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Actionable alterations in glioblastoma: Insights from the implementation of genomic profiling as the standard of care from 2016 to 2023

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Abstract

Background. In 2016, genomic profiling was implemented for patients with grade 4 primary brain tumors at Rigshospitalet, Denmark. The aim of this study was to discover actionable alterations and to match these with targeted therapies.

Methods. Between January 2016 and December 2023, 483 brain tumor patients were profiled. We retrieved clinical data and molecular data. Whole exome, whole genome, or panel sequencing, along with SNP array analyses, and RNA-seq were performed on resected primary tumor tissue. Alterations were classified according to the European Society for Medical Oncology (ESMO) Scale for Clinical Actionability of Molecular Targets (ESCAT) following the European Association of Neuro-Oncology (EANO) guideline on rational molecular testing.

Results. A total of 200 (41.4%) patients' tumors harbored an alteration of interest according to the EANO guideline. Twenty (4.1%) patients had an ESCAT high-tier alteration (tier I or II), while 155 patients (32.1%) had an alteration corresponding to ESCAT IIIA. Thirty-five patients (7.2%) had an actionable alteration, and 15 (3.1%) received targeted therapy. The treated targets were BRAFV600E mutations, FGFR alterations, NTRK fusions, PDGFRA fusions, PTPRZ1-MET fusions, and TMB-high. The overall response rate was 20%, with a median duration of response of 12 months, and 47% achieved stable disease as the best response.

Conclusions. Genomic profiling uncovers alterations of interest in a substantial number of patients, but only a minority are considered by the Danish National Molecular Tumor Board to have actionable alterations, and even fewer receive targeted therapy. Nevertheless, factors, such as promising targets and the increasing availability of trials, may contribute to a future increase in the number of patients benefiting from targeted therapies based on genomic profiling.

Keywords

actionable alterations | ESCAT | genomic profiling | targeted therapy

Glioblastoma is the most prevalent malignant adult brain tumor.[1] The standard treatment for glioblastoma patients in good performance status is surgery, concurrent temozolomide and radiation therapy, and adjuvant temozolomide. Despite years of research and numerous clinical trials with novel agents, glioblastoma patients have a dismal prognosis with

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a median overall survival of 15 months.[1–4] Almost every patient will experience recurrence of their cancer, and the 5-year overall survival is less than 10%.[5]

Genomic profiling, including DNA and RNA sequencing, copy number variation analysis, and RNA expression have enabled the discovery of frequent genomic alterations that drive cancer. Genomic analysis and treatments targeting molecular alterations have become standard therapy for several tumor types, such as lung, breast, or colon cancer.[6–8] In comparison, no commonly applicable targeted treatments have been able to improve overall survival in glioblastoma.[1] In the hope of finding a molecular target that can be matched with a targeted therapy, clinicians have increasingly performed genomic profiling in patients who have failed standard-of-care options.[9] The impact of this expanded genomic profiling on treatment decisions is still emerging, and optimal use remains an area of ongoing investigation.[10–14] Previous reports show that, in glioblastoma, genomic profiling may reveal actionable targets where matched targeted therapies can improve clinical outcomes.[10–14] However, the choice of genomic analysis and optimal timing of sequencing remain unclear. Other known challenges, such as low trial availability, low enrollment rates, and the unknown clinical relevance of various targetable alterations, also need to be amended to justify the potential implementation of sequencing as standard of care for glioblastoma patients.

In 2016, in-house genomic profiling was introduced for patients with primary brain tumors at the Department of Oncology at Rigshospitalet, Denmark. The aim of this study was to discover actionable alterations and to match these with targeted therapies. The target patient population was patients with glioblastoma, or other WHO grade 4 primary brain tumors, fit enough to receive standard therapy with the Stupp regimen.[15] Later, in 2022, as one of the focus areas of the newly established Danish National Genome Center, genomic profiling became a standard option for primary brain tumor patients in Denmark.[16]

This paper is a single-center retrospective study of the 483 profiled patients between January 2016 and December 2023, contributing to the expanding evidence on the clinical value of profiling glioblastoma patients. We provide an overview of clinical characteristics and genomic profiles for all patients. We also classify possible targets for therapy according to the European Society for Medical Oncology (ESMO) Scale for Clinical Actionability of Molecular Targets (ESCAT) and present clinical outcomes for the segment that received matched targeted therapy.[17]

