Neuro-Oncology Advances

6(1), 1–13, 2024 | https://doi.org/10.1093/noajnl/vdae019 | Advance Access date 8 February 2024

Long-term survivors of glioblastoma: Tumor molecular, clinical, and imaging findings

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Abstract

Background. Glioblastoma (GBM) is the most aggressive primary brain malignancy with <45% living a year beyond diagnosis. Previously published investigations of long-term survivors (LTS) provided clinical data but rarely incorporated a comprehensive clinical and molecular analysis. Herein, we identify clinical, imaging, molecular, and outcome features for 23 GBM-LTS patients and compare them with a matched cohort of short-term survivors (STS).

Methods. Molecularly confirmed Isocitrate Dehydrogenase *(IDH)* wildtype GBM patients living ≥3 years post-diagnosis ($N_{LTS} = 23$) or <3 years ($N_{STS} = 75$) were identified from our Natural History study. Clinical and demographic characteristics were compared. Tumor tissue was analyzed with targeted next generation sequencing (NGS) ($N_{LTS} = 23$; $N_{STS} = 74$) and methylation analysis ($N_{LTS} = 18$; $N_{STS} = 28$). Pre-surgical MRI scans for a subset of LTS ($N = 14$) and STS control (N = 28) matched on sex, age, and extent of resection were analyzed.

Results. LTS tended to be younger. Diagnostic MRIs showed more LTS with T1 tumor hypointensity. LTS tumors were enriched for *MGMT*p methylation and tumor protein 53 (*TP53*) mutation. Three patients with classic GBM histology were reclassified based on NGS and methylation testing. Additionally, there were LTS with typical poor prognostic molecular markers.

Conclusions. Our findings emphasize that generalized predictions of prognosis are inaccurate for individual patients and underscore the need for complete clinical evaluation including molecular work-up to confirm the diagnosis. Continued accrual of patients to LTS registries that contain
comprehensive clinical, imaging, tumor molecular data, and outcomes measures may pro\vide important insights about individual patient prognosis.

Key Points

- Long-term survivors (LTS) show poor prognostic markers *MGMT*p unmethylated, mesenchymal subtype, and *TERT*p mutations.
- Next generation sequencing (NGS) and methylation are critical additions to histological review to confirm diagnosis.
- GBM-LTS studies should use a centralized definition for ease of comparison.

Importance of the Study

Previous long-term survivor (LTS) studies of patients with glioblastoma (GBM) did not incorporate the WHO 2021 criteria for diagnosis, tumor sequencing and methylation, or comprehensive clinical information for potential predictors of improved survival. Recent guidelines for GBM diagnosis integrate molecular testing which provides a more accurate determination of LTS-GBM by removing potential "contaminating" findings from patients harboring tumors that mimic the histology of GBM but have an improved prognosis (eg, *IDH* mutated grade 4 astrocytoma, anaplastic pleomorphic xanthoastrocytoma). Not only is this diagnostic accuracy important for research studies but is critical for patient treatment management and prognosis. Incorporation of the methylation classifier enabled the identification of 3 non-GBM patients in our LTS cohort who were classified as histological GBM. In addition to advanced molecular techniques, our study incorporates robust clinical data, patient reported outcomes and evaluated potential MRI characteristics as a biomarker of survival.

Glioblastoma (GBM) is the most common primary brain malignancy and comprises over 50% of all primary CNS tumors.[1] The median survival is reported to range from 12 to 15 months when patients are treated with the standard of care therapy, however population-based studies estimate a median survival of 8–10 months.[1,2] Despite their poor prognosis, around 7% of GBM patients live at least 5 years beyond their diagnosis, termed long-term survivor (LTS) in some studies.[1,3,4]

Depending on the study, GBM-LTS describes any patient that has survived from 2 to 10 years (or more) beyond their initial histological diagnosis. Inclusion criteria in these studies have typically relied on histological findings and have not usually incorporated molecular testing of these tumors. Part of this lack of molecular diagnostics relates to the evolution of the definition of GBM in the WHO guideline over the past 2 decades.[1,3,5] The most important changes have been the exclusion of tumors with Isocitrate dehydrogenase (*IDH)* mutation and/or 1p19q co-deletion and additional refinement of histological and molecular criteria.[5–8] Some studies of LTS do not test for and therefore have likely included *IDH* mutant (*IDH*-mt) astrocytoma or 1p19q co-del *IDH*-mt oligodendrogliomas,[4,9] diagnoses known to have better overall survival.[10] Additionally, the introduction of the methylation classifier has markedly improved diagnostic accuracy thereby providing a more definitive diagnosis where the histology or sequencing findings are inconclusive.[11,12]

We interrogated the National Cancer Institute (NCI) Center for Cancer Research (CCR) Neuro-Oncology Branch (NOB) Natural History Study (NHS) and uncovered 26 GBM-LTS, which we defined as ≥3 years of survival from initial diagnoses with a centrally confirmed histological diagnosis of GBM. This cutoff was based on the clinical relevance and larger number of studies with a similar definition. We utilized advanced molecular testing including next generation sequencing and tumor methylation to first restrict our analysis to *IDH*-wildtype (*IDH*-wt) GBM resulting in 23 confirmed cases. Clinical prognostic factors, common molecular mutations, MR imaging studies, and outcomes measures were reviewed to determine if there were features enriched in this cohort that could be examined in future studies to predict a LTS at the time of diagnosis.

Materials and Methods

Patients were identified from the NCI-NOB NHS (NCT02851706, PI: TS Armstrong), an IRB-approved protocol with written informed consent. The NCI-NOB-NHS is an observational protocol designed to collect clinical information from central nervous system (CNS) patients throughout their disease trajectory. Exclusion criteria include being <18 years of age. Patients with a histological diagnosis of GBM enrolled between September 2016 and May 2020 were retrospectively identified. Pre-surgical MRIs were obtained from the NIH imaging database and analyzed by 2 blinded clinicians (J.S., E.KP) based on preset criteria for commonly reviewed characteristics. Formalin-fixed paraffin-embedded (FFPE) tumor tissue was requested for molecular profiling using a targeted NGS panel, methylation classifier, O-6-methylguanine-DNA methyltransferase promoter methylation status, copy number variant (CNV) analysis, and 1p/19q co-deletion status.

