Abstract #1439

Epigenetic priming by hypomethylation enhances the immunogenic potential of tolinapant in T-cell lymphoma

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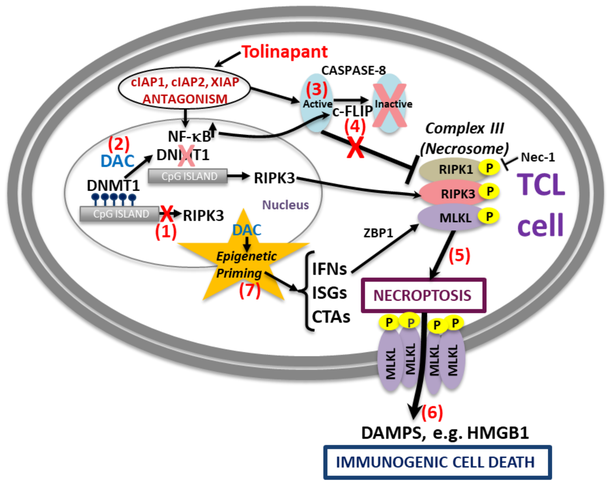
INTRODUCTION

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], suggesting that hypermethylation plays a role in PTCL pathology. HMAs have shown 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).

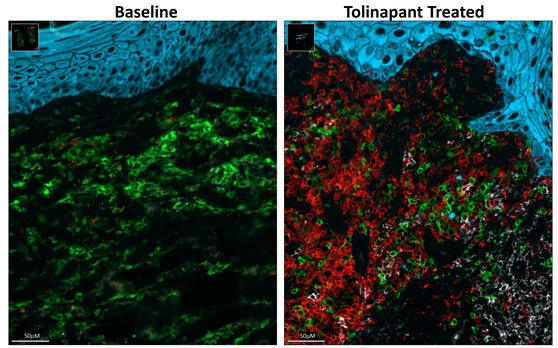
FIGURE 1 COMBINATION MECHANISM OF ACTION



1. RIPK3 and MLKL can be silenced by promoter methylation
2. DAC (decitabine) can reverse the hypermethylation and lead to re-expression of RIPK3 and MLKL
3. Caspase-8 activation during apoptosis blocks necroptosis
4. Tolinapant treatment leads to upregulation of c-FLIP (via NF-κB signalling) and inactivation of caspase-8
5. RIPK1, RIPK3 and MLKL phosphorylation leading to Complex III formation, enabling necroptosis
6. Necroptosis leads to release of DAMPS and ICD
7. DAC treatment leads to further transcriptional changes which favour ICD, including upregulation of interferons (IFNs) and interferon stimulated genes (ISGs), plus upregulation of cancer testis antigens (CTAs)

TOLINAPANT IS AN IMMUNOMODULATOR IN THE CLINIC (NCT02503423 CTCL/PTCL TRIAL)

FIGURE 2: TOLINAPANT-INDUCED CHANGE IN TME OF CTCL LESION



Multiplex immunofluorescence analysis of CTCL patient skin biopsies taken at screening (baseline images on left) and after 1 cycle of tolinapant treatment (right).

