TMET-26

Allosteric ClpP agonist ONC206 alters mitochondrial metabolism and stress response to elicit apoptosis in meningioma

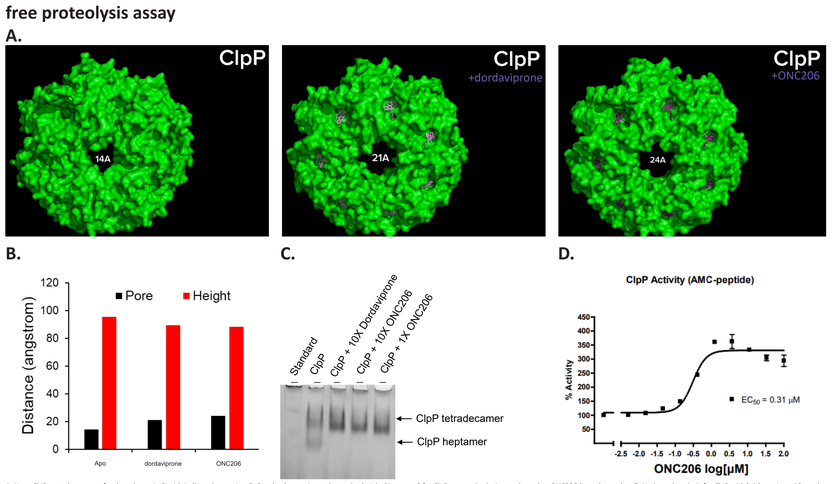
Andrew K. Lee, Cristina Maranto, Scott Foster, Varun V. Prabhu and Joshua E. Allen

Chimerix, Inc., a Jazz Pharmaceuticals company, Durham, NC

Introduction

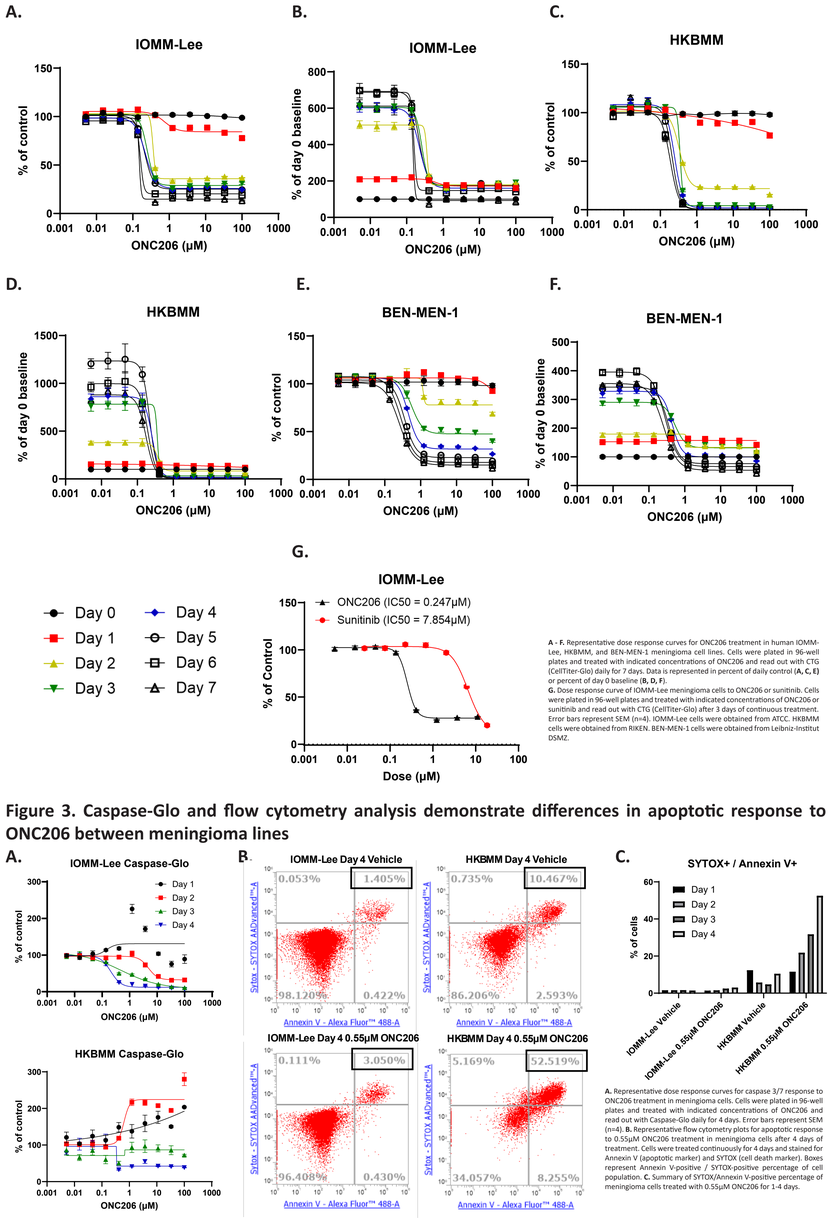
- Dordaviprone (ONC201), a first-in-class imipridone, is an oral, blood-brain barrier penetrating, selective small molecule antagonist of dopamine receptor D2 (DRD2) and agonist of the mitochondrial protease caseinolytic mitochondrial matrix peptidase proteolytic subunit (ClpP).[1-5]
- Dordaviprone is FDA-approved for the treatment of adult and pediatric patients with H3 K27M-mutant diffuse midline glioma. Dordaviprone has demonstrated tolerability and durable tumor regressions in patients with H3 K27M-mutant glioma.[6,7]
- ONC206, a chemical derivative of dordaviprone, is the second imipridone to be developed and is currently in Phase 1 clinical development.[8]
- Relative to dordaviprone, ONC206 has demonstrated differentiated DRD2 receptor pharmacology, improved potency, enhanced absorption/tissue distribution, and preclinical anti-cancer activity in vitro and in vivo.[9-13]
- Reduced abundance of H3K27 trimethylation in meningioma clinical samples is significantly correlated with rapid progression and highly aggressive meningioma subtypes.[14]
- Mitochondrial metabolism and oxidative phosphorylation is associated with poor survival in hypermetabolic meningiomas.[15]

