Aminopeptidase Inhibition as a Targeted Treatment Strategy in Myeloma

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Hypothesis.
As myeloma cells produce large quantities of monoclonal immunoglobulin and are reliant on protein production and the unfolded protein response, we hypothesised that disrupting intracellular protein turnover using an aminopeptidase inhibitor would result in myeloma cell death.

Introduction.
- The aminopeptidase enzyme system catalyses the hydrolysis of amino acids from the N-terminus of protein and peptide substrates. These enzymes are essential to many physiologically important processes e.g. protein maturation, degradation of peptides and cell cycle control.
- Inhibiting these enzymes disrupts protein turnover leading to an accumulation of peptides and a reduction in the free amino acid content of the cells, resulting in cell death.
- CHR-2797 (Chroma Therapeutics) is a novel inhibitor which is targeted to the M1 family of aminopeptidases, a group of metalloenzymes that contain a central Zn²⁺ ion.
- CHR-2797 is a water-soluble compound which once inside the cell is susceptible to intracellular esterases that generate the charged acid metabolite CHR-79888. This also has inhibitory effects against aminopeptidases and has low membrane permeability resulting in intracellular accumulation.

CHR-2797 inhibits myeloma cell proliferation and induces apoptosis.
A. Treatment of MM cell lines over a 96hr period with CHR-2797 inhibited proliferation as determined by a WST-1 assay: H929, ∆: MM1s, ▲: U266, ▼: JJN3, *: MM1r).
B. The acid metabolite CHR-79888 failed to inhibit proliferation in the same assay.
C. CHR-2797 was able to induce cell death in CD138+ plasma cells isolated from patient samples over a 72hr time course.
D. Cell death in MM cell lines was validated over a 96hr time course using annexin V/PI staining and trypan blue exclusion.

CHR-2797 is synergistic with other anti-myeloma therapies.
A. The combination of 100nM of dexamethasone and a range of CHR-2797 doses demonstrates synergy in the dexamethasone sensitive cell line MM1s after 96 hours.
B. The combination of CHR-2797 and bortezomib demonstrates an additive effect when CHR-2797 is added 24 hours prior to the bortezomib.

Aminopeptidase inhibition effects bone marrow stromal cells / myeloma cell interactions.
A. CHR-2797 caused minimal inhibition of proliferation of BMSCs.
B. CHR-2797 is able to overcome the protective effects of the BMS and induce inhibition in myeloma cell growth when myeloma cells were cultured with BMSCs compared to when myeloma cells were cultured alone.
C&D. ELISA analysis of supernatants demonstrate treatment with 500nM CHR-2797 caused depletion in VEGF levels in MM and BMS co-cultures but no change in IL-6 levels.

CHR-2797 induces non-caspase dependent myeloma cell death.
Western blot analysis demonstrates:-
No induction of caspase mediated cell death.
An increase in AIF and endoG consistent with non-caspase cell death.
An increase in Noxa and Puma with the cleavage of Mcl-1.

Aminopeptidase inhibition induces the unfolded protein response.
Treatment of myeloma cells with CHR-2797 induced the UPR.
A. Splicing of XBP1s to XBP1 determined by RT-PCR.
B. Real-time ‘Taqman’ PCR levels of the pro-apoptotic factor CHOP increased.
C. Immunoblotting of nuclear fractions of cell lysates demonstrated the translocation of the cleaved fragment of ATF6α to the nucleus.
D. Cytoplasmic inclusions were induced in response to treatment (24 hrs, 1c50 doses). These inclusions contain immunoglobulin light chain

Conclusion.
- Inhibiting protein turnover using the aminopeptidase inhibitor CHR-2797 results in myeloma cell apoptosis.
- Cell death occurs in a non-caspase dependent manner and is associated with activation of the UPR.
- This represents a novel and promising therapeutic approach.
- A phase I/II clinical trial is underway (see oral presentation 443).

Acknowledgments.
- This work was funded by Kay Kendall Leukaemia Fund, the General Clinical Research Fund of The Royal Marsden Hospital and the Department of Health.