Blue: cytokeratin in skin cells

Green: $CD4^+$ non-transformed T cells and lymphoma cells

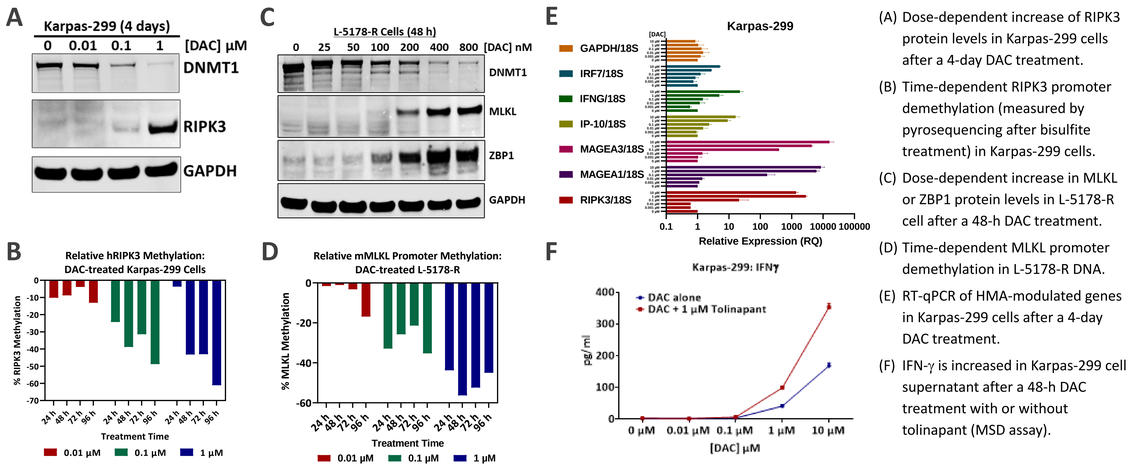
Red: $CD8^+$ T cells;

White: $CD20^+$ B cells

Customized mIF assay on Lunaphore COMET™ platform (Propath UK)

IN VITRO ACTIVITY OF TOLINAPANT AND DECITABINE IN TCL CELL LINES

FIGURE 3: DAC-INDUCED HYPOMETHYLATION INCREASES NECROSOME PROTEIN LEVELS, INTERFERON SIGNALLING AND NEOANTIGEN EXPRESSION



(A) Dose-dependent increase of RIPK3 protein levels in Karpas-299 cells after a 4-day DAC treatment.

(B) Time-dependent RIPK3 promoter demethylation (measured by pyrosequencing after bisulfite treatment) in Karpas-299 cells.

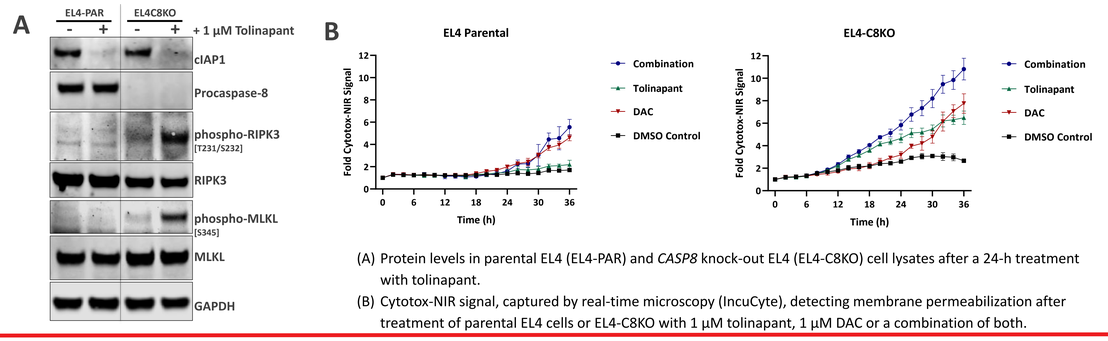
(C) Dose-dependent increase in MLKL or ZBP1 protein levels in L-5178-R cell after a 48-h DAC treatment.

(D) Time-dependent MLKL promoter demethylation in L-5178-R DNA.

(E) RT-qPCR of HMA-modulated genes in Karpas-299 cells after a 4-day DAC treatment.

(F) IFN-γ is increased in Karpas-299 cell supernatant after a 48-h DAC treatment with or without tolinapant (MSD assay).