Figure 1. Co-crystallization with ClpP revealed distinctions in the ONC206-ClpP resolved X-ray crystal structure relative to the dordavidone-bound or apo complex with nanomolar potency of ONC206 in cell-free proteolysis assay



A. X-ray ClpP crystal structure 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). **D.** Cell-free proteolysis assay for ONC206.

Figure 2. ONC206 exhibits nanomolar potency against meningioma cell lines with greater potency than sunitinib



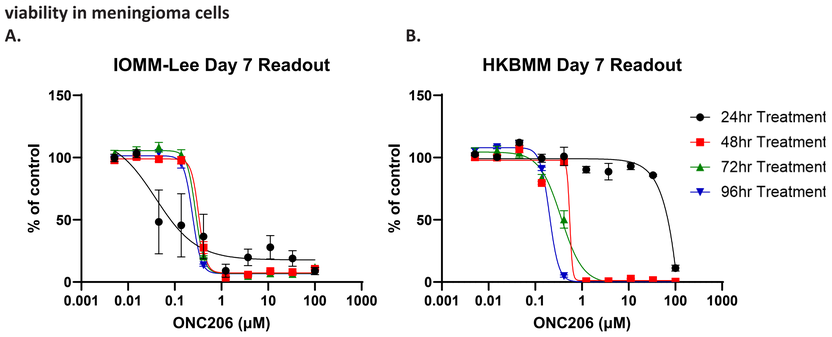
A - F. Representative dose response curves for ONC206 treatment in human IOMM-Lee, HKBMM, and BEN-MEN-1 meningioma cell lines. Cells were plated in 96-well plates and treated with indicated concentrations of ONC206 and read out with CTG (CellTiter-Glo) daily for 7 days. Data is represented in percent of daily control (**A, C, E**) or percent of day 0 baseline (**B, D, F**).
G. Dose response curve of IOMM-Lee meningioma cells to ONC206 or sunitinib. Cells were plated in 96-well plates and treated with indicated concentrations of ONC206 or sunitinib and read out with CTG (CellTiter-Glo) after 3 days of continuous treatment. Error bars represent SEM (n=4). IOMM-Lee cells were obtained from ATCC. HKBMM cells were obtained from RIKEN. BEN-MEN-1 cells were obtained from Leibniz-Institut DSMZ.

