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Molecular profiling-based decision for targeted therapies in *IDH* wild-type glioblastoma

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Abstract

Background. Molecular profiling allows tumor classification as well as assessment of diagnostic, prognostic, and treatment-related molecular changes. Translation into clinical practice and relevance for patients has not been demonstrated yet.

Methods. We analyzed clinical and molecular data of isocitrate dehydrogenase wild-type glioblastoma patients with sufficient clinical follow-up from the Heidelberg Neuro-Oncology Center and with molecular analysis of tumor tissue that consisted of DNA methylation array data, genome-scale copy number variations, gene panel sequencing, and partly *mTOR* immunohistochemistry between October 2014 and April 2018.

Results. Of 536 patients screened, molecular assessment was performed in 253 patients (47%) in a prospective routine clinical setting with further clinical appointments. Therapy decision was directly based on the molecular assessment in 97 (38%) patients. Of these, genetic information from *MGMT* ($n = 68$), *EGFR* ($n = 7$), *CDKN2A/B* ($n = 8$), alterations of the PI3K–AKT–mTOR pathway ($n = 5$), and *BRAF* ($n = 3$) have been the most frequently used for decision making with a positive overall survival signal for patients with glioblastoma harboring an unmethylated *MGMT* promoter treated according to the molecular assignment. Based on detected molecular alterations and possible targeted therapies, we generated an automated web-based prioritization algorithm.

Conclusion. Molecular decision making in clinical practice was mainly driven by *MGMT* promoter status in elderly patients and study inclusion criteria. A reasonable number of patients have been treated based on other molecular aberrations. This study prepares for complex molecular decisions in a routine clinical decision making.

Key Points

- Molecular treatment decisions can be based on molecular profiling of recently obtained tissues.

Importance of the Study

The improvements in molecular profiling of glioblastoma allow personalized targeted treatments based on detected molecular changes. This study demonstrated the translation of broad molecular assessment into molecular-based treatments in a prospective clinical nonstudy setting. The molecular assessment was performed in 253 patients in a prospective routine clinical setting. Therapy decision was directly based on the molecular assessment in 97 of the patients and was mainly driven by *MGMT* promoter methylation status or genetic information from *EGFR*, *CDKN2A/B*, alterations of the PI3K–AKT–mTOR pathway, and *BRAF*. Revision of molecular data identified 213 potentially targetable alterations in 183 of 253 patients indicating that targeted treatments could increasingly influence clinical treatment decisions for future patients. A web-based application was implemented to provide comprehensive information about possible treatment allocations based on individually uploaded molecular data to support complex molecular decision making for glioblastoma patients.

The unfavorable prognosis of glioblastoma had not greatly improved in recent years since the introduction of radiochemotherapy.[1] Advances in understanding of glioblastoma at the molecular level[2–5] enable new opportunities for molecular diagnostics and targeted treatments of glioma patients. Molecular markers have been implemented in the updated World Health Organization (WHO) classification of brain tumors[6] and are increasingly used to guide treatment decisions. *Isocitrate dehydrogenase (IDH) 1* and *2* mutations[7] and 1p/19q co-deletion are routinely tested in glioma patients In addition, *O*^{6}*-methylguanine DNA methyltransferase* (*MGMT*) promoter methylation status has been shown to be a predictive biomarker for response to alkylating chemotherapy such as temozolomide or lomustine[8,9] and was therefore integrated as predictive biomarker into the current European guidelines for diagnosis and treatment of glioblastoma, for consideration at least in elderly patients.[1] At present, the *MGMT* promoter methylation status mainly guides treatment decisions in elderly patients in whom combined radiochemotherapy might be too burdensome due to age and comorbidities.[10] Most other patients are still treated with radio-chemotherapy although patients with an unmethylated *MGMT* promoter are unlikely to benefit from temozolomide demonstrating the particular need for new treatment strategies in this patients' subgroup, which was shown to not define a molecularly distinct subgroup.[11] Prior clinical trials demonstrated the feasibility of replacing temozolomide by targeted treatments (eg, temsirolimus, bevacizumab, or enzastaurin).[12,13] These treatments did not result in worse survival outcome compared to temozolomide but also failed to improve survival in these molecularly unselected patient cohorts. Therefore, further clinical studies in molecularly selected patient populations may help to set the next steps. In non-*IDH*-mutant gliomas, targetable alterations represent the variant III of epidermal growth factor receptor (EGFRvIII) mutation[14] and v-Raf murine sarcoma viral oncogene homolog B (*BRAF*) mutations.[15] Phosphorylation of mechanistic target of rapamycin (*mTOR*) at Ser2448 is suggested as a biomarker for response to treatment with temsirolimus[12] and a hypermutator phenotype might predict response to checkpoint inhibition[16] or not.[17]

The NCT Neuro Master Match (N^2M^2)/(Neurooncology Working Group of the German Cancer Society [NOA]-20) phase I/IIa umbrella trial intends to molecularly direct therapy for patients with glioblastoma harboring an unmethylated *MGMT* promoter. Allocation to specific targeted treatments is based on molecular alterations.[18] The feasibility to perform extensive molecular diagnostics in a timely fashion to inform clinical decision making was demonstrated in the N^2M^2 pilot study.[19] However, translation of extensive molecular diagnostic into clinical practice and resulting targeted treatments has not been demonstrated so far.