FIGURE 4: LOSS OF CASPASE-8 DRIVES NECROPTOSIS IN TCL CELLS AND DAC ENHANCES IMMUNOGENIC CELL DEATH INDUCED BY TOLINAPANT

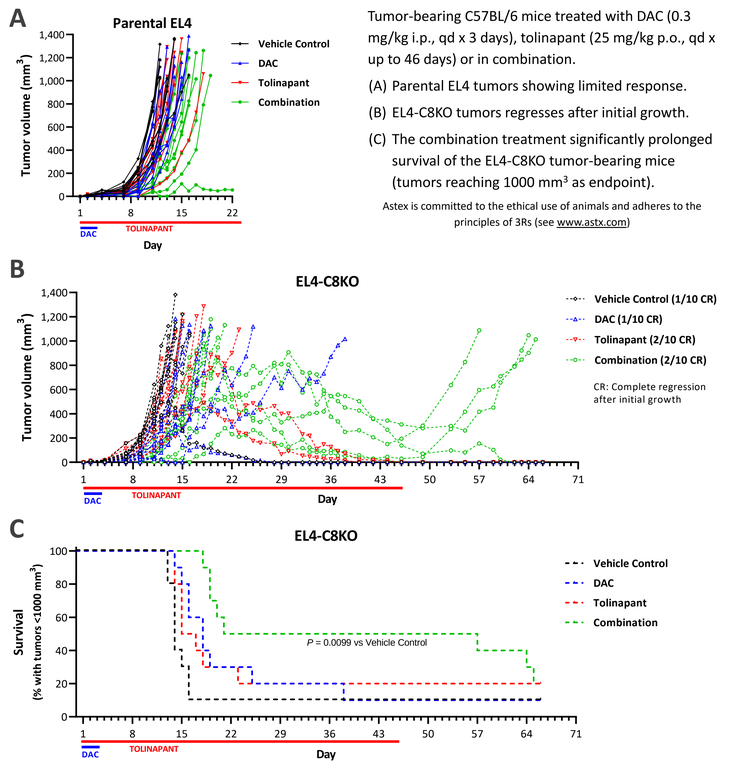


(A) Protein levels in parental EL4 (EL4-PAR) and *CASP8* knock-out EL4 (EL4-C8KO) cell lysates after a 24-h treatment with tolinapant.

(B) Cytotox-NIR signal, captured by real-time microscopy (IncuCyte), detecting membrane permeabilization after treatment of parental EL4 cells or EL4-C8KO with 1 μM tolinapant, 1 μM DAC or a combination of both.

IN VIVO COMBINATION ACTIVITY IN A MOUSE SYNGENEIC TCL MODEL

FIGURE 5: A COMBINATION OF TOLINAPANT PLUS DAC (DECITABINE) DRIVES INCREASED CONTROL IN NECROPTOSIS-ENABLED MODEL OF TCL (EL4-C8KO)



Tumor-bearing C57BL/6 mice treated with DAC (0.3 mg/kg i.p., qd x 3 days), tolinapant (25 mg/kg p.o., qd x up to 46 days) or in combination.

(A) Parental EL4 tumors showing limited response.

(B) EL4-C8KO tumors regresses after initial growth.

(C) The combination treatment significantly prolonged survival of the EL4-C8KO tumor-bearing mice (tumors reaching 1000 mm^3 as endpoint).

Astex is committed to the ethical use of animals and adheres to the principles of 3Rs (see www.astx.com)

CONCLUSIONS

- CTCL tumor infiltration with $CD8^+$ T cells in clinical trial samples from PTCL patients confirms tolinapant's immunomodulatory modality.
- *In vitro* HMA treatment of TCL cell lines leads to promoter demethylation and re-expression of RIPK3 as described for other cancer cell lines [6].
- Increased interferon signalling and neoantigen expression (e.g., MAGEA1 and MAGEA3) by HMA treatment of TCL cell lines highlights potential for driving immunomodulatory activity in the tumor microenvironment [7].
- The combination of tolinapant and decitabine enhanced lytic cell death *in vitro* and significantly prolonged survival of the EL4-C8KO tumors *in vivo*.
- Collectively, the data presented here suggest a mechanistic rationale for the current clinical trial testing the combination of tolinapant and ASTX727 (oral decitabine/cedazuridine) in PTCL (ASCERTAIN-P, NCT05403450).

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