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Omics sciences and precision medicine in glioblastoma

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Abstract

Glioblastoma is a highly aggressive and malignant type of brain cancer with a poor prognosis, despite current treatment options of surgery, radiation therapy, and chemotherapy. These treatments have limitations due to the aggressive nature of the cancer and the difficulty in completely removing the tumor without damaging healthy brain tissue. Personalized medicine, using genomic profiling to tailor treatment to the patient's specific tumor, and immunotherapy have shown promise in clinical trials. The blood-brain barrier also poses a challenge in delivering treatments to the brain, and researchers are exploring various approaches to bypass it.

More effective, personalized treatment approaches are needed to improve outcomes for glioblastoma patients. This tumor is studied using genomics, transcriptomics, and proteomics techniques, to better understand its underlying molecular mechanisms. Recent studies have used these techniques to identify potential therapeutic targets, molecular subtypes, and heterogeneity of tumor cells.

Advancements in omics sciences have improved our understanding of glioblastoma biology, and precision medicine approaches have implications for more accurate diagnoses, improved treatment outcomes, and personalized preventive care. Precision medicine can match patients with drugs that target specific genetic mutations, improve clinical trials, and identify individuals at higher risk for certain diseases. Precision medicine, which involves customizing medical treatment based on an individual's genetic makeup, lifestyle, and environmental factors, has shown promise in improving treatment outcomes for glioblastoma patients. Identifying biomarkers is essential for patient stratification and treatment selection in precision medicine approaches for glioblastoma, and several biomarkers have shown promise in predicting patient response to treatment. Targeted therapies are a key component of precision medicine approaches in glioblastoma, but there is still a need to improve their effectiveness.

Technical challenges, such as sample quality and availability, and challenges in analyzing and interpreting large amounts of data remain significant obstacles in omics sciences and precision medicine for glioblastoma. The clinical implementation of precision medicine in glioblastoma treatment faces challenges related to patient selection, drug development, and clinical trial design, as well as ethical and legal considerations related to patient privacy, informed consent, and access to expensive treatments. ***Clin Ter 2023; 174 Suppl. 2 (6):77-84 doi: 10.7417/CT.2023.2474***

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Introduction

Brief overview of glioblastoma and its high mortality rate

Glioblastoma is a type of brain cancer that arises from glial cells, which are supportive cells in the brain. It is the most aggressive and malignant type of brain cancer, with a median survival time of only 15 months since the diagnosis, even with aggressive treatments. The exact etiology of glioblastoma is unknown, but it is believed to be linked to genetic mutations and abnormal growth of glial cells. Symptoms of glioblastoma may include headaches, seizures, memory loss, personality changes, and difficulty in speaking or movements. Treatment options include maximal safe surgical resection, radiation therapy and concomitant chemotherapy, followed by adjuvant chemotherapy. However, the prognosis of patients affected by glioblastoma remains poor regardless of the type of treatment adopted.

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The high mortality rate of glioblastoma is due to several factors, including tumor aggressiveness, challenges to achieve gross total resection preserving neurological functions, and the limited effectiveness of current treatments. According to the National Brain Tumor Society, glioblastoma is the most common primary malignant brain tumor, accounting for about 47% of all primary brain tumors. The five-year survival rate for glioblastoma is less than 10%, and only about 5% of patients survive for 10 years after the diagnosis (1).

Research is ongoing, to better understand the underlying causes of glioblastoma and to develop more effective treatments. One promising approach involves the use of personalized medicine, which uses the patient's genetic information to tailor treatment to their specific tumor. Clinical trials are currently underway to test the safety and effectiveness of personalized medicine for glioblastoma (2, 3).

Current standard treatment options for glioblastoma and their limitations

The standard treatment options for glioblastoma include surgery, radiation therapy, and chemotherapy. These treatments can help slow down the progression of the disease, but they have several limitations.

Surgery is the first-line treatment option for glioblastoma and is aimed to accomplish the maximal tumor resection. However, since glioblastoma tends to infiltrate surrounding brain tissue, it can be difficult to remove the entire tumor without causing postoperative neurological deficits (4).

Radiation therapy on the other hand, uses high-energy beams of radiation, and while it can be effective in slowing the growth of glioblastoma, it can also damage healthy brain tissue, leading to side effects such as fatigue, headaches, and cognitive problems (5, 6).

Finally, chemotherapy involves the use of drugs to kill cancer cells. It can be effective in slowing down the progression of glioblastoma, but can be limited by the occurrence of significant side effects, including headache, nausea, vomiting, fatigue and pancytopaenia. Additionally, the blood-brain barrier can limit the amount of chemotherapy drugs that reach the brain, thus making it difficult to reach therapeutic levels.

