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Gliomatosis cerebri (GC) growth pattern: A single-center analysis of clinical, histological, and molecular characteristics of GC and non-GC glioblastoma

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Abstract

Background. The biological understanding of glioblastoma (GB) with gliomatosis cerebri (GC) pattern is poor due to the absence of GC-specific studies. Here, we aimed to identify molecular or clinical parameters that drive GC growth.

Methods. From our methylome database of IDH (isocitrate dehydrogenase)-wildtype GB, we identified 158 non-GC and 65 GC cases. GC cases were subdivided into diffuse-infiltrative (subtype 1), multifocal (subtype 2), or tumors with 1 solid mass (subtype 3). We compared clinical, histological, and molecular parameters and conducted a reference-free tumor deconvolution of DNA methylation data based on latent methylation components (LMC).

Results. GC subtype 1 less frequently showed contrast-enhancing tumors, and more frequently lacked morphological GB criteria despite displaying GB DNA methylation profile. However, the tumor deconvolution did not deliver a specific LMC cluster for either of the GC subtypes. Employing the reference-based analysis MethylCIBERSORT, we did not identify significant differences in tumor cell composition. The majority of both GC and non-GC patients received radiochemotherapy as first-line treatment, but there was a major imbalance for resection. The entire GC cohort had significantly shorter overall survival (OS) and time to treatment failure (TTF) than the non-GC cohort. However, when filtering for cases in which only stereotactic biopsy was performed, the comparison of OS and TTF lost statistical significance.

Conclusions. Our study offers clinically relevant information by demonstrating a similar outcome for GB with GC growth pattern in the surgically matched analysis. The limited number of cases in the GC subgroups encourages the validation of our DNA methylation analysis in larger cohorts.

Key Points

- In treatment-matched IDH-wildtype glioblastoma, gliomatosis cerebri is not associated with worse overall survival.
- No differences in latent methylation patterns or tumor composition were found between GC and non-GC cases.

Importance of the Study

This study demonstrates a similar outcome for glioblastoma with gliomatosis cerebri growth pattern in treatment-matched cohorts. No distinct pattern in latent methylation components or tumor composition was identified for GC glioblastoma.

The term gliomatosis cerebri (GC) describes infiltrative glioma growth, expanding over 3 or more cerebral lobes. Despite its distinct radiological features, studies have failed to identify histopathological or molecular patterns that could establish GC as a glioma subgroup.[1] Consequently, GC was erased from the WHO classification of CNS tumors.[2] However, from a clinical point of view, the presence of GC growth pattern remains particularly challenging, as the wide extent of tissue infiltration often limits essential therapeutic options, in particular surgery and focal radiotherapy.[3] This, along with biological attributes that drive the GC growth pattern, may contribute to the worse OS of patients with GC compared to patients with astrocytic non-GC tumors of corresponding WHO grade.[4,5] In a recent study of WHO grade II/III glioma, we showed worse OS for WHO grade II/III glioma with GC pattern. This was partly due to imbalances in first-line treatment.[5] Of note, our analysis showed worse OS for GC patients who received radiotherapy. These findings stand in contrast to clinical trials that previously confirmed the beneficial role of combined radiochemotherapy in low-grade gliomas[6,7] and underline the need for GC-specific analyses to help to evaluate the safety and effectiveness of current adjuvant treatment options for GC gliomas.

With regard to glioblastoma (GB), studies on cases with GC growth patterns are sparse and are often designed to identify prognostic factors or molecular subgroups associated with the presence of GC.[1,8–10] To the best of our knowledge, studies combining both contemporary molecular markers and clinical data have not yet been conducted. Hence, the aim of our study was to fill that gap. In a retrospective analysis of IDH-wildtype glioblastoma from our epigenetically characterized cohort, we collected radiological and clinical data aiming to identify clinicopathological parameters that could help to: (i) understand the biological factors that drive the diffuse tumor extension, (ii) guide treatment for this challenging radiological GB subgroup.

Materials and Methods

Patient Cohort and Data Acquisition

The study, clinical data collection, histological, immunohistochemical, and molecular pathological analyses were approved by the Institutional Review Boards of the University Cancer Center Frankfurt (UCT) and the Ethical Committee at the University Hospital Frankfurt (project number SNO-02-2017). Between January 2017 and July 2021, 451 glioma samples were subjected to human methylation EPIC array at the Institute of Neurology of the Goethe University Frankfurt, Germany. At our brain tumor center, all cases of diffuse gliomas undergo methylome profiling. Clinical data were collected by an experienced clinician (I.D.). The KPS score was assessed from the neurological examination as documented prior to treatment initiation. We defined OS as the time from the time of biopsy or surgical resection to death from any cause, estimated by the Kaplan–Meier method. As comprehensive follow-up MRI was not available for all-patients, we used time to treatment failure (TTF) as a surrogate parameter for PFS. TTF was defined as the interval from the initiation of one therapy to the initiation of any following therapy, or death from any cause. Patients who did not reach an endpoint were censored to the date of last contact.

