Updated EANO guideline on rational molecular testing of gliomas, glioneuronal, and neuronal tumors in adults for targeted therapy selection – Update 1

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Abstract

The standard of care for adult patients with gliomas, glioneuronal and neuronal tumors consists of combinations of surgery, radiotherapy, and chemotherapy. For many systemic cancers, targeted treatments are a major part of the standard treatment, however, the predictive significance of most of the targets for treatment in systemic cancer are less well established in central nervous system (CNS) tumors . In 2023 the EANO Guideline Committee presented evidence based recommendations for rational testing of molecular targets for targeted treatments. From all targets reviewed, only testing for BRAF V600E mutations was of proven clinical benefit; despite regulatory approvals for tumor agnostic treatment of NTRK gene fusions and high Tumor Mutational Burden (TMB) for patients with adult brain tumors, the evidence of clinical benefit for patients was still limited . This guideline has a modular structure, allowing regular updating of individual sections and adding new ones. The present version (Update 1) presents a review of the rationale of testing for PTEN, H3F3A, MTAP, RET and IDH, and presents an update of the text on TMB high and mismatch repair deficiency. It also presents an overview of therapeutic yield of routine next generation sequencing for mutations and fusion detection. The supplement accompanying this version contains the in depth review of all targets, whereas in the main manuscript the final recommendations of the revised and new targets are presented. Updates will be made on a regular basis.

Key words

Diffuse glioma, (glio)neuronal tumors, targeted treatment, EANO guideline, IDH

Key points

- Testing for actionable alterations in patients with glial or (glio)neuronal tumors should be driven by rational considerations on the frequency of the target, the actionability of the target, a realistic estimate of the patient benefit and the presence of trial options for that patient.

- With the recent approval by the FDA of vorasidenib for use in newly diagnosed grade IDFH mutated diffuse glioma, the assessment of IDH status has now also therapeutic implications.

- Apart from assessing BRAF and IDH alterations, routine platform sequencing in adult patients with glial and (glio)neuronal tumors has still limited clinical impact .

Introduction.

In May 2023 the "EANO guideline on rational molecular testing of gliomas, glioneuronal, and neuronal tumors in adults for targeted therapy selection" was published.[1] This guideline aimed at providing a rational approach to molecular testing for targeted treatment in adult patients with primary brain tumors, both with respect to which tumor types to test and to which targets provide a rational treatment target. For target selection and evaluation, use was made of the ESMO Scale for Clinical Actionability of molecular Targets (ESCAT) and the ESMO Magnitude of Clinical Benefit Scale (MCBS) which provide levels of evidence of a target and a scoring system to assess the clinical benefit of the target inhibition.[2] The guideline also presented background on the interpretation of what is to be considered a pathogenetic variant and the various approaches to assess pathogenetic variants. A companion EANO guideline provided evidence how molecular testing should be conducted to diagnose brain tumors according to the 5th edition of the WHO Classification of Tumours of the Central Nervous System published in 2021 (WHO CNS5).[3,4] The EANO guideline for rational molecular testing of glioneuronal, and neuronal tumors in adults for targeted therapy selection" has a modular structure, allowing regular updates both by adding and by updating chapters, without the need to update the entire text. In part because of perceived omissions, identified after the publication but also because of the need to present the information on novel targets we now present the first update. This update covers PTEN/PI3K, IDH as a therapy target, *RET* fusions, *MTAP* deletions, and an update of the chapter of tumor mutational burden (TMB). In addition, we provide an overview of studies evaluating the implementation of Next Generation Sequencing panels in daily practice with the aim to identify treatment targets for treatment in cohorts of brain tumor patients. In this main manuscript, only the conclusion sections of the individual targets are presented. In supplement I, we present the full text with all in depth reviews on the targets described in the present update. In the supplemental file II all the targets are presented in full, either as described in the

first guideline or in the present revision. This is therefore the essential EANO document with all the current recommendations on testing for molecular targets. For the rationale of the ESCAT score and ESMO-MCBS, we refer the reader to the essential first 2023 publication of this guideline that details the background of molecular testing, assigning pathological significance to variants, and how to assign clinical significance to these variants.[1] Figure 1 presents the relative frequency of the targets in the various glial and (glio)neuronal tumors, and the ESCAT score attributed to this target. This figure and the accompanying supplemental text give guidance when further testing is rational and the status of the target. .The aim of the EANO Guideline Committee is to continue updating this guideline with regular intervals. Table 1 provides an overview of the targets reported on, and in which version of the guideline they were reported or an update.was presented

Review of the therapeutic yield of panel sequencing for target identification and clinical outcome

