The Clinical and Multi-Omic Prognostic Factors of Glioblastoma Multiforme

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I. Introduction

Glioblastoma multiforme (GBM) is an advanced-stage central nervous system tumor of frequent primary origin via aggressive, spontaneous oncogenesis (Urbańska, 2014). It has a frequency of approximately 5-6 individuals per 100,000 and accounts for over a half of all malignant gliomas, totalling to 250,000 cases annually worldwide (Alifieris et al., 2015). Though rare in incidence, GBMs have a very poor prognosis of an average of 14-15 after diagnosis with a peak incidence of diagnosis at ages 55-60 (Hanif et al., 2017). Other epidemiological factors for GBMs include patient sex, race, ethnicity, country of residence, and in a few cases, family history. Risk factors for GBMs, along with other gliomas, include prior extensive exposure to radiation therapy, employment in synthetic rubber manufacturing or petroleum refining, exposure to vinyl chloride or pesticides, diagnosis of rare hereditary diseases including neurofibromatosis, and more (Brandes et al., 2008). Clinical indicators of GBMs include but are not limited to headaches, focal neurologic deficits, confusion, memory loss, personality changes and/or seizures. A traditional treatment course for GBM entails maximal surgical resection with radiotherapy with concomitant and adjuvant DNA-alkylating and cross-linking agents.

It is germane to identify potential prognostic factors for GBMs to develop personalized treatment regimes for patients and potentially achieve enhanced prognostic outcomes. Although a vast majority of GBM cases present with a constrained and poor prognosis, there still exists significant variation in morbidity and mortality among patients of varying clinical profiles. Previous literature notes differential therapeutic efficiency and overall prognostic outcomes

based on patient sex, age at initial diagnosis, race, and other epidemiological factors. However, little has been investigated regarding the molecular basis of why these epidemiological factors can contribute to varying prognoses. This study aims to test the veracity of such clinical factors as prognostic indicators on a novel cohort of GBM patients, as well as to uncover multi-omic insights into the potential molecular mechanisms underlying differences in prognosis among GBM patients. The Clinical Genome Atlas (TCGA) Data Bank and Clinical Proteomic Tumor Analysis Consortium (CPTAC) operated by the National Cancer Institute offers a trove of relevant clinical, genomic, transcriptomic, and proteomic datasets attributed to 590 cases of GBM for TCGA and 115 cases for CPTAC (The Cancer Genome Atlas Network, 2013; Rodriguez et al., 2021). Access to pertinent patient clinical, genomic, transcriptomic and proteomics profiles for GBM patients via TCGA and CPTAC offers a promising avenue for the identification of genes (and their implicated biomolecular pathways) that are differentially expressed among GBM patients of varying clinical profiles. Herein, we will utilize the clinical and mutation annotated format (MAF) databases of the TCGA-GBM Project to identify commonly mutated genes or mutation types associated with different clinical factors and their impact on patient survival rates as obtained via co-lollipop and mutational co-occurrence plots. We will construct transcriptomic-proteomic heatmaps and UMAP plots to further explore multi-omic variations in GBM molecular profiles based upon patient clinical factors. We will also utilize the drug sub-dataframe of the clinical database to observe the effects of various clinical factors on patient survival rates when administered various chemotherapeutics as observed in Kaplan-Meier plots. Overall, exploring the genetic and biomolecular basis of GBMs can be fruitful in regards to developing new diagnostic and prognostic biomarkers and determining the patient's therapeutic course. These analyses will corroborate previous findings

that patient age and race can serve as potential prognostic factors for most GBMs (Kokudo, N. et al., 2019). However, our findings will challenge the notion that patient sex can be a statistically robust prognostic factor for GBM. Finally, our findings support the hypothesis that such prognostic clinical factors indicate underlying differences in patient multi-omic profiles, meaning that there exist variations within the multi-step oncogenesis underlying GBM and contributing to differential mutation frequencies and molecular pathways impacted.

II. Methods

All statistical analyses were conducted locally utilizing R statistical software and the Python programming language. The ggplot2, BiocManager, TCGAbiolinks, maftools, survival, survminer and dplyr R libraries were installed and loaded prior to any analysis. For Python, the cptac, pandas, numpy, scipy, seaborn, matplotlib, and sklearn were installed and imported. Clinical, drug and MAF data from NCI's TCGA database was queried, downloaded and processed into a local dataframe utilizing the accession code "TCGA-GBM". Proteomic data was accessed via the accession code of "Gbm " on NCI's CPTAC database. The R library ggplot2 was used to construct a boxplot comparing the patient's age at initial pathologic diagnosis and sex/tumor site for each sample, as well as to visualize the distribution of Karnosfky scores among all samples. The clinical dataframe was processed by the R libraries survival and survminer to construct several Kaplan-Meier survival plots based on patient age, sex, tumor site and Karnofsky score classification. The R library maftools was used on the MAF database to construct an oncoplot of all GBM samples, as well as to construct a co-oncoplot of the samples stratified by age. The maftools library was also utilized to construct several co-lollipop plots of genes of interest and several somaticInteractions plots comparing the overall GBM samples to age-stratified samples. Utilizing the Python library cptac, GBM clinical and multi-omic data was

obtained and processed to plot an overall transcriptomic-proteomic heatmap, as well as heatmaps stratified by age and race. Further Python analysis entailed processing patient clinical and transcriptomic data to construct UMAP plots with the sklearn library using age and race as separate parameters. The drug data dataframe was counted via the R library dplyr and the top 4 most common drug names were saved into a vector. The surv and survminer libraries used this list to construct Kaplan Meier plots of the overall GBM sample set, and the two age-based subsets, each containing four curves corresponding to each of the top four drugs.

III. Results

The five primary clinical parameters of interest included the patient's age, sex, race, Karnofsky evaluation score, and tumor site. First, it was found that both patient sex and malignant neoplasm site did not demonstrate a statistically significant relation with age at initial pathologic diagnosis, allowing for survivorship comparisons (Figures 1 & 2).

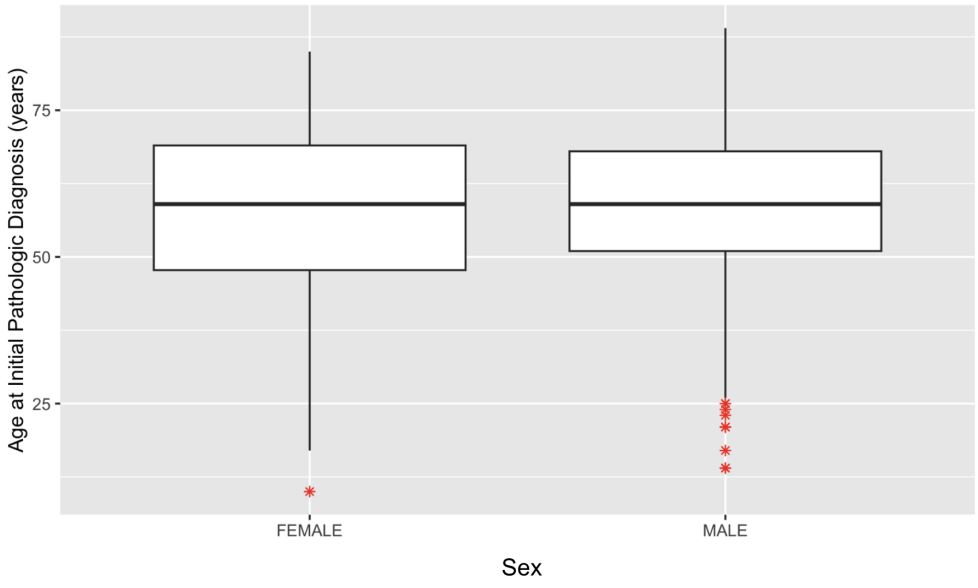

Figure 1. Boxplots representing the distribution of the age at initial pathologic diagnosis stratified by patient sex. There is clear statistical overlap among both plots, indicating no significant difference in age at diagnosis among the sexes.

