#667

Identification of biomarkers of response to MDM2 inhibition in solid tumours using computational, multi-omics approaches

Harpreet Saini, Maria Ahn, George Ward, Justyna Kucia-Tran, Christina Gewinner, Nicola Ferrari, Jessica Brothwood, Luke Bevan, **Matthew Davis**, Lynsey Fazal, Martin Sims, Marc O'Reilly, Gianni Chessari, Roberta Ferraldeschi, John Lyons, Nicola Wallis, Neil Thompson

[1]Astex Pharmaceuticals, 436 Cambridge Science Park, Cambridge, CB4 0QA, UK

INTRODUCTION

- *TP53* is a tumour suppressor gene that negatively controls many key hallmarks of cancer[1,2]. The *TP53* pathway is frequently inactivated via mutation or an p53-MDM2 interaction.[1,2,3]
- Inhibition of the p53-MDM2 interaction leads to activation of *TP53* in *TP53* wild-type tumours.[3,4]
- *MDM2* antagonists have shown modest anti-tumour activity in the clinic and have dose limiting haematological toxicities.[5,6]
- ASTX295 is an oral, potent inhibitor of the p53-MDM2 protein-protein interaction with bone marrow sparing characteristics[7], which modulates the *TP53* pathway and induces apoptosis in in-vitro and in-vivo TP53 wild-type models.[8]
- ASTX295 is currently being evaluated in a Phase 1/2 study in patients with advanced solid tumours (NCT03975387).[9]
- *TP53* wild-type status may be insufficient to predict sensitivity to ASTX295. Multi-omics based computational approaches were used to predict potential biomarkers of response to ASTX295 in *TP53* wild-type tumours.

Figure 1: Targeting the p53-MDM2 interaction

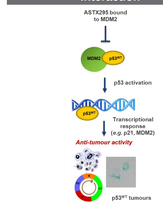
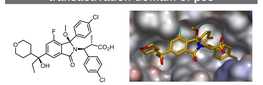


Figure 2: Structure of ASTX295, an isoindolinone-based MDM2 inhibitor. ASTX295 occupies three subpockets on MDM2 involved in the recognition of the residues Phe19, Trp23, and Leu26 of the transactivation domain of p53

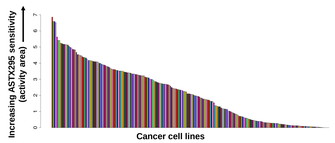


METHODS

Cell panel drug screening

- ASTX295 sensitivity was quantified by cell viability for 219 *TP53*-wild type cancer cell lines from 28 different tumour types.
- Genomic features of cell lines such as mutations, copy number and hypermethylation were obtained from Iorio *et al.*[10]
- ANOVA was used to identify genomic features significantly associated to ASTX295 response in cancer cell lines.

Figure 3: Activity area of ASTX295 across 219 *TP53* type-wild cancer cell lines (colours denote different tumour types)



Apoptotic effects in mesothelioma primary cell lines

- ASTX295 induced anti-proliferative and apoptotic effects were assessed in an independent panel of 12 patient-derived mesothelioma primary cell lines.
- Apoptosis induced by ASTX295 was measured as percentages of cells with activated caspase-3 at 1µM concentration following 72-hr treatment (apoptotic cut-off = >40% caspase-3 activation).
- Differential gene expression and pathway enrichments between apoptotic and non-apoptotic cell lines performed using DESeq2[12] and Gene Set Enrichment Analysis (GSEA)[13].

Table 1: Anti-proliferation and apoptotic effects of ASTX295 in human patient-derived mesothelioma cell lines. All cell lines were obtained from Mesobank UK[11]

Cell line	Subtype	IC50 (µM)	Apoptotic classification
#40	Epitheloid	0.0092	apoptotic
#35	Biphasic	0.045	non-apoptotic
#2	Biphasic	0.062	apoptotic
MESO_50T	Biphasic	0.067	non-apoptotic
#52	Epitheloid	0.076	apoptotic
#12	Biphasic	0.078	apoptotic
#24	Sarcomatoid	0.094	non-apoptotic
#18	Biphasic	0.11	non-apoptotic
#19	Biphasic	0.17	apoptotic
#26	Biphasic	0.36	non-apoptotic
MESO_7T	Biphasic	0.36	apoptotic
MESO_29T	Biphasic	>10	non-apoptotic

