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Discovery of potent dual inhibitors of both XIAP and cIAP using fragment based drug discovery

Gianni Chessari, Ildiko Buck, Elisabetta Chiarparin, James Day, Martyn Frederickson, Keisha Hearn, Tom Heightman, Petra Hillmann, Aman Iqbal, Chris Johnson, Jon Lewis, Vanessa Martins, Caroline Richardson, Tomoko Smyth, Emiliano Tamanini, Neil Thompson, Marc Vitorino, George Ward, Glyn Williams, Pamela Williams, Nicola Wilsher, Alison Woolford

Astex Pharmaceuticals, 436 Cambridge Science Park, Milton Road, Cambridge, CB4 0QA, United Kingdom

INTRODUCTION

The inhibitor of apoptosis (IAP) proteins are important regulators of cancer cell survival, which makes them attractive targets for cancer therapy.

IAP proteins are characterized by one to three baculovirus IAP repeat (BIR) domains, and most of them also possess a carboxyterminal ubiquitin ligase RING domain. BIRs are small (~70 aa) Zn-coordinated domains, which are necessary for the antiapoptotic activity of most IAPs. The majority of BIR domains present a surface groove with affinity toward N-terminal epitopes of defined sequence. A variety of proteins use their N-terminal region to interact with BIR domain grooves. Some of these protein-protein interactions contribute to oncogenesis and resistance to therapy.

X-Chromosome-linked IAP (XIAP) has antiapoptotic activity as a result of its potent inhibition of caspases 3, 7 and 9 via its BIR domains.

Cellular IAP proteins, cIAP1 and cIAP2, are also able to interact with tumour necrosis factor receptor-associated factor 2 (TRAF2). This unique property among IAP proteins enables recruitment of cIAP1 and cIAP2 to TNFR-signaling complexes where they regulate the activation of caspase-8.

Small molecule BIR antagonists that mimic the N-terminal sequence of SMAC (an endogenous inhibitor of the IAPs) have the ability to sensitise and/or promote apoptosis in cancer cells and inhibit tumour growth in vivo. Binding of IAP antagonists to the BIR domains of cIAP1/2 and XIAP leads to the release of caspases from XIAP inhibition and also to the induction of c-IAP autoubiquitination activity and rapid proteasomal degradation of the c-IAP proteins. Besides neutralizing these antiapoptotic proteins, the IAP antagonists activate canonical and non-canonical NF-κB pathways and induce cell death that is dependent on TNF-α signaling.

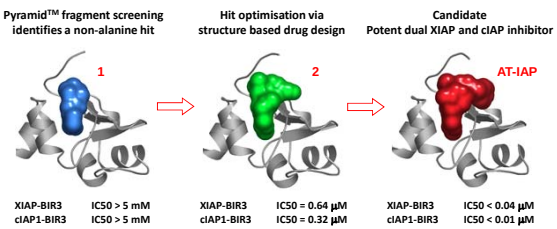
References

- 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-kappaB, inflammation and cancer, Gyrd-Hansen & Meier, P., Nat Rev. Cancer, 2010, 10(8):561-74

FRAGMENT BASED DRUG DISCOVERY OF AT-IAP

Fragment-based drug discovery is a rapidly growing alternative to high throughput screening in which very small molecules are screened by specialised techniques such as NMR and X-ray crystallography. Only relatively small libraries of fragments are required and observed fragment hits often possess high potency when normalised to their size (high ligand efficiency).

Fragment to Candidate evolution



	Assay	AT-IAP
XIAP (cellular activity)	HEK293-X-C9 (I.P.)	**0.003 µM**
cIAP1 (cellular activity)	EVSA-T (proliferation)	**0.001 µM**
	MDA-MB-231 (proliferation)	**0.005 µM**
	MDA-MB-231 (cIAP1 degradation)	**0.0004 µM**
	HCT-116 (control)	**inactive**
Selectivity (cIAP1 vs XIAP)	HEK-293/MDA-MB-231 (cIAP1 degr.)	**<10 fold**

- Non-peptidic and non-alanine fragments were identified from application of Astex Pyramid™ fragment screening to BIR3 domains of XIAP and cIAP1.
- Fragment **1** 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 cIAP-BIR3 and XIAP-BIR3 and chemically distinct from SMAC mimetics in the clinic.

PK and Safety

- **AT-IAP** is orally available in mouse and rat, and it shows low/moderate clearance in both species.
- No P450 or hERG liabilities are associated with **AT-IAP**.
- No liver toxicity nor weight loss has been observed in mouse at MTD for 21 days (levels of ALT and AST in plasma were normal and comparable to control).
- No significant cytokine elevation has been observed in human PBMC at 10 µM conc.

