



**HAL**  
open science

## Molecular profile to guide personalized medicine in adult patients with primary brain tumors: results from the ProfiLER trial

Alice Bonneville-Levard, Didier Frappaz, Olivier Tredan, Olivier Trédan, Emilie Lavergne, Véronique Corset, Vincent Agrapart, Sylvie Chabaud, Daniel Pissaloux, Qing Wang, et al.

### ► To cite this version:

Alice Bonneville-Levard, Didier Frappaz, Olivier Tredan, Olivier Trédan, Emilie Lavergne, et al.. Molecular profile to guide personalized medicine in adult patients with primary brain tumors: results from the ProfiLER trial. *Medical Oncology*, 2021, 39 (1), pp.4. 10.1007/s12032-021-01536-4. hal-03915456

**HAL Id: hal-03915456**

**<https://inria.hal.science/hal-03915456>**

Submitted on 29 Dec 2022

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



# Molecular profile to guide personalized medicine in adult patients with primary brain tumors: results from the ProfILER trial

Alice Bonneville-Levard<sup>1</sup> · Didier Frappaz<sup>1</sup> · Olivier Tredan<sup>1</sup> · Emilie Lavergne<sup>2</sup> · Véronique Corset<sup>2</sup> · Vincent Agrapart<sup>2</sup> · Sylvie Chabaud<sup>2</sup> · Daniel Pissaloux<sup>3</sup> · Qing Wang<sup>3</sup> · Valery Attignon<sup>3</sup> · Stéphanie Cartalat<sup>4</sup> · François Ducray<sup>4</sup> · Laure Thomas-Maisonneuve<sup>4</sup> · Jérôme Honnorat<sup>4</sup> · David Meyronet<sup>5</sup> · Luc Taillandier<sup>6</sup> · Marie Blonski<sup>6</sup> · Alain Viari<sup>7</sup> · Christian Baudet<sup>7</sup> · Emilie Sohier<sup>7</sup> · Sylvie Lantuejoul<sup>8</sup> · Sandrine Paindavoine<sup>8</sup> · Isabelle Treilleux<sup>8</sup> · Christine Rodriguez<sup>8</sup> · David Pérol<sup>2</sup> · Jean-Yves Blay<sup>1</sup>

Received: 23 December 2020 / Accepted: 15 June 2021 / Published online: 5 November 2021  
© Springer Science+Business Media, LLC, part of Springer Nature 2021

## Abstract

Immunohistochemistry and recent molecular technologies progressively guided access to personalized anti-tumoral therapies. We explored the feasibility, efficacy, and the impact of molecular profiling in patients with advanced brain tumors. This multicentric prospective trial ProfILER enrolled patients with primary brain tumors, who have been pre-treated with at least one line of anti-cancer treatment, and for whom molecular profiles had been achieved using next-generation sequencing and/or comparative genomic hybridization on fresh or archived samples from tumor, relapse, or biopsies. A molecular tumor board weekly analyzed results and proposed molecular-based recommended therapy (MBRT). From February 2013 to December 2015, we enrolled 141 patients with primary brain tumor and analyzed 105 patients for whom tumor genomic profiles had been achieved. Histology mainly identified glioblastoma ( $N=46$ , 44%), low-grade glioma ( $N=26$ , 25%), high-grade glioma ( $N=12$ , 11%), and atypical and anaplastic meningioma ( $N=8$ , 8%). Forty-three (41%) patients presented at least one actionable molecular alteration. Out of 61 alterations identified, the most frequent alterations occurred in *CDKN2A* ( $N=18$ ), *EGFR* ( $N=12$ ), *PDGFRa* ( $N=8$ ), *PTEN* ( $N=8$ ), *CDK4* ( $N=7$ ), *KIT* ( $N=6$ ), *PIK3CA* ( $N=5$ ), and *MDM2* ( $N=3$ ). Sixteen (15%) patients could not be proposed for a MBRT due to early death ( $N=5$ ), lack of available clinical trials ( $N=9$ ), or inappropriate results ( $N=2$ ). Only six (6%) of the 27 (26%) patients for whom a MBRT had been proposed finally initiated MBRT (everolimus ( $N=3$ ), erlotinib ( $N=1$ ), ruxolitinib ( $N=1$ ), and sorafenib ( $N=1$ )), but discontinued treatment for toxicity ( $N=4$ ) or clinical progression ( $N=2$ ). High-throughput sequencing in patients with brain tumors may be routinely performed, especially when macroscopic surgery samples are available; nevertheless delays should be reduced. Criteria for clinical trial enrollment should be reconsidered in patients with brain tumors, and a panel of genes specifically dedicated to neurologic tumors should be developed to help decision-making in clinical practice.

**Keywords** Primary brain tumor · Molecular profiling · Precision medicine · Targeted therapy · Decision-making

✉ Alice Bonneville-Levard  
alice.bonneville-levard@lyon.unicancer.fr

<sup>1</sup> Department of Medical Oncology, Léon Bérard Cancer Centre, 28, rue Laennec, 69373 Lyon, France

<sup>2</sup> Department of Clinical Research and Innovation, Léon Bérard Cancer Centre, Lyon, France

<sup>3</sup> Department of Translational Research and Innovation, Léon Bérard Cancer Centre, Lyon, France

<sup>4</sup> Department of Neuro-Oncology, Hospices Civils de Lyon, Lyon, France

<sup>5</sup> Department of Neuropathology, Hospices Civils de Lyon, Lyon, France

<sup>6</sup> Department of Neuro-Oncology, Central Hospital, Nancy, France

<sup>7</sup> Synergie Lyon Cancer, Bio-Informatics Platform, Léon Bérard Cancer Centre, Lyon, France

<sup>8</sup> Department of BioPathology, Léon Bérard Cancer Centre, Lyon, France

## Introduction

Up to 2016, diagnosis and treatment of brain tumors were based on histopathology classifications [1]. In the last decade, oncogene mutations, amplifications, or deletions for various types of cancers was highly considered to guide targeted therapy development and improve overall survival. Trastuzumab and pertuzumab inhibit HER-2 overexpression in breast cancer [2], and erlotinib especially inhibits *EGFR exon 19 or 21* mutation or deletion in non-small cell lung cancers [3]. Anti-EGFR of third generation investigated how to overcome resistance [4].

