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Sequential EGFR Inhibition Sensitizes Glioblastoma to Temozolomide

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Abstract

Glioblastoma (GBM) remains a devastating disease where standard temozolomide chemotherapy is limited by O^6-methylguanine-DNA methyltransferase (MGMT)-mediated DNA damage repair. By targeting EGFR, the major driving oncogene of GBM, Guo, Habib, and their colleagues demonstrate that EGFR inhibition prior to temozolomide induces an adaptive response that downregulates MGMT, enhancing therapeutic efficacy.

Keywords

O^6-methylguanine-DNA methyltransferase; activator protein-1; nuclear factor κB; miR-616

Glioblastoma (GBM) is the most common malignant primary brain tumor in adults, characterized by an aberrantly poor prognosis and severely limited therapeutic options [1]. Among current treatment strategies, chemotherapy with the DNA-alkylating agent **temozolomide (TMZ)** (see Glossary) is a critical part of the current standard care for GBM [1]. Mechanistically, TMZ induces **O^6-methylguanine** DNA lesions that trigger futile cycles of the DNA mismatch repair machinery, culminating in lethal DNA double-strand breaks and cell apoptosis [2,3]. However, in clinic, only approximately 40% of GBM patients show a measurable response before developing therapeutic resistance and tumor relapse inevitably occur [2].

O^6-methylguanine-DNA methyltransferase (MGMT)-mediated DNA damage repair is the most critical and well-characterized mechanism for therapeutic resistance to TMZ. This

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Declaration of interests

All authors declare they have no competing interests.

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crucial DNA repair enzyme directly removes the TMZ-induced methyl adduct from the guanine base, thereby counteracting the therapeutic effect of the chemotherapy [3]. The expression of MGMT is epigenetically regulated by methylation of its gene promoter. In the clinic, approximately 40% to 45% of GBM patients harbor ***MGMT* promoter hypermethylation**, which silences the gene expression and correlates with enhanced TMZ sensitivity [3,4]. Conversely, the remaining patients present with an unmethylated promoter that typically sustains active MGMT transcription, which is frequently associated with diminished TMZ sensitivity and a worse clinical prognosis [1,3].

In parallel to the challenges of chemoresistance, targeting the epidermal growth factor receptor (EGFR) has emerged as another prominent therapeutic strategy. EGFR is a major oncogenic driver of GBM malignancy, with gene amplification or hyperactivating mutations occurring in approximately 50% of clinical GBMs [5–8]. However, EGFR-targeting clinical trials for GBM historically remains ineffective, despite whether the EGFR inhibitor was administrated as monotherapies or when concurrently combined with the standard TMZ chemotherapy regimen [5,7,8].

To circumvent this therapeutic bottleneck, a recent study published in Science Translational Medicine by Guo, Habib, and their colleagues offers a compelling proof-of-concept strategy to link small-molecule EGFR inhibitors with TMZ sensitivity [4] (Figure 1). The authors uncovered a previously unrecognized mechanistic interplay, revealing that small-molecule **EGFR tyrosine kinase inhibitors (TKIs)**, such as erlotinib and afatinib, robustly decrease MGMT expression at both the mRNA and protein levels across human GBM patient-derived xenograft (PDX) lines, patient-derived glioma spheres, and mouse xenograft models.

Mechanistically, this downregulation is orchestrated by a complex signaling crosstalk between two downstream transcription factors with opposing regulatory roles at the *MGMT* locus: **activator protein-1** (AP-1, a c-Jun/c-Fos protein complex) and nuclear factor κB (NF-κB). While EGFR inhibition activates both pathways, their downstream impacts on *MGMT* transcription are opposed: a c-Jun/c-Fos (AP-1) complex acts as a transcriptional repressor that inhibits MGMT expression, whereas p65 (a primary subunit of the NF-κB) drives *MGMT* transcription. Chromatin immunoprecipitation analysis revealed that upon EGFR TKI exposure, enhanced AP-1 binding at the *MGMT* promoter displaces p65 from the locus. Crucially, the authors discovered that AP-1 activation concurrently drives the transcription of a **microRNA (miR)**-616. miR-616 suppresses *MGMT* expression through a coordinated, dual-action mechanism: it directly binds to the 3’ untranslated region (UTR) of *MGMT* mRNA to inhibit its translation, while concurrently dampening NF-κB activation by downregulating p65. Ultimately, the dominance of this AP-1/miR-616 axis over the opposing NF-κB pathway leads to diminished expression of MGMT, thus increasing GBM sensitivity to TMZ.

