#361

Allosteric ClpP agonist ONC206 alters mitochondrial metabolism, stress response, chromatin accessibility and cell cycle to elicit apoptosis in pheochromocytoma

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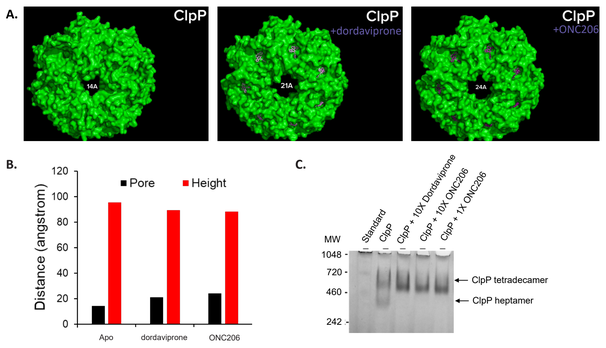
Introduction

- 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]
- Dordaviprone demonstrated responses in patients with neuroendocrine pheochromocytoma-paraganglioma (PC-PG) including tumors with SDHB/FH loss-of-function driver mutations.[14]

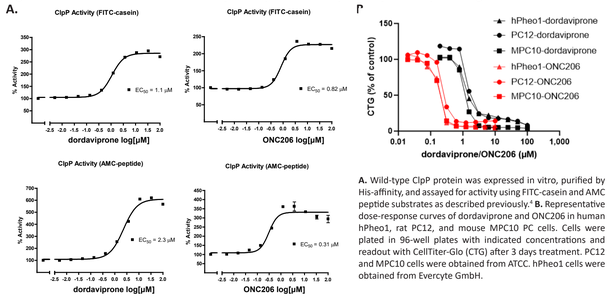
Results

Figure 1. Co-crystalization 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. MW = molecular weight (kDa)

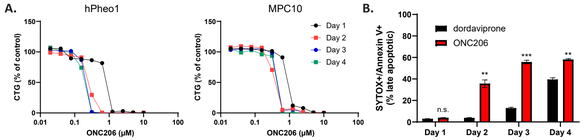
Figure 2. ONC206 exhibits nanomolar potency in cell-free proteolysis assays and against PC cell lines



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.** Representative dose-response curves of dordaviprone and ONC206 in human hPheo1, rat PC12, and mouse MPC10 PC cells. Cells were plated in 96-well plates with indicated concentrations and readout with CellTiter-Glo (CTG) after 3 days treatment. PC12 and MPC10 cells were obtained from ATCC. hPheo1 cells were obtained from Evercyte GmbH.

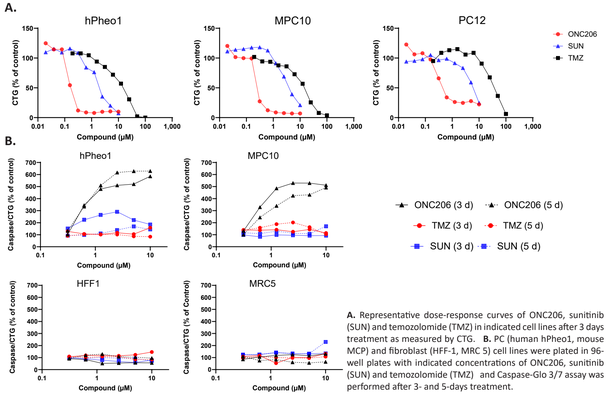
Results

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



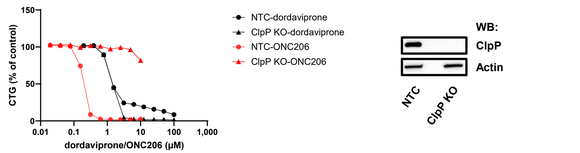
A. hPheo1 and MPC10 PC cells plated in 96-well plates were treated with ONC206 at concentrations ranging from 19.5 nM to 10 µM for 24, 48, 72 or 96 hours. At the end of the designated exposure timepoints, wells were replenished with fresh media (no ONC206). ATP content in cells was measured by CTG luminescence as a readout of cellular survival. All readouts were performed 7 days after the addition of ONC206. Cell survival after washout was measured as percent survival relative to control (vehicle treated) cells. **B.** hPheo1 were treated with 0.55µM ONC206 and 3µM dordaviprone for 1, 2, 3, 4-days and wells were replenished with fresh media (no drug). Apoptosis was measured with flow cytometry using SYTOX (cell death) and Annexin V (apoptosis) stains on Day 7. ** p<0.001 *** p<0.0001 n=3