Methods

Patients

Patients were 18 years and older and were diagnosed with a WHO grade 4 primary brain tumor from January 2016 to December 2023. We included the following diagnoses according to WHO Classification of Tumors of the Central Nervous System (2016 (4th edition) and 2021 (5th edition)): glioblastoma, IDH-wildtype, WHO grade 4; astrocytoma, IDH-mutant WHO grade 4; diffuse hemispheric glioma, H3 G34-mutant, WHO grade 4; and diffuse midline glioma, H3 K27M-mutant, WHO grade 4.[18,19] Patients with astrocytoma, IDH-mutant WHO grade 4 who previously had a lower grade glioma were included based on their date of grade 4 tumor diagnosis. We reviewed and retrieved clinical data from the electronic patient records. All patients were diagnosed and treated at Rigshospitalet in Denmark. Rigshospitalet is a university hospital in Copenhagen and a referral center. Neuro-oncology is centralized at our institution, and our catchment area is 2.75 million people. All ethical and data management approvals have been obtained through the Neurogenome protocol (Danish National Medical Research Ethics Committee approval number H-21023801).[20]

Danish National Molecular Tumor Board

The Danish National Molecular Tumor Board (DNMTB) was introduced in 2013 and is now being held as a weekly, national, multidisciplinary tumor board that involves molecular biologists, geneticists, pathologists, oncologists, and biochemists. The DNMTB discusses the results of comprehensive genomic profiling of patients with the goal of guiding patients' treatment based on their molecular profile. Incidental germline findings were reported back to the patient based on their information preference.

Molecular Data

We retrospectively evaluated all genomic profiles presented at the DNMTB. Variants presented at the DNMTB were classified in agreement with relevant guidelines.[21] From these profiles, we then extracted data on molecular alterations based on these criteria: (1) pathogenic or likely pathogenic single-nucleotide variants' insertions/deletions (indels) and fusions, (2) amplifications, (3) biallelic deletions, (4) monoallelic deletions for mutated tumor suppressor genes, and (5) genome-wide targets such as high tumor mutational burden (TMB-high) and homologous recombination deficiency (HRD).

We categorized single-nucleotide variants and insertions/deletions as mutations. For each gene, we reported only one instance of a specific alteration type, regardless of how many identical alterations occurred within that same gene.

ESCAT and Glioma-Relevant Targets

We classified the molecular alterations according to the ESCAT scale.[17] The ESCAT scale is developed by ESMO to rank genomic alterations as targets for cancer precision medicine. It assigns 6 tiers of evidence (Table 1) based on the available evidence of clinical benefit for the alteration-drug match. The scale aims to offer a common language for the stakeholders in oncology and drug development.

In this study, we have chosen to classify our alterations in the same ESCAT tiers as the recent European Association of Neuro-Oncology (EANO) guideline on rational molecular testing by Capper et al.[9] (Table 1). This was done for data congruence and does not necessarily reflect the recommendations of the DNMTB, which also take into account available trials, performance status, and other factors.[22]

Table 1. Description of ESCAT Tiers and List of Glioma-Relevant Targets

ESMO Scale for Clinical Actionability of Molecular Targets (ESCAT)	
Tier	Short description of tiers
I	Alteration-drug match is associated with improved clinical outcomes in a specific tumor type in randomized (IA), nonrandomized (IB) or clinical trials across tumor types (IC).
II	Alteration-drug match is associated with antitumor activity in a specific tumor type but with unknown magnitude. Evidence of clinical benefit from retrospective studies (IIA), or higher response rates in prospective trials without survival data (IIB).
III	Alteration-drug match is associated with clinical meaningful evidence but from other tumor types. Limited evidence for specific tumor types or broadly across tumor types (IIIA). Similar molecular alteration as a tier I target but without supportive clinical data (IIIB).
IV	Alteration-drug match is associated with supportive preclinical data. In vivo or in vitro data available (IVA). Only supportive in silico data (IVB).
V	Alteration-drug match is associated with objective responses, but no improved outcomes.
X	Lack of supportive evidence
List of Glioma-Relevant Targets According to EANO	
Gene	Alteration type
ALK	Fusions, mutations
BRAF	V600E mutations, KIAA1549:BRAF fusions
CDK4/6	Amplifications
EGFR	Mutations in the intracellular domain
FGFR	*FGFR3* (fusions, mutations), *FGFR1* mutations (N546K, K656E)
HRD	Homologous recombination deficiency
MDM2/4	Amplifications, fusions, mutations
MET	Exon 14 skipping, fusions, amplifications
MMR	De novo and treatment-induced mismatch repair deficiency
NF1	Loss-of-function alterations
NTRK1-3	Fusions
PDGFRA	Amplifications, fusions, mutations
POLE	De novo and treatment-induced *POLE* alterations
ROS1	Fusions
TMB-high	De novo and treatment-induced TMB-high
TSC1/2	Mutations

Adapted from Mateo et al. and Capper et al., respectively.[9,17]

To match the actionable targets reviewed by the EANO guideline, we grouped fusions and identical alterations in the following genes: *CDKN2A*, *CDKN2B*, and *CDKN2A/B* as *CDKN2A/B*; *CDK4*, *CDK6*, and *CDK4/6* as *CDK4/6*; and *MDM2*, *MDM4*, and *MDM2/4* as *MDM2/4*.