The access to molecular biology platforms, high-throughput sequencing, and improvement in bioinformatics [5] have extensively evolved [6] and progressively modified clinical practices and management of patients with cancer [7]. Despite similar results in terms of tolerance are reported in phase I and II trials for patients with brain tumors, neuro-oncology patients are still often excluded from large multicohort basket trials [8]. The multicentric prospective program ProfiLER designed at the Centre Léon Bérard aimed to determine the molecular profile from archived or fresh biopsy in 2579 patients with metastatic, locally advanced solid cancers, or hematologic cancers [9]. The main objective was to identify the panel of molecular characteristics in each histopathologic subgroup for each type of cancer. A molecular multidisciplinary tumor board (MTB) referred patients to early-phase clinical trials with appropriate biological inclusion criteria for personalized targeted therapy. We report results in the subpopulation of 141 patients with brain tumors.

## Materials and methods

### Study design and patients

The French prospective multicentric trial ProfiLER (NCT01774409) enrolled patients with a metastatic solid or hematologic tumor disease with no initial limitation on Eastern Cooperative Oncology Group Performance Status (ECOG-PS), and inclusions have been later restricted from October 2013 to patients with ECOG-PS of 0 or 1. The study received local approval (Ethics committee of Lyon Sud-Est IV) and was conducted in accordance with the Good Clinical Practice guidelines of the International Conference on Harmonization, and the Declaration of Helsinki, and relevant French and European laws and directives. All patients provided written informed consent. Results on the global cohort have been published [9].

## Procedures

The genomic molecular profiles are established using formalin-fixed paraffin-embedded (FFPE) tumor specimen containing  $\geq 10\%$  of tumor cells, emerged from primary tumor, relapse, or metastasis from archival samples or de novo biopsies. Genetic profiling was obtained by next-generation sequencing (NGS) on predefined genes [10, 11] and/or genome-wide microarray-based Comparative Genomic Hybridization (aCGH). Assessment of mutations was performed using the platform Ion Torrent Personal Genome Analyzer (Thermo Fisher Scientific, MA).

The size distribution of the DNA amplicons was analyzed on the 2200 TapeStation (Agilent Technologies, Santa Clara, USA) using the High Sensitivity DNA Reagent Kit (Agilent Technologies Santa Clara, USA). Template preparation, emulsion PCR, and Ion Sphere Particle 8 (ISP) enrichment were performed using the Ion One-Touch™ 2 kit (Thermo Fisher Scientific, MA) according to manufacturer's instructions. The ISPs were loaded onto an Ion 318™ Chip Kit V2 (Thermo Fisher Scientific, MA) and sequenced using an Ion PGM™ Sequencing 200 Kit V2 (Thermo Fisher Scientific, MA) on the Ion Torrent PGM™ (Thermo Fisher Scientific, MA) for 500 cycles. The raw signal data were analyzed using NextGENe Software Suite v3.4.2 (SoftGenetics, PA) and in-house bioinformatics pipeline. The samples with limited DNA quantity not suitable for standard aCGH were analyzed using Cytoscan HD according to the manufacturer's protocol (Affymetrix®, Santa Clara, CA).

The pipeline includes quality score assignment, alignment to the human genome 19 reference, mapping quality QC, coverage analysis, and variant calling. After completion of the primary data analysis, lists of detected sequence variants [single nucleotide variants (SNVs) and INDELs] were compiled in the variant call format (VCF) files. For downstream analysis, variants with a minimum coverage of 50 reads containing at least 10 of the mutant reads were selected. Variant calls were further analyzed using variant filtering and annotation using COSMIC v.64 released on March 26, 2013, [11] and dbSNP build 135, released on June 6, 2012 [12].

The variants were filtered according to their frequency ( $> 5\%$  for SNVs and  $> 10\%$  for INDELs), strand ratio ( $> 0.2$ ), and reads coverage ( $> 50X$  for SNVs and  $100X$  for INDELs).

### Weekly molecular tumor board (MTB)

A molecular multidisciplinary tumor board including medical oncologists, molecular biologists, and pathologists

reviewed weekly all the available analyses (CGH and/or NGS results according to initial disposable tumor quantity) and identified, prioritized, and selected alterations as actionable when genomic molecular alteration (mutation/deletion/amplification) allowed to assign a molecular-based recommended therapy (MBRT). Alterations are used as key criteria for inclusion in on-going clinical trials, or as target for MBRT, defined as molecular-targeted agents (MTA) outside EMA approval. The characterization of alterations as actionable was time dependent, according to rapidly developing state of the art, recent relevant publications and/or ongoing trials, and drug availability. For patients with molecular alteration identified as target for a specific therapy, or meeting inclusion criteria for an early clinical trial, the MTB referred the patient to his medical oncologist for current health status evaluation and proposed treatment recommendations. If multiple targets were identified, treatments currently available through clinical trials were proposed. In the absence of identified mutation/deletion/amplification, or for alteration with no matching MTA available, no specific recommendations were issued. Patients who have initiated an MBRT were followed according to the clinical trial protocol in which they had been included or according to the routine medical practice for off-label use of MTAs.

