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Role of ClpP in the Anti-cancer Effects of Imipridone ONC201 and ONC206

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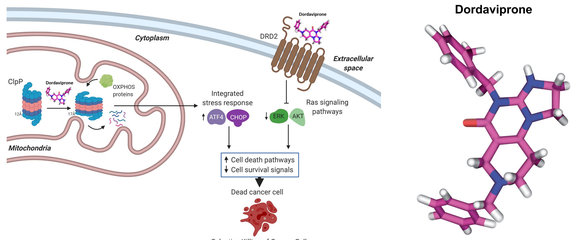
Introduction

- ONC201 (dordaviprone), 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]
- Dordaviprone has demonstrated tolerability and durable tumor regressions in patients with H3 K27M-mutant glioma.[6]

Figure 1. Dordaviprone 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 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.[9-12]

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.

Genomics of Drug Sensitivity in Cancer (GDSC) Cell Line Screening and Gene Expression Analysis

- Cell viability assays with 1088 GDSC cell lines for ONC206 were performed as previously described.[13] 620 human cancer cell lines were analyzed for correlations between ClpP expression and ONC206 IC90.

ClpP profiling

- ClpP FITC-casein assay was performed as described previously.[4]

TET-system

- SF8628 (MOI 1) cells were continuously exposed to Doxycycline at dose concentrations 5, 10 and 25 ng/ml for 1 day for ClpP induction.

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.

Methods

Resistant Clones (cont)

- 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.

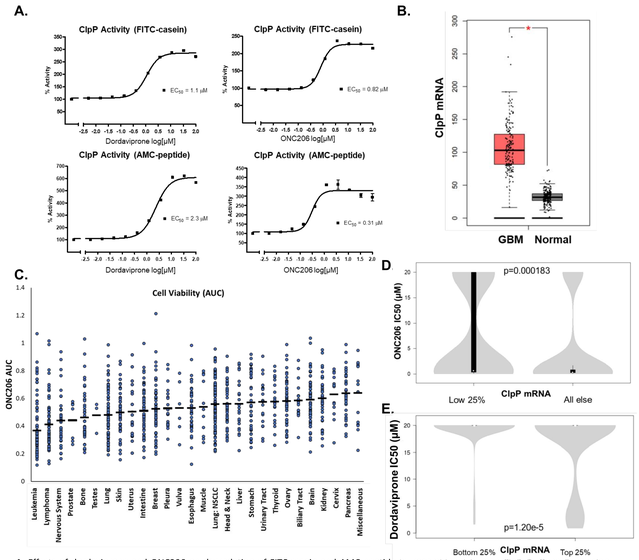
- After DNA purification, samples were divided into replicates for sequencing at Novogene.

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, dordaviprone resistant, ONC206 resistant) was performed in triplicate for indicated treatments (vehicle, dordaviprone IC50, ONC206 IC50) and timepoints (4hr, 24hr, 48hr continuous, 24hr-treat/24hr-washout).

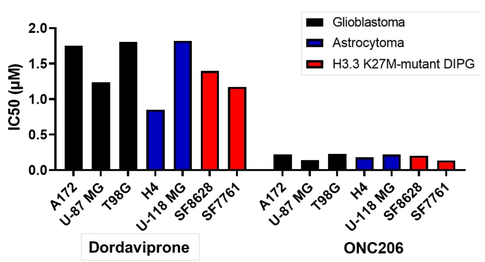
Results

Figure 2. Dordaviprone/ONC206 Act as ClpP Agonists and ClpP Expression Correlates with *In Vitro* Anti-cancer Efficacy



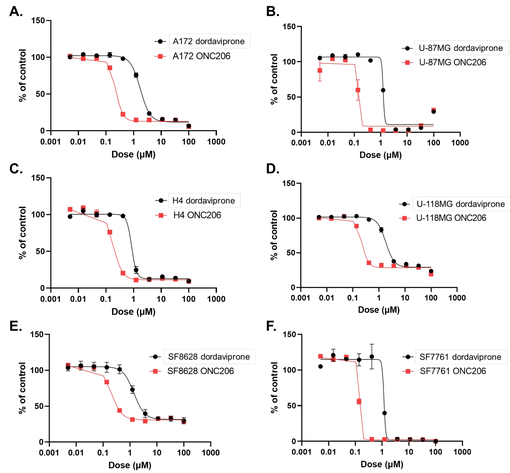
A. Effects of dordaviprone and ONC206 on degradation of FITC-casein and AMC-peptide by recombinant human ClpP. **B**. Gene Expression Profiling Interactive Analysis (GEPIA) database analysis showed ClpP mRNA was overexpressed in glioblastoma cells relative to normal cells. **C**. *In vitro* sensitivity of 1088 GDSC human cancer cell lines to ONC206 (78 nM - 20 µM, 72 hr) organized by tumor type. The results are shown as average ONC206 area under the dose response curve (AUC) (black bars) with representation of all cell lines in each tumor type. Violin plot for correlation of **D.** ONC206 and **E.** dordaviprone IC50 (µM) in GDSC cell lines with ClpP mRNA expression.