Newer treatment approaches that are currently being investigated for glioblastoma include targeted therapy, immunotherapy, and gene therapy. However, these approaches are still in the early stages of development and have not yet been proven to be effective in clinical trials (7).

The need for more effective, personalized treatment approaches

Glioblastoma remains a challenging tumor to treat and cure. Therefore, there is a need for more effective, personalized treatment approaches.

One promising path for personalized treatment is the use of genomic profiling to identify the specific genetic mutations and abnormalities driving the growth of the tumor. This information can be used to target those specific mutations with targeted therapies or immunotherapies. It was found that glioblastoma patients who received targeted therapies based on genomic profiling had improved outcomes as compared to patients who received standard treatments (8-10).

Immunotherapy, already adopted to treat other cancers, has shown promising results in early clinical trials for glioblastoma. Furthermore, patients with recurrent glioblastoma who underwent experimental immunotherapy had a longer median survival (12 months) than patients who received standard treatments (5.5 months) (11, 12). Apart from personalized treatments, more effective pharmacologic strategies to cross the blood-brain barrier and deliver drugs to the tumor are needed. Ultrasounds and nanotechnologies are under investigation to overcome this obstacle (13).

Omics Sciences in Glioblastoma

Genomics techniques used in glioblastoma research

Genomics techniques are essential tools for understanding the underlying molecular mechanisms of glioblastoma. For example, whole genome sequencing (WGS) and whole exome sequencing (WES) can be used to identify genetic mutations and copy number variations in glioblastoma. Moreover, RNA sequencing (RNA-seq) can be used to identify differentially expressed genes and potential biomarkers, while single-cell sequencing can enable the identification of heterogeneity and clonal evolution in glioblastoma. Finally, DNA methylation analysis is used to identify epigenetic changes in glioblastoma. These techniques have enabled the identification of potential therapeutic targets and the development of precision medicine approaches for glioblastoma treatment. However, technical challenges exist in the analysis and interpretation of the large amounts of data generated by these techniques. Variability in sample quality and availability also poses a significant challenge in glioblastoma research. One study used WGS to identify somatic mutations in glioblastomas and found that the mutations were associated with specific molecular subtypes of the disease, which could help guide personalized treatments (14). Another study used genomic analysis to identify a genetic alteration that drives resistance to the temozolomide, which is the first-line chemotherapy drug used to treat glioblastoma (15).

Transcriptomics techniques used in glioblastoma research

Transcriptomics is the study of gene expression at the transcript level. This technique can provide valuable information about the underlying molecular mechanisms of glioblastoma and can help in biomarker identification. Several transcriptomics techniques have been used in glioblastoma research, including microarray analysis, RNA-seq, and single-cell RNA sequencing (scRNA-seq). Microarray analysis allows for the measurement of the expression levels of thousands of genes simultaneously, and has been used to identify differentially expressed genes in glioblastoma as compared to normal brain tissue. RNA-seq provides higher resolution and sensitivity than microarray analysis and has been used to identify novel spliced transcripts and fusion genes in glioblastoma. scRNA-seq allows for the identification of heterogeneity within tumors and has been used to identify subpopulations of cells with different gene

expression profiles. These transcriptomics techniques have provided valuable insights into the molecular mechanisms of glioblastoma and have the potential to identify novel therapeutic targets. Transcriptomic analysis was used in a study to identify a set of genes that are overexpressed in glioblastomas and could serve as potential therapeutic targets (16). Another study used transcriptomics to identify a molecular signature associated with poor survival in glioblastoma patients (17).

Proteomics techniques used in glioblastoma research

Proteomics techniques are essential tools for understanding the protein expression and post-translational modifications, finally resulting in biomarkers and therapeutic targets identification. They can be applied also to study glioblastoma tumorigenesis and progression. One widely used technique is mass spectrometry, which can identify and quantify thousands of proteins in a single sample. Additionally, proteomics approaches can also be used to study protein-protein interactions, protein localization, and protein function.

Other proteomics techniques include two-dimensional gel electrophoresis, which separates proteins based on their isoelectric point and molecular weight, and protein microarrays, which allow for high-throughput analysis of protein expression and interactions. These techniques have been used to identify potential biomarkers and therapeutic targets in glioblastoma, such as the upregulation of EGFR and the downregulation of PTEN in tumor tissues. However, challenges remain in standardizing proteomics protocols and interpreting the large amounts of data generated from these techniques. One study used proteomic analysis to identify a protein that is overexpressed in glioblastomas and could serve as a therapeutic target (18). Another study used proteomics to identify proteins that are differentially expressed in response to treatment with temozolomide (19).