The aims of the present retrospective study were (1) to analyze the translation of prospective broad molecular diagnostics of *IDH* wild-type gliomas into clinical decision making and treatment with molecular-guided therapy in clinical routine, (2) to outline the current usage and potential for targeted therapies, and (3) to provide a web tool for automated allocation of patients to possible targeted therapies.

Methods

Patient Cohort

As of April 4, 2018, we screened the Heidelberg Neuropathology database. Clinical and molecular data of 536 adult patients with the diagnosis of glioblastoma from the Heidelberg Neuro-Oncology Center with molecular analysis of tumor tissue consisting of at least methylation array allowing for assessment of global methylation profiles and copy number variations (CNVs) ± additional gene panel sequencing between October 2014 and April 2018 were identified. The cohort was retrospectively revised for following inclusion criteria: (1) patients aged ≥18 years, (2) integrated diagnosis of *IDH* wild-type glioblastoma, (3) neuropathological report about results of molecular analysis available for treating physicians, and (4) further clinical appointments and treatment in the Department of Neuro-Oncology after molecular analysis was reported. Two hundred fifty-three of the 536 patients (47%) were finally included in this study (Figure 1). The remaining excluded patients had either primarily research-related molecular

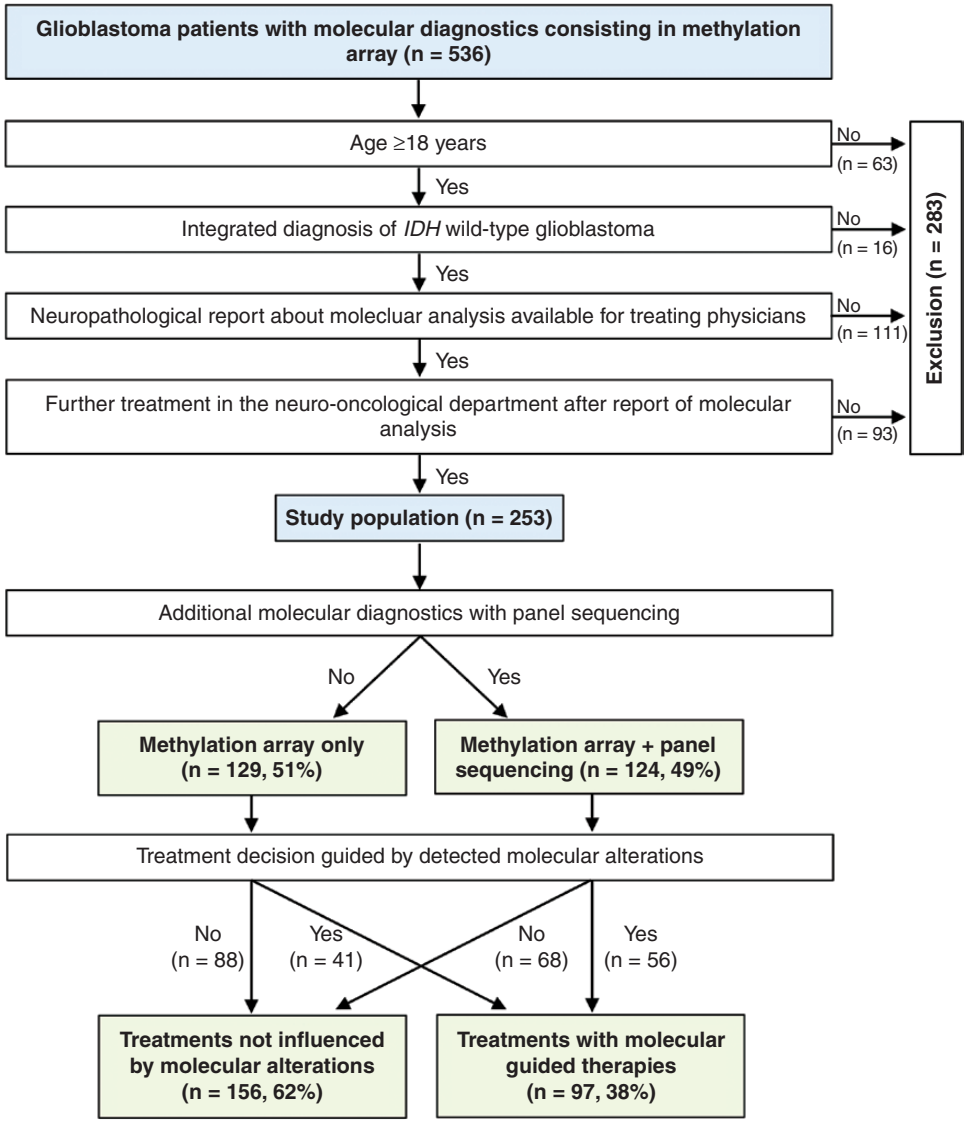

Figure 1. Study inclusion criteria and targeted treatment decisions.

analysis, were further treated outside Heidelberg, had an *IDH* mutated tumor or a non-glioma methylation classifier assignment (Figure 1). The concept of the investigation was approved by the local ethics committee (No. 206/2005).

Clinical characteristics of the patients were retrieved from electronic medical reports. The day of primary surgery was defined as the day of primary diagnosis. Follow-up was closed for included patients on October 1, 2019. Detected molecular alterations were obtained from neuropathological reports and by retrospective analysis of raw data of respective methylation and sequencing diagnostics.