Tissue Samples

During diagnostic surgery, the excised tumor was sent to the Department of Pathology. A frozen section from a sample from the excised tumor was performed, and a preliminary diagnosis was given. The sample was subsequently frozen and stored in the biobank at −80°C. A final diagnosis was obtained from the remaining paraffin-embedded FFPE tissue. Genomic analysis was performed on either frozen or FPPE tissue, from the biobank, sent to the Department of Genomic Medicine, or on FFPE at the Department of Pathology. Blood samples were obtained for germline testing.

Genomic Analysis

Whole exome sequencing (WES), whole genome sequencing (WGS), or panel sequencing with Trusight Oncology (TSO500), along with SNP array analyses, and RNA-seq were performed on DNA/RNA extracted from tumor tissues. Tumor tissues from the patients enrolled before 2021 were analyzed by WES, and from January 2021 and forth, WGS was used for the frozen tissue and TSO500 for the paraffin-embedded tissue. The turnaround time from a patient's consent to a genomic profile is between 4 and 6 weeks. Two patients solely had the targeted panel Archers FusionPlex Solid Tumor Panel (v1.1) performed on their tissue (Supplementary Methods).

Whole exome sequencing.—Whole exome sequencing was performed on DNA isolated from whole blood and tumor tissue DNA using SureSelect Clinical Research Exome

(Agilent) with fragmentation to 300 bp using Covaris S2 (Agilent) and adaptor ligation using KAPA HTP Library Preparation Kit (Roche). Sequencing libraries were paired-end sequenced on Illumina NextSeq500, HiSeq2500, or NovaSeq6000 platforms. Reads were aligned to the human reference genome (hg19/GRCh37) using CLC Biomedical Genomics Workbench (Qiagen), and variant calling was performed above 10% frequency in the tumor DNA. Identification of somatic mutations was performed using a tumor/normal analysis in which germline variants were subtracted from the tumor variants. The identified somatic mutations were further filtered using Ingenuity Variant Analysis (IVA) from Qiagen to identify cancer-associated variants.

Mutational load was assessed in IVA as nonsynonymous variants (missense, nonsense, insertions, deletions, and splice site mutations) after filtering for sequencing quality and excluding common variants (>1% in 1000 Genomes, ExAC, and NHLBI ESP databases).

Whole genome sequencing.—PCR-free library preparation and fragmentation for Illumina were performed on DNA isolated from the whole blood and fresh frozen tissue. The average sequencing depth was minimum ≥60× for tumor and ≥30× for germline DNA sequencing with minimum 95% of the genome covered ≥10×. Sequencing reads were mapped to the human reference genome (hg38/GRCh38), and variants were called using GATK (best practice guidelines) and Haplotype caller or Mutect2 for germline and somatic variants, respectively. Somatic mutations were reported as standard with variant allele frequency (VAF) ≥ 10%. For low tumor cell content in the tissue sample, obvious pathogenic tumor mutations were examined without a VAF cutoff. Analysis software was Qiagen Clinical Insight Interpret v.8.0.

TMB was based on the total number of somatic mutations from WGS. TMB-high was defined as more than 10 nonsynonymous mutations per megabase. No international standardized technique for mutational load exists.

Trusight oncology 500.—DNA isolated from FFPE tissue was used for TSO500 HT analysis (Illumina). The TSO500 HT gene panel includes >500 cancer-associated genes and was optimized to identify cancer hotspot mutations in sparse/fragmented DNA. Sequencing reads were mapped to the human reference genome (hg38/GRCh38), and mutations were called using GATK Mutect2 (best practice guidelines). In addition, mutations, TMB, and microsatellite instability were also called using the Illumina TSO500 HT analysis pipeline. Somatic mutations were reported by default at VAF ≥ 5%. In the report, missense, nonsense, frameshift, minor insertions/deletions, and intron mutations (±2 bp) were reported. Inversions, translocations, and larger deletions and duplications (eg, multiple exons) were not included in the analysis. Average sequencing depth (×) was minimum ≥ 250×. Analysis software was Qiagen Clinical Insight Interpret v.8.0.

SNP array.—CytoScan HD/OncoScan array (ThermoFisher) was performed on DNA isolated from tissue. Optimal analysis was obtained with a tumor content of ≥20%.

