NOVEL SMALL MOLECULE DUAL ANTAGONISTS OF XIAP AND CIAP1 GENERATED BY FRAGMENT-BASED DRUG DISCOVERY (FBDD) ARE EFFECTIVE IN PRE-CLINICAL MODELS

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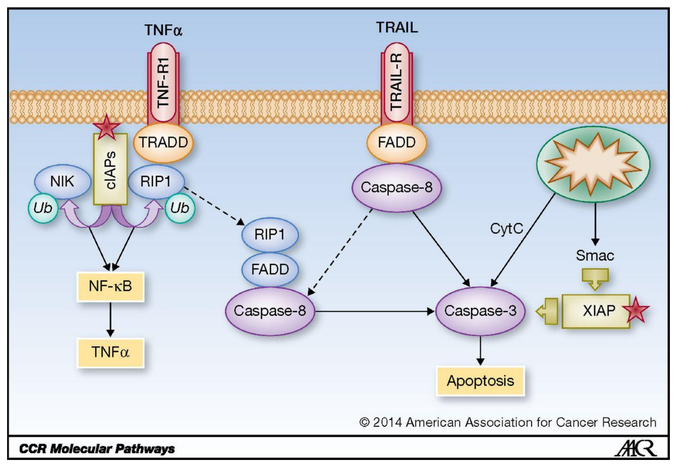
INTRODUCTION

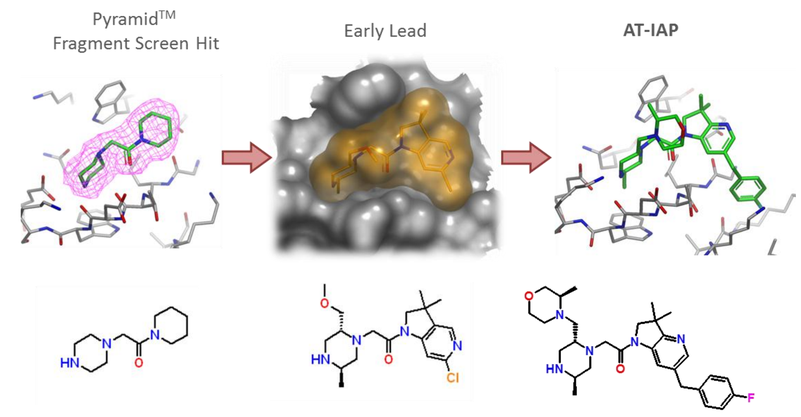
The inhibitor of apoptosis proteins (IAPs) are widely de-regulated in many tumours and contribute to cancer drug resistance. The targeted inhibition of IAPs can switch TNF-alpha signaling in cancer cells from pro-survival to pro-apoptotic. Therefore, IAPs represent an attractive target for cancer therapy.

The IAP family member cellular IAP1 (cIAP1) is involved in the regulation of TNF-alpha signaling and X-linked IAP (XIAP) directly interacts with and inhibits caspases. IAP family members are characterized by BIR (baculoviral IAP repeat) domains, to which the endogenous inhibitor of IAPs SMAC (second mitochondria derived activator of caspases) binds. Peptidomimetic compounds based on the SMAC sequence have been developed, but they show high selectivity for cIAP1. We used our fragment based-drug discovery approach to generate a non-alanine, non-peptidomimetic IAP antagonist, which has dual potency for XIAP and cIAP1. Here we describe the characterization of this compound in *in vitro* and *in vivo* models of melanoma and breast cancer.