Radiological Assessment

Of the resulting cases, tumor extension and the presence of contrast enhancement were evaluated independently by 3 investigators (E.S., E.H., and M.W.) by reviewing the MRI scan prior to biopsy/resection. In case of disagreement, images were discussed in detail to reach a consensus. GC growth pattern was defined as tumor lesion affecting 3 or more cerebral lobes as detected by MRI on T2-weighted sequences. Of note, the cerebellum, brain stem, and basal ganglia were considered separate lobes.

To account for radiological heterogeneity within our GC cohort, we defined 3 GC subtypes (Figure 1). Tumors with diffuse-infiltrative, frequent bilateral growth were denominated subtype 1. Tumors with ≥2 noncontiguous lesions expanding over 3 lobes were termed "multifocal" (subtype 2). From the latter, we distinguished tumors displaying only 1 large, solid mass ("large tumor," subtype 3). Note that we opted for using the term "multifocal" as the vast majority of the subtype 2 cases showed anatomical continuity of the separate lesions. In contrast to that, the term "multicentric" is frequently used to describe distant lesions, often located in opposite hemispheres or separated by the tentorium.

Human Methylation EPIC Array

Punch biopsies from FFPE gliomas were collected and subjected to DNA isolation followed by bisulfite conversion. The samples were prepared for the Human Methylation EPIC array (Illumina, San Diego, USA) covering 850 000 CpG sites according to the manufacturer's recommendations. The O6-methyl guanin-DNA methyltransferase (MGMT) gene promoter methylation was investigated by use of the MGMT–STP27 algorithm provided by https://www.molecularneuropathology.org. Epigenetic molecular GB subclasses were determined by the brain tumor classifier version 11b4 provided by molecularneuropatholog.org. platform.

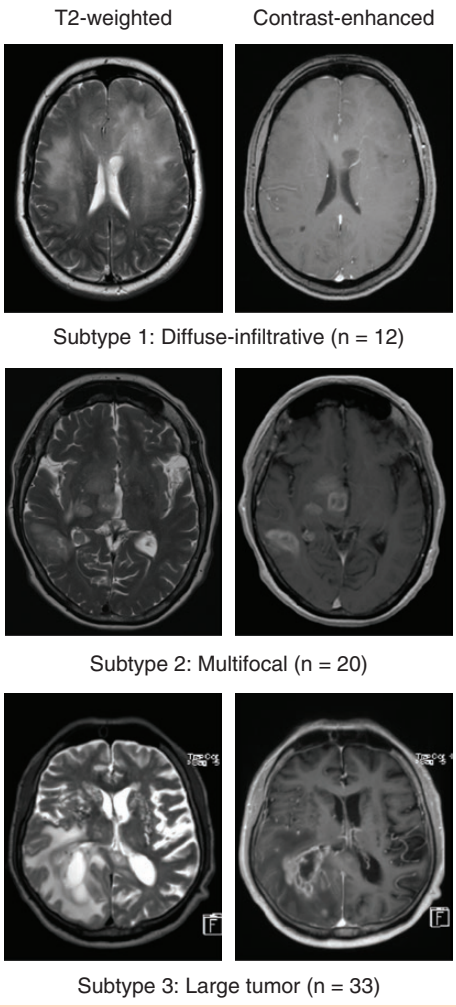

Figure 1. Subtype classification of gliomatosis cerebri (GC) cases. Representative MRI scans for GC subtype 1 (diffuse-infiltrative), 2 (multifocal), and 3 (large tumor).

Reference-Free MeDeCom Analysis

We investigated the DNA methylation data of the bulk tumor samples with the reference-free MeDeCom algorithm that dissects DNA methylation data into major components of variation, called latent methylation components (LMC).[11] DNA methylation data were processed according to a recently published protocol that selects the 5000 most variably methylated CpG sites across the samples as input to MeDeCom. Following investigation of the cross-validation error and of the objective value for the parameter number of LMCs (kappa) and the regularization parameter (lambda), LMCs resulted. Aiming to prevent a strong dependence of the clustering on the LMC with the highest proportion across the samples, we standardized the LMC proportions using *z*-scores. LMC proportion values were standardized by subtracting the respective column mean and dividing by the column SD. Standardization was performed for LMCs 1–6 of all samples collectively. For hierarchical cluster analysis, we used Ward's minimum variance method.