Molecular Analysis

Molecular assessment was performed by the Department of Neuropathology, Heidelberg University Hospital. Besides confirmation of diagnosis, tissue was evaluated with a focus on tumor cell content (>40% needed) as well as necrosis. In parallel, blood was taken as germline control from suitable patients. Nucleic acid extraction from the tumor as well as blood control samples was performed using standard protocols (Maxwell 16 LEV Blood DNA Kit; Invitek Invisorb Genomic DNA Kit; Macherey Nagel NucleoSpin RNA Kit).

Illumina Methylation 450k/EPIC Array

For methylation arrays the Illumina HumanMethylation450 (450k) or MethylationEPIC (EPIC) kits were used to obtain the DNA methylation status at >450 000 and >850 000 CpG sites, respectively, according to the manufacturer's instructions at the Genomics and Proteomics Core Facility of the

German Cancer Research Center in Heidelberg, Germany and at the Department of Neuropathology as described previously.[19]

Methylation Data Processing and Copy Number Analysis

Raw methylation files were quality checked and methylation classifier assignment was performed (https://www.molecularneuropathology.org).[20] Samples were further analyzed using custom scripts based on the methylation pipeline "ChAMP" (version 2.10.1)[21] in R (version 3.5.1, www.r-project.org). In brief, filtering was done for multihit sites, SNPs, and XY chromosome-related CpGs, then data were normalized with a Beta-Mixture Quantile-based method and analyzed for batch effects with a singular value decomposition algorithm. *MGMT* promoter methylation status was determined by the method of Bady et al.[22] Custom scripts based on the R packages "minfi" (version 1.26.2) and "conumee" (version 1.14.0) were implemented for CNV profiling and visualization. A predefined set of 26 genes related to glioma characteristics or potential targeted treatment were annotated in detailed regions and particularly analyzed.

Panel Sequencing of 130 Brain Tumor Relevant Genes

Panel sequencing of 130 genes was performed as described previously.[23] In brief, DNA was extracted from formalin-fixed paraffin-embedded tissue and blood if available and quality checked. Paired-end sequencing was performed on a NextSeq 500 instrument (Illumina). Subtraction of germline variants was performed whenever blood was available (34 of 110 patients, 30.9%).

For assessment of potentially targetable mutations, single-nucleotide variants (SNVs) were filtered by the snp138 database whenever germline subtraction from blood was not available. Further filtering was done including only exonic alterations with either deletion/insertion, nonsynonymous SNV or stop-gain SNV. Mutation allele frequency was calculated as follows: (altered reads)/(all read at this position). Nonsynonymous SNV was tested for potential functional impact using the online tool mutationassessor.org.[24]

mTOR Immunohistochemistry

In 6 patients additional mTOR immunohistochemistry was performed as described previously using heat antigen retrieval procedure (citrate buffer) and the phospho-mTOR antibody (Ser-2448, #2976, Cell Signaling Technology, 1:100) according to the manufacturer's protocol.[12] A scoring system was based on the percentage of p-mTOR positive tumor cells and their staining intensity. A score of 180 and above is considered a high mTOR phosphorylation.

Statistical Analysis and Prioritization Application

All computational and statistical analysis was done in R (version: 3.5.1, www.r-project.org) with the extension of open source packages. The prioritization application is coded with the "shiny" package (version 1.1.0) for R. Source code can be made available on reasonable request. Sequencing and copy number data are loaded from text files. Both sources are integrated and cross-checked with an exchangeable biomarker–drug combination table (Supplementary Table 4), taking also combinational biomarkers and negative biomarkers that exclude the use of this drug into account. Targeted therapies with the highest evidence are presented. The prioritization application is freely available online (www.targetglioma.dkfz.de).

Results

Characteristics of the Patient Cohort

Two hundred fifty-three of 536 screened patients with detailed molecular assessment were included in this study (Figure 1). Most patients ($n = 238$, 94%) were classified by methylation classification into the 3 main glioblastoma subgroups: receptor tyrosine kinase (RTK) I, RTK II, or mesenchymal (Supplementary Table 1). Fifteen patients belonged to rare *IDH* wild-type groups such as midline ($n = 4$), *MYCN* ($n = 2$), *H3K27* mutant ($n = 2$), anaplastic pleomorphic xanthoastrocytoma ($n = 1$), anaplastic pilocytic astrocytoma ($n = 1$), and ganglioglioma ($n = 1$). In 3 patients, classification was not conclusive. The *MGMT* promoter was methylated in 117 patients (46%), unmethylated in 135 patients (53%), and the methylation status was not determinable with certainty in one patient. One hundred ten patients (43%) were female and the median age at first diagnosis was 61.1 years (21–85 years) (Supplementary Table 1). Molecular assessment was performed in a prospective routine clinical setting. Decisions to perform detailed molecular analysis apart from routinely assessed *MGMT* methylation status to guide treatment decisions were made individually by treating physicians. Methylation arrays (450k or EPIC) were performed in all patients included in this study. In 124 patients (49%) additional sequencing of predefined, potential targetable genes was carried out using a 130-gene panel sequencing[23] approach (Figure 1 and Supplementary Table 2). Molecular assessment was mainly performed with tumor tissues from primary tumors (234 patients, 92%) whereas tumor tissues from re-resection of recurrent tumors were used in 19 patients (8%).