Regulation of death receptor signaling by Smac mimetic (★).
Fulda S Clin Cancer Res 2014;20:3915-3920

OPTIMISATION OF FRAGMENTS

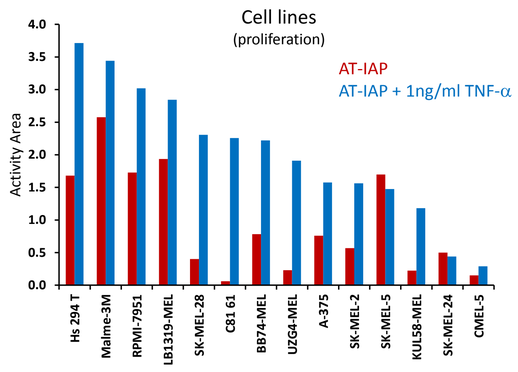


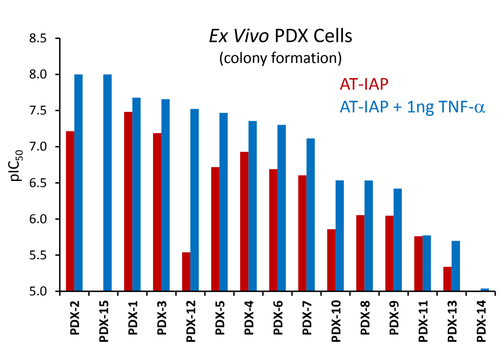
- Balanced cIAP1/XIAP profile
- Non peptidomimetic
- Non alanine warhead
- Potent cellular activity
- Oral activity in *in vivo* models

CELLULAR ACTIVITY OF AT-IAP

Assay	Description	EC_{50} (nM)
XIAP Cell Assay	HEK293-XIAP-Caspase-9 (I.P)	**5.1**
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 MDA-MB-231 HCT-116 (insensitive control)	**0.83** **4.4** **>10,000**

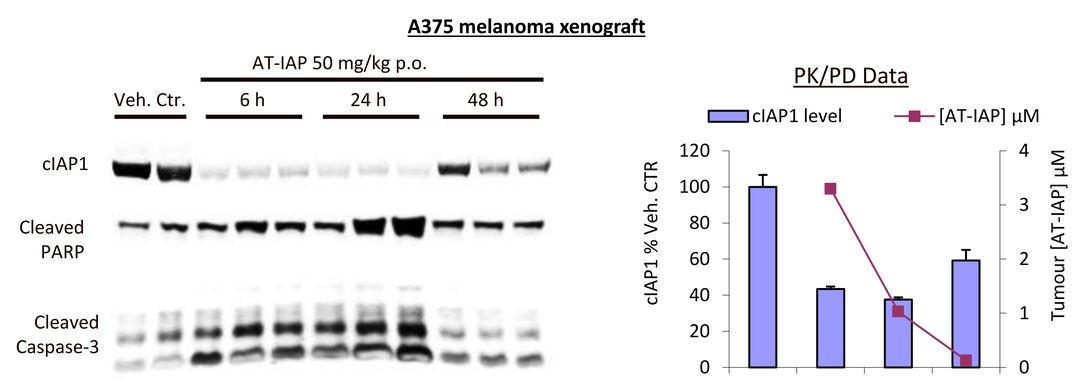
AT-IAP ACTIVITY IN MELANOMA CELL LINES AND PRIMARY TUMORS



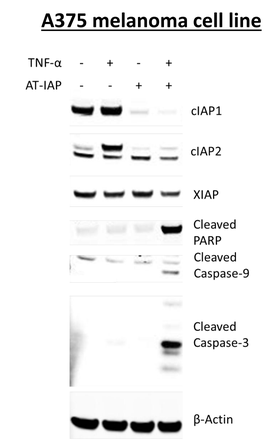


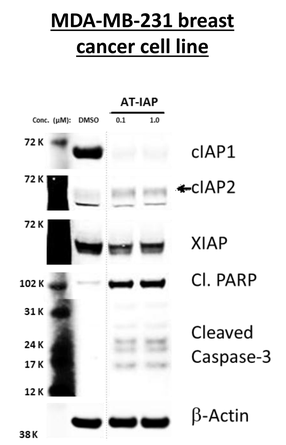
- Cell viability assay screen of cell lines for **AT-IAP** sensitivity +/- TNF-α

