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

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INTRODUCTION

The inhibitor of apoptosis (IAP) proteins are important regulators of cancer 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 IAPs 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 tumor 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 caspases.

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 cIAP12 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-kB pathways and induce cell death that is dependent on TNFα signaling.

Fragment-based drug discovery

Fragment-based drug discovery is a rapidly growing alternative to high throughput screening in which very small molecules are screened by specialized 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).

Fragment to Candidate evolution

Pyramid™ fragment screening identifies a non-peptide hit

Hit optimisation via structure based drug design

Candidate Potential dual XIAP and cIAP inhibitor

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:
- Autoliquification and proteasomal degradation of cIAP
- Disruption of XIAP-Caspase-9 complex
- Induction of cleaved PARP and cleaved Caspase-3

IMMUNOCOMPELLATION (IP) Assay for XIAP Inhibition

An engineered HBB202 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 selectivity and to select dual antagonists of both IAPs.

PK and Safety

- AT-IAP is orally available in mouse and rat, and it shows low/moderate clearance in both species.
- No Pgp or NER liabilities are associated with AT-IAP.
- No liver toxicity or 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 cytochrome elevation has been observed in human PBMC at 10 μM conc.

REFERENCES


ACTIVITY IN A PANEL OF TUMOUR CELL LINES

Malignancies Colon Breast Ovary Leukemia

In vivo PD in Mouse Models

XIAP Inhibition

cIAP1 and cIAP2 overexpressed xenograft

cIAP1 degradation

Casp9 degradation

Cleaved Caspase-3

XIAP/cIAP1 Dual Antagonist

AT-IAP (labeled at 30 mg/kg po)

AT-IAP (labeled at 30 mg/kg po)

AT-IAP (labeled at 30 mg/kg po)

AT-IAP (labeled at 30 mg/kg po)

CONCLUSIONS

- Successful use of FBDD to a PPI target and generation of a non-peptide XIAP 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