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Identification of Biomarkers Predictive of Response to ASTX295, a Next-Generation MDM2 Antagonist, in Solid Tumors Carrying Wild-type p53

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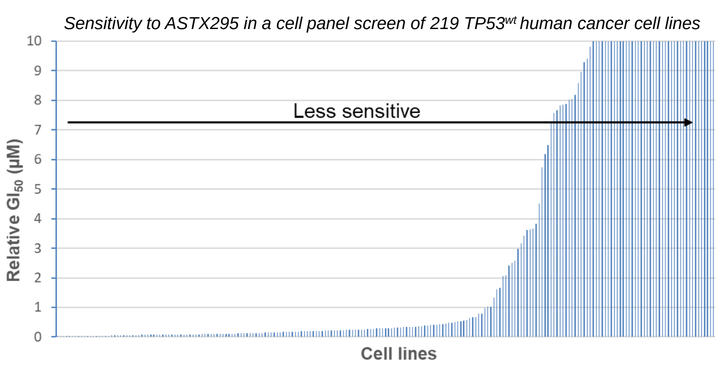
Introduction

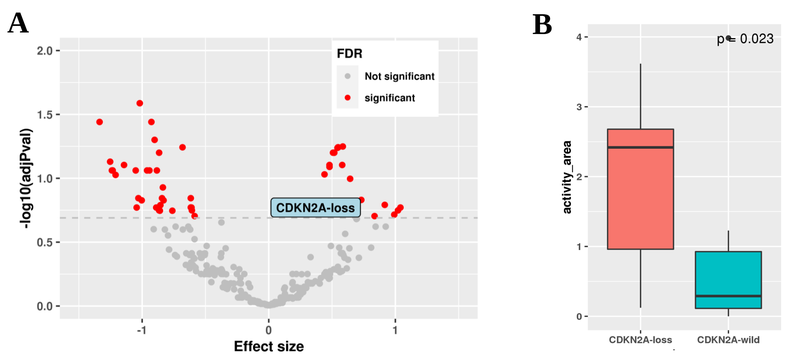
- Inhibition of the MDM2-p53 interaction in tumors carrying wild-type p53 prevents p53 degradation and reactivates it to elicit an anti-cancer effect [1, 2]. Targeting the p53-MDM2 interaction therefore remains a promising strategy for cancer therapy.
- ASTX295 is a next-generation MDM2 antagonist designed to have a shorter plasma half-life and avoid the on-target bone marrow toxicities [3].
- To further increase the therapeutic index, biomarkers predictive of response to ASTX295 in p53 wild-type solid tumors were identified.

Fig 1. *TP53*^{WT} status alone may be insufficient to predict single agent sensitivity to MDM2 antagonist



- ASTX295 showed anti-proliferative activity in a panel of 219 p53 wild-type cell lines, with 143 of them showing GI_{50} values less than 1 μM and 50 showing values less than 0.1 μM.
- A wide range of sensitivity to ASTX295 among cancer cell lines suggested that additional biomarkers may be needed to improve patient selection strategy.

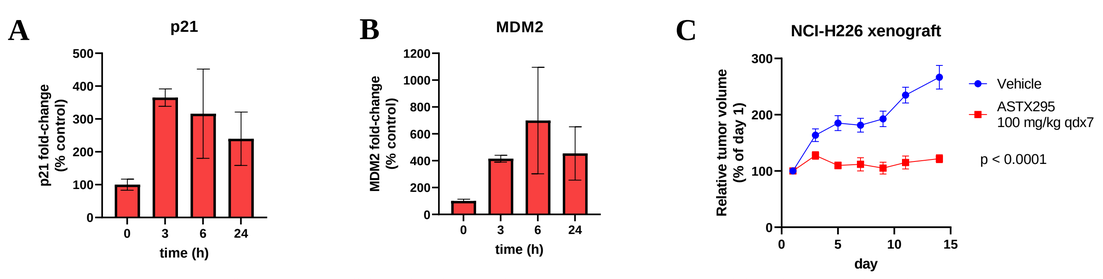
Fig 2. ASTX295 sensitivity is significantly associated with *CDKN2A*-loss in the cell panel screen



A) Volcano plot showing association of molecular features to ASTX295 sensitivity in TP53 wild-type cell lines
B) CDKN2A-loss identified as a statistically significant biomarker of sensitivity to ASTX295

- Bioinformatics analyses of the cell line panel screen data identified *CDKN2A* loss as a marker predictive of sensitivity to ASTX295.
- The mesothelioma was selected as potential indication for follow-up experimental validation due to high frequency of *CDKN2A*-loss (based on TCGA).