LTS were defined as patients with histologically defined GBM living ≥3 years beyond their initial diagnosis ($N = 43$). Short-term survivors (STS) were defined as patients with histological GBM living <3 years ($N = 80$). Project eligibility was then refined by the availability of *IDH* status by NGS ($N_{LTS} = 23$, $N_{STS=}75$) and methylation classifier identification as GBM ($N_{LTS} = 18$, $N_{STS} = 28$). Samples without next-generation sequencing (NGS) or confirmation of *IDH*-wt status were excluded from the study as well as *IDH*-mt tumors. An additional analysis of clinical characteristics and variant profiling was done comparing STS living at most 18 months ($N = 49$) and LTS that lived at least 60 months ($N = 20$) from diagnosis (Supplementary Table 1).

AllPrep DNA/RNA FFPE kit (Qiagen) was used to extract genomic DNA (gDNA) and total RNA from 5 µm FFPE slides. cDNA was created using random hexamer reverse PCR Libraries were constructed using an Ampliseq custom panel of 858 DNA amplicons assessing 56 genes and 69 RNA primer pairs assessing 25 gene pairs (Supplementary Figure 1).This was used to assess variants in DNA, relevant gene fusions, and gain or loss of specific genes through full chromosome profiling. Sequencing was completed using the Ion S5™ XL Sequencing system. Signal processing,

base calling, and alignment to GRCH37/hg19 human genome assembly were done using Torrent Suite™ software. All variants were manually reviewed and called using Integrative Genomic Viewer and Ion Reporter Software v 5.0. Multiple bioinformatics databases were used for the complete interpretation of the variants.

For methylome analysis, gDNA was bisulfite converted using an EZ DNA methylation kit (Zymo Research), processed using an Infinium MethylationEPIC kit, and beadchip scanned on an iScan reader (Illumina). Signal processing, *MGMT* promoter methylation, and methylation-based classifier analysis was carried out using IDAT files uploaded to the DKFZ pipeline.[11] Chromosome 7 gain and chromosome 10 loss and zygosity of *CDKN2A* loss was determined through full chromosome methylation patterns.

Telomerase reverse transcriptase promoter *(TERTp)* C.1-124C >T (COSM1716558) and C.1-146C >T (COSM1716559) ddPCR assay used PrimePCR ddPCR *TERT* C228T_113 Assay/*TERT* C250T_113 Assay (BIO-RAD, Hercules, CA). Each reaction included 10 µl of 2× ddPCR supermix (no dUTP), 1 µl of *TERT*p mutant primers/probes mix (FAM/HEX), 0.5M Betaine, and 1 mM EDTA. All tests were performed in duplicate with 40–100 ng DNA input per reaction. Thermal cycling conditions followed manufacture protocol. Mutational analysis was performed on a BIO-RAD QX200 ddPCR system and fractional abundance of the mutant allele was determined with QuantaSoft v.1.7 (BIO-RAD).

For comparison of NGS results, the MSKCC genomic study was accessed and analyzed through www.cbioportal.org/study?id=glioma_mskcc_2019 (Supplementary Table 2).[13]

The "LTS-imaging" group ($N = 14$) was comprised of LTS with available pre-surgical diagnostic MRIs. A control group matched for sex, age and extent of resection ($N = 28$) was generated from the larger STS cohort. Imaging including T1, T1 post-contrast, T2, T2/FLAIR, DWI, and ADC were jointly reviewed by 2 blinded clinicians, 1 neuro-oncologist (EKP) 1 neuroradiologist (JS) and analyzed based on 13 characteristics defined as either present or absent by pre-defined criteria. These characteristics were (1) T1 white matter mass, (2) T1 intensity (hypointense, isointense, hyperintense, mixed intensity), (3) T1 central heterogeneous signal, (4) T1 post-contrast irregular enhancement, (5) T1 post-contrast peripheral enhancement, (6) T1 post-contrast gray matter enhancement, (7) number of lobes involved on T2/FLAIR, (8) T2/FLAIR intensity (hypointense, isointense, hyperintense, mixed intensity), (9) T2/FLAIR central heterogeneous signal, (10) T2/FLAIR mass effect, (11) T2/FLAIR perilesional signal abnormality and gray matter signal, (12) gray matter signal on T2/FLAIR (13) restricted diffusion (DWI vs ADC) (Supplemental Figure 2).

At the time of NHS entry, patients were asked to report their symptoms and duration of symptoms at presentation and were also administered a series of patient-reported outcomes (PROs) questionnaires to measure their symptom burden, mood, and general health status. The MDASI-BT instrument reports on the severity of 22 symptoms, and 6 measures of interference with daily life, experienced in the previous 24 h on a 0–10 severity scale, with 0 being "not present and 10 being "as bad as you can imagine."[14] PROMIS Depression 8a Short Form is a 8-item instrument that measures the severity of depression symptoms from 1 "never" to 5 "always" during the past 7 days.[15,16] Raw scores are converted to a *t*-score with *t*-scores greater than 1 SD (>60) indicating moderate-severe depression symptoms. Similarly, the PROMIS Anxiety Short Form 8a is an 8-item measure which rates anxiety symptoms from 1 to 5 with 1 being "never" and 5 being "always." Scoring is similar to the PROMIS Depression 8a. General health status was measured using the EQ-5D-3L, which assess 5 dimensions including mobility, self-care, usual activities, pain/discomfort, and anxiety/depression with each dimension having 3 levels to the dimensions: 1 or no problems, 2 or some problems, and 3 or extreme problems.[17]

Statistical Analysis

Clinical characteristics, molecular data, and imaging characteristics were summarized using descriptive statistics. Association between clinical, molecular, and imaging characteristics with survivorship was evaluated via chi-square or Fisher's exact tests of association with odds ratios (OR) and their 95% confidence intervals (95% CI) reported. Group differences were evaluated through independent samples *t*-tests. Significance was set at $P < .05$. Statistical tests were conducted on IBM SPSS Statistics.[18]

Results

Between September 2016 and May 2020, 123 patients with a histological diagnosis of GBM enrolled in the NOB-NHS (Figure 1). Forty-three patients had lived at least 3 years beyond their diagnosis, of which 26 were confirmed *IDH*-wt by NGS. Three were determined to not be GBM from methylation testing (tissue available for testing, $N = 21$) so were removed from the clinical analysis, resulting in 23 LTS. Of the 80 GBM patients that lived <3 years, 75 were confirmed *IDH*-wt and were termed short-term survivors (STS) and used as a comparison for clinical characteristics and development of control groups for imaging and molecular analysis. Trends seen in the larger cohorts were reflected in the "extreme" STS and LTS cohorts (Supplementary Table 1).

