Predictive Biomarker Evaluation and Molecular Differentiation for Imipridones ONC201 and ONC206

Sara Morrow[1], Kirti Nath[2], Yiqun Zhang[3], Matthew J. Garnett[4], Ultan McDermott[4], Cyril H. Benes[5], Joel Basken[6], Wafik S. El-Deiry[3], Joshua E. Allen[1], Varun V. Prabhu[1]

[1]Chimerix, Inc., Durham, NC; [2]Oncoceutics, Inc., Philadelphia, PA; [3]Brown University, Providence, RI; [4]Wellcome Trust Sanger Institute, Hinxton, UK; [5]Massachusetts General Hospital, Boston, MA; [6]Arpeggio Biosciences, Boulder, CO

Abstract

Imipridone ONC201 is a first-in-class dopamine receptor D2 (DRD2) antagonist and mitochondrial protease ClpP agonist that is well tolerated and induces durable tumor regressions in H3 K27M-mutant glioma patients. ONC206, a chemical derivative of ONC201 currently in Phase I trials for central nervous system tumors, is also a DRD2 and ClpP dual targeting imipridone with differentiated target engagement and nanomolar potency. We explored molecular differentiation and predictive biomarker studies for ONC201 and ONC206 using gene expression profiling, development of resistance clones and RNA-seq/proteomics in large cancer cell line panels including the 1000-cell line GDSC panel. Gene expression profiling revealed that ONC206 and ONC201 induce partially overlapping signatures in U87 glioblastoma cells. Similarly, T98G glioblastoma cells with acquired resistance to ONC201 or ONC206 revealed partial cross resistance and combinatorial efficacy, supporting distinct functional effects. Next, we evaluated a curated 35-gene panel corroborated from prior literature on ONC201 including DRD2- (DRD2, DRD5, TH, EGFR), ClpP- (ClpP, NDUFS7, NDUFA12, SDHA, SDHB, POLD2, POLDIP2), epigenetic- (EZH2, EED, EZHIP, KDM1A, KDM3A, KDM4A, KDM6A, KDM6B), hypoxia- (HIF1, HIF2, HIF3, VHL, VEGFA, HES1, TGM2, CELF2, P4HA2, CEBPB) and downstream signaling-related (ATF3, ATF4, MYC, MYCN, GNA11, GNA15) genes as predictive biomarkers for ONC201/ONC206 efficacy (IC50, IC90, AUC and mRNA fold change) in the GDSC panel using CCLE RNA-seq data. Optimized thresholds for predictive power revealed that the top molecular correlatives prioritized for ONC201 (ClpP, TGM2, NDUFS7, EZH2, EGFR, POLD2, HIF2A, CEBPB, HES1) and ONC206 (ClpP, TGM2, NDUFS7, EZH2, EGFR, HIF1, VHL, ATF4, MYC, KDM6B) in GDSC partially overlap. Biomarker combinations with the prioritized markers improved predictive power compared to single markers. Dual (both markers must be co-expressed) or independent combinations involving EGFR, NDUFS7, EZH2 and ClpP were most predictive for ONC206, while those involving ClpP, EGFR and EZH2 were most predictive for ONC201. Results from GDSC for ONC201 were further confirmed using the PRISM dataset with RNA-seq and proteomics that revealed similar results. Hypoxic tumor cell cultures confirmed GDSC findings that elevated tumor cell hypoxia can impart ONC201- or ONC206- resistance. Thus, ONC206 is a distinct agent that may be uniquely poised to address tumors that are not addressed by ONC201 or have developed acquired resistance. Our results indicate that for accurate prediction of ONC201 and ONC206 clinical benefit, a curated combinatorial biomarker approach for each tumor type, using diverse detection approaches such as protein expression, genomics, and transcriptomics, may be used in ongoing clinical trials.

