Neuro-Oncology Advances
6(1), vdae143, 2024 | https://doi.org/10.1093/noajnl/vdae143 | Advance Access date 19 August 2024

Oligodendroglioma patient survival is associated with circulating B-cells and age

Jennie W. Taylor, Gayathri Warrier, Helen M. Hansen, Lucie McCoy, Terri Rice, Geno Guerra, Stephen S. Francis, Jennifer L. Clarke, Paige M. Bracci, Sara Hadad, Karl T. Kelsey, Margaret Wrensch, Annette M. Molinaro, and John K. Wiencke

All author affiliations are listed at the end of the article

Corresponding Author: Jennie W. Taylor MD, MPH, University of California, San Francisco, 400 Parnassus Ave, A808, San Francisco, California 94143, USA (jennie.taylor@ucsf.edu).

Abstract

Background. Variations in survival among patients with oligodendroglioma are unexplained by known prognostic factors. To assess the impact of peripheral immune profiles on prognosis, we applied immunomethylomics analyses—DNA methylation of archived whole blood samples, to characterize immune cells.

Methods. We compared the proportions of immune cells from patients with oligodendroglioma to other glioma subtypes and controls. We used recursive partitioning analysis (RPA) within the oligodendrogliomas to correlate with survival.

Results. Patients with oligodendrogliomas (141) were median age at diagnosis of 44 years; 57% male; 75% White; 60% prior chemotherapy; and 25% on dexamethasone at sample collection. Patients with oligodendrogliomas had immune profiles more similar to controls than other glioma subtypes, though with notably lower B-cells. RPA of patients with oligodendrogliomas delineated 2 survival groups based on an interaction between age and B-naïve cells. Patients with longer survival (median 24.2 years) were ≤42 years of age with higher B-naïve cells versus worse survival (median 16.9 years) who were ≤42 years of age with lower B-naïve cells or >42 years of age ($P = .00032$). Patients with worse survival also had lower CD4- and CD8-naïve T-cells. Similar immune profiles were observed in an independent cohort of oligodendroglioma patients prior to surgery.

Conclusions. Peripheral blood immune profiles in oligodendroglioma suggested that younger patients with lower B-naïve cells experienced shorter survival. Though our findings lack of validation cohort and use a heterogenous patient population, they suggest peripheral blood immune profiles may be prognostic for patients with glioma and warrant further investigation.

Key Points

- Blood immune profiles in patients with oligodendrogliomas are distinct from other gliomas and controls.
- Oligodendroglioma patient survival is associated with the interaction of age and B-naïve cells.
- Younger patients with oligodendrogliomas with lower B-naïve cells have shorter survival.

Importance of the Study

Patients with oligodendroglioma live longer than other glioma subtypes, though their disease is ultimately fatal. Identifying prognostic factors in this rare disease with prolonged survival is challenging, and few are known. The peripheral immune system may play a role in glioma prognosis, and immunomethylomics is a powerful tool for characterizing immune cells in archived whole blood using DNA methylation. In a large cohort of patients with oligodendrogliomas and extensive follow-up time, we observed unique immune cell profiles compared to other glioma subtypes and controls. Within patients with oligodendrogliomas, we identified an interaction between age and B-naïve cells correlated with overall survival. In younger patients, those with lower B-naïve cells had a shorter survival. This hypothesis-generating finding suggests that immune cell subtypes, particularly B-cells, may be an unexplored prognostic marker in patients with oligodendrogliomas and warrants further investigation.

Background

Oligodendrogliomas are a rare subtype of diffuse gliomas seen in adults with an annual age-adjusted incidence in the United States ranging from 0.11–0.23/100 000.[1] Oligodendrogliomas are defined by mutations in the isocitrate dehydrogenase gene (*IDH*) and telomerase (*TERT*) promoter and codeletion of chromosomes 1p and 19q.[2] For grade 2 oligodendroglioma, the median age at diagnosis is 44 years, with a median overall survival of 205 months (95% CI: 196–209) versus 49 years of age and median survival of 108 months (95% CI: 101–116) for grade 3 tumors.[1] Genome-wide association studies demonstrate that genetic factors contribute to the etiology of oligodendroglioma.[3,4] Even though oligodendrogliomas comprise <5% of all adult diffuse gliomas, their young age at diagnosis and prolonged survival translate into a sizable morbidity burden (ie, in 2019, there were 24 710 individuals living with oligodendroglioma).[5]

Few definitive prognostic factors are known for oligodendrogliomas, likely due to the challenges of disease heterogeneity, low incidence, and longevity. The distinction between grades 2 and 3 oligodendrogliomas remains purely histologic and not defined by any molecular factors, unlike *IDH* mutant astrocytomas, wherein homozygous deletion of *CDKN2A/B* confers an adverse prognostic influence and is sufficient to upgrade these tumors to grade 4 regardless of histologic appearance.[2] In addition to grade, age at diagnosis[1,6] and extent of resection[7] are the most substantial prognostic factors.

Immune factors have been associated with the risk of developing oligodendrogliomas in patients with a history of asthma and allergy.[8] Based on tumor immune phenotypes, 2 prognostic groups were described in oligodendrogliomas using several large data sets with differences in survival associated with tumor lymphocyte and macrophage infiltration, antigen-presenting cell and T-cell coinhibition, and inhibition of checkpoint gene expression.[9] However, little is known about the role of the peripheral immune system and survival outcomes in oligodendrogliomas.

Recent advances in epigenetics provide new tools to examine circulating immune cells. One method, immunomethylomics, uses DNA methylation to quantify immune cell subtypes.[10–13] DNA-based immune profiling is reproducible, does not require intact cells, and permits the use of archived whole blood. Thus, immunomethylomics allows for prospective studies of survival in cancer populations that are not otherwise possible with conventional flow cytometry.[14]

In a previous analysis of immune profiles in a heterogeneous group of gliomas, we found an interaction between age, neutrophils, and CD4 T-cells that distinguished 4 survival groups.[15] We also identified differences in immune profiles in an independent cohort of patients with oligodendrogliomas recruited to our ongoing prospective Immune Profiles Study (IPS).[16] Here, we apply these same immunomethylomics methods to archived whole blood samples from patients with oligodendrogliomas with a median follow-up time of 12 years (95% CI: 11.2–14.3). The results reveal peripheral immune profiles that distinguish oligodendrogliomas both from other glioma subtypes and healthy controls. In addition, we found immune subtypes that, interacting with age at diagnosis, were prognostic for survival in patients with oligodendrogliomas.

Methods

Study Populations

San Francisco Adult Glioma Study. The Adult Glioma Study (AGS) is a case–control study that includes 3164 patients newly diagnosed with glioma who were residents of the San Francisco Bay Area and/or patients of the University of California San Francisco (UCSF) Neuro-oncology clinic and 2140 people without glioma who were residents of the San Francisco Bay Area and/or seen in the UCSF phlebotomy clinic between 1991 and 2012.[17] This study was approved by the institutional review board of the UCSF Human Research Protection Program. Informed consent was obtained from all study participants. Data collection and details of the AGS have been previously described.[15,17–19] In brief, adults at least 18 years of age with a newly diagnosed glioma who resided in the San Francisco Bay Area from 1991–1994 (series 1); 1997–1999 (series 2); 2001–2004 (series 3); 2006–2010 (series 4); 2010–2012 (series 5) were eligible to participate. Series 3–5 (2001–2012) also recruited adult glioma patients seeking care at UCSF Neuro-Oncology, regardless of their residence.

