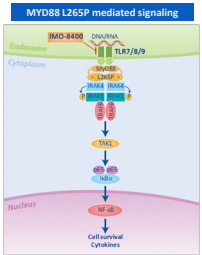


Introduction

- Emerging biology has identified novel therapeutic targets for the treatment of B-cell lymphomas
 - Constitutive activation of nuclear factor (NF)- κ B and JAK/STAT signaling has been shown to promote cell survival in the activated B-cell-like (ABC) subtype of diffuse large B-cell lymphoma (DLBCL)
- A highly recurrent oncogenic mutation affecting MYD88 in ABC-DLBCL tumors has been identified¹
 - MYD88 is an adaptor protein that mediates Toll-like receptor (TLR) signaling; L265P was the most prevalent MYD88 mutation observed
 - Reported in 29% of patients with ABC-DLBCL, in over 90% of patients with Waldenström's macroglobulinemia (WM)² and in other B-cell lymphomas
- The MYD88 L265P oncogenic mutation is shown to promote cell survival by initiating signaling through IRAK-1, IRAK-4, NF- κ B, JAK/STAT, and secretion of IL-6 and IL-10
 - Recently, it has been shown that knockdown of TLR7 or TLR9 expression, but not other TLRs, blocked NF- κ B signaling and survival of cells expressing MYD88 L265P oncogenic mutation⁴
- TLRs 7 and 9 are expressed on B-cells and dendritic cells



Research Objectives: Validate the rationale for targeting TLR7 and TLR9 for treatment of B-cell lymphomas harboring MYD88 L265P oncogenic mutation

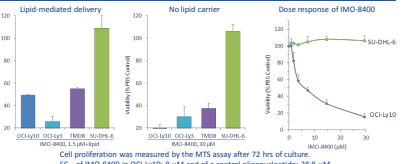
- Evaluate effect of IMO-8400, an antagonist of TLR7, TLR8, and TLR9,^{5,6} on signaling pathways in human lymphoma cells with the MYD88 L265P oncogenic mutation
- Evaluate effect of IMO-8400 treatment on tumor cell proliferation and survival
 - In vitro and in vivo in mouse xenograft models
- Evaluate specificity of TLR7/9 inhibition to the MYD88 L265P oncogenic mutation
- Rationale: Upstream inhibition of the TLR/MYD88 pathway may be more appropriate to lymphomas characterized by MYD88 L265P compared to downstream inhibition of specific proteins such as IRAK-4 and BTK
 - IRAK-4 is employed by all TLRs except TLR3; the blocking of IRAK-4 may have an impact on all TLR functions in other cells, including B-cells
- Patients harboring MYD88 L265P mutation as the only identified oncogenic mutation showed no response to treatment with ibrutinib, a BTK inhibitor⁷

Characteristics of cells employed in the studies

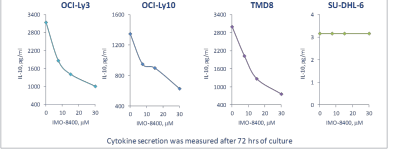
Cell-line	Culture	MYD88 L265P mutation	TLR7/9
OCI-19	ABC-DLBCL	+	+
OCI-19#1	ABC-DLBCL	+	+
TH96	ABC-DLBCL	+	+
SO-DHL-6	GCB-DLBCL	-	-
MYD88 L1	Waldenström's Macroglobulinemia	+	+
MYD88 L2	Waldenström's Macroglobulinemia	+	+
Human primary cells		+	+

In vitro Studies in MYD88 L265P-Positive ABC-DLBCL Cell Lines

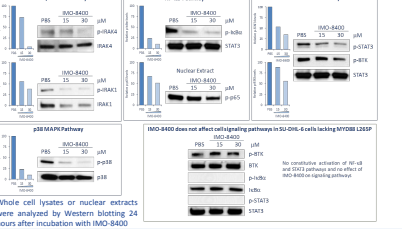
IMO-8400, an antagonist of TLRs 7, 8 and 9, inhibits survival of cells harboring MYD88 L265P oncogenic mutation



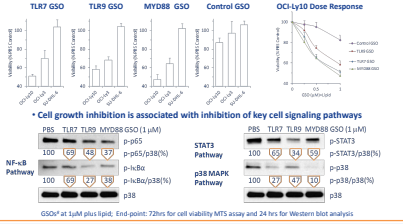
Decreased cell viability is associated with inhibition of cytokines



