benefit assessments in single-arm studies, the ESMO developed an assessment tool for quantifying the magnitude of the clinical benefit of anticancer treatments. This ESMO Magnitude in Clinical Benefit Scale (ESMO-MCBS) for single-arm studies in "orphan diseases" and for diseases with "high unmet need" comprises a 2-step evaluation resulting in adjusted ESMO-MCBS grades 1–5 (Supplementary Table ST7).[24] ESMO-MCBS grading is based on response evaluation criteria in solid tumors.

We propose here a modified version of ESMO-MCBS for neuro-oncology patients, designated as Neuro-Oncology Magnitude in Clinical Benefit Scale (Neuro-MCBS) (Table 1). We incorporated radiological assessment in neuro-oncology criteria[19,20,25,26] and defined four grades based on PFS, radiological assessment in neuro-oncology criteria and duration of clinical benefit, indicating highest (grade 1), intermediate (grade 2), rather incremental (grade 3), and lack of (grade 0) clinical benefit for the individual patient.

An overview of all clinical outcome parameters of MTB therapies in the present study is provided in Supplementary Table ST6. Based on part 1 of the ESMO-MCBS (Supplementary Table ST7). In 36 glioblastoma we determined 14% Neuro-MCBS grade 1 ($n = 5$), 33% grade 2 ($n = 12$), and 14% and grade 3 ($n = 5$), whereas, 39% ($n = 14$) did not have any clinical benefit from MTB therapy. In the group of 10 other gliomas ($n = 5$ astrocytoma, IDH mutant, CNS WHO grade 4; $n = 3$ astrocytoma, IDH mutant, CNS WHO grade 3; $n = 1$ astrocytoma, IDH mutant, CNS WHO grade 2; $n = 1$ diffuse midline glioma, H3 K27-altered, CNS WHO grade 4), we detected Neuro-MCBS grade 1 in 0%, grade 2 in 20% ($n = 2$), grade 3 in 10% ($n = 1$). 70% of this subgroup did not benefit from MTB therapy ($n = 7$). Within meningioma patients ($n = 8$, one patient with meningioma CNS WHO grade 1, 4 patients with meningioma CNS WHO grade 2, and 3 patients with meningioma CNS WHO grade 3), we detected clinical benefit in all patients: Neuro-MCBS grades 1 ($n = 5$, 63%), 2 ($n = 2$, 25%), 3 ($n = 1$, 13%) (Supplementary Table ST6).

Discussion

Biomarker-based treatment strategies have the potential to improve clinical outcomes and quality of life.[21,22] We focus here on adult neuro-oncology patients within the ongoing observational MTB@ZPM trial (Figure 1), a group of cancer patients with a very high unmet clinical need that is underrepresented in previous studies.

Our molecular profiling workflow used standardized steps for variant classification[27–29] (Supplementary Methods) and further emphasized the value of RNA-seq in clinical routine, for example, for the detection of gene fusions (Figure 2). Still, the evaluation of transcriptional effects of amplifications and deletions are challenging due to a lack of corresponding normal nervous system tissue from the same patient. We met this challenge by utilizing RNA data from other patients as controls ("outlier detection") and anticipate that the increasing implementation of RNA-seq within the clinical setting and collaborations in larger networks will contribute to an increasing data foundation in this regard.

Until the data cutoff, 86 patients (22.6% of the MTB recommendation cohort) started MTB therapy. This percentage reflects that the MTB recommendation cohort represents a heavily pretreated group of patients (>2 lines of prior therapy), a majority of which with progressive glioblastoma, where PFS is typically in the order of 2–3 months only.[30] We realize, that our median turnaround time for molecular diagnostics of 81 days (2 and 8 months) from patient identification to MTB therapy recommendation (supplementary data) needs further improvement. In comparison, we acknowledge that the MASTER program[17] and the INFORM trial[18] achieved a median turnaround time of 44 days and 25.4 days, respectively, in a multicenter setting. To address this and in an effort to further optimize the diagnostic workflows in MTB@ZPM, we initiated molecular diagnostics for neuro-oncology patients as early as possible to ensure availability of MTB therapy recommendations within a clinically relevant time frame. With that, our median latency between confirmed tumor progression and initiation of MTB therapy remained 30 days, an interval that actually meets the mandatory washout times for many therapeutic agents (prior therapies). It must be taken into account, that this workflow (Figure 1A) is designed for biomarker-guided treatments outside clinical trials and after the failure of registered treatment lines. Consequently, all registered treatments (eg, lomustine in patients with progressive glioblastomas) must have been applied before the initiation of the (off-label MTB therapy). Therefore, although we performed molecular profiling in

