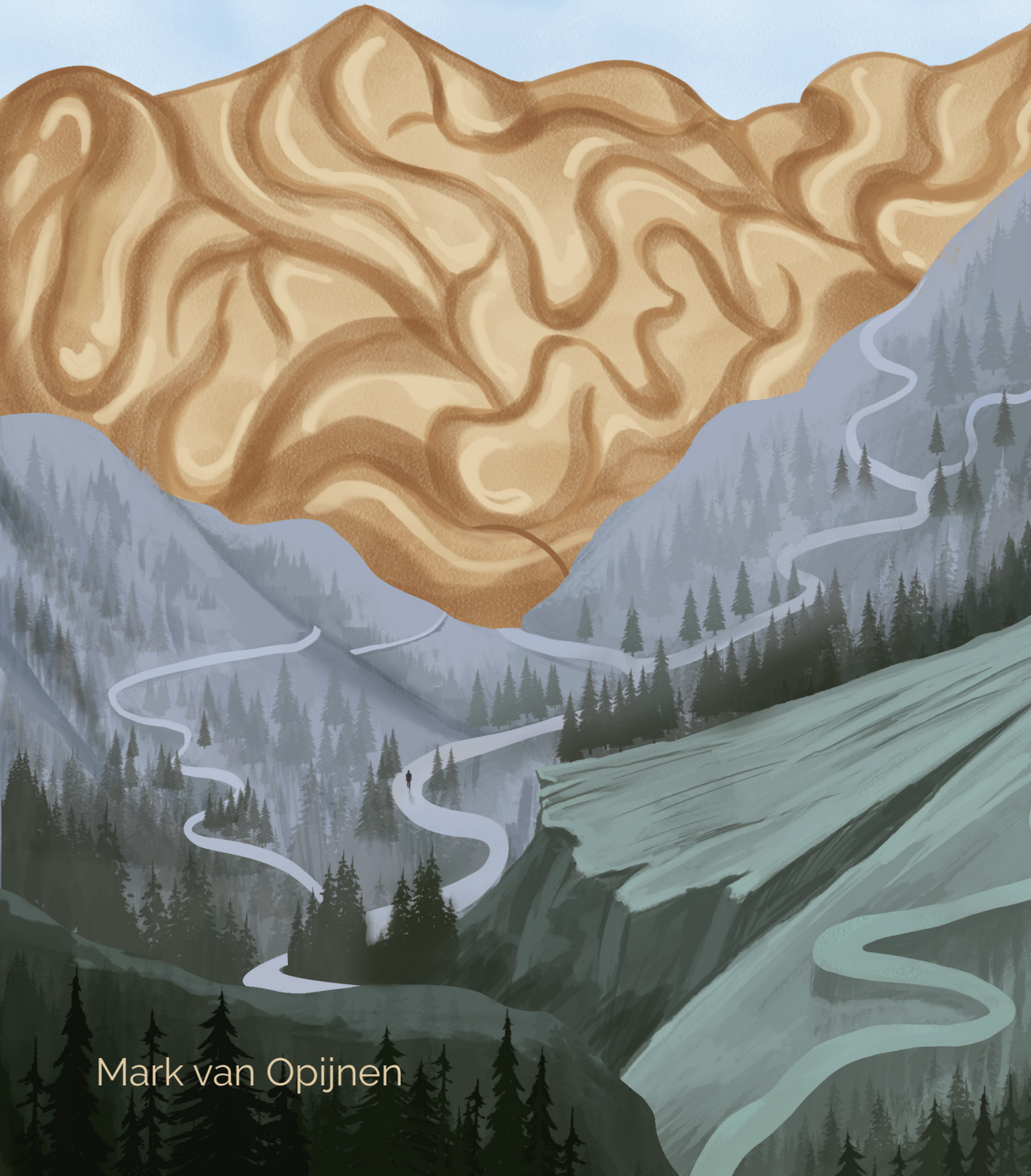
Recurrent glioblastoma in the era of molecular diagnostics: practice variation and practical implications

Opijnen, M.P. van

RECURRENT GLIOBLASTOMA IN THE ERA OF MOLECULAR DIAGNOSTICS

Practice variation and practical implications



Mark van Opijnen

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About the cover: Patient's and personal perspectives are reflected by the cover illustration. The patient's journey, from the moment of glioblastoma recurrence, is characterized by an uncertain path with lots of decision moments. The horizon is blocked by gyri-sulci mountains. The author's journey, during his PhD program, was characterized by uncertainties and unexpected twists too, with the love for the mountains being a constant and inspiring factor.

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I will lift up my eyes to the hills, from whence comes my help?
My help comes from the LORD, who made heaven and earth.

Psalm 121:1 and 2

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Chapter 1

General introduction and outline

"We have done everything we could, and at this point there is nothing more we can do for you." Giving a patient a message like this will promptly take the patient's hope – physicians should therefore never say anything like this. More importantly, the second part of this phrase is invalid either way. Actually, there is always something a physician could do for the patient, for instance regarding symptom management or palliative care. Therefore, this is one of the most valuable lessons a physician must keep in mind, especially while caring for the sickest of the sick. Indeed, this thesis is about the sickest of the sick: patients with a recurrent glioblastoma. And for these patients, the current challenge is to prove the first part of the phrase (i.e. the 'everything-part') wrong as well by redefining 'everything'. Now, whole genome sequencing (WGS) is not standard-of-care for patients with a glioblastoma. This thesis partly focusses on the feasibility and potential benefit of WGS in patients with a recurrent glioblastoma. Diagnostically speaking, WGS is very close to 'doing everything'. Patients with such a dismal prognosis like patients with a recurrent glioblastoma tend to have, ultimately deserve physicians whose goal is to go the extra mile. Physicians who are open to acknowledge and reduce unwanted practice variation. Who are eager to consider diagnostics and treatments that are not (yet) standard-of-care. And who take ethical considerations into account while caring for their patients on their precarious journey.

GLIOBLASTOMA

Glioblastoma, the most common glial primary brain tumor, is almost always lethal. In the Netherlands, every year approximately 1000 patients are diagnosed with this heterogeneous disease.(1) According to the most recent taxonomy, the term glioblastoma refers to an isocitrate dehydrogenase (*IDH*) wildtype tumor that is classified World Health Organization (WHO) grade 4.(2) Symptomatology depends on the affected brain location(s) and thus include a wide range of possible neurologic deficits like headache, nausea, muscle weakness, speech deficits, new onset of epileptic seizures, vision problems or neuropsychological symptoms.(3) Standard treatment for patients with newly diagnosed glioblastoma consists of maximal safe surgical resection followed by postoperative radiotherapy with concomitant and adjuvant temozolomide chemotherapy.(4, 5) Despite this intensive treatment scheme, the prognosis of this incurable disease remains poor with a median survival of less than fifteen months.(6) Moreover, the benefit from temozolomide is only pronounced in patients with a tumor with methylation of the O6-methylguanine-DNA methyltransferase (MGMT) promoter.(7) Unfortunately, the tumor almost inevitably recurs.

SEMANTIC DISCUSSION

One can debate the proper formulation of ‘recurrence’ of the glioblastoma. Some people favour ‘relapse’ or ‘regrowth’ instead, since the infiltrative growth pattern of glioblastoma makes complete (i.e. on microscopic level) surgical resection impossible. Consequently, the glioblastoma never went away after initial treatment and can therefore, strictly seen, not recur. In literature, however, ‘recurrence’ and its conjugations are by far the most common terms, followed by ‘relapse’ and ‘regrowth’. More importantly, these semantics do not hamper the consensus on the clinical meaning: disease progression characterised by new signs of vital tumor tissue on clinical and/or radiological examination after initial antitumor treatment, ultimately confirmed by histopathological analysis. For uniformity, ‘recurrence’ and its conjugations will be used throughout this thesis.

RECURRENT GLIOBLASTOMA

In the recurrent setting, evidence on the best treatment strategy becomes scarce and highly relies on individual patient characteristics. As the guideline of the European Association for Neuro-Oncology (EANO) states, ‘standard-of-care treatments for patients with recurrent glioblastoma are not well-defined.’(5) Only a small number of 20-30% of the patients with well-localized tumors are eligible for re-resection, commonly with a symptomatic lesion seen not earlier than six months after initial resection.(5) In general, this selected group of patients may have a survival benefit from re-resection, especially when adjuvant treatment will follow the surgical procedure.(8, 9) However, given that patient-specific factors such as Karnofsky Performance Status (KPS), extent of resection, and radiological findings impact the survival benefit as well, the decision whether or not to perform a re-resection is everything but straightforward.(10-15) When it comes to systemic treatment, nitrosoureas or retreatment with temozolomide are most commonly used, with limited progression-free survival rates at six months (15-20%) and objective response rate of less than 10%.(16-20) Patients with a methylated MGMT promoter may benefit from a temozolomide rechallenge, from lomustine or even from the combination of both.(21-23) Outside of the European Union, bevacizumab has been approved for recurrent glioblastoma.(24, 25) The European evidence-based opinion, however, is that there is no survival benefit of bevacizumab for the treatment of recurrent glioblastoma.(5, 26, 27) Some patients undergo re-irradiation, which may result in local disease control in a proportion of patients.(28-32) However, a second

course of radiotherapy is not always feasible due to the hazards of cumulative (cognitive) neurotoxicity.

PRACTICE VARIATION

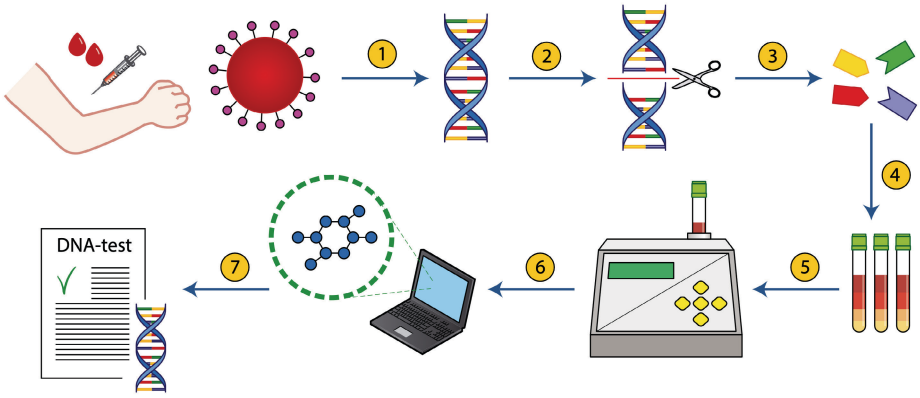
Practice variation in medicine has been studied before and is a well-known phenomenon.(33, 34) Likewise in neuro-oncology, practice is subject to variation, for instance in mapping procedures in glioma surgery, neuroimaging after glioblastoma surgery, or perioperative laboratory testing.(35-37) Despite the need to reduce practice variation in medicine, health professionals are not sure about the feasibility of such a reduction.(38) Generally, two main factors can be identified to explain the variability in treatment decisions: the lack of guidelines/large prospective studies, and the concept of noise. Both are covered by Kahneman et al., who described noise as the 'unwanted variability of judgements' with the property that the true answer may be even unknowable.(39) Kahneman also concluded that medicine is a noisy profession in which the interrater reliability could be powerfully reduced by guidelines.(40) In the absence of clear guidelines on recurrent glioblastoma treatment, one should be aware of the risk of unwanted practice variation. Simultaneously, randomized or prospective clinical trials are urgently needed and should also contribute to the development of evidence-based guidelines on the treatment of recurrent glioblastoma.

GENOME SEQUENCING

The entire human genome was first cataloged in 2001, after thirteen years of international effort.(41) Nowadays, the turnaround time of sequencing and analyzing the entire genome is normally within five to ten days. Ever since that first complete genome sequencing more than two decades ago, genome sequencing is increasingly integrated into clinical practice, with reduction of costs and increase of availability as a result. Started with diagnostic applications, genome sequencing is currently used for therapeutic strategies as well. Theoretically, fighting cancer by treating at DNA level holds great promise for individualized therapy. In a heterogenetic disease like glioblastoma, WGS will provide all molecular information through a single test within a reasonable time of one to two weeks *(Figure 1).* The potential of WGS in the area of personalized medicine for patients with cancer has been demonstrated before, with high sensitivity and precision to detect molecular alterations.(42, 43)

The actionability of a given molecular alteration is based on information in public knowledgebases, including the Clinical Knowledgebase (CKB), Oncology Knowledge Base (OncoKB) or the Clinical Interpretation of Variants in Cancer (CIViC). It can be split by evidence levels according to stablished classification levels, including the six level ESCAT (ESMO Scale for Clinical Actionability of molecular Targets) classification.(44) According to the ESCAT classification, treatment based on hypothetical targets should only be considered in the context of early clinical trials. To demonstrate that such hypothesized treatments are effective, downstream clinical studies are required. These trials should also investigate and link pharmacodynamics to the clinical utility of the targeted therapy, since not all drugs will effectively cross the blood-brain barrier. A successful example of molecular therapy is dabrafenib/trametinib in patients with progressive *BRAF* p.V600E mutant gliomas.(45)

Figure 1. A simplified overview of the process of whole genome sequencing (WGS).



Step 1: DNA extraction from blood and tumor tissue. Step 2 and 3: fragmentation and amplification of the DNA. Step 4: adding chemicals to sequence the DNA. Step 5: replication of the DNA fragments and sequencing preparation. Step 6: reading of the DNA fragments (sequencing) and processing the sequencing information. Step 7: building the WGS report.

ETHICS IN NEURO-ONCOLOGY

The increased use of (broad) genome sequencing in neuro-oncology necessitates a renewed conversation about informed consent procedures. There is currently no consensus on how to obtain informed consent for WGS in patients with (recurrent) glioblastoma. Notwithstanding, it is the physician's moral duty to ensure that the patient understands both the potentials and downsides of genome sequencing.

For instance, since WGS analysis requires not only tumor DNA but also a control DNA sample (e.g. from blood) to discriminate somatic mutations (tumor sample) from germline mutations (control sample), unravelling the germline DNA potentially reveals genetic predisposition. This knowledge might be relevant for patients and their relatives, and the question whether the disease runs in the family is often an important question in the consulting room. Therefore, the potential consequences of the germline analysis must be kept in mind and should be discussed with patients before WGS diagnostics are performed. Regarding these 'unsolicited findings', it is important to be aware of the patient's right not to know, as it applies to family members as well.(46) Finally, patients should be made aware of the fact that, despite the therapeutic potential following WGS, the analysis itself does not impact individual outcomes.

Next to ethical considerations regarding informed consent procedures, the governance structures for sharing personal health data should be adapted to (broad) genome sequencing too. For instance, WGS generates extensive data on the genomic alterations in the cancer cells, as well on normal cells. This data is per definition highly personal and therefore require strict regulations. In the European Union, data handling is safeguarded by the General Data Protection Regulation (GDPR).(47) The recurrent glioblastoma population is characterized by its vulnerability and, in case of cognitive impairments, abstract themes such as data sharing warrant extra meticulous communication. Indeed, physicians have the responsibility to explain details about the benefits and risks of WGS in an accurate and understandable manner.(48) Notwithstanding the increasing role of molecular diagnostics, the abovementioned considerations should frame the shared decision making process between physician and patient.

AIMS AND OUTLINE OF THIS THESIS

In **part I** of this thesis, we study several aspects of **practice variation**. First, we review the literature on mapping-guided re-resection in recurrent glioma (**chapter 2**). In **chapter 3** we study the practice variation in re-resection between neuro-oncology specialists in the Netherlands. In international guidelines, recommendations for the treatment of recurrent glioblastoma are limited. In **chapter 4**, we investigate the availability of national guidelines and recommendations throughout Europe. Once a re-resection is planned, this offers the opportunity to expand the histopathological analysis with molecular tissue analysis to look for treatment targets. We study the

current practice of genome sequencing in **chapter 5**, in the primary as well as in the recurrence setting.

In **part II** of this thesis, we describe the role of **molecular diagnostics** to personalize and optimize systemic treatment for patients with a recurrent glioblastoma. To uniform practice and to maximize treatment options, we present the protocol of our prospective, nationwide trial on complete genome diagnostics in recurrent glioblastoma patients in **chapter 6**. We describe the results of the interim analysis of this study in **chapter 7**. Since it is important to study new targeted treatments in the context of available clinical trials, we review the current clinical trial landscape in **chapter 8**.

Part III of this thesis covers some **practical implications**. Applying whole genome sequencing potentially reveals genetic predisposition to glioblastoma. Therefore, we study the prevalence of pathogenic germline variants in **chapter 9**. In **chapter 10**, we provide an ethical framework analysis of obtaining informed consent for whole genome sequencing.

Finally, we summarize and discuss the findings of these studies in **chapter 11** and provide future directions.

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PART I

Practice variation

2

Chapter 2

The impact of intraoperative mapping during re-resection in recurrent gliomas: a systematic review

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ABSTRACT

Purpose. Previous evidence suggests that glioma re-resection can be effective in improving clinical outcomes. Furthermore, the use of mapping techniques during surgery has proven beneficial for newly diagnosed glioma patients. However, the effects of these mapping techniques during re-resection are not clear. This systematic review aimed to assess the evidence of using these techniques for recurrent glioma patients.

Methods. A systematic search was performed to identify relevant studies. Articles were eligible if they included adult patients with recurrent gliomas (WHO grade 2-4) who underwent re-resection. Study characteristics, application of mapping, and surgical outcome data on survival, patient functioning, and complications were extracted.

Results. The literature strategy identified 6,372 articles, of which 125 were screened for eligibility. After full-text evaluation, 58 articles were included in this review, comprising 5,311 patients with re-resection for glioma. Of these articles, 17% (10/58) reported the use of awake or asleep intraoperative mapping techniques during re-resection. Mapping was applied in 5% (280/5,311) of all patients, and awake craniotomy was used in 3% (142/5,311) of the patients.

Conclusion. Mapping techniques can be used during re-resection, with some evidence that it is useful to improve clinical outcomes. However, there is a lack of high-quality support in the literature for using these techniques. The low number of studies reporting mapping techniques may, next to publication bias, reflect limited application in the recurrent setting. We advocate for future studies to determine their utility in reducing morbidity and increasing extent of resection, similar to their benefits in the primary setting.

Keywords. Glioma, recurrence, re-resection, intraoperative mapping, survival

INTRODUCTION

Adult-type diffuse gliomas are the most common primary malignant brain tumors in adults.[1] Maximal safe resection to prolong survival is the mainstay of the treatment in the newly diagnosed setting, with extent of resection (EOR) and residual tumor volume as important prognostic factors.[2, 3] For tumors located in or near functional tissue, maximal safe resection can be challenging. Intraoperative mapping (i.e. electrophysiology) has the potential to achieve a maximal safe resection without causing neurological deterioration by locating important functions such as motor or, in case of awake mapping, language function.[4, 5] Compared to general anesthesia without mapping, intraoperative mapping has been demonstrated effective in glioma populations in terms of neurological, functional, cognitive, radiological and survival outcomes.[4, 6-8]

There is some controversy on standard-of-care in the recurrent setting.[9, 10] Re-resection is one of the possibilities, as are (re-)challenge chemotherapy, (re-) irradiation, targeted therapy (e.g. vorasidenib[11] or dabrafenib/trametinib[12]), recruitment into clinical trials, or best supportive care.[2] Treatment decisions are influenced by several factors including overall performance (Karnofsky Performance Status (KPS) or World Health Organization (WHO) functioning scale), tumor location and size, and prior treatment.[2] For glioma WHO grade 2, there is little debate on the importance of maximal safe re-resection.[13-15] Also, patients with glioma WHO grade 4 might benefit from re-resection, albeit limited to selected patients on the favorable side of the spectrum.[16-19]

Although the surgical goal for recurrent gliomas is often the same as for newly diagnosed tumors, the impact of intraoperative mapping in this recurrent setting is poorly understood. Studies on this either failed to stratify between glioma WHO grade 2 and 3-4[20, 21], included grade 2 tumors that had progressed to grade 3 or 4[22, 23] or failed to stratify between use/non-use of intraoperative mapping[24-26], resulting in mixed results that are hard to interpret. In the absence of solid evidence, it is likely that intraoperative mapping is currently omitted in most of the cases or not seriously considered in many departments. Therefore, to maximize safe re-resection in patients with recurrent glioma WHO grade 2-4, the current lack of evidence and treatment recommendations needs to be solved.

This systematic review aimed to investigate the impact of intraoperative mapping during re-resection on survival, neurological, functional and radiological outcomes

in patients with recurrent glioma WHO grade 2-4. The results of this review may help neurosurgeons in the delicate process of surgical decision making in these patients.

METHODS

Search strategy

This systematic review was performed in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines.[27] A computer-aided search of PubMed, Embase, Web of Science, Medline (OvidSP), Cochrane and Google Scholar was performed with the help of the biomedical information specialist to identify relevant studies (*Supplemental S1*). The databases were searched up to August 2023. All identified abstracts were screened on title and abstract by two authors (YS and JKWG). Full-text screening of potentially relevant publications was performed according to predefined criteria (see Study selection). Any discrepancies were resolved by discussion. Reference lists of included articles were screened for additional references to be included.

Study selection

Inclusion criteria for eligible studies were (1) study population consisted of adult patients with recurrent gliomas WHO grade 2-4 who had undergone re-resection, (2) 15 or more participants, and (3) written in English. Exclusion criteria were (1) no stratification between gliomas WHO grade 2 and 3-4, (2) no stratification between awake and asleep craniotomy, (3) secondary malignant progression from WHO grade 2 to grade 3 or 4, and (4) book chapters, case reports, letters to editors, technical reports, review articles.

Quality assessment and risk of bias

The quality of the included articles was evaluated using the Newcastle–Ottawa scale for observational cohort studies[28] by one reviewer (MPvO) and verified by the senior authors (AJPEV, JKWG). The Newcastle-Ottawa score for cohort studies is divided in three domains: selection, comparability and outcome. The selection category consisted of four items: representativeness of the exposed cohort, selection of the non-exposed cohort, and ascertainment of exposure. The comparability category assessed the comparability of cohorts based on the design or analysis. Finally, the outcome category contained three scoring items: assessment of outcome, whether follow-up was long enough for outcomes to occur, and adequacy of follow-up for cohorts. According to this scale, studies were qualified as ‘good quality’ if they had 3-4 points in the selection domain, 1-2 points in the comparability domain, and 2-3

points in the outcome/exposure domain. 'Fair quality' comprised studies which had 2 points in the selection domain, 1-2 points in the comparability domain, and 2-3 points in outcome/exposure domain. Studies were qualified as 'poor quality' if they had 0-1 point in the selection domain, 0 points in the comparability domain, or 0-1 point in outcome/exposure domain.

Data extraction

Study characteristics that were extracted included study design, number of patients undergoing re-resection, patient demographics, anesthesia technique (awake or asleep), application of intraoperative mapping, WHO classification, pre- and postoperative KPS, EOR, procedure-related complications, postoperative treatment, and survival. Survival was divided in the time between primary diagnosis and death (overall survival) and the time between re-resection and death (post-progression survival).

Statistical analysis

Categorical variables were reported as absolute numbers (*n*) and percentages of the total. Data was stratified for recurrent glioma WHO grade 2-4 and the intraoperative mapping techniques were compared. Medians or percentages for different outcomes were calculated based on the number of patients included in each study or treatment arm. Medians were weighted to control for different sample sizes. P-values of <0.05 were considered statistically significant.

RESULTS

Search results

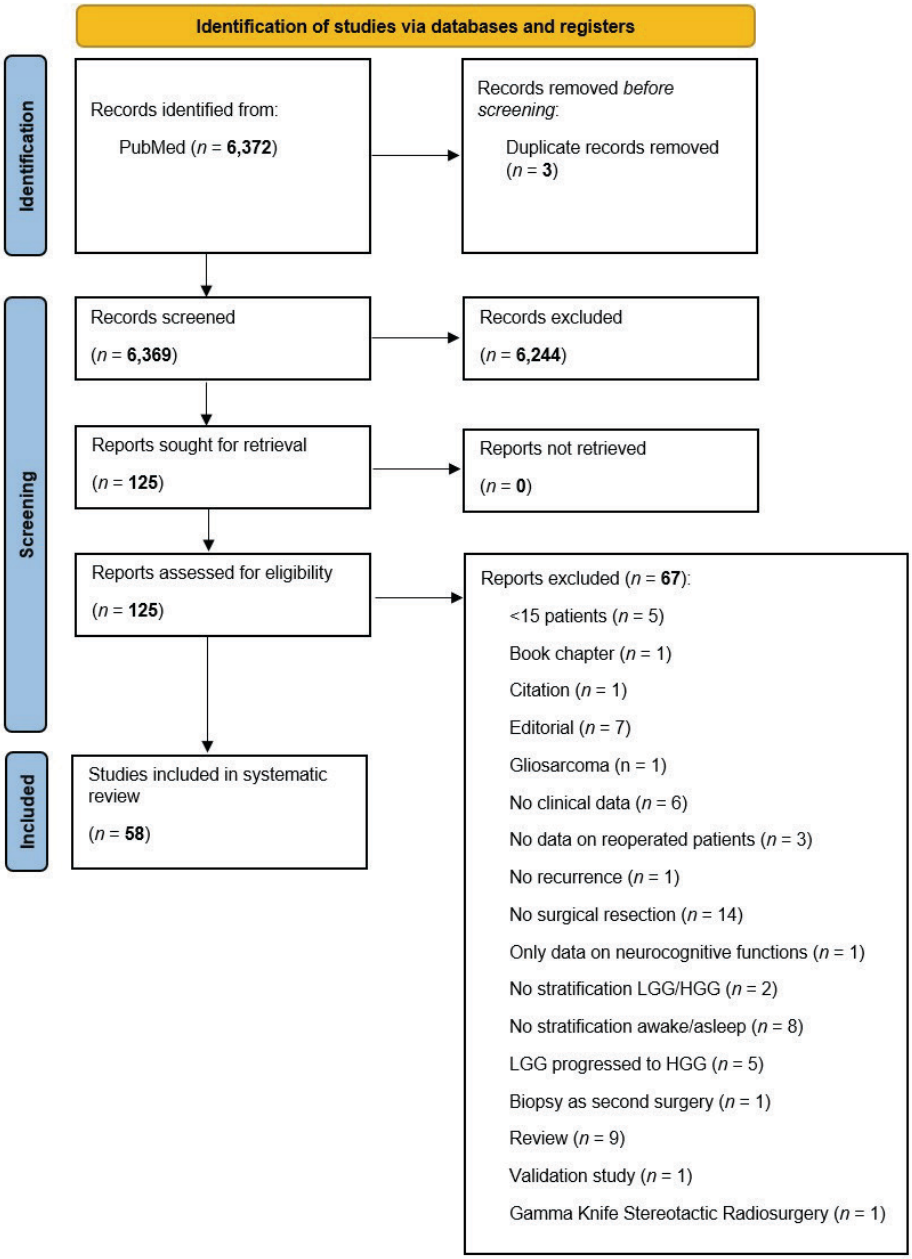
The search strategy resulted in 6,372 abstracts, of which 6,369 remained after removing duplicates. Of these, 6,244 articles were excluded during the initial screening round based on title and abstract. Of the remaining 125 abstracts that were full-text screened, 58 articles were classified eligible according to the predefined criteria. See *Figure 1* for an overview of the selection process.

Study characteristics

A total of 5,311 patients were included in this systematic review. Of the 58 included articles, six articles were of prospective design and 52 articles were of retrospective design. The year of publication ranged from 1981-2023, with the majority (46 [79%] of 58) of the studies published within the last 10 years. Two studies (2 [3%] of 58) included only patients with glioma WHO grade 2 (without progression to grade 3

or 4), 55 studies (55 [95%] of 58) included only patients with glioma WHO grade 3-4 and one study (1 [2%] of 58) included both gliomas WHO grade 2 and 3-4. The weighted median age of the patients was 56 years (range 45.5-72), for those studies that reported the median (33 [57%] of 58). The study characteristics of the included studies can be found in *Table 1* (*Supplemental S2*).

Figure 1. Schematic breakdown of literature search results.



Abbreviations: HGG: high-grade glioma WHO grade 3-4, LGG: low-grade glioma WHO grade 2.

General findings on mapping

The first main finding was that only 10 studies (10 [17%] of 58) mentioned whether the re-resection was performed under awake/asleep conditions and/or whether intraoperative mapping techniques were used.[20, 29-37] All other articles did not provide details on awake/asleep craniotomy and/or mapping technique during re-resection.[17, 38-84] Regarding the anesthesia technique (awake/asleep) used in the 10 studies, two studies included re-resections in which awake craniotomy was applied[31, 34], five studies described re-resection under general anesthesia[29, 30, 32, 36, 37], two studies included both awake and asleep re-resections (but did not stratify the outcomes by either awake or asleep)[20, 35], and one study did not specify awake/asleep approach.[33] On a total of 58 studies, re-resection in an awake setting was performed in 3% (142/5,311) of the patients. See *Figure 2* for a schematic overview of studies with information on awake/asleep craniotomy and intraoperative mapping.

The second main finding was that, next to information on awake/asleep conditions, additional information on the use of intraoperative mapping was not routinely included: only six studies described this.[20, 31-34, 36] In general, out of a total of 58 studies, intraoperative mapping was applied in 5% (280/5,311) of the patients. Intraoperative electrophysiology techniques that were described included awake speech mapping, direct cortico-subcortical electrostimulation, and motor-evoked or somatosensory-evoked potentials.

Outcomes for recurrent glioma WHO grade 2

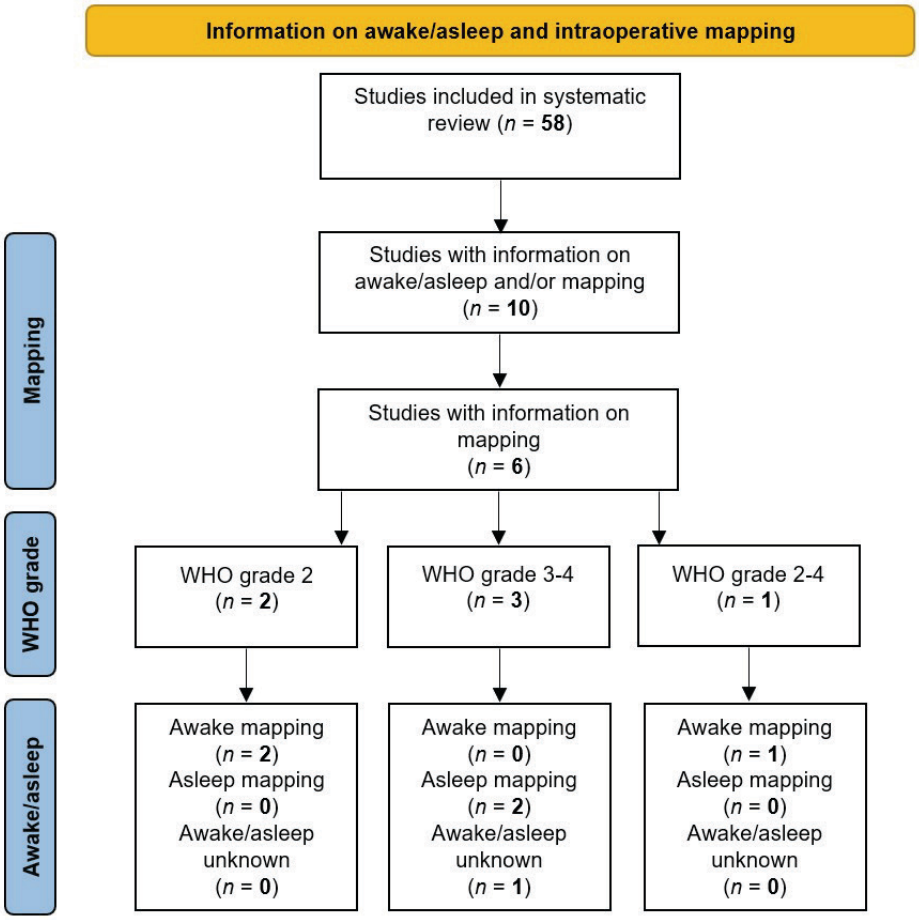
Intraoperative mapping during re-resection for glioma WHO grade 2 was described in three of the six studies, all under awake conditions (*Figure 2*).[20, 31, 34] Although survival outcomes were not reported in these studies, information on the extent of resection was available. The percentage of complete resections was reported in all three studies and ranged from 5% (1/20) in one study on recurrent insular glioma WHO grade 2[34] to 21% (13/62) in one study that was not limited to tumors in specific locations.[31] The third study found a complete resection rate of 65% (17/26) after awake craniotomy with intraoperative mapping but did not stratify between recurrent gliomas WHO grade 2 and 3-4.[20] Only one study mentioned the treatment after mapping-guided re-resection, showing that 95% (59/62) of the patients did not receive postoperative treatment.[31]

Information on the safety of the procedure was available in all three studies. The percentage of perioperative complications (e.g. surgical-site infections or transient neurological deficits) ranged between 4% to 36%, although definitions of complications immediately after surgery differed among the included studies.

Focusing on the clinical examination three months postoperatively, 89-100% of the patients recovered from initial postoperative worsening of their neurological condition.[31, 34] The third study, including both patients with recurrent gliomas WHO grade 2 and 3-4, also included a study arm with general anesthesia without intraoperative mapping.[20] This allowed comparisons in neurological deficits after either awake or asleep craniotomy. One week after re-resection, significantly more neurological deficits were seen in the asleep group compared to the awake group (22% versus 4%, p=0.032), but three months postoperatively no significant difference was observed (12% versus 4%, p=0.231).[20]

Studies on re-resection for glioma WHO grade 2 using general anesthesia with or without intraoperative mapping were not included in the final selection of this review.

Figure 2. Schematic breakdown of studies with information on intraoperative mapping.



Outcomes for recurrent glioma WHO grade 3, astrocytoma grade 4, and glioblastoma

Intraoperative mapping was mentioned in four studies on patients with recurrent glioma WHO grade 3-4, either during awake craniotomy[20], under general anesthesia[32, 36] or with unknown awake/asleep setting (*Figure 2*).[33] Two studies reported the survival after mapping-guided re-resection. The results from both these studies indicated that mapping was associated with non-inferior post-progression survival (PPS) (10.3 months, 95% CI 7.6-10.4[33], odds ratio 0.9, 95% CI 0.6-1.3[36]). Complete resection, in this study defined as surgical resection of >90% of the pre-operative tumor volume, was achieved in 75% (48/64) of the patients.[33] In the same

study, new neurological deficits occurred in 13% (8/64) of the patients, but the timing of this observation was not described. Adjuvant treatment after mapping-guided re-resection was also reported by two studies, showing that 74-88% of the patients received postoperative treatment.[33, 36]

In two studies, patients with glioma WHO grade 3-4 were operated in an awake setting although the application of any type of intraoperative mapping was not mentioned.[20, 35] The association between awake/asleep re-resection and survival was investigated in one of these articles using Cox proportional hazard models, which showed no significant difference between awake and asleep re-resection for overall survival (OS) (hazard ratio 1.82, 95% confidence interval 0.99-3.34) or PPS (hazard ratio 1.02, 95% confidence interval 0.58-1.8).[35] No details were reported in both these studies on the impact of awake craniotomy on postoperative KPS, perioperative complications or postoperative treatment. In contrast to awake craniotomy, the survival after re-resection under general anesthesia was detailed by several studies. Taking these studies together, a weighted median OS of 16.9 months (range 16.7-31.0)[30, 32, 36, 37] and a weighted median PPS of 11.0 months (range 5.0-11.0)[29, 30, 36, 37] was observed, although mapping was not taken into account in this analysis. For those studies providing the endpoint GTR, GTR was achieved in 55% (302/551).[29, 32, 36] Perioperative complications were detailed for 599 patients, with events, regardless of grade, in 19% (111/599) of the patients.[29, 30, 32, 36, 37]

Quality assessment

The median quality assessment score of the 58 studies was 7 out of 9 with a range of 3-9. The mean score was 6.7 out of 9.0 with a standard deviation of 1.6. Thirty-six percent (21/58) of studies could be classified as ‘good quality’, 24% (14/58) as ‘fair quality’, and 40% (23/58) as ‘poor quality’. Most studies failed on the representativeness of the exposed cohort (i.e., they selected re-resection candidates only without including a nonsurgical control arm, therefore increasing the risk of selection bias) and/or showed no sufficient comparability (i.e., they did not control for important factors such as age and/or EOR, KPS, time to recurrence, both within and between groups). An overview of the quality assessment per study is displayed in *Table 2* (*Supplemental S3*).