## Study endpoints

The primary endpoint of the study was the incidence of molecular alterations detected in tumor sample. Secondary endpoints included a description of patients with actionable alterations with MBRT initiated and responses to MBRT.

## Statistical population and analysis

Analyses were performed on clinical data from patients enrolled up to December 1, 2015 with molecular analyses completed and examined by the MTB before April 21, 2016. Statistical analysis was performed with SAS version 9.3.

## Results

### Population

Between the February 26, 2013 and the December 1, 2015, 1822 patients were enrolled in the ProfiLER program including 141 patients with primary brain tumors. Molecular analyses were performed in 105 of these 141 patients (Fig. 1). Technical failures ( $N=36$ ) occurred because of insufficient quantity or quality of tumoral samples ( $N=30$ ), or other technical problems ( $N=6$ ).

Characteristics of the 105 patients are shown in Table 1. The main representative histologic type of tumors were glioblastoma ( $N=46$ , 43.8%), low-grade glioma ( $N=26$ , 24.8%), high-grade glioma ( $N=12$ , 11.4%), and atypical and anaplastic meningioma ( $N=8$ , 7.6%).

Median delay between the diagnosis of the primary tumor and the inclusion in ProfiLER program was 2.7 (0.2–29) years [glioblastoma: 14 (0.5–62) months; low-grade glioma: 93 (14.2–257) months; anaplastic gliomas: 77 (29–234) months]. Median delay from written informed consent to MTB recommendations was 2.8 (1–7.1) months.

The samples were mainly obtained from surgical resection ( $N=104$ ), but some biopsies were also performed [stereotactic biopsy ( $N=33$ ), open biopsy ( $N=4$ )]. Molecular profiles were mostly successfully achieved using samples from surgical resection with only 12/104 failures, and the rate of screen failures for molecular analyses was higher in samples issued from biopsies [open biopsy (1/4); stereotactic biopsy (21/33)].

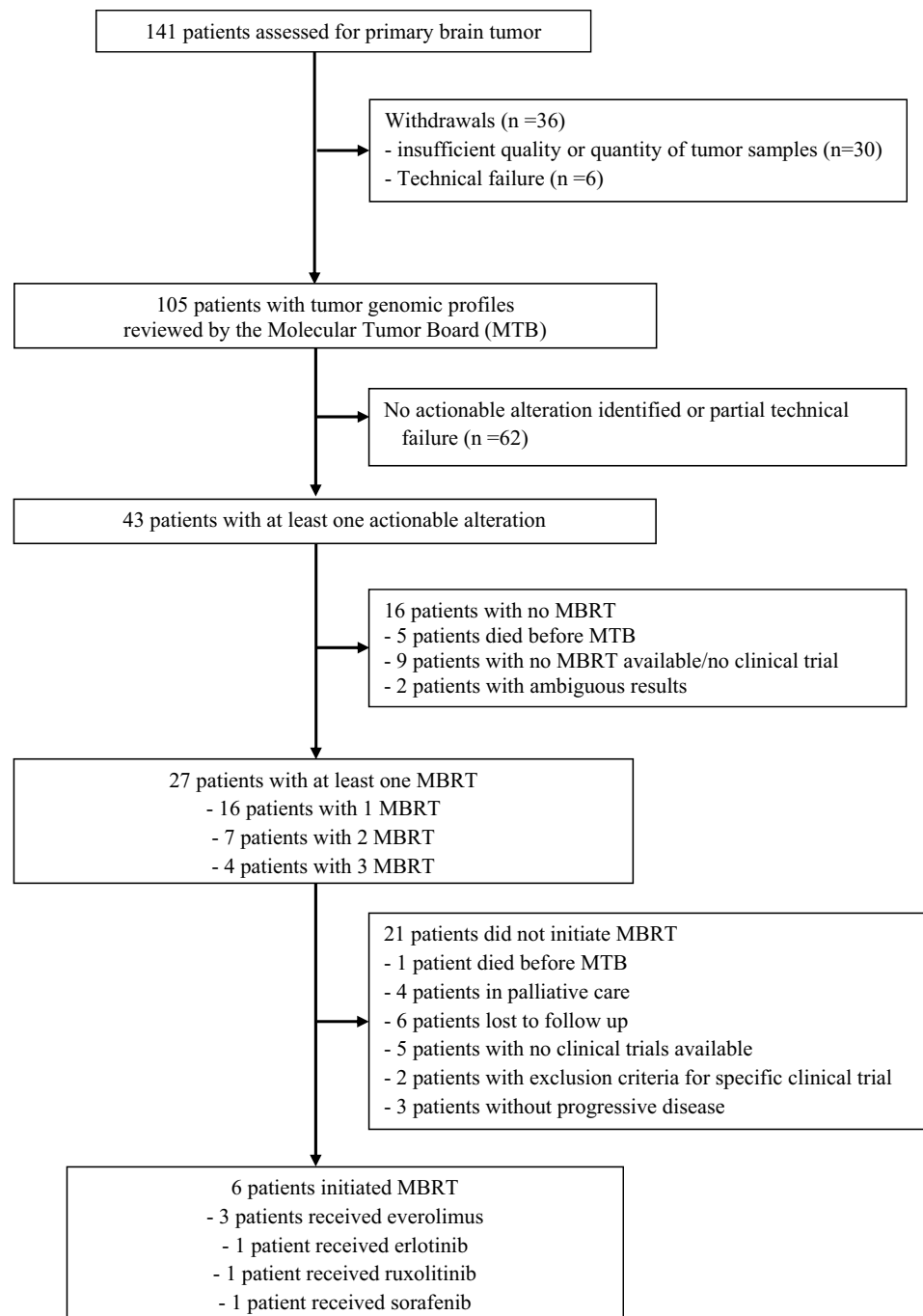
Of the 105 patients, 78 (74.3%) patients had complete molecular profile based on both CGH array and NGS results, 10 patients (9.5%) had only CGH array results, and 13 patients (12.4%) had exclusively NGS results. Four patients had no results with any of these procedures, and results have been later achieved using Affymetrix and not reported in the present analysis.

