P1308 SELECTIVE SMALL MOLECULE INHIBITION OF CTP SYNTHASE 1 (CTPS1) SUPPRESSES T CELL PROLIFERATION AND CYTOKINE RELEASE, HIGHLIGHTING A NOVEL THERAPEUTIC TARGET FOR GRAFT-VERSUS-HOST DISEASE

Topic: Stem cell transplantation - Experimental

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Background: Graft-versus-host disease (GvHD) significantly increases both the morbidity/mortality and economic cost of allogeneic haematopoietic stem cell transplantation. Key to successful therapy is effective disease control whilst maintaining a competent anti-infection response. Acute GvHD (aGvHD) is driven by the rapid proliferation of, and cytokine release from, donor-derived T cells. Standard of care therapy with systemic steroids is associated with broad and deleterious immunosuppression, and fails to achieve adequate disease control in a third of patients. There is currently a lack of consensus around therapy beyond steroids [1]. CTP synthases catalyse the conversion of UTP to CTP, the rate limiting step in pyrimidine synthesis. Humans have two isoforms of this enzyme (CTPS1 and CTPS2); human genetic studies have identified an essential and non-redundant role for CTPS1 in the proliferation of B and T cells [2].

[1] Blood. 2020 May 7;135(19):1630-1638. [2] Nature. 2014 Jun 12;510(7504):288-92. [3] J Transl Med. 2010 Oct 26;8:104.

Aims: To assess the impact of selective CTPS1 inhibition on the proliferation of, and cytokine release from, human T cells.

Methods: Aliquots of whole blood or isolated peripheral blood mononuclear cells (PBMC) from healthy volunteer donors underwent T cell activation with CD3/CD28 beads and incubation with or without a selective CTPS1 inhibitor for 24-72 hours. Levels of intracellular CTP, ATP and GTP nucleotides were quantitated by liquid chromatography–mass spectrometry; cell proliferation was assessed by tritiated thymidine incorporation, Ki-67 by flow cytometry and cytokine release by analysis of plasma using the Luminex platform.

Results: STP938 is a potent and reversible low molecular weight small molecule inhibitor of CTPS1, which shows >1,300-fold selectivity for human CTPS1 over CTPS2. Exposure of stimulated PBMCs to STP938 resulted in a concentration dependent depletion of CTP but had no effect on the levels of GTP or ATP, confirming selective inhibition of CTP synthase by STP938. CTPS1 inhibition by STP938 elicited a concentration dependent inhibition of CD3/CD28-induced T cell proliferation in whole blood samples after 72 hours incubation, resulting in full suppression of proliferation at the highest concentration tested (1 µM) (Figure A). Exposure to STP938 also reduced the proportion of T cells in cell cycle, as evidenced by a decrease in the expression of Ki-67 (Figure B). STP938 exposure induced a concentration dependent inhibition of T cell cytokine release in whole blood samples, with higher STP938 concentrations producing near-complete suppression (Figure C). Notably, significant inhibition of T cell cytokine release was observed after only 24 hours’ exposure of stimulated T cells to STP938 (Figure D, 1 µM STP938). Given that human T cells do not undergo cell division until at least 40 hours after CD3/CD28 stimulation is initiated [3], these findings indicate that the ability of CTPS1 inhibition to prevent cytokine release is independent of its effects on cell proliferation.

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Summary/Conclusion: CTPS1 inhibition by STP938 induces a rapid suppression of T cell cytokine release along with suppression of T cell proliferation; both of these effects are concentration dependent and near maximal at exposures of STP938 that are likely to be achievable in the clinic. CTPS1 represents a novel target with the potential to effect rapid and specific disease control in aGvHD. STP938 is currently on the path to clinical development in lymphoid malignancy.