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).1,2 Dordaviprone has demonstrated tolerability and durable tumor regressions in patients with H3 K27M-mutant glioma.3

Figure 1. Dordaviprone Mechanism of Action

Methods

Cell Lines

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

Cell Viability

- Cells (1000-5000) were seeded in a 96-well plate containing compound dilutions in DMEM/F12 media supplemented with 10% FBS.
- 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 indetermined treatments (vehicle, dordaviprone IC50, ONC206 IC50) and timepoints (4h, 24h, 48h continuous, 24hr-treat/24hr-washout).

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

Results

Resistant Clones (cont)

- Time-viability of acquired resistance was confirmed by growing cells in the absence of drug for 2 to 4 weeks and retesting.
- Two polynomial resistant lines were generated for each compound.

Whole Genome/Transome 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 indetermined treatments (vehicle, dordaviprone IC50, ONC206 IC50) and timepoints (4h, 24h, 48h continuous, 24hr-treat/24hr-washout).

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

Glioblastoma (GBM; T98G, A172, U-87MG) and astrocytoma (H4, U-118MG) lines were obtained from American Type Culture Collection (ATCC).

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

A. ClpP Knockout Impairs Dordaviprone and ONC206 Efficacy

B. ClpP overexpression

C. ClpP Knockout Impairs Dordaviprone and ONC206 Efficacy

D. ClpP overexpression

Figure 5. ClpP Knockout Impairs Dordaviprone and ONC206 Efficacy

E. ClpP overexpression

F. ClpP Knockout Impairs Dordaviprone and ONC206 Efficacy

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

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

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

Figure 9. ClpP Overexpression Enhances Sensitivity to Dordaviprone/ONC206 in Acquired Resistance Cells

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 dordaviprone/ONC206 exposure.
- Studies on the role of DRD2, an additional binding target, in dordaviprone/ONC206 response, suggested by prior published work, are ongoing.

Acknowledgements

- This work was supported by the National Institutes of Health (K01CA235742, T32CA221056, R01CA233849, and U54CA224844-01) and the Prabhu Foundation. The authors wish to acknowledge the contribution of the authors' and their colleagues' work in the understanding of ClpP expression and function.

Disclosures

- This study was supported by Chimerix, Inc.
- CM, JEA, RL, PS, VVP, OJ, and DMD are employees of and have stock ownership in Chimerix, Inc.
- AL, CM, JEA, RL, PS, VVP, and DMD are employees of and have stock ownership in Chimerix, Inc.
- ONC201, a chemical derivative of dordaviprone, is the second imipridone to be developed and is currently in Phase I clinical development.1
- Relative to dordaviprone, ONC206 has demonstrated differentiated DRD2 receptor pharmacology, improved potency, enhanced absorption/toxicity distribution, and preclinical anti-cancer activity in vitro and in vivo.2,3

References