Astex Pharmaceuticals

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

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Disclosures

I am an employee of Astex Pharmaceuticals

I will present pre-clinical data from the IAP program

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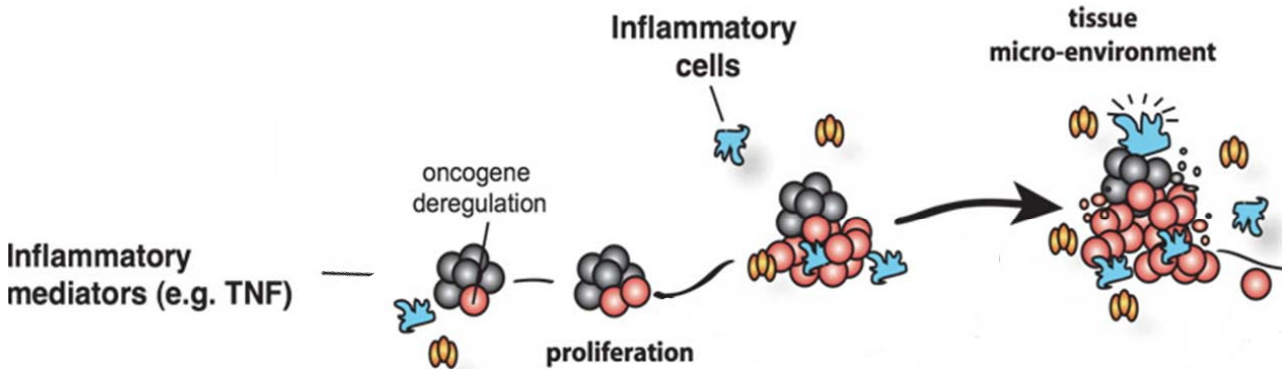
Inflammation and Tumor Progression

- **Strong clinical unmet need for inflammatory tumour types**
  - Melanoma, Breast, Colorectal, Mesothelioma, Ovarian
- **Inflammatory mediators (e.g. TNFα) can initiate tumorigenesis and subsequent tumour progression**
  - TNFα released by the macrophage activates Nuclear Factor-Kappa B (NF-κB) pathway.
  - NF-κB activates production of pro-survival proteins that promote cell proliferation and inflammation



http://www.breakthroughresearch.org.uk/the_research_centre/research_teams/apoptosis/index.html

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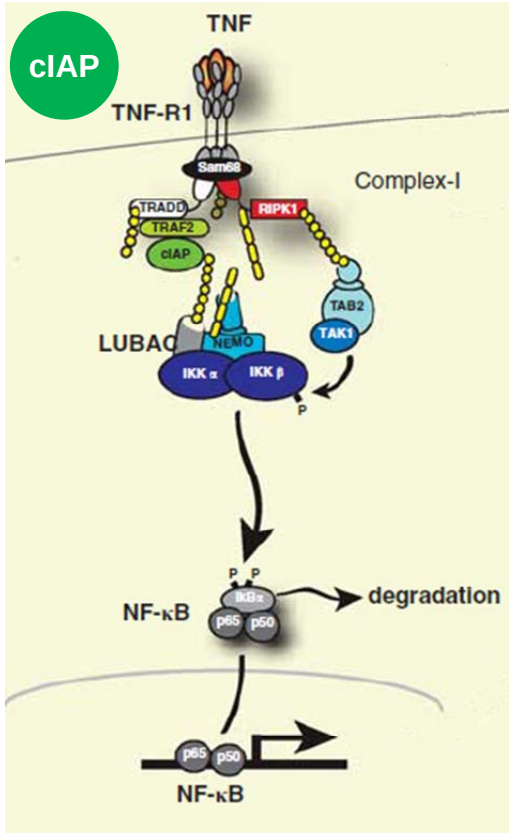
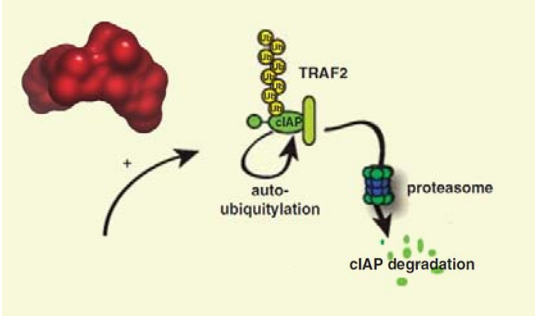
TNFα Signaling and IAP Antagonism

