Supplemental Information

INTRAOPERATIVE MASS SPECTROMETRY BASED PLATFORM FOR IDH MUTATION STATUS, GLIOMA DIAGNOSIS, AND ESTIMATION OF TUMOR CELL INFILTRATION

Hannah Marie Brown[1‡], Clint M. Alfaro[1‡], Valentina Pirro[1‡], Mahua Dey[2#], Eyas M. Hattab[3], Aaron A. Cohen-Gadol[2], R. Graham Cooks[1*]

[1]Department of Chemistry, Purdue University, West Lafayette, Indiana; and [2]Department of Neurological Surgery, Indiana University School of Medicine, Goodman Campbell Brain and Spine, Indianapolis, Indiana; [3]Department of Pathology and Laboratory Medicine, University of Louisville, Kentucky

‡Authors contributed equally

Present address Department of Neurological Surgery, University of Wisconsin Medical School, Medical School, Madison, Wisconsin

Running Title: Intraoperative DESI-MS for glioma diagnosis

Contents

Supplemental Methods

Intraoperative Patient Cohort

A total of 58 human subjects were enrolled in our study. Of those, six subjects were clinical screenfails and subsequently excluded. Data from three subjects were excluded due to poor desorption electrospray ionization (DESI) signal. This resulted in 49 human subjects from whom tissue was collected and analyzed with desorption electrospray ionization-mass spectrometry (DESI-MS).

A total of 247 biopsies was obtained; the average number of biopsies per subject was five. Some biopsies were smeared multiple times when additional tissue was available, resulting in 334 total tissue smears. Of these smears, 62 were excluded due to poor DESI signal or inadequate quality for histopathology, resulting in 272 smears to be used for statistical analysis for disease status and tumor cell percentage (TCP) and for comparison of DESI and histopathology. See the Supplemental Methods Data Analysis section (below) for our definition of smears with poor DESI-MS signal and why they were excluded.

The method for IDH mutation status was developed midway through the study. Therefore, it could not be used for all subjects, but it was used to analyze tissue smears (n = 164) obtained from 28 human subjects.

The average patient age was 46 ± 16 years; 24 subjects were female and 25 subjects were male; the number of grade I, II, III, and IV gliomas were 1, 15, 6, and 25, respectively, with two subjects for whom WHO grades were not determined; 41 subjects had primary gliomas and eight had recurrent gliomas; 23 subjects were IDH-mut, 25 were IDH-wt, and one subject had unknown IDH status.

Intraoperative DESI-MS

The DESI spray solvent consisted of 1:1 DMF-ACN, Nitrogen gas pressure of 180 psi, solvent flow rate of 1.2 µL / minute, spray voltage of 5kV, negative ion polarity was used. In order

to obtain a high-quality average spectrum of the sample, the DESI spray was rastered across the tissue smear in a zigzag pattern using a precision moving stage. Under certain conditions, as used in this study, DESI-MS analyzed tissue samples can be H&E stained and then interpreted by pathologists, allowing for correlations between tissue disease status as determined by these complementary methods.(24) Negative ion mode metabolite, lipid, and MS/MS data were collected as different segments in the MS over a total time of three minutes. The same smears were re-analyzed using a solvent consisting of 37:38:25 ACN-EtOH-DMF and using multiple-stage mass spectrometry for the detection of 2-hydroxyglutarate (2-HG), for an additional three minutes of data acquisition. We filled a syringe with each solvent outside the operating room (OR) prior to each case, and when analyzing the smears, we switched the syringe to the appropriate one for the method that we were running. We typically analyzed all the smears with DMF-ACN using the first method, switched the syringe, and then analyzed them all again with the 2-HG optimized solvent system using the second method.

Data Analysis

Scan Filtering and Data Normalization for the Full-Scan Lipid and Metabolite Profiles

The collected lipid data were filtered using standard deviation thresholds of the absolute signal intensity compared to background spectra collected from a blank glass slide. Lipid scans with signal three-standard deviations greater than the blank were retained and averaged to generate a representative lipid profile for the sample. The metabolite profiles were filtered by multivariate modeling of the background and selecting MS scans that were statistically different from the background. Metabolite scans that were significantly different to those of the blank glass slide were retained and averaged to generate a representative metabolite profile for the sample. The lipid and metabolite profile scans that were retained were normalized using standard normal variate (SNV) transformation prior to PCA to correct for baseline shifts and fluctuations in total ion count.

For some tissue smears, no scans were selected after using these data filtering criteria due to low DESI-MS signal. Average DESI-MS spectra of tissue smears were used to provide molecular diagnosis of smeared tissue biopsies on a glass slide. MS scans from areas of the glass slide containing no smeared tissue and smears giving signals of insufficient intensity were excluded by applying a cut-off value to the absolute signal intensity. For the lipid profiles, ion counts at maximum peak intensity for ions *m/z* 768, 788, 794, 834, and 888 were summed and

plotted over the time of acquisition; these ions correspond to glycerophospholipids present in brain tissue but absent in glass. Only scans with mass spectra having summed ion counts greater than the cutoff value were used for chemical predictions. For the metabolite profile, ion counts for ions m/z 89 and 175, present in diseased tissue, were summed and subtracted from the intensity of ions m/z 119, 143, and 163, present on the glass slide. Likewise for the lipid profiles, only scans with mass spectra having summed ion counts greater than the cutoff value were used for chemical predictions. For each selected mass spectrum (both for lipid and metabolite profiles), the full width at half maximum (FWHM) was calculated for the base peak. All spectra with mass resolution < 1000 were excluded. Smears for which no scans were selected in either the lipid or metabolite profile were excluded from further statistical analysis. The smears that provided poor signal and were excluded tended to contain acellular tissue, have sparse smear cell density, be smears with overall low cellularity, or have significant air-drying artifacts.

IDH Mutation Status Using MS^3 Detection of 2-HG

For IDH mutation status, the average TIC normalized intensity of the MS^3 fragment ions (*m/z* 101 and 85) detected from sequential fragmentation of the 2-HG precursor ion (*m/z* 147 in the negative mode) was used as a measure of IDH mutation status. These data were collected using the DESI-MS method outlined in Figure 1, denoted as Method 2. The MS^3 data for 2-HG (147->129->O) was collected over 1.1 minutes while scanning the sample under the DESI spray in a zigzag raster pattern, similar to the method utilized for the full-scan lipid and metabolite profiles. A significant fraction of the scans contained no signal intensity, or did not contain signal for either of the 2-HG MS^3 product ions. For each tissue smear, scans were filtered to include only scans that contained signal for either *m/z* 85, *m/z* 101, or both. The retained MS^3 scans were normalized using the average total ion count measured in the full-scan acquisition taken in segment 3 of the 2-HG method (the MS method is shown schematically in Figure 1B and 1C in the main text.) After TIC normalization, the intensities of the two MS^3 product ions were summed together to provide an IDH mutation score for each smear. In instances where more than one smear was made for the same tumor core biopsy, the average normalized and summed 2-HG product ion intensity of all smears was calculated and used to predict the IDH mutation status of that biopsy. The TIC normalized and summed MS^3 product ion intensities were analyzed using ROC curve analysis, the Wilcoxon rank-sum test, and t-tests using Tukey HSD to account for multiple comparisons. The IDH mutation status was categorized as either IDH-mutant (IDH-mut) or IDH-wildtype (IDH-wt).

TCP from Full-Scan NAA Abundance

The SNV normalized intensity of *m/z* 174, corresponding to N-acetyl-aspartic acid (NAA), measured in the full-scan MS metabolite profile, was used to estimate (approximately) the tumor cell percentage, using a previously published data set as a training set. TCP predictions via NAA intensity used categories of low (TCP ≤50%) and high (TCP ≥ 51%).

TCP from MS^2 Monitoring of NAA

The MS^2 scan include in Method 1 (Figure 1C in the main text) targeted the deprotonated ion of NAA. The MS^2 of NAA produces fragment ions at *m/z* 88, 114, 130, and 156 utilizing our LTQ mass spectrometer and the given fragmentation conditions. The MS^2 scans were filtered to only include scans that contained intensity for all of the NAA fragment ions. After MS^2 scan filtering, the data were normalized using the average TIC detected in the metabolite profile scan for each tissue smear. The normalized intensities of each of the NAA MS^2 fragment ions were summed to calculate the NAA MS^2 intensity. NAA product ion intensities below the cut-off value of 5 in the MATLAB code were assigned a random value of one to five as an intensity value. The normalized and summed NAA MS2 intensities were analyzed with ROC curve analysis and the Wilcoxon rank-sum test.

Lipid Profile Deconvolution

Principal component regression analysis was used to calculate the percentage of white matter, grey matter, and glioma contributing to the lipid profiles of the new samples using a linear regression model built from data collected in a previous study. The model was based on the presumption that the observed lipid profile for the glioma samples were composed of a ternary mixture of white-matter, grey-matter, and glioma. The summed percentages of these three categories is 100%. The regression model was built from data collected from DESI-MS imaging of banked glioma and normal human brain specimens. Histologically correlated mass spectra were compiled based on histopathological assessment, compressed with PCA, and the average PC1 and PC2 scores for samples of pure WM, GM, and G were calculated. The three extremes (PC1 and PC2 scores corresponding to 100%WM, 100%GM, 100%G) were used to calculate the predicted PC1 and PC2 scores for each possible mixture (Supplemental Figure 1). To predict the composition of new samples, the PCA lipid profile loading matrix from the previous study was used to calculate the scores of the new samples. The calculated PC1 and PC2 scores of the new samples were matched to predicted PC1 and PC2 scores (the predicted PC1 and PC2 scores were each associated

with specific percentages of GM, WM, and G). The percentages of GM:WM:G associated with the PC1 and PC2 scores that most closely matched the calculated PC1 and PC2 scores of the new samples were used as the TCP prediction for the new samples. GM and WM were categorized as infiltrative margins (IM) to correspond with histopathological categorization. The percentage of G calculated for each unknown sample was used as an estimate of the TCP. TCP using the lipid deconvolution was categorized as low (TCP $\leq$ 50%) or high (TCP $\geq$ 51%).

Disease Diagnosis

Diagnosis was accomplished using principal component analysis-linear discriminant analysis (PCA-LDA). First, the lipid and metabolite data were combined using mid-level data fusion on their respective PCA scores. These PCs were multiplied by the training set lipid and metabolite loading matrices to generate combined PC scores. These combined PC scores were subsequently multiplied by the training set fused loading matrix eigenvectors to calculate the fusion PC scores of the new samples. The PC scores of the new samples were then used to classify their disease status using linear discriminant analysis (LDA) eigenvectors from the training set. The disease diagnosis provided by PCA-LDA on the fused lipid and metabolite profiles obtained from DESI are glioma (G), grey matter (GM), or white matter (WM). G is indicative of diseased tissue; GM and WM are indicative of low infiltration brain tissue, referred to as infiltrative margins (I.M.).