Fig 3. ASTX295 modulates p53 pathway in *CDKN2A*-loss *TP53*^{WT} mesothelioma cells and confers anti-tumor activity *in vivo*



ASTX295 was dosed at 100 mg/kg qd PO to Balb/c nude mice bearing NCI-H226 xenograft. p21 (A) and MDM2 (B) levels were measured using MSD assay (n=3 mice per group) and anti-tumor activity was assessed in mice (n=8) treated with ASTX295 for 7 consecutive days (qdx7) (C). Compound was well tolerated.

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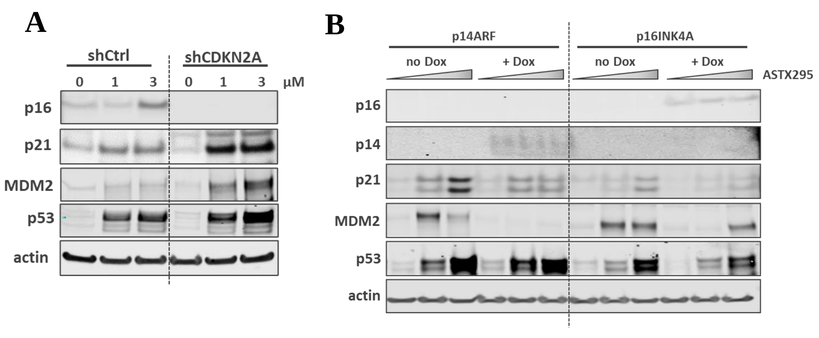
- ASTX295 induced expression of p53 target genes (p21 and MDM2) (A-B) and conferred significant anti-tumour activity *in vivo* in the NCI-H226 (mesothelioma) p53 wild-type *CDKN2A*-loss xenograft model (C).

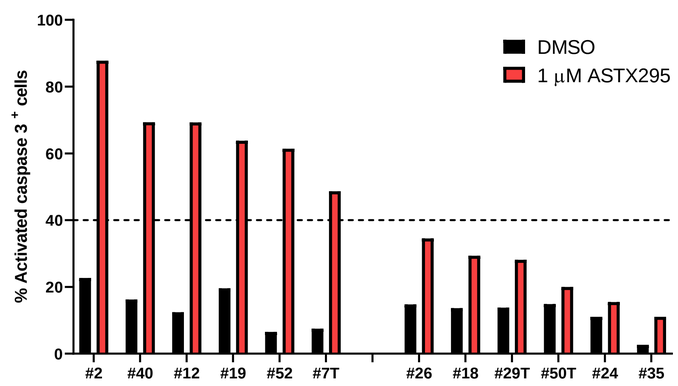
Fig 4. The sensitivity to ASTX295 in p53^{WT} cell lines is modulated by genetic manipulation of *CDKN2A*



Western Blot analysis of p53 pathway after ASTX295 treatment for 24h in vitro. A) MCF7 (breast cancer) TP53^{WT}, CDKN2A^{WT} cells expressing shRNA hairpins directed against CDKN2A or non-targeting control. B) Mero95-p14ARF and Mero95-p16INK4A (mesothelioma) TP53^{WT}, CDKN2A-loss cells treated with 0.2 μM doxycycline to induce p14 and p16 expression, respectively, followed by treatment with 0.1 μM or 1 μM ASTX295.

- Knock-down of CDKN2A in *CDKN2A* wild-type cells enhanced the activation of p53 pathway (A), while re-expression of p14 or p16 encoded by *CDKN2A* reduced it (B).

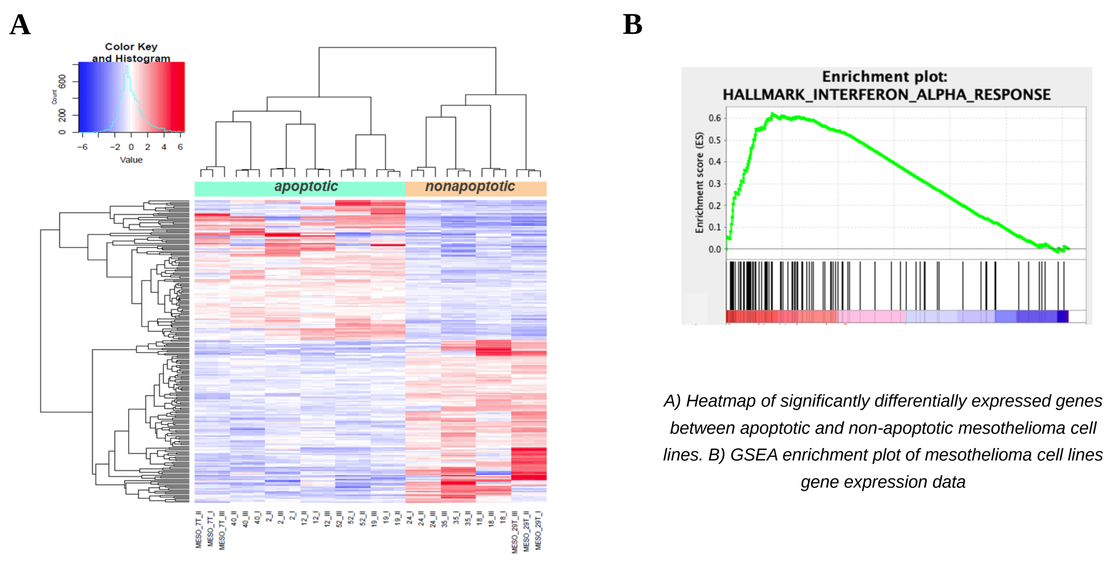
Fig 5. Apoptotic response to ASTX295 in patient-derived mesothelioma cells