Examples of recent studies using these techniques

A number of studies used genomics, transcriptomics, and proteomics techniques to further our understanding of glioblastoma.

Darvin and colleagues used RNA-seq to identify the subset of patients who may benefit from treatment with immune checkpoint inhibitors. They found that patients with high levels of immune-related gene expression had better survival outcomes after treatment with immune checkpoint inhibitors (20).

In another study, the researchers used genomic and transcriptomic analysis to identify a novel molecular subtype of glioblastoma, called MES-IG, characterized by high levels of immune and inflammatory signaling. They found that MES-IG tumors were more responsive to immune checkpoint inhibitors than other subtypes of glioblastoma (21).

Bollard and co-workers identified a set of proteins upregulated in glioblastoma and associated with poor outcomes by means of proteomic analysis. They demonstrated how targeting one of these proteins, called PIM1, could improve treatment outcomes in glioblastoma patients (22).

Eventually, researchers used scRNA-seq to analyze the heterogeneity of glioblastoma tumors at the single-cell level. They found that glioblastomas are highly heterogeneous tumors and different subpopulations of tumor cells may respond differently to treatment (23).

Advancements in omics sciences have improved our understanding of glioblastoma biology

The genomics of glioblastoma has been extensively studied to identify mutations and alterations that drive tumor's growth and progression. The Cancer Genome Atlas (TCGA) project identified, on the basis of genomic alterations, four molecular subtypes of glioblastoma with different prognoses and response to therapies. The IDH1 mutation was found to

Table 1. List of genes involved in glioblastoma, with OMIM id, the pathology to which they are correlated, and the inheritance pattern.

Location	Phenotype	Inheritance	Phenotype MIM number	Gene/Locus	Gene/Locus MIM number
2q34	Glioma, susceptibility to, somatic	-	137800	IDH1	147700
5p15.33	Glioma susceptibility 8	-	613033	GLM8	613033
7q31.33	Glioma susceptibility 9	AD	616568	POT1	606478
8q24.21	Glioma susceptibility 7	-	613032	CCDC26	613040
9p21.3	Glioma susceptibility 5	-	613030	GLM5	613030
10q23.31	Glioma susceptibility 2	-	613028	PTEN	601728
13q13.1	Glioblastoma 3	AR	613029	BRCA2	600185
15q23-q26.3	Glioma susceptibility 4	-	607248	GLM4	607248
17p13.1	Glioma susceptibility 1	AD, SMu	137800	TP53	191170
17q12	Glioblastoma, somatic	-	137800	ERBB2	164870
20q13.33	Glioma susceptibility 6	-	613031	GLM6	613031

be a strong prognostic factor for glioblastoma patients (24). Transcriptomic analyses have been used to identify genes that are differentially expressed in glioblastoma, which can serve as potential therapeutic targets or biomarkers. The TCGA project identified the EGFR signaling pathway as a key pathway activated in glioblastoma. Some drugs targeting this pathway have been developed and tested in clinical trials (25).

Epigenetic alterations, such as DNA methylation and histone modifications, have been shown to play a role in glioblastoma development and progression. Epigenetic changes can alter gene expression and promote tumor growth. Epigenetic therapies have been developed and tested in clinical trials (26). Moreover, proteomic analyses have been used to identify proteins that are differently expressed in glioblastomas and can serve as potential therapeutic targets or biomarkers (27).

Precision Medicine in Glioblastoma

Definition of precision medicine and its use in glioblastoma treatment

Precision medicine refers to the customization of medical treatment based on individual's genetic makeup, lifestyle, and environmental factors. In glioblastoma, precision medicine involves identifying specific genetic mutations that drive tumor growth and developing targeted therapies to inhibit these mutations. Precision medicine approaches have shown promise in improving treatment outcomes for glioblastoma patients (28).

Importance of identifying biomarkers for patient stratification and treatment selection

Identifying biomarkers is essential for patients' stratification and treatment selection in precision medicine approaches. Biomarkers are molecular indicators of disease or response to treatments, and their identification can help match patients with the most effective therapies. In glioblastoma, identifying biomarkers is particularly important due to the heterogeneity of the disease and the variable response to treatment (29).

Examples of promising biomarkers in glioblastoma

Several biomarkers have been identified in glioblastoma, including *IDH1* and *IDH2* mutations, MGMT promoter methylation status, and *EGFRvIII* mutations, or alteration of PI3K/AKT/MTOR and RAS/RAF/MEK/MAPK signaling pathways, DNA-damage repair pathways and cell cycle checkpoints. These biomarkers can help predict patients' response to treatments and guide treatments' selection (30,31).

