EXTH-104

Role of ClpP and Mitochondrial Metabolism in the Anti-cancer Effects of Imipridone ONC201 and ONC206 in Glioblastoma and DIPG

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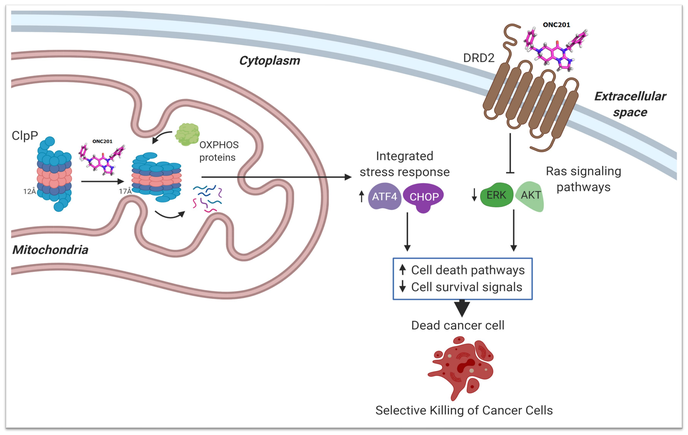
Introduction

- ONC201, a first-in-class imipridone, is an oral, blood-brain barrier penetrating, selective small molecule antagonist of dopamine receptor D2 (DRD2) and agonist of the mitochondrial protease caseinolytic mitochondrial matrix peptidase proteolytic subunit (ClpP) (**Figure 1**).[1-5]
- ONC201 has demonstrated tolerability and durable tumor regressions in H3 K27M glioma patients.[6]

Figure 1. ONC201 Mechanism of Action



AKT, protein kinase B; ATF4, activating transcription factor 4; CHOP, C/EBP-homologous protein; ClpP, caseinolytic protease P; DRD2, dopamine receptor D2; ERK, extracellular-regulated kinase. Originally published in Anderson, et al. *Clin Cancer Res*. 2022;28:1773–82.

- ONC206, a chemical derivative of ONC201, is the second imipridone to be developed and is currently in Phase 1 clinical development.[7]
- Relative to ONC201, ONC206 has demonstrated differentiated DRD2 receptor pharmacology[8], improved potency, enhanced absorption/tissue distribution, and preclinical anti-cancer activity *in vitro* and *in vivo*.[9-12]

Methods

Cell Lines
- Glioblastoma (GBM; T98G, A172, U-87MG) and astrocytoma (H4, U-118MG) lines were obtained from American Type Culture Collection (ATCC).
- H3.3 K27M mutant diffuse intrinsic pontine glioma cell lines (DIPG; SF8628, SF7761) were acquired from Millipore.

Cell Viability
- Cells (1000-5000) were seeded in a 96-well plate containing compound dilutions in the appropriate range. Cell viability was determined using CellTiter-Glo (Promega) at day five unless specified.

CRISPR
- Edit-R All-in-one Lentiviral sgRNA vector (Dharmacon, Horizon) was used to generate ClpP knockout cell lines.
- The lentiviral vector contains sgRNA that targets the ClpP gene in exon 1 or exon 4, a human Cas9 codon, and a puromycin resistance marker.
- sgRNA sequences are as follows:
  - ClpP gene in exon 1: ATGTGGCCCGGAATATTGGT
  - ClpP gene in exon 4: AGGGCTGGTGATCATGATA

Transduction
- SF8628 cell lines were transduced with lentiviral particles with a multiplicity of infection (MOI) of 0.03 or 0.3 for 2 days, then selected with puromycin (1 µM) for 14 days and harvested for further protein detection and analysis.

Resistant Clones
- Treatment to generate acquired resistance was initiated at approximately 0.2 to 1 times the IC50 and drug concentration was doubled when cells were able to proliferate in a given concentration.
- Acquired resistance was defined as cell proliferation in the presence of drug as determined by CellTiter-Glo.
- Time-stability of acquired resistance was confirmed by growing cells in the absence of drug for 2 to 4 weeks and retesting.
- Two polyclonal resistant lines were generated for each compound.

