EXTH-37

Allosteric mitochondrial ClpP agonist ONC206 alters stress response, metabolic and epigenetic profiles to elicit anti-cancer efficacy in high-grade gliomas

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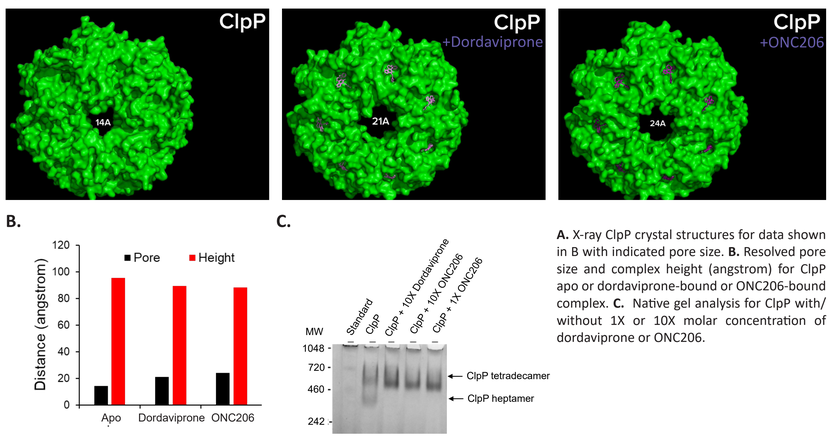
Introduction

- Dordaviprone (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).[1-5]
- Dordaviprone has demonstrated tolerability and durable tumor regressions in patients with H3 K27M-mutant glioma.[6]
- ONC206, a chemical derivative of dordaviprone, is the second imipridone to be developed and is currently in Phase 1 clinical development.[7]
- Relative to dordaviprone, ONC206 has demonstrated differentiated DRD2 receptor pharmacology[8], improved potency, enhanced absorption/tissue distribution, and preclinical anti-cancer activity in vitro and in vivo.[9-13]

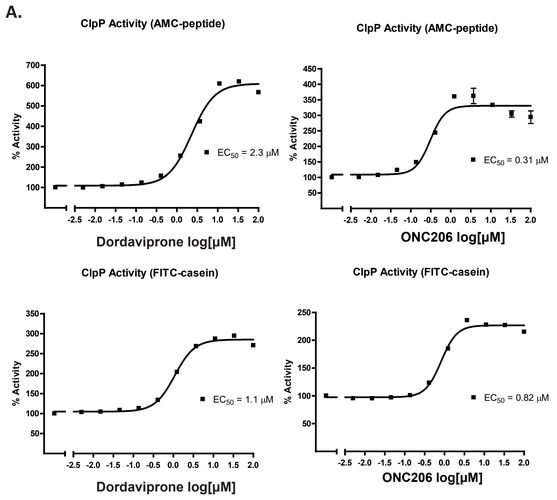
Results

Figure 1. Co-crystallization with ClpP revealed distinctions in the ONC206-ClpP resolved X-ray crystal structure relative to the dordaviprone-bound or apo complex



A. X-ray ClpP crystal structures for data shown in B with indicated pore size. **B.** Resolved pore size and complex height (angstrom) for ClpP apo or dordaviprone-bound or ONC206-bound complex. **C.** Native gel analysis for ClpP with/ without 1X or 10X molar concentration of dordaviprone or ONC206.

Figure 2. ONC206 exhibits nanomolar potency in cell-free proteolysis assays and against glioma cell lines



A. Wild-type ClpP protein was expressed in vitro, purified by His-affinity, and assayed for activity using FITC-casein and AMC peptide substrates as described previously.[4] **B.** For IC50 determination, cells were treated with dordaviprone or ONC206 at various concentrations and viability was measured on Day 5 using CellTiter-Glo. Glioblastoma (GBM; T98G, A172, U-87MG) and astrocytoma (H4, U-118MG) lines were obtained from ATCC. H3.3 K27M-mutant diffuse intrinsic pontine glioma cell lines (DIPG; SF8628, SF7761) were acquired from Millipore. **C.** Patient-derived high-grade glioma cells with indicated H3 K27M status were treated with ONC206 at indicated concentrations and viability was measured on Day 4 or 7 using CellTiter-Glo. Cells were obtained from Children's Brain Tumor Network/Children's Hospital of Philadelphia.

