AACR Poster # 5542

ASTX660, a non-peptidomimetic antagonist of cIAP1/2 and XIAP, promotes an anti-tumor immune response in pre-clinical models of T-cell lymphoma.

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INTRODUCTION

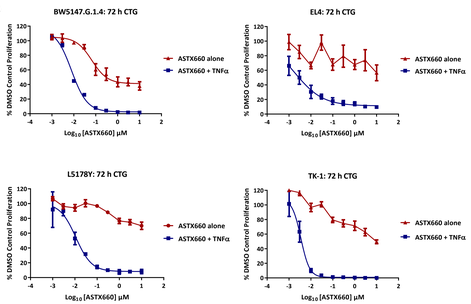
ASTX660 is a potent, non-peptidomimetic antagonist of the cellular and X-linked inhibitors of apoptosis proteins (cIAP1/2 and XIAP), which is currently being tested in a first in human phase I-II study in patients with advanced solid tumors and lymphomas (NCT02503423) where preliminary activity has been described in a group of T-cell lymphomas (1)

Herein, together with its well-characterized pro-apoptotic effect (2), we describe a new role for ASTX660 as an immunomodulatory molecule capable of promoting an anti-tumor immune response in pre-clinical models of T-cell lymphoma. These data add to the description of ASTX660's mode of action and our ongoing understanding of the preliminary clinical efficacy that has been reported.

References:
1. Samaniego F, et al., Hematological Oncology. 2019;37(S2):527.
2. Ward GA et al., Mol Can Ther. 2018;17(7):1381-91

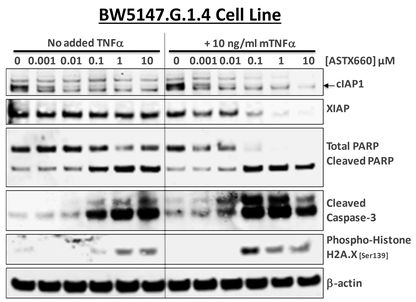
ASTX660 induces cell death in a panel murine T-cell lymphoma cell lines

1A



- The effect of ASTX660 on *in vitro* cell proliferation was investigated in a panel of murine T cell lymphoma cell lines (1A). BW5147.G.1.4 cells exhibit ASTX660 single-agent activity while addition of 10 ng/ml mouse TNFα sensitizes all 4 cell lines to ASTX660.

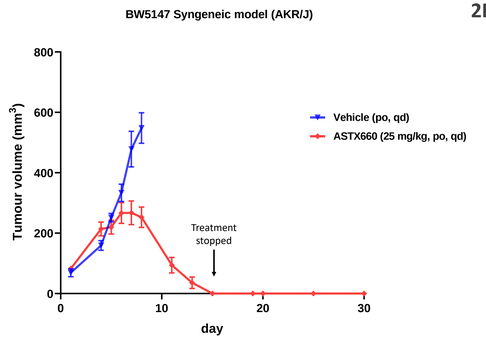
1B



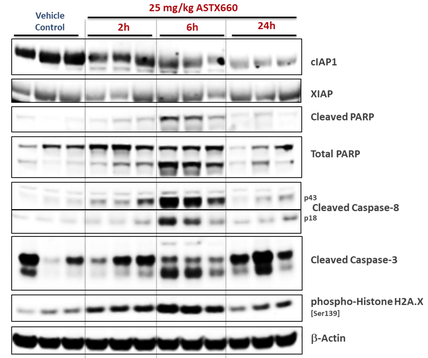
- ASTX660 treatment of BW5147.G.1.4 cells leads to degradation of cIAP1 in absence or presence of TNFα, and induction of apoptotic markers (cleaved PARP and cleaved caspase-3) which are augmented on addition of TNFα (1B).