Whole Genome/Exome Sequencing
- DNA was purified with QIAamp DNA Blood Midi from approximately 5 million cells.
- After DNA purification, samples were divided into replicates for sequencing at Novogene.

RNAseq analysis
- RNA extraction, library prep, and sequencing using NEBNext library prep and Illumina sequencing (1x75bp reads, avg 15M reads) on extracted RNA from T98G glioblastoma cells (parental, ONC201 resistant, ONC206 resistant) was performed in triplicate for indicated treatments (vehicle, ONC201 IC50, ONC206 IC50) and timepoints (4hr, 24hr, 48hr continuous, 24hr-treat/24hr-washout).

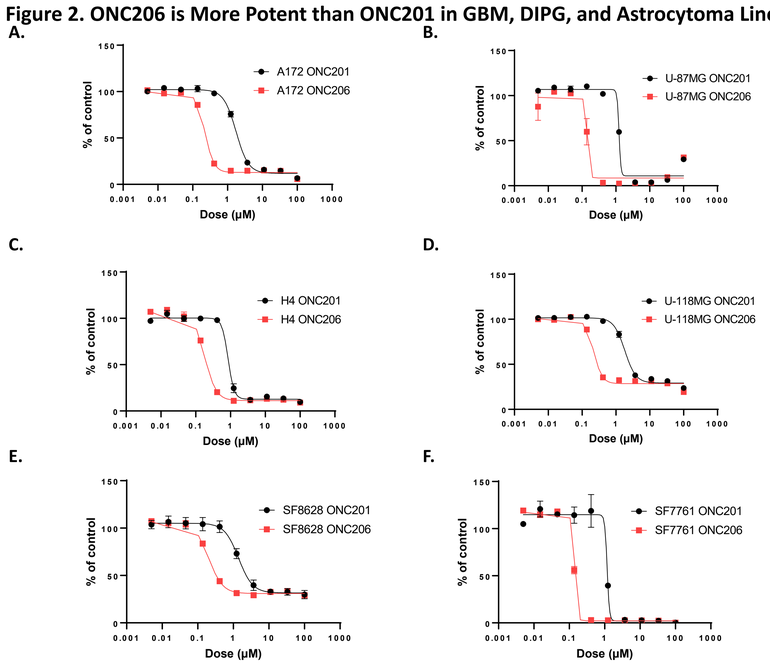
Results

Table 1. IC50 for ONC201 and ONC206 in GBM, DIPG, and Astrocytoma Lines (Day 5)

Cell line	Classification	ONC201 IC50 (µM)	ONC206 IC50 (µM)
A172	Glioblastoma	1.751	0.223
U-87 MG	Glioblastoma	1.237	0.140
T98G	Glioblastoma	1.807	0.225
H4	Astrocytoma	0.851	0.180
U-118 MG	Astrocytoma	1.821	0.218
SF8628	H3.3 K27M-mutant DIPG	1.398	0.201
SF7761	H3.3 K27M-mutant DIPG	1.172	0.135

