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RESEARCH ARTICLE

Tumor Markers and Signatures

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Profiling of circulating glial cells for accurate blood-based diagnosis of glial malignancies

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Abstract

Here, we describe a blood test for the detection of glial malignancies (GLI-M) based on the identification of circulating glial cells (CGCs). The test is highly specific for GLI-M and can detect multiple grades (II–IV) and subtypes including gliomas, astrocytomas, oligodendrogliomas, oligoastrocytomas and glioblastomas, irrespective of gender and age. Analytical validation of the test was performed as per Clinical and Laboratory Standards Institute (CLSI) guidelines. Real-world performance characteristics of the test were evaluated in four clinical (observational) studies. The test has high analytical sensitivity (95%), specificity (100%) and precision (coefficient of variation [CV] = 13.7% for repeatability and CV = 23.5% for within laboratory precision, both at the detection threshold) and is not prone to interference from common drugs and serum factors. The ability of the test to detect and differentiate GLI-M from non-malignant brain tumours (NBT), brain metastases from primary epithelial malignancies (EPI-M) and healthy individual donors (HD) was evaluated in four clinical cohorts. Across these clinical studies, the test showed 99.35% sensitivity (95% confidence interval [CI]: 96.44%–99.98%) and 100% specificity (95% CI: 99.37%–100%). The performance characteristics of this test support its clinical utility for diagnostic triaging of individuals presenting with intracranial space-occupying lesions (ICSOL).

KEYWORDS

blood test, brain tumour, central nervous system, circulating glial cells, diagnosis, glial malignancy, liquid biopsy, non-invasive, triaging

What's new?

While most intracranial masses are non-malignant, it is critical to get a prompt, accurate diagnosis. However, obtaining brain tissue for evaluation is unpleasant and carries significant risks. Here, the authors describe a blood test for glial malignancies based on identification of circulating glial cells. The test successfully detects multiple grades and subtypes, including gliomas, astrocytomas,

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oligodendrogliomas, oligoastrocytomas and glioblastomas. The test was evaluated in four clinical studies and showed 100% specificity and 99.35% sensitivity. This blood-based approach could be particularly useful in cases where the lesion is unresectable or a biopsy is impossible.

1 | INTRODUCTION

Brain tumours account for 85%–90% of all primary central nervous system (CNS) tumors[1] and ~300,000 (~1.6%) of the total ~19,300,000 annual cancer incidences and 250,000 (~2.5%) of the total 10,000,000 annual cancer-related deaths globally.[2] In patients presenting with radiological intracranial space-occupying lesions (ICSOL), non-malignant pathologies are more common[3] accounting for ≥70% of all cases, while the differential diagnosis among the malignant conditions include primary glial malignancy (GLI-M), metastases from other solid tumours and non-glial subtypes of CNS malignancies (NGCM) which are rarer. Glioblastoma multiforme (GBM) is the most common (49%) subtype of GLI-M.[3] As malignant and non-malignant conditions have different management pathways, the expeditious establishment of diagnosis is of critical importance.

The standard of care (SoC) for establishing a diagnosis in individuals presenting with ICSOL is histopathological evaluation (HPE) of tumour tissue specimens obtained from surgical excision or biopsy. Surgical resection or biopsy is more challenging in patients with poor performance, comorbidities or patients' reluctance.[4] Procedural risks are well-documented and include pain and discomfort, intracranial haemorrhage, cerebral oedema, infections, morbidity and mortality.[5] Furthermore, the anatomical site of the lesion may be associated with increased procedural risks and complications. Prior studies also suggest that ~70% of patients with intracranial lesions have benign conditions[3] indicating that in a sizeable population of symptomatic individuals, the ability to obtain the same inference non-invasively may reduce the requirement for and risks associated with invasive tissue sampling procedures.

Therefore there is considerable benefit in the non-invasive detection of GLI-M including risk mitigation, resource optimization, cost benefits and avoidance of delays in time to diagnosis and time to treatment, especially in unresectable cases where tissue sampling is unviable. Previous attempts at non- or minimally invasive detection of GLI-M and differentiation of GLI-M from non-malignant brain tumours (NBT) and brain metastases have examined the profiling of gene variants[6] or CpG island methylation[7] in cell-free deoxyribonucleic acid (cfDNA) and profiling of exosomal messenger-/micro-ribonucleic acid (mRNA/miRNA) transcripts.[8] However, these approaches are limited by their low sensitivity and specificity.[9] Circulating tumour cells (CTCs) are viable malignant cells in circulation, the molecular evaluation of which may be an alternative to or comparable with that of the tumour tissue from which they originate.[10–12] CTCs are rarely detected in the peripheral blood of healthy individuals (or among those with non-malignant conditions) and their detection in asymptomatic populations may indicate an underlying malignancy.[13,14] Such malignant cells shed by a primary GLI-M are referred to as circulating glial cells (CGCs) and are identified based on the expression of glial fibrillary acid protein (GFAP) and oligodendrocyte transcription factor-2 (OLIG2). In this article, 'CGCs' refers to malignant glial tumour cells in circulation while 'CTCs' refers to malignant cells from other primary solid tumours (non-CNS).

We previously described the functional enrichment of CTCs from peripheral blood using a proprietary CTC enrichment medium (CEM) which selectively induces apoptosis in non-malignant cells and permits the survival of malignant cells. This method yields sufficient viable CTCs for downstream applications including multiplexed immunocytochemistry (ICC).[13,15] In the present study we used this enrichment process to harvest CGCs and CTCs from blood samples, which were then profiled via ICC to determine the expression of markers including GFAP, OLIG2, cytokeratins (CK) and the common leukocyte antigen (CD45) (Figure 1). Here, we describe the performance characteristics of the blood-based test to detect GLI-M and differentiate it from NGCM, NBT and epithelial malignancies with brain metastases (EPI-M).

2 | METHODS

2.1 | Patients and samples

All biological samples reported in this article were obtained from participants in the following clinical studies which support the sponsor's efforts to develop and validate non- or minimally invasive technologies for detection of various types of malignancies.

The GlioLENS study (https://ctri.nic.in/Clinicaltrials/login.php, keyword search for Trial ID 017663, completed) enrolled patients with benign and malignant central nervous system (CNS) conditions as well as symptomatic individuals with intracranial space-occupying lesions (ICSOL) suspected of CNS malignancies. The TRUEBLOOD study (https://ctri.nic.in/Clinicaltrials/login.php, keyword search for Trial ID 017918, ongoing) enrols patients diagnosed with all types of cancers or benign conditions as well as symptomatic individuals with suspected cancers. The RESOLUTE study (https://ctri.nic.in/Clinicaltrials/login.php, keyword search for Trial ID 017219, ongoing) enrols healthy asymptomatic adults with no prior diagnosis of cancer and no current symptoms or clinical features of cancer. The prospective study at Imperial College London (ongoing, not registered at any clinical trial repository) enrols surgery- and biopsy-naive adults with ICSOLs to determine concordance between the detection of CGCs in pre-surgery/pre-biopsy blood and subsequent HPE diagnosis on tumour tissue. For all samples in the above studies, as well as all samples considered in the current article, HPE of tissue sample was the reference/gold standard for diagnosis.