Supplemental Discussion

Glioma Diagnosis using Tumor Core Biopsies

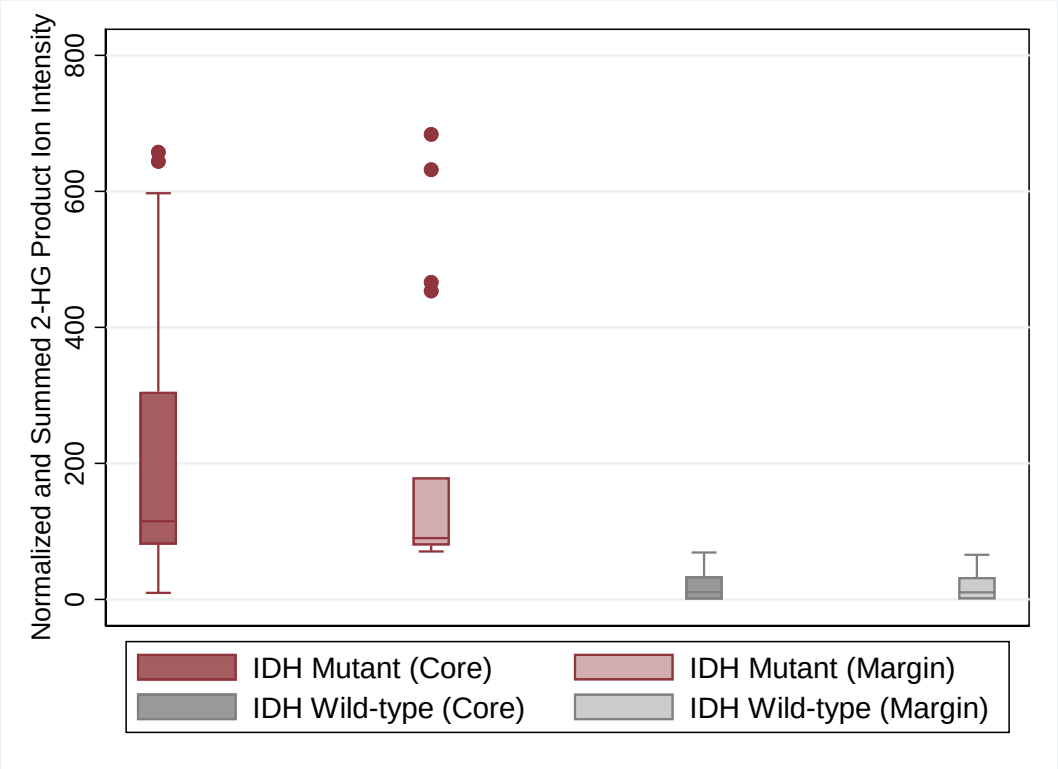
As an intraoperative diagnostic tool to assist neurosurgery, the capability to recognize glioma when present would be useful. In some cases, a single biopsy of tumor area might be sufficient for this diagnostic information. When including a single stereotactic biopsy obtained from a T2 weighted MRI enhancing area for each case, and histopathology assessed the tissue smear as glioma, the agreement between the PCA-LDA diagnosis and histopathology was 92% (n=38). Cases 30, 32, 39, 41, 50, 52, 53 were excluded due to histopathology assessment providing a diagnosis of I.M. for all tissue smears; cases 28, 29, and 35 were excluded for lack of adequate DESI-MS signal obtained from smears for which histopathology provided a diagnosis of glioma; case 45 was excluded due to its histological diagnosis as tumor but not sufficient for diagnosis of glioma. These excluded cases are discussed in the next two subsections.

Heterogeneity of Tumor Core Biopsies

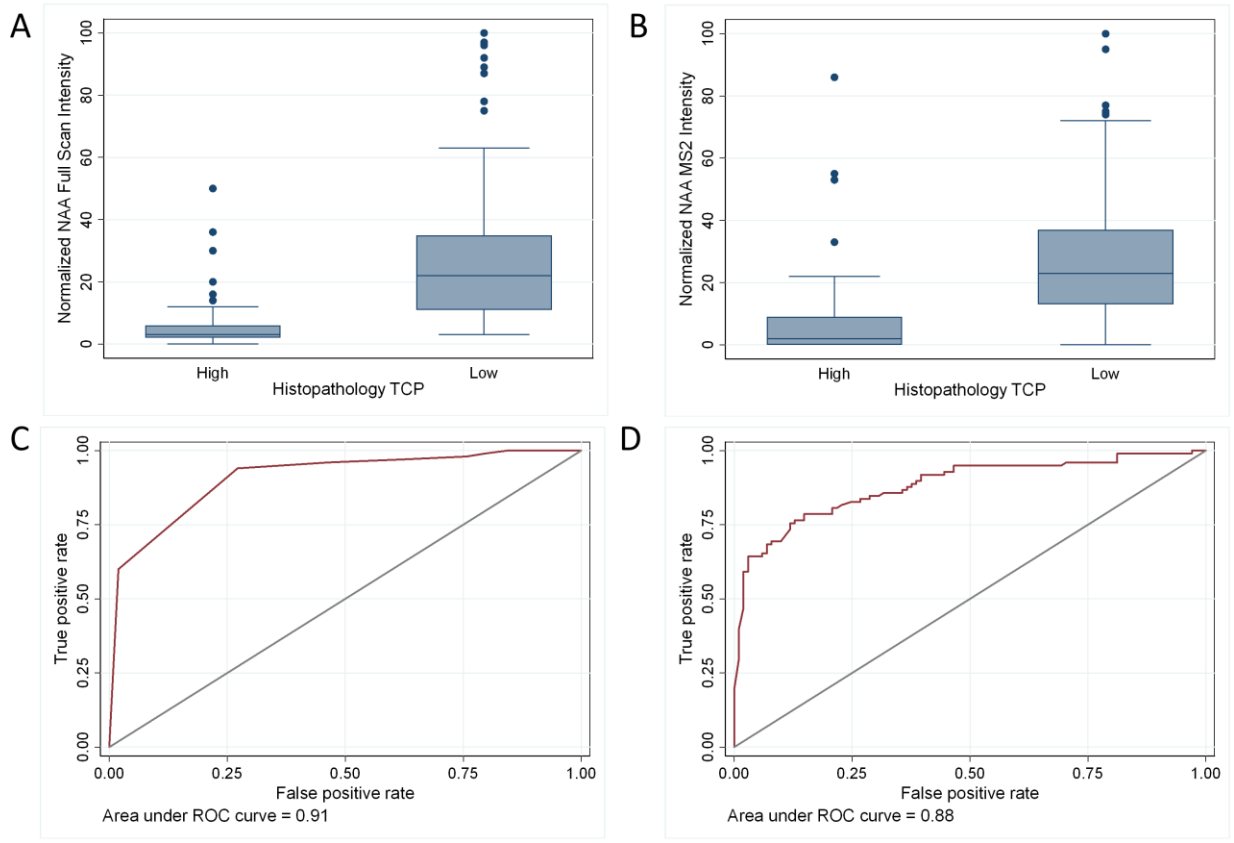
Several of the tumor core biopsies provided histopathological assessments indicating I.M. rather than G. No samples from case 41 were taken from enhancing areas based on the MRI snapshots. The DESI-MS diagnosis for these smears agrees with the histopathological assessment (DESI predicted either WM or GM and histopathology found I.M.), except for smears from case 52 and 53, which DESI predicted G rather than I.M. These cases highlight the complexity and heterogeneity of gliomas, even in tumor cores. DESI-MS methods may be useful in assessing such heterogeneity during surgery.

Special Cases in Which DESI-MS Provided Potential Clinical Utility

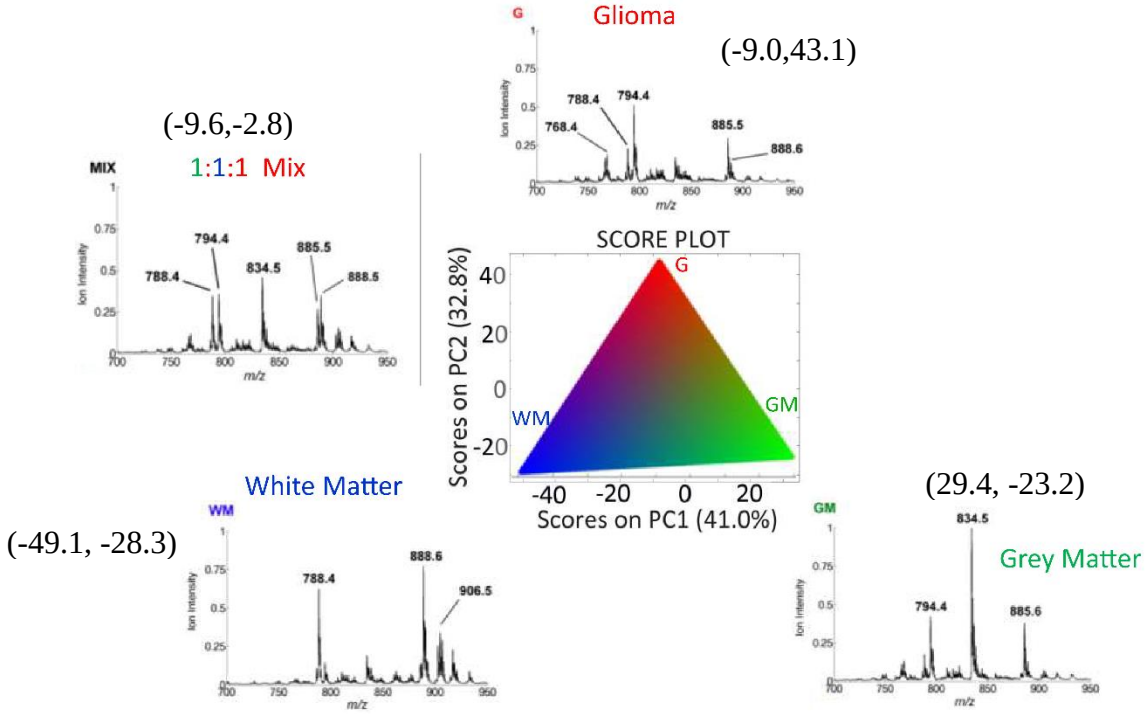
Tissue smears containing large amounts of necrosis and necrotic tumor are indicative of a brain pathology but not specific for glioma in the absence of viable tumor cells. Smears from case 51 and 40 show significant necrosis and necrotic tumor. The DESI-MS diagnosis of these smears was consistent with glioma. For comparison, smears from case 47, which is a high TCP glioma smear, is shown. Case 45 was suspected for lymphoma during the frozen section consultation due to the high nuclear-to-cytoplasmic ratio and discohesive nature of the tumor cells. The biopsy from this case was analyzed by DESI and provided a PCA-LDA diagnosis of G, and histopathology identified tumor but could not confidently diagnose glioma due to the morphology of this subtype (Supplemental Figure 4). The final pathology provided a diagnosis of glioblastoma based on molecular tests. These cases show that DESI-MS during surgery could provide diagnostic information during surgery that can assist in confirming a glioma diagnosis.