TCGA mesothelioma patient dataset

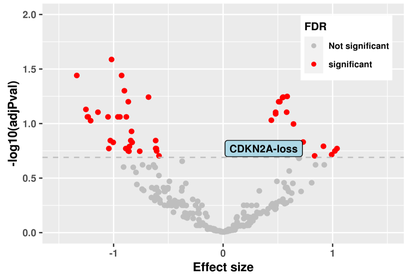
- TCGA mesothelioma patient gene expression data was obtained from Xena[14].
- Weighted gene co-expression network analysis was performed using the WGCNA[15] to identify expression modules and hub genes from the mesothelioma expression data (81 samples & 27,501 protein coding genes).
- Co-expression modules were correlated to mesothelioma clinical characteristics and genetics features.
- R package anRichment[16] was used for functional enrichment of significantly correlated expression modules.

RESULTS

1. Cell panel analysis

- ASTX295 sensitivity is significantly associated with *CDKN2A*-loss in the cell panel drug screening data.
- The mesothelioma indication was selected as potential indication for follow-up experimental validation due to high frequency of *CDKN2A*-loss (based on TCGA).

Figure 4: Volcano plot showing association of molecular features to ASTX295 sensitivity in TP53 wild-type cell lines



2. Differential gene expression of mesothelioma primary cell lines

- 105 and 123 genes were predicted up-regulated and down-regulated respectively in apoptotic compared to non-apoptotic cell lines (more than 2-fold expression & adjusted p-value < 1e-7).
- The "Interferon Signalling" pathway was predicted as significantly up-regulated in apoptotic cell lines (Normalised enrichment score = 1.87 and FDR q-value < 0.002).
- Using QIAGEN's Ingenuity pathway analysis[17], upstream regulator analysis and network analysis, we identified 15 activated transcription factors (activation score >2 & p-values <0.05) including many Interferon Response Proteins (IRFs) and Interferon Inducible Genes (IFIs).

Figure 5a: Heatmap of significantly differentially expressed genes between apoptotic and non-apoptotic mesothelioma cell lines

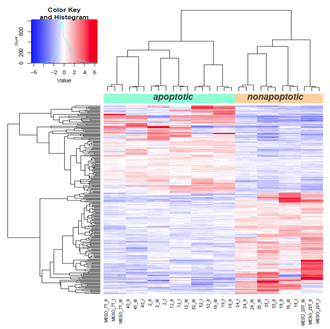
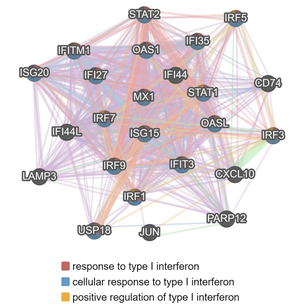


Figure 5b: GSEA enrichment plot of mesothelioma cell lines gene expression data

Figure 5c: Genemania[18] network analysis of significantly up-regulated genes in apoptotic cell lines



3. Confirmation in mesothelioma patient dataset

- Identified 25 co-expression modules (M1-M25) and the module M10 was identified as positively correlated (p-value = 4e-04) to *TP53*-wild and *CDKN2A*-loss group of patients.
- Genes in module M10 were significantly enriched in Interferon signalling pathway. Hub genes in module M10 identified based on high intramodular connectivity were comprised of IRF genes.

Figure 6a: The cluster dendrogram of 25 co-expression modules each uniquely defined by a distinct colour. Each colour represents a module

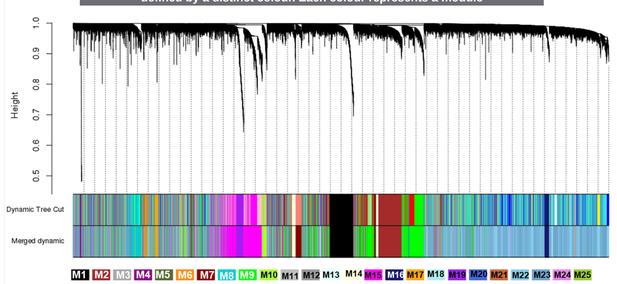


Figure 6b: Schematic representation of correlation of expression modules to clinical and genetic features of mesothelioma

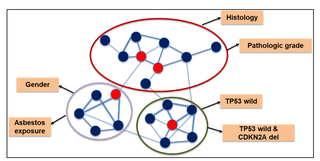


Figure 6c: Heatmap showing correlation of expression modules (vertical axis) to different genetic features (horizontal axis). Module M10 positively correlated to *TP53*-wild and *CDKN2A*-loss