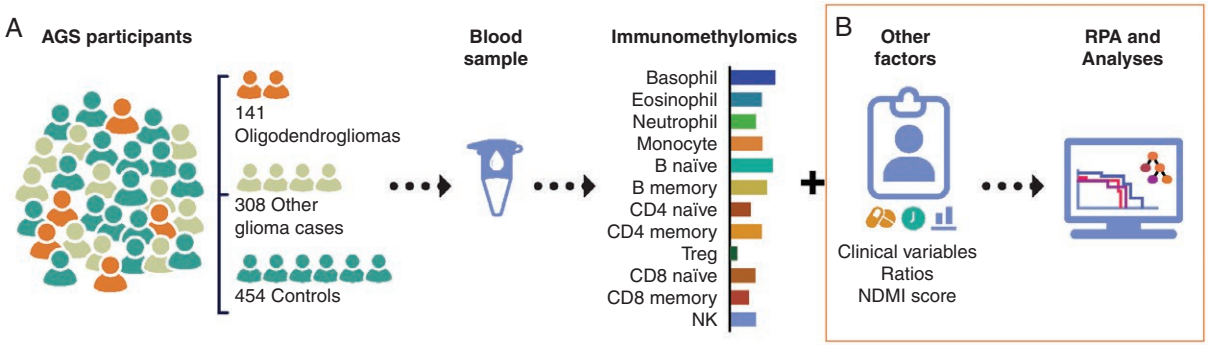


Figure 1. Study schema: (A) Participants enrolled in the AGS underwent blood sampling after diagnosis. Immunomethylomic arrays were run on archived blood samples to generate proportions of 12 immune cell subtypes. (B) Immune profiles from patients with oligodendrogliomas were combined with key clinical variables (ie, age at diagnosis), ratios generated from immune cell subtypes, including NDMI, and analyzed for survival using RPA. AGS, Adult Glioma Study; Treg, -regulatory cells; NK, natural killer cells; NDMI, neutrophil dexamethasone methylation index; RPA, recursive partitioning analysis.

Nonglioma controls were recruited through random digit dialing within the San Francisco Bay Area (series 1–4) (72%) and clinic-based methods from the phlebotomy lab at UCSF (series 5; 38%). Controls were frequently matched to cases by sex, age, and race (Figure 1). Participants provided single blood specimens a median of 110 days (IQR 40–217) for oligodendrogliomas and 103 (IQR 55–167) for other gliomas after diagnosis and were interviewed about various factors, including treatment information and dexamethasone use.[19]

Gliomas were defined by the WHO 2016,[20] with oligodendrogliomas harboring mutations in *IDH* and *TERT* and codeletion of chromosomes 1p and 19q.[2,20,21] Since patients were diagnosed from 1991 to 2012, and CDKN2A/B homozygous deletion status was only available for a limited number of patients, we could not update the classification by WHO 2021.[2] This study used a subset of these patients including 141 who were classified as *IDH* mutant/1p19q codeleted oligodendrogliomas; 308 patients with other WHO 2016 classifications (9 *IDH* mutant astrocytomas; 2 IDH mutant GBMs; 127 *IDH* wildtype diffuse astrocytomas; 170 *IDH* wildtype GBMs) as well as 454 nonglioma AGS controls.[15]

Immune Profiles Study

This is an ongoing, prospective study examining longitudinal blood immune profiles of patients with newly diagnosed gliomas, including grades 2 and 3 oligodendrogliomas, who were recruited between March 2018 and January 2021. Patients were identified and consented before surgery, with the first blood sample collected at least one day before surgery at UCSF. We analyzed the immune profile of presurgical blood samples collected at least one day before surgery to minimize glucocorticoids' impact on immune profiles. Note that this time point differs from the 141 patients with oligodendrogliomas from the AGS, whose blood was retrospectively collected at a median of 3 months after surgery. Methylation arrays and immunomethylomics analysis were performed as below.[16]

DNA Methylation Arrays

As previously described, frozen (–80°C) anticoagulated whole blood was processed for DNA isolation and bisulfite conversion.[13] The Illumina 850K EPIC DNA methylation platform (Illumina, Inc) was used.[15] All samples and array experiments were blinded to clinicopathologic variables. Illumina DNA methylation EPIC 850K BeadChip arrays were performed on approximately 200–500 ng of DNA. The probe intensity data (IDATs) obtained were processed using R software and the minfi[22] and Enmix[23] packages. Background correction and dye-bias normalization were performed using the preprocess Noob function, followed by identifying and filtering low-quality probes and samples, wherein low-quality samples and probes were defined as those with >5% missing values across the sample or the CpG data. Sex chromosome-associated probes and CpH probes were filtered, followed by general masking of cross-reactive, polymorphic, and single nucleotide polymorphism (SNP)-associated probes identified by Zhou et al.[24]

The leukocyte cell-type proportions were estimated using the cell-type reference library from the FlowSorted.BloodExtended.EPIC package.[11] This resulted in 12 cell-type proportions and additional derived ratios: neutrophils, eosinophils, basophils, monocytes, total lymphocytes, total and subset of B cells (memory and naïve), total and subsets of CD4 and CD8 cells (memory and naïve), T regulatory cells, and natural killer (NK) cells. Calculated ratios included: neutrophil-to-lymphocyte (NLR); lymphocyte-to-monocyte (LMR); B-cell naïve-to-memory; B-cell naïve-to-total; CD4-to-CD8; CD4 naïve-to-memory and CD4 naïve-to-total; CD8 naïve-to-memory; and CD8 naïve-to-total. We also calculated the blood-based neutrophil dexamethasone methylation index (NDMI), measuring a patient's epigenetic response to dexamethasone.[25]

We evaluated 3 DNA methylation-based epigenetic clocks described previously to assess the impact of epigenetic changes on age acceleration[15]: (1) a blood-based methylation model by Hannum (HannumAge)[26]; (2) a

multi-tissue algorithm to predict chronological age by Horvath (HorvathAge)[27]; and (3) an additional algorithm to predict phenotypic age to differentiate morbidity and mortality risk among same-age individuals by Levine (DNAmPhenoAge)[28]—that we had previously investigated in a larger cohort of patients with glioma.[15] All 3 methylation ages were calculated using the methylAge function in the Enmix Bioconductor package.[23]

Given the potential impact of environmental allergies and prior viral exposures on immune profiles and the known association of allergies and glioma risk,[29] we also examined IgE antibody titers and IgE food and respiratory allergies, as well as IgG antibodies for several viruses in a subset of patients, as previously described.[19,29] We also investigated the relationship of the SNP rs55705857 on chromosome 8q24.21, which confirms a 6-fold increase in the risk of developing an *IDH* mutant glioma (including oligodendrogliomas),[3,4,30] and is suggested to be prognostic.[31]

Statistical Analysis

Using AGS patients, we compared the blood cell characteristics of patients with oligodendrogliomas to those of other glioma cases and controls and adjusted for age. Overall survival (OS) was calculated from diagnosis to death or last follow-up for patients with oligodendrogliomas. Due to nonlinearity and nonproportional hazards, part DSA,[32,33] a recursive partition analysis (RPA) method, was used to explore interactions among all clinical and immune markers and build survival risk groups in patients with oligodendrogliomas using censored data. Restricted mean survival time and restricted mean time lost were used to analyze the model results further.[34] Prediagnostic surgery immune profiles were assessed in the independent IPS data using summary measures. Two-sided tests ($P < .05$) determined statistical significance without correction for multiple comparisons. All analyses were performed in R version 4.2.0.([R Foundation for Statistical Computing, Vienna, Austria]).

