B-12

Allosteric ClpP agonist JZP3507 (formerly ONC206) alters mitochondrial metabolism, stress response and chromatin accessibility to elicit apoptosis in pheochromocytoma

Cristina Maranto[1], Scott Foster[1], Andrew Lee[1], Varun V. Prabhu[1], Joshua E. Allen[1]

[1]Chimerix, Inc. (a Jazz Pharmaceuticals company), Durham, NC, USA

BACKGROUND

JZP3507 (formerly ONC206), a second generation imipridone, is a caseinolytic protease P (ClpP) agonist and dopamine receptor D2/3 (DRD2/3) antagonist with nanomolar potency. Dordaviprone demonstrated responses in patients with pheochromocytoma-paraganglioma (PCPG). We characterized the mechanism of action, in vitro efficacy, response determinants and acquired resistance for JZP3507 in PC.

METHODS

ClpP was expressed in E. coli and purified by His-tag affinity for biochemical assays. PC (hPheo1, PC12, MPC10) and fibroblast (HFF-1, MRC-5) cells were commercially sourced. Cell viability (CellTiter-Glo, CyQuant), apoptosis (Caspase Glo, annexin V) assays, seahorse analysis, and multi-omics were conducted in PC cells treated with vehicle/JZP3507. Resistant hPheo1 cells were generated by passaging with increasing JZP3507 concentrations.

RESULTS

Co-crystallization with ClpP revealed an allosteric ligand interaction with distinctions in the JZP3507-ClpP resolved crystal structure relative to the dordaviprone-bound or apo complexes. JZP3507 was a more potent agonist than dordaviprone in cell-free human ClpP casein/peptide assays. Adrenal gland tumors emerged as most sensitive when JZP3507 cytotoxicity was assessed in a panel of 432 human cancer cell lines. JZP3507 induced dose- and time-dependent apoptosis in PC but not fibroblast cells. JZP3507 demonstrated stronger cell viability inhibition and/or apoptosis induction in PC lines relative to dordaviprone, temozolomide, sunitinib, and belzutifan at equivalent and/or therapeutically relevant concentrations. In hPheo1 cells, CRISPR-mediated SDHB or FH knockout and DRD2 overexpression did not impact JZP3507 sensitivity while ClpP knockout impaired JZP3507 sensitivity. Proteomics indicated inhibition of mitochondrial metabolism by JZP3507, including OXPHOS/TCA cycle while metabolomics revealed elevated α-ketoglutaric acid, 2-hydroxyglutaric acid and reduced succinic acid, fumaric acid in a ClpP-dependent manner. Consistent with epigenetic regulation by metabolites,

ATACseq revealed JZP3507 altered chromatin accessibility while RNAseq demonstrated upregulated stress response, apoptosis and downregulated metabolism-related pathways. Western blot confirmed JZP3507 downregulated mitochondrial proteins, neuroendocrine markers and upregulated stress response. JZP3507 reduced chromogranin A secretion by ELISA in hPheo1 cells that underwent neuroendocrine differentiation. PCR and western blot of acquired resistant cells revealed ClpP silencing in some resistant lines, confirmed by ATACseq. Whole exome sequencing revealed diverse ClpP missense and/or termination mutations. Seahorse analysis showed JZP3507 inhibited mitochondrially derived ATP in parental but not acquired resistant lines. Overexpression of ClpP missense mutations in wt cells conferred resistance while overexpression of wt ClpP restored JZP3507 sensitivity in acquired resistant lines.

CONCLUSIONS

JZP3507 is a potent novel agent that is superior to SOC and dordaviprone in PC. ClpP mediates response and acquired resistance to JZP3507 in PC.

ABSTRACT ID #36607