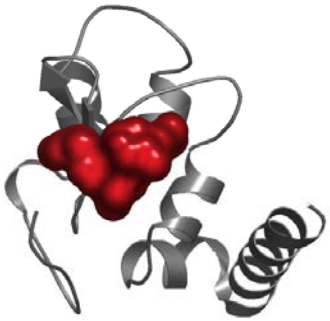
Canonical NF-κB pathway



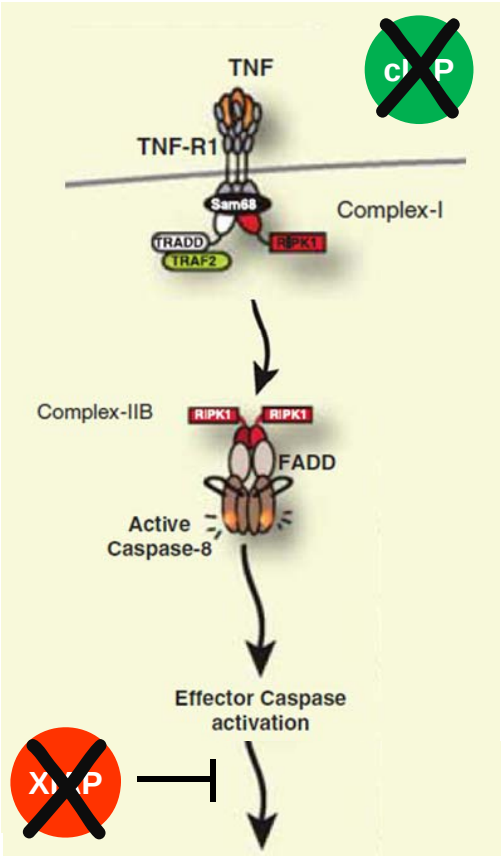
cIAP antagonists promote cIAP auto-ubiquitylation and proteosomal degradation



XIAP antagonists promote activation of Caspase-3, -7, -9



TNF signalling in absence of cIAP



Inflammation

Apoptosis

The full pro-apoptotic action of cIAP1 loss cannot be achieved without sustained XIAP inhibition

Darding, M. & Meier, P. Cell Death Differ, (2012), pp.58-66

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Astex Target Product Profile

- **Molecular Profile**
  - Dual XIAP and cIAP inhibitors
    - Switch TNFα signalling from inflammation to apoptosis
    - Strong apoptotic signal: activation of Caspase-3, -7, -8, -9
    - Non peptidomimetics and non alanine as the warhead
  - Well tolerated at therapeutic dose
    - No overproduction of cytokines (side effect due to cIAP1 inhibition)
    - No liver toxicity
  - Once daily oral

- **Disease Area**
  - Clinical unmet need in inflammatory tumor types (single agent)
    - Melanoma (also breast, colorectal, mesothelioma, ovarian)

