A Potent XIAP And cIAP1 Dual Antagonist Is Effective In Melanoma Models

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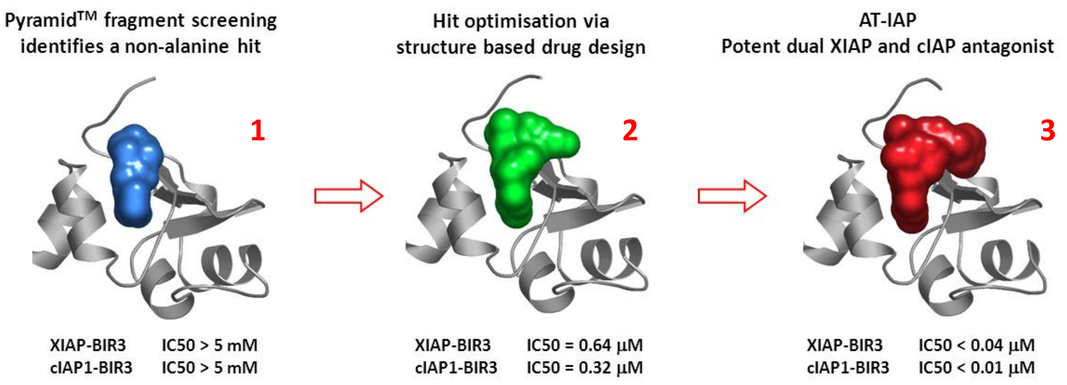
INTRODUCTION

The inhibitor of apoptosis (IAP) proteins are attractive targets for cancer therapy as they are deregulated in many tumours and contribute to resistance to anticancer therapies. These proteins are characterised by baculoviral IAP repeat (BIR) domains, to which the cell's own IAP antagonist SMAC (second mitochondria-derived activator of caspases) binds. Peptidomimetic compounds based on the N-terminal AVPI sequence of SMAC have been developed, but these are largely selective for cIAP1 over XIAP.

More recently it has been suggested that an ideal antagonist will have equal activity against both cIAP1 and XIAP and that dual antagonism of cIAP1 and XIAP in inflammatory tumours can lead to a switch in TNF-α signalling away from being pro-survival towards being pro-apoptotic. We used our fragment based-drug discovery approach to generate non-peptidomimetic IAP antagonists, which have dual potency for XIAP and cIAP1. Here we describe the characterisation of these compounds in a range of *in vitro* and *in vivo* models, including models of melanoma, an indication which often has an inflammatory phenotype.