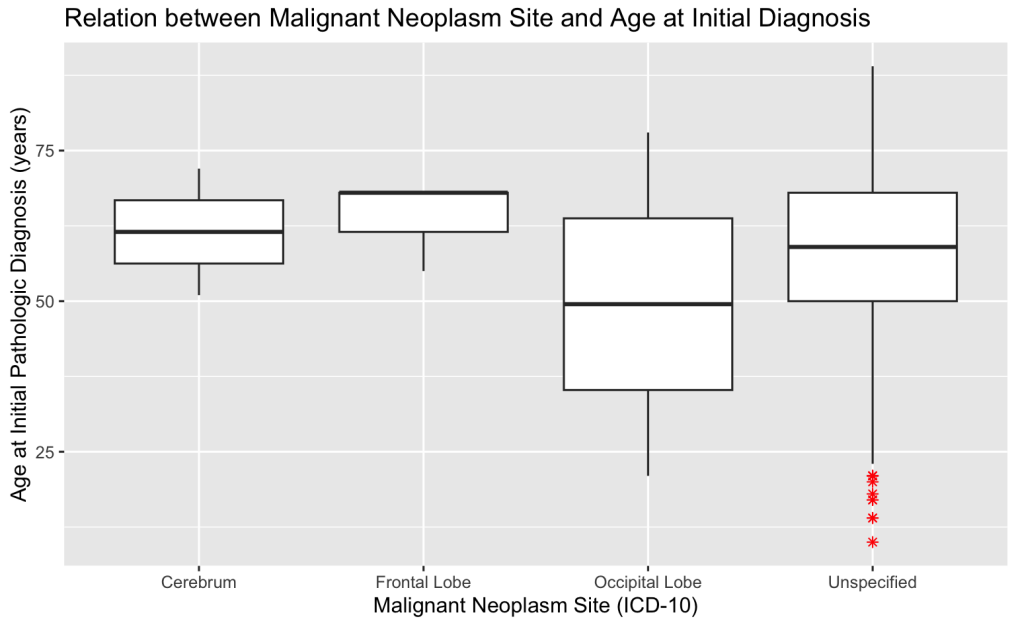

Figure 2. Boxplots representing the distribution of the age at initial pathologic diagnosis stratified by tumor site (based on ICD-10 classification system). There is clear statistical overlap among both plots, indicating no significant difference in age at diagnosis based upon the location of the neoplasm.

After conducting survivorship analysis for each of the five pertinent clinical factors, we observed no statistically significant differences in survivorship when stratifying by patient sex and tumor site (Figures 3 & 4).

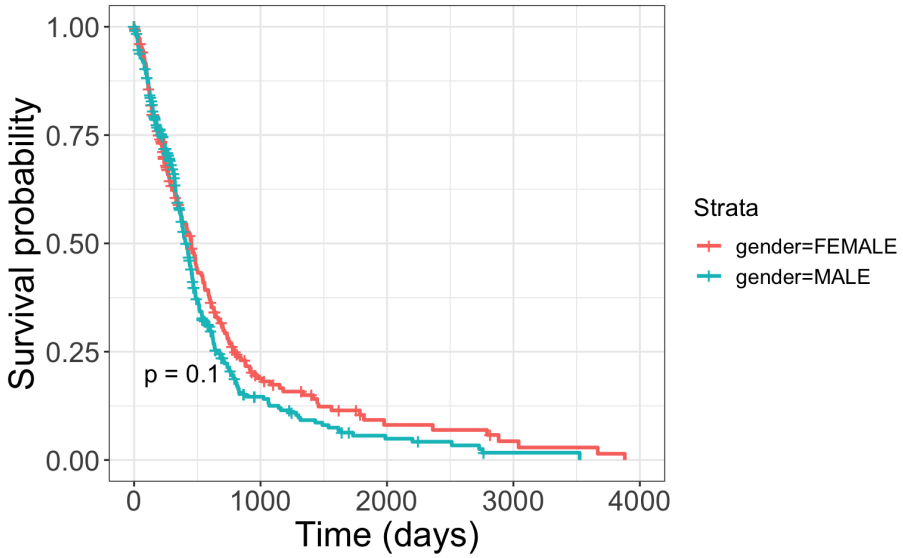

Figure 3. Kaplan-Meier survivorship curve stratified by patient's biological sex shows no statistically significant differences in GBM prognosis. P-value of 0.1 is larger than 0.05, meaning that we fail to reject the null hypothesis that there is no significant difference in patient survivorship based upon their sex.

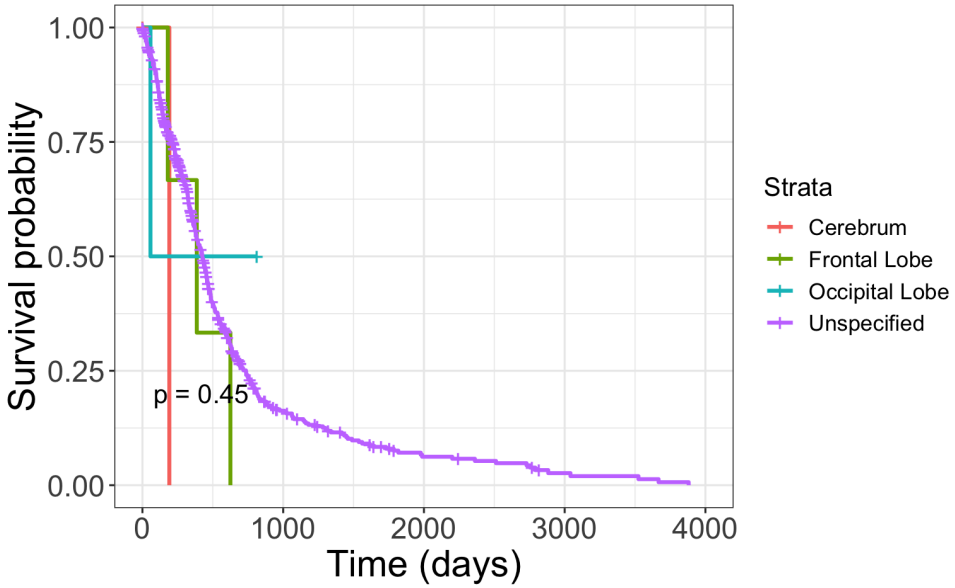

Figure 4. Kaplan-Meier survivorship curve stratified by patient's tumor site shows no statistically significant differences in GBM prognosis. P-value of 0.45 is larger than 0.05, meaning that we fail to reject the null hypothesis that there is no significant difference in patient survivorship based upon the location of their neoplasm.

However, when stratifying based upon the patient's race, Karnofsky scores and age, we observed statistically significant differences in prognostic outcome favoring younger, Asian patients with excellent Karnofsky scores (indicating little to no morbidity) [Figures 5, 7 & 8]. It must be noted that the relationship observed for race is not entirely conclusive due to the scant sampling of only 13 Asian patients within our cohort. Age classifications were done on the condition of whether the patients exceeded the age of 50 years old, with old classified as >50 and young classified as <= 50. Prior analysis of Karnofsky scores demonstrated a skewed right distribution that was fair for survivorship analysis (Figure 6). Karnofsky scores were binned into three categories: poor (indicating scores < 50), moderate (scores < 80), and excellent (scores of 80 or above). Patients

lacking one or more of these factors were either excluded in the survivorship analysis or denoted as "Unspecified".

