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Targeting the MDM2-p53 interaction: time- and concentration-dependent studies in tumour and normal human bone marrow cells reveal strategies for an enhanced therapeutic index

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Introduction

p53 activity is regulated by its interaction with MDM2, which acts as an E3 ubiquitin ligase, but also inhibits the transcriptional activity of p53. In tumour cells, activated p53 elicits a transcriptional response that may block proliferation and induce apoptosis. However, prolonged p53 reactivation can also induce thrombocytopenia and neutropenia [1, 2] .

We aimed to design an MDM2-p53 antagonist with a differentiated tolerability profile that could be used to treat patients with wild-type *TP53* malignancies.

As part of an alliance between Newcastle University, Astex Pharmaceuticals and Cancer Research UK, we discovered ASTX295, a potent inhibitor of the MDM2-p53 interaction that is currently in clinical studies for patients with solid tumours (NCT03975387).

Objective

ASTX295 has a shorter plasma half-life, which we hypothesised would help to mitigate the neutropenia and thrombocytopenia observed with earlier MDM2-p53 antagonists in clinical studies. To examine this hypothesis *in vitro*, we determined time- and concentration-dependent responses to ASTX295 treatment in healthy volunteer-derived human bone marrow cells, megakaryocytes, and in a panel of human tumour cell lines.

Methodology

- SRB & XTT assays were used for cell line growth Inhibition assays (72h)
- Western blotting was performed on whole-cell lysates
- Activity against MDM2 was assessed using a previously described ELISA method [3]
- Colony-forming assays were performed on cell lines that had been treated with ASTX295 (washed out after 6, 12, or 24h) and seeded at low density to allow colony formation, and subsequent calculation of LC_{50} values.
- For human bone marrow assays, samples containing bone marrow cells from healthy patients undergoing hip surgery were obtained under the ethical approval of the Newcastle Biobank (REC 12/NE/0395). Following Lymphoprep™ , cells were treated *ex vivo* with ASTX295 (which was washed out after 6, 12, or 24h) and seeded for Granulocyte-macrophage (GM) colony-forming assays in methylcellulose.
- In megakaryocyte studies, human bone marrow derived CD34+ progenitor cells were cultured for 12 days in media containing supplements to promote megakaryocyte expansion and differentiation. Prior to treatment, cells were characterized by flow cytometry to detect expression of markers CD34, CD41 and CD42 and shown to be differentiating down the megakaryocyte lineage. Treatments were given for 6h, washed out for 18h, and repeated daily for a total of 3 days.

Results

Fig 1. ASTX295 - structure

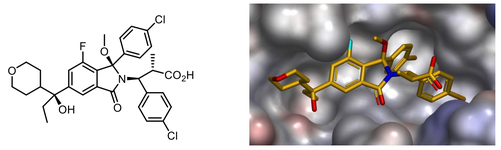

Figure 1: Structure of ASTX295, an isoindolinone-based MDM2 inhibitor. The compound inhibits MDM2 with an IC_{50} of <1nM (by MDM2 ELISA). ASTX295 occupies three subpockets on MDM2 involved in the recognition of the residues Phe19, Trp23, and Leu26 of the transactivation domain of p53.

Fig 2. ASTX295 - a potent and selective inhibitor of the MDM2-p53 pathway

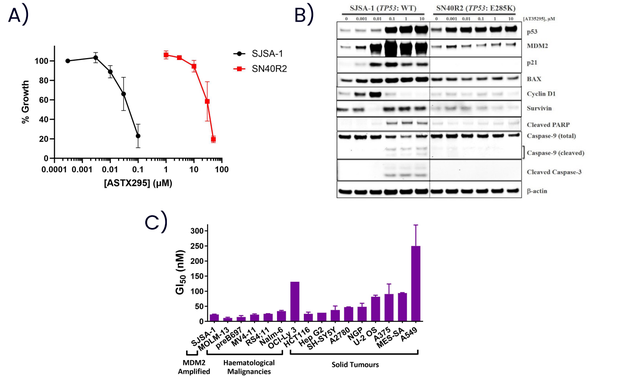

Figure 2: (A) ASTX295 growth inhibition in SJSA-1 (*TP53* wild-type) *versus* SN40R2 (*TP53* mutant) tumour cell lines (72h), (B) ASTX295-induced protein expression (p53, its transcriptional targets and pro-apoptotic proteins) after 6h exposure in SJSA-1 and SN40R2 cells, (C) Growth inhibition at 50% (GI_{50}) for a tumour cell line panel