Supplemental Figure 1. Box plots of summed 2-HG product ion intensities, separated by IDH mutation status and biopsy location (core or margin). Biopsies from the tumor core and margins of IDH-mut tumors produced significantly higher 2-HG compared to the IDH-wt tumors ($p < 0.0001$ using the Tukey-HSD test for statistical significance). The IDH mutant tumors produced slightly higher 2-HG in the cores compared to margins, but the difference was not statistically significant.



Supplemental Figure 2. Assessment of tumor cell percentage based on N-acetyl-aspartic acid (NAA) measurements. A) Box plot showing dispersion in the normalized full-scan *m/z* 174 intensity (corresponding to NAA) as a function of TCP. High indicates TCP greater than or equal to 51%; Low indicates TCP less than or equal to 50% TCP. B) Box plots showing dispersion in normalized MS^2 product ion intensity for NAA. C) ROC curve for A (predicting TCP high or low based on full-scan NAA intensity) provided AUC of 0.91. D) ROC curve for B (predicting TCP high or low based on MS^2 NAA intensity) provide AUC of 0.88.



Supplemental Figure 3. Scheme illustrating the lipid deconvolution approach. A ternary mixture model was fitted to the lipid profile PCA data obtained from a previous study. The continuous ternary mixture is indicated by the colored triangle on the PC score plot. In the illustration, RGB color coding was used to make a continuous mixture between red (glioma (G)), blue (white matter (WM)), and green (grey matter (GM)). PC1 and PC2 coordinates corresponding to each of these RGB color codes were created, such that each pair of PC1 and PC2 coordinates corresponds to a unique combination of [G GM WM]. After projection of the new samples onto this PCA space, the model was used to predict the percentages of the reference glioma, grey matter, and white matter PCA scores that best matched the unknown spectra. The average lipid profile of the pure classes along with the (PC1, PC2) coordinates are shown around the mixture model at the corresponding vertex, along with the 1:1:1 mixture G:GM:WM. For the present study, the PCA-LDA classifications of GM and WM were recategorized as infiltrative margin (IM) to match the histopathology categories.

Supplemental Table 1. Confusion matrix for assessing correlation between histopathology assessments and the DESI-MS estimates of TCP and disease status of tissue biopsies from 10 subjects whose data was previously published in reference 1 (Pirro et. al. PNAS 2017).

		Histopathology[a]	
		High TCP/Glioma[b]	Low TCP/Infiltrative Margin[b]
Lipid Deconvolution TCP Estimate	High TCP/Glioma[b]	29	4
Full-scan NAA Intensity TCP Estimate		32	5
PCA-LDA Diagnosis		28	4
Lipid Deconvolution TCP Estimate	Low TCP/Infiltrative Margin[b]	6	19
Full-scan NAA Intensity TCP Estimate		3	18
PCA-LDA Diagnosis		9	17

Lipid Deconvolution TCP Estimate:
- Sensitivity: 83%
- Specificity: 83%
- Accuracy: 83%

Full-scan NAA Intensity TCP Estimate:
- Sensitivity: 91%
- Specificity: 78%
- Accuracy: 86%

PCA-LDA Diagnosis:
- Sensitivity: 76%
- Specificity: 81%
- Accuracy: 78%

[a]Histopathology assessments of TCP were used for correlation with lipid deconvolution and NAA intensity classifications of TCP. Histopathology assessments of disease status were used for correlation with PCA-LDA diagnosis of the tissue smears.
[b]For lipid deconvolution and NAA intensity classification, histopathology TCP categories were High ≥51% and Low ≤50%. Histopathology categories of glioma and infiltrative margin were used for correlation with PCA-LDA diagnosis of glioma and infiltrative margin.

Supplemental Table 2. Summary of subjects recruited, if excluded from study or analysis, and total biopsies/smears

Study subject number	Screenfail	Reason for Screenfail	Outlier	Reason for outlier	Previously Published*	Total Biopsies	Total Smears
001	No		No	-	1	9	9
002	No		Yes	Poor DESI signal	1	0	0
003	Yes	Not glioma	No	-	1	0	0
004	No		Yes	Poor DESI signal	1	0	0
005	No		No	-	1	7	8
006	No		No	-	1	6	7
007	No		No	-	1	5	6
008	Yes (withdrawn)	Resection unsafe	No	-	1	0	0
009	No		No	-	1	7	9
010	No		No	-	1	6	7
011	No		Yes	Poor DESI signal	1	0	0
012	No		No	-	1	7	8
013	No		No	-	1	7	10
014	No		No	-	1	6	8
015	No		No	-	1	6	9
016	No		No	-	1	7	7
017	No		No	-	N	4	4
018	No		No	-	N	5	8
019	No		No	-	N	7	10
020	No		No	-	N	5	8
021	No		No	-	N	6	10
022	Yes	Not glioma	No	-	N	0	0
023	No		No	-	N	4	9
024	No		No	-	2	2	3
025	No		No	-	2	7	10
026	No		No	-	2	5	5
027	No		No	-	2	6	10
028	No		No	-	2	5	8
029	No		No	-	2	6	10
030	No		No	-	2	5	6
031	No		No	-	2	5	6
032	No		No	-	2	5	7
033	No		No	-	2	6	6
034	No		No	-	2	3	6

Supplemental Table 2. Continued

035	No		No	-	2		6	6
036	Yes	Not glioma	No	-	N		0	0
037	No		No	-	2		4	5
038	No		No	-	2		6	6
039	No		No	-	2		3	3
040	No		No	-	2		3	6
041	No		No	-	2		5	5
042	No		No	-	2		3	3
043	Yes	Not glioma	No	-	N		0	0
044	No	-	No	-	2		6	10
045	No	-	No	-	2		1	1
046	No	-	No	-	N		6	9
047	No	-	No	-	2		4	8
048	No	-	No	-	2		3	7
049	Yes	Not glioma	No	-	N		0	0
050	No	-	No	-	N		6	6
051	No	-	No	-	2		6	6
052	No	-	No	-	2		3	4
053	No	-	No	-	2		6	9
054	No	-	No	-	2		3	7
055	No	-	No	-	N		4	4
056	No	-	No	-	N		2	4
057	No	-	No	-	N		2	4
058	No	-	No	-	N		6	7
Total Subjects Included (Excluding Screenfails and Outliers) **49**	Total Screenfails (Subject) **6**		Total Outliers (Subject) **3**		New to Glioma Diagnosis and TCP (Subject) **38**	New to IDH Mutation Assay (Subject) **6**	Total Biopsies **247**	Total Smears **334**
	Average Screenfail Rate **10.3%**		Average Outlier Rate (Subject) **6.1%**				Average Biopsies per Subject **5.0**	Average Smears per Biopsy **1.4**

*"1" and "2" indicates that data was used in SI references 1(Pirro. et al. PNAS 2017) and 2 (Alfaro. et al. J. Neurosurg. 2019), respectively. No subjects included in Reference 1 were included in Reference 2. "N" indicates that the data was not utilized in a previous publication. For biopsies that were utilized in Reference 2, only the 2-HG MS^n data was utilized; lipid and metabolite profile data was collected using Method 1 (Figure 1, main text), but that data was not analyzed or included in the previous publication.

Supplemental Table 3. Study subject demographics, diagnosis, and treatment information.

Study subject number	Age	Gender	Race	Ethnicity	Tumor location	Integrated diagnosis	Primary/ Recurrent	F	Awake	Mapping	IMRI
001	30	M	W	N.H.	Left insula	Oligodendendroglioma, IDH-M,WHO grade II	P	Y	N	Y	N
005	23	M	B	A.A.	Left frontal lobe	Dysembryoplastic neuroepithelial tumor, IDH-WT, WHO grade I	P	N	N	N	N
006	24	M	W	N.H.	Left frontal intra-axial	Astrocytoma, IDH-M, WHO grade III	P	N	N	N	N
007	65	M	W	N.H.	Right parietal lobe	GBM, IDH-WT, WHO grade IV	P	N	N	N	N
009	44	F	W	N.H.	Left parietal intra-axial	Oligodendendroglioma, IDH-M and 1p/19q codeleted,WHO grade II	P	N	N	Y	N
010	47	M	W	N.H.	Left frontotemporal	GBM, IDH-WT, WHO grade IV	R	Y	N	N	N
012	30	M	W	N.H.	Left insula	GBM, IDH-M, WHO grade IV	R	N	N	N	N
013	73	F	W	N.H.	Right temporoparietal	GBM, IDH-WT, WHO grade IV	P	Y	N	N	N
014	52	F	W	N.H.	Left parietal lobe	GBM, IDH-WT, WHO grade IV	P	Y	N	N	N
015	33	F	W	N.H.	Right parietal lobe	Oligodendendroglioma, IDH-M,WHO grade II	P	N	N	N	N
016	68	M	W	N.H.	Right frontal intra-axial	Diffuse astrocytoma, IDH-M, WHO grade II	P	N	N	N	N
017	38	M	W	N.H.	Left posterior frontal	Diffuse glioma, IDH-M, WHO grade II	P	N	Y	Y	N
018	25	M	M.R.	N.H.	Left frontotemporal lobe	Diffuse glioma, IDH-WT, WHO grade II	P	N	N	N	N
019	39	F	W	N.H.	Left temporal lobe	Anaplastic glioma, IDH-M, WHO grade III	R	N	N	N	N
020	53	F	W	H.	Right parietal lobe; 2 nodules	GBM, IDH-WT, WHO grade IV	P	Y	N	N	N
021	45	F	W	N.H.	Left frontal lobe	Diffuse glioma, IDH-M, WHO grade II	P	N	N	N	N
023	63	F	W	N.H.	Left parietooccipital lobe	GBM, IDH-WT, WHO grade IV	P	N	N	N	Y
024	58	M	W	N.H.	Right temporal lobe	GBM and gliosarcoma, IDH wild-type, WHO grade IV	P	N	N	N	N

