#289 Pulsatile induction of the p53 pathway by MDM2 antagonist ASTX295 shows an enhanced therapeutic index *in vivo*

Lynsey Fazal[1], Maria Ahn[1], Luke Bevan[1], Andrea Biondo[1], Gianni Chessari[1], Aurelie Courtin[1], Keisha Hearn[1], Steven Howard[1], Simone Jueliger[1], Harold Keer[3], Justyna Kucia-Tran[1], David R Newell[2], Aram Oganesian[3], Alpesh Shah[1], Martin Sims[1], Huw Thomas[2], Nicola Wallis[1], Stephen R Wedge[2], Elaine Willmore[2], Yan Zhao[2], Nicola Wilsher[1]

[1]Astex Therapeutics, 436 Cambridge Science Park, Milton Road, Cambridge, CB4 0QA, UK; [2]Cancer Research Horizons Therapeutic Innovation, Newcastle Drug Discovery Group, Newcastle University, Newcastle upon Tyne, UK; [3]Taiho Oncology, Inc., Pleasanton, CA

Background

- The tumour suppressor transcription factor p53 is regulated by one of its transcriptional targets, MDM2, in a negative feedback loop. In tumours the p53 pathway is often inactivated either via *TP53* mutation or an increase in MDM2.
- MDM2 antagonists reactivate p53 in *TP53* wild-type tumours and are being developed as a potential cancer treatment. However, dose-limiting bone marrow toxicities, particularly thrombocytopenia and neutropenia[1,2,3], are commonly observed.
- *In vitro* studies suggested longer activation of the p53 pathway was required in normal cells to trigger cell death compared with *TP53* wild-type tumour cell lines[4].
- ASTX295 is a potent, next generation MDM2 antagonist designed to have a shorter plasma half-life to deliver short pulses of exposures *in vivo*.
- Preclinical *in vivo* data were consistent with the hypothesis, that shorter exposures of an MDM2 antagonist can reduce bone marrow toxicity but retain efficacy, as were subsequent observations in patients with solid tumours (ASTX295-01 phase I study; NCT03975387)[5].

Materials and Methods

- ASTX295 IV or oral single dose plasma PK was measured in wild type female CD-1 mice.
- ASTX295 plasma and tumour concentrations measured by LCMS and tumour p21 measured by MSD analysis were determined at multiple time points following oral administration of ASTX295 to female mice bearing SJSA-1 (CD-1 nude) or NCI-H226 (male BALB/c nude) human tumour xenografts.
- Non-clinical efficacy of ASTX295 was assessed following daily oral doses of ASTX295 or vehicle to tumour bearing mice for up to 14-days. Tumour volumes were measured every 2 to 3 days until study completion.
- In a GLP toxicology study groups of WT mice were dosed with ASTX295 or vehicle for 28-days and samples collected for TK and blood cell counts on days 1 and 28.
- *In vitro* cell data and clinical data was generated as previously described[4,5].
- The care and treatment of animals were in accordance with the UK coordinating Committee for Cancer Research guidelines and with the UK Animals (Scientific Procedures) Act 1988 law. Astex is committed to the ethical use of animals and adheres to the principles of 3Rs (see www.astx.com).

Results

In vitro

- ASTX295 is a potent antagonist of the MDM2-p53 interaction.
- Reduced exposure to ASTX295 (< 12 hours), may help spare healthy bone marrow cells whilst retaining significant tumour cell killing[4].

Table 1: In vitro Potency of ASTX295

Species	Human	Mouse	Cell Line	SJSA-1	NCI-H226	MOLM13
[A]ITC Kd (nM)	1.3	0.54	[B]EC50 (nM)	27	220	9

A) Isothermal Titration Calorimetry (ITC) used to determine the equilibrium dissociation constant (KD) of ASTX295 binding. B) ASTX295 growth inhibition in SJSA-1 (osteosarcoma, TP53 WT, MDM2 amp), NCI-H226 (Mesothelioma, *TP53* WT, *CDKN2A* loss) and MOLM13 (AML, *TP53* WT) tumour cell lines.