Figure 3. Caspase-Glo and flow cytometry analysis demonstrate differences in apoptotic response to ONC206 between meningioma lines

A. Representative dose response curves for caspase 3/7 response to ONC206 treatment in meningioma cells. Cells were plated in 96-well plates and treated with indicated concentrations of ONC206 and read out with Caspase-Glo daily for 4 days. Error bars represent SEM (n=4). **B.** Representative flow cytometry plots for apoptotic response to 0.55μM ONC206 treatment in meningioma cells after 4 days of treatment. Cells were treated continuously for 4 days and stained for Annexin V (apoptotic marker) and SYTOX (cell death marker). Boxes represent Annexin V-positive / SYTOX-positive percentage of cell population. **C.** Summary of SYTOX/Annexin V-positive percentage of meningioma cells treated with 0.55μM ONC206 for 1-4 days.

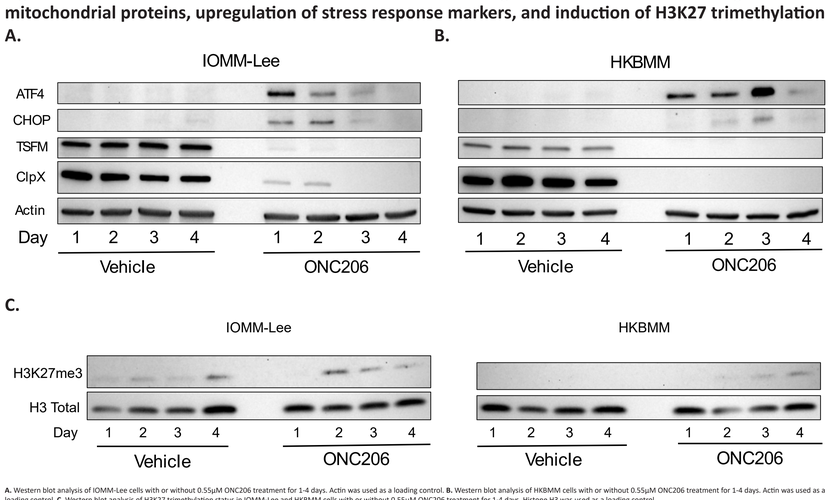
Results

Figure 4. Treatment with ONC206 for 48 hours was sufficient to induce a durable impairment of cellular viability in meningioma cells



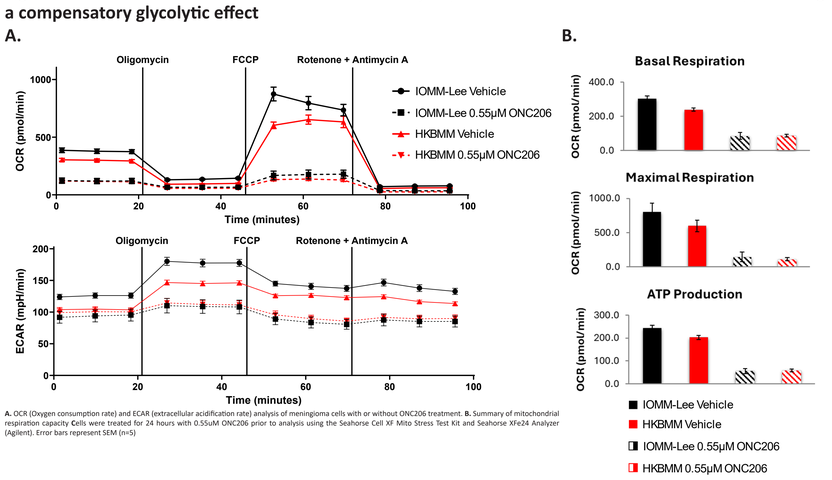
Washout CTG results for IOMM-Lee (**A**) and HKBMM (**B**) cells treated with ONC206. Cells were plated in 96-well plates and treated with ONC206 at indicated concentrations for the indicated durations before drug-containing media was removed and cells replenished with fresh media. All cells were read out with CTG at 7 days post-initial treatment. Error bars represent SEM (n=4).