Before 2022, HRD was estimated based on the number of LOH segments greater than 15 Mb according to Marquard et al.[23] From 2022, HRD was estimated based on the score developed by Telli et al.[24]

RNA-seq.—TruSeq-stranded total RNA (Illumina) or Illumina stranded total RNA library preparation was performed on RNA isolated from tissue. Proliferation index was calculated based on the expression level of 100 selected biomarkers. For a highly proliferating tumor, the proliferation index was >5.5. For fusion analysis, Oshell's pipeline FusionMap v.10.0.1.29, Arriba, or Starfusion was used.[25–27]

Telomerase reverse transcriptase promotor region mutation.—In contrast to 2021 and beyond, telomerase reverse transcriptase (TERT) promoter mutations were not routinely analyzed in the years when WES was the preferred method of genomic analysis. Between 2016 and 2018, it was determined using Sanger sequencing for the 2 most common mutations: c.124C>T and c.-146C>T.[15] In brief, primers were designed to produce PCR products covering the sites. The purified PCR products were sequenced by Sanger sequencing using an ABI 3730 DNA Analyzer according to the manufacturer's instructions (Applied Biosystems).

Germline alterations.—We started to use a panel for germline variants in the molecular reports from March 2021, and before that, we did not test for germline alterations systematically. The panel tested the following genes: *BRCA1*, *BRCA2*, *ATM*, *MLH1*, *MSH2*, *MSH3*, *MLH2*, *MLH3*, *MSH6*, *PMS2*, *PALB2*, *RAD51C*, *RAD51D*, and *MBD4*. In April 2022, we added genes from Miller et al. (2021) to the panel.[28] The new panel tested the following genes: *APC*, *ATM*, *BMPR1A*, *BRCA1*, *BRCA2*, *CHEK2*, *MAX*, *MEN1*, *MLH1*, *MSH2*, *MSH6*, *MUTYH*, *NF2*, *PALB2*, *PMS2*, *PTEN*, *RAD51C*, *RB1*, *RET*, *SDHAF2*, *SDHB*, *SDHC*, *SDHD*, *SMAD4*, *STK11*, *TMEM127*, *TP53*, *VHL*, and *WT1*. The findings were reported in accordance with AMCG recommendations.[29]

Statistical Analysis and Survival

Progression-free survival was defined as the time from diagnosis or start of experimental therapy to either progressive disease (PD) or death from any cause, and overall survival was defined as the time from diagnosis to death from any cause. Response assessment of PD, stable disease (SD), partial response (PR), and complete response (CR) was done according to the Response Assessment in Neuro-Oncology (RANO) criteria.[30]

All statistical analysis and generating of plots were made in RStudio (version 2022.12.0 + 353). For survival analysis with 95% confidence intervals (95% CI), the package survival was used. For patient characteristics, the package tableone was used. Other packages used were tidyverse, ggplot, and ComplexHeatmap.

Results

Patient Characteristics

For patients diagnosed between January 2016 and December 2023, genomic profiling was performed on 483 patients' primary tumors. Of these patients, 435 (90.1%) had glioblastoma, IDH-wildtype, WHO grade 4; 39 (8.1%) had

astrocytoma, IDH-mutated WHO grade 4; and 9 (1.8%) had diffuse hemispheric glioma, H3 G34-mutant, WHO grade 4 or diffuse midline glioma, H3 K27M-mutant, WHO grade 4. The most frequent DNA analysis performed on the primary tissue was WGS with 268 samples (55.5%), followed by WES with 199 samples (41.2%), TSO500 with 14 samples (2.9%), and 2 samples (0.4%) were only analyzed with Archers Fusionplex.

Standard therapy with the Stupp regimen was administered to 409 (84.7%) of the patients. The remaining 74 (15.3%) patients' treatment regimens and other characteristics are described in Table 2.

Half of the patients in the cohort had their genomic profiles discussed at the DNMTB within 4.1 months of their diagnosis. When looking selectively at the patients diagnosed in the last 3 years of the study, this time period was reduced to 2.5 months after diagnosis.

Genomic Results

Of the 483 patients, 472 (97.7%) patients had tumors with genomic alterations that met our selection criteria (described in Methods under Molecular Data). The resected tumor material was insufficient for genomic analysis in 9 patients (1.9%) and 2 patients (0.4%) had no alterations that met our selection criteria. *TERT*-promoter mutations (62%) are the most common alterations followed by *CDKN2A/B* deletions (51%), *EGFR* alterations (41%), *PTEN* alterations (38%), and *TP53* mutations (31%) (Figure 1). Supplementary Figures 1 and 2 show the alterations found in samples that underwent WES and WGS, respectively.

Glioblastoma-Relevant Actionable Targets

A total of 200 (41.4% of total cohort) patients' tumors harbored a glioma-relevant alteration, according to the EANO guideline by Capper et al.[9] We identified 274 alterations in 200 patients (Table 3 and Figure 2). Fifty-four (11.2%) patients had 2 relevant alterations and 10 (2.1%) patients had 3. However, only 20 (4.1%) patients had a high-tier alteration (tier IB or IIB). The highest ESCAT tier for most patients was IIIA, found in 155 patients. This represents 77.5% of the 200 patients with a glioma-relevant target (Figure 3).