Clinical Characteristics

LTS patients were, on average, 5 years younger at diagnosis compared to STS, but the difference did not reach the established significance level (Table 1). The median overall survival was 45 months (range 37–186) for LTS and 16 months (range 6–32) for STS. More LTS were female (48% vs 28%; OR = 2.36, 95% CI: 0.90, 6.16, $P = .080$), did not have progression by imaging at the last visit (61% vs 32%; OR = 0.27, 95% CI: 0.10, 0.73, $P = .010$) and were not on treatment (74% vs 27%; OR = 0.15, 95% CI: 0.05, 0.45, $P = <.001$). STS tended to present more often at an emergency facility (71%, OR = 2.53, 95% CI: 0.83, 6.34, $P = .110$) while more LTS presented at outpatient clinics (39%). More LTS had frontal and parietal brain lobe involvement at presentation compared to STS (43% vs 31% and 43% vs 29%). Some STS

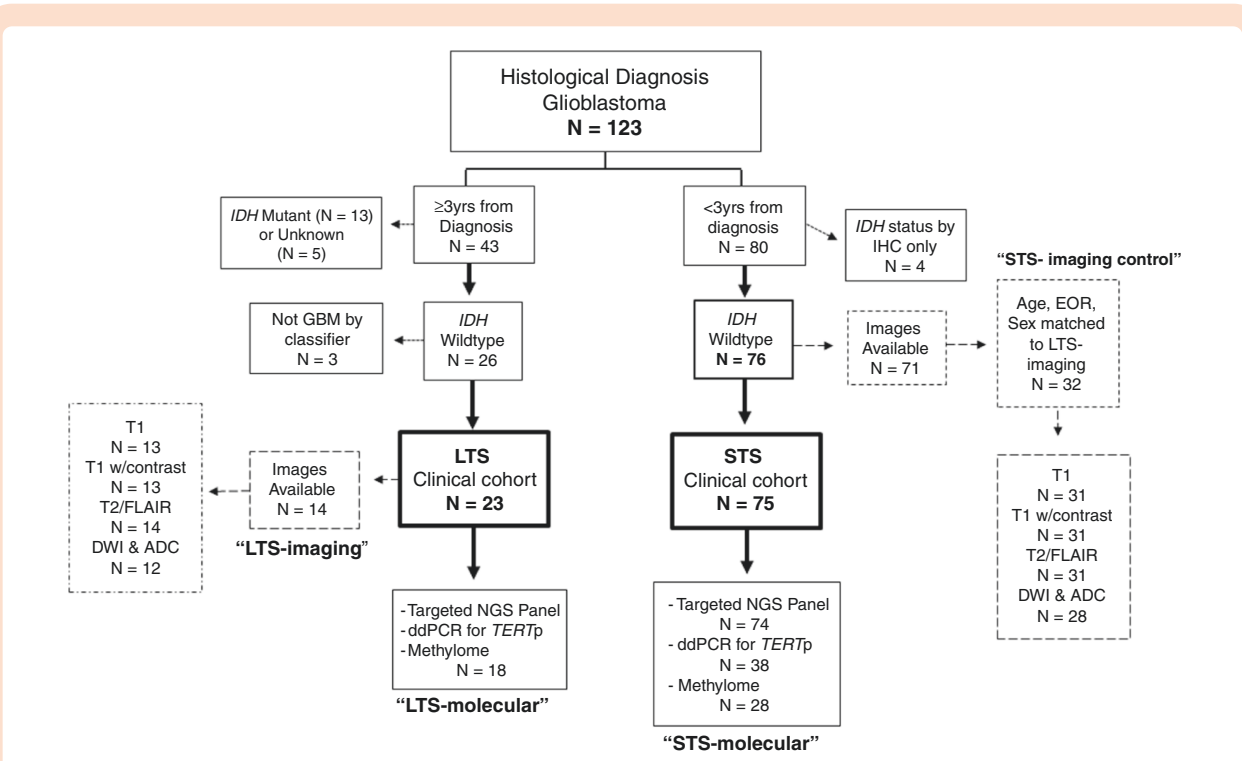

Figure 1. Group criteria for clinical, molecular and imaging evaluation. Patients were screened for histological diagnosis of glioblastoma and then length of survival from diagnosis. Those with *IDH* wildtype status confirmed by NGS living ≥3 years comprise the LTS group ($N = 23$), those <3 years the STS group ($N = 75$). Groups were further refined by methylation diagnosis for molecular analysis ($N_{LTS} = 18$, $N_{STS} = 28$). For imaging, the availability of diagnostic imaging was required for both groups ($N_{LTS} = 14$, $N_{STS} = 71$). The "STS-imaging control" group was age, sex, and extent of resection matched 2:1 to the "LTS-imaging group" ($N = 28$; extent $P = .342$, CV = 0.21, OR = 2.5 E).

tumors had tumor involvement of the occipital lobe, basal ganglia, and insula while no LTS presented with tumor in these brain regions. Though statistical significance was not reached, there was a trend toward more LTS having had a gross total tumor resection at initial diagnosis (57% vs 39%; OR = 2.06, 95% CI: 0.80, 5.31, $P = .134$) and received chemoradiation followed by maintenance temozolomide (Stupp protocol)[2] (87% vs 65%; OR = 2.52, 95% CI: 0.78, 8.19, $P = .124$). Recurrent tumor was confirmed in 83% of LTS with more than 1 surgery (Supplementary Table 3). Signs of treatment effect were found instead of recurrent tumor in the remaining patients.

