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Characterization of a novel, potent small molecule MDM2 antagonist which activates wild-type p53 and induces apoptosis in AML

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INTRODUCTION

In the presence of various stress signals, p53 acts as a tumor suppressor by regulating the expression of a multitude of genes to elicit cellular responses such as cell cycle arrest and apoptosis. The activity of p53 is tightly regulated by MDM2, an E3 ubiquitin ligase that acts as a primary inhibitor of p53 function by, for example, targeting p53 for proteasomal degradation. Early studies have demonstrated that blocking the MDM2-p53 interaction in tumors carrying wild-type p53 prevents p53 degradation and reactivates its transcriptional activity. Small molecule MDM2 antagonists that inhibit the MDM2-p53 interaction, therefore, present a promising strategy for cancer therapy and a number of these compounds are in clinical development.

Herein, we describe the characterization of a novel, potent small molecule MDM2 antagonist in AML *in vitro* and *in vivo* pre-clinical models and in patient-derived AML blast cells.

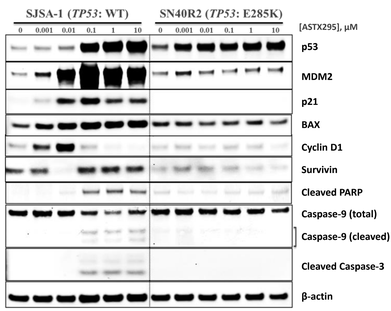
A novel small molecule MDM2 antagonist which activates wild-type p53

A

Cell line	*TP53* Status	Mean IC_{50} (nM)
SJSA-1	Wild-type	23
SN40R2	Mutant	20,000
A2780	Wild-type	47
A2780CP	Mutant	8,700
HCT116	Wild-type	250
HCT116 $p53^{-/-}$	Deleted	15,000

- The effect of ASTX295 on in vitro cell proliferation was investigated in a range of cell lines with wild-type or mutant *TP53*. ASTX295 potently inhibited cell proliferation in the *TP53* wild-type cell line but not the *TP53* mutant. (A)

B



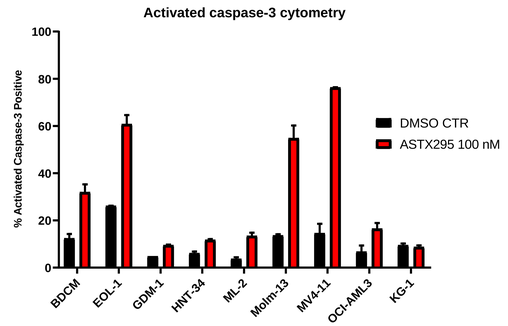
- ASTX295 treatment in SJSA-1 (*TP53* wild-type cell line) was able to induce the expression of well-known p53 transcriptional targets such as MDM2 and p21, together with induction of apoptotic markers (such as BAX, cleaved PARP, cleaved caspase-3). As expected no induction was seen in the *TP53* mutant cell line SN40R2. (B)

ASTX295 inhibits proliferation and induces apoptosis in AML cell lines

A

Cell Line	TP53 Status	Mean IC_{50} (nM)
BDCM	Wild-type	24
EOL-1	Wild-type	8.8
GDM-1	Wild-type	120
HNT-34	Wild-type	29
ML-2	Wild-type	9.0
MOLM-13	Wild-type	8.8
MOLM-14	Wild-type	3.8
MV4-11	Wild-type	5.3
OCI-AML2	Wild-type	18
OCI-AML3	Wild-type	36
OCI-AML5	Wild-type	9.4
KG-1	Mutant	>10,000

B



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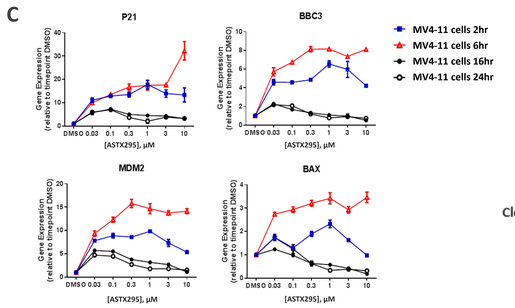

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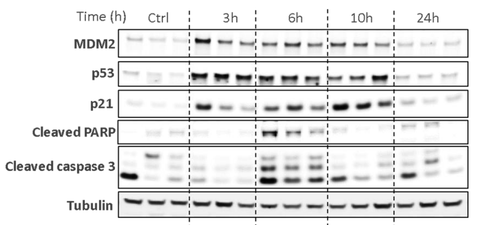
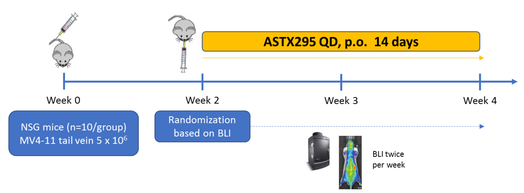