Supplemental Table 3. Continued

025	47	F	W	N.H.	Right occipital lobe	GBM, IDH-WT, WHO grade IV	P	Y	N	N	N
026	46	F	W	N.H.	Right parietal lobe	Anaplastic astrocytoma, IDH-M, WHO grade III	P	N	N	Y	N
027	52	F	W	N.H.	Left frontoparietal lobe	Complex anaplastic astrocytoma, IDH-M, WHO grade III	P	N	Y	Y	N
028	63	M	W	N.H.	Right temporal lobe	Gliosarcoma,IDH-WT, WHO grade IV	R	N	N	N	N
029	53	F	W	N.H.	Right temporal lobe	Diffuse astrocytoma, IDH-WT, WHO grade II	P	N	N	N	N
030	38	F	W	N.H.	Right frontal lobe	Diffuse astrocytoma, IDH-WT, WHO grade II	P	N	N	N	Y
031	68	M	W	N.H.	Right parietal lobe	GBM, IDH-WT, WHO grade IV	P	N	N	N	N
032	54	F	W	N.H.	Left parietal lobe	GBM, IDH-WT, WHO grade IV	P	Y	Y	Y	N
033	20	M	W	N.H.	Left temporal lobe	Diffuse astrocytoma, IDH-M, WHO grade II	P	N	N	N	Y
034	66	F	W	N.H.	Right temporal lobe	GBM, IDH-WT, WHO grade IV	P	N	N	N	N
035	46	M	B	A.A.	Right posterior frontal periventricular	GBM, IDH-WT, WHO grade IV	P	Y	N	N	N
037	59	M	W	N.H.	Bifrontal	GBM, IDH-WT, WHO grade IV	P	N	N	N	N
038	23	M	W	N.H.	Left frontal lobe	Anaplastic astrocytoma, IDH-mutant, WHO grade III	P	N	N	N	N
039	59	M	W	N.H.	Left temporal lobe	GBM, IDH-WT, WHO grade IV	R	N	N	N	N
040	57	F	W	N.H.	Right frontal lobe	GBM, IDH-WT, WHO grade IV	P	N	N	N	N
041	26	F	W	N.H.	Right frontal lobe	Diffuse glioma, IDH-M	P	N	N	N	N
042	35	F	W	N.H.	Right frontal lobe	GBM, IDH-mutant, WHO grade IV	P	N	N	Y	N
044	30	M	W	N.H.	Left temporal lobe and insula	Diffuse astrocytoma, IDH-M,WHO grade II	R	N	N	N	N
045	62	F	W	N.H.	Right frontal lobe	GBM, IDH-WT, WHO grade IV	P	N	N	N	N
046	66	F	A	N.H.	Left temporal lobe	Diffuse glioma, IDH-WT	P	N	N	N	N
047	48	M	W	N.H.	Right frontal lobe	Anaplastic astrocytoma, IDH-mutant, WHO grade III	P	N	N	Y	N

Supplemental Table 3. Continued

048	68	M	W	N.H.	Right frontal lobe	GBM with granular cell features, IDH-WT, WHO grade IV	P	Y	N	N	N
050	32	F	W	N.H.	Left insular and mesial temporal	Diffuse astrocytoma, IDH-M,WHO grade II	P	N	N	N	Y
051	30	F	A.A.	N.H.	Right frontal lobe	GBM, IDH-M, WHO grade IV	R	N	N	N	N
052	61	M	W	N.H.	Right temporal lobe	GBM, IDH-WT, WHO grade IV	P	Y	N	N	N
053	27	M	A	N.H.	Right temporal lobe and insula	Diffuse astrocytoma, IDH-M,WHO grade II	P	N	N	N	N
054	68	M	W	N.H.	Right parietal-occipital lobe	GBM, IDH-M, WHO grade IV	P	N	N	N	Y
055	73	M	W	N.H.	Right frontal lobe	GBM, IDH-WT, WHO grade IV	P	N	N	N	N
056	35	F	W	N.H.	Left parietal lobe	GBM, IDH-WT, WHO grade IV	P	N	N	N	N
057	56	F	W	N.H.	Left frontal lobe	Oligodendroglioma, IDH-M and 1p/19q codeleted,WHO grade II	R	N	N	N	N
058	26	M	W	N.H.	Right frontoparietal region	Oligodendroglioma, IDH-M and 1p/19q codeleted,WHO grade II	P	N	Y	Y	N
	Average Age	Gender Balance	Race Balance	Hispanic Balance	Tumor Location Balance	Tumor Grade and IDH status Balance					
	47.0	25 Males/24 Females	43 W/ 3 B / 2 A / 1 MR	1 H/ 48 NH		1 Grade I/ 17 Grade II/ 6 Grade III/ 25 Grade IV	23 IDH-M / 26 IDH-WT				

Race categories: W = White; N.H. = Non-Hispanic; B = Black; A = Asian; MR = multi-racial
GBM = Glioblastoma
Primary/Recurrent: P = Primary; R = Recurrent
F = Fluorescein
IMRI = Intraoperative MRI used (yes/no)
Mapping = Brain mapping used (yes/no)
Awake = Awake craniotomy (yes/no)

Supplemental Table 4. Additional histopathology results for the study subjects.

Study subject number	GFAP	ATRX	P53	Ki67	1p/19q	MGMT
001	1	0	1 (20%)	1 (1%)	0	--
002	-	-	-	-	-	-
003	-	-	-	-	-	-
004	-	-	-	-	-	-
005	1	--	--	1 (1-5%)	0	--
006	1	0	1 (80%)	1 (4%)	0	--
007	1	1	1 (3%)	1 (4%)	--	0
008	-	-	-	-	-	-
009	1	1	1 (<1%)	1 (5%)	1	--
010	1	--	--	1 (20%)	0	--
011	-	-	-	-	-	-
012	1	0	1	1	0	0
013	1 (large %)	0	1	1 (30%)	--	0
014	1	--	1 (40%)	1 (30%)	--	0
015	1	1	1	1 (< 5%)	0	--
016	1	0	1 (<1%)	1 (<1%)	0	--
017	1	0	1 (40%)	1 (3%)	--	--
018	1	0	1 (15%)	1 (1-2%)	--	--
019	1	0	1 (75%)	1 (40%)	--	--
020	1	1	1 (<10%)	1 (30%)	0	1
021	0	1	1 (rare cells)	1 (10%)	--	--
022	-	-	-	-	-	-
023	1	1	1 (80%)	1 (50%)	0	1
024	1	1	1 (scattered tumor cells) & 1 (<10% spindle cell and glial component)	1 (5% spindle cell component) & 1 (40% of glial component)	--	--
025	1	1	1 (<1%)	1 (40%)	--	1
026	1	0	1 (90%)	1 (5%)	0	1
027		1	1 (<1%)	1 (10% in anaplastic gliomatous component; <5% in remaining tumor)	0	--
028	1	--	--	1 (20%)	0	0
029	1	1	1 (<1%)	1 (1-2%)	--	--
030	1	2	1 (15-20%)	1 (1-2%)	0	--

Supplemental Table 4. Continued

031	1	1	1 (<1%)	1 (approx. 50%)	--	--
032	1	1	1 (<5%)	1 (>60% in regions 1 ATRXl; as low as 10-15% in regions 0 ATRX)	--	1
033	1	2(partial loss of staining)	1 (20%)	1 (10%)	0	--
034	1	1	1 (10%)	1 (3-5%)	0	1
035	1	1	1 (10-15%) with faint staining	1 (30%)	--	1
036	-	-	-	-	-	-
037	1	1	1 (<5%)	1 (30%)	--	--
038	1	0	1 (>75%)	1 (up to 5-10%)	0	--
039	1	--	--	--	--	--
040	1	0	1 (25%)	1 (20%)	--	1
041	1	0	1 (positive in the majority of the tumor cells)	1 (approx. 5%)	--	--
042	1	0	1 (>80%)	1 (5%)	0	Ind.
043	-	-	-	-	-	-
044	1	0	1 (>95%)	1 (approx 3%)	0	--
045	1	0	0	1 (50-75%)	0	--
046	1	1	1 (<5%)	1 (5-10%) in most hypercellular regions	--	--
047	1	0	1 (>50%)	1 (approx. 3-5%)	0	--
048	1	1	1 (>50%)	1 (approx 10%)	--	0
049	-	-	-	-	-	-
050	1	0	1 (40-50%)	1 (<3%)	--	--
051	1	--	1 (>80%)	--	--	1
052	1	0	1 (<1%)	1 (5-10%)	--	--
053	1	--	1 (30%)	1 (3-4%)	0	--
054	--	1	1	--	--	0
055	1	1	1 (<5%)	1 (40-50%)	--	1
056	1	1	1 (20%)	1 (variably immunoreactive in up to 30% of tumor cells)	0	1
057	1	--	--	--	1	--
058	1	1	--	1(1%)	1	--

This table reports IHC staining results. 1 = immunoreactive; 0 = non-immunoreactive. For Ki-67, the percentage indicates the labelling index (percentage of tumor cells that are immunoreactive).

Supplemental Table 5. DESI-MS predictions of disease status and TCP for the smears obtained for each subject

Sample Information						Selected DESI-MS Scans		PCA-LDA Diagnosis	NAA (Full-Scan) TCP Prediction		Lipid Deconvolution TCP Prediction		IDH Prediction from 2-HG	
Subject	**Biopsy # (Subject)**	**Core/Margin**	**Smear # (Subject)**	**Smear # (Overall)**	**Included/ Excluded in statistical predictions**	**# Lipid Scans**	**# Metabolite Scans**	**Classification**	**Mean TCP Value**	**TCP Category**	**Mean TCP Value**	**TCP Category**	**Normalized 2-HG Value**	**IDH Prediction**
1	1	Margin	1	1	I	62	81	G	64	High	100	High	N/A	N/A
	2	Margin	2	2	E	-	120	-	91	High	-	-	N/A	N/A
	3	Core	3	3	I	74	27	G	81	High	100	High	N/A	N/A
	4	Core	4	4	I	72	110	G	72	High	100	High	N/A	N/A
	5	Margin	5	5	I	28	63	G	82	High	98	High	N/A	N/A
	6	Core	6	6	I	64	95	G	80	High	100	High	N/A	N/A
	7	Core	7	7	I	43	112	G	70	High	100	High	N/A	N/A
	8	Core	8	8	I	55	106	G	74	High	100	High	N/A	N/A
	9	Margin	9	9	E*	58	94	G	44	Low	100	High	N/A	N/A
5	1	N/A	1	10	I	13	140	G	81	High	100	High	N/A	N/A
		N/A	2	11	I	24	115	G	72	High	100	High	N/A	N/A
	2	Margin	3	12	I	38	72	WM	55	High	32	Low	N/A	N/A
	3	Core	4	13	I	23	60	G	72	High	100	High	N/A	N/A
	4	Margin	5	14	I	29	109	WM	66	High	52	High	N/A	N/A
	5	Margin	6	15	I	40	93	WM	57	High	40	Low	N/A	N/A
	6	Margin	7	16	I	21	79	G	65	High	91	High	N/A	N/A
	7	Core	8	17	I	30	137	G	79	High	100	High	N/A	N/A