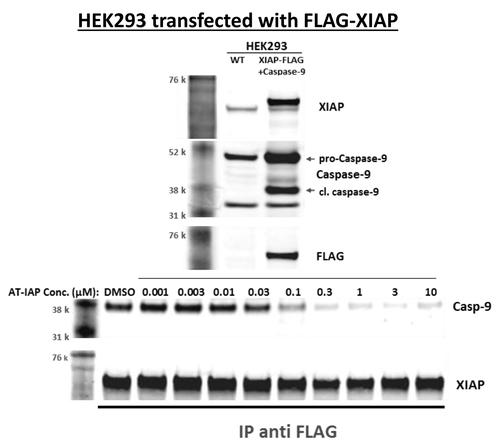
MELANOMA AND BREAST CANCER XENOGRAFT PK/PD DATA



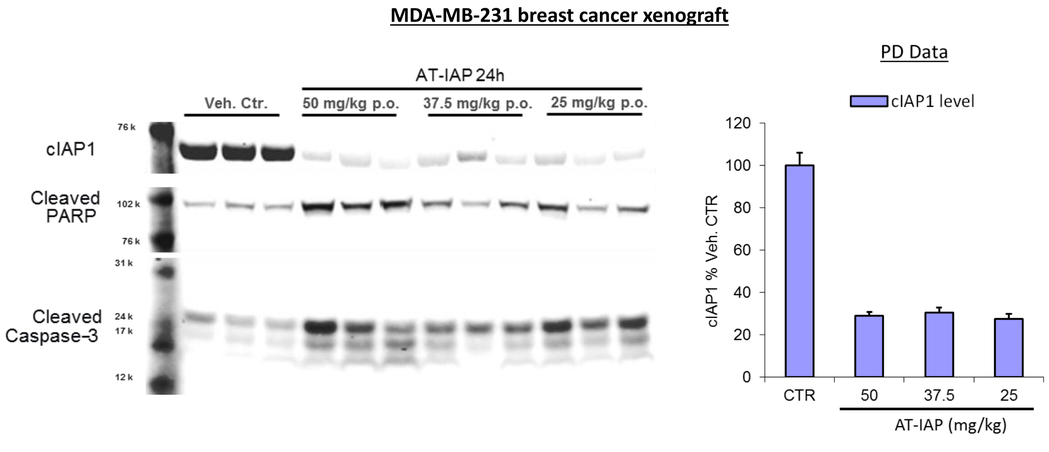
BIOMARKER EVALUATION IN VITRO BY WESTERN BLOTTING