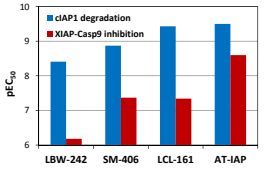
XIAP AND CIAP1 ANTAGONISM

In vitro binding assays are not sensitive enough to differentiate potent compounds. Cell based assays have to be used to assess the effect of XIAP/cIAP1 dual antagonism:

- Autoubiquitination and proteasomal degradation of cIAP1
- Disruption of XIAP:Caspase-9 complex
- Induction of cleaved PARP and cleaved Caspase-3

Immunoprecipitation (IP) Assay for XIAP Inhibition

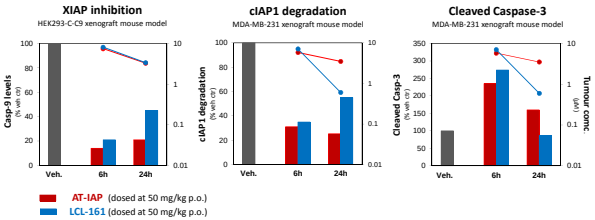
- An engineered HEK293 cell line was stably co-transfected with full length FLAG-tagged human XIAP cDNA and full length (untagged) human caspase-9 cDNA. Inhibition of caspase-9 binding to XIAP was measured in IP assays.
- The IP assay gives a sensitive read-out for XIAP antagonism in cells which could be plotted against cIAP1 degradation to establish relative XIAP vs cIAP1 selectivities and to select dual antagonists of both IAPs.



Compound	Chemistry	Status	Selectivity cIAP/XIAP
AT-IAP	FBDD Non-Ala warhead	Preclin	< 10 fold
LBW-242 Novartis	Peptidomimetic Ala-like warhead	Preclin	> 150 fold
LCL-161 Novartis	Peptidomimetic Ala-like warhead	Phase I/II	> 100 fold
SM-406 Ascenta	Peptidomimetic Ala-like warhead	Phase I/II	> 30 fold

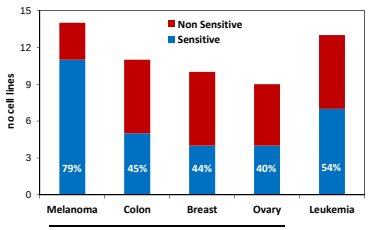
- **AT-IAP** is a potent dual antagonist of XIAP and cIAP1
- Alanine-based compounds tend to be cIAP1 selective

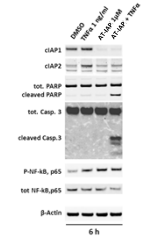
In vivo PD in Mouse Models



- **AT-IAP** achieved high concentration in tumours over 24h which ensured excellent antagonism of both XIAP and cIAP1 with consequent reduction of cIAP1 levels and induction of the apoptosis markers cleaved caspase-3 and cleaved PARP (data not shown).

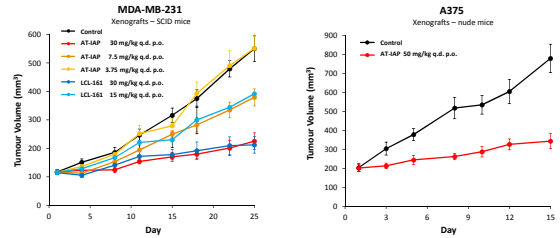
ACTIVITY IN A PANEL OF TUMOUR CELL LINES





- 57 inflammatory tumour cell lines tested in 72h proliferation assays with AT-IAP +/-1 ng/ml TNFα
- High proportion of melanoma cell lines (11/14) displayed sensitivity to AT-IAP in the presence of TNFα
- Example of Western blot profiling of lysates from a sensitive melanoma cell line (Sk-Mel2) is shown on the left above, showing:
  - reduction in cIAP1 and cIAP2 levels with AT-IAP treatment
  - induction of apoptosis markers (cl. PARP and cl. caspase-3) with AT-IAP + TNFα
  - induction of NF-κB marker, phospho-p65, with TNFα and/or AT-IAP

ANTI-TUMOUR ACTIVITY IN XENOGRAFT STUDIES



CONCLUSIONS

- Successful use of FBDD to a PPI target and generation of a non-alanine, non peptidomimetic IAP antagonist, which is chemically distinct from SMAC mimetics in the clinic
- **AT-IAP** represents a novel class of IAP antagonists with unique pharmacological profile
  - Potent dual antagonist of cIAP and XIAP
  - In vivo single agent efficacy demonstrated in two xenograft models
  - No toxicities observed in preliminary studies

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Poster presented at AACR Annual Meeting, March 31st – April 4th, 2012 • Chicago (USA).

Poster can be downloaded from www.astx.com