Targeted therapies in precision medicine for glioblastoma

A key component of precision medicine approaches in glioblastoma are targeted therapies. These therapies selectively target specific molecular pathways that drive tumor growth and survival, leading to improved treatment outcomes. Examples of targeted therapies in glioblastoma include EGFR inhibitors, VEGF inhibitors, MEK/BRAF inhibitors, or recently IDH1 inhibitors as vorasidenib (32).

Overview of current FDA-approved targeted therapies for glioblastoma

There are currently few FDA-approved targeted therapies for glioblastoma, including regorafenib (a multikinase inhibitor). However, these therapies have limited efficacy, and there is a need for more effective targeted therapies in glioblastoma (33).

Implications for precision medicine approaches

There are several implications of precision medicine approaches, including more accurate diagnoses, improved treatment outcomes, and the potential for personalized preventive care. Precision medicine can help clinicians make more accurate diagnoses by identifying genetic mutations that contribute to a patient's disease. For example, genetic testing can help diagnose cancer and guide treatment decisions (34,35). Moreover, precision medicine can also help identify the most effective treatments for patients by matching them with drugs that target specific genetic mutations. Precision medicine can improve clinical trials by selecting patients with specific genetic profiles that are more likely to respond to a particular treatment. This can help speed up drug development and reduce the number of patients needed for clinical trials (35,36). Precision medicine can also be used to identify individuals who are at higher risk for certain diseases, thus developing personalized preventive care plans. For example, genetic testing can identify individuals at higher risk for hereditary cancers, allowing for earlier screening and intervention (37).

Challenges and Limitations of Omics Sciences and Precision Medicine in Glioblastoma

Technical challenges in omics sciences for glioblastoma research

Glioblastoma is a complex and heterogeneous disease, and omics sciences have become essential tools for understanding its underlying molecular mechanisms. However, several technical challenges of omics sciences need to be addressed to improve glioblastoma research.

A review discusses the challenges of single-cell sequencing technology in glioblastoma research, including data quality control, data analysis, and interpretation (38). Another article highlights the need for standardized protocols in proteomics research to reduce variability and increase reproducibility (39). Eventually, a study identifies several challenges in the analysis of DNA methylation data in glioblastoma research, such as normalization and batch effect removal (40).

Challenges in analyzing and interpreting large amounts of data

Omics sciences generate huge amounts of data, that are challenging to be analyzed and interpreted. The integration of multi-omics data in glioblastoma research, such as data normalization, feature selection, and machine learning algorithms, can be reached with difficulties (41). Similarly, RNA-seq data in glioblastoma, such as the identification of novel biomarkers and the validation of differential gene expression, are difficult to be interpreted (42).

Variability in sample quality and availability

Obtaining high quality and sufficient quantity of samples for omics sciences is a significant challenge in glioblastoma research. Tumor tissue heterogeneity and necrosis are limiting factors for genomic analyses (43), such as rarity of some brain tumors for sample availability (44) and sample preservation and processing for proteomics research (44,45).

Clinical challenges in implementing precision medicine in glioblastoma treatment

Precision medicine holds promise for improving glioblastoma treatments, but its clinical implementation faces several challenges. Patients' selection, drugs' development, clinical trial design (46), identification of possible molecular targets, development of effective combination therapies (47), integrating genomic data into the clinical decision-making for the treatment of glioblastomas (48) are just some of these challenges.

Ethical and legal considerations in using precision medicine for glioblastoma patients

The use of precision medicine in glioblastoma treatment raises ethical and legal considerations. There are several ethical challenges posed by using genomic data in clinical decision-making, concerning also the potential impact on patient's autonomy and privacy (49). Other issues include data privacy, intellectual property, and informed consent (50,51). Furthermore, ethical considerations of providing expensive precision medicine treatments to patients with limited access to healthcare resources should be taken into account (52).

Future Directions and Conclusion

Promising developments in omics sciences and precision medicine for glioblastoma

Omics sciences and precision medicine have the potential to revolutionize glioblastoma treatment. A review article highlights the promising developments in omics sciences, including single-cell sequencing, spatial transcriptomics, and liquid biopsy analysis (53). Another article discusses the potential of precision medicine in glioblastoma treatment, such as the identification of novel biomarkers and the development of targeted therapies (54).