Molecular-Guided Decisions

Decisions to perform detailed molecular analysis were mostly made before or during first-line therapy (in 212 patients, 84%) guiding first-line treatment or evaluating possible treatment strategies in case of progression. However, in 41 patients (16%), decisions for molecular analysis were taken after progressive disease to investigate treatment options for recurrent tumors (Supplementary Table 2). Molecular assessments resulted in a molecular-guided therapy in 97 of the 253 patients (38%) (Supplementary Table 2). Fifty-six of the 124 patients (45%) with molecular analysis including methylation array and panel sequencing

and 41 of the 129 patients (32%) with molecular analysis including methylation array without further sequencing received treatment guided by detected molecular alterations. However, treatment decisions were mostly based on data obtained from methylation array (methylation and CNV) or occasionally performed immunohistochemistry of mTOR phosphorylation (3%). Panel sequencing was used to allocate patients with targetable *BRAF* V600E mutations (3%) and *EGFR* mutation (1%).

Molecular analysis frequently guided treatments at first-line therapy (in 72 patients, 74%, Supplementary Table 2). Twenty-five patients (26%) received molecular-guided treatments at progressive disease. In these patients, the molecular assessment was performed with tissue from primary resection in 17 patients (68%) and with freshly dissected tissue from re-resection of recurrent tumors in 8 of these patients (32%) (Supplementary Table 1).

Molecular-guided treatment decisions were based on *MGMT* methylation status in 68 patients (70%). *MGMT* methylation status was mostly used to guide first-line therapy in elderly patients toward a monotherapy with temozolomide in case of methylated *MGMT* promoter or toward radiotherapy in patients with unmethylated *MGMT* promoter due to the presumed limited efficacy of temozolomide in the latter patient group.[9,10,25,26] In 10 patients, decisions about treatments with bevacizumab and nivolumab, for which no generally accepted biomarker exists so far, were mostly based on an unmethylated *MGMT* promoter providing alternative treatment options for patients not likely benefitting from alkylating chemotherapy.

Apart from *MGMT* promoter status, genetic information about *EGFR*, *CDKN2A/B*, and *BRAF* was most frequently used for clinical decision making (Table 1). The detection of an *EGFR* amplification or mutation resulted in *EGFR* inhibitor treatment strategies in 7 patients and the detection of *CDKN2A/B* deletions in treatments with the CDK4/6 inhibitor palbociclib in 8 patients. Three patients with *BRAF* V600E mutations received further targeted treatment with dabrafenib and trametinib. In addition, *H3K27M* mutations, *PDGFR* amplifications, *PTEN* status, *NF1* mutation, and the phosphorylation of mTOR at Ser2448 were used for molecular-guided treatment decisions. The detection of an *H3K27M* mutation led to treatment with panobinostat in one patient. One patient was treated with imatinib based on *PDGFR* amplification and 2 patients were treated with the PI3K inhibitor Buparlisib and the cMET inhibitor INC280 based on negative *PTEN* status. Pembrolizumab was added to first-line therapy in one patient due to the detection of a hypermutator phenotype. The phosphorylation of mTOR resulted in treatments with the mTOR inhibitor temsirolimus in 4 patients.[12] In addition, one patient was treated with temsirolimus based on a PIK3CA mutation and one patient received further treatment with the MEK inhibitor trametinib based on the detection of an NF1 mutation.

Molecular-guided treatments were initiated after a median of 29.5 days (range: 11–88) after primary diagnosis in patients receiving molecular-guided treatments as first-line therapy and after a median of 600 days (range: 112–2735) in patients with molecular-guided treatments at recurrent disease. In the group of patients with molecular-guided treatments at recurrent disease, 40% of these patients (10 of 25) received molecular-guided treatments after first progression, 36% (9) of these patients after second progression, and 24% (6) of these patients were treated with molecular-guided treatments after more than 2 prior progressions (2 patients after third progression, 3 patients after fourth progression, and one patient after fifth progression). All of these patients were treated with radiotherapy and all but one patient additionally with temozolomide chemotherapy prior to molecular-guided treatments. Forty-four percent (11) of the patients received further chemotherapy with lomustine at progressive disease prior to initiation of molecular-guided treatments. The median Karnofsky performance score (KPS) at the start of molecular-guided treatments was 80% (range: 60%–100%). The overall survival (OS) was similar in the patients' groups with and without molecular-guided treatments with a median of 416 days in patients with and 414 days in the patients without molecular-guided treatments, with a high rate of censored patients (32 patients [33%] and 49 patients [31%], respectively). For further survival analysis, only patients with treatments based on molecular alterations other than *MGMT* methylation status were compared with the patients without molecular-based treatments. Patients with molecular-guided treatments based on *MGMT* methylation status were excluded from further survival analysis as this patients' subgroup mostly consisted of elderly patients who received either monotherapy with temozolomide or monotherapy with radiotherapy and were therefore not comparable with the other patients. After stratification for *MGMT* promoter status, there was a prolonged OS with molecular-based treatments in patients with tumors harboring an unmethylated *MGMT* promoter, whereas this effect on OS was not observed in patients with glioblastomas harboring a methylated *MGMT* promoter (Supplementary Figure 1).

Patient Examples

An 81-year-old patient was diagnosed with a right temporal glioblastoma in February 2017. Molecular diagnostics revealed an *IDH* wild-type glioblastoma with an unmethylated *MGMT* promoter, the patient was treated with radiotherapy alone as a first-line treatment after tumor resection in March 2017. First disease progression occurred in December 2018. Then re-radiotherapy and temozolomide chemotherapy were discussed as further treatment options and the decision for a re-radiotherapy was made. Re-radiotherapy was performed in January 2019. However, the patient died in March 2019.