Background and Methods

ONC201 and ONC206 Overview

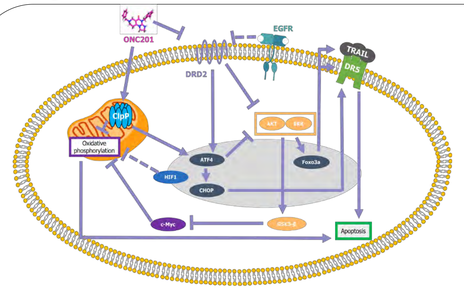
- ONC201: first-in-class bitopic DRD2/3 antagonist and allosteric ClpP agonist that crosses the blood-brain barrier and exhibits clinical efficacy in recurrent H3 K27M-mutant glioma
- ONC206: Chemical derivative of ONC201 and second imipridone in clinical development with differentiated receptor pharmacology, improved potency and robust preclinical anti-cancer activity in vitro and in vivo

Biomarker Rationale

- ONC206 was recently shown to be an allosteric agonist of ClpP, a mitochondrial protease responsible for degradation of electron transport chain members that carry out OXPHOS. ONC201 inhibits OXPHOS via ClpP
- ONC206 activates the integrated stress response pathway, triggering ATF4 induction (Ishida et al., Clinical Cancer Research, 2018)
- Proteasomal degradation of c-myc by ONC201 is associated with DRD2 antagonism and inhibition of OXPHOS in GBM (Ishida et al., Clinical Cancer Research, 2018)
- Low EGFR expression is associated with increased DRD2 expression in GBM (He Y et al., Neuro-oncology, 2020); EGFR activation is associated with glycolysis (Babic et al., Cell Metabolism, 2013)
- Upregulation of HIF1 drives tumor cells toward glycolysis (Semenza et al., Semin Cancer Biol, 2009)

Methods

- The PRISM dataset within the Cancer Dependency Map Project was used to analyze correlations between gene expression and ONC201 growth inhibitory activity
- ONC201- and ONC206-resistant T98G cell lines were generated by growing cells in drug-containing media until resistance, defined as cell proliferation at 3-fold above initial IC_{50}, was achieved.
- T98G (GBM), U251 (GBM), HT29 (colon cancer) and MDA-MB-361 (breast adenocarcinoma) cells were treated with increasing concentrations of ONC201 or ONC206 under hypoxic (0.2 or 0.5% CO_2) and normoxic (5% CO_2) conditions
- 620 human cancer cell lines were analyzed for correlations between biomarker expression and anticancer efficacy
- Efficacy data were obtained from the Genomics of Drug Sensitivity in Cancer panel (0-20μM ONC201 or ONC206, 72h treatment)
- mRNA data were obtained from the Cancer Cell Line Encyclopedia
- U87 cells were treated with ONC201 (200nM, 10μM), ONC206 (200nM) or ONC212 (200nM), after which GSEA was performed (GSE104273 and GSE103963)



Predictive biomarkers
ClpP ATF4
MYC DRD2
EGFR DRD5
HIF1

Pharmacodynamic biomarkers
ATF4 TUNEL
CHOP NK
DR5

Figure 1. Overview of predictive and pharmacodynamic biomarkers based on the ONC201 mechanism of action (Wagner et al, 2017; Ishida et al, 2018; Hu W et al, 2020).

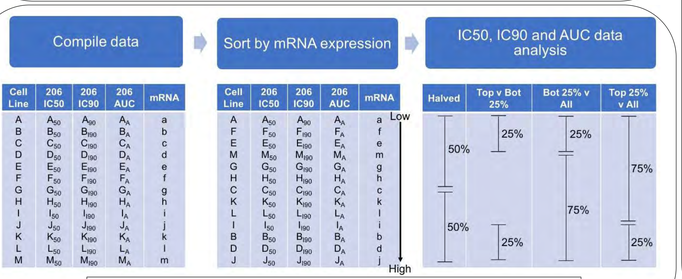


Figure 2. Representative methods for categorizing expression and sensitivity for single biomarker analysis.

Results

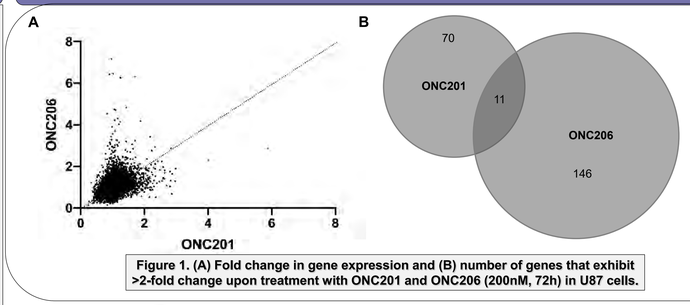


Figure 1. (A) Fold change in gene expression and (B) number of genes that exhibit >2-fold change upon treatment with ONC201 and ONC206 (200nM, 72h) in U87 cells.

