REVIEW ARTICLE

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The Evolving Relationship: Impact of Combined Radiotherapy and Temozolomide Treatment on Critical Biomarkers in Glioblastoma Multiforme Patients – A Comprehensive Analysis

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Abstract

Background: The established first-line treatment for newly diagnosed Glioblastoma Multiforme (GBM) involves maximal surgical removal of the tumor, followed by a regimen of radiotherapy (RT) together with concurrent and maintenance temozolomide (TMZ) chemotherapy. Patient response to this combined approach varies widely and is closely associated with the tumor's molecular characteristics.

Objective: This analysis compiles current research on how the RT/TMZ combination modifies crucial GBM biomarkers over time, focusing on therapy-induced alterations rather than their initial prognostic significance.

Methods: A systematic review of literature from January 2000 to July 2024 was performed using PubMed, Scopus, and Web of Science. Search keywords included "glioblastoma," "radiotherapy," "temozolomide," "MGMT," "IDH," "biomarker," and related terms. Emphasis was placed on clinical trials and key preclinical studies.

Results: The RT/TMZ protocol imposes significant selective pressure, dynamically influencing GBM biomarkers. MGMT promoter methylation is the primary predictor of TMZ efficacy, but treatment often leads to the expansion of MGMT-active, resistant tumor clones at recurrence. IDH1/2 mutations are strong prognostic indicators, and their associated metabolic changes may increase tumor sensitivity to DNA-damaging therapies. Treatment substantially reshapes the tumor immune microenvironment; RT can stimulate anti-tumor immune responses but also increase PD-L1 expression, while TMZ often causes severe lymphocyte depletion. Additionally, therapy promotes the selection of cells with enhanced DNA damage repair mechanisms and activates survival pathways such as EGFR, fostering treatment resistance.

Conclusion: RT and TMZ induce continuous, adaptive changes in GBM biomarkers. Recognizing this dynamic process is essential for personalizing treatment, assessing response, and developing new combination therapies to combat resistance.

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Introduction

Glioblastoma Multiforme (GBM) is the most frequent and aggressive primary brain tumor in adults, known for its invasive growth, cellular diversity, and inevitable recurrence [1]. The current standard treatment, established by Stupp et al., combines maximal safe surgery with RT and TMZ chemotherapy [2]. This protocol offers a limited but meaningful extension in average survival time.

The benefit of this treatment, however, differs greatly among patients. The molecular profile of a GBM tumor is a key factor influencing both its natural progression and its reaction to therapy [3]. Biomarkers like the methylation status of the O^6-methylguanine-DNA methyltransferase (MGMT) gene promoter and mutations in the isocitrate dehydrogenase (IDH1/2) genes are now vital clinical diagnostic tools, incorporated into the WHO brain tumor classification system [4].

While these biomarkers provide a baseline assessment at diagnosis, a deeper insight reveals that the RT/TMZ regimen itself acts as a significant evolutionary driver, actively modifying the tumor's molecular and cellular environment [5]. Treatment eliminates susceptible cell populations while allowing resistant clones to proliferate, ultimately leading to therapeutic failure [6]. This review examines the changing effects of RT and TMZ on major GBM biomarkers, describing how therapy influences the tumor's genetics, characteristics, and immune setting. By integrating this evidence, we emphasize the need to view biomarkers as fluid, therapy-responsive elements—a perspective crucial for future treatment advances.

Methods

Search Strategy

A thorough search for relevant English-language studies published between January 2000 and July 2024 was conducted using PubMed, Scopus, and Web of Science. Keywords and MeSH terms included "glioblastoma," "radiotherapy," "temozolomide," "MGMT," "IDH," "biomarker," "immune microenvironment," "PD-L1," "DNA damage response," "EGFR," and "therapy resistance." References from retrieved articles were also examined.

Study Selection Criteria

Eligible studies included original preclinical and clinical research and meta-analyses focusing on how RT and/or TMZ affect molecular or cellular biomarkers in GBM, or which investigate the predictive/prognostic value of biomarkers within the standard treatment protocol. Exclusions were case reports, editorials, non-English papers, studies on pediatric gliomas, and research not directly connecting biomarker changes to RT/TMZ.Information on authors, study design, models, treatments, biomarkers studied, key findings on biomarker changes, and conclusions was extracted. Findings were organized and discussed narratively by biomarker theme.

Results

MGMT Promoter Methylation: The Key Predictive Biomarker

The DNA repair protein MGMT causes resistance to TMZ. Its activity is controlled by promoter methylation, which turns the gene off [7].