The use of next generation sequencing (NGS) panel diagnostics for the classification of gliomas and (glio)neuronal tumors is increasing, as it offers the possibility of both mutational analysis, assessment of fusions and copy number alterations in one run.[5-7] Apart from their use for routine diagnostics according to the WHO 2021 brain tumor classification, these panels can also be used for the identification of targets for target treatments. Indeed, these reports frequently state the numbers of patients with identified targets for treatment, often with percentages varying from to 50% - 80% suggesting major patient benefit of these diagnostic procedures.[5,8-10] The used databases to call the pathogenicity of the molecular alteration vary, and different databases may results in different interpretations.[11] Not all reports use scales that reflect the level of actionability of the target, such as the on FDA approvals based Tier I and II approach

and the ESMO ESCAT scale.[2,12] The percentage of patients with a target identified remains however theoretical if no analysis of outcome of the target identification is provided. This can be done at two levels, at the level of target therapy actually provided ("treatment change because of the NGS result") or at the level of a target successfully treated ("durable response"). It is the latter part that reflects true patient benefit, but most studies do not report that. For a good understanding of the patient benefit of the NGS panel diagnostics for this purpose beyond routine CNS tumor diagnostics, randomized clinical trials should be performed in specific populations (umbrella trials) according to NGS results. Another way that could be helpful in suggesting a potential usefulness of biomarkers based therapy could be the percentage of patients in whom clinical benefit was achieved in terms of either an objective response, the percentage of patients presenting progression-free survival (PFS) on matched therapy (PFS2) 1.3-fold longer than the PFS on prior therapy (PFS1) or long-term survival (PFS2/PFS1 ratio).[13] This requires meticulous follow-up of the patient cohort, which is unfortunately only provided in a few reports.[8,9,14] In studies providing such analyses, clinical benefit is reported in 0.25 to 4% of patients.[8,9,14] The largest series on 442 glioblastoma IDH-wildtype (wt) patients identified in 3.4% of patients a target classified as ESCAT IB–IC ('ready for routine use') and in 6.7% a target classified as ESCAT IIB (investigational).[2,8] Thirty-six patients (10.5%) of 343 candidates (8.6% of the total population) for targeted therapy were actually treated with a targeted therapy. Three responses (8.3%) were observed (2 with dabrafenib/trametinib, 1 with entrectinib), and in a total of 8 patients a PFS2/PFS1 ratio of more than 1.3 was observed (including 2 out of 4 patients treated with erdafitinib; 1 of 1 treated with capmatinib). Thus, patient benefit was achieved in 6 out of 343 which sums up to approximately 2.6% of the tested population. However, the rate of benefit is also influenced by other factors including the tumor type (i.e., glioblastoma IDH-wt is less likely to respond than other diffuse glioma or glioneuronal tumor types as the former commonly harbor multiple pathways activation), availability of a potent brain-penetrant drug or clinical trial (*i.e.*, a potential target is identified but no effective therapy is available) and the disease stage at

which the therapy is proposed (*i.e.*, results only available at the advanced, refractory setting where responses are less likely to occur). In IDH-wt glioblastoma, the most common alterations (e.g., *EGFR* amplification, *PTEN* loss, *PIK3CA* mutations, *CDK4/6* amplifications) have so far not been targeted successfully.

Several series have systematically assessed fusion analysis in glioma patients. Among 390 glioma patients, one series found that 11% (25/235) of glioblastomas, 12% (5/42) of grade 3 astrocytomas, 8% (2/25) of grade 2 astrocytomas, and 33% (2/6) of pilocytic astrocytomas (WHO 2007) harbored potentially targetable fusions.[7] The occurrence of fusions was significantly higher in IDH-wt tumors (12%, n = 31/261) compared to IDH mutant tumors (4%; n = 4/109) (p = 0.011). The most common potentially targetable fusions were in FGFR (n = 12), MET (n = 11), and NTRK (n = 8). In IDH mutant tumors 2 MET fusions, 2 NTRK fusions and one other fusion were observed. Besides the pathognomonic chromosomal 1p/19q alteration, no additional fusions were observed in oligodendrogliomas (n=15 with known 1p/19q status). Another series on 356 patients with diffuse glioma reported fusions in 53 out of 166 histologically grade 4 gliomas (151 IDH-wt, 15 IDH-mt) in: *MET* (n = 18), *EGFR* (n = 14), *FGFR* (n = 12), *NTRK* (n = 5), *RET* (n = 2), *AKT3* (n = 1), and *PDGFRA* (n = 1) [15]. Gene fusions were observed in both IDH-wt (48/151, 31%) and IDH-mutant grade 4 tumors (5/15 tumors, 33%). Numerous novel gene fusions were identified in this cohort. Their biological (i.e. oncogenicity, false positive in view of the rather unexpected high rate of fusions) and clinical relevance (i.e. targetability) require confirmation in future studies. While high-throughput targeted RNA sequencing offers a broader path to diagnosis, it can also increase the false-positive rate at which fusion genes are detected.[16] In a third study using a mRNA fusion panel testing 56 genes on 647 diffuse glioma patients, the authors identified 52 tumors (8%) exhibiting a potentially targetable fusion (*FGFR3*: 16, *MET:* 14*, EGFR*: 7, *NTRK1*: 2, *NTRK2*: 6, *BRAF*: 6, *ROS1*: 4, and *PIK3CA*: 1).[17] These potentially targetable fusions were identified in 9% (40/458) of