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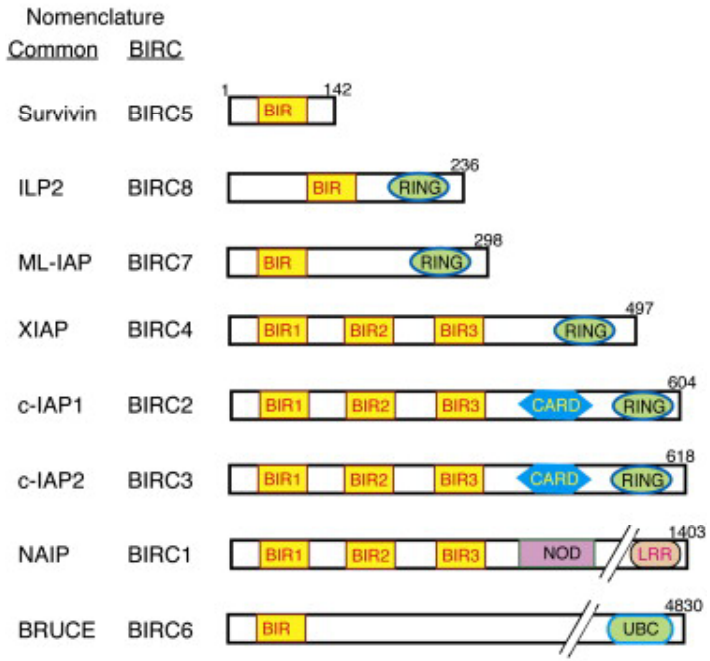
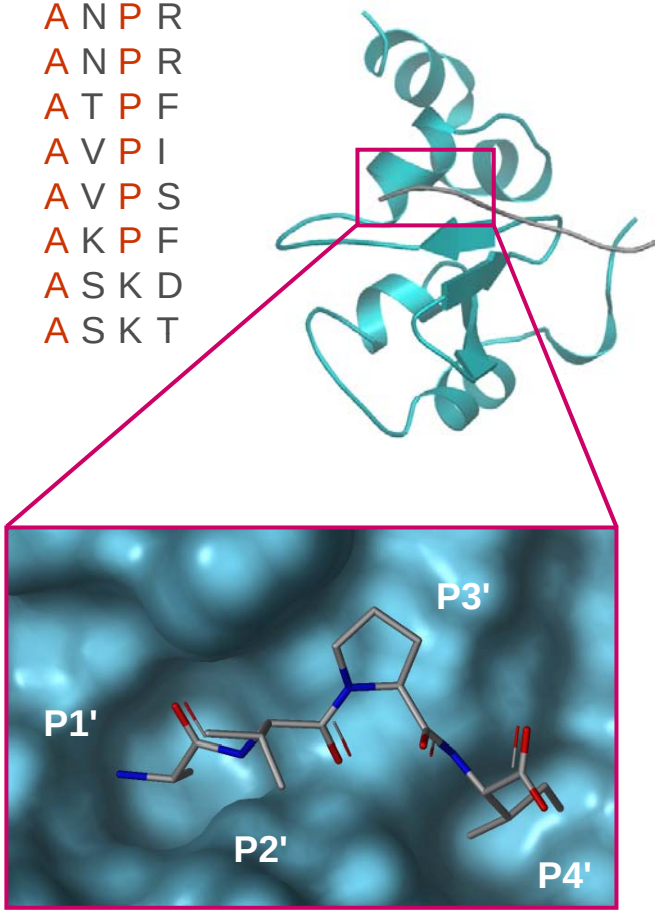
IAP Protein Family

Human IAPs



Caspase-3	A N P R
Caspase-7	A N P R
Caspase-9	A T P F
SMAC	A V P I
HtrA2	A V P S
GSPT	A K P F
CLPX	A S K D
3HB	A S K T



XIAP-BIR3 SMAC complex

- **First generation of IAP antagonists**
  - Based on SMAC peptide sequence AVPI
  - Ala-like warhead
  - Tendency for cIAP1 selectivity

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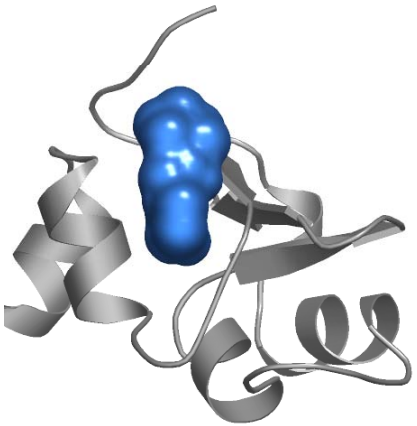
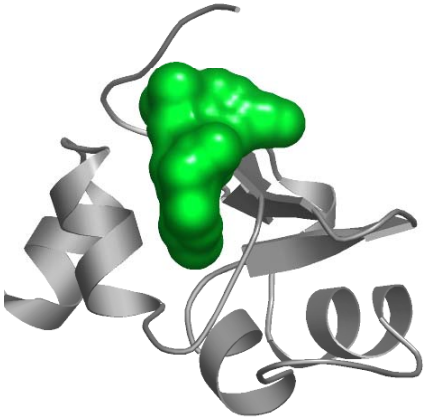
Fragment to Candidate Progression