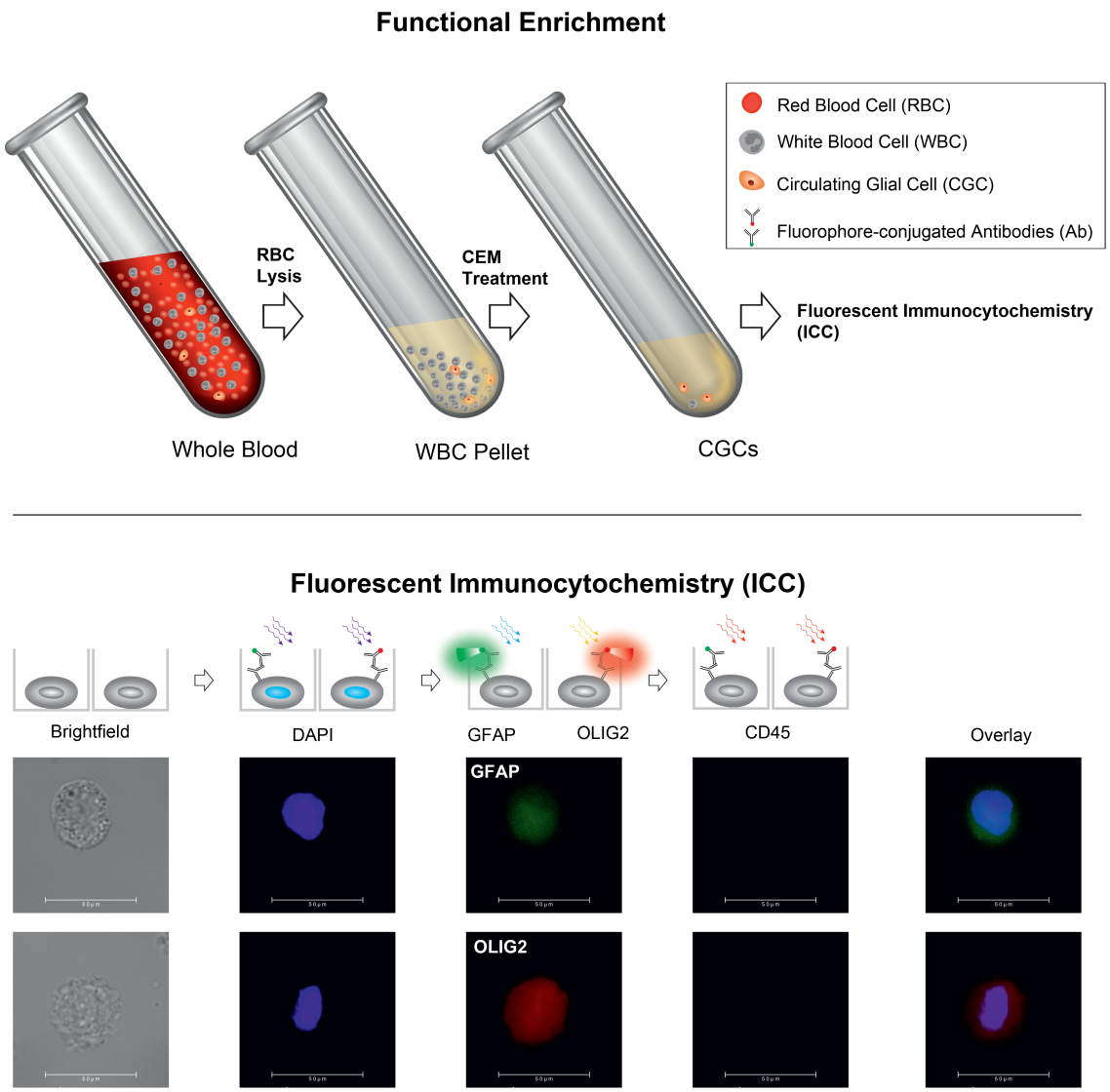

FIGURE 1 Schema of test. Functional enrichment of CTCs is achieved using a proprietary CGC/CTC enrichment medium (CEM) which eliminates all non-malignant cells and permits tumour derived malignant cells to survive. Subsequently, multiplexed immunocytochemistry (ICC) identifies CGCs based on positive expression of GFAP and/or OLIG2.

Up to 20 mL of peripheral blood was collected from all adult study participants using EDTA vacutainers. In patients aged <18 years, up to 10 mL of blood was collected. For patients who underwent tissue sampling (surgery or biopsy), the blood collection was performed before the procedure. The amount of blood sample used for each type of study is described in the respective sub-sections. Leftover blood samples were used for research activities beyond the scope of this article. Where possible, leftover fresh tissue samples were also obtained (in appropriate tumour transport media which maintains the viability of tumour cells) from patients. All samples (blood and tissue) were stored at 2°C–8°C during transport to reach the sponsor's laboratory within 48 h where they were processed. The sponsor's laboratory is accredited for College of American Physicians (CAP) and Clinical Laboratory Improvement Amendments (CLIA) and also adheres to the ISO 9001:2015, ISO 27001:2013 and ISO 15189:2012 quality standards. The status of all samples was blinded to the operators (those who performed the enrichment and ICC) as well as the analysts (those who analysed the data) by assigning unique 10-digit alphanumeric barcodes to minimize potential biases arising from prior knowledge of the sample status. The reporting of observational studies in this article complies with the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) guidelines.[16]

2.2 | Antisera and reference cells

Antisera and reference cells used in the methods are described in Methods S1 and Table S1. All human cell lines used have been authenticated using short tandem repeat (STR) profiling within the last 3 years. All experiments were performed with mycoplasma-free cells.

2.3 | Enrichment of circulating tumour/glial cells from peripheral blood

Blood samples (5 or 7.5 mL) were processed for the enrichment of circulating malignant cells (CGCs/CTCs) as described previously.[13,15] Comprehensive details of this process are provided in the Methods S2.

2.4 | Isolation of viable tumour cells

The isolation of viable tumour cells (TCs) from a tumour sample (malignant/benign) has been described previously.[17] Comprehensive details of this process are provided in Methods S3.