Supplemental Table 5. Continued

6	1	Core	1	18	I	40	78	WM	63	High	18	Low	N/A	N/A
	2	Core	2	19	I	65	85	G	74	High	97	High	N/A	N/A
	3	Margin	3	20	I	78	60	WM	14	Low	77	High	N/A	N/A
	4	Core	4	21	I	65	44	WM	63	High	48	Low	N/A	N/A
	5	Core	5	22	I	39	72	WM	58	High	52	High	N/A	N/A
		Core	6	23	I	87	45	WM	55	High	30	Low	N/A	N/A
	6	Core	7	24	I	99	53	WM	26	Low	0	Low	N/A	N/A
7	1	Core	1	25	I	92	121	G	65	High	64	High	N/A	N/A
	2	Margin	2	26	I	125	127	GM	6	Low	21	Low	N/A	N/A
	3	Core	3	27	I	125	120	WM	38	Low	0	Low	N/A	N/A
	4	Core	4	28	I	80	86	WM	35	Low	0	Low	N/A	N/A
	5	Margin	5	29	I	87	91	WM	14	Low	0	Low	N/A	N/A
		Margin	6	30	I	98	93	WM	5	Low	0	Low	N/A	N/A
9	1	N/A	1	31	I	9	34	G	57	High	74	High	N/A	N/A
		N/A	2	32	I	37	41	G	47	Low	78	High	N/A	N/A
	2	Core	3	33	I	124	127	WM	1	Low	0	Low	N/A	N/A
	3	Margin	4	34	I	111	120	WM	2	Low	0	Low	N/A	N/A
	4	Margin	5	35	I	92	65	WM	11	Low	0	Low	N/A	N/A
		Margin	6	36	I	105	90	WM	3	Low	0	Low	N/A	N/A
	5	Core	7	37	I	117	109	WM	27	Low	0	Low	N/A	N/A
	6	Core	8	38	I	61	84	G	58	High	99	High	N/A	N/A
	7	Core	9	39	I	72	72	WM	0	Low	0	Low	N/A	N/A

Supplemental Table 5. Continued

10	1	Core	1	40	I	48	109	GM	28	Low	0	Low	N/A	N/A
	2	Core	2	41	I	120	106	G	25	Low	68	High	N/A	N/A
	3	Core	3	42	I	55	93	G	49	Low	98	High	N/A	N/A
	4	Core	4	43	I	94	129	G	62	High	96	High	N/A	N/A
		Core	5	44	I	70	96	G	40	Low	74	High	N/A	N/A
	5	Margin	6	45	I	48	65	G	48	Low	98	High	N/A	N/A
	6	Margin	7	46	I	104	127	WM	27	Low	38	Low	N/A	N/A
12	1	Core	1	47	I	42	83	G	76	High	100	High	N/A	N/A
		Core	2	48	I	50	121	G	78	High	100	High	N/A	N/A
	2	Core	3	49	I	52	87	G	48	Low	65	High	N/A	N/A
	3	Core	4	50	I	62	107	G	71	High	91	High	N/A	N/A
	4	N/A	5	51	I	45	92	G	48	Low	82	High	N/A	N/A
	5	N/A	6	52	I	53	93	WM	66	High	50	Low	N/A	N/A
	6	N/A	7	53	I	43	95	WM	58	High	50	Low	N/A	N/A
	7	N/A	8	54	I	47	89	WM	63	High	26	Low	N/A	N/A
13	1	Margin	1	55	I	22	20	G	76	High	83	High	N/A	N/A
		Margin	2	56	I	12	22	G	85	High	87	High	N/A	N/A
	2	Core	3	57	I	20	23	G	81	High	92	High	N/A	N/A
		Core	4	58	I	24	25	G	80	High	87	High	N/A	N/A
	3	Margin	5	59	E*	19	22	WM	78	High	47	Low	N/A	N/A
		Margin	6	60	I	27	23	WM	65	High	32	Low	N/A	N/A

Supplemental Table 5. Continued

13	4	Margin	7	61	E*	12	-	-	-	-	21	Low	N/A	N/A
	5	Margin	8	62	E*	11	-	-	-	-	8	Low	N/A	N/A
	6	Margin	9	63	E	18	-	-	-	-	1	Low	N/A	N/A
	7	Margin	10	64	I	4	25	WM	49	Low	0	Low	N/A	N/A
14	1	Margin	1	65	E	-	-	-	-	-	-	-	N/A	N/A
	2	Core	2	66	E*	24	17	WM	30	Low	21	Low	N/A	N/A
	3	Margin	3	67	E	-	-	-	-	-	-	-	N/A	N/A
		Margin	4	68	E*	14	22	G	79	High	98	High	N/A	N/A
	4	Core	5	69	I	9	24	G	88	High	97	High	N/A	N/A
	5	Core	6	70	I	24	25	G	91	High	99	High	N/A	N/A
	6	Margin	7	71	I	5	24	G	88	High	100	High	N/A	N/A
		Margin	8	72	I	15	25	G	86	High	100	High	N/A	N/A
15	1	Core	1	73	I	5	18	G	74	High	78	High	N/A	N/A
		Core	2	74	I	7	24	G	79	High	100	High	N/A	N/A
	2	Core	3	75	I	5	23	G	81	High	79	High	N/A	N/A
		Core	4	76	I	14	22	G	75	High	80	High	N/A	N/A
	3	Core	5	77	I	5	25	G	85	High	87	High	N/A	N/A
	4	Margin	6	78	I	33	21	G	40	Low	87	High	N/A	N/A
		Margin	7	79	I	43	25	WM	7	Low	3	Low	N/A	N/A

Supplemental Table 5. Continued

15	5	Margin	8	80	I	18	23	WM	69	High	13	Low	N/A	N/A
	6	Margin	9	81	I	28	24	WM	48	Low	0	Low	N/A	N/A
16	1	Core	1	82	I	14	15	WM	1	Low	0	Low	N/A	N/A
	2	Core	2	83	I	9	22	G	21	Low	78	High	N/A	N/A
	3	Core	3	84	I	26	25	WM	43	Low	29	Low	N/A	N/A
	4	Margin	4	85	I	24	18	WM	54	High	17	Low	N/A	N/A
	5	Margin	5	86	I	28	25	WM	41	Low	17	Low	N/A	N/A
	6	Margin	6	87	I	25	21	WM	23	Low	0	Low	N/A	N/A
	7	Core	7	88	E	21	-	-	-	-	33	Low	N/A	N/A
17	1	Core	1	89	I	8	20	G	68	High	89	High	N/A	N/A
	2	Core	2	90	I	15	25	WM	71	High	54	High	N/A	N/A
	3	Core	3	91	I	32	25	WM	69	High	23	Low	N/A	N/A
	4	Margin	4	92	I	32	17	WM	52	High	28	Low	N/A	N/A
18	1	Core	1	93	E*	20	25	G	88	High	92	High	N/A	N/A
		Core	2	94	E*	23	23	G	88	High	91	High	N/A	N/A
	2	Core	3	95	I	20	25	WM	84	High	58	High	N/A	N/A
	3	Core	4	96	I	27	25	WM	89	High	68	High	N/A	N/A
	4	Margin	5	97	I	23	10	WM	11	Low	16	Low	N/A	N/A
		Margin	6	98	I	32	19	WM	26	Low	14	Low	N/A	N/A
	5	Core	7	99	I	23	25	WM	80	High	33	Low	N/A	N/A

Supplemental Table 5. Continued

18	5	Core	8	100	I	28	25	WM	76	High	30	Low	N/A	N/A
19	1	Core	1	101	I	27	24	G	85	High	94	High	N/A	N/A
	2	Margin	2	102	E*	24	25	WM	80	High	45	Low	N/A	N/A
	3	Core	3	103	E	-	25	-	88	High	-	-	N/A	N/A
		Core	4	104	I	23	25	G	90	High	96	High	N/A	N/A
	4	Core	5	105	I	14	25	G	89	High	92	High	N/A	N/A
	5	Margin	6	106	I	23	25	GM	26	Low	0	Low	N/A	N/A
		Margin	7	107	I	24	25	GM	26	Low	2	Low	N/A	N/A
	6	Margin	8	108	I	25	25	WM	52	High	0	Low	N/A	N/A
	7	Margin	9	109	I	13	25	WM	68	High	43	Low	N/A	N/A
		Margin	10	110	I	28	25	WM	36	High	3	Low	N/A	N/A
20	1	Margin	1	111	I	13	25	WM	72	High	40	Low	N/A	N/A
		Margin	2	112	I	30	25	WM	62	High	38	Low	N/A	N/A
	2	Margin	3	113	I	30	25	WM	70	High	59	High	N/A	N/A
		Margin	4	114	I	31	25	WM	56	High	38	Low	N/A	N/A
	3	Margin	5	115	I	32	25	WM	22	Low	0	Low	N/A	N/A
	4	Margin	6	116	I	33	25	G	72	High	83	High	N/A	N/A
	5	Margin	7	117	I	27	25	WM	31	Low	0	Low	N/A	N/A

Supplemental Table 5. Continued

20	5	Margin	8	118	I	33	25	WM	12	Low	0	Low	N/A	N/A
21	1	Margin	1	119	I	8	25	G	32	Low	64	High	N/A	N/A
		Margin	2	120	I	18	25	G	24	Low	69	High	N/A	N/A
	2	Core	3	121	I	30	22	GM	17	Low	57	High	N/A	N/A
		Core	4	122	I	20	25	GM	12	Low	50	Low	N/A	N/A
	3	N/A	5	123	I	26	25	GM	24	Low	70	High	N/A	N/A
		N/A	6	124	I	27	25	GM	25	Low	70	High	N/A	N/A
	4	Core	7	125	I	29	22	WM	32	Low	40	Low	N/A	N/A
		Core	8	126	I	30	24	G	37	Low	68	High	N/A	N/A
	5	Margin	9	127	I	24	22	GM	18	Low	36	Low	N/A	N/A
	6	Margin	10	128	I	30	21	WM	33	Low	11	Low	N/A	N/A
23	1	Core	1	129	E*	3	25	G	71	High	83	High	N/A	N/A
	2	Core	2	130	I	2	25	WM	70	High	88	High	N/A	N/A
		Core	3	131	E*	32	25	WM	75	High	23	Low	N/A	N/A
	3	Core	4	132	I	32	25	WM	69	High	47	Low	N/A	N/A
		Core	5	133	I	32	25	WM	76	High	54	High	N/A	N/A
	1	Core	6	134	E	-	25	-	79	High	-	-	N/A	N/A
		Core	7	135	E	-	25	-	83	High	-	-	N/A	N/A
	4	Core	8	136	I	18	25	G	82	High	83	High	N/A	N/A
		Core	9	137	I	10	25	G	80	High	81	High	N/A	N/A
24	1	Margin	1	138	I	17	25	G	75	High	84	High	5.4	WT
		Margin	2	139	I	23	25	G	75	High	83	High	0	WT