Figure 4. ONC206 demonstrates superior cell viability inhibition and apoptosis in PC cell lines relative to temozolomide and sunitinib



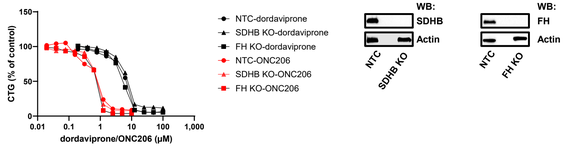
A. Representative dose-response curves of ONC206, sunitinib (SUN) and temozolomide (TMZ) in indicated cell lines after 3 days treatment as measured by CTG. **B.** PC (human hPheo1, mouse MCP) and fibroblast (HFF-1, MRC 5) cell lines were plated in 96-well plates with indicated concentrations of ONC206, sunitinib (SUN) and temozolomide (TMZ) and Caspase-Glo 3/7 assay was performed after 3- and 5-days treatment.

Figure 5. ClpP knockout impairs dordaviprone and ONC206 efficacy in PC cells in vitro



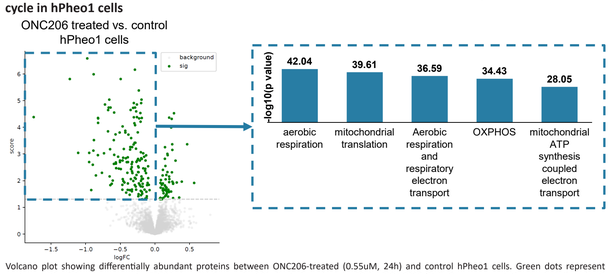
Representative dose-response curves of dordaviprone and ONC206 in human hPheo1 cells with or without CRISPR mediated ClpP knockout. Cells were plated in 96-well plates with indicated concentrations of ONC206 and readout with CTG (measures cell ATP content) after 5 days treatment. KO, knockout; NTC, non-targeting control.

Figure 6. ONC206 Inhibits Cell Viability in PC Cells with SDHB or FH Knockout In Vitro



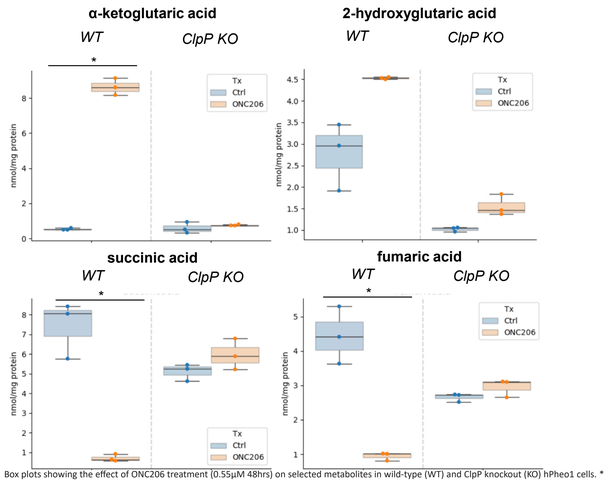
Representative dose-response curve of ONC206 (after 96-hour treatment) in human hPheo1 cells with or without CRISPR mediated SDHB or FH knockout. Cells were plated in 96-well plates with indicated concentrations of ONC206 and readout with CTG (measures cell ATP content) after 4 days treatment.

Figure 7. Proteomics indicates ONC206 inhibits mitochondrial metabolism including OXPHOS and TCA cycle in hPheo1 cells



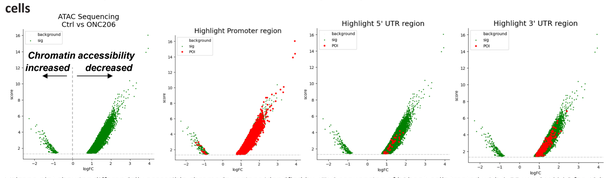
Volcano plot showing differentially abundant proteins between ONC206-treated (0.55uM, 24h) and control hPheo1 cells. Green dots represent proteins with a false discovery rate (FDR) below 5%. Score: -log10(adjusted p-value); logFC: log2(ONC206/Ctrl). Overrepresentation analysis (bar chart) of depleted proteins, highlighting the most significantly enriched biological terms.

