Precision Targeting of MYD88 Mutant DLBCL Using the Novel Combination of IRAKIMiDs and BCL2 Inhibition

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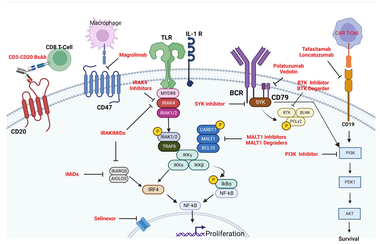
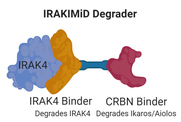
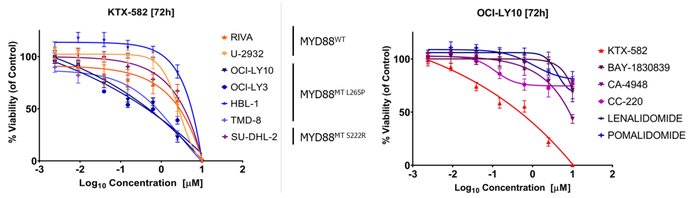
INTRODUCTION

- MYD88 mutations constitutively activate both NF-kB and AP1 pathways, promoting B-cell proliferation and survival
- Based on new genetic DLBCL classifications, co-mutations in MYD88 and CD79B (C5 and MCD subgroups) are associated with an inferior survival after R-CHOP
- IRAK4 is an integral component of MYD88 signaling, and has both kinase activity and scaffolding function
- **IRAKIMiDs** are novel heterobifunctional degraders that utilize an IMiD as a CRBN binder to drive the degradation of both IRAK4 and IMiD substrates in a single molecule





KTX-582 Demonstrates Potent *In Vitro* Activity in MYD88^{Mut} Lymphoma Cell Lines



MYD88 mut ABC-DLBCL cell lines are more sensitive to IRAKIMiDs as compared to wildtype cell lines

IRAKIMiDs have superior activity in MYD88 mut cell line as compared to IRAK4 inhibitors and IMiD compounds

OCI-LY10 [MYD88 mut]

KTX-582

Venetoclax (nM)	24 HRS 10	24 HRS 50	24 HRS 100	48 HRS 10	48 HRS 50	48 HRS 100	72 HRS 10	72 HRS 50	72 HRS 100	96 HRS 10	96 HRS 50	96 HRS 100
0.75	1	4	16	1	7	21	5	13	29	11	19	40
1	8	13	21	11	19	35	18	30	41	25	42	50
1.25	10	17	23	20	28	42	23	34	39	33	43	44

Ibrutinib (nM)	24 HRS 10	24 HRS 50	24 HRS 100	48 HRS 10	48 HRS 50	48 HRS 100	72 HRS 10	72 HRS 50	72 HRS 100	96 HRS 10	96 HRS 50	96 HRS 100
0.25	-5	-4	-6	-9	-12	-10	-5	-9	-8	-13	-11	-4
0.5	-3	-2	-7	-6	-7	-9	-5	-4	-5	-11	-4	4
0.75	-7	-6	-9	-5	-6	-3	-5	-2	-1	-7	-1	9

Umbralisib (nM)	24 HRS 10	24 HRS 50	24 HRS 100	48 HRS 10	48 HRS 50	48 HRS 100	72 HRS 10	72 HRS 50	72 HRS 100	96 HRS 10	96 HRS 50	96 HRS 100
500	-8	-10	-5	-4	-7	-4	-9	-10	-10	-11	-12	-10
1000	-9	-8	-9	-6	-7	-10	-12	-13	-10	-11	-9	-5
5000	-7	-10	-8	-4	-8	-4	-6	-11	-8	-9	-6	-3

Potential synergistic drug combinations in conjunction with KTX-582 were evaluated to simultaneously inhibit several pathways contributing to NF-kB activation and downstream effects.

Venetoclax was found to be the most potent in combination.

