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

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Chapter 7

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

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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.

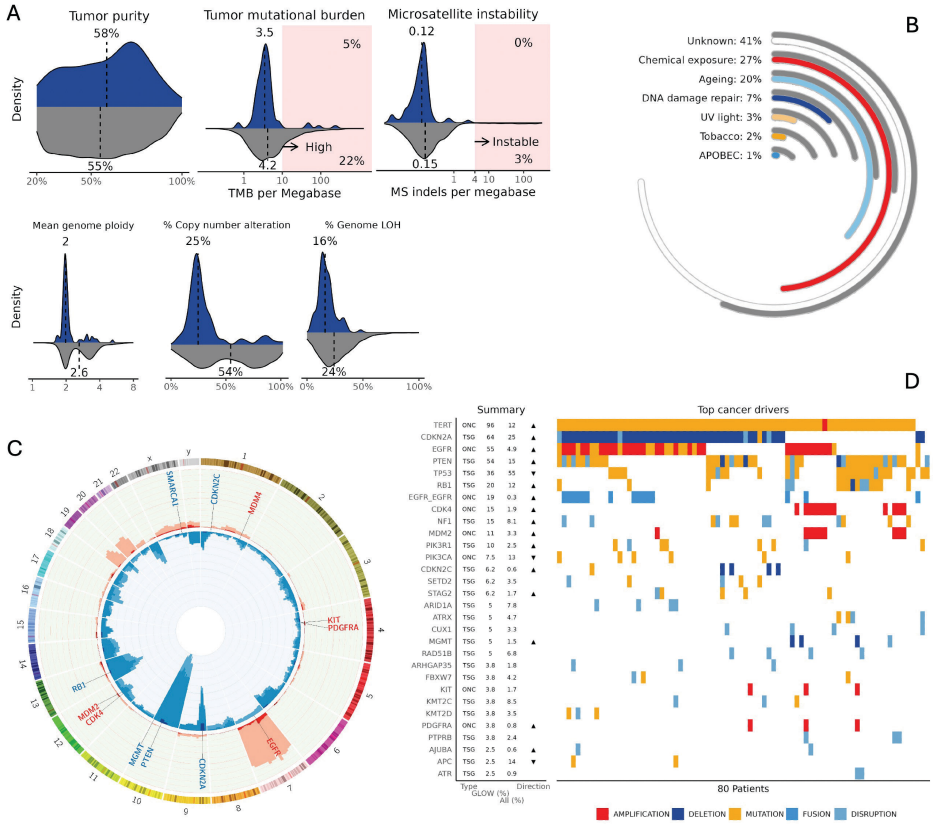
Characteristics	Total cohort ***n*** **= 100**
Gender, no.	
Male	74
Female	26
Median age at recurrence, years (IQR)	60.0 (51.3-68.0)
Hemisphere, no.	
Left	41
Right	2
Bilateral	57
Tumor lobe involvement, no.	
Cerebrum (incl. basal ganglia, insula, thalamus)	0
Frontal	44
Temporal	42
Parietal	26
Occipital	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

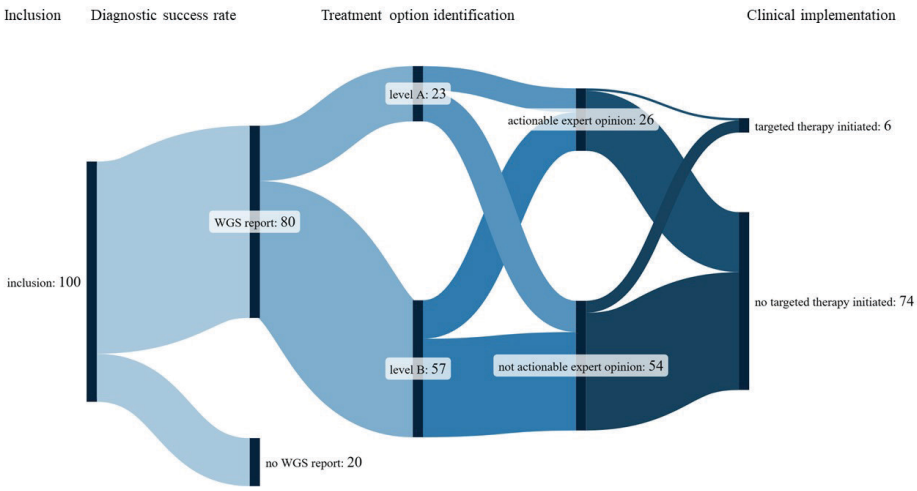
evidence found. In these 23 patients with one or more level A targets, ten patients had at least one actionable variant according to the expert panel opinion. Another sixteen patients with maximum level B variants were classified as having at least one actionable variant by the experts, resulting in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

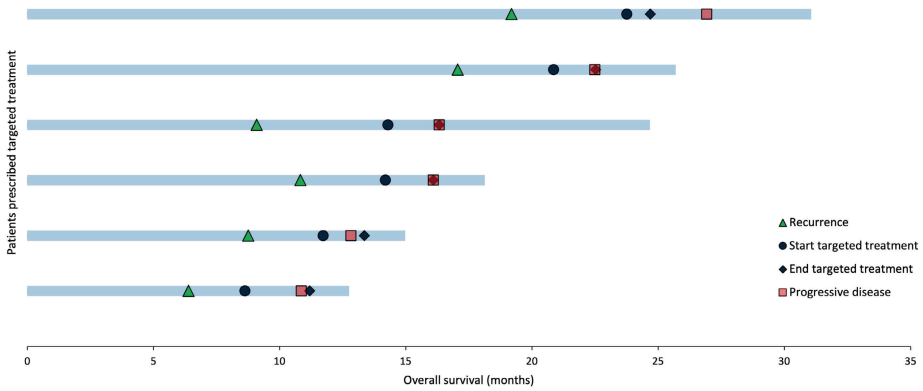


Level A: Food and Drug Administration (FDA) approved therapy and/or guidelines based on the Clinical Knowledgebase, Level B: late clinical trials based on the Clinical Knowledgebase, WGS: whole genome sequencing.

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