Abstract No. 380

Induction of apoptosis with a novel dual cIAP1/XIAP antagonist in models of melanoma

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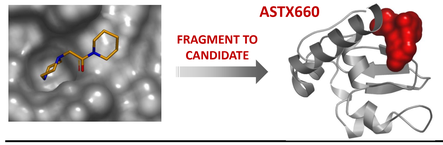
INTRODUCTION

Inhibitor of apoptosis (IAP) antagonists are being tested in the clinic for the treatment of cancer as they can switch cancer cell TNFα signalling from being pro-survival to being pro-apoptotic, and relieve the block on effector caspase activation (1,2). Astex has used fragment based-drug discovery to develop a first-in-class, dual XIAP/cIAP1 antagonist (**ASTX660**), which is non-peptidomimetic, does not contain an alanine as a warhead and demonstrates prolonged antagonism of both XIAP and cIAP1 *in vivo*.

Melanoma is a highly aggressive malignancy with an exceptional ability to develop resistance to targeted therapies. Targeting IAP proteins in melanoma is a promising strategy to overcome this resistance (3), and **ASTX660** represents a novel approach because of the enhanced potency against XIAP (4).

IAP ANTAGONISTS DERIVED FROM FRAGMENT-BASED DRUG DISCOVERY

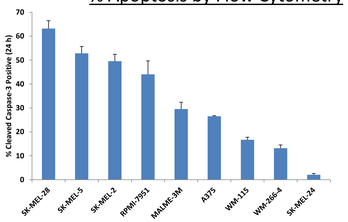


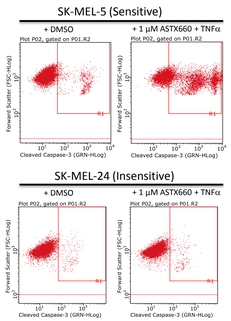
FRAGMENT TO CANDIDATE

- Balanced cIAP1/XIAP profile
- Non peptidomimetic
- Non alanine warhead
- Oral

	Assay (EC_{50} in nM)	ASTX660	CUDC-427	LCL-161	Debio-1143	Birinapant (bivalent)
XIAP cellular activity	HEK293-XIAP-Caspase-9 (I.P.)	**2.8**	**10**	**35**	**34**	**23**
cIAP1 cellular activity	MDA-MB-231 (cIAP1 degradation)	**0.22**	**0.044**	**0.40**	**0.88**	**0.23**
Proliferation	MDA-MB-231	**1.8**	**3.0**	**7.8**	**19**	**0.98**
	HCT-116 (control)	**inactive**	**inactive**	**inactive**	**inactive**	**inactive**

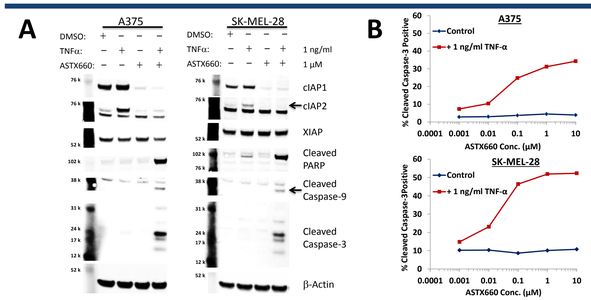
EVALUATION OF APOPTOSIS IN MELANOMA CELL LINES BY FLOW CYTOMETRY





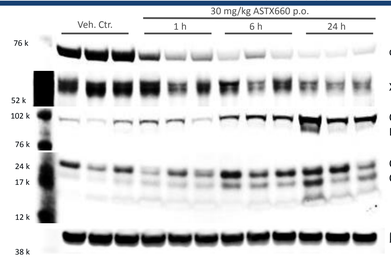
- Levels of apoptosis measured by flow cytometry in melanoma cell lines after addition of a fluorogenic caspase-3 substrate (NucView – Biotium) after 24 h treatment with 1 µM **ASTX660** + 1 ng/ml TNF-α

INDUCTION OF APOPTOSIS MARKERS IN TWO MELANOMA CELL LINES



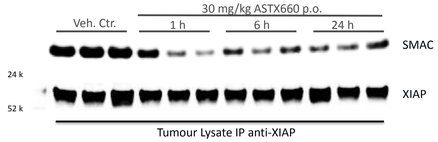
- TNFα-dependent induction of apoptosis markers was measured by Western blotting (A) or cleaved caspase-3 substrate flow cytometry (B) in two melanoma cell lines (A375 & SK-MEL-28)

cIAP1 ANTAGONISM & APOPTOSIS *IN VIVO* (MDA-MB-231 TUMOURS)