Supplemental Table 5. Continued

24	2	Core	3	140	I	26	25	G	79	High	85	High	6	WT
25	1	Margin	1	141	I	25	25	G	82	High	74	High	0	WT
	2	Margin	2	142	I	21	21	GM	63	High	52	High	N/A	N/A
	3	Margin	3	143	I	21	25	GM	67	High	59	High	N/A	N/A
		Margin	4	144	I	22	25	WM	58	High	19	Low	N/A	N/A
	4	Margin	5	145	E	-	25	-	79	High	-	-	N/A	N/A
	5	Margin	6	146	I	26	22	WM	33	Low	4	Low	N/A	N/A
	6	Margin	7	147	I	21	14	WM	44	Low	0	Low	N/A	N/A
	7	Margin	8	148	I	30	18	WM	39	Low	0	Low	N/A	N/A
	5	Margin	9	149	I	30	25	WM	22	Low	0	Low	0	WT
	7	Margin	10	150	I	29	16	WM	33	Low	0	Low	8.2	WT
26	1	Core	1	151	I	15	9	G	64	High	76	High	26	WT
	2	Margin	2	152	I	26	16	WM	7	Low	0	Low	61.4	WT
	3	Margin	3	153	I	31	19	WM	22	Low	0	Low	70.5	Mut
	4	Margin	4	154	I	32	22	WM	16	Low	0	Low	82.5	Mut
	5	Core	5	155	I	25	11	G	52	High	71	High	62.3	WT
27	1	Core	1	156	E	-	-	-	-	-	-	-	N/A	N/A

Supplemental Table 5. Continued

27	1	Core	2	157	I	2	6	G	81	High	100	High	N/A	N/A
	2	Margin	3	158	E	-	-	-	-	-	-	-	N/A	N/A
	3	Core	4	159	E	-	-	-	-	-	-	-	N/A	N/A
		Core	5	160	E	-	19	-	88	High	-	-	N/A	N/A
	4	Core	6	161	E	-	23	-	87	High	-	-	N/A	N/A
		Core	7	162	E	-	-	-	-	-	-	-	N/A	N/A
	5	Core	8	163	E	-	13	-	83	High	-	-	N/A	N/A
	6	Core	9	164	I	7	22	WM	76	High	31	Low	N/A	N/A
	5	Core	10	165	I	14	9	WM	73	High	36	Low	N/A	N/A
28	1	Core	1	166	E	-	23	-	75	High	-	-	16.9	WT
	2	Margin	2	167	E	-	-	-	-	-	-	-	0	WT
	3	Margin	3	168	I	16	20	GM	34	Low	0	Low	0	WT
		Margin	4	169	I	25	15	GM	33	Low	0	Low	61.2	WT
	4	Core	5	170	E	-	-	-	-	-	-	-	0	WT
		Core	6	171	E	-	24	-	74	High	-	-	16.4	WT
	5	N/A	7	172	I	24	25	GM	44	Low	35	Low	27.5	WT
		N/A	8	173	E	-	-	-	-	-	-	-	0	WT
29	1	Core	1	174	E	-	-	-	-	-	-	-	0	WT
		Core	2	175	E	-	-	-	-	-	-	-	0	WT
	2	Margin	3	176	E	-	-	-	-	-	-	-	0	WT
		Margin	4	177	E	-	-	-	-	-	-	-	0	WT
	3	Margin	5	178	E	-	-	-	-	-	-	-	10.5	WT

Supplemental Table 5. Continued

29	4	Core	6	179	I	30	26	WM	24	Low	0	Low	0	WT
	5	Core	7	180	I	30	25	WM	50	Low	49	Low	21.9	WT
	6	Margin	8	181	I	19	25	WM	61	High	51	High	0	WT
		Margin	9	182	I	29	25	WM	58	High	31	Low	14.1	WT
		Core	10	183	I	27	17	WM	46	Low	35	Low	0	WT
30	1	Core	1	184	I	28	26	WM	55	High	8	Low	57.1	WT
		Core	2	185	I	25	15	WM	60	High	46	Low	36.9	WT
	2	Margin	3	186	I	34	22	WM	1	Low	0	Low	12.6	WT
	3	Margin	4	187	I	36	28	WM	1	Low	0	Low	0	WT
	4	Margin	5	188	I	19	15	WM	7	Low	0	Low	56.2	WT
	5	Margin	6	189	I	18	29	GM	1	Low	0	Low	26.5	WT
31	1	Margin	1	190	I	14	29	G	79	High	72	High	0	WT
		Margin	2	191	I	22	29	GM	80	High	66	High	4.6	WT
	2	Core	3	192	E	-	-	-	-	-	-	-	0	WT
	3	Margin	4	193	I	27	29	WM	46	Low	8	Low	0	WT
	4	Core	5	194	E	-	-	-	-	-	-	-	0	WT
	5	Core	6	195	I	12	12	WM	50	Low	0	Low	0	WT
32	1	Margin	1	196	I	18	25	WM	9	Low	0	Low	0	WT
	2	Core	2	197	I	20	26	WM	44	Low	13	Low	0	WT
		Core	3	198	I	25	16	WM	31	Low	0	Low	N/A	N/A

Supplemental Table 5. Continued

32	3	Margin	4	199	I	27	20	WM	48	Low	0	Low	N/A	N/A
	4	Margin	5	200	I	20	19	WM	44	Low	0	Low	N/A	N/A
	5	Core	6	201	I	7	13	WM	37	Low	0	Low	0	WT
		Core	7	202	I	17	8	WM	40	Low	2	Low	0	WT
33	1	Margin	1	203	I	7	21	WM	55	High	30	Low	N/A	N/A
	2	Margin	2	204	E	-	-	-	-	-	-	-	5.5	WT
	3	Margin	3	205	E	-	-	-	-	-	-	-	59.7	WT
	4	Core	4	206	I	14	27	WM	77	High	21	Low	87.6	Mut
	5	Core	5	207	E	-	-	-	-	-	-	-	43.8	WT
	6	Core	6	208	E	-	-	-	-	-	-	-	71.4	Mut
34	1	Margin	1	209	I	21	19	GM	13	Low	0	Low	4.3	WT
		Margin	2	210	I	29	23	GM	17	Low	0	Low	3	WT
	2	Core	3	211	I	24	24	WM	59	High	10	Low	9.3	WT
		Core	4	212	I	16	21	WM	59	High	1	Low	0	WT
	3	Core	5	213	I	9	22	WM	57	High	0	Low	0	WT
		Core	6	214	I	13	23	WM	52	High	0	Low	10.5	WT
35	1	Core	1	215	I	23	8	WM	29	Low	0	Low	0	WT
	2	Margin	2	216	E	-	-	-	-	-	-	-	22.4	WT
	3	Margin	3	217	E	12	-	-	-	-	11	Low	0	WT
	4	Margin	4	218	E	20	-	-	-	-	0	Low	0	WT

Supplemental Table 5. Continued

35	5	Margin	5	219	E	9	-	-	-	-	9	Low	0	WT
	6	Margin	6	220	E	-	-	-	-	-	-	-	12.9	WT
37	1	Core	1	221	I	10	16	G	84	High	71	High	0	WT
		Core	2	222	I	7	16	G	85	High	70	High	0	WT
	2	Core	3	223	I	9	22	G	85	High	99	High	0	WT
	3	Core	4	224	I	8	23	G	81	High	92	High	0	WT
	4	Core	5	225	I	12	7	G	77	High	73	High	0	WT
38	1	Core	1	226	I	6	29	G	87	High	100	High	33.5	WT
	2	Core	2	227	I	4	28	G	75	High	89	High	N/A	N/A
	3	Core	3	228	I	28	27	WM	40	Low	0	Low	101.7	Mut
	4	Margin	4	229	I	7	22	WM	65	High	56	High	N/A	N/A
	5	Margin	5	230	I	23	22	WM	78	High	36	Low	15.1	WT
	6	Core	6	231	I	22	25	WM	84	High	54	High	73.5	Mut
39	1	Margin	1	232	I	50	46	WM	17	Low	6	Low	38.3	WT
	2	Margin	2	233	I	62	64	WM	30	Low	0	Low	16.3	WT
	3	Core	3	234	I	67	66	WM	38	Low	0	Low	19.7	WT
40	1	Margin	1	235	E	-	60	-	79	High	-	-	32.9	WT
		Margin	2	236	E	-	60	-	82	High	-	-	0	WT
	2	Core	3	237	I	24	62	G	76	High	93	High	15.1	WT
		Core	4	238	I	33	65	G	65	High	84	High	44.3	WT

Supplemental Table 5. Continued

40	3	Margin	5	239	I	64	67	WM	19	Low	0	Low	65.6	WT
		Margin	6	240	I	64	65	WM	22	Low	0	Low	17.3	WT
41	1	Margin	1	241	I	49	64	WM	37	Low	22	Low	90.2	Mut
	2	Margin	2	242	I	64	61	WM	7	Low	0	Low	N/A	N/A
	3	Margin	3	243	I	53	65	G	19	Low	71	High	26.1	WT
	4	Margin	4	244	I	53	49	WM	20	Low	71	High	10.1	WT
	5	Margin	5	245	I	38	37	WM	57	High	53	High	N/A	N/A
42	1	Core	1	246	E*	21	59	G	89	High	86	High	71	Mut
	2	Core	2	247	I	20	64	WM	71	High	50	Low	180.3	Mut
	3	Core	3	248	E*	37	64	WM	74	High	39	Low	88.9	Mut
44	1	Core	1	249	I	20	64	G	95	High	94	High	70.5	Mut
		Core	2	250	I	9	64	G	91	High	91	High	91.3	Mut
		Core	3	251	I	22	63	G	96	High	88	High	97.4	Mut
	2	Core	4	252	I	39	63	G	89	High	83	High	76.4	Mut
	3	Margin	5	253	I	26	63	G	80	High	93	High	78.1	Mut
		Margin	6	254	I	18	63	G	81	High	92	High	80.7	Mut
	4	Margin	7	255	I	60	64	G	97	High	83	High	83.4	Mut
	5	Margin	8	256	I	46	65	GM	8	Low	50	Low	79.3	Mut
	6	Core	9	257	I	36	65	G	66	High	89	High	94.7	Mut
		Core	10	258	I	49	65	G	26	Low	78	High	76.7	Mut