Pyramid™ fragment screening identifies a non-alanine hit



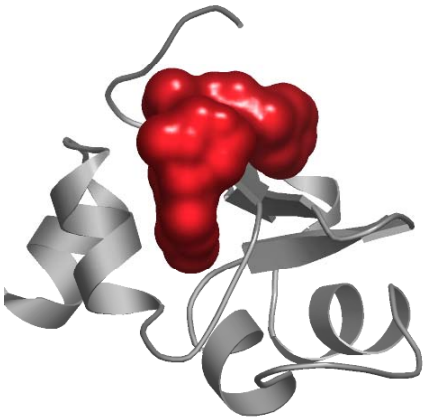
XIAP-BIR3 **IC50 > 5 mM**
cIAP1-BIR3 **IC50 > 5 mM**

Hit optimisation via structure based drug design



XIAP-BIR3 **IC50 = 0.64 μM**
cIAP1-BIR3 **IC50 = 0.32 μM**

Candidate (ASTX)
True dual XIAP and cIAP inhibitor



XIAP-BIR3 **IC50 < 0.04 μM**
cIAP1-BIR3 **IC50 < 0.01 μM**

- **ASTX** is well tolerated in mouse at efficacious dose (50 mg/kg p.o.)
  - No liver toxicity (levels of ALT and AST in plasma were normal and comparable to control).
  - No weight loss
- **ASTX** is orally available in mouse and rat and it shows low/moderate clearance in both species

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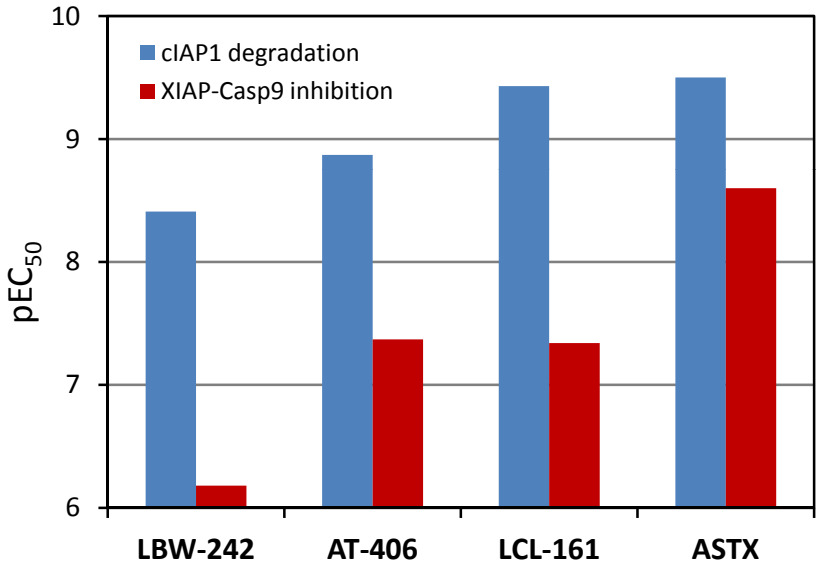
Astex Candidate Shows Potent Pan-Inhibition in Cell

- **Cell based assays give more accurate reflection of XIAP and cIAP1 potency**
  - In vitro binding assays not sensitive enough to differentiate potent compounds



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

ASTX is a potent dual inhibitor of XIAP and cIAP1

Alanine-based compounds tend to be cIAP1 selective

cIAP1 degradation measured in MDA-MB-231 cell line
XIAP-Casp9 inhibition measured in HEK293 engineered cell line

