

Introduction

Tolinapant (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 clinical trial in patients with advanced solid tumours and lymphomas (NCT02503423) [2]. IAP antagonists have been reported to exhibit broad immuno-modulatory effects on both the innate and adaptive immune systems. We have investigated the profile of tolinapant in preclinical T cell lymphoma models and evaluated tolinapant's ability to enhance immune mediated killing of T cell lymphoma cells, both *in vitro* and *in vivo*.

References:

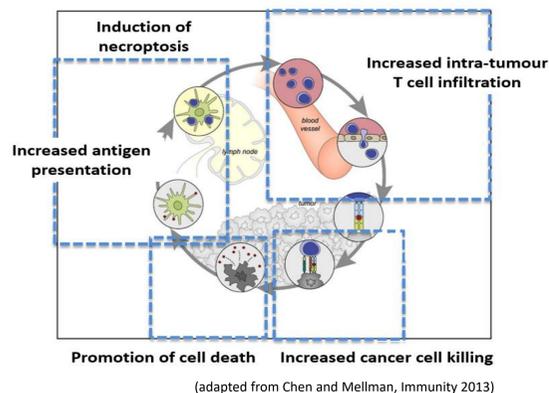
- G Ward et al., 2018, Mol Cancer Therapeutics Jul;17(7):1381-1391
- A Hollebecque et al., 2019, AACR-NCI-EORTC International Conference on Molecular Targets and Cancer Therapeutics

Results and Conclusions

We describe a novel role for tolinapant as an immunomodulatory molecule, capable of enhancing an anti-tumour immune response against preclinical models of T-cell lymphoma *in vitro* and *in vivo*, as evidence by:

- Tolinapant treatment enhanced killing of tumour cells by anti-CD3 antibody-stimulated PBMCs or CD8⁺ cells, or by IL-15-stimulated NK92 cells.
- Tolinapant-dependent target cell killing required direct cell-cell contact.
- Tolinapant treatment of HH-PBMC co-cultures resulted in increased secretion of the pro-inflammatory cytokines, Interleukin (IL)-2, tumour necrosis factor (TNF)- α and interferon (IFN)- γ .
- Immune-directed HH cell killing is at least partially dependent on TNF- α and IFN- γ .
- In vivo* administration of tolinapant resulted in complete regression of mouse syngeneic T cell lymphoma tumours (BW5147.G.1.4) but not in the absence of active adaptive immune system.
- Tolinapant treatment led to immunogenic cell death and an increase in infiltration of immune effector cells in syngeneic BW5147.G.1.4 tumours.

Tolinapant promotes the Cancer-Immunity cycle at multiple steps



Tolinapant promotes immune-mediated tumour cell killing

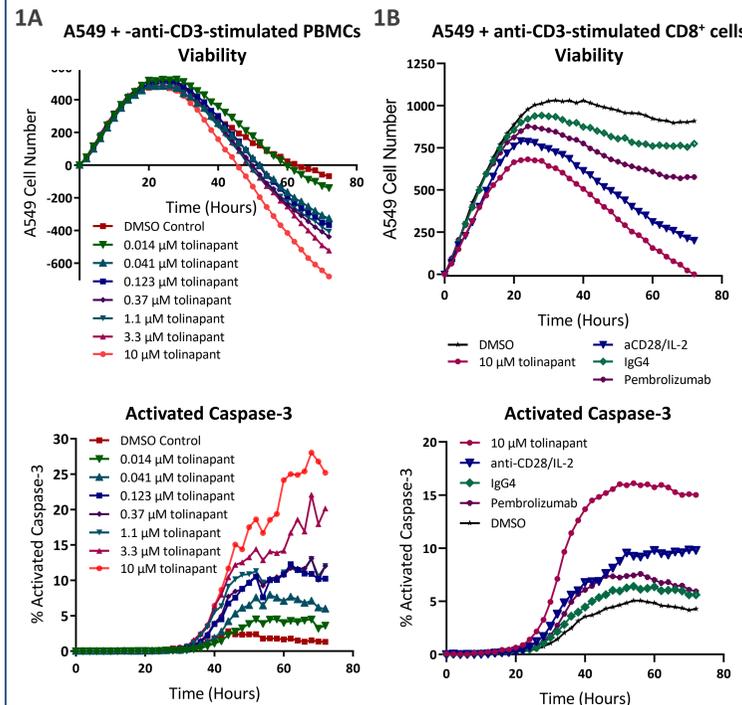


Figure 1: Tolinapant enhanced immune cell killing of A549-NucRed cells. (A) A549 cells were co-cultured with anti-CD3 stimulated PBMCs using increasing concentrations of tolinapant and A549 cell viability and enhanced caspase-3 activation was assessed over time. (B) Tolinapant enhanced anti-CD3 activated CD8⁺ T cell killing of A549 cells which was more pronounced than pembrolizumab treatment. Experiments performed at Charles River, UK.

