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POSTER ABSTRACTS

802.CHEMICAL BIOLOGY AND EXPERIMENTAL THERAPEUTICS

Development of Novel Protein-Drug Conjugates for the Treatment of Chronic Myelomonocytic Leukemia

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Introduction:
Chronic myelomonocytic leukemia (CMML) is a myeloid stem cell neoplasm with few effective therapies and poor prognosis. We have developed CCL2-drug conjugates that specifically bind, internalize and eliminate cells of the disease clone via inhibition of the key metabolic enzyme NAMPT. CCL2 is a chemokine which binds CCR2, a receptor expressed predominantly on the classical monocytes canonically expanded in CMML, while CMML stem and progenitors display ~100-fold higher CCR2 expression compared with healthy equivalents *(Ferrall-Fairbanks et al, Blood Cancer Discov 2022 3(6):536-553)*. CCL2-NAMPTi conjugates thus represent an attractive and novel strategy, co-opting both lineage specificity and therapeutic window to deliver payload drugs for efficient and targeted cell killing.

Methods:
Four NAMPTi warheads were made in adaptable seven-step synthetic route and covalently conjugated to human CCL2 though TGase. We screened the range of CCL2-NAMPTi conjugates on CCR2-expressing cell lines (THP-1; MV411) for their ability to reduce NAD/NADH and induce cytotoxicity. The lead CCL2-NAMPTi conjugate was identified based on its ability to reduce NAD/NADH levels, induce cell death, and show impermeability when using the unconjugated warhead alone. Plasma stability studies confirmed conjugate stability in mouse and human plasma with no release of unconjugated active warhead. The lead compound was then tested on CD14+ cells isolated from CMML patient peripheral blood (PB); examining NAD/NADH levels, cell viability and induction of caspase 3/8. To better understand its specificity and target cell population, we conducted *in vivo* studies using fluorescently labelled CCL2 in wild type and CCR2 ^{KO} mice. Therapeutic effectiveness of the lead conjugate was evaluated by means of NAD/NADH reduction in THP1 subcutaneous models.

Results:
All CCL2-NAMPTi tested induced cell death in THP-1 cells (IC50 1.1-7.2 nM) following 72 hours incubation. The lead compound, MM015, effectively reduced NAD/NADH levels in CCR2+ cell lines (THP-1; MV411) 24-48 hours following treatment (IC $_{50}$ 0.1-0.8 nM), with a corresponding decrease in cell viability at 72 and 96 hours (IC $_{50}$ 1.1- 2.1 nM). No effect was observed on CCR2- cell lines (Jurkat; PC9). Specificity of the lead conjugate was confirmed by measuring NAD/NADH levels and cytotoxicity in presence of 100-fold excess of unconjugated CCL2. Efficacy studies on primary CMML PB samples confirmed that MM015 exposure specifically reduced NAD/NADH levels (n=6, IC $_{50}$ 0.5-15 nM) and cell viability (n=6, IC $_{50}$ 1.9-250 nM), and induced caspase 3/8 activation. Flow cytometry analysis of CMML PB confirmed CCR2 expression was largely restricted to monocytes and precursors, as compared with other immune cell subsets (n=6). Accordingly, efficacy measures in treated whole PB were exclusively seen in the CD14+ population, sparing lymphoid and other lineage cells, demonstrating ability of CCL2-NAMPTi to selectively target the CMML disease clone.
In vivo experiments using fluorescently labelled CCL2 confirmed selective uptake of intravenously administered human CCL2 by CCR2+ cells within mouse blood, bone marrow and in THP-1 subcutaneous tumour cells. No uptake of fluorescently labelled CCL2 was observed in CCR2 ^{KO} mice, further confirming CCR2 specific uptake. Additionally, measurement of human CCL2 in CCR2 ^{KO} mouse peripheral blood by ELISA confirmed CCR2 dependent CCL2 uptake, 1 hour following intravenous administration. *In vivo* efficacy studies showed consistent reduction of NAD/NADH levels in xenografted tumor cells, and monocytic cells in the murine BM compartment, 24 hours following IV injection of MM015.

Conclusion:

We have developed a novel drug delivery strategy utilizing CCL2-NAMPTi conjugates to selectively target and eliminate key CMML cell populations. CCL2-NAMPTi can effectively reduce NAD levels and induce subsequent cell death in CCR2-expressing cell lines, primary CMML cells and *in vivo* models. This innovative approach holds therapeutic promise to address this major unmet clinical need.

Disclosures No relevant conflicts of interest to declare.

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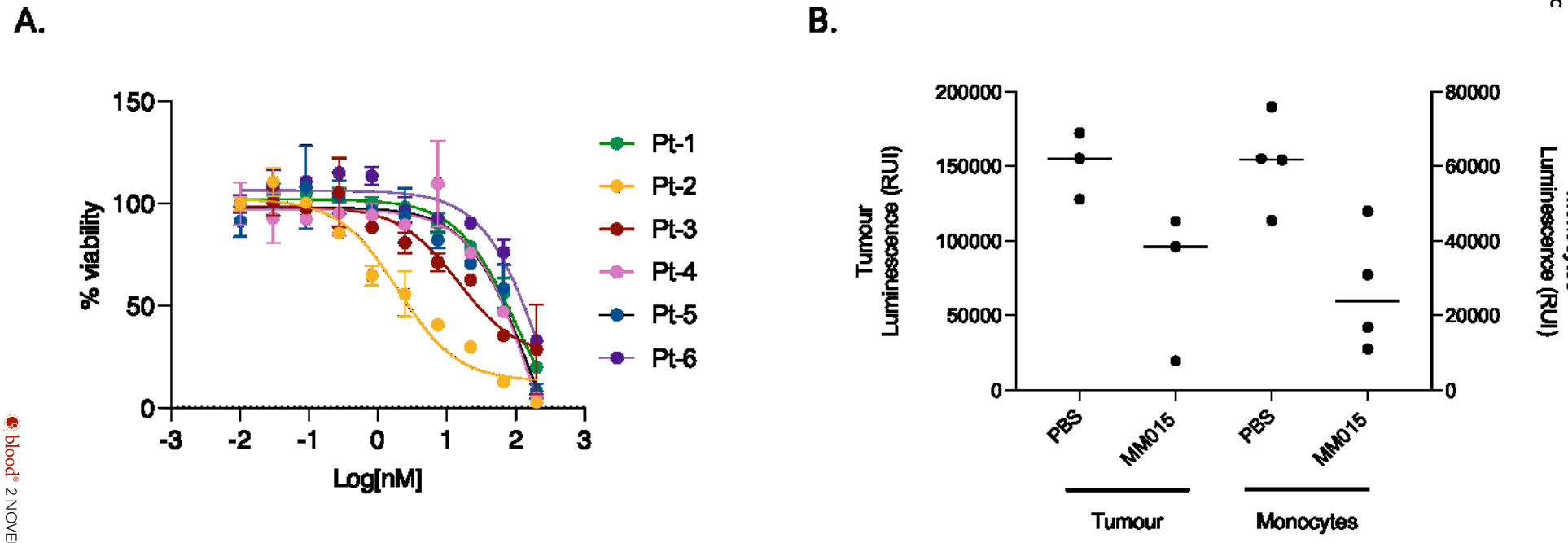

A. Percentage viability of CD14+ cells isolated from six CMML patients following treatment with MM015

B. THP-1 subcutaneous tumour mice were treated with either PBS or 5mg/kg CCL2-MM015. 24 hours following treatment, relative NAD/NADH levels in cells isolated from THP-1 subcutaneous tumors or monocytes isolated from bone marrow was determined

Figure 1