Abstract # EP1326

Characterisation of ASTX660, an antagonist of cIAP1/2 and XIAP, in mouse models of T cell lymphoma

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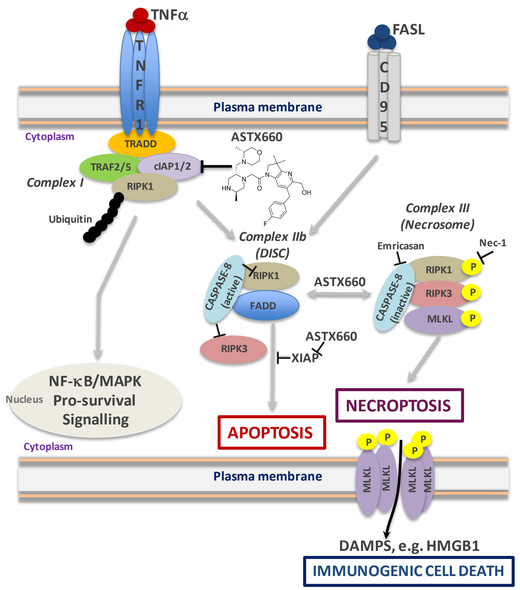
INTRODUCTION

ASTX660 is a potent, non-peptidomimetic antagonist of the cellular and X-linked inhibitors of apoptosis proteins (cIAP1/2 and XIAP) [1], which is currently being tested in a first in human phase I-II study in patients with advanced solid tumor and lymphomas (NCT02503423), with preliminary clinical efficacy having been reported in T cell lymphoma [2]. IAP antagonists have been reported to exhibit broad immuno-modulatory effects on both the innate and adaptive immune systems, leading to them currently being tested in the clinic in combination with cancer immunotherapeutics, such as checkpoint antibodies [3].

OBJECTIVE

Our aim was to profile the activity of ASTX660 in preclinical T cell lymphoma models and to explore, both *in vitro* and *in vivo*, the mechanism of action of ASTX660 in driving direct tumour cell death or immune-cell mediated cell death, and hence in initiating and potentiating a robust and durable anti-tumour immune response.

MECHANISM



ASTX660 has the potential to switch TNFR1 pro-survival signalling towards an apoptotic **or** necroptotic form of programmed cell death. Tumour cells have evolved various checkpoint mechanisms to evade entry into programmed cell death pathways.

ASTX660 treatment can override these checkpoints, leading to apoptotic cell death under conditions in which caspase-8 is in an active form. If caspase-8 activity is blocked, ASTX660 treatment can lead to tumour cells being killed via the necroptotic pathway [4].

Induction of necroptosis in a tumour can drive a potent innate and adaptive immune response which drives the cancer immunity cycle [5], and can lead to robust immune cell-mediated eradication of a tumour.

REFERENCES

1. **Ward GA, Lewis EJ, Ahn JS, Johnson CN, Lyons JF, Martins V, et al.** ASTX660, a Novel Non-peptidomimetic Antagonist of cIAP1/2 and XIAP, Potently Induces TNFα-Dependent Apoptosis in Cancer Cell Lines and Inhibits Tumor Growth. Molecular Cancer Therapeutics. 2018;17(7):1381-91.
2. **Samaniego F, Hollebecque A, Foss F, Lister J, Mita M, Wagner-Johnston N, et al.** Preliminary results of ASTX660, a novel non-peptidomimetic cIAP1/2 and XIAP antagonist, in relapsed/refractory peripheral T cell lymphoma and cutaneous T cell lymphoma. Hematological Oncology. 2019;37(S2):527.
3. **Michie J, Kearney CJ, Hawkins ED, Silke J, Oliaro J.** The Immuno-Modulatory Effects of Inhibitor of Apoptosis Protein Antagonists in Cancer Immunotherapy. Cells. 2020;9(1).
4. **Legrand AJ, Konstantinou M, Goode EF, Meier P.** The Diversification of Cell Death and Immunity: Memento Mori. Molecular cell. 2019;76(2):232-42.
5. **Chen D, Mellman I.** Oncology meets immunology: the cancer-immunity cycle. Immunity. 2013;39(1):1-10