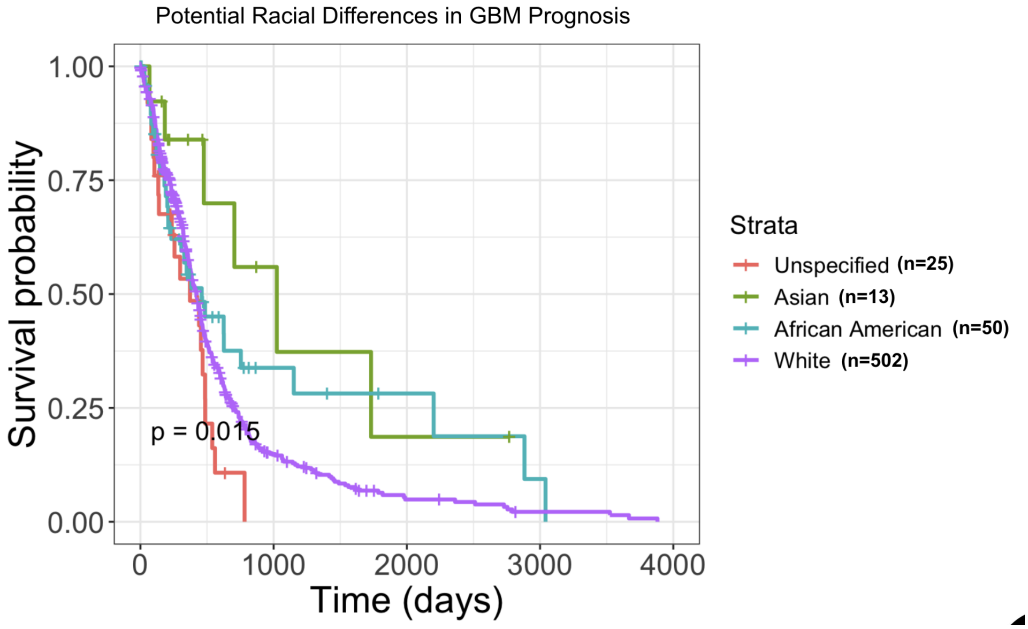

Figure 5. Kaplan-Meier survivorship curve stratified by patient's race shows some statistically significant, but not entirely conclusive differences in GBM prognosis. P-value of 0.015 is less than 0.05, meaning that we can reject the null hypothesis that there is no significant difference in patient survivorship based upon the patient's race, with Asian patients exhibiting enhanced survivorship compared to White and African American cohorts. However, the small sample size of only 13 Asian patients precludes a definitive conclusion that such a relationship is robustly significant.

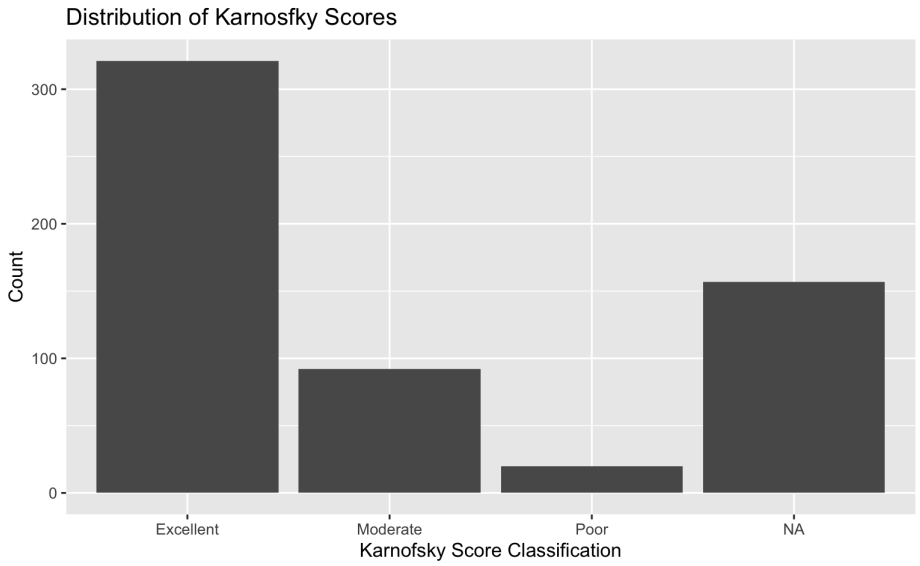

Figure 6. Histogram of Karnofsky scores within the 590 patient GBM cohort show a skewed right distribution that does not preclude further survivorship analysis, as classification of "Poor" still has an adequate sample size of 20 patients.

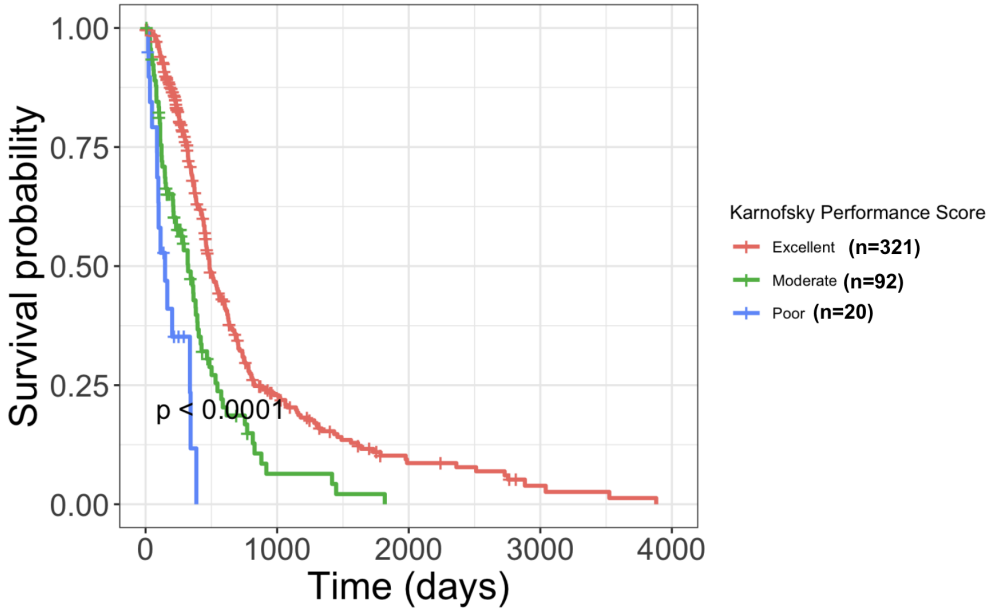

Figure 7. Kaplan-Meier survivorship curve stratified by patient's Karnofsky score classification shows statistically significant differences in GBM prognosis, favoring patients achieving excellent morbidity scores. P-value of 0.0001 is less than 0.05, meaning that we can reject the null hypothesis that there is no significant difference in patient survivorship based upon the patient's morbidity score.

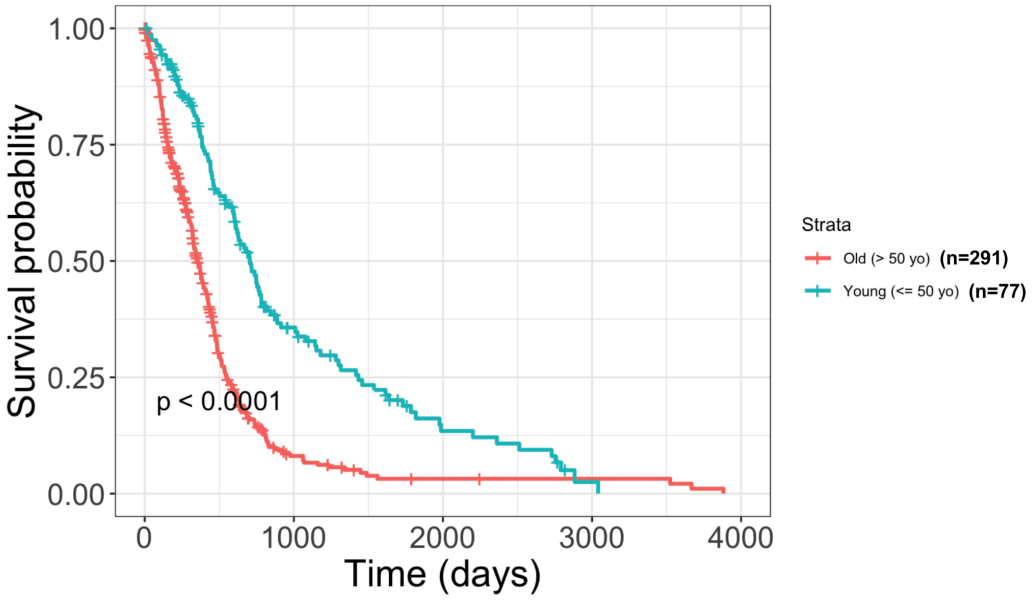

Figure 8. Kaplan-Meier survivorship curve stratified by patient's age classification shows statistically significant differences in GBM prognosis, favoring patients of younger age. "Young" patients were classified as 50 years of age or younger, while patients older than 50 were designated as "Old". P-value of 0.0001 is less than 0.05, meaning that we can reject the null hypothesis that there is no significant difference in patient survivorship based upon the patient's morbidity score.