OPTIMISATION OF FRAGMENTS: BINDING ASSAY DATA

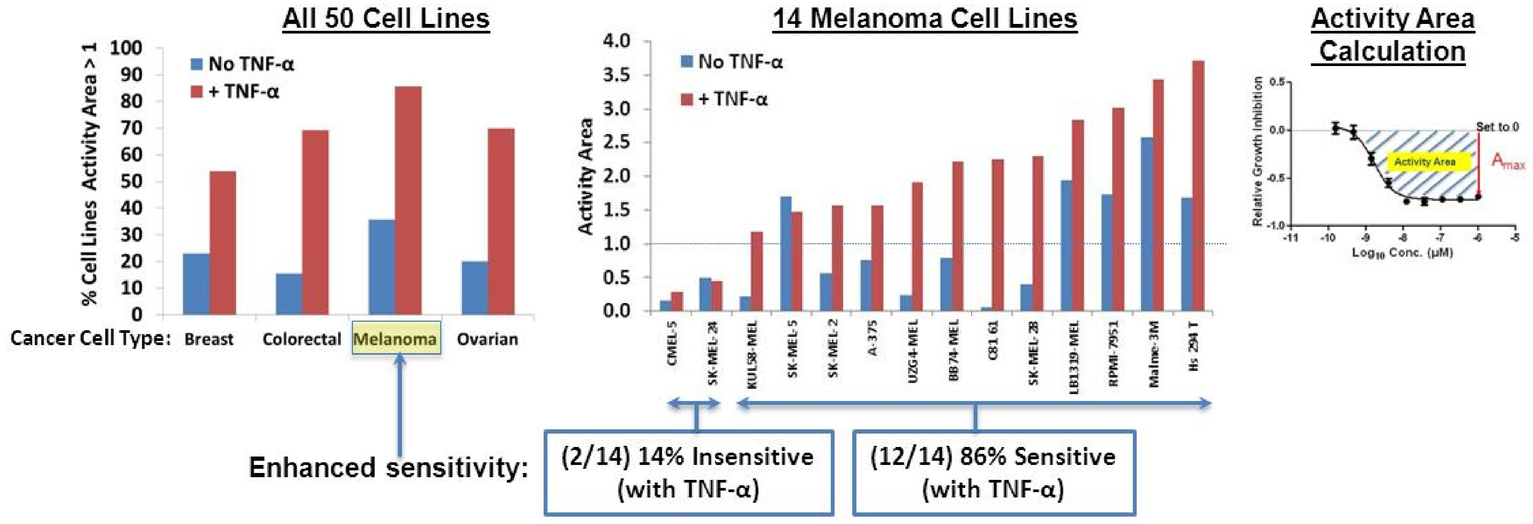


CELLULAR ACTIVITY PROFILE OF AT-IAP

Assay	Description	EC_{50} (µM)
XIAP Cell Assays	HEK293-XIAP-Caspase-9 (I.P.) *	**0.0054**
	HEK293-XIAP-SMAC (I.P.) *	**0.022**
ML-IAP Cell Assay	HEK293-ML-IAP-SMAC (I.P.) *	**0.011**
cIAP1 Cell Assay	MDA-MB-231 (cIAP1 degradation) *	**0.00037**
Cell Proliferation Assays	EVSA-T	**0.00094**
	MDA-MB-231	**0.0045**
	HCT-116 (insensitive control)	**inactive**

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

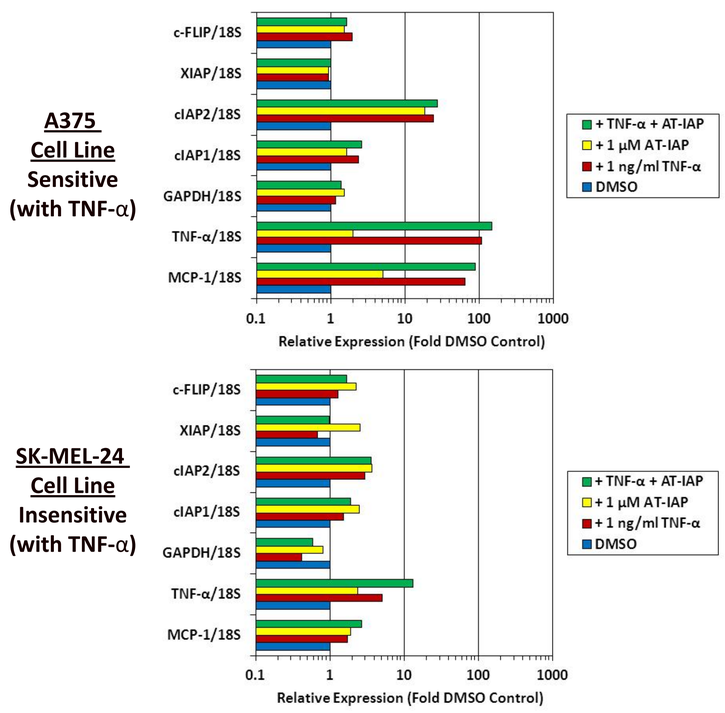
CELL LINE PROLIFERATION ASSAY SCREEN: MELANOMA AND OTHER INFLAMMATORY SOLID TUMOUR TYPE CELL LINES



