Characterisation Of The Activity Of Potent XIAP/cIAP1 Dual Antagonists In Melanoma Models

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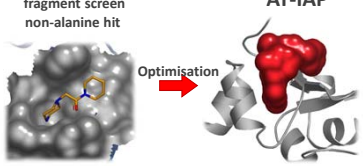
INTRODUCTION

Inhibitor of apoptosis (IAP) antagonists are being tested in the clinic for the treatment of cancer as they can switch cancer cell TNF-α signalling from being pro-survival to being pro-apoptotic, and relieve the block on effector caspase activation. Astex has used fragment based-drug discovery to develop a second generation of IAP antagonists, which are non-peptidomimetic and do not contain an alanine as a warhead.

Melanoma is a highly aggressive malignancy with an exceptional ability to develop resistance to targeted therapies. Here we report data from models of melanoma in which our IAP antagonists have demonstrated potent *in vitro* and *in vivo* activity. Using a predictive biomarker strategy, we have also demonstrated activity in a patient-derived xenograft (PDX) model. Cancer Stem Cell (CSC) fractions within melanoma cell lines are more apoptosis-resistant than the bulk cell population and they have been associated with resistance to cancer therapy, relapse and disease progression. We have demonstrated that our compounds induce enhanced levels of apoptosis in such CD133$^+$ cancer stem cell (CSC) populations, thereby demonstrating the potency of our dual antagonists.

IAP ANTAGONISTS DERIVED FROM FRAGMENT-BASED DRUG DISCOVERY



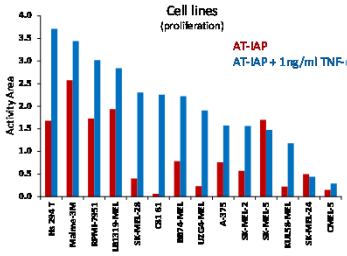
- Balanced cIAP1/XIAP profile
- Non peptidomimetic
- Non alanine warhead
- Potent cellular activity
- Oral activity in *in vivo* models

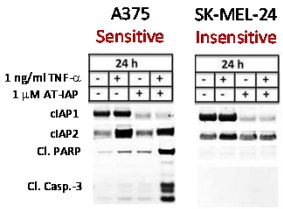
CELLULAR ACTIVITY OF AT-IAP

Assay	Description	EC_{50} (nM)
XIAP Cell Assays	HEK293-XIAP-Caspase-9 (I.P.) *	5.1
	HEK293-XIAP-SMAC (I.P.) *	22.0
ML-IAP Cell Assay	HEK293-ML-IAP-SMAC (I.P.) *	11.0
cIAP1 Cell Assay	MDA-MB-231 (cIAP1 degradation) *	0.32
Cell Proliferation Assays	EVSA-T	0.83
	MDA-MB-231	4.4
	HCT-116 (insensitive control)	>10,000

* IAP immunoprecipitation (I.P.) and cIAP1 degradation assays set up using the Meso Scale Discovery (MSD) platform

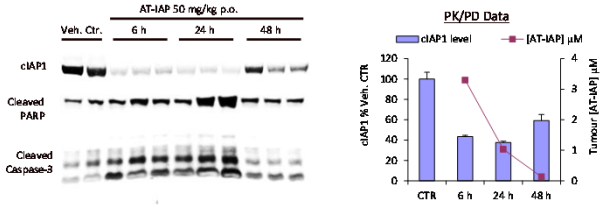
AT-IAP ACTIVITY IN MELANOMA CELL LINES AND *EX VIVO* PDX CELLS





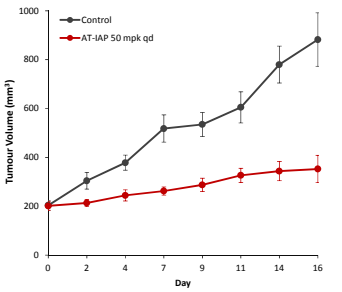
- cIAP1 degradation observed in all cell lines – sensitive or insensitive
- Sensitivity in melanoma cell lines is driven by the ability of AT-IAP to switch TNF-α signalling from pro-survival to pro-apoptotic
- TNF-α can be endogenously produced by the cell line
- TNF-α can also be produced by inflammatory cells present in tumour micro-environment