Results

Clinical and Demographic Characteristics of Oligodendrogliomas, Other Glioma Subtypes, and Controls

We identified 141 patients with oligodendrogliomas from the AGS. Clinical and demographic data are described in Table 1. The median time from diagnosis to blood draw was 110 days (IQR 40 – 217). At the last follow-up, 42 (29.8%) patients were deceased, and the median follow-up time was 12.6 years (95% CI: 11.2–14.3). The median overall survival was 17.5 years (95% CI: 16.9—NA).

Patients with oligodendroglioma were significantly younger than other glioma cases and controls. The median age of 44 years (IQR 37–51 years) is similar to what is reported by the CBTRUS of 44 years for oligodendrogliomas, grade 2, and 49 years for anaplastic oligodendrogliomas.[1] Compared to the other gliomas (mostly IDH wildtype), more patients with oligodendrogliomas underwent resection versus biopsy; fewer had received either chemotherapy or radiation before blood draw; and fewer were taking dexamethasone at the time of blood draw (Table 1).

Blood Immune Profiles of Patients With Oligodendrogliomas Are Distinct From Other Gliomas and Controls

The proportion of immune cells in patients with oligodendrogliomas differed from the other glioma subtypes (Table 2). Patients with oligodendrogliomas had a higher proportion of basophils and eosinophils, lower neutrophils, and higher total lymphocytes across all fractions of all fractions of B-cells, CD4, and CD8 T-cells except for Tregs. Patients with oligodendrogliomas also had a higher proportion of NK cells and significantly lower NLR and NDMI. These findings persisted after age adjustment, except for the proportions of CD8-memory and naïve cells (Table 2), and are also likely influenced by differences in exposure to prior systemic therapies and dexamethasone between patients with other gliomas (predominately IDH wildtype) versus oligodendrogliomas.

Though significant differences were also seen between patients with oligodendrogliomas and controls, even after adjusting for age, the immune profiles of the oligodendrogliomas were more similar to controls than other gliomas (Table 2). We also compared immune profiles of patients with oligodendrogliomas without dexamethasone at the time of blood draw (n = 104) to controls, and found that that, again after adjusting for age, patients with oligodendrogliomas had a significantly lower proportion of total naïve cells and all subtypes of B-cells (Supplementary Table 1).

Survival of Patients With Oligodendrogliomas is Associated With an Interaction Between B-naïve Cells and Age

An RPA survival model identified 2 significantly different survival groups in the 141 patients with oligodendrogliomas based on an interaction between age at diagnosis and the proportion of B-naïve cells. The group with the worst outcome had a median survival of 16.9 years (14.8—NR). This group included younger patients (≤42 years of age) with a lower proportion of B-naïve cells (≤1.91) or older patients (>42 years of age; $N = 104$). The group with the better outcome had a median survival of 24.2 years (95% CI: 20.4—NR); this group included younger patients (≤42 years of age) with a higher proportion of B-naïve cells (>1.91; $N = 37$; Figure 2).

When comparing the other immune profiles between the 2 survival groups, we found that patients in the group with worse survival had a significantly lower proportion of all naïve cells—including total, CD4, and CD8 T-cells—compared to patients with better survival. Similarly, the ratio of naïve-to-memory and naïve-to-total cells was lower in the group with poorer survival across all immune profile subsets compared to the group with better survival (Supplementary Table 2). Though lower B naïve cells were accompanied by lower CD4 and CD8 naïve cells, RPA

Table 1. Demographic and Clinical Characteristics of the AGS Oligodendrogliomas, Other Glioma Cases, and Controls

	OG (*N* = 141)	Other gliomas (*N* = 308)	OG v/s other gliomas *P*-value[a]	Controls (*N* = 454)	OG v/s Controls *P*-value[a]
Chronological age (years), median (IQR)	44.0 (37, 51)	53.0 (43, 64)	<.001	52.0 (41, 63)	<.001
Days between diagnosis and blood draw, median (IQR)	110 (40, 217)	101 (55, 167)	.303	—	
Sex					
Female	60 (42.6%)	127 (41.2%)	—	207 (45.6%)	—
Male	81 (57.4%)	181 (58.8%)	.999	247 (54.4%)	.306
Smoking status					
Missing	2	0		1	
Ever	53 (38.1%)	172 (55.8%)	—	225 (49.7%)	—
Never	86 (61.9%)	136 (44.2%)	.030	228 (50.3%)	.127
Alcohol drinking status					
Missing	1	1	—	1	
Ever	127 (90.7%)	284 (92.5 %)	.410	386 (85.2%)	—
Never	13 (9.3%)	23 (7.5%)		67 (14.8%)	.027
Body mass index, median (IQR)	25.8 (23.1, 29.1)	25.8 (23.3, 29.5)	.876	25.8 (23.3, 29.8)	.539
Race					
Asian	13 (9.2%)	22 (7.1%)	.613	30 (6.6%)	.811
Black	0 (0.0%)	8 (2.6%)	.983	48 (10.6%)	.976
Hispanic	20 (14.2%)	23 (7.5%)	.369	46 (10.1%)	.702
Native American	1 (0.7%)	1 (0.3%)	.743	2 (0.4%)	.728
Other	1 (0.7%)	5 (1.6%)	.370	5 (1.1%)	.538
Pacific Islander	0 (0.0%)	0 (0.0%)	NA	1 (0.2%)	.997
Non-Hispanic White	106 (75.2%)	249 (80.8%)	—	322 (70.9%)	—
Vital status					
Alive	99 (70.2%)	39 (12.7%)			
Dead	42 (29.8%)	269 (87.3%)			
Median follow-up time, years (95% CI)	12.6 (11.2, 14.3)	15.7 (13.2, 17.1)			
WHO 2016 glioma classification					
Glioblastoma, IDH wt	0 (0.0%)	170 (55.2%)			
Astrocytoma, IDH wt	0 (0.0%)	127 (41.2%)			
Glioblastoma, IDH mt	0 (0.0%)	2 (0.6%)			
Astrocytoma, IDH mt	0 (0.0%)	9 (2.9%)			
Oligodendroglioma, IDH mt	141 (100.0%)	0 (0.0%)			
WHO 2016 grade					
Grade II	103 (73.0%)	46 (14.9%)	—		
Grade III	38 (27.0%)	90 (29.2%)	<.001		
Grade IV	0 (0.0%)	172 (55.8%)	.980		
Molecular subtype (based on IDH, 1p19q, and TERT)[b]					
Missing	1 (0.7%)	24 (7.8%)			
IDH only	0 (0.0%)	9 (2.9%)			
TERT only	0 (0.0%)	78 (25.3%)			
Triple-negative	0 (0.0%)	197 (64.0%)			
Triple positive	140 (99.3%)	0 (0.0%)			