the early phase of the disease, clinical deterioration under second or third-line therapies often made it impossible to initiate the MTB therapy.

In our experience, the addition of selected immunohistochemistry panels (Figure 3, Supplementary Figure S3) was helpful in further informing target decisions and prioritization. Concordant IHC might be useful to predict clinical benefits from matching molecular therapies. Yet, the sample size of this specific subcohort (Supplementary Figure S3) is small (n = 13). Thus, validation in a larger sample size would be necessary.

We detected germline variants in 54/661 cases (Figure 1D, Table 2). While rare inherited syndromes including neurofibromatosis 1 and 2 or Li-Fraumeni syndrome are associated with increased risk for glioma, the vast majority of tumors in the nervous system occur in patients without significant family history.[31,32] Some case reports reported germline variants in different tumors within the nervous system, for example, germline pathogenic *PMS2* in oligodendroglioma.[33] The *International Gliogene Consortium* investigated non-syndromic families with at least 2 glioma patients. It seemed that these families comprise clusters of 2 cases suggesting rather low penetrance and low risk of developing additional gliomas.[34] Recent genome-wide association studies indicated risk variants and particularly putative new associations between glioma and autoimmune conditions.[35] The relevance of the germline variants that we detected in our study (Table 2) still needs to be evaluated in future (pre)clinical studies.

In total, 380/408 patients (93.1%) received one or more biomarker-guided recommendations, similar to a rate of 88.0% within the MASTER trial.[17] Most of our priority 1 recommendations (41.5 %) fell into ESCAT tier III followed by ESCAT tier V 19.2% (Figure 2C). This primarily reflects the lack of data from molecularly stratified clinical trials for many neuro-oncological tumor entities and highlights the importance of currently ongoing basket and umbrella trials in neuro-oncology, for example, the umbrella trial NOA-20/N^{2}M^2 (NCT03158389), the platform trial GBM Agile (NCT03970447) or INSIGhT (NCT02977780) and calls for similar clinical trials in other neuro-oncology entities beyond glioblastoma.

The main focus of the present study was the assessment of the clinical outcome of adult neuro-oncology patients within MTB@ZPM. In order to avoid the issue of comparing patients with various neuro-oncological tumor diagnoses, we have used an intra-patient clinical outcome assessment tool. We detected a PFS2/PFS1 >1.3 in 31.3% of our evaluable patients. This is in line with the MOSCATO 01 trial, in which a PFS2/PFS1 ratio >1.3 was observed in 33% (4) of patients, and the MASTER trial which reported a PFS2/PFS1 ratio >1.3 in 35%[17] of patients. The WINTHER trial had aimed for a ratio >1.5, this was achieved at 22.4%.[36] When analyzing the largest patient group within the MTB therapy cohort, those patients with a progressive glioblastoma, a PFS2/PFS1 ratio >1.3 was achieved in 11 of 35 patients (31%) (Figure 4B). In line with the results from the other studies mentioned above, our data indicate that a subset of patients with glioblastoma might indeed benefit from molecular-targeted therapy. We believe it is unlikely that pseudoprogressions may have influenced our PFS2/PFS1 cohort, given that pseudoprogression typically occurs 3–6 months after initial radiochemotherapy, and most of these patients received MTB therapy at or beyond second relapse and were well beyond that time window (Supplementary Table ST2). In addition to the intra-patient PFS2/PFS1 ratio, we proposed a novel assessment parameter, Neuro-MCBS (Table 1) based on the ESMO-MBCS. We believe that Neuro-MCBS might be more suitable for neuro-oncology patients. This modified tool certainly requires validation in future prospective studies.