Sixty-two (59%) patients had no molecular alterations, or alteration with no matching targeted treatment available. Forty-three (41%) patients presented at least one actionable molecular alteration (mutation/deletion/amplification), including 20 patients who had a single alteration, 10 patients had 2 alterations, 8 patients had 3 alterations, and 5 patients had  $\geq 4$  alterations. Altogether, 61 alterations were described (Table 2). Twenty-two patients had at least one gene amplification ( $N=22$ ), 22 had at least one homozygous gene deletion ( $N=22$ ), and 17 patients had at least one pathogenic mutation ( $N=17$ ). The most frequent alterations were *CDKN2A* homozygous deletion ( $N=18$ ), *EGFR* amplification ( $N=12$ ), *PDGFRa* amplification ( $N=6$ )/mutation ( $N=2$ ), *PTEN* homozygous deletion ( $N=5$ )/mutation ( $N=3$ ), *CDK4* amplification ( $N=7$ ), *KIT* amplification ( $N=6$ ), *PIK3CA* mutation ( $N=5$ ), and *MDM2* amplification ( $N=3$ ) (Table 2).

Among the 43 (41%) patients with at least one actionable molecular alteration, 16 (15%) patients did not benefit from MTB recommendation due to early death ( $N=5$ ) or unavailability of clinical trials ( $N=9$ ) despite actionable alterations had been identified, such as *EGFR* amplification ( $N=4$ ), *CDK4* amplification ( $N=2$ ) and/or *CDKN2A* homozygous deletion ( $N=7$ ), or unexploitable results ( $N=2$ ).

In the 27 (26%) patients who have been proposed a targeted therapy, only six patients ultimately received the MBRT (Fig. 1).

**Fig. 1** Study profile. *MBRT* molecular-based recommended therapy, *MTB* molecular tumor board



The characteristics of the 6 patients who received a MBRT are described in Table 3. The six patients who initiated MBRT were patients with glioblastoma, who had been treated at least with the standard first-line therapy combining radiotherapy and temozolomide. The median delay between glioblastoma diagnosis and inclusion in ProFiLER trial was 21 (18–60) months. Four of the six patients were treated in the multicenter basket trial MOST (NCT02029001) linked to ProFiLER program, investigating nilotinib, everolimus, sorafenib, lapatinib, and pazopanib, in patients with

identified adequate molecular alteration at the time of our study. Three of them with identified activation of the Pi3K/AKT/mTOR pathway received everolimus, one patient with *PDGFRa* amplification received sorafenib, one patient with *JAK* mutation was treated with ruxolitinib, and the last patient with *EGFR* mutation received erlotinib. Treatment discontinuation occurred for toxicity [persistent thrombocytopenia grade 3 ( $N=2$ ), cerebral hemorrhage grade 2 ( $N=1$ ), hypertriglyceridemia grade 4 ( $N=1$ )] or for clinical progression ( $N=2$ ).

**Table 1** Patient characteristics

	N= 105
Median age at inclusion in ProfILER trial (years)	49.0 (19–78)
Gender	
Male	70 (66.3%)
Female	35 (33.7%)
Performance status (ECOG-PS)	21 (20%)
Missing data	16 (19%)
0	49 (58.3%)
1	16 (19%)
2	3 (3.6%)
3	
Tumor histology	
Glioblastoma	46 (43.8%)
Low-grade glioma	26 (24.8%)
Anaplastic glioma	12 (11.4%)
Grade II meningioma	4 (3.8%)
Grade III meningioma	4 (3.8%)
Ependymoma (NOS)	2 (1.9%)
Anaplastic ependymoma	2 (1.9%)
Medulloblastoma	2 (1.9%)
Angiomatoid histio-cyto-fibroma	1 (1%)
Anaplastic ganglioglioma	1 (1%)
Pinealoma	1 (1%)
Pinealoblastoma	1 (1%)
Primitive neuro-ectodermal tumor (NOS)	1 (1%)
Median delay from diagnosis of primary brain tumor to inclusion in ProfILER trial (months)	32.4 (2.4–352.2)
Glioblastoma (months)	14.2 (0.5–62.2)
Low-grade glioma (months)	93.6 (14.2–257.1)
Anaplastic glioma (months)	77 (29–234)
Median delay from written inform consent to MTB results (months)	2.8 (1–7.1)
Number of patients with $\leq 1$ alteration	43
1	20
2	10
3	8
$\geq 4$	5

*ECOG-PS* Eastern Cooperative Oncology Group Performance Status, *MTB* molecular tumor board; *NOS* not otherwise specified

## Discussion

We aim to explore the use of personalized medicine to treat patients with malignant brain tumors and especially for patients with high-grade gliomas who have an extremely poor prognosis.

We report a long delay between diagnosis and inclusion in the ProfILER program. Most of the enrolled patients have already been heavily treated with several lines of treatment and did not meet highly selective criteria for inclusion in potential clinical trials. High-grade gliomas rarely received more than 2 or 3 lines, including as first-line post-operative strategy, radiation with concomitant monthly administration of temozolomide. At time of relapse, patients fit enough may experience further surgery and may receive several other

treatments such as nitrosoureas and antiangiogenic, but reduced life expectancy is generally observed. In addition, increased number of relapses associated with new alterations compared to primary tumors. Primary glioma cells culture demonstrate large genomic and transcriptional changes during the first 30 cell passages [13], and tumor landscape differs between diagnosis and relapse [14].