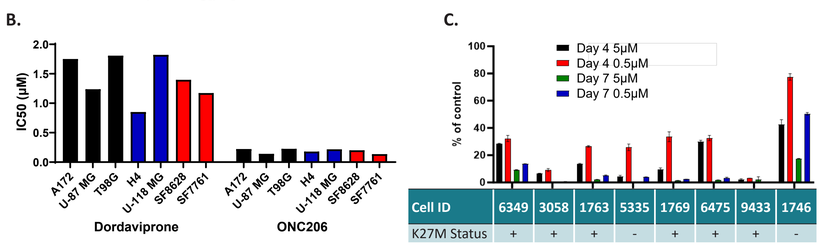
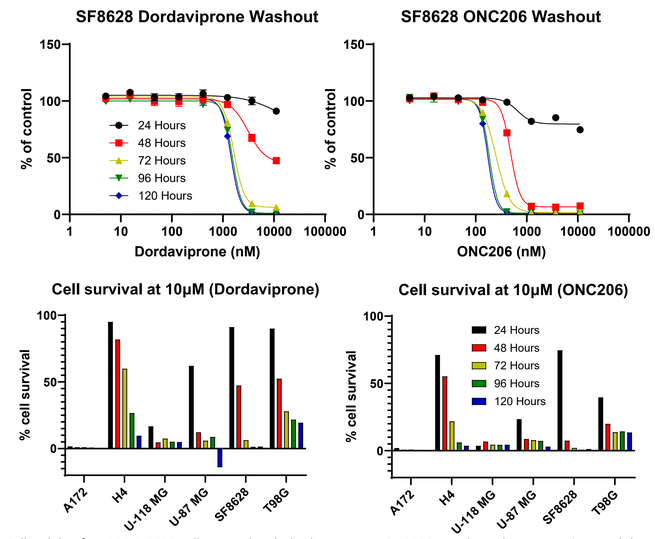


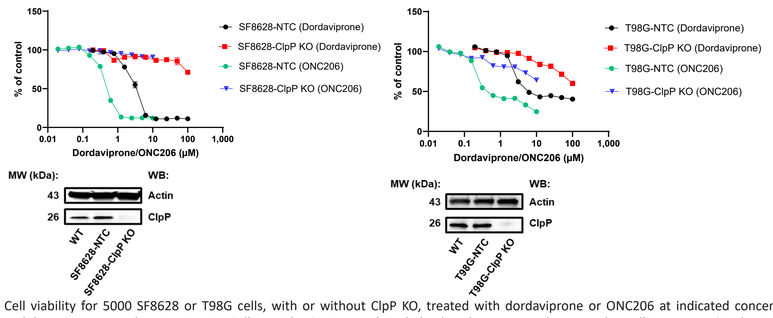
Figure 3. Sustained ONC206 exposure needed to impair glioma cell viability



Cell viability for 5000 SF8628 cells treated with dordaviprone or ONC206 at indicated concentrations and duration before removal of drug-containing media and replenishment with standard media. Viability was measured on Day 7 using CellTiter-Glo. Bar graphs indicate % cell viability after treatment with 10μM dordaviprone or ONC206 for the same duration across multiple cell lines.