glioblastomas, in 4% of IDH-mutant astrocytomas (4/78), and in none of 51 patients with oligodendrogliomas. Eleven (21%) of these patients (*FGFR*: 4; *MET*: 1; *EGFR*: 2; *BRAF*: 1; *NTRK*:3) received treatment with a fusion-targeted inhibitor. Except for three patients staying free from progression between 7 and 12 months, all others relapsed within 6 months and no response was observed.

Conclusion panel diagnostics for targeted treatment

The diagnostic yield of NGS panel analysis for diagnosis in gliomas is high, allowing a precise classification with most NGS panels routinely used. The yield in terms of identification of therapeutic targets with successful treatment interventions is however much lower. From a therapeutic perspective, the most relevant alteration that can be identified with most available panels are *BRAF V600E* mutations. Fusions are more robustly detectable by RNA-NGS than by DNA-NGS, which may require a second run using specific RNA-NGS panels or favor the use of a combined DNA/RNA panel approach.[1] Gene fusions are more frequent in glioblastomas, IDH-wt (~10%) compared to IDH-mutant astrocytomas. In oligodendrogliomas IDHmt and 1p/19q co-deleted, they appear to be exceedingly rare. The clinical and biological significance of many fusions require further investigations. Most fusion targets identified are below ESCAT level II (*FGFR*, *EGFR*, *MET*), with data on *NTRK* in adult patients still very limited. With the recent results obtained with the type II RAF inhibitor tovorafenib and the MEK inhibitor selumetinib, testing for *BRAF::KIAA* fusions may become therapeutically relevant in tumors known to harbor this type of fusion (*e.g.*, high grade astrocytoma with piloid features, diffuse leptomeningeal glioneuronal tumor or pilocytic astrocytoma; ESCAT 1B).[18,19]

Recommendation: specific NGS panel testing for fusions is to be considered in patients with adult-type diffuse glioma that are still in a good clinical condition in whom standard treatment options are exhausted, that are still in a good enough clinical condition and with clinical trial options available for the most likely to be detected fusions. (April 2024)

H3 K27 Alteration: Integrated recommendations on testing and treatment for H3 K27-alterations

Diffuse gliomas in midline structures should be tested for H3 p.K28 (K27) alteration as part of the standard diagnostics as specified in the WHO CNS5 classification. To date, H3 K27 mutation cannot yet be considered a direct target for specific molecular drugs, although early trials on ONC201 in recurrent disease indicate activity (**ESCAT IIB; ESMO MCBS grade 2**) and phase II and III trials in recurrent and newly diagnosed tumors are ongoing. For patients diagnosed with a tumor with an H3 p.K28 (K27) alteration referral to a center of excellence with trials options available should be considered, either at first diagnosis (preferably) or at recurrence. (April 2024)

Phosphatase and tensin homologue (*PTEN*)/ phosphatidylinositol 3-kinase (PI3K) alterations in cancer: Integrated recommendations on testing and treatment for of PTEN/PI3K alterations

Despite the frequency of PTEN/PI3K pathway activation in gliomas, especially glioblastomas, routine testing for these alterations is not recommended given the lack of effective therapies (ESCAT IIIA). Testing for *PTEN/PI3K* alterations should be limited to patients who are in a good clinical condition with clinical trial options available. (April 2024)

Rearranged during transfection *(RET)* alterations: Integrated recommendations on testing and treatment for *RET* alterations

In adult patients with gliomas, glioneuronal or neuronal tumors, RET alterations are classified as **ESCAT IIIA** (mutations and fusions) and **ESCAT IIIB** (amplifications) targets; therefore, testing for these targets should only be considered in patients who have exhausted standard treatment options, are in good enough clinical condition, and have clinical trial options available. If a RET alteration is identified as part of a broader, more general NGS screening, treatment should be considered in a clinical trial or prospective registry. (April 2024)

