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AT-IAP, a dual cIAP1 and XIAP antagonist with oral antitumor activity in melanoma models

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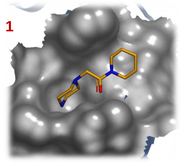
INTRODUCTION

Melanoma is a highly aggressive malignancy with an exceptional ability to develop resistance and no curative therapy is available for patients with metastatic disease who lack a BRAF mutation. Inhibitor of apoptosis proteins (IAP) play a key role in preventing cell death by apoptosis. IAPs are highly regulated by endogenous antagonists (e.g. SMAC) but in melanoma expression levels of IAPs are generally high and depleting IAPs by siRNA tended to reduce cell viability, with XIAP reduction being the most efficient [1]. IAP antagonists have the ability to switch IAP-controlled pro-survival pathways towards apoptosis and cell death. Recent evidence suggests that a true dual antagonist of both cIAP1 and XIAP will promote strong apoptotic response via generation of ripoptosome complexes, with resultant caspase activation [2, 3].

DISCOVERY OF AT-IAP, A DUAL cIAP1/XIAP ANTAGONIST

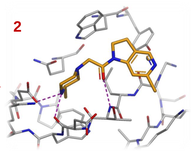
- Astex performed a fragment screen against XIAP-BIR3 domain via X-ray crystallography and 1D-NMR.
- Compound **1** is a non-alanine fragment hit which binds very weakly to both cIAP1-BIR3 and XIAP-BIR3. Hit optimisation using a structure based approach led to compound **2** with submicromolar affinities.
- Further optimisation yielded **AT-IAP**, which is a potent dual antagonist of cIAP1 and XIAP, chemically distinct from SMAC mimetics in the clinic.
- The interactions between the piperazine warhead and the BIR3 domains of XIAP and cIAP1 are distinct and allow the development of antagonists with balanced profiles.

Pyramid™ fragment screening identifies a non-alanine hit



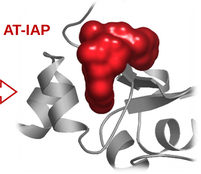
XIAP-BIR3 IC_{50} > 5 mM
cIAP1-BIR3 IC_{50} > 5 mM

Hit optimisation via structure based drug design



XIAP-BIR3 IC_{50} = 640 nM
cIAP1-BIR3 IC_{50} = 320 nM

Potent dual XIAP and cIAP inhibitor



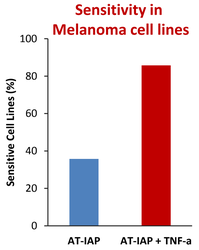
XIAP-BIR3 IC_{50} < 40 nM
cIAP1-BIR3 IC_{50} < 10 nM

	Assay (IC_{50} in nM)	AT-IAP	LCL-161	Debio-1143	Birinapant
XIAP (cellular activity)	HEK293-XIAP-Caspase9 (I.P.)	5.1	35.0	37.0	15.0
cIAP1 (cellular activity)	EVSA-T (proliferation)	0.8	1.1	1.9	0.1
	MDA-MB-231 (proliferation)	3.9	7.8	17.0	1.0
	MDA-MB-231 (cIAP1 degradation)	0.3	0.4	1.0	0.2
	HCT-116 (control)	inactive	inactive	inactive	inactive

OBJECTIVES

- Previously, we have described the characterisation of **AT-IAP** in a range of in vitro and in vivo models, showing activity against a panel of melanoma and leukemia cell lines.
- The objective of this work is to identify a set of biomarkers which can predict in vivo activity of **AT-IAP** in cell lines and patient derived melanoma models.
- Establish the basis of clinical patient selection strategy.

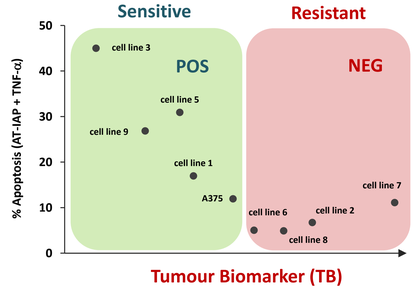
AT-IAP *IN VITRO* ACTIVITY IS ENHANCED WITH THE ADDITION OF TNF-α



- Sensitivity 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

A TUMOR BIOMARKER PREDICTS SENSITIVIY *IN VITRO* TO AT-IAP IN THE PRESENCE OF TNF-α

- Not all cell lines/tumors respond to **AT-IAP** in presence of TNF-α.

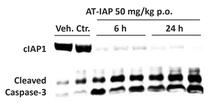


- We have identified a genetic profile for tumours where TNF-α signalling can be switched from pro-survival to pro-apoptotic after treatment with **AT-IAP**.

