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Low Skp2 expression is predictive of sensitivity to an MDM2 antagonist in p53 wild-type AML

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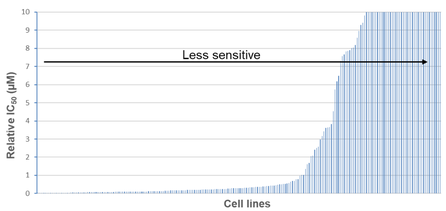
INTRODUCTION

- In the presence of various stress signals, p53 acts as a tumour suppressor by regulating expression of a multitude of genes leading to different cellular responses such as apoptosis and cell cycle arrest. As the p53 pathway is often dysregulated through increased MDM2 activity, inhibition of MDM2-p53 interactions is a promising strategy to target tumours carrying wild-type p53
- Astex in collaboration with the University of Newcastle has discovered a Murine double minute 2 (MDM2) antagonist that potently activates the p53 pathway[1]
- Here, we present the discovery of a novel patient selection strategy for MDM2 antagonist-induced apoptosis in p53 wild-type AML

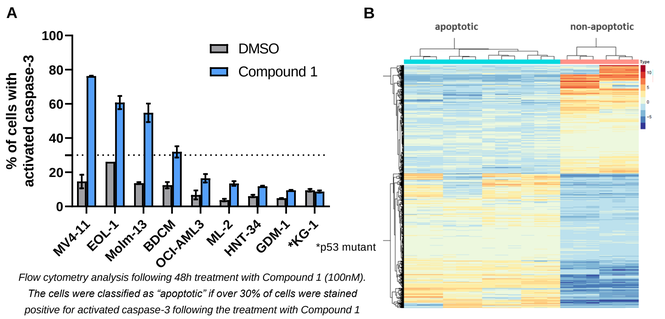
Fig 1. p53^{WT} status alone is not sufficient to predict single agent sensitivity to MDM2 antagonist



Sensitivity to Compound 1 in a cell panel screen of 219 p53^{wt} human cancer cell lines

- An MDM2 antagonist (Compound 1) was tested in a panel of 219 p53 wild-type cancer cell lines derived from a range of tumour tissues
- A wide range of sensitivity to Compound 1 was observed among p53 wild-type cancer cell lines suggesting that additional biomarkers are important to improve patient selection strategy

Fig 2. Further refinement of patient selection strategy in p53^{WT} AML based on apoptotic potential of cancer cell lines

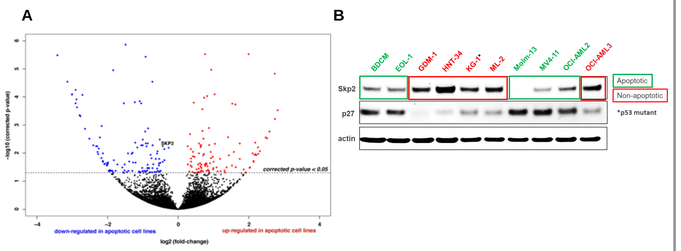


Flow cytometry analysis following 48h treatment with Compound 1 (100nM). The cells were classified as "apoptotic" if over 30% of cells were stained positive for activated caspase-3 following the treatment with Compound 1

(A) Compound 1 induced a range of apoptotic responses in several p53 wild-type AML cell lines. No effect was seen on p53 mutant KG-1 cells

(B) Differential gene expression analysis of apoptotic vs. non-apoptotic AML cell lines showed clear difference in gene expression profiles

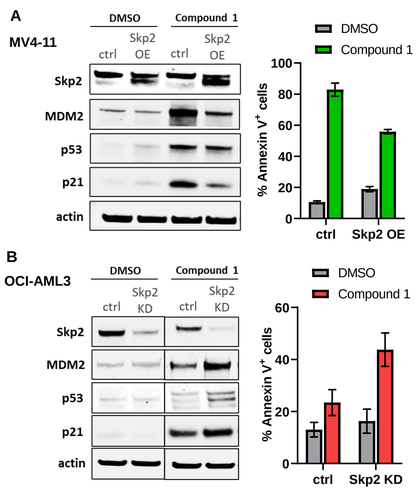
Fig 3. Basal Skp2 expression is lower in apoptotic p53^{WT} AML cell lines



(A) Bioinformatics analysis of differentially expressed genes between apoptotic vs. non-apoptotic samples identified Skp2 as significantly down-regulated in apoptotic AML cell lines. Skp2 has been reported to limit p53-induced apoptosis through inhibiting p53 acetylation[2]