Table 1. Continued

	OG (*N* = 141)	Other gliomas (*N* = 308)	OG v/s other gliomas *P*-value[a]	Controls (*N* = 454)	OG v/s Controls *P*-value[a]
Surgery					
Biopsy only	5 (3.5%)	49 (15.9%)	—		
Surgery	136 (96.5%)	259 (84.1%)	.002		
Chemotherapy missing	3	0			
No	55 (39.9%)	77 (25.0%)	—		
Yes	83 (60.1%)	231 (75.0%)	.001		
Radiation					
No	93 (66.0%)	41 (13.3%)	—		
Yes	48 (34.0%)	267 (86.7%)	<.001		
Treatment within 6 months of diagnosis					
Missing	3	0	—		
Both chemotherapy and radiation	34 (24.6%)	214 (69.5%)	<.001		
Chemotherapy only	49 (35.5%)	17 (5.5%)	.157		
Radiation only	12 (8.7%)	53 (17.2%)	<.001		
Neither	43 (31.2%)	24 (7.8%)			
Treatment prior to blood draw					
Presurgery	0 (0.0%)	13 (4.2%)	.987		
No prior treatment	71 (50.4%)	80 (26.0%)	—		
During radiation	0 (0.0%)	29 (9.4%)	.981		
Prior radiation only	13 (9.2%)	45 (14.6%)	.020		
Prior chemotherapy only	39 (27.7%)	24 (7.8%)	.028		
Radiation and chemotherapy only	18 (12.8%)	117 (38.0%)	<.001		
Dexamethasone at time of blood draw					
Missing	2	3		6	
No	104 (74.8%)	161 (52.8%)	—	447 (99.8%)	—
Yes	35 (25.2%)	144 (47.2%)	<.001	1 (0.2%)	<.001

[a]Age-adjusted logistic regression models.
[b]Patients were categorized into 5 glioma groups based on 3 tumor molecular markers: IDH, 1p19q, and TERT. Details of categories can be found in Eckel-Passow JE, Lachance DH, Molinaro AM, et al. *Glioma Groups Based on 1p/19q, IDH, and TERT promoter mutations in tumors. NEJM* 2015; 372(26):2499-2508.
Abbreviations: AGS, adult glioma study; OGs, Oligodendroglioma; IQR, Interquartile Range; IDH, Isocitrate dehydrogenase; WT, wild type; MT, mutant; TERT, Telomerase reverse transcriptase.

identified B-cell populations as being the most prognostic variables (Supplementary Figure 1).

Other clinical variables including grade, prior treatment, and dexamethasone were not different between the 2 survival groups. There was a slightly longer median time from diagnosis to blood draw of 125 days (IQR 21–269) for patients in the better survival group (Part 2) versus 108 days (IQR 46–194) for those with worse survival ($P = .036$; Supplementary Table 3).

Younger Patients With Lower Proportions of B-naïve Cells Are Associated With Shorter Survival

The patient group with worse survival (Part 1) is comprised of 2 distinct groups of patients, largely separated on age at diagnosis. To better understand the differences and similarities of this survival subgroup, we further separated the patients into the younger patients ≤42 years of age with a lower proportion of B-naïve cells ($N = 28$; Part 1a) and >42 years of age ($N = 76$) (Part 1b). We compared this group with patients in Part 2, defined as those ≤42 years of age with a higher proportion of B-naïve cells ($N = 37$; Figure 3). Patients in Part 1a had a markedly lower proportion of B-naïve cells compared to Part 2 (1.5 vs 2.9), suggesting the effect of lower B-naïve cells cannot be explained by

Table 2. Immune Profiles

		OGs (*N* = 141)	Other gliomas (*N* = 308)	OGs vs other glioma *P*-value		Controls (*N* = 454)	OGs vs controls *P*-value	
				Un-adjusted	Age-adjusted		Un-adjusted	Age-adjusted
Cell proportions, median (IQR)								
Granulocytes	Basophil	0.7 (0.5, 1.3)	0.5 (0.5, 0.8)	*.012*	*.003*	1.1 (0.6, 1.8)	*<.001*	*<.001*
	Eosinophil	0.8 (0.5, 1.9)	0.5 (0.5, 0.8)	*.016*	*.019*	1.0 (0.5, 2.2)	.332	.609
	Neutrophils	58.7 (51.5, 64.4)	66.6 (55.9, 76.4)	*<.001*	*<.001*	54.7 (47.4, 61.8)	*.011*	*.014*
	Monocyte	7.4 (6.0, 8.8)	7.3 (5.6, 9.5)	.553	.480	7.6 (6.4, 9.3)	.691	.721
Total Lymphocytes		28.6 (23.8, 35.9)	21.6 (14.3, 29.6)	*<.001*	*<.001*	32.3 (25.5, 38.6)	*.002*	*.001*
Total Naïve		7.2 (4.6, 10.7)	3.7 (2.1, 7.0)	*<.001*	*.004*	7.5 (4.9, 11.1)	.130	*<.001*
B-cells	B-total	3.8 (2.8, 4.8)	2.5 (1.6, 3.8)	*<.001*	*<.001*	5.2 (3.8, 6.4)	*<.001*	*<.001*
	B-memory	1.7 (1.3, 2.2)	1.4 (1.0, 1.9)	*.001*	*<.001*	2.1 (1.6, 2.7)	*<.001*	*<.001*
	B-naïve	2.0 (1.1, 2.8)	0.9 (0.5, 2.1)	*<.001*	*.017*	2.7 (1.9, 3.9)	*<.001*	*<.001*
CD4 T-cells	CD4-total	13.3 (9.9, 16.8)	8.9 (5.1, 13.1)	*<.001*	*<.001*	13.5 (10.3, 17.2)	.335	.220
	CD4-memory	7.6 (5.9, 10.7)	6.0 (3.4, 8.8)	*<.001*	*<.001*	8.5 (5.9, 11.6)	.308	.908
	CD4-naïve	3.7 (1.8, 5.8)	1.5 (0.5, 3.5)	*<.001*	*.009*	3.3 (1.3, 5.4)	.757	.149
	Treg	0.9 (0.5, 1.6)	0.8 (0.5, 1.4)	.090	.842	1.1 (0.5, 1.8)	*.023*	*.007*
Cell proportions, median (IQR)								
CD8 T-cells	CD8-total	6.8 (4.6, 10.3)	5.2 (2.5, 9.3)	*<.001*	*.019*	7.7 (5.0, 11.4)	.162	.996
	CD8-memory	5.2 (2.9, 8.9)	4.1 (1.8, 7.6)	*.036*	.051	6.3 (3.3, 9.9)	.078	.221
	CD8-naïve	1.0 (0.5, 2.7)	0.5 (0.5, 1.3)	*<.001*	.213	0.8 (0.5, 2.2)	.208	.108
Natural killer		3.5 (2.5, 4.9)	2.5 (1.4, 3.9)	*<.001*	*<.001*	3.5 (2.3, 4.8)	.444	.719
Cell ratios, median (IQR)								
B-naïve/ B-memory		1.2 (0.6, 1.8)	0.7 (0.5, 1.5)	.414	.753	1.3 (0.8, 1.9)	.051	*.012*
B-naïve/ B-total		53.5 (38.6, 64.8)	42.6 (31.8, 60.6)	*.007*	.269	56.3 (44.4, 66.4)	*.034*	*.002*