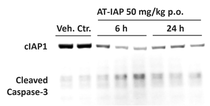
AN ADDITIONAL BIOMARKER IS REQUIRED TO PREDICT SENSITIVITY *IN VIVO*

- Xenograft PK/PD data have been used to test the relevance of the tumor biomarker *in-vivo*.
- Degradation of cIAP1 over a period of 24 h was observed in all models.
- Higher level of apoptosis was observed in the A375 model as predicted by the Tumor Biomarker.
- The lack of apoptosis marker induction in the Cell Line 11 model cannot be predicted by Tumor Biomarker.

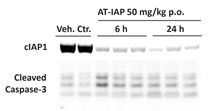
PD in A375 Mouse Model



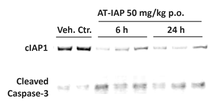
PD in Cell Line 8 Mouse Model



PD in Cell Line 11 Mouse Model



PD in Cell Line 6 Mouse Model

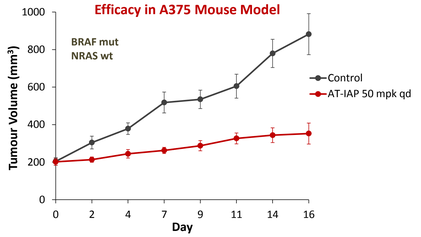


- Poor in-vivo efficacy could be linked to lack of TNF-α in the tumour xenograft micro- environment.
- The short half-life of TNF-α presents a major technical challenge to its quantitation in tumours.
- TNF-α RNA levels measured in untreated tumour samples do not correlate with sensitivity to IAP antagonists (data not shown).
- We have identified a biomarker to reliably assess the inflammatory status of the tumor microenvironment.

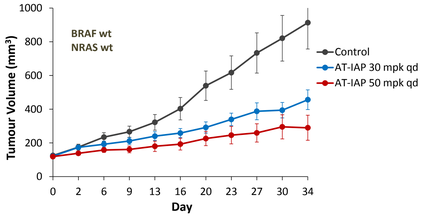
Cell Line	Biomarker Tumor	Biomarker Microenvironment	Activation of Apoptosis
A375	Positive	High	YES
Cell Line 8	Negative	High	NO
Cell Line 11	Positive	Normal	NO
Cell Line 6	Negative	Normal	NO

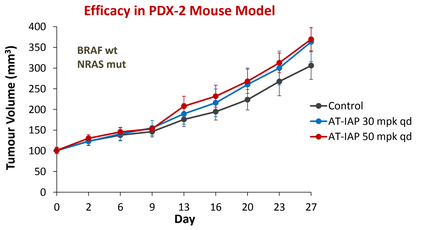
TUMOR AND ENVIRONMENT BIOMARKERS PREDICT AT-IAP IN VIVO EFFICACY

- The link between apoptosis and tumour growth inhibition was confirmed by demonstrating A375 xenograft growth inhibition.



- The significance of the microenvironment biomarker was confirmed in PDX models.
- Both PDX1 and PDX2 had positive Tumor Biomarker and were sensitive to **AT-IAP** in *ex-vivo* colony formation studies in presence of TNF-α (data not shown). However, only PDX1 had elevated microenvironment biomarker and showed in-vivo sensitivity.





CONCLUSIONS

- **AT-IAP** is a potent, balanced non-peptidic antagonist of both XIAP and cIAP1. This dual activity is a consequence of the novel non-alanine scaffold which is derived from a hit found in the fragment screen.
- In vitro cell line testing suggested significant activity against a panel of melanoma cell lines and PDXs, which was enhanced on addition of exogenous TNF-α.
- A biomarker analysis demonstrated clear differences between sensitive and insensitive melanoma cell lines. However, in vivo single agent efficacy was predicted much better by coupling the tumor biomarker with a microenviroment biomarker.
- Preliminary studies suggest that the two identified biomarkers could be used to predict single agent activity in other tumor types (see table below).

Tumor type	Cell line/ PDX	Tumor Biomarker	Microenvironment Biomarker	*In vivo* Efficacy
Melanoma	A375	Positive	High	YES
Melanoma	PDX-1	Positive	High	YES
Melanoma	PDX-3	Positive	High	YES
Breast	MDA-MB-231	Positive	High	YES
Lymphoma	cell line 12	Positive	High	YES
Melanoma	cell line 2	Negative	High	YES
Melanoma	PDX-2	Positive	Normal	NO
Melanoma	cell line 10	Negative	High	NO
Melanoma	cell line 3	Positive	High	NO
Colon	cell line 19	Negative	Low	NO

REFERENCES

[1] Engesaeter et al., Cancer Biology & Therapy, 2011, 12 (1), 47
[2] Ndubaku et al., ACS Chem Biol., 2009, 4 (7), 557
[3] Meier, P., Nat Rev. Cancer, 2010, 10 (8), 561

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