DISCUSSION

This systematic review showed that there is a limited amount of evidence to assess the impact of intraoperative mapping during re-resection for patients with

recurrent glioma WHO grade 2-4. A minority of the included articles (10 [17%] of 58) reported the use on awake/asleep craniotomy and/or mapping technique during re-resection, with awake re-resection described in only 3% (142/5,311) of the patients. Intraoperative mapping in general was described in a mere 5% (280/5,311) of the patients. A possible explanation for this limited number of studies reporting mapping in the recurrent setting could be that few surgeons apply mapping in this setting, next to factors like publication bias and inconsistent reporting. The limited amount of evidence for these mapping techniques is in stark contrast with the situation for patients with newly diagnosed tumors. For these patients, these techniques have proven to be effective for improving outcomes by increasing extent of resection, decreasing postoperative deficits, and consequently, prolonging survival.[4, 6-8] Although some reports indicate that these techniques might have the same benefits in the recurrent setting, high-quality evidence is needed to assess this comprehensively.

A first reason for the lack of evidence in the recurrent setting is the low number of cases that have been carried out using intraoperative mapping techniques in the literature. We also observed that articles often did not differentiate between glioma WHO grade 2 and grade 3-4, or included patients with glioma WHO grade 2 that had progressed to WHO grade 3 or 4 at the time of re-resection. Moreover, almost all included studies lacked proper stratification: outcomes were not stratified by awake/sleep, use/non-use of intraoperative mapping, or WHO tumor grade. This made a comprehensive evaluation of the prognostic impact of mapping during re-resection difficult.

A second reason for the lack of evidence is the overall low quality of studies. As demonstrated in the quality assessment (*Table 2)*, 17% (10/58) of the included studies did not show comparability of the cohorts on the basis of the design or analysis since they do not control for one or two important factors such as age and/or O6-methylguanine-DNA methyltransferase (MGMT) promotor methylation, EOR, KPS or time to recurrence. Studies also failed on the selection of the exposed cohort (45%, 26/58) since selection bias frequently led to the inclusion of optimal surgical candidates only, which is not representative of the average condition of patients with recurrent glioma WHO grade 2-4.

These limitations of the current evidence illustrate the need for carefully designed high-quality studies. This need is underlined by the fact that currently, international guidelines leave treatment decisions for recurrent glioma WHO grade 3-4 up to individual decision with little to no guidance.[2, 85, 86] As a result, treatment preferences

for the use or non-use of intraoperative mapping differ between surgeons and centers, as does the indication for re-resection in general.[87] Importantly, since there is evidence that eloquent areas might have been reorganized at the time of re-resection, the possibility of this 'functional reshaping' may warrant the use of intraoperative mapping during re-resection to achieve maximal safe re-resection.[88-90]

Studies should not only apply stratification between different patient subgroups, but factors such as predefined endpoints and adequate power analysis should be considered to generate high-quality evidence. Ideally, these studies are carried out prospectively. Examples are the ongoing RESURGE (NCT02394626) and RECSUR (NCT06283927) studies investigating re-resection versus best supportive care, and the RECMAP study (NCT06273176) investigating the impact of intraoperative mapping during re-resection. However, since prospective design is not always feasible, retrospectively designed studies should control for selection bias and confounding with techniques such as propensity score matching with multivariate regression or stratification of subgroups and outcomes.

Limitations

This systematic review has some limitations. First, several outcome variables were not comparable between the articles included in this study. For instance, the definition of GTR varied and the KPS was either on a continuous scale or categorized, making comparisons difficult. Another limitation is the large percentage of retrospective studies and the small percentage of studies focusing on recurrent glioma WHO grade 2. Third, the included studies did often not explain their indication setting for using mapping techniques. The results, therefore, have to be interpreted with caution since we were not able to assess the presence of selection bias in our congregate results.

CONCLUSIONS

Previous studies indicate that re-resection of recurrent tumors may improve clinical outcomes for glioma patients. Furthermore, mapping techniques have been proven to be effective in increasing extent of resection while decreasing postoperative deficits in newly diagnosed tumors. In this systematic review, we investigated the effect of these mapping techniques when used during resection for recurrent tumors. We hypothesized that these mapping techniques can be beneficial as well in the recurrent setting to make the surgery safer and more extensive. However, there was insufficient evidence to adequately assess the comprehensive impact of

these techniques during re-resection on neurological, functional, radiological and survival outcomes in recurrent glioma patients. This lack of high-quality evidence may have been caused by the relatively low number of surgeons currently using it, and the overall low quality of studies included in this review. We are concerned that the current lack of strong evidence for, and the reluctance to use these techniques in daily practice may cause a vicious circle, while their potential benefits remain unknown. We advocate, therefore, for well-designed studies to comprehensively determine their potential utility in reducing morbidity and increasing extent of resection, similar to their benefits in the primary setting. The results from these studies could improve the indication setting for these techniques and consequently, the clinical outcomes for recurrent glioma patients.

SUPPLEMENTARY INFORMATION

The online version contains supplementary material available at https:// doi. org/ 10. 1007/ s11060- 024- 04874-1.

AUTHOR CONTRIBUTIONS

MPvO, YS, AJPEV and JKWG contributed to the study conception and design. Material preparation, data collection and analysis were performed by MPvO, YS, MED, AJPEV and JKWG. The first draft of the manuscript was written by MPvO and all authors commented on previous versions of the manuscript. Supervision was provided by AJPEV and JKWG. All authors read and approved the final manuscript.

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DATA AVAILABILITY

No datasets were generated or analysed during the current study.

CONFLICTS OF INTEREST

The authors have not disclosed any competing interests.

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3

Chapter 3

Practice variation in re-resection for recurrent glioblastoma: a nationwide survey among Dutch neuro-oncology specialists

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ABSTRACT

Background. Despite current best treatment options, a glioblastoma almost inevitably recurs after primary treatment. However, in the absence of clear evidence, current guidelines on recurrent glioblastoma are not well defined. Re-resection is one of the possible treatment modalities, though it can be challenging to identify those patients who will benefit. Therefore, treatment decisions are made based on multidisciplinary discussions. This study aimed to investigate the current practice variation between neuro-oncology specialists.

Methods. In this nationwide study among Dutch neuro-oncology specialists, we surveyed possible practice variation. Via an online survey, four anonymized recurrent glioblastoma cases were presented to neurosurgeons, neuro-oncologists, medical oncologists, and radiation oncologists in the Netherlands using a standardised questionnaire on whether and why they would recommend a re-resection or not. The results were used to provide a qualitative analysis of the current practice in the Netherlands.

Results. The survey was filled out by 56 respondents, of which 15 (27%) neurosurgeons, 26 (46%) neuro-oncologists, 2 (4%) medical oncologists, and 13 (23%) radiation oncologists. In two of the four cases, there appeared to be clinical equipoise. Overall, neurosurgeons tended to recommend re-resection more frequently compared to the other specialists. Neurosurgeons and radiation oncologists showed opposite recommendations in two cases.

Conclusions. This study showed that re-resection of recurrent glioblastoma is subject to practice variation both between and within neuro-oncology specialties. In the absence of unambiguous guidelines, we observed a relationship between preferred practice and specialty. Reduction of this practice variation is of importance; to achieve this, adequate prospective studies are essential.

Keywords. Glioblastoma, recurrence, re-resection, practice variation, survey

INTRODUCTION

Glioblastoma is a devastating primary malignant brain tumor with a median survival of 15 months. Despite current best treatment options the tumor inevitably recurs [1, 2]. International guidelines on the diagnosis and treatment of diffuse gliomas in adulthood do not provide well defined standard-of-care treatments for patients with a recurrent glioblastoma [3]. According to these guidelines, re-resection remains an option for about 20-30% of the patients, typically patients with symptomatic but circumscribed lesions and symptomatic patients with progression exceeding six months after initial surgery. In general, there is little discussion that re-resection can improve overall survival, provided that patient and tumor specific factors such as Karnofsky Performance Status (KPS), extent of resection and radiological findings are on the favourable end of the spectrum [4-9]. A consensus on re-resection has been shown difficult to obtain and patients discussed in multidisciplinary meetings still depend on expert opinions. It is exactly this deliberation, however, together with treatment specific and future specific factors, that makes the decision whether or not to perform a re-resection everything but straightforward and even controversial instead. And although some patients with a recurrent glioblastoma could benefit from a re-resection, for a larger group an optimal treatment paradigm remains not clear. What do different neuro-oncology specialists recommend in those cases? What are decisive factors and which considerations are taken into account when recommending re-resection in specific cases of recurrent glioblastoma?

This study aimed to investigate the current practice variation between neuro-oncology specialists by surveying their recommendations in four different cases. Given the lack of support in international guidelines, the results of this study might offer new insights in areas of consensus and controversy regarding re-resection for recurrent glioblastoma, and contribute to more consensus in the treatment of these patients.

MATERIALS AND METHODS

Study design

In the Netherlands, there are fourteen neurosurgical centers and seventeen radiotherapy centers, including seven academic hospitals, that treat patients with glioblastoma. Patients are referred to these centers from smaller, regional hospitals that do not have the expertise or the optimal neurosurgical facilities. To assess possible practice variation in re-resection for patients with a recurrent glioblastoma,

four cases were presented to practicing neurosurgeons, neuro-oncologists (i.e. neurologists with neuro-oncology expertise), radiation oncologists and medical oncologists throughout the Netherlands (selection process described below). The cases were selected based both on their representativeness and variability with respect to patient characteristics (such as age and clinical performance), radiological findings and the course over time (especially the time between initial surgery and recurrence). The first case is an example of a resectable tumor with considerable risks of post-operative neurological deficits in a patient who is in a good clinical condition and for whom adjuvant/other treatment options are available. The second case illustrates a diffuse, multifocal recurrence in a young patient, with very limited adjuvant treatment options. Third, we show a case of a small, asymptomatic, and late recurrence in a patient for whom reasonable adjuvant/other treatment options are available. Finally, the fourth case describes an early and multifocal recurrence in a young patient with a preference not to have surgery. All four patients had already died at moment of selection, and family was not consulted to ask for consent in order to avoid increasing their emotional burden. Furthermore, we anonymized the images and added fictitious, non-relevant patient characteristics to create four illustrative but anonymous vignettes. All images shown in the cases were T1-weighted MRI images after contrast administration. Relevant T2-features are described as well. The vignettes of the cases can be found in *Figure 2-5*.

Survey design and distribution

Respondents were contacted with an online survey: for every case we asked whether the respondent would recommend a re-resection [yes/no]. Following questions were asked for the considerations taken into account and subsequently for decisive factors (Supplementary *Table 1)*. The decisive factors that were asked for were subdivided in patient, tumor characteristics, treatment characteristics and future specific factors, without any further definition. Multiple answers were possible for these considerations and decisive factors, as well as the option to specify. Baseline characteristics of the respondents included specialty, type of department [general hospital/academic hospital/private practice], age in years [30-39/40-49/50-59/60-70], gender [male/female/would rather not say] and years of experience as a medical specialist (i.e. time since finishing residency) [0-5/6-10/11-20/21-30/>30]. Finally, the respondents were asked for the minimum overall survival (in months) from the date of re-resection for a re-resection to be generally considered proportional.

The survey was distributed via e-mail invitations, primarily to the members of the Dutch Neuro-Oncology Society (Landelijke Werkgroep Neuro-Oncologie, LWNO), a society with approximately two hundred active members. Additional responses were collected by personal invitations to neuro-oncology specialists throughout the Netherlands. Subsequent distribution to members of their local neuro-oncology boards was done by some of them. As a result, response rates could not be reliably assessed. The survey was open for response between July 11th and September 2nd 2022 and we sent multiple reminders to respond. By responding, the participants consented to the anonymous publication of the results. The online survey was made by using the web survey tool SurveyMonkey (Momentive Inc., San Mateo, California, USA, www.surveymonkey.com).

Statistical analysis

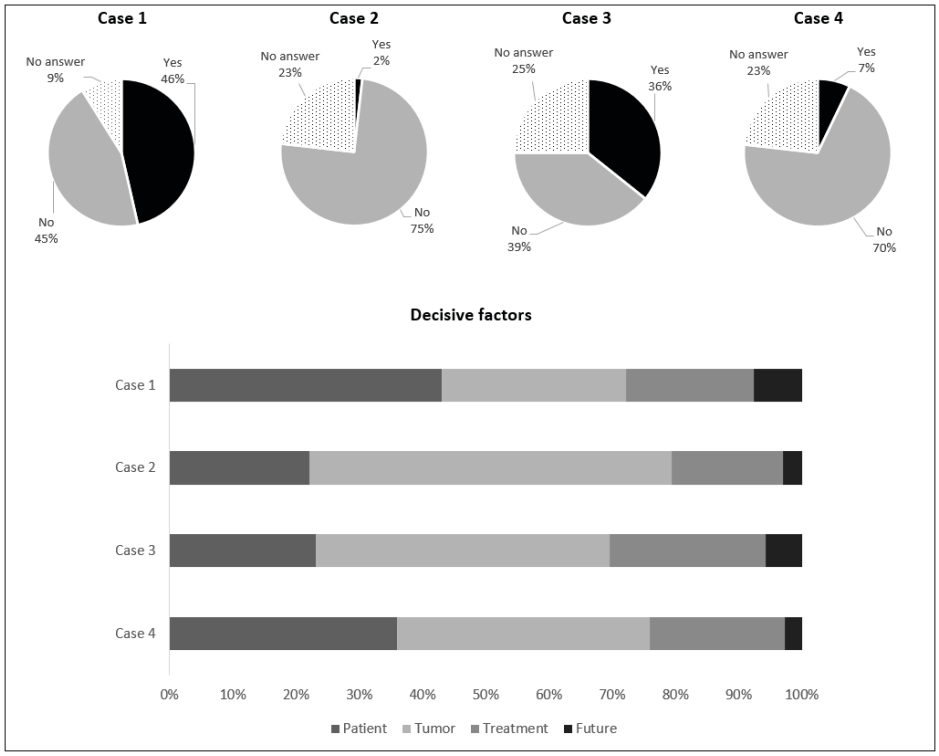
Categorical variables were reported using percentages and counts, taking different subgroup sizes into account. Continuous variables were described using the median and range. Formal statistics were not further applied because of the relatively small numbers, particularly for subgroup analyses, what would lead to unreliability of the conclusions. No separate analyses of the medical oncologists' answers could be done because of the very limited response of these specialists (*n*=2). Statistical analyses were performed using statistical package *IBM SPSS Statistics for Windows* version 28.0.

RESULTS

Respondents' characteristics

The survey was filled out by 56 respondents, of which 43 (77%) completed all four cases. Of all respondents, 27% (15/56) were neurosurgeons, 46% (26/56) neuro-oncologists, 4% (2/56) medical oncologists and 23% (13/56) radiation oncologists. No major numerical differences between medical specialties were observed for age, type of department and years of experience as a medical specialists; we noted some differences in gender distribution, see *Table 1*.

Figure 1. Case-specific answers on the question 'Would you recommend a re-re-section in this case?' together with decisive factors.



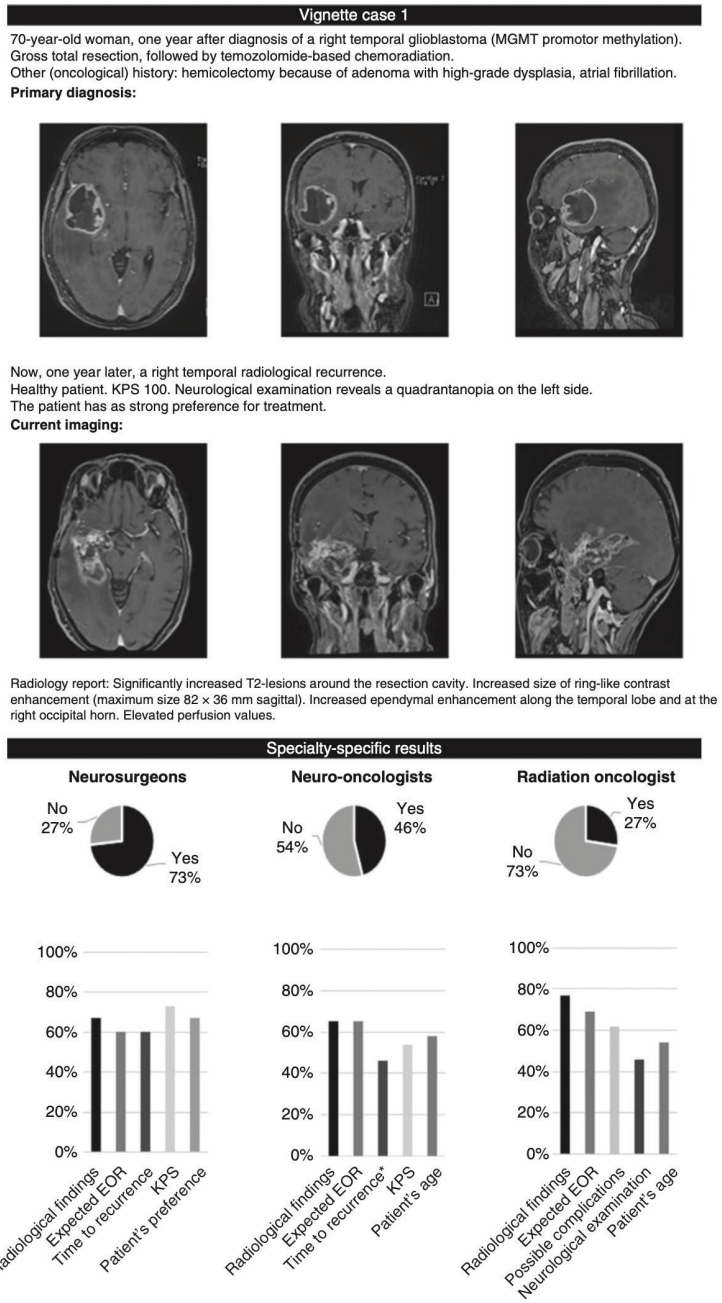
General trends

In two of the four cases, there appeared to be clinical equipoise among the respondents. In case 1, 46% recommended a re-resection whereas 45% did not recommend re-resection, and the remainder did not answer. Likewise, 36% of the respondents was in favour of a re-resection in case 3, compared to 39% who was not in favour. In contrast, case 2 and 4 showed almost unanimity with only 2% and 7% of the respondents recommending a re-resection, respectively (*Figure 1*). Regardless of type of medical speciality, a median of 6 months (range 3-15) of estimated overall survival from the date of re-resection was considered the minimum for a re-resection to be proportional.

Overall, tumor characteristics were most frequently (67%) decisive in the recommendation to perform a re-resection or not. The second most common decisive aspect was patient characteristics (50% of the respondents). Treatment

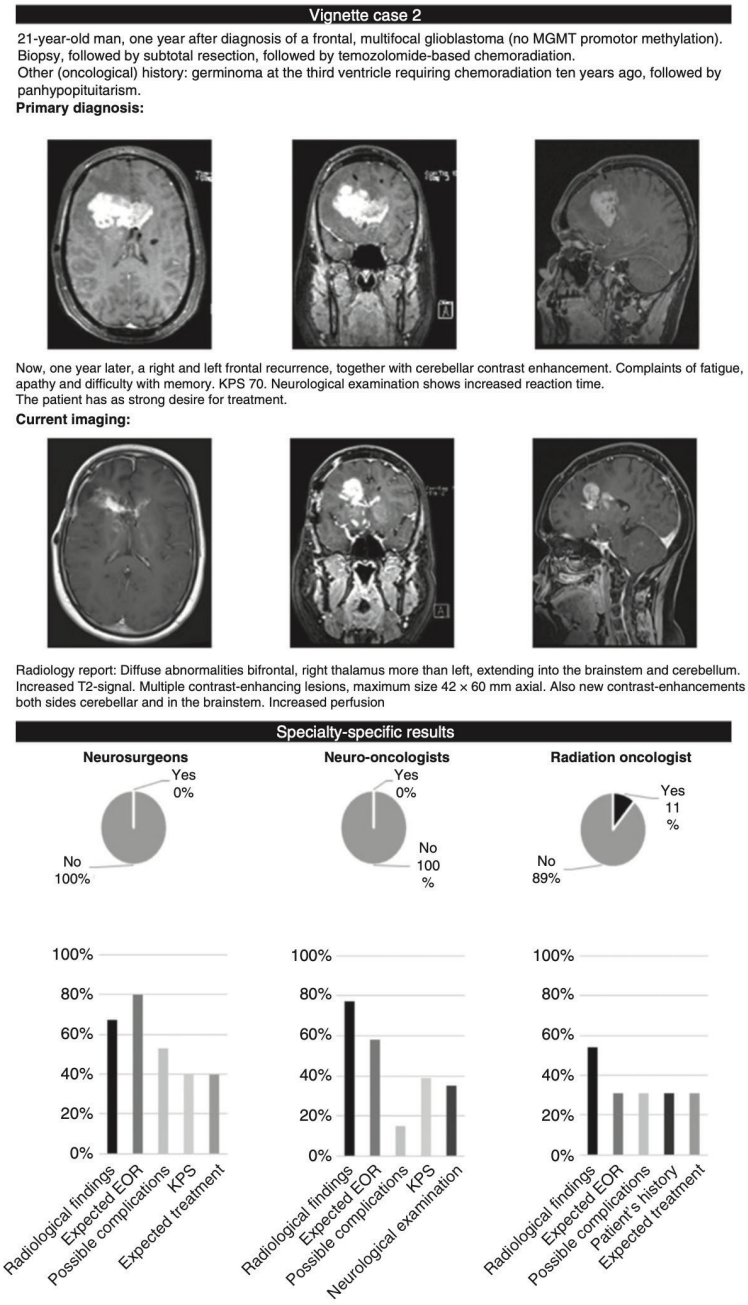
characteristics and future specific factors were less often decisive in the decision: overall 33% and 8%, respectively (see also *Figure 1*). Overall, the radiological findings at the time of recurrence and the expected extent of re-resection were the two most common considerations in all specialties. Interestingly, the patient's preference was a strong case-dependent consideration, ranging from 4% in case 2 to 57% in case 4.

Figure 2. Vignette of case 1 with specialty-specific answers on the question 'Would you recommend a re-resection in this case?' and underlying considerations. MRI images shown are T1-weighted images after contrast administration.



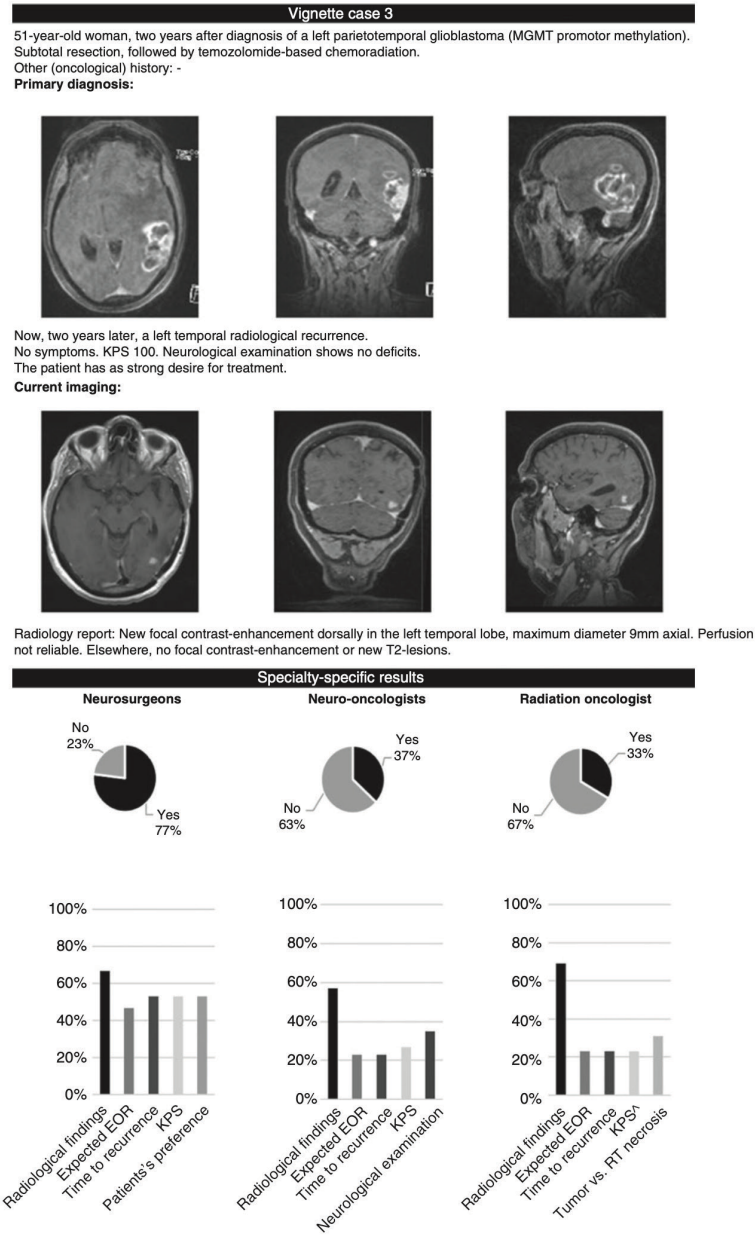
EOR: extent of re-resection, KPS = Karnofsky performance status, MGMT = O^6-methylguanine-DNA methyltransferase, MRI = magnetic resonance imaging, RT necrosis: radiation necrosis. The five most frequently chosen considerations per specialty are depicted. Equal proportions that are not shown: *the findings on the neurological examination.

Figure 3. Vignette of case 2 with specialty-specific answers on the question 'Would you recommend a re-resection in this case?' and underlying considerations. MRI images shown are T1-weighted images after contrast administration.



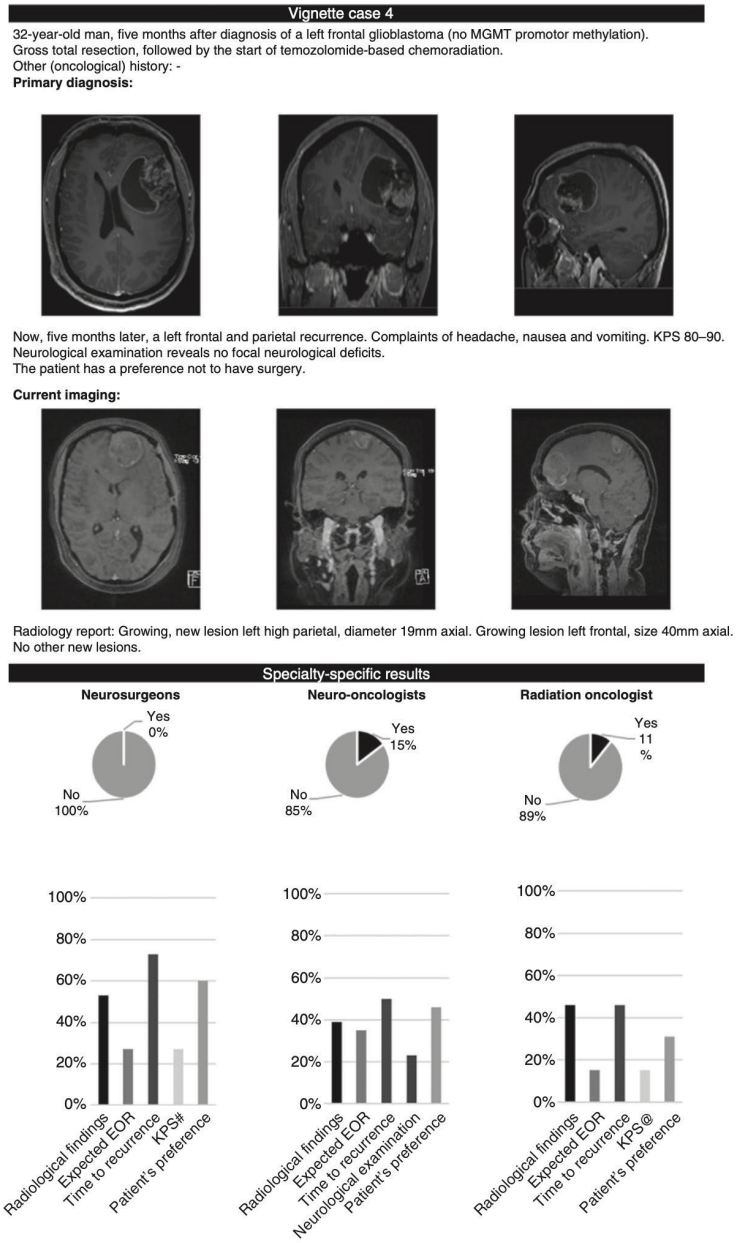
EOR: extent of re-resection, KPS = Karnofsky performance status, MGMT = O^6-methylguanine-DNA methyltransferase, MRI = magnetic resonance imaging, RT necrosis: radiation necrosis.

Figure 4. Vignette of case 3 with specialty-specific answers on the question 'Would you recommend a re-resection in this case?' and underlying considerations. MRI images shown are T1-weighted images after contrast administration.



EOR: extent of re-resection, KPS = Karnofsky performance status, MGMT = O^6-methylguanine-DNA methyltransferase, MRI = magnetic resonance imaging, RT necrosis: radiation necrosis. The five most frequently chosen considerations per specialty are depicted. Equal proportions that are not shown: ^possible complications, expected treatment following re-resection.

Figure 5. Vignette of case 4 with specialty-specific answers on the question 'Would you recommend a re-resection in this case?' and underlying considerations. MRI images shown are T1-weighted images after contrast administration.



EOR: extent of re-resection, KPS = Karnofsky performance status, MGMT = O^6-methylguanine-DNA methyltransferase, MRI = magnetic resonance imaging, RT necrosis: radiation necrosis. The five most frequently chosen considerations per specialty are depicted. Equal proportions that are not shown: #expected treatment follow re-resection, @patient's history, treatment already given.

Practice variation by specialty

Neurosurgeons leaned more often towards performing a re-resection in the patients with a recurrent glioblastoma. In case 1 and 3, cases in which the 'yes/no-ratio' was equal (see *Figure 1*): 73% and 77% of the neurosurgeons recommended a re-resection in these cases, compared to 46% and 37% of the neuro-oncologists and 27% and 33% of the radiation oncologists, respectively. See *Figure 2-5* for specialty- and case-specific trends. Two specialty-specific trends can be observed, the first being a neurosurgeons' tendency to consider KPS more often than the other specialists. A similar trend was noticed for the patient's preference, which was taken into account more often by the neurosurgeons compared to the other specialists. No differences were found for decisive factors between specialists, with tumor-specific factors followed by patient-specific factors as the two most common.

The most eminent examples of practice variation between specialties can be found in case 1 and 3: almost opposite recommendations between neurosurgeons and radiation oncologists, with neuro-oncologists being more equally distributed in their preferences (*Figure 2 and 4*). Of note, practice variation can also be seen within the same specialty when it comes to the same case. For example, some neurosurgeons, with no more than ten years of experience, opted for re-resection because it was a "superficially circumscribed location" and "gross-total resection very well possible" while other neurosurgeons, with more than ten years of experience, looked at the same tumor being "too small" with "limited oncological benefit of re-resection".

Table 1. Respondent's characteristics. Separate results of medical oncologists (n=2) were omitted.

Characteristics	Neurosurgeons *n* = 15	Neuro-oncologists *n* = 26	Radiation oncologists *n* = 13	Total cohort *n* = 56
Gender, no. (%)				
Male	14 (93%)	8 (31%)	6 (46%)	29 (52%)
Female	1 (7%)	17 (65%)	7 (54%)	26 (46%)
Unknown	0 (0%)	1 (4%)	0 (0%)	1 (2%)
Age in years, no. (%)				
30-39	6 (40%)	5 (19%)	4 (31%)	16 (29%)
40-49	4 (27%)	8 (31%)	4 (31%)	16 (29%)
50-59	3 (20%)	8 (31%)	3 (23%)	15 (27%)
60-70	2 (13%)	4 (15%)	2 (15%)	8 (14%)
Unknown	0 (0%)	1 (4%)	0 (0%)	1 (2%)
Department, no. (%)				
General hospital	8 (53%)	14 (54%)	5 (38%)	28 (50%)
Academic hospital	7 (47%)	12 (46%)	6 (46%)	26 (46%)
Private practice	0 (0%)	0 (0%)	1 (8%)	1 (2%)
Unknown	0 (0%)	0 (0%)	1 (8%)	1 (2%)
Years of experience, no. (%)				
0-5	3 (20%)	4 (15%)	4 (31%)	12 (21%)
6-10	6 (40%)	6 (23%)	1 (8%)	13 (23%)
11-20	3 (20%)	9 (35%)	4 (31%)	17 (30%)
21-30	3 (20%)	5 (19%)	3 (23%)	11 (20%)
>30	0 (0%)	2 (8%)	1 (8%)	3 (5%)

No.: number of patients

DISCUSSION

This study surveyed the practice variation in re-resection for recurrent glioblastoma among neuro-oncology specialists throughout the Netherlands. In two of the four cases presented to them, we found equal proportions of specialists in favour and not in favour of a re-resection. Numeric differences suggested that neurosurgeons recommend a re-resection more often than neuro-oncologists and radiation

oncologists. The largest interspecialty variation was seen in case 1 and 3 between neurosurgeons and radiation oncologists, with almost opposite recommendation proportions (see *Figure 2 and 4*). Overall, tumor specific factors were the most frequently (67%) decisive in the decision to perform a re-resection or not.

Practice variation in medicine has been studied before and is a well-known phenomenon [10, 11]. Likewise in neuro-oncology, practice is subject to variation, for instance in mapping procedures in glioma surgery, neuroimaging after glioblastoma surgery or perioperative laboratory testing [12-14]. The need to reduce practice variation in medicine being out of debate, but health professionals are not sure about the feasibility of a reduction [15].

Two main factors can be identified to explain the variability in treatment decisions: the lack of guidelines/large prospective studies and the concept of noise. Both are covered by Kahneman et al, who described noise as the 'unwanted variability of judgements' with the property that the true answer may be even unknowable [16]. This is exactly what happened in our study: one can observe the scattering of the answers while the true answer is unknown or unknowable. Kahneman et al. conclude that medicine is a noisy profession in which the interrater reliability could be powerfully reduced by guidelines [17]. The lack of clear guidelines on recurrent glioblastoma treatment can be explanatory for the findings in the current study. This lack of clear guidelines, in turn, is largely due to the absence of high-quality evidence, e.g. from randomized clinical trials, or from prospective, population-based (registry-based) cohort studies.

More specific explanations for the variation in re-resection as found in our study include the following. First, clinicians have to deal with discrepancies, sometimes subtle, between population-based guidelines and the individual patient in front of them. To decide whether an individual belongs to the 20-30% mentioned in the guidelines [3], is a matter of careful multidisciplinary deliberation, resulting in patient-tailored treatment. The applicability of those guidelines could therefore be fairly questionable, resulting in opposed recommendations on re-resection. Second, more risk-averse specialists, whether or not related to the number of years of experience, may be inclined to not recommend re-resection because of the still ongoing debate about the benefit of re-resection, supported by some studies opposing re-resection [18-20]. What is more, a relationship between specialty and preference can be observed in our results. Neurosurgeons recommended re-resection more often than the other specialists, what might be a reflection of their specific expertise and consulting role in multidisciplinary discussions. Neuro-oncologists most frequently considered the

findings of the neurological examination. Radiation oncologists, in turn, took the radiological findings into account most commonly. The observed case-dependency of considering the patient's preference can be fairly explained by specialists' strong opinion to not perform a re-resection in certain cases (e.g. multifocality). On the other hand, clinical equipoise can be seen especially in those cases in which re-resection is considered one of the realistic options.

This study has some limitations to be mentioned. First, this online survey intended not to be more than a reflection of the actual practice. Given four anonymized cases, it can be challenging for respondents to deliberately give a recommendation without being able to ask for additional details and without knowing the clinical nuances. Second, small numbers hampered subgroup analyses and subsequent quantification of the results. Third, this survey could only have triggered specialists with strong opinions on this topic to respond. This could have led to response bias. Finally, in the Dutch practice, decisions on the treatment of brain tumor patients are made based on multidisciplinary discussion, something that was not accounted for in the current study design. Because of these limitations, the results of this study have to be interpreted with caution.

The focus of this article was to demonstrate that there is practice variation in recommendation of re-resection. Of course, other therapies might be considered (much more) appropriate in specific cases and the presence/absence of adjuvant therapy options could affect the choice to offer the patient surgery as well. Indeed, the clinical benefit of surgery is limited in the absence of adjuvant therapy.[4] Conversely, in patients with good adjuvant treatment options, no consensus exists on whether (cytoreductive) surgery prior to adjuvant treatment improves prognosis. In this setting, our results suggest that different specialists have different views on the added value of surgery. The ongoing randomized controlled RESURGE trial (NCT02394626) aims to further identify the impact of re-resection on the overall survival of glioblastoma patients.

To conclude, our study showed that re-resection of recurrent glioblastoma is subject to practice variation both between and within neuro-oncology specialties. Future research would be of interest to reveal whether this scenario is the same in other countries and how practice variation in this field can be reduced. Due to the different angles these different specialist groups have on patient care, our results underline the crucial function of multidisciplinary tumor board discussion.