The ProfILER trial planned to use any available archived samples and did not require de novo biopsy for histologic and molecular confirmation. Although this issue is currently being debated, this was a pragmatic decision at time of trial conception. Our series showed that analyses required extensive delays and results were lately available. Several difficulties should be addressed through earlier obtention of patient written consent to transfer samples mainly stored outside

**Table 2** Molecular alterations (amplification, deletion, mutation) identified as potential target for molecular-based recommended therapy (MBRT)

Molecular alterations		N= 105 (43 patients with 61 altera- tions)
<b>Amplification</b>	<b>22</b>	<b>(21.0%)</b>
<i>CCNE (cyclin E)</i>	1	(1.0%)
<i>CDK4</i>	7	(6.7%)
<i>CDK6</i>	1	(1.0%)
<i>EGFR</i>	12	(11.4%)
<i>KDR (VEGFR2)</i>	5	(4.8%)
<i>KIT</i>	6	(5.7%)
<i>MDM2</i>	3	(2.9%)
<i>PDGFRA</i>	6	(5.7%)
<b>Deletion</b>	<b>22</b>	<b>(21.0%)</b>
<i>CDKN2A (P16/INK4)</i>	18	(17.1%)
<i>PDGFA</i>	1	(1.0%)
<i>PTEN</i>	5	(4.8%)
<i>TSC1</i>	1	(1.0%)
<b>Mutation</b>	<b>17</b>	<b>(16.2%)</b>
<i>BRAF</i>	1	(1.0%)
<i>FLT1 (VEGFR1)</i>	1	(1.0%)
<i>FLT3 (STK1)</i>	1	(1.0%)
<i>HRAS</i>	1	(1.0%)
<i>JAK</i>	1	(1.0%)
<i>KDR (VEGFR2)</i>	1	(1.0%)
<i>MET</i>	1	(1.0%)
<i>MTOR</i>	1	(1.0%)
<i>PDGFRA</i>	2	(1.9%)
<i>PIK3CA</i>	5	(4.8%)
<i>PTEN</i>	3	(2.9%)

N=105 patients, N=43 patients with at least one molecular alteration, n=61 alterations. MBRT: molecular-based recommended therapy. To note, one patient with similar alterations (mutation, amplification or deletion) was considered only once

our center, and earlier molecular biology results issued from high-throughput technologies leading to ideally propose inclusion in clinical trials in the first-line of treatment. Appropriate procedures were not systematically implemented in each hospital when the study was initiated, and even if specific medical consultations were planned, access to samples and molecular analyses was not systematically possible, and better addressing scheme of anatomopathologic samples between laboratories and hospitals would participate in reducing delays. Similar observations were reported in more recent trials which also faced difficulties in shortening delay to achieve molecular profile results on fresh biopsy [15, 16]. A delay of eight weeks from biopsy to medical consultation for results report is usually necessary. The feasibility of a semi-emergency complex multilayer

molecular diagnostics with a median time of 4–5 weeks from surgery to the molecular tumor board would be required to address the ongoing need of potential eligibility for MBRT for 35% of newly diagnosed glioblastomas [17]. Neuro-oncologists currently follow the 2016 WHO classification and integrated diagnosis involving molecular results has been implemented in the routine practice [7].

This paves the way to molecular target front-line assessment, and decision should be taken shortly in the case of de novo biopsy requirement at time of relapse. Sampling impacts the results of molecular analyses, and we noted that samples issued from macroscopic resection provide the most appropriate samples for analysis. Repeated open biopsy for diagnostic purposes is cumbersome, and alternative procedure should be favored. As for other tumor sites, iterative biopsies should be avoided, and liquid biopsies are preferred if such samples allow to appropriately access relevant data for clinical decisions [18]. Nevertheless, liquid biopsies are generally not contributive enough in neuro-oncology, especially for glioma. A second issue to overcome is the intra-tumoral heterogeneity. As glioblastomas usually did not harbor a single actionable driver mutation, efficacy of a monotherapy may be hampered by subclonal evolution. *EGFR* amplification and/or mutation have been detected in more than half of glioblastomas. These alterations have guided the development of treatment strategies targeting *EGFR*. However, investigations using *EGFR* as target in clinical trials did not demonstrated efficacy so far [19–21]. This may partially be explained by the clonal and functional heterogeneity of *EGFR* in glioblastoma development [22]. Despite the lack of efficacy of anti-*EGFR* therapy, one patient received erlotinib as compassionate treatment.

The concept of “actionable” target has evolved over time, and patient recruitments are only allowed for restricted period; therefore improved access to appropriate studies justifies the requirement for expertise of a dedicated MTB. Moreover, among the actionable alterations initially identified, early-phase trials allowing the enrollment of patients with primary brain tumors were scarce. Indeed, primary or secondary brain tumors are often exclusion criteria for phase I trials. Yet, inclusion of patients with brain tumor in early-phase trials have been considered as feasible [8]. Less stringent criteria should be applied to allow inclusion of neuro-oncological patients for whom neurological deficit is the single barrier to inclusion [23].

So far, specific trials investigating targeted therapies in patients with brain tumor harboring molecular alterations, such as rindopepimut in patients with glioblastoma carrying *EGFRvIII* mutation [24] or anti-*EGFR* for recurrent or newly diagnosed glioblastomas [25, 26], did not demonstrate efficacy. Another phase III study INTELLANCE investigating the antibody–drug conjugate depatuxizumab mafodotin prematurely stopped for futility (NCT02573324).