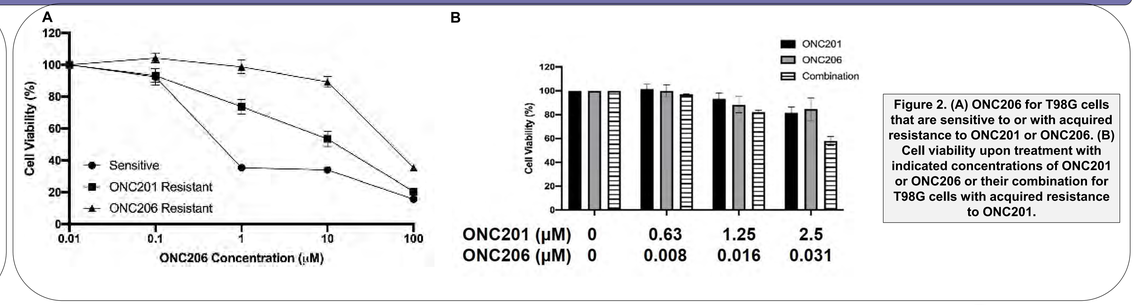
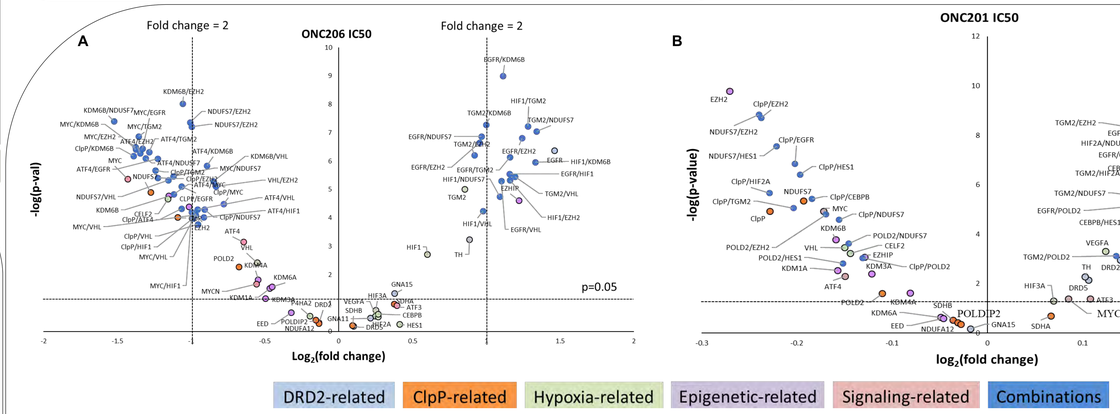
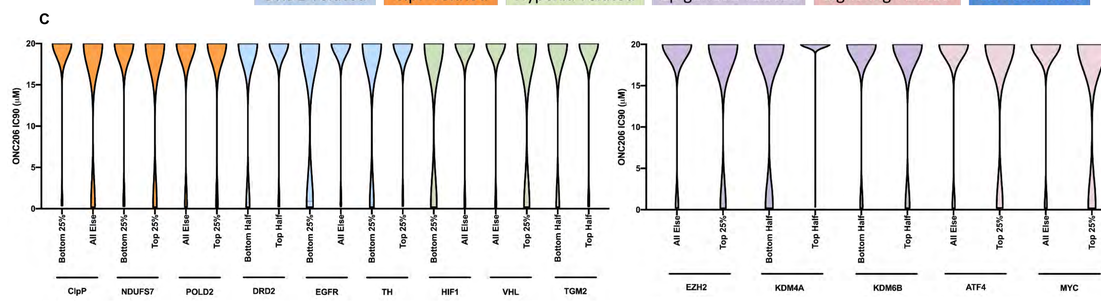


Figure 2. (A) ONC206 for T98G cells that are sensitive to or with acquired resistance to ONC201 or ONC206. (B) Cell viability upon treatment with indicated concentrations of ONC201 or ONC206 or their combination for T98G cells with acquired resistance to ONC201.