AUTHOR CONTRIBUTIONS

MPvO, FYFvV and MLDB contributed to the study conception and design. Material preparation, data collection and analysis were performed by MPvO, FYFdV, RJAN, TJS, RDSNT, WT, JJCV, JJMvdH and MDLB. The first draft of the manuscript was written by MPvO and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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AVAILABILITY OF DATA AND MATERIALS

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

CONFLICT OF INTEREST STATEMENT

None of the authors declare a conflict of interest.

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SUPPLEMENTARY MATERIAL

Supplementary Table 1. Considerations taken into account and decisive factors asked after the question whether the respondent would recommend a re-resection or not.

Considerations	Decisive factors
The patient's current age	Patient specific factors
The patient's medical history	Tumor specific factors
The current KPS	Treatment specific factors
The findings on the neurological examination	Future specific factors
The patient's preference	Please specify
To differentiate between tumor recurrence or radiation necrosis	
The molecular tumor profile	
The current radiological findings	
The time between initial resection and recurrence	
The extent of resection at initial resection	
The treatment already given to the patient	
The expected extent of resection at re-resection	
The possible complications as a result of re-resection	
The possible complications as a result of re-resection	
The expected treatment following re-resection and its possible effect	
The possibility to find new targets for treatment	
The alternatives of a re-resection	
Too little information available	
Other (please specify)	

KPS = Karnofsky performance status.

4

Chapter 4

Recurrent glioblastoma in national guidelines on the diagnosis and treatment of gliomas: A matter of European practice variation

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ABSTRACT

Introduction. The optimal treatment for recurrent glioblastoma patients remains not well-defined in international guidelines. On top of that, the availability of national guidelines is uncharted.

Research question. This study aimed to investigate the availability of national guidelines on the diagnosis and treatment of adult glioma throughout Europe, specifically focusing on recurrent glioblastoma.

Material and Methods. Medical specialists with neuro-oncology expertise from all European countries were asked for the availability of official national guidelines. The primary outcome was whether guidelines provided recommendations on the treatment of recurrent glioblastoma in adults. Secondary outcomes included treatment specific recommendations and the role of clinical trials in the treatment of recurrent glioblastoma. The quality of the guidelines was assessed using the AGREE II instrument.

Results. Of the 50 countries in Europe, information on guideline availability was obtained for 38 countries (76%). In twelve countries (24%) national guidelines on the diagnosis and treatment of glioma in adults exist. Focusing on recurrent glioblastoma, nine (18%) of the European countries provided any recommendations on the treatment of recurrent glioblastoma. In four (33%) guidelines it was explicitly stressed that there is currently no standard or evidence-based treatment for these patients.

Discussion and Conclusion. National guidelines on the treatment of glioblastoma in adults are not uniformly available in Europe. In addition, and in contrast with international guidelines, the national guidelines differ profoundly in their recommendations regarding recurrent glioblastoma. This could contribute to unwanted practice variation. Efforts are needed to not only optimize, but also harmonize treatment for recurrent glioblastoma patients.

Keywords. Glioblastoma, recurrence, treatment, guideline, practice variation

INTRODUCTION

The optimal diagnosis and treatment for primary glioblastomas in adults is well-defined in (inter)national guidelines. For instance, the guideline of the European Association for Neuro-Oncology (EANO) on the diagnosis and treatment of diffuse gliomas of adulthood provides clear, evidence-based recommendations for the treatment of newly diagnosed *IDH*-wild-type glioblastoma.[1] However, in the recurrent setting, an inevitable and dismal scenario, evidence on the best treatment strategy becomes scarce and highly relies on individual patient characteristics. As the EANO guideline states, 'standard-of-care treatments for patients with recurrent glioblastoma are not well-defined.'[1] The only comment on recurrent glioblastoma in the guideline of the European Society for Medical Oncology (ESMO) is the lack of efficacy of erlotinib and imatinib.[2] The guideline of the American Society of Clinical Oncology (ASCO) and Society for Neuro-Oncology (SNO) is in line with the European tendency: 'no recommendation for or against any therapeutic strategy can be made for treatment of recurrent glioblastoma.'[3]

Previously, we have shown that practice variation regarding recurrent glioblastoma re-resection even exists within one country.[4] This observation raised the question to what extent national guidelines in Europe, if any, provide recommendations on the treatment of recurrent glioblastoma. To help physicians and their patients find the best treatment option for recurrent glioblastoma, in the absence of clear international recommendations, national guidelines could play a role. National guidelines are of particular interest since availability of (experimental) therapies may vary per country as the implementation of current scientific evidence may differ.

This study aims to investigate the availability of national guidelines on the diagnosis and treatment of adult glioma throughout Europe, specifically focusing on recurrent glioblastoma. Since optimal treatment in the recurrent phase of the disease is not well-defined in international recommendations, we want to explore whether recommendations on the treatment of adult patients with recurrent glioblastoma are provided on a national level.

MATERIAL AND METHODS

Study design

Medical specialists from all European countries were asked by email or in person for the availability of national guidelines in their country on the diagnosis and treatment

of gliomas in adults. These medical specialists were neurosurgeons, neurologists with neuro-oncology expertise, medical oncologists or radiation oncologists, all involved in the care for patients with brain tumors in their country. When available, the document of what they currently use as a guideline was shared with us or could be downloaded directly from the Internet. Additional online mining was performed to retrieve information from the countries of which the contacted persons did not respond to our messages. Informal or incomplete documents such as letters, patient information folders, expert opinions or presentations were not included in this guideline study. For all other, official guidelines or consensus documents, the latest version available was used. Non-English and non-Dutch documents were carefully translated using online translation tools and were subsequently read. Various synonyms of 'recurrent' (e.g. 'regrowth', 'relapse', 'recurrence') were used to retrieve all information about recurrent glioblastoma or recurrent glioma in general.

Outcomes

The primary outcome was whether national guidelines provided recommendations on the treatment of recurrent glioblastoma in adults, regardless treatment modality or specific treatment details. This was divided into 'No national guideline available', 'No recommendations' and 'Treatment recommendations'. Secondary outcomes included treatment specific recommendations and the role of clinical trials in the treatment of recurrent glioblastoma (divided into 'No national guideline available', 'No recommendations' and 'Trial recommendations').

Guideline quality assessment

The quality of the entire guidelines was assessed using the Appraisal of Guidelines for Research and Evaluation II (AGREE II).[5] The AGREE II is an internationally widely used and validated instrument for guideline appraisal.[6] Using this instrument, six relevant domains, comprising 23 different items, were scored separately for each guideline. These domains were: scope and purpose, stakeholder involvement, rigour of development, clarity of presentation, applicability, and editorial independence. Each domain was scored using a scale ranging from 1 (strong disagreement) to 7 (strong agreement). This scoring was independently done by two appraisers (MPvO and MTRR) to improve quality assessment.

Statistical analysis

Categorical variables were reported by using counts and percentages while continuous variables were described by using the medians and ranges. Instructions in the user's manual of the AGREE II were followed to properly calculate the domain scores. For each domain, the maximum possible score was: *number of items per*

domain x *highest possible score* (7) x *number of appraisers* (2). Likewise, the minimum possible score for each domain was: *number of items per domain* x *lowest possible score* (1) x *number of appraisers* (2). The following equation was used to scale the scores for each domain:

$$\frac{\textit{Obtained score-Minimum possible score}}{\textit{Maximum possible score-Minimum possible score}} \text{ X } 100\%$$

If an item was not included in the guideline, this absence of information was scored with a 1 out of 7. Total domain scores of ≥60% were deemed acceptable and scores ≥80% were deemed high quality.[7-9] Figures were created using the open software environment *R*, version 4.2.1.

RESULTS

General results

Of the 50 European countries (geographically defined and transcontinental countries included)[10], information on the availability of national guidelines was obtained for 38 countries (76%). 26 (52%) of these countries did not and twelve (24%) did have a national guideline, respectively. The twelve countries from which their national guidelines on the diagnosis and treatment of gliomas in adults was shared were: Belgium, Denmark, France, Germany, Italy, the Netherlands, Norway, Russia, Spain, Sweden, Turkey and United Kingdom. The latest versions of the guidelines differed between 2008 and 2023 (median 2020). The guidelines either discussed neurological diseases in general (1/12), or neuro-oncological diseases (5/12), or gliomas (4/12), or glioblastomas specifically (2/12). See *Figure 1* for a visualization of the guideline availability.

Treatment recommendations

Of all 50 European countries, in nine (18%) national guidelines recommendations on the treatment of recurrent glioblastoma were provided – regardless of the comprehensiveness of the recommendations. The guidelines of two countries (Denmark and United Kingdom) reported only on recurrent high-grade glioma in general while the guideline of Turkey only reported on recurrent gliomas in general (*Figure 2*).

For those twelve countries with national guidelines, in four of them (33%) it was explicitly stressed that there is currently no standard or evidence-based treatment

for patients with recurrent glioblastoma. Multidisciplinary consultation to discuss treatment upon recurrence was recommended in seven (58%) guidelines, for the other countries it was not clear if this was so obvious that it was not stated, or that it was not common practice. As a time-dependent cut-off for treatment upon progression, a progression free period between initial tumor treatment and recurrence of at least six months was suggested in six (50%) of these guidelines. Palliative care and symptom management at first recurrence was suggested in all but the Turkish guideline (92%).

Figure 1. Guideline availability in Europe.

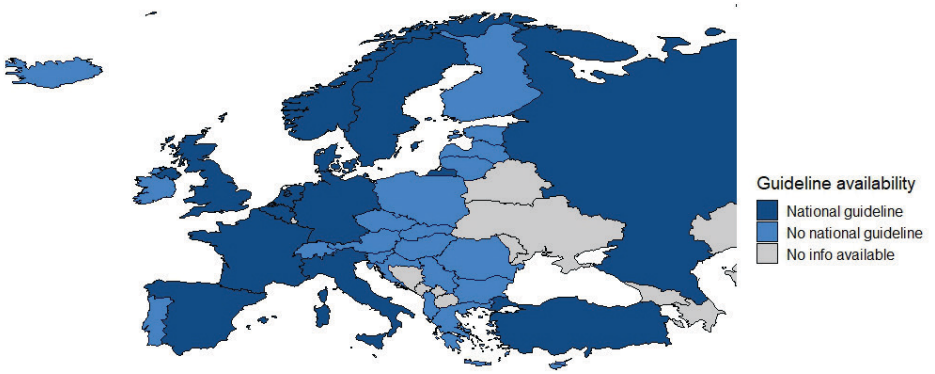
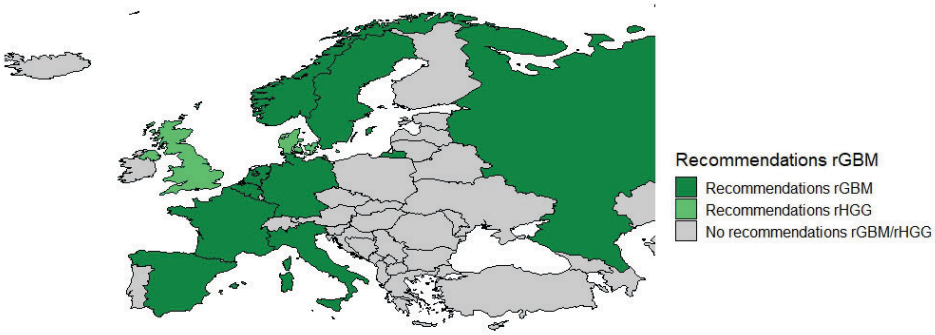


Figure 2. National recommendations on recurrent glioblastoma.



rGBM: recurrent glioblastoma; rHGG: recurrent high-grade glioma.

Re-resection as one of the treatment modalities was recommended for selected patients only in all available guidelines. Selection criteria for re-resection were generally the same across the countries, with Karnofsky performance status (KPS, e.g. ≥70), time to recurrence (e.g. more than six months) and the age of the patient (e.g. <70) as the most frequently mentioned prognostic factors to be taken into

account. The specific cut-offs of these factors were not provided in all guidelines. The Belgian guideline stated that 'selected patients with a focal recurrence' might benefit from a second resection but did not further specify the selection criteria. Re-resection combined with the implementation of carmustine-impregnated wafers (Gliadel®) was considered as an option in the French and Spanish guidelines.

Although the majority of the guidelines mentioned the potential of either a rechallenge temozolomide after a temozolomide-free interval (e.g. of more than four to six months) or treatment with CCNU (lomustine), more variation was seen regarding other systemic treatment options. The anti-vascular endothelial growth factor (anti-VEGF) antibody bevacizumab, for instance, was suggested as an anti-tumor treatment option in the Danish, German and Russian guidelines. Regorafenib, an oral multi-targeted tyrosine kinase inhibitor, was considered in the Italian guideline as a first therapeutic option for patients with recurrent glioblastoma and with a good performance status (defined as KPS ≥80). The German guideline briefly referred to regorafenib, but none of the other guidelines mentioned this regimen. Likewise, dendritic cell based immunotherapy was suggested in a single guideline (Belgium). Treatment with tumor treating fields (TTFields) in case of recurrence was actively not recommended, as stated in the English, French, Italian, Norwegian, Spanish and Swedish guideline.

Regarding re-irradiation, there was a general consensus that only patients with a small, focal recurrence, and taken into account the previously administered dose and radiation-free interval (e.g. of six to twelve months), can be offered a second course of radiation therapy. Specific definitions of the factors to be taken into account were not provided in all guidelines.

Role of clinical trials

Regarding the role of clinical trials in the recurrent setting, five (42%) of the available guidelines considered enrollment into clinical trial to be an option. For example, the Spanish guideline stated that 'the best option [for recurrent glioblastoma] is the enrollment into clinical trials'. If that is not an option, a second-line treatment should be considered according to this guideline. Genomic profiling in the context of enrollment into clinical trials was recommended in the Danish guideline. The guideline of the United Kingdom, however, stated that 'the point at which to use genomic biomarker-based therapy' is uncertain.

Quality assessment

Scope and purpose

This domain is 'concerned with the overall aim of the guideline, the specific health questions, and the target population'.[5] The median score for this domain was 58.3% with a range of 13.9-100%. The Turkish guideline scored the lowest score. The English guideline had the highest score for this domain.

Stakeholder involvement

This domain 'focuses on the extent to which the guideline was developed by the appropriate stakeholders and represents the views of its intended users'.[5] The median score for this domain was 58.3% with a range of 0.0-86.1%. The French guideline scored the lowest score. The English guideline scored the highest score for this domain.

Rigour of development

This domain 'relates to the process used to gather and synthesize the evidence, the methods to formulate the recommendations, and to update them'.[5] The median score for this domain was 26.6% with a range of 13.5-79.2%. The French guideline scored had the lowest score. The Italian guideline scored highest for this domain.

Clarity of presentation

This domain 'deals with the language, structure, and format of the guideline'.[5] The median score for this domain was 76.4% with a range of 44.4-97.2%. The Turkish guideline scored lowest. The Danish guideline scored highest for this domain.

Applicability

This domain 'pertains to the likely barriers and facilitators to implementation, strategies to improve uptake, and resource implications of applying the guidelines'. [5] The median score for this domain was 20.8% with a range of 2.1-45.8%. The Belgian guideline had the lowest score. The English guideline scored highest.

Editorial independence

This domain is 'concerned with the formulation of recommendations not being unduly biased with competing interests'.[5] The median score for this domain was 25.0% with a range of 0.0-79.2%. The French and Turkish guidelines scored lowest. The English guideline had the highest score for this domain.

Overall assessment

According the AGREE II instrument manual, the abovementioned domain scores are independent and should not be aggregated into a single quality score.[5] However, it is evident that the UK guideline had overall the highest scores (*Table 1*). Applicability of the different guidelines was generally low, while the clarity of presentation was generally good.

Table 1. Domain scores for the available guidelines using the AGREE-II instrument[5].

Country	Domain 1: scope and purpose (%)	Domain 2: stakeholder involvement (%)	Domain 3: rigour of development (%)	Domain 4: clarity of presentation (%)	Domain 5: applicability (%)	Domain 6: editorial independence (%)
Belgium	25.0%	41.7%	27.1%	61.1%	2.1%	29.2%
Denmark	58.3%	58.3%	28.1%	**97.2%**	33.3%	50.0%
France	19.4%	0.0%	13.5%	**86.1%**	14.6%	0.0%
Germany	77.8%	72.2%	49.0%	**88.9%**	12.5%	79.2%
Italy	**88.9%**	58.3%	79.2%	75.0%	31.1%	75.0%
The Netherlands	**97.2%**	66.7%	59.4%	75.0%	20.8%	20.8%
Norway	58.3%	58.3%	21.9%	75.0%	20.8%	33.3%
Russia	36.1%	38.9%	26.0%	50.0%	33.3%	20.8%
Spain	55.6%	50.0%	16.7%	77.8%	10.4%	20.8%
Sweden	69.4%	58.3%	21.9%	**86.1%**	35.4%	12.5%
Turkey	13.9%	38.9%	26.0%	44.4%	12.5%	0.0%
United Kingdom	**100%**	**86.1%**	74.0%	77.8%	45.8%	79.2%
Median score (range)	58.3% (13.9-100)	58.3% (0.0-86.1)	26.6% (13.5-79.2)	76.4% (44.4-97.2)	20.8% (2.1-45.8)	25.0% (0.0-79.2)

High quality scores (≥80%) depicted in bold, acceptable scores (≥60-79%) are underlined.

DISCUSSION

This study investigated the availability of national guidelines on the diagnosis and treatment of adult glioma throughout Europe. Of the 50 European countries, twelve (24%) shared their national guidelines publicly online or through personal correspondence. Focusing on recurrent glioblastoma, we found that only nine (18%) of the European countries provide any recommendations on the treatment of recurrent glioblastoma. The quality of the twelve available guidelines assessed by the AGREE II method showed remarkable differences between countries and domains, with the guideline of the United Kingdom showing overall the highest scores.

Information on the treatment of recurrent glioblastoma varied from the statement that there is currently no standard-of-care for these patients, to more detailed descriptions of the (lack of) evidence for different treatment modalities. This was not explicitly taken into account in this study, since the first statement (i.e. 'there is currently no standard-of-care') might as well provide guidance to clinicians. Moreover, the body of recurrent glioblastoma recommendations did not appear to be related to the quality of the guideline: some guidelines clearly provided different treatment options but showed marginal scores on the quality assessment, and vice versa. In general, this study did not intend to include sociodemographic characteristics or economic status to compare different guidelines and different countries, although it is not unlikely that this could affect the content of national recommendations.

Interestingly, the administration of bevacizumab as a treatment for glioblastoma recurrence was mentioned in some guidelines. However, this drug has only been approved for that indication outside the European Union, like in Canada, Switzerland and the United States, based on two uncontrolled phase 2 studies showing objective response rates of around 30% for the treatment with bevacizumab alone or in combination with irinotecan.[11, 12] The European evidence-based opinion, however, is that there is no survival benefit of bevacizumab for the treatment of recurrent glioblastoma.[1, 13, 14] Likewise, the application of carmustine wafers, as considered in two guidelines, is currently not common practice in Europe.[1, 3]

Attention should be paid when presence of guidelines becomes synonymous to good clinical practice. As mentioned before, even in the presence of national guidelines remarkable differences in re-resection practice have been observed between neuro-oncology specialists.[4] Thus, national guidelines do not necessarily

rule out the phenomenon of practice variation. Similarly, the absence of national guidelines does not necessarily mean suboptimal practice, especially when considering the availability of international guidelines. Indeed, some respondents stated that, in the absence of a national guideline, international guidelines (e.g. the EANO guideline) are used. Here, the adage ‘absence of evidence does not mean evidence of absence’ seems applicable. Nevertheless, the discrepancy in treatment uniformity between the primary setting and the recurrent setting, as observed in glioblastoma patients, remains worrisome. More importantly, prioritizing the collection of evidence in the recurrent setting should precede the development of guidelines, since the increasing number of guidelines is currently not paralleled by an equal increase in evidence. The development of more guidelines should therefore be viewed critically in the absence of more data and evidence on the treatment of recurrent glioblastoma.

The ASCO-SNO guideline strongly recommends the participation of recurrent glioblastoma patients in clinical trials were possible.[3] The EANO guideline agrees on this, albeit less pronounced, with the statement that appropriate clinical trials ‘should be considered'.[1] However, only five (42%) of the available guidelines in our study considered enrollment into clinical trials as an option, with varying degrees of strength of that recommendation. Based on the lack of evidence for standard systemic treatment options and low availability for suited patients, we strongly advocate the enrollment of recurrent glioblastoma patients in clinical trials. As effective treatment options are still limited, identification of new clinically relevant targets is of urgent importance and should be done in the context of clinical trials and prospective registries.[15, 16]

Some limitations of this study have to be considered when interpreting our findings. The design of the study potentially resulted in the retrieval of only those guidelines of countries with known or findable contact information. Details on the guideline availability of the twelve countries for which we have not been able to obtain any information would have been of added value. Second, the language in which the guidelines are written may have influenced proper interpretation, although careful reading and translation was pursued. Another limitation is the absence of country-specific clinical outcome data, that might have made the correlation possible between (presence of) national recommendations and clinical outcomes. Generally put, quantification of our data would be of interest.

CONCLUSION

In conclusion, this study shows that national guidelines on the treatment of recurrent glioblastoma in adults are widely unavailable in Europe. This, among other factors including education, patient volume, lack of evidence, and the role of multidisciplinary consultations, could contribute to unwanted (inter)national practice variation and should therefore force more (experimental) research into the optimal treatment for these patients. When comparing national guidelines, cultural and educational differences should be taken into account. Future research should investigate whether national guideline availability correlates with clinical outcomes and with sociodemographic characteristics and economic status of countries, in order to further study the impact and origins of unwanted (inter)national practice variation.

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CONFLICT OF INTEREST STATEMENT

None of the authors declare a conflict of interest.

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Chapter 5

Next generation sequencing of high-grade adult-type diffuse glioma in the Netherlands: interlaboratory variation in the primary diagnostic and recurrent setting

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ABSTRACT

Purpose. Next generation sequencing (NGS) is an important tool used in clinical practice to obtain the required molecular information for accurate diagnostics of high-grade adult-type diffuse glioma (HGG). Since individual centers use either in-house produced or standardized panels, interlaboratory variation could play a role in the practice of HGG diagnosis and treatment. This study aimed to investigate the current practice in NGS application for both primary and recurrent HGG.

Methods. This nationwide Dutch survey used the expertise of (neuro)pathologists and clinical scientists in molecular pathology (CSMPs) by sending online questionnaires on clinical and technical aspects. Primary outcome was an overview of panel composition in the different centers for diagnostic practice of HGG. Secondary outcomes included practice for recurrent HGG and future perspectives.

Results. Out of twelve neuro-oncology centers, the survey was filled out by eleven (neuro)pathologists and seven CSMPs. The composition of the diagnostic NGS panels differed in each center with numbers of genes ranging from 12 to 523. Differences are more pronounced when tests are performed to find therapeutic targets in the case of recurrent disease: about half of the centers test for gene fusions (60%) and tumor mutational burden (40%).

Conclusion. Current notable interlaboratory variations as illustrated in this study should be reduced in order to refine diagnostics and improve precision oncology. In-house developed tests, standardized panels and routine application of broad gene panels all have their own advantages and disadvantages. Future research would be of interest to study the clinical impact of variation in diagnostic approaches.

Keywords. Next generation sequencing, high-grade glioma, adult, variations, practice

INTRODUCTION

The final diagnosis of a high-grade adult-type diffuse glioma is increasingly based on molecular characteristics of the tumor [1]. This dependence on molecular alterations has increased with the release of the fifth edition of the World Health Organization (WHO) classification of tumors of the central nervous system (WHO CNS5) in 2021 [2], which is largely based on the evidence provided by the Consortium to Inform Molecular and Practical Approaches to CNS Tumor Taxonomy – Not Officially WHO (cIMPACT-NOW) [3-5]. Consequently, the European Association of Neuro-Oncology (EANO) updated its guidelines for the clinical management of adult patients with diffuse gliomas and provided extensive recommendations on diagnosis and treatment, based on immunohistochemistry and additional molecular testing [6, 7]. Molecular characteristics can now overrule the diagnosis based on morphological characteristics, clearly illustrated in isocitrate dehydrogenase 1 and 2 (*IDH1/2*) and H3-wildtype diffuse gliomas where in adult patients regardless of the histology the presence of a *TERT*-promotor mutation, *EGFR*-amplification and/or a gain of chromosome 7 together with a loss of chromosome 10 (so called "+7/-10"), warrants the diagnosis of a glioblastoma, IDH wild-type (CNS WHO grade 4) [1, 3, 8-10]. This clinical value of molecular characteristics is also demonstrated in IDH-mutant astrocytomas in which the general favorability of low grade histology is overruled by the presence of homozygous *CDKN2A/B* deletion resulting in a grade 4 diagnosis [4, 11].

Besides methylome profiling, next generation sequencing (NGS) of tumor DNA is used in clinical practice to determine the molecular characteristics of a malignant brain tumor. Depending on the exact setup and protocol, NGS allows testing for mutations, gene fusions (especially RNA-based), copy number aberrations (CNAs) including loss of heterozygosity (LOH), and small insertions/deletions (InDel) [12-14]. To keep the costs of the NGS-tests reasonable, most molecular pathology laboratories currently apply targeted panels focusing on genes of interest for glioma diagnostics. These panels were generated and updated over time to keep up with the ever-evolving scientific literature and recommendations. Moreover, laboratories may use either lab developed tests (LDTs), i.e., custom-made panels, or commercial, standardized panels (also known as in vitro diagnostics (IVDs)). Hence, panels may vary significantly between centers, even when located within the same region. The interpretation of the molecular alterations occurs by the use of general oncogenetic concepts and the multiple databases. Although the workflows for these diagnostics are similar in different centers, reported genes and outcomes are not necessarily identical. The variation in NGS panel platforms

as well as interpretation workflows can be expected to contribute to interlaboratory variation in the diagnostic work-up of and perhaps even in the treatment of adult patients with a malignant brain tumor.

This study aimed to evaluate the current practice in the application of NGS for patients with a high-grade adult-type diffuse glioma (HGG), both primary and recurrent, in the Netherlands in order to make recommendations on the clinical practice of genome-based diagnostics in patients with an HGG.

MATERIALS AND METHODS

Data collection

In the Netherlands, patients with a (suspected) brain tumor are referred to centers with neuro-oncological expertise. There are 14 neurosurgical centers that treat patients with glioblastoma, a diagnosis that is made approximately 1000 times a year in the Netherlands [15]. The diagnosis is definitive after the histological and molecular ('histomolecular') assessment by a (neuro)pathologist. Most often NGS is performed locally, but sometimes it is centralized, resulting in discrepancy between total number of neuro-oncological centers and specialized pathology departments involved in this study. The molecular reports are integrated in the morphology reports by the (neuro)pathologist, who is responsible for making accurate, 'histomolecular' diagnoses. Clinical scientists in molecular pathology (CSMPs) are responsible for the proper execution and interpretation of molecular assays. Together with (neuro)pathologists and clinical oncologists, CSMPs are important stakeholders in the molecular tumor board (MTB) in which rare and/or complex molecular information is being discussed and taken into account in a treatment advice for each patient [16, 17].

From April 2022 until July 2022, questionnaires were sent to (neuro)pathologists and CSMPs and qualitative data was collected on current NGS panel practice in the Netherlands. All (neuro)pathologists and CSMPs with experience with NGS were eligible for participation. Participants were selected from twelve centers in the Netherlands providing neuro-oncological pathology services, including seven academic centers, four peripheral hospitals and one independent pathology laboratory. One (neuro)pathologist and one CSMP (if any) were selected per center. Respondents were assured that answers on the questionnaires would be kept confidential and that the answers would be processed anonymously.

The questionnaire was designed in two different versions: one was sent to CSMPs to assess technical details on NGS panels, the other was sent to (neuro)pathologists to assess clinical aspects related to the ordering and reporting of NGS results. Part one of the questionnaire was about the practice for HGG at initial diagnosis, the second part was about the practice for HGG at recurrence and the final part evaluated future perspective regarding genome-based diagnostics. Questionnaires were sent via e-mail, and reminders were sent by e-mail or given by phone call up to two times to potential participants if they had not yet responded.

Outcomes

The primary outcome was NGS panel practice for HGG at initial diagnosis, e.g. genes included in the different centers for diagnostic practice. Secondary outcomes were NGS panel practice for recurrent HGG (including the role of the MTB), and future perspectives (including expectations on future replacement of NGS by whole genome sequencing (WGS)).

Statistical analysis

Categorical variables were reported using percentages and counts with the intention to qualitatively analyze the results. Calculations were based on total number of respondents for the specific questions; missing answers were taken out from the analyses. Therefore, total counts might vary per outcome. Figures were created using the open software environment *R*, version 4.2.1.

RESULTS

Questionnaire response

Of the twelve centers, nine of them had their own CSMP services. The questionnaire was filled out by eleven (11/12, 92%) (neuro)pathologists and seven (7/9, 78%) CSMPs. In total, 78% (14/18) of the respondents answered all questions of the questionnaire, the remainder skipped only one or two questions. See *Table 1* for a summary of the most important results.

Initial tumor

In the diagnostic process of an HGG, in 4/11 (36%) centers NGS is always applied by default, and in another 5/11 (46%) it is only used for specific patient groups, for instance patients aged under 55 or 60 years, when immunohistochemistry is not sufficient for the diagnosis of an IDH1 R132H wild-type glioblastoma. In 2/11 (18%) of the centers, NGS is not used by default, but rather methylome profiling for

instance. When NGS is applied, most centers (9/11, 82%) always explicitly reported diagnostic markers (e.g., *IDH1/2*, *ATRX*, *TERT*), regardless the mutational status of the marker (e.g., 'No mutation in *IDH1/IDH2* found'). Likewise, prognostic markers (e.g. *CDKN2A/B*) were always reported in 8/11 (73%) of the laboratories, in contrast to (not exclusively) predictive markers (e.g. *BRAF*, *EGFR*) (3/11, 27%) and details on actionability (0%).

All but one (10/11, 91%) center used LDTs by default for the diagnosis of an HGG. The composition of the NGS gene panels for diagnosis of the initial tumor was different in each center (*Figure 1,* panel composition obtained from the seven CSMPs), and numbers of genes included in the different panels ranged from 12 to 49 for the LDTs. One of the centers used a broad gene panel (TruSight Oncology 500, TSO500) containing 523 genes in the diagnostic setting; other centers would be able to do this by indication. No correlation was observed between the size of a center (based on national quality registries) and size of a panel. Regarding the genes essential for the diagnoses of adult-type diffuse gliomas according the WHO CNS5 classification [18], 2/7 (29%) covered all these eight genes (*Figure 2*). Of the most relevant of these genes, *IDH1/2, TP53* and *EGFR* are covered by all panels whereas two panels did not cover mutations in the *TERT*-promotor. However, these centers test for *TERT*-promotor mutation via a separate test such as droplet digital polymerase chain reaction (ddPCR), whether or not at the request of the (neuro) pathologist. Only the broad gene panel covered complete genes, the LDTs were limited to hotspots.

Table 1. Summary of the most important results from the questionnaire.

Primary lesions	
Neuro-oncology NGS panels per week per center, no. (%)[a]	
0-5	6 (86%)
5-10	1 (14%)
Panel origination, no. (%)	
Lab developed test	6 (86%)
Commercial test	1 (14%)
Latest panel update (%)	
Before 2019	1 (14%)
2019 or later	5 (71%)
Unknown	1 (14%)

CNAs analysed, no. (%)	
Yes	6 (86%)
No	1 (14%)
NGS applied by default, no. (%)	
Yes	4 (36%)
No	2 (18%)
Only in specific cases	5 (46%)
Markers always reported, no. (%)	
Diagnostic markers	11 (100%)
Prognostic markers	8 (73%)
Predictive markers	3 (27%)
Actionability	0 (0%)
Recurrent lesions	
Neuro-oncology NGS panels per week per center, no. (%)	
0-5	7 (100%)
5-10	0 (0%)
Composition molecular tumor board, no. (%)	
Clinical scientist in molecular pathology	6 (100%)
(Neuro)pathologist	2 (33%)
Neurologist	3 (50%)
Neurosurgeon	1 (17%)
Medical oncologist	5 (83%)
Other (e.g. clinical geneticist)	4 (67%)
CNAs analysed, no. (%)	
Yes	5 (83%)
No	1 (17%)
Goal(s) molecular diagnostics, no. (%)	
Diagnostic markers	1 (10%)
Therapeutic targets	8 (80%)
Gene fusions	6 (60%)
Tumor mutational burden	4 (40%)
Methylome profiling	1 (10%)
Other (e.g. microsatellite instability)	3 (30%)

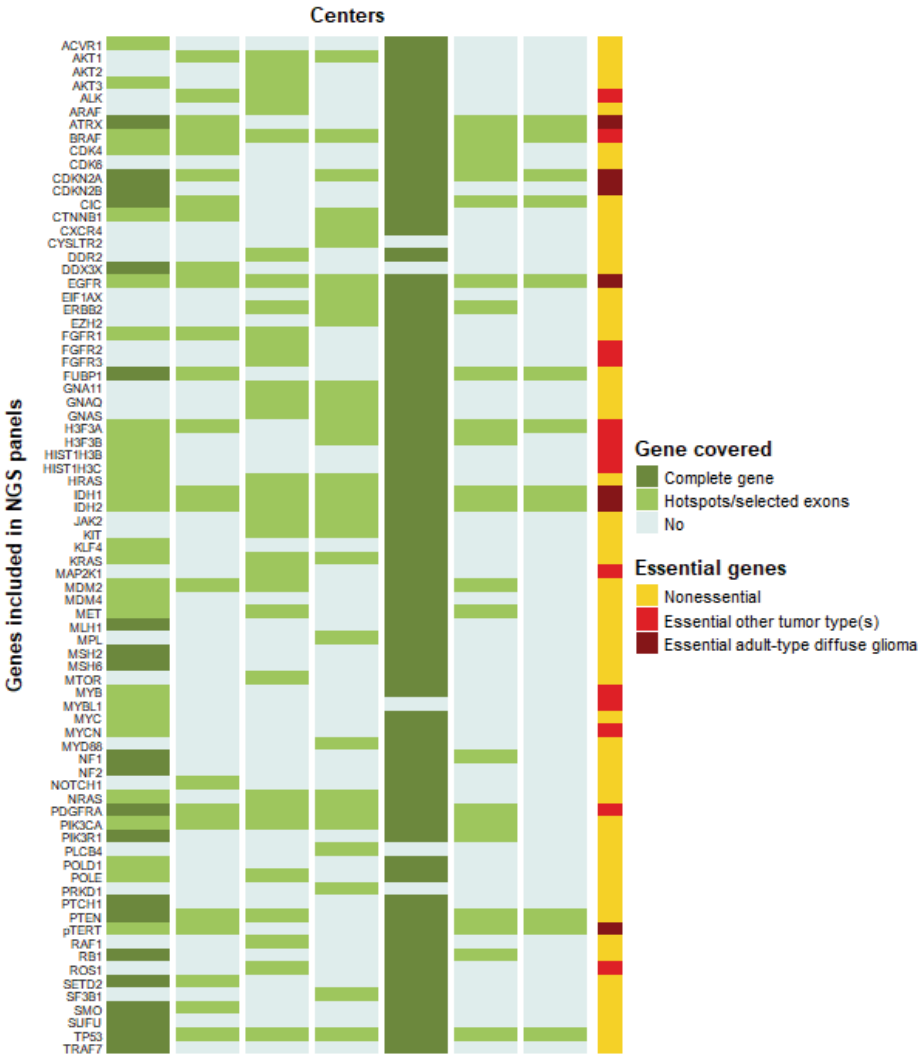
[a]Total counts vary because the total number of respondents differed per question. CNAs: copy number aberrations. NGS: next generation sequencing.

Recurrent tumor

In the case of molecular diagnostics for recurrent HGGs, 8/10 (80%, one respondent missing) of the centers apply genome sequencing to identify potential therapeutic targets. All centers have access to an MTB (whether it be in or outside their own infrastructure), but none of them discuss every patient after analysis of potential therapeutic targets. Selection is based on the molecular findings, for instance to discuss targeted treatment options, and discussion in MTBs is almost exclusively at the request of the treating physician. The composition of the MTB differs in each center, but always CSMPs and medical oncologists are members of the MTB [17].

