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ASTX295, a novel small molecule MDM2 antagonist, demonstrates potent activity in AML in combination with decitabine

Luke Bevan[1] (luke.bevan@astx.com), Nicola Ferrari[1], Juan Castro[1], Gianni Chessari[1], Lynsey Fazal[1], Ian Hardcastle[2], Steven Howard[1], Justyna Kucia-Tran[1], Herbie Newell[2], Martin Noble[2], Huw Thomas[2], George Ward[1], Steve Wedge[2], Elaine Willmore[2], Yan Zhao[2], Maria Ahn[1]

[1]Astex Pharmaceuticals, 436 Cambridge Science ParkMilton Road, Cambridge, CB4 0QA, UK,
[2]Cancer Research UK Newcastle Drug Discovery Unit, Newcastle University, Newcastle upon Tyne, United Kingdom

Introduction

The tumour suppressor p53 is activated in response to various stress signals to induce transcriptional changes leading to cellular responses such as cell cycle arrest and apoptosis. Activity of p53 is tightly regulated by the E3 ubiquitin ligase MDM2, which inhibits p53 function by, for example, targeting it for proteasomal degradation. Targeting the MDM2-p53 interaction to restore p53 function, is therefore, a promising strategy for cancer therapy and a number of these compounds are in clinical development including ASTX295 (NCT03975387). ASTX295 is a novel, orally bioavailable MDM2 antagonist developed through structure-based drug design that has demonstrated potent activity in a range of p53 wild-type pre-clinical models.

We investigated the therapeutic potential of ASTX295 alone and in combination with decitabine, a DNA-hypomethylating agent, in acute myeloid leukaemia (AML).

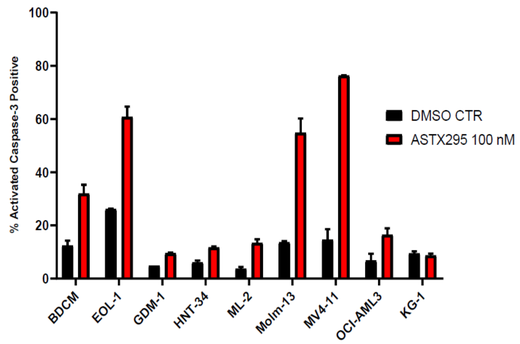
ASTX295 inhibits proliferation and induces apoptosis in AML cell lines

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 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).

Cell line	*TP53* status	Mean GI_{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
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

Treatment with ASTX295 for 48 hours induced a strong pro-apoptotic response in a number of *TP53* wild-type AML cell lines.

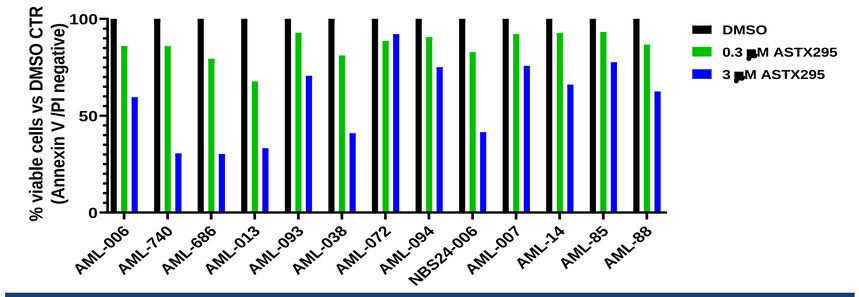
Figure 1: ASTX295 induced apoptosis in AML cell lines



ASTX295 induces apoptosis in primary AML samples

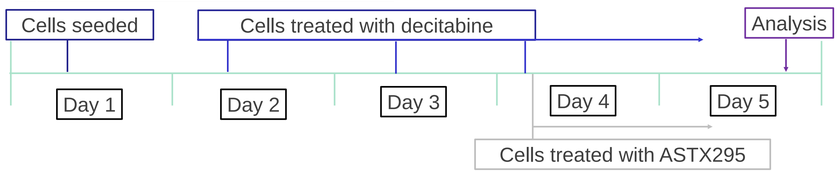
Primary AML blast samples with a high blast content were cultured in selected expansion media. 13 samples were treated with ASTX295 at two concentrations for 24 hours prior to flow cytometry analysis for Annexin V /PI staining.
ASTX295 induced a strong pro-apoptotic response in a number of AML blast samples.

Figure 2: ASTX295 induced apoptosis in primary AML samples



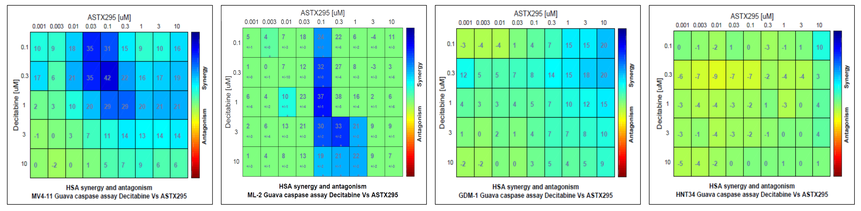
Activity of ASTX295 on AML cell lines was further enhanced by combining with decitabine

Figure 3: Treatment Schedule for cell lines



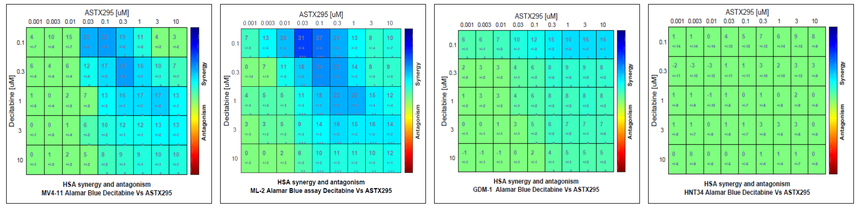
Treatment of AML cell lines with ASTX295 and decitabine in combination showed an increased pro-apoptotic induction compared to respective single agent treatments in 3 of 4 cell lines tested (MV4-11, ML-2, GDM-1). Combenefit analysis was performed using Combenefit software (1); demonstrating that this increase was synergistic.