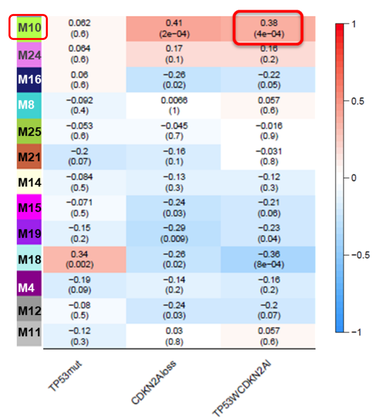
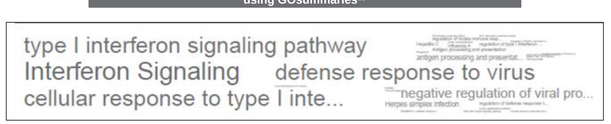


Figure 6d: Word cloud summaries of GO enrichment analysis of module M10 using GOsummaries[19]



CONCLUSIONS

- In-vitro cell line screening combined with molecular features of cell lines identified *CDKN2A* loss as a marker of sensitivity to ASTX295 in *TP53* wild type cell lines.
- Sensitivity due to *CDKN2A*-loss was observed in an independent dataset of patient-derived human mesothelioma cell lines.
- Assessment of apoptosis and differential gene expression between apoptotic and non-apoptotic mesothelioma cell lines provided an additional way to further refine the potential patient population.
- Using transcriptomics and integrated computational approaches, including WGCNA and upstream regulator analyses, identified the Interferon Signalling pathway and IRFs as potential regulators associated with response to ASTX295, both in mesothelioma cell lines and patient data.
- Overall, the approach helped to identify novel biomarkers associated with ASTX295 sensitivity, and could provide new insights into the underlying mechanism of ASTX295 response.

REFERENCES

1) Mantovani et al. (2019) Mutant p53 as a guardian of the cancer cell. Cell Death and Differentiation 26, 199-212.
2) Hassin and Oren (2023) Drugging p53 in cancer: one protein, many targets. Nature Review Drug Discovery 22(2), 127-144.
3) Patrick Chene (2003) Inhibiting the p53–MDM2 interaction: an important target for cancer therapy. Nature Reviews Cancer 3, 102-109.
4) Zhu et al (2022) Targeting p53–MDM2 interaction by small-molecule inhibitors: learning from MDM2 inhibitors in clinical trials. Journal of Hematology and Oncology 15, Article number 91.
5) Ray-Coquard et al (2012) Potent and orally active small-molecule inhibitors of the MDM2-p53 interaction. Lancet Oncology 13, 1133-1140.
6) Pi et al (2019) Evaluating dose-limiting toxicities of MDM2 inhibitors in patients with solid organ and hematologic malignancies: A systematic review of the literature. Leukemia Research 86, 106222.
7) AACR Annual Meeting 2024 – Poster 21, abstract 3333.
8) AACR Annual Meeting 2024 – Poster 12, abstract 666.
9) AACR Annual Meeting 2024 – CT066/16.
10) Iorio et al (2016) A landscape of pharmacogenomic interactions in cancer. Cell 3, 740-754.
11) Rintoul et al (2016) Mesobank UK: an international mesothelioma bioresource. Thorax 71, 380-382.
12) Love et al (2014) Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. Genome Biology 15, Article number: 550.
13) Subramanian et al (2005) Gene set enrichment analysis: A knowledge-based approach for interpreting genome-wide expression profiles. PNAS 102 (43), 15545-15550.
14) https://xenabrowser.net/datapages/?cohort=GDC%20TCGA%20Mesothelioma%20(MESO)&removeHub=https%3A%2F%2Fxena.treehouse.gi.ucsc.edu%3A443.
15) Langfelder and Horvath (2008) WGCNA: an R package for weighted correlation network analysis. BMC Bioinformatics 9, Article number: 559.
16) https://labs.genetics.ucla.edu/horvath/htdocs/CoexpressionNetwork/GeneAnnotation/.
17) https://qiagen.my.salesforce-sites.com/KnowledgeBase/articles/Knowledge/Upstream-Regulator-Analysis
18) Warde-Farley (2010) The GeneMANIA prediction server: biological network integration for gene prioritization and predicting gene function. Nucleic Acids Research 38, 214-220.
19) Kolde and Vilo (2015) GOsummaries: an R Package for Visual Functional Annotation of Experimental Data. F1000 Research 4, 574.

Poster presented at AACR Annual Meeting April 5-10, 2024 (Poster available to download from Astex's website at www.astx.com)