

## Glioblastoma as an age-related neurological disorder in adults

Miri Kim<sup>®</sup>, Erik Ladomersky, Andreas Mozny, Masha Kocherginsky, Kaitlyn O'Shea, Zachary Z. Reinstein, Lijie Zhai, April Bell, Kristen L. Lauing, Lakshmi Bollu, Erik Rabin, Karan Dixit, Priya Kumthekar<sup>®</sup>, Leonidas C. Plataniias, Lifang Hou, Yinan Zheng, Jennifer Wu, Bin Zhang, Maya Hrachova, Sarah A. Merrill<sup>®</sup>, Maciej M. Mrugala, Vikram C. Prabhu, Craig Horbinski, Charles David James, Bakhtiar Yamini, Quinn T. Ostrom, Margaret O. Johnson, David A. Reardon, Rimas V. Lukas, and Derek A. Wainwright<sup>®</sup>

*Department of Neurological Surgery, Loyola University Medical Center, Maywood, Illinois, USA (M.K., V.C.P.); Department of Neurological Surgery, Northwestern University Feinberg School of Medicine, Chicago, Illinois, USA (E.L., A.M., Z.Z.R., L.Z., A.B., K.L.L., L.B., E.R., C.H., C.D.J., D.A.W.); Department of Preventive Medicine, Northwestern University Feinberg School of Medicine, Chicago, Illinois, USA (M.K., K.O., L.H., Y.Z.); Department of Neurology, Northwestern University Feinberg School of Medicine, Chicago, Illinois, USA (K.D., P.K., R.V.L.); Department of Medicine, Division of Hematology–Oncology, Northwestern University Feinberg School of Medicine, Chicago, Illinois, USA (L.C.P., B.Z., D.A.W.); Department of Urology, Northwestern University Feinberg School of Medicine, Chicago, Illinois, USA (J.W.); Division of Neuro-Oncology, Department of Neurology, Mayo Clinic, Phoenix, Arizona, USA (M.H., S.A.M., M.M.M.); Department of Neurological Surgery, University of Chicago Medical Center & Biological Sciences, Chicago, Illinois, USA (B.Y.); Department of Neurosurgery, Duke University School of Medicine, Durham, North Carolina, USA (Q.T.O., M.O.J.); Dana-Farber/Harvard Cancer Center, Harvard Medical School, Boston, Massachusetts, USA (D.A.R.); Department of Microbiology and Immunology, Northwestern University Feinberg School of Medicine, Chicago, Illinois, USA (D.A.W.)*

**Corresponding Author:** Derek A. Wainwright, PhD, Departments of Neurological Surgery, Medicine–Hematology/Oncology, Microbiology–Immunology, Northwestern University Feinberg School of Medicine, 300 East Superior St., Tarry Building, Room 2-703, Chicago, IL 60611, USA ([Derekwainwright@northwestern.edu](mailto:Derekwainwright@northwestern.edu)).

### Abstract

**Background.** Advanced age is a major risk factor for the development of many diseases including those affecting the central nervous system. Wild-type isocitrate dehydrogenase glioblastoma (IDH<sup>wt</sup> GBM) is the most common primary malignant brain cancer and accounts for ≥90% of all adult GBM diagnoses. Patients with IDH<sup>wt</sup> GBM have a median age of diagnosis at 68–70 years of age, and increasing age is associated with an increasingly worse prognosis for patients with this type of GBM.

**Methods.** The Surveillance, Epidemiology, and End Results, The Cancer Genome Atlas, and the Chinese Glioma Genome Atlas databases were analyzed for mortality indices. Meta-analysis of 80 clinical trials was evaluated for log hazard ratio for aging to tumor survivorship.

**Results.** Despite significant advances in the understanding of intratumoral genetic alterations, molecular characteristics of tumor microenvironments, and relationships between tumor molecular characteristics and the use of targeted therapeutics, life expectancy for older adults with GBM has yet to improve.

**Conclusions.** Based upon the results of our analysis, we propose that age-dependent factors that are yet to be fully elucidated, contribute to IDH<sup>wt</sup> GBM patient outcomes.

### Key Points

- The aging brain and body contribute to especially poor outcomes for older adult patients.
- The mechanism of GBM progression and response to treatment in the context of aging remain as understudied areas of research.

## Importance of the Study

IDH<sup>wt</sup> glioblastoma (GBM) is an aggressive primary brain cancer for which patient outcomes remain poor despite the use of multimodal therapies. This study was undertaken to address a gap in knowledge regarding especially poor outcomes for older GBM patients. Age-associated changes including

decreased immune system function and chronic neuroinflammation are potential contributors to the reduced posttreatment survival of older adult ( $\geq 65$  years of age) GBM patients and represent areas of need for an increased study to inform personalized approaches for treating elderly patients.

## Advanced Age Enhances the Risk for Human Disease

Wild-type isocitrate dehydrogenase glioblastoma (IDH<sup>wt</sup> GBM) is the most common aggressive primary brain tumor in adults and has a rapidly progressing course with a median age of diagnosis of 68–70 years.<sup>1</sup> GBM consists of distinct subclonal cell populations with a high heterogeneity that contributes to poor survival outcomes irrespective of the treatment used.<sup>2–4</sup> Age- and gender-adjusted incidence of GBM is highest among adults 75–84 years of age.<sup>5</sup> The age-adjusted incidence rate of GBM among adults 35–44 years of age per 100 000 people is 1.25 and increases to 8.05, 12.99, and 15.13 among adults 55–65, 65–74, and 75–84 years, respectively.<sup>6</sup> The basis for the increased incidence of GBM among elderly individuals is poorly understood and underexplored.