2.5 | Immunocytochemistry profiling

Immunocytochemistry (ICC) profiling of samples for the identification of CTCs/CGCs was performed as described previously.[15] Comprehensive details of this process are provided in Methods S4. Table 1 is the interpretation matrix for the classification of samples based on the expression status of GFAP, OLIG2, CD45 and CK.

2.6 | Method development

Method development studies described in Methods S5 and S6 evaluated the expression levels (fluorescence intensity, FI) of GFAP and OLIG2 in various cell types including reference cells, white blood cells (WBCs), malignant tumour cells, non-malignant cells, CGCs from various subtypes of glial malignancies and CTCs from patients with various (non-CNS) malignancies. The findings of these studies are represented in Figures S1–S3. In addition, the concordance of CGCs with malignant glial tumour tissue for amplification of the epidermal growth factor receptor (EGFR) gene was evaluated as an orthogonal study to establish equivalence of CGCs with malignant glial tumour tissue as described in Methods S7. The findings of our study are represented in Figure S4.

TABLE 1 Inference matrix.

GFAP	OLIG2	CK[a]	Classification
+	+	−	GLI-M
+	−	−	
−	+	−	
−	−	+	EPI-M
−	−	−	NGCM/NBT/HD
	(Any other findings)		Indeterminate

Note: The inference matrix shows the various marker expression statuses and the predictions. Primary classification of samples is based on expression of GFAP and OLIG2. The matrix also explains the classification in a subset of samples where CK was used in addition to the above markers.

Abbreviations: EPI-M, epithelial malignancy with brain metastases; GLI-M, glial malignancy; HD, healthy (asymptomatic) donors; NBT, non-malignant brain tumour; NGCM, non-glial central nervous system malignancy.

[a]For the subset of samples where CK was evaluated.

2.7 | Analytical validation

Analytical validation established the performance characteristics of the test with U87MG (glioblastoma reference cell line) cells which have been previously verified for high expression (FI) of GFAP and OLIG2 as well as absence of CD45 expression. A master spike of U87MG reference cells (10^6 cells/mL) was generated and serially diluted (10 fold or 2 fold) as required in ×1 phosphate-buffered saline (PBS). Measured amounts of the diluted U87MG cells were added into healthy donor blood (HDB) samples to generate the required cell density following which the samples were processed for enrichment of apoptosis-reluctant cells using the CEM. Enriched cells were used for ICC profiling to determine the status of GFAP+ and OLIG2+ cells.

2.7.1 | Analyte stability

To determine the analyte stability, 36 × 5 mL aliquots of HDB were spiked with ~15 U87MG cells. Of these 36 spiked samples, 6 each were either processed immediately or after 24, 48, 72, 96 and 120 h storage at 2°C–8°C. Of the six aliquots evaluated at each time point, three aliquots each were used to determine counts of GFAP+ cells and OLIG2+ cells. The stability period was defined as the maximum period of time up to which the observed count of GFAP+ and OLIG2+ cells were ≥80% of the seeded number of cells, that is, ≥12 cells were detected as indicated in Table S3.

In addition, 30 mL of blood was collected from a known case of glial malignancy and split into six aliquots of 5 mL each; one sample was processed immediately (0 h), and the others were processed after 24, 48, 72, 96 and 120 h storage at 2°C–8°C. The observed counts of GFAP+ and OLIG2+ cells at 0 h were normalized as 100% and cell counts observed in the other aliquots at 24, 48, 72, 96 and 120 h were expressed as relative (%) to the count at 0 h. The stability period was defined as the maximum period of time up to which the observed count of GFAP+ and OLIG2+ cells were ≥80% of the number of cells detected at 0 h (Table S3).

2.7.2 | Linearity

U87MG cells were spiked into 176 × 5 mL aliquots of healthy donor blood samples. The 176 aliquots comprised two sets of 88 aliquots (11 spikes × 8 replicates). The study also included 16 × 5 mL aliquots (2 sets × 8 replicates) of healthy donor blood samples which were not spiked. Each set was assigned for detection of one of the two marker positive cell types (GFAP+/OLIG2+). Linearity was evaluated between 1 and 1280 cells/5 mL (Table S4). The Linearity Interval was

evaluated as per the approach described in Clinical and Laboratory Standards Institute (CLSI) approved guideline EP06.[18] In addition, the linear response characteristics were also evaluated by Linear Regression to determine the coefficient of correlation (R^2) and are depicted in Figure S5.

2.7.3 | Limits of blank, detection and quantitation

The limit of blank (LoB) was determined from the 8 × 5 mL unspiked healthy donor blood samples per marker in the Linearity study. The limit of detection (LoD) of each marker was determined from a subset of the Linearity study which included 24 × 5 mL samples spiked with 1, 3 or 5 U87MG cells (8 of each). The limit of quantitation (LoQ) was determined from a subset of 32 × 5 mL samples from the Linearity study which were spiked with 1, 3, 5 or 10 U87MG cells (8 of each) per marker. The LoB, LoD and LoQ were determined according to CLSI guideline EP17-A2.[19]

2.7.4 | Sensitivity, specificity and accuracy

Sensitivity (Table S5) was determined from a subset of the Linearity study samples which included 40 × 5 mL samples spiked with 5, 10, 20, 40 and 80 U87MG cells (8 of each) per marker type. Specificity was determined from the 8 × 5 mL unspiked healthy donor blood samples (per marker type) in the Linearity study. Accuracy was the combined proportion of samples with true positive and true negative findings per marker type.

2.7.5 | Precision

The precision of the test was evaluated at 5 cells/5 mL (sample positivity threshold) as well as at 15 cells/5 mL (3× threshold) using a 10 × 2 × 8 design which yielded a total of 160 observations over 10 days, by two users each with eight replicate samples. Samples for the precision study were generated by User 1 by spiking 5 and 15 U87MG cells into separate 8 × 5 mL aliquots of healthy donor blood (HDB) per day for 10 days. Samples were processed by CEM treatment and ICC profiling to determine counts of GFAP+ and OLIG2+ cells. User 2 repeated the above study at both spike densities on the same 10 days. The mean of observed recoveries was used to calculate standard deviation (SD) and coefficient of variation (CV, %) for repeatability and within-laboratory precision (since this was a single site study) as per the two-factor nested analysis of variance (ANOVA) approach described in CLSI guideline EP05-A3 (Table S6).[20]

2.7.6 | Interfering substances

The ability of the test to detect GFAP+ and OLIG2+ cells was evaluated in the presence of commonly used drugs (except anticancer drugs) and serum parameters as potential interfering agents (Table S7). Pure (analytical grade) molecules for each of these agents were obtained from commercial vendors and stored under recommended conditions until use. All substances were reconstituted according to the manufacturer's instructions in appropriate solvents to prepare working stock solutions which were immediately used for spiking studies. All drugs were used at the reported Peak Plasma Concentrations (C_{max}), while serum parameters were evaluated at elevated concentrations as indicated in CLSI guideline EP07. Blood from a healthy donor (120 mL) who was not under any medication (last 14 days) was procured from a blood bank and spiked with approximately 1200 U87MG cells (to achieve 10 cells/mL). The spiked sample was split into 24 × 5 mL aliquots; 23 aliquots were spiked with each of the above substances at the indicated concentrations and 1 aliquot was used as an unspiked control. Samples were processed by CEM treatment and ICC profiling to determine count of marker positive cells.