As evident by the survivorship curves, race and age are two primary clinical factors that can potentially serve as indicators of prognosis for a given GBM patient. However, the scant nature of the Asian cohort within our GBM patient population challenges the robustness of race as a prognostic factor. Future investigations with an adequately sized Asian GBM patient cohort is necessary for a definitive conclusion. Therefore, we primarily analyzed age-stratified patient data for our multi-omic analyses due to adequate sample sizes for both the old and young subsets. An overall oncoplot of non-stratified patient mutation data shows frequently-implicated cancer genes including PTEN, TP53, EGFR, and PIK3CA (Figure 9). There are few neurodevelopmental and musculoskeletal genes involved such as TTN, which is a large protein component of skeletal muscle.

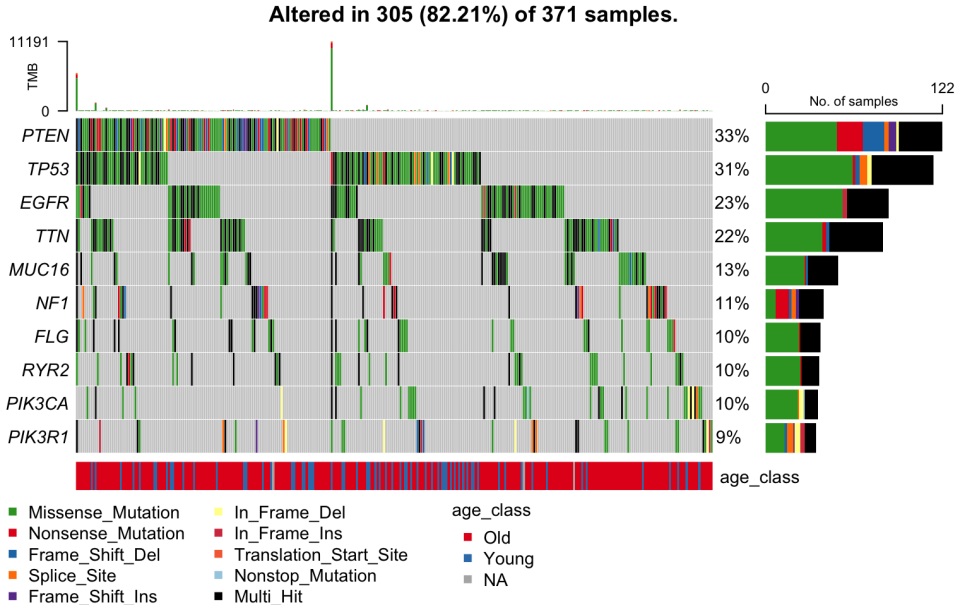

Figure 9. An oncoplot of all tissue samples of GBM patients demonstrates mutations affecting mainly general cell growth/proliferation pathways and tissue-specific pathways in the CNS. Only the top 10 most prevalent mutated genes are shown on this plot.

We observe significant differences in mutation profiles when stratifying the patient population by age, as TP53, ATRX, and IDH1 are expressed by a differential of greater than 10% in favor of younger patient populations (Figure 10). Other genes among the top 7 shown are expressed in similar frequencies and entail general cell growth and proliferation genes that are commonly implicated in cancer.

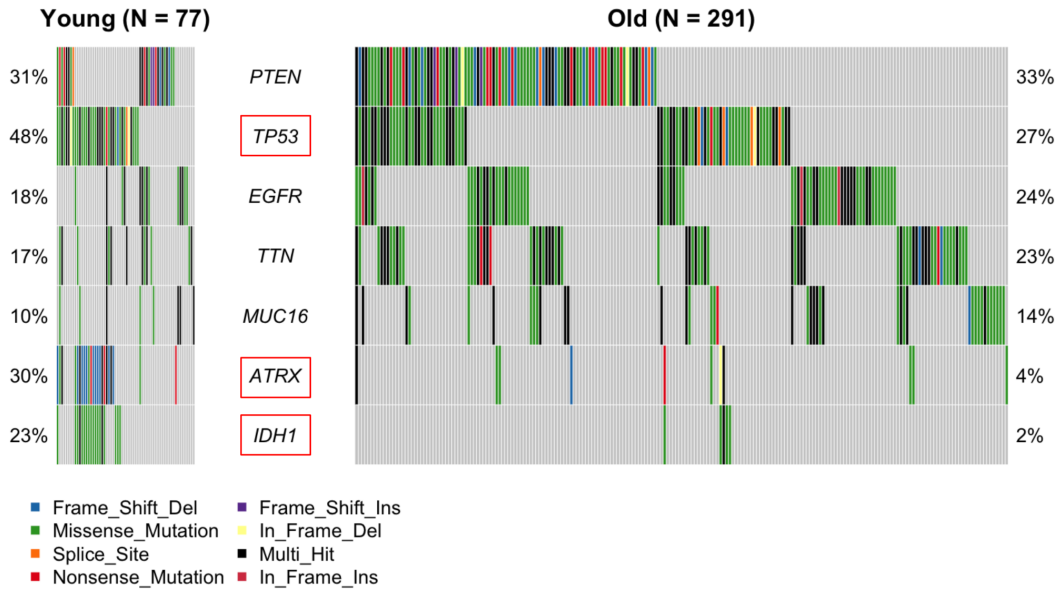

Figure 10. An oncoplot of age-stratified tissue samples of GBM patients demonstrates significantly different mutation frequencies for various general cell growth and CNS-specific genes. Only the top 7 most prevalent mutated genes among both cohorts are shown on this plot.

We can analyze each of the three differentially expressed somatic mutations to make inferences regarding potentially different molecular pathways that may be implicated in glioblastomas among younger and older patient populations. The co-lollipop plot for ATRX demonstrates similar mutational sites and types across the gene.

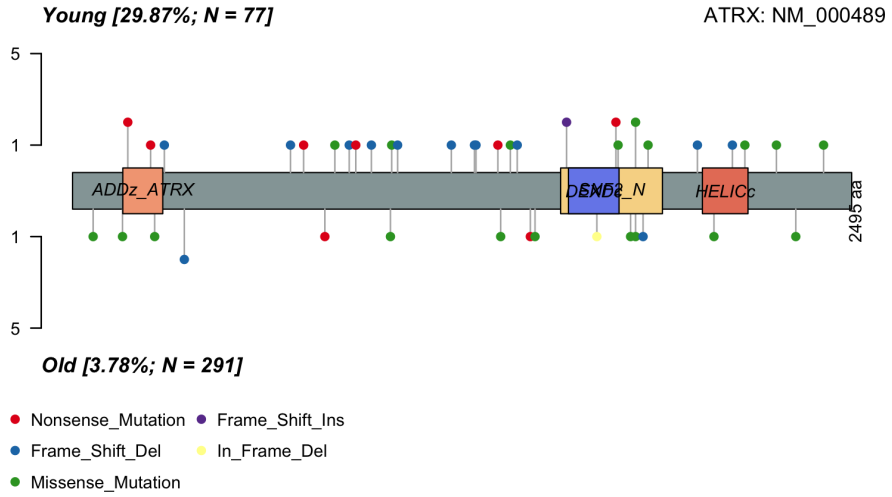

Figure 11. Co-lollipop plot of ATRX stratified by patient age shows no marked difference in both mutation site and type for major sites. The large differential expression of this gene may indicate a more extensive involvement of this molecular pathway in glioblastomas affecting younger patients.