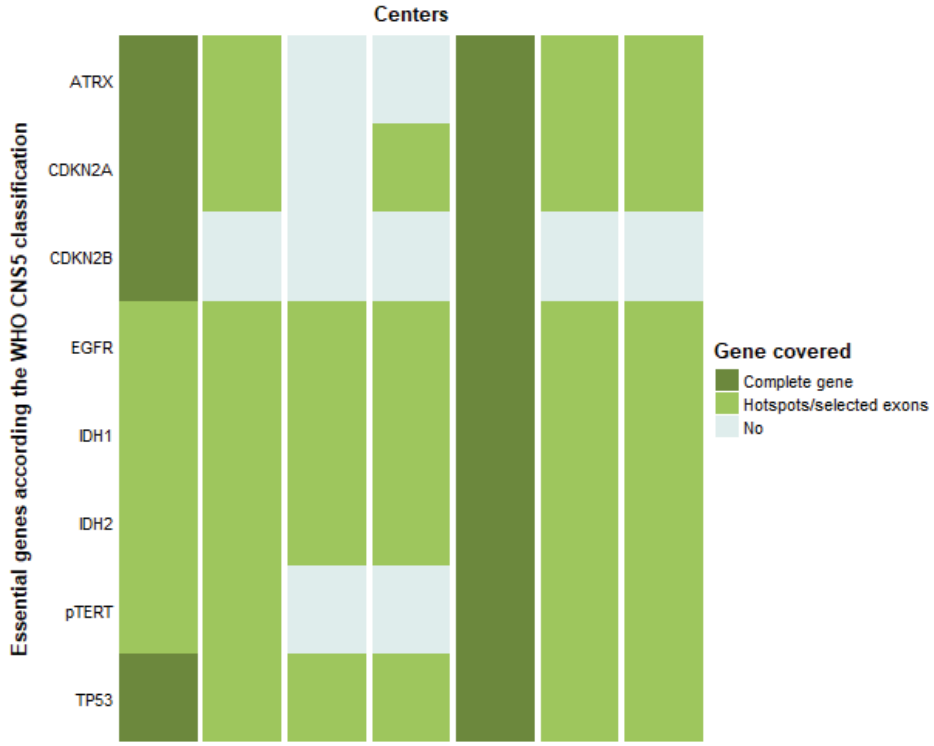
Regarding the testing for potential therapeutic targets in recurrent lesions, the decision to apply these molecular diagnostics is a multidisciplinary decision, for instance made during regular multidisciplinary discussion attended by clinicians and (neuro)pathologists. Reasons for the use of additional molecular analysis in the case of a recurrent HGG include the absence of NGS in the primary setting, ambiguity in previous test results, the introduction of new molecular markers since the primary diagnosis, or a relatively young patient in a good condition (Karnofsky Performance Status (KPS) $\geq$70).

Figure 1. Heatmap overview of next generation sequencing (NGS) gene panels in different centers.



Seven centers provided detailed panel information. For the center with the broad 500 gene panel by default, only those genes present in at least one of the other panels are depicted. Essentiality is based on the fifth edition of the World Health Organization classification of tumors of the central nervous system (WHO CNS5)[2, 18].

Figure 2. Heatmap overview of next generation sequencing (NGS) platforms in different centers regarding genes listed as essential for adult-type diffuse glioma diagnosis [2, 18].



WHO CNS5: fifth edition of the World Health Organization classification of tumors of the central nervous system.

Future perspectives

The majority (5/7, 71%) of the CSMPs expect updates of the current NGS panel within two years, with both diagnostic and therapeutic targets in small (17%) or broad (83%) NGS panels. 7/11 (64%) of the (neuro)pathologists do not expect a replacement of NGS by WGS for the diagnostics of adult HGG within five years, while 3/11 (27%) do expect this, and 1/11 (9%) do not know. Most important arguments for this skepticism towards WGS include the cost-effectiveness (7/8, 88%) and too much/irrelevant data to analyze (6/8, 75%). However, maximizing treatment options by WGS based diagnostics was an important argument for three (neuro)pathologists to see future importance of WGS within five years.

DISCUSSION

This study investigated the current practice in the application of NGS for patients with a high-grade adult-type diffuse glioma in the Netherlands. Of the seven centers that shared their information via the CSMPs, NGS panels were different in each center, with a wide range in the number of genes per panel.

In a country where molecular testing is relatively widely reimbursed, financial incentives are not likely to play an important role in the interlaboratory variation as found in our study. Explanatory factors could be, for example, local protocols or variable interest in experimental, molecularly targeted, therapeutic options. The variability in the composition of the panels as found in our study can also be explained by the finding that six of the seven panels were LDTs. These in-house produced tests result by definition in practice variation between different centers and frequently updating LDTs is difficult. A Dutch interview-based research investigated the application of diagnostics in hospital practice and found no straightforward explanation for the use of either LDTs or commercial tests [19]. However, that study showed that explanatory features of LDTs include the lower costs and the tailoring to the specific laboratory practices, compared to commercial panels. Importantly, commercial tests are not by definition superior to LDTs since commercial tests could not easily or quickly be updated (i.e., adapted to the newest molecular criteria), and they do not rule out the possibility of practice variation when it comes to the interpretation of test results.

Practice variation in the application of NGS for patients with HGG could possibly result in diagnostic variability and delayed diagnosis. Even though different centers most often end up with the same molecular information for the primary diagnosis after sequential, layered testing, this would be time and eventually cost consuming. Differences are more pronounced when tests are performed in order to find therapeutic targets in the case of recurrent disease. For example, about half of the centers test for gene fusions (60%) and tumor mutational burden (40%). Although the occurrence of targetable gene fusions in glioblastoma is low and treatment effectiveness in the context of expediency is still being investigated, patient selection for potential trial participation is reduced when testing is omitted [20, 21].

The variable, layered diagnostic process could potentially be solved by routine application of broad gene panels, supplemented by broad gene fusion tests for instance in the case of recurrent disease. Considerable advantages of generic,

broad gene panels over LTDs include less need for updates, significantly less risks of omitting to test certain biomarkers, and time-efficiency. These advantages must be weighed against higher costs, potential difficulties with reimbursement, increased risk of unsolicited findings and the fact that broad gene panels are sometimes inferior in detecting CNAs (and especially deletions like *CDKN2A*).

In May 2022, the new In Vitro Diagnostic Medical Devices Regulation (IVDR) came into effect in the European Union with the goal to improve patient safety and to ensure that innovative medical devices remain available [22]. This IVDR, the implementation of which will gradually unfold, will also affect in-house produced tests leading to more standardization of the diagnostic practice. Although more strictly regulated, IVDR requirements should not impede the application of LDTs [23]. However, professionals express their worries about the impact of the IVDR possibly resulting in decreased innovativeness and increased costs and administrative work [19].

This study has some limitations to be mentioned. First, this online survey is a reflection of the current laboratory practice, of both initial and recurrent HGG, and standard protocols per center, and left little room for discussion. For instance, a center with a smaller diagnostic panel might deploy broader diagnostics by indication. Second, local approaches possibly will slightly differ between (neuro) pathologists and/or CSMPs, but our study did not require more than one (neuro) pathologist and one CSMP per center to test for this inter- and intraspecialty variation. Another limitation is that the current study design did not account for the multidisciplinary setting in which decisions on the treatment of brain tumor patients are made in Dutch practice. Finally, this study did not assess the impact on clinical practice after NGS analysis in the different centers.

To conclude, our study illustrates the current interlaboratory variation in the application of NGS panels for patients with a high-grade adult-type diffuse glioma, both at first diagnosis and in the recurrent setting. Reducing this practice variation by applying broad gene panels as a standard has the dual potential of refining the diagnostics and improving precision oncology. Future research would be of interest to study the clinical impact of variation in diagnostic approaches.

AUTHOR CONTRIBUTIONS

MPvO, MLDB, WdL and SLNM contributed to the study conception and design. Material preparation, data collection and analysis were performed by MPvO, MLDB, WdL and SLNM. The first draft of the manuscript was written by MPvO and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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AVAILABILITY OF DATA AND MATERIALS

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

CONFLICT OF INTEREST STATEMENT

The authors declare no competing interests.

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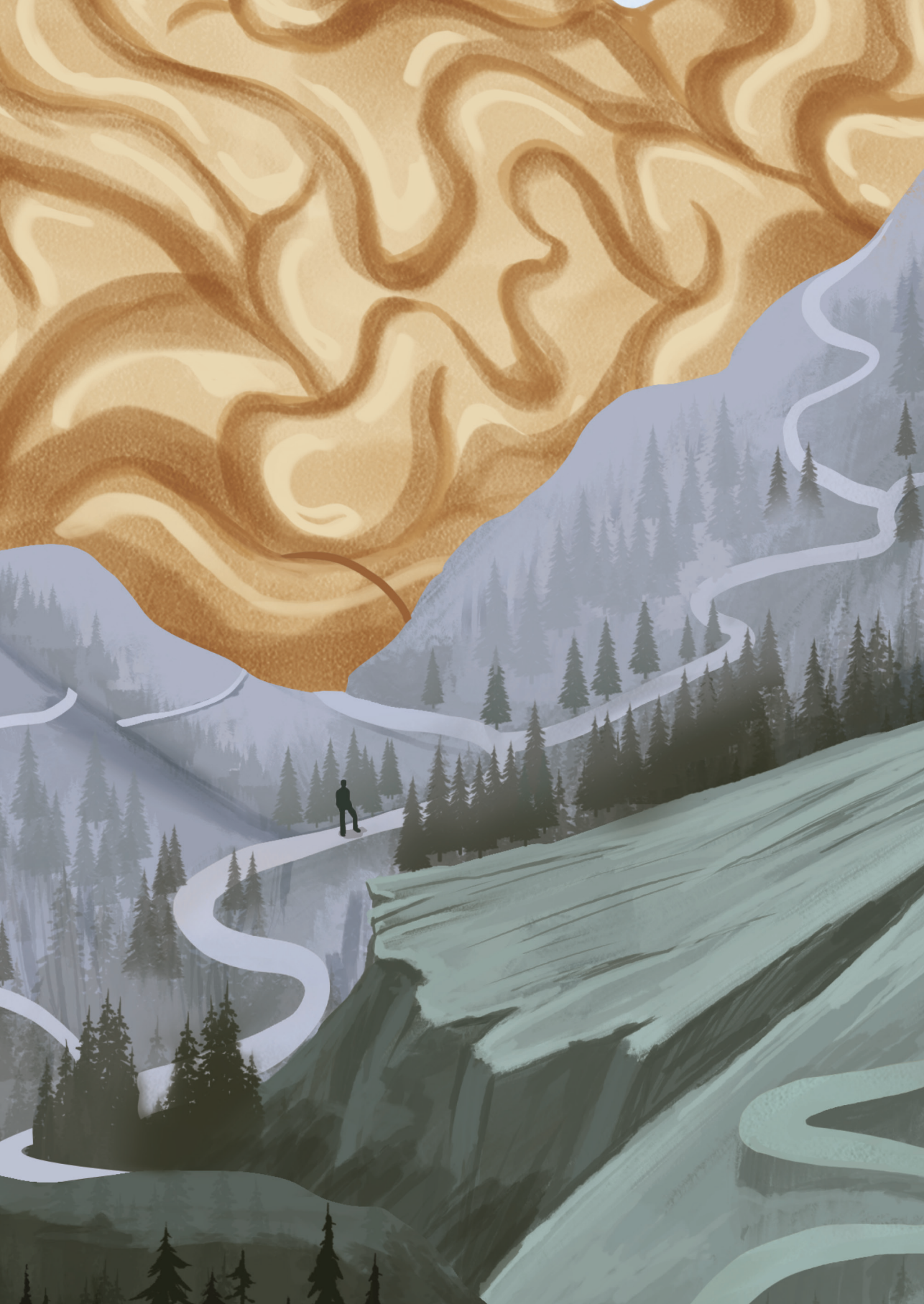

PART II

Molecular diagnostics

6

Chapter 6

Study protocol of the GLOW study: maximising treatment options for recurrent glioblastoma patients by whole genome sequencing-based diagnostics – a prospective multicenter cohort study

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ABSTRACT

Background. Glioblastoma (GBM), the most common glial primary brain tumour, is without exception lethal. Every year approximately 600 patients are diagnosed with this heterogeneous disease in The Netherlands. Despite neurosurgery, chemo -and radiation therapy, these tumours inevitably recur. Currently, there is no gold standard at time of recurrence and treatment options are limited. Unfortunately, the results of dedicated trials with new drugs have been very disappointing. The goal of the project is to obtain the evidence for changing standard of care (SOC) procedures to include whole genome sequencing (WGS) and consequently adapt care guidelines for this specific patient group with very poor prognosis by offering optimal and timely benefit from novel therapies, even in the absence of traditional registration trials for this small volume cancer indication.

Methods. The GLOW study is a prospective diagnostic cohort study executed through collaboration of the Hartwig Medical Foundation (Hartwig, a non-profit organisation) and twelve Dutch centers that perform neurosurgery and/or treat GBM patients. A total of 235 patients with a first recurrence of a glioblastoma will be included. Dual primary endpoint is the percentage of patients who receive targeted therapy based on the WGS report and overall survival. Secondary endpoints include WGS report success rate and number of targeted treatments available based on WGS reports and number of patients starting a treatment in presence of an actionable variant. At recurrence, study participants will undergo SOC neurosurgical resection. Tumour material will then, together with a blood sample, be sent to Hartwig where it will be analysed by WGS. A diagnostic report with therapy guidance, including potential matching off-label drugs and available clinical trials will then be sent back to the treating physician for discussing of the results in molecular tumour boards and targeted treatment decision making.

Discussion. The GLOW study aims to provide the scientific evidence for changing the SOC diagnostics for patients with a recurrent glioblastoma by investigating complete genome diagnostics to maximize treatment options for this patient group.

Trial registration. ClinicalTrials.gov, NCT05186064. Registered 11th January, 2022.

Keywords. Glioblastoma, whole genome sequencing, treatment options, diagnostics, recurrence

BACKGROUND

Glioblastoma (GBM), the most common glial primary brain tumour, is almost always lethal. In the Netherlands, every year approximately 600 patients are diagnosed with this heterogeneous disease. Standard treatment for patients with newly diagnosed GBM consists of maximal safe surgical resection followed by postoperative radiation with concomitant and adjuvant temozolomide therapy.(1) Despite this intensive treatment scheme, these tumours inevitably recur and the prognosis of patients remains poor with a median survival of 14 months.(2) At the time of recurrence, only a small number of patients with well-localized tumours are eligible for re-resection. Systemic treatment is commonly suggested for recurrence, of which nitrosoureas or retreatment with temozolomide being mostly used with limited progression-free survival rates at 6 months (15-20%) and objective response rate of less than 10%. (3-7) Patients with an O6-methylguanine DNA methyltransferase (MGMT) promoter-methylated recurrent tumour may benefit from a temozolomide rechallenge, from lomustine or even the combination of both.(8-10) Outside of the European Union, bevacizumab has been approved for relapsed GBM.(11, 12) Some patients with relapsed GBM undergo re-irradiation, which may result in local disease control in a proportion of patients.(13-17) However, this is not always feasible due to the hazards of cumulative (cognitive) neurotoxicity.

Unfortunately, the results of dedicated trials with new drugs have been very disappointing. Target pre-screening, if applicable, was usually performed on archival tumour material, limited gene panels were used and not in every case a central review was performed. Targeted treatment options are becoming increasingly available for cancer patients, however studies on molecular targets for recurrent GBM patients have not yet led to clinical advantages.(18) Still, there is a major unmet need for this patient category as demonstrated by the limited treatment options and very poor survival. Furthermore, the organisation of standard-of-care (SOC) molecular testing for GBM is suboptimal. First, molecular tests are currently performed sequentially, which takes more time, especially in absence of gene panels. Second, because of this organization, tissue might become scarce. Third, different centers use different molecular panels, which are not all tailored towards identifying relevant biomarkers for (experimental) targeted treatments. Whole genome sequencing (WGS) will provide all molecular information in a single test and within a limited time of ten to fourteen days. Furthermore, additional stratification biomarkers for treatments can be identified using WGS. Although WGS is validated as a clinical diagnostic test(19, 20), its implementation in routine care environments is still slowly growing, although in the Netherlands, the non-

profit organisation Hartwig provides access to WGS based testing to all hospitals. The potential of WGS in the area of personalised medicine for patients with cancer has been demonstrated before, but it has never been prospectively studied as a SOC procedure in patients with a recurrent GBM.(20, 21)

Actionability of a molecular alteration is based on information in public knowledge bases, including the Clinical Knowledgebase (CKB), Oncology Knowledge Base (OncoKB), the Clinical Interpretation of Variants in Cancer (CIViC), and can be split by evidence levels according to stablished classification levels: including the six level ESMO Scale for Clinical Actionability of Molecular Targets (ESCAT) classification of the European Society for Medical Onoclogy (ESMO).(22) Hypothetical target molecular alterations are those that, at minimum, are associated with preclinical evidence linking the alteration with drug activity. According to the ESCAT classification, treatment should then only be considered in the context of early clinical trials and lack of clinical data should be stressed to patients. To demonstrate that such hypothesized treatments are effective, down-stream clinical studies are required which are facilitated by effective and comprehensive identification of these molecular events without repeating past experiences with drugs that were proven to be ineffective. These trials should also investigate and link pharmacodynamics to the clinical utility of the targeted therapy, since not all drugs will effectively cross the blood-brain barrier.

The GLOW (GLioblastoma targeted treatment Option maximization by Wgs) study aims to evaluate the diagnostic value of extensive molecular diagnostics based on complete genome sequencing for patients with a first recurrence of their glioblastoma undergoing surgery for the recurrence. Consequently, this might result in the adaption of care guidelines by offering optimal and timely benefit from novel therapies, even in the absence of traditional registration trials for this small volume cancer indication.

METHODS/DESIGN

Study design

The GLOW study is a prospective diagnostic cohort study executed through collaboration of the Hartwig Medical Foundation (Hartwig, a non-profit organisation) and twelve Dutch centers that perform neurosurgery and/or treat GBM patients. The study aims to obtain, besides surgery, a more accurate pre-treatment stratification of recurrent GBM patients by obtaining fresh tumour samples and

a blood sample (obtained during reresection as part of SOC) for WGS analysis leading to targeted treatment and eventual better progression free and overall survival. The patient outcomes of the prospective cohort will be compared with a similar-sized multicenter historical cohort of patients, who have not received routine WGS, seen between 2019 and 2020 in Utrecht University Medical Center (UMCU) and Haaglanden Medical Center (HMC). An independent data monitoring committee (DMC) is established to ensure independent trial supervision. The DMC will monitor the recruitment, the reported adverse events and the data quality after inclusion of the tenth patient, and at least once a year. The study design is summarised in *Figure 1*. The study is registered on ClinicalTrials.gov with number NCT05186064.

OBJECTIVES

Primary objective

The primary objective of the GLOW study is to determine the percentage of patients who receive targeted therapy after surgery, including experimental therapy based on the WGS report, which should ultimately result in more effective treatment (not part of the study) and improved survival, which will be measured as overall survival (OS) within GLOW.

Secondary objectives

There are several secondary objectives in this study. First, improvement of progression-free survival and overall survival by three months for patients that are treated based on WGS results. Second, to determine the percentage of tumour samples with sufficient quality for WGS analysis obtained during routine neurosurgical reresection. Third, to determine the percentage of tumour samples with an informative mutational profile, i.e. the number of patients with actionable mutations and number of actionable mutations per patient. Finally, to determine access to registered drugs for non-registered indications (i.e. off-label use) for these patients in The Netherlands.

Study population

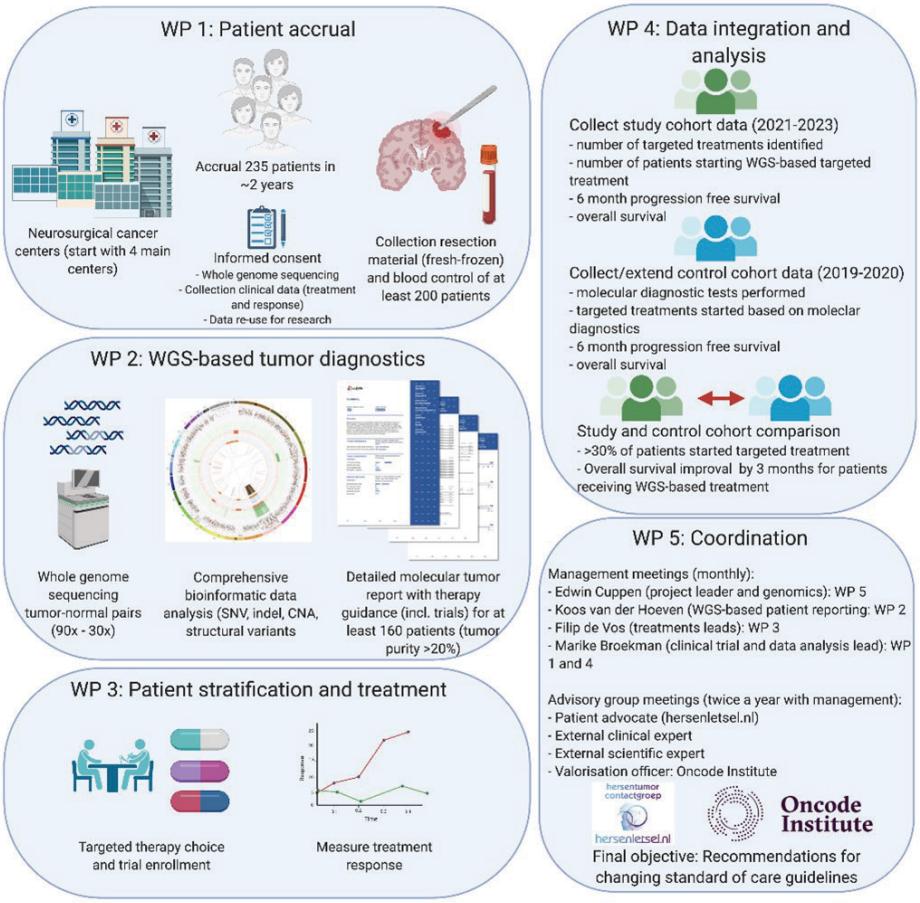
Within two years from the clinical phase, 235 patients will be recruited. Adult patients with a histopathologically confirmed isocitrate dehydrogenase (IDH) wildtype (wt) glioblastoma with a first recurrence after radiotherapy and/or systemic therapy and who are suited for SOC reresection, are eligible to participate in this study. The patients should have a life expectancy of at least three months, allowing adequate follow-up of toxicity and antitumour activity, together with a Karnofsky

Performance Status (KPS) of at least seventy, since the patients should be deemed eligible for targeted treatment options, also in a clinical trial setting. Finally, the patients have to be able and willing to give written informed consent. Potential subjects who currently receive antitumour treatment will be excluded, although patients may enter other studies after WGS based treatment decision making is completed. Patients with any other clinically significant medical condition which, in the opinion of the treating physician, makes it undesirable for the patient to participate in medication studies or which could jeopardize compliance with study requirements including, but not limited to, ongoing or active infection, significant uncontrolled hypertension, or severe psychiatric illness/social situations, will be excluded as well.

Statistical analysis

There are no formal statistical considerations that underlie this study as the study assesses the impact of using WGS in diagnostics versus current standard of care and patients will receive potentially a broad range of treatments with variable outcome expectations. First interim analysis of the results, on which premature termination or modification of the study will be based, will be started when the clinical follow-up data of 100 WGS analysed patients is available.

Figure 1. Design of the GLOW study with work packages (WP) overview.



Sample size calculation

The aim is to include a total of 235 patients in this study. Based on clinical expertise, around 15% of the initially included patients are expected to not be able to undergo the planned reresection because of medical conditions or personal choices, resulting in a total of 200 patients who will be included in the GLOW study. Based on previous experience, for about 20% of patients the obtained material is unfortunately not suited for WGS due to insufficient harvest of tumour cells. Collecting procedures aimed for avoiding necrotic and low tumour purity regions and prioritizing the best suited material for molecular diagnostics should minimise this rate. Over the complete project, on average a maximum of 20% of samples will be expected to drop out due to insufficient quality for WGS, mainly due too low

tumour purity. This means that a WGS based patient report will be generated for a minimum of 160 patients.

Sample collection and processing

Study participants will undergo standard reresection of the tumour by the neurosurgeon as part of SOC. The collection of fresh frozen material will be done according to the standard operation protocol. Upon tissue collection, multiple samples will be sent to the pathology department of the neurosurgical center. After confirmation of the diagnosis recurrent glioblastoma, samples including information regarding the tumour cell percentage will be shipped to Hartwig for processing. Although the aim is to use 200ng of DNA as input for WGS, all tumour samples with a minimum of 50ng of DNA will be processed. Although not used in this study, RNA will simultaneously be isolated from the same tumour tissue and biobanked for later usage like whole transcriptome sequencing. In addition, a 10mL blood sample will be collected from the patients to isolate normal germline DNA (i.e., not only from the tumour) in order to be able to discriminate somatic mutations from the patient's germline DNA background variations. After diagnostic procedures by Hartwig, the samples will be stored in the local biobanks of the corresponding centers.

DNA sequencing

Only tumours with at least 20% tumour purity will be further processed for deep sequencing by WGS. The tumour purity will be maximised by collecting multiple samples from different regions of the tumour to avoid radionecrotic samples. WGS of the tumour DNA will be performed according to the previously described standard procedures.(21) Samples with the required tumour purity will be deep-sequenced on Illumina Novaseq to an average depth of 90-100x and the blood control samples to a depth of 30-35x. Thus, a total of four 'standard 30x' genome equivalents are generated per patient to be able to filter for abundantly present germline variants and to deal with tumour heterogeneity and presence of non-tumour cells in the tumour sample. This enables the reporting of somatic variants and therapeutically actionable mutations. Hartwig has established procedures for WGS under ISO17025 accreditation and the WGS based test is already used in routine diagnostics for other indications (e.g. Cancer of Unknown Primary) and in various hospitals in The Netherlands.

Treatment decision

The WGS report that will be made available by Hartwig (see *Supplement 1* for an example) will be sent to the local pathologist and local study coordinator, who will add the report to the electronic patient files and enters relevant information to a

nationwide network and registry of histo- and cytopathology in The Netherlands (PALGA: Pathologisch-Anatomisch Landelijk Geautomatiseerd Archief).(23) In addition, patient reports will be returned to the treating medical specialist as well as to central and local principal investigators. The neuro-oncology team will discuss the results and allocate subsequent treatment accordingly. If needed, the local neuro-oncologist can consult a centralized molecular tumour board which will also receive the anonymised report for central data management. In case of a persistent discordance between the results of WGS and SOC diagnostics, the SOC findings will be leading in the treatment decision. Such discrepancies will be followed up with revalidation of the results (e.g. to exclude sample heterogeneity as a cause) including the use of an independent orthogonal assay when needed.

Ethical considerations

Every patient will be extensively informed about the study goals and (potential) patient impact by a local research nurse, nurse practitioner or clinical specialist, and will have to sign an informed consent before participating in the study. Potential study participants will get one to two weeks, the time between planning surgery and the operation date, to decide on participating and will get the opportunity to ask additional questions or consult the independent expert of the study. Apart from consenting to the collecting, storage and use of their tumour and blood material, the patients will be asked for their consent to being informed about relevant inherited findings in germline DNA and, if so, under which conditions. Participants can limit this choice to disease that are preventable or treatable and can provide their preference for family to obtain access to heritable information after being deceased. This germline consenting model is optimized based on patient preferences(24) and also was applied in the CPCT-02 (open, NCT01855477), WIDE (closed)(25) and DRUP (open, NCT02925234) studies. All adverse events (AEs) reported spontaneously by the subject or observed by the investigator or his staff will be recorded. All AEs will be followed until they have ended, or until a stable situation has been reached. Depending on the AE, follow-up may require additional tests or medical procedures.

Primary endpoints

Dual primary endpoint is the percentage of patients who receive targeted therapy based on the WGS report and OS. The OS of these patients will be compared to the OS of patients in the historical cohort, who have not had WGS based treatment, and should be improved by three months at least.

Secondary endpoints

Tissue collection and reports

The aim is that at least in 85% of all patients included tumour and blood collection will be successful. Feasibility of routine WGS analysis in this patient population will be measured by the percentage of patients for whom a successful WGS report can be generated. The aim is that at least 80% of the patients for which tumour and blood material was collected will receive a WGS report. Reasons for not being able to produce a patient report based on WGS include low or no tumour cellularity of the available tumour material (expected 15 to 20% based on previous experiences), low DNA yield or quality (e.g. due to necrosis, <3%), and technical failures (<2%).

Targeted treatment options

Another important endpoint is the added value of WGS indicated by the number of targeted treatment options identified. As mentioned before, actionability is based on information in public knowledge bases and can be split by ESCAT classification evidence levels.(22) Because the ESCAT levels are not yet available in public knowledge bases, Food and Drug Administration (FDA) approved drugs and drugs for which a trial is currently available, based on the JAX CKB clinical knowledgebase, will be reported by Hartwig. Interpretation of the genomic variants in terms of pathogenicity and actionability will be done by using criteria for classifying pathogenic variants(26) and expert interpretation in molecular tumour boards.

The expectation is that at least one potentially actionable DNA alteration should be identified in at least 75% of the patients with a WGS report. Consequently, the number of experimental treatments available for these patients with a recurrent GBM will be measured. At least 50% of the identified indications should be available (albeit off-label drugs) through a study, including the DRUP study. A third endpoint regarding targeted treatment options is a doubling of the number of patients starting a targeted treatment in presence of one or more actionable variants (i.e. from 16% to 32%). We aim to dissect this increase for improvements due to diagnostics and/or availability of novel drugs by both comparing historic diagnostic yields as well as treatments given and outcomes.

Progression free survival

Finally, data about the median progression free survival after reresection will be collected by calculating the time between the date of the reresection and the date of clinical and/or radiological progression. The aim of the GLOW study is to improve the median progression free survival by at least three months for the patients who

are treated based on WGS results compared to patients in the historical cohort who are not treated based on WGS results.

DISCUSSION

The GLOW study is a unique trial since it is the first time that patients with a recurrent glioblastoma will prospectively obtain a standard-WGS analysis to identify targeted treatment options that could help treatment decision after reresection. The prognosis in this patient population remains very poor, and several questions about the best treatment strategy at the time of first recurrence of the tumour are still unanswered. This study aims to generate evidence for the added value of WGS as a routine diagnostic in this patient population. If a significant benefit is demonstrated, this will show cost effectiveness. However, it is important to be aware of the limitations of this study.

From a patient's perspective, it can be essential to know everything is done to give them an opportunity of a targeted treatment, whether experimental or not. Notwithstanding, it is crucial to remember that the GLOW study will not investigate the treatments itself, but focusses on the clinical effect of a different diagnostic strategy. We do fully realise that with today's knowledge and available drugs, this study may not reach successful endpoints due to limited effectiveness of the mostly experimental treatments that will be given based on WGS. Secondary endpoints, as the feasibility of routine WGS diagnostics, are therefore also important for determining next steps as the future targeted drug portfolio is likely to be expanded significantly.(27, 28) Another potential limitation could be the situation in which an actionable target is found in absence of a recruiting drug study. However, previous studies on WGS based diagnostics in cancer, i.e. the beforementioned CPCT-02 and WIDE studies, do not support this potential objection. Moreover, experimental targets will not be reported to avoid these situations. At the same time, a close monitoring of the expanded use of existing anticancer drugs could lead to new treatments.(29) Finally, the heterogeneity of glioblastoma, tumour penetrating issues and pathway redundancy are all limitations that could hamper successful targeted treatments and should therefore be kept in mind when analysing the results of this study.

In conclusion, the GLOW study aims to investigate the feasibility, validity, utility and value of WGS for recurrent GBM patients. This will allow for disclosure of potentially novel targets for therapy for these patients.

ABBREVIATIONS

AEs: adverse events; CIViC: Clinical Interpretation of Variants in Cancer; CKB: Clinical Knowledgebase; DMC: data monitoring committee; ESCAT: ESMO Scale for Clinical Actionability of Molecular Targets; ESMO: European Society for Medical Oncology; FDA: Food and Drug Administration; GBM: Glioblastoma; GLOW: GLioblastoma targeted treatment Option maximization by Wgs; HMC: Haaglanden Medical Center; IDH: isocitrate dehydrogenase; KPS: Karnofsky Performance Status; MGMT: O6-methylguanine DNA methyltransferase; OncoKB: Oncology Knowledge Base; OS: overall survival; PALGA: Pathologisch-Anatomisch Landelijk Geautomatiseerd Archief; SOC: Standard of care; UMCU: Utrecht University Medical Center; WGS: Whole genome sequencing; wt: wildtype

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

The study has been approved by the Medical Ethical Committee of Leiden/The Hague/Delft (METC LDD), and by the participating sites, at the start of the study. All patients will be asked for written informed consent before they can participate in this study.

AUTHOR CONTRIBUTIONS

MPvO, MLDB, JJMvdH, EC and FYFV are responsible for the conception and design of the study and drafting of the manuscript. MJvdB, JAFK, SLNM, LVB, AC, MEvL, MJV, HBF, AH, KHK, CMLD, HRJ and PAJTR are responsible for drafting and revision of the manuscript. All authors have read and approved the manuscript.

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AVAILABILITY OF DATA AND MATERIALS

The datasets obtained during the current study, data management procedures or the full protocol will be available from the corresponding author upon reasonable request. Genomics data and certain clinical data of patients that have given consent for re-use of their data are made readily available through the standard controlled access mechanism of the Hartwig Medical Foundation (see https://www.hartwigmedicalfoundation.nl/applying-for-data/ for details and application forms).

CONFLICT OF INTEREST STATEMENT

JJMvdH and EC are employed by Hartwig Medical Foundation.

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7

Chapter 7

Glioblastoma targeted treatment option maximization by whole genome sequencing (GLOW): an interim analysis

Under review (2025)

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ABSTRACT

Background. At the time of glioblastoma recurrence, treatment options remain limited. This study presents the results of the interim analysis of the GLOW study: a study investigating the potential added value of routine whole genome sequencing (WGS) diagnostics in patients with recurrent glioblastoma to identify potentially actionable variants for targeted therapy.

Methods. The GLOW study is a prospective, diagnostic, multicenter cohort study for adult patients undergoing surgery for glioblastoma *IDH*wt recurrence. We analyzed the results of the first 100 patients. Primary outcomes were the percentage of patients who received targeted therapy based on the WGS reports, and overall survival of all patients. Secondary outcomes included, among others, the diagnostic success rate and targeted treatment options identified.

Results. In 80% of the patients a successful WGS report was delivered. Targeted treatment options as assessed by relevant medical experts were identified in 29% of these patients, and targeted treatment was eventually initiated in 7.5%. Several reasons for not starting treatment were identified. The median progression free and overall survival for these six patients were 1.87 months (95% CI 1.40-2.34) and 18.1 months (95% CI 6.48-29.8), respectively. No ESCAT level I-II variants were found.

Discussion. Although the diagnostic success rate for WGS analysis was high and potentially actionable variants were identified, the clinical impact in terms of targeted therapy initiation was low, especially in the absence of targeted drugs. Genome-driven trials are urgently needed to create the evidence for (in)efficacy of molecularly matched treatments in patients with recurrent glioblastoma.

Keywords. Glioblastoma, recurrence, whole genome sequencing, targeted therapy

INTRODUCTION

Glioblastoma, the most common malignant primary brain tumor, inevitably recurs despite intensive initial treatment consisting of maximal safe surgical resection followed by chemoradiation and adjuvant chemotherapy with temozolomide.(1) At the time of recurrence, evidence regarding the optimal treatment strategy is limited and highly relies on individual patient characteristics, resulting in unspecified standard-of-care treatment.(2) Commonly suggested therapies include re-resection followed by radiation and/or chemotherapy(3, 4), chemotherapy alone (re-challenge temozolomide, or nitrosoureas)(5, 6) or radiotherapy alone.(7, 8) However, limited effectiveness illustrates the urgent need for new treatment strategies. While targeted treatment options are increasingly available for cancer patients in general, studies on molecular targets for patients with recurrent glioblastoma are not yet translated into clinical advantages.(9) In an attempt to boost the strategy of targeted treatment by evaluatating the diagnostic value of extensive molecular diagnostics, the *Glioblastoma targeted treatment Option maximization by Wgs* (GLOW) study has been initiated. Patients with a first recurrence of glioblastoma and who undergo standard-of-care surgery are included and receive whole genome sequencing-based diagnostics (WGS). The main goal of this study is to determine the percentage of patients for whom targeted therapy could be initiated based on the WGS results.(10) Here, we present the results of the interim analysis of the GLOW study.

METHODS

Study population and procedures

The GLOW study is a prospective, diagnostic, single arm, multicenter cohort study in which adult patients participate who undergo neurosurgery for first recurrence of glioblastoma isocitrate dehydrogenase 1 and 2 wildtype (*IDH*wt).(10) The entire study will close after the inclusion of 235 patients. Here, we present the results after inclusion of the first 100 patients in relation to predefined key drivers for success. These patients underwent re-resection or re-biopsy of the tumor as part of standard-of-care. Tumor samples have been analyzed by WGS at Hartwig Medical Foundation, Amsterdam.(11) Subsequently, the results of these WGS analyses have been returned to the local team of the patients' treating physicians. Tumor samples with a tumor cell percentage (TCP) of <15% were not deep-sequenced as false negative rates for variant detection will become too high at the standard 100x sequencing depth for the tumor.

