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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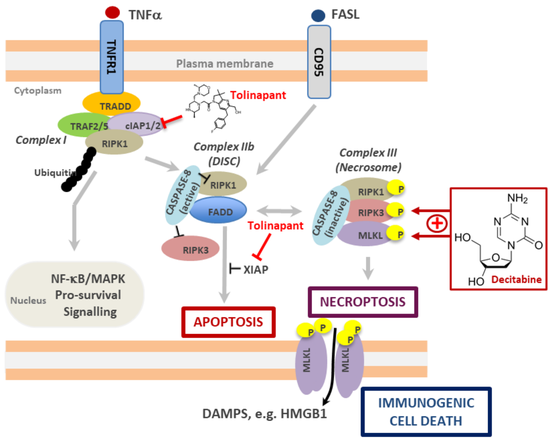
INTRODUCTION

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 that 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 TCL.

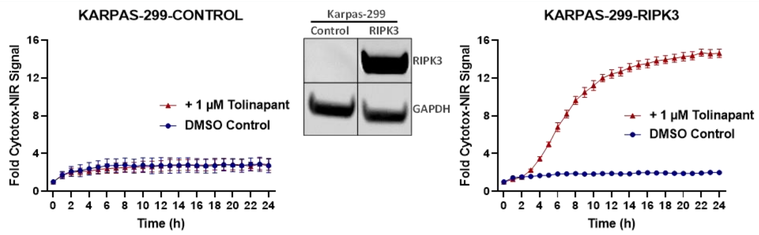
FIGURE 1 COMBINATION MECHANISM OF ACTION



- Tolinapant induces an immunogenic form of cell death (necroptosis) when necrosome components are expressed.
- Decitabine (DAC) 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.

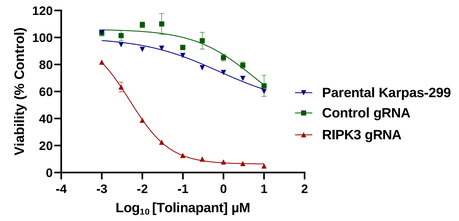
IN VITRO EXPRESSION OF RIPK3 IN TCL CELL LINES

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



- Genetically-manipulated RIPK3 expression in Karpas-299 enables lytic cell death (cytotox-NIR 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

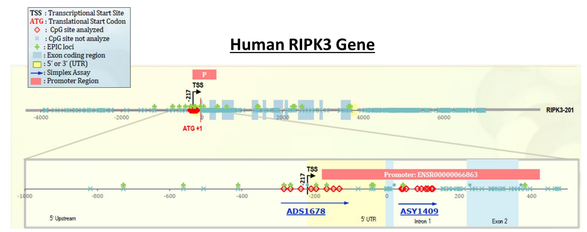
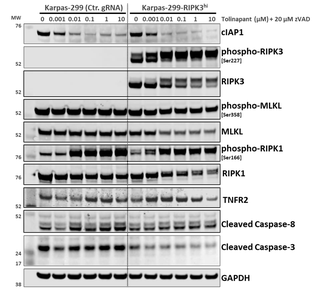
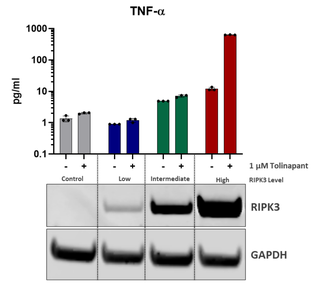


FIGURE 3A NECROPTOSIS INDUCTION ON TREATMENT OF KARPAS-299-RIPK3 CELLS WITH TOLINAPANT



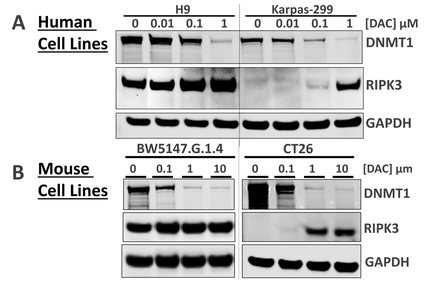
- Karpas-299-RIPK3 cells undergo necroptosis when treated with tolinapant as demonstrated by an increase in phospho-RIPK3.

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

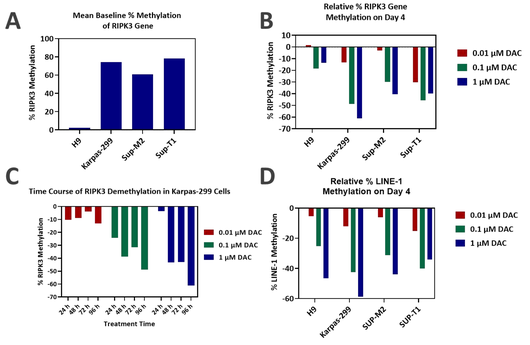


- Karpas-299 cells expressing more RIPK3 secrete more TNF-α on treatment with tolinapant compared to the control Karpas-299 cell line.

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

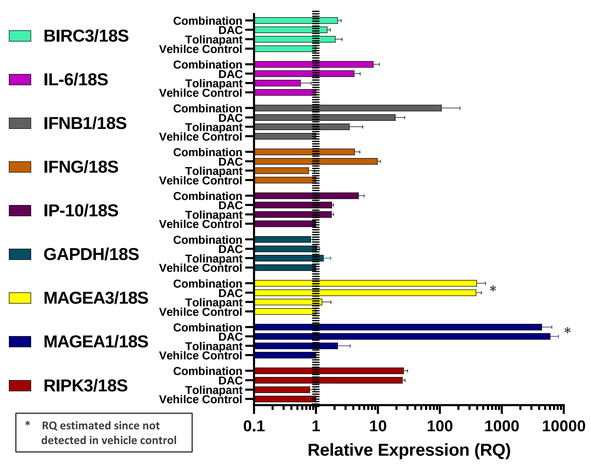
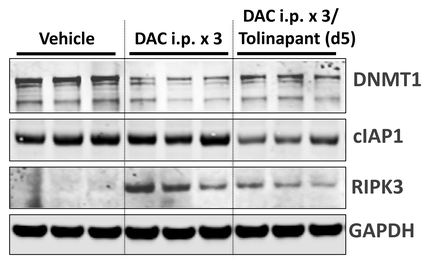


FIGURE 6B DAC DOSING INDUCES RIPK3 RE-EXPRESSION IN KARPAS-299 XENOGRAFTS *IN VIVO*



- 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

- Genetically-manipulated expression of RIPK3 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 clinically testing the combination of tolinapant and decitabine in TCL.
- A tolinapant and ASTX727 (oral decitabine and cedazauridine) clinical study in PTCL is planned.

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Related poster at EHA 2022: P1278 Manavalan *et al.*

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

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