A 54-year-old patient was diagnosed with a right parieto-occipital glioblastoma in April 2015. Molecular diagnostics demonstrated an *IDH* wild-type glioblastoma with an unmethylated *MGMT* promoter, phosphorylation of *mTOR* at Serin2448, a *CDK4* and *MDM2* amplification, and a deletion of *CDKN2A*. The patient was treated with combined radiochemotherapy with temozolomide and adjuvant temozolomide treatment. After disease progression in October 2015, the decision for a treatment with temsirolimus was made based on the phosphorylation of *mTOR* at Serin2448. Temsirolimus was given until further progressive disease in MRI in February 2016, when a

Table 1. Molecular-Guided Treatment Decisions

	Molecular-Guided Therapy (n = 97)
Molecular alterations for decision of molecular-guided therapy	97
MGMT (methylated/unmethylated)	68 (31/37)
*BRAF*V600E mutation	3
EGFR amplification/*EGFR*vIII mutation	7
CDKN2A/B deletion	8
H3K27M mutation	1
Hypermutated phenotype	1
phospho-mTOR Ser2449	4
PIK3CA mutation	1
PTEN negative	2
PDGFR amplification	1
NF1 mutation	1
Targeted therapy	
Palbociclib	8
EGFR inhibitor	7
Dabrafenib +Trametinib	3
Trametinib	1
Panobinostat	1
Temsirolimus	5
Pembrolizumab	2
Nivolumab	6
Bevacizumab	4
Buparlisib (PI3K inhibitor), INC280 (cMET inhibitor)	2
Imatinib	1
KPS prior to start of targeted therapies (median; range)	80 (60–100)

Detected molecular alterations, its frequency, and guided molecular treatments are listed in this table. In addition, Karnofsky Performance Scores prior to the start of targeted therapy were retrieved from electronic medical reports.

treatment with bevacizumab was initiated due to the presumed limited efficacy of further alkylating chemotherapy based on the unmethylated *MGMT* promoter. However, in March 2016 MRI revealed further disease progression. Based on the detected molecular alterations with a *CDK4* amplification and deletion of *CDKN2A*, a treatment with palbociclib was initiated and given until progressive disease in August 2016, which was also the last consultation in our department. At this time a recommendation for further treatment with lomustine was made.

Hypothetic Allocation to Targeted Treatments Based on Automated Allocation

Future studies for targeted therapies aim to include a broader range of patients based on molecular profile. We aimed at identifying patients in our cohort for possible treatment allocation. CNV profiling from methylation array (100% of cases) identified amplifications of *EGFR* in 116 patients (46%). Less common amplifications occurred in *PDGFRA*, *MYCN*, *CDK4*, and *MDM2*. Most frequent deletions have been observed in *CDKN2A/B* (58%), *NF1* (18%), and *PTEN* (12%) (Figure 2A). One hundred thirty-gene panel sequencing identified 305 SNVs and insertions/deletions in 91 of the 110 (83%) profiled tumors (mean: 3.42, range 0–43). We additionally assessed mutation allele frequency and potential impact of the mutation on protein function with mutationassessor.org.[24] *PTEN* mutations or frameshift deletions were detected in 35 patients (32%) and *TP53* mutations and mutations of *EGFR* in 18% and 9% of patients, respectively. Twenty mutations (7%) were rated as potentially high impact on protein function, of these have been 8 *PTEN* and 3 *EGFR* mutations (Figure 2B and Supplementary Table 3). Two of the patients had >15 mutations in 130-gene panel sequencing, suggesting a hypermutation phenotype. An updated treatment allocation algorithm (www.targetglioma.dkfz.de) based on our recent suggestion[27] was implemented and used to identify putative biomarkers (Figure 3 and Supplementary Table 4). This application is currently capable of calculating potential targets from the combination of methylation, sequencing, and p-mTOR immunohistochemistry data as used in the N^2M^2 trial.[28] Resistance biomarkers (eg, *RB* mutation for CDK4/6 inhibition) are included. It will be constantly updated according to new knowledge and to the capability