**Table 3** Characteristics of the 6 patients for whom MBRT was proposed and initiated

Patient	Tumor histology	Age (years)	Previous lines of treatment	Alterations	Delay (months) from diagnosis to inclusion in ProfiLER	MBRT, treatment duration, reason for discontinuation	Delay (months) from treatment initiation to death)
#1	Glioblastoma	47	-*stupp -temozolomide -lomustine -stereotaxic radiotherapy	<i>JAK</i> mutation	21	Ruxolitinib, 22 days, Hematologic toxicity (thrombocytopenia grade 3)	3
#2	Glioblastoma	45	-*stupp -stereotaxic radiotherapy -lomustine -bevacizumab	Amplification 4q: <i>PDGFRa</i> , <i>KIT</i> , <i>KDR</i>	22	Sorafenib, 9 days, Cerebral hemorrhage grade 2	4
#3	Glioblastoma	73	-*stupp -lomustine + bevacizumab	<i>pTEN</i> homozygote deletion	18	Everolimus, 18 days, Hematologic toxicity (thrombocytopenia grade 3)	2
#4	Glioblastoma	51	-*stupp -lomustine + bevacizumab	<i>EGFR</i> amplification	20	Erlotinib, 46 days, Clinical progression	1
#5	Glioblastoma	64	-*stupp -temozolomide	<i>MTOR</i> exon 53 mutation	60	Everolimus, 87 days, Clinical progression	6
#6	Glioblastoma	54	-*stupp -lomustine + bevacizumab -stereotaxic radiotherapy -temozolomide + bevacizumab -rindopepimut	<i>pTEN</i> Heterozygous deletion + 2nd allele mutation (bi-allelic inactivation)	21	Everolimus, 17 days, Toxicity (grade 4 hypertriglyceridemia)	2

MBRT: molecular-based recommended therapy; \*stupp: radiotherapy + concomitant and adjuvant administration of temozolomide

Although our patient did not show actionable targets such as BRAF, some targeted therapies in brain tumors have been reported to be efficient. The multicohort trial VE-BASKET revealed promising results in glioblastoma and anaplastic astrocytoma with BRAFV600E mutation [27]: median progression-free survival was 5.3 (95% CI 1.8–12.9) months with an objective response rate of 9.1% and a median survival of 11.9 (95% CI 8.3–40.1) months. The bi-therapy dabrafenib-trametinib (BRAF and MEK inhibitors) led to improved response rates [28]. This BRAFV600E mutation is scarce in high-grade glioma but may represent a high rate of pleomorphic xanthoastrocytoma epithelioid glioblastoma [29].

The basket trial MOST enrolled patients with any tumors, and high-throughput technologies from ProfiLER trial were not specifically dedicated to patients with brain tumors. Only 7.7% of the patients in the ProfiLER program had neurological tumors [9]. The current gene panel excluded

some genes which may be relevant for targeted therapies and may favor inclusions in brain tumor-specific trials. For example, patients with medulloblastoma harboring SHH-activation pathway were eligible in other ongoing trials such as MEVITEM investigating the efficacy of the Sonic Hedgehog pathway inhibitor vismodegib [30–32]. Similarly, the phase I evaluating the IDH-mutant inhibitor ivosidenib demonstrated good tolerance and promising results in terms of efficacy in patients with low-grade glioma [33]. The results of the trial TARGET investigating anti-FGFR3 (NCT02824133) are pending.

In our series, patients may have several targets identified but only one therapy was available at the time of MTB decision, and no prioritization had to be performed. Three patients with mTOR pathway activation were treated with everolimus in the basket trial MOST for less than three months and discontinued for progression or toxicity. Such result could be explained by glioblastoma natural resistance to mammalian



target of rapamycin (m-TOR) inhibitors [34], although activation of the PI3K-AKT-mTOR signaling pathway in glioblastomas has been described [35]. Three other patients had mutation or amplification in genes encoding for tyrosine kinases and received off-label-targeted therapies. Early discontinuations occurred for toxicity ( $N=2$ ) or for clinical progression ( $N=1$ ). The patient treated with ruxolitinib had a *JAK p.T756A* mutation, identified only once in the COSMIC database (COSM240271) which did not allow to predict sensitivity to the anti-JAK ruxolitinib. Indeed, monodrug-targeted therapies did not only fail to demonstrate efficacy but also reported unacceptable toxicities. The randomized study SHIVA investigating the use of molecular-targeted agents outside their indication in heavily pre-treated patients with cancer did not improve progression-free survival compared with other treatments at physician's choice [16].

This study proposed molecular-targeted therapy to patients already pre-treated with potentially toxic drugs, such as temozolomide [36] and lomustine [37], based on hematologic parameters. Discontinuations occurred for toxicity in patients who had already received long and aggressive treatments. MTA should be proposed as adjuvant treatment, alone or in association with standard therapies [38].

We reported the results of a high-throughput technology in patients with brain tumors. The trial ProfiLER-2 (NCT03163732) planned to explore how many patients could benefit from a MBRT based on a broader panel of genes compared to our standard panel genes. A panel of genes dedicated to brain tumor should be developed and inclusion criteria for appropriate enrollment of patients with neurologic cancers in clinical trials should be reconsidered.

**Acknowledgements** The authors thank the participating patients, staff at each of the study sites, the MBT members, as well as Sophie Darnis, PhD, employee of the Centre Leon Bérard, for medical editorial assistance with this manuscript.

**Funding** This work was supported by Institut National du Cancer (INCa) LYric [DGOS-INCa-4664], Bpifrance Financement abounded by European Community [E8983-PREDICTIV], Agence Nationale de la Recherche (ANR)-LabEx DEvweCAN [10-LABX-0061], European Union [Project EURACAN 739521], la Fondation ARC, Ligue de l'Ain contre le Cancer, and Centre Leon Bérard (no grant number). The funders had no role in study design, data collection, data analysis, data interpretation, or writing of the report.

