Abstract No: PS1322

ASTX660, a non-peptidomimetic antagonist of cIAP1/2 and XIAP, induces apoptosis in T-cell lymphoma by enhancing immune mediated and death receptor dependent killing

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INTRODUCTION

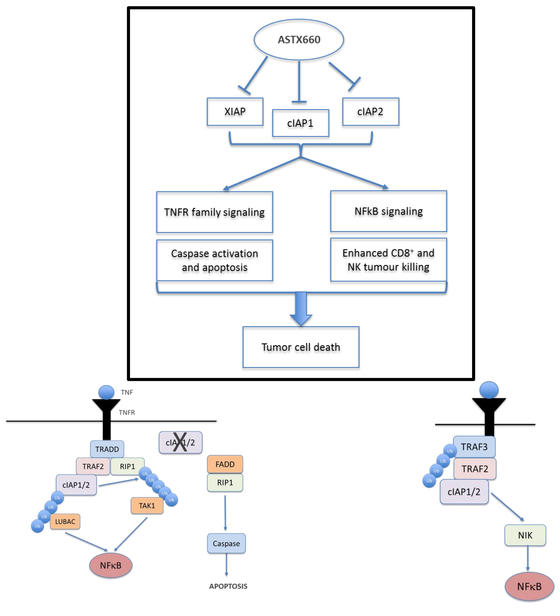
ASTX660 is a potent, non-peptidomimetic antagonist of the cellular and X-linked inhibitors of apoptosis proteins (cIAP1/2 and XIAP)[1-2] , which is currently being tested in a first in human phase I-II study in patients with advanced solid tumors and lymphomas (NCT02503423, Abstract PS1073).
IAPs play a major role in regulating apoptosis as well as modulating NF-κB pathways with IAP antagonists enhancing tumor necrosis factor (TNF) receptor superfamily mediated apoptosis[3] and have been reported to be effective anti-tumor immune enhancers[4].

In response to TNFα, cIAPs ubiquitinate RIP1, promoting the formation of complexes which activate survival signaling through the canonical NF-kB pathway. Simultaneously, formation of the death-inducing signaling complex (DISC), which drives apoptosis, is prevented. Removal of cIAP1/2 allows the DISC to form, leading overall to a switch in TNFα signaling from pro-survival to proapoptotic. Once loss of cIAP1/2 is combined with release of the XIAP-mediated block on caspases, which is essential for full activation of apoptosis, a sustained proapoptotic effect in the presence of TNFα is obtained. Antagonism and subsequent degradation of the cIAP1/2 also leads to the stabilization of NIK (NF-κB-inducing kinase), which activates the noncanonical NF-kB pathway, resulting in enhancement of T-cell co-stimulation [4].

We describe the preliminary characterization of ASTX660 in *in vitro* and *in vivo* pre-clinical models of relevance to T-cell lymphoma, in support of an ongoing clinical study (see poster PS1073) recruiting T-cell lymphoma subjects.

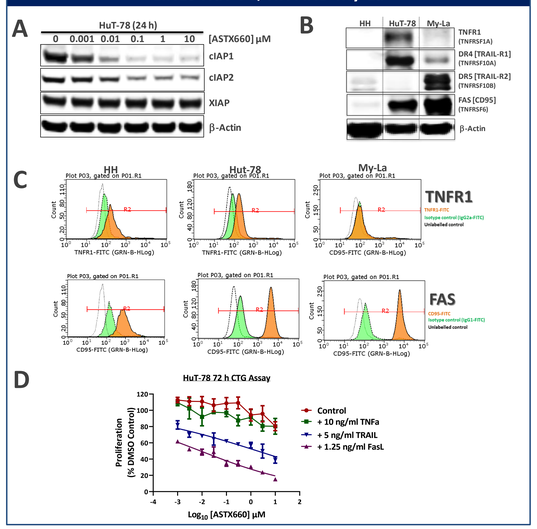
MECHANISM OF ASTX660

- ASTX660 antagonism of cIAP1/2 and XIAP can have direct cytotoxic effects on T-cell lymphoma cells through enhancement of apoptotic signaling initiated by TNFR signaling.
- ASTX660 antagonism in non-transformed T-cells can lead to enhanced activation via the non-canonical NF-κB pathway



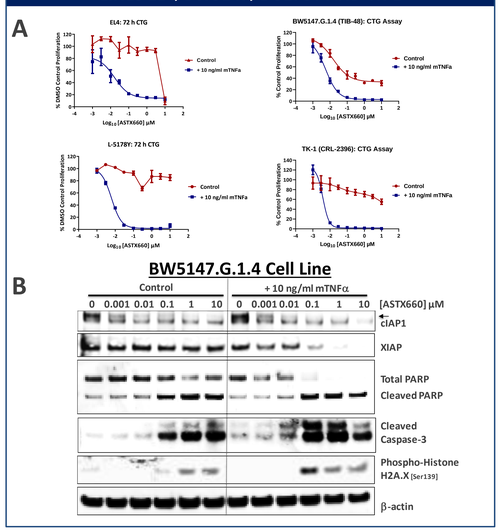
ASTX660 POTENTIATES EXTRINSIC APOPTOTIC SIGNALING TRIGGERED BY DEATH RECEPTOR LIGANDS

IAP AND DEATH RECEPTOR EXPRESSION IN HUMAN CTCL CELL LINES HuT-78, HH AND My-La



- cIAP1 and cIAP2 levels are reduced in HuT-78 cells after treatment with ASTX660 (A).
- CTCL cell lines HuT-78 and My-La have high basal FAS expression and low TNFR1 (B & C).
- HuT-78 cells undergo apoptosis in the presence of ASTX660 treatment with FAS ligand and TRAIL, but not TNFα (D).

