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P1248 CTP SYNTHASE 1 IS A NOVEL TARGET IN T CELL CANCERS, WITH SMALL MOLECULE INHIBITION INDUCING DEATH OF NEOPLASTIC HUMAN T CELLS IN VITRO AND INHIBITION OF THEIR GROWTH IN AN IN VIVO XENOTRANSPLANT MODEL

Topic: 20. Lymphoma Biology & Translational Research

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Background: T cell lymphomas are rare haematological cancers characterised by poor response to cytotoxic chemotherapy with resulting unmet clinical need. Recent studies have identified targeted therapies as a promising approach to improve outcomes. CTPS1, which catalyses the rate limiting step in pyrimidine synthesis, plays an essential and non-redundant role in B and T cell proliferation but is complemented by the homologous CTPS2 isoform outside of the haemopoietic system [1].

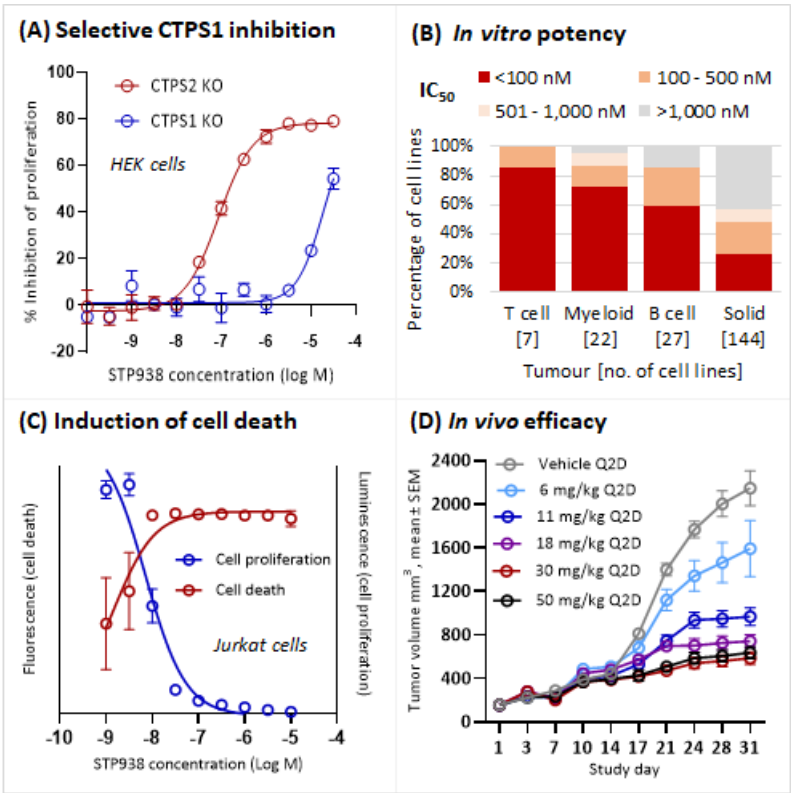
[1] E. Martin *et al.*, Nature. 2014 Jun 12; 510(7504):288-292.

Aims: To assess the impact of selective CTPS1 inhibition on the proliferation and survival of human neoplastic T cells.

Methods: Recombinant CTPS1 and CTPS2 enzymatic activity was assessed by quantitating cytidine triphosphate by RapidFire Mass Spectrometry. HEK cells lacking either CTPS1 or CTPS2 were generated using CRISPR technology; cell viability was measured after 3-5 days incubation with test compounds by CellTiter-Glo 2.0. For the cellular complementation assay, Jurkat cells lacking CTPS1 and CTPS2 were transduced with lentivirus carrying different CTPS1 and CTPS2 constructs cloned into a pLVX-EF1α-IRES-mCherry backbone; transduced cells were cultured with or without test compound and cell viability was assessed 72 hours later by CellTiter-Blue. For *in vitro* cancer cell line studies, cells were incubated for 72 hours with or without test compound; cell viability was assessed by CellTiterGlo and cell death by CellTiterTox Green. For *in vivo* experiments, sub-lethally irradiated female NOD-SCID mice were transplanted subcutaneously with 10^7 Jurkat cells embedded in Matrigel. Mice were randomised to n=8 per treatment group when tumours reached mean 150 mm^3. Test compound and vehicle control were administered subcutaneously every two days. Tumour volume was measured twice weekly.

Results: STP938 is a potent and reversible low molecular weight inhibitor of CTPS1, which shows >1,300-fold selectivity for human CTPS1 over CTPS2. Selectively for CTPS1 inhibition was confirmed in a cellular system using HEK cells lacking either CTPS1 or CTPS2, which are consequently dependent on CTPS2 or CTPS1, respectively, for proliferation (Figure A). A cellular complementation assay identified the ATP pocket of CTPS1 as the binding site for the compound series and highlighted residues critical for the selectivity over CTPS2. STP938 exhibited anti-proliferative activity against a broad range of human cancer cells when tested *in vitro*; haematological malignancies were significantly more sensitive than solid tumours cell lines, with T cell malignancies being the most sensitive (Figure B). Exposure of human neoplastic T cells (Jurkat and MOLT-4) to STP938 resulted in concentration dependent induction of cell death (Figure C). In an *in vivo* model of T cell neoplasia, where human Jurkat cells were transplanted subcutaneously into immunodeficient mice, STP938 showed dose dependent inhibition of tumour growth (Figure D).

Image:



Summary/Conclusion: CTPS1 inhibition by STP938, a novel small molecule with favourable pharmaceutical properties, inhibits the growth of human neoplastic T cells *in vitro* and *in vivo*. CTPS1 represents a novel target in T cell cancers. STP938 is currently being prepared for a first in human study in patients with relapsed or refractory lymphoma.