Figure 5. Western blot analysis of ONC206-treated meningioma cells reveals downregulation of mitochondrial proteins, upregulation of stress response markers, and induction of H3K27 trimethylation



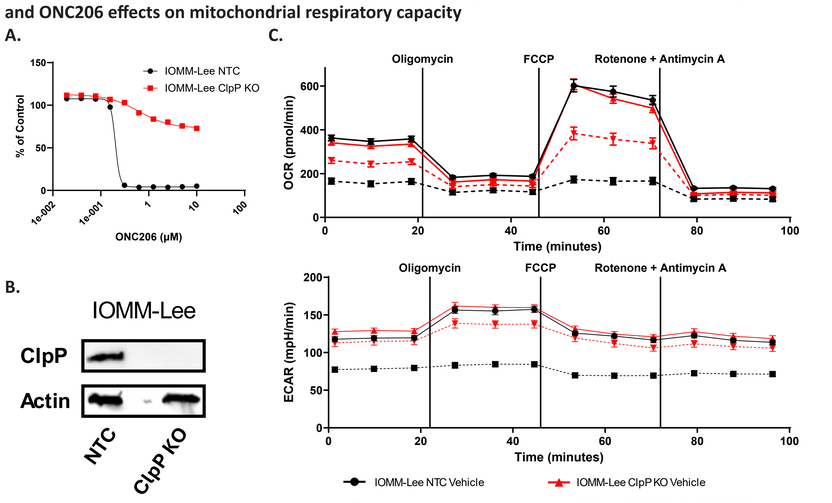
A. Western blot analysis of IOMM-Lee cells with or without 0.55μM ONC206 treatment for 1-4 days. Actin was used as a loading control. **B.** Western blot analysis of HKBMM cells with or without 0.55μM ONC206 treatment for 1-4 days. Actin was used as a loading control. **C.** Western blot analysis of H3K27 trimethylation status in IOMM-Lee and HKBMM cells with or without 0.55μM ONC206 treatment for 1-4 days. Histone H3 was used as a loading control.

Figure 6. ONC206 treatment for 24 hours substantially impairs mitochondrial function without promoting a compensatory glycolytic effect



A. OCR (Oxygen consumption rate) and ECAR (extracellular acidification rate) analysis of meningioma cells with or without ONC206 treatment. **B.** Summary of mitochondrial respiration capacity Cells were treated for 24 hours with 0.55uM ONC206 prior to analysis using the Seahorse Cell XF Mito Stress Test Kit and Seahorse XFe24 Analyzer (Agilent). Error bars represent SEM (n=5)

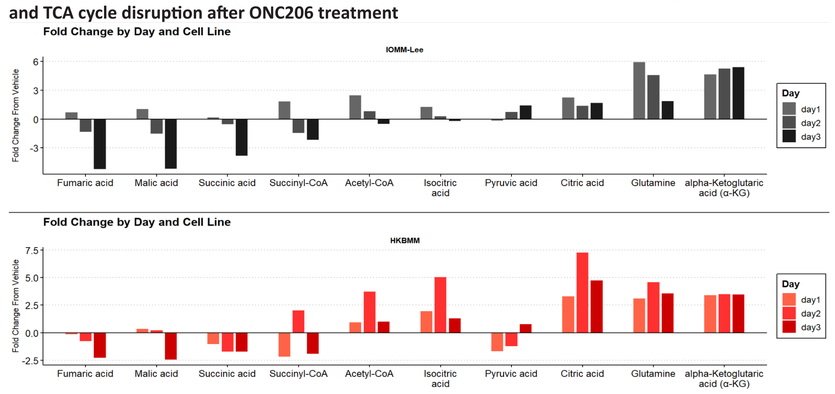
Figure 7. CRISPR Cas9-mediated knockout (KO) of ClpP abrogates ONC206 sensitivity in meningioma cells and ONC206 effects on mitochondrial respiratory capacity



