Targeting the CBM Signalosome with a MALT1 Signalosome Glue for Treatment of NFκB Driven Solid Tumors

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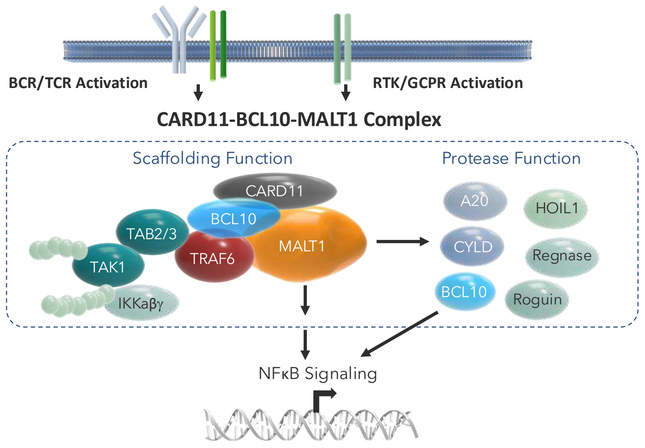
Introduction

The CBM signalosome (CARDs/BCL10/MALT1) is an evolutionarily conserved key regulator of canonical NFκB signaling. Activating mutations of the CBM signalosome constitutively activate NFκB, driving tumorigenesis including B-cell Non-Hodgkin Lymphoma (B-NHL). Depletion of MALT1 was shown preclinically to suppress NFκB 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 NFκB signaling. Secondarily, MALT1 is a paracaspase, fine-tuning multiple signaling pathways, including NFκB, via substrate cleavage. Targeting MALT1 scaffolding effectively suppresses NFκB activity while minimizing impact on the immune system.

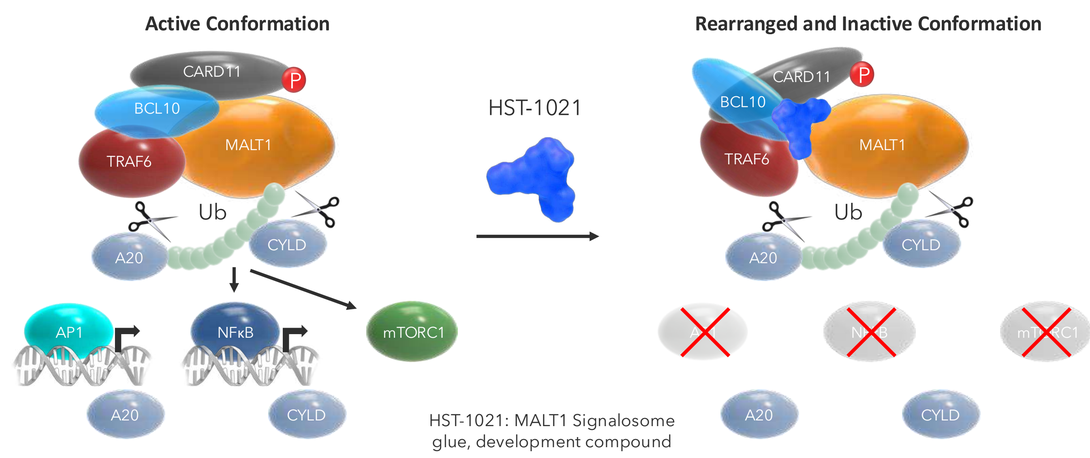
Using Smart Allostery™ platform, we have discovered a potentially first-in-class MALT1 signalosome glue with potent and broader activity against NFκB driven tumors. Here we discuss the differential pharmacological profile of MALT1 signalosome glue and protease inhibitor and the evidence of why scaffolding inhibition may be a superior MoA for oncology.

MALT1 Scaffolding Activity Governs CBM-Mediated Canonical NFκB Signaling



1. Upstream receptors signaling converge on CBM complex
2. MATL1 signals through CBM complex as scaffolding protein, and via its protease function
3. Results in canonical NFκB activation

The Discovery of the Potentially First-In-Class Allosteric MALT1 Non-degrading Glue to Inhibit CBM Signalosome Activity Without Affecting Protease Activity

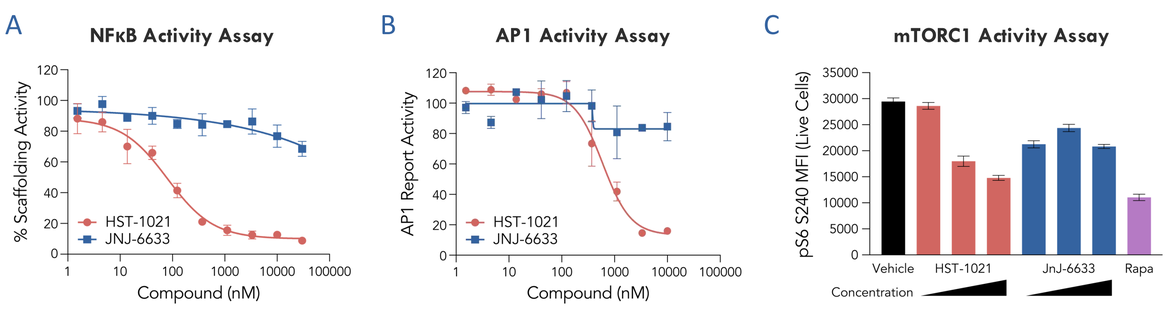


HST-1021: MALT1 Signalosome glue, development compound