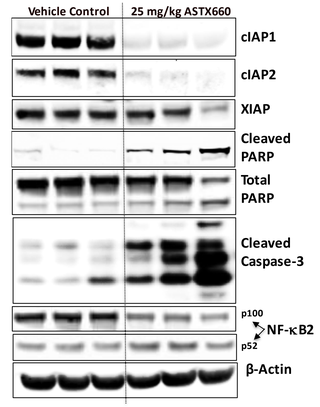
EFFECTS OF ASTX660 ON MURINE T-CELL LYMPHOMA CELL LINES EL4, BW5147, L-5178Y AND TK-1



- Addition of 10 ng/ml mouse TNFα sensitizes all 4 murine T-cell lymphoma cell lines to ASTX660 (A).
- BW5147.G.1.4 cells exhibit sensitivity to single agent ASTX660 (A) and (B).

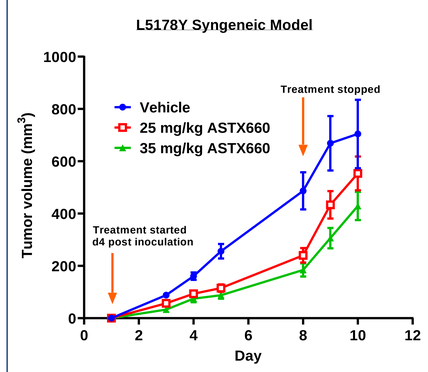
IN VIVO ACTIVITY, PD AND MODULATION OF APOPTOSIS

PHARMACODYNAMIC BIOMARKER MODULATION IN HUMAN HH XENOGRAFTS



- ASTX660 dosing leads to cIAP1/2 depletion, increased apoptosis markers and effects on NF-κB signaling in HH-xenograft bearing SCID mice.

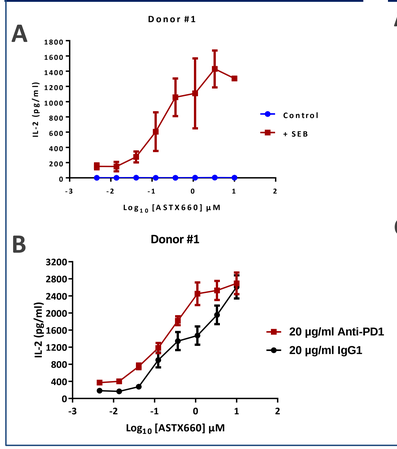
ASTX660 ACYIVTY IN AN L5178Y SYNGENEIC MOUSE T-CELL LYMPHOMA MODEL



- ASTX660 dosing leads to a significant reduction in rate of growth of L5178Y xenografts in DBA/2 mice.

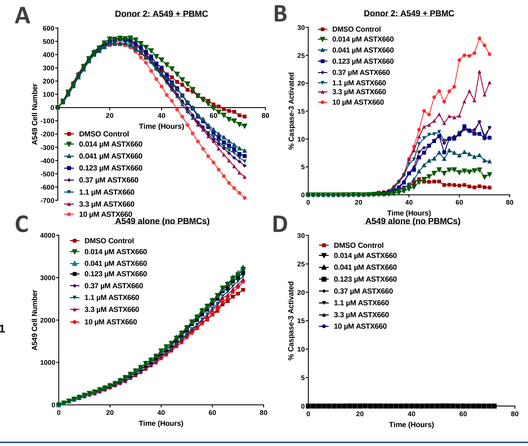
ASTX660 MODULATES IMMUNE CELL KILLING

ASTX660 AUGMENTS IL-2 EXPRESSION FROM SEB-STIMULATED PBMC



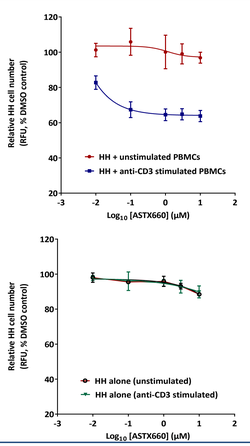
- Staphylococcal enterotoxin B (SEB) treatment leads to polyclonal activation of T-cells.
- ASTX660 potentiates IL-2 release from SEB-stimulated PBMCs (A).
- Anti-PD1 + ASTX660 combination has additive effect on IL-2 release from stimulated PBMCs (B).

ASTX660 AUGMENTS ACTIVATED PBMC KILLING OF A549 CELLS



- ASTX660 enhances killing of the lung cancer cell line A549 when co-cultured with anti-CD3 activated PBMCs (A) with increased caspase-3 activation (B).
- ASTX660 does not enhance killing (C) or caspase-3 activity (D) in A549 cells cultured without PBMCs.

ASTX660 AUGMENTS ACTIVATED PBMC KILLING OF HH CELLS AFTER 48 HOURS



- ASTX660 enhances killing of HH cells (engineered to stably express luciferase) co-cultured with anti-CD3 activated PBMCs for 48 hours (A).
- ASTX660 does not enhance killing of HH cells cultured without PBMCs or with PBMCs in the absence of anti-CD3 stimulation for 48 hours (B).

CONCLUSIONS

- ASTX660 demonstrates anti-tumor activity in T-cell lymphoma models.
- ASTX660 can enhance apoptosis induced by death receptor ligands *in vitro*.
- Co-culture experiments have demonstrated that ASTX660 potentiates tumor killing by PBMCs.
- ASTX660 shows anti-tumor activity in a mouse syngeneic model.
- ASTX660 is currently being evaluated in a Phase I/II study in relapsed/refractory peripheral T-cell lymphoma and cutaneous T-cell lymphoma (NCT02503423) (EHA Poster PS1073).

REFERENCES

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(4) Dougan S.K. and Dougan M. Immunotherapy, 2018, **10**(9):787-796

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