- MTT proliferation assays on 50 cancer cell panel selected from inflammatory solid tumour types with or without 1 ng/ml TNF-α (Oncodesign)
- Docetaxel, included as an assay standard, yielded an average activity area of 3.9 across all 50 cell lines
- Improved activity on this selected panel of 50 cancer cell lines was noted compared to other IAP antagonist screens (Cheung *et al.*, Can. Res., 2009)

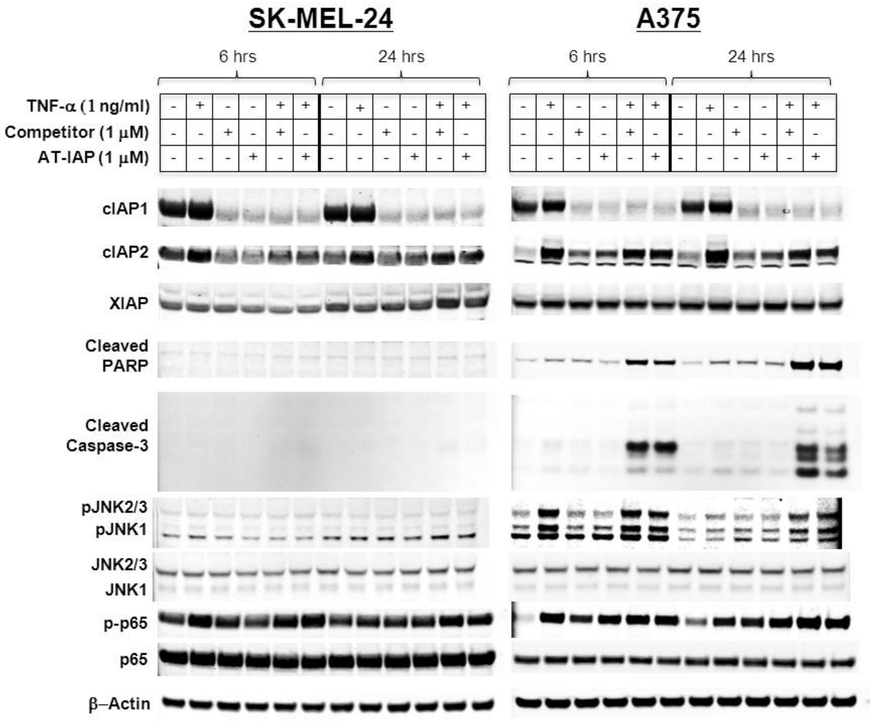
BIOMARKER EVALUATION OF INSENSITIVE AND SENSITIVE MELANOMA CELL LINES

COMPARATIVE TAQMAN PCR ($\Delta\Delta C_T$)



- A higher level of induction of MCP-1, TNF- α and cIAP2 was observed with **AT-IAP** + TNF-α in A375 cells compared to SK-MEL-24 cells

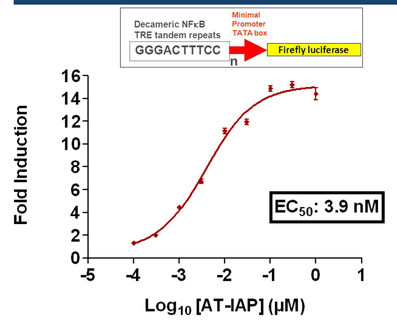
WESTERN BLOTTING



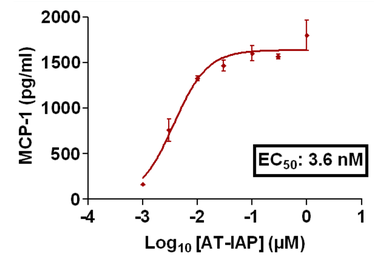
- The insensitive SK-MEL-24 cell line has higher basal NF-κB signalling compared to the sensitive A375 cell line

NF-κB ACTIVATION IN TRANSFORMED CELLS

HT1080 REPORTER ASSAY (6 h)