Next Generation Sequencing Panel and CNV Analysis

For analysis of molecular results, we report on any surgery tissue as studies show the median number of mutations for pre/post-treatment tissue within patients does not change significantly.[19,20] However, if diagnostic or pre-treatment tissue was available this was prioritized for testing. In our LTS cohort, based on a targeted glioma gene panel (Supplementary Figure 1), the most commonly mutated gene was tumor protein 53 (*TP53*) at 43% (Table 2). *TERT*p mutation was present in 57%, Epidermal growth factor receptor (*EGFR*) amplification and *EGFR* fusion variant 3 (*EGFR*viii) was found in 43% and 14% of tumors, respectively. Retinoblastoma transcriptional co-repressor 1 (*RB1*) mutations were found in 3 cases and H3 histone family 3A (*H3F3A*) in 1. Homozygous or heterozygous cyclin dependent kinase inhibitor 2A (*CDKN2A*) deletion, measured using methylome pipeline, was present in 39% of cases assayed ($n = 18$). Full chromosome 7 gain with chromosome 10 loss, also measured by methylome was present in 61% of LTS ($n = 18$). The most common mutation in those with full gain/loss was *EGFR* copy number gain (55%) while most of those with partial or no loss had phosphatase and tensin homolog gene (*PTEN)* mutations (71%).

Molecular testing of tumors from the STS group revealed that the most common mutation was *TERT*p (92%) while B-Raf proto-oncogene serine/threonine kinase (*BRAF)* and MutS homolog 6 (*MSH6)* were found in 1 case each. Copy number alterations were present in 49% of tumors; *CDKN2A* loss in 57% ($n = 27$) and *EGFR* amplification in 34%. Although only a limited number of gene fusions were evaluated (Supplementary Figure 1) 1 of these fusions was found in 24% ($n = 74$) of cases, specifically *EGFRviii* in 15% and fibroblast growth factor and transforming acidic coiled-coil containing protein 3 gene fusion (*FGFR-TACC3)* in 7%. Chromosome 7 gain with 10 loss was seen in 89% of STS ($n = 28$) with only 1 having neither characteristic and 2 with partial 10 loss only.

Patients whose tumor was positive for *TERT*p were less likely to be LTS compared to those whose tumor was

Table 1. Clinical Characteristics Collected Through Natural History Study

Demographics		LTS	STS	OR [95%] P-value	LTS- Imaging	STS- Imaging (control)	LTS- Methylation	STS- Methylation
		N = 23	*N* = 75		*N* = 14	*N* = 28	*N* = 18	*N* = 28
Age at diagnosis[a]	Average, median (range)	50, 53 (20–69)	56, 58 (28–78)	– *P* = .037*	54, 58 (23–69)	54, 58 (28–68)	52, 56 (20–69)	54, 57 (28–69)
		n (%)	*n* (%)		*n* (%)	*n* (%)	*n* (%)	*n* (%)
Sex	Male	12 (52)	54 (72)	1.00	7 (50)	14 (50)	11 (61)	8 (29)
	Female	11 (48)	21 (28)	2.36 [0.90, 6.16]	7 (50)	14 (50)	7 (39)	20 (71)
Race	White	17 (74)	60 (80)	0.57 [0.19, 1.73]	12 (86)	23 (82)	13 (87)	22 (79)
	African American/Black	3 (13)	5 (7)	1.00	–	2 (7)	2 (11)	2 (7)
	Asian	3 (13)	5 (7)	1.00	2 (14)	2 (7)	3 (17)	2 (7)
	Other	-	2 (2)	1.00				
	Unknown	-	3 (4)	--	–	1 (4)	–	2 (7)
Clinical characteristics								
Vital status (as of Feb 2021)	Alive	10 (43)	–	–	6 (43)	–	10 (56)	-
	Dead	11 (48)	75 (100)	–	7 (50)	28 (100)	7 (39)	28 (100)
	Lost to follow-up	2 (9)	–	–	1 (7)	–	1 (6)	-
Institution type at presentation	ER	13 (57)	53 (71)	1.00				
	Outpatient clinic	9 (39)	16 (21)	2.53 [0.83, 6.34]				
	Not known	1 (4)	6 (8)	–				
Overall survival (months)	Average, median (range)	71, 45 (37–186)	17, 16 (6–32)		57, 47 (40–90)	17, 17 (6-32)	76, 51 (40-189)	25, 24 (18-37)
Extent of resection	Biopsy	1 (4)	12 (16)	1.00	1 (7)	2 (7)	1 (6)	6 (21)
	Subtotal resection	6 (26)	34 (45)	1.00	4 (29)	12 (43)	4 (27)	11 (39)
	Gross total resection	13 (57)	29 (39)	2.06 [0.80, 5.31]	9 (64)	14 (50)	10 (56)	11 (29)
	Resection, NOS	3 (13)	–	1.00	–	–	3 (17)	–
Number of surgeries	1	8 (35)	45 (60)	–	5 (36)	20 (71)	7 (39)	14 (50)
	2	10 (43)	25 (33)	–	6 (43)	7 (25)	6 (33)	10 (36)
	≥3	5 (22)	5 (7)	–	3 (21)	1 (4)	5 (28)	4 (14)
Number of radiation treatments	0	–	3 (4)	–	–	1 (4)	–	2 (7)
	1	14 (61)	59 (78)	–	11 (79)	20 (71)	12 (67)	18 (64)
	≥2	8 (35)	14 (18)	–	3 (21)	7 (25)	6 (33)	8 (29)
Time from 1st surgery to start of radiation (weeks)	Mean (SD) min, max	*N* = 23 4.4 (2.0) 0, 9	*N* = 74 4.5 (1.8) 1, 10					
Number of treatments	0	–	11 (15)	–	–	3 (11)	–	2 (7)
	1	9 (39)	23 (31)	–	7 (50)	7 (25)	6 (33)	3 (11)
	2	6 (26)	17 (23)	–	4 (29)	8 (29)	5 (28)	5 (18)
	≥3	8 (35)	24 (32)	–	3 (21)	10 (36)	7 (39)	18 (64)
Number of recurrences	0	5 (22)	14 (19)	–	4 (29)	4 (14)	4 (27)	3 (11)
	1	5 (22)	32 (43)	–	5 (36)	12 (43)	5 (28)	9 (32)