Outcomes

The primary outcomes were the percentage of patients who received targeted therapy based on the WGS reports, and overall survival (OS) of all patients. OS was defined as the time between the first histopathological diagnosis and death. Secondary outcomes included the diagnostic success rate (i.e. the percentage samples in which the tumor cell percentage was ≥15% and a WGS report could be delivered), the targeted treatment options identified, targeted therapy initiation, and the median progression free survival (PFS) and OS for patients who were treated based on the WGS results. The PFS was defined as the time between the start of targeted therapy and the magnetic resonance imaging (MRI) on which new progression was seen.

Biomarker actionability

The number of targeted treatment options identified was based on potential actionability on biomarker level. The potential actionability was first based on the variants reported by Hartwig, which was in turn based on information in public knowledge bases, including the Clinical Knowledgebase (CKB, Genomenon) and Oncology Knowledge Base (OncoKB). Interpretation of potential clinical actionability in the clinical context of these reported variants was done by an expert team of clinical oncologists (HHN, JJMvdH and FYFdV) and a clinical scientist in molecular pathology (PR). To translate the 'potentially actionable variants' to 'actionable variants', these experts annotated all reported, potentially actionable variants for every patient, individually and blinded for the other experts' annotations. Disagreements were solved in consensus, resulting in a list of actionable variants in the current study population. This list was not shared with local treatment teams, so treatment initiation was independent of our experts' annotations. Finally, the variants were split by evidence levels according to the six level ESCAT (ESMO Scale for Clinical Actionability of molecular Targets) classification.(12) The classification from another recent study could be used for some of the variants.(13)

Statistical analysis

Sociodemographic and clinical characteristics of the patients were described. Continuous variables were reported using the median together with the interquartile range (IQR). Median survival rates were calculated using Kaplan-Meier curves and reported with the 95% confidence interval (95% CI). The time to recurrence was defined as the time between the first resection and the first radiological recurrence. The post-progression survival was calculated from the date of re-resection. Patients for whom the date of death was unknown at the time of this interim analysis were censored at the moment of last follow-up. Since patients will potentially receive a

broad range of treatments with variable outcome expectations, no formal statistical comparisons of survival rates between patient subgroups were made. Statistical analyses were performed using statistical package *IBM SPSS Statistics for Windows* version 28.0.

RESULTS

Patient characteristics

The patients were included between August 2022 and September 2024, in nine different Dutch hospitals. The median age at recurrence was 60.0 years (IQR 51.3-68.0), and the male/female ratio was 2.8:1. The median time to recurrence, calculated from the first resection, was 14.8 months (IQR 9.91-22.7). At recurrence, 12% had a Karnofsky performance status (KPS) of 70 and 88% had a KPS of 80 or higher. The median follow-up after re-resection was 6.65 months (IQR 3.55-9.64). 59% of the patients had died at the end of this follow-up, with a median post-progression survival of 8.94 months (95% CI 7.92-9.96). Overall, the median OS in the cohort was 29.9 months (95% CI 26.3-33.5). More details of the patient characteristics can be found in *Table 1*.

Table 1. Patient characteristics.

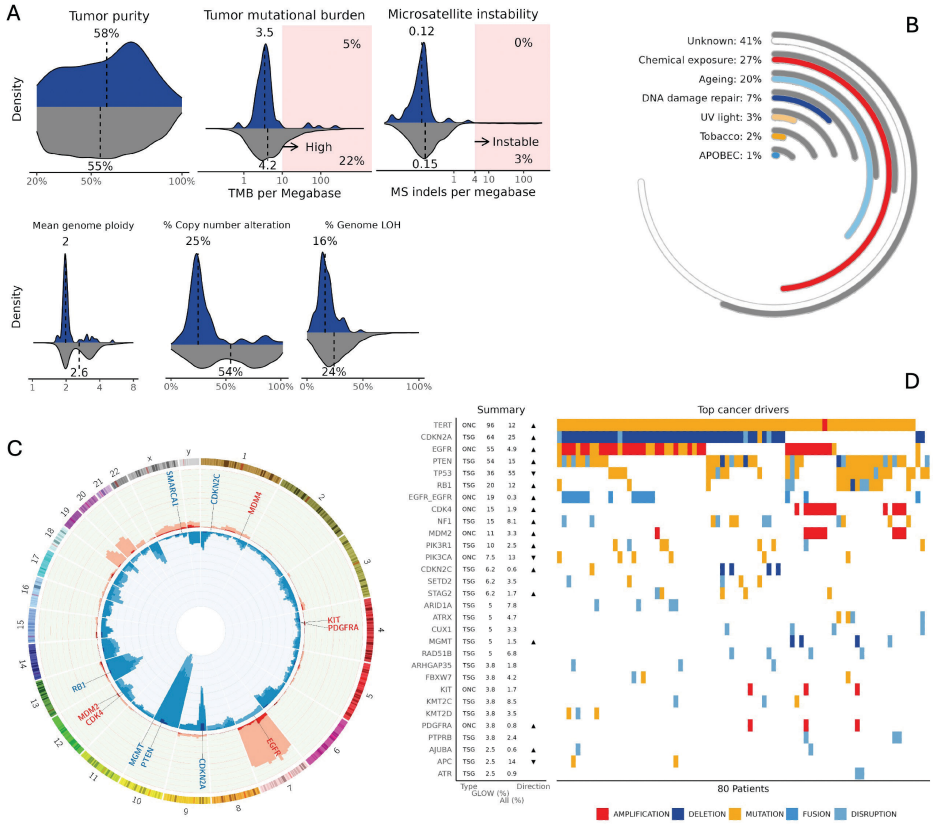
Characteristics	Total cohort ***n*** **= 100**
Gender, no.	
Male	74
Female	26
Median age at recurrence, years (IQR)	60.0 (51.3-68.0)
Hemisphere, no.	
Left	41
Right	2
Bilateral	57
Tumor lobe involvement, no.	
Cerebrum (incl. basal ganglia, insula, thalamus)	0
Frontal	44
Temporal	42
Parietal	26
Occipital	13
Ventricles	1
Cerebellum	0
Brainstem	0
Corpus callosum	2
Extent of resection, no.	
Biopsy	6
Subtotal resection	36
Gross total resection	58
MGMT promoter methylation, no.	
Yes	40
No	40
Unknown	20
First line treatment, no.	
Stupp chemoradiation	77
Elderly scheme chemoradiation	16
Radiotherapy monotherapy	2
Chemotherapy monotherapy	1
Other	4

Characteristics	Total cohort *n* = 100
First line treatment completed, no.	
Yes	85
No	15
Median time to recurrence, months (IQR)	14.8 (9.91-22.7)
Extent of re-resection, no.	
Biopsy	9
Subtotal resection	36
Gross total resection	43
Unknown	12
KPS at recurrence, no.	
70	12
80	24
90	39
100	25

Diagnostic WGS

The diagnostic success rate was 80%, meaning that 80 WGS reports were delivered (*Figure 1*). Main reason for failure was an insufficient TCP to obtain reliable WGS results. The median overall turnaround time between blood and tumor tissue arrival at Hartwig and return of the WGS report date was 9.0 working days (IQR 7.0-10.0). This was 9.0 working days (IQR 8.0-10.0) for successful WGS reports (which includes a shallow-sequencing procedure (8x depth) to assess tumor purity, followed by deep-sequencing (100x depth)) and 6.0 working days (IQR 5.0-7.0) for samples in which the TCP appeared to be too low (i.e. <15%) at quality checks (only shallow-sequencing procedure). More details on the genomic landscape of 80 out of the 100 GLOW patients can be found in *Figure 1A-D*.

Figure 1. The genomic landscape of the GLOW study patients.



Genomic characteristics of the 80 GLOW patients compared to overall Hartwig database characteristics from 7462 metastatic pan-cancer cancers. (**A**) Mutational landscape characteristics: Sample tumor purity, tumor mutational burden (TMB), microsatellite instability, mean genome ploidy, % of copy number alteration, % of genome loss-of-heterozygosity (LOH). Data of GLOW patients shown in blue, pan-cancer reference data in grey. (**B**) Processes underlying mutations based on single base substitutions and trinucleotide contexts (cosmic v3.4) and grouped based on proposed etiology. (B) Copy number alteration profile of the combined 80 GLOW tumors. Inner ring shows the percentage of tumors with observed LOH (light blue), LOH and significant copy number loss (<0.6x ploidy, blue), and full gene deletion (0 copies, dark blue). Outer ring shows percentage of tumors with high level amplification (>3x ploidy, dark red), moderate amplification (2-3x, red) and low level amplification (1.4-2x, light red). Frequently observed high-level driver genes are indicated in red (amplification) and blue (loss). (**D**) Oncoplot of the top cancer driver genes. The % of GLOW patients with an observed aberration and compared to the pan-cancer database. An arrow (up- or downwards) indicates a statistical difference in incidence rate of the 80 GLOW patients. This figure is based on output provided in the Cancer Vignettes (https://www.hartwigmedicalfoundation.nl/en/data/vignettes/).

Treatment option identification

In the 80 patients with a WGS report, at least one CKB level A target was identified in 23 patients (29%). In the remainder (57/80, 71%), CKB level B was the highest

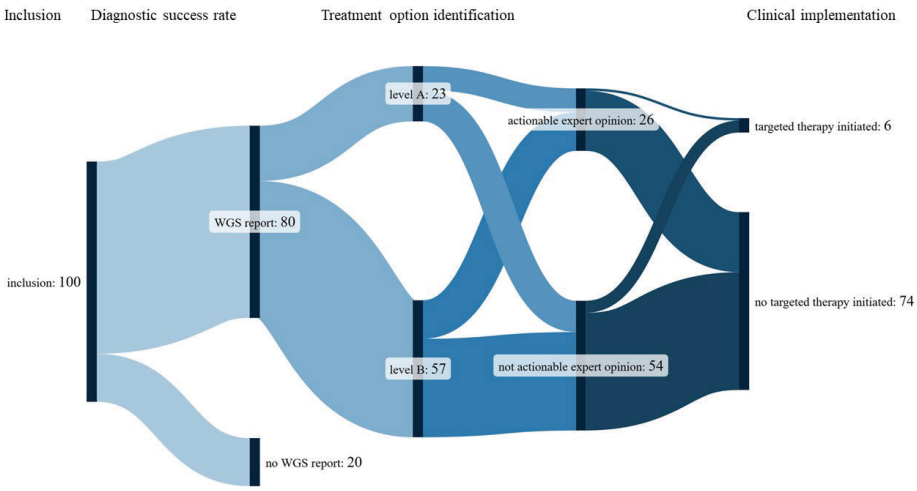
evidence found. In these 23 patients with one or more level A targets, ten patients had at least one actionable variant according to the expert panel opinion. Another sixteen patients with maximum level B variants were classified as having at least one actionable variant by the experts, resulting in a total of 26 patients with one or more actionable variants (*Figure 2*).

The variants in this cohorts classified as actionable according to the expert panel at the time of WGS report, were found in the following genes: *BRAF*, *BRCA2*, *CHEK2*, *FGFR1*, *KDR*, *MDM2*, *MET*, *MSH6*, *NF1*, *POLE*, *RAD51B* and *ROS1*. In addition, a high tumor mutational burden (TMB) was considered actionable (accounting for temozolomide associated mutational signature 11). See *Supplemental S1* for an overview of the experts' individual annotations and consensus list of reported variants. No ESCAT level I-II variants were found. The most prevalent variant was an inactivating mutation in the *NF1* gene, observed in 10% of the patients (ESCAT level IIIA(14, 15)). See *Table 2* for the specific events, treatment examples and the population frequency.

Table 2. Actionable targets in total cohort (n=100).

Gene	Event	Example	ESCAT level	% in cohort
NF1	inactivating mutation	trametinib	IIIA (14, 15)	10%
MDM2	amplification	milademetan	IV (16, 17)	5%
-	High TMB	nivolumab	IIIB (18-20)	4%
KDR	overexpression	sunitinib	IV (21, 22)	2%
MSH6	inactivating mutation	nivolumab	IIIB (18, 20, 23)	2%
BRAF	fusion	trametinib binimetinib (compassionate use)	IIIA (24)	1%
BRCA2	inactivating mutation	PARP inhibitors	IIIB (25-27)	1%
CHEK2	inactivating mutation	PARP inhibitors	IV (28)	1%
FGFR1	activating mutation	erdafitinib	IIB (29-31)	1%
MET	fusion	cabozantinib (compassionate use)	IIIA (32-34)	1%
POLE	mutation	nivolumab	IIIB (35, 36)	1%
RAD51B	inactivating mutation	PARP inhibitors	IIIB (37, 38)	1%
ROS1	fusion	entrectinib	IIIA (32, 39)	1%

Figure 2. Sankey diagram visualizing flows per study phase.

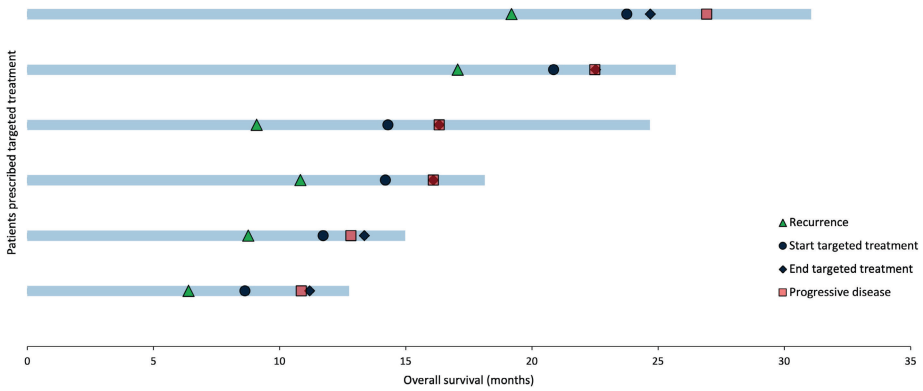


Level A: Food and Drug Administration (FDA) approved therapy and/or guidelines based on the Clinical Knowledgebase, Level B: late clinical trials based on the Clinical Knowledgebase, WGS: whole genome sequencing.

Targeted therapy initiation

Overall, 6/80 (7.5%) of the patients were prescribed targeted treatment based on the WGS results. This was done by local physicians' decisions, independent of the expert panel annotation. These treatments consisted of the following: erlotinib for *EGFR* amplification/p.Ala289Val activating mutation/p.Ala289Thr activating mutation, abemaciclib for *CDK4* amplification, dacomitinib for *EGFR* p.Ala289Val/p.Ser229Cys activating mutations and entrectinib for *ROS1-GOCP* fusion. The median duration on these drugs was 1.76 months (IQR 1.44-2.14), with main reasons for discontinuation being adverse events and further tumor progression. As per drug repurposing protocol, the effectiveness was evaluated by MRI two months after targeted treatment initiation. The median PFS for these six patients was 1.87 months (95% CI 1.40-2.34) and the median OS was 18.1 months (95% CI 6.48-29.8). See *Figure 3* for a visualization of the course of the disease in these patients.

Figure 3. Swimmers plot visualizing course after targeted treatment.



As can be seen in *Figure 2*, the large majority (96%, 25/26) of the patients in whom one or more targeted treatment option(s) were identified according to the expert panel opinion, did not start with targeted treatment. Evaluating the physicians' arguments (that were given in about a quarter of the cases), three main reasons could be identified to clarify this discrepancy between treatment option identification and not starting targeted treatment. First, it appeared that physicians hesitated to start experimental therapy at the time of recurrence. Instead, they opted for (rechallenge) chemotherapy or re-irradiation (initiated in 62% [16/26] of these patients), while "saving the WGS results for the time of a probable future second recurrence." Second, the variant-drug combination was deemed not meaningful in the local tumor board. The third observation was that, when the physician was willing to initiate targeted therapy, drug repurposing programs required measurable disease at the start of the treatment for assessment of treatment response, thereby excluding patients in whom gross total re-resection was achieved.

On the other hand, for five out of the 54 patients for whom WGS could not identify an actionable target according to the experts' opinion, targeted treatment was initiated by the treating physician. In these cases, variants were deemed meaningful in the local tumor boards. The treatments in these five patients were: abemaciclib (for *CDK4* amplification), dacomitinib (for *EGFR* activating mutations) and three times erlotinib (for *EGFR* amplification/ activating mutations). Afterwards, these variants were deemed not meaningful in the expert panel**.**

DISCUSSION

This study presented the results of the interim analysis following the inclusion of the first 100 patients in the GLOW study, that aims to investigate the clinical value of WGS analysis in patients with a recurrent glioblastoma. Of these 100 patients, targeted treatment options were identified in 23 patients, and targeted treatment was eventually initiated in six patients. No ESCAT level I-II variants were found.

Various lessons have been learned after the analysis of the results of the first 100 patients of the GLOW study. First, we show the feasibility of routine WGS analysis in this patient population, based on the diagnostic success rate as we showed in this study. Moreover, the majority of the WGS reports is sent to the local tumor board within two weeks. That is, in our opinion, a fast and effective diagnostic process to obtain a large amount of genomic information about the patient's tumor. Third, several potentially actionable variants were identified that deserve serious and careful evaluation for clinical implementation.

Several factors for the poor targeted therapy initiation rate can be identified. For instance, the clinical implementation of the WGS results was hampered by the prevalent physicians' opinion that upon recurrence, 'standard therapies' like lomustine and rechallenge temozolomide should be preferred. A substantial number of times, the WGS results were "preserved for potential future recurrence" However, in none of the cases in our cohort WGS-based targeted therapy was actually initiated at the moment of progression after re-resection. Currently, we are performing a follow-up study to describe the barriers in the used of targeted therapies in our patient population, based on a multi-disciplinary panel discussion with the local treating physicians. A second major limitation for targeted therapy initiation in this recurrent glioblastoma population, has to do with the clinical features of glioblastoma. For most experimental approaches in solid tumors a measurable lesion needs to be identified at the entry of the study. After neurosurgery for recurrent glioblastoma, this is mostly not the case since the goal of neurosurgical intervention is maximal safe resection (i.e. dissection of all 'measurable disease'). As a result, patients in the GLOW study were frequently excluded from the DRUP, in which patients can be treated with off-label anti-cancer drugs. To circumvent this for future patients, we decided to prepare a drug repurposing program, specifically designed for glioma patients, to bridge the gap between treatment option identification and available therapies for this population. In the future, the results of this project, called glioma individualized molecular treatment program

(GLIMP), should also synergistically improve clinical implementation of WGS-based treatment option identification.

Our results are comparable with another recent study on actionable molecular alterations in *IDHwt* glioblastoma patients.(13) In this study, there were also limited clinically relevant targets, and only 10.5% (36/442) patients received personalized treatment. The authors described that 10% of their patients had at least one ESCAT IB/IC/IIB variant, identified after next generation sequencing (NGS). Interestingly, they reported one recurrent glioblastoma patient with a *ROS1-GOCP* fusion, who maintained a complete response for 11.3 months on entrectinib. In our study, there was also a patient with a *ROS1-GOCP* fusion on entrectinib, but this patient had a PFS of only two months. Entrectinib was initiated three months after gross total re-resection, when regrowth was visible on the MRI.

One of the main limitations of this study was the lack of a central molecular tumor board annotating all the variants found in this study. Currently, there is an undesirable separation between the assessment of pathogenicity (by clinical scientists in molecular pathology) and actionability (by clinicians like medical oncologists) making actionability interpretation subjective, as also observed in our expert panel. It also appeared that a binary distinction for expert actionability annotation (yes/no) was not as straightforward as it might seem. Another limitation of this study was, as beforementioned, the limited enrollment in clinical drug trials because of inclusion criteria not matched to recurrent glioblastoma patients. As a result, fewer patients were provided experimental drugs than anticipated at time of setup of the study.

The results of this study underline that we are still anything but close to success of targeted therapy in glioblastoma patients. With only 6.0% of the patients receiving targeted therapy, discontinuation after a median of 1.76 months and with a median PFS of 1.87 months, these numbers illustrate that there are still many opportunities for thorough exploration of the potential benefits of targeted treatments for recurrent glioblastoma patients and subsequent treatment strategy optimization.

To conclude, the results of the interim analysis of the GLOW study showed various valuable lessons on the routine use of WGS analysis in recurrent glioblastoma patients. Routine WGS analysis was feasible, fast and generated a large amount of genomic information on potentially actionable variants. Simultaneously, a remarkable drop was observed from high diagnostic success rates (WGS analysis) and potentially actionable variants to poor clinical implementation of the WGS

results and targeted therapy initiation. Well accessible biomarker-driven trials with targeted drugs are urgently needed to create the evidence for (in)efficacy of molecularly matched treatments in patients with recurrent glioblastoma.

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AUTHOR CONTRIBUTIONS

MPvO, JJMvdH, EC, FYFdV and MLDB contributed to the study design. Material preparation, data collection and analysis were performed by MPvO, PR, HHN, JJMvdH, EC, FYFdV and MLDB. The first draft of the manuscript was written by MPvO and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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AVAILABILITY OF DATA AND MATERIALS

The datasets generated during and/or analysed during the current interim analysis are available on reasonable request.

CONFLICT OF INTEREST STATEMENT

None of the authors declare a conflict of interest

Supplemental S1. Experts' individual annotations and consensus list of reported variants.

Gene, event	Expert #1	Expert #2	Expert #3	Expert #4	Consensus, actionable?
CCND2 overexpression	No	Maybe	Yes	No	No
CDK4 amplification	No	Maybe	No	No	No
CDK6 overexpression	No	Maybe	Yes	No	No
CDKN2A loss	No	No	No	No	No
CREBBP loss	Maybe	No	No	No	No
EGFR Activating mutation	Yes	Maybe	Yes	Yes	Yes
EGFR amplification	Maybe	No	Yes	No	No
EGFR overexpression	Maybe	No	Yes	No	No
FGFR1 *activating mutation*	Yes	Maybe	Yes	Yes	Yes
KDR overexpression	Maybe	Maybe	Yes	No	Yes
KIT amplification	Maybe	Maybe	Yes	No	No
KMT2D mutation	No	No	Maybe	No	No
MDM2 amplification	Maybe	No	Yes	Yes	Yes
MSH6 Inactivating mutation	Maybe	No	Yes	Yes	Yes
NF1 inactivating mutation	Maybe	Maybe	Yes	Yes	Yes
PBRM1 inactivating mutation	No	Maybe	Yes	No	No
PDGFRA amplification	Maybe	Maybe	Yes	No	No
PIK3CA activating mutation	Maybe	No	Maybe	No	No
PIK3R1 inactivating mutation	Maybe	Maybe	Maybe	No	No
POLE mutation	Yes	No	Yes	Yes	Yes

Gene, event	Expert #1	Expert #2	Expert #3	Expert #4	Consensus, actionable?
PTEN inactivating mutation	Maybe	Maybe	Yes	No	No
PTEN (partial) loss	Maybe	No	Yes	No	No
RB1 loss/mutation	Maybe	Maybe	Maybe	No	No
TSC1 inactivation mutation/ loss	Maybe	Maybe	Yes	No	No
ARID1A inactivating mutation	No	No	Maybe	No	No
BRCA2 inactivating mutation	Yes	Yes	Yes	Yes	Yes
BRAF-DTD1 fusion	Yes	Yes	Maybe	No	Yes
CHEK2 inactivating mutation	Yes	Yes	Maybe	Yes	Yes
EGFR-EGFR vIII fusion	No	Maybe	Maybe	Yes	No
EP300 inactivating mutation	No	No	No	No	No
ERBB4 activating mutation	No	Yes	No	No	No
High tumor mutational burden	Maybe	Yes	Yes	Yes	Yes
MET-RPH3A/PTN fusion	Yes	Yes	Yes	Yes	Yes
RAD51B inactivating mutation	Yes	Yes	Maybe	Yes	Yes
ROS1-GOPC fusion	Yes	Yes	Yes	Yes	Yes

8

Chapter 8

The role of molecular biomarkers in recurrent glioblastoma trials: an assessment of the current trial landscape of genome-driven oncology

Medical Oncology. 2024;41(11):250.

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ABSTRACT

For glioblastoma patients, the efficacy of targeted therapy is limited to date. Most of the molecular therapies previously studied are lacking efficacy in this population. More trials are needed to study the actual actionability of biomarkers in (recurrent) glioblastoma. This study aimed to assess the current clinical trial landscape to assess the role of molecular biomarkers in trials on recurrent glioblastoma treatment. The database ClinicalTrials.gov was used to identify not yet completed clinical trials on recurrent glioblastoma in adults. Recruiting studies were assessed to investigate the role of molecular criteria, which were retrieved as detailed as possible. Primary outcome was molecular criteria used as selection criteria for study participation. Next to this, details on moment and method of testing, and targets and drugs studied, were collected. In 76% (181/237) of the included studies, molecular criteria were not included in the study design. Of the remaining 56 studies, at least one specific genomic alteration as selection criterium for study participation was required in 33 (59%) studies. Alterations in *EGFR*, *CDKN2A/B* or *C*, *CDK4/6*, and *RB* were most frequently investigated, as were the corresponding drugs abemaciclib and ribociclib. Of the immunotherapies, CAR-T therapies were the most frequently studied therapies. Previously, genomics studies have revealed the presence of potentially actionable alterations in glioblastoma. Our study shows that the potential efficacy of targeted treatment is currently not translated into genome-driven trials in patients with recurrent glioblastoma. An intensification of genome-driven trials might help in providing evidence for (in)efficacy of targeted treatments.

Keywords. Recurrent glioblastoma, clinical trial, molecular testing, targeted treatment, genome-driven oncology

INTRODUCTION

At the inevitable time of glioblastoma recurrence, re-resection, chemotherapy, radiotherapy or combinations of these are still the most commonly used treatment modalities.[1-3] The introduction of targeted therapies and immunotherapies has led to new optimism in other, systemic cancers, although drug resistance and side effects remain challenging drawbacks.[4, 5] New targets and treatments are being investigated, highlighting a continuing interest in precision oncology. In neuro-oncology however, the success rate of targeted therapy is limited to date.[6] This is largely explained by fact that the blood-brain barrier and the blood-tumor barrier hamper effective drug delivery and penetration.[7, 8] The *BRAF p.V600E* mutation is currently the only example of an evidence-based target for recurrent glioma, targeted by dabrafenib/trametinib and with response rates around 30%.[6, 9] In patients with isocitrate dehydrogenase (*IDH*) mutant gliomas, the *IDH* inhibitor vorasidenib showed a significant improvement in the progression-free survival.[10] Other molecular therapies previously suggested in neuro-oncology are either tumor agnostic and/or lacking efficacy in brain tumors, and are therefore not standard-of-care.[6] Thus, although several targets have been studied before, there is still a knowledge gap of potentially actionable targets without solid evidence for either efficacy or inefficacy in glioblastoma *IDH* wildtype (IDHwt) patients.

Therefore, this current lack of evidence of the efficacy of genome-driven oncology in glioblastoma patients should not paralyze the exploration of new potentially actionable targets. For instance, hypothetical druggable alterations were found in all but one of the 42 glioblastoma samples analysed by whole genome sequencing (WGS).[11] At the same time, it was shown that the glioblastoma driver instability after standard-of-care primary treatment affects the design of genome-driven trials.[12] Hence, the feasibility of routinely sequencing the whole genome of patients with recurrent glioblastoma in order to maximize targeted treatment options is currently being explored.[13]

To better address challenges regarding implementation of genome-driven oncology for patients with glioblastoma, (confirmatory) studies are needed to further study the actionability of biomarkers in this population.[1, 6, 14] This study aimed to assess the current clinical trial landscape to describe the role of genome-driven treatment in the trials on recurrent glioblastoma treatment by picturing the specific potentially actionable targets or systemic therapies that are now being investigated.

METHODS

Search strategy

A search in the online database of clinical research studies ClinicalTrials.gov was conducted up to June 13, 2024, to identify clinical trials on recurrent glioblastoma in adults. The search terms ‘glioblastoma’ and ‘recurrent’ were combined with filtering on adult patients. No additional filters were applied. This search strategy on ClinicalTrials.gov automatically included other tumor types, which required manual and record by record screening according to the following criteria.

Selection criteria

This study included all studies on recurrent glioblastoma, primarily based on ClinicalTrials.gov classification and subsequently based on description of the inclusion criteria provided by the investigators. Studies solely on newly diagnosed glioblastoma (in which experimental therapies are not applied) or other tumor types, or studies including pediatric patients or medical devices were excluded. Likewise, studies on imaging, radiotherapy, surgery or anti-cancer diet were also excluded. Diagnostic molecular criteria were not part of the selection criteria. Subsequent selection was based on the current recruitment status: completed, terminated, withdrawn, suspended or no longer available studies were excluded since details on previously studied molecular targets were beyond the scope of this study. Instead, next to recruiting studies, trials with status ‘available’, ‘not recruiting’ or ‘unknown’ were included as well to secure a comprehensive overview of the current and upcoming trial landscape.

Data extraction

The role of molecular criteria in studies included in the final analysis was assessed by reading the detailed description, eligibility criteria and study plan (including design and outcome measures) of the study. For those studies with at least one specific genomic alteration as a selection criterium for study participation, details on target(s) and/or drugs studied and moment of molecular diagnostic (i.e. testing on fresh or archival tissue) were then retrieved. Next to this, details on target analysis method (e.g. DNA or RNA sequencing, immunohistochemistry (IHC) or fluorescent in situ hybridization (FISH)), study phase, number of study participants, and recurrence (first or second) were collected.

RESULTS

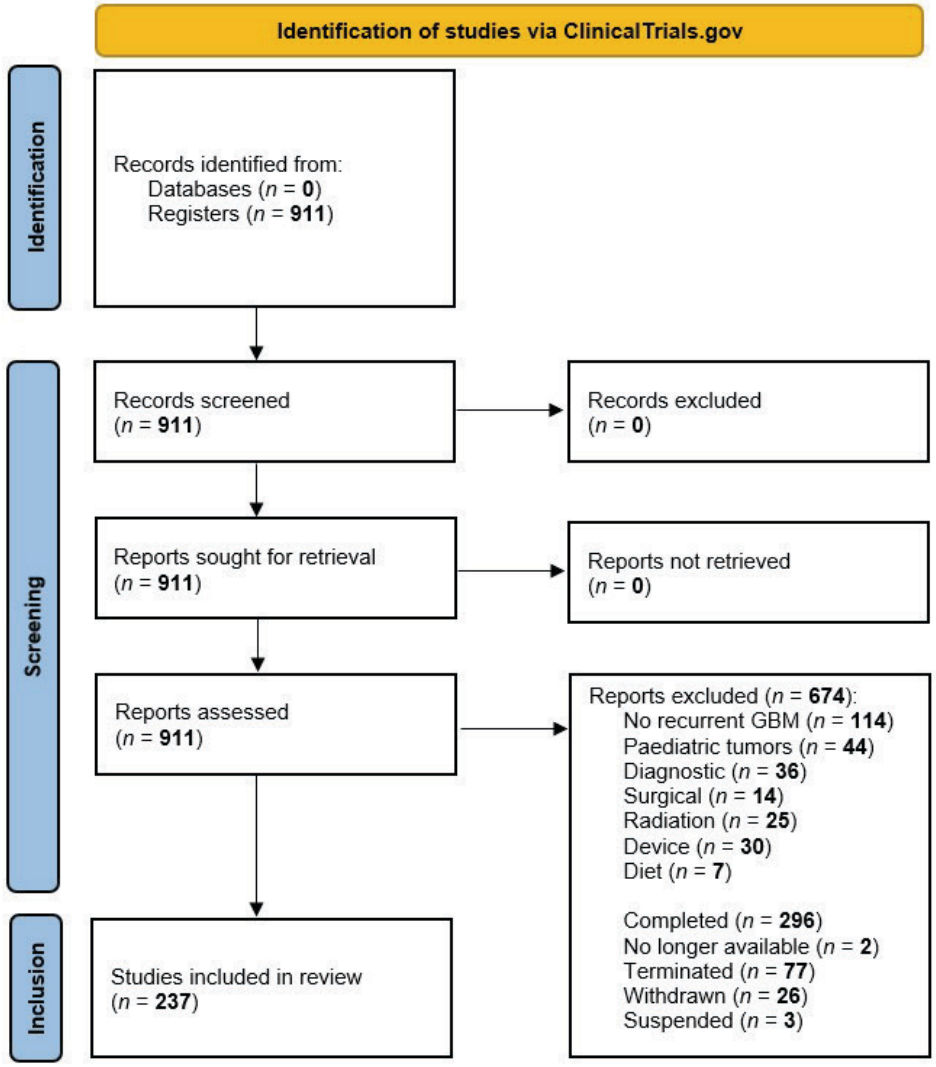
Search results

The search strategy resulted in a total of 911 records. Of these, 270 records were excluded based on the objective and/or design of the study. Subsequently, another 404 records were excluded based on the recruitment status of the study. As a result, a total of 237 records were classified eligible and included for molecular criteria assessment. See *Figure 1* for an overview of the selection process.

Study characteristics

In 181 (76%) of the 237 included studies, molecular criteria (other than diagnostic) were not included in the study design. Of the remaining 56 studies, at least one specific genomic alteration as an upfront inclusion criterium for study participation, was required in 33 (59%) of those studies (*Table 1*). The remaining 23 (41%) studies applied molecular criteria after patient inclusion, for instance for drug response correlation. The mean number of study participants in these 33 studies was 38 (range 10-200). The most frequent study phase was 1 (64%, 21/33), followed by phase 2 (24%, 8/33) and phase 1-2 (12%, 4/33). Looking to the in-/exclusion criteria, in most of these studies the glioblastoma recurrence was not specified (73%, 24/33), but was occasionally limited to first (21%, 7/33) or 'first or second' (6%, 2/33) recurrence. The requirement that molecular testing was performed on fresh tumor material (i.e. at recurrence) was not provided in most studies. In two studies fresh material was used (6%, 2/33), while in 8 studies archival (i.e. from primary setting) and/or fresh tissue was used for molecular testing (24%, 8/33). In the remaining studies either archival tissue sufficed (30%, 10/33) or a requirement regarding the moment of molecular testing was not provided (39%, 13/33). Testing was done by next generation sequencing (NGS) or RNA sequencing (RNAseq) in 8 and 1 of the 33 studies, respectively. IHC, FISH and sequencing of DNA via tumor in situ fluid (TISF) collection were used in 14, 3 and 1 of the studies, respectively, while the target analysis method was not specified in 11 of the 33 (33%) studies.

Figure 1. Study selection process.



GBM: glioblastoma

Table 1. Details of studies including molecular criteria in the study design.

Category	Number (%)
Studies with specific variant(s) as inclusion criterium (*n*=33)	
Genes	
EGFR	11 (33%)
CDK4/6	4 (12%)
CDKN2A/B/C	4 (12%)
RB	4 (12%)
HER2	3 (9%)
PTEN	3 (9%)
ATRX	1 (3%)
BRCA	1 (3%)
FGFR	1 (3%)
FGFR-TACC	1 (3%)
IDH	1 (3%)
KIT	1 (3%)
TERT	1 (3%)
VEGFR	1 (3%)
Proteins	
B7-H3	2 (6%)
MMP2	2 (6%)
CD147	1 (3%)
CND1/2	1 (3%)
mTOR	1 (3%)
p53	1 (3%)
PD-L1	1 (3%)
PDGFRa	1 (3%)
pERK	1 (3%)
Studies investigating systemic therapies (*n*=48)[a]	
Targeted treatment	
Other	15 (31%)
Protein kinase inhibitor	8 (17%)
Tyrosine kinase inhibitor	8 (17%)
PARP inhibitor	5 (10%)
EGFR inhibitor	3 (6%)
Immunotherapy	
CAR-T	10 (21%)

Table 1. *Continued*

Category	Number (%)
Monoclonal antibody	6 (13%)
Other	4 (8%)
Other	
Acetazolamide	1 (2%)
Mycophenolate mofetil	1 (2%)

[a]Total number of therapies is 61 in these studies together. Chemotherapy in studies combining therapy with chemotherapy is not shown.

Targets and therapies investigated

Looking somewhat further into detail, *EGFR* (mutation or amplification**,** *n*=11) was the most frequently investigated gene, followed by *CDKN2A/B or C* (deletion)*, CDK4/6* (amplification) and *RB* (wildtype status), each being investigated in 4 studies. Of the protein targets, B7-H3 and MMP2 were the most frequently (*n*=2 each) studied, both in the context of chimeric antigen receptor T-cell (CAR-T) therapy *(Table 1)*. All these alterations were used as a selection criterium for study participation.

Systemic therapies were investigated in 48 of the 56 studies on molecular criteria, but not all these studies required upfront matching based on at least one genomic alteration (*Table 2*). The majority (*n*=27) of these therapeutic studies investigated one or more targeted therapies. Within the targeted therapy group, abemaciclib was the most frequently studied target-matched (*CDKN2A/B/C, CDK4/6, RB*) drug. Ribociclib, targeting the same genomic alterations, was the second most frequently studied drug. Focusing on immunotherapies, CAR-T therapies were the most frequently studied therapies that, inherently to the principle of CAR-T therapy, required upfront matching based on a genomic alteration. Other therapies being studied in recurrent glioblastoma included acetazolamide and mycophenolate mofetil, both known for potentiating chemosensitivity. In the study on acetazolamide, patients receive concomitant temozolomide, and Bcl-3 expression level is determined to examine the ability of Bcl-3 to predict treatment response. Mycophenolate is studied in combination with temozolomide and/or radiation therapy, and as an exploratory objective, molecular characterization of all glioblastoma tissues by RNAseq is performed.