For the prevalent tumor suppressor gene TP53, we observe a distinct mutational profile between the two cohorts as younger patients are impacted with a greater number of in-frame deletions while older patients exhibit greater frequencies of splice sites and nonsense mutations (Figure 12). Mutation sites are more or less similar for both cohorts, while there is perhaps a slightly more uniform distribution for the older patient cohort.

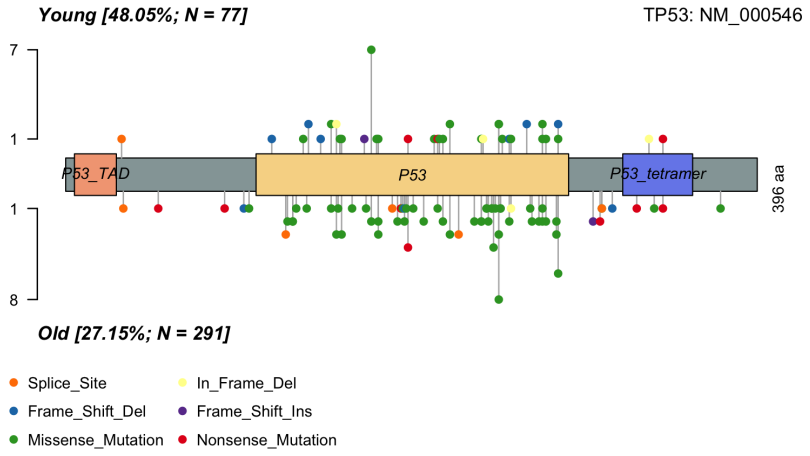

Figure 12. Co-lollipop plot of TP53 stratified by patient age shows slight differences in mutation types for major sites. Older patients are impacted by a greater number of splice site and nonsense mutations, while younger patients experience more in-frame deletions.

For IDH1, we observe nearly identical mutation site and type among both cohorts (Figure 13). However, there are evidently different expression rates favoring younger patients, indicating that IDH1 may be a novel non-general molecular pathway that could be underlying prognostic differences based on age.

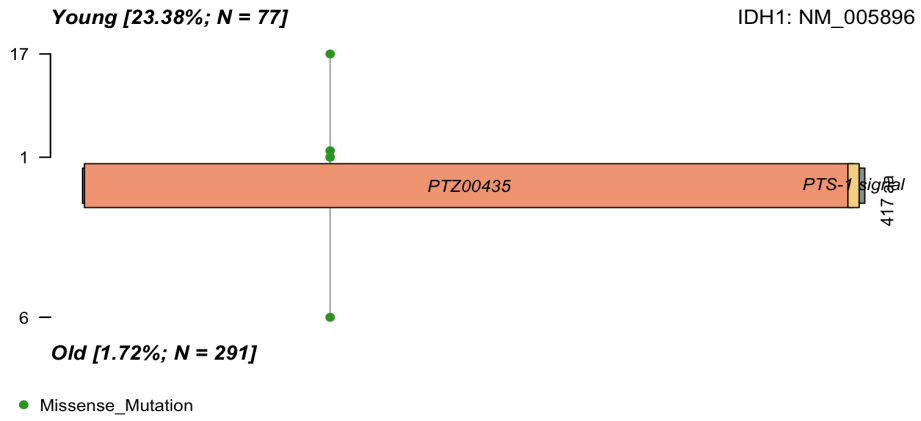

Figure 13. Co-lollipop plot of IDH1 stratified by patient age shows nearly identical mutation types and sites. However, differences in expression rate indicate that IDH1 may be implicated in a novel molecular pathway that impacts prognostic outcomes between the two cohorts.

While there are slight differences in individual gene expression frequency, mutation site, and mutation type, it is also imperative to analyze gene expression relationships utilizing somaticInteractions plots. We first constructed a somatic interaction plot for the general patient cohort, revealing few significant mutually exclusive relations and numerous co-occurrences (Figure 15a).

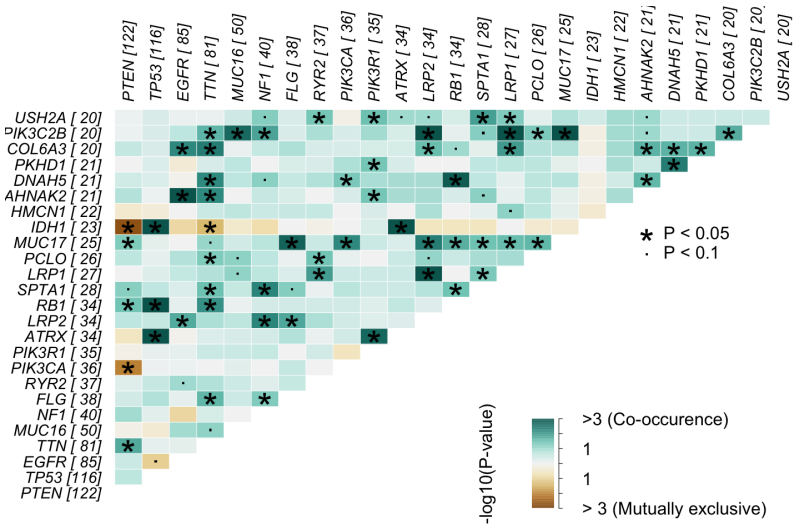

Figure 15a. Somatic interaction plot of GBM mutation data NOT stratified by age (generated via somaticMutations) shows a mix of statistically significant co-incidences and mutually exclusive relationships.

Analyzing mutual exclusivities can be a strong differentiating factor since they are relatively sparse and variable. When stratified by age, our somatic interaction plots demonstrate a unique signature of co-occurrences and mutual exclusivities that are easily discernible (Figures 15b & c). For younger patients, there is a lone mutual exclusive relation between IDH1 and PTEN that is unique to this subset. There is an abundance of moderate and strong co-occurrences that outnumber the older patient cohort. For older patients, we observe unique mutually exclusive relations between PIK3CA & PTEN and NF1 & EGFR that are unique to this subset. Co-occurrences are abundant and not easily distinguishable from younger patients.

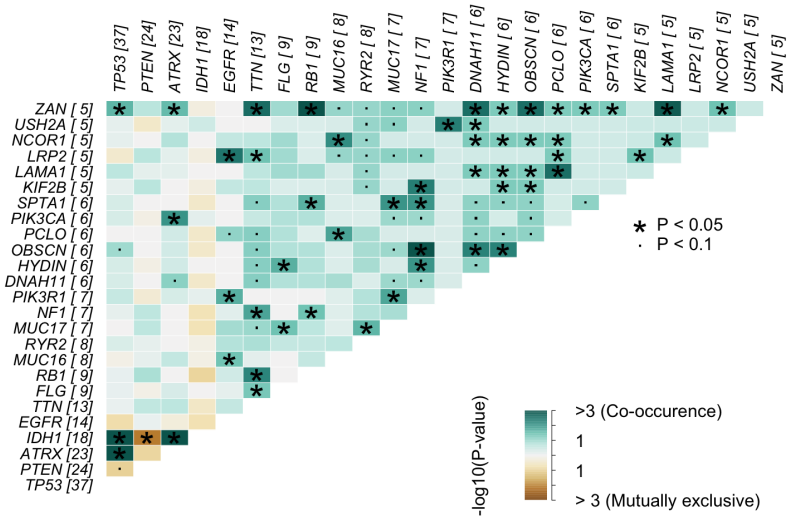

Figure 15b. Somatic interaction plot of GBM mutation data for the younger patient cohort (generated via somaticMutations) shows an abundance of statistically significant co-occurrences and a singular mutually exclusive relationship between IDH1 & PTEN. Analyzing mutual exclusivities can be a strong differentiating factor since they are relatively sparse and variable.