Life expectancy has been increasing in the United States and many other developed countries for more than 40 years. Increasing life expectancy is attributed to scientific, medical, technological, and/or sociological discoveries that have contributed to a growing population that survive to  $\geq 65$  years of age. For an individual in the United States born in 2018, life expectancy is 76 and 81 years of age for men and women, respectively.<sup>7,8</sup>

Advanced age of  $\geq 65$  years is associated with a high risk of disease incidence with greater than 80% of this age group being afflicted with diabetes, hypertension, heart disease, cancer, or other condition requiring medical management.<sup>9–11</sup> With respect to cancer, 55% of newly diagnosed cancer and 70% of cancer-related deaths occur in individuals  $\geq 65$  years of age.<sup>12</sup> Advanced age is therefore the most common risk factor associated with a cancer diagnosis and also the most common negative prognostic factor.<sup>9,13</sup>

Here we explore the hypothesis and evidence that IDH<sup>wt</sup> GBM primarily arises due to age-dependent changes that take place in the older adult central nervous system (CNS) that includes declining immune system function. We hypothesize that age-dependent processes enhance GBM cell initiation, progression, and resistance to therapeutic approaches. A better understanding of age-dependent changes in the brain and immune system may lead to the development of personalized therapeutic approaches that provide better outcomes for older adults with GBM.

## Methods

### Surveillance of Epidemiology and End Results Database

All population, incidence, and mortality data from the Surveillance of Epidemiology and End Results Database (SEER) database were accessed through SEER \* Stat (Version 8.3.5). Population-level data were accessed through a Frequency Session. Variables examined were (1) age recode with  $\leq 1$ -year olds and (2) year. Incidence and mortality data were accessed through a Rate Session. Variables examined for incidence include (1) age recode with  $\leq 1$ -year olds, (2) year of diagnosis, (3) histology recode—broad groupings, (4) histology recode—brain groupings, and (5) cause of death (COD) to site recode. The COD to site recode was used to analyze the mortality rate of GBM. Variables examined for mortality include (1) age recode with  $\leq 1$ -year olds, (2) year of death, and (3) COD recode. All rate data were crude/non-age-adjusted. Data were accessed on March 16, 2021.

### The Cancer Genome Atlas and Chinese Glioma Genome Atlas

Survival data for GBM patients were analyzed from the cancer genome atlas and the Chinese Glioma Genome Atlas. The data were accessed using the UCSC Xena portal. Data were accessed on March 16, 2021.

### Meta-analysis

PubMed search of “glioblastoma” that was limited to phase III randomized trials between 1985 and 2020. Each study was reviewed independently to assess the contribution of aging to tumor survivorship. Log hazard ratio (HR) and standard error from the HR and confidence intervals for age were extracted and reported HRs for age. Confidence intervals are reported “as is” for studies that reported HRs and confidence intervals by age group. Four studies represented with blocks reported only the HR point estimate without the corresponding confidence intervals or standard error. In the Levin et al. (2000) and Brem (1995) papers, HR was reported on the log scale per either a decade of age or 1 year of age and was converted to HR scale by exponentiating the estimate. For the Elliot

et al. (1997) and Malmström et al. (2017) studies, 1/HR is reported to have the younger age group as the reference group. Forest plot was done using *metaviz* package in R v. 4.0.2 statistical software.

## Results

### Advanced Age and IDH<sup>wt</sup> GBM

SEER database analysis between 1975 and 2017 of patients diagnosed with GBM shows the age-related increase of the incidence for this cancer, with a peak incidence between 70 and 79 years old. GBM has a poor prognosis as indicated by a mortality to incidence ratio consistently more than 0.8 for all patients older than the age of 35 (Figure 1A). Percent mortality versus age plot developed from SEER data reveals the highest value for the 65–69 years of age group. Analysis of the same data recorded by The Cancer Genome Atlas shows a peak at 60–64 years of age. Chinese Glioma Genome Atlas data are distinct in showing a peak in a younger population of GBM patients; possibly as a result of a patient cohort that includes a surprisingly low percentage of adults  $\geq 65$  years of age: 16.25% (Figure 1B and C).

### Clinical Trials and the Aging Population

A review of GBM clinical trials finds that many limit enrollment to patients  $\leq 70$  years of age (Supplementary Table 1). In addition, many studies do not report outcomes with respect to patient age. Eighty publications were identified that report phase III clinical trial outcomes and of these, only 10 report HRs for age; although the median age of patients enrolled in those trials was in the 50s. Of the clinical trials that allow for age stratification of data, an increased HR of mortality clearly associates with increasing age (Figure 2). Though limited in number, these studies show that patients in the 60 and older demographic group consistently have reduced survival irrespective of treatment used. The studies that are for identifying treatment options for the aged population include de-escalation regimens.

## Discussion

### The Relationship Between Advanced Age and the Onset of Neuropathological Disease

The aged brain is more susceptible to local insults from reactive oxygen species within specific CNS regions including the hippocampus, substantia nigra, and the spinal cord.<sup>14</sup> Stalled or impaired cerebral blood flow in the aged cingulate, insular, prefrontal gyri, as well as hippocampus contribute to brain volume loss in those regions and have been associated with the cognitive decline with primary and secondary neurodegenerative conditions.<sup>15</sup> The median age of patients with Alzheimer's disease (AD), Parkinson's disease (PD), and stroke falls at or between 75–84, 72, and 69 years of age, respectively (Table 1). This

