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ONC201 and ONC206: Metabolically ClipPing the wings of diffuse midline glioma

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It has long been clear that effective treatment of diffuse intrinsic pontine gliomas and other diffuse midline gliomas (DMG)—primarily in children and young adults—represents a critical challenge in neuro-oncology. These aggressive brain tumors have an extremely poor prognosis and lack any durably effective treatments. The discovery several years ago of histone 3.3 mutations, most commonly K27M and G34R/V,[1] as drivers within many of these tumors has offered the prospect for better biological understanding and more targeted therapies, but to date, there have not been resulting breakthroughs in treatment. However, recent studies have indicated at least two promising lines of attack for the more common H3.3K27M-mutated gliomas. Chimeric antigen receptor (CAR) T cells targeting the ganglioside GD2 are showing early potential in patients in pediatric care,[2] and in addition the small-molecule imipridone ONC201 was found to have potential activity against H3.3K27M-mutated gliomas in early trials testing its efficacy in glioma (serendipitously in the first patient).[3–5] Fully leveraging the latter discovery has been hampered by inadequate understanding of ONC201's mechanism of action against H3.3K27M-mutated glioma, but a new study by Przystal et al in this issue provides substantial new insights into this.[6]

The authors focused on the activities and mechanisms of ONC201 and ONC206, a more recent analog, in multiple settings and models of pediatric DMG with H3.3K27M mutation. Both drugs showed activity against DMG in vitro and modestly extended survival in vivo in DMG PDX mouse models. It was confirmed that both drugs bind to the mitochondrial serine protease ClpP (caseinolytic protease proteolytic subunit), supporting earlier work suggesting ClpP activation as a potential mechanism for ONC201's effects[7]—and in contrast to prior assumptions that dopamine receptor antagonism was mediating ONC201 activity against DMG. The primary target is a critical question for maximizing the benefits of ONC201 against DMG, and Przystal et al provide important new evidence for ClpP as the key mediator. They demonstrate that ClpP mRNA and protein levels correlate with pediatric brain tumor grade and with overall survival. Importantly, DMG cells with CRISPR/Cas9 knockout of the *CLPP* gene were resistant to ONC201 and ONC206, providing strong evidence that ClpP is the relevant target.

ONC201 and ONC206 were found for the first time to affect mitochondrial activity in DMG cells, which could well follow from the mitochondrial protease ClpP being the primary target. These metabolic effects may be the dominant late-stage mediator of these drugs' activity against DMG cells. Both drugs were found to decrease mitochondrial membrane potential, increase mitochondrial generation of reactive oxygen species (ROS), and induce mitochondrial morphologic aberrations—with ONC206 generally having stronger effects. ONC201 and ONC206 reduced mitochondrial oxidative phosphorylation, oxygen consumption rate, and ATP production. In addition, both drugs were found to trigger apoptosis and the integrated stress response (ISR), with the latter evident in vivo in the subcutaneous setting as well as in vitro.

The authors also showed the effects of ONC201 on the differentiation of DMG cells, which may be triggered by the mitochondrial activities of this drug. Single-cell RNA sequencing (scRNA-seq) experiments demonstrated that ONC201 treatment of DMG cells reduced cycling cells and those with an oligodendrocyte precursor cell (OPC)-like state, while increasing those with a differentiated astrocyte cell (AC)-like state.

This report also provided noteworthy findings on similarities, differences, and combinatorial effects of ONC201 and ONC206. For the most part, ONC201 and ONC206 had similar downstream effects. ONC206 was significantly more potent in most settings, with slightly stronger binding to ClpP. However, intriguing differences in the effects of ONC201 and ONC206 emerged. ONC201 reduced glycolysis, while ONC206 promoted it. Furthermore, each drug and the combination yielded some differences in transcriptomic effects, including ONC206 causing an up-regulation of the hypoxia-inducible factor (HIF) pathway. Despite having differential effects on glycolysis and the transcriptome, the combination of ONC201 and ONC206 had stronger overall effects on mitochondria. In addition, combining the two drugs generally yielded at least additive and sometimes synergistic activity against DMG in vitro (synergistic with the Bliss and ZIP algorithms but not by Loewe) and slightly stronger activity than the single agents in vivo. The authors hypothesize that this is due to the differences in the activities of ONC201 and ONC206, but further work should be done on whether it stems from what is effectively higher dosing of these similar

drugs with the combination; this will help determine whether the combination should be pursued in the clinic.

In addition to its findings on the activity and mechanism of ONC201 and ONC206 against DMG, this report also added to growing information on low toxicity of these drugs—which cannot be taken for granted given their effects on mitochondria and on dopamine receptors. ONC201 and ONC206 were found to have negligible toxicity in zebrafish larvae, consistent with their general safety and tolerability in early clinical trials.

While this study adds to our knowledge of the effects of ONC201 and ONC206 on DMG cells, critical questions remain. It is particularly important to determine why these agents have preferential activity against H3.3K27M-mutated gliomas. These gliomas may be particularly sensitive to ClpP activation due to its over-expression, their sensitivity to the downstream metabolic effects, or perhaps some degree of both. In addition, given the mitochondrial and metabolic effects of these drugs on glioma, it is conceivable that combining them with other agents targeting mitochondria and metabolism may yield synergistic activities. Answering these questions, as well as following up on the work of this study, will help maximize the impact of these agents—both as single agents and as combinations—on DMG and potentially other cancers as well.

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