Fig 3. Tumour cell lines show time-dependent cytotoxicity

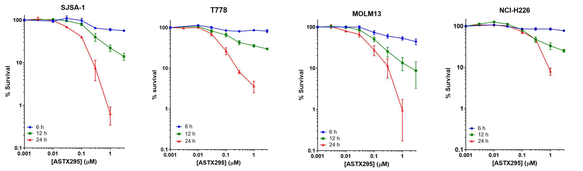

Figure 3: Colony-forming assays in *MDM2*-amplified osteosarcoma (SJSA-1) and liposarcoma (T778), AML (MOLM-13), and Lung carcinoma (NCI-H226) cells. Cells were exposed to ASTX295 for 6, 12 and 24h.

Fig 4. Human bone marrow time-dependency *versus* tumour cell lines

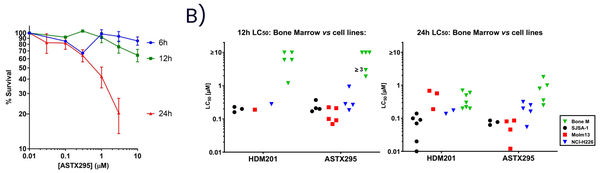

Figure 4: (A) Colony-forming assay in human bone marrow cells, after treatment with ASTX295, (B) Summary of LC_{50} values derived from colony forming assays with human bone marrow *versus* tumor cell lines after treatment with HDM201 or ASTX295.

Fig 5. CD34+ stem cell-derived megakaryocytes

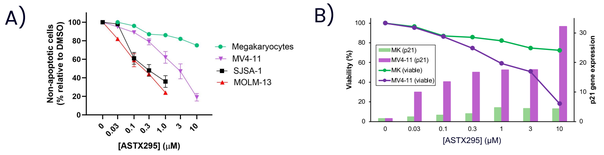

Figure 5: (A) Flow cytometric analysis of apoptotic cells following 6h exposure to ASTX295, daily for 3 days, (B) Viability and induction of p21 gene expression in megakaryocytes (MK) and MV4-11 AML cells, following a 6h daily exposure exposure to ASTX295 for 3 consecutive days

Conclusions

- ASTX295 is a potent antagonist of the MDM2-p53 interaction
- ASTX295 demonstrates approximately 1000-fold selectivity for wild-type *TP53* cell lines *versus* mutant *TP53* cells (Fig. 2A)
- ASTX295 treatment of wild-type *TP53* tumour cells resulted in increases in the level of p53, its transcriptional targets MDM2 and p21, and apoptotic markers (cleaved PARP, caspase-3 and caspase-9) in tumour cells (Fig. 2B)
- Our *in vitro* data suggest that a shorter exposure to ASTX295 (6h - 12 hours), may help to spare healthy bone marrow cells and megakaryocytes whilst retaining significant tumour cell killing (Figs. 3 – 5). Consequently, intermittent exposure to an MDM2-p53 antagonist could favourably modulate its therapeutic index.
- The short plasma half-life of ASTX295 provides flexibility in controlling the duration of exposure *in vivo*, potentially enabling a more bone-marrow sparing approach to MDM2-p53 antagonism to be utilised in future combination strategies.
- Additional presentations on ASTX295 (#666, 667, 6588 & CT066) have been presented at this meeting, the latter reporting preliminary clinical data.

References

1. Ray-Coquard I *et al.*, Lancet Oncol, 13(11), 1133-40, 2012
2. Iancu-Rubin C *et al.*, Exp Hematol, 42(2), 137-45, 2014
3. Chessari G *et al.*, J Med Chem, 64(7), 4701-88, 2021