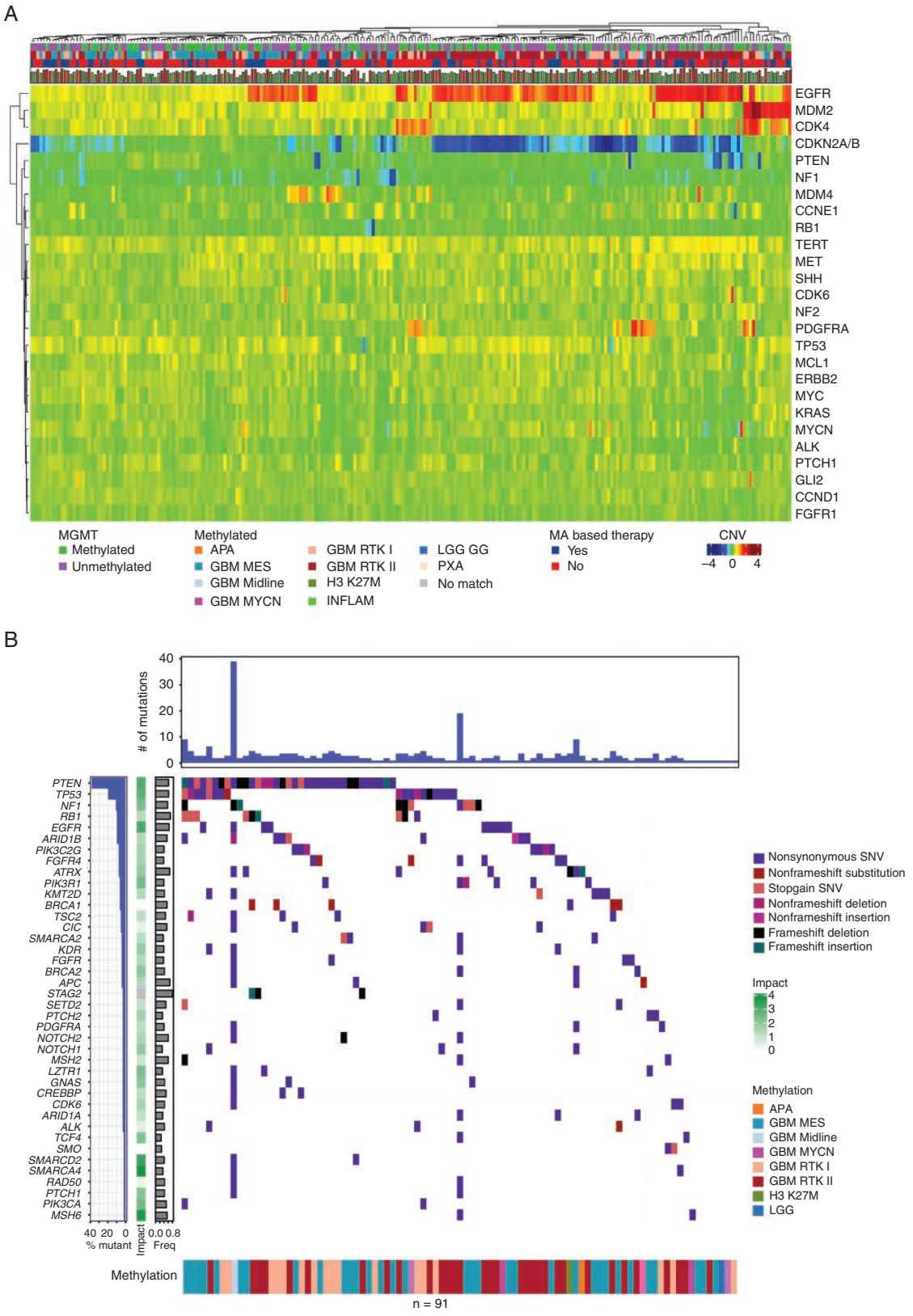

Figure 2. Genetic alterations in the patient cohort. (A) Copy number alterations derived from 450k/EPIC methylation array of 26 frequently altered glioblastoma relevant genes in the 253 patients. (B) Oncoplot of the panel sequencing derived mutations in the cohort with sequencing data. The top 40 most frequent mutations in the cohort are shown. APA, anaplastic pilocytic astrocytoma; MES, mesenchymal; RTK, receptor tyrosine kinase; INFLAM, inflammatory; LGG GG, low-grade glioma ganglioglioma; PXA, pilocytic xanthoastrocytoma.

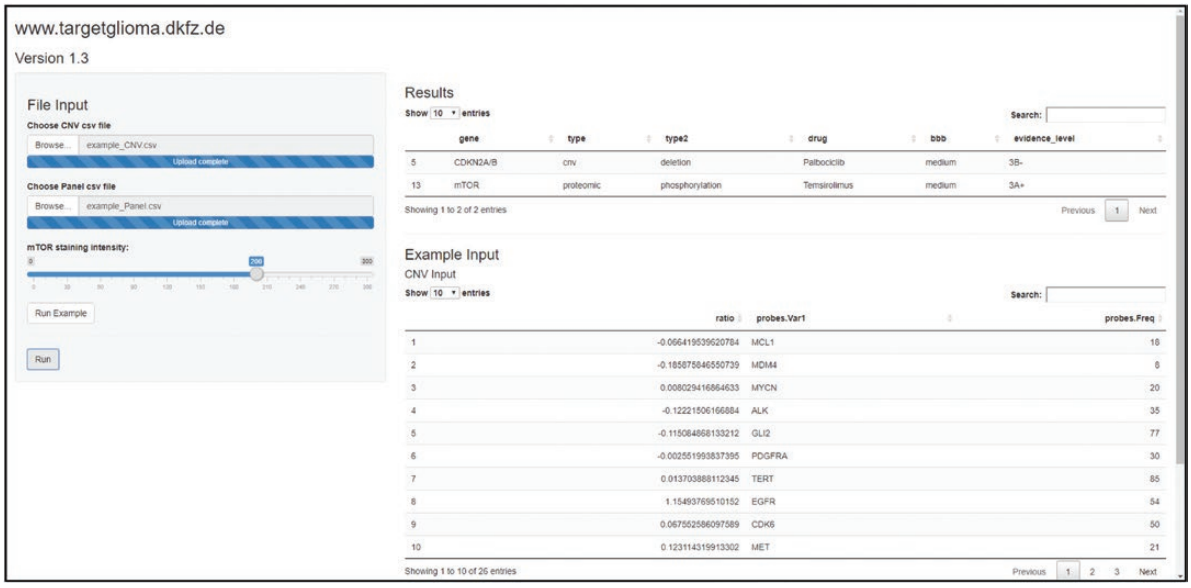

Figure 3. Molecular decision application. The molecular decision application shows potentially targetable alterations.