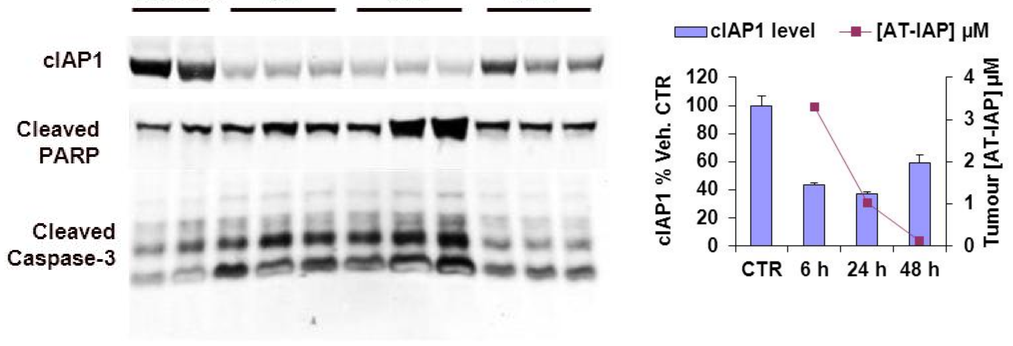


EVSA-T MCP-1 ELISA (24 h)



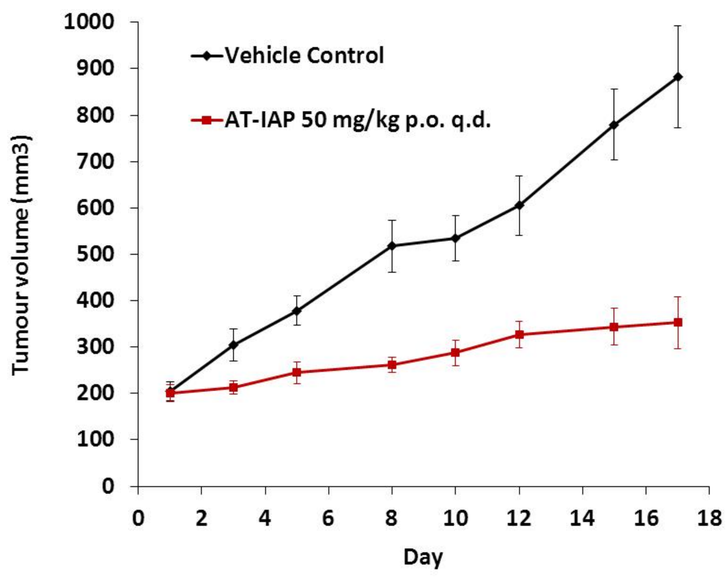
- No significant levels of cytokines (TNF-α, IL-1β, IL-6, IL-8 or MCP-1) were measured by Luminex in human PBMC supernatants after 24 h incubation with concentrations of **AT-IAP** up to 10 µM (KWS Biotest)

A375 MELANOMA XENOGRAFT PK/PD DATA



- 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

INHIBITION OF A375 MELANOMA XENOGRAFT GROWTH



- **AT-IAP** is currently being evaluated in patient derived melanoma xenograft models (Oncotest)

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 significant activity against a panel of melanoma cell lines (and other types), which was enhanced on addition of exogenous TNF-α (1ng/ml)
- Biomarker analysis demonstrated clear differences between sensitive and insensitive melanoma cell lines
- *In vivo* single agent efficacy demonstrated in a melanoma xenograft model (A375)
- Current work to establish potential serum biomarkers to predict sensitivity to IAP antagonist, **AT-IAP**

REFERENCES

- Targeting IAP proteins for therapeutic intervention in cancer, Fulda S. & Vucic D., Nat. Rev. Drug Disc., 2012, 1;11(2):109-24
- IAPs: from caspase inhibitors to modulators of NF-κB, inflammation and cancer, Gyrd-Hansen & Meier, P., Nat Rev. Cancer, 2010, 10(8):561-74

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This poster can be downloaded at www.astx.com