BACKGROUND: Diffuse midline glioma (DMG) is a universally fatal CNS tumor, with a median overall survival of <12-months. Recent studies, including our own, have identified overexpression of the mitochondrial protease ClpP in DMG patient samples. ClpP plays a pivotal role in regulating energy production. Brain-penetrant ClpP agonists, including ONC021, ONC206, and TR107, induce non-specific degradation of the mitochondrial electron transport chain. However, there is considerable variability in the response of DMG cell lines to these therapies, with only about half reaching IC50. Notably, TP53-mutant cell lines exhibit reduced sensitivity.

METHODS/RESULTS: Further analysis using quantitative proteomics of TP53-mutant DMG cell lines exhibited hallmark activation of ClpP, increased mitochondrial stress and dysfunction. Importantly, ClpP agonism increased the activity of the transcription factor NRF2, driving expression of NQO1 (p=0.0017). NQO1 is a cytoprotective enzyme, promoting redox balance and cell survival. This highlights a potential adaptive response to ClpP agonist therapy in DMG cells. Analysis of DMG patient samples through publicly available RNA sequencing data identified ubiquitously high NRF2 expression compared to other pediatric CNS tumors. This led us to hypothesize that a clinically relevant, selective NQO1 inhibitor could elicit a strong response to ClpP agonism. Therefore, we examined ARQ761, a CNS-active therapy that disrupts NQO1 leading to the formation of an unstable hydroquinone, causing oxidative DNA damage and apoptosis. When ARQ761 was used concurrently with a ClpP agonist, the combination showed additive effects, average Bliss Score of 9.91 (n=4). Interestingly, administering ARQ761 24 h after ClpP agonist exposure combined synergistically, average Bliss Score of 15.87 (n=4). This synergy was further potentiated using ARQ761 48 h after ClpP agonism, when NQO1 expression peaked, resulting in an average Bliss Score of 20.30 (n=4). CONCLUSIONS: This study has unveiled an intrinsic antioxidant defense mechanism in DMG, which facilitates evasion from treatments inducing oxidative stress. This defense mechanism appears particularly robust in cell lines harboring TP53-mutations, which are also known to exhibit resistance to radiotherapy.

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DIPG-88. TARGETING THE ANTIOXIDANT RESPONSE ELEMENT AXIS SENSITIZES DIFFUSE MIDLINE GLIOMA CELLS TO ONC201
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