DUAL mTORC1/2 INHIBITION BY TORIN 2 INDUCES CYTOTOXIC AND CYTOSTATIC EFFECTS IN *IN VITRO* MODELS OF CHRONIC LYMPHOCYTIC LEUKAEMIA

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Poster #1806

Background

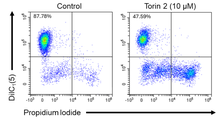
- The PI3K/AKT/mTOR axis has been implicated in the pathogenesis of multiple haematological malignancies including CLL [1, 6].
- Clinical trials and pre-clinical research has shown that targeting PI3K or AKT results in intolerable toxicity or diminished efficacy, respectively [2-4, 8].
- Inhibitors against mTORC1 or pan-mTOR inhibitors with higher affinity for mTORC1 have shown varied biological effects in CLL clinical trials [5, 7].

We aimed to investigate the potential efficacy of other pan-mTOR inhibitors in CLL

Methods

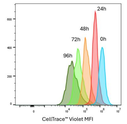
Flow cytometry

Viability



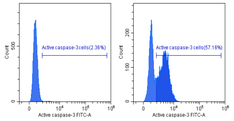
DiIC$_1$(5), Propidium iodide

Proliferation



CellTrace™ Violet kit

Active caspase-3



Antibody staining

Western blotting P-p70S6K (Thr389), Total p70S6K, P-Akt (Ser473), Total Akt

Results

1. PI3K, AKT, and other mTOR inhibitors were less effective compared to Torin 2

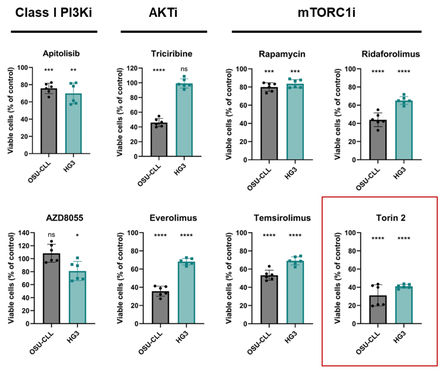


Figure 1. Torin 2 outperformed other inhibitors of PI3K, AKT, and mTOR.
CLL cell lines (OSU-CLL and HG3) were incubated with 10 μM of each drug for 48 hours with six technical replicates per cell line. Viable cells were measured using a CellTiter™ Glo assay and EnSight™ plate reader. Data were normalised to vehicle (DMSO) controls and are presented as mean ± S.D. Statistical significance was determined by unpaired t-test (*, p<0.05; **, p<0.01; ***, p<0.001; ****, p<0.0001; ns, not significant).

Results

2. Torin 2 exhibits cytotoxic and cytostatic effect against CLL cell lines

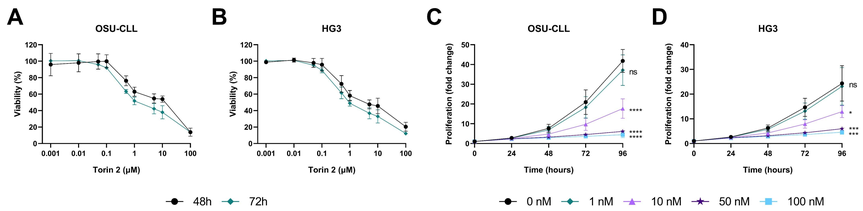


Figure 2. Torin 2 reduces survival and proliferation of CLL cells.
CLL cell lines (OSU-CLL and HG3) were incubated with varying doses of Torin 2 for up to 96 hours. Viability (A-B) was determined by DiIC$_1$(5) and propidium iodide staining and were normalised to untreated controls. Proliferation (C-D) was determined using the CellTrace™ Violet proliferation kit and normalised to 0h fluorescence. Data for both assays was acquired using flow cytometry. An ordinary one-way ANOVA was performed to determine statistically significant differences in proliferation between Torin 2-treated cells and untreated controls at 96 hours (*, p<0.05; ***, p<0.001; ****, p<0.0001; ns, not significant).

3. Cell death induced by Torin 2 is caspase-mediated

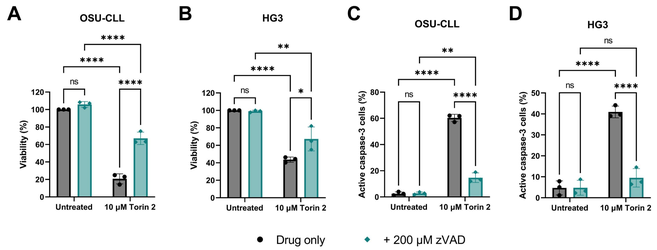


Figure 3. Effects of Torin 2 are caspase-mediated.
CLL cell lines (OSU-CLL and HG3) were preincubated with media only or 200 μM Z-VAD-FMK (zVAD) for 1 hour prior to treatment with 10 μM Torin 2 for 48 hours. Viability (A-B) was determined by DiIC$_1$(5) and propidium iodide staining and normalised to untreated controls. Percentage of cells with active caspase-3 (C-D) was determined by antibody staining on fixed and permeabilised cells. Data for both assays was acquired using flow cytometry. Data are mean ± S.D of three independent experiments, and statistical significance was determined by two-way ANOVA with multiple comparisons (*, p<0.05; **, p<0.01; ****, p<0.0001; ns, not significant).