Table 2. Continued

	OGs (*N* = 141)	Other gliomas (*N* = 308)	OGs vs other glioma *P*-value		Controls (*N* = 454)	OGs vs controls *P*-value	
			Un-adjusted	Age-adjusted		Un-adjusted	Age-adjusted
$CD4^+T/CD8^+T$	1.9 (1.3, 2.7)	1.8 (1.0, 3.0)	.331	.770	1.8 (1.1, 2.8)	.302	.816
CD4-naïve/ CD4-memory	0.5 (0.2, 0.8)	0.4 (0.1, 0.7)	.889	.094	0.4 (0.1, 0.7)	.953	.212
CD4-naïve/ CD4-total	28.9 (14.3, 39.3)	23.1 (10.2, 34.9)	*.008*	.582	24.7 (10.2, 36.6)	.147	.220
CD8-naïve/ CD8-memory	0.2 (0.1, 0.6)	0.2 (0.1, 0.7)	.993	.208	0.2 (0.1, 0.5)	.846	.274
CD8-naïve/CD8-total	18.1 (8.2, 37.4)	17.8 (7.7, 42.2)	.937	.111	15.9 (5.9, 34.3)	.293	.330
CD8-memory/ Treg	5.8 (2.3, 11.8)	4.4 (1.6, 11.5)	.518	.510	5.3 (2.2, 12.4)	.586	.823
LMR	3.9 (2.9, 5.1)	3.0 (2.1, 4.4)	*.043*	.062	4.1 (3.2, 5.5)	.160	.036
NLR	2.0 (1.4, 2.7)	3.0 (2.1, 4.4)	*<.001*	*<.001*	1.7 (1.2, 2.4)	*.003*	*.006*
Total Naïve/ Total lymphocyte	0.3 (0.2, 0.4)	0.2 (0.1, 0.3)	*.008*	.777	0.3 (0.2, 0.4)	.870	.011
NDMI score	−2.3 (−3.4, −0.9)	−0.2 (−2.1, 2.1)	*<.001*	*<.001*	−3.3 (−4.2, −2.1)	*<.001*	*<.001*

Italics, statistically significant.
Abbreviations: OGs, oligodendrogliomas; IQR, Interquartile Range; Treg, T-regulatory cells; LMR, Lymphocyte monocyte ratio; NLR, Neutrophil lymphocyte ratio; NDMI, Neutrophil dexamethasone methylation index.

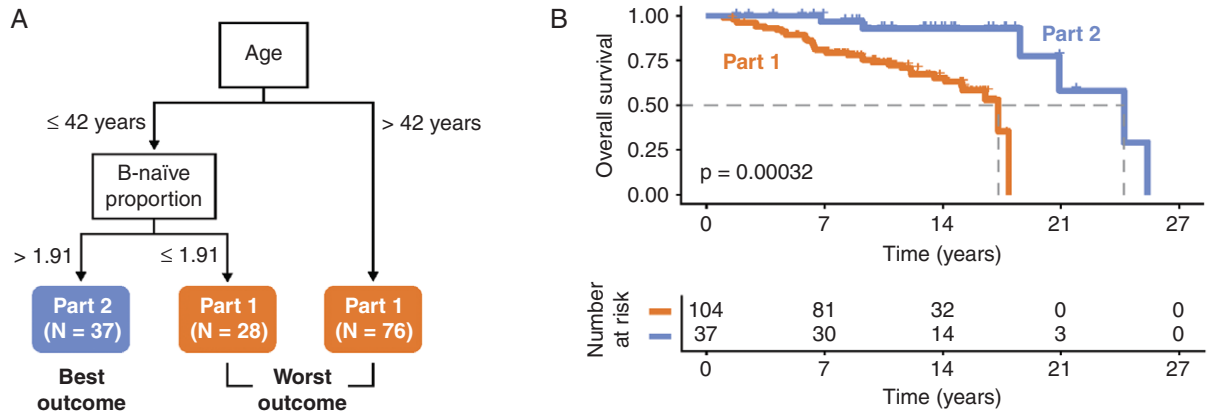
age alone. The patients in Part 1a were also noted to have a lower proportion of all naïve cells (total, CD4, CD8, and B) and ratios compared to those in Part 2 (Figure 4 and Supplementary Table 4). There was no significant difference in prior treatments, dexamethasone exposure, or other clinical characteristics between the 2 younger groups (Supplementary Table 5).

Differences in Epigenetic Clocks, Environmental Exposures, Genetic Alterations, or Prior Treatment Do Not Explain Survival Groups

We investigated several possible factors to address whether alternative explanations were driving differences in survival between the groups. Based on epigenetic clock analyses, patients in Part 1a were found to have age acceleration that differed from part 2 only for DNAmPhenoAge (Supplementary Table 6). Incorporating each epigenetic clock into the part DSA analysis with chronological age and all the clinical and immune profiles, we found a very similar interaction between age and B-cells (data not shown), suggesting similar epigenetic and chronological ages in patients with oligodendrogliomas.

In a subset of patients, we also found similar total IgE titers, IgE food, or respiratory allergies across the different survival groups. IgG antibodies for common CMV, EBV, HSV, and VZV viruses were also similar across survival groups. Similarly, no significant differences were seen for different allele frequencies of the risk SNP rs55705857 or CDKN2A deletion in tumor samples among the different survival groups (Supplementary Table 7).

Patients who received chemotherapy before their blood draw were noted to have lower B-naïve and total B-cells compared to patients without prior chemotherapy exposure (Supplementary Figure 2). However, the survival groups showed no significant difference in treatments (Supplementary Tables 3 and 5). Additionally, we found that the proportion of B-naïves did not decrease in the controls with increasing age and, therefore, the interaction between age and proportion of B-naïves cells seen in the oligodendrogliomas is not explained by increasing age alone (Supplementary Figure 3).