(B) Western Blot analysis demonstrated that all 5 apoptotic cell lines have low levels of Skp2 basal protein expression when compared to 4 non-apoptotic p53^{WT} AML cell lines. An inverse correlation was also observed between Skp2 and p27 expression levels in these cell lines which is in line with p27 being a target of Skp2-mediated degradation[2]

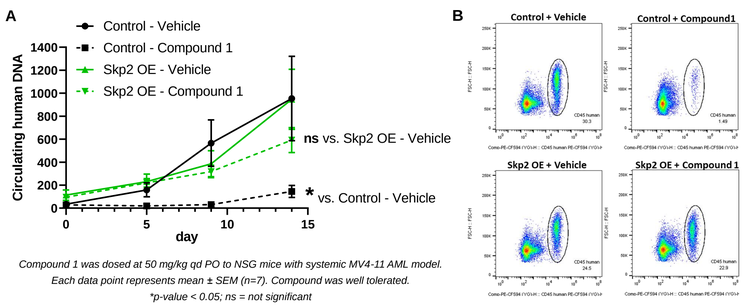
Fig 4. The sensitivity to MDM2 antagonist in p53^{WT} AML cell lines is modulated by genetic manipulation of Skp2



(A) Skp2 over-expression (OE) in 'apoptotic' MV4-11 cells resulted in decreased p53 pathway activation (decreased induction of MDM2 and p21) and reduced activation of apoptosis after treatment with Compound 1

(B) Skp2 knock-down (KD) in 'non-apoptotic' OCI-AML3 cells resulted in stronger p53 pathway activation (increased induction of MDM2 and p21) and increased activation of apoptosis after treatment with Compound 1

Fig 5. Skp2 expression modulates sensitivity to MDM2 antagonist *in vivo*



*Compound 1 was dosed at 50 mg/kg qd PO to NSG mice with systemic MV4-11 AML model. Each data point represents mean ± SEM (n=7). Compound was well tolerated. *p-value < 0.05; ns = not significant*

(A) Compound 1 conferred significant anti-tumour activity *in vivo* in the systemic MV4-11 AML model as determined by PCR analysis of circulating human DNA. Over-expression of Skp2 in this model led to resistance to treatment with Compound 1

(B) The effects of Skp2 over-expression on tumour burden after Compound 1 treatment were confirmed by flow cytometry analysis of human CD45^{+} MV4-11 cells in the mouse bone marrow

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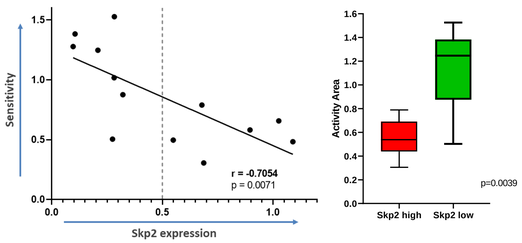
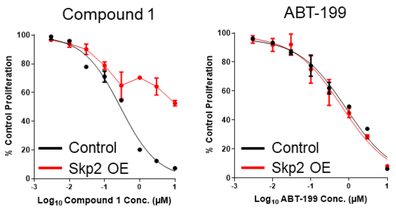
Fig 6. Skp2 expression correlates with sensitivity to MDM2 antagonist in primary AML blasts isolated from patients



- Primary AML samples with high blast content (>80%) were cultured *in vitro.* The effects of Compound 1 on apoptosis after 24h treatment were determined by flow cytometry. The Skp2 basal protein expression was assessed by capillary western analysis
- Analysis of 13 independent samples showed that Skp2^{low} primary AML blasts are significantly more sensitive to the treatment with Compound 1 than Skp2^{high} blasts

Fig 7. Skp2 is a specific biomarker for MDM2 antagonist



- Over-expression of Skp2 in MV4-11 cells induced resistance to Compound 1 but did not modulate the sensitivity to another key regulator of apoptosis as tested with Bcl-2 inhibitor (ABT-199)

DISCUSSION

- This work demonstrates *in vitro* and *in vivo* activity of MDM2 antagonist in AML models with low basal Skp2 levels. These pre-clinical data validate low levels of Skp2 as a novel patient selection approach in AML and support the clinical investigation of MDM2 antagonist as therapeutic strategy for the treatment of SKP2^{low} p53^{wt} AML

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References: [1] Chessari, Hardcastle *et al.* (2021) J. Med. Chem
[2] Reed (2008) Cancer Cell