Tolinapant promotes immune-mediated lymphoma cell killing in co-culture assays which is contact-dependent

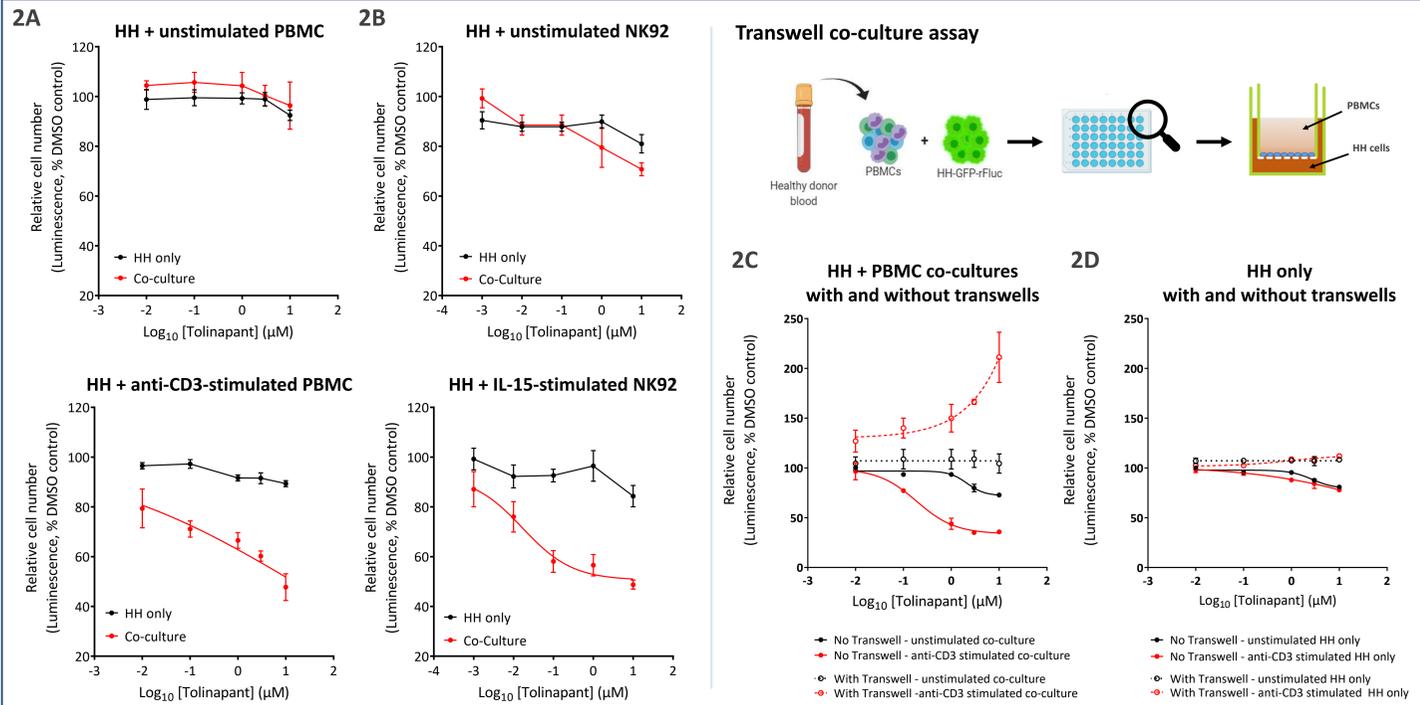


Figure 2: Tolinapant treatment led to reduced viability of the firefly luciferase-expressing human lymphoma cell line, HH, in the presence of anti-CD3-activated PBMCs (A) or IL-15-stimulated NK92 cells (B). No loss of viability was observed when unstimulated PBMCs or NK92 were used. Tolinapant-enhanced HH cell killing required immune cell contact with tumour cells. Anti-CD3 activated PBMCs were either mixed with HH cells or co-cultured with HH cells separated by transwell inserts. (C) Mixed PBMC-HH cultures showed decreased HH cell viability in a dose-dependent manner, while HH cells separated from PBMCs by transwell inserts showed no loss of viability (D). For all experiments, cells were treated with tolinapant for 48 hours.

Tolinapant results in increased proinflammatory cytokine secretion and directs immune-mediated cell killing