KTX-582 In Combination with Venetoclax is Synergistic in MYD88^{Mut} Lymphoma Cell Lines

U2932 [MYD88 wt]

KTX-582

Venetoclax (nM)	24 HRS 1000	24 HRS 1500	24 HRS 2000	48 HRS 1000	48 HRS 1500	48 HRS 2000	72 HRS 1000	72 HRS 1500	72 HRS 2000	96 HRS 1000	96 HRS 1500	96 HRS 2000
0.75	-2	-3	9	-3	-5	10	2	4	22	-1	-1	21
1	0	1	10	2	4	11	4	4	19	-3	-3	21
1.25	-5	-4	5	1	-4	12	2	3	18	1	-3	20

OCI-LY10 [MYD88 mut]

KTX-582

Venetoclax (nM)	24 HRS 10	24 HRS 50	24 HRS 100	48 HRS 10	48 HRS 50	48 HRS 100	72 HRS 10	72 HRS 50	72 HRS 100	96 HRS 10	96 HRS 50	96 HRS 100
0.75	1	4	16	1	7	21	5	13	29	11	19	40
1	8	13	21	11	19	35	18	30	41	25	42	50
1.25	10	17	23	20	28	42	23	34	39	33	43	44

OCI-LY3 [MYD88 mut]

KTX-582

Venetoclax (nM)	24 HRS 50	24 HRS 100	24 HRS 250	48 HRS 50	48 HRS 100	48 HRS 250	72 HRS 50	72 HRS 100	72 HRS 250	96 HRS 50	96 HRS 100	96 HRS 250
1250	13	17	21	17	20	23	17	25	17	33	31	22
2500	11	10	20	17	21	22	17	24	16	29	26	18
5000	19	21	23	20	23	22	14	18	10	19	16	11

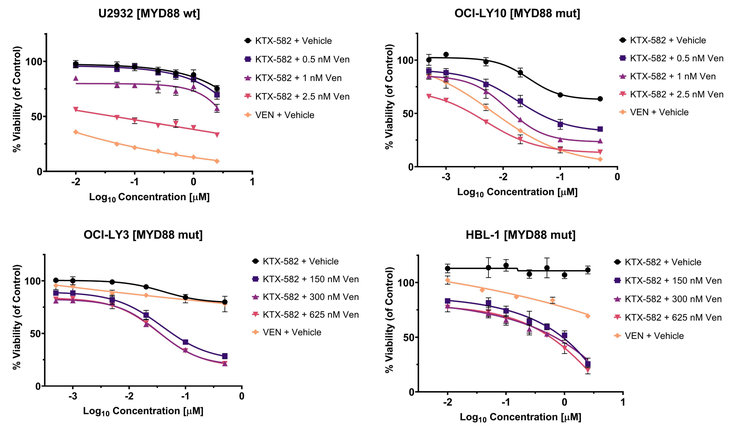
HBL-1 [MYD88 mut]

KTX-582

Venetoclax (nM)	24 HRS 1250	24 HRS 2500	24 HRS 5000	48 HRS 1250	48 HRS 2500	48 HRS 5000	72 HRS 1250	72 HRS 2500	72 HRS 5000	96 HRS 1250	96 HRS 2500	96 HRS 5000
625	16	21	27	34	37	49	38	52	59	35	37	65
1250	21	20	19	36	38	46	49	63	59	44	66	63
2500	21	25	21	36	35	48	49	64	59	48	70	63

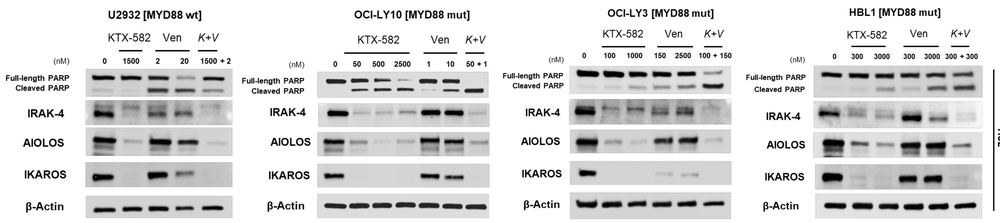
The combination of KTX-582 with venetoclax demonstrated potent synergy as assessed by excess over Bliss (EOB) in all MYD88 mut DLBCL cell lines, with maximum values peaking at 72-96 hours.

