Inhibition of TLR7 and TLR9 Blocks MYD88 L265P Oncogenic Mutation-mediated Signaling

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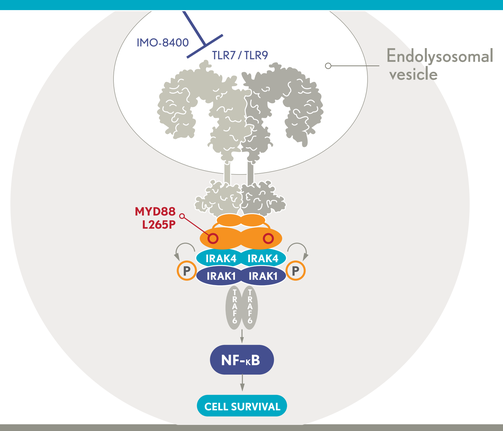
INTRODUCTION

- The MYD88 L265P oncogenic mutation is present in various B-cell malignancies, including in 29% of patients with ABC-DLBCL[1] and 90% of patients with Waldenström's macroglobulinemia[2] (WM)
- MYD88 is an adaptor molecule in TLR-mediated signaling; the MYD88 L265P oncogenic mutation promotes tumor cell survival by over-activation of the TLR signaling pathway,[3] which includes IRAK1/IRAK4, TRAF6, BTK, NF-KB and JAK/STAT
- B-cells express TLR7 and TLR9; inhibition of TLR7 and TLR9 reduces MYD88 L265P-driven signaling and inhibits tumor cell survival[4]
- Therefore TLR antagonism is an intriguing novel approach to the treatment of B-cell malignancies harboring the MYD88 L265P mutation
- In the current study, we evaluated two modalities to suppress MYD88 L265P-driven over-activation of TLR signaling: gene-silencing oligonucleotides[5] (GSOs) targeted to MYD88, TLR7, and TLR9; and IMO-8400, a selective antagonist of TLR7, TLR8, and TLR9

IMO-8400 INHIBITS MYD88 L265P-DRIVEN SIGNALING AND CELL SURVIVAL



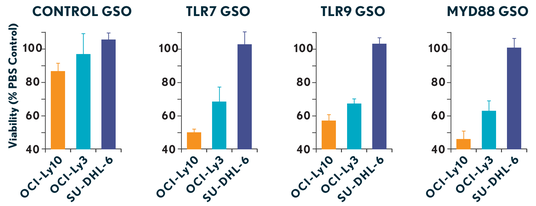
CHARACTERIZATION OF LYMPHOMA CELL LINES

Cell Line	DLBCL Subtype	MYD88 L265P
OCI-Ly3	ABC	Homozygous
OCI-Ly10	ABC	Heterozygous
TMD8	ABC	Heterozygous
SU-DHL-6	GCB	Absent

RESULTS

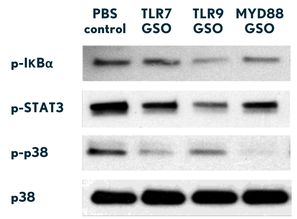
GSOs ELUCIDATE THE ROLE OF TLR7, TLR9 AND MYD88 ON SURVIVAL OF DLBCL CELLS HARBORING MYD88 L265P

GSO KNOCKDOWN OF TLR7, TLR9 OR MYD88 DECREASES VIABILITY OF LYMPHOMA CELLS



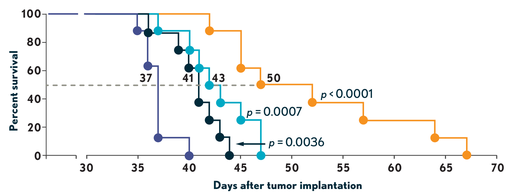
Cells were treated with 1 μM TLR7, TLR9 or MYD88 GSOs plus lipid for 72 h. Cell viability was evaluated by the MTS assay.

DECREASED CELL VIABILITY IS ASSOCIATED WITH INHIBITION OF KEY CELL SIGNALING PATHWAYS



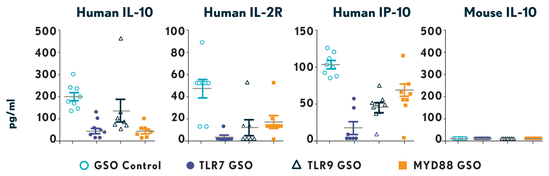
OCI-Ly10 cells were treated with 1 μM TLR7, TLR9 or MYD88 GSOs plus lipid for 24 h and whole cell lystaes were analyzed by western blotting.

GSO KNOCKDOWN OF TLR7, TLR9 OR MYD88 PROLONGS MOUSE SURVIVAL IN OCI-LY10 DISSEMINATED TUMOR MODEL



SCID mice (n = 8/group) bearing disseminated OCI-Ly10 xenografts were treated with 15 mg/kg TLR7, TLR9, MYD88 GSO or mismatch control by i.p. injection twice per week for three weeks followed by weekly injection until the end of the study. Treatment with TLR7, TLR9 and MYD88 GSO significantly prolongs survival compared to GSO mismatch control.