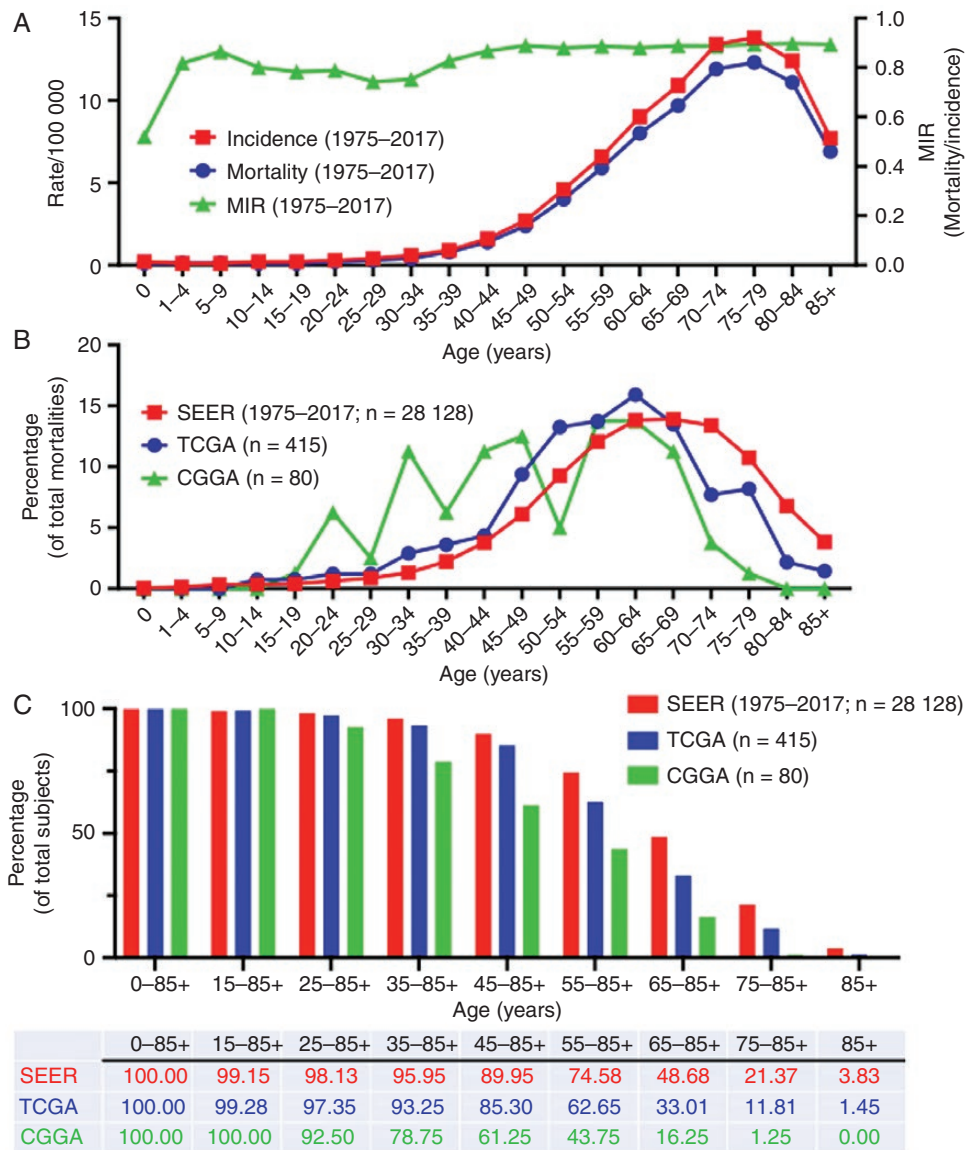
is within a similar age range to the median age of an IDH<sup>wt</sup> GBM patient diagnosis. However, the age-dependent mechanistic relationship between IDH<sup>wt</sup> GBM and other age-related neurodegenerative diseases remains unexplored (Figure 3A).

Advanced age in the CNS is associated with an enhancement of pro-inflammatory processes and the upregulation of immunosuppressive factors that can impair antitumor immune system functions. Microglia are the resident macrophages of the brain and contribute to the immunosuppressive microenvironment as they skew toward an M2 phenotype and secrete matrix metalloproteinases.<sup>16</sup> Major histocompatibility complex II and CD11b are upregulated while<sup>17,18</sup> bone marrow-derived dendritic cells (DCs) infiltrate and accumulate inside the brain parenchyma of older adult mice.<sup>19–21</sup> Immunosuppressive indoleamine 2,3 dioxygenase 1 (IDO) levels increase in the normal brain during advanced age and decrease the benefit of immunotherapy in older adult mice with brain tumors.<sup>22–25</sup> Unexpectedly, the age-dependent increase of brain IDO activity acts non-enzymically since pharmacologic IDO enzyme inhibitor treatment fails to reverse its immunosuppressive effects in older adults.<sup>24</sup> This raises an intriguing consideration of whether there are additional immunosuppressive factors that increase in the brain and act non-canonically during advanced age (Figure 3B).

### Aging and Senescence

Cellular senescence, chronic sterile inflammation, and the development of the inflammasome play a critical role in the development of age-related pathologies. Cellular senescence is a hallmark of aging and affects the outcome of subjects with neurodegenerative disease and cancer.<sup>26</sup> In contrast to an acute response to extrinsic factors, senescence is triggered by exposure to genotypic stress and damage to intracellular components. Senescent cells are permanently growth-arrested and reflect a mechanism of defense against the transformation into an oncogenic cell that divides uncontrollably. Although cellular senescence is presumed to confer an antitumorigenic function, the senescent cells release pro-inflammatory factors referred to as the secretory associated senescent phenotype (SASP) that exacerbates a variety of different age-related pathologies including AD, PD, and cancer.<sup>27–29</sup> Brain aging and neurodegenerative changes are associated with an enrichment of gene expression for SASP factors including TGF- $\beta$ , IL-6, IL-8, VEGF, matrix metalloproteinase-2 (MMP-2), and MMP-9—all of which have been implicated to also possess a role in cancer, neuroinflammation, and neurodegeneration.<sup>28–31</sup> The SASP enhances the invasion of tumor cells and accelerates the rate of cancer progression by changing DNA methylation and gene expression patterns.<sup>32–34</sup>

