Targeting the CBM Signalosome with a MALT1 Scaffolding Inhibitor for Treatment of Non-Hodgkin Lymphomas

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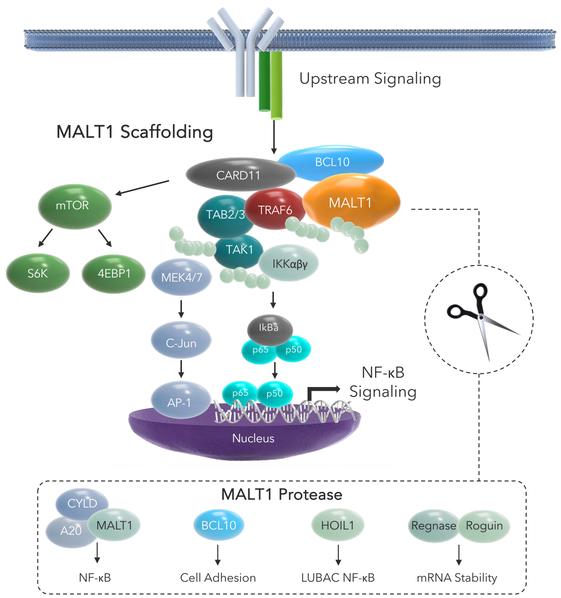
Introduction

The CBM signalosome (CARDs/BCL10/MALT1) is an evolutionarily conserved key regulator of canonical NF-κB signaling. Activating mutations of the CBM signalosome constitutively activate NF-κB, driving tumorigenesis including B-cell Non-Hodgkin Lymphoma (B-NHL). Depletion of MALT1 was shown preclinically to suppress NF-κB driven Activated B-Cell Diffuse Large B-Cell Lymphoma (ABC-DLBCL), inspiring many efforts to develop MALT1 inhibitors for therapeutic use.

MALT1 is a dual function protein with two independent roles. Primarily, MALT1 is a scaffold protein organizing the CBM complex and directly transducing canonical NF-κB signaling. Secondarily, MALT1 is a paracaspase, fine-tuning multiple signaling pathways, including NF-κB, via substrate cleavage. Targeting MALT1 scaffolding effectively suppressed NF-κB activity while minimizing impact on the immune system.

Using the Smart Allostery™ platform, we have discovered a first-in-class MALT1 scaffolding inhibitor with potent and broader activity against NF-κB driven tumors. Here we discuss the differential pharmacological profile of scaffolding inhibitors and protease inhibitors and the evidence that supports the rationale for scaffolding inhibition as a superior MoA for oncology.

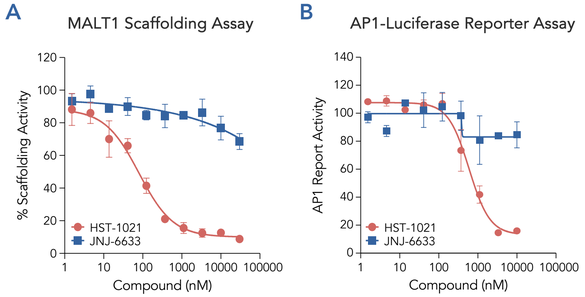
MALT1 Scaffolding Activity Governs CBM-Mediated Canonical NF-κB and AP1 Signaling



MALT1's scaffolding function and MALT1's protease function can be uncoupled and function independently.

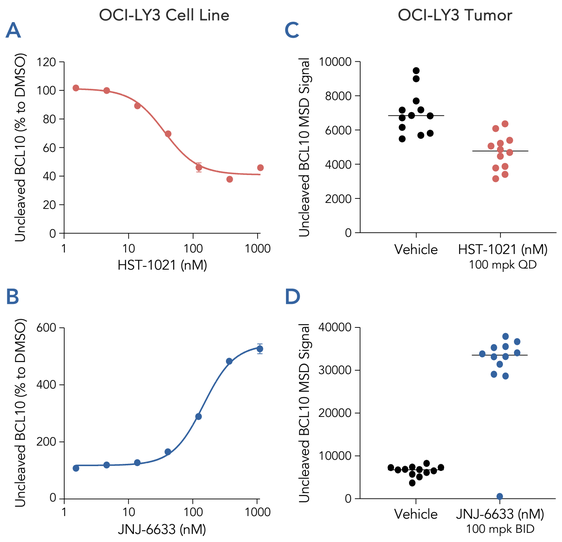
Results

Figure 1. HST-1021, a First-in-class MALT1 Scaffolding Inhibitor, Potently Inhibits NF-κB and AP1 Activity