Recently, the SHIVA investigators had rather discouraged off-label use of molecularly targeted agents and emphasized the importance of clinical trial enrollment for investigating predictive biomarker efficacy.[3] In our view, one does not exclude the other within the setting of an observational trial. Without any doubt, there is room for improvement and further steps are crucial to further increase the clinical benefit including (1) constant refinement and optimization of comprehensive molecular profiling, (2) careful case-by-case evaluations to determine if initiation of comprehensive molecular profiling earlier in the course of the disease might be helpful, (3) molecular-instructed combination therapies with an outcome assessment adapted to neuro-oncological patients Neuro-MCBS and specific patient-reported outcome evaluation, and (4), of utmost importance, the design and initiation of more molecularly instructed clinical trials.

Taken together, our neuro-oncology cohort within MTB@ZPM offers unprecedented insights into molecular profiles and clinical outcomes of a diverse spectrum of neuro-oncology entities within a large-scale "real world" experience. The molecular-guided MTB therapy recommendations expanded treatment options and may lead to clinically meaningful benefits in a subset of patients as assessed by intra-patient outcome measures. These molecular profiles and clinical outcome data can inform future clinical trials and preclinical basic science projects and thereby contribute to the forward and backward translation cycle.

Supplementary Material

Supplementary material is available online at *Neuro-Oncology Advances* online.

Keywords

Molecular tumor board | MTB@ZPM-001 (NCT03503149) | precision medicine | real-world data | targeted therapy

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Conflict of Interest

BB is the co-founder, shareholder, and CTO of AIRAmed GmbH. DZ, EH, CG, and FP received financial and technical support from Elekta AB, Philips, Siemens, Kaiku Health, and TheraPanacea under research agreements from Dr. Sennewald Medizintechnik GmbH for educational events. CS received an institutional grant from Illumina and research grants from BMS Stiftung Immunonkologie outside the submitted work. LZ is co-founder, board member, and consultant for HepaRegeniX GmbH. AF served as consultant to Roche, Novartis, MSD, BMS, Pierre-Fabre; received travel support from Roche, Novartis, BMS, Pierre-Fabre, received speaker fees from Roche, Novartis, BMS, MSD, and CeGaT. She reports institutional research grants from BMS "Stiftung Immunonkologie". CL has served on advisory boards and received speaker and consulting fees from AbbVie, Bristol-Myers Squibb, and Pfizer MB served on advisory boards of Bayer, Bristol-Myers Squibb, Incyte, MSD Sharp & Dome, and Roche. GhT has served on advisory boards of AbbVie, Bayer, Boehringer Ingelheim, and Bristol-Myers Squibb; received consulting fees from AbbVie, Bayer; received speaker fees from Medac and Novocure. The other authors do not have any conflicts of interest to disclose related to this work.

Author Contribution

Data acquisition and assembly: MR, SCK, JR, BW, HB, HH, PB, DR, LG, LH, MS, DJM, FP, EH, CG, MN, RB, OR, CR, CS, SO, SAE, AG, SB, MS, FF, SS, JS, OK, SN, GG, SF, BB, UE, MB, NM, MT, DZ, and GhT. Bioinformatic analysis: OK, SN, GG, SF, AG, SO. Interdisciplinary discussions at MTB: all authors. Project and data management: MR, SCK, JR, HH, KR, ÖÖ, SF, SN, SB, JS, RB, GhT. Data analysis and interpretation: MR, SCK, JR, BW, HB, HH, PB, DR, LG, LH, MS, DJM, FP, RB, OR, LZ, CL, SYB, TE, HM, MB, NM, DZ, GhT; Display Items: BW, HB, MR, SCK, JR, JS, GhT; Manuscript writing: GhT wrote the first draft, next versions by MR, SCK, JR, CS, OR, RB, JS, and SN. Manuscript review, editing, and final approval before submission: all authors. Conception and design of the study: GhT

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