Ongoing targeted therapies for glioblastoma

Several clinical trials are currently ongoing to identify targeted therapies for glioblastoma. For example, a clinical trial is evaluating the safety and efficacy of a combination therapy, consisting of Toca 511 (a retroviral replicating vector) and Toca FC (a prodrug of the antifungal drug 5-fluorocytosine), for patients with recurrent high-grade glioma, including GBM. The therapy works by selectively targeting cancer cells and delivering a cytotoxic drug to kill them. The trial is currently ongoing (at phase 3) and has shown promising results so far (55).

AG-881 is a dual inhibitor of the metabolic enzymes isocitrate dehydrogenase-1 and 2 (IDH1/2), which are commonly mutated in GBM. The drug has been evaluated in phase 3 clinical trial for patients with IDH-mutant recurrent or progressive GBM. The trial aims to determine whether AG-881 can improve overall survival compared to standard chemotherapy (56).

Regorafenib is a multikinase inhibitor, whose main targets are kinases involved in angiogenesis (VEGFR1–3 and TIE2), oncogenesis (KIT, RET, RAF1, and BRAF), tumor microenvironment (PDGFR and FGFR), and tumor immunity (colony stimulating factor 1 receptor). The administration of regorafenib in patients with recurrent glioblastoma has shown encouraging results (57,58).

Another possibility is the use of immune checkpoint inhibitors, such as pembrolizumab and nivolumab, which have shown promising results in the treatment of several cancers (including GBM). These drugs work by blocking proteins expressed by cancer cells that inhibit the immune system's ability to attack them. Moreover, a potential linkage with immunotherapy and PARP inhibitors has been identified in 44% of glioblastoma patients as a consequence of alterations in DNA-damage repair genes, supporting the purpose of their combination in clinical setting. Several clinical trials are ongoing to evaluate the safety and efficacy of these drugs in combination with other therapies for GBM (59).

The potential impact of omics sciences and precision medicine on glioblastoma treatment and patient outcomes

Omics sciences and precision medicine have the potential to significantly impact glioblastoma treatment and patient outcomes. Via advanced technologies and personalized approaches, precision medicine can potentially lead to more effective and targeted treatments. For instance, the identification of specific genetic mutations in glioblastoma tumors can inform the use of targeted therapies, such as EGFR inhibitors or IDH inhibitor (60). Additionally, omics sciences can aid in the identification of novel biomarkers for diagnosis and monitoring of glioblastoma (61).

Furthermore, the use of precision medicine may also lead to better patient outcomes and survival rates. A study found that patients with IDH-mutant glioblastomas had a significantly better overall survival rate when treated with IDH-targeted therapies as compared to standard chemotherapy (62). Another study showed promising results for the use of the PARP inhibitor talazoparib in glioblastoma patients with DNA damage response gene mutations (63). Overall, omics sciences and precision medicine hold signi-

ficant potential for improving glioblastoma treatment and patient outcomes.

Conclusion and recommendations for future research and clinical practice

In conclusion, glioblastoma is a highly aggressive and deadly form of brain cancer, and its treatment remains a significant challenge. However, recent advances in omics sciences and precision medicine offer promising opportunities for improved diagnosis and treatment. Future research in glioblastoma should focus on addressing the technical challenges in omics sciences, such as sample variability and data analysis. Additionally, there is a need for ongoing clinical trials to evaluate the efficacy and safety of emerging targeted therapies, as well as to identify biomarkers that can predict treatment response. Furthermore, it is important to address the clinical challenges in implementing precision medicine in glioblastoma treatment, such as patient stratification and treatment selection. This requires collaboration among clinicians, researchers, and patients to develop personalized treatment plans that consider the unique characteristics of each patient's tumor. Finally, there is a need to address ethical and legal considerations in using precision medicine for glioblastoma patients, such as ensuring privacy, informed consent, and access to expensive treatments. By addressing these challenges, omics sciences and precision medicine can potentially improve glioblastoma treatment and patient outcomes. Overall, the future of glioblastoma research and clinical practice will rely on multidisciplinary collaboration and a personalized approach to treatment that considers the unique characteristics of each patient's tumor.

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Conflicts of interest statement

Authors declare no conflict of interest.