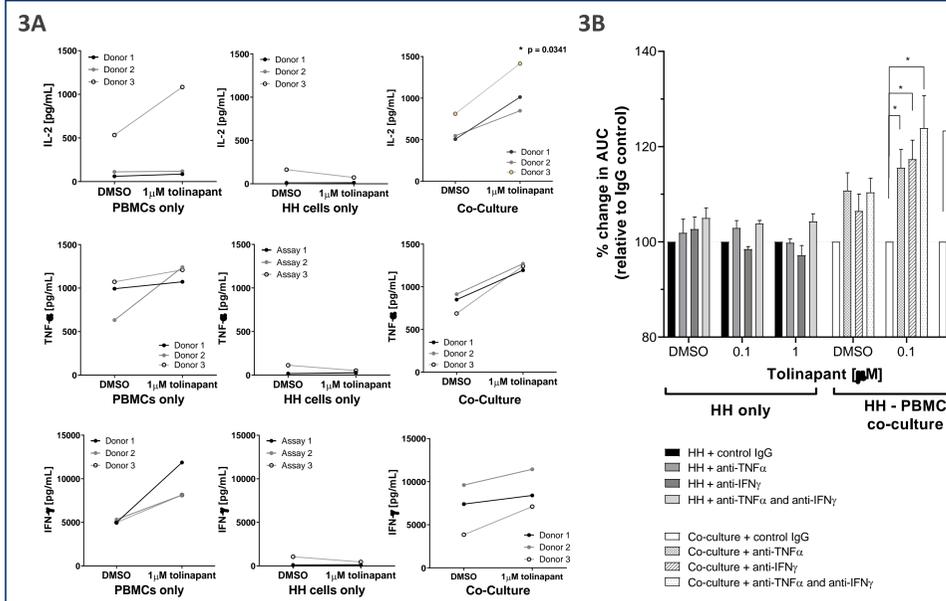


Figure 3: Tolinapant treatment to increased secretion of pro-inflammatory cytokines which modulate immune-directed cell killing. (A) Supernatants from anti-CD3-stimulated HH-PBMC co-cultures were assessed for pro-inflammatory cytokine secretion using MSD (T= 72hrs). (B) Addition of anti-TNF- α or anti-IFN- γ antibodies or a combination of both to HH-PBMC co-cultures resulted in significantly decreased HH target cell killing. HH cell only cultures were not affected. The graph shows relative change in AUC from viability assays similar to Fig 1A (T= 48hrs, n=3).

Tolinapant demonstrates anti-tumour activity in *in vivo* models of T-cell Lymphoma and requires an active adaptive immune system

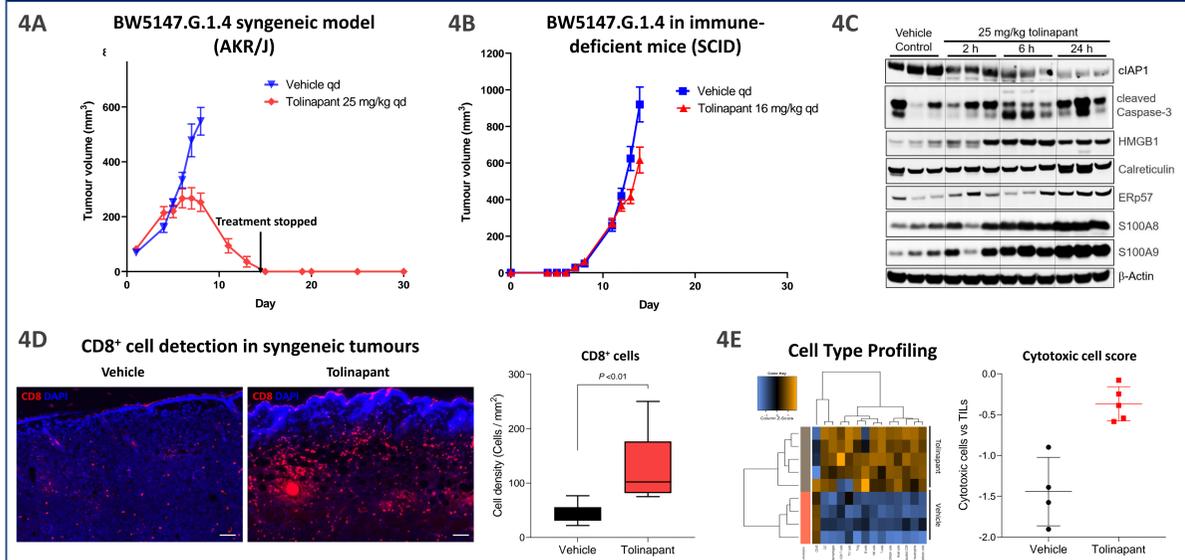


Figure 4: Tolinapant treatment causes regression in syngeneic murine T cell lymphoma but not in the absence of adaptive immune system. (A) Immunocompetent AKR/J mice bearing syngeneic BW5147.G.1.4 tumour were treated with 25 mg/kg via oral gavage once daily for 14 days. Tolinapant treatment led to complete tumour regression in 100% of mice. (B) Immunodeficient SCID mice bearing BW5147.G.1.4 tumour treated similarly showed no tumour regression. (C) Western blots of BW5147.G.1.4 syngeneic tumour lysates prepared after a single dose of 25 mg/kg tolinapant showed an increase in immunogenic cell death biomarkers (alarmin expression, e.g., HMGB1, S100 proteins). (D) Increased tumour infiltration of CD8-positive cells in tolinapant-treated syngeneic tumours were detected with immune fluorescence. (E) Increased infiltration of cytotoxic cells into tolinapant-treated mouse tumours was also detected using the Nanostring IO360 platform.