Figure 4: Combenefit analysis of caspase 3 activation in cell lines



Treatment of MV4-11, ML-2 and GDM-1 cells with ASTX295 and decitabine in combination also showed an increase in growth inhibitory effect via Alamar Blue assay, and was indicated as a synergistic interaction by Combenefit analysis.

Figure 5: Combenefit analysis of viability (by Alamar Blue assay) in cell lines



Combinatory effect of ASTX295 in combination with decitabine on primary AML blasts

Treatment of primary AML blasts with ASTX295 and decitabine in combination showed an increased pro-apoptotic induction compared to respective single agent treatment in 7 out of 11 different donors. Data not shown for those without combinatory effect confirmed through Combenefit analysis (example of combinatory effect in Combenefit grid displayed in Figure 7 H).

Figure 6: Treatment Schedule for AML Blasts

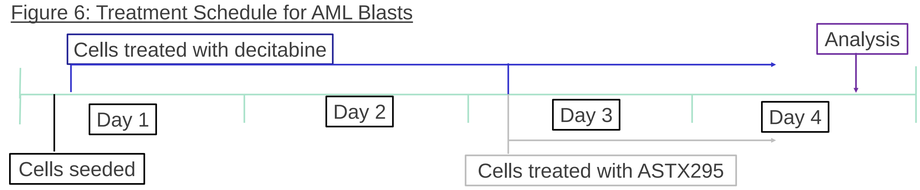
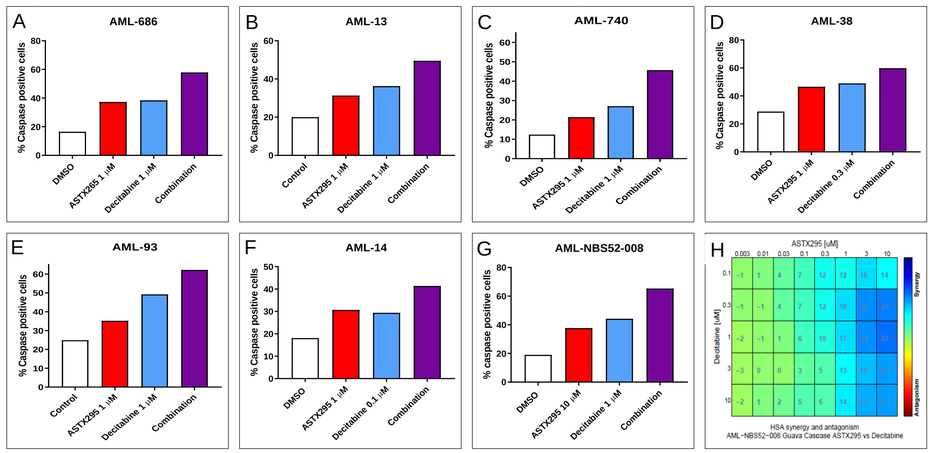


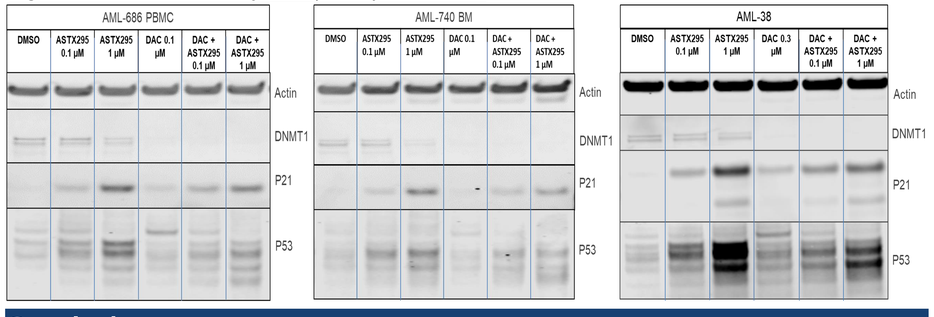
Figure 7: Caspase activation in primary AML blast following single agent and combination treatment



Target engagement confirmed by western blotting

Western blots of samples from 3 different AML blast donors confirm target engagement of both ASTX295 and decitabine by modulation of p53 target proteins and DNMT1 levels, respectively.

Figure 8: Western blot analysis of primary AML blasts



Conclusion

Our findings demonstrate that the combination of ASTX295 with decitabine exhibits potent activity against p53 wild-type AML cells, and thus merits further investigation.

Reference: 1) Di Veroli GY, Fornari C, Wang D, et al. Combenefit: an interactive platform for the analysis and visualization of drug combinations. *Bioinformatics*. 2016;32(18):2866-2868. doi:10.1093/bioinformatics/btw230

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