Table 2. Systemic therapies currently being investigated in recurrent glioblastoma.

Systemic therapy	Molecular matching criterium	ClinicalTrials.gov ID	Study phase
Targeted therapy			
Abemaciclib	*CDKN2A/B/C* inactivation or *CDK4/6* amplification and *RB* wild-type	NCT02981940	Phase 2
	CDKN2A/B/C inactivation or *CDK4/6* amplification and *RB* wild-type	NCT04391595	Early phase 1
	CDKN2A/B inactivation or *CDK4/6* amplification	NCT04074785	Early phase 1
Abexinostat	-	NCT05698524	Phase 1
Afatinib	*EGFR* amplification	NCT05432518	Early phase 1
Anlotinib	*VEGFR/PDGFR/FGFR/Kit* mutation (not specified)	NCT04004975	Phase 2
BDTX-1535	*EGFR* amplification/ mutation/variant	NCT05256290	Phase 1-2
Bevacizumab	-	NCT05540275	Phase 2
	-	NCT02974621	Phase 2
	-	NCT03890952	Phase 2
	-	NCT04074785	Early phase 1
	-	NCT02142803	Phase 1
Cabozantinib	-	NCT05039281	Phase 1-2
Cediranib	-	NCT02974621	Phase 2
Cetuximab	*EGFR* overexpression	NCT02800486	Phase 2
CM93	*EGFR* mutation/ amplification	NCT04933422	Phase 1
Dasatinib	*PDGFR* amplification	NCT05432518	Early phase 1
Everolimus	PI3K/*PTEN*/mTOR activated pathways	NCT05432518	Early phase 1
Lapatinib	*EGFR* amplification	NCT02101905	Phase 1
LY3214996	pERK positivity >30%	NCT04391595	Early phase 1

Table 2. *Continued*

Systemic therapy	Molecular matching criterium	ClinicalTrials.gov ID	Study phase
Navtemadlin	p53 wild-type	NCT03107780	Phase 1
Niraparib	- *ATRX* loss	NCT05297864 NCT05076513	Phase 2 Early phase 1
Olaparib	*TP53* mutation -	NCT05432518 NCT02974621	Early phase 1 Phase 2
Osimertinib	*EGFR* amplification/ mutation	NCT03732352	Phase 2
Palbociclib	*CDK4/6* amplification	NCT05432518	Early phase 1
Ribociclib	*RB* positivity *RB* wild-type and *CDKN2A/B/C* loss or *CDK4/6* amplification or CND1/2 amplification or 9p21.3 deletion	NCT02345824 NCT02933736	Phase 1 Early phase 1
Sapanisertib	- -	NCT02133183 NCT02142803	Phase 1 Phase 1
Selinexor	-	NCT05432804	Phase 1-2
Sorafenib	PDGFRa expression	NCT01817751	Phase 2
Talazoparib	*IDH* mutation, *PTEN* mutation, *"BRCA*ness" signature	NCT04740190	Phase 2
Temsirolimus	mTOR activation	NCT05773326	Early phase 1
Trastuzumab-deruxtecan	*HER2* expression	NCT06058988	Phase 2
Verteporfin	*EGFR* amplification/ mutation	NCT04590664	Phase 1-2
Immunotherapy			
Anti-PD-L1 CSR T cells	PD-L1 positivity	NCT02937844	Phase 1
Atezolizumab	- -	NCT06069726 NCT05039281	Phase 2 Phase 1-2

Systemic therapy	Molecular matching criterium	ClinicalTrials.gov ID	Study phase
CAR-T B7-H3	B7-H3 positivity	NCT04385173	Phase 1
	B7-H3 positivity	NCT04077866	Phase 1-2
CAR-T CD147	CD147 positivity	NCT04045847	Early phase 1
CAR-T Chlorotoxin	MMP2+ expression	NCT04214392	Phase 1
CAR-T CHM-1101	MMP2+ expression	NCT05627323	Phase 1
CAR-T EGFR-IL13Ra2 cells	*EGFR* amplification	NCT05168423	Phase 1
CAR-T EGFRvIII	EGFRvIII expression	NCT05802693	Early phase 1
	EGFRvIII expression	NCT02844062	Phase 1
	EGFRvIII expression	NCT06186401	Phase 1
CARv3-TEAM-E T cells	EGFRvIII mutation/*EGFR* amplification	NCT05660369	Phase 1
	-	NCT05024175	Phase 1
Erdafitinib	*FGFR-TACC* fusion	NCT05859334	Phase 2
Erlotinib	-	NCT00054496	Phase 2
Ezabenlimab	-	NCT03383978	Phase 1
Lerapolturev	-	NCT04479241	Phase 2
Memory-enriched T-cells	*HER2* expression	NCT03389230	Phase 1
Nivolumab	-	NCT03890952	Phase 2
NK-92/5.28.z	*HER2* expression	NCT03383978	Phase 1
Pembrolizumab	-	NCT04479241	Phase 2
	-	NCT03277638	Phase 1-2
Tislelizumab	*PTEN/TERT* mutation (not specified)	NCT05540275	Phase 2
Other			
Acetazolamide	-	NCT03011671	Phase 1
Mycophenolate mofetil	-	NCT05236036	Phase 1

DISCUSSION

This study aimed to assess the current clinical trial landscape to assess the role of molecular biomarkers in trials on recurrent glioblastoma treatment. In 76% (181/237) of the included studies, molecular criteria (other than diagnostic) are not included in the study design. *EGFR* amplifications/mutations are the most frequently investigated genomic alterations, followed by *CDKN2A/B or C* deletion*, CDK4/6* amplification and *RB* wildtype status. Abemaciclib and ribociclib are the most frequently studied targeted therapies, while CAR-T therapies form the majority of our selection of the current trials on immunotherapy.

Currently, the established treatment options for patients with recurrent glioblastoma remain limited and far from being targeted to individual molecular characteristics.[1] Despite several attempts, the results of genome-driven oncology in the glioblastoma population so far are mixed and mostly disappointing.[15] First, the role of the blood-brain barrier and the blood-tumor barrier in relation to the efficacy of targeted treatments is an important factor to take into account.[7, 8] In addition, presence of a potential target does not automatically means initiation of targeted treatment: an implementation gap is noticed between the finding of hypothetical druggable targets and the acting on that finding.[16] Challenges for genome-driven oncology as observed in that study include target credentialing and validation, tumor heterogeneity and clinical trial design. Notwithstanding these challenges, experts emphasize the need for (confirmatory) studies to further study the actual actionability of biomarkers in glioblastoma patients.[1, 6] An excellent example is the N2M2 study in patients with newly diagnosed glioblastoma without methylation of the O6-methylguanine-DNA methyltransferase (MGMT) promoter, a phase I/IIa umbrella trial of molecularly matched targeted therapies.[17] The recently presented results of this N2M2 study (NCT03158389) show clinical activity of temsirolimus in patients demonstrating mTOR activation while palbociclib has no clinical activity in patients with *CDK4* amplification or *CDKN2A/B* codeletion.

Our assessment of the clinical trial landscape shows that the majority (76%) of the current trials aim to treat recurrent glioblastoma regardless the molecular characteristics of the tumor. More specifically, studies with upfront selection based on molecular alteration(s) to study the efficacy of certain drugs form a minority (14%) of the current clinical trial landscape. These early phase studies, in turn, are weakened by the fact that molecular testing on fresh tumor material at recurrence is required in less than 30 percent of the studies. Reflecting on these outcomes, some comments need to be made. First of all, the yield of extensive molecular

screening for potentially actionable alterations and subsequent targeted treatment is not undebated. For instance, after NGS analysis in more than 400 glioblastoma patients, personalized treatment was initiated in only 11% of the patients.[18] At the same time, WGS analyses showed that glioblastomas harbor potentially actionable alterations in the majority of the cases.[19, 20] A second remark is that trials with extensive molecularly analyzed glioblastomas require good access to molecular tests, which is not the case all over the world. Third, the observation that fresh tumor material at recurrence is not required in the majority of the studies, may be indicative of the fact that current standard practices prove difficult to adapt to optimal molecular diagnostics.

This study has some limitations to be considered. First, the selection of the clinical trials was purely based on the registration on ClinicalTrials.gov, which allows for an incomplete snapshot of the trials going on since new studies can be registered on ClinicalTrials.gov on a daily basis. A second limitation is that the recruitment status of a study could be outdated since actual status is dependent on update information provided by the research team itself. As a result, studies no longer recruiting may have been erroneously included in this assessment of the current trial landscape. On the other hand, our study design ruled out studies no longer recruiting, potentially resulting in the loss of interesting new information on treatment targets. Nevertheless, the methods used in this assessment ensure a fair assessment and indication of the current clinical trial landscape. Finally, this study did not investigate (recently) completed or terminated trials, which would have been interesting to compare previously studied targeted drugs with currently experimental therapies. As a result, our study does not allow any conclusions about past efforts in the field of genome-driven oncology for patients with recurrent glioblastoma.

To conclude, this study provided an insight into the current trials on the role of molecular biomarkers in trials on recurrent glioblastoma treatment. Currently, the need for new studies with upfront selection based on molecular alteration(s) to study the efficacy of certain drugs is not yet translated into genome-driven trials being conducted. Our results emphasize that, in order to move the field of neuro-oncology into the direction of personalized medicine and to bridge the knowledge gap, an intensification of genome-driven trials is needed.

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AUTHOR CONTRIBUTIONS

MPvO and MLDB contributed to the study conception and design. Data collection and analysis were performed by MPvO and MLDB. The first draft of the manuscript was written by MPvO and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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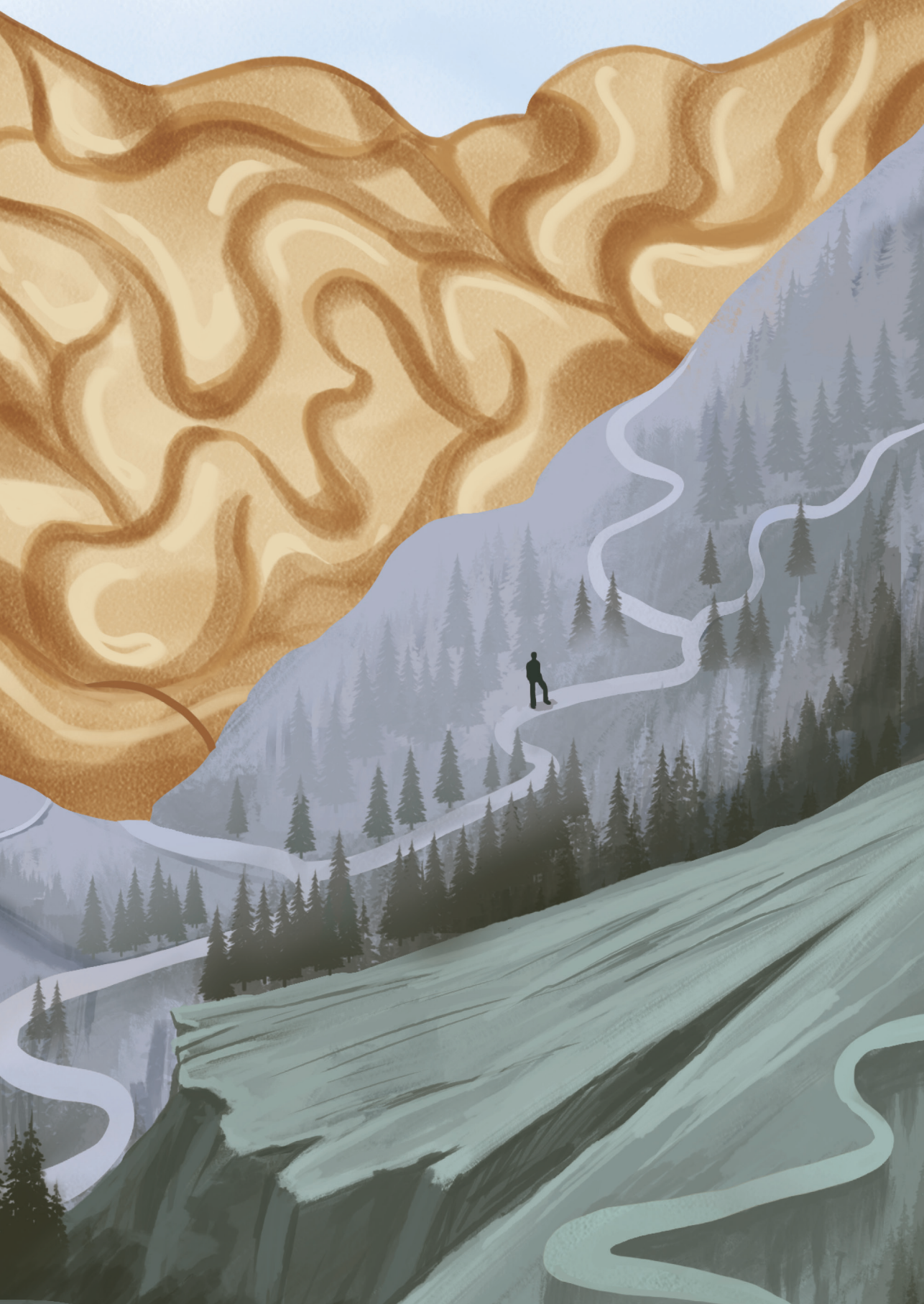
AVAILABILITY OF DATA AND MATERIALS

The datasets used and/or analysed during the current study available from the corresponding author on reasonable request.

CONFLICT OF INTEREST STATEMENT

None of the authors declare a conflict of interest



PART III

Practical implications

9

Chapter 9

Genome sequencing-based analysis of genetic predisposition to adult glioblastoma

Under review (2025)

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ABSTRACT

The hereditary of adult glioblastoma is still largely unexplored. With the option of broad molecular testing, it is crucial that clinicians are aware of the a priori probability of finding germline predisposition in glioblastoma patients. Here, we studied the genetic predisposition to adult glioblastoma using paired tumor-normal WGS data in an unselected, average cohort of 98 glioma WHO grade 4 patients. In 11 patients (11%), 13 PGVs were found in genes strongly associated with familial glioblastoma (*MSH6* (3x), *PMS2* (5x), *MSH2*, *TP53*, *NF1*, *BRCA1*) or medulloblastoma (*SUFU*). In eight of these patients (73%), causality was supported by a second (somatic) event and/or a matching genome-wide mutational signature. Thus, germline predisposition does play a role in the development of adult glioblastoma, with mismatch repair deficiency being the main mechanism. Our results also highlight the benefits of tumor-normal WGS for glioblastoma patients and their families, beyond identifying actionable mutations for therapy.

Keywords. Glioblastoma, genome sequencing, genetic predisposition, germline

INTRODUCTION

Glioblastoma, a primary brain tumor, is the most common and most aggressive malignant brain tumor in adults. Despite intensive treatment consisting of surgical resection followed by radiotherapy with concurrent and sequential chemotherapy, the prognosis remains poor with a median survival of 15 months.[1, 2] One of the contributing factors challenging effective treatment strategies is the inter- and intratumoral heterogeneity of this devastating disease.[3] This becomes also apparent in the complexity revealed by genomics[4] and single-cell RNA sequencing.[5, 6] Nevertheless, genomic analysis of the tumor is considered a promising technological development that could enable personalized treatment strategies. The most comprehensive approach for genomic analysis is genome sequencing (GS), which has been clinically validated for diagnostic purposes.[7, 8] GS is not yet widely used in routine settings, especially for glioblastoma, mostly because of lack of evidence of clinical utility, costs, or both. Therefore, we have initiated the GLOW trial, a clinical study to explore potential added value of GS for recurrent glioblastoma patients.[9] As GS analyses typically include a control normal DNA sample (e.g. from blood) to distinguish somatic variants (acquired in the tumor cell) from germline variants (inherited), they may also reveal potential genetic predisposition to glioblastoma. This knowledge might be relevant to patients and their relatives, and the presence of familial predisposition is often an important question in the consulting room. Furthermore, variants in several predisposition genes are increasingly important for (immune- or targeted) therapy selection.[10-16]

In contrast to other (sub)types of cancer, for instance breast cancer and colon cancer, but also to pediatric gliomas, the prevalence of heredity in adult glioblastoma patients is still largely unexplored, mainly due to lower incidence and limited datasets that are available to investigate this topic.[17-19] In general, an estimate of approximately 5% of all glioma patients have a positive family history for glioma, with twofold to elevenfold increased incidence ratios in those families.[20-22] These cases show similarity to sporadic cases in terms of demographics (age, gender), morphology and tumor grade, and penetrance of hereditary glioma is suggested to be low.[23] Hereditary glioblastoma, also called familial glioblastoma, caused by single-gene hereditary disorders is very rare[24] and often involves predisposition of a range of tumor types. Current knowledge is limited to a few syndromes including neurofibromatosis type 1 (*NF1* mutation, autosomal dominant), Li Fraumeni syndrome (*TP53* mutation, autosomal dominant), Turcot syndrome type 1 (mismatch repair genes [MLH1 & PMS2] mutations, autosomal dominant) and Lynch or constitutional mismatch repair deficiency (mismatch repair genes

mutations, autosomal dominant [Lynch] or recessive [constitutional mismatch repair deficiency]).[25-27] Furthermore, in enriched cohorts (i.e. selected for personal and/or family history) pathogenic variants in *BRCA 1* and *2*, *CHEK2*, *HERC2*, *MUTYH*, *NF1*, *POT1* and *TERF2* have been associated with glioblastoma[20, 28-30], although their contribution to glioblastoma development remains unclear, since second-hit somatic variants were not observed for many.[29] Apart from these syndromes, familial glioblastoma is thought to be multifactorial and autosomal recessive.[31-33] Genome-wide association studies (GWAS) have identified several risk loci for glioblastoma, but causality of specific variants or genes in these regions remains unclear.[24, 34]

Taken together, in sporadic and/or late onset glioblastoma cases the prevalence and contribution of pathogenic germline variants (PGVs) remains unclear. It is, therefore, of interest to systematically analyze the complete germline genome of unselected glioblastoma patients, including small and structural variants, to identify genes with PGVs as potential candidates for cancer predisposition. This study thus aimed to gain novel insight into the prevalence of genetic predisposition to glioblastoma by retrospectively analyzing germline data of an unselected, adult glioblastoma patient population.

MATERIALS AND METHODS

Patient inclusion

For this retrospective, germline analysis study, genome sequencing data from the Hartwig Medical Foundation (Amsterdam, the Netherlands) database was used. All patients that contributed to this database have consented to reuse of their data, including germline data, for cancer research purposes. All adult patients (i.e. from 18 years and older) diagnosed with nervous system cancer (disease ontology ID: 3093) and whose data was stored in the database before November 1, 2023, were eligible. Patients were mainly collected in the context of the CPCT-02 (NCT01855477) and GLOW (NCT05186064) studies. Hereafter, the patient selection was further filtered based on tumor type, and only gliomas WHO grade 4 were included in final analyses ($n = 98$). Sampling in these patients was performed after recurrent disease. Family history of malignant neoplasms was not taken into account. Patient consent was based on a broad consent intending publicly available access-controlled data for academic cancer research related requests. For this study, a Data Access Request (DR-310) was signed to obtain the genome sequencing germline and somatic data. All samples were de-identified and keys between study number and patient number were stored solely locally in the hospitals.

Genome sequencing

All samples were sequenced at Hartwig Medical Foundation as per ISO-accredited diagnostic standards (ISO17025), as described previously.[35] Shortly, tumor samples with at least 20% tumor purity were deep-sequenced on Illumina Novaseq 6000 to an average depth of 90-100x. The blood control samples were sequenced to a depth of 30-35x. Somatic and germline variant calling was done using the open-source Hartwig WiGiTS toolset (https://github.com/hartwigmedical/hmftools v5_33). Also, tumor heterogeneity and presence of non-tumor cells in the tumor sample were computed (https://github.com/hartwigmedical/hmftools/tree/master/purple) and accounted for. The strategy for this germline analysis has been validated previously.[36]

Selection of relevant genes

Because of interpretation challenges and limited statistical power associated with the number of available patients compared to the vast search space of the genome, as well as the expected limited penetrance of individual genes, it was considered not feasible to perform a sufficiently powered genome-wide association study for analysis of variants that might be involved in glioblastoma predisposition. Hence, a manually curated list of known cancer-associated genes was created to first explore potential involvement of candidate genes. As a basis, the reportable germline gene list used as part of the pan-cancer routine diagnostic analysis pipeline from Hartwig was used.[36] This gene panel is based on national guidelines[37] and experience at the Netherlands Cancer Institute and was for this study expanded with genes from several other cancer predisposition gene panels: a germline driver catalogue previously described and curated by Priestley et al.[35], a subset of genes from the American College of Medical Genetics and Genomics (ACMG)[38], the Hereditary Cancer Gene Curation Expert Panel from ClinGen[39], the adult solid tumors cancer susceptibility panel created by National Health Service (NHS) and Genomics England[40], and from the literature.[20,30] After comparing these different gene lists, a comprehensive list of 170 genes was generated for the current germline predisposition analysis. For all of these genes the likely mechanism of action was determined as either oncogene or tumor suppressor gene (*Supplementary Table 1*).

Small variant calling

Small variants include stop-gain mutations, frameshifts due to small insertions or deletions, inframe deletions, inframe insertions, missense mutations and splice site mutations. Within the standard pipeline workflow of Hartwig (https://github.com/hartwigmedical/pipeline5), small variants in both tumor and germline are called by the algorithm ‘Somatic Alterations in Genome’ (SAGE; v3.2) (https://github.com/

hartwigmedical/hmftools/tree/master/sage). SAGE is a precise and highly sensitive caller for single nucleotide variants (SNVs), multiple nucleotide variants ≤32 base pairs (MNVs) and small insertions and deletions (InDels). In the standard data processing workflow of Hartwig, SAGE is given a panel containing the regions of genes of interest for germline analysis in a Browser Extensible Data (BED) format (*Supplementary Table 1*). For our selected gene panel, a custom BED file (https://github.com/MvOglow/germlineGBM.git) was created using the in-house tool HMF Gene Utilities (v1.1, https://github.com/hartwigmedical/hmftools/tree/master/gene-utils) which used the GENCODE coordinates for the Genome Reference Consortium Human Build 37 (GRCh37) definitions. All raw compressed reference-oriented alignment map (CRAM) files containing the aligned sequencing reads for the included patients were re-processed with SAGE using the default germline run parameters (v3.4; *Supplementary Figure 1*) and these custom gene regions. Subsequently, this data was annotated and filtered using 'Prediction and Annotation of Variant Effects' (PAVE) germline (v1.6) (https://github.com/hartwigmedical/hmftools/tree/master/pave) using the default germline parameters (*Supplementary Figure 2*).

Hereafter, variants annotated as having only synonymous canonical coding effects were removed from the output files. To reduce inclusion of common variants and potential false positives, additional filters were used next to the default SAGE filters: (1) variants with a Genome Aggregation Database (gnomAD; v2.1.1[41]) population frequency >1% were removed and classified as population variance; (2) germline variants with a low recalibrated quality score (see below) were removed and (3) germline variants with a frequency ≥5% in the Hartwig database (n = 5,778, excluding the patients included in this study) were removed as these are likely population variants specific to the Dutch population. SAGE accounted for false positive calls or poor sensitivity by recalibrating the empirical base quality score provided by the sequencer. The ad-hoc cut-off based on these recalibrated Phred-scaled quality scores was determined using a density plot of the recalibrated Phred quality of all obtained variants for the included patients and set at 235.6 for variants to be included in further analyses (*Supplementary Figure 3*).

Structural variants and copy number variations calling

By default, structural variants (SVs) and copy number variations (CNVs) were called genome-wide by GRIDDS2 in the Hartwig pipeline.[42] After processing, this data was annotated and filtered by GRIPPS germline and stored in a SQL database (pipeline release v5.33). All SVs and CNVs within the regions defined in the BED file were obtained from the SQL database. Because gnomAD does not provide population

frequencies for SVs, the data was filtered based on the variant frequency within the Hartwig database (excluding the patients included in this study). All obtained SVs occurring in ≥5% of all other patients in the Hartwig database were excluded. Since the Hartwig databases contained 5,778 patients next to the patients included in this study, SVs occurring in ≥289 patients were discarded.
The interpretability of copy number gains is low as there is no international consensus on the significance of differences between the exact number of copies, e.g. three versus more than three copies. Moreover, most genes are tumor suppressor genes. Therefore, we assessed only copy number losses and no copy number gains.

Clinical significance

The clinical significance of variants was based on their annotation in ClinVar, a public archive of human genetic variants and interpretations of their significance to disease.[43] The main conclusions in our study were based on 'pathogenic' and 'likely pathogenic' variants. Variants of unknown significance (VUS) were not studied. To direct the potential effect of these variants on the functional protein, the Ensembl Variant Effect Predictor (VEP) was used.[44] All shortlisted variants were manually reviewed by a clinical laboratory geneticist (RK) to determine pathogenicity according to routine diagnostic procedures, all likely-pathogenic (class 4) and pathogenic variants (class 5) were considered as pathogenic germline variants (PGVs). As a second step to assess the clinical significance of PGVs, tumor type-specific manual curation and tumor genome analysis was performed. The following subdivision was used: category 1 were causal events (gene associated with glioblastoma + matching tumor findings), category 2 were known predisposition genes but without demonstrated causality (gene associated with glioblastoma without matching tumor findings, or gene not associated but having matching tumor findings), and category 3 contained variants less likely to contribute to glioblastoma.

Tumor sample analysis

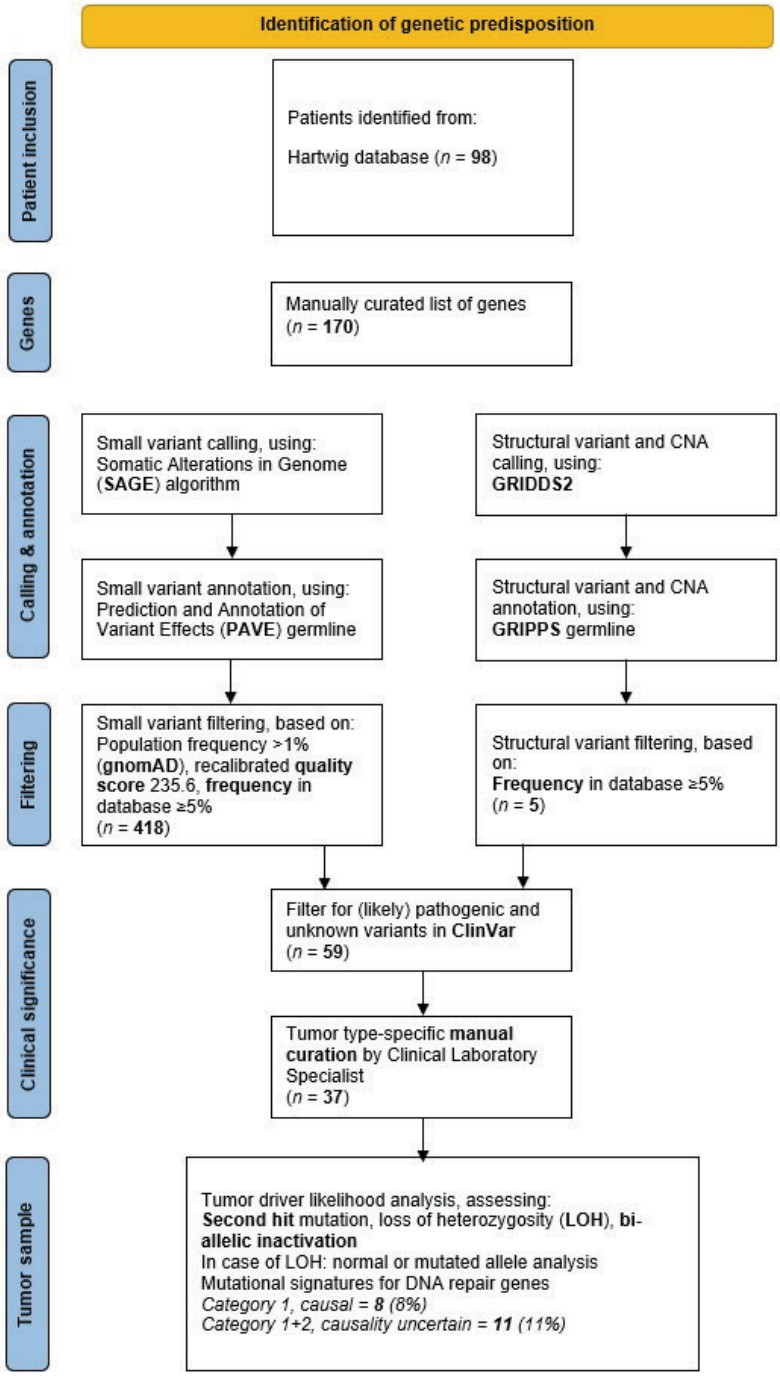
For tumor suppressor genes, the common model for pathogenicity is that both alleles of the gene become inactivated in the tumor. In case of germline predisposition, the second allele is typically inactivated by a second mutation or loss of heterozygosity (LOH, although epi-genetic inactivation through methylation is also possible). Therefore, we assessed all candidate genes for somatic events, and, in case of LOH, determined if the normal or mutated germline allele was lost. In addition, we explored if any of the candidate genes was also a common somatic driver in glioblastoma patients, i.e. inactivated bi-allelic by somatic events. Finally,

mutational signatures were studied for DNA repair genes. In case of splice site variants, RNA sequencing data (which was available for approximately 80% of the patients) was used to validate the impact of the variant at transcript levels. A graphic overview of the methods for identification of predisposition can be found in *Figure 1*.

Statistics

Sociodemographic characteristics were compared by using chi-square test for categorical variables and t-test for continuous variables. In case of violation of the normality assumption, a non-parametric test was used for the continuous variables.

Figure 1. Flowchart methods.



RESULTS

Patient characteristics

A total of 98 patients met the inclusion criterium of 'adult glioma WHO grade 4' and were included in this study for germline predisposition analysis. Of these, 70.4% (69/98) were male and the median age for males and females was 61 years. Most of the patients had a primary, isocitrate dehydrogenase (*IDH*) wildtype glioblastoma (93.9%, 92/98) while 6.1% (6/98) of the tumors had a somatic *IDH* mutation (classifying them as astrocytoma WHO grade 4). The family history, particularly regarding the occurrence of malignant neoplasms, was unidentified.

Germline findings in an average glioblastoma population

After filtering for canonical coding effects, gnomAD population frequency and quality score, a total of 418 small variants and five structural variants (SVs) were detected in 107 of the 170 different genes. Filtering for variants that were annotated as '(likely) pathogenic' or 'unknown' in ClinVar following manual curation, resulted in a total of 30 (including three SVs) PGVs in 18 different genes in 25 unique patients (25.5% of all patients). Of these 30 PGVs, 11 were observed in genes with an explicitly recessive inheritance and 19 in genes having dominant inheritance. All 11 PGVs in recessive genes were monoallelic and, therefore, excluded from overall prevalence, because only biallelic or compound heterozygous germline variants in such genes are considered as having associated hereditary risks (*Table 1*).

The 19 dominant inheritance PGVs were present in 11 different genes in 16 unique patients (16% of all patients). Six of these PGVs were in cancer predisposition genes (*ATR*, *CHEK2* (3x), *SDHA* and *MITF*) without an established association with familial glioblastoma. Interestingly, the majority, 13 PGVs in 11 patients, were in established cancer predisposition genes with a strong association with familial glioblastoma (*MSH6* (3x), *PMS2* (5x), *MSH2*, *TP53*, *NF1* and *BRCA1*) or with medulloblastoma (*SUFU*). Thus, the prevalence of known genetic predisposition to glioblastoma was 11% (11/98) in our unselected cohort, with additional candidates in another 5.1% of patients (5/98).

Genetic predisposition driving glioblastoma oncogenesis

As most predisposition genes involve tumor suppressors, all candidate causal events were assessed for second hit (somatic) events in the tumor data. PGVs with a second (somatic) event are considered causal for glioblastoma oncogenesis. For all six PGVs (*ATR*, *CHEK2* (3x), *SDHA* and *MITF*) without an established association with familial glioblastoma and for 10 out of 11 PGVs in recessive genes (*BLM* (4x),

ERCC3, *MUTYH* (2x), *FANCF*, *SBDS* and *WRN*), no second (somatic) event (small variant or structural variant resulting in LOH) or matching mutational signature was detected in the tumor. Thus, those variants, except for possibly *BUB1B*, were unlikely to contribute to the development of glioblastoma in our cohort (category 3 – see *Table 1*). Additionally, for three patients with PGVs in genes with a strong association with familial glioblastoma (*NF1*, *MSH6* and *MSH2*) also no second (somatic) event or expected matching mutational signature was detected, indicating that for these variants the causality for tumorigenesis in these patients remains unclear (category 2 – see *Table 1*).

Importantly, for the remaining 10 PGVs that were identified in genes with a strong association with familial glioblastoma or medulloblastoma (*SUFU*, *MSH6* (2x), *PMS2* (5x), *TP53* and *BRCA1*), a second (somatic) event and/or a matching mutational signature was identified in the tumor. These variants were present in eight different patients, resulting in a proven germline predisposition rate of 73% in the patients with relevant PGVs (8/11). Of interest, two of these patients most likely have constitutional mismatch repair deficiency (CMMRD) syndrome, since they each harbored two PGVs in *PMS2* and both were microsatellite instable with a high tumor mutational burden (*Table 1*).

DNA damage response – significant role for mismatch repair (MMR) in glioblastoma

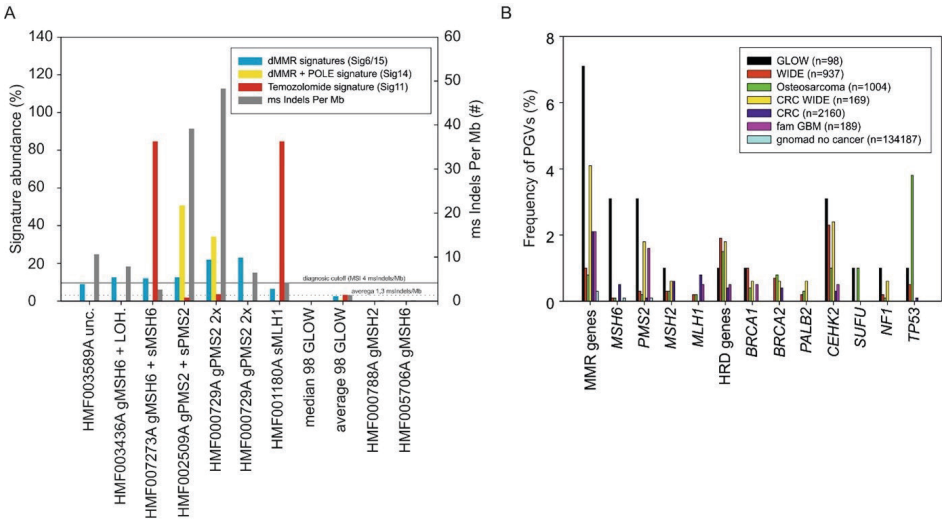
The known pathogenic predisposition variants in 11 patients could be divided in two main mechanisms. First, two patients had PGVs in genes involved in cell proliferation/survival (Ras/mitogen-activated protein kinase pathway; NF1 & Shh signaling pathway; *SUFU*). Second, nine patients had 11 PGVs in genes involved in the DNA damage response or cell cycle pathway (*TP53*, *BRCA1*, *PMS2*, *MSH6* and *MSH2*). These included a patient showing LOH for *TP53* and another patient showing LOH for *BRCA1* along with a homologous recombination deficiency (HRD) footprint. The tumor in this patient underwent whole genome duplication after LOH (*Supplement Figure 4)*.

The majority of patients was thus found to harbor a PGV in one of the mismatch repair (MMR: MSH2, PMS2, MLH1, MSH6) genes (7 out of 11). By measuring microsatellite instability (MSI) based on GS, we observed that, within the total cohort, seven patients had > 1.3 microsatellite Indels Per Mb (overall average 1.3, median 0.12) and six of seven patients had ≥ 4 microsatellite Indels Per Mb (diagnostic cutoff of WGS handled by Hartwig Medical Foundation – see *Figure 2A*). For one of the seven patients with MSI no evidence for either germline or somatic

mutations in any of the four MMR genes was found. For the remaining six patients with MSI, one patient with somatic loss of function of *MLH1* and five patients with germline loss of function of *MSH6* (2x) or *PMS2* (3x) matched with MSI (*Figure 2A*).