Reference-Based MethylCIBERSORT Algorithm

For a detailed deconvolution of the cellular composition of bulk GB samples, we applied the reference-based analysis MethylCIBERSORT that relies on DNA methylome-based reference data to identify distinct cellular contents (cancer cells, CD14-positive, CD19-positive, CD56-positive and CD8-positive cells, T-regulatory cells, CD4-positive effector cells, eosinophils, fibroblasts, and neutrophils). Methylation patterns of the sample of interest are compared with deposited cell-type-specific DNA methylomes. MethylCIBERSORT analysis was carried out according to the respective protocols.[12] Briefly, EPIC array IDAT sets were imported in R's "minfi" package to perform quality checks, Noob normalization, and acquisition of beta values. Using the "MethylCIBERSORT" R package, a mixture file was built whose matrix consisted of beta values for comparison to a reference matrix (provided by TRF). This reference file contained signature methylation beta values of defined cell types. After generating the mixture file, mixture, and reference files were uploaded onto the CIBERSORT portal and deconvoluted (provided by the Alizadeh Lab, Stanford University, USA, developed by Newman et al.[13]).

Statistical Analysis and Data Visualization

Statistical significance between 2 subgroups was calculated by univariate analysis using the log-rank (Mantel–Cox) test. A *P*-value of <.05 was considered statistically significant. Statistical analysis and data illustrations were performed with SPSS Statistics version 22.0 (IBM Corp., Armonk, NY, USA). Figures were prepared for publication with CorelDRAW version 21 (Corel, Ottawa, Ontario, Canada).

Results

Study Overview and Cohort Characteristics

From our 850 k methylome database ($n = 451$), we identified 230 cases of the methylation class GB with a calibrated score of 0.84 or higher (Figure 2), the average score being ≥0.95 in all subcohorts. By review of MRI prior to biopsy/resection, we identified 65 cases with GC growth pattern and 158 non-GC cases. As detailed above (Figure 1), the GC cohort was further subclassified into subtype 1 (diffuse-infiltrative, $n = 12$), subtype 2 (multifocal, $n = 20$), and subtype 3 (large tumor, $n = 33$).

In all cases, we collected comprehensive data on clinical, radiological, histological, and molecular parameters (Table 1). Between non-GC and GC cohorts, we found no significant imbalances with regard to age. Patients belonging to GC subtype 3 showed the lowest median KPS at diagnosis, and male patients were slightly overrepresented in this subgroup. GC subtype 1 stood out by showing the highest percentage of tumors lacking morphologic signs of malignancy. Furthermore, the presence of contrast enhancement was least frequently observed in this subgroup. With regard to MGMT promoter methylation, GC subtype 1 also

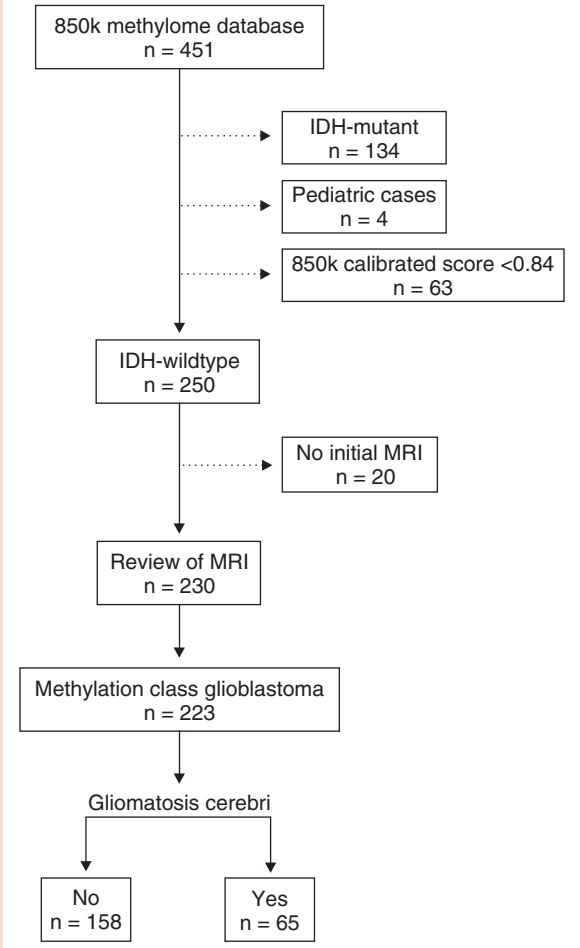

Figure 2. Consort diagram.

showed the highest percentage of unmethylated tumors. In contrast, MGMT methylation was present in roughly two-thirds of the subtype 3. However, due to the low numbers in some groups, these results need to be considered with caution.

It has been suggested that methylation subclasses correspond with growth patterns.[14,15] For this reason, we compared methylation subclasses of all cohorts using a calibrated score of ≥0.5 as a cutoff. The average score was 0.81 for non-GC, 0.76 for GC subtype 1, 0.78 for subtype 2, and 0.76 for subtype 3. Mesenchymal, RTK I, and RTK II were the predominating subclasses of non-GC, GC subtypes 2 and 3 tumors, whereas GC subtype 1 did not show enrichment for RTK II.