- **Prediction and Initial Benefit:** Strong evidence shows patients with methylated *MGMT* promoters experience significantly greater survival benefit from TMZ chemotherapy added to RT [8, 9].
- **Therapy-Driven Change and Resistance:** TMZ treatment creates strong selective pressure. Cells with unmethylated promoters and high MGMT activity survive, leading to recurrent tumors populated by TMZ-resistant clones [11, 12]. Some initially unmethylated tumors can also develop methylation at recurrence as an adaptive response [13].

IDH Mutations: A Core Prognostic Indicator Mutations in IDH1/2 genes identify a GBM subtype with a notably better prognosis [15].

- **Strong Prognostic Value:** The improved outcomes for IDH-mutant GBM patients are consistent across treatments, including RT/TMZ [16], leading to its separate classification by the WHO [4].
- **Potential for Synergistic Action:** The metabolite D-2-hydroxyglutarate (2-HG), produced by mutant IDH, can inhibit DNA repair enzymes [17, 18]. This induces a "BRCAness" state, potentially making IDH-mutant cells more vulnerable to DNA-damaging agents like RT and TMZ [19].

Alterations in the Tumor Immune Microenvironment

The immunosuppressive GBM microenvironment is significantly changed by RT/TMZ [21].

- **Radiotherapy's Dual Role:** RT can promote an anti-tumor immune response by causing immunogenic cell death and attracting T-cells [22, 23]. A major

counteracting effect is the increased expression of PD-L1 on tumor cells post-RT [24, 25].

- **Temozolomide's Lymphocyte Impact:** A major side effect of standard TMZ is severe lymphocytopenia, particularly affecting CD4+ T-cells, which can persist long-term [27, 28]. Interestingly, alternative (e.g., metronomic) TMZ schedules may more selectively reduce regulatory T-cells (Tregs), possibly improving immune responses [29, 30].

Stimulation of DNA Repair and Survival Mechanisms

Tumor cells counteract the DNA damage from RT and TMZ by activating repair and survival pathways.

- **Increased DNA Damage Repair:** Treatment selects for clones with enhanced DNA repair capacity, such as base excision repair (for TMZ damage) and homologous recombination (for RT damage) [31, 32]. Proteins like PARP1 are often elevated in recurrent GBM, contributing to resistance [33].
- **EGFR Pathway Activation:** EGFR is altered in most GBM cases [35]. Both RT and TMZ can stimulate EGFR signaling as a survival response, aiding cell repair and growth [36, 37].

Discussion

This review highlights a major shift in understanding: GBM biomarkers are dynamic components that interact with and are shaped by therapy. The standard RT/TMZ regimen acts as an evolutionary force, molding the tumor's biology and leading to resistant recurrence. Grasping this dynamic is vital for progress.

MGMT promoter methylation is the foremost predictor of TMZ benefit [8, 9]. However, treatment selects for MGMT-expressing resistant cells [11, 12], underscoring that a single diagnostic biopsy may not guide later therapy. Liquid biopsies tracking circulating tumor DNA (ctDNA) offer promise for monitoring these changes over time [39, 40].

IDH1/2 mutations define a prognostically favorable group [15, 16]. The induced "BRCAness" state explains their better response to DNA-damaging therapy [17, 18] and suggests potential for combination with agents like PARP inhibitors [19].

The effects on the tumor immune microenvironment are complex. RT's ability to act as an in-situ vaccine [22, 23] supports combination with immunotherapy, but is offset by PD-L1 upregulation [24, 25]. The significant lymphodepletion from standard TMZ may hinder immunotherapy [27, 28], making alternative dosing schedules worth exploring [29, 30].

Finally, therapy induces adaptive DNA damage repair and pro-survival signaling [31, 32, 36, 37]. Targeting these adapted pathways—for example, with DDR or next-generation EGFR inhibitors—requires innovative combination strategies.

Limitations include the heterogeneity of studies and difficulty isolating effects of RT versus TMZ. Most data compare primary and first recurrent tumors; evolution through multiple treatment lines is less known.

Conclusion

In summary, radiotherapy and temozolomide profoundly and dynamically alter GBM biomarkers. Standard treatment actively selects for resistance and remodels the tumor's molecular and immune landscape. Advancing GBM care requires moving from a static to a dynamic, adaptive treatment model that anticipates and addresses the tumor's evolution under therapeutic pressure. Embracing this complexity is key to improving outcomes.

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The authors contributed to the data analysis. Drafting, revising and approving the article, responsible for all aspects of this work.

Conflict of Interest

None

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