Supplemental Table 5. Continued

45	1	N/A	1	259	E*	59	65	G	83	High	89	High	N/A	N/A
46	1	Core	1	260	I	36	64	G	70	High	75	High	48.5	WT
		Core	2	261	I	31	64	G	62	High	85	High	69	WT
	2	Core	3	262	I	33	65	G	68	High	68	High	4.9	WT
	3	Core	4	263	I	29	65	G	61	High	87	High	15.3	WT
		Core	5	264	I	31	66	G	62	High	83	High	78.7	Mut
		Core	6	265	I	39	64	G	59	High	79	High	27.7	WT
	4	Margin	7	266	I	38	62	G	56	High	49	Low	40.6	WT
	5	Margin	8	267	I	30	64	G	59	High	68	High	29	WT
	6	Margin	9	268	I	60	65	WM	29	Low	10	Low	56.1	WT
47	1	Core	1	269	I	63	60	WM	45	Low	33	Low	41.1	WT
		Core	2	270	I	66	62	WM	40	Low	33	Low	53.3	WT
	2	Core	3	271	I	49	63	G	92	High	85	High	82.7	Mut
		Core	4	272	I	52	61	G	79	High	73	High	131.9	Mut
	3	Core	5	273	I	55	57	G	33	Low	56	High	91.1	Mut
		Core	6	274	I	53	59	G	37	Low	64	High	83.4	Mut
	4	Margin	7	275	I	57	61	G	57	High	38	Low	77.8	Mut
		Margin	8	276	I	62	63	WM	46	Low	28	Low	63.1	WT
48	1	Core	1	277	I	26	48	WM	43	Low	25	Low	0	WT
		Core	2	278	I	18	58	G	30	Low	71	High	16.8	WT
	2	Core	3	279	I	19	63	WM	64	High	19	Low	14.3	WT
		Core	4	280	I	2	60	WM	81	High	11	Low	46.8	WT
	3	Core	5	281	I	62	64	WM	28	Low	16	Low	28.3	WT

Supplemental Table 5. Continued

48	3	Core	6	282	I	60	63	WM	30	Low	10	Low	9.6	9.6
		Core	7	283	I	63	65	WM	26	Low	6	Low	10.8	10.8
50	1	Core	1	284	I	70	75	WM	52	High	12	Low	106.7	Mut
	2	Margin	2	285	I	73	77	WM	4	Low	25	Low	62.8	WT
	3	Core	3	286	I	75	74	WM	51	High	9	Low	154.5	Mut
	4	Core	4	287	I	62	74	G	17	Low	54	High	116.9	Mut
	5	Core	5	288	I	41	72	WM	84	High	36	Low	76.9	Mut
	6	Core	6	289	I	69	73	WM	54	High	32	Low	89.8	Mut
51	1	Core	1	290	I	6	71	G	100	High	72	High	393.9	Mut
	2	Margin	2	291	E*	11	73	G	82	High	75	High	466.2	Mut
	3	Margin	3	292	E*	2	72	G	83	High	89	High	631.8	Mut
	4	Core	4	293	E*	2	69	G	94	High	84	High	657.6	Mut
	5	Margin	5	294	E*	9	72	G	71	High	83	High	683.8	Mut
	6	Core	6	295	E*	15	73	G	90	High	81	High	524.1	Mut
52	1	Margin	1	296	I	50	41	GM	43	Low	27	Low	0	WT
	2	Margin	2	297	I	61	42	WM	38	Low	17	Low	28.6	WT
	3	Core	3	298	E*	23	39	G	96	High	58	High	53.2	WT
		Core	4	299	I	60	41	G	96	High	35	Low	N/A	N/A
53	1	Core	1	300	I	60	41	WM	59	High	20	Low	113.1	Mut
	2	Margin	2	301	I	54	44	WM	36	Low	25	Low	179.6	Mut
		Margin	3	302	I	68	41	WM	49	Low	16	Low	109.3	Mut

Supplemental Table 5. Continued

53	3	Core	4	303	I	57	44	G	45	Low	55	High	95.5	Mut
		Core	5	304	I	37	44	G	46	Low	76	High	185.2	Mut
	4	Core	6	305	I	58	43	WM	65	High	20	Low	135.9	Mut
	5	Core	7	306	I	55	43	WM	65	High	26	Low	195.3	Mut
	6	Margin	8	307	I	32	40	G	67	High	63	High	43	WT
		Margin	9	308	I	40	43	G	52	High	49	Low	57.3	WT
54	1	Margin	1	309	E	-	-	-	-	-	-	-	N/A	N/A
		Margin	2	310	E	-	-	-	-	-	-	-	97.5	Mut
	2	Core	3	311	I	16	75	WM	57	High	25	Low	78.4	Mut
		Core	4	312	I	25	73	WM	51	High	11	Low	363	Mut
	3	Core	5	313	I	16	74	G	85	High	78	High	147.4	Mut
		Core	6	314	I	11	73	G	90	High	92	High	144.4	Mut
		Core	7	315	I	15	75	G	87	High	90	High	129.8	Mut
55	1	Margin	1	316	I	25	75	G	83	High	42	Low	9.2	WT
	2	Core	2	317	I	50	60	WM	38	Low	7	Low	0	WT
	3	Core	3	318	E*	22	67	G	86	High	63	High	0	WT
	4	N/A	4	319	I	39	71	WM	72	High	27	Low	0	WT
56	1	Core	1	320	I	4	74	G	100	High	67	High	7.9	WT
	2	Core	2	321	I	9	74	G	97	High	61	High	40.2	WT
		Core	3	322	I	37	76	G	91	High	52	High	34.3	WT
		Core	4	323	I	46	74	G	83	High	60	High	63.1	WT
57	1	Core	1	324	I	5	72	G	72	High	82	High	403.1	Mut
	2	Core	2	325	I	15	74	G	57	High	76	High	588.5	Mut

Supplemental Table 5. Continued

57	2	Core	3	326	I	11	72	G	62	High	86	High	597.2	Mut
		Core	4	327	I	9	73	G	77	High	91	High	367.5	Mut
58	1	Core	1	328	I	14	74	G	39	Low	87	High	644	Mut
		Core	2	329	I	7	72	G	50	Low	98	High	579.1	Mut
	2	Margin	3	330	I	5	74	WM	48	Low	51	High	453.5	Mut
	3	Margin	4	331	I	24	74	WM	11	Low	9	Low	125.1	Mut
	4	Core	5	332	I	19	74	WM	17	Low	16	Low	247.9	Mut
	5	Margin	6	333	I	33	73	WM	0	Low	5	Low	72.5	Mut
	6	Core	7	334	I	23	73	WM	21	Low	16	Low	436.4	Mut

Cells with hyphens indicate that no MS scans met the inclusion criteria and were excluded from statistical analyses.
I = Included
E = Excluded
G = glioma
WM = White matter
GM = Grey Matter
For NAA predictions of TCP, the TCP categories were High ≥51% and Low ≤50%.
For lipid deconvolution TCP predictions, the TCP categories were High ≥51% and Low ≤50%.
WT = IDH-wildtype
Mut=IDH-mutant
Samples with “N/A” for the IDH assessment indicate that the smears were not analyzed with the 2-HG DESI-MS method.

Supplemental Table 6. Pathological evaluation of the tissue smears obtained from each study subject

Sample Information						
Subject	**Biopsy # (Subject)**	**Smear # (Subject)**	**Smear # (Overall)**	**Diagnosis**	**TCP-Estimate**	**Smear Quality**
1	1	1	1	G	100	--
	2	2	2	G	100	--
	3	3	3	G	100	--
	4	4	4	G	100	--
	5	5	5	G	100	--
	6	6	6	G	100	--
	7	7	7	G	100	--
	8	8	8	G	100	--
	9	9	9	I.M.	10	BMA
5	1	1	10	G	90	--
		2	11	G	90	--
	2	3	12	G	60	--
	3	4	13	G	80	--
	4	5	14	G	50	--
	5	6	15	G	60	--
	6	7	16	G	80	--
	7	8	17	G	80	--
6	1	1	18	G	50	--
	2	2	19	G	90	--
	3	3	20	GM	0	A
	4	4	21	G	70	--
	5	5	22	G	50	--
		6	23	I.M.	25	MA
	6	7	24	I.M.	15	--
7	1	1	25	G	90	--
	2	2	26	I.M.	10	--
	3	3	27	G	50	--
	4	4	28	G	50	--
	5	5	29	I.M.	10	--
		6	30	G	30	--

Supplemental Table 6. Continued

9	1	1	31	G	90	--
		2	32	G	90	--
	2	3	33	I.M.	30	--
	3	4	34	I.M.	50	--
	4	5	35	G	60	--
		6	36	I.M.	30	--
	5	7	37	I.M.	15	--
	6	8	38	G	95	--
	7	9	39	I.M.	20	--
10	1	1	40	I.M.	30	--
	2	2	41	GM	0	MA
	3	3	42	G	80	--
	4	4	43	G	80	--
		5	44	G	50	--
	5	6	45	G	60	--
	6	7	46	I.M.	50	--
12	1	1	47	G	70	--
		2	48	G	70	--
	2	3	49	GM	0	BMA
	3	4	50	G	70	--
	4	5	51	I.M.	40	--
	5	6	52	G	80	--
	6	7	53	I.M.	50	--
	7	8	54	G	70	--
13	1	1	55	G	90	--
		2	56	G	90	--
	2	3	57	G	90	--
		4	58	G	90	--
	3	5	59	WM	0	BMA
		6	60	WM	0	MA
	4	7	61	WM	0	BMA
	5	8	62	WM	0	BMA
	6	9	63	I.M.	5	--
	7	10	64	I.M.	5	--

Supplemental Table 6. Continued

14	1	1	65	--	--	IA
	2	2	66	I.M.	30	--
	3	3	67	A.C.T.	100	--
		4	68	A.C.T.	100	--
	4	5	69	G	70	--
	5	6	70	G	90	--
	6	7	71	G	90	--
		8	72	G	90	--
15	1	1	73	G	75	--
		2	74	G	75	--
	2	3	75	G	75	--
		4	76	G	80	--
	3	5	77	G	80	--
	4	6	78	WM	10	--
		7	79	GM	20	--
	5	8	80	WM	10	--
	6	9	81	WM	15	--
16	1	1	82	I.M.	30	--
	2	2	83	G	70	--
	3	3	84	G	75	MA
	4	4	85	I.M.	30	MA
	5	5	86	I.M.	30	--
	6	6	87	I.M.	40	--
	7	7	88	I.M.	40	MA
17	1	1	89	G	70	--
	2	2	90	G	80	--
	3	3	91	WM	0	A
	4	4	92	G	50	--
18	1	1	93	G	50	BMA
		2	94	G	50	IA
	2	3	95	I.M.	10	MA
	3	4	96	I.M.	10	MA
	4	5	97	I.M.	20	--
		6	98	I.M.	10	--
	5	7	99	WM	0	A
		8	100	WM	0	MA

Supplemental Table 6. Continued

19	1	1	101	G	90	--
	2	2	102	G	40	IA
	3	3	103	G	90	--
		4	104	G	90	--
	4	5	105	G	90	--
	5	6	106	WM	5	--
		7	107	WM	5	--
	6	8	108	WM	30	--
	7	9	109	GM	5	--
		10	110	GM	5	--
20	1	1	111	G	70	--
		2	112	G	70	--
	2	3	113	G	70	--
		4	114	G	90	MA
	3	5	115	WM	5	--
	4	6	116	G	70	--
	5	7	117	WM	5	--
		8	118	WM	5	--
21	1	1	119	I.M.	20	MA
		2	120	G	50	--
	2	3	121	G	40	--
		4	122	G	80	A
	3	5	123	G	60	--
		6	124	G	70	MA
	4	7	125	G	40	--
		8	126	G	60	--
	5	9	127	I.M.	5	--
	6	10	128	I.M.	5	--
23	1	1	129	--	--	--
	2	2	130	G	90	--
		3	131	G	90	IA
	3	4	132	G	90	--
		5	133	G	90	--
	1	6	134	--	--	--
		7	135	--	--	--
	4	8	136	G	90	--
		9	137	G	90	--