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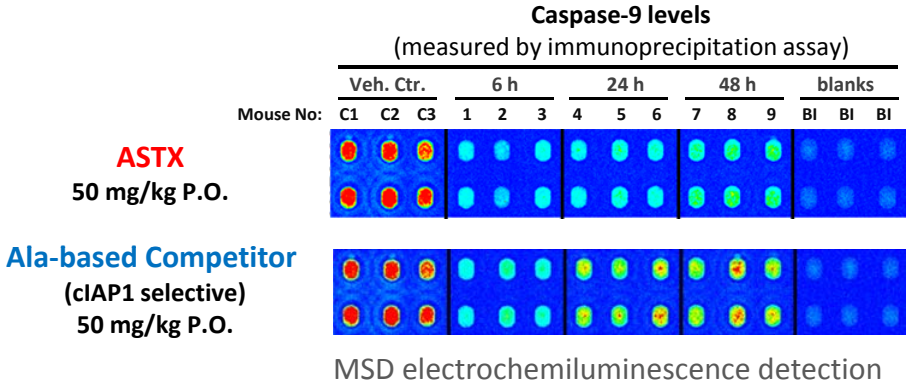
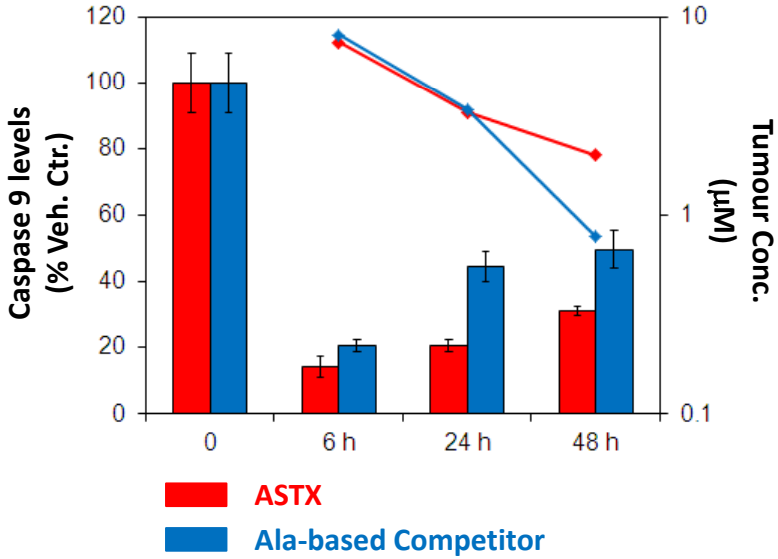
In Vivo XIAP Inhibition HEK293-X-C9

HEK293-X-C9 xenograft mouse model





- ASTX strongly binds to XIAP inhibiting the formation of XIAP/Caspase-9 complex over 48 h
- ASTX PK/PD profile in the HEK293-X-C9 xenograft mouse model is superior to Alanine-based SMAC mimetics competitors