Latent Methylation Components and Tumor Cell Composition

Considering the distinct growth patterns of the 3 GC subgroups, we asked if these could originate from differences in tumor heterogeneity, and hypothesized that the diffuse-infiltrative GC subtype could contain more normal brain cells. Hence, we aimed to further decipher the DNA methylation patterns of GC tumors. We employed a reference-free deconvolution of large-scale DNA methylation data that relies on superordinate DNA methylation patterns described as latent methylation components (LMC).[11] Through this analysis, 6 LMCs were identified (Figure 3A). Importantly, no clusters were found to be specific for either of the GC subtypes in the hierarchical cluster analysis. As GB is a heterogenous tumor containing both neoplastic and non-neoplastic cells, such as glial cells, we proceeded to investigate the cellular composition of the GC tumors by using the reference-based analysis MethylCIBERSORT. With regard to tumor cells, neurons, fibroblasts, glia, or immune cells, we found no statistically significant differences in tumor cell composition among the 3 GC subtypes (Figure 3B).

Clinical Management of GC and Non-GC Glioblastoma

As stated above, the presence of GC complicates the clinical management of a given glioma entity as it may reduce the feasibility and/or safety of particular treatment options and, thus, negatively influence patient outcomes. Therefore, we analyzed the clinical data of the GC and non-GC cohort with regard to the first-line treatment. We found that radiochemotherapy was most frequently administered in all patient groups (Figure 4A). While in all groups a subset of patients received radiotherapy alone, treatment with chemotherapy alone was only observed in GC cases of subtypes 2 and 3. In those cases, chemotherapy was administered prior to radiotherapy with the aim of reducing tumor mass and to enabling involved field radiotherapy, thus reducing the irradiated brain volume, but treatment was discontinued due to clinical worsening. GC subtype 3 showed the highest percentage of cases in which no treatment occurred, possibly linked to the lower median KPS seen in this group due to the high tumor volume (see Table 1).

As the extent of resection of contrast-enhanced tumor has been shown to correlate with patient survival,[16] we investigated the extent of contrast-enhanced tumor resection for all-GC tumors (Figure 4B). Subtype 1 showed the highest percentage of complete resection of the contrast-enhanced tumor. In these cases ($n = 3$), cytoreductive surgery was performed to reduce tumor bulk.

Given the central role of gross total resection for OS, we proceeded to analyze how often surgical treatment was combined with chemotherapy and/or radiotherapy in the first-line treatment setting (Figure 4C). As expected, gross total resection was found only in the non-GC cohort. Moreover, widespread resection (>90%) was more frequently performed than in the GC cohorts. Here, stereotactic biopsy was the dominating surgical approach. Lastly, we compared chemotherapy protocols among the GC and non-GC cohorts (Figure 4C). While in all groups most patients received temozolomide, a combination of temozolomide with lomustine was not used for GC subtype 1. In this subgroup, only 3 cases showed MGMT methylation. In one case with MGMT methylation, lomustine was not given due to low platelet count prior to treatment initiation to avoid increased myelotoxicity. One

Table 1. Cohort characteristics

	NonGliomatosis (*n* = 158)		GC Subtype 1 Diffuse-Infiltrative (*n* = 12)		GC Subtype 2 Multifocal (*n* = 20)		GC Subtype 3 Large Tumor (*n* = 33)	
	n	%	*n*	%	*n*	%	*n*	%
Sex								
Male	94	60	8	67	10	50	23	70
Female	64	40	4	33	10	50	10	30
Age								
Median	65		62		65		69	
Range	25–86		38–82		34–85		51–85	
KPS								
Median	80		90		80		70	
Origin of tissue								
First diagnosis	148	94	12	100	19	95	33	100
Recurrence	10	6	–	–	1	5	–	–
Contrast medium enhancement								
No	4	3	2	17	3	15	1	3
Yes	154	97	10	83	17	85	32	97
Morphological criteria of GB								
No	5	3	4	33	2	10	4	12
Yes	152	96	8	67	17	85	29	88
n.n.	1	1			1	5		
Methylation subclass								
Mesenchymal	62	41	5	46	7	37	10	34
Midline	3	2	1	9	–		1	3
MYCN			–		1	5	–	
RTKI	34	23	4	36	5	26	7	23
RTKII	52	34	1	9	6	32	12	40
MGMT promoter								
Unmethylated	86	54	9	75	10	50	12	36
Methylated	71	45	3	25	10	50	21	64
n.n.	1	1						

case did not receive any treatment, and one case was lost to follow-up.

Treatment-Matched Analysis of OS and Time to Treatment Failure

Considering our previous findings on histopathological and clinical data, we asked whether the presence of GC influences the OS and TTF of glioblastoma patients. First, we compared all-GC cases versus the non-GC cohort (Figure 5A and B). We found that the GC cohort had a significantly shorter OS with a median of 246 days as opposed to 414 days in the non-GC group (log-rank test, $P = .011$) (Figure 5). Furthermore, the GC cohort showed shorter TTF (median TTF 225 vs. 332 days, log-rank test, $P = .024$) (Figure 5B). However, in the pairwise comparison of non-GC versus each GC subgroup, only GC subtype 3 (large tumor) showed significantly shorter OS (142 vs. 414 days, log-rank test, $P = .001$) (Supplementary Figure 1). GC subtype 1 had shorter OS (329 days vs. 414 days), but values did not reach significance ($P = .171$).