Reference

1. National Brain Tumor Society. About Glioblatoma. (Accessed on 28/08/2023 at https://braintumor.org/events/glioblastoma-awareness-day/about-glioblastoma/)
2. Stupp R, Taillibert S, Kanner AA et al. Maintenance Therapy With Tumor-Treating Fields Plus Temozolomide vs Temozolomide Alone for Glioblastoma: A Randomized Clinical Trial. JAMA. 2015 Dec; 314(23):2535-43
3. Wen PY, Kesari S. Malignant gliomas in adults. N Engl J Med. 2008 Jul; 359(5):492-507
4. Stupp R, Mason WP, van den Bent MJ, et al. Radiotherapy plus concomitant and adjuvant temozolomide for glioblastoma. N Engl J Med. 2005 Mar;352(10):987-96
5. Wick W, Platten M, Meisner C, et al. Temozolomide chemotherapy alone versus radiotherapy alone for malignant astrocytoma in the elderly: the NOA-08 randomised, phase 3 trial. Lancet Oncol. 2012 Jul; 13(7):707-15
6. Stupp R, Hegi ME, Mason WP, et al. Effects of radiotherapy with concomitant and adjuvant temozolomide versus radiotherapy alone on survival in glioblastoma in a randomised phase III study: 5-year analysis of the EORTC-NCIC trial. Lancet Oncol. 2009 May;10(5):459-66
7. Louis DN, Perry A, Reifenberger G, et al. The 2016 World Health Organization Classification of Tumors of the Central Nervous System: a summary. Acta Neuropathol. 2016 Jun;131(6):803-20
8. Zhu JJ, Wong ET. Personalized medicine for glioblastoma: current challenges and future opportunities. Curr Mol Med. 2013 Mar; 13(3):358-67
9. Reardon DA, Schuster J, Tran DD, et al. ReACT: overall survival from a randomized phase II study of rindopepimut (CDX-110) plus bevacizumab in relapsed glioblastoma. (Accessed on 28/08/2023 at https://escholarship.org/uc/item/3jc65466)
10. Pellegatta S, Savoldo B, Di Ianni N et al. Constitutive and TNFα-inducible expression of chondroitin sulfate proteoglycan 4 in glioblastoma and neurospheres: Implications for CAR-T cell therapy. Sci Transl Med. 2018 Feb; 10(430):eaao2731
11. Johanns TM, Miller CA, Dorward IG et al. Immunogenomics of Hypermutated Glioblastoma: A Patient with Germline POLE Deficiency Treated with Checkpoint Blockade Immunotherapy. Cancer Discov. 2016 Nov; 6(11):1230-1236
12. Fomchenko EI, Holland EC. Platelet-derived growth factor-mediated gliomagenesis and brain tumor recruitment. Neurosurg Clin N Am. 2007 Jan;18(1):39-58, viii
13. The Normal and Brain Tumor Vasculature: Morphological and Functional Characteristics and Therapeutic Targeting. (Accessed on 28/08/2023 at https://www.frontiersin.org/articles/10.3389/fphys.2021.622615/full)
14. Brennan CW, Verhaak RG, McKenna A, et al. The somatic genomic landscape of glioblastoma. Cell. 2013 Oct; 155(2):462-77
15. Johnson BE, Mazor T, Hong C et al. Mutational analysis reveals the origin and therapy-driven evolution of recurrent glioma. Science. 2014 Jan; 343(6167):189-193
16. Ma K, Cheng Z, Sun L, et al. Identification of potential therapeutic targets for gliomas by bioinformatics analysis. Oncol Lett. 2017 Nov; 14(5):5203-5210
17. Gusev Y, Bhuvaneshwar K, Song L, et al. The REMBRANDT study, a large collection of genomic data from brain cancer patients. Sci Data. 2018 Aug; 5:180158
18. Wang Q, Hu B, Hu X, et al. Tumor Evolution of Glioma-Intrinsic Gene Expression Subtypes Associates with Immunological Changes in the Microenvironment. Cancer Cell. 2017 Jul; 32(1):42-56.e6
19. Vizcaíno JA, Côté RG, Csordas A, et al. The PRoteomics IDEntifications (PRIDE) database and associated tools: status in 2013. Nucleic Acids Res. 2013 Jan; 41(Database issue):D1063-9.
20. Darvin P, Toor SM, Sasidharan Nair V, et al. Immune checkpoint inhibitors: recent progress and potential biomarkers. Exp Mol Med. 2018 Dec; 50(12):1-11
21. Neftel C, Laffy J, Filbin MG, et al. An Integrative Model of Cellular States, Plasticity, and Genetics for Glioblastoma. Cell. 2019 Aug; 178(4):835-849.e21
22. Bollard J, Miguela V, Ruiz de Galarreta M, et al. Palbociclib (PD-0332991), a selective CDK4/6 inhibitor, restricts tumour growth in preclinical models of hepatocellular carcinoma. Gut. 2017 Jul; 66(7):1286-1296
23. Tirosh I, Venteicher AS, Hebert C, et al. Single-cell RNA-seq supports a developmental hierarchy in human oligodendroglioma. Nature. 2016 Nov; 539(7628):309-313