- ASTX295 exerted a strong anti-proliferative effect when tested on a panel of *TP53* wild-type AML cell lines, with GI_{50} values of <30 nM being observed in 9 out of 11 cell lines. In contrast, the compound had little effect on *TP53* mutant KG-1 cells (GI_{50} >10 µM). (A)
- ASTX295 induced a strong pro-apoptotic effect after 48h of treatment on several *TP53* wild-type AML cell lines. No effect was seen on *TP53* mutant KG-1 cells. (B)
- The effect of ASTX295 on activation of the p53 pathway and apoptosis was confirmed *in vitro* by qRT-PCR on p53 transcriptional targets in MV4-11 cells (C) and *in vivo* at the protein level by western blot of MV4-11 tumor xenograft tissue after a single dose of ASTX295. (D)

ASTX295 treatment demonstrates efficacy in an *in vivo* systemic model of AML (1)

A



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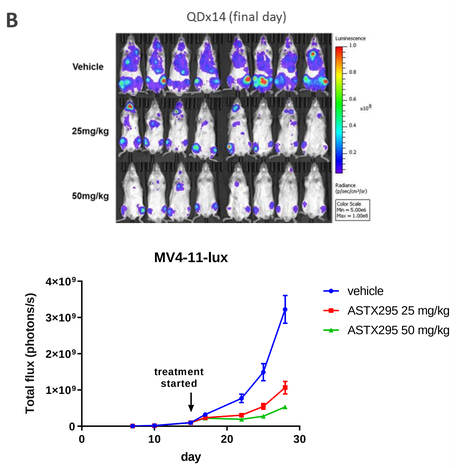
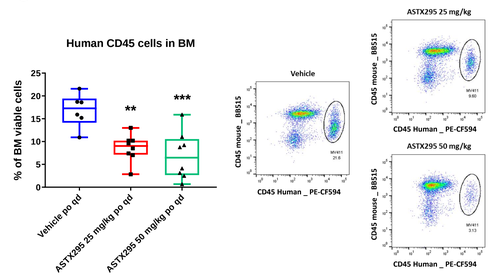


- Schematic representation of the *in vivo* experiment using the MV4-11 systemic AML model engineered to express luciferase (MV4-11-lux) coupled with *in vivo* bioluminescence imaging to monitor tumour growth in real-time. (A)
- ASTX295 displayed significant *in vivo* efficacy. QDx14 oral dosing at well tolerated doses demonstrated a clear reduction in tumour growth. (B)

ASTX295 treatment demonstrates efficacy in an *in vivo* systemic model of AML (2)

C



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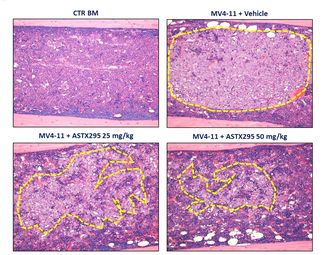
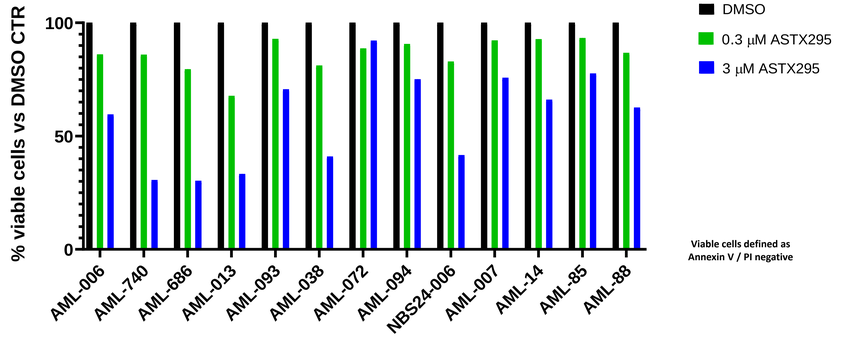


- The reduction of tumour burden after ASTX295 treatment (QDx14) was confirmed by flow cytometry for human CD45+ MV4-11 cells in the bone marrow (C) and by H&E staining of bone marrow sections. (D)

ASTX295 induces apoptosis in primary AML samples

- 13 primary AML samples with high blast content (>80%) were analysed
- AML blasts were cultured in selected expansion media
- 24h apoptosis assay was set up with two ASTX295 concentrations (flow cytometry using Annexin V / PI staining)



- ASTX295 triggered apoptosis when tested on primary AML blast cells. Interestingly some primary lines showed a strong apoptotic response (AML-740, AML-686), while others were more resistant to the compound (AML-85).

SUMMARY & CONCLUSIONS

- ASTX295 potently inhibits cell proliferation and induces cell death *in vitro* on a panel of *TP53* wild-type AML cell lines.
- ASTX295 shows *in vivo* efficacy when tested in an AML systemic mouse model.
- ASTX295 induces apoptosis in primary AML blast cells isolated from patients.
- Targeting the MDM2-p53 interaction using ASTX295 is a potential therapeutic strategy for AML.

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