Abstract P1293

# Combining the IAP antagonist, tolinapant, with a DNA hypomethylating agent enhances anti-tumour mechanisms in preclinical models of T-cell lymphoma

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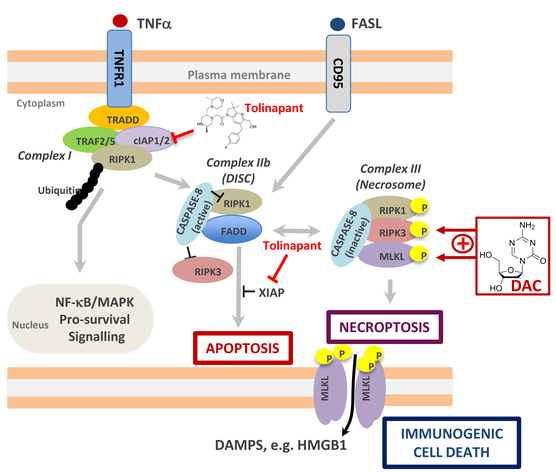
## INTRODUCTION

Tolinapant (ASTX660) is a potent, non-peptidomimetic antagonist of cIAP1, cIAP2 and XIAP [1,2], and has demonstrated immunomodulatory properties in pre-clinical models of T cell lymphoma (TCL) [3]. In an ongoing Phase 2 trial (NCT02503423), tolinapant has shown activity against highly pre-treated peripheral and cutaneous T-cell lymphoma [4].

Hypomethylating agents (HMAs) have also shown clinical responses in some subsets of PTCL [5,6], suggesting thar reduction of methylation can deliver efficacy in PTCL. In addition, HMAs and IAP antagonists show immunomodulatory anti-cancer potential in pre-clinical studies.

Here we have investigated the potential for HMA-induced reversal of epigenetic silencing or altered cell signalling to promote the induction of immunogenic forms of cell death (ICD), such as necroptosis, driven by tolinapant treatment in pre-clinical models of T-cell lymphoma (TCL).

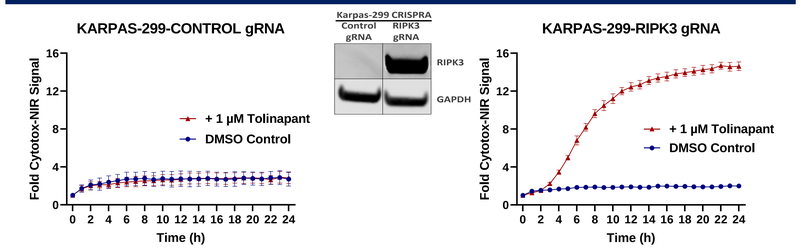
## FIGURE 1 COMBINATION MECHANISM OF ACTION




- Tolinapant induces an immunogenic form of cell death (necroptosis) when necrosome components are expressed.
- Decitabine treatment leads to upregulation of key proteins (e.g. RIPK3 [7]) within the necrosome by direct promoter demethylation or altered interferon signalling.
- The combination of tolinapant and decitabine enhances immunogenic forms of cell death in TCL.

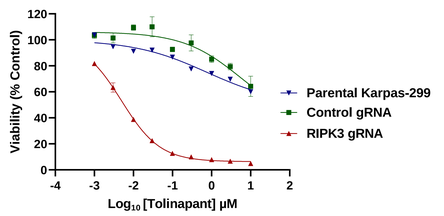
## *IN VITRO EXPRESSION OF RIPK3 IN TCL CELL LINES*

### FIGURE 2A EXPRESSION OF RIPK3 INCREASES LYTIC CELL DEATH IN KARPAS-299 HUMAN TCL CELL LINE



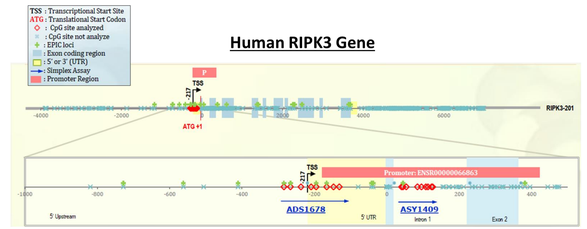

- CRISPRa-directed RIPK3 expression in Karpas-299 enables lytic cell death (cytox staining) on tolinapant treatment.

### FIGURE 2B RIPK3 EXPRESSION REDUCES VIABILITY OF KARPAS-299 CELLS

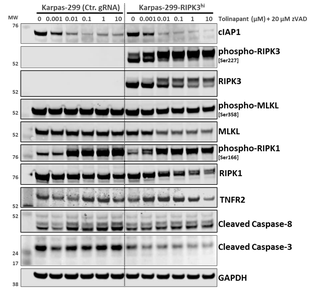



- Karpas-299 cells expressing RIPK3 lose viability on treatment with tolinapant.

### FIGURE 5 DAC TREATMENT REDUCES HUMAN RIPK3 PROMOTER METHYLATION

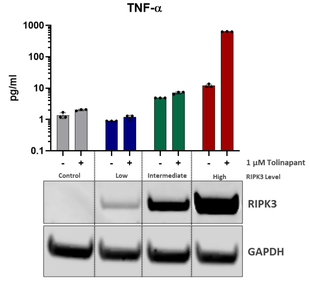



### FIGURE 3A NECROPTOSIS INDUCTION ON TREATMENT OF KARPAS-299-RIPK3 CELLS WITH TOLINAPANT



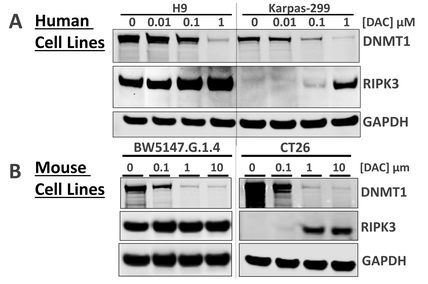

- Karpas-299-RIPK3 cells undergo necroptosis when treated with tolinapant as demonstrated by an increase in phospho-RIPK3.