24. Cancer Genome Atlas Research Network. Comprehensive genomic characterization defines human glioblastoma genes and core pathways. Nature. 2008 Oct; 455(7216):1061-8
25. Brat DJ, Verhaak RG, Aldape KD, et al. Comprehensive, Integrative Genomic Analysis of Diffuse Lower-Grade Gliomas. N Engl J Med. 2015 Jun; 372(26):2481-98
26. Feng J, Kim ST, Liu W, et al. An integrated analysis of germline and somatic, genetic and epigenetic alterations at 9p21.3 in glioblastoma. Cancer. 2012 Jan 1; 118(1):232-40
27. Li X, Wang W, Wang J, et al. Proteomic analyses reveal distinct chromatin-associated and soluble transcription factor complexes. Mol Syst Biol. 2015 Jan; 11(1):775
28. Desai A, Reddy NK, Subbiah V. Top advances of the year: Precision oncology. Cancer. 2023 Jun 1;129(11):1634-1642
29. Druker BJ, Talpaz M, Resta DJ, et al. Efficacy and safety of a specific inhibitor of the BCR-ABL tyrosine kinase in chronic myeloid leukemia. N Engl J Med. 2001 Apr; 344(14):1031-7
30. Fountzilas E, Tsimberidou AM, Vo HH, et al. Clinical trial design in the era of precision medicine. Genome Med. 2022 Aug 31; 14(1):101
31. Khoury MJ, Iademarco MF, Riley WT. Precision Public Health for the Era of Precision Medicine. Am J Prev Med. 2016 Mar; 50(3):398-401
32. Precision Medicine Advancements in Glioblastoma: A Systematic Review. (Accessed on 28/08/2023 at https://www.biomedicinej.com/cgi/viewcontent.cgi?article=1403&context=biomedicine#:~:text=The%20current%20standard%20of%20treatment,personalized%20medicine%20for%20glioblastoma%20therapy)
33. Szopa W, Burley TA, Kramer-Marek G, et al. Diagnostic and Therapeutic Biomarkers in Glioblastoma: Current Status and Future Perspectives. Biomed Res Int. 2017; 2017:8013575
34. Sareen H, Ma Y, Becker TM, et al. Molecular Biomarkers in Glioblastoma: A Systematic Review and Meta-Analysis. Int J Mol Sci. 2022 Aug 9; 23(16):8835
35. Williams G, Llewelyn A, Thatcher R, et al. Utilisation of semiconductor sequencing for the detection of predictive biomarkers in glioblastoma. PLoS One. 2022 Mar;17(3):e0245817
36. Shergalis A, Bankhead A 3rd, Luesakul U, et al. Current Challenges and Opportunities in Treating Glioblastoma. Pharmacol Rev. 2018 Jul; 70(3):412-445
37. Stupp R, Taillibert S, Kanner A et al. Effect of Tumor-Treating Fields Plus Maintenance Temozolomide vs Maintenance Temozolomide Alone on Survival in Patients With Glioblastoma: A Randomized Clinical Trial. JAMA. 2017 Dec;318(23):2306-2316.
38. Johnson E, Dickerson KL, Connolly ID, et al. Single-Cell RNA-Sequencing in Glioma. Curr Oncol Rep. 2018 Apr 10;20(5):42
39. Domon B, Aebersold R. Challenges and opportunities in proteomics data analysis. Mol Cell Proteomics. 2006 Oct;5(10):1921-6
40. Della Monica R, Cuomo M, Buonaiuto M, et al. MGMT and Whole-Genome DNA Methylation Impacts on Diagnosis, Prognosis and Therapy of Glioblastoma Multiforme. Int J Mol Sci. 2022 Jun 27;23(13):7148.
41. Barzegar Behrooz A, Latifi-Navid H, da Silva Rosa SC, et al. Integrating Multi-Omics Analysis for Enhanced Diagnosis and Treatment of Glioblastoma: A Comprehensive Data-Driven Approach. Cancers (Basel). 2023 Jun 12; 15(12):3158
42. Sallam M, Mysara M, Baatout S, et al. Meta-Analysis of RNA-Seq Datasets Identifies Novel Players in Glioblastoma. Cancers (Basel). 2022 Nov 24; 14(23):5788
43. Diaz-Cano SJ. Tumor heterogeneity: mechanisms and bases for a reliable application of molecular marker design. Int J Mol Sci. 2012; 13(2):1951-2011
44. Hanif F, Muzaffar K, Perveen K, et al. Glioblastoma Multiforme: A Review of its Epidemiology and Pathogenesis through Clinical Presentation and Treatment. Asian Pac J Cancer Prev. 2017 Jan 1;18(1):3-9