**Data availability** Research data will be shared upon request to the corresponding author.

## Declarations

**Conflict of interest** The authors have declared no potential conflicts of interest.

**Ethical approval** The study received local approval (Ethics committee of Lyon Sud-Est IV) and was conducted in accordance with the Good Clinical Practice guidelines of the International Conference on Har-

monization, and the Declaration of Helsinki, and relevant French and European laws and directives.

**Consent to participate** All patients provided written informed consent.

**Consent for publication** All authors wrote, have read, and approved the final manuscript.

## References

- Louis DN, Ohgaki H, Wiestler OD, Cavenee WK, Burger PC, Jouvet A, et al. The 2007 WHO classification of tumours of the central nervous system. *Acta Neuropathol.* 2007;114(2):97–109.
- Swain SM, Baselga J, Kim SB, Ro J, Semiglazov V, Campone M, et al. Pertuzumab, trastuzumab, and docetaxel in HER2-positive metastatic breast cancer. *N Engl J Med.* 2015;372(8):724–34.
- Rosell R, Taron M, Reguart N, Isla D, Moran T. Epidermal growth factor receptor activation: how exon 19 and 21 mutations changed our understanding of the pathway. *Clin Cancer Res.* 2006;12(24):7222–31.
- Mok TS, Wu Y-L, Ahn M-J, Garassino MC, Kim HR, Ramalingam SS, et al. Osimertinib or platinum-pemetrexed in EGFR T790M-positive lung cancer. *N Engl J Med.* 2017;376(7):629–40.
- Sboner A, Mu XJ, Greenbaum D, Auerbach RK, Gerstein MB. The real cost of sequencing: higher than you think! *Genome Biol.* 2011;12(8):125.
- Marino P, Touzani R, Perrier L, Rouleau E, Kossi DS, Zhaomin Z, et al. Cost of cancer diagnosis using next-generation sequencing targeted gene panels in routine practice: a nationwide French study. *Eur J Hum Genet.* 2018;26(3):314–23.
- Louis DN, Perry A, Reifenberger G, von Deimling A, Figarella-Branger D, Cavenee WK, et al. The 2016 World Health Organization classification of tumors of the central nervous system: a summary. *Acta Neuropathol.* 2016;131(6):803–20.
- Gounder MM, Nayak L, Sahebjam S, Muzikansky A, Sanchez AJ, Desideri S, et al. Evaluation of the safety and benefit of phase I oncology trials for patients with primary CNS tumors. *J Clin Oncol.* 2015;33(28):3186–92.
- Tredan O, Wang Q, Pissaloux D, Cassier P, de la Fouchardiere A, Fayette J, et al. Molecular screening program to select molecular-based recommended therapies for metastatic cancer patients: analysis from the ProfiLER trial. *Ann Oncol.* 2019;30(5):757–65.
- O'Leary NA, Wright MW, Brister JR, Ciufo S, Haddad D, McVeigh R, et al. Reference sequence (RefSeq) database at NCBI: current status, taxonomic expansion, and functional annotation. *Nucleic Acids Res.* 2016;44(D1):D733–45.
- Forbes SA, Beare D, Boutselakis H, Bamford S, Bindal N, Tate J, et al. COSMIC: somatic cancer genetics at high-resolution. *Nucleic Acids Res.* 2017;45(D1):D777–83.
- Sherry ST, Ward MH, Kholodov M, Baker J, Phan L, Smigielski EM, et al. dbSNP: the NCBI database of genetic variation. *Nucleic Acids Res.* 2001;29(1):308–11.
- Baskaran S, Mayrhofer M, Kultima HG, Bergstrom T, Elfineh L, Cavellier L, et al. Primary glioblastoma cells for precision medicine: a quantitative portrait of genomic (in)stability during the first 30 passages. *Neuro-Oncol.* 2018;20(8):1080–91.
- Chen X, Wen Q, Stucky A, Zeng Y, Gao S, Loudon WG, et al. Relapse pathway of glioblastoma revealed by single-cell molecular analysis. *Carcinogenesis.* 2018;39(7):931–6.
- Laskin J, Jones S, Aparicio S, Chia S, Ch'ng C, Deyell R, et al. Lessons learned from the application of whole-genome analysis to the treatment of patients with advanced cancers. *Cold Spring Harb Mol Case Stud.* 2015;1(1):a000570.