A. Dose response curves of ClpP KO IOMM-Lee cells to ONC206 treatment compared to NTC (non-targeting control). Cells were plated in 96-well plates and treated with indicated concentrations of ONC206 and read out with CTG after 3 days of continuous treatment. **B.** Western blot analysis of ClpP KO IOMM-Lee cells compared to NTC. Actin was used as a loading control. **C.** OCR (Oxygen consumption rate) and ECAR (extracellular acidification rate) analysis of ClpP KO IOMM-Lee cells with ONC206 treatment compared to NTC. Cells were treated for 24 hours with 0.55μM ONC206 prior to analysis using the Seahorse Cell XF Mito Stress Test Kit and Seahorse XFe24 Analyzer (Agilent). Error bars represent SEM (n=5).

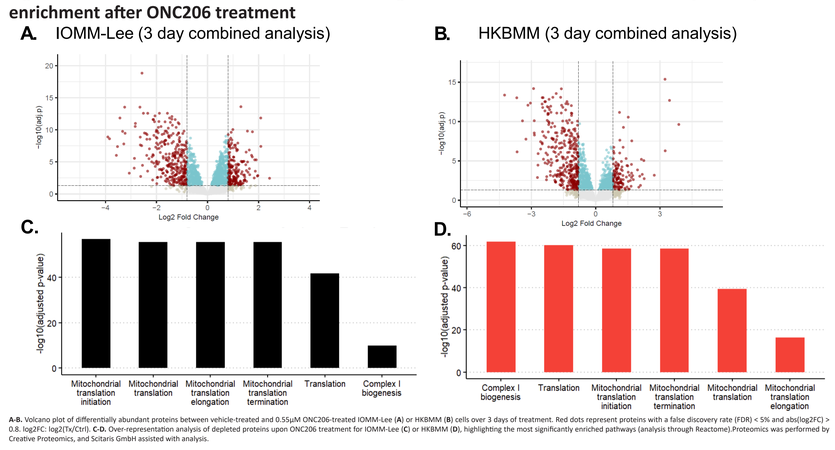
Results

Figure 8. Metabolomics comparison between meningioma lines confirms alpha-ketoglutarate induction and TCA cycle disruption after ONC206 treatment



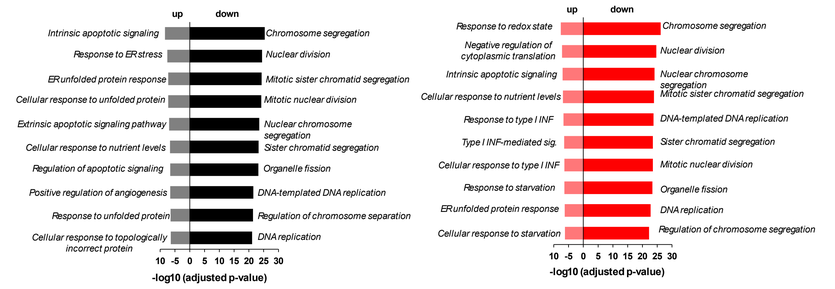
Metabolomics analysis of key TCA cycle-related central carbon metabolites (out of 89 metabolites) represented as fold change from vehicle control at same timepoints. Cells were treated with vehicle or 0.55μM ONC206 for 1-3 days prior to submission for metabolomics analysis. Central carbon pathway metabolomics was performed by Creative Proteomics, and Sciteris GmbH assisted with analysis.