Supplemental Table 6. Continued

24	1	1	138	G	70	--
		2	139	G	80	--
	2	3	140	G	80	--
25	1	1	141	G	60	--
	2	2	142	G	70	--
	3	3	143	G	80	--
		4	144	G	50	--
	4	5	145	G	90	--
	5	6	146	WM	5	--
	6	7	147	WM	5	--
	7	8	148	WM	5	--
	5	9	149	WM	5	--
	7	10	150	WM	5	--
26	1	1	151	G	80	--
	2	2	152	I.M.	10	--
	3	3	153	WM	5	--
	4	4	154	WM	10	--
	5	5	155	G	60	--
27	1	1	156	G	90	MA
		2	157	G	90	MA
	2	3	158	G	90	MA
	3	4	159	G	90	MA
		5	160	G	90	A
	4	6	161	G	90	MA
		7	162	G	90	MA
	5	8	163	G	90	MA
	6	9	164	G	90	A
	5	10	165	G	90	A
28	1	1	166	G	90	A
	2	2	167	I.M.	0	MA
	3	3	168	GM	10	A
		4	169	GM	10	A
	4	5	170	G	50	MA
		6	171	G	60	MA
	5	7	172	GM	20	A
		8	173	GM	0	A

Supplemental Table 6. Continued

29	1	1	174	--	--	--
		2	175	G	60	MA
	2	3	176	I.M.	20	MA
		4	177	I.M.	20	BMA
	3	5	178	WM	0	BMA
	4	6	179	WM	10	MA
	5	7	180	WM	10	MA
	6	8	181	WM	10	A
		9	182	WM	10	A
		10	183	WM	10	MA
30	1	1	184	I.M.	30	MA
		2	185	I.M.	15	MA
	2	3	186	WM	5	A
	3	4	187	WM	5	A
	4	5	188	WM	5	A
	5	6	189	GM	10	A
31	1	1	190	G	90	A
		2	191	G	90	A
32	2	3	192	G	90	MA
	3	4	193	WM	15	MA
	4	5	194	--	--	--
	5	6	195	G	50	MA
	1	1	196	GM	10	A
	2	2	197	GM	10	MA
		3	198	GM	10	A
	3	4	199	WM	5	A
	4	5	200	WM	5	A
	5	6	201	I.M.	5	MA
		7	202	I.M.	5	MA
33	1	1	203	GM	10	A
	2	2	204	GM	10	A
	3	3	205	G	60	MA
	4	4	206	G	60	MA
	5	5	207	G	--	IA
	6	6	208	I.M.	30	MA

Supplemental Table 6. Continued

34	1	1	209	GM	10	A
		2	210	GM	10	A
	2	3	211	G	70	A
		4	212	G	70	MA
	3	5	213	G	70	MA
		6	214	G	70	MA
35	1	1	215	WM	10	A
	2	2	216	G	90	A
	3	3	217	WM	10	A
	4	4	218	WM	10	A
	5	5	219	WM	0	MA
	6	6	220	WM	0	MA
37	1	1	221	G	90	MA
		2	222	G	70	MA
	2	3	223	G	80	A
	3	4	224	G	80	A
	4	5	225	G	80	A
38	1	1	226	G	80	MA
	2	2	227	G	80	A
	3	3	228	WM	30	A
	4	4	229	GM	20	MA
	5	5	230	WM	0	MA
	6	6	231	I.M.	10	MA
39	1	1	232	I.M.	0	A
	2	2	233	WM	0	A
	3	3	234	WM	0	A
40	1	1	235	G	100	A
		2	236	G	100	A
	2	3	237	G	100	A
		4	238	G	100	A
	3	5	239	WM	20	MA
		6	240	WM	20	MA
41	1	1	241	WM	0	A
	2	2	242	WM	0	A
	3	3	243	GM	0	A
	4	4	244	I.M.	5	A
	5	5	245	I.M.	5	MA
42	1	1	246	G	0	A
	2	2	247	G	80	A
	3	3	248	--	--	IA

Supplemental Table 6. Continued

44	1	1	249	G	100	A
		2	250	G	100	A
		3	251	G	100	A
	2	4	252	G	100	A
	3	5	253	G	100	A
		6	254	G	100	A
	4	7	255	G	100	A
	5	8	256	GM	0	A
	6	9	257	GM	60	A
		10	258	GM	0	A
45	1	1	259	T	100	A
46	1	1	260	G	60	MA
		2	261	G	80	A
	2	3	262	G	80	A
	3	4	263	G	60	A
		5	264	G	80	A
		6	265	G	80	A
	4	7	266	G	60	A
	5	8	267	I.M.	0	A
	6	9	268	WM	5	A
47	1	1	269	WM	20	MA
		2	270	WM	30	A
	2	3	271	G	100	A
		4	272	G	100	A
	3	5	273	I.M.	50	A
		6	274	I.M.	50	A
	4	7	275	WM	40	A
		8	276	WM	40	A
48	1	1	277	GM	10	A
		2	278	GM	0	A
	2	3	279	WM	60	A
		4	280	G	50	A
	3	5	281	GM	50	MA
		6	282	G	50	A
		7	283	G	70	A

Supplemental Table 6. Continued

50	1	1	284	WM	20	MA
	2	2	285	GM	15	A
	3	3	286	WM	20	MA
	4	4	287	GM	0	A
	5	5	288	WM	50	MA
	6	6	289	WM	50	MA
51	1	1	290	G	100	A
	2	2	291	T	100	A
	3	3	292	T	100	A
	4	4	293	T	100	A
	5	5	294	T	100	A
	6	6	295	T	100	A
52	1	1	296	GM	20	MA
	2	2	297	I.M.	30	--
	3	3	298	WM	--	IA
		4	299	I.M.	40	MA
53	1	1	300	WM	10	MA
	2	2	301	GM	10	MA
		3	302	GM	10	A
	3	4	303	I.M.	20	A
		5	304	GM	40	A
	4	6	305	WM	30	MA
	5	7	306	WM	30	MA
	6	8	307	GM	0	MA
		9	308	GM	0	A
54	1	1	309	--	--	IA
		2	310	--	--	IA
	2	3	311	GM	10	MA
		4	312	GM	10	MA
	3	5	313	WM	40	MA
		6	314	G	60	MA
		7	315	G	70	MA
55	1	1	316	G	100	A
	2	2	317	WM	10	A
	3	3	318	--	--	IA
	4	4	319	GM	30	A
56	1	1	320	G	100	MA
	2	2	321	G	100	MA
		3	322	G	100	A
		4	323	G	100	A

Supplemental Table 6. Continued

57	1	1	324	G	80	A
	2	2	325	G	80	A
		3	326	G	80	MA
		4	327	G	80	A
58	1	1	328	G	80	A
		2	329	G	50	MA
	2	3	330	I.M.	10	A
	3	4	331	WM	0	A
	4	5	332	WM	0	A
	5	6	333	WM	0	A
	6	7	334	WM	0	A

G = Glioma
WM = White matter
GM = Grey Matter
A = Adequate
MA = Marginally adequate
BMA = Barely marginally adequate
IA = Inadequate
Tissue smears with inadequate quality (IA) were not evaluated by EMH. The Smear Quality datapoint was not evaluated for all of the smears in the study; Smear Quality cells with hyphens indicate that smear was not evaluated for smear quality.

Supplemental Table 7. Confusion matrix showing correlation between histopathology and PCA-LDA cross-validation predictions of TCP category using the lipid profiles obtained from the n=272 intraoperative tissue smears. Four principal components and five cross-validation groups were used.

		Histopathology TCP		
		Low	High	Total
PCA-LDA TCP Prediction	Low	108	13	121
	High	45	106	151
	Total	153	119	272
	Sensitivity	70.6%		
	Specificity	89.6%		
	Accuracy	78.6%		

Supplemental Table 8. Confusion matrix showing correlation between histopathology and PCA-LDA cross-validation predictions of TCP category using the metabolite profiles obtained from the n=272 intraoperative tissue smears. Six principal components and five cross-validation groups were used.

		Histopathology TCP		
		Low	High	Total
PCA-LDA TCP Prediction	Low	127	42	169
	High	26	77	103
	Total	153	119	272
	Sensitivity	83.0%		
	Specificity	64.7%		
	Accuracy	75.0%		

Supplemental Table 9. Confusion matrix comparing DESI and histopathology TCP predictions for the grade I-III margin biopsies.

		Histopathology[a]	
		High TCP/Glioma[b]	Low TCP/Infiltrative Margin[b]
Full-scan NAA Intensity TCP Estimate	High TCP/Glioma[b]	10	9
Lipid Deconvolution TCP Estimate		5	6
Full-scan NAA Intensity TCP Estimate	Low TCP/Infiltrative Margin[b]	0	26
Lipid Deconvolution TCP Estimate		5	29

Full-scan NAA Intensity TCP Estimate	**Lipid Deconvolution TCP Estimate:**
• Sensitivity: 100%	• Sensitivity: 50%
• Specificity: 74%	• Specificity: 83%
• Accuracy: 80%	• Accuracy: 76%

[a]Histopathology assessments of TCP were used for correlation with lipid deconvolution and NAA intensity classifications of TCP.
[b]For lipid deconvolution and NAA intensity classification, histopathology TCP categories were High ≥51% and Low ≤50%.

Supplemental Table 10. Confusion matrix comparing DESI and histopathology TCP predictions for the grade IV margin biopsies.

		Histopathology[a]	
		High TCP/Glioma[b]	Low TCP/Infiltrative Margin[b]
Full-scan NAA Intensity TCP Estimate	High TCP/Glioma[b]	12	0
Lipid Deconvolution TCP Estimate		8	0
Full-scan NAA Intensity TCP Estimate	Low TCP/Infiltrative Margin[b]	1	19
Lipid Deconvolution TCP Estimate		5	19

Full-scan NAA Intensity TCP Estimate	**Lipid Deconvolution TCP Estimate:**
• Sensitivity: 92%	• Sensitivity: 62%
• Specificity: 100%	• Specificity: 100%
• Accuracy: 97%	• Accuracy: 84%

[a]Histopathology assessments of TCP were used for correlation with lipid deconvolution and NAA intensity classifications of TCP.
[b]For lipid deconvolution and NAA intensity classification, histopathology TCP categories were High ≥51% and Low ≤50%.