2.8 | Clinical studies

The clinical sensitivity of the test for the detection of GLI-M and the specificity of the test for differentiating GLI-M from non-malignant brain tumours (NBT), brain metastases from epithelial malignancies (EPI-M), non-glial subtypes of central nervous system (CNS) malignancies (NGCM) and healthy individual donors (HD, neither prior diagnosis nor current symptoms or clinical features of cancer) were evaluated in samples from four clinical cohort studies. For all samples (Table S2) in the above studies, the confirmatory diagnosis was based on HPE of tumour tissue (gold standard). In all studies, the operators and analysts were blinded to the clinical status of the samples during sample processing. The (detected) expression status of GFAP, OLIG2, CD45 and CK was used to predict the clinical status of the samples. The concordance of the prediction model with the actual clinical status (diagnosis based on HPE) formed the basis of the sensitivity and specificity of the test.

The first study evaluated the ability of the test to identify and differentiate GLI-M from NBT based on GFAP and OLIG2 status in 189 samples from 145 GLI-M, and 44 NBT cases. All samples were initially assigned to Training and Test Sets in a 70%:30% ratio. Status of the Training Set samples was always unmasked to the analysts to verify the FI thresholds and algorithm.

The second study evaluated the ability of the test to identify and differentiate GLI-M from NBT and EPI-M based on the assessment of GFAP, OLIG2 and CK in a cohort of 586 samples which included 500 HD, 24 cases of EPI-M, 40 cases of GLI-M and 22 cases of NBT.

The third (prospective) study evaluated the ability of the test to detect and differentiate GLI-M from NBT based on assessment of GFAP and OLIG2 in a prospective multicentre cohort of 68 individuals with ICSOL suspected of GLI-M.

The fourth study evaluated the ability of the test to differentiate GLI-M, NBT and NGCM based on the assessment of GFAP and OLIG2 in a cohort of 31 individuals presenting with intra-axial ICSOL on brain imaging.

TABLE 2 Summary of analytical validation.

Parameter	Value
Analyte stability	48 h
Linearity	$R^2 \geq .99$
Linearity interval	5–1280 cells/5 mL
Limit of blank	0 cells/5 mL
Limit of detection	1 cell/5 mL
Limit of quantitation	6 cells/5 mL
Specificity	100% (85.8%–100%)
Sensitivity	95.0% (83.1%–99.4%)
Accuracy	96.9% (89.2%–99.6%)
Repeatability	13.7% (at 5 cells/5 mL), 10.0% (at 15 cells/5 mL)
Within laboratory precision	23.5% (at 5 cells/5 mL), 13.7% (at 15 cells/5 mL)

Note: Analytical validation was performed using control samples generated by spiking measured amounts of U87MG reference cells into healthy donor blood (HDB). The findings of the Analytical Validation indicate that the Test provides consistent, accurate and reproducible results with little or no interference from routine endogenous or exogenous factors when samples are obtained, stored and processed under the recommended conditions. Numbers within parentheses indicate 95% confidence intervals (95% CI). The two values for repeatability and within laboratory precision are the CV (%) at 5 cells/5 mL (positivity threshold) and 15 cells/5 mL (3 × positivity threshold), respectively.

3 | RESULTS

3.1 | Method development

Comprehensive findings of Method Development Studies are provided in Methods (S2–S7). Figures S1–S4 are a graphical representation of the findings in the method development and verification studies described in Methods S5–S7.

3.2 | Analytical validation

Table 2 summarizes the findings of the analytical validation study, which shows that the test detects CGCs with high reliability.

3.2.1 | Analyte stability

In the spiked samples, ≥80% of the seeded GFAP+ and OLIG2+ cells were detected ('recovered') for up to 72 h after the initial sample preparation (Table S3).

In the aliquots of the clinical sample, ≥80% GFAP+ and OLIG2+ cells were detected ('recovery') for up to 48 h after the initial sample collection when the cell counts at 0 h were normalized as 100%. The lower of these findings (in clinical samples) is hence reported as the analyte stability period. The findings of the stability studies indicate that clinical samples can be stored at 2°C–8°C for up to 48 h with a ≤15% loss of cells.

3.2.2 | Linearity

The linearity of the test was determined according to the Clinical and Laboratory Standards Institute (CLSI) guideline EP06. In addition, a regression analysis was also performed to determine the coefficient of correlation (R^2). The linearity interval was determined to be 5–1280 cells/5 mL based on the lower limit of the linear interval (LLLI) being 5 cells/5 mL and the upper limit of the linear interval (ULLI) being 1280 cells/5 mL for both markers. Similarly, $R^2 \geq .99$ for both markers demonstrated the linear response characteristics of the method (Figure S5, Table S4). At the sample positivity threshold (5 cells/5 mL), the observed deviation from linearity was −17% for GFAP and −19% for OLIG2, which were within the permissible range of −26% to +22% for 15% Allowable Deviation from Linearity (ADL), as specified in CLSI EP06. Though the test is not intended for the quantitation of CGCs, the linear characteristics of the test indicates its ability to yield observations proportionate to the number of marker positive cells.

3.2.3 | Limits of blank, detection and quantitation

The LoB, LoD and LoQ were determined according to CLSI guideline EP17-A2. GFAP+ or OLIG2+ cells were not detected in any of the unspiked healthy donor (HD) blood samples; zero (0) cell counts indicated the absence of false positives. Thus, the LoB was determined to be 0 cells/mL for GFAP as well as for OLIG2. The LoD was determined as 1 cell/5 mL for GFAP and OLIG2. Based on a 15% prespecified ADL, the LoQ was determined to be 6 cells/5 mL for GFAP and 5 cells/5 mL for OLIG2 (the overall LoQ was 6 cells/5 mL). The LoD and LoQ study findings indicate the ability of the test to detect and quantitate samples with lower amounts of marker positive cells at the detection threshold.