KTX-582 and Venetoclax Leads To Enhanced Anti-Proliferative Activity *in vitro* in MYD88^{Mut} Lymphoma Cell Lines



Combinational therapy enhanced the antiproliferative activity in MYD88 mut lymphoma cell lines when compared to either compound alone.

KTX-582 and Venetoclax Induced Increased Apoptosis in MYD88^{Mut} Cell Lines

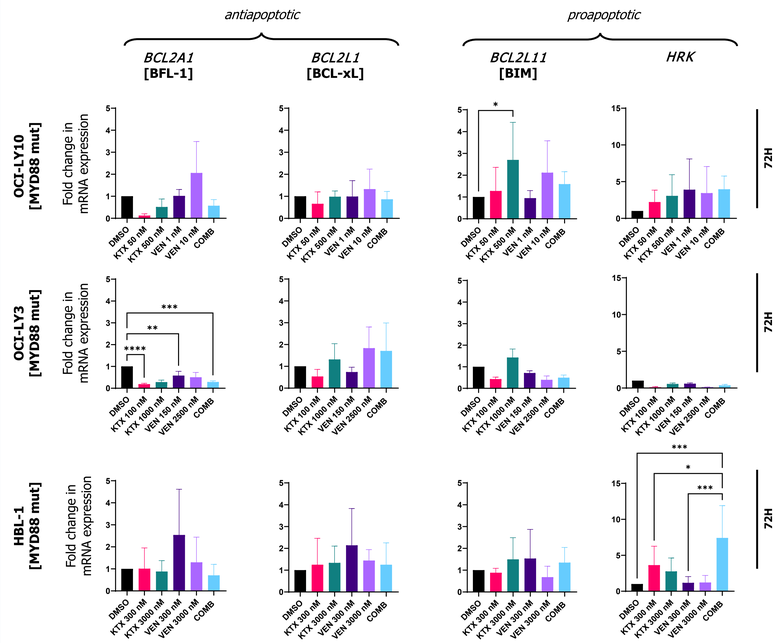


Increase apoptosis with the combination was evaluated by western blot and flow cytometry.

KTX-582 activity leads to degradation of IRAK4 and Aiolos/Ikaros.

After dual exposure, the activity of KTX-582 was not impaired with the addition of venetoclax.

KTX-582 and Venetoclax Leads to Differential Expression of *BCL2* Family Members



HBL-1 demonstrated highly significant induction of the proapoptotic sensitizer, HRK, following the combinatory treatment with KTX-582 and venetoclax

CONCLUSION

- At nanomolar concentrations, KTX-582 and venetoclax synergistically suppressed cell growth and leads to increased apoptotic cell death
- KTX-582 in conjunction with venetoclax can differentially regulate expression of proapoptotic and antiapoptotic BCL2 family members in MYD88^{MT} DLBCL
- These data suggest that inhibiting multiple pathways upstream and downstream of NF-kB activity is a viable and rational strategy for MYD88^{MT} DLBCL
- Evaluating the molecular mechanisms underlying the activity of this novel combination is in progress, with the aim of identifying additional biomarkers that can predict for sensitivity to this combination, as well as translation and validation in mouse models.
- KT-413, an IRAKIMiD compound, is currently being evaluated in a Phase 1 clinical trial for Relapse/Refractory Non-Hodgkin Lymphoma (NCT05233033).
- Our studies also support a potential combination with venetoclax in the future

ACKNOWLEDGEMENTS

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