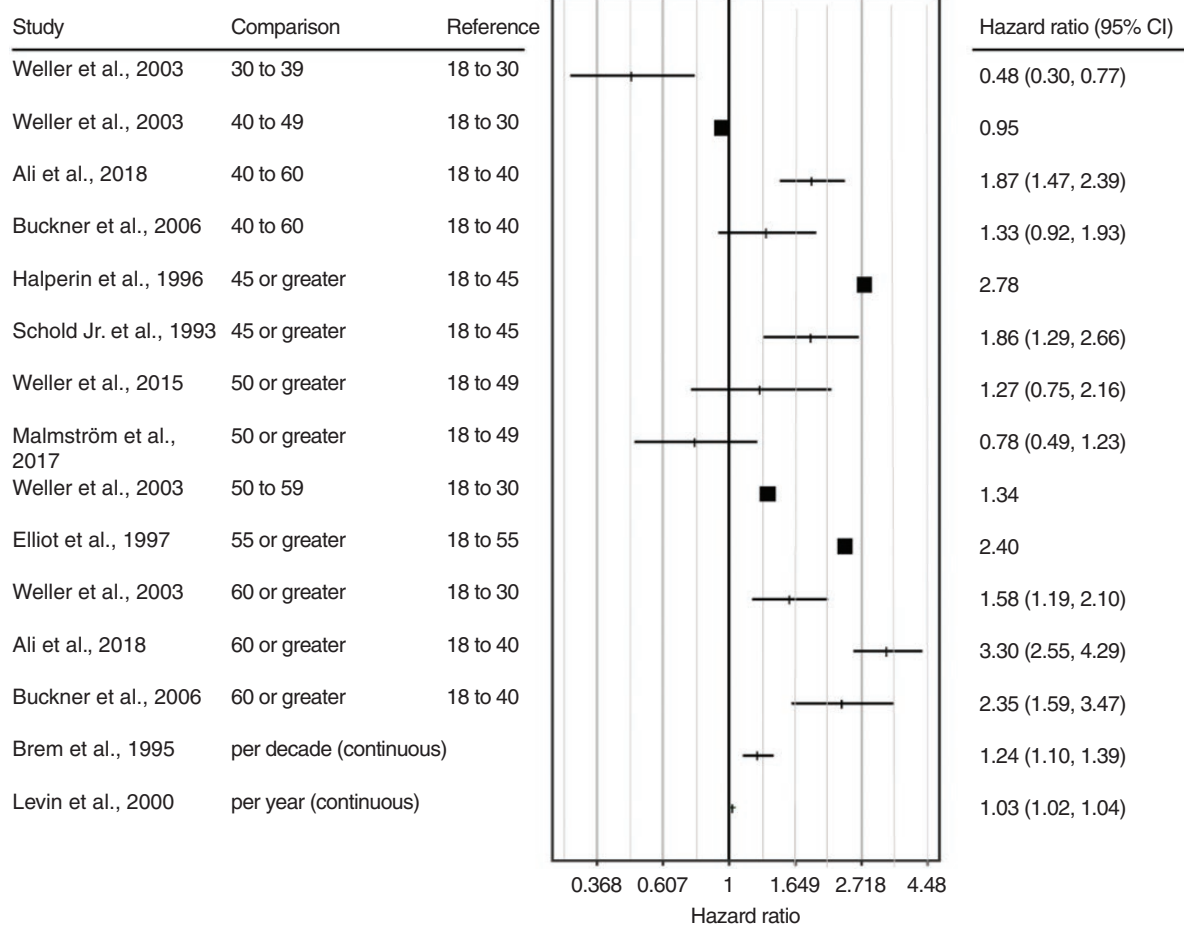
Inflammaging represents age-associated changes that result from chronic exposure to pro-inflammatory factors and the consequential effects that result in tissue damage.<sup>35</sup> Secondary pathological mechanisms that arise due to inflammaging contribute to type II diabetes, atherosclerosis, and a weakened immune system that is incapable of clearing infectious agents,



**Figure 1.** Age-dependent stratification of glioblastoma (GBM) patient incidence, mortality, and frequency across different databases. The Surveillance, Epidemiology, and End Results (SEER), the Cancer Genome Atlas (TCGA), and the Chinese Glioma Genome Atlas (CGGA) databases were analyzed. (A) The incidence of GBM per 100 000 individuals (red), mortality due to GBM (blue), and the mortality/incidence ratio (green) of patients between 1975 and 2017 were binned by 5-year intervals of age groups. (B) Comparison across databases for percentage mortality due to GBM among the SEER (red), TCGA (blue), and CGGA (green) between 1975 and 2017 binned by 5-year intervals of age groups. (C) Age distribution among the SEER (red), TCGA (blue), and CGGA (green) databases as assessed across different GBM patient age groups, demonstrating increased representation of elderly individuals (65–85+ bin) in the SEER database relative to CGGA and TCGA databases (inside open bracket).

infected cells, and malignant cells.<sup>36,37</sup> Implicated in neurodegenerative conditions, activated microglia release the inflammasome-derived cytokines, IL-1 $\beta$  and IL-8, which are toxic to neurons and contribute to neurotoxicity.<sup>38</sup> Age-dependent inflammasome activation in microglia, macrophages, and dendritic cells also contribute to tumorigenesis.<sup>39</sup> Immunosenescence is the derivative result of inflammaging that leads to decreased immune cell effector functions and the inability to adequately address infections, cancer, and antigenic challenges.<sup>40,41</sup>

P53 and p16<sup>INK4A</sup> play critical roles in regulating the phenotype of senescent cells. Older adult mouse hippocampal cells with increased p16<sup>INK4A</sup> have an associated cognitive decline that is rescued by eradicating p16<sup>INK4A+</sup> senescent cells.<sup>42</sup> The p16<sup>INK4A</sup> expression model has proven to be one of the most useful in vivo markers of senescent cells to date. With this in mind, p16 reporter mice were generated to allow for the detection of senescent cells through bioluminescence, localization, and isolation of live p16<sup>INK4A+</sup> cells, as well as the inducible eradication of those cells with



**Figure 2.** Forest plot of a meta-analysis evaluating hazard ratios as stratified by age of published phase III clinical trials involving patients with glioblastoma. Among the 10 publications available for meta-analysis based on age, 6 unique reference groups were identified for comparison. Hazard ratios comparing each age group versus the youngest group were obtained from reported univariable analyses or from multivariable analyses which adjusted for age. Overall, most hazard ratios were greater than 1 among older age groups, suggesting worse overall survival.

the benign drug, ganciclovir. This model was previously used to confirm that senescent cell eradication decreases inflammation, tumor metastasis, and relapse of non-CNS models of cancer.<sup>43,44</sup>

Targeting cellular senescence has been an area of research and development in both animal models and humans. Dasatinib and quercetin are senolytics that target the PI3K/Akt/mTOR pathway and are approved for use in humans. Treatment with dasatinib and quercetin enhances the longevity of animal models and alleviates aging-related phenotypes<sup>45,46</sup> by decreasing senescent cell numbers, enhancing cardiovascular function, and even ameliorating neurocognitive effects of AD plaque burden.<sup>47,48</sup> Initial studies using mouse brain tumor models treated with radiation (RT), anti-PD-1 mAb, IDO enzyme inhibitor, dasatinib, and quercetin improved long-term survival in older adults as compared to mice treated with RT, anti-PD-1 mAb, and IDO enzyme inhibitor without senolytics.<sup>49</sup> Although a phase II clinical trial in recurrent GBM for selective treatment with dasatinib was previously found to be ineffective at improving overall survival,<sup>50</sup> this result is in line with

recent studies in older adult mice when it is not further combined with both quercetin and immunotherapy. The consideration of the complex environment, inside of the tumor, as well as outside of the tumor but within the brain of older adults, should be evaluated for fully addressing the factors that promote GBM outgrowth and contribute to therapeutic resistance (Figure 3).