3.2.4 | Sensitivity, specificity and accuracy

Based on the detection of marker positive cells in the 40 spiked samples (5–80 cells/5 mL), the overall analytical sensitivity was determined to be 92.5% for GFAP and 95.0% for OLIG2. At the detection threshold (5 cells/5 mL), the sensitivities were 62.5% for GFAP and 75% for OLIG2. At 10 cells/5 mL and above, the sensitivity was 100% for GFAP as well as for OLIG2. Since GFAP+ and OLIG2+ cells were undetectable in any of the unspiked samples (as observed in the LoB study), the analytical specificity was deemed to be 100%. Based on the overall sensitivity and the specificity, the overall accuracy was 95.3% for GFAP and 96.9% for OLIG2 (Table S5).

3.2.5 | Precision

The precision of the test was evaluated at 5 cells/5 mL (detection threshold) and 15 cells/5 mL (3× detection threshold) via two-factor

nested analysis of variance (ANOVA) as described in CLSI EP05-A3. The observed mean, standard deviation (SD) and coefficient of variation (CV, %) along with the 95% confidence interval (CI) for repeatability and within laboratory precision are provided in Table S6. The % CVs were 13.7% for repeatability and 23.5% for within laboratory precision at 5 cells/5 mL and 10.0% for repeatability and 13.7% for within laboratory precision at 15 cells/5 mL. These findings suggest acceptable repeatability considering that the analytes (whole cells) are prone to significant random variations (as compared to soluble analytes) especially at lower densities.

3.2.6 | Interfering substances

The ability of the test to detect GFAP+ and OLIG2+ cells was evaluated in the presence of potentially interfering substances including common drugs (excluding anticancer drugs) as well as serum parameters that may be elevated under various disease conditions. Table S7 lists the various agents tested, their concentrations (in 5 mL volume) and the observed number of GFAP+ and OLIG2+ cells based on an initial spike density of 10 cells/5 mL. As shown in the same table, the presence of drugs at medically relevant peak plasma concentrations (C_{max}) or the deranged (clinically high) serum parameters did not significantly affect the sensitivity of the test for detection of spiked U87MG cells. Our study established the ability of the test to remain unaffected and detect CGCs in the presence of systemic treatment agents (drugs) and elevated serum parameters.

3.3 | Clinical study findings

The ability of the test to detect GLI-M and differentiate GLI-M from NBT, NGCM, EPI-M and HD were established in four stringent, blinded clinical studies.

The inclusion criteria and demographics of the first study are provided in Tables S8 and S9, respectively. The observations for the training and test set samples are provided in Table S10. Among the 101 GLI-M cases in the Training Set, 100 were positive (99%) and 1 was negative (1%) for CGCs. Among the 31 cases of NBT, 1 (3.2%) was positive and 30 (96.8%) were negative for CGCs. In the absence of follow-up data demonstrating a diagnosis of GLI-M, the positive NBT case was considered a false positive. In the Test Set ($n = 57$), 44 samples had positive findings and 13 samples had negative findings. All 44 positive samples were determined as GLI-M indicating 100% sensitivity. All negative samples were determined to be NBT indicating 100% specificity (Table S11).

The inclusion criteria and demographics of the second study are provided in Tables S12 and S13, respectively. The observations of the study samples and the performance characteristics are provided in Table S14. Among the 40 GLI-M samples, none were positive for CTCs whereas all were positive for CGCs. Among the 24 EPI-M samples, none were positive for CGCs but all were positive for CTCs. Among the samples from NBT cases ($n = 22$) and healthy individuals ($n = 500$), none were positive for CGCs or CTCs. In our study, the test had 100% sensitivity for detecting GLI-M and 100% specificity for differentiating GLI-M from EPI-M and HD.

The inclusion criteria and demographics of the third study are provided in Tables S15 and S16, respectively. The observations of the samples are summarized in Table S17. Of the 68 cases, 56 were positive for CGCs and 12 were negative as per the Inference Matrix. After unblinding, it was revealed that all 56 positive samples were GLI-M and all 12 samples were NBT. In our study, the test had 100% sensitivity for detecting GLI-M and 100% specificity for differentiating GLI-M from NBT (Table S18).

The inclusion criteria and demographics of the fourth study are provided in Tables S19 and S20, respectively. Of the 31 cases, 13 were positive and 18 were negative for CGCs. After unblinding, all 13 positive samples were determined as GLI-M. Of the 18 negative samples,

TABLE 3 Clinical performance characteristics.

	Sensitivity	Specificity	Accuracy
Study-1 (validation set)	100% (95% CI: 91.96%–100%) ($n = 44$)	100% (95% CI: 75.29%–100%) ($n = 13$)	100% (95% CI: 93.73%–100%) ($n = 57$)
Study-2	100% (95% CI: 91.19%–100%) ($n = 40$)	100% (95% CI: 99.33%–100%) ($n = 546$)	100% (95% CI: 99.37%–100%) ($n = 586$)
Study-3	100% (95% CI: 93.62%–100%) ($n = 56$)	100% (95% CI: 73.54%–100%) ($n = 12$)	100% (95% CI: 94.72%–100%) ($n = 68$)
Study-4	92.86% (95% CI: 66.13%–99.82%) ($n = 14$)	100% (95% CI: 80.49%–100%) ($n = 17$)	96.77% (95% CI: 83.30%–99.92%) ($n = 31$)
Cumulative	*99.35% (95% CI: 96.44%–99.98%)* *($n = 154$)*	*100% (95% CI: 99.37%–100%)* *($n = 588$)*	*99.87% (95% CI: 99.25%–100%)* *($n = 742$)*

Note: The performance characteristics of the test were determined in four clinical studies using blinded samples. Sensitivity was determined as the proportion of true positive findings in samples from patients with glial malignancies (GLI-M) and specificity was determined as the proportion of true negative findings in samples from patients with non-malignant brain tumours (NBT), samples from patients with non-glial central nervous system (CNS) malignancies (NGCM), samples from patients with epithelial malignancies with CNS metastases (EPI-M) or samples from healthy (asymptomatic) donors (HD) with no prior diagnosis or current suspicion of any cancer. Accuracy was determined as the combined proportion of true positives and true negatives. The 95% confidence interval (CI) and the number of samples for each type of determination are provided in parentheses.

1 was GLI-M, 8 were NGCM and 9 were NBT (Table S21). The test had 92.9% sensitivity for detection of GLI-M and 100% specificity for differentiating GLI-M from NBT (Table S22).

Table 3 provides a summary of the study-wise performance characteristics as well as the cumulative performance characteristics.