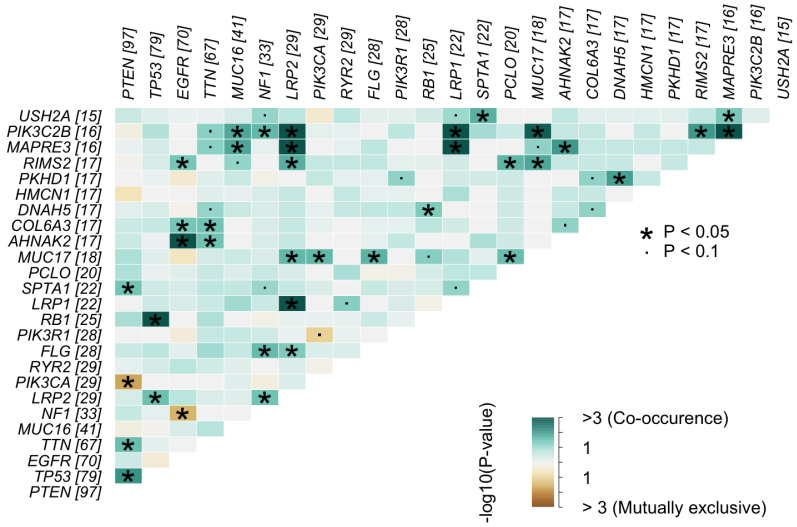

Figure 15c. Somatic interaction plot of GBM mutation data for the older patient cohort (generated via somaticMutations) shows an abundance of statistically significant co-occurrences and a pair of mutually exclusive relationships between PIK3CA & PTEN and NF1 & EGFR. Analyzing mutual exclusivities can be a strong differentiating factor since they are relatively sparse and variable.

Having obtained several genes of interest from our co-oncoplot and somatic interaction plot analyses, we can now construct general and age-stratified heatmaps evaluating the relation between transcriptomic and proteomic expression of these genes. First, we constructed a heatmap of the non-stratified total data to reveal a fair composition of positive and negative correlations (Figure 16). When stratifying our heatmaps by patient age, we observe nearly identical heatmaps indicating that the transcriptomic and proteomic profiles for older and younger patients are very similar (Figure 17). However, when stratifying by race, we observe slightly different expression correlations for the gene IDH1, which was identified earlier for its unique genomic mutation frequency in younger patient populations (Figure 18).

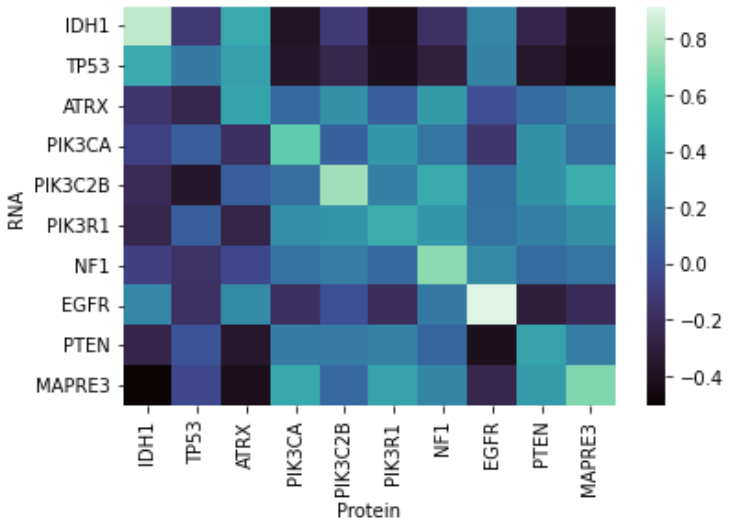

Figure 16. Transcriptome-proteome correlation heatmap for the overall patient cohort shows a fair balance of positive and negative expression correlations for ten identified genes of interest.

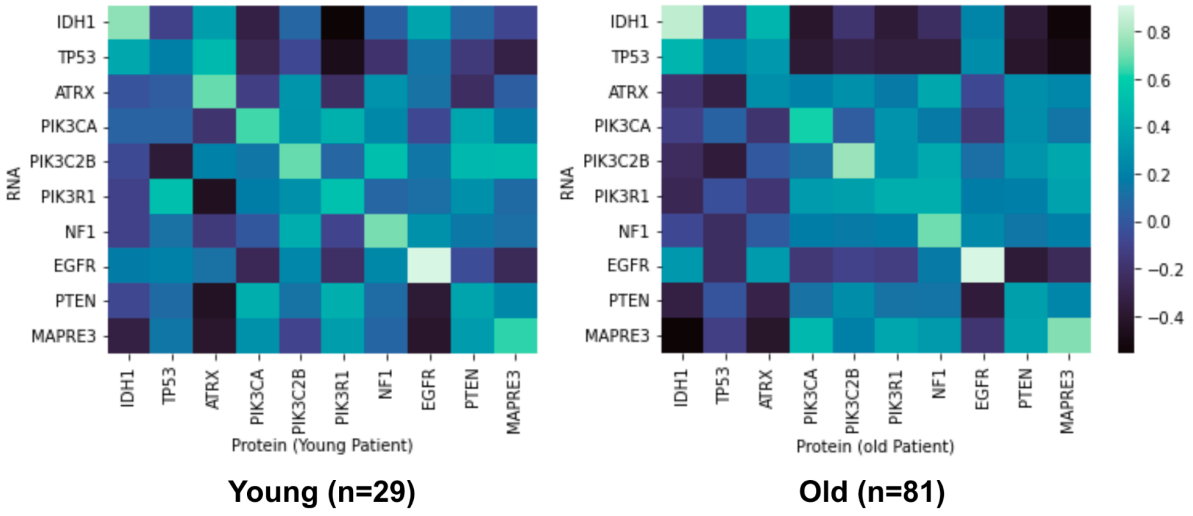

Figure 17. Transcriptome-proteome correlation heatmap for patient cohorts stratified by age demonstrates nearly identical expression profiles for older and younger patient populations, indicating minimal transcriptomic and proteomic differences.

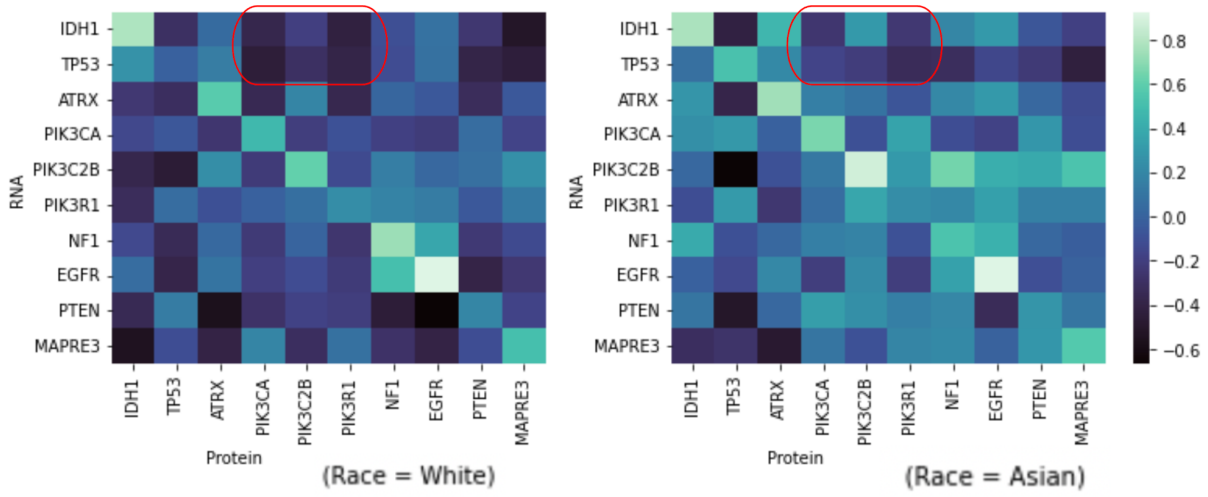

Figure 18. Transcriptome-proteome correlation heatmap for patient cohorts stratified by race (primarily White and Asian due to scarcity of African American and other race patients) demonstrates minor differences in specifically IDH1 and TP53 expression correlations. Younger patients demonstrate a more positive correlation between IDH1/TP53 transcriptome and proteome expression levels, indicating a potential upregulation of the molecular pathways underlying these genes compared to White patients.

