**P864 CTPS1 IS A NOVEL THERAPEUTIC TARGET IN MYELOMA - SELECTIVE SMALL MOLECULE INHIBITION DELIVERS SINGLE AGENT ACTIVITY AND SYNERGISES WITH ATR INHIBITION**

**Topic:** 13. Myeloma and other monoclonal gammopathies - Biology & Translational Research

Christina Pfeiffer¹, Philip Beer², Hélène Asnagli², Arnold Bolomsky³, Alexander Grandits⁴,⁵, Anja Schneller¹, Julia Huber¹, Niklas Zojer¹, Martin Schreder¹, Andrew Parker², Heinz Ludwig¹

¹ Department of Medicine I, Klinik Ottakring, Wilhelminen Cancer Research Institute, Vienna, Austria; ² Step Pharma, Saint-Genis-Pouilly, France; ³ National Cancer Institute, Center for Cancer Research, Lymphoid Malignancies Branch, National Institutes of Health, Bethesda, United States; ⁴ Department of Medicine I, Division of Oncology, Medical University of Vienna, Vienna, Austria; ⁵ Comprehensive Cancer Center, Vienna, Austria

**Background:** Recent years have seen major improvements in the treatment of myeloma; however, relapse occurs in the majority of patients. Novel treatment approaches are moving away from cytotoxic chemotherapy towards targeted therapies with defined mechanisms of action. Malignant cells have an increased demand for key cellular components and a reliance on de novo synthesis pathways. CTP synthetase 1 (CTPS1) plays a pivotal role in pyrimidine production, by catalysing the rate limiting step in the de novo synthesis of CTP, which is required for DNA, RNA and phospholipids. Human genetic studies have identified an essential and non-redundant role for CTPS1 in lymphoid cell proliferation which is complemented by the homologous CTPS2 isoform outside the haemopoietic system.

**Aims:** To evaluate the role of CTPS1 as a novel target in myeloma, elucidate the molecular consequences of CTPS1 inhibition and test rationally designed combination therapy.

**Methods:**

STP938 is a potent small molecule CTPS1 inhibitor with >1,300-fold selectivity for CTPS1 over CTPS2. The effects of STP938 on cell proliferation (metabolic activity, tetrazolium indicator), apoptosis (annexin V) and cell cycle (propidium iodide) were assessed in vitro using 12 myeloma cell lines. The role of CTPS1 was further assessed in CRISPR knock-out (KO) experiments. Activation of proteins in the replication stress and DNA damage response pathways was analysed by western blotting. Additive/synergistic effects of combining STP938 with two different ATR inhibitors were assessed on cell line proliferation and enumerated by Bliss score (Synergyfinder 2.0).

**Results:**

STP938 showed single agent activity against 6 of 12 cell lines tested, with IC₅₀ values for sensitive lines ranging from 19 to 128 nM (Figure A). STP938 showed cytotoxic activity against sensitive lines, evidenced by induction of apoptosis. KO experiments confirmed that MM cell lines depend on CTPS1 for proliferation. Exposure to STP938 was associated with accumulation of cells in S phase, induction of a replication stress response as evidenced by activation (phosphorylation) of CHEK1, and induction of the DNA damage response pathway as evidenced by activation of CHEK2 (albeit to a lesser extent than activation of CHEK1). Importantly, S phase accumulation, induction of replication stress and induction of DNA damage response was observed in cell lines where STP938 did not produce significant anti-proliferative activity (Figure B). Given the ability of STP938 to activate CHEK1, STP938 was tested in combination with an ATR inhibitor, as ATR is the main upstream activator of CHEK1. The ATR inhibitor ceralasertib demonstrated additive or synergistic activity when combined with STP938 (Figure C). Notably, synergy between STP938 and ATR inhibition, along with induction of apoptosis, was observed in cell lines resistant to single agent STP938. Results were confirmed with a second ATR inhibitor (VE-821, Figure C).
Summary/Conclusion:

Inhibition of CTPS1 by single agent STP938 showed anti-proliferative activity against 6 of 12 myeloma cell lines, and induced replication stress in all cell lines. Combined STP938 and ATR inhibition showed anti-proliferative activity in all cell lines, including those resistant to single agent STP938. These data suggest a model whereby myeloma cells with high background replication stress are sensitive to killing by single agent STP938, whereas cells with lower background replication stress are sensitised by STP938 to cell death induced by ATR inhibition (Figure D). STP938 will shortly enter clinical development for patients with late stage lymphoid neoplasms.