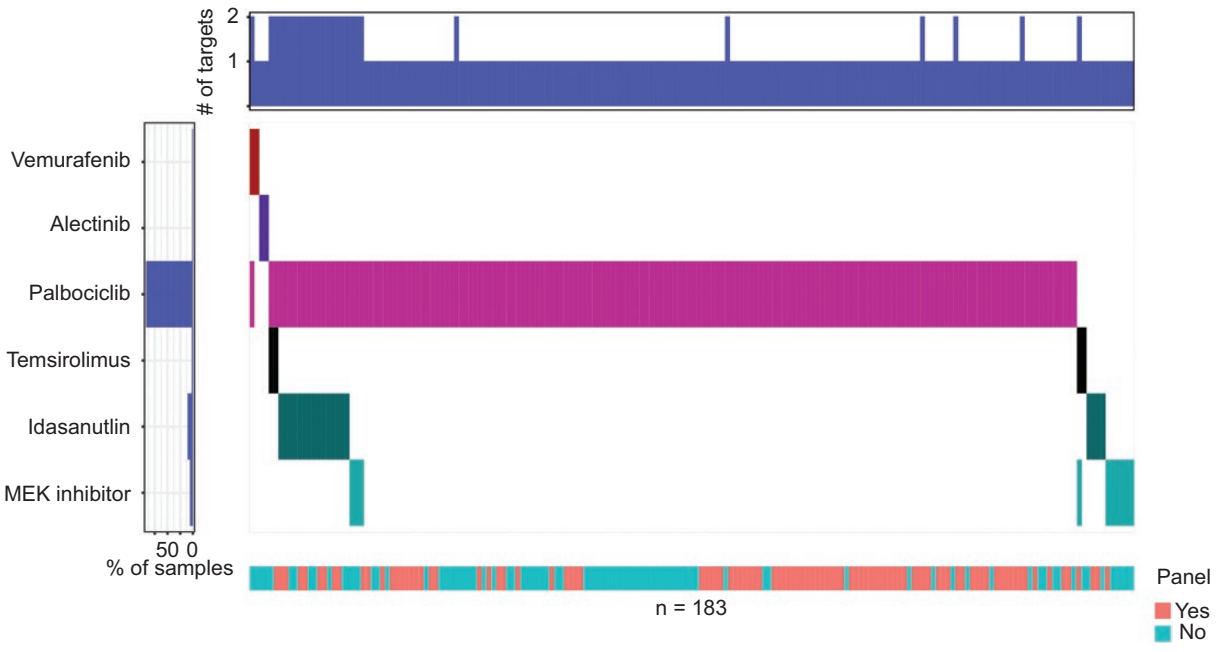

Figure 4. Distribution of potentially targetable alterations. Potentially targetable alterations grouped by potential treatment are shown for 183 patients where at least one potential target was identified.

of including all kinds of multiomic data including whole genome sequencing, expression, and proteomic data. According to the current application, we identified 213 potentially targetable alterations in 183 of the 253 patients (72%, Figure 4). Most of the alterations might confer sensitivity to a CDK4/CDK6 inhibitor with a lower confidence level. Also, higher confidence (2B) *BRAF* V600E mutations have been detected in 2 cases. We furthermore detected 2 mutations and one non-frameshift substitution in the *ALK* gene, but the impact of these mutations

is unknown and allele frequencies only range from 32% to 47% (Figure 2B, Supplementary Table 4), questioning the usability of *ALK* mutations in treatment decision for glioma. Of note, 9 of the 110 patients with panel sequencing harbored *NF1* frameshift deletions or stop-gain SNVs potentially sensitizing for MEK and mTOR inhibitors with allele frequencies between 30% and 100%.

Patients With Multiple Molecular Targets and Possibilities for Combined Therapies

As single targeted therapies are unlikely to promote durable responses, we aimed to identify possible combination therapies. Twenty-seven patients (11%) of our cohort had 2 targetable alterations. Most frequent co-occurring targetable alterations were *CDK4* amplifications or *CDKN2A/B* co-deletions in combination with *MDM2* amplifications (in 12 patients), which might predict a response to treatments with palbociclib and idasanutlin, respectively. In addition, *CDKN2A/B* co-deletions were detected in combination with *NF1* deletions/mutations, suggesting a response to MEK/CDK4/6 inhibitor treatment strategies, in 3 patients and in combination with TSC2 mutations, which might indicate a response to treatment with temsirolimus in one patient. Moreover, in 5 patients, *CDKN2A/B* co-deletions co-occurred with *CDK4* or *CDK6* amplifications, which might predict a response to treatment with a CDK4/6 inhibitor (Figures 3 and 4).

Discussion

Comprehensive molecular assessment was performed in 253 patients in a prospective clinical routine setting leading to molecular-guided treatments in a reasonable number of these patients (97 patients, 38%).

