Preclinical characterization of LOXO-338, a novel, oral and selective BCL2 inhibitor

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Preclinical characterization of LOXO-338, a novel, oral and selective BCL2 inhibitor

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Background

- Dysregulation of BCL2 family proteins is implicated in the pathophysiology of various hematologic malignancies.
- Venetoclax is the only BCL2 inhibitor approved for the treatment of chronic lymphocytic leukemia/small lymphocytic lymphoma and acute myeloid leukemia.
- LOXO-338 is a novel, orally bioavailable BCL2 small molecule inhibitor, designed to achieve selectivity over BCL-xL to avoid dose-limiting thrombocytopenia associated with BCL-xL inhibition.[1]
- LOXO-338 is being developed for the treatment of patients with previously treated advanced hematologic malignancies.
- Combination therapy of LOXO-338 with pirtobrutinib (LOXO-305), the next generation, non-covalent, highly selective and potent BTK inhibitor, provides a promising strategy to enhance therapeutic efficacy and overcome resistance of covalent BTK inhibitors.
- Here we describe the *in vitro* and *in vivo* pharmacological profile of LOXO-338, in comparison to venetoclax, to support its advancement into clinical trials.

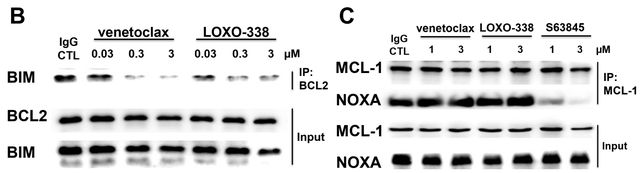
Materials and Methods

- *Homogeneous time-resolved fluorescence (HTRF):* Compounds were incubated with the target proteins for 15 mins at room temperature. HTRF donor and acceptor (Cisbio binding assay kit) were added for a 2 hr reaction and then fluorescence was measured.
- *Co-immunoprecipitation:* RS4;11 and Karpas 299 cells were treated with compounds for 4 hrs. Cell lysates were collected for co-immunoprecipitation.
- *Mouse xenograft models:* Tumors were established by subcutaneous injection of: DOHH2 cells into BALB/c-nude mice; MV4-11 cells into BALB/c-nude mice; RS4;11 cells into NOD-SCID mice; and TMD8 cells into NOD SCID mice. When tumors reached a volume of 100–150 mm^3 for DOHH2 and MV4-11, 100–200 mm^3 for RS4;11, and 400 mm^3 for TMD8, mice were randomized into control and treatment groups.
- *In vitro combination:* REC-1 and TMD8 cells were treated with compounds for 72 hrs. Cell viability was measured using CellTiter-Glo (Promega).

Fig 1. LOXO-338 selectively inhibits BCL2 interaction with pro-apoptotic proteins BAK and BIM

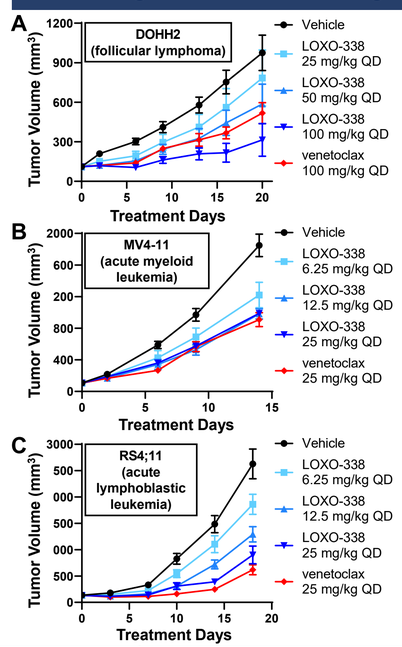
A

Target	LOXO-338 IC_{50} nM (SD)	Venetoclax IC_{50} nM (SD)
BCL2/BAK	4.5 (0.4)	3.3 (0.0)
MCL-1/BAK	>10,000	>10,000
BCL-xL/BAK	49.6 (0.9)	32.6 (0.9)



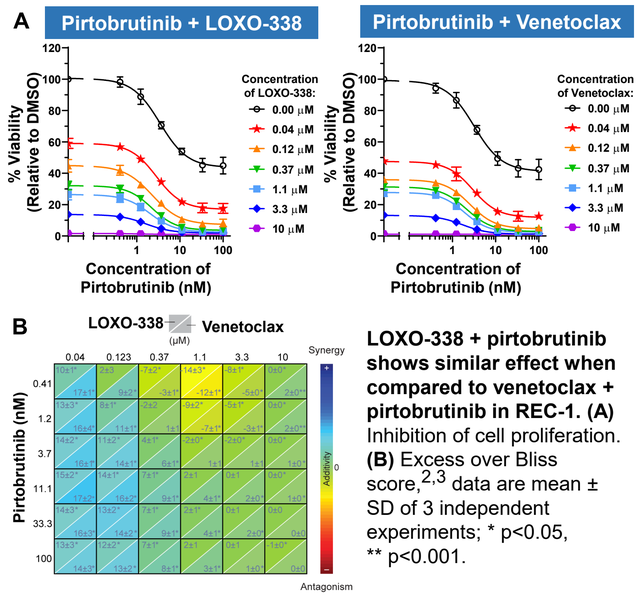
LOXO-338 shows comparable activity as venetoclax to selectively inhibit BCL2 over other BCL2 family members. (A) LOXO-338 inhibits BCL2/BAK (>10-fold selectivity) compared to BCL-xL/BAK interaction and has no effect on MCL-1/BAK interaction in HTRF assays. **(B-C)** LOXO-338 inhibits BCL2/BIM interaction in a dose-dependent manner and has no effect on MCL-1/NOXA interaction in co-immunoprecipitation assays (**B** - RS4;11 and **C** - Karpas 299 cells). MCL-1 inhibitor, S63845, was used as an assay control.