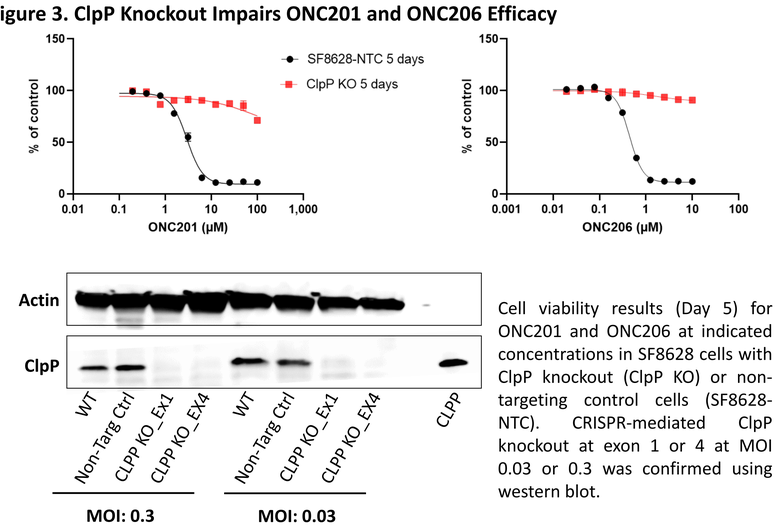
Results

Figure 2. ONC206 is More Potent than ONC201 in GBM, DIPG, and Astrocytoma Lines



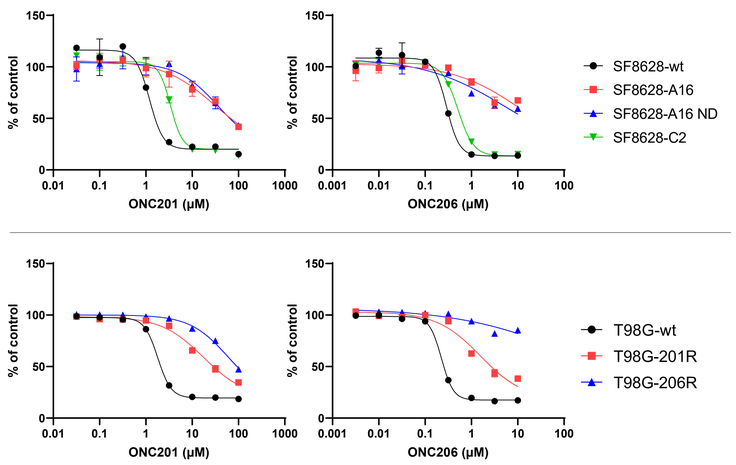
Cell viability results for ONC201 and ONC206 at indicated concentrations/timepoints in glioblastoma (**A**, A172; **B**, U-87MG), astrocytoma (**C**, H4; **D**, U-118MG) and H3.3 K27M-mutant DIPG (**E**, SF8628; **F**, SF7761) lines at day 5 (n=4). Red indicated ONC206, black indicates ONC201.

Figure 3. ClpP Knockout Impairs ONC201 and ONC206 Efficacy



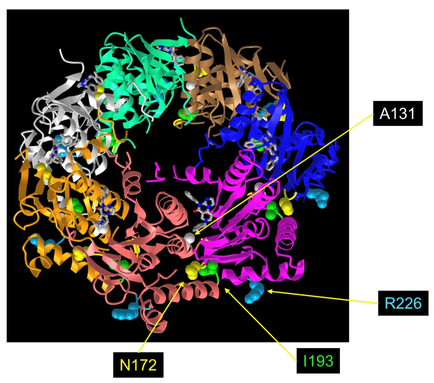
Cell viability results (Day 5) for ONC201 and ONC206 at indicated concentrations in SF8628 cells with ClpP knockout (ClpP KO) or non-targeting control cells (SF8628-NTC). CRISPR-mediated ClpP knockout at exon 1 or 4 at MOI 0.03 or 0.3 was confirmed using western blot.

Figure 4. Cells with Acquired Resistance to ONC201 Showed Cross-resistance to ONC206



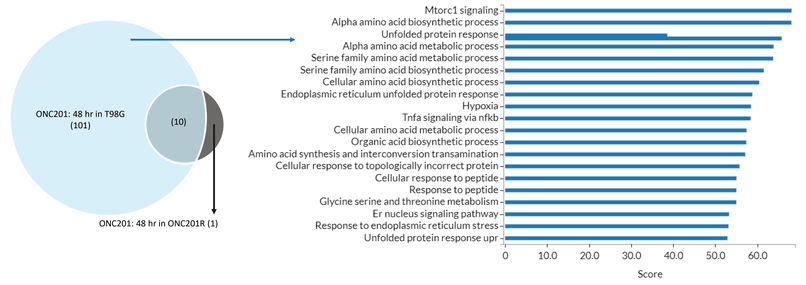
Cell viability results (Day 5) for ONC201 and ONC206 in SF8628 and T98G parental and resistant cells. SF8628-A16 and SF8628-C2 = cells selected under 16 or 2 µM ONC201. SF8628-A16 ND = A16 cells without ONC201 treatment for 25 days. T98G-201R and T98G-206R = cells selected under 16 µM ONC201 or 1.6 µM ONC206.