4. mTORC1 and mTORC2 signalling is blocked by Torin 2

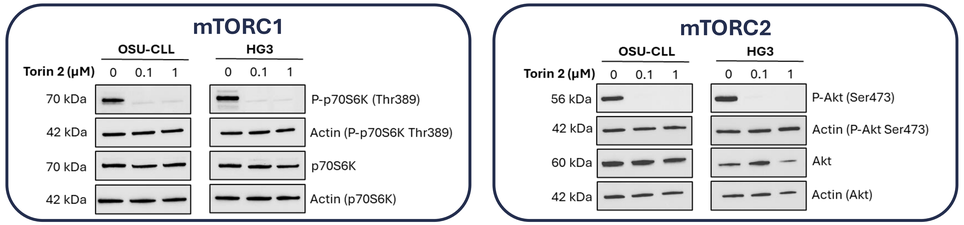


Figure 4. Torin 2 blocks mTORC1 and mTORC2 signalling.
Western blot analysis of CLL cell lines (OSU-CLL and HG3) treated for 1 hour with 0, 0.1, or 1 μM Torin 2. Cells were lysed in the presence of protease and phosphatase inhibitors before 20 μg protein from each lysate was resolved on a stain-free gel. Proteins were transferred to a nitrocellulose membrane and blocked in TBST + 5% skim milk powder. Membrane was probed overnight with primary antibodies of interest then incubated with HRP-conjugated secondary antibodies. Membranes were developed with Clarity Western ECL substrate and imaged using a ChemiDoc Imaging system.

5. Torin 2 is toxic to patient CLL cells in an *in vitro* model of the lymph node microenvironment

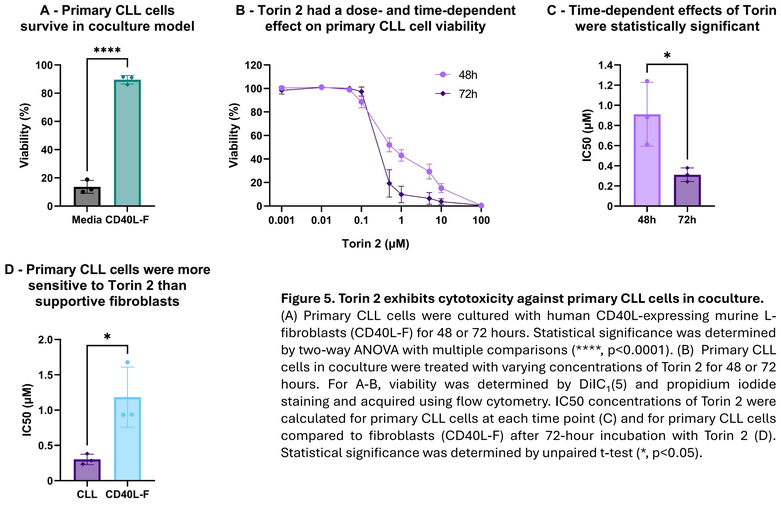


Figure 5. Torin 2 exhibits cytotoxicity against primary CLL cells in coculture.
(A) Primary CLL cells were cultured with human CD40L-expressing murine L-fibroblasts (CD40L-F) for 48 or 72 hours. Statistical significance was determined by two-way ANOVA with multiple comparisons (****, p<0.0001). (B) Primary CLL cells in coculture were treated with varying concentrations of Torin 2 for 48 or 72 hours. For A-B, viability was determined by DiIC$_1$(5) and propidium iodide staining and acquired using flow cytometry. IC50 concentrations of Torin 2 were calculated for primary CLL cells at each time point (C) and for primary CLL cells compared to fibroblasts (CD40L-F) after 72-hour incubation with Torin 2 (D). Statistical significance was determined by unpaired t-test (*, p<0.05).

Conclusions

- Torin 2 outperformed other inhibitors targeting the PI3K/Akt/mTOR axis, some of which had been previously explored in CLL.
- Torin 2 induced both cytotoxic and cytostatic effects in CLL cell lines.
- mTORC1 and mTORC2 signalling was blocked by Torin 2.
- Primary mechanism of Torin 2-induced cell death was caspase-mediated apoptosis.
- Torin 2 had significant cytotoxic effects on primary CLL cells despite supportive fibroblast layer.

References

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