Table 1. Continued

Demographics		LTS	STS	OR [95%] P-value	LTS- Imaging	STS- Imaging (control)	LTS- Methylation	STS- Methylation
		N = 23	*N* = 75		*N* = 14	*N* = 28	*N* = 18	*N* = 28
	2	7 (30)	14 (19)	–	4 (29)	6 (21)	4 (27)	6 (21)
	≥3	6 (26)	15 (20)	–	1 (7)	6 (21)	5 (28)	10 (36)
Received stupp protocol	Yes	20 (87)	49 (65)	2.52 [0.78, 8.19]	13 (93)	22 (79)	16 (89)	19 (68)
	No	3 (13)	26 (35)	1.00	1 (7)	6 (21)	2 (11)	9 (32)
Progression on last visit	Yes	8 (35)	51 (68)	0.27 [0.10, 0.73]* *P* = .010	5 (36)	18 (64)	5 (28)	19 (68)
	No	14 (61)	24 (32)	1.00	9 (64)	10 (36)	12 (67)	9 (32)
	Unknown	1 (4)	–	–		–	1 (6)	–
On treatment at last visit	Yes	6 (26)	46 (61)	0.15 [0.05, 0.45]* *P* < .001	2 (14)	17 (61)	4 (27)	20 (71)
	No	17 (74)	20 (27)	1.00	12 (86)	5 (18)	14 (78)	8 (29)
	Unknown	–	9 (12)	–		6 (21)	–	2 (7)
Brain lobe involved at presentation	Frontal	10 (43)	23 (31)	–				
	Temporal	11 (48)	35 (47)	–				
	Occipital	0 (0)	4 (5)	–				
	Parietal	10 (43)	22 (29)	–				
	Basal Ganglia	0 (0)	2 (3)	–				
	Insula	0 (0)	1 (1)	–				
	None provided	0 (0)	2 (3)	–				

Abbreviations: LTS = long-term survivor, STS = short-term survivor, OR = odds ratio, 95% CI = 95% confidence interval, Sig. = significance level.
*Significant at *P* < .05.
[a]Independent samples *t*-test.

negative (OR = 0.14, 95% CI: 0.04, 0.45, *P* < .001). Full chromosome 7 gain and full chromosome 10 loss was more likely in tumors in the STS group (OR = 0.20, 95% CI: 0.04, 0.91, *P* = .037). Though not statistically significant, the *TP53* variant was present in more LTS tumors compared to STS (OR = 1.82, 95% CI: 0.69, 4.77, *P* = .224).

To verify the relevance of these results, we compared our findings with those from a MSKCC study of genomic variants in glioma.[13] After filtering on similar inclusion criteria to our study, there were 63 LTS and 401 STS. Overall, the trends were comparable except they found *EGFR* amplification at a similar rate between the 2 groups (Supplementary Table 2).

Methylation Analysis: *MGMT* Promoter, CNS Classifier

The 850K Illumina methylation chip covers 23 CpGs of *MGMT*p, of which 2 are used to determine promoter methylation level based on an algorithm tested for correlation using expression data.[11,21] In our LTS cases, 89% of the samples were *MGMT*p methylated compared to 29% of control (OR = 20, 95% CI: 3.72, 107.66, *P* < .001, Table 2).

Methylation classifier matched a majority of LTS (53%) and STS (46%) to the RTKII subtype (Table 2). Through classifier and UMAP analysis, 2 cases were identified as Anaplastic Pleomorphic Xanthoastrocytoma and 1 as ganglioglioma. Three of the LTS (17%) did not match to any entity due to DNA quality (*n* = 1) or <50% tumor cells present in the sample (*n* = 2). One STS did not match due to low tumor content.

Imaging

Pre-diagnostic MRI analysis of T1 images revealed that LTS were more likely to have a hypointense lesion (OR = 9.52, 95% CI: 2.02, 44.91, *P* = .004, Table 3) while the control group more frequently had a lesion with mixed intensity on T1 images (Table 2). Central heterogeneity tended to be more common in the control group than in LTS patients. All lesions were located within white matter and the number of mean lobes involved on T2/FLAIR were 1.79 for both groups. Almost all lesions had irregular and peripheral enhancement on T1 post-contrast images. Gray matter involvement on either T1 post-contrast images or on T2/FLAIR images were slightly more commonly seen in the control group. T2/FLAIR hyperintensity was more common in LTS than in controls. No difference in the T2/FLAIR heterogenous signal

Table 2. Molecular Characteristics of LTS and STS-Molecular Group

Methylation Analysis		LTS (N = 18)	STS-Molecular (N = 28)	OR [95% CI] Sig.
		n (%)	***n*** (%)	
MGMT status	Methylated	16 (89)	8 (29)	20.00 [3.72–107.65]* $P < .001$
	Unmethylated	2 (11)	20 (72)	
Classifier category	Matched to entity	15 (83)	26 (96)	
	GBM, H3.3 G34 mutant	1 (7)	–	–
	GBM, midline	–	1 (4)	–
	GBM, RTK 1 (proneural)[a]	2 (13)	5 (19)	0.68 [0.11–4.00]
	GBM, RTK 2 (classic)[b]	8 (53)	12 (46)	1.43 [0.40–5.07]
	GBM, mesenchymal[c]	4 (27)	9 (33)	0.73 [0.18–2.94]
	No match	3 (17)	1(4)	
	DNA quality	1 (33)	–	
	<50% tumor	2 (67)	1 (100)	
CNV analysis	Chr7/Chr10			
	Full chr7 gain/Full chr10 loss	11 (61)	24 (89)	0.20 [0.04–0.91]* $P = .037$
	Partial chr7 gain/ Full chr10 loss	1 (6)	–	1.00
	Partial chr7 gain only	1 (6)	-	1.00
	Chr 10 loss only	4 (22)	2 (7)	1.00
	Neither	1 (6)	1 (4)	1.00
	Unknown		1	
	CDKN2A loss	7 (39)	$n = 27$ 16 (57)	0.44 [0.13-1.48]
	Homozygous loss	6 (33)	8 (25)	-
	Heterozygous loss	1 (11)	8 (32)	-
	No loss	11 (61)	11 (43)	1.00
Variant analysis				
		LTS *N* = 23	**STS molecular *N* = 74**	
		N (%)	*N* (%)	
	TP53 mt	10 (43)	22 (30)	1.82 [0.69, 4.77]
	*TERT*p mt	13 (57)	68 (92)	0.14 [0.04, 0.45]* $P < .001$
	EGFR amplification	10 (43) $N = 22$	25 (34)	1.63 [0.62, 4.30]
	EGFRviii	4 (14) $N = 22$	11 (15) $N = 73$	1.25 [0.36, 4.41]