### Dynamic Interactions Between the Aging Brain, the Aging Immune System, and GBM

A robust anti-GBM immune response in adults requires (1) a fully competent immune system including both the CD4<sup>+</sup> and CD8<sup>+</sup> T cell lineages, (2) immune cell activation and specificity to GBM-specific antigens, (3) effective infiltration into the GBM microenvironment, and (4) the ability to eradicate GBM cells (Figure 4). Major limitations to maximal immunotherapeutic efficacy in older adults with GBM are due to the intratumoral immunosuppression that increases in part due to tumor-infiltrating T cells,<sup>51,52</sup> as well as due to

**Table 1.** CNS Disease States, SEER Database Analysis, TCGA, and Norwegian Database Analysis MSBASE.org

Condition	Median Age of Diagnosis (years)	Age-Adjusted Incidence (per 100 000)	Median Survival (months)	Standard of Care
<i>Glioma (astrocytoma)</i>	57	4.99	11.6	
Grade II (diffuse)	48	0.46	104.4	Resection, radiation
Grade III (anaplastic)	53	0.41	9.9	Resection, radiation, temozolomide
Grade IV (glioblastoma)	65	3.21	10.0	Resection, radiation, temozolomide
IDH WT GBM	68–70	NR	12.1 (mean = 14.0)	See above
IDH MT GBM	45–48	NR	24.2 (mean = 39.7)	
<i>Oligodendroglioma</i>	45	0.34	129.7	
Grade II	43	0.23	147.7	Surgery, radiation
Grade III (anaplastic)	50	0.11	63.8	Surgery, radiation, PCV
<i>Ependymoma</i>	44	0.43	150+	Resection, radiation, combo. Chemotherapy
<i>Meningioma</i>	66	8.33	78.4	
Grade I	66	8.23	116	Surgery
Grade II–III	65	0.10	76.4	Surgery, radiation
<i>Pituitary tumors</i>	51	3.94	120+	Surgery, radiation
<i>Metastatic lesions to the brain</i>				
Lung	61–80	8.17	5.8	Targeted therapy for driver mutation, surgery, stereotactic radiosurgery, whole-brain radiation therapy, immune checkpoint inhibitors, chemotherapies
Breast	61–80	0.33	10.0	
Colon	61–80	0.12	6.0	
Renal	61–80	0.33	5.0	
Melanoma	61–80	0.19	6.0	
<i>CNS lymphoma</i>	66	0.43	7.9	Methotrexate, radiation, rituximab
<i>Neurodegenerative disorders</i>				
Alzheimer's disease	75–84	148	48–96	Cholinesterase inhibitors, NMDA antagonist
Parkinson's disease	72.3	8–18	9 years	Dopamine agonists, NMDA antagonist, MOA B inhibitors,
Amyotrophic lateral sclerosis	34.0	1.6	20–48	Riluzole, edaravone
Huntington's disease	53.4	0.38	10–20 years	Tetrabenazine, deutetabenazine
<i>Stroke</i>	69.2	249.5	5–10 years	Thrombolytics, mechanical thrombectomy
<i>Autoimmune disorders</i>				
Multiple sclerosis	20–30	7.5	40.6 years	Ocrelizumab, Siponimod, Cladribine, interferon beta
Transverse myelitis	10–19, 30–40	0.134	Not reported	Glucocorticoids, IVIG, plasmapheresis
Neuromyelitis optica	40.1	0.053–0.4	Not reported	Methylprednisolone, PLEX, Eculizumab, Ocrelizumab, Inebilizumab, Satralizumab

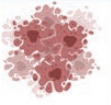
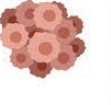


IVIG, intravenous gamma globulin; NMDA, N-methyl-D-aspartate; PCV, procarbazine, lomustine, vincristine; PLEX, plasma exchange. All info from UpToDate unless otherwise noted. See [Supplementary Material](#) for references.

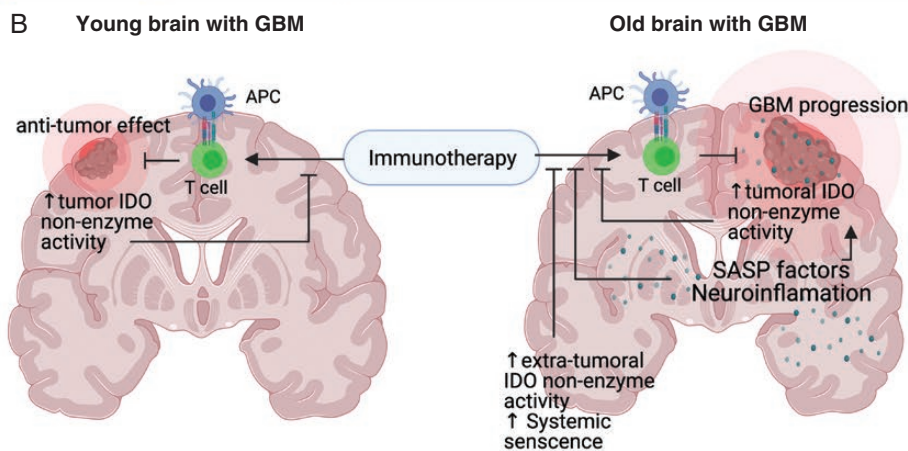
the immunosuppression in the brain that arises from aging-dependent mechanisms, and are the subject of an ongoing investigation by our group.<sup>24,25</sup> Compounding the impaired systemic and local antitumor response effects is the conservative option with respect to chemotherapeutic dose intensity, extent of resection, and radiation based on clinical evaluation of patient treatment tolerance among older adults, which places them at a therapeutic disadvantage. The complexity of managing a newly diagnosed GBM in older adults presents as a multidimensional challenge due to the consideration of

additional risk factors including frailty, medical comorbidities, compromised immune system status, and an increased risk and susceptibility to neurotoxicity with standard treatments.<sup>53</sup>