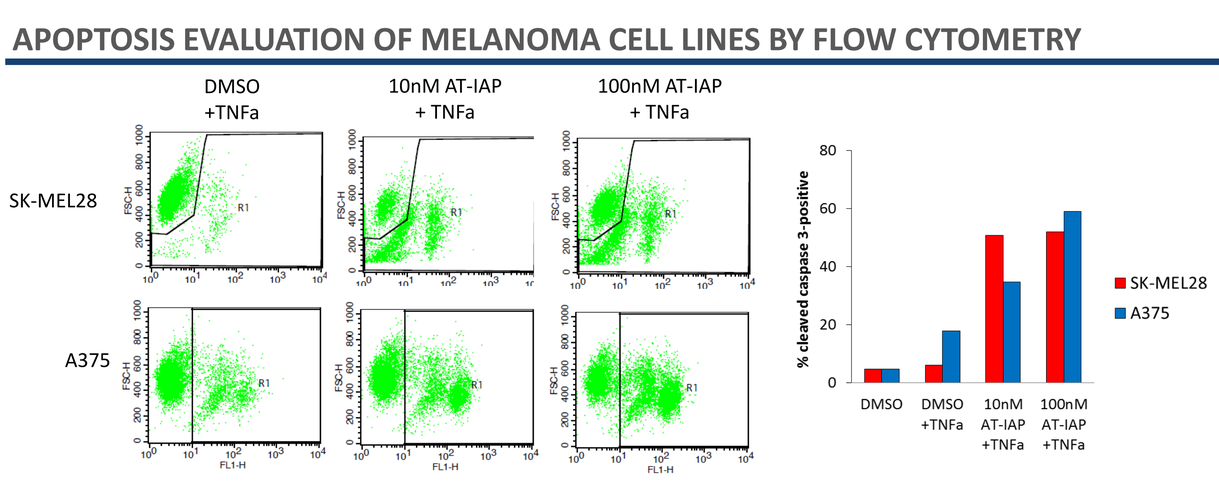


- cIAP1/2 degradation and apoptosis marker 24h after **AT-IAP** addition in sensitive cancer cell lines
- XIAP-Caspase-9 interaction disrupted 2h after **AT-IAP** addition



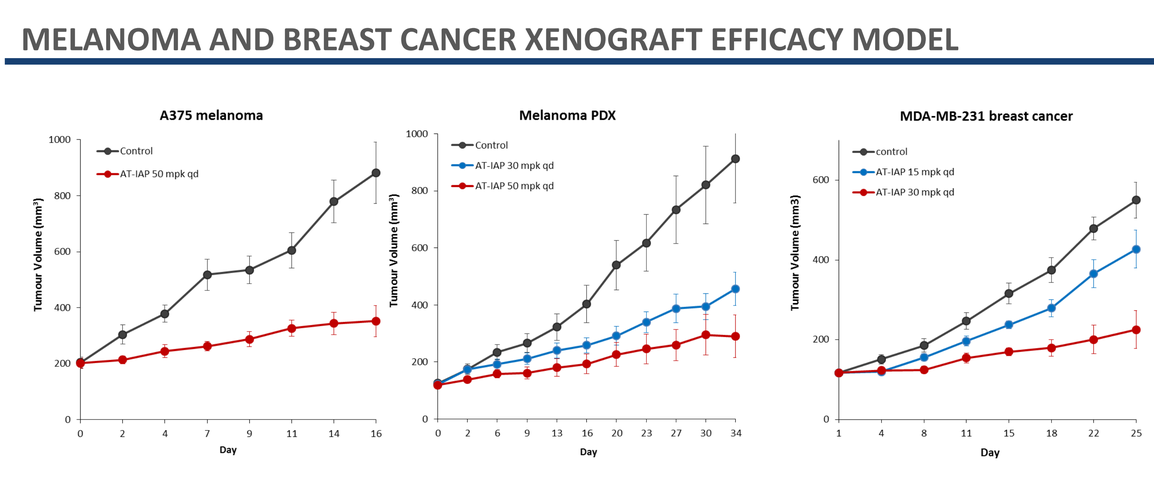
- Degradation of cIAP1 and induction of apoptosis markers observed in both tumour types after single doses of **AT-IAP**

APOPTOSIS EVALUATION OF MELANOMA CELL LINES BY FLOW CYTOMETRY



- Increased caspase-3-substrate staining after 48h treatment with **AT-IAP** + 1ng/ml TNF-α

MELANOMA AND BREAST CANCER XENOGRAFT EFFICACY MODEL



- **AT-IAP** was well tolerated up to 50 mg/kg. p.o. q.d. and shows significant *in vivo* activity in xenograft models.

SUMMARY AND CONCLUSIONS

- **AT-IAP** represents a novel IAP antagonist with a potent dual cIAP1 and XIAP antagonist profile.
- *In vitro* cell line testing suggests significant activity against a panel of melanoma and primary tumor cell lines, which is enhanced on addition of exogenous TNF-α (1ng/ml).
- *In vitro* biomarker evaluation shows robust inhibition of cIAP1 and XIAP-Caspase-9 interaction and up-regulation of apoptosis marker.
- **AT-IAP** induces cIAP1 inhibition and up-regulation of apoptosis marker *in vivo.*
- *In vivo* single agent efficacy can be demonstrated in the A375 melanoma and MDA-MB-231 breast cancer xenograft models.

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Poster presented at "Cancer Pharmacogenomics and Targeted Therapies 2014" 17-19 September 2014, Cambridge, UK