Abbreviations: LTS = long-term survivor, STS = short-term survivor, OR = odds ratio, 95% CI = 95% confidence interval, Sig. = significance level.
*Significant at $P < .05$.
[a]Reference group is not RTK I subcategory.
[b]Reference group is not RTK II subcategory.
[c]Reference group is not mesenchymal subcategory.

was seen between the groups. Almost all lesions had a mass effect and perilesional signal abnormality on T2/FLAIR sequences. GRE and SWI images were also assessed but limited availability made analysis non-informative

LTS Study Comparison

Clinical and molecular characteristics from various LTS studies were compared where data were available (Table 4).[4,9,22–27] Most of the studies did not have a complete clinical and molecular work-up; 2 studies did not perform any NGS to verify 1p/19q or *IDH* status.[9] The importance of *IDH* sequencing is evident in the large cohort ETERNITY study where 30% of the histologically GBM-LTS tumors were reclassified to *IDH*-mt, so no longer a GBM by WHO 2021 definition.[27] Across these studies, a majority of LTS patients were white, had a gross total resection and received standard chemoradiation. Sex

Table 3: MRI characteristics for LTS and STS

		LTS-Imaging N = 14 n (%)	STS-Imaging Control N = 28 n (%)	OR [95% CI] Sig.
T1 white matter mass				
	Yes	13 (100)	27 (100)	-
	No	0	0	
	Unknown	1	1	
T1 hypointensity				
	Yes	10 (91)	7(30)	9.52 [2.02-44.91]* *P* = .004
	No (mixed)	3 (27)	20 (70)	1.00
	Unknown	3	1	-
T1 center heterogeneity				
	Yes	7 (54)	19 (73)	0.43 [0.11–1.73]
	No	6 (46)	7 (27)	1.00
	Unknown	1	2	--
T1 irregular enhancement				
	Yes	13 (100)	25 (93)	-
	No	0	2 (7)	
	Unknown	1	1	
T1 post-contrast peripheral enhancement				
	Yes	13 (100)	24 (89)	-
	No	0	3 (11)	
	Unknown	1	1	
T1 post-contrast gray matter enhancement				
	Yes	8 (62)	16 (59)	1.10 [0.28–4.27]
	No	5 (38)	11 (41)	1.00
	Unknown	1	1	
T2/FLAIR, #of lobes				-
	mean	1.79	1.79	
T2/FLAIR hyperintensity				
	Yes	4 (29)	4 (15)	2.30 [0.48–11.08]
	No (mixed)	10 (71)	23 (85)	1.00
	Unknown	0	1	
T2/FLAIR heterogeneity				
	Yes	12 (86)	24 (89)	0.75 [0.11–5.11]
	No	2 (14)	3 (11)	1.00
	Unknown	0	1	
T2/FLAIR mass effect				
	Yes	13 (93)	28 (100)	-
	No	1 (7)	0	
T2/FLAIR perilesional signal abnormality				
	Yes	13 (100)	22 (92)	-
	No	0	2 (8)	
	Unknown	1	4	
T2/FLAIR gray matter signal				
	Yes	11 (79)	21 (78)	1.05 [0.29–5.02]
	No	3 (21)	6 (22)	1.00
	Unknown	0	1	
Restrictive Diff				
	Yes	6 (67)	20 (80)	0.5 [0.09–2.73]
	No	3 (33)	5 (20)	1.00
	Unknown	5	3	

Abbreviations: LTS = long-term survivor, STS = short-term survivor, OR = odds ratio, 95% CI = 95% confidence interval, Sig. = significance level, T1=, T2 = (may not needed if they are accepted abbreviations for the journal).
*Significant at $P < .05$.

Table 4: Comparison of Clinical and Molecular Characteristics Between Similar Studies

	NOB-NHS	1	2	3	4	5	6	7	TCGA
Year published	2022	2014	2019	2020	2016	2018	2023	2023	2013
N	23	7	12	8757	8	29 LTS	16	189	543
Study cohort	NIH NOB	MSKCC	Mayo	NCDB	OBTS	HVS	Belgium	International	TCGA
Dates used	2016–2020	1995–2008	1995–2013	2004–2013	N/A	2000–2015	2013–2018	2005–2014	1989–2011
WHO definition	2021	2007	2016	Multiple	unclear	2007	2021	2016	multiple
LTS definition	≥3 yrs	>2 yrs	≥5 yrs	≥3 yrs	≥33 mo	>36 mo	≥3 yrs	≥5 yrs	N/A
	Clinical Characteristics								
Age (years) Mean (range)		57 (32–70)	48 (19–70)	55 (46–63)	64 (55–83)	51 (22–72)	58.2 (45–67)	56 (24–78)	58.4 (median)
	***n* (%)**	***n* (%)**	***n* (%)**	***n* (%)**	***n* (%)**	***n* (%)**	***n*(%)**	***n* (%)**	***n* (%)**
Female	11 (48)	2 (29)	7 (58)	3910 (44.6)	6 (75)	Not collected	7 (44)	96 (50.8)	209 (38.6)
White	17 (74)	Not collected	Not collected	7152 (82.8)	6 (75)	Not collected	Not collected	Not collected	Not collected
Gross total resection	13 (57)	6 (86)	7 (58)	Not collected	5 (63)	17 (57)	7 (44)	134 (76.6)	Not collected
Received Stupp Protocol	20 (87)	7 (100)	3 (27)	6298 (75.1)	8 (100)	23 (79)	16 (100)	130 (76.4) *N* = 170	217 (40)
Overall survival in months Mean, median (range)	71, 45 (37–186)	75.5, 62.5 (50.6–138.3)	Not reported	Not reported, 53.4 (43.1–72.9)	33, not reported (18–57)	Not reported, 62 (36–205)	Not reported, 52.8 (36–unclear)	Not reported, 108 (94.8–142.8)	Not reported, 12.2 (0–127)
	Molecular Characteristics								
Sequencing method	Targeted panel (56 genes)	Sanger/ microarray	Targeted panel (50 genes)	N/A	WES/ WGS	Targeted panel (50 genes)	*IDH* only (*N* = 36)	*IDH* only	WES/WGS
MGMTp unmethylated	2 (11) N = 18	2 (29)	0 (0)	283 (43.7)	3 (38)	13 (45)	2 (13)	48 (25.7)	174 (48.5) *N* = 359
*IDH*1/2 mutant	0 (0)	0 (0)	0 (0)	Not collected	0 (0)	5 (17)		189 (67.5) *N* = 280	28 (6.6) *N* = 423
TP53	10 (43)	–	4 (33)	–	2 (25)	11 (38)		–	70 (28) *N* = 251
*TERT*p	13 (57)	–	9 (75)	–	Not reported	15 (52)		–	21 (84) *N* = 25
EGFR amp	10 (43) *N* = 22	–	8 (64)	–	4 (50)	8 (28)		–	"Decreased frequencies"
*EGFR*viii	4 (14) *N* = 22	–	Not reported	–	Not reported	Not assessed		–	5 (11) *N* = 42