GSO SUPPRESSION OF HUMAN CYTOKINES AND CHEMOKINES IN OCI-LY10 DISSEMINATED TUMOR MODEL

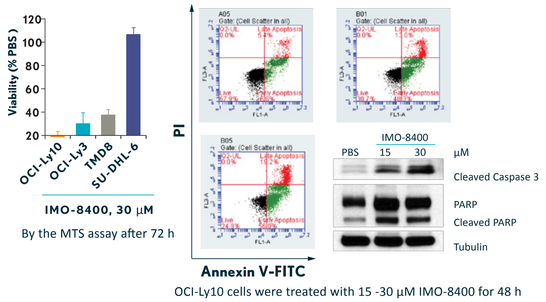


Serum samples collected on day 28 after tumor implantation were evaluated by human and mouse multiplex assays.

IMO-8400, A TLR ANTAGONIST, AS A THERAPEUTIC APPROACH FOR DLBCL WITH MYD88 L265P MUTATION

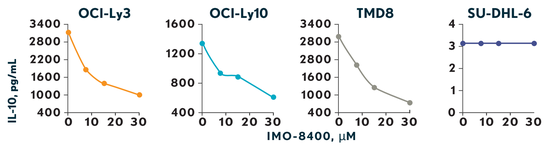
IN VITRO CELL CULTURE STUDIES

IMO-8400 INDUCES APOPTOSIS AND INHIBITS SURVIVAL OF MYD88 L265P + CELLS



OCI-Ly10 cells were treated with 15 -30 μM IMO-8400 for 48 h

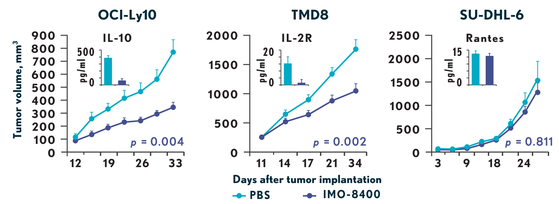
DECREASED CELL VIABILITY IS ASSOCIATED WITH INHIBITION OF CELL SIGNALING



Cytokine secretion was measured after 72 hrs of culture

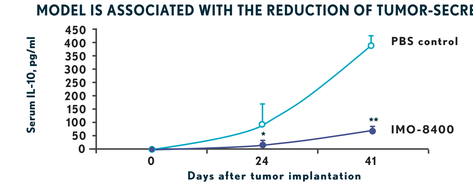
IN VIVO XENOGRAFT STUDIES

SYSTEMIC IMO-8400 TREATMENT LEADS TO INHIBITION OF TUMOR GROWTH AND ASSOCIATED CYTOKINES IN MYD88 L265P-POSITIVE TUMOR MODELS



SCID mice (n = 10/group) bearing s.c. implanted xenograft tumor were treated with 25 mg/kg IMO-8400 by i.p. administration twice per week for 3 weeks followed once per week until the end of the study. Treatment was initiated when tumor nodules reached 50 to 200 mm³.

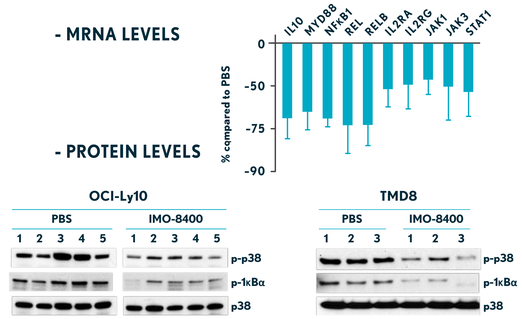
IMO-8400 SUPPRESSION OF TUMOR GROWTH IN OCI-LY10 XENOGRAFT MODEL IS ASSOCIATED WITH THE REDUCTION OF TUMOR-SECRETED IL-10



Serum samples collected after s.c. OCI-Ly10 implantation were analyzed for secreted human IL-10. Correlated to tumor growth inhibition, treatment with IMO-8400 inhibits circulating tumor-associated IL-10.

IN VIVO XENOGRAFT STUDIES, CONTINUED

IMO-8400 DECREASES MYD88 L265P MUTATION-DRIVEN SIGNALING PATHWAYS



*RNA and protein were extracted from OCI-Ly10 or TMD8 xenografts 41 or 29 days, respectively, after tumor implantation.

CONCLUSIONS

- The TLR antagonist IMO-8400 and gene silencing oligonucleotides targeted to TLR7, TLR9, or MYD88 inhibit over-activation of TLR-induced signaling and decrease tumor cell viability in B-cell lymphoma cell lines harboring the MYD88 L265P mutation
- Tumor growth inhibition in xenograft models is correlated to reduced tumor-secreted cytokines and with inhibition of MYD88 L265P mutation-driven signaling pathways including JAK/STAT, NF-κB and p38
- IMO-8400 and gene silencing oligonucleotides show no impact on a lymphoma cell line lacking the MYD88 L265P mutation
- Based on promising preclinical data and clinical safety, IMO-8400 is currently being evaluated in a Phase 1/2 clinical trials in patients with DLBCL and Waldenström's macroglobulinemia harboring the MYD88 L265P mutation. Please visit **www.iderapharma.com** for further information

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4. Bhagat L, Agrawal S et al. IMO-8400, a selective antagonist of TLRs 7, 8 and 9, inhibits MYD88 L265P mutation-driven signaling and cell survival: A potential novel approach for treatment of B-cell lymphomas harboring MYD88 L265P mutation. AACR annual meeting 2014. Abstract #2570.
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There are no relevant conflicts of interest to disclose.