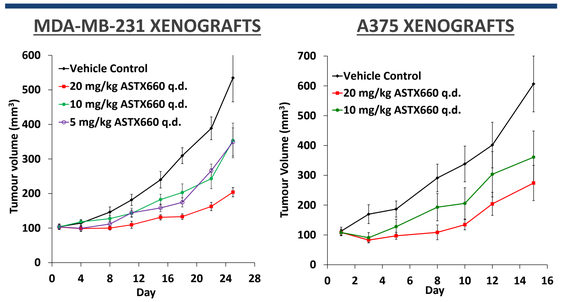
- Rapid cIAP1 degradation and induction of apoptosis markers were measured in MDA-MB-231 xenograft lysates after a single dose of **ASTX660** by Western blotting

XIAP ANTAGONISM *IN VIVO* (MDA-MB-231 TUMOURS) BY I.P. ASSAY



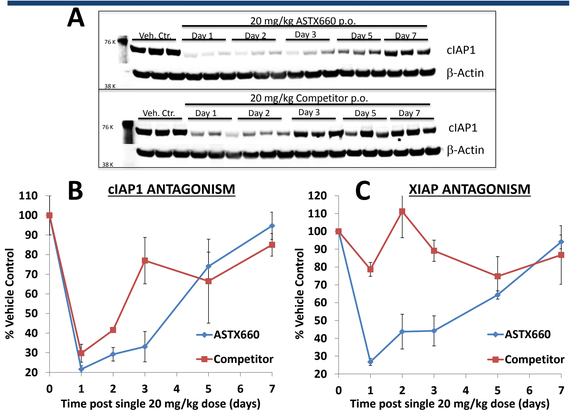
- Rapid XIAP antagonism after **ASTX660** dosing was measured in MDA-MB-231 xenograft lysates by anti-XIAP immunoprecipitation (I.P.) and measurement of SMAC:XIAP complex levels by Western blotting

IN VIVO CELL LINE XENOGRAFT EFFICACY STUDIES



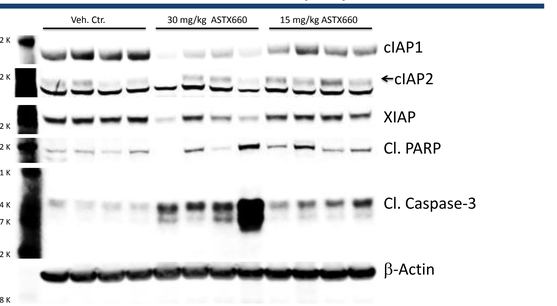
- Significant dose-dependent efficacy was obtained in two cell line xenograft models: MDA-MB-231 (triple negative breast cancer) and A375 (BRAF mutant melanoma)

PROLONGED DUAL ANTAGONISM *IN VIVO* (MDA-MB-231 TUMOURS)



- Western blot analysis of MDA-MB-231 xenograft lysates (A) indicates prolonged cIAP1 depletion after a single dose of 20 mg/kg **ASTX660** p.o., with a comparison shown to a competitor compound dosed at 20 mg/kg p.o.
- The single 20 mg/kg **ASTX660** dose induced a prolonged level of antagonism of both cIAP1 and XIAP for 72 h post dose, whilst the competitor IAP antagonist compound, dosed at the same dose (20 mg/kg p.o.), only antagonised cIAP1 – measured by MSD assays of cIAP1 levels in tumour lysates (B) or XIAP:SMAC complex levels (C)

MELANOMA PATIENT DERIVED XENOGRAFT (PDX) STUDY WESTERN BLOTS



- Levels of cIAP1 were reduced 24 h after final dose with **ASTX660** and there was an increase in apoptosis markers (Cl. PARP and cl. caspase-3) in PDX tumour lysates

CONCLUSIONS

- **ASTX660** represents a novel class of IAP antagonists with a potent dual cIAP1 and XIAP antagonist profile.
- *In vitro* cell line testing suggested that **ASTX660** has significant activity against a panel of melanoma cell lines, which is enhanced on addition of exogenous TNFα.
- After oral dosing with A**STX660** there is prolonged antagonism of both cIAP1 and XIAP *in vivo*, representing a differentiated profile to cIAP1-selective competitor compounds.
- Significant *in vivo* activity has been seen in the A375 melanoma cell line xenograft model and in a melanoma PDX model after dosing with **ASTX660**.
- **ASTX660** is currently undergoing pre-clinical evaluation.

REFERENCES

[1] Targeting IAP proteins for therapeutic intervention in cancer, Fulda S. & Vucic D., Nat. Rev. Drug Disc., 2012, 1;11(2):109-24

(2) IAPs: from caspase inhibitors to modulators of NF-κB, inflammation and cancer, Gyrd--Hansen & Meier P., Nat. Rev. Cancer, 2010, 10(8):561-74

(3) The novel SMAC mimetic Birinapant exhibits potent activity against human melanoma cells, Krepler C. *et al.*, Clin. Can. Res, 2013, 19(7):1784-94

(4) X-linked inhibitor of apoptosis protein – a critical resistance regulator and therapeutic target for personalized cancer therapy, Obexer P. & Ausserlechner M.J., Frontiers in Oncol., 2014, 4, 1-9

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