16. Le TC, Delord JP, Goncalves A, Gavaille C, Dubot C, Isambert N, et al. Molecularly targeted therapy based on tumour molecular profiling versus conventional therapy for advanced cancer (SHIVA): a multicentre, open-label, proof-of-concept, randomised, controlled phase 2 trial. *Lancet Oncol.* 2015;16(13):1324–34.
17. Pfaff E, Kessler T, Balasubramanian GP, Berberich A, Schrimpf D, Wick A, et al. Feasibility of real-time molecular profiling for patients with newly diagnosed glioblastoma without MGMT promoter hypermethylation—the NCT neuro master match (N2M2) pilot study. *Neuro-Oncol.* 2018;20(6):826–37.
18. Figueroa JM, Skog J, Akers J, Li H, Komotar R, Jensen R, et al. Detection of wild-type EGFR amplification and EGFRvIII mutation in CSF-derived extracellular vesicles of glioblastoma patients. *Neuro-Oncol.* 2017;19(11):1494–502.
19. Raizer JJ, Abrey LE, Lassman AB, Chang SM, Lamborn KR, Kuhn JG, et al. A phase II trial of erlotinib in patients with recurrent malignant gliomas and nonprogressive glioblastoma multiforme postirradiation therapy. *Neuro-Oncol.* 2010;12(1):95–103.
20. Yung WK, Vredenburgh JJ, Cloughesy TF, Nghiemphu P, Klencke B, Gilbert MR, et al. Safety and efficacy of erlotinib in first-relapse glioblastoma: a phase II open-label study. *Neuro-Oncol.* 2010;12(10):1061–70.
21. van den Bent MJ, Brandes AA, Rampling R, Kouwenhoven MC, Kros JM, Carpentier AF, et al. Randomized phase II trial of erlotinib versus temozolomide or carmustine in recurrent glioblastoma: EORTC brain tumor group study 26034. *J Clin Oncol.* 2009;27(8):1268–74.
22. Eskilsson E, Rosland GV, Solecki G, Wang Q, Harter PN, Graziari G, et al. EGFR heterogeneity and implications for therapeutic intervention in glioblastoma. *Neuro-Oncol.* 2018;20(6):743–52.
23. Frappaz D, Bonneville-Levard A, Ricard D, Carrie S, Schiffler C, Xuan KH, et al. Assessment of Karnofsky (KPS) and WHO (WHO-PS) performance scores in brain tumour patients: the role of clinician bias. *Support Care Cancer.* 2021;29(4):1883–91.
24. Weller M, Butowski N, Tran DD, Recht LD, Lim M, Hirte H, et al. Rindopepimut with temozolomide for patients with newly diagnosed, EGFRvIII-expressing glioblastoma (ACT IV): a randomised, double-blind, international phase 3 trial. *Lancet Oncol.* 2017;18(10):1373–85.
25. Brown PD, Krishnan S, Sarkaria JN, Wu W, Jaeckle KA, Uhm JH, et al. Phase I/II trial of erlotinib and temozolomide with radiation therapy in the treatment of newly diagnosed glioblastoma multiforme: North Central Cancer Treatment Group Study N0177. *J Clin Oncol.* 2008;26(34):5603–9.
26. Uhm JH, Ballman KV, Wu W, Giannini C, Krauss JC, Buckner JC, et al. Phase II evaluation of gefitinib in patients with newly diagnosed grade 4 astrocytoma: Mayo/North Central Cancer Treatment Group Study N0074. *Int J Radiat Oncol Biol Phys.* 2011;80(2):347–53.
27. Kaley T, Touat M, Subbiah V, Hollebecque A, Rodon J, Lockhart AC, et al. BRAF inhibition in BRAF(V600)-mutant gliomas: results from the VE-BASKET study. *J Clin Oncol.* 2018;36(35):3477–84.
28. Wen PY, Weller M, Lee EQ, Alexander BM, Barnholtz-Sloan JS, Barthel FP, et al. Glioblastoma in adults: a Society for Neuro-Oncology (SNO) and European Society of Neuro-Oncology (EANO) consensus review on current management and future directions. *Neuro-Oncol.* 2020;22(8):1073–113.
29. Furuta T, Miyoshi H, Komaki S, Arakawa F, Morioka M, Ohshima K, et al. Clinicopathological and genetic association between epithelioid glioblastoma and pleomorphic xanthoastrocytoma. *Neuropathology.* 2018;38(3):218–27.
30. Frappaz D, Sunyach MP, Le RE, Blonski M, Laurence V, Bonneville LA, et al. Adolescent and Young Adults (AYAS) brain tumor national Web conference. On behalf of ANOCEF, GO-AJA and SFCE societies. *Bull Cancer.* 2016;103(12):1050–6.
31. Robinson GW, Orr BA, Wu G, Gururangan S, Lin T, Qaddoumi I, et al. Vismodegib exerts targeted efficacy against recurrent sonic hedgehog-subgroup medulloblastoma: results from phase II pediatric brain tumor consortium studies PBTC-025B and PBTC-032. *J Clin Oncol.* 2015;33(24):2646–54.
32. Frappaz D, Barritault M, Montane L, Laigle-Donadey F, Chinot O, Le Rhun E, et al. MEVITEM—a phase I/II of vismodegib + temozolomide vs temozolomide in patients with recurrent/refractory medulloblastoma with Sonic Hedgehog pathway activation. *Neuro-Oncol.* 2021. <https://doi.org/10.1093/neuonc/noab087>.
33. Mellinghoff IK, Ellingson BM, Touat M, Maher E, De La Fuente MI, Holdhoff M, et al. Ivosidenib in isocitrate dehydrogenase 1-mutated advanced glioma. *J Clin Oncol.* 2020;38(29):3398–406.
34. Iwanami A, Gini B, Zanca C, Matsutani T, Assuncao A, Nael A, et al. PML mediates glioblastoma resistance to mammalian target of rapamycin (mTOR)-targeted therapies. *Proc Natl Acad Sci USA.* 2013;110(11):4339–44.
35. Aldape K, Zadeh G, Mansouri S, Reifenberger G, von Deimling A. Glioblastoma: pathology, molecular mechanisms and markers. *Acta Neuropathol.* 2015;129(6):829–48.
36. Stupp R, Mason WP, van den Bent MJ, Weller M, Fisher B, Taphoorn MJ, et al. Radiotherapy plus concomitant and adjuvant temozolomide for glioblastoma. *N Engl J Med.* 2005;352(10):987–96.
37. Taal W, Oosterkamp HM, Walenkamp AM, Dubbink HJ, Beerepoot LV, Hanse MC, et al. Single-agent bevacizumab or lomustine versus a combination of bevacizumab plus lomustine in patients with recurrent glioblastoma (BELOB trial): a randomised controlled phase 2 trial. *Lancet Oncol.* 2014;15(9):943–53.
38. Lesueur P, Lequesne J, Grellard JM, Dugue A, Coquan E, Brachet PE, et al. Phase I/IIa study of concomitant radiotherapy with olaparib and temozolomide in unresectable or partially resectable glioblastoma: OLA-TMZ-RTE-01 trial protocol. *BMC Cancer.* 2019;19(1):198.

**Publisher's Note** Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.