Targets and Targeted Therapy

Since the introduction of genomic profiling, we have matched several patients with molecular targets to available experimental targeted therapies. The targets include: *BRAF*V600E mutation, *FGFR3* fusions, *FGFR1* mutation, *NTRK* fusions, *PDGFRA* fusion, *PTPRZ1-MET* fusion, and TMB-high. Among the 483 patients who underwent genomic sequencing, 35 patients (7.2%) had one or more of these actionable alterations, and 15 (3.1%) of them received targeted therapy. Of the 15 patients who received targeted therapies, 11 were enrolled in a clinical trial, 3 accessed the drug through compassionate use or named patient programs, and 1 received the drug off-label. The majority of patients, 12 (80%), started their targeted therapy in the last 3 years of the study. The main reason for not receiving targeted therapy despite having a target was poor performance status or other signs of clinical deterioration. This occurred in 10 of 35 patients (29%). In 4 (11%) of these patients, delayed genomic analysis or investigators waiting for measurable disease were factors that may have contributed to the clinical deterioration at the time of potential treatment. Five patients had still not relapsed at the time of writing and are therefore still candidates for treatment. Supplementary Table 1 provides patient characteristics and clinical outcomes for the 35 patients with actionable targets.

Response and Survival

Among the 15 patients who received targeted therapies, the overall response rate was 20%, consisting of PRs, and 47% (7 patients) achieved SD as the best response. The median number of cycles administered was 6. All PRs were in ESCAT high-tier targets, one patient with BRAFV600E mutation, one patient with FGFR-TACC3 fusion and one patient with NTRK fusion. The duration of response was 11.9, 14, and 12.2 months, respectively (Figure 3; Supplementary Table 1). The median progression-free survival, from start of experimental therapy, was 3.9 months (range: 0.9–14 months).

The median progression-free survival and overall survival, from time of diagnosis, for the whole sequenced group were 8.5 months ($n = 483$, 95% CI: 7.6–10.3) and 18.4 months ($n = 483$, 95% CI: 17.4–20.4), respectively. Subgrouping for MGMT promoter methylation status, the methylated and unmethylated sequenced patients had a median OS of 25.9 months ($n = 223$, 95% CI: 23.1–29.8) and 16.0 months ($n = 257$, 95% CI: 15.0–17.5), respectively.

Germline Mutations

We found pathogenic germline mutations in 11 (2.2%) of our patients. Nine cases were associated with hereditary cancer, with only one being previously identified. We had 3 patients with *MUTYH* mutations associated with familial adenomatous polyposis syndrome, 2 with *TP53* mutations associated with Li-Fraumeni syndrome, 2 with MSH2 mutations associated with Lynch syndrome, 1 with *LZTR1* mutation associated with schwannomatosis, and 1 with a *PTEN* mutation associated with Cowden syndrome. The other patients had mutations in the known tumor suppressor genes *BRCA1* (1 patient) and *RAD51C* (1 patient). The patient with the RAD51C mutation had an astrocytoma and the others had glioblastomas. The age of one of the TP53-mutated patients was 29 years when diagnosed, and the remaining patients were older than 50 years. The median age was 60 years (range: 29–80). Six patients were referred to genetic counseling, while 3 patients did not have any indication for a referral. The referral status of the remaining 2 patients was unclear from the electronic records.

Discussion

In this study, we have presented the clinical and molecular data from 483 sequenced patients with grade 4 primary brain tumors. Our cohort resembles a common spread of

Table 2. Patient Characteristics

	Glioblastoma	Astrocytoma	Diffuse Glioma
n	435	39	9
Sex = male (%)	271 (62.3)	18 (46.2)	5 (55.6)
Age (median [range])	61 [18, 89]	39 [23, 68]	35 [18, 53]
MGMT methylation status (%)			
Methylated	191 (43.9)	31 (81.6)	1 (11.1)
NA	1 (0.2)	0 (0.0)	1 (11.1)
Performance status (%)			
0–1	401 (92.6)	37 (94.9)	8 (88.9)
2	27 (6.2)	0 (0.0)	1 (11.1)
3–4	3 (0.7)	0 (0.0)	0 (0.0)
NA	2 (0.5)	2 (5.1)	0 (0.0)
Corticosteroid use (%)			
No	244 (56.2)	29 (74.4)	4 (44.4)
Yes	190 (43.8)	9 (23.1)	4 (44.4)
NA	0 (0.0)	1 (2.6)	1 (11.1)
Extent of resection (%)			
Macrototal resection	254 (58.5)	25 (64.1)	2 (22.2)
Partial resection	95 (21.9)	10 (25.6)	3 (33.3)
Stereotactic biopsy	84 (19.4)	4 (10.3)	4 (44.4)
NA	1 (0.2)	0 (0.0)	0 (0.0)
First-line treatment (%)			
Standard therapy	375 (86.2)	28 (71.8)	6 (66.7)
Standard therapy variations	21 (4.8)	0 (0.0)	0 (0.0)
3 Gy × 10 only	16 (3.7)	0 (0.0)	1 (11.1)
Standard therapy + trial drug	10 (2.3)	0 (0.0)	0 (0.0)
2 Gy × 30 only	6 (1.4)	0 (0.0)	1 (11.1)
Temozolomide only	5 (1.1)	8 (20.5)	0 (0.0)
Other	2 (0.5)	3 (7.7)	1 (11.1)
Completed standard therapy (%)			
No	223 (59.6)	6 (21.4)	4 (66.7)
Yes	129 (34.3)	21 (75.0)	2 (33.3)
In treatment	23 (6.1)	1 (3.6)	0 (0.0)
Genomic analysis (%)			
WGS	246 (56.6)	19 (48.7)	3 (33.3)
WES	177 (40.7)	18 (46.2)	4 (44.4)
Targeted panel	12 (2.8)	2 (5.1)	2 (22.2)
Relapse surgery = yes (%)	166 (45.9)	9 (34.6)	2 (25.0)