4 | DISCUSSION

The presentation of patients with intracranial malignancy is frequently symptomatically non-specific and differentiating such patients from those with non-malignant conditions or absent pathology is challenging. Indicative of this, GBM presents as a medical emergency more frequently than any other common cancer, implying that effective strategies for the rapid diagnostic stratification of patients presenting with suspicious symptoms are urgently required. Furthermore, it is critical to differentiate GLI-M from NBT or metastases from other solid tumours. Obtaining a tissue diagnosis is often challenging and has well-described risks.

Here we describe a blood-based test for the detection of GLI-M in individuals presenting with ICSOL, based on the detection of CGCs by multiplexed fluorescence ICC profiling (Figure 1). The test can detect common subtypes that account for approximately 97% of all GLI-M, irrespective of age, gender, subtype and grade. The analytical validation of our platform confirmed accuracy and reliability of the test. The clinical validation studies demonstrated an overall (cumulative) >99% sensitivity and 100% specificity for detection of GLI-M. The performance characteristics of the test favour the clinical adoption of this technology to support more effective diagnosis in individuals presenting with ICSOL, especially among patients with unresectable or non-biopsiable ICSOLs. To our knowledge, there are currently no non-invasive or non-radiological tests for the detection of GLI-M in individuals with ICSOLs.

Our test is based on the detection of CTCs, which in the context of a glial malignancy are called CGCs. In primary solid organ cancers, the existence of CTCs is linked to dissemination and metastatic spread. Extracranial metastases, although rare in GLI-M, have been reported previously.[21–25] The detection of CGCs in blood samples from patients with GLI-M appears to indicate that while CGCs can enter circulation, they may be unable to find a target tissue where they can egress, survive and grow.[26] Zhang et al hypothesized that the inability to detect extracranial metastasis may be a consequence of the low survival (shorter life span) of patients with GLI-M, and that the probability of detecting extracranial metastases may be higher in patients who survive longer.[27]

Prior studies have shown the presence of CGCs in low and high grade gliomas and glioblastomas, as well as their absence in healthy individuals and those with non-malignant brain tumours. Bang-Christensen et al reported 0.5–42 CGCs/3 mL blood irrespective of grade or subtype of GLI-M[28] via a novel immunocapture method. MacArthur et al used density-gradient centrifugation followed by telomerase assay and Nestin expression to detect CGCs in 8 out of 11 (72%) cases of radiation-naive glioma with an average of 8.8 CGCs/mL of blood.[10] Sullivan et al demonstrated that the mesenchymal like properties of CGCs could contribute to their invasiveness, allowing them to enter into circulation.[29] Based on chromosome eight polyploidy and immunostaining for GFAP (positive) and CD45 (negative), Gao et al reported CGCs in the peripheral blood of 24 out of 31 (77%) patients with GLI-M with no correlation between the number of CGCs and the subtype/grade of malignancy.[30] Similarly, Krol et al reported CGC clusters in 7 of 13 (53.8%) cases of glioblastoma.[31]

Our test detects CGCs based on cellular GFAP and OLIG-2 expression, and provides unambiguous evidence of the underlying malignancy in the form of directly visualized malignant cells. The detection of GFAP and OLIG2 positivity in malignant cells is not prone to confounding, as may be observed in case of various serum cancer antigens that are often elevated in patients with non-malignant conditions. In our test, the positive marker expression in cells is determined based on standardized fluorescence intensities (FI) detected using a sensitive and automated high content screening platform that minimizes the risk of false negatives. Our test showed high sensitivity and specificity for the detection of CGCs in analytical validations as well as in the clinical studies.

Our study shows that it is possible to obtain sufficient viable CGCs in peripheral blood samples for the detection of GLI-M and differentiation of GLI-M from NBT and brain metastases of solid tumours. To our knowledge, the test described in this article is the first of its kind that uses a hallmark property of malignancy for enrichment of CGCs. Applications of this core technology for the detection of breast and prostate cancers have been previously described.[32,33] Our test is minimally invasive and is performed on a peripheral (venous) blood sample. This test is not currently intended to replace standard diagnostic imaging or tissue sampling. Contemporary brain imaging such as magnetic resonance imaging (MRI) offers diagnostic guidance in ICSOLs with some capability to distinguish malignant and non-malignant ICSOLs based on radiological morphology. We envisage that our test would provide an additional layer of high quality evidence that can potentially support diagnostic and disease management decisions before lifting the scalpel. The CGC-based approach described in our study may be especially relevant in cases of unresectable or non-biopsiable ICSOLs which can pose a diagnostic roadblock. Surgical resection may not be viable due to the proximity of the lesion to regions associated with vital functions or comorbidities; up to 40% of cases with advanced or high-grade brain lesions are reported to be unresectable.[34] Furthermore, brain biopsies have been reported to be unviable, inconclusive or non-diagnostic in up to 20% of cases.[35–37]

In such cases, the test findings have the potential to mitigate any risks of overtreatment in individuals with non-malignant brain tumours as well as the potential to reduce the risks associated with delayed diagnosis and treatment in individuals with GLI-M. In addition, the detection and differentiation of CGCs and (epithelial) CTCs based on marker expression profiles can also aid the differentiation of primary CNS (glial) malignancy and brain metastases of non-CNS primary tumours; albeit rare, prior reports have described cancers presenting with brain metastases[38] and such cases represent yet another

subset of patients who may benefit from our approach. The strength of our study is the multiple clinical studies with blinded sample analysis, all of which demonstrated high concordance between test findings and clinical diagnosis and support the clinical application of the test.

The high clinical sensitivity indicates a very low risk of missing GLI-M and the high specificity indicates an imperceptible (if any) risk of false positive findings in individuals without a primary GLI-M. Although the test has high performance characteristics for the detection of GLI-M, it is not intended for detection of rarer subtypes such as CNS lymphoma and gliosarcoma. The test is also not intended to provide diagnostic information such as the subtype or grade of malignancy. The 2021 World Health Organization (WHO) guidance for the classification of CNS tumors[39] describes molecular features (gene variants) for diagnosis. Advances in next generation sequencing (NGS) technology platforms suggest a potential for molecular profiling of glial malignancies using the limited yields of tumour nucleic acids (TNA) isolated from CGCs.[40] We envisage future iterations of our test to include immune-profiling of CGCs as well as molecular profiling of CGC-derived TNA for a more holistic role in diagnostic work-up with reduced dependence on tumour tissue. However, the current scope of the test is limited to the detection/identification of CGCs as an indicator of an underlying glial malignancy and is not intended to replace standard tissue sampling based diagnosis in patients where it is recommended, necessary and feasible.

The approach described in this article requires only a peripheral blood drawn from the patients. The simplicity of a blood based test makes it amenable to integration within the standard of care diagnostic pathways in most healthcare systems. Blood collection is a simple, low risk procedure that can be performed at any primary healthcare centre, physician's clinic or pharmacy. From the patient's perspective, there are no additional visits to advanced healthcare facilities or additional wait times. From the healthcare provider's perspective, no additional resources or infrastructural investments are required.