This regulatory network provides a potential explanation for why historical clinical trials combining TMZ and EGFR inhibitors failed. Guo, Habib, and their colleagues observed that TMZ exposure itself actively induces *MGMT* expression in GBM models. Consequently, concurrent co-administration of EGFR TKIs and TMZ fails to reduce MGMT levels because the intended suppression is offset by the opposing upregulation driven by TMZ. Conversely,

a sequential schedule-treating GBM with an EGFR inhibitor prior to TMZ exposure-successfully downregulates MGMT levels and increases sensitivity to TMZ in orthotopic GBM PDX mouse models. The translational relevance of this novel scheduling was validated using human GBM specimens derived from prior clinical trials (NCT04197934 and NCT00727506). Resected post-treatment tissues from patients treated with the brain-penetrant EGFR inhibitor WSD-0922 exhibited a substantial reduction in MGMT expression compared to their primary/naive tumors (NCT04197934). In contrast, paired primary and recurrent specimens utilizing concurrent afatinib and TMZ combinations showed no reduction in MGMT levels (NCT00727506). These findings reinforce that **sequential treatment** with an EGFR inhibitor prior to TMZ could be a promising therapeutic strategy.

This work underscores a crucial pharmacological principle: when combining therapeutic agents, the timing or sequence of administration can be critical. This concept is exemplified by a successful precedent in non-small-cell lung cancer, where sequential rather than concurrent administration of EGFR inhibitors and chemotherapy successfully bypassed cell cycle antagonism [9]. Mechanistically, concurrent scheduling yielded suboptimal outcomes, potentially because EGFR inhibitors induce G1 phase cell cycle arrest, which shields tumor cells from the cytotoxic effects of phase-dependent chemotherapeutic agents [9]. Similarly, in this study [4], the findings by Guo, Habib, and their colleagues suggest that the sequential administration is also vital in GBM, indicating that the administration of EGFR inhibitor prior to TMZ may be more effective by downregulating MGMT expression.

To advance the translational potential of this promising sequential regimen, a crucial next step will be the precise mapping of the target patient population. While EGFR activation and high baseline MGMT expression are both highly prevalent features in GBM patients, further characterization of the exact molecular intersection of these patient cohorts within clinical datasets will be highly valuable. Identifying this overlapping subpopulation, characterized by concurrent EGFR activation and unmethylated MGMT status will, in theory, define the candidate pool that are most likely to benefit from this sequential scheduling.

Second, discovery of the underlying pharmacological mechanisms of this EGFR TKI-induced MGMT downregulation is an exciting and promising future direction. An intriguing observation emerging from this work is that the suppression of MGMT operates independently of EGFR signaling, raising the compelling possibility of either additional alternative signaling cascades or a selective, class-wide off-target mechanism shared among these small molecules. While Guo, Habib, and their colleagues robustly demonstrated this phenomenon across multiple distinct inhibitors—including erlotinib, afatinib, and WSD-0922—systematically determining whether this is a universal property inherent to all clinical-grade EGFR TKIs remains an open and highly instructive question for future research. Defining this pharmacological response will provide the necessary rationale to select the most pharmacologically optimized agents for subsequent trial designs.

Finally, delineating the exact kinetics and durability of this MGMT suppression will be critical for clinical translation. While these preclinical models provide a robust foundation, the precise longevity of this molecular window of vulnerability in human patients remains to be determined. It is plausible for future studies to evaluate whether this molecular

downregulation persists long enough to sensitize GBM tumor response throughout the entire courses of standard TMZ treatment, or to define the precise window of TMZ exposure that actively benefits from this prior priming effect. Establishing these detailed profiles will offer important guidance for optimizing the scheduling of this therapeutic regimen. Ultimately, this study underscores that optimizing the order of administration may offer a viable path to successfully integrating EGFR inhibitors into current standard care for patients with GBM.

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Glossary

Activator protein-1 (AP-1)
a family of dimeric transcription factors, commonly composed of a c-Jun and c-Fos protein complex, that regulate gene expression in response to growth factors, cellular stress, and other extracellular signals.

Epidermal growth factor receptor tyrosine kinase inhibitor (EGFR TKI)
a small-molecule drug that inhibits the kinase activity of EGFR and thereby alters its downstream signaling pathways.

***MGMT* promoter hypermethylation**
an epigenetic modification whose high level is associated with transcriptional silencing of the *MGMT* gene and increased sensitivity to temozolomide in glioblastoma.

microRNAs (miR)
small, non-coding RNA molecules with 21–25 nucleotides in length that regulate gene expression in cells.

O^6-methylguanine-DNA methyltransferase (MGMT)
a DNA repair enzyme that removes alkyl groups from the O^6 position of guanine and thereby counteracts the cytotoxic effects of alkylating chemotherapy.

O^6-methylguanine
a mutagenic DNA lesion produced by alkylating agents such as temozolomide, which can trigger mismatch repair–dependent DNA damage when not removed by MGMT.

Sequential treatment
a therapeutic strategy in which two or more agents are administered in a defined order rather than simultaneously.

Temozolomide (TMZ)
an orally administered DNA-alkylating agent used as standard chemotherapy for glioblastoma and other glioma subtypes.