MGMT = O^6-methylguanine-DNA methyltransferase; SD = standard deviation.
Corticosteroids are whether patients received 10 mg at the first meeting with the oncologist. Extent of resection is as judged by the surgeon. Regarding treatment (1) standard therapy + trial drug: 10 patients had extra treatment added on to their standard therapy as part of a clinical trial (5 nivolumab or placebo, 4 trotabresib, and 1 marizomib). (2) Standard therapy variations include 4 patients who received 2 Gy × 30 but nivolumab instead of temozolomide, 15 patients received 3 Gy × 10 only but also adjuvant temozolomide, 3 patients received regular standard therapy but nontargeted immunotherapy instead of the adjuvant temozolomide, and 1 patient received 2 Gy × 30 only and adjuvant temozolomide. (3) Other includes single patients receiving bevacizumab/irinotecan, bevacizumab/nivolumab, lomustine, NTRK-inhibitor, and 2 untreated patients. Regarding genomic analysis, targeted panel includes TSO500 and archers Fusionplex.

Figure 1. Oncoprint of the alterations found in at least 4% of the patients (n = 468 patients). X-axis: patients; y-axis left side: percentage of patients with the alteration. Y-axis right side: alteration name (deep deletions are biallelic deletions and shallow deletions are monoallelic deletions); right side bar chart along y-axis: number (bar length) and type (color) of alteration per alteration (note: if a patient harbored several alterations in the same gene, only one is visualized, except for monoallelic deletions and mutations in tumor suppressor genes).

Table 3. Targetable Alterations and Their Corresponding ESCAT Tiers

ESCAT Tier	Molecular Target	Total Target Count (n = 274)	% of All Cohort Patients (n = 483)
IB	BRAFV600E	3	0.6
IIB	FGFR3 fusion	11	2.3
IIB	FGFR1 mutation	2	0.0
IIB	NTRK fusion	4	0.8
IIIA	CDK4/6 amplification	62	12.8
IIIA	EGFR mutation; intracellular domain	6	1.2
IIIA	HRD	7	1.4
IIIA	MET amplification	17	3.5
IIIA	MET fusion	1	0.2
IIIA	NF1 alteration	56	11.6
IIIA	PDGFRA amplification	42	8.7
IIIA	PDGFRA fusion	1	0.2
IIIA	TMB-high; pretreatment	4	0.8
IIIB	TMB-high; posttreatment	3	0.6
IIIB	TSC1 mutation	1	0.2
IV	MDM2/4-amp	54	11.2

The targets were selected and allocated to ESCAT tiers following the EANO guideline on rational molecular testing by Capper et al.[9]

grade 4 diagnosis for an outpatient clinic with 90.1% glioblastoma; 8.1% astrocytoma, IDH mutant; and 1.8% diffuse gliomas. The median OS of 18.4 months also matches other cohorts of sequenced patients.[10,11]

The vast majority (97.7%) of the sequenced patients provided a tumor sample with sufficient quality to find tumor-associated molecular alterations. This success rate matches other studies.[12,13]

Our cohort resembles previous reports regarding the most common alterations. The most frequent alterations were *CDKN2A/B* deletions, *EGFR* amplifications and mutations, *PTEN* deletions and mutations, *TERT*-promoter

mutations, and *TP53* mutations.[31,32] The frequency of TERT-promoter mutations was lower than reported in the literature because they were not routinely analyzed in the early years of this study.[31]