### Intratumoral Immune Suppression Is Independent of Age-Related Changes in the Brain

Factors expressed and cells that reside within the GBM microenvironment influence the strength of

A		Features	Young	Elderly
 Antitumor factors	Circulating Lymphocytes	Immune function, adaptive immunity, reduced in GBM patients	↓	↓↓
	CD8+ T Eff	PD1 expression, CTLA-4, LAG-3, Tim-3	↓	↓↓
	NK	Innate immune system, tumor lysis	↓	?
 Protumoral, immunosuppressive factors	Hypoxia	Promotion of VEGF and angiogenesis, reduces T cell activation and activity	↑	↑
	Microglia	Resident brain macrophages, can skew to M2 phenotype, promotes tumor proliferation and migration, MMP expression	↑	?
	CD4+PD1+CD62L- Exhausted T cell	PD1 expression, CTLA-4, LAG-3, Tim-3	↑	?
	TAM, MDSC	M2 phenotype, anti-inflammatoxy, PDL1 expression immune suppression	↑↑	?
	CD4+FOXP3+CD25+ T Regulatory cells	Suppress antitumor immunity by T Effector cells	↑	?
	moDCs and pbDCs	Antigen presentation, T cell anergy by low antigen presentation	↑	?
 Brain specific factors	IDO	PD1 expression, CTLA-4, LAG-3, Tim-3	↑	↑↑↑
	SASP factors	TGF- $\beta$ , IL-6, IL-8, MMPs	↑	↑↑↑
	Immunosuppressive Cytokines	IL-10, reduced IFN-g, CSF-1, reduce T cell recruitment and activation	↑	↑↑
	Neuroinflammation	PD-1, CTLA-4, LAG-3, Tim-3	↑	↑↑
	Surgical debulking	Reduced number of peripheral lymphocytes		
 Systemic factors	Thymic involution	Absolute decrease in naïve CD4+ and CD8+ T cells, decreased TCR diversity	—	↑
	Bone marrow sequestration	Reduced circulating lymphocytes	—	↑
	Systemic senescence, inflammaging	p16INK4a expression, chronic inflammation, neurotoxicity	—	↑↑
	Systemic steroids	Reduced number of peripheral lymphocytes, inhibits IL1 $\beta$ ,		
	Chemotherapy	Chronic treatment mediated lymphopenia, reduced circulating CD4+ counts		



**Figure 3.** Immunological factors associated with aging, GBM progression, and/or resistance to treatment. (A) Antitumor and pro-tumorigenic factors at the cellular level in young versus elderly patients. Specific factors at the level of the tumor microenvironment have not been fully examined in aging populations. Extratumoral brain-specific factors within young versus elderly patients and systemic features associated with young

anti-GBM immune-mediated tumor eradication potential. Immunosuppressive factors within the GBM microenvironment are primarily regulated by tumor cells<sup>54,55</sup> that are further enhanced by progressively increasing levels of tumor-infiltrating T cells.<sup>51,52</sup> Indications to date suggest that the immune suppression inside of the tumor is independent of age-dependent immunosuppressive mechanisms.<sup>25</sup> Due to this, the GBM can be considered in some ways to act as a time capsule that is insulated to ongoing age-dependent changes that are ongoing in the surrounding brain stroma. It is tempting to hypothesize that age-dependent changes also contribute to the initiation of precursor cells during a GBM cell-transforming event.

The cellular composition of the tumor microenvironment including surface ligands, such as the immune checkpoint inhibitor PD-L1, secreted cytokines including IL-10 and TGF $\beta$ , as well as senescence-associated proteins, create a complex landscape of immunosuppressive interactions between the tumor cells, stroma, antigen-presenting cells, and infiltrating immune cells. Furthermore, this landscape is dynamic in time. Tumor-infiltrating T cells induce immunosuppressive factors including IDO and PD-L1 in human GBM.<sup>52</sup> In humanized mouse models with intracranial human GBM, GBM-infiltrating T cells also promote the expression of interferon-sensitive immunosuppressive factors including PD-L1, PD-L2, and IDO.<sup>51,56</sup> IDO is canonically characterized as an interferon-inducible tryptophan metabolic enzyme that suppresses the immune response by facilitating immunosuppressive Treg recruitment and accumulation, which in turn, suppresses CD8<sup>+</sup> cytolytic T-cell effector actions.<sup>57</sup> Increased IDO expression in patient-resected glioma is inversely associated with patient survival.<sup>58</sup>

Since the intratumoral immunosuppressive environment appears to be age-independent, it is unknown as to how the age-dependent changes in the CNS parenchyma surrounding bulk tumor have such a dramatically negative effect on GBM patient outcomes. It remains possible that contributions by the increased levels of senescent cells, increased levels of neuroinflammation, and increased vascular abnormalities arising in the older adult brain have a collectively synergistic negative effect that enhances GBM cell migration and/or GBM margin invasion into the nontumor brain parenchyma. With respect to immune checkpoint blockade (ICB) that works well in a subset of patients with a variety of malignancies and age groups such as in melanoma, it is now critical to determine whether the resistance of ICB in GBM patients is the result of intra-GBM factors or primarily due to age-dependent extra-GBM factors in the brain parenchyma.<sup>59</sup>

