# Targeting Bone Marrow with CCL2 conjugated cytotoxic NAMPT inhibitors

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## Introduction

Chronic myelomonocytic leukaemia (CMML) is characterised by the aberrant proliferation of monocytic cells. The initial mutations occur within the bone marrow, and treatment is palliative due to current drugs only being able to kill cells in the peripheral blood stream. Slow proliferation of bone marrow cells also results in widely used chemotherapy agents that target proliferation being ineffective. Herein we report a novel CCL2 conjugated drug delivery system that can selectively target cancerous cells involved in CMML, including in bone marrow by utilizing a Nampti inhibitor as the cytotoxic payload. These compounds are metabolic cytotoxins, allowing induction of apoptosis and cell death in non-proliferating cells.

## Methods and Materials

Click protein conjugation was carried out using standard conditions and protein purified and isolated by PD size exclusion chromatography. Samples were analysed by MALDI- TOF and MCP-1 ELISA assay. Conjugate compounds were incubated with THP1 and Jurkat monocytic cells for 48 hrs and analysed using a WST-1 cell proliferation assay. *In Vivo* mouse studies were undertaken by injecting CCL2- SCY5 into WT and CCR2 KO mice at 1 mg/kg (10 µM in PBS, 0.1% BSA). After three hours samples were taken from bone marrow and blood, labelled for standard markers and analysed by FACS.

# Results

## Nampti as cytotoxic payload

High (10% FBS) and low (2.5% FBS) proliferative THP1 were treated with FK866 or the typical cytotoxic ADC payload DM-1. DM-1 showed a large drop off in potency in the slower proliferating THP1 cells in 2.5% FBS, whereas FK866 showed minimal reduction in efficacy.

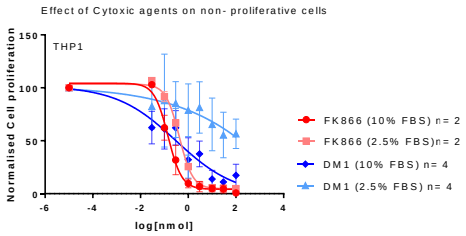


**Figure 1 Comparison of FK866 (Nampti) with DM1 (anti- mitotic agent) in both high proliferative (10% FBS) and low proliferative (2.5% FBS) THP1 cells. DM1 shows large drop off in potency in low proliferative cells whilst FK866 shows no significant change.**

Following earlier studies on selective uptake of CCL2 conjugates by CCR2+ cells. THP1 and Jurkat cells were incubated for 48h with a novel CCL2-NAMPTi and WST-1 was used as cell proliferation assay, showing that CCL2-NAMPTi is high selectivity for CCR2-expressing cells.

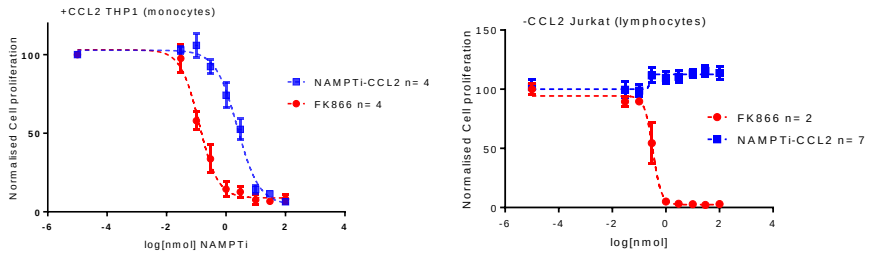


**Figure 2 dose response curve of CCL2- Nampti in CCL2 positive THP1 cells (left) and CCL2 negative Jurkat cells (right). CCL2- Nampti is shown to be selective towards THP1 cells.**

In *in vivo* studies WT mice showed high levels of Cy5 uptake in the CCR2 positive population in bone marrow as well as in the blood, while no uptake is observed in any cell type for the CCR2 -/- mice.

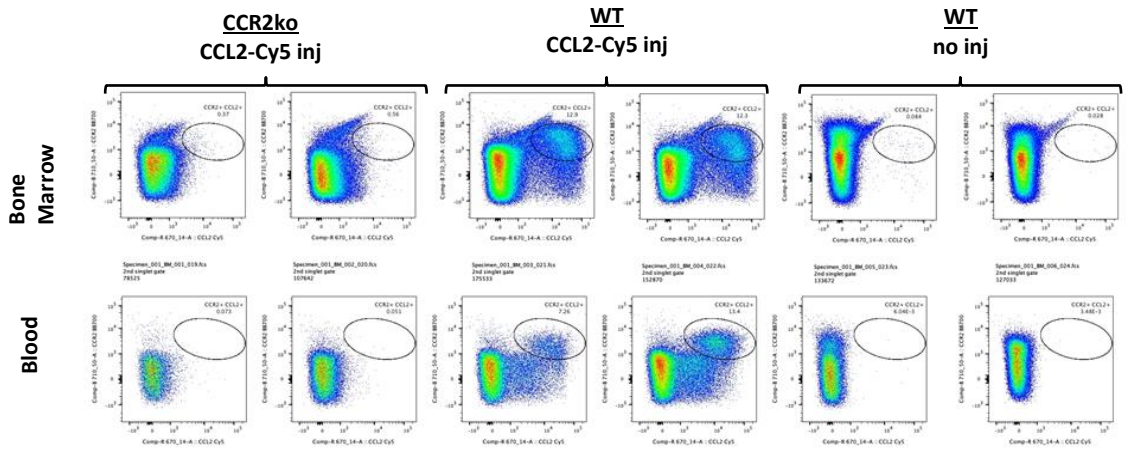


**Figure 3 Flow cytometry data of *in vivo* mouse study. The data depicts significant uptake of CCL2- SCy5 into bone marrow and blood of wild type mice.**

## Conclusion

NAMPT inhibitors are metabolic cytotoxins that can target non-proliferating cells, however as a result they have no useful therapeutic window. We have developed CCL2 conjugates that are selectively internalized by CCR2+ cells, and are able to target the bone marrow *in vivo*.

Combining these findings with the initial THP1 results gives a very promising basis that a CCL2-Nampti inhibitor will be able to target and kill cancerous bone marrow cells in CMML.

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