Molecular-guided treatments were frequently based on *MGMT* methylation status guiding treatment decisions at first-line therapy in elderly patients and thereby following the European guidelines for the treatment of glioblastoma in this patients' group.[1] In addition, the *MGMT* promoter status was used for treatment decisions of therapies with so far unknown biomarkers such as bevacizumab and nivolumab to provide treatment alternatives for patients not likely benefitting from alkylating chemotherapy. Apart from *MGMT* promoter status, alterations in *EGFR*, *BRAF* mutations, *CDKN2A/B* co-deletions, and phosphorylation of *mTOR* at Ser2448 were most frequently used for molecular-guided treatment decisions. Molecular-guided treatments at first-line therapy were mainly based on *MGMT* methylation status or molecular alterations used as study inclusion criteria. Decisions for targeted treatments based on other genetic alterations were mostly taken at progressive disease as these treatments do not represent standard therapy and treatment costs have to be applied for at medical insurances. However, along with further improvements of molecular diagnostics, increasing translation of molecular alterations into molecular-guided treatment decisions, and data about the effectivity of targeted therapies, these treatments might be better assessable also for treatments in the early stage of disease in near future. In this context, one limitation of this study is that it was not meant and powered for survival analysis. Survival analysis for patients with molecular-guided treatments based on molecular alterations other than *MGMT* promoter status suggested that particularly patients with an unmethylated *MGMT* promoter had a longer OS if molecular-guided treatments were used. However, the patient population might be biased by patients' selection for extensive molecular analysis and various treatments had been administered in different disease stages. In addition, interpretation of survival data is limited due to small numbers of patients with molecular-guided treatments in the patients' subgroup for survival analysis, the different molecular-guided treatments, and the retrospective nature of the study. In this regard, the recently started N^2M^2 trial will provide important data about the effectivity of molecular-guided targeted therapies in first-line treatment. Until that, this current study offers an exploratory approach to the current use of targeted drugs and the usage of molecular data. Others have conducted similar trials with a focus on repurposed drugs and the option for multiple therapies at recurrence.[29]

Although a reasonable number of patients have already received treatments based on molecular markers in clinical practice beyond *MGMT* promoter methylation status for chemotherapy decision, we identified targetable alterations in many more patients (29 vs. 185 patients) demonstrating that personalized treatments are just at the beginning to influence clinical decision making. Nevertheless, at present molecular-guided treatments based on molecular markers other than *MGMT* promoter remain as treatment options for recurrent disease due to the limited accessibility at the early stage of disease outside clinical studies. Therefore, patients were treated with molecular-guided treatments only at the late stages of disease in this study limiting the efficacy of the treatments and its translation into clear survival benefits. Furthermore, patients need to have a reasonable KPS prior to treatment initiation and also after (multiple) progressions, which limits the potential for molecular-guided treatments to a small subgroup of patients. Therefore, studies demonstrating the feasibility of molecular-guided treatment decisions and its translation into clinical practice help to increase the accessibility of targeted treatments at an early stage of the disease also outside of clinical trials. In this regard, it is important to select targeted treatments with a presumed high blood–brain barrier penetration to ensure that the treatment is reaching its target.

However, molecular-guided clinical decision making remains challenging as molecular analysis often results in the detection of several potential targetable alterations with many options for single or combined treatments and clinical data providing evidence for the efficacy of different molecular-guided treatments in selected patients' populations are still missing. In this regard, the N^2M^2 trial will provide important information in the upcoming years. The prioritization algorithm is helpful in guiding treatment allocations to single targeted treatments based on the level of evidence for respective biomarkers and treatments. However, in cases with the detection of several biomarkers for different targeted treatments combined

therapy with multiple targeted treatments might be even more effective especially with regard to acquired resistance mechanisms. We identified 30 patients in our cohort who had at least 2 potentially targetable molecular alterations. Among them, *CDK4* and *MDM2* amplifications were the most frequently detected co-occurring alterations that are targetable by palbociclib and idasanutlin. In addition, *NF1* deletion, which might predict a particular response to MEK inhibitors, was frequently found in combination with *CDKN2A/B* deletion, which might predict response to palbociclib. One patient displayed *CDK4* and *MDM2* amplification as well as a nonsynonymous SNV in the *NF1* gene. Therefore, treatments with palbociclib, idasanutlin, or MEK inhibitors might represent effective treatment options for this patient. Based on recent preclinical studies, combined treatment of idasanutlin and MEK inhibition demonstrated superior efficacy than respective monotherapies.[30] In addition, MEK inhibition was demonstrated to be effective in tumors with *NF1* loss.[31,32] Thus, it could be speculated that the combination of idasanutlin with a MEK inhibitor could be the better choice over a combination with a CDK4 inhibitor for this patient. These data also demonstrate the challenge of molecular-guided clinical decision making and the urgent need for further data about the effectivity and tolerability of combined treatments. In addition, co-occurrences of *CDK4* or *CDK6* amplification and *CDKN2A/B* co-deletion, which both represent potential biomarkers for treatment with palbociclib, were detected in 6 patients. As alterations of multiple genes of the same pathway might lead to higher confidence in the prediction of potential response to targeted therapies, further molecular understanding of altered pathways will improve the prediction of treatment efficacy in the near future.

In conclusion, the data demonstrate that broad molecular assessment already led to molecular-guided treatments in a reasonable number of patients in prospective clinical nonstudy settings. Molecular decision making in clinical practice was mainly driven by *MGMT* promoter status in elderly patients or study inclusion criteria for first-line therapy and by detection of various molecular alterations at the treatment of recurrent disease. In addition, revision of molecular data identified relevantly more patients with potentially targetable alterations indicating that targeted treatments could increasingly influence clinical treatment for future patients. To support molecular clinical decision making, we implemented a web application providing comprehensive information about possible treatment allocations based on individually uploaded molecular data. This tool will be constantly updated and further developed based on the newest clinical and preclinical findings and integration of a broad range of multiomics data will be performed. This study complements the molecular N^2M^2 pilot study in a routine clinical decision making and prepares for complex molecular decision making for glioblastoma patients.

Supplementary Material

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

Keywords

glioblastoma | molecular profiling | targeted therapies.

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