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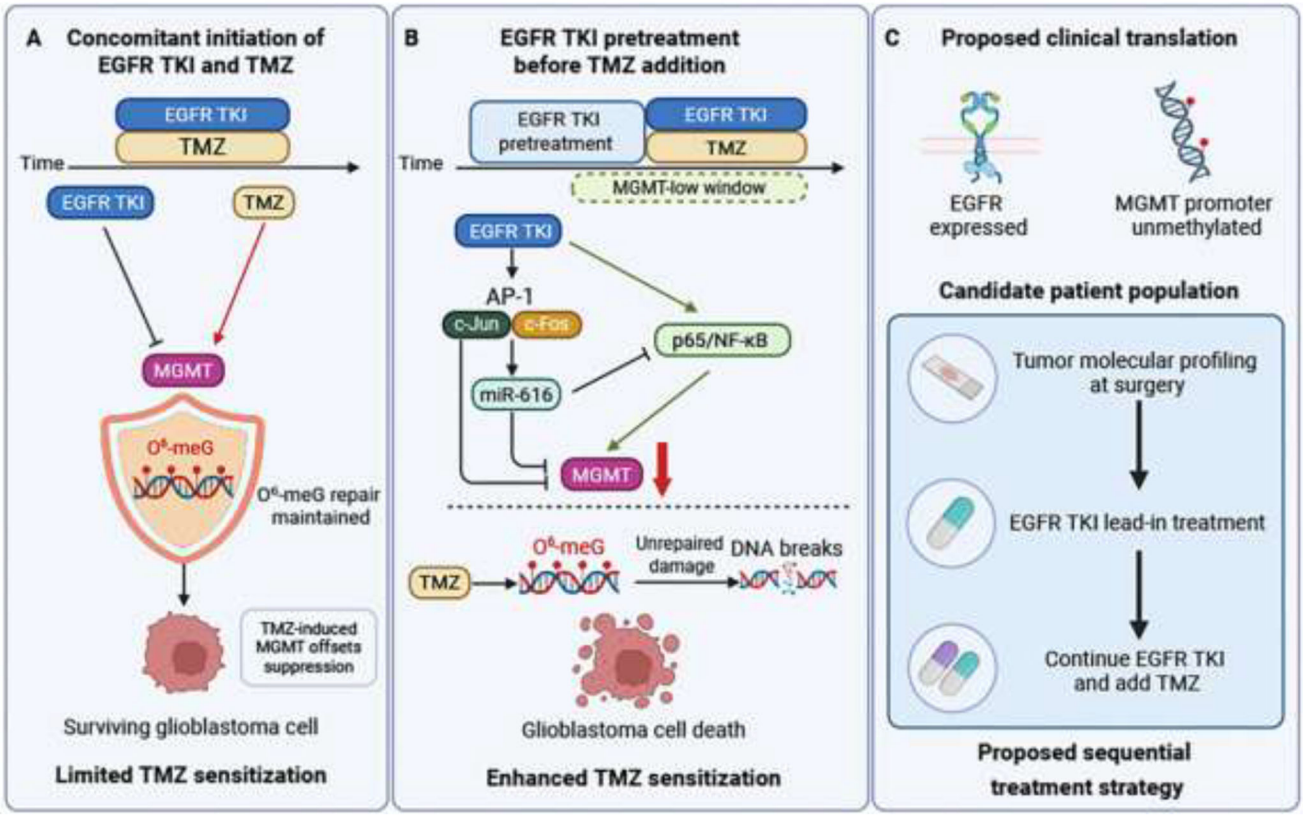

Figure 1. Treatment sequence determines MGMT suppression and temozolomide sensitization in glioblastoma.

(A) During concomitant treatment, temozolomide (TMZ)-induced O^6-methylguanine-DNA methyltransferase (MGMT) upregulation counteracts MGMT suppression mediated by epidermal growth factor receptor tyrosine kinase inhibitor (EGFR TKI) treatment, thereby preserving MGMT-mediated DNA damage repair activity and limiting TMZ sensitization. (B) EGFR TKI pretreatment activates activator protein-1 (AP-1, a c-Jun/c-Fos complex), which directly represses MGMT transcription and induces miR-616 expression. miR-616 further suppresses MGMT expression by targeting the 3′ untranslated region of MGMT mRNA and by attenuating p65/NF-κB signaling, which is activated by EGFR TKI and promotes MGMT expression. Subsequent TMZ administration during this MGMT-low window promotes the accumulation of unrepaired O^6-methylguanine lesions and tumor cell killing. (C) Proposed clinical translation. A rational clinical translation strategy should involve first determining tumor EGFR expression and *MGMT* promoter methylation status at surgery to select candidate patients, followed by a sequential treatment. The figure was created in BioRender (https://BioRender.com/).