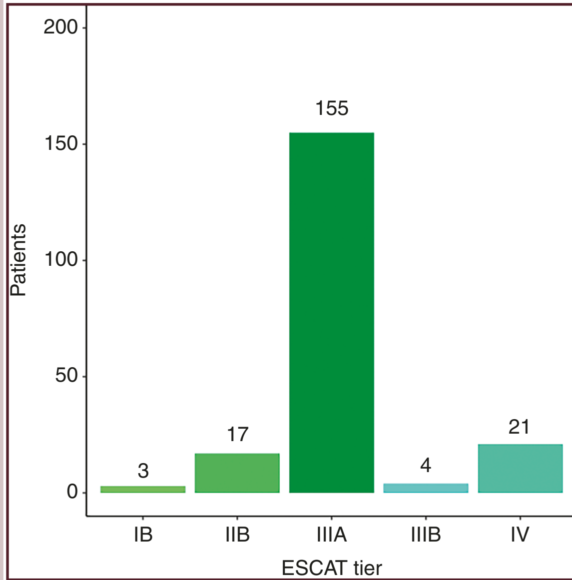

Figure 2. Distribution of patients by ESCAT tiers (n = 200 patients).[9] This figure shows the highest tier for each patient. For patients harboring multiple ESCAT targets, the highest ESCAT tier was selected.

The incidence of germline alterations in our cohort, at 2.2%, was also lower than that reported in the literature, where it is around 5%.[1] This is due to the fact that germline analysis was not routinely performed in all patients in the years before 2021.

Our institution adopted WGS as the standard method for molecular diagnosis in 2021, whereas other institutions favor WES due to its lower cost and comparable clinical benefit.[33] Supported by the Danish National Genome Center, we were able to choose WGS for all patients because it offers more opportunities to discover new potential molecular targets for future therapeutic interventions and to identify resistance mechanisms.[34–36]

The logistical setup and the awareness among treating physicians regarding the need to request genomic profiles in a timely manner for patients who could benefit from them have improved over time. In the last 3 years of the study, half of the patients had a genomic profile presented at the DNMTB within 2.5 months of pathologic diagnosis. This short time is significant because it allows timely access to experimental therapies not only at relapse but also in the first-line adjuvant setting. In later years, researchers have increasingly moved trials of targeted therapies to the first-line setting, where the likelihood of treatment benefit could be higher.[36] This design feat requires rapid genomic analysis, which we show is feasible.

We detected a relevant molecular alteration, according to the EANO guideline on rational testing of gliomas, in 41.4% of the patients.[9] Only 4.1% of the patients had alterations belonging to a high tier (IB or IIB). This proportion is lower than the one reported by Padovan et al., who found a high-tier alteration in 10.1% of their 417 glioblastoma

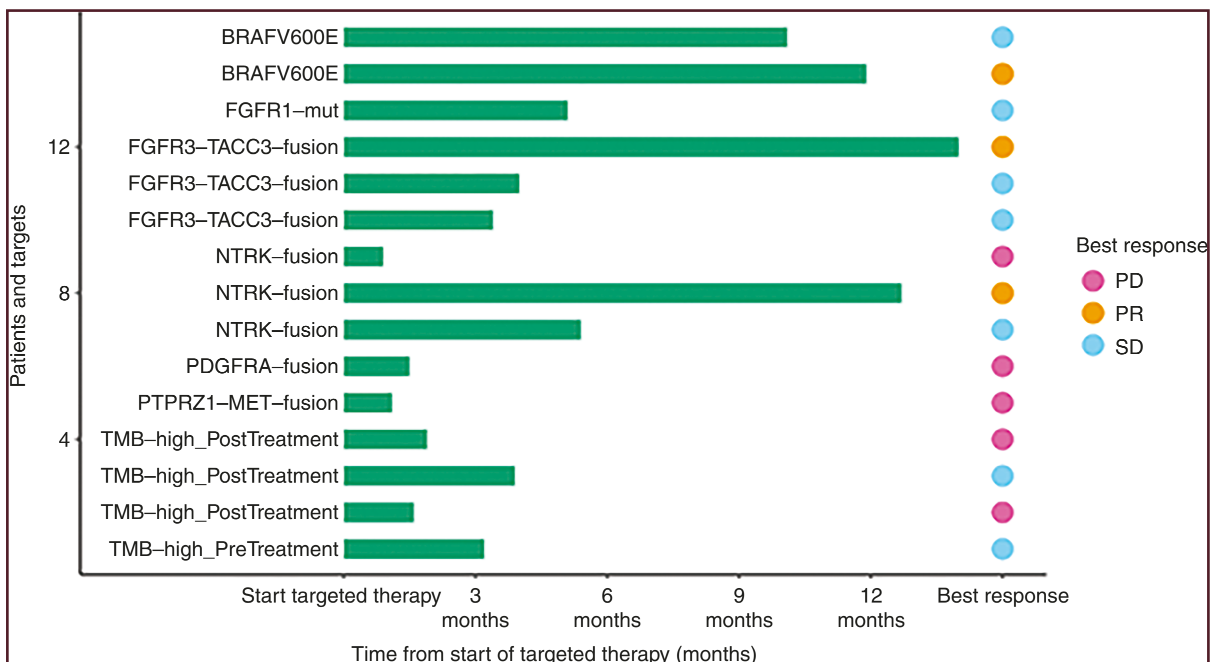

Figure 3. Swimmers plot showing targets treated, progression-free survival (PFS) and best responses in the group of patients treated with targeted therapies. PD = progressive disease; PR = partial response; SD = stable disease.

