Abstract No. 2570

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

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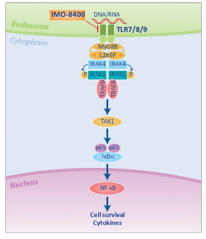
Introduction

- Emerging biology has identified novel therapeutic targets for the treatment of B-cell lymphomas
  - Constitutive activation of nuclear factor (NF)-κB 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 identified[1]
  - 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-κB, 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-κB signaling and survival of cells expressing MYD88 L265P oncogenic mutation[3,4]
- TLRs 7 and 9 are expressed on B-cells and dendritic cells

MYD88 L265P mediated signaling



Characteristics of cells employed in the studies

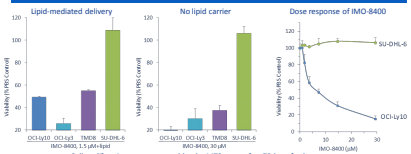
Cell lines	Cell type	Mutation status	MYD88 L265P expression
OCI-Ly3	ABC-DLBCL	+	+
OCI-Ly10	ABC-DLBCL	+	+
TMD8	ABC-DLBCL	+	+
SU-DHL-6	GCB-DLBCL	–	+
MWCL-1	Waldenström's Macroglobulinemia	+	+
Patient primary cells	Waldenström's Macroglobulinemia	+	+

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 inhibitor[7]

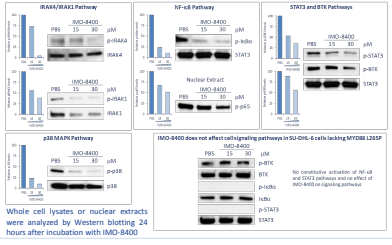
In vitro Studies in MYD88 L265P-Positive ABC-DLBCL Cell Lines

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



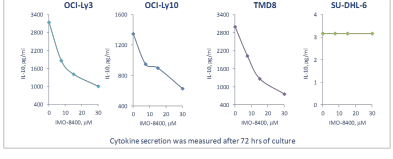
Cell proliferation was measured by the MTS assay after 72 hrs of culture.
EC_{50} of IMO-8400 in OCI-Ly10: 8 μM and of a control oligonucleotide: 28.8 μM

IMO-8400 inhibits key cell signaling pathways in OCI-Ly10 cells



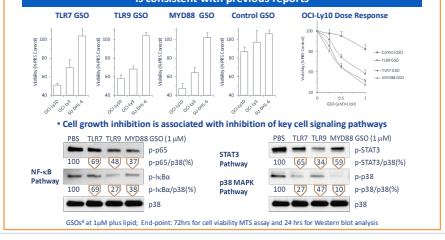
Whole cell lysates or nuclear extracts were analyzed by Western blotting 24 hours after incubation with IMO-8400

Decreased cell viability is associated with inhibition of cytokines



Cytokine secretion was measured after 72 hrs of culture

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

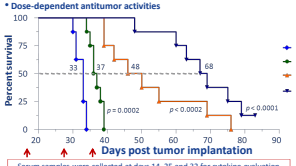


GSOs* at 1μM plus lipid; End-point: 72hrs for cell viability MTS assay and 24 hrs for Western blot analysis

Tumor Models of MYD88 L265P-Positive ABC-DLBCL

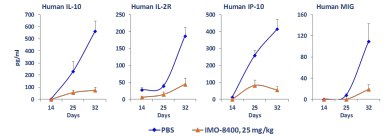
Treatment with IMO-8400 prolongs mouse survival in OCI-Ly10 disseminated xenograft model

• Dose-dependent antitumor activities

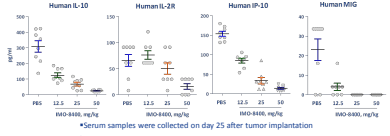


Serum samples were collected at days 14, 25 and 32 for cytokine evaluation

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



• Suppression of tumor-associated cytokines is dose-dependent

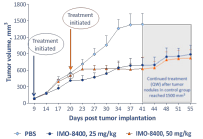


•Serum samples were collected on day 25 after tumor implantation

SCID mice (n = 8/group) bearing disseminated OCI-Ly10 xenografts were treated with 12.5, 25 or 50 mg/kg IMO-8400 by i.p injections twice per week for three weeks followed by weekly injection until the end of the study. Treatment with IMO-8400 resulted in dose-dependent extension of survival and cytokine inhibition.

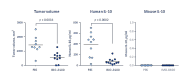
Treatment with IMO-8400 inhibits tumor growth in OCI-Ly10 solid tumor xenograft model

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

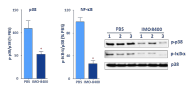


SCID mice (n = 10/group) were implanted s.c. with 5 × 10^6 OCI-Ly10 on day 0. Treatment was initiated on day 9 when tumor volumes reached average of 85 mm³ with 25 mg/kg IMO-8400. In a second group of mice, treatment was initiated when tumor volumes reached average of 500 mm³ with 50 mg/kg IMO-8400. IMO-8400 was administered by i.p. injection, BIW for 3 weeks followed by QW till the end of study.

• OCI-Ly10 growth is associated with elevated cytokines; IMO-8400 inhibits tumor-secreted IL-10 while has no impact on mouse IL-10



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

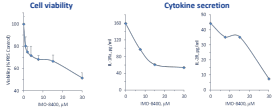


Day 41 serum and tumor samples from mice in the PBS control and 25 mg/kg IMO-8400 groups were analyzed.

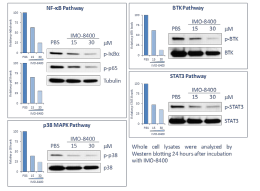
Waldenström's Macroglobulinemia

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

MWCL-1 cell line

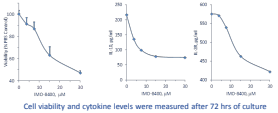


Cell viability and cytokine levels were measured after 72 hrs of culture



Whole cell lysates were analyzed by Western blotting 24 hours after incubation with IMO-8400

Patient primary bone marrow cells



Cell viability and cytokine levels were measured after 72 hrs of culture

Gene array analysis performed after 48 hrs of culture

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-κB and JAK/STAT pathways
  - Inhibition of IRAK1/4, NF-κB, STAT3, p38, and BTK signaling pathways
- Furthermore, treatment of established tumors (500 mm^3) 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-Ly10	TMD8	SU-DHL-6
DLBCL subtype	ABC	ABC	GCB
MYD88 L265P mutation	+	+	–
Tumor growth inhibition	Yes	Yes	No
Cytokine/Chemokine	Inhibited	Inhibited	No change
NF-κB 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 to the FDA

References

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