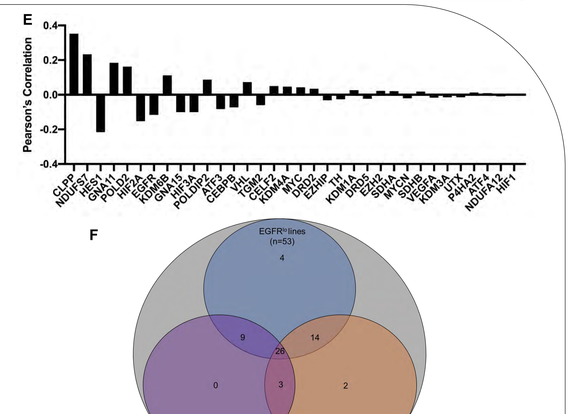


Figure 4. Single and combinatorial biomarker correlations with ONC201 and ONC206 efficacy in GDSC panel with RNA seq data available (n = 620). Volcano plots for (A) ONC206 and (B) ONC201 IC50 (center color code for both plots). (C) Violin plots of best representative IC90 data for ONC206 (center color code for both plots) (D) Heatmap summary of p-values by biomarker for each method of data analysis. Red: high expression correlates with improved efficacy (p<0.05). Yellow: Low expression correlates with improved efficacy (p<0.05). Black: expression does not correlate with efficacy (p>0.05). (E) Biased analysis of biomarker panel expression and ONC201 efficacy. (F) Venn diagram depicting the distribution of ONC206 IC90 lines according to biomarker expression. (G) Bar chart showing the percentage of ONC206 IC90 and non-IC90 cell lines captured by biomarkers either alone or in dual/independent combinations. 'IC90' denotes cell lines with IC90 < 20, whereas 'non-IC90' denotes cell lines with a measured IC90 of 20, the maximum concentration in the cell viability assay. '+' denotes optimal expression of each biomarker, whereas '-' denotes non-optimal expression.

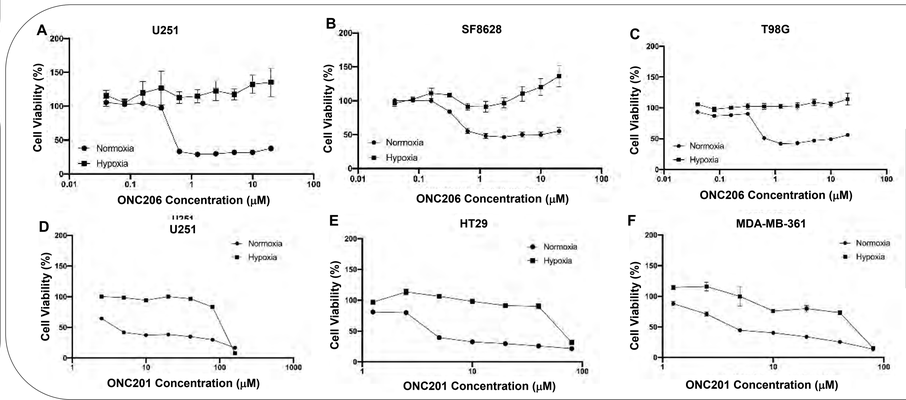


Figure 3. Cell viability of (A) U251, (B) SF8628 and (C) T98G cells cultured under normoxia or hypoxia and treated with ONC206 (72h) at indicated concentrations. Cell viability of (D) U251, (E) HT29 and (F) MDA-MB-361 cells cultured under normoxia or hypoxia and treated with ONC201 (72h) at indicated concentrations.

Conclusions and Future Directions

- Gene expression profiling, GDSC biomarker prioritization, acquired resistance and combinatorial synergy suggest that ONC206 exhibits distinct therapeutic properties relative to ONC201
- ONC206 may be uniquely poised to address tumors that are not addressed by ONC201 or have developed acquired resistance.
- GDSC analyses indicate that single biomarkers do not completely capture the sensitivity profiles of ONC201 and ONC206 across tumor types and that combinations of biomarkers increase predictive power
- Hypoxia-related gene expression in GDSC and hypoxic tumor cultures reveal that tumor hypoxia could be a mechanism of resistance to ONC201 and ONC206
- Combinations of predictive biomarkers evaluated at the RNA or protein level optimized for each tumor type could be explored in future clinical studies
- There is a need to further understand the biological relevance of correlations observed and mechanistic interplay between the prioritized biomarkers for each compound

Disclosures: SM, VVP, WSED and JEA are shareholders of Oncoceutics, Inc and/or Chimerix Inc