A375 MELANOMA XENOGRAFT PK/PD DATA



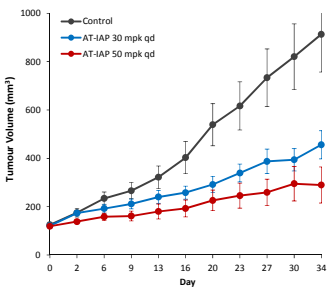
- Reduced levels of cIAP1 were detected in A375 xenograft tumour lysates up to 24 h after dosing, and elevated levels of apoptosis markers (cleaved PARP and cleaved caspase-3) were evident
- Significant tumour concentrations of **AT-IAP** were measured at 6 and 24 h, suggesting daily 50 mg/kg p.o. dosing achieves good coverage of cIAP1 and XIAP targets

A375 MELANOMA XENOGRAFT



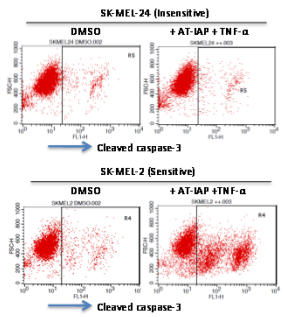
- Significant *in vivo* activity seen in the A375 melanoma cell line xenograft model after oral dosing with **AT-IAP**

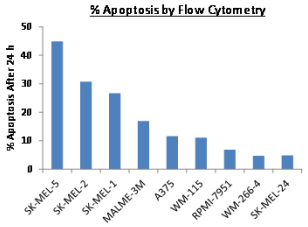
MELANOMA PATIENT-DERIVED XENOGRAFT (PDX)



- Significant dose-dependent efficacy seen in a melanoma PDX model as predicted by Astex's dual biomarker selection strategy

EVALUATION OF APOPTOSIS IN MELANOMA CELL LINES BY FLOW CYTOMETRY



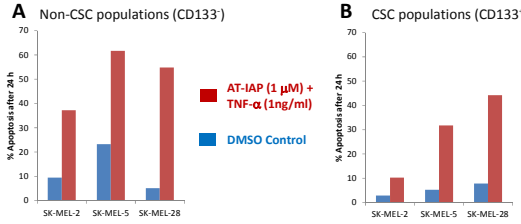


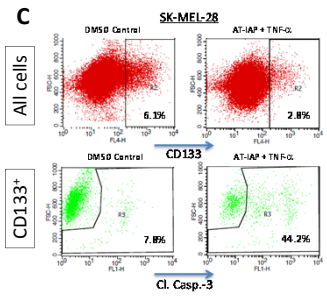
- Levels of apoptosis measured by flow cytometry in melanoma cell lines after addition of a fluorogenic caspase-3 substrate (NucView – Biotium) after 24 h treatment with **AT-IAP** + 1 ng/ml TNF-α

DUAL cIAP1/XIAP ANTAGONIST TARGETS CANCER STEM CELL (CSC) POPULATIONS

Cell line	CD133$^+$ cells
SK-MEL-2	1-2 %
SK-MEL-5	0.5-1 %
SK-MEL-28	2-6 %
A375	none

- Small sub-populations of CD133$^+$ CSCs were found in SK-MEL-5, SK-MEL-2 and SK-MEL-28 (but not A375) cell lines





- **AT-IAP** + TNF-α induces apoptosis after 24 h in non-CSC populations (**A**)
- The more resistant CSC CD133$^+$ populations are induced to undergo apoptosis by **AT-IAP** + TNF-α (**B**)
- **AT-IAP** + TNF-α treatment for 24 h results in a drop in % SK-MEL-28 cells that are CD133$^+$ **(C)**

SUMMARY AND CONCLUSIONS

- **AT-IAP** represents a novel class of IAP antagonist with a potent dual cIAP1 and XIAP antagonist profile
- *In vitro* cell line testing suggested that **AT-IAP** has significant activity against a panel of melanoma cell lines and *ex vivo* PDX cells, which was enhanced on addition of exogenous TNF-α
- Significant *in vivo* activity has been seen in A375 melanoma cell line xenograft model after oral dosing with **AT-IAP,** and also in a melanoma PDX model predicted to be sensitive by Astex's dual biomarker selection strategy
- Our preliminary studies have shown that **AT-IAP** has potent apoptosis-inducing capacity in melanoma CSC-fractions

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