The percentage of patients with PGVs in MMR genes within this unselected glioblastoma cohort were compared to the percentage of patients with PGVs in MMR genes within other unselected cancer cohorts[20, 36, 45, 46] and the gnomAD v2.11 (non-cancer) cohort[41]. Although numbers remain small, a higher than expected frequency of patients with glioblastoma carrying a PGV in MMR genes was seen, with the biggest difference for *MSH6* and *PMS2* (*Figure 2B*).

Figure 2. Significant role for mismatch repair (MMR) in glioblastoma.



(A) Cosmic single base substitution (SBS) signatures for dMMR (Sig 6+15) dMMR+POLE (Sig14) and Temozolomide (Sig11) and number of ms Indels per Mb are depicted for seven patients within the total cohort having > 1.3 microsatellite Indels Per Mb. (B) Frequency of pathogenic germline variants (PGVs) in the genes as described in the GLOW study versus other cohorts.

CRC: colorectal cancer[46], CRC WIDE: subgroup WIDE colorectal cancer patients[36], fam GBM: familial glioblastoma cohort[20], GLOW: current composite cohort, gnomad: non-cancer reference cohort[41], HRD genes: homologous recombination deficiency genes (BRCA1/2 & PALB2), dMMR genes: deficient mismatch repair genes (MSH6, PMS2, MSH2 & MLH1), MSI: microsatellite instability, osteosarcoma[45], WIDE: metastatic cancer[36]

Table 1. Findings after manual curation of possibly interesting variants.

Patient	Age	Gene	c.HGVS[1]	p.HGVS[2]	Classification	Inheritance	Mechanism	Tumor status	Category	TMB	MSI	IDH1/2
HMF002821A	55	*SUFU*	c.436C>T	p.Arg146*	P	dominant	LOF	LOH	1	2,1	0,1	WT
HMF003436A	72	*MSH6*	c.467C>G	p.Ser156*	P	dominant	LOF	MSI & second hit(s) c.1444C>T, p.Arg482* c.3254delC, p.Phe1088fs	1	37.9	**7.8**	WT
HMF002509A	45	*PMS2*	c.1882C>T	p.Arg628*	P	dominant	LOF	MSI & second hit(s) c.943C>T, p.Arg315* c.1732C>T, p.Arg578Cys	1	339	**39**	IDH2 p.A370T
HMF003795A	53	*BRCA1* *SDHA*	c.2210delC c.91C>T	p.Thr737fs p.Arg31*	P P	dominant dominant	LOF LOF	LOH, HRD signature no second hit	1 3	8,6	0,2	WT
HMF001910A	19	*PMS2*	c.137G>T c.736_741delins TGTGTGTGAAG	p.Ser46Ile p.Pro246fs	LP P	dominant	LOF	MSI	1	11,7	**6,4**	IDH2::IDH2 fusion
HMF000729A	33	*PMS2*	c.325dupG c.825A>G	p.Glu109fs p.Gly275= (splice)[5]	P P[5]	dominant	LOF	MSI	1	279	**48,2**	IDH1 p.T325M
HMF007273A	50	*MSH6*	c.742delC	p.Arg248fs	P	dominant	LOF	MSI, LOH	1	89	**2,6**	WT
HMF006786A	N/A	*TP53*	c.711G>A	p.Met237Ile	P	dominant	LOF	biallelic	1	3,1	0,1	IDH1 p.R132C
HMF006763A	55	*NF1*	complex event[&] (see below)	unbalanced	P	dominant	LOF	no second hit[#]	2	3,5	0,1	WT
HMF005706A	N/A	*MSH6*	c.3514dupA	p.Arg1172fs	P	dominant	LOF	MSS, no second hit	2	3,2	0,1	WT
HMF000788A	51	*MSH2*	c.942+2delT	p.? (splice?)[6]	LP[6]	dominant	LOF	MSS, no second hit variant lost in tumor	2	3	0,1	WT
HMF000649A	73	*BUB1B*	c.2210T>G	p.Leu737*	P	recessive	LOF	second hit, large deletion including *BUB1B*	3	3,7	0,1	WT
HMF000655A	57	*ATR*	inversion (see below)	Inversion exon 42-47 + XRN1	P	dominant	LOF	no second hit	3	4,4	0,2	WT

Table 1. *Continued*

Patient	Age	Gene	c.HGVS[1]	p.HGVS[2]	Classification	Inheritance	Mechanism	Tumor status	Category	TMB	MSI	IDH1/2
HMF000925A	71	*CHEK2*	c.1229delC	p.Thr410fs	P	dominant±	LOF	no second hit	3	3,5	0,1	WT
HMF000842A	54	*CHEK2*	c.1229delC	p.Thr410fs	P	dominant±	LOF	no second hit	3	2,7	0,1	WT
HMF007010A	77	*CHEK2*	c.1229delC	p.Thr410fs	P	dominant±	LOF	no second hit	3	4	0,1	WT
HMF001235A	47	*ERCC3*	c.760C>T	p.Gln254*	P	recessive	LOF	no second hit	3	2,5	0,1	WT
HMF001434A	68	*BLM*	deletion (see below)	deletion exon 2-22	P	recessive	LOF	no second hit	3	3	0,1	WT
HMF003406A	39	*BLM*	c.3558+1G>T	p.? (splice)	P	recessive	LOF	no second hit	3	2,9	0,1	WT
HMF001701A	54	*BLM*	c.1642C>T	p.Gln548*	P	recessive	LOF	no second hit	3	1,7	0	WT
HMF001787A	55	*MUTYH*	c.1178G>A	p.Gly393Asp	P	recessive	LOF	no *MUTYH* signature, no second hit	3	1,5	0,1	WT
HMF007027A	73	*SBDS*	c.258+2T>C	p.? (splice)	P	recessive	LOF	no second hit	3	4,1	0,2	WT
HMF006506A	65	*FANCF*	c.484_485delCT	p.Leu162fs	P	recessive	LOF	no second hit	3	2,8	0,1	WT
HMF000329A	55	*BLM* *MITF* *WRN*	c.1933C>T c.1255G>A c.1105C>T	p.Gln645* p.Glu419Lys p.Arg369*	P P P	recessive dominant + recessive	LOF GOF LOF	no second hit	3	2,6	0,1	WT
HMF006895A	72	*HERC2* *MUTYH*	c.8002G>C c.1138delC	p.Val2668Leu p.Ala382fs	VUS P	dominant- recessive	LOF LOF	no second hit no *MUTYH* signature, no second hit	3	4	0,1	WT
HMF003589A	55							MSI	n/a	216	**11**	WT
HMF001180A	73							MSI, somatic *MLH1*	n/a	80	**4**	WT

[1]Coding reference sequence, [2]Protein reference sequence, [&]14:102878068-17:29458864, G: gain of function, HGVS: Human Genome Variation Society, LOF: loss of function, WT: wildtype

Classifications: LP: likely pathogenic, P: pathogenic, VUS: variant of uncertain significance

Inheritance: ±Risk factor for breast cancer, [+]Risk factor for melanoma, [-]Associated with glioblastoma

5Out-of-frame skipping of the first 22 nucleotides of exon 8[67, 68] and observed with RNAseq, see supplemental Figure 5

6Splicing could not be proved with RNAseq data. This could be because the variant is lost in the tumor. The expert panel InSiGHT consideres this variant as Likely pathogenic

Tumor status: #Somatic gain 17:29421945-29709134; complete gene is amplified in tumor but amplification involved a large part of the chromosome (non-focal), HRD/HRP: homologous recombination deficient/proficient, MSI/MSS: microsatellite instability/stability

Category: 1: causal event, 2: known predisposition gene but causality not demonstrated, 3: less likely to contribute to glioblastoma

NC_000014.8:g.pter_102878068delins[NC_000017.10:g.29458864_qter] / p.? (deletion of chr14pter_1st exon NF1)

NC_000003.11:g.142091960_142182304inv / p.? (inv exon 42-47 ATR + XRN1)

NM_001287246.2(BLM):c.-4-1619_*16491del / p.?

DISCUSSION

This study showed the germline predisposition in a cohort of 98 adult glioblastoma patients. In 11% of the patients, pathogenic germline variants (PGVs) were observed in genes previously associated with familial glioblastoma; thus these PGVs likely contributed to the oncogenesis of these unselected glioblastoma patients. PGVs were found in the following genes: *BRCA1*, *MSH6*, *PMS2, TP53, NF1* and *SUFU*. Furthermore, for ten PGVs in *SUFU*, *MSH6* (2x), *PMS2* (5x), *TP53* and *BRCA1*, in eight different patients causality was proven, since second (somatic) events and/or matching mutational signature were detected. Several of these PGVs were in predisposition genes that are increasingly important for (targeted) therapy selection and for all findings counseling by a clinical geneticist is indicated. Mismatch repair deficiency formed the main mechanism of the unselected cohort, with 7.1% of the patients harboring a PGV in one of the mismatch repair (MMR) genes, including five patients with microsatellite instability.

The results of this study are unique in several aspects. First, no preselection based on personal and/or family history of malignant neoplasms was applied to the study cohort. Second, the pairing of both blood and tumor tissue samples allowed for verification of the causality of potentially interesting events. Third, since all patients underwent paired GS testing combined with RNA sequencing (~80%), we were able to not only study point mutations (which is a limitation in most of the cancer predisposition research) but also copy number variations, structural variants, splice site variants (*supplementary Figure 5*) and mutational signatures.

We detected a number of PGVs in dominant and recessive genes without proven causality for glioblastoma, since the tumor sample analyses did not show second hit mutations in almost all of these cases. In the Netherlands, observed putative PGVs in dominant genes that do not match the tumor type (*ATR*, *CHEK2* (3x), *SDHA* and *MITF*) are normally not reported back to the patient, except if there is a matching personal and/or familial history. Unfortunately, in the current retrospective study design, we were not able to identify the pedigrees of the patients with PGVs, making further details of the inheritance pattern and possible consequences for family members impossible. In recessive genes, all 11 PGVs were monoallelic and considered low/no risk for cancer predisposition. Still, these variants potentially modified the genesis of the tumor as risk loci associated with susceptibility to glioblastoma. Unfortunately, our study lacked sufficient power to study these monoallelic PGVs in recessive genes in a statistically sound manner. Interestingly, in one patient with a PGV in *BUB1B*, the remaining wildtype allele was somatically

lost due to a large deletion. The causality of the PGV in this recessive gene could not been demonstrated, although there is evidence for the role of *BUB1B* as a (pan-)cancer predisposing gene[47], including glioblastoma.[48] When variants like these are identified, they are normally not reported back to the patient. Because these variants do not have any relevance for the patient nor for the patient's family except if there is consanguinity in the family, no genetic counselling and testing is recommended.

Currently, the international guideline of the European Association of Neuro-Oncology (EANO) on the diagnosis and treatment of diffuse gliomas of adulthood recommend genetic counselling in patients with 'relevant germline variants or suspected hereditary cancer syndromes.'[49] This recommendation is based on low level evidence (i.e. class IV, level C evidence) and did not specify which germline variants are considered relevant. The familial tumor syndromes associated with gliomagenesis named in this EANO guideline include neurofibromatosis type I, tuberous sclerosis, Turcot syndrome, Li-Fraumeni syndrome and Lynch syndrome. Other international guidelines of neuro-oncology or medical oncology societies lack recommendations on germline testing and genetic counseling of gliomas in adults.[50, 51] However, the more recent EANO guideline on molecular testing of gliomas in adults recommend genetic counseling prior to germline testing, as for instance specific attention is paid to MMR gene deficiencies.[52] Yet, most of the PGVs found in our study are currently not tested for in most of the Dutch laboratories.[53]

As the use of comprehensive tumor genetic and genomic diagnostic tests continues to grow, the detection of PGVs is occurring more frequently than previously expected.[36, 48, 54, 55] In our unselected cohort, many PGVs are identified in genes such as *BRCA1*, *MSH6*, *PMS2*, and *NF1*, which are crucial not only for germline follow-up but also for selecting appropriate therapies, particularly immune-based or targeted treatments, as observed in other tumor types. For example, melanoma, MMR deficient colorectal cancer, and other non-colorectal MMR deficient tumors have shown remarkable responses to immunotherapy.[13-16, 56] While some glioblastoma patients exhibit long-term responses to immunotherapy, this treatment has shown limited efficacy in over 90% of unselected glioblastoma cases.[57-60] Among those who responded (partially or fully), most likely were patients with hypermutated tumors, possibly due to MMR deficiency or MMR deficiency + *POLE* defects.[57, 61-65] Our findings indicate that most of these hypermutated tumors harbor a PGV in one of the MMR genes. Thus, comprehensive tumor genetic and genomic profiling for glioblastoma patients requires an integrated approach that facilitates appropriate referral to clinical geneticists.

This study has some limitations that have to be considered. First, this type of research cannot be done without making assumptions. Assumptions were not only made when defining the pathogenicity of variants[38], but essentially every single step in our methods, e.g. variant calling, annotation, filtering, curation involved choices based on assumptions. Although these are based on generally accepted international standards, changes over time based on progressive insights may impact outcomes. A second limitation is the relatively small sample size, which hampered statistically powered analyses of the PGVs. Third, our cohort contained six patients (6.1%) with a somatic *IDH* mutation, which might be extra relevant, in terms of prognostic relevance, in the context of MMR deficiency.[66] Lastly, due to consent and privacy regulation limitations, we were not able to assess the pedigrees of the patients with PGVs, making assessment of the inheritance pattern and possible consequences for family members impossible.

To conclude, this study investigated the germline predisposition to glioblastoma in an average adult glioblastoma population. 11% of these patients had a pathogenic germline variant that (likely) predisposed to the development of the glioblastoma, with potential associated therapy options. The results could guide clinicians who have to inform patients about broad molecular tests for personalized medicine and its associated putative germline findings, once current gene panels are adapted to these findings.

AUTHOR CONTRIBUTIONS

Study concept and design: MPvO, DRvV, MLDB, EC. Material preparation, data collection, and analysis: MPvO, DRvV, JdL, EC, RK. Writing and revision of manuscript: MPvO, DRvV, JdL, FYFdV, MLDB, EC, RK.

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None

AVAILABILITY OF DATA AND MATERIALS

The underlying research are partly facilitated by Hartwig Medical Foundation and the Center for Personalized Cancer Treatment (CPCT) which have generated,

analysed and made available data for this research. Data can be requested via https://www.hartwigmedicalfoundation.nl/en/data/data-access-request/. Hartwig Medical Foundation is willing to share with external qualified researchers access to patient-level data and supporting clinical documents. These requests are reviewed and approved by an independent review committee on the basis of scientific merit. All data provided are anonymized to respect the privacy of patients who have participated in the study, in line with applicable laws and regulations.

CONFLICT OF INTEREST STATEMENT

None of the authors declare a conflict of interest.

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SUPPLEMENTARY

Supplementary Figure 1. Example of command-line arguments that were used to run SAGE in default germline mode: see https://github.com/MvOglow/germlineGBM/blob/66c02a570c85c630038f3c5644346cbaf25385ac/Supp%20Figure%201.png

Supplementary Figure 2. Example of command line arguments that were used to run PAVE in default germline mode: see https://github.com/MvOglow/germlineGBM/blob/621feeac129493396680c14c6bd848b306df926f/Supp%20Figure%202.png

Supplementary Figure 3. Quality density plot of small variant scores**:** see https://github.com/MvOglow/germlineGBM/blob/57955240ee192e8239c7c00da93dee9195834570/Supp%20Figure%203.png

Supplementary Figure 4. Circos plot of HMF006786A: see https://github.com/MvOglow/germlineGBM/blob/080f00ba444cd6e6f8b817b08cdf7147beba2ac8/Supp%20Figure%204.png

Supplementary Figure 5. In silico predictions and RNAseq analysis of *PMS2* exon 1-12 for HMF000729A: see https://github.com/MvOglow/germlineGBM/blob/c36c7c4d636c9d681eec358cca0cd8308fed1ff0/Supp%20Figure%205.png

Supplementary Table 1. Overview of the genes included in the gene panel that was used: see https://github.com/MvOglow/germlineGBM/blob/b97de47f93218de719e216bc7ce8e38a3b42d78b/Supp%20Table%201.docx

10

Chapter 10

Whole genome sequencing in (recurrent) glioblastoma: challenges related to informed consent procedures and data sharing

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ABSTRACT

Increased use of whole genome sequencing (WGS) in neuro-oncology for diagnostics and research purposes necessitates a renewed conversation about informed consent procedures and governance structures for sharing personal health data. There is currently no consensus on how to obtain informed consent for WGS in this population. In this narrative review, we analyze the formats and contents of frameworks suggested in literature for WGS in oncology and assess their benefits and limitations. We discuss applicability, specific challenges, and legal context for patients with (recurrent) glioblastoma. This population is characterized by the rarity of the disease, extremely limited prognosis, and the correlation of the stage of the disease with cognitive abilities. Since this has implications for the informed consent procedure for WGS, we suggest that the content of informed consent should be tailor-made for (recurrent) glioblastoma patients.

Keywords. Whole genome sequencing, recurrent glioblastoma, cognitive impairment, informed consent, data sharing

INTRODUCTION

The understanding of tumor genesis and -progression is improving due to the combined use of advanced data analysis techniques with next generation sequencing (NGS) and whole genome sequencing (WGS).[3] Results may facilitate personalized medicine through the identification of therapeutically relevant alterations and pharmacogenetics, realizing the assessment of genomic variants impacting therapeutic potential or side-effects.[19] Simultaneous development of targeted therapies steadily increases the relevance of genomic essays in clinical cancer care of patients with solid tumors.[16] However, the use of WGS and subsequent targeted therapies is not (yet) standard-of-care for patients with tumors of the central nervous system.[8] Various papers have described the genomic landscape of glioblastoma[7, 12, 27], the most common primary malignant brain tumor. Currently, NGS is used for diagnosis and identification of molecular alterations with potential therapeutical implications in glioblastoma.[8] The benefit of routine application of WGS for patients with recurrent glioblastoma is currently being explored in a prospective clinical trial.[44] WGS provides a wealth of information that could contribute to a better understanding of pathogenesis and to the development of novel therapies, therapy monitoring and treatment optimization.[25] Sharing genome-wide genomics data in combination with clinical information with databanks has the potential to improve future care for patients.

Compared to NGS, which uses a predefined gene panel, WGS sequences the whole genome including non-coding areas. Moreover, WGS is a reliable technique for detecting structural variants such as gene fusions. The 'completeness' of WGS has the additional potential of minimizing interlaboratory variations in NGS panel composition.[43] WGS reports present extensive data on the genomic alternations in cancer cells, as well as a comprehensive view of normal tissue and tumor clonality. It therewith provides immediate clarity on whether alterations are somatic or germline and could reveal additional (hereditary) information unrelated to the tumor. Such results are called unsolicited findings (UF) and may have practical and ethical consequences that are difficult to predict upfront.

Current European Association of Neuro-Oncology (EANO) guidelines address how and when to test for predictive genetic alterations, how to report findings, and how to attribute pathogenic and clinical relevance.[8, 45] However, there are no international recommendations on the format of informed consent procedures for WGS in neuro-oncology. Insecurities surrounding the sensitivity of genomic data and difficulties in predicting the impact of findings amplify the importance of

patient counseling on informed consent procedures for WGS and data sharing. We investigate whether traditional standards of informed consent are clinically feasible in the context of WGS for patients with (recurrent) glioblastoma, who often suffer neurocognitive impairment. We further explore ethical implications described for WGS in oncology; the legal frame of existing models of informed consent; and the role of patient characteristics on their preferences regarding the receiving and sharing of genomic data.

METHODS

Study purpose and search strategy

In this narrative review, the primary purpose of this study was to examine the current literature on ethical implications related to informed consent procedures and data sharing in the context of WGS in (recurrent) glioblastoma. The PubMed database was used and the search strategy was not restricted to brain tumors or oncology, because of the limited literature available. Therefore, the search strategy was composed of the following keywords:

("Whole Genome Sequencing"[MeSH] OR "whole genome sequencing" OR "WGS") AND (("Informed Consent"[MeSH] OR "informed consent" OR "consent") OR ("Data Sharing"[MeSH] OR "Data Management"[MeSH] OR "Confidentiality"[MeSH] OR "data sharing" OR "data management" OR "data privacy") OR ("Ethics"[MeSH] OR "Bioethical Issues"[MeSH] OR "ethical implications" OR "ethical considerations" OR "bioethics") OR ("Legislation as Topic"[MeSH] OR "Jurisprudence"[MeSH] OR "legal implications" OR "legal considerations" OR "law" OR "regulations"))

Articles of potential interest were screened for their relevance based on the following criteria. First, they should address either one or more of the next topics related to the use of WGS in humans: ethical implications, legal implications, issues regarding informed consent, issues regarding data sharing. Second, articles focusing solely on technical aspects of WGS without discussing ethical implications were not included. Finally, articles not written in English were excluded.

Data extraction and analysis

Data extraction focused on key themes related to ethical considerations, including (1) patient autonomy and informed consent, (2) privacy and data sharing practices, (3) legal frameworks and regulations, and (4) broader bioethical discussions to WGS in oncology. Subsequently, the ethical implications of WGS were explored by

synthesizing data on currently described issues with the use of WGS in oncology, ethical principles of autonomy in the context of participant comprehension and data sharing, and ethical dilemmas arising from the potential for incidental findings, genetic privacy concerns and implications for family members. To explore the legal frameworks governing WGS, data was synthesized on relevant legal precedents and case law, and international policies on data sharing, storage and protection on the context of genetic information.

The results were synthesized to provide a comprehensive overview of the ethical and legal implications of WGS in (recurrent) glioblastoma. Themes were organized into sections covering informed consent, data sharing, privacy concerns, and legal considerations. To assess whether traditional standards of informed consent are clinically feasible in the context of WGS, this study included a focused examination of articles discussing limitations and benefits of different models of informed consent procedures as used in medical research as well.

RESULTS

Ethical implications of informed consent for WGS in oncology

WGS analysis could result in the disclosure of sensitive information, which may have (psychological) consequences for patients and their relatives. Informed consent procedures can significantly endorse patient autonomy and should carefully be considered. Factors that affect informed consent procedures for WGS analysis and data sharing include privacy concerns and preconditions for autonomy, such as information disclosure and participant comprehension.[41]

Information disclosure and relevance of findings

Unclear relevance of findings makes disclosure about potential risks and consequences of WGS challenging and could result in misguided perceptions of beneficence and harm.[22] Genomic alterations may have a different clinical relevance across cancer types and the evidence of actionability can range from hypothetical target for treatment to established therapeutic efficacy.[8] Clinical relevance of findings is based on their predictive value in relation to disease progression, the probability of treatment response, actionability in terms of consequential interventions and whether there are immediate consequences for patients. Guidelines provided by the American College of Medical Genetics and Genomics (ACMG)[36] and joint recommendations of Clinical Genome Resource (ClinGen), Cancer Genomics Consortium (CGC) and Variant Interpretation for

Cancer Consortium (VICC)[23] can be used to classify pathogenicity of germline and somatic variants, respectively. Following, there are scoring systems that assess the levels of evidence supporting the clinical value of pathogenic variants as targets for treatment. The European Society for Medical Oncology (ESMO) Scale for Clinical Application of molecular Targets (ESCAT-classification system)[30], OncoKB[10] and CIViC[21] assess the degree of actionability of somatic variants, while ClinVar[26] provides interpretations of germline variants. However, the majority of genomic data available in databases used to assess biological significance of variants include only information on non-central nervous system tumor types. The value of these scales is dependent on international differences in regulatory approval of drugs and the availability of trials.[33] Ideally, an interdisciplinary tumor board discusses the degree of actionability of variants per case.

There is no international consensus about a specific list of genomic variants that should be communicated back to patients.[6, 48] Nor is it obligatory to communicate back any genomic UFs in the European Union (EU). Yet, the ACMG recommends that clinicians should report back genomic variants that are actionable or have phenotypes that are highly penetrant, disease causing or of other medical relevance. [32] Reporting back a default list of findings may violate the ethical norm of 'the right not to know'.[15] The question should be raised whether potential clinical benefit and the clinicians' duty to prevent harm supersedes the principle of autonomy of the patient. Dutch guidelines advice against reporting back genomic findings to patients who have expressed their unwillingness to receive them during informed consent procedures.[37]

Participant comprehension

The complexity of genomic concepts may hamper patient comprehension during counseling for WGS analysis, which challenges clinicians to review if autonomous decision-making has taken place. A quantitative multicenter study found that patients who declared to have sufficient knowledge and experience with genomic testing, changed their consent after watching educational videos on receiving information about UFs.[4] Moreover, a survey of patients with refractory, metastatic cancer undergoing WGS analysis further found that their expectations regarding direct benefits of study participation are largely unfulfilled.[38] Despite contrary clinical counseling, the survey concluded that patients expected written reports of sequencing findings, a greater understanding of the causes of their cancer, results making them eligible for participation in clinical trials and disclosure of UFs.

Protecting patient autonomy and data sharing

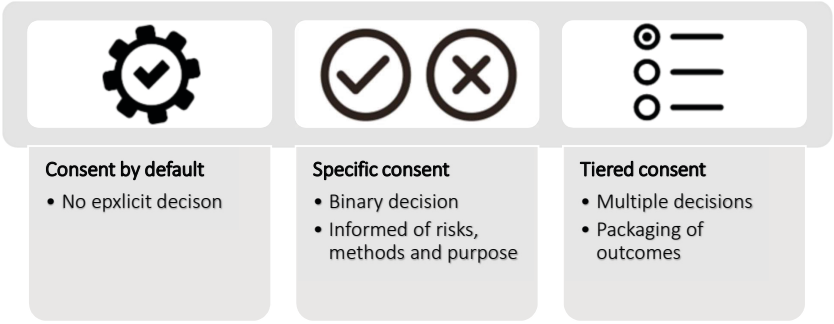
Patients may hesitate to share their genomic data due to concerns about potential misuse. Databanks generally secure the patients' right to protection of personal data technically and in data licenses. Efforts are made to de-identify data by the replacement of personal details with an automatically generated code and through aggregation of data into big data sets. Nevertheless, genomic sequences are per definition unique to an individual and these measures will therefore never eliminate the theoretical possibility of patient reidentification. Regardless, legislation mandates only that sufficient measures need to be taken to ensure reidentification is not possible with reasonable efforts.[35] The sensitivity of data depends on context and its relation to other information, patient interests, and consequential decision-making.

Different countries often have different data protection regulation, which makes sharing data in international research teams challenging. Concerns about unwarranted disclosure of genetic information extend the patient-physician relationship and is further influenced by societal factors, encompassing politics, law, and health care. In general, a higher data protection standard can be expected when there are more institutional and political safeguards in place.[46] An example would be the protection of genetic information in France and Canada, where findings are exclusively allowed to be used for medical and scientific purposes. In the EU discrimination based on genetics is forbidden by law and genetic data is classified as sensitive data under the General Data Protection Regulation (GDPR).[35] In stark contrast, in the United Kingdom, findings can be used to determine insurance thresholds if policy exceeds a certain financial limit, while in the United States (US) patients may need to disclose genetic findings for certain kinds of insurance.[2, 18] The Genetic Information Nondiscrimination Act (GINA) in the US, which excludes employers with under 15 employees, does not protect against genetic discrimination by disability-, long term care- and life insurance.[11] Accordingly, patients undergoing germline testing have reported fear for discrimination based on genetics, for example by insurance companies or employers.[18] Regulations protecting personal data and conditions allowing for secondary use differ regionally in both the US[20] and the EU.[24] This complicates data transfers between the US and the EU, despite the US-EU privacy shield.[5] Moreover, notwithstanding the necessity of these regulations, they may impact the feasibility of clinical research in which sharing genomics and proteomics data is crucial.[13]

Models of informed consent

Under the influence of legislation different models of informed consent were developed for consent to treatment, research, disclosure of genomic results and data storage.[9] Examples of models available for consideration are *consent by default, specific consent, tiered consent*, and *broad consent*.[46] Each model is characterized by its own legal context, advantages, and limitations *(Figure 1)*.

Figure 1. Overview of the types and characteristics of informed consent.



Consent by default

Consent by default is applied when in consenting to participation in a study, patients automatically consent to publicly sharing the results and data of that study.[31] Although this option could limit the administrative burden on researchers, *consent by default* is not legally valid for sharing personal data under the GDPR.[35] Permission for the processing of personal data in the context of providing medical treatment is not necessary. However, explicit consent must be obtained if healthcare providers wish to lawfully use the genetic data for further processing, such as research.

Specific informed consent

Specific informed consent refers to the binary decision of a patient after being informed of potential risks, the methods and purpose of a study or treatment. The *specific informed consent* that is given in daily clinical care or medical randomized controlled trials is not sufficient to handle large scale genomic data, because the clinical relevance of the wide range of possible- and potentially UFs in WGS is not apparent in advance and can be difficult to express in terms of risk or consequences. However, recital 33 of the GDPR recognizes that it is not always possible to describe the purpose of research at the moment of data collection.[35] Specific informed consent could be used to offer patients who consented to WGS

analysis the option of *opting out* on receiving any genetic information, in protection of their right not to know.

Tiered consent

In solution to overwhelming patients with excessive amounts of information and limiting the administrative burden imposed on researchers, *tiered consent* wields a binning approach. Patients are presented with categorized packages to which they can choose to *opt in*. *Table 1* depicts an example of pre-arranged packages of results based on relevance.[4]

Table 1. Example of pre-arranged packages of results used in tiered consent.

Categories of unsollicited findings	
Actionable	Findings regarding a genetic predisposition for disease with available treatment or prevention.
Non-actionable	Findings regarding a genetic predisposition for disease for which no effective treatment or prevention has been established yet.
Heritable	Findings regarding a genetic predisposition with reproductive relevance and relevance to relatives. These findings do not necessarily have direct consequences for the patient.
Unknown relevance	Findings with no known genetic or clinical relevance.

Organizations and studies have made recommendations for returning genomic findings in oncology. An example of current practice would be the combination of *specific informed consent* and *tiered consent*[28], corresponding with recent suggestions by the Dutch guideline on molecular tumor diagnostics.[37] Primarily, patients should be offered the option to *opt out* of the disclosure of any genetic information. If they are open to receiving genetic information, a default package of solicited findings that are actionable, valid, and accurate will be disclosed. Subsequently, patients can *opt* in on distinct categories of UFs through the *tiered consent* approach. This opportunity to differentiate between options could improve expectation management in counseling and enhance patient autonomy. Research showed that participants enrolled through *tiered consent* were less likely to change their consent for sharing genetic information post-debrief in comparison to through *consent by default and specific consent*.[31] Heedful selection of consent procedures and design of bio-informatic analysis that are selective for specific

genomic findings could further provide solutions in the dilemma of selecting which findings to report back to patients.

Broad consent

In addition to consenting to primary research, patients could be asked to share their genomic data with biobanks or databanks. The Office of Human Research Protections revised the Common Rule in 2018[39] and effectively introduced a new category of informed consent in January 2019: *broad consent*. This option endeavors to increase transparency with advancing technology and big data, where personally identifiable data is accumulated into databanks and biobanks.[17] Widespread participation and accumulation of genomic data sets may give rise to global research networks, sequence reference libraries and connectivity between scientists and their discoveries. To maximize public profit, health data should be made findable, accessible, interoperable, and reusable, conform the FAIR-principles.[47] Factors complicating broad consent are the limited control over unspecified future use of data, indefinite storage and use of material and the limited ability for participants to withdraw. It could be argued that patient interests are not thoroughly being safeguarded by consent at the moment of data collection.[5]

Focus points in a population with (recurrent) glioblastoma

Patients with glioblastoma have a very limited prognosis and many patients are suffering cognitive or neurological impairments as a result of treatment or disease related factors.[40] This, combined with the relative rarity of the disease and the lack of standard-of-care in the recurrent setting, makes this patient population different from other patients with cancer and might require a tailored approach to informed consent for WGS. Since the presence of cognitive or neurological impairments may be seen in the primary setting as well, the following considerations apply to both primary and recurrent glioblastoma.

Previously, it has been shown in solid tumor patients that specific patient characteristics and personal context, such as demographics and stage of disease, affect preferences regarding disclosure of genomic findings through *tiered consent* [4]. These characteristics include experienced quality of life, depressive feelings, and having a college degree. Patients with first- and second-degree relatives were more interested in UFs of reproductive relevance. Notably, patients with curative treatment options were less willing to receive UFs in general than advanced care patients. Age, health literacy, experience with tumor profiling, and sociodemographic factors play a crucial role in the decision-making process.[4] These findings demonstrate that next to the potential actionability and clinical relevance of genomic

findings, patient characteristics might impact preferences in receiving findings and sharing genomic data.

In relating these characteristics to patients with (recurrent) glioblastoma, it should be noted that there are no curative treatment options for glioblastoma. Determining heredity with germline research does therefore not have consequences in terms of preventive treatment options for relatives. Experienced quality of life and feelings of depression are relevant factors in patients with incurable disease. Clinicians obtaining informed consent should be aware of neurocognitive impairments magnifying the previously described challenges that arise in counseling for WGS. Patient autonomy should be valued and preserved as much as possible. Next to experienced quality of life, the importance attributed to quality of life is an important factor in decision making for (clinicians treating) patients with (recurrent) glioblastoma. This population may need more guidance than other oncological populations. Digital tools, such as educational videos for patients and e-learnings for health care professionals[37], could increase the focus on patient education and improve management of patient expectations.

While WGS reports may identify potentially actionable molecular alterations, there are no registered genotype-phenotype correlations with defined clinical consequences known for glioblastoma and ESCAT-scores are still low. Currently, the number of studies initiated for targeted therapies in the recurrent glioblastoma population is limited. Nevertheless, in case an actionable target is identified, recurrent glioblastoma patients might be offered targeted treatment therapy.

In patients with a limited prognosis, like (recurrent) glioblastoma, managing hopes and expectations is important. Especially since this might affect the information (such as UFs) they would like to receive. Indeed, there is a risk that consent procedures are biased by *therapeutic misconception* or *therapeutic hope*[1]. *Therapeutic misconception* means that patients have a false belief that they will obtain clinical benefit from participating in research. This can be resolved by identifying and correcting the patients' false beliefs and providing tailored information, but there is no evident solution to *therapeutic hope*, which exists when there is even the slightest chance at benefit for the patient.

Subjecting patients to further interventions, especially an invasive procedure to obtain fresh frozen tumor samples with the sole purpose to perform WGS, is currently not justifiable, because chances at medical benefit are small and the actionability of potential targets is uncertain. It is crucial for clinicians who provide

tailored information to be transparent about difficult topics, such as limitations in predicting immediate consequences based on clinical relevance and the lack of evidence for treatments in early experimental phase I trials. The alternative option of best supportive care should be considered.

In addition to patient characteristics and unknown actionability of findings, rarity of disease may play a role in decision making. Patients with (recurrent) glioblastoma, as well as patients with other (rare) diseases, might hope to benefit other patients with the same disease. A survey exploring motives for participation in the LeukoTreat program for genetically inherited neurodegenerative disease showed that patients and their families both hoped that their participation would contribute to a better understanding of the progress and causes of the disease, discoveries with (non-)therapeutic impact and more efficient diagnostic tests.[14] These altruistic motives were also observed in a survey by the Dutch Federation of Cancer Patient Organizations, which showed that most cancer patients agree to secondary use of their personal health data without separate consent.[34] This reveals a compassion for future patients. Rare disease communities have the tendency to be more engaged in comparison to populations with more common diseases.

CONCLUSION

NGS and WGS are increasingly being used in neuro-oncology, yet there is no global consensus regarding informed consent for WGS and sharing genomic data in (neuro-)oncology.[29] There are several models available for consideration, of which the benefits, limitations and legal context were discussed. We conclude there are many specific challenges for the population of patients with (recurrent) glioblastoma, related to the rarity of the disease, its' extremely limited prognosis, and the correlation of the stage of the disease with cognitive abilities. Especially cognitive impairments magnify the challenges that arise during counseling for WGS, such as information disclosure and participant comprehension. From an ethical perspective, it is important to recognize vulnerability in cohorts. This vulnerability, that is not exclusive to recurrent glioblastoma patients, may point to a limited capacity to consent and increased sensitivity towards coercion or exploitation.

We suggest that the content of informed consent should be specific to patient populations. A combined model[37] of specific- and tiered consent was proposed for WGS in (recurrent) glioblastoma. The binning approach used in tiered consent has been demonstrated to enhance patient autonomy and it can be adjusted according

to the interests of specific populations. Broad consent is suggested in the context of sharing personally identifiable data with databanks, though it raises concerns about patient autonomy. In parallel, development of meta-governance solutions should be prioritized to facilitate widespread use of genomic data and international collaborations.[13, 42]

Future studies determining the preferences of vulnerable cohorts, such as (recurrent) glioblastoma, could further enhance preservation of autonomy prior to standardization of informed consent procedures. Understanding how patient characteristics influence patient preferences in receiving findings could influence categorization based on relevance in tiered consent. It would be interesting to explore whether patients with a limited prognosis and rarity of disease are more prone to an altruistic approach in comparison to people with common disease. For example, to investigate their interest in possible consequences for relatives or the benefit of the patient population; whether they are more willing to donate their data to databases for research; and whether a limited prognosis of disease influences the fear for genetic discrimination. Determining the preferences of vulnerable cohorts upfront could help patients, physicians, and science.