### Treatment-Related Immune Changes in GBM Patients and Consequences for the Elderly

Standard of care treatment for GBM patients includes surgical debulking, radiation, and systemic chemotherapy

which generates lymphopenic conditions to a level that is comparable with HIV<sup>+</sup> AIDS patients. These lymphopenia-inducing effects may be amplified in older adults with GBM.<sup>60,61</sup> At the systemic level, immune cells from patients with high-grade glioma display systemic dysfunction of effector cells as compared to control patients. However, it is important to note that age-matched controls have not been carefully considered as exemplified by a study that prospectively evaluated GBM-diagnosed patients at a median age of 68 as compared to a healthy cohort with a median age of 56.<sup>61,62</sup> In a study of 219 newly diagnosed GBM patients with a mean age of 54.2 years, over 25% developed grade 1 lymphopenia (<1500 cells/mm<sup>3</sup>) and grade 3 lymphopenia (200–500 cells/mm<sup>3</sup>) was noted in 15–25% of GBM patients even prior to the initiation of the standard of care, with sustained lymphopenia lasting several months after the final cycle of chemotherapy.<sup>63</sup> In this study, 75% of patients were predicted to fully recover lymphocyte counts at a median of 240 days after final temozolomide dosing.<sup>62–64</sup>

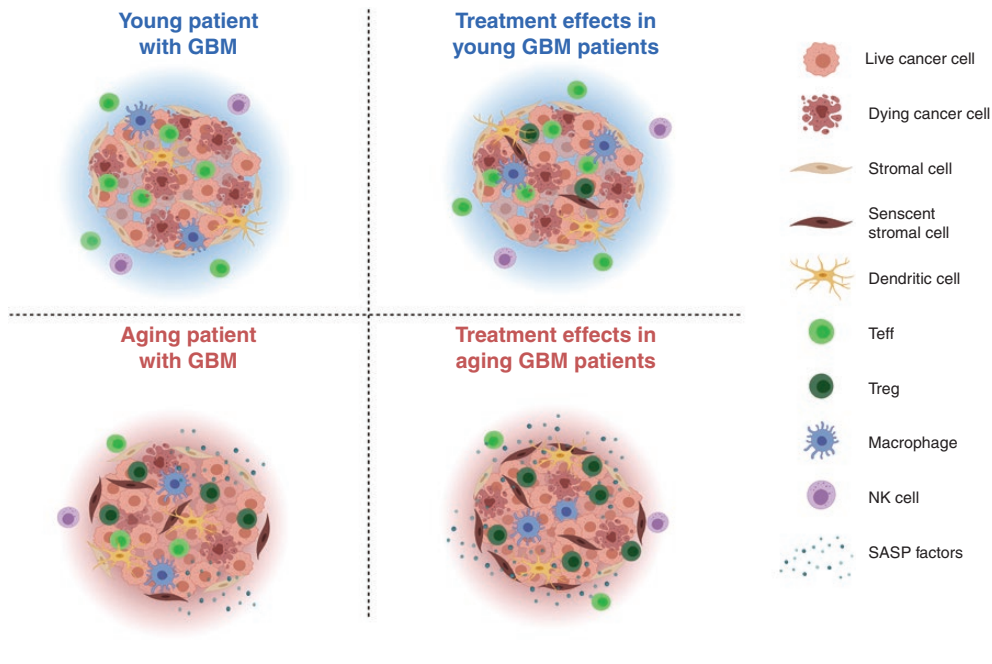
Aside from lymphopenia caused by chemoradiotherapy, corticosteroids that are routinely administered for ameliorating tumor-related symptoms or treatment-related edema may further exacerbate lymphopenia.<sup>65</sup> GBM patients who are treated with dexamethasone to address symptomatic cerebral edema were found to have a striking CD4<sup>+</sup> T-cell deficiency without a significant increase in the proportion of Tregs.<sup>66</sup> The combination of surgical resection with steroid use has also been demonstrated to impact the number of circulating T lymphocytes in animal models of surgical resection.<sup>67</sup> Evaluation of 65- to 86-year-old GBM patients found that only 57% had normal baseline total lymphocyte counts with a 41% reduction of total lymphocyte counts after beginning chemoradiation that persisted for at least a 12-month duration.<sup>68</sup> The older adult patients with high-grade lymphopenia demonstrated a significantly worse overall survival of 4.6 months as compared to patients with mild or moderate lymphopenia of 11.6 months.<sup>63,68</sup> Combining chemoradiotherapy while increasing tumor immunogenicity also affects circulating CD4<sup>+</sup> counts in the peripheral immune system.<sup>69</sup>

### Systemic Immune Changes in the Elderly and Implications for Antitumor Immunity

Outside the CNS, the aging body undergoes significant immunological changes that reduce the ability (1) to counter pathogens, (2) mount significant immune responses to standard vaccinations, and (3) to survey the landscape for neoplastic cells. Aging causes progressive thymic involution that decreases the formation of nascent T cells whereby up to 20% are generated by the thymus in younger individuals that decreases to <1% in

and elderly. Question marks indicate unexplored biology. (B) Working hypothesis of aging-dependent factors affecting antitumor immune responses. T-cell effector function is inhibited in young brains through intratumoral IDO expression. In contrast, aging brain T-cell effector function is impaired by tumor-expressing IDO, SASP factors, and associated neuroinflammatory changes within the brain parenchyma, as well as other extratumoral factors including nonenzymatic IDO activity and systemic senescence. Number of arrows indicates abundance with increases indicated by upward-facing and decreases indicated by down-facing. Created with BioRender.com.





**Figure 4.** The intra- and extratumoral environment changes with age and treatment. A hypothetical schema for describing factors in and around the brain tumor which contribute to malignant progression and response to therapy with age-dependent changes and therapy-related changes. Blue indicates a more immunocompetent antitumor response with increased  $T_{eff}$  (bright green) response and adequate tumor killing. Red indicates a progressively immunosuppressive tumor environment with increased age, recruiting more  $T_{reg}$  cells (dark green), increasing SASP factors, and treatment-related immunosuppressive changes including the reduced activity of  $T_{eff}$  cells and reduced tumor killing. Adapted from “Cold vs Hot Tumors” BioRender.com (2021). Retrieved from <https://app.biorender.com/biorender-templates>. Created with BioRender.com.

individuals older than the age of 50.<sup>70</sup> Additionally, there is a relative and absolute decrease in naïve  $CD4^+$  and  $CD8^+$  T cells coincident with a decrease of TCR diversity in older adults.