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	Part 2	Part 1
Overall survival, years median (95% CI)	24.2 (95% CI 20.4 – NR)	16.9 (14.8 – NR)
Age, years median (IQR)	33.0 (30, 39)	47.5 (41, 53)
B naïve proportion, median (IQR)	2.6 (2.2, 3.5)	1.6 (0.8, 2.3)
	Best outcome	**Worst outcome**

Figure 2. Clinical and immune profiles RPA delineates 2 survival groups for patients with oligodendrogliomas based on an interaction between age at diagnosis and B naïve cell proportions. (A) Clinical variables and immune profiles RPA from 141 patients with oligodendrogliomas, identified primary node as age and B-naïve cell proportion as secondary node. Patients fell into 2 survival groups. Part 1 were patients with worse outcome, and included patients ≤42 years of age with a lower proportion of B-naïve cells or patients >42 years of age. Part 2 were patients with better outcome included patients who were ≤42 years of age with a higher proportion of B-naïve cells (>1.91). (B) Kaplan–Meier curves are shown for Part 1 and Part 2 (P = .00032). (C) Table of median overall survival, age, and B-naïve cell proportion for the 2 parts.

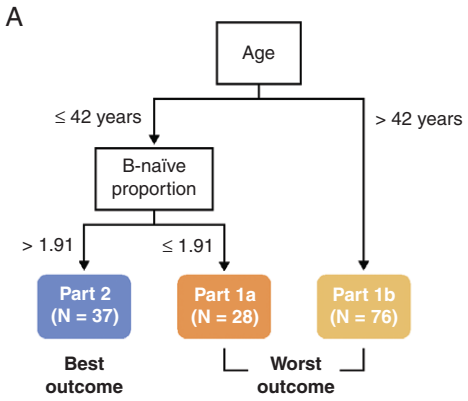
Phenotypes of Lower Naïve Cells are Also Seen in Blood Samples Prior To Diagnosis.

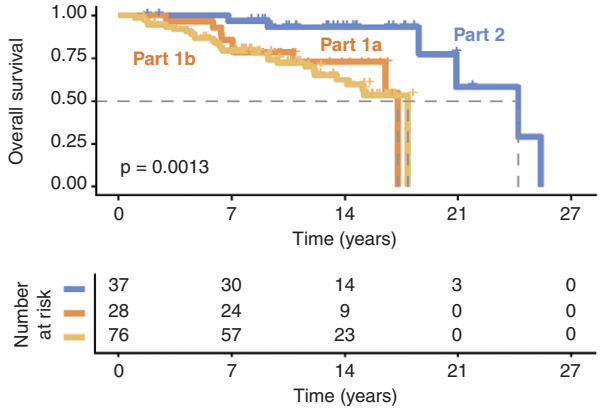
To better understand if the interaction between age and proportion of B-naïve cells is present at the time of diagnosis, we investigated if a similar phenotype of immune profiles from the partDSA model above was observed in a cohort of 49 newly diagnosed patients with oligodendroglioma as part of the ongoing IPS at UCSF. We identified 3 patients who were young with a lower proportion of B-naïve cells (part 1a); 23 who were older (part 1b) and 23 patients who were younger with a higher proportion of B-naïve cells (part 2). Interestingly, we saw a similar pattern of a lower proportion of naïve cells (total, CD4, CD8) and a ratio of CD4 naïve-to-total and total naïve-to-lymphocyte (Supplementary Tables 8) without significant difference in key clinical characteristics (Supplementary Tables 9). With follow-up time limited thus far (median of 2.4 years [95% CI: 1.6–3.1]), these patients will need to be followed to determine if the interaction between age and proportion of B-naïve cells that we saw in the AGS will be validated in the patients with oligodendrogliomas in IPS for whom blood was collected and analyzed prior to surgery.

Discussion

Patients with oligodendrogliomas have longer survival compared to patients with many other glioma subtypes. However, this illness is still life-limiting with few known prognostic factors which may, in part, be secondary to the rarity of the disease and the need for prolonged follow-up to define survival outcomes. Using immunomethylomics from archived peripheral blood samples, we found patients with oligodendrogliomas to have somewhat similar immune profiles, after adjusting for age, to a control population but markedly different compared to other glioma subtypes, though differences in exposures to systemic therapy and dexamethasone likely also contributed to this finding. However, all subtypes of B-cells, were still significantly lower in patients with oligodendrogliomas versus controls, even in those without dexamethasone at the time of blood draw, suggesting that changes in the immune profiles are not driven by corticosteroids and may be a biologic effect that warrants further exploration.

This study of 141 patients with oligodendrogliomas and extensive long-term follow-up, identified a striking interaction between age and B-naïve cells resulting in 2 distinct survival groups. Younger patients with oligodendrogliomas and a higher proportion of peripheral B-naïve cells lived 7.3 years longer than younger patients with a lower proportion of B-naïve cells. The age cutoff of 42 years is a younger





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	Part 2	Part 1a	Part 1b
	Younger with higher B naïve	Younger with lower B naïve	Older
Overall survival, years median (95% CI)	24.2 (CI 20.4 – NR)	16.9 (16.2 – NR)	17.5 (13.8 – NR)
Age, years median (IQR)	33.0 (30 – 39)	37.5 (34 – 39)	50.0 (47 – 56)
B naïve proportion, median (IQR)	2.6 (2.2 – 3.5)	1.2 (0.8 – 1.6)	1.9 (0.9 – 2.8)
	Best outcome	**Worst outcome**	

Figure 3: Clinical and immune profiles RPA distinguishing the younger patients by B-naïve cell proportions identifying 3 survival groups for patients with oligodendrogliomas. (A) Clinical variables and immune profiles RPA from 141 patients with oligodendrogliomas, identified primary node as age and B-naïve cell proportion as secondary node. Part 1a were younger patients with worse outcome and included 28 patients ≤42 years of age with a lower proportion of B-naïve cells (≤1.91). Part 1b were older patients with worse outcome and included 76 patients >42 years of age. Part 2 were patients with better outcome included 37 patients who were ≤42 years of age with a higher proportion of B-naïve cells (>1.91). (B) Kaplan–Meier curves are shown for Part 1a, Part 1b, and Part 2 (P = .0013). (C) Table of median overall survival, age, and B-naïve cell proportion for the 3 parts.

age and near the median for patients with oligodendroglioma,[1] and the younger patients with longer survival had higher B-naïve cell proportions.

In contrast, the patients with lower B-naïve cell proportions fell far below reference values and had shorter survival similar to patients who were older. The phenotype of lower naïve cells across several cell types in these younger patients may serve as a novel, noninvasive, prognostic marker and could factor into treatment decisions along the disease trajectory. While we acknowledge these findings were seen in blood samples from varying time points and exposure to different treatments, we investigated several known immunomodulators including epigenetic clocks, history of allergies, viral exposures, SNPs, CDKN2A deletion in tumor samples, prior treatments, and dexamethasone exposure, which failed to support an alternative explanation. However, statistical power was limited due to smaller sample sizes. Intriguingly, preliminary findings from our prospective, longitudinal study of immune profiles in patients with oligodendrogliomas suggest this phenotype of lower naïve cells may be present from the time of diagnosis, suggesting that changes in immune profiles are early events in the course of the disease.