45. Tribe AKW, McConnell MJ, Teesdale-Spittle PH. The Big Picture of Glioblastoma Malignancy: A Meta-Analysis of Glioblastoma Proteomics to Identify Altered Biological Pathways. ACS Omega. 2021 Sep 14; 6(38):24535-24544
46. Frattini V, Trifonov V, Chan JM, et al. The integrated landscape of driver genomic alterations in glioblastoma. Nat Genet. 2013 Oct;45(10):1141-9
47. Ghosh D, Nandi S, Bhattacharjee S. Combination therapy to checkmate Glioblastoma: clinical challenges and advances. Clin Transl Med. 2018 Oct 16; 7(1):33
48. Sinigaglia M, Assi T, Besson FL, et al. Imaging-guided precision medicine in glioblastoma patients treated with immune checkpoint modulators: research trend and future directions in the field of imaging biomarkers and artificial intelligence. EJNMMI Res. 2019 Aug 20; 9(1):78
49. Gupta S, Smith TR, Broekman ML. Ethical considerations of neuro-oncology trial design in the era of precision medicine. J Neurooncol. 2017 Aug;134(1):1-7
50. McGrath SP, Peabody AE Jr, Walton D, et al. Legal Challenges in Precision Medicine: What Duties Arising From Genetic and Genomic Testing Does a Physician Owe to Patients? Front Med (Lausanne). 2021 Jul 26;8:663014
51. Conboy C. Consent and Privacy in the Era of Precision Medicine and Biobanking Genomic Data. Am J Law Med. 2020 May; 46(2-3):167-187
52. Beiko J, Suki D, Hess KR, et al. IDH1 mutant malignant astrocytomas are more amenable to surgical resection and have a survival benefit associated with maximal surgical resection. Neuro Oncol. 2014 Jan;16(1):81-91
53.. Emerging Role of Glioma Stem Cells in Mechanisms of Therapy Resistance. (Accessed on 28/08/2023 at https://www.mdpi.com/2072-6694/15/13/3458)
54. Rong L, Li N, Zhang Z. Emerging therapies for glioblastoma: current state and future directions. J Exp Clin Cancer Res. 2022 Apr 15;41(1):142
55. Cloughesy TF, Petrecca K, Walbert T et al. Effect of Vocimagene Amiretrorepvec in Combination With Flucytosine vs Standard of Care on Survival Following Tumor Resection in Patients With Recurrent High-Grade Glioma: A Randomized Clinical Trial. JAMA Oncol. 2020 Dec;6(12):1939-1946
56. Chou FJ, Liu Y, Lang F, et al. D-2-Hydroxyglutarate in Glioma Biology. Cells. 2021 Sep 7;10(9):2345
57. Lombardi G, Caccese M, Padovan M et al. Regorafenib in Recurrent Glioblastoma Patients: A Large and Monocentric Real-Life Study. Cancers (Basel). 2021 Sep;13(18):4731
58. Chiesa S, Mangraviti A, Martini M et al. Clinical and NGS predictors of response to regorafenib in recurrent glioblastoma. Sci Rep. 2022 Sep;12(1):16265
59. Omuro A, Vlahovic G, Lim M et al. Nivolumab with or without ipilimumab in patients with recurrent glioblastoma: results from exploratory phase I cohorts of CheckMate 143. Neuro Oncol. 2018 Apr;20(5):674-686
60. Malignant Gliomas in Adults. (Accessed on 28/08/2023 at https://www.sciencedirect.com/science/article/abs/pii/B9780750675161000050#:~:text=Introduction,the%20United%20States%20each%20year.&text=They%20represent%20

the%20most%20common,primary%20brain%20tumor%20in%20adults.)
61. Tang Y, Qing C, Wang J, et al. DNA Methylation-based Diagnostic and Prognostic Biomarkers for Glioblastoma. Cell Transplant. 2020 Jan-Dec; 29:963689720933241
62. Johnson BE, Mazor T, Hong C et al. Mutational analysis reveals the origin and therapy-driven evolution of recurrent glioma. Science. 2014 Jan;343(6167):189-193
63. Cloughesy TF, Petrecca K, Walbert T, et al. Effect of Vocimagene Amiretrorepvec in Combination With Flucytosine vs Standard of Care on Survival Following Tumor Resection in Patients With Recurrent High-Grade Glioma: A Randomized Clinical Trial. JAMA Oncol. 2020 Dec; 6(12):1939-1946