CONCLUSIONS

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**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 baculoviral IAP repeat (BIR) domains, and most of them also possess a caspase-recruitment domain (CARD) or a baculoviral IAP-repeat (BIR) domain.
- XIAP, the most prominent member of the IAP family, is involved in the regulation of multiple cellular processes, including cell survival, proliferation, and apoptosis.

**AXEST PYRAMID™ FRAGMENT SCREENING PLATFORM FOR INHIBITOR OF APOPTOSIS PROTEIN FAMILY**

- **X-Ray Crystallography and NMR Spectroscopy**
  - Fragment 1
  - Optimised Fragment 1
  - Lead 1 (cIAP-1 selective)
  - Lead 2 (pain selective)

- **R&D and Lead Optimisation**
  - The Fragment Library was a target set and was screened against XIAP-BIR domain via X-ray crystallography and NMR. The binding mode of the hits was investigated using X-ray crystallography and 2D-NMR.
  - The ligands were selected based on X-ray crystallographic and NMR data and validated against the XIAP-BIR domain.

- **Cell-based data**
  - **Compounds tested in 72h-proliferation assays**: using two sensitive human breast cancer cell lines, EVSA-T and MDA-MB-231, and in the invasive human colon cancer cell line, HCT116, to control for off-target effects.
  - **Both Lead 1 and Lead 2** in 72h-proliferation assays show IC50 values of 230 nM and 300 nM on the more sensitive EVSA-T cell line. The Novartis lead, LBW424, was included as a control.

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**FRAGMENTS TO LEADS**

- **Structure based drug design** has been used to develop fragment hits into lead molecules.
- **Fragment 1** has an intrinsic selectivity towards the BIR3 domain of cIAP-1. Its optimisation generated Lead 1, which is a selective cIAP-1/BIR3 antagonist.
- **Fragment 2** binds very weakly to both cIAP-1/BIR3 and XIAP-BIR3. However, its X-ray crystal structure suggested areas of improvement, which allowed us to improve the potency. Further optimisation provided Lead 2, which is equipotent to cIAP-1/BIR3 and XIAP-BIR3 antagonist.

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**Pyramid™ Fragment Screening**

- **Lead optimisation** on both series is ongoing.

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**Novel Small Molecule Antagonists of XIAP, cIAP1/2 Generated by Fragment-Based Drug Discovery**

Gianni Chessari, Ildiko Buck, Joe Coyle, James Day, Tom Davies, Keisha Heam, Finn Holding, Steven Howard, Aman Iqbal, Chris Johnson, Caroline Richardson, Andrew Sharp, Emiliano Taminani, George Ward, Glyn Williams, Pamela Williams, Alison Woolfold, Marc Vitorino, Charlotte Griffiths-Jones, Mike Reader, Astex Therapeutics Ltd., 436 Cambridge Science Park, Milton Road, Cambridge, CB4 0QA, UK.

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**X-Ray Crystallography and NMR Spectroscopy**

- **Fragment Library**
- **Targeted hits**
- **Lead Library**

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**TOXICITY ASSAYS**

- **IC50 values of 230 nM and 300 nM** on the more sensitive EVSA-T cell line. The Novartis lead, LBW424, was included as a control.

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**Cell-based data**

- **Compounds tested in 72h-proliferation assays** using two sensitive human breast cancer cell lines, EVSA-T and MDA-MB-231, and in the invasive human colon cancer cell line, HCT116, to control for off-target effects.
- **Both Lead 1 and Lead 2** in 72h-proliferation assays show IC50 values of 230 nM and 300 nM on the more sensitive EVSA-T cell line. The Novartis lead, LBW424, was included as a control.

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**Lead optimisation** on both series is ongoing.

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**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 baculoviral IAP repeat (BIR) domains, and most of them also possess a caspase-recruitment domain (CARD). BIRs are small (~70 aa), Zn-coordinated domains, which are necessary for the endoplasmic reticulum 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 domains. Some of these protein-protein interactions contribute to oncogenesis and resistance to therapy.

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

**Caspase IAP proteins, cIAP1 and cIAP2, are also able to interact with tumor necrosis factor receptor-associated factor 2 (TRAF2).** This unique property among IAP proteins enables recruitment of cIAP1 and cIAP2 to TNFα 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 sensitize and/or promote apoptosis in cancer cells and inhibit tumor growth in vivo.** Binding of IAP antagonists to the BIR domains of cIAP1 and XIAP leads to the release of caspase from IAP inhibition and also to the induction of a-IAP autoubiquitination activity and rapid proapoptotic 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 (which is dependent on TNF signaling).

Fragment-based drug discovery is a rapid 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 normalized to their size (high ligand efficiency).

To describe an example of Astex Pyramid™ fragment screening to BIR domains, which led to the identification of novel, non-peptidic and non-alanine lead series.**