Finally, the UMAP algorithm was executed on patient transcriptomic data stratified by race and sex respectively to ultimately show no discernable clustering pattern for either plot (Figures 19 & 20).

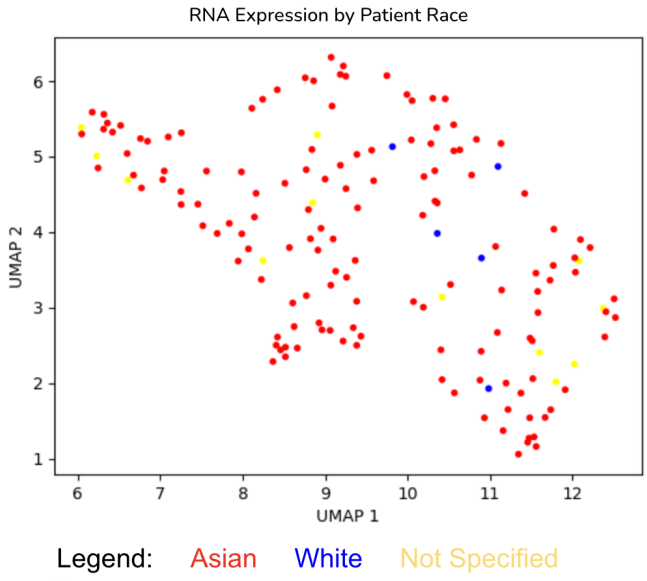

Figure 19. UMAP plot constructed based upon patient transcriptomic data and race shows no distinct clustering pattern, potentially indicating no latent underlying pattern between these dimensions.

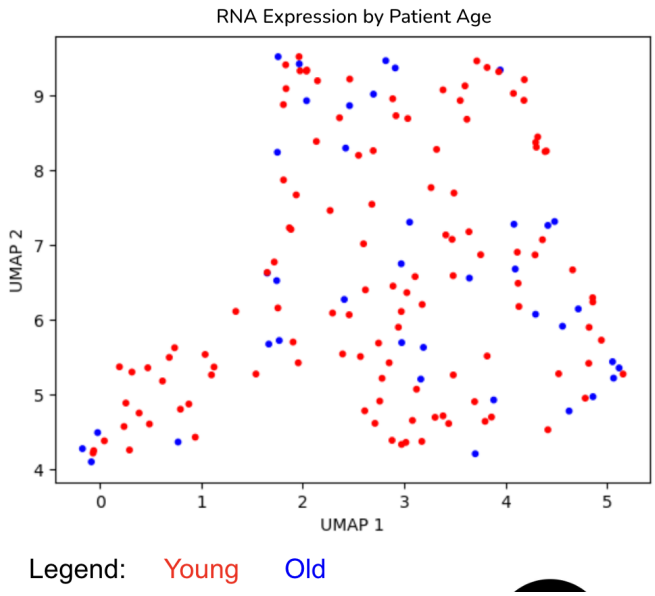

Figure 20. UMAP plot constructed based upon patient transcriptomic data and age shows no distinct clustering pattern, potentially indicating no latent underlying pattern between these dimensions.

Now equipped with the notion that patient age can serve as a significant clinical prognostic factor for glioblastomas, we can compare the efficacy of the top four most commonly administered glioblastoma drugs among younger and older patient cohorts to see if we observe differential efficacies as potentially indicated by the differential prognoses. For the non-stratified overall patient cohort, we observe some statistically significant difference in the efficacy of the top four administered drugs, specifically favoring Carmustine over Lomustine (Figure 21b). Interestingly, however, we only observe the statistically significant difference in drug efficacy in the young cohort particularly with the drug Carmustine, an alkylating agent that crosslinks with DNA to prevent its replication and transcription (Figure 21b). Meanwhile, we observe no

statistically significant difference among the efficacies of the four drugs in the older patient cohort (Figure 21b).

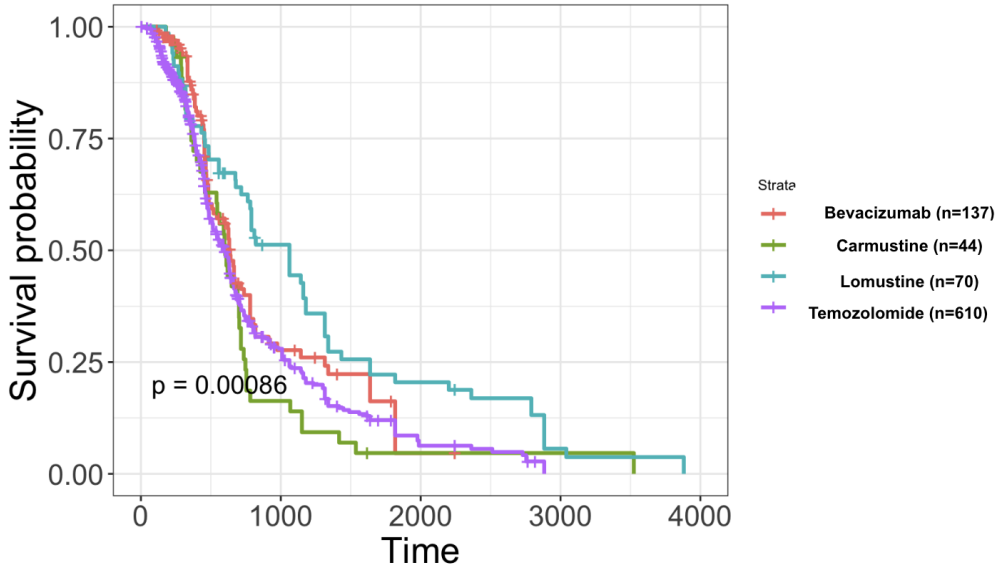

Figure 21a. Kaplan-Meier survivorship curve NOT stratified by patient age classification shows statistically significant difference in treatment efficacies of top four most administered GBM drugs, favoring Carmustine.