Fig 2. LOXO-338 is well-tolerated and inhibits tumor growth in mouse xenograft models



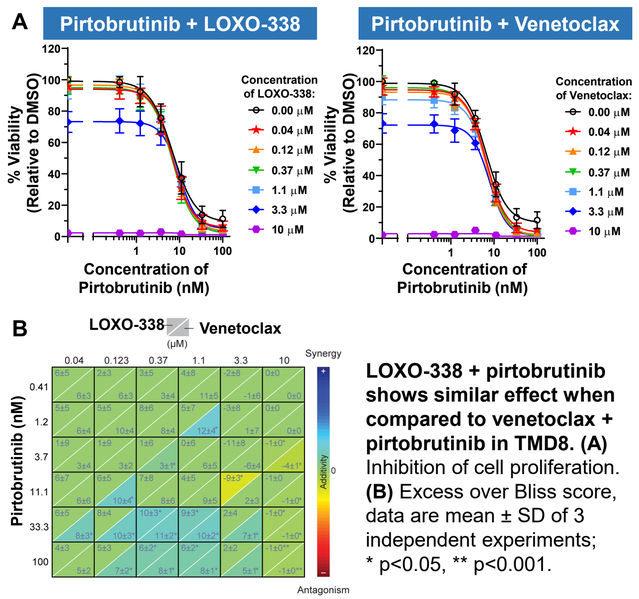
LOXO-338 was well-tolerated by the tumor-bearing mice and no significant weight loss was observed (data not shown)**. The anti-tumor potency of LOXO-338 is similar to comparable doses of venetoclax. (A-C)** Dose-dependent tumor growth inhibition by oral administration of LOXO-338 in DOHH2, MV4-11, and RS4;11 mouse xenograft models. Data are mean ± SEM (n≥6 mice/group).

Fig 3. *In vitro* combination of LOXO-338 + pirtobrutinib demonstrates mild synergy in REC-1 human MCL model



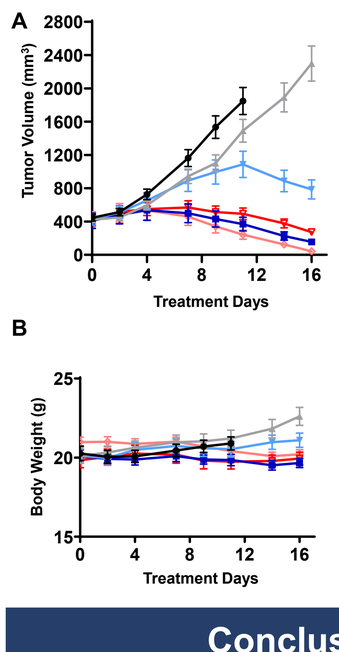
LOXO-338 + pirtobrutinib shows similar effect when compared to venetoclax + pirtobrutinib in REC-1. (A) Inhibition of cell proliferation. **(B)** Excess over Bliss score,[2,3] data are mean ± SD of 3 independent experiments; * p<0.05, ** p<0.001.

Fig 4. *In vitro* combination of LOXO-338 + pirtobrutinib demonstrates additivity in TMD8 human DLBCL model



LOXO-338 + pirtobrutinib shows similar effect when compared to venetoclax + pirtobrutinib in TMD8. (A) Inhibition of cell proliferation. **(B)** Excess over Bliss score, data are mean ± SD of 3 independent experiments; * p<0.05, ** p<0.001.

Fig 5. Combination of LOXO-338 + pirtobrutinib in TMD8 xenograft model shows greater tumor growth inhibition



(A) LOXO-338 + pirtobrutinib (15 mg/kg or 30 mg/kg) combinations show a statistically significant greater than additive effect on tumor growth inhibition (Bliss independence analysis on day 11 data), p<0.001 and p=0.025, respectively. Data are LS mean ± SEM. **(B) LOXO-338 + pirtobrutinib is well-tolerated.** Animal body weights are mean ± SD. n≥8 mice/group. Dosing initiated on day 1.

Conclusions

- LOXO-338
  - Is a novel, orally bioavailable BCL2 small molecule inhibitor, with a favorable preclinical pharmacological profile comparable to venetoclax.
  - Selectively inhibits BCL2 over other BCL2 family members in biophysical and cellular assays.
  - Is well-tolerated *in vivo* and demonstrates dose-dependent tumor growth inhibition in various murine xenograft models.
  - Shows greater efficacy in combination with pirtobrutinib, a BTK inhibitor.
- This preclinical profile of LOXO-338 supports its nomination as a novel BCL2 inhibitor clinical candidate.
- A first-in-human Phase 1 clinical trial is planned for 2021.

References

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