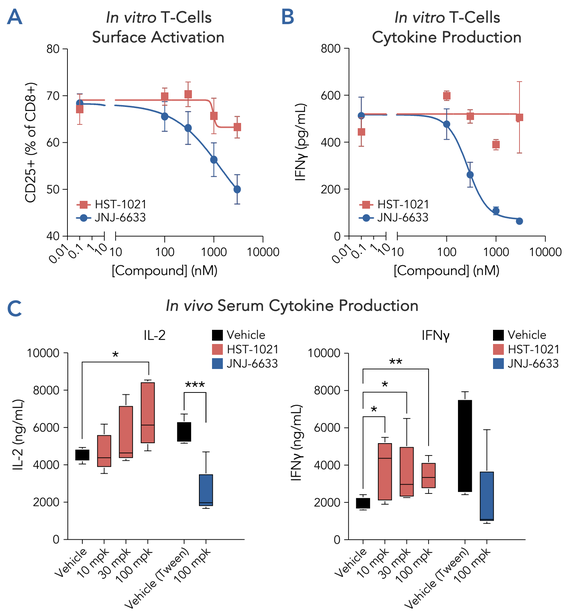
(A) OCI-LY3 cells were treated with HST-1021 or JNJ-6633 for 24hr, the modulation of p-IκBα was monitored. (B) HEK293-AP1 cells were pre-treated with HST-1021 or JNJ-6633 for 1hr, followed by stimulation with 10nM PMA for 6hr, AP1 reporter activity was then measured using a luciferase assay. JnJ-6633 (Safimaltib), a clinical stage MALT1 protease inhibitor.

Figure 2. HST-1021 Enhances MALT1 Protease Activity *in vitro* and *in vivo*



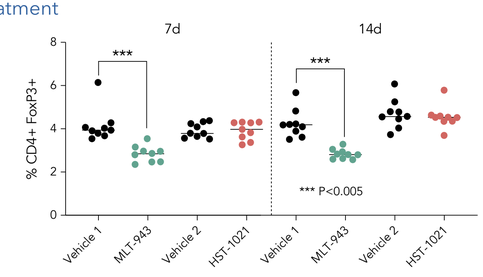
MALT1 protease activity was measured using an uncleaved BCL10 MSD assay. For the *in vitro* activity, OCI-LY3 cells were treated with HST-1021 (A) or JNJ-6633 (B) for 24hr. For the *in vivo* activity, uncleaved BCL10 was determined from the OCI-LY3 CDX tumors 2 hours after a 5-day dosing regiment of HST-1021 at 100 mpk QD (C) or JNJ-6633 at 100mpk BID (D).

Figure 3. HST-1021 Does Not Perturb T-Cell Activation and Increases Serum Cytokine Levels *in vivo*



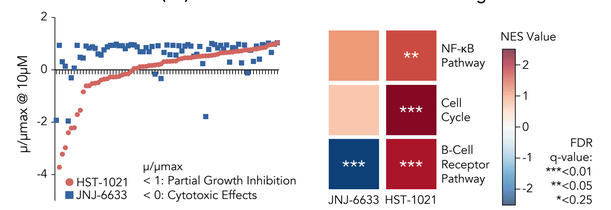
T-cells were isolated from frozen PBMCs and pretreated with compound for 1hr, followed by a 72hr stimulation with 10μg/mL aCD3 + 3μg/mL aCD28 in presence of the compound. The cells were analyzed by flow cytometry for the T-cell activation marker CD25 (A). The medium was collected to measure IFNγ production (B). To measure *in vivo* cytokine production, mice were dosed with the compound at different doses for 2hrs, followed by a 4hr aCD3 stimulation. Blood was collected, the cytokine production was measured using the MSD assay (C).

Figure 4. HST-1021 Avoided Treg Depletion After 14-Days Treatment



Mice were dosed with vehicle, MLT-943 at 40 mg/kg BID or HST-1021 at 50 mg/kg QD for 7 or 14 days. The frequency of Foxp3 +CD25+ Tregs in the blood was analyzed by flow cytometry. MLT-943, a protease inhibitor developed by Novartis.

Figure 5. HST-1021 Demonstrates Broader and More Potent Activity Than MALT1 Protease Inhibitor



(A) A lymphoma cell line panel was treated with HST-1021 or JNJ-6633 at dose ranging from 30 μM to 1.5 nM for 4 days. Proliferation was analyzed using μ/μmax, where μ/μmax < 0 indicates cytotoxicity, and 0 < μ/μmax < 1 indicates partial growth inhibition. (B) Heatmap of pathway enrichment signatures.

Summary

Scaffolding Inhibition Has Unique and Differential Pharmacology

	CBM Scaffolding Activity	Protease Activity	Downstream Signaling	Biological Manifestation
MALT1 dual inhibitor or degrader	↓	↓	• Protease related pathway inhibited ↓ • NF-κB ↓ AP1 ↓ mTOR ↓	• Complete inhibition of NF-κB • Immune suppression
MALT1 protease inhibition	No change	↓	• Protease related pathway inhibited ↓ • NF-κB - AP1 - mTOR ↑	• Partial inhibition of NF-κB • Autoimmune (IPEX syndrome)
MALT1 scaffolding inhibition	↓	↑	• Protease related pathway ↑ • NF-κB ↓ AP1 ↓ mTOR ?	• Complete inhibition of NF-κB • No T-cell suppression, no IPEX syndrome • Combinable with T-cell mediated cancer immune therapies

Conclusions

- The potential first-in-class MALT1 scaffolding inhibitor offers a best-in-class approach to targeting CBM-complex-driven NF-κB signaling.
- Scaffolding inhibitor effectively blocked both NF-κB and AP1 signaling without impairing protease activity and avoided negative effects on T-cell function.
- Scaffolding inhibitor offers a promising therapeutic approach for NF-κB driven cancers.

References

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