Figure 8. Metabolomics reveals ONC206 elevated α-ketoglutaric acid, 2-hydroxyglutaric acid and reduced succinic acid, fumaric acid in a ClpP-dependent manner in hPheo1 cells



Box plots showing the effect of ONC206 treatment (0.55µM 48hrs) on selected metabolites in wild-type (WT) and ClpP knockout (KO) hPheo1 cells. * p<0.05 (Welch's t-test)

Figure 9. ATACseq shows altered chromatin accessibility in response to ONC206 treatment in hPheo1 cells



Volcano plot showing differentially accessible chromatin regions identified by ATAC sequencing of hPheo1 cells treated with 0.55 µM ONC206 for 48 h vs. control untreated cells. Green dots represent regions with an FDR below 5%. Score: -log10(p-value); logFC: log2(Ctrl/ONC206).

Figure 10. RNAseq demonstrates ONC206 upregulated stress response, apoptosis and downregulated cell cycle, metabolism-related pathways in hPheo1 cells

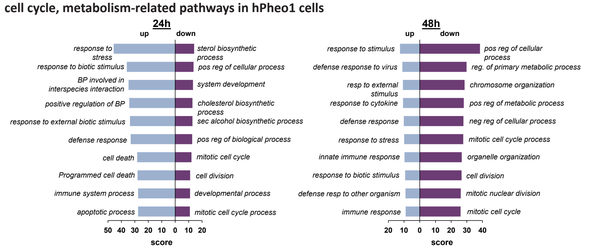
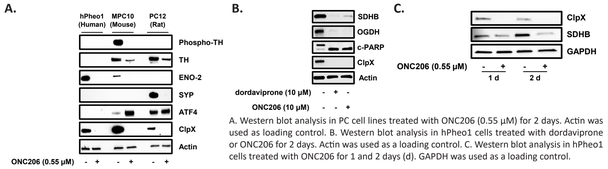
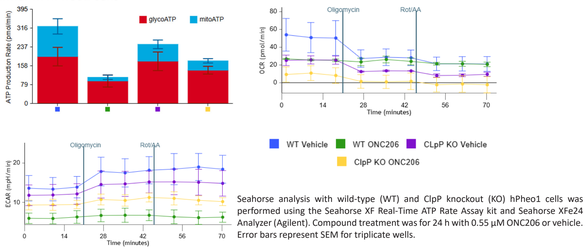


Figure 11. ONC206 Downregulates Mitochondrial Proteins, Neuroendocrine Markers and Upregulates Stress Response in PC Cells



A. Western blot analysis in PC cell lines treated with ONC206 (0.55 µM) for 2 days. Actin was used as loading control. B. Western blot analysis in hPheo1 cells treated with dordaviprone or ONC206 for 2 days. Actin was used as a loading control. C. Western blot analysis in hPheo1 cells treated with ONC206 for 1 and 2 days (d). GAPDH was used as a loading control.

Figure 12. Seahorse analysis shows ONC206 impairs mitochondrial metabolism in a ClpP-dependent manner in hPheo1 cells



Seahorse analysis with wild-type (WT) and ClpP knockout (KO) hPheo1 cells was performed using the Seahorse XF Real-Time ATP Rate Assay kit and Seahorse XFe24 Analyzer (Agilent). Compound treatment was for 24 h with 0.55 µM ONC206 or vehicle. Error bars represent SEM for triplicate wells.

Conclusions

- ONC206 exhibits enhanced nanomolar potency and superior apoptosis relative to dordaviprone, temozolomide and sunitinib in PC cell lines
- ClpP expression and agonism is key for the anti-cancer efficacy of ONC206 in vitro
- ONC206 treatment is associated with alterations in mitochondrial metabolism, chromatin accessibility, stress response, neuroendocrine markers and cell cycle in PC cells

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; 13. Anderson PM, et al. *Clin Cancer Res.* 2022;28(9):1773-1782.

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