Next-Generation Sequencing for Glioblastoma Diagnosis

- Molecular Biomarkers of Glioma
- Challenges in implementing 2021 WHO CNS tumor classification in a resource-limited setting
- Medulloblastoma associated with Lynch syndrome: a case report of germline MLH1 variant and tumor molecular characterization
- Improved Diagnosis of Glioblastoma, IDH-Wildtype, Metastasis through Molecular and DNA Methylation Profiling: Two Case Reports
- Glioblastoma with metastasis to the bone marrow
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- Favorable Response to Conventional Chemoradiotherapy in Radiation-induced Glioma Harboring Coamplification of PDGFRA, KIT, and KDR: A Case Report and Literature Review
- Adult diffuse IDH-wildtype lower-grade gliomas with PDGFRA gain/amplification should be upgraded as glioblastoma

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Molecular profiling of GBM has become crucial for improving diagnosis, predicting prognosis, and guiding personalized therapies. Next-generation sequencing (NGS) has emerged as a powerful tool to comprehensively analyze the genetic landscape of GBM, providing insights into its heterogeneity and potential therapeutic targets.

NGS enables the simultaneous detection of multiple genetic alterations, including single nucleotide variants (SNVs), copy number variations (CNVs), structural variations (SVs), and epigenetic modifications.

Key molecular features identified in GBM include:

IDH1/IDH2 Mutations: IDH-mutant glioblastomas (previously classified as secondary GBM) have a better prognosis than IDH-wildtype GBM. TERT Promoter Mutations: Common in primary GBM and associated with poor prognosis. EGFR Amplifications and Mutations: EGFRvIII, a common variant, is associated with tumor progression and resistance to therapy. PTEN Mutations and Deletions: Linked to PI3K/AKT pathway activation and poor response to targeted therapies. TP53 Mutations: Frequently found in IDH-mutant gliomas, contributing to tumorigenesis. MGMT Promoter Methylation: Predicts response to temozolomide (TMZ) chemotherapy and is an important prognostic biomarker. ATRX Mutations: Associated with alternative lengthening of telomeres and commonly found in IDH-mutant tumors. H3F3A Mutations: Characteristic of pediatric gliomas, especially diffuse midline gliomas. Clinical Applications of NGS in GBM Accurate Molecular Classification: NGS supports the 2021 WHO classification of CNS tumors by integrating molecular markers into GBM diagnosis. Identification of Actionable Targets: Mutations in pathways such as RTK/PI3K, DNA repair, and epigenetic regulation may inform targeted therapy choices. Prognostic Stratification: Molecular profiles identified by NGS help predict disease progression and response to therapy. Detection of Tumor Evolution and Resistance Mechanisms: Longitudinal NGS monitoring can reveal acquired resistance to therapy and guide treatment adjustments. Liquid Biopsy Applications: NGS can be applied to circulating tumor DNA (ctDNA) in cerebrospinal fluid (CSF) and plasma to enable non-invasive monitoring of GBM. Challenges and Future Directions Despite its promise, the use of NGS in GBM diagnosis faces several challenges:

Tumor Heterogeneity: Intratumoral genetic variability may lead to sampling bias. Cost and Accessibility: High costs limit widespread adoption in routine clinical practice. Data Interpretation: Large-scale sequencing generates complex data requiring bioinformatics expertise. Future advancements in single-cell sequencing, multi-omics integration, and artificial intelligence-driven data analysis are expected to enhance the diagnostic and therapeutic utility of NGS in GBM.

Conclusion NGS is revolutionizing the molecular diagnosis and management of glioblastoma by providing a comprehensive genetic landscape that informs classification, prognosis, and therapeutic strategies. As technology advances, integrating NGS into routine clinical practice will improve personalized treatment approaches and potentially extend survival in patients with GBM.

Retrospective observational studies

The purpose of a study by Vivancos Sánchez et al. was to assess the clinical impact of next-generation sequencing (NGS), as an increasingly available and advantageous tool, for glioblastoma patients. Adult patients aged less than 65, and surgically treated for glioblastoma between 2010-2021, were included. Tumor samples were analyzed with NGS using the Oncomine Comprehensive v3 (OCA) panel and Ion Reporter Genexus v5.9.1 (Thermo Fisher Scientific). Thirty-two patients were included, with a median age of 47.7 years and a median overall survival of 25 months. Identification of mutations by NGS resulted in a change in diagnosis in two cases. In all patients but one, at least one genetic alteration was detected (median of three per patient), most commonly EGFR amplification. In 93.7% of patients, biomarkers that make them potentially eligible for a clinical trial were found. No survival differences were seen regarding genetic alterations, although a trend towards better survival for those patients without CDK4 mutation was observed ($p = 0.088$). The use of NGS provides useful information for diagnosis, especially in young patients, and it will probably become valuable for clinical decision-making as more therapeutic targets and treatments emerge. For the moment, it is crucial for scientific progress to happen [1)].

This study reinforces the diagnostic and research utility of NGS in GBM, particularly in identifying trial-eligible patients. However, its clinical impact remains marginal, as no clear therapeutic benefit is demonstrated. Future research should focus on larger, multi-center cohorts, integrative molecular profiling (RNA-seq, methylation, proteomics), and prospective studies linking NGS findings to actual therapeutic interventions.

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Vivancos Sánchez C, Esteban Rodríguez MI, Peláez García A, Taravilla-Loma M, Rodríguez-Domínguez V, Rodríguez-Antolín C, Rosas-Alonso R, Losantos-García I, Isla Guerrero A, Gandía-González ML. Clinical Impact of a Next-Generation Sequencing Approach for Glioblastoma Patients. Cancers (Basel). 2025 Feb 22;17(5):744. doi: 10.3390/cancers17050744. PMID: 40075591.