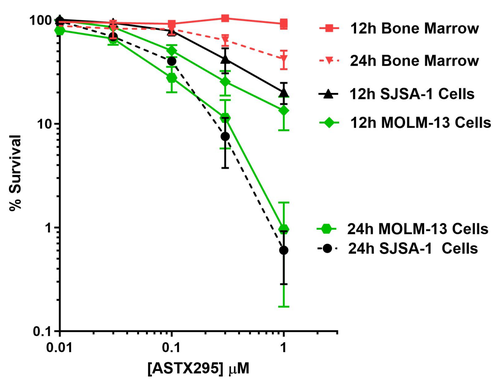

Figure 1: Time-dependency of cytotoxicity in human bone marrow versus tumour cell lines

Colony forming assays with human bone marrow versus tumour cell lines after treatment with ASTX295 for 12 and 24h.

Mouse *In Vivo*

- ASTX295 has a low clearance, good oral bioavailability and short plasma half-life in mouse.
- ASTX295 caused significant tumour growth inhibition in the SJSA-1 and NCI-H226 xenograft models.

Table 2: Pharmacokinetics of ASTX295 in mice

Route	Dose (mg/kg)	Half-life (h)	Vss (L/kg)	Cl (mL/min/kg)	Route	Dose (mg/kg)	Half-life (h)	% F
IV	1	1.8	2.2	24	PO	5	2.8	53

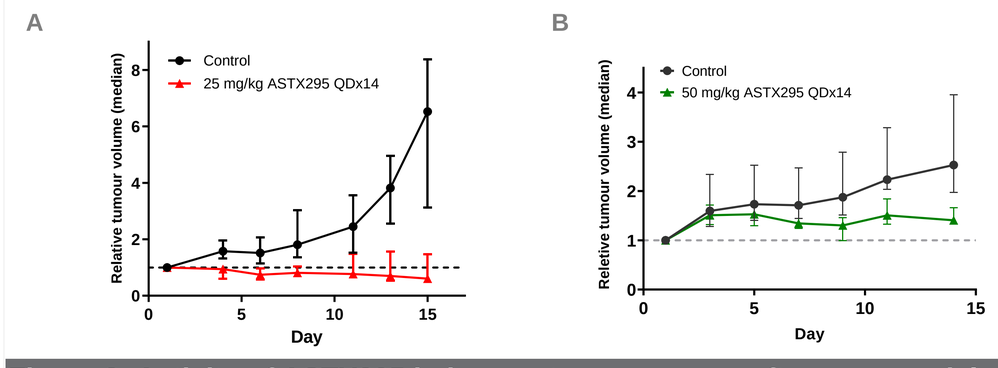

Figure 2: Activity of ASTX295 in human tumour xenograft mouse models

A) SJSA-1 xenograft model[6] B) HCI-H226 xenograft model

- Oral administration of ASTX295 led to a short duration of induction of p21, the p53 transcriptional target, in human tumour xenografts. Maximum induction was achieved at 6 hours post dose, however, the magnitude was cell line dependent.
- There were no significant changes in platelet and neutrophil counts after 28-days of daily oral dosing at 30 mg/kg ASTX295.
- ASTX295 exposure was comparable between PKPD and toxicology studies.
- In human tumour xenograft mouse models ASTX295 showed marked activity at exposures which did not induce thrombocytopenia and neutropenia in mice.