samples. However, relative to other studies in the literature with matching populations, we observed comparable frequencies of high-tier alterations: *BRAF*V600E (0.6% vs 1%–2%), *FGFR3-TACC3* alterations (2.3% vs 3.1%), and *TRK* fusions (1.1% vs 0.56%–1.69%).[37–39]

Only 3.1% of the cohort received targeted therapy based on their genomic analysis. This is lower than in other comparable papers, such as by Lim-Fat et al. and Padovan et al.[10,13] However, this number largely depends on the number of available clinical trials. For instance, in our institution, available trials with targeted therapies for grade 4 brain tumors were limited before 2020, and 12 of our 15 patients began their targeted therapy after 2021. Therefore, if we look selectively at the later years, the numbers of included patients treated are more like other centers.

We might have been able to treat some more patients if we had performed systematic upfront profiling from the beginning in 2016. Four of the 35 patients with targetable alterations were profiled after they had relapsed and had already started their relapse treatment. However, despite timely profiling, 6 out of 35 (17%) patients' performance status at relapse still did not allow enrollment in the trial, demonstrating the potential rapid deterioration of this disease. Another barrier to treatment was the lack of measurable disease, which is a common inclusion criterion in glioblastoma trials with response-based endpoints. However, if trials were to move to survival-based endpoints, it would be appropriate to include these patients.

Our response results are in line with the literature, as patients with high-tier targets, according to the ESCAT framework (*BRAFV600E*, *NTRK* fusion, and *FGFR* fusion), demonstrated the best outcomes.[9] The median progression-free survival for the treated group was 3.9 months (range: 0.9–14 months). This result should be interpreted with caution, as the group had diverse molecular profiles and clinical characteristics that may have influenced survival and response to therapy.

Our predominantly glioblastoma cohort has a lower proportion of patients treated with targeted therapy than other genomically profiled solid tumor and pan-cancer cohorts, which report 5%–27% of such cases.[40–43] Our cohort also has fewer high-tier ESCAT targets than other pan-cancer cohorts. A recent study of 516 sequenced patients across tumor types found that 34% of tumors had an ESCAT tier I or II target.[40] Most of our targets were ESCAT IIIA, meaning that there is clinically meaningful evidence in other cancer types, but it has yet to be shown in glioblastoma. There have been many trials with targeted therapies for glioblastoma; however, all but a few have been failures.[3] Some of the reasons for this are efficacy trials run without biomarker-enrichment nor data on blood-brain barrier penetrance, and the not fully understood tumor heterogeneity. These and other reasons are discussed elsewhere.[3,36]

Another difference between our cohort and most pan-cancer cohorts is that we used primary tissue to identify alterations for drug matching in the relapse setting. Previous studies have shown that genomic alterations in relapsed glioblastoma were mostly similar to those in primary tumors.[13,35,44] However, it is still unclear whether sequencing at diagnosis is enough for future clinical decisions, and more studies on paired samples are needed to address this question.

Conclusion

This study shows that genomic profiling uncovers alterations of interest in a substantial number of patients, but only a minority are considered by the DNMTB to have actionable alterations, and an even smaller fraction ultimately receive targeted therapy. Nevertheless, this paper also highlights how various factors—such as promising drug targets, the shortened time from diagnosis to tumor board recommendations; the increased availability of clinical trials for brain tumor patients; and the recent recommendations regarding actionable alterations in society guidelines from ESMO and EANO—may contribute to a future increase in the number of patients benefiting from targeted therapies based on genomic profiling.

Supplementary material

Supplementary material is available online at *Neuro-Oncology Practice* (https://academic.oup.com/nop/).

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Conflict of interest

None declared.

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Authorship statement

Writing of the manuscript (V.F.). Inclusion of patients from the outpatient clinic (D.N., T.U., H.S.P., and B.H.). Treatment with targeted therapies and trial PIs (B.H., M.H., K.S.R., and I.S.). Oncologists responsible for the molecular tumor board meetings (B.H., M.H., K.S.R., and I.S.). Responsible for all genomic analyses (C.Y.W.). Help with data collection (L.B. and K.G.) Responsible for pathological diagnoses and analyses (K.B.L. and L.C.M.). All authors have contributed to the concept of the study and have critically read and approved the manuscript.

Data availability

De-identified clinical and molecular data and scripts used for figures and analyses can be provided by authors upon request.

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