Figure 5. Positions of ClpP Mutations Identified in GBM and DIPG ONC201/206 Resistant Cells Relative to ONC201 Binding Site (derived from NCBI Molecular Modeling Database (MMDB) ID: 175107)



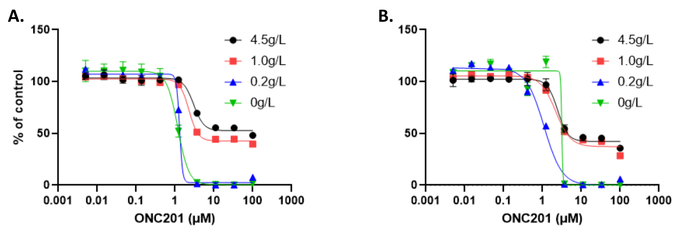
- ONC201 and ONC206-resistant T98G GBM clones (3-fold above initial IC50)
  - ONC201 resistant cells had ClpP A131D/R226G mutations (~32-38% prevalence)
  - ONC206 resistant cells had a ClpP R226G mutation (~43-54% prevalence)
- ONC201-resistant SF8628 DIPG K27M clones
  - ONC201 (16 µM) resistant cells (A16) had a ClpP I193V mutation (~50-67% prevalence)
  - ONC201 (2 µM) resistant cells (C2) had a ClpP N172Y mutation (~22-33% prevalence)

Figure 6. RNAseq Analysis in T98G Parental Versus ONC201-resistant Cells



RNAseq analysis revealed upregulation (101 genes) of the integrated stress response and amino acid metabolism with ONC201 (IC50) treatment (48h) in T98G parental cells but not ONC201-resistant cells (ONC201R). Each circle represents a comparison. When area proportion is set, the size of the circle is proportional to the number of genes or features that are differentially expressed at a given p-value cutoff. Overlap is proportional to the number of shared genes between those two comparisons.

Figure 7. Glioma Cells are More Sensitive to ONC201 with Lower Glucose Concentrations in the Media



Cell viability results (Day 3) for ONC201 at indicated concentrations in U-118MG (A) and SF8628 (B) cells with varying glucose levels in the media (n=2).

Summary

- Effects on ClpP and mitochondrial metabolism are key aspects for the mechanism of action of ONC201 and ONC206 *in vitro*.
- ClpP could also play a role in the evolution of glioma cell resistance upon prolonged exposure to ONC201 or ONC206.
- ONC206 exhibits improved nanomolar potency relative to ONC201 in GBM, DIPG, and astrocytoma cells.
- Studies on the role of DRD2, suggested by prior published work, are ongoing.

References

1. Allen JE, et al. *Sci Transl Med*. 2013;5(171):171ra17; **2.** Free RB, et al. *Mol Pharmacol*. 2021;100(4):372-387; **3.** Madhukar NS, et al. *Nat Commun*. 2019;10(1):5221. **4.** Ishizawa J, et al. *Cancer Cell*. 2019;35(5):721-737 e9; **5.** Graves PR, et al. *ACS Chem Biol*. 2019;14(5):1020-1029; **6.** Chi AS, et al. J Neurooncol. 2019 Oct;145(1):97-105. **7.** Theeler BJ, et al. *J Clin Oncol*. 2020;38(15_suppl):TPS2576-TPS2576; **8.** Prabhu VV, et al. Cancer Res 2020;80(16 Suppl):Abstract nr 5688; **9.** Wagner J, et al. *Cell Cycle*. 017;16(19):1790-1799; **10.** Staley A, et al. *Am J Cancer Res*. 2021;11(11):5374-5387; **11.** Zhang Y, et al. *Front Oncol*. 2020;10:577141; **12.** Tucker K, et al. *Am J Cancer Res*. 2022;12(2):521-536.

Disclosures

SF, AL, CM, JEA, RL, PS, and **VVP** are employees of and have stock ownership in Chimerix, Inc. **JEA** and **VVP** are shareholders of Oncoceutics, Inc.

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Poster PDF

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