Regarding TTF, both subtypes 1 and 3 had significantly shorter TTF as opposed to the non-GC cohort (184 vs. 332 days, log-rank test, $P = .009$; 143 vs. 331 days, log-rank test, $P = .040$). Lastly, to account for the imbalances of surgical options mentioned above, we conducted a sub-analysis by filtering for cases in which no surgical treatment other than stereotactic biopsy was performed. In this treatment-matched comparison, the analysis lost statistical significance, both for OS (Figure 5C) as well as for TTF (Figure 5D).

Discussion

In this analysis of a contemporary cohort of patients with an epigenetic profiles of IDH-wildtype glioblastoma,

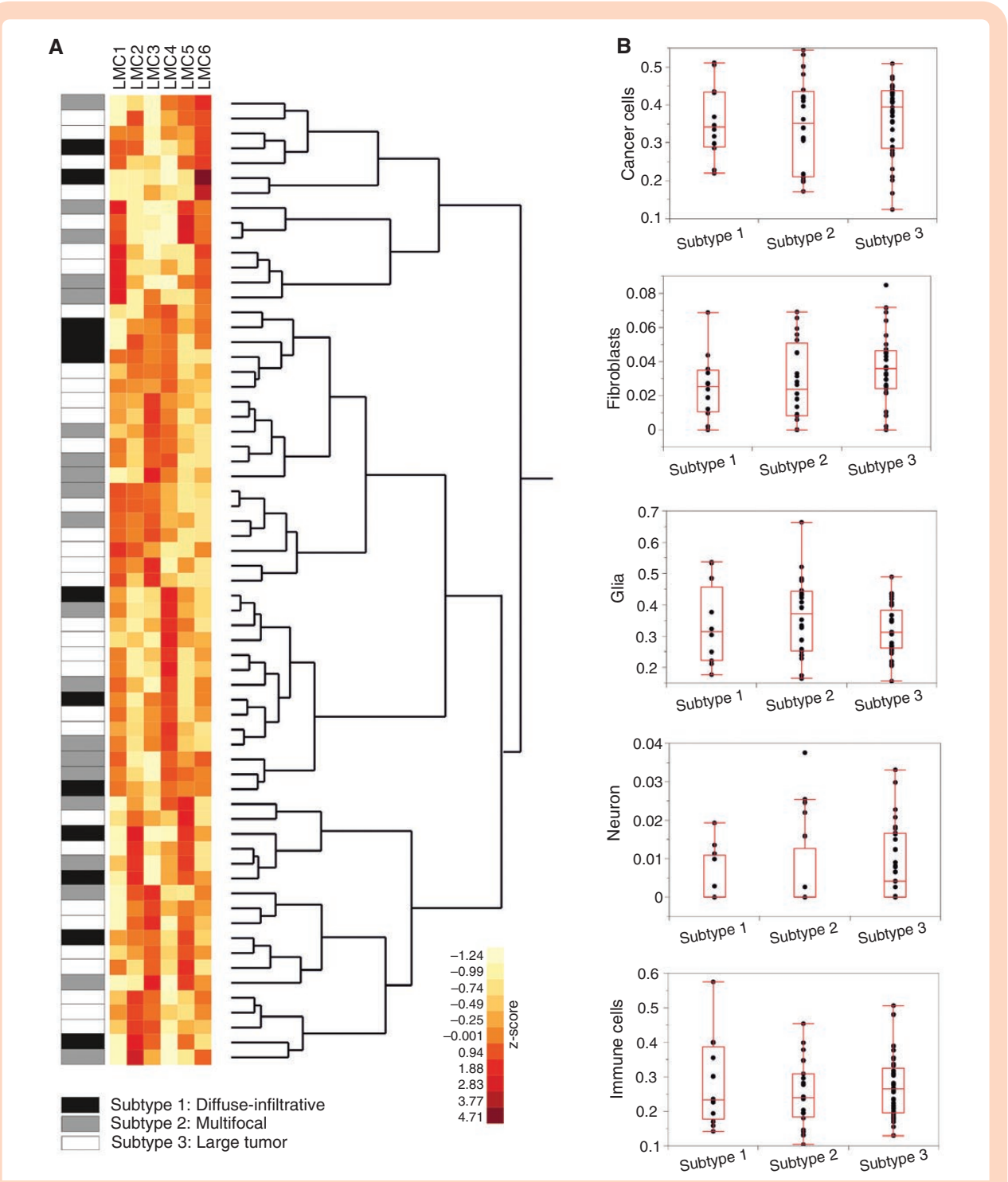

Figure 3. Analysis of latent methylation components and cellular components of the gliomatosis cerebri (GC) tumors. (A) A reference-free deconvolution of large-scale DNA methylation data for computation of latent methylation components (LMC) was used to search for clusters among the GC subtypes. (B) By using the reference-based MethylCIBERSORT analysis, we compared the GC subtypes with regard to their cellular components.