### FIGURE 3B INCREASED CYTOKINE SECRETION IN KARPAS-299-RIPK3 CELLS ON TREATMENT WITH TOLINAPANT

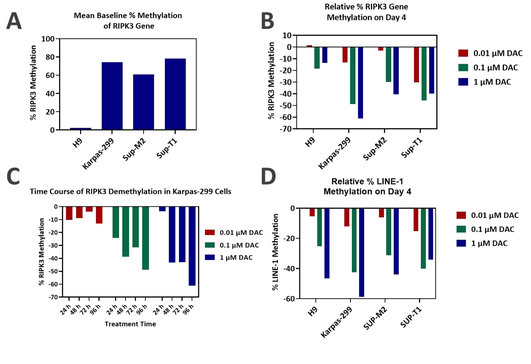



- Karpas-299 cells expressing more RIPK3 secrete more TNF-α on treatment with tolinapant compared to the control Karpas-299 cell line.

### FIGURE 4 DAC TREATMENT LEADS TO RIPK3 RE-EXPRESSION IN HUMAN AND MOUSE TCL CELL LINES



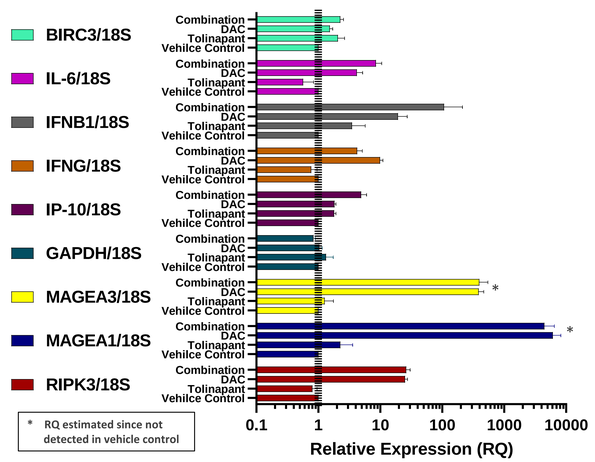

- Human (A) and mouse (B) cell lines were treated with DAC for 4 or 2 days, respectively. RIPK3 was detected in DAC-treated Karpas-299 (A) or CT-26 cells (B) in which RIPK3 is normally silenced; whilst H9 (A) and BW5147.G.1.4 cells (B) have high RIPK3 basal expression.



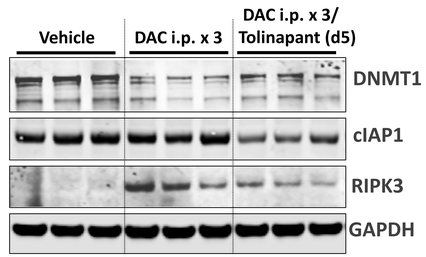

- Human H9 cells have low basal methylation of the RIPK3 gene promoter (A).
- Decitabine treatment of human TCL cell lines leads to RIPK3 gene promoter (B & C) and LINE-1 (D) demethylation by pyrosequencing (EpigenDX).

## *IN VIVO RE-EXPRESSION OF RIPK3 IN KARPAS-299 XENOGRAFTS*

### FIGURE 6A DOSING WITH DAC AND/OR TOLINAPANT LEADS TO GENE EXPRESSION CHANGES IN KARPAS-299 XENOGRAFTS *IN VIVO* BY QPCR




### FIGURE 6B DAC DOSING INDUCES RIPK3 RE-EXPRESSION IN KARPAS-299 XENOGRAFTS *IN VIVO*




- Comparative expression by real-time qPCR of RNA isolated from Karpas-299 xenografts demonstrates upregulation of interferons, other cytokines/chemokines and cancer testis antigens by decitabine (Figure 6A). Some of the biomarkers are further enhanced by the combination.
- Decitabine treatment of mice bearing Karpas-299 xenografts leads to RIPK3 re-expression detected by Western blotting of tumour lysates (Figure 6B).

## CONCLUSIONS

- Re-expression of RIPK3 using CRISPR activation in the Karpas-299 cell line led to increased immunogenic cell death after treatment with tolinapant. This not only highlights the importance of RIPK3 in tolinapant-driven cell death, but also provides rationale for combining tolinapant with agents that can increase RIPK3 expression.
- We confirmed that re-expression of RIPK3 in TCL cell lines can be achieved by decitabine (hypomethylating agent) treatment of TCL cell lines.
- Necroptosis signalling in TCL is induced by decitabine and tolinapant alone and by the combination [8].
- HMA has the potential to drive an immunomodulatory activity [9] and we demonstrate further enhancement with tolinapant.
- Collectively, the data presented here suggest a mechanistic rationale for testing the combination of tolinapant and decitabine in TCL.
- A tolinapant and ASTX727 (oral decitabine) clinical study in PTCL is planned.

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Poster presented at the EHA Congress, Vienna, Austria, 9 - 12th June 2022

MS, GW, SJ, ZZ, MD, AB, HS, JT, IP, AB, JL & TS are full-time employees of Astex Pharmaceuticals

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