Primary mesothelioma cells (from Mesobank UK) treated with 1 μM ASTX295 for 72h. Flow cytometry analysis of percentage of positive cells with activated caspase 3

- ASTX295 induced apoptotic effects were assessed in an independent panel of 12 patient-derived mesothelioma primary cell lines [4].
- 6 out of 12 cell lines showed >40% apoptosis following treatment with ASTX295.

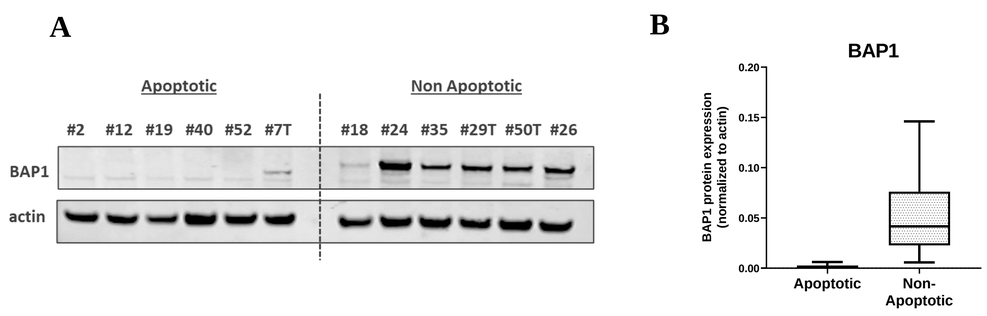
Fig 6. Apoptotic mesothelioma cells are enriched for interferon signalling



A) Heatmap of significantly differentially expressed genes between apoptotic and non-apoptotic mesothelioma cell lines. B) GSEA enrichment plot of mesothelioma cell lines gene expression data

- The "Interferon Signalling" pathway was identified as significantly up-regulated in ASTX295-induced apoptotic patient-derived mesothelioma cell lines

Fig 7. Loss of BAP1 correlates with increased ASTX295-induced apoptosis



A-B) Western Blot analysis of basal BAP1 protein expression in primary mesothelioma cell lines (A) and the densitometry quantification of BAP1 levels in apoptotic and non-apoptotic cell lines (B).

- BAP1 loss is one of the features linked to enrichment of core interferon pathway genes [5]. Western Blotting analysis revealed that all 6 patient-derived apoptotic mesothelioma cell lines had showed loss of detectable BAP1 protein expression.

Conclusions

- Cell panel screen identified loss of *CDKN2A* as a marker of sensitivity to ASTX295 in p53 wild-type cancer cell lines. Sensitivity to ASTX295 in *CDKN2A*-loss cells was validated in patient-derived mesothelioma cell lines.
- Assessment of apoptosis and differential gene expression between apoptotic and non-apoptotic primary mesothelioma cell lines provided an additional way to further refine the potential patient population and increase sensitivity to ASTX295. The studies identified enrichment of interferon signalling and loss of BAP1 expression in ASTX295-induced apoptotic cell lines.
- These data highlight the potential to apply a biomarker-based approach to further refine patient selection strategies. Together with its bone marrow sparing characteristics, improved patient stratification could increase the therapeutic index for ASTX295 (NCT03975387) [6].

References:
[1]Chene (2003). Nature Reviews Cancer; [2]Zhu *et al.* (2022) J. Hematol. Oncol ; [3]Ahn, Oral presentation at ENA Annual Meeting 2024; [4]Rintoul *et al.* (2016). Thorax; [5]Hmeljak, *et al.* (2018) Cancer Discov., [6]Dumbrava, *et al.*: Poster #CT066, AACR Annual Meeting 2024

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