IDH mutant gliomas, including grade 2 and 3 oligodendrogliomas, are felt to be immunologically "cold" tumors, in part from the oncometabolite 2-hydroxygluterate (2-HG) producing an immunosuppressive tumor microenvironment. Flow cytometry of peripheral blood from 20 oligodendroglioma patients prior to surgery, identified higher Tregs compared to controls and more immunosuppression in grade 2 versus 3 tumors.[35] With slower-growing tumors and younger patients with more intact immune systems, immunotherapy has been considered an attractive approach in IDH mutant gliomas.[36] However, immunotherapy strategies, such as peptide vaccines, have yet to demonstrate efficacy or convincing evidence of systemic or intratumoral immune response in this population.[36–38] The immunological privilege of the brain is a popular hypothesis to explain the ineffectiveness of immune strategies in IDH mutants. The broad study of tumor-infiltrating lymphocytes has generally focused on T-cells, yet a growing body of research suggests that B-cells are strong prognostic indicators, especially in the context of immune checkpoint inhibitors.[39] In tumor tissue, B-cells are shown to produce immunostimulatory cytokines for recruitment, influence T-cell responses, and activate innate immune responses.[40] B-cells can have antitumor properties by recognizing tumor-specific antigens, antibody production, serving as antigen processing cells, and directly killing cancer cells. However, they can also have pro-tumorigenic properties through activating myeloid-derived suppressor cells, pro-tumorigenic cytokine production, and activating immunosuppressive regulatory T-cells.[41] Little is known about the

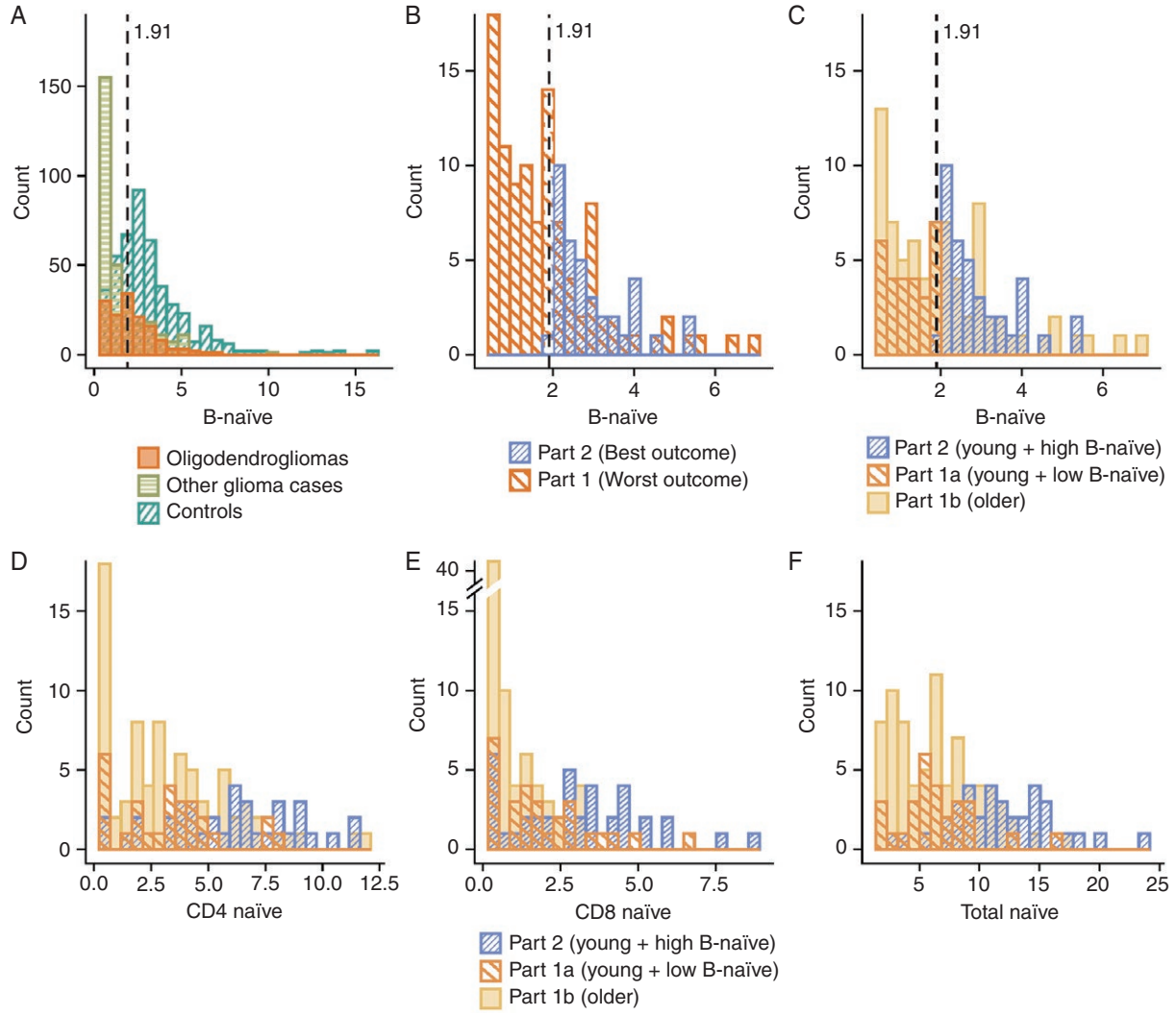

Figure 4. Comparisons of immune cell proportions (A) B-naïve cell proportions from 141 patients with oligodendrogliomas compared to 308 patients with other glioma subtypes and 454 controls. (B) B-naïve cell proportions from patients with oligodendrogliomas by 2 parts and (C) by 3 parts. (D-F) Cell proportions by 3 parts for other naïve subtypes including CD4 (D), CD8 (E), and total (F).

role of B-cells, either in the tumor microenvironment or peripherally, in IDH mutant gliomas. Our results in patients with oligodendrogliomas harboring an IDH mutation suggest that changes in the B- and other naïve cells delineate survival subsets is an intriguing hypothesis. Further evaluation in other IDH mutant gliomas, which were underrepresented in this data set, is warranted to better characterize the relationship of the peripheral immune system in both glioma genesis and response to treatment.

Age is one of the few known prognostic factors across all gliomas, including oligodendrogliomas, though the underlying mechanisms are not well understood. Normal aging results in a decrease in B- and T-lymphocyte production and an increase in neutrophils and monocytes. The proportion of naïve to terminally differentiated memory lymphocytes also decreases, and this immunosenescence is associated with a higher risk of infection, inflammation, certain autoimmune diseases, and cancers. A decrease in the naïve compartment of immune cells with increasing age has also been shown using immunomethylomics.[42] This makes our finding of a markedly lower proportion of naïve cells across all patients with oligodendrogliomas, though specifically in younger patients with poor survival even more striking. Our prospective IPS study will allow us to further interrogate the potential dynamic changes of immune subtypes with different treatments and time points across the disease trajectory.