CONTACT INFORMATION

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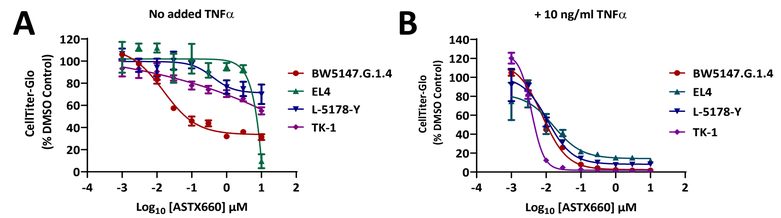
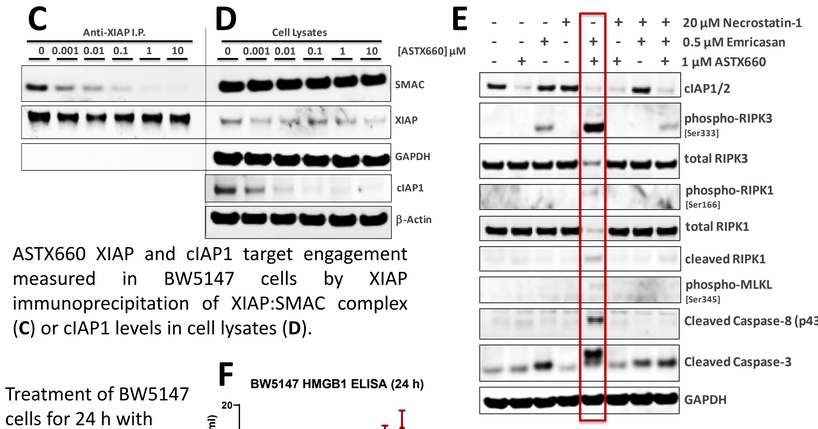
RESULTS: Activity of ASTX660 *in vitro*

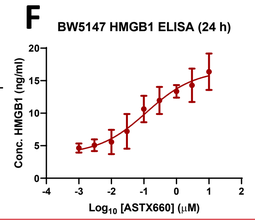


ASTX660 treatment inhibits viability measured in 72 h proliferation (CTG) assay of BW5147 cells in the absence of added TNFα (**A**). The viability of all 4 mouse T cell lymphoma lines is inhibited by ASTX660 in the presence of added mouse TNFα (**B**).



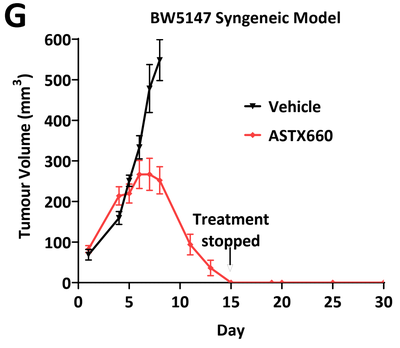
ASTX660 XIAP and cIAP1 target engagement measured in BW5147 cells by XIAP immunoprecipitation of XIAP:SMAC complex (**C**) or cIAP1 levels in cell lysates (**D**).

Treatment of BW5147 cells for 24 h with ASTX660 led to a dose-responsive increase in HMGB1 measured in cell supernatants by ELISA (**F**).

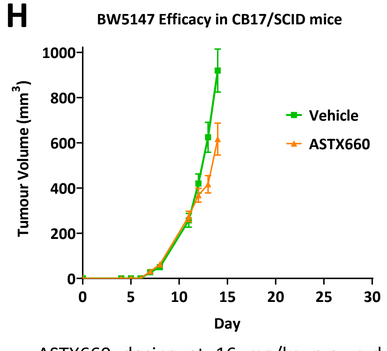


Inhibition of caspase-8 by combination of emricasan along with ASTX660 for 48 h led to induction of necroptosis in BW5147 cells – this was inhibited by RIPK1 inhibition with Necrostatin-1 (**E**).