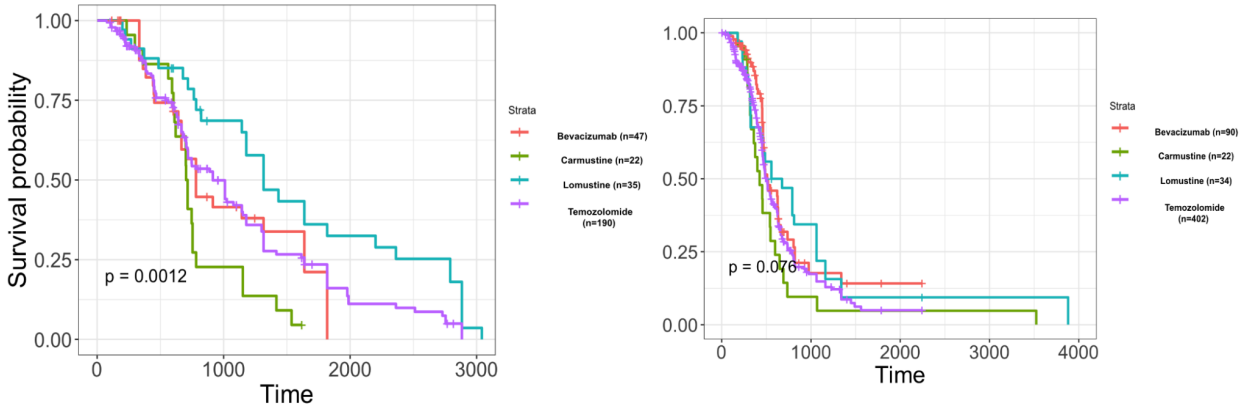

Figure 21b. Kaplan-Meier survivorship ship stratified by patient age classification shows a statistically significant difference in the treatment efficacy of Carmustine compared to its counterparts in YOUNGER patient cohort. However, no statistically significant difference in treatment efficacy is observed in the OLDER patient cohort.

IV. Discussion

Our analysis mostly supports the validity of age at initial pathologic diagnosis as a clinical prognostic factor for glioblastomas affecting the brain, as made evident by our strong p-value obtained from the age-stratified KM plot. While patient age at initial pathologic diagnosis has already been recognized in literature as a robust prognostic factor for GBM (Ladomersky et al., 2019), we can further elucidate the molecular basis for such differences in long-term patient survivorship through analyzing the similarities and differences in our age-stratified multi-omic analyses. Our age-stratified co-oncoplot unearthed three primary genes that may explain such a divergence in prognosis for younger and older patients: TP53, ATRX, and IDH1. TP53 is a classical tumor suppressor gene that has been widely investigated in the realm of cancer biology. It is partly responsible for the complex process of tumorigenesis in virtually all cancers, contributing to the oncogenic function of cancer cells and the aggression of proliferating tumors (Rivlin et al., 2011). The higher mutation frequency of TP53 may be associated with more aggressive variants of GBM due to the higher possibility of enhanced tumorigenesis, however younger populations may nonetheless experience better survival due to the recognition of TP53 as an attractive therapeutic target in numerous cancer drugs. ATRX is another gene with a higher mutation frequency for younger patient populations. This gene is responsible for chromatin remodeling, specifically with the addition of histone H3.3 that is required for the alternative lengthening of telomeres (ALT) as well as epigenetic control (Haase et al., 2018). When ATRX is compromised as is often observed in younger patients, we expect increased genomic instability for the patient due to telomere shortening and decreased epigenetic

control. Therefore, ATRX mutation can drive further genetic mutations and recombination that are favorable for cancer proliferation and mutagenesis. Finally, IDH1 is a gene that encodes for isocitrate dehydrogenase, an enzyme responsible for the generation of alpha-ketoglutarate and replenishing NADPH in the Krebs cycle. IDH1 mutation is associated with lower grade gliomas alongside TP53, with EGFR upregulation and PTEN downregulation being associated with higher grade gliomas (Yan et al., 2009). IDH1 mutation has been documented to produce 2-hydroxyglutarate, an oncometabolite, in the early onset of gliomagenesis along with depressing NADPH cycling in the Krebs cycle (Cohen et al., 2013). Given that IDH1 is associated with the early stages of glioma development, it is reasonable that its mutation may be implicated in enhanced prognostic outcomes since it indicates a non-advanced histochemical stage glioma that is more amenable to treatment. Overall, ATRX, TP53 and IDH1 are all genes that are associated with early-stage glioblastoma, which may account for enhanced prognostic outcomes since less advanced cancers are more likely to be effectively treated with modern therapeutic approaches.

Somatic interaction plots for age-stratified patient cohorts further our understanding of the underlying dynamics of key genes involved in gliomagenesis. In younger patients, the lone mutually exclusive relationship observed between IDH1 and PTEN is characteristic of early-stage glioblastomas. In fact, PTEN loss or repression is a very common phenomenon in gliomas that promotes the PI 3-kinase cascade via the inactivation of second messengers (Gont et al., 2013). With simultaneous mutations promoting the expression of EGFR, PIK3CA, and PIK3R1, this cocktail of gene mutations is capable of unleashing the PI3K-PKB pathway to promote unchecked cell growth and proliferation in younger patients. Meanwhile, older patients couple this downregulation of PTEN and upregulation of PIK3CA and EGFR with another gene that is associated with more advanced gliomas–NF1. NF1 mutation in older patients is a genetic

marker for neurofibromatosis type-1 (NF-1), which is an autosomal dominant syndrome with a primary symptom of predisposition to the most aggressive and high-mortality gliomas of the CNS (Lobbous et al., 2020). When analyzing age-stratified heatmaps based upon transcriptomic and proteomic data, we observe similar up- and downregulation patterns with the genes IDH1, ATRX, EGFR, NF1, PIK3CA, and other genes of interest as the somatic interaction plots. This indicates that key cancer metabolic and proliferation pathways such as the PI3K-PKB pathway are affected across all omic domains, resulting in tumorigenesis.

Given the conclusion that age can serve as a robust prognostic factor for glioblastomas, we can formulate actionable hypotheses surrounding the development of various cancer therapies specific for younger and older patient populations. In fact, a simple survivorship analysis of young and old GBM patient cohorts can reveal the fact that different cancer drugs can have statistically significant efficacies depending on patient age. As demonstrated by our age-stratified KM comparing the top four most administered GBM treatments, we observed the powerful effect of Carmustine on young patients compared to its relatively in-line efficacy when administered to old patients. This notable discrepancy between Carmustine's efficacy in the younger and older patient cohorts indicates that Carmustine may be a more suitable drug for early-stage gliomas such as those often affecting younger patients. More broadly, it demonstrates that personalized medicine may dictate the future of cancer biology and therapeutics, as a one-drug-fits-all approach falls prone to inefficacy and perhaps unfailingly results in glioblastoma's hallmark poor prognosis.

While not extensively investigated in our studies due to relatively scant sample sizes, patient morbidity evaluations via Karnofsky score classifications, as well as patient race, can potentially be equally robust prognostic factors for glioblastoma. The reasoning for the

importance of patient morbidity in predicting patient mortality is a simple one: patients who experience greater distress as a result of their condition are often the ones who are the farthest along the course of their glioblastoma progression. The aggressive nature of glioblastomas means that neoplasms are rapidly able to proliferate to more regions of the CNS and even metastasize to other body regions, resulting in a systemic cancer environment that is frequently associated with poor patient morbidity. However, such a clinical factor may not be as powerful as race or age due to the fact that there will be a delay in the accumulation of patient symptoms resulting in overall discomfort, which may indicate that it may already be tardy to render effective treatment. With regards to patient race, it is necessary to collect a uniform large sampling of Asian, White, African American, and other races to be able to fairly compare all subsets and make robust statistical conclusions. While previous literature corroborates the notion that race significantly impacts patient mortality, specifically when comparing the White and African American populations (Wu et al., 2019), there has yet to be a thorough, systematic analysis of the multi-omic profile underlying such prognostic differences among the races.

V. Conclusion

After extensive statistical analysis of glioblastoma patient clinical and multi-omic profiles, it is evident that patient age at initial pathologic diagnosis, race, and Karnofsky classification scores are potentially statistically significant prognostic factors. Further investigation with larger, more uniform GBM patient clinical and multi-omic datasets are required to reach a definitive conclusion regarding the robustness of the two aforementioned factors. However, even with solely patient age at initial pathologic diagnosis, we can make more informed calculations of a patient's long term survivorship as well as their response to various

therapies including Carmustine. Further analysis of the multi-omic underpinnings of clinical prognostic factors can be fruitful in this initiative towards personalized medicine, where we can pin down a patient's exact cancer environment and administer specialized drugs to target particular molecular pathways that are most implicated.

VI. References

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