In conclusion, we present a blood-based, non-radiological test for the detection of glial malignancies with potential clinical applications in symptomatic individuals who have been advised to undergo an invasive biopsy as part of standard diagnostic work-up as well as diagnostic support in individuals with unresectable and/or non-biopsiable ICSOLs. Our test has the potential to enable more effective clinical decision making by providing direct evidence for the presence of GLI-M in these cases.

AUTHOR CONTRIBUTIONS

The work reported in the article has been performed by the authors, unless clearly specified in the text. **Kevin O'Neill:** Conceptualization, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Writing – review & editing. **Nelofer Syed:** Conceptualization, Data Curation, Formal Analysis, Investigation, Methodology, Project administration, Resources, Writing – review & editing. **Timothy Crook:** Conceptualization, Investigation, Methodology, Project administration, Resources, Writing – review & editing. **Sudhir Dubey:** Conceptualization, Investigation, Data Curation, Writing – review & editing. **Mahadev Potharaju:** Conceptualization, Investigation, Data Curation, Writing – review & editing. **Sewanti Limaye:** Conceptualization, Investigation, Writing – review & editing. **Anantbhushan Ranade:** Conceptualization, Investigation, Writing – review & editing. **Giulio Anichini:** Conceptualization, Investigation, Data Curation, Formal Analysis, Project administration, Writing – review & editing. **Darshana Patil:** Conceptualization, Investigation, Methodology, Data Curation, Formal Analysis, Project administration, Software, Validation, Visualization, Writing – original draft. **Vineet Datta:** Conceptualization, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Visualization, Writing – original draft. **Rajan Datar:** Conceptualization, Funding acquisition, Methodology, Data Curation, Formal Analysis, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – original draft. All authors have read the article and agreed to its contents, the author list and its order as well as the above author contribution statements.

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CONFLICT OF INTEREST STATEMENT

KN, NS, SD, MP, AR, GA have declared no conflicts of interest. SAL declares consulting role/advisory roles/speaker's bureau for Astra Zeneca, Merck, Bristol-Myers Squibb, Merck Sharp & Dohme (MSD), Boehringer Ingelheim, Roche and Novartis and is also a co-founder of Iylon Precision Oncology, having stock and other ownerships interests in this company – all conflicts are outside of the current study. TC is a fractional equity holder of the study sponsor (Datar Cancer Genetics, DCG). VD is a fractional equity holder and full time employee of the study sponsor. DP is a full time employee of the study sponsor and receives professional fees from the study sponsor. RD is the founder, major shareholder (funder) and a Director of the study sponsor.

DATA AVAILABILITY STATEMENT

The data that support the findings of our study are available from the corresponding author upon reasonable request.

ETHICS STATEMENT

All studies with human participants were approved by an Ethics Committee of the study sponsor and/or the participating site. The Institutional Ethics Committee (IEC) of Datar Cancer Genetics (DCG, sponsor) gave ethical approval for GlioLENS, TRUEBLOOD and RESOLUTE studies. The IEC – Clinical Studies of Apollo Hospitals, Hyderabad gave ethical approval for the GlioLENS study. The Imperial College Ethics Committee (EC)/Institutional Review Board (IRB)

approved the prospective study conducted at Imperial College, London. All studies with human participants were performed in accordance with the Declaration of Helsinki and applicable regulatory guidelines. Written informed consent was obtained from all adult study participants, or from the parents of adolescent and paediatric patients (i.e., patients aged less than 18 years) before participation and sample collection.