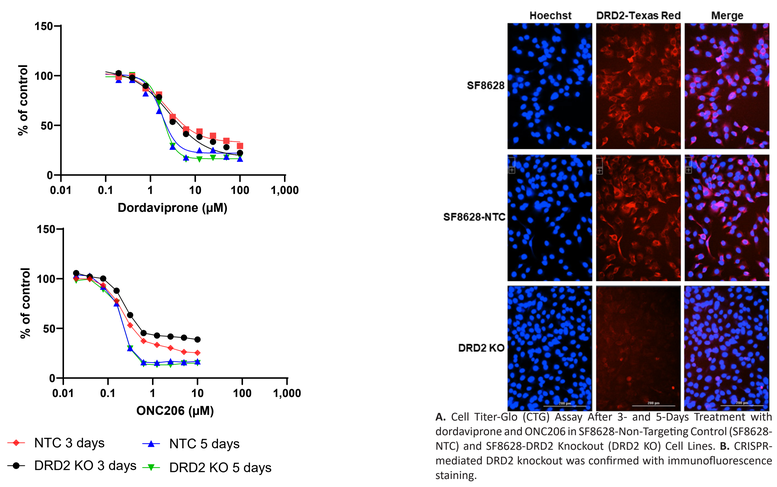
Results

Figure 4. ClpP knockout impairs dordaviprone and ONC206 efficacy in glioma cells in vitro



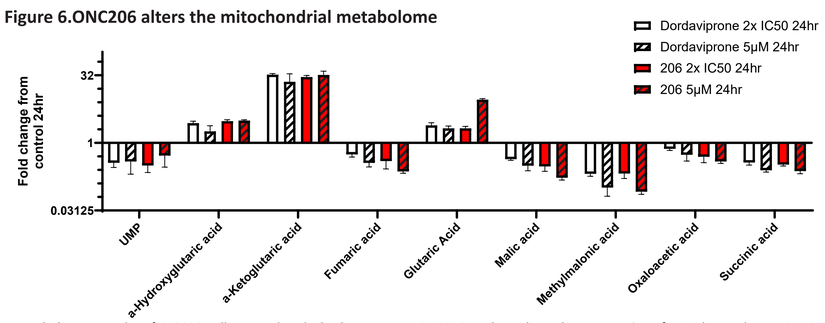
Cell viability for 5000 SF8628 or T98G cells, with or without ClpP KO, treated with dordaviprone or ONC206 at indicated concentrations. Viability was measured on Day 5 using CellTiter-Glo. CRISPR-mediated ClpP knockout was made using Edit-R All-in-one Lentiviral sgRNA vector (Dharmacon, Horizon) and confirmed using Western blot. ClpP antibody was from AbCam. Actin antibody was from Santa Cruz Biotechnology. KO, knock-out; NTC, non-targeted control; WB, western blot; WT, wild-type.

Figure 5.DRD2 knockout does not impair dordaviprone and ONC206 efficacy in glioma cells in vitro



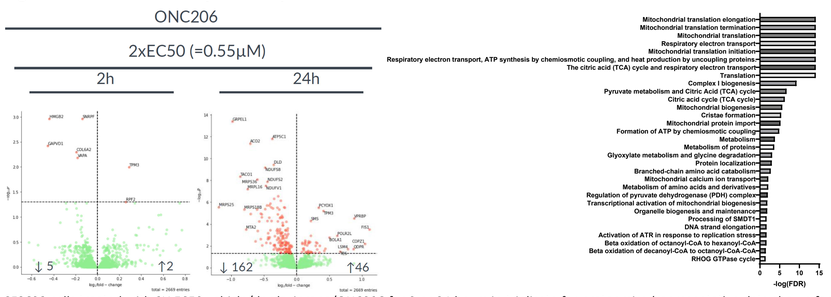
A. Cell Titer-Glo (CTG) Assay After 3- and 5-Days Treatment with dordaviprone and ONC206 in SF8628-Non-Targeting Control (SF8628-NTC) and SF8628-DRD2 Knockout (DRD2 KO) Cell Lines. **B.** CRISPR-mediated DRD2 knockout was confirmed with immunofluorescence staining.