we compared key clinicopathological parameters of GC versus non-GC cases. First, in our comparison of histological and molecular features, we sought to identify parameters that may be correlated with the distinct GC growth patterns. We found that GC subtype 1 less frequently showed contrast-enhanced tumors, and more frequently lacked morphological GB criteria despite displaying GB DNA methylation pattern. This is of relevance as contrast

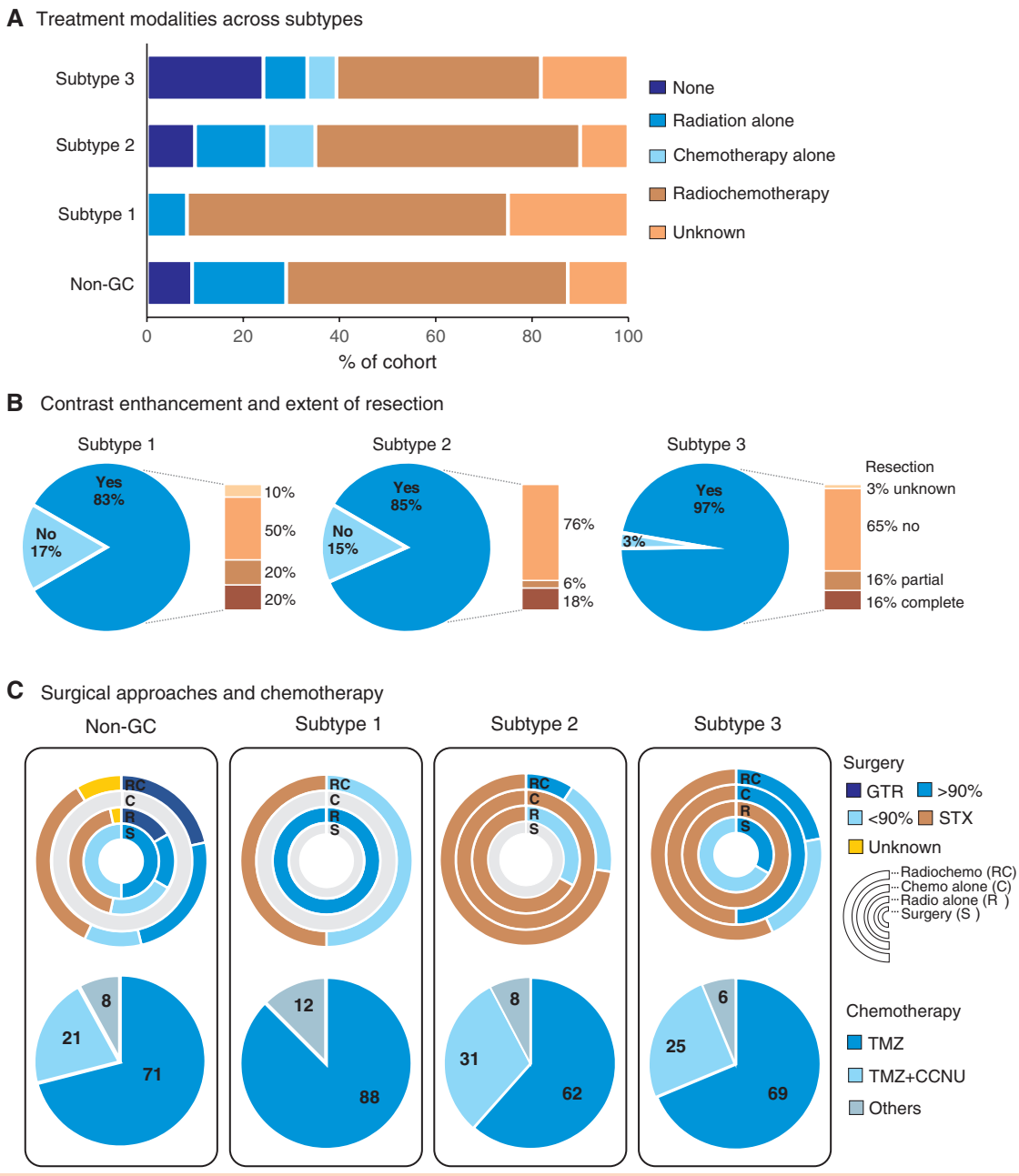

Figure 4. Distribution of first-line treatment options between GC and non-GC cases. (A) First-line adjuvant treatment included radiation, chemotherapy, or radiochemotherapy. For cases marked as unknown, no clinical data was available. Numbers are given as a percentage of the respective cohort. For absolute numbers, please refer to Supplementary Table 1. (B) Comparison of the presence of contrast enhancement (yes vs. no) among the 3 GC subtypes. Bar charts represent the extent of resection of the contrast-enhanced tumor. Numbers correspond to the percentage of the respective GC subtype. (C) Upper panel: Visualization of the use of surgical procedures (S) either alone (inner circle), or in combination with radiotherapy (R), chemotherapy (C), or radiochemotherapy (RC) (from inner to outer circle). Numbers are visualized as percentages of the respective cohort. Light grey circles are set as placeholders for null values. Lower panel: Frequency of first-line chemotherapy given as a percentage of the respective cohort.

