EO-4426: A Brain-Penetrant Dual DNA Polymerase α and Ribonucleotide Reductase Inhibitor for High-Grade Gliomas and Other Aggressive Cancers

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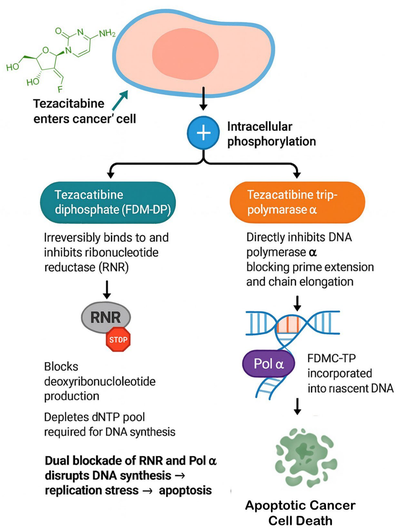
BACKGROUND

High-grade gliomas (HGG) and other CNS malignancies are marked by replication stress, genomic instability, and resistance to standard-of-care therapies. EO-4426 (tezacitabine, FDMC) is an orally bioavailable, brain-penetrant small molecule that inhibits both DNA polymerase alpha (Pol α) and ribonucleotide reductase (RNR)—critical enzymes for DNA replication and repair. This dual-targeting mechanism induces replication fork collapse and accumulation of DNA damage, selectively impacting rapidly proliferating tumor cells.

Mechanism of Action: EO4426
(tezacitabine)

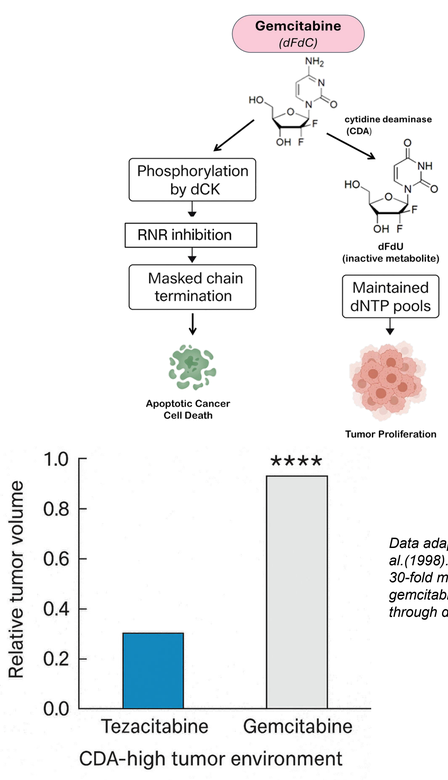


EO4426 Overcomes CDA-Mediated Resistance

EO4426 maintains antitumor activity in cytidine deaminase (CDA)–high tumor environments where cytidine analogs such as gemcitabine are rapidly inactivated. Unlike gemcitabine, which is converted by CDA to inactive dFdU, EO4426's modified 5,6-dihydro-2′,2′-difluorocytidine structure resists deamination while retaining phosphorylation by deoxycytidine kinase (dCK). The active metabolites—FDMC-DP and FDMC-TP—inhibit ribonucleotide reductase (RNR) and DNA polymerase α, respectively, leading to depletion of deoxynucleotide pools, replication fork collapse, and apoptosis.

Together, these studies establish that tezacitabine resists CDA-mediated degradation, retains activation by dCK, and sustains dual RNR + Pol α inhibition—overcoming a key metabolic resistance pathway in CDA-overexpressing tumors.

Mechanism of CDA-mediated resistance to RNR inhibitors



Data adapted from Takahashi et al.(1998). EO4426 is approximately 30-fold more resistant than gemcitabine to metabolic inactivation through deamination by CDA

PRIOR CLINICAL EVALUATION OF EO4426

EO-4426 has been studied in over 400 patients across multiple Phase 1 and Phase 2 trials as both i.v. and oral formulations, alone and in combination with 5-FU or cisplatin. The drug showed favorable tolerability, with reversible neutropenia as the primary dose-limiting toxicity, and clinical activity across diverse cancers, including CNS tumors.

Indication	Phase
Advanced solid tumors	Phase 1
Hematologic malignancies (NCT00061620)	Phase 1
Metastatic colorectal cancer (NCT00051688)	Phase 2
Advanced esophageal or gastric adenocarcinoma (NCT00054873)	Phase 2

Safety Parameter	Summary of Findings
Dose-Limiting Toxicity (DLT)	Myelosuppression, primarily neutropenia.
Most Common AEs	Neutropenia, thrombocytopenia, anemia, fatigue, nausea/vomiting.
Grade ≥3 AEs	Severe neutropenia, thrombocytopenia, infections related to cytopenias.
Non-Hematologic AEs	Mostly mild GI symptoms, fatigue, anorexia, rare mucositis.
Organ Toxicity	No consistent hepatic, renal, or cardiac toxicity reported.
Reversibility	Hematologic toxicity reversible with dose interruption.
Overall Safety Profile	Predictable cytotoxic-class myelosuppression.