Figure 6.ONC206 alters the mitochondrial metabolome



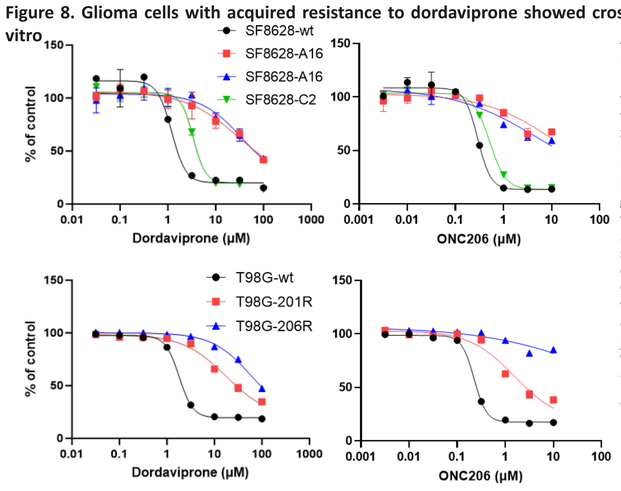
Metabolomics results of SF8628 cells treated with dordaviprone or ONC206 at the indicated concentrations for 24 h are shown. 2X IC50 concentrations were 4.5, 0.55, and 0.12 μM, respectively. Only metabolites with consistent directional change, fold change >2, and significant difference by t-test ($p<0.05$) from control for at least 2 out of 3 imipridones are shown.

Figure 7. Proteomics analysis in response to ONC206 treatment in SF8628 cells[14]



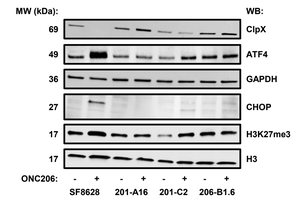
SF8628 cells treated with 2X EC50 vehicle/dordaviprone/ONC206 for 2 or 24 hours in triplicate for proteomics (representative data shown for ONC206). Reactome pathway analysis for significantly ($p<0.05$) downregulated proteins with dordaviprone and ONC206 treatment for 24h compared to vehicle treatment shown (FDR: false discovery rate)

Figure 8. Glioma cells with acquired resistance to dordaviprone showed cross-resistance to ONC206 in vitro



Treatment to generate acquired resistance was initiated at 0.2 to 1x the IC50 and drug concentration was doubled when cells were able to proliferate. SF8628-A16 and SF8628-C2 lines were selected up to 16 or 2 μM dordaviprone, respectively. The SF8628-A16 ND line was derived from A16 and grown without dordaviprone treatment for 25 days. T98G-201R and T98G-206R lines were selected up to 16 μM dordaviprone or 1.6 μM ONC206, respectively. Cell viability for 5000 cells treated with dordaviprone or ONC206 at indicated concentrations was measured on Day 5 using CellTiter-Glo.

Figure 9. ONC206 induces expression of ATF4, CHOP, and H3 K27me3 while reducing ClpX in parental, but not resistant cells



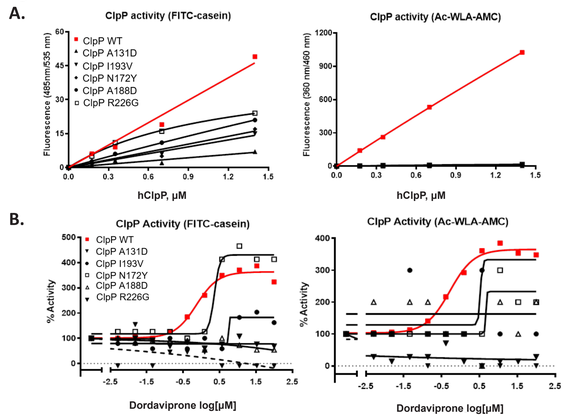
Western blot results for SF8628 parental and resistant lines. 201-A16 were selected with 16 μM dordaviprone, 201-C2 were selected with 2 μM dordaviprone, 206-B1.6 were selected with 1.6 μM ONC206. Cells were exposed to vehicle or 0.55 μM ONC206 for 72 h prior to protein extraction and Western blotting. ClpX and GAPDH antibodies were from AbCam. ATF-4, CHOP, and K27me3 antibodies were from Cell Signaling Technology.