1. Gerber, et al. Transcriptional diversity of long-term glioblastoma survivors. Neuro Oncol. 2014; 16(9):1186–1195.
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Table 5: Patient Reported Outcomes at Presentation via Risk survey

		LTS	STS
		N **= 20** ***n*** **(%)**	***N*** **= 61** ***n*** **(%)**
Number of symptom categories reported by patient at presentation	1	4 (20)	18 (30)
	2 or more	16 (80)	39 (64)
Time with symptoms before presentation		***N*** **= 23**	***N*** **= 72**
	No symptoms	0 (0)	1 (1)
	<6 months	17 (74)	53 (71)
	6 months to 1 year	5 (22)	5 (7)
	>1 year	0 (0)	3 (4)
	Don't recall	1 (4)	10 (14)
Symptom category			
		N **= 20**	***N*** **= 61**
	Seizures	3 (15)	13 (23)
	Headaches	10 (50)	23 (38)
	Vision problems	1 (5)	6 (10)
	Hearing loss	1 (5)	1 (2)
	Taste/smell changes	1 (5)	3 (5)
	Facial numbness	0 (0)	4 (7)
	Memory problems/confusion	5 (25)	22 (36)
	Nausea/vomiting	4 (20)	4 (7)
	Personality/behavior changes	3 (15)	6 (10)
	Difficulty with balance/coordination/walking	6 (30)	15 (25)
	Speech difficulties	2 (10)	12 (20)
	Difficulty swallowing	2 (10)	1 (2)
	Weakness in arms or legs	5 (25)	12 (20)
	Sensory changes in arms or legs	2 (10)	9 (15)
	Fatigue	1 (5)	0 (0)
	Pain	0 (0)	1 (2)
	Dizziness/loss of consciousness	0 (0)	2 (3)
	Other	0 (0)	1 (2)
	No symptoms	0 (0)	2 (3)
	Don't recall	0 (0)	1 (2)

distribution varied among the studies with 2 of the 4 studies, with available data, reporting females as the majority of LTS. Four of the 6 compared LTS studies incorporated some type of NGS but varied from small gene panels to whole exome sequencing. Chehade et al. was the only study to include methylation classifier to analyze the LTS but did not incorporate any other molecular information.[24]

When compared to total GBM cases from The Cancer Genome Atlas (TCGA) total GBM cases, we found that our LTS group had a lower median age (54 yr vs 58 yr) and similar race distribution but a higher percentage of females (50% vs 39%, Table 5).[28] These limited data sets do raise the possibility that other factors may be important to consider but remain uncertain as they were not included in these analyses.

Patient Reported Outcomes

A majority of both STS and LTS reported 2 or more symptoms at presentation with these symptoms lasting <6 months (Table 5). However, STS more commonly could not recall how long symptoms lasted compared with LTS (14% and 4%). More STS presented with seizures, vision problems, facial numbness, memory problems/confusion, speech difficulties when compared to LTS. A larger proportion of LTS presented with headaches, nausea, behavior changes, difficulty with balance/coordination/walking and weakness in arms or legs. Data from the MDASI-BT at the time of study entry revealed that both groups had fatigue as their top moderate-severe rated symptom (LTS = 64%, STS = 53%, Supplementary Table 5). Overall mean interference was lower for LTS at 3.2 (SD = 2.7) compared to 4.0

(SD = 3.0) for STS. The highest symptom burden category was the cognitive factor (consisting of difficulty remembering, speaking, understanding and concentrating) for LTS (mean = 3.2, SD = 2.8, min = 0, max = 8) and affective factor (consisting of distress, fatigue, disturbed sleep, sadness, irritability) for STS (mean = 3.2, SD = 2.3) min = 0, max = 8.4).

General health status measured by EQ-5D-3L showed more LTS had some problems walking (Supplementary Table 6; LTS = 64%, STS = 50%), washing or dressing (LTS = 46%, STS = 31%) and were unable to perform usual activities (LTS = 32%, STS = 18%). Most LTS were not anxious or depressed (64%), which was also shown in the PROMIS Anxiety Short Form and PROMIS Depression Short Form results (Supplementary Table 7). LTS had lower scores for both measures (anxiety: LTS mean = 48.7, SD = 10.5, max = 69; STS mean = 51.6, SD = 10.6, max = 82.2; depression: LTS mean = 51.5, SD = 9.8, max = 69.7, STS mean = 51.5, SD = 9.6, max = 81.1) with 18% reporting moderate-severe anxiety or depression compared to 24% and 18% for STS, respectively.