PRECLINICAL ACTIVITY AGAINST CNS TUMORS

EO-4426 (tezacitabine) demonstrated potent activity in preclinical intracranial models, significantly extending survival in both primary brain tumors—including GBM—and aggressive CNS-engrafting neuroblastoma, outperforming standard agents such as BCNU. In addition, its CNS penetration and cytidine-deaminase–resistant design support its potential utility in tumor types with a high incidence of brain metastases, such as lung and breast cancer.

Primary Brain Tumor	ELO4426 Dosing	Results
D45 glioblastoma	100mg/kg ip 2x week	Median Survival EO4426: 46.5d Control: 20d p<0.0001
SK-N-C neuroblastoma	200mg/kg ip 2x week	Median survival: EO4426: 39d BCNU: 23d p<0.0001 90d survival EO4426: 90% Controls: carmustine 26%; untreated 23%
U87 MG glioblastoma	10-20mg/kg q.d. x5	>OS vs. control (p<0.01)
MDA-MB-231 (TNBC) *(high CNS mets)*	15mg/kg ip d x2weeks	90-100% CR

FUTURE DIRECTIONS

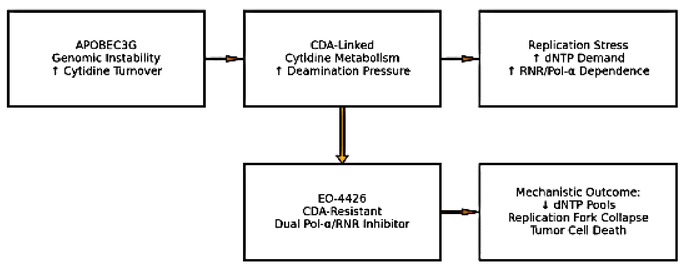
EO-4426 in CDA-High Tumors and CNS Metastases

- Many solid tumors with high or inducible cytidine deaminase (CDA)—including NSCLC, TNBC, ovarian cancer, and HNSCC—rapidly inactivate cytidine analogs such as gemcitabine, limiting drug exposure and therapeutic effect.
- EO-4426 (tezacitabine) is *designed to resist CDA-mediated deamination*, maintaining prolonged active drug levels even in CDA-rich microenvironments.
- EO-4426 also demonstrates CNS penetration and potent intracranial activity in preclinical GBM and neuroblastoma models, providing strong rationale for use in cancers with high CNS-metastatic burden, where both BBB penetration and metabolic resistance restrict current treatment options.
- Together, these properties position EO-4426 as a differentiated therapy for systemic tumors prone to CNS colonization.

Cancer Type	Brain Met Incidence	RNR Standard of Care	CDA Expression Prevalence	
NSCLC	25–45%	Gemcitabine	~25–40%	CDA often upregulated in tumors with prior exposure to nucleoside analogs; higher in squamous NSCLC
SCLC	50–80%	Gemcitabine	~20–30%	
Breast (HER2+, TNBC)	20–50%	Gemcitabine	induced in TNBC	Low baseline CDA, but TNBC shows higher CDA induction under chemotherapy (adaptive resistance).
Melanoma	40–60%	Hydroxyurea	Low	
Ovarian Cancer	2–5%	Gemcitabine	~30–40%	Highest prevalence platinum-resistant disease
Head & Neck SCC	1–5%	Hydroxyurea	~40–60%	

EO-4426 in Mesenchymal GBM

- MES GBM displays APOBEC3G-driven genomic instability and heightened cytidine-metabolism activity, including pathways associated with CDA-mediated deamination and nucleoside-analog resistance.
- APOBEC3G enrichment increases replication stress, dNTP turnover, and dependence on RNR and Pol-α, creating a targetable vulnerability in DNA synthesis and repair.
- EO-4426 is a brain-penetrant, CDA-resistant dual Pol-α/RNR inhibitor that bypasses cytidine-deaminase inactivation while collapsing dNTP pools and inducing replication-fork failure.
- Demonstrated intracranial activity in GBM and neuroblastoma models supports its potential to overcome MES-associated radio-resistance and DNA-repair dependence.
- Collectively, these features position EO-4426 as a differentiated therapeutic strategy for MES-enriched GBM, a subtype defined by high replication stress, APOBEC-linked genomic plasticity, and poor response to current therapies



CONCLUSIONS AND NEXT SETPS

- EO-4426 is a brain-penetrant, CDA-resistant dual Pol-α/RNR inhibitor that drives replication-fork collapse and DNA damage, with demonstrated intracranial activity and preserved efficacy in CDA-high tumor environments that inactivate other cytidine analogs.
- Its mechanistic profile and preclinical data support development in CDA-high solid tumors with high CNS-metastatic risk and MES-enriched GBM (APOBEC/CDA-linked replication stress) where EO-4426 addresses both metabolic resistance and blood–brain barrier limitation
- GMP manufacture of EO-4426 drug product to enable initiation of new clinical trials, alongside supporting CMC work required for regulatory filings is planned.
- Advance translational and clinical readiness through preclinical studies in CDA-high metastatic models (NSCLC, TNBC, ovarian, HNSCC) and GBM subtypes including evaluation of the APOBEC3G→CDA axis, dNTP-pool depletion and replication-stress biomarkers (γH2AX, pRPA), CNS PK/PD modeling, and synergy with RT or DDR-targeting agents; followed by biomarker-enriched Phase 1b/2 cohorts in, CDA-high tumors, and CNS-metastatic disease and MES GBM.

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