Table 1. Positions of ClpP mutations identified in dordaviprone/ONC206 resistant glioma cells

Parental Line	Name	Selection (μM)	ClpP Mutation(s)	Mutation Location	Mutant Proportion
T98G, GBM	201-R	Dordaviprone (16 μM)	A131D	ClpP monomer interfaces	17-20%
			R226G	ClpX interface	12-21%
T98G, GBM	206-R	ONC206 (1.6 μM)	R226G	ClpX interface	43-54%
SF8628, DIPG K27M	201-A16	Dordaviprone (16 μM)	I193V	ClpX interface	50-67%
SF8628, DIPG K27M	201-C2	Dordaviprone (2 μM)	N172Y	ClpP monomer interfaces	22-33%
SF8628, DIPG K27M	206-B1.6	ONC206 (1.6 μM)	A188D	ClpX interface	~50%

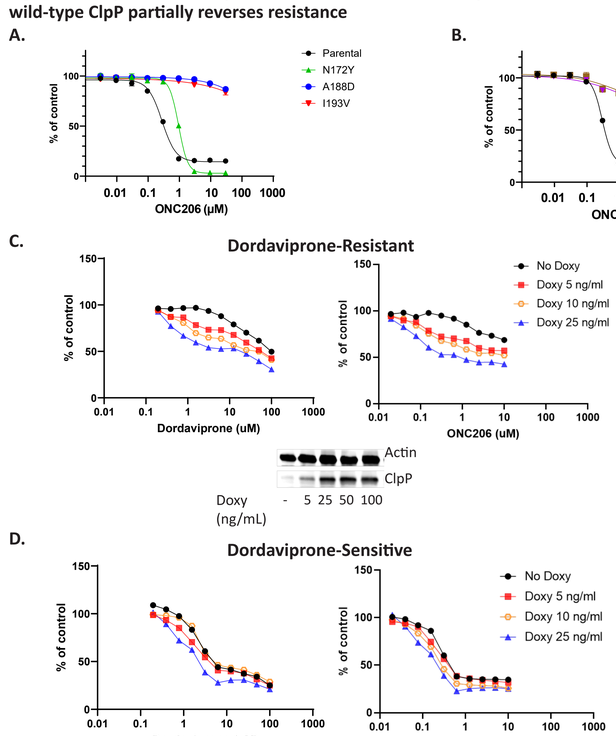
T98G cells are pseudopentaploid. SF8628 cells are ~diploid.
ClpP crystal structure is from PDB ID: 6DL7 and displayed using iCn3D (NCBI). Whole genome/whole exome sequencing was performed at Novogene.

Figure 10. ClpP with resistance mutations are catalytically impaired and reduce dordaviprone-mediated agonism



A. Cell-free degradation activities of recombinant human wild-type and mutated ClpP on FITC-casein and AMC-peptide substrates were determined. **B.** Agonism of wild-type and mutated ClpP was determined in the same assay with dordaviprone added at increasing concentrations.

Figure 11. Expression of ClpP mutants is sufficient for imipridone resistance while over-expression of wild-type ClpP partially reverses resistance



A and B. Cell viability for parental cells and in cells transduced with lentiviral vectors (GenScript) expressing ClpP proteins with the indicated mutations. Cells were treated with ONC206 at the indicated concentrations and viability was measured on Day 4 using CellTiter-Glo. **C and D.** Cell viability for dordaviprone and ONC206 at indicated concentrations in SF8628-A16, dordaviprone resistant cells **(C)** and sensitive parental cells **(D)** with or without ClpP overexpression using a TET-inducible lentiviral expression system (TET Systems, Takara). Expression of ClpP was induced by doxycycline treatment for 24 h prior to dordaviprone or ONC206 treatment. Viability was measured on Day 3 using CellTiter-Glo. Doxycycline-mediated ClpP overexpression was confirmed using western blot.

Conclusions

- ONC206 exhibits nanomolar potency in glioma cell lines
- ClpP expression and agonism is key for the anti-cancer efficacy of ONC206 in vitro
- Acquired resistance to ONC206 in vitro is associated with alterations in ClpP
- ONC206 treatment is associated with alterations in metabolism, ISR, and histone methylation

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;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_Supplement):5688-5688; 9. Wagner J, et al. Cell Cycle. 2017;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; 13. Prabhu VV, et al. Clin Cancer Res. 2019;25(7):2305-2313; 14. Jassal B, et al. Nucleic Acids Res. 2020 Jan 8;48(D1):D498-D503.

Conflicts of Interest

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

Poster PDF