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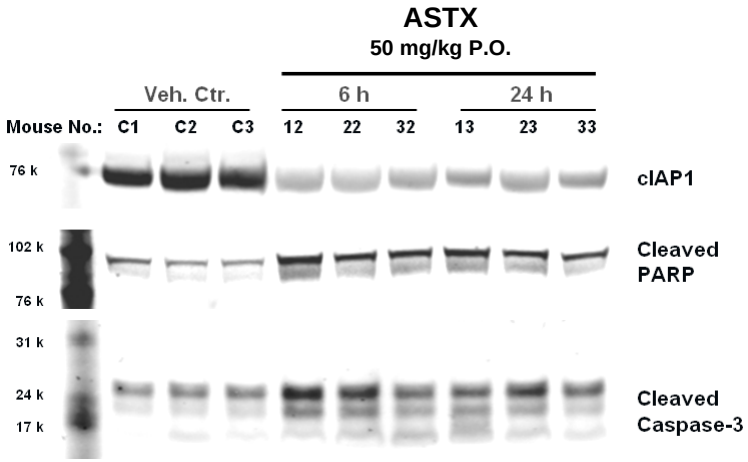
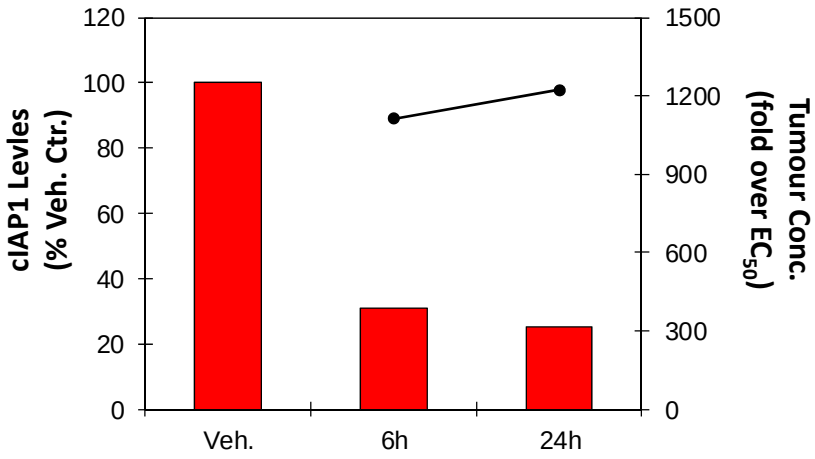
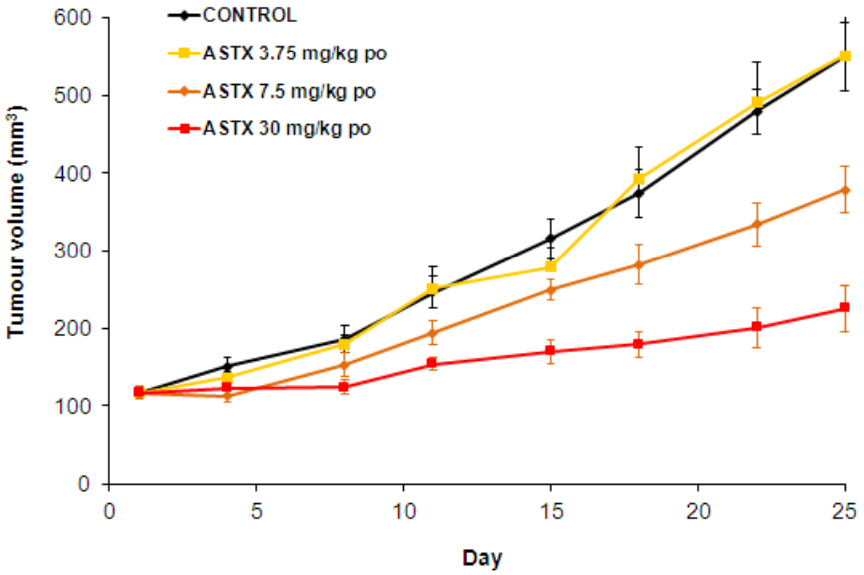
In Vivo Efficacy in MDA-MB-231 mouse model

PK/PD





Efficacy Study



- ASTX achieves high concentration in tumor and plasma over 24 h
- Excellent inhibition cIAP1 and XIAP
- Induction of apoptosis markers (cleaved PARP, cleaved Caspase-3)
- Strong inhibition of tumour growth.