enhancement has been shown to correspond to cellularity, necrosis, perfusion, and vascular alterations in glioblastoma.[17–19] Thus, the presence of contrast enhancement could be considered a surrogate parameter of locally altered metabolism, which, consequently, could translate into differences in tumor biology and, potentially, therapy resistance. In fact, GC subtype 1 had significantly shorter TTF compared to non-GC (Figure 5D). Interestingly, the CATNON trial has shown that GB without morphological features of GB do not benefit from the addition of temozolomide to radiotherapy,[20] underlining the potential relevance of these aspects for treatment guidance. We are

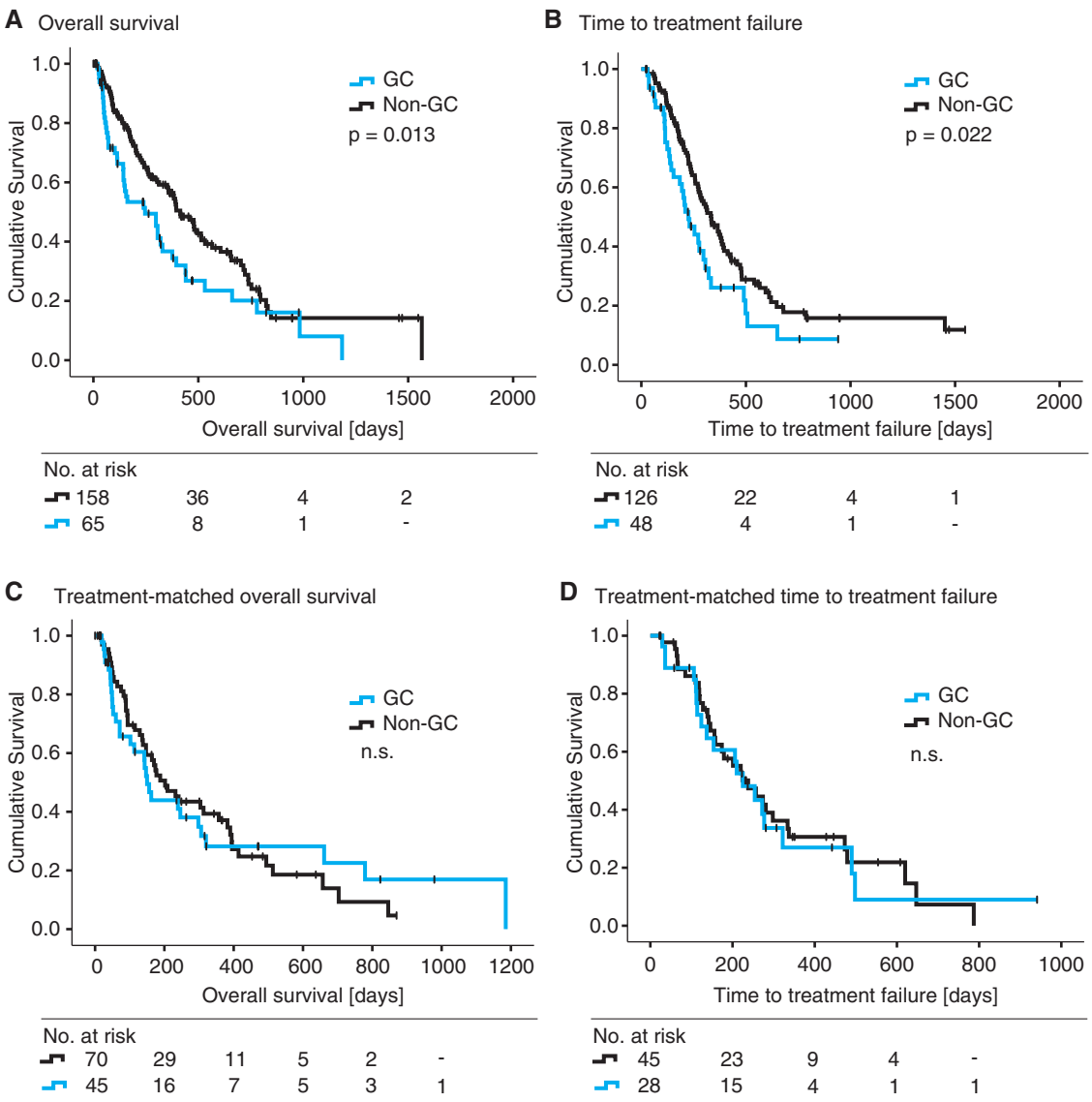

Figure 5. Impact of tumor extension on OS and TTF. (A and B) OS and TTF were calculated for the GC and non-GC cohorts regardless of the surgical method employed. (C and D) OS and TTF were calculated for cases without surgical resection (stereotactic biopsy only), independent of adjuvant treatment.

aware that due to tumor heterogeneity a sampling bias has to be taken into account.