Figure 9. Proteomics comparison between meningioma lines reveals commonalities in pathway enrichment after ONC206 treatment



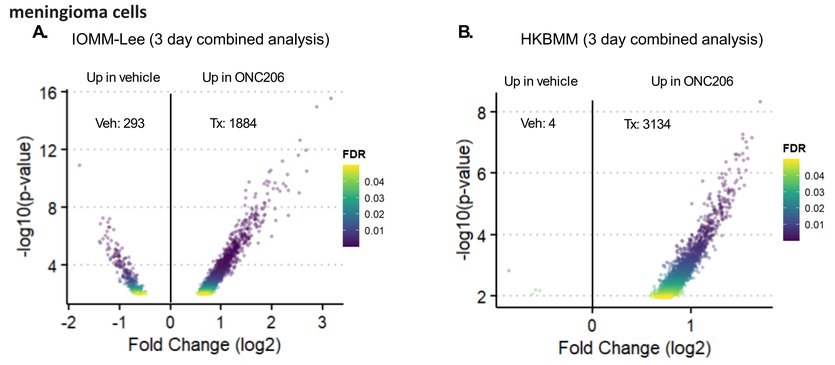
A-B. Volcano plot of differentially abundant proteins between vehicle-treated and 0.55μM ONC206-treated IOMM-Lee (**A**) or HKBMM (**B**) cells over 3 days of treatment. Red dots represent proteins with a false discovery rate (FDR) < 5% and abs(log2FC) > 0.8. log2FC: log2(Tx/Ctrl). **C-D.** Over-representation analysis of depleted proteins upon ONC206 treatment for IOMM-Lee (**C**) or HKBMM (**D**), highlighting the most significantly enriched pathways (analysis through Reactome).Proteomics was performed by Creative Proteomics, and Sciteris GmbH assisted with analysis.

Figure 10. RNA-seq demonstrates ONC206 upregulates stress response and apoptotic response, and downregulates cell cycle-related pathways in meningioma cells



Over-representation analysis of significantly enriched or depleted GO biological processes (FDR < 5%) in significantly altered mRNAs following treatment of IOMM-Lee or HKBMM cells with 0.55μM ONC206 over 3 days. Graphs show the most significantly enriched pathways as –log10(adjusted p-value). Differential expression transcription counts was filtered by p < 0.05 and abs(logFC) > 1. RNA-seq was performed by Novogene, and Sciteris GmbH assisted with analysis.

Figure 11. ATAC-seq shows altered chromatin accessibility in response to ONC206 treatment in meningioma cells



A-B. Volcano plot showing differentially accessible chromatin regions identified by ATAC sequencing of vehicle-treated or 0.55μM ONC206-treated IOMM-Lee (**A**) or HKBMM (**B**) cells combined over 3 days. log2FC: log2(Tx/Ctrl). ATAC-seq was performed by CD Genomics, and Sciteris GmbH assisted with analysis.

Conclusions

- ONC206 exhibits improved nanomolar potency relative to sunitinib, durable cell viability inhibition, and induction of apoptosis in meningioma cell lines
- ClpP expression is key for the anti-cancer efficacy of ONC206 in vitro
- ONC206 treatment is associated with alterations in mitochondrial metabolism, chromatin accessibility, cellular stress response, and cell cycle in meningioma 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. Arrillaga-Romany I, et al; Journal of Clinical Oncology 42:1542-1552, 2024; 7. Venneti S, et al. Cancer Discov 13:2370-2393, 2023; 8. Theeler BJ, et al. J Clin Oncol.2020;38(15_suppl):TPS2576-TPS2576; 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. Cancer Res (2020) 80 (16_Supplement): 5688.14. Katz LM, et al. Acta Neuropathol. 2018;135(6):955-963; 15. Landry AP, et al. Neuro Oncol. 2025;24:27(8):2047-59.

Conflicts of Interest
AKL, CM, SF, VVP, and JEA are employees and shareholders of Chimerix, Inc, A Jazz Pharmaceutical Company.

Poster PDF

Society of Neuro-Oncology (SNO) Annual Meeting; November 19-23, 2025; Honolulu, HI, USA