High tumor mutational burden, DNA mismatch repair and polymerase proofreading deficiency: Integrated recommendations on testing and treatment for high tumor mutational burden, DNA polymerase and MMR deficiency

In patients with newly diagnosed tumors, testing for MMR mutations should be considered in young adults (< 50 years), tumors with unusual histological or molecular features (e.g., high grade glioma IDH wildtype with ATRX loss, tumors with severe pleomorphism and/or giant cell features, tumors not falling into classic molecular subtypes, or associated with a DNA methylation pattern suggestive of MMR deficiency (PMMRDIA, "Diffuse pediatric-type high grade glioma, RTK1 subtype, subclass A", and "Adult-type diffuse high-grade glioma, IDH-wildtype, subtype E"), and patients with a personal or familial history suggestive of germline DNA polymerase or MMR deficiency. Since prospective and retrospective cohorts of adult glioma patients with de novo TMB-H or MMR deficiency treated with immune checkpoint blockade have so far not resulted in significant response rates (**ESCATIIIA**), treatment should preferably be given within prospective registries or clinical trials, despite the tumor agnostic approval in some countries of checkpoint blockade for TMB-high tumor).[20] For post-treatment TMB-H in glioma, reports of benefit with

immune checkpoint blokade are anectdotal (**ESCATIIIB**) and treatment is best limited to trial enrolment after standard of care is exhausted. In patients with recurrence of the tumor after alkylating agents, testing of the recurrent tumor for TMB/MMR deficiency is relevant only in the context of available clinical trials for patients with IDH-mutant gliomas, *MGMT* promoter methylated IDH-wildtype glioblastoma, or patients who initially responded to alkylating agents, but the current reports on efficacy do not justify a biopsy for the sole reason to obtain tissue for TMB/MMR deficiency analysis. Testing should also be considered for IDH mutant glioma relapsing after prior temozolomide chemotherapy, if chemotherapy with an alkylating agent is considered. (April 2024)

Isocitrate dehydrogenase (IDH) mutations: Integrated recommendations on testing and treatment for IDH alterations

All diffuse gliomas should be tested for IDH mutations to meet standard diagnostic requirements.[4,21] IDH mutations have been established as **ESCAT I-A** molecular treatment target in patients with grade 2 IDH mutant gliomas treated with surgery but not with radiotherapy or chemotherapy and its use has been approved by FDA.[22] Further regulatory approvals will influence drug access in the clinical setting. (August 2024)

Methylthioadenosine phosphorylase (MTAP) deletion: Integrated recommendations on testing and treatment

In all, there is currently no robust evidence for a diagnostic, prognostic or predictive role of *MTAP* deletion in gliomas. Screening for *MTAP* deletion in glioma patients should be considered in the context of available clinical trials (ESCAT IV). If testing is decided, there is no consensus on optimal assay to use and testing should be decided depending on trial requirement and tissue

availability. Both IHC, arrays, and NGS have been reported in this setting and seem robust. If a genomic assay is required for trial participation but not directly available, IHC can be considered as a pre-screening strategy to identify potential candidates before genomic confirmation of *MTAP* loss. (April 2024)

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Figure 1. **Overview of molecular targets found in gliomas, glioneuronal tumors, and neuronal tumors of adults and associated ESCAT score.**

Notes: Numbers are rough estimates based on literature and public databases, data on rare or new subtypes for which only a few samples have been characterized may evolve. Whenever feasible, results have been translated into tumor types as they are defined according to the WHO 2021 central nervous system tumor classification. This may be responsible for variations in biomarker prevalence compared to past studies. Definitions of variants may vary between different studies (eg, rare mutations outside known hotspots of which the somatic status is unknown and oncogenic potential has not been determined). Single cases or discordant reports regarding target prevalence are not included in the table.

Table 1. Targets described and the EANO guideline version in which they were published: the first guideline or the present August 2024

BRAF	May 2023
NF1	May 2023
ALK	May 2023
EGFR	May 2023
ALK	May 2023
FGFR/MET	May 2023
NTRK	May 2023
PDGFRA	May 2023
ROS1	April 2024
CDK4/6	May 2023
MDM2/4	May 2023
PI3K/PTEN	April 2024
mTOR/TSC1/TSC	May 2023
RET alterations	April 2024
IDH	August 2024
H3 K27M	April 2024
MTAP	April 2024
TMB, MMR, POLE	Revised in August 2024
HRD	May 2023

Figure 1