Figure 3. IC50 for Dordaviprone and ONC206 in glioma cell line cell viability assays (Day 5)



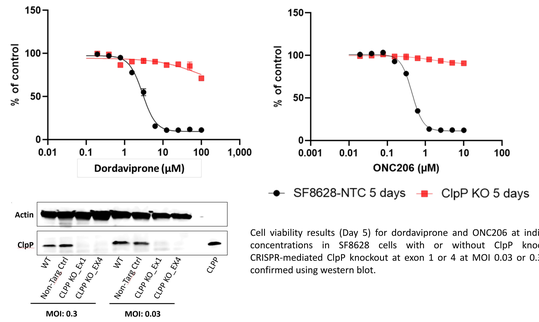
Results

Figure 4. ONC206 and Dordaviprone *In Vitro* Efficacy in Glioma Cell Lines



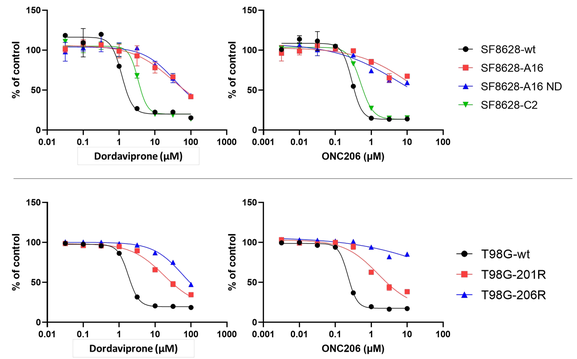
Cell viability results for dordaviprone and ONC206 at indicated concentrations/timepoints in glioblastoma (**A**, A172; **B**, U-87MG), astrocytoma (**C**, H4; **D**, U-118MG) and H3.3 K27M-mutant DIPG (**E**, SF8628; **F**, SF7761) lines at day 5 (n=4). Red indicates ONC206, black indicates dordaviprone.

Figure 5. ClpP Knockout Impairs Dordaviprone and ONC206 Efficacy



Cell viability results (Day 5) for dordaviprone and ONC206 at indicated concentrations in SF8628 cells with or without ClpP knockout. CRISPR-mediated ClpP knockout at exon 1 or 4 at MOI 0.03 or 0.3 was confirmed using western blot.

Figure 6. Glioma Cells with Acquired Resistance to Dordaviprone Showed Cross-resistance to ONC206



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

Figure 7. Positions of ClpP Mutations Identified in Dordaviprone/ ONC206-resistant Glioma Cells

Parental Line	Type	Ploidy	Selection (µM)	ClpP Mutation(s)	Mutant Proportion
T98G	GBM	hyper-pentaploid	Dordaviprone (16 µM)	A131D + R226G	32-38%
T98G	GBM	hyper-pentaploid	ONC206 (1.6 µM)	R226G	43-54%
SF8628	DIPG K27M	~diploid	Dordaviprone (16 µM)	I193V	50-67%
SF8628	DIPG K27M	~diploid	Dordaviprone (2 µM)	N172Y	22-33%
SF8628	DIPG K27M	~diploid	ONC206 (1.6 µM)	A188D	~ 50%

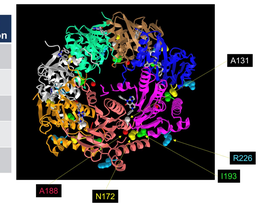
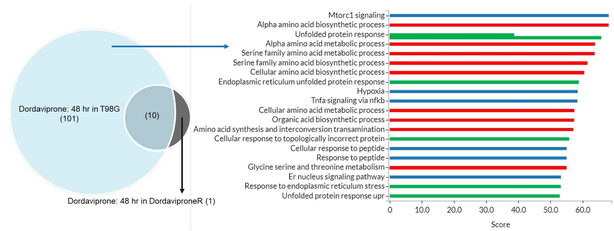
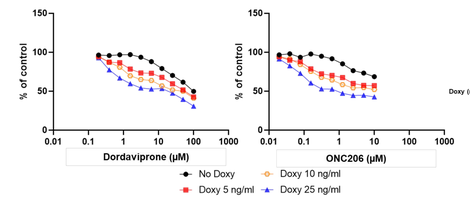


Figure 8. RNAseq Analysis in Response to Dordaviprone Treatment in T98G Parental versus Dordaviprone-resistant Cells



RNAseq analysis revealed upregulation (101 genes) of the integrated stress response (green) and amino acid metabolism (red) with dordaviprone (IC50) treatment (48h) in T98G parental cells but not dordaviprone-resistant cells (dordaviproneR). 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 9. ClpP Overexpression Enhances Sensitivity to Dordaviprone/ONC206 in Acquired Resistance Cells



Cell viability results (Day 3) for dordaviprone and ONC206 at indicated concentrations in SF8628 A16 (dordaviprone resistant) cells with or without ClpP overexpression using a TET-inducible system. Doxycycline-mediated ClpP overexpression at MOI 1 was confirmed using western blot.

Summary

- ONC206 exhibits nanomolar potency in glioma cell lines
- ClpP expression and agonism is key for the anti-cancer efficacy of dordaviprone and ONC206 in vitro.
- ClpP could also play a role in the evolution of glioma cell resistance upon prolonged dordavirpone/ONC206 exposure.
- Studies on the role of DRD2, an additional binding target, in dordaviprone/ONC206 response, 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;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.

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.

Acknowledgements

Editorial assistance was provided by Meghan Sullivan, PhD of Chimerix, Inc.

Poster PDF

Presented at the American Association for Cancer Research (AACR) Annual Meeting; April 14-19, 2023