Immunomethylomics is a powerful tool for interrogating the peripheral immune system in patients with gliomas. It overcomes many of the challenges posed by flow cytometry, including better scalability and the ability to analyze archived samples. As the library of methylation profiles for immune cells expands, it will allow more in-depth analysis of specific subtypes. When total leukocyte counts are available, which was not the case in this study, absolute counts can be calculated in addition to cell proportions. Though the expectation is for absolute counts to track with cell proportions,[42] being able to further quantify

and compare immune subtypes across different diagnoses and time may provide further insight into the interaction between age, immune profiles, and survival.

We acknowledge there are several limitations of this study, including the relatively small sample size and the cross-sectional nature of the study with variable times from diagnosis to blood draw and heterogeneity in treatment before blood draw. Our other glioma cases primarily harbored IDH wildtype tumors, therefore restricting broader conclusions on the relationship between IDH mutational status and peripheral immune profiles. We also acknowledge that we could not correlate DNA methylation from the peripheral immune system with immune characteristics from the tumor. Most notably, we lack an independent cohort to validate these findings. Our previous work in IDH wildtype gliomas, which are both more common with shorter survival, highlights the potential power of immunomethylomics.[15] Oligodendrogliomas are rare tumors with long survival times, making validating prognostic markers challenging. This study is hypothesis-generating and particularly timely, with a likely shift in the treatment of oligodendrogliomas and an urgent need to develop prognostic markers, particularly early in the disease course. Our ongoing, prospective, longitudinal study of immunomethylomics across several glioma subtypes, including oligodendrogliomas, will provide an opportunity to better understand how immune profiles change over time, with treatment, and with progression. In addition to assessing the proportions of immune cells, we will be able to determine absolute counts and, with our collaborators, continue to extend the deconvolution library to analyze more subtypes of immune cells.

Oligodendrogliomas reduce the lifespan in those affected, despite patients' significantly longer relative survival than patients with other gliomas, and an evolving landscape of treatment options with the recent success of IDH inhibitors only heightens the need for prognostic markers.[43] We identified B-naïve cells in the peripheral blood as a possible prognostic factor that warrants validation in larger cohorts of patients with oligodendrogliomas. To facilitate biomarker discovery in this rare disease, there is a need for large-scale international collaboration to develop well-annotated repositories of both tumor and peripheral blood specimens.

Supplementary material

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

Keywords

epigenetics | glioma | immune factors | immunomethylomics | oligodendroglioma

Funding

Work at University of California, San Francisco was supported by the National Institutes of Health (grant numbers R01CA52689, P50CA097257, R01CA126831, R01CA139020, and R25CA112355), as well as the Ioglio Collective, the National Brain Tumor Foundation, the Stanley D. Lewis and Virginia S. Lewis Endowed Chair in Brain Tumor Research, the Robert Magnin Newman Endowed Chair in Neuro-oncology, and by donations from families and friends of John Berardi, Helen Glaser, Elvera Olsen, Raymond E. Cooper, and William Martinusen. This project was supported by the National Center for Research Resources and the National Center for Advancing Translational Sciences, National Institutes of Health, through UCSF-CTSI Grant Number UL1 RR024131. Its contents are solely the responsibility of the authors and do not necessarily represent the official views of the NIH.

Acknowledgments

The authors wish to acknowledge study participants, the clinicians, and research staff at the participating medical centers; the UCSF Helen Diller Family Comprehensive Cancer Center Genome Analysis Core, which is supported by a National Cancer Institute Cancer Center Support Grant (5P30CA082103); the UCSF Cancer Registry (for updating UCSF glioma case survival and vital status); Epigenomics Profiling Services division of Diagenode SA; Avera Institute for Human Genetics, Sioux Falls, USA; QB3 Genomics, UC Berkeley, Berkeley, CA, RRID:SCR_022170; the UCSF Neurosurgery Tissue Bank; and, the UCSF Brain Tumor Center Database for data management and collection. We thank Noel Sirivansanti and Ken Probst for their artistic contributions. The collection of cancer incidence data used in this study was supported by the California Department of Public Health pursuant to California Health and Safety Code Section 103885; Centers for Disease Control and Prevention's (CDC) National Program of Cancer Registries, under cooperative agreement 5NU58DP006344; the National Cancer Institute's Surveillance, Epidemiology and End Results Program under contract HHSN261201800032I awarded to the University of California, San Francisco, contract HHSN261201800015I awarded to the University of Southern California, and contract HHSN261201800009I awarded to the Public Health Institute, Cancer Registry of Greater California. The ideas and opinions expressed herein are those of the author(s) and do not necessarily reflect the opinions of the State of California, Department of Public Health, the National Cancer Institute, and the Centers for Disease Control and Prevention or their Contractors and Subcontractors. All analyses, interpretations, and conclusions reached in this manuscript from the mortality data are those of the author(s) and not the State of California Department of Public Health. Study data were collected and managed using REDCap electronic data capture tools hosted at UCSF.[1,2] REDCap (Research Electronic Data Capture) is a secure, web-based software platform designed to support data capture for research studies, providing (1) an intuitive interface for validated data capture, (2) audit trails for tracking data manipulation and export procedures, (3) automated export procedures for seamless data

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Conflict of interest statement

J.K.W. and K.T.K. are cofounders of Cellintec, which played no role in the current study. The remaining authors declare no competing interests. J.W.T. has received institutional grant support from Servier Pharmaceuticals and Bristol Myers Squibb; advisory board support from Servier Pharmaceuticals. J.L.C. has received institutional grant support from Servier Pharmaceuticals.

Authorship statement

Conception and drafting: J.W.T., G.W., A.M.M., and J.K.W.. Revising the article: J.W.T., G.W., A.M.M., J.K.W., J.L.C., S.S.F., G.G., P.M.B., S.H., K.T.K., L.M., T.R., H.M.H., and M.W.. Data collection/analysis: G.W., L.M., T.R., H.M.H., A.M.M., and M.W.. Final approval of the version to be published: J.W.T., G.W., H.M.H., L.M., T.R, G.G., S.S.F., J.L.C., P.M.B., S.H., K.T.K., M.W., A.M.M., and J.K.W..

Data availability

Methylation and phenotype data used in this manuscript are available through dbGaP--controlled access. Methylation and phenotype data from the Adult Glioma Study are available through dbGaP Study Accession phs001497.v2.p1. Methylation and phenotype data for a subset of the presurgery samples from the Immune Profiles Study are available through dbGaP Study Accession phs002998.v1.p1. Additional data may be made available upon reasonable request to the corresponding author.

Affiliations

Department of Neurological Surgery, University of California San Francisco, San Francisco, California, USA (J.W.T., G.W., H.M.H., L.M.C., T.R., G.G., S.S.F., J.L.C., S.H., M.W., A.M.M., J.K.W.); Department of Neurology, University of California San Francisco, San Francisco, California, USA (J.W.T., J.L.C.); Weill Institute for Neurosciences, University of California San Francisco, San Francisco, California, USA (J.W.T., S.F., J.L.C., A.M.M.); Department of Epidemiology and Biostatistics, University of California San Francisco, San Francisco, California, USA (S.F., P.M.B., A.M.M., J.K.W.); Departments of Epidemiology; Pathology and Laboratory Medicine, Brown University, Providence, Rhode Island, USA (K.T.K.)

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