Immunosuppressive Tregs increase in the peripheral circulation of GBM patients and have an increased migratory potential toward tumor-conditioned media in vitro. Strikingly, this effect was skewed to favor younger GBM patients with a median age of 54.<sup>58,71,72</sup> Immunosuppressive myeloid-derived suppressor cells are increased in the circulating peripheral blood, bone marrow, spleen, and tumors of patients with various types of cancers including glial neoplasms, melanoma, and pancreatic tumors. However, their distribution across the different age groups has not been well studied.<sup>73–75</sup>

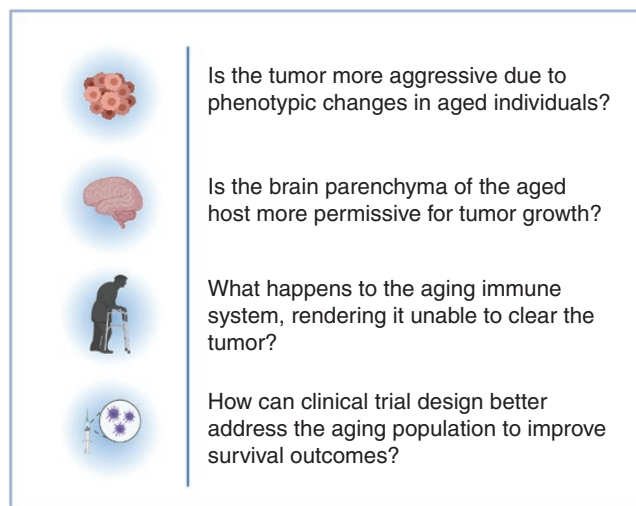
Within the bone marrow, fat deposition increases in an age-dependent manner,<sup>76</sup> and hematopoietic stem cell differentiation shifts from a predominantly lymphoid- to myeloid-based developmental output.<sup>77</sup> Peripheral blood DCs (pbDCs) from aged humans have an increased activation state as demonstrated by their increased CD83 and CD86 expression<sup>78</sup> and conversely, myeloid DCs (mDCs) produce less pro-inflammatory cytokines including IL-6, IL-12, and TNF- $\alpha$  with age.<sup>78,79</sup> Monocyte-derived DCs (moDCs) have a limited ability to activate lymphocytes<sup>80</sup> and both moDCs and pbDCs have a decreased ability to produce IFN- $\gamma$  in older adults.<sup>79,81–85</sup> The composition of peripheral blood  $CD4^+$  T cells declines with age<sup>86</sup> and becomes enriched with Tregs while exhausted  $CD4^+PD1^+CD62L^-$  T cells

become more common and the absolute number of circulating naïve  $CD4^+$  T cells declines.<sup>87</sup> The effects of aging inside of the bone marrow, on the adaptive immune system, and their combined relationship to changes on host neuroimmunology have yet to be investigated (Figure 4).

## Conclusions and Future Directions

Older adults with GBM pose a unique challenge to clinicians and researchers as preclinical animal models have yet to provide a vehicle that translates beneficial results into human clinical trials. Some of this failure is likely due to the lack of analogous ages between older human adults with GBM and the age of the mouse with a brain tumor undergoing experimental evaluation.<sup>24</sup> The analogous age of a 65-year-old human patient with GBM is equivalent to ~90 weeks of age for a C57BL/6 mouse with a brain tumor. Studying the age-relevant animal brain tumor model to recapitulate the physiology of advanced age in the brain can become costly and time-intensive as survivorship and care of these animals can extend into the timeline of years. Although these studies require an increased length of time and financial support, the preclinical brain tumor modeling outcomes may better predict which therapies will benefit the large majority of IDH<sup>wt</sup> GBM patients.

A gradual functional decline that accompanies the aging process impacts multiple organ systems and influences



**Figure 5.** Age-specific questions that remain to be explored in the setting of glioblastoma.

the extent of treatment that elderly patients with GBM receive.<sup>88,89</sup> Optimal treatment for GBM has not changed significantly and the extent of resection at initial presentation and tumor recurrence has been shown to have a significant impact on both progression-free survival and overall survival in both younger and older patients.<sup>90</sup> Preexisting medical comorbidities often confer elderly patients as less than optimal candidates for major surgical procedures and treatment modalities. In particular, cardiopulmonary disease, the use of anticoagulant or antiplatelet agents, and the presence of cardiac implanted devices can make surgery less safe and render the patient as a biopsy candidate rather than for consideration of complete resection. These issues also complicate eligibility criteria for enrolling in clinical trials due to the need to perform MR imaging over time. Our analysis of published phase III clinical trials collectively demonstrated that current studies do not adequately evaluate treatment options for the elderly despite the evolution of care as it has expanded into immune-based therapies and chemotherapeutic agents. Limitations of our observations and evaluations of the database include the difficulty in isolating IDH<sup>wt</sup> tumors from the SEER database over the course of study and also the lack of clinical trial data that routinely stratify patient outcomes by age, thus yielding a descriptive analysis of the listed clinical trials. These results beg the question: How do clinicians and scientists address phenotypic changes of older adult patients with GBM to aim for better outcomes (Figure 5)?

Future studies of GBM should go beyond the understanding of intratumoral changes and should also incorporate the age-dependent changes in the brain that affect subject outcomes. As further genetic biomarkers of aging are identified, patient stratification and the identification of easily targeted proteins, surface markers, or immune cell populations may yield improved results. Studies to fully evaluate the immunologic landscape of older adults with GBM in the periphery and in the tumor microenvironment may help elucidate targetable pathways for improved overall survival

and quality of life. Multimodal combination therapies that target senescence, inflammaging, and the immunosuppressive environment of elderly patients will likely become necessary during future therapeutic approaches that address the negative effects of tumor and brain aging, simultaneously, for improving overall survival in IDH<sup>wt</sup> GBM patients.

## Supplementary Material

Supplementary material is available at *Neuro-Oncology Advances* online.

## Keywords

aging | CD4 | glioma | IDO | immunotherapy | senescence

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