AUTHOR CONTRIBUTIONS

Mira C. Hasner, Mark P. van Opijnen and Marike L. D. Broekman contributed to the study conception and design. The first draft of the manuscript was written by Mira C. Hasner and Mark P. van Opijnen and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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CONFLICT OF INTEREST

None of the authors declare a conflict of interest.

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11

Chapter 11

Summary and general discussion

Before we started writing this thesis, we had the idea that there was little uniformity in the treatment of recurrent glioblastoma, and there was a limited role for targeted therapies, let alone for routine WGS-based diagnostics. With this thesis, we aimed to assess practice variation (part 1), molecular diagnostics (part 2) and practical implications of whole genome sequencing (WGS, part 3) in adult patients with recurrent glioblastoma. By covering these subjects, we aimed to contribute to high-value care for these patients in the era of molecular diagnostics.

In **chapter 2** we showed that there is a lack of high-quality support in the literature for using mapping during glioma re-resection. Systematically reviewing the literature led to the finding that only 17% (10/58) of the included articles reported information about awake/asleep setting or intra-operative mapping during re-resection. Moreover, six out of these ten studies provided details on the use of mapping. Lastly, only one study compared overall survival in patients with awake re-resection versus patients with asleep re-resection. This study included patients with glioma World Health Organization (WHO) grade 3-4, and showed no significant difference in overall survival (hazard ratio 1.82, 95% confidence interval 0.99-3.34) or post-progression survival (hazard ratio 1.02, 95% confidence interval 0.58-1.8).[1] Overall, the main limitation of the current literature is that they are lacking to report details on intra-operative techniques or that they do not stratify between patient subgroups. Therefore, based on the results of our systematic review, a comprehensive evaluation of the prognostic impact of mapping-guided re-resection turned out to be difficult. However, the need of this is extra important since international guidelines provide little to no guidance when it comes to treatment decisions for recurrent glioma WHO grade 3-4.[2-4] A second important argument is the evidence in the newly diagnosed setting, in which intra-operative mapping has proven to contribute to better survival rates and fewer neurological deficits.[5-8] To rule out the possibility of undertreatment in patients with recurrent glioma, our study underlines the urgent need for future, well-designed studies addressing the beforementioned limitations. Fortunately, initiatives have been launched with international studies like the RECMAP study (NCT06273176) and the RECSUR study (NCT06283927).

In **chapter 3** we demonstrated that re-resection of recurrent glioblastoma is subject to practice variation both between and within Dutch neuro-oncology specialists. By presenting different cases of recurrent glioblastoma to neuro-oncology specialists and asking them the simple (main) question whether they would recommend a re-resection in the specific cases, we aimed to assess possible practice variation. The survey was filled out by 56 respondents, of which 15 (27%)

neurosurgeons, 26 (46%) neuro-oncologists, 2 (4%) medical oncologists, and 13 (23%) radiation oncologists. The results of this study were disconcerting. In the absence of unambiguous guidelines, we observed a relationship between preferred practice (whether to recommend a re-resection or not) and specialty. For instance, in one case 73% of the neurosurgeons recommended a re-resection compared to an opposite 73% of the radiation oncologists who not recommended a re-resection. Overall, in two of the four cases there appeared to be clinical equipoise, with neurosurgeons tending to recommend re-resection more frequently compared to the other specialists. Of note, practice variation was also seen within the same specialty, with one specialist recommending a re-resection because “gross-total resection is very well possible” while a colleague refrained from re-resection since “there is limited oncological benefit”, talking about the same lesion. As said, these results are worrisome but not surprising at the same time. Worrisome, because the survival benefit of re-resection[9] will be unequally allocated to patients, depending on a physician’s preference. Yet, health professionals agree on the need to reduce practice variation.[10] Simultaneously, our results are not quite surprising since the psychologist Daniel Kahneman already concluded that medicine is a ‘noisy’ profession (i.e. with unwanted variability of judgements) in which the interrater reliability could be powerfully reduced by guidelines.[11] We add that our results underline the crucial function of multidisciplinary tumor board discussion.

In **chapter 4** we illustrated that the abovementioned need for (inter)national guidelines on the treatment of recurrent glioblastoma is currently not met. Of the twelve European countries with national guidelines on the diagnosis and treatment of adult glioma (24% of the 50 European countries), nine provided any recommendations on the treatment of recurrent glioblastoma. Moreover, these recommendations differed profoundly from each other. Regarding the role of clinical trials in the recurrent setting, five (42%) of the available guidelines considered enrollment into clinical trial to be an option. It is important to note that the presence of guidelines should not become synonymous to good clinical practice. As seen in **chapter 3**, even in the presence of national guidelines remarkable differences in re-resection practice have been observed between neuro-oncology specialists. Thus, national guidelines do not necessarily rule out the phenomenon of practice variation. Similarly, the absence of national guidelines does not necessarily mean suboptimal practice, especially when considering the availability of international guidelines. More importantly, prioritizing the collection of evidence in the recurrent setting should precede the development of guidelines, since the increasing number of guidelines is currently not paralleled by an equal increase in evidence. Intensification of generation of more evidence should also discriminate between

practice variation that is unwanted and that which is not necessarily unwanted. Future research should investigate whether national guideline availability correlates with clinical outcomes and with sociodemographic characteristics and economic status of countries, in order to further study the impact and origins of unwanted (inter)national practice variation.

A final example of practice variation was seen in **chapter 5**. Here, the interlaboratory variation in next generation sequencing (NGS) of high-grade adult-type diffuse glioma in the Netherlands was surveyed. Our results showed that the composition of diagnostic NGS panels differed in each center, with numbers of genes in the different panels ranging from 12 to 523. Differences were more pronounced when tests are performed to find therapeutic targets in the case of recurrent disease: about half of the centers test for gene fusions and tumor mutational burden. Even though different centers most often end up with the same molecular information for the primary diagnosis after sequential, layered testing, this would be time and eventually cost consuming. In addition, the practice variation in the tests for therapeutic targets could reduce patient selection for potential trial participation when testing for targets is omitted.[12, 13] Without having studied the clinical impact of this practice variation, it is clear that in-house developed tests, standardized panels and routine application of broad gene panels all have their own advantages and disadvantages. Nevertheless, applying broad gene panels as a standard has the dual potential of refining the diagnostics and improving precision oncology.

In **chapter 6**, the protocol of the GLOW (GLioblastoma targeted treatment Option maximization by Wgs) study was presented. This prospective multicenter cohort study aims to investigate the feasibility, validity, utility and value of WGS for recurrent glioblastoma patients. This will allow for disclosure of potentially novel targets for therapy for these patients. Through collaboration of the Hartwig Medical Foundation and twelve Dutch centers, a total of 235 patients with a first glioblastoma recurrence will be included. This trial is registered under the identifier NCT05186064.

The interim results of the GLOW study were presented in **chapter 7**. After inclusion of the first 100 patients, a diagnostic success rate of 80% was found. Based on these 80 WGS reports, targeted therapy was initiated in 6 patients (7.5%). The following targeted therapies were initiated: abemaciclib (*CDK4/6* inhibitor), dacomitinib (*EGFR* inhibitor), entrectinib (*TRK/ROS1/ALK* inhibitor) and erlotinib (3x, *EGFR* inhibitor). The median duration on these experimental drugs was 1.76 months (interquartile range 1.44-2.14), with further progression and adverse events being reasons for discontinuation. Several factors for the poor targeted therapy initiation rate can

be identified. For instance, the clinical implementation of the WGS results was hampered by the prevalent physicians' opinion that upon recurrence, 'standard therapies' like lomustine and rechallenge temozolomide should be preferred. A considerable number of times, the WGS results were "preserved for potential future recurrence". A second major limitation for targeted therapy initiation in this recurrent glioblastoma population, was the following. Once the treating physician wanted to initiate experimental therapy, the DRUP (Drug Rediscovery Protocol) team was accessed and asked to disclose the specific drug for the specific patient. However, one of the criteria for participation in the DRUP is 'measurable disease' at the time of treatment initiation. Since maximal safe resection (i.e. cutting away all measurable disease) is the goal of neurosurgical intervention, our recurrent glioblastoma patients were then refused to participate in the DRUP. The latter made us prepare a DRUP-like program especially designed for glioma patients, to bridge the gap between treatment option identification and available therapies for this population. In the future, the results of this project, called glioblastoma individualized molecular treatment program (GLIMP), should also synergistically improve clinical implementation of WGS-based treatment option identification.

In **chapter 8**, the current clinical trial landscape was assessed to investigate the role of molecular biomarkers in trials on recurrent glioblastoma treatment. After screening the database ClinicalTrials.gov, we found that 76% (181/237) of the current studies did not include molecular criteria in the study design. In the remaining 56 studies, *EGFR* amplifications/mutations, *CDKN2A/B* or *C* deletion, *CDK4/6* amplification, and *RB* wildtype status were most frequently investigated, as were the corresponding drugs abemaciclib and ribociclib. Our study showed that the potential efficacy of targeted treatment is currently not yet translated into genome-driven trials in patients with recurrent glioblastoma. We therefore advocate an intensification of genome-driven trials in an attempt to provide more evidence for the (in)efficacy of targeted treatments and to bridge this knowledge gap. An excellent example is the N2M2 study, a phase I/IIa umbrella trial of molecularly matched targeted therapies.[14] The recently presented results of this N2M2 study (NCT03158389) show clinical activity of temsirolimus in patients demonstrating mTOR activation while palbociclib has no clinical activity in patients with *CDK4* amplification or *CDKN2A/B* codeletion. Currently, the acting on potentially druggable targets is challenged by target credentialing and validation, tumor heterogeneity and clinical trial design.[15] Efforts are needed to overcome these challenges and, as said, bridge the knowledge gap regarding genome-driven oncology in glioblastoma patients. The current lack of evidence and past results should not paralyze the exploration of new potentially actionable targets.

In **chapter 9**, the genetic predisposition to adult glioblastoma based on whole genome sequencing analysis was studied. In an unselected cohort of 98 patients, pathogenic germline variants (PGVs) were observed in 11% (11/98) of the patients. PGVs were found in the following genes: *BRCA1*, *MSH6*, *PMS2, TP53, NF1* and *SUFU*. In eight of these patients (73%) causality was supported by a second (somatic) event and/or a matching genome-wide mutational signature. Our study showed that germline predisposition does also play a role in the development of adult glioblastoma (as is more commonly known for pediatric gliomas), with mismatch repair deficiency being the main mechanism. This finding might have some consequences and can be integrated in the discussion about the application of WGS-based diagnostics. First, several of these PGVs were in predisposition genes that are increasingly important for (targeted) therapy selection.[16-19] Second, most of the PGVs found in our study are currently not tested for in most of the Dutch laboratories, as we have seen in **chapter 5**. Our findings do also underline the importance of genetic counseling prior to germline testing, with specific attention for mismatch repair gene deficiencies, as recommended in the EANO guideline on molecular testing of gliomas in adults.[12] As the use of comprehensive tumor genetic and genomic diagnostic test continues to grow, the detection of PGVs is occurring more frequently than previously expected.[20, 21] Thus, comprehensive tumor genetic and genomic profiling for glioblastoma patients requires an integrated approach that facilitates appropriate referral to clinical geneticists.

In **chapter 10**, the challenges related to informed consent procedures and data sharing regarding WGS in (recurrent) glioblastoma were discussed. The increased use of WGS in neuro-oncology for diagnostic and research purposes necessitates a renewed conservation about informed consent procedures and about governance structures for sharing personal health data, illustrated by the findings from **chapter 9**. There is currently no consensus on how to obtain informed consent for WGS in this population. In this chapter, we analyzed the formats and contents of frameworks suggested in literature. Since (recurrent) glioblastoma is characterized by the rarity of the disease, extremely poor prognosis and impact on cognitive abilities, we suggested that the informed consent procedure should be tailormade for these patients. A combined model of specific and tiered consent was proposed, and in parallel, the development of meta-governance solutions should be prioritized to facilitate widespread use of genomic data and international collaborations.[22] It is important to understand how patient characteristics influence patient preferences in receiving WGS findings, which in turn could influence categorization based on relevance in tiered consent.

FUTURE DIRECTIONS

Taking the evidence from **chapters 2 to 10** together, we conclude that the journey of a patient with recurrent glioblastoma is subject to practice variation in diagnostics and treatments, in which the clinical implementation of WGS results in the context of precision oncology has currently little support from treating physicians, accompanied by some ethical objections that need to be considered. Another important conclusion is that routine WGS-based diagnostics might help the (future) patient, since WGS – which was proven fast and feasible in our population – has a great potential to not only create a lot of new knowledge about the biology of glioblastomas, but also to unravel novel targets for treatment.

The results of this thesis lead to the following future directions. First, we endorse future studies on the survival benefit of re-resection and the development of prediction models to be able to better discriminate which individual patients will benefit from (mapping-guided) re-resection. This could reduce practice variation in re-resection and might further improve the concept of precision oncology. Simultaneously, while the costs continue to decrease, routine WGS-based diagnostics should gain more prominence upon glioblastoma recurrence. The advantages of WGS are multiple, with the uniformity and completeness on the diagnostic hand, and the accumulation of tumor specific knowledge on the scientific hand. To facilitate access to targeted therapies for recurrent glioblastoma patients, we are eager to initiate the beforementioned GLIMP study in the near future. We are convinced that these patients deserve equal changes, acknowledging the specific characteristics and associated hurdles in this entity. To this end, a second project we are about to start is charting the neuro-oncology specialists' individual attitudes and beliefs towards clinical implementation of WGS-based therapies. What are the ideas, thoughts and assumptions behind the reluctance to prefer targeted therapy over 'standard' treatment? Finally, more molecularly matched targeted therapy trials are urgently needed to collect target specific evidence for efficacy, as some recent successful stories in other glioma populations were published.[23, 24]

This thesis was started with the statement that "there is actually always something a physician can do for the patient", referring to symptom management and palliative care. At the end of this thesis, we may now conclude that 'doing everything' in terms of diagnostics and treatments should be redefined once WGS and WGS-based treatments become clinical practice. Fortunately, science is characterized by curiosity and not by cynicism, therefore leaving us hopeful for the future in which new and effective treatments for recurrent glioblastoma patients will be discovered. A long way might be ahead, yet the potential is all the greater.

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APPENDICES

Nederlandse samenvatting

List of publications

Curriculum Vitae

Dankwoord

NEDERLANDSE SAMENVATTING

Voordat we aan het schrijven van dit proefschrift begonnen, hadden we het idee dat er weinig uniformiteit bestond in de behandeling van recidief glioblastoom, en dat de rol van gerichte therapie beperkt is, laat staan die van routinematige WGS-diagnostiek. In dit proefschrift is getracht om de praktijkvariatie (deel 1), moleculaire diagnostiek (deel 2) en praktische implicaties van volledige genoomanalyse (*whole genome sequencing*, WGS, deel 3) in volwassen patiënten met een recidief glioblastoom te onderzoeken. Door deze onderwerpen te onderzoeken, hopen we bij te dragen aan hoogwaardige zorg voor deze patiënten in een tijd waarin moleculaire diagnostiek een steeds belangrijkere rol krijgt.

In **hoofdstuk 2** hebben we aangetoond dat er in de literatuur een gebrek is aan hoogwaardig bewijs voor het gebruik van *mapping* tijdens de re-resectie van een glioom. Door het systematisch beoordelen van de beschikbare literatuur stuitten we op de bevinding dat slechts 17% (10/58) van de geïncludeerde artikelen informatie rapporteerde over wakkere/slapende setting of intra-operatieve *mapping* tijdens re-resectie. Bovendien gaven maar zes van deze tien studies details over het gebruik van *mapping*. Uiteindelijk vergeleek maar een studie de totale overleving van patiënten die een wakkere re-resectie ondergingen met die van patiënten die een slapende re-resectie ondergingen. Deze laatste studie bevatte patiënten met een glioom WHO graad 3-4 en liet geen significant verschil zien in totale overleving (hazard ratio 1.82, 95% betrouwbaarheidsinterval 0.99-3.34) of in overleving na ziekteprogressie (hazard ratio 1.02, 95% betrouwbaarheidsinterval 0.58-1.8).[1] Alles bij elkaar bezien is de belangrijkste beperking in de huidige literatuur dat details over intra-operatieve technieken ontbreken, of dat er niet gestratificeerd wordt tussen subgroepen patiënten. Daarom bleek een nauwkeurige evaluatie van de prognostische impact van *mapping* tijdens re-resectie, op basis van de resultaten van dit systematisch literatuuronderzoek, moeilijk om weer te geven. De noodzaak hiervan is echter extra belangrijk aangezien internationale richtlijnen weinig tot geen richting geven als het gaat om behandelbeslissingen voor recidief gliomen WHO graad 3-4.[2-4] Een tweede belangrijk argument is het bewijs dat er is bij nieuw gediagnosticeerde gliomen, waarbij intra-operatieve *mapping* bewezen bijdraagt aan betere overlevingskansen en minder neurologische complicaties.[5-8] Onze studie onderstreept het urgente belang van toekomstige, goed vormgegeven studies om de voorgenoemde beperkingen aan te pakken en om de kans op onderbehandeling in patiënten met een recidief glioom te minimaliseren. Gelukkig zijn er initiatieven onderweg met internationale studies zoals de RECMAP-studie (NCT06273176) en de RECSUR-studie (NCT06283927).

In **hoofdstuk 3** hebben we laten zien dat re-resectie van recidief glioblastoom onderhevig is aan praktijkvariatie, zowel onder als tussen Nederlandse specialisten op het gebied van neuro-oncologie. Door hen verschillende cases van een recidief glioblastoom voor te leggen en hen de simpele (hoofd)vraag voor te leggen of zij in die specifieke casus een re-resectie zouden voorstellen, hebben we geprobeerd om mogelijke praktijkvariatie in kaart te brengen. De vragenlijst werd ingevuld door 56 respondenten, waaronder 15 (27%) neurochirurgen, 26 (46%) neuro-oncologen, 2 (4%) internist-oncologen en 13 (23%) radiotherapeut-oncologen. De resultaten van deze studie waren verontrustend. In de afwezigheid van eenduidige richtlijnen bleek er een verband te bestaan tussen de voorkeur voor behandeling (wel of niet een re-resectie aanbevelen) en het specialisme van de respondent. In een van de cases bijvoorbeeld, raadde 73% van de neurochirurgen een re-resectie aan, terwijl een tegenovergestelde 73% van de radiotherapeut-oncologen een re-resectie afraadde. In totaal bleek er in twee van de vier cases onzekerheid over de juiste behandeling, waarbij neurochirurgen geneigd waren om re-resectie vaker aan te bevelen dan andere specialisten. Overigens werd er ook praktijkvariatie gezien binnen hetzelfde specialisme. De ene specialist bijvoorbeeld raadde re-resectie aan omdat “volledige resectie goed mogelijk is” terwijl een collega over dezelfde tumor sprekend een re-resectie afraadde “omdat het oncologische voordeel beperkt is”. Zoals gezegd zijn deze resultaten zorgelijk, en tegelijkertijd ook niet verrassend. Zorgelijk, omdat het overlevingsvoordeel van re-resectie[9] ongelijk wordt toebedeeld aan patiënten, afhankelijk van de voorkeur van de behandelaar. Ondertussen zijn gezondheidsprofessionals het eens over de noodzaak om praktijkvariatie te reduceren.[10] Tegelijkertijd zijn de resultaten van onze studie niet erg verrassend aangezien de psycholoog Daniel Kahneman al geconcludeerd heeft dat geneeskunde een beroep is met veel ruis (verschil in oordelen die identiek zouden moeten zijn). Kahneman betoogt dat dit fenomeen sterk gereduceerd zou kunnen worden door richtlijnen.[11] We voegen hier aan toe dat onze resultaten de cruciale functie van multidisciplinaire tumorbesprekingen onderstrepen.

In **hoofdstuk 4** illustreren we dat de bovengenoemde noodzaak voor (inter) nationale richtlijnen over recidief glioblastomen momenteel niet gehaald wordt. Van de twaalf Europese landen met nationale richtlijnen over de diagnose en behandeling van gliomen bij volwassenen (24% van de 50 Europese landen) gaven er negen enige aanbeveling(en) over de behandeling van recidief glioblastoom. Bovendien verschilden deze aanbevelingen sterk van elkaar. Wat betreft de rol van klinische onderzoeken in de recidiefsetting werd in vijf (42%) van de beschikbare richtlijnen overwogen om de patiënt te laten deelnemen in een klinisch onderzoek. Het is belangrijk om op te merken dat de beschikbaarheid van richtlijnen niet

synoniem wordt aan goede klinische zorg. Zoals in **hoofdstuk 3** is aangetoond, worden er zelfs in de aanwezigheid van nationale richtlijnen opmerkelijke verschillen tussen neuro-oncologie specialisten waargenomen als het gaat over re-resecties. Nationale richtlijnen sluiten dus niet per se het fenomeen van praktijkvariatie uit. Omgekeerd geldt hetzelfde: de afwezigheid van nationale richtlijnen betekent niet noodzakelijkerwijs dat de geleverde zorg suboptimaal is, zeker niet gelet op de beschikbaarheid van internationale richtlijnen. Belangrijker is dat het ontwikkelen van richtlijnen voorafgegaan moet worden door het verzamelen van meer bewijs over de recidiefsetting, aangezien het stijgende aantal richtlijnen momenteel niet parallel loopt met eenzelfde stijging in bewijskracht. Intensivering van het creëren van meer bewijs zou ook een onderscheid moeten maken tussen praktijkvariatie die ongewenst is en die niet per se ongewenst is. Toekomstig onderzoek moet uitwijzen of de beschikbaarheid van nationale richtlijnen correleert met klinische uitkomsten en met sociodemografische karakteristieken en economische status van landen, om de impact en oorzaken van ongewenste (inter)nationale praktijkvariatie verder te onderzoeken.

Een laatste voorbeeld van praktijkvariatie zagen we in **hoofdstuk 5**. Daar werd de variatie tussen laboratoria in *next generation sequencing* (NGS) van hooggradige diffuus gliomen bij volwassenen in Nederland onderzocht. Onze resultaten lieten zien dan de samenstelling van diagnostische NGS-panels in elk centrum verschillend was, met het aantal genen per panel variërend tussen de 12 tot 523. De verschillen waren nog meer uitgesproken wanneer getest werd om therapeutische aanknopingspunten te vinden in het geval van progressieve ziekte: ongeveer de helft van de centra test op genfusies en aantal mutaties per tumorcel (TMB). Ondanks dat verschillende centra uiteindelijk toch op dezelfde moleculaire informatie voor de primaire diagnose uitkomen na sequentieel, gelaagd testen, kan dit toch tijd- en kostenrovend zijn. Bovendien kan de praktijkvariatie in de testen voor therapeutische aanknopingspunten de patiëntselectie voor potentiële deelname aan onderzoek reduceren wanneer het testen voor aanknopingspunten achterwege wordt gelaten.[12, 13] Zonder de klinische impact van deze praktijkvariatie te hebben onderzocht, is het duidelijk dat in-huis ontwikkelde testen, gestandaardiseerde panels en routinematige toepassing van brede genpanels allemaal hun eigen voor- en nadelen hebben. Desalniettemin heeft het standaard toepassen van brede genpanels het tweeledige potentieel van het gelijktrekken van diagnostiek en het verbeteren van precisieoncologie.

In **hoofdstuk 6** werd het protocol van de GLOW-studie (*GLioblastoma targeted treatment Option maximization by Wgs*) gepresenteerd. Deze prospectieve

multicenter cohortstudie heeft als doel om te onderzoek wat de haalbaarheid, validiteit, bruikbaarheid en waarde zijn van WGS-diagnostiek bij patiënten met een recidief glioblastoom. Dit maakt het mogelijk om eventuele nieuwe aanknopingspunten voor behandeling voor deze patiënten te ontrafelen. Door samenwerking tussen de Hartwig Medical Foundation en twaalf Nederlandse ziekenhuizen wordt een totaal van 235 patiënten met een eerste recidief van het glioblastoom geïncludeerd. Dit onderzoek is geregistreerd onder het nummer NCT05186064.

De tussentijdse resultaten van de GLOW-studie werden besproken in **hoofdstuk 7**. Na inclusie van de eerste 100 patiënten werd een diagnostisch succespercentage van 81% gevonden. Op basis van deze 81 WGS-rapporten werd er bij 6 patiënten (7.4%) gerichte therapie gestart. De volgende gerichte therapieën werden gestart: abemaciclib (*CDK4/6*-remmer), dacomitinib (*EGFR*-remmer), entrectinib (*TRK-/ ROS1-/ALK*-remmer) en erlotinib (3x, *EGFR*-remmer). De mediane behandelduur met deze experimentele behandelingen was 1.76 maanden (interkwartielafstand 1.44-2.14), met verdere progressie en bijwerkingen als redenen om de behandeling te staken. Verschillende factoren voor de slechte uitkomst wat betreft het starten van gerichte therapie kunnen worden geïdentificeerd. Zo werd de klinische implementatie van de WGS-resultaten bijvoorbeeld belemmerd door de veelvoorkomende opvatting van behandelend artsen dat ten tijde van het recidief, ‘standaardbehandelingen’ zoals lomustine en opnieuw temozolomide de voorkeur moeten hebben. Een aanzienlijk aantal keer werden de WGS-resultaten “bewaard voor eventuele nieuwe progressie”. Een tweede belangrijke beperking voor het starten van gerichte behandeling in deze populatie met recidief glioblastoom was de volgende. Wanneer de behandelend arts eenmaal gerichte therapie wilde starten, werd het DRUP-team (*Drug Rediscovery Protocol*) benaderd en gevraagd om het specifieke medicijn voor deze specifieke patiënt beschikbaar te stellen. Echter, een van de criteria voor deelname aan de DRUP is ‘meetbare ziekte’ ten tijde van het starten van de behandeling. Omdat maximaal veilige resectie (oftewel: het wegsnijden van alle meetbare ziekte) het ultieme doel van neurochirurgische interventie is, werd onze patiënten met een recidief glioblastoom vervolgens geweigerd om deel te nemen aan de DRUP. Dit laatste heeft ertoe geleid dat wij een DRUP-achtig programma zijn gaan voorbereiden, specifiek bedoeld voor glioompatiënten om zo het gat te dichten tussen identificatie van behandelopties en beschikbare therapieën voor deze populatie. In de toekomst moeten de resultaten van dit project, *glioblastoma individualized molecular treatment program* (GLIMP) genoemd, middels een synergistisch effect de klinische implementatie van WGS-gebaseerde identificatie van behandelopties verbeteren.

In **hoofdstuk 8** werd het huidige klinische onderzoekslandschap bekeken om te onderzoeken wat de rol is van moleculaire biomarkers in onderzoeken naar de behandeling van recidief glioblastoom. Na het screenen van de database ClinicalTrials.gov vonden we dat 76% (181/237) van de huidige studies geen moleculaire criteria meeneemt in het ontwerp van de studie. In de overige 56 studies werden *EGFR*-amplificaties/-mutaties, *CDKN2A/B/C*-deletie, *CDK4/6*-amplificatie en *RB*-wildtype het vaakst onderzocht, net als de bijbehorende medicijnen abemaciclib en ribociclib. Onze studie liet zien dat de potentiële effectiviteit van gerichte behandeling momenteel nog niet vertaald wordt naar genoomgedreven onderzoeken bij patiënten met recidief glioblastoom. Daarom betogen we een intensivering van genoomgedreven onderzoeken in een poging om meer bewijs te leveren voor de (in)effectiviteit van gerichte behandeling en om dit kennishiaat te overbruggen. Een mooi voorbeeld is de N2M2-studie, een fase I/IIa-studie naar moleculair gematchte gerichte behandelingen.[14] De recent gepresenteerde resultaten van deze N2M2-studie (NCT03158389) laten klinische activiteit zien van temsirolimus in patiënten met mTOR-activatie, terwijl palbociclib geen klinische activiteit heeft in patiënten met *CDK4*-amplificatie of *CDKN2A/B*-codeletie. Op dit moment wordt het handelen naar potentiële aanknopingspunten voor behandeling bemoeilijkt door het toekennen van de juiste waarde aan een aanknopingspunt, het valideren daarvan, tumorheterogeniteit en het ontwerp van klinische onderzoeken.[15] Om deze uitdagingen te overkomen is veel inspanning nodig. Hiermee kan echter ook het kennishiaat wat betreft genoomgedreven oncologie in glioblastoompatiënten overbrugd worden. Het huidige gebrek aan bewijs en resultaten uit het verleden moeten het zoeken naar nieuwe potentiële aanknopingspunten voor behandeling niet platleggen.

In **hoofdstuk 9** werd op basis van WGS-analyse de genetische predispositie voor glioblastoom bij volwassenen bestudeerd. In een niet-geselecteerd cohort van 98 patiënten werden in 11% (11/98) van de patiënten pathogene kiembaanvarianten (PGVs) gevonden. Deze PGVs werden in de volgende genen aangetroffen: *BRCA1*, *MSH6*, *PMS2, TP53, NF1* en *SUFU*. In acht van deze patiënten (73%) werd het vermoeden op causaliteit ondersteund door een tweede (somatische) afwijking en/of een matchend genoombreed mutatieprofiel. Onze studie liet zien dat kiembaanpredispositie een rol speelt in het ontstaan van glioblastoom bij volwassenen (zoals algemener bekend voor pediatrische gliomen), met *mismatch repair* deficiëntie als het belangrijkste mechanisme. Deze bevinding kan een aantal consequenties hebben en kan geïntegreerd worden in de discussie over de toepassing van WGS-diagnostiek. Ten eerste werden verschillende van de PGVs in predispositiegenen gevonden die steeds belangrijker zijn voor selectie van

(doelgerichte) therapie.[16-19] Ten tweede wordt op de meeste van de PGVs die in deze studie zijn gevonden niet getest in de meeste Nederlandse laboratoria, zoals we hebben gezien in **hoofdstuk 5**. Onze resultaten onderstrepen ook het belang van genetische counseling voorafgaand aan kiembaanonderzoek, met speciale aandacht voor *mismatch repair* deficiënties, zoals aanbevolen in de EANO-richtlijn over moleculaire testen bij gliomen bij volwassenen.[12] Terwijl het gebruik van uitgebreide genetische en genomische diagnostische testen toeneemt, gebeurt het vaker dan gedacht dat er PGVs gevonden worden.[20, 21] Uitgebreide genetische en genomische profilering van glioblastomen vereist dus een geïntegreerde benadering met goede verwijzing naar klinisch genetici.

In **hoofdstuk 10** werden de uitdagingen bediscussieerd die verbonden zijn aan het verkrijgen van toestemming voor WGS en het delen van die data van patiënten met een (recidief) glioblastoom. Het toegenomen gebruik van WGS in de neuro-oncologie voor diagnostische en onderzoeksdoeleinden vraagt om een hernieuwd gesprek over het afnemen van geïnformeerde toestemming en over overheidsstructuren voor het delen van persoonlijke gezondheidsdata, zoals de bevindingen uit **hoofdstuk 9** laten zien. Er is op dit moment geen consensus over hoe toestemming voor WGS moet worden afgenomen in deze populatie. In dit hoofdstuk hebben we onderzocht welke vormen van toestemmingsmodellen er in de literatuur worden voorgesteld en wat hun inhoud is. Aangezien (recidief) glioblastoom wordt gekenmerkt door de zeldzaamheid van de ziekte, de extreem slechte prognose en de impact op cognitieve vermogens, stelden we voor dat het afnemen van geïnformeerde toestemming voor deze patiënten op maat gemaakt moet worden. Een gecombineerd model van specifieke en gelaagde toestemming werd voorgesteld, en tegelijkertijd moeten overheidsoverstijgende oplossingen ontwikkeld worden om breder gebruik van genomische data en internationale samenwerkingen mogelijk te maken.[22] Het is belangrijk om te begrijpen hoe patiëntkarakteristieken invloed hebben op patiëntvoorkeuren wat betreft het geïnformeerd worden over WGS-bevindingen, wat vervolgens weer invloed kan hebben op categorisatie op basis van relevantie bij gelaagde toestemming.

AANBEVELINGEN VOOR DE TOEKOMST

Het bewijs uit de **hoofdstukken 2 tot en met 10** samennemend, concluderen we dat de reis van een patiënt met een recidief glioblastoom onderhevig is aan praktijkvariatie in diagnostiek en behandelingen, waarin de klinische implementatie van WGS-resultaten in de context van precisieoncologie momenteel weinig steun

heeft van behandelend artsen, vergezeld door enkele ethische bezwaren die overwogen moeten worden. Een andere belangrijke conclusie dat routinematige WGS-analyse de (toekomstige) patiënt zou kunnen helpen, aangezien WGS – dat bewezen snel en haalbaar is in onze populatie – een groot potentieel heeft om niet alleen veel nieuwe kennis te creëren over de biologie van glioblastomen, maar ook om nieuwe aanknopingspunten voor behandeling te ontdekken.

De resultaten van dit proefschrift leiden tot de volgende aanbevelingen voor de toekomst. Ten eerste moedigen we toekomstige studies naar het overlevingsvoordeel van re-resectie en de ontwikkeling van predictiemodellen aan om beter onderscheid te kunnen maken welke individuele patiënten baat kunnen hebben van (*mapping*-gestuurde) re-resectie. Dit kan praktijkvariatie in re-resectie verminderen en kan het concept van precisieoncologie verder verbeteren. Tegelijkertijd, terwijl de kosten blijven dalen, zou routinematige WGS-diagnostiek een belangrijkere rol moeten krijgen bij recidief glioblastoom. Er zijn verscheidene voordelen van WGS, met aan de diagnostische kant de uniformiteit en volledigheid en met aan de wetenschappelijke kant de toenemende tumorspecifieke kennis. In een poging om de toegang tot gerichte behandelingen voor patiënten met een recidief glioblastoom te faciliteren, zijn we erop gebrand om de voorgenoemde GLIMP-studie in de nabije toekomst te starten. We zijn ervan overtuigd dat deze patiënten gelijke kansen verdienen, erkennend dat dit type tumor specifieke kenmerken en bijbehorende obstakels kent. Daarom staan we op het punt een tweede project te starten, namelijk het in kaart brengen van de opvattingen en overtuigingen van individuele specialisten in de neuro-oncologie met betrekking tot de klinische implementatie van op WGS-gebaseerde behandeling. Wat zijn de ideeën, gedachten en aannames achter de terughoudendheid om gerichte behandeling te verkiezen boven ‘standaard’ behandeling? Ten slotte zijn er meer studies met moleculair afgestemde gerichte behandelingen nodig om targetspecifiek bewijs voor effectiviteit te verzamelen. Recent zijn hier enkele succesvolle voorbeelden bij andere glioompopulaties over gepubliceerd.[23, 24]

Dit proefschrift begon met de opmerking dat er “eigenlijk altijd iets is wat een arts kan doen voor de patiënt”, verwijzend naar symptoommanagement en palliatieve zorg. Aan het eind van dit proefschrift zouden we nu kunnen concluderen dat ‘alles doen’ in termen van diagnostiek en behandelingen opnieuw gedefinieerd moet worden zodra WGS en op WGS-gebaseerde behandelingen toegepast worden in de klinische praktijk. Gelukkig wordt de wetenschap gekenmerkt door nieuws-gierigheid en niet door cynisme, wat ons hoopvol maakt voor de toekomst, waarin nieuwe en effectieve behandelingen voor patiënten met recidief glioblastoom ontdekt zullen worden. Een lange weg ligt er voor, maar des te groter is het potentieel.

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CURRICULUM VITAE

Martinus Peter van Opijnen was born on March 29, 1995 in Gouda, the Netherlands. After finishing secondary school at Driestar College Gouda, Mark started studying Biomedical Engineering in 2013 at Eindhoven University of Technology. During his first year, he found he wanted to become a medical doctor instead of an engineer. In 2014 he started his medical study at Leiden University, which he completed in March 2021. During the third year of his study, he became interested in research and started doing research in the field of neuro-oncology, supervised by dr. Linda Dirven, dr. Johan Koekkoek and prof. dr. Martin Taphoorn. After obtaining his medical degree, Mark started working as a resident not in training at the neurosurgical department at the Haaglanden Medisch Centrum, The Hague. Simultaneously, he got the opportunity to combine his clinical work with a PhD trajectory supervised by prof. dr. mr. Marike Broekman, dr. Filip de Vos and prof. dr. ir. Edwin Cuppen. After working for two and a half years at the neurosurgical department, he decided to switch gears and started his training as a general practitioner in September 2024. Mark is married to Hanja Zuidijk and together they have a daughter and a son: Lauren and Boaz.

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