RESULTS: ASTX660 *in vivo* efficacy



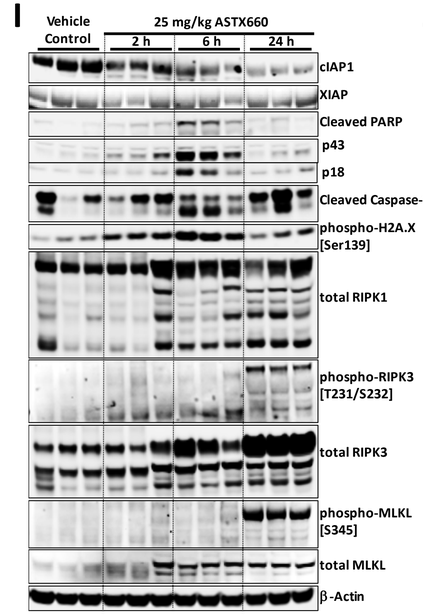
25 mg/kg ASTX660 p.o. q.d. dosing led to complete BW5147 tumour regression in AKR/J immune competent mice (**G**).

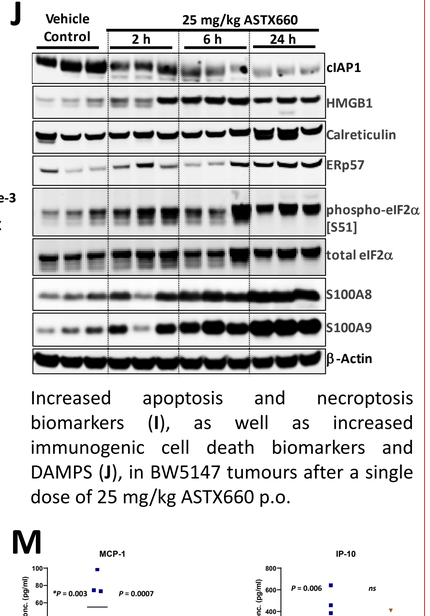


ASTX660 dosing at 16 mg/kg p.o. q.d.* in immunodeficient CB17/SCID bearing BW5147 tumours does not alter tumour growth (**H**).

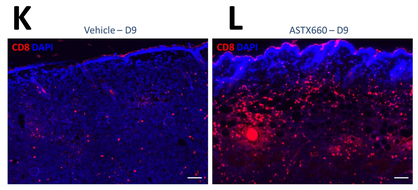
* dose gave equivalent exposure

RESULTS: *In vivo* PD analysis

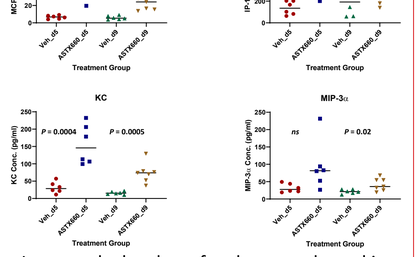




Increased apoptosis and necroptosis biomarkers (**I**), as well as increased immunogenic cell death biomarkers and DAMPS (**J**), in BW5147 tumours after a single dose of 25 mg/kg ASTX660 p.o.



Anti-CD8 immunofluorescence staining of BW5147 tumours after 9 days of 25 mg/kg ASTX660 (**L**) or vehicle (**K**) dosing, showing an increased level of CD8⁺ T cell infiltrate in the ASTX660-dosed mice.



Increased levels of plasma chemokines measured by MSD assay at day 5 or day 9 of ASTX660 compared to vehicle dosing (**M**).

* Two tailed t-test between vehicle and ASTX660 treated groups (ns = $p > 0.05$)

CONCLUSIONS

- ASTX660 can induce necroptosis as a result of IAP antagonism *in vitro* and *in vivo*.
- ASTX660 treatment can cure mice of syngeneic BW5147 tumours *in vivo*, and this requires an intact adaptive immune system.
- ASTX660 treatment increases CD8⁺ T cells infiltration into tumours and systemic chemokine biomarkers.
- We hypothesize that ASTX660 causes tumour cell death by necroptosis, which increases antigen presentation and leads to recruitment of CD8⁺ T cells to the tumour.
- We are describing 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.

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