ASTX660 treatment demonstrates efficacy in *in vivo* models of T-cell Lymphoma

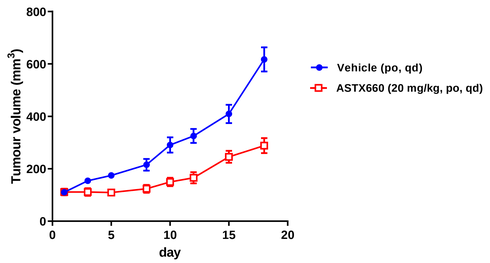
2A



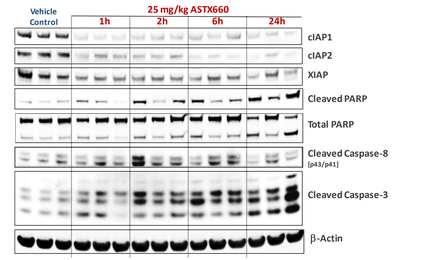
2B



2C



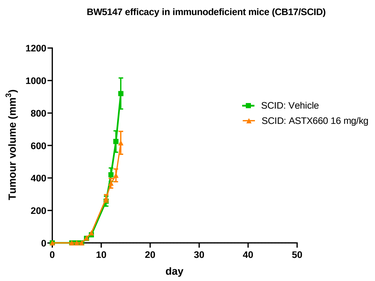
2D



- ASTX660 was efficacious in the BW5147.G.1.4 *in vivo* syngeneic model. Once daily oral dosing for 14 days, at well tolerated doses, led to complete and stable tumor regressions in 100% of mice (2A).
- The effect of ASTX660 on target engagement and apoptosis was confirmed *in vivo* at the protein level by western blot of BW5147.G.1.4 tumor xenograft tissue. A single dose of ASTX660 resulted in cIAP1 protein depletion and an increase of apoptosis markers (2B).
- ASTX660 was also efficacious in the HH human xenograft model of T-cell lymphoma. Once daily oral dosing for 21 days, at a well tolerated dose, led to a reduction in tumor growth (2C).
- The effect of ASTX660 on target engagement and apoptosis was confirmed *in vivo* at the protein level by western blot of HH tumor xenograft tissue. A single dose of ASTX660 resulted in cIAP1 protein depletion and an increase of apoptosis markers (2D).

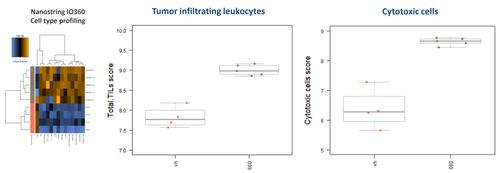
ASTX660 in vivo efficacy requires an intact adaptive immune system

3A

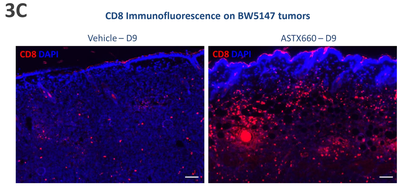


- ASTX660 treatment had no effect on tumor growth of the BW5147.G.1.4 murine T-cell lymphoma cell line in immunodeficient CB17/SCID mice (3A). These data demonstrate an involvement of the adaptive immune system in ASTX660-induced tumor regressions.
- ASTX660 dosing in the syngeneic model increases tumor infiltrating leukocytes and cytotoxic cells as assessed by the Nanostring IO360 platform (3B). CD8 positive cells, assessed by IF, are also increased after ASTX660 dosing (3C).

3B

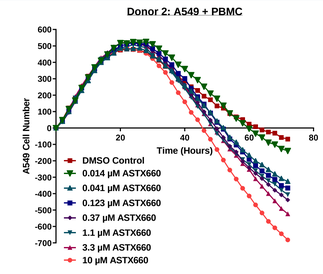


3C

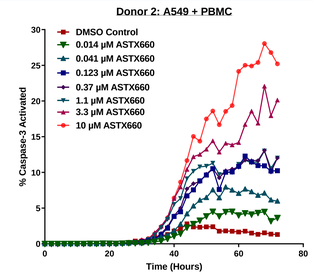


ASTX660 promotes immune-mediated tumor killing

4A

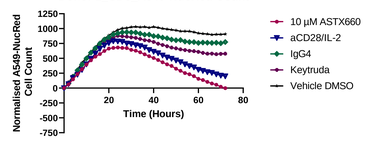


4B

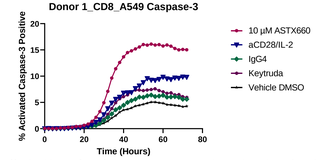


- ASTX660 potentiates anti-CD3 activated PBMC killing of A549-NucRed cells (4A) and enhances caspase-3 activation (4B) in a dose responsive fashion.

4C

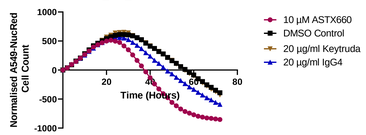


4D

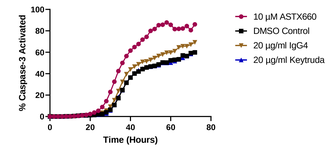


- ASTX660 potentiates anti-CD3 activated $CD8^+$ T cell killing of A549-NucRed cells (4C) and enhances caspase-3 activation (4D).

4E

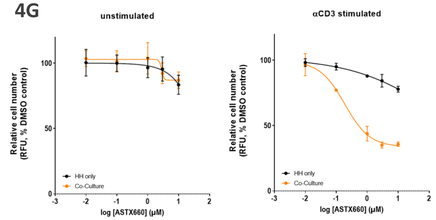


4F



- ASTX660 potentiates IL-15 activated NK cell killing of A549-NucRed cells (4E) and enhances caspase-3 activation (4F).

4G



- ASTX660 potentiates anti-CD3 activated PBMC killing of HH human lymphoma cells (4G) while it has no effect on unstimulated PBMC cultures.

SUMMARY & CONCLUSIONS

- ASTX660 inhibits cell proliferation and induces cell death *in vitro* on a panel of murine T-cell lymphoma cell lines.
- ASTX660 shows *in vivo* efficacy when tested in T-cell lymphoma mouse models. Interestingly, an intact adaptive immune system is required for an effective anti-tumor response.
- *In vitro* co-culture experiments show that ASTX660 potentiates tumor killing by PBMCs.
- These results describe an additional role for ASTX660 as an immunomodulatory molecule capable of promoting an anti-tumor immune response in pre-clinical models of T-cell lymphoma. These insights may allow better understanding of observed clinical response in the T-cell lymphoma patient population.

CONTACT INFORMATION

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Poster presented at AACR 2020 Virtual Annual Meeting II (June 22-24)