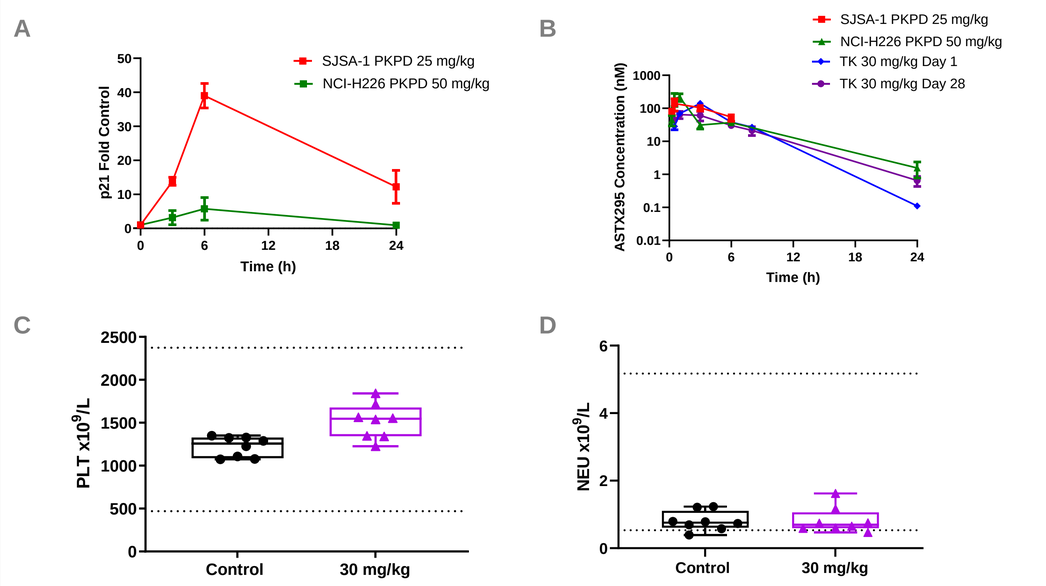

Figure 3: ASTX295 PKPD and haematology

A) p21 induction in SJSA-1 and NCI-H226 xenografts following a single oral dose of ASTX295, B) Free ASTX295 plasma concentrations in mice following the indicated oral doses in SJSA-1 and NCI-H226 PKPD and toxicology studies, C + D) Blood cell counts following 28-days dosing of vehicle and ASTX295, dotted lines indicate the normal range.

Clinical

- In the ASTX295-01 phase 1 clinical study[5] target PK exposure levels of ASTX295 associated with biological activity based on preclinical models were achieved. ASTX295 had a relatively short human half-life of ~4-6 hours.
- Induction profile of p21 and MDM2 gene expression in surrogate tissue was consistent with a shorter pulsatile modulation of the p53 pathway.
- ASTX295 was well tolerated at clinically effective doses while avoiding significant thrombocytopenia and neutropenia. Gastrointestinal adverse events were dose-limiting.
- Objective clinical responses were seen in heavily pre-treated subjects, including liposarcoma and lung cancer. Suggestion of clinical activity was observed in some GBM patients.

Table 2: Clinical Pharmacokinetics of ASTX295 from Phase IA

Cohort	Dose (mg)	N	AUC	Cmax	Tmax	%AUC target
5	420 (QD)	10	4061 (123)	1083 (189)	3 (2 – 4)	15 – 49%
12	660 (Q2W)	10	13097 (118)	2580 (127)	3 (1 – 6)	47 – 160%

PK data of dose regimes selected for the Phase IB dose expansion

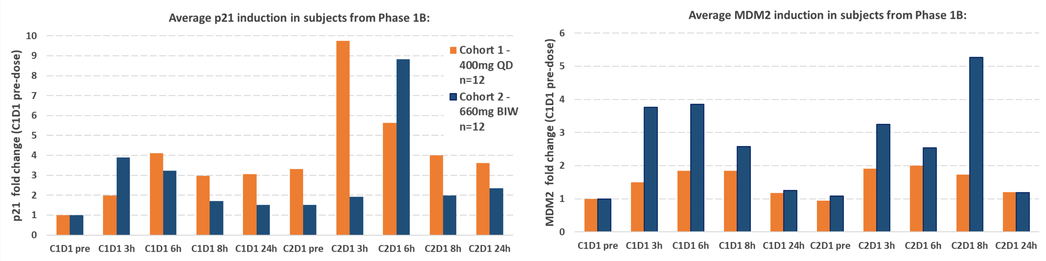

Figure 4: Mean p21 and MDM2 Induction in subjects from Phase IB

p21 and MDM2 gene expression analysis in PBMCs using quantitative and high-sensitivity probe-based Droplet Digital PCR (ddPCR) Technology (Bio-Rad Laboratories, Inc.)[5].

Conclusions

- ASTX295 showed a shorter duration of p53 pathway modulation *in vivo* whilst maintaining efficacy and avoiding myelosuppression in preclinical studies consistent with clinical observations.
- Pulsatile exposure of ASTX295 may be a strategy to achieve an improved therapeutic index for the MDM2 antagonist mechanism.
- The 660 mg dose given twice weekly achieved target levels associated with biological activity with minimal haematological effects and was proposed as the optimal RP2D.

References

1. Italiano A: Cancer Discov 2023;13:1765-7.
2. Ray-Coquard I, et al: Lancet Oncology 2012; 13:1133-40
3. Iancu-Rubin C et al., Exp Hematol, 42(2), 137-45, 2014
4. Willmore E, et al.: Poster #3333, AACR Annual Meeting 2024
5. Dumbrava E, et al.: Poster #CT066, AACR Annual Meeting 2024
6. Ahn M: Oral presentation at ENA Annual Meeting 2024

 Poster presented at 36th EORTC-NCI-AACR, Barcelona, 23-25 Oct 2024. (Poster available to download from Astex's website at www.astx.com)