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REFERENCES

1. Cancer.Net. Brain Tumor: Statistics. Accessed November 30, 2023. https://www.cancer.net/cancer-types/brain-tumor/statistics
2. Cancer Stat Facts: Brain and Other Nervous System Cancer. Accessed November 30, 2023. https://seer.cancer.gov/statfacts/html/brain.html
3. Miller K, Ostrom Q, Kruchko C, et al. Brain and other central nervous system tumor statistics, 2021. *CA Cancer J Clin*. 2021;71(5):381-406. doi:10.3322/caac.21693
4. Malone H, Yang J, Hershman DL, Wright JD, Bruce JN, Neugut AI. Complications following stereotactic needle biopsy of intracranial tumors. *World Neurosurg*. 2015;84(4):1084-1089. doi:10.1016/j.wneu.2015.05.025
5. Koszewski W, Kroh H, Kunert P. Trudności w biopsjach stereotaktycznych guzów mózgu [Difficulties in stereotactic biopsies of brain tumors]. *Neurol Neurochir Pol*. 2002;36(3):481-488. Polish.
6. Ramkissoon L, Pegram W, Haberberger J, et al. Genomic profiling of circulating tumor DNA from cerebrospinal fluid to guide clinical decision making for patients with primary and metastatic brain tumors. *Front Neurol*. 2020;19(11):544680. doi:10.3389/fneur.2020.544680
7. Nassiri F, Chakravarthy A, Feng S, et al. Detection and discrimination of intracranial tumors using plasma cell-free DNA methylomes. *Nat Med*. 2020;26(7):1044-1047. doi:10.1038/s41591-020-0932-2
8. Ebrahimkhani S, Vafaee F, Hallal S, et al. Deep sequencing of circulating exosomal microRNA allows non-invasive glioblastoma diagnosis. *npj Precis Oncol*. 2018;12(2):28. doi:10.1038/s41698-018-0071-0
9. Pickles J, Fairchild A, Stone T, et al. DNA methylation-based profiling for paediatric CNS tumour diagnosis and treatment: a population-based study. *Lancet Child Adolesc Health*. 2020;4(2):121-130. doi:10.1016/S2352-4642(19)30342-6
10. Macarthur K, Kao G, Chandrasekaran S, et al. Detection of brain tumor cells in the peripheral blood by a telomerase promoter-based assay. *Cancer Res*. 2014;74(8):2152-2159. doi:10.1158/0008-5472.CAN-13-0813
11. Müller C, Holtschmidt J, Auer M, et al. Hematogenous dissemination of glioblastoma multiforme. *Sci Transl Med*. 2014;6:247ra101. doi:10.1126/scitranslmed.3009095
12. Kan L, Drummond K, Hunn M, et al. Potential biomarkers and challenges in glioma diagnosis, therapy and prognosis. *BMJ Neurol Open*. 2020;2(2):e000069. doi:10.1136/bmjno-2020-000069
13. Akolkar D, Patil D, Crook T, et al. Circulating ensembles of tumor-associated cells: a redoubtable new systemic hallmark of cancer. *Int J Cancer*. 2020;146(12):3485-3494. doi:10.1002/ijc.32815
14. Ranade A, Bhatt A, Page R, et al. Hallmark circulating tumor-associated cell clusters signify 230 times higher one-year cancer risk. *Cancer Prev Res (Phila)*. 2021;14(1):11-16. doi:10.1158/1940-6207.CAPR-20-0322
15. Gaya A, Crook T, Plowman N, et al. Evaluation of circulating tumor cell clusters for pan-cancer noninvasive diagnostic triaging. *Cancer Cytopathol*. 2021;129(3):226-238. doi:10.1002/cncy.22366
16. von Elm E, Altman D, Egger M, et al. Strengthening the reporting of observational studies in epidemiology (STROBE) statement: guidelines for reporting observational studies. *BMJ*. 2007;335(7624):806-808. doi:10.1136/bmj.39335.541782.AD
17. Crook T, Gaya A, Page R, et al. Clinical utility of circulating tumor-associated cells to predict and monitor chemo-response in solid tumors. *Cancer Chemother Pharmacol*. 2021;87(2):197-205. doi:10.1007/s00280-020-04189-8
18. CLSI. *Evaluation of Linearity of Quantitative Measurement Procedures. CLSI Guideline EP06*. 2nd ed. Clinical and Laboratory Standards Institute; 2020.
19. CLSI. *Evaluation of Detection Capability for Clinical Laboratory Measurement Procedures. Approved Guideline. CLSI Document EP17-A2*. 2nd ed. Clinical and Laboratory Standards Institute; 2012.
20. CLSI. *Evaluation of Precision of Quantitative Measurement Procedures. Approved Guideline. CLSI Document EP05-A3*. 3rd ed. Clinical and Laboratory Standards Institute; 2014.
21. Hamilton J, Rapp M, Schneiderhan T, et al. Glioblastoma multiforme metastasis outside the CNS: three case reports and possible mechanisms of escape. *J Clin Oncol*. 2014;32(22):e80-e84. doi:10.1200/JCO.2013.48.7546
22. Pasquier B, Pasquier D, N'Golet A, et al. Extraneural metastases of astrocytomas and glioblastomas: clinicopathological study of two cases and review of literature. *Cancer*. 1980;45(1):112-125. doi:10.1002/1097-0142(19800101)45:13.0.co;2-9
23. Chang H, Ding Y, Wang P, Wang Q, Lin Y, Li B. Cutaneous metastases of the glioma. *J Craniofac Surg*. 2018;29(1):e94-e96. doi:10.1097/SCS.0000000000004204
24. Pérez-Bovet J, Rimbau-Muñoz J. Glioblastoma multiforme metastases to the masticator muscles and the scalp. *J Clin Neurosci*. 2018;53:237-239. doi:10.1016/j.jocn.2018.04.021
25. Rosen J, Blau T, Grau SJ, Barbe MT, Fink GR, Galldiks N. Extracranial metastases of a cerebral glioblastoma: a case report and review of the literature. *Case Rep Oncol*. 2018;11(2):591-600. doi:10.1159/000492111
26. Eibl RH, Schneemann M. Liquid biopsy and primary brain tumors. *Cancers (Basel)*. 2021;13(21):5429. doi:10.3390/cancers13215429
27. Zhang H, Yuan F, Qi Y, Liu B, Chen Q. Circulating tumor cells for glioma. *Front Oncol*. 2021;11:607150. doi:10.3389/fonc.2021.607150
28. Bang-Christensen S, Pedersen R, Pereira M, et al. Capture and detection of circulating glioma cells using the recombinant VAR2CSA malaria protein. *Cell*. 2019;8(9):998. doi:10.3390/cells8090998
29. Sullivan J, Nahed B, Madden M, et al. Brain tumor cells in circulation are enriched for mesenchymal gene expression. *Cancer Discov*. 2014;4(11):1299-1309. doi:10.1158/2159-8290.CD-14-0471
30. Gao F, Cui Y, Jiang H, et al. Circulating tumor cell is a common property of brain glioma and promotes the monitoring system. *Oncotarget*. 2016;7(44):71330-71340. doi:10.18632/oncotarget.11114
31. Krol I, Castro-Giner F, Maurer M, et al. Detection of circulating tumour cell clusters in human glioblastoma. *Br J Cancer*. 2018;119(4):487-491. doi:10.1038/s41416-018-0186-7
32. Crook T, Leonard R, Mokbel K, et al. Accurate screening for early-stage breast cancer by detection and profiling of circulating tumor cells. *Cancers (Basel)*. 2022;14(14):3341. doi:10.3390/cancers14143341
33. Limaye S, Chowdhury S, Rohatgi N, et al. Accurate prostate cancer detection based on enrichment and characterization of prostate cancer specific circulating tumor cells. *Cancer Med*. 2023;12(8):9116-9127. doi:10.1002/cam4.5649
34. Fazeny-Dörner B, Wenzel C, Veitl M, et al. Survival and prognostic factors of patients with unresectable glioblastoma multiforme. *Anticancer Drugs*. 2003;14(4):305-312. doi:10.1097/00001813-200304000-00008
35. Air E, Warnick R, McPherson C. Management strategies after nondiagnostic results with frameless stereotactic needle biopsy:

retrospective review of 28 patients. *Surg Neurol Int*. 2012;3(Suppl 4): S315-S319. doi:10.4103/2152-7806.103026

36. Woodworth G, McGirt M, Samdani A, et al. Frameless image-guided stereotactic brain biopsy procedure: diagnostic yield, surgical morbidity, and comparison with the frame-based technique. *J Neurosurg*. 2006;104(2):233-237. doi:10.3171/jns.2006.104.2.233
37. Khatab S, Spliet W, Woerdeman P. Frameless image-guided stereotactic brain biopsies: emphasis on diagnostic yield. *Acta Neurochir*. 2014;156(8):1441-1450. doi:10.1007/s00701-014-2145-2
38. Füreder L, Widhalm G, Gatterbauer B, et al. Brain metastases as first manifestation of advanced cancer: exploratory analysis of 459 patients at a tertiary care center. *Clin Exp Metastasis*. 2018;35(8): 727-738. doi:10.1007/s10585-018-9947-1
39. Louis D, Perry A, Wesseling P, et al. The 2021 WHO classification of tumors of the central nervous system: a summary. *Neuro Oncol*. 2021; 23(8):1231-1251. doi:10.1093/neuonc/noab106
40. Kolostova K, Pospisilova E, Pavlickova V, et al. Next generation sequencing of glioblastoma circulating tumor cells: non-invasive solution for disease monitoring. *Am J Transl Res*. 2021;13(5):4489-4499.

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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