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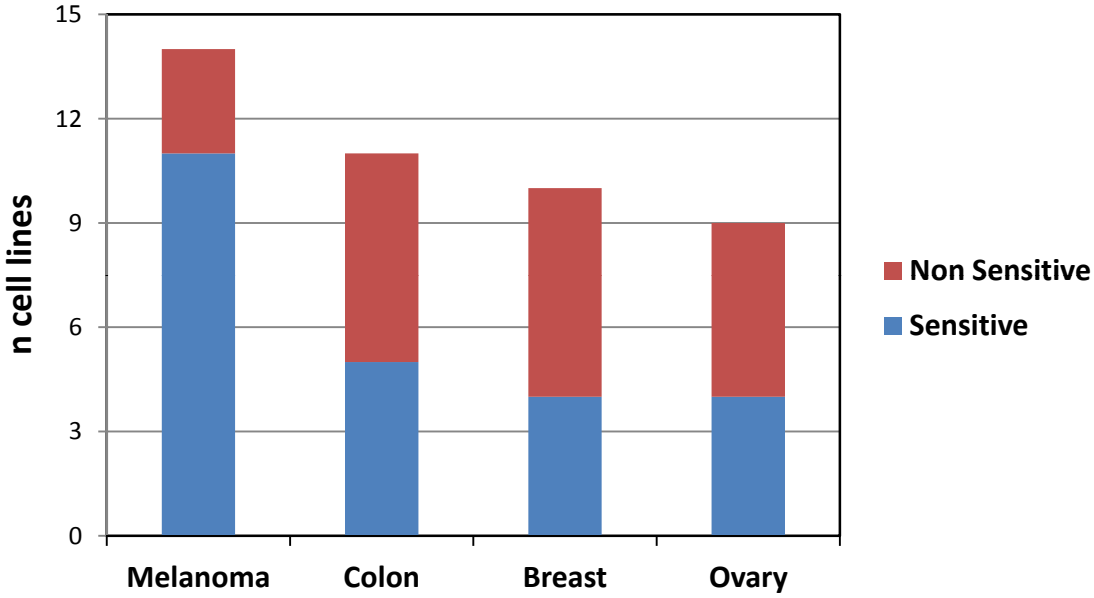
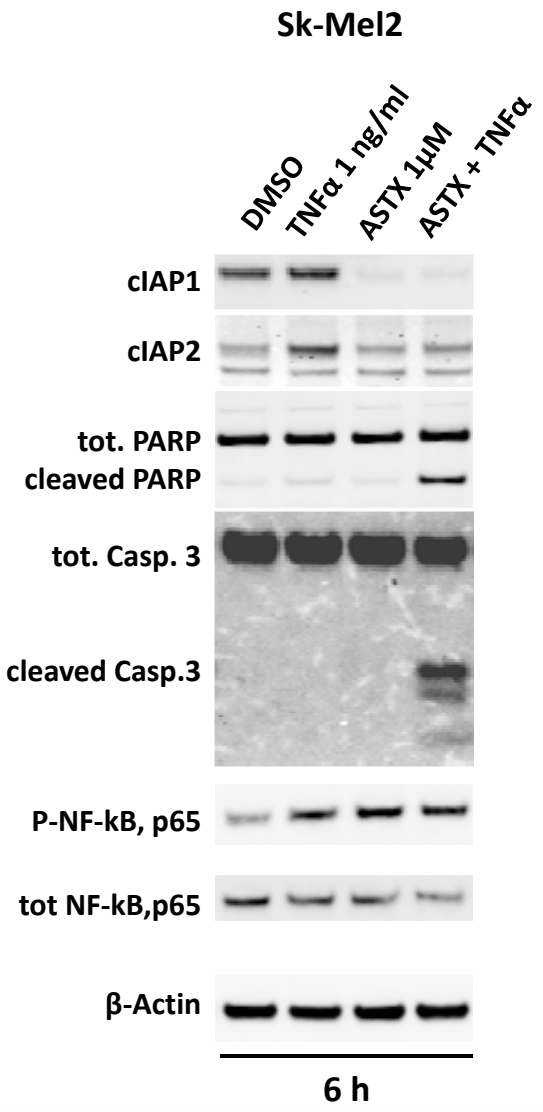
Cell Lines Panel of Inflammatory Tumours

31 inflammatory tumour cell lines where tested with ASTX + 1ng/ml of TNFα





- Inflammatory tumours cell lines shows good sensitivity to **ASTX** when TNF is present (>50% overall sensitivity)
- Sensitivity is driven by the ability of **ASTX** to switch the TNFα signalling from pro-survival to pro-apoptotic

Summary

- **Potent dual XIAP and cIAP1/2 candidate**
  - Novel non-alanine and non-peptidic chemotype identified by Pyramid™ fragment screening
  - Structure based drug design approach led to candidate molecule:
    - Good physico-chemical properties and in vivo profile
    - Well tolerated (no liver tox nor elevation of cytokines levels)
    - No P450 or hERG liabilities
    - Structurally distinct from any known IAP inhibitors
- **Oral pharmacodynamic activity and tumour growth inhibition demonstrated**
- **Targeting inflammatory tumour types**

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Acknowledgements

IAP Team
Based in Cambridge (UK) & Dublin (US)

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