Discussion

Glioblastoma is a devastating disease that has a severe impact on the patient's functional status as well as a poor prognosis. Nevertheless, there are patients who live long beyond the expected median survival.[4,9,22,24–28] Studies of LTS sought to uncover molecular or clinical indicators that would identify these patients at diagnosis, however, the variation in LTS definition, reporting of clinical data and extent of molecular testing made any comparison between studies challenging. Additionally, the diagnosis of GBM historically relied strictly on histological criteria of pseudopalisading necrosis, microvascular proliferation, and high mitotic activity, while the current definition incorporates molecular data requiring *IDH* wildtype status, lack of H3K27 or H3G34 mutation and intact 1p19q.[5–8] Many of the previous GBM-LTS studies have used outdated definitions and thus have data "contaminated" by patients who would now be diagnosed with tumors with better survival such as an *IDH* mutated tumor or even an oligodendroglioma.

In our study, we utilized our routine clinical pipeline to describe our GBM-LTS cohort, defined here as *IDH*-wt methylation classifier confirmed GBM patients that had lived at least 3 years beyond initial the diagnosis. This work builds on our prior work analyzing outcomes for NOB LTS across glioma diagnoses,[29] by adding clinical characteristics, pre-surgical MRI analysis, and comprehensive molecular testing to the outcomes data on our GBM-LTS. To support the critique on the variation among GBM-LTS studies, we compared similar studies to identify any common clinical and molecular parameters.

Incorporation of the methylation classifier into the clinical workflow has been shown to improve accuracy and even reclassify some cases.[11] In this study, we re-classified 3 of the initial 26 histologically defined GBM-LTS patient's tumors as anaplastic pleomorphic xanthroastrocytoma and ganglioglioma, 2 diagnoses that have very different treatment and survival rates from GBM. The inclusion of such cases in large LTS studies can mask the true characteristics of this group and complicate any proposed markers. Recently, Chehade et al. utilized the classifier to analyze their LTS cohort and found most were RTK I subtype (43%),[25] while our assayed cases were mostly RTK II (53%) or "classic," which is defined by *EGFR* alterations and *CDKN2A* loss. The mesenchymal subtype, which is typically associated with chemotherapy and radiation resistance,[30] was found in similar percentages in both studies (27% of our LTS, 29% of Chehade et al.). However, there have been studies showing that the mesenchymal subtype has an increased level of immune markers in their microenvironment[31] possibly helping them to respond to certain treatments preferentially and improve survival outcomes.

The status of *MGMT*p methylation is a very well-established prognostic factor in GBM.[21,32] The tumor tissue in our study had *MGMT*p status determined from tumor methylation analysis, which incorporates CpG probes shown critical to *MGMT* expression to provide a more accurate measure of MGMT activity to correlate better with true response to temozolomide.[21,33] Interestingly, 11% of LTS tumors were unmethylated which suggests that, while unmethylated *MGMT*p is associated with a lower likelihood of LTS, it is not an absolute.

Using MRI to identify survival subgroups of GBM patients is a practical approach of clinical interest. To further improve the utility of diagnostic radiology exams in prognosis, the prediction of tumoral *IDH*-mt, *MGMT*p, and *EGFR* alterations based on advanced imaging analyses has been attempted in research studies and clinical trials.[34–36] Our retrospective analysis we found that LTS more commonly presented with a T1 hypodense lesion compared to STS, however, this finding is not adequately predictive to use in a clinical setting. Although the etiology of this difference is unknown, it is possible that there may be some differences in tumor biology such as tumor cellularity, metabolism, angiogenesis, or immunogenicity that alters features on MRI and ultimately determines the growth and treatment response of the tumor. It would be interesting to use automatic imaging tools in subsequent studies as our finding highlights the promising future of radiomics in neuro-oncology and supports the need for further research.

An important aspect of the disease trajectory is the patient experience and outcome, critical factors in survivorship. A majority of LTS presented at an outpatient clinic with seizures or focal deficits usually presenting, like STS with symptoms for <6 months before discovery of their brain tumor, although patients were more likely to have difficulty reporting the duration of symptoms. These are symptoms that may lead to earlier presentation and imaging analysis, representing a lead time bias for this group. Conversely, most STS present with symptoms such as headache, nausea, and generalized behavioral changes, which are more consistently associated with increased intracranial pressure. This may represent tumors that may have a higher velocity of growth at presentation or a tumor that may have been in a less eloquent area of the brain lending itself to growth before discovery. Both groups had activity-related interference rated as their highest factor, indicating that symptoms in both groups are associated with a significant impact on both activity and mood

related components of their lives. Interestingly, both the percentage of patients reporting anxiety and depression associated symptoms were not different between groups and the ED-5D-3L results showed LTS rated all dimensions except anxiety or depression as having "some problems" as compared to none in the STS group, which may indicate the impact of the duration of symptoms on patients perception of their health status, but that mood-related disturbance is important in a subset in both groups. The use of multiple instruments to measure different dimensions and factors is essential to a proper assessment of the impact of the disease and treatment on how the patient feels and functions to inform the clinical care and needs of patients in these cohorts.

Our study, like prior reports, was limited by the sample number. A larger patient population with comprehensive clinical and molecular data may enable the discovery of new factors that would better identify this uncommon group of patients whose survival exceeds expectation. These insights may help to better understand tumor biology and potentially, therapeutic vulnerabilities that may help improve treatments for the larger population of patients with GBM. The creation of an international registry with a standard clinical data collection and tumor molecular testing would greatly facilitate these analyses.

Supplementary material

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

Keywords

glioblastoma | long-term survivor | methylation | predictors

Funding

The Natural History Study Project is supported by Intramural Project 1ZIABC011768-03 (T.S.A.).

Conflict of interest statement

None declared.

Authorship statement

Data acquisition: N.J.B., E.V., E.K.P., Z.A., A.C., E.G., T.K., J.L., M.L., K.M., J.R., L.B., E.B., M.P., L.P., M.P.P., T.P., B.L.T., J.W., K.W., A.P.S., and M.Q. Data review and analysis: N.J.B., E.V., E.K.P. , Z.A., K.A., J.S., T.A., and M.R.G. Manuscript preparation: N.J.B., E.V., E.K.P., T.S.A., and M.R.G.

Disclaimer

The views expressed in this article are those of the authors and do not necessarily reflect the official policy or position of the Uniformed Services University, Department of the Army, Department of Defense or the US Government.

Acknowledgments

We would like to thank the patients and their families for their contribution to this work.

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