In contrast to the histopathological findings, the comparison of DNA methylation pattern by the MeDeCom algorithm did not reveal a distinct signature for either GC subtype (Figure 3A). As cell type-specific patterns may not be represented by bulk-tissue DNA methylome, we additionally performed a computational tumor deconvolution. Comparing the proportions of the indicated cell fractions, we found no differences between the GC subtypes (Figure 3B). However, given the limited number of cases analyzed in our study, and in the GC subtype 1 cohort in particular, these data have to be interpreted with caution. Nevertheless, we believe that it is important to clarify which biological properties drive the diffuse-infiltrative growth pattern of GC subtype 1 in order to identify new therapeutic options. For this, multicentric studies appear necessary and should include additional analysis, such as mass spectrometry of the tumor proteome and mutational data, which were not available for our samples. Another interesting approach is the sampling of tumor tissue from more than one site in order to gain deeper knowledge of the infiltrative nature of GC.[3]

Second, regarding clinical management, we found that radiochemotherapy, the current standard of care for glioblastoma,[21] was the most frequent adjuvant treatment in all-cohorts (Figure 4A). This stands in contrast to our previous study on WHO grade II/III gliomas, in which we found major imbalances regarding adjuvant treatment, in particular the use of combined radiochemotherapy.

Interestingly, in the current study, the cohorts differed in that chemotherapy alone was only administered in GC cases (subtypes 2 and 3; Figure 4A) with the aim of reducing tumor mass prior to radiation. Unsurprisingly, surgical resections were much less frequently performed in the GC cohort, and that gross total resection was not achieved in any of the GC cases (Figure 4C). Our group has previously reported similar findings on low-grade glioma.[5]

Third, while the comparison of the total cohorts showed worse OS and treatment response for GC (Figure 5A and B), the values lost statistical significance in the treatment-matched analysis (Figure 4C and D). Of note, the treatment-matched cohorts both had a median KPS of 70. While it seems tempting to simply attribute the shorter OS of the GC cohort to the unfeasibility of surgery, previous studies have reported an ambivalent role of radiotherapy for glioma with GC growth pattern,[8,22,23] thus highlighting the need to re-evaluate adjuvant treatment for this radiological subgroup. Furthermore, due to the lack of prospective studies of molecularly well-defined cohorts, it is unclear if gliomas with GC growth patterns may be a target for other strategies, such as immune therapies or antiangiogenesis treatment.[24]

Conclusion

In summary, in our comprehensive clinicopathological comparison of GC and non-GC glioblastoma, we found no distinct methylation signature for GC tumors. However, GC subtype 1 stood out by less frequently matching histological GB criteria. As the underlying biology is poorly understood, the factors driving the diffuse, infiltrative growth remain unknown. Our study offers clinically relevant information by demonstrating worse OS and TTF for patients with GC pattern on the one hand, but also by showing similar outcomes for GC and non-GC cohorts with matching first-line treatment. Our findings underline the need for GC-specific evaluation of treatment options and strongly encourage their validation in a prospective, multicenter study design.

Supplementary material

Supplementary material is available online at *Neuro-Oncology* (https://academic.oup.com/neuro-oncology).

Keywords

glioblastoma | gliomatosis cerebri | overall survival | treatment

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Conflict of interest statement

J.P.S. has received honoraria for lectures or advisory board participation from Boehringer, Medac, Roche, Seagen, Med-Update, and Novocure. U.H. has received lecture and/or advisory board honoraria from Medac and Bayer. E. Fokas has received honoraria from Celgene, and trial funding support from AstraZeneca that are unrelated to the present study. All other authors declare that they have no conflict of interest.

Ethics approval

The study, clinical data collection, histological, immunohistochemical, and molecular pathological analyses were approved by the Institutional Review Boards of the University Cancer Center Frankfurt (UCT) and the Ethical Committee at the University Hospital Frankfurt (project no. SNO-02-2017). Patients registered in the UCT database signed informed consent regarding publishing their data in anonymized form.

Authorship

Conceptualization: I.D. and J.P.S; Data acquisition: I.D., E.S., E.H., M.W., K.J.W., M.W.R., P.N.H. and J.P.S.; Analysis and interpretation of data: all authors. Data visualization: I.D. and K.J.W. Writing of the original draft: I.D. Review and final editing of the manuscript: all authors.

Data availability

All data generated or analyzed during this study are included in anonymized form in this article. Further enquiries can be directed to the corresponding author.

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