IMO-8400 inhibits key cell signaling pathways in OCI-19 cells

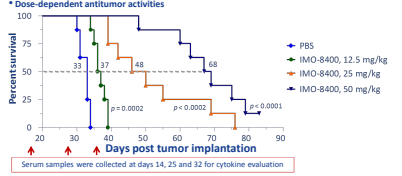


Effect of Gene-Silencing Oligonucleotide (GSO) knockdown of TLR7, TLR9 or MYD88 on cells harboring MYD88 L265P oncogenic mutation is consistent with previous reports

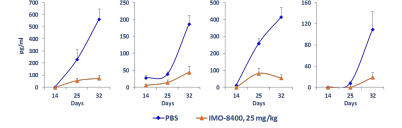


Tumor Models of MYD88 L265P-Positive ABC-DLBCL

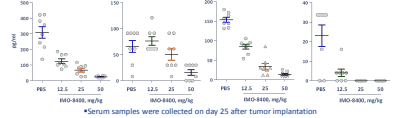
Treatment with IMO-8400 prolongs mouse survival in OCI-19 Ly10 disseminated xenograft model



Inhibition of tumor growth is associated with suppression of key tumor-associated cytokines

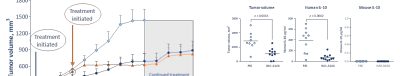


Suppression of tumor-associated cytokines is dose-dependent

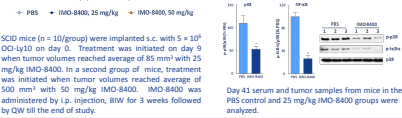


Treatment with IMO-8400 inhibits tumor growth in OCI-19 Ly10 solid tumor xenograft model

IMO-8400 inhibits tumor growth in both early and late stages; shows therapeutic effect on developed tumors (500 mm³)

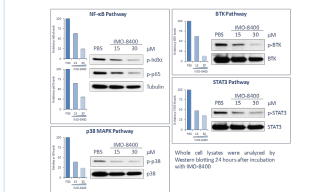
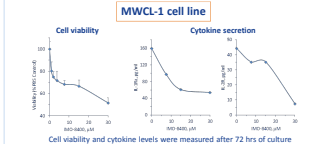


Antitumor activity is associated with the inhibition of key tumor survival signaling pathways

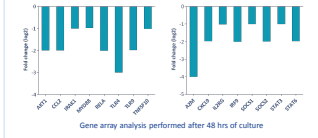
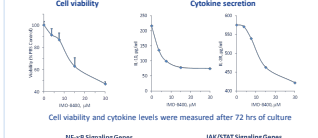


Waldenström's Macroglobulinemia

IMO-8400 inhibits cell viability, cytokine production and key signaling pathways in MYD88 L265P positive WM cells



Patient primary bone marrow cells



Summary

- Knockdown of TLR7 and TLR9 in MYD88 L265P mutant cells led to decreased cell signaling and inhibition of cell survival, in agreement with previous reports
- Treatment with IMO-8400, an antagonist of TLRs 7, 8, and 9, of ABC-DLBCL cells expressing MYD88 L265P oncogenic mutation in vitro and in mouse xenograft models led to inhibition of cell survival and tumor growth, respectively, and:
 - Inhibition of cytokine induction including IL-10
 - Down-regulation of gene expression in the NF- κ B and JAK/STAT pathways
 - Inhibition of IRAK1/4, NF- κ B, STAT3, p38, and BTK signaling pathways
- Furthermore, treatment of established tumors (500 mm³) with IMO-8400 led to delayed tumor growth
- IMO-8400 treatment of Waldenström macroglobulinemia cells expressing MYD88 L265P oncogenic mutation led to similar inhibitory effects on cell signaling and survival
- No activity was seen in a GCB-DLBCL cell line, SU-DHL-6, lacking MYD88 L265P oncogenic mutation, in vitro or in vivo

IMO-8400 antitumor activity is specific to tumors with MYD88 L265P oncogenic mutation

Tumor cell line	OCI-19	TH96	SU-DHL-6
DLBCL subtype	ABC	ABC	GCB
MYD88 L265P mutation	+	+	-
Tumor growth inhibition	Yes	Yes	No
Cytokine/Chemokine	Inhibited	Inhibited	No change
NF- κ B signaling	Inhibited	Inhibited	No change

Current Clinical Development

- IMO-8400 is in clinical development for the treatment of B-cell lymphomas harboring MYD88 L265P oncogenic mutation
- Patient enrollment is now open in a Phase 1/2 trial of IMO-8400 in Waldenström's macroglobulinemia
- A protocol for Phase 1/2 trial in patients with DLBCL harboring the MYD88 L265P oncogenic mutation has been submitted by the FDA

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