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Characterization of a Potent XIAP and cIAP1 Dual Antagonist in Models of Melanoma and Leukemia

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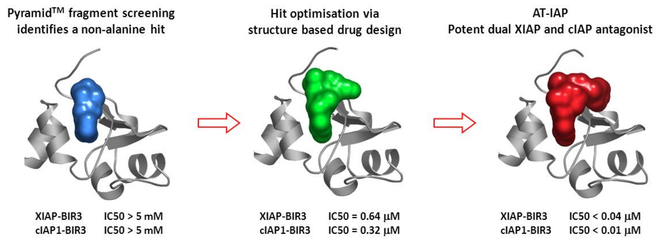
INTRODUCTION

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 characterized 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-alanine, non-peptidomimetic IAP antagonists, which have dual potency for XIAP and cIAP1. Here we describe the characterization of these compounds in a range of *in vitro* and *in vivo* models, including models of melanoma and leukemia where we have seen enhanced sensitivity in cell line testing.

OPTIMISATION OF FRAGMENTS: BINDING ASSAY DATA



CELLULAR ACTIVITY OF AT-IAP

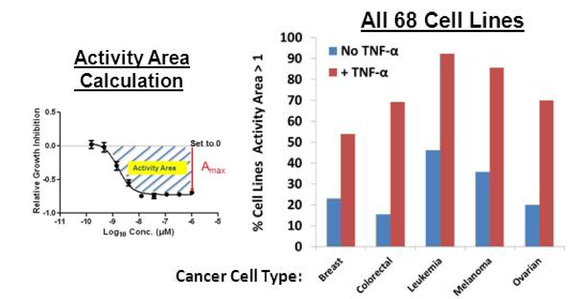
Assay	Description	EC_{50} (nM)
XIAP Cell Assays	HEK293-XIAP-Caspase-9 (I.P.) *	**5.7**
	HEK293-XIAP-SMAC (I.P.) *	**22.0**
ML-IAP Cell Assay	HEK293-ML-IAP-SMAC (I.P.) *	**11.0**
cIAP1 Cell Assay	MDA-MB-231 (cIAP1 degradation) *	**0.37**
Cell Proliferation Assays	EVSA-T	**0.89**
	MDA-MB-231	**4.2**
	HCT-116 (insensitive control)	**>10,000**

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

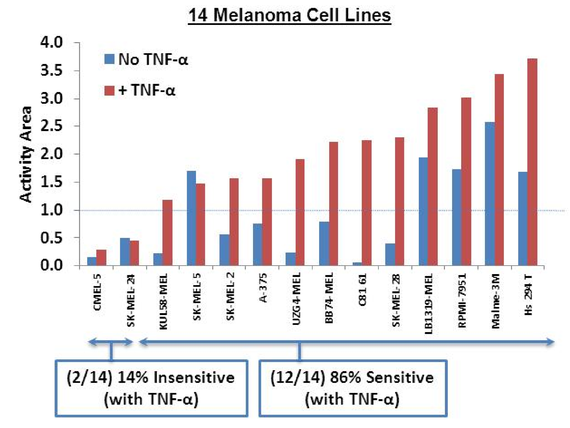
REFERENCES

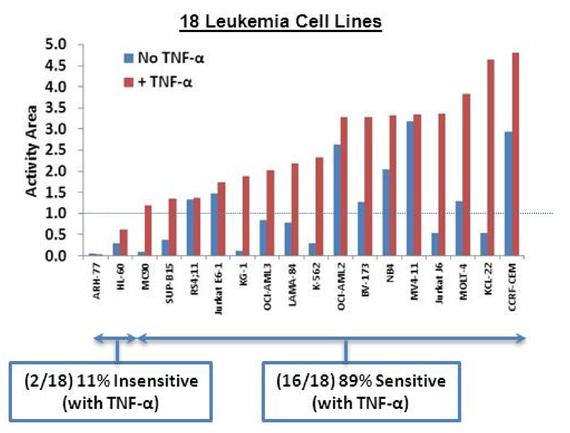
- Targeting IAP proteins for therapeutic intervention in cancer, Fulda S. & Vucic D., Nat. Rev. Drug Disc., 2012, 1;11(2):109-24
- IAPs: from caspase inhibitors to modulators of NF-κB, inflammation and cancer, Gyrd-Hansen & Meier, P., Nat Rev. Cancer, 2010, 10(8):561-74

CELL LINE SCREENING: PROLIFERATION ASSAY DATA

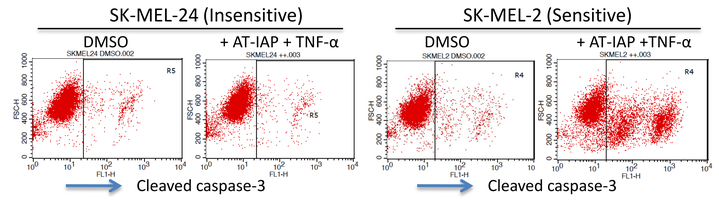


- Melanoma and leukemia cell lines show enhanced sensitivity to **AT-IAP** in a 68 cancer cell line MTT proliferation assay screen setup with or without 1 ng/ml TNF-α

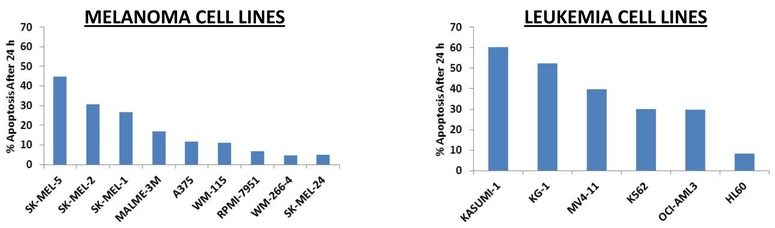




APOPTOSIS (CLEAVED CASPASE-3) EVALUATION BY FLOW CYTOMETRY

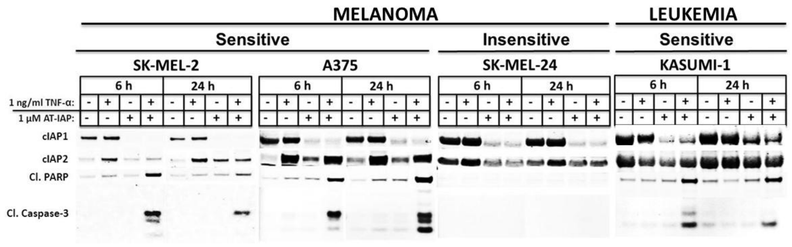


- Increased NucView™ 488 caspase-3 substrate (Biotium) staining in SK-MEL-2 cells compared to SK-MEL-24 cells after 24 h treatment with **AT-IAP** + 1 ng/ml TNF-α



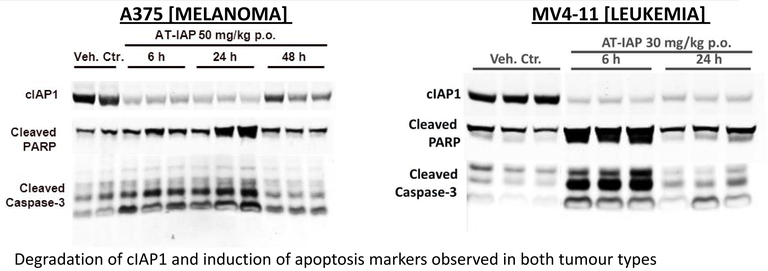
- Levels of apoptosis measured in cells by flow cytometry after 24 h treatment with **AT-IAP** + 1 ng/ml TNF-α

BIOMARKER EVALUATION OF SENSITIVE/INSENSITIVE CELL LINES BY WESTERN BLOTTING



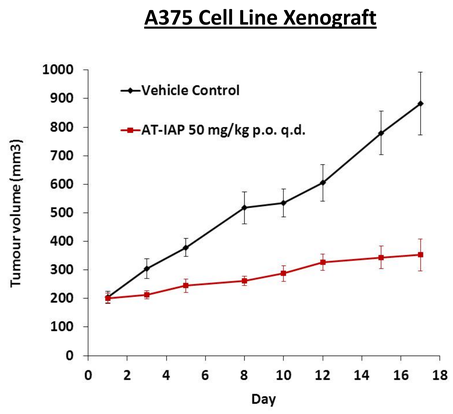
- cIAP1 degradation observed in all cell lines – sensitive or insensitive
- No induction of apoptosis markers at either time point in the insensitive SK-MEL-24 cell line

MELANOMA & LEUKEMIA XENOGRAFT PD DATA



- Degradation of cIAP1 and induction of apoptosis markers observed in both tumour types

MELANOMA XENOGRAFT EFFICACY MODEL



- **AT-IAP** was well tolerated 50 mg/kg. p.o. q.d. – no significant body weight loss

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 and leukemia cell lines, which was enhanced on addition of exogenous TNF-α (1ng/ml)
- Biomarker analysis demonstrated clear differences between sensitive and insensitive cell lines
- *In vivo* single agent efficacy demonstrated in the A375 melanoma cell line xenograft model, and is being tested in other cell line xenograft models (melanoma and leukemia)
- **AT-IAP** sensitivity is currently being assessed against a cell panel from 20 different melanoma patient-derived xenografts (PDX) in colony formation assays set up in the presence or absence of added TNF- α
- Current work is focussed on the validation of a biomarker strategy to predict single agent activity of **AT-IAP** in patients
- **AT-IAP** is being tested in melanoma PDX efficacy studies predicted to be sensitive based on prior biomarker analysis

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Poster presented at 24th EORTC-NCI-AACR Symposium on 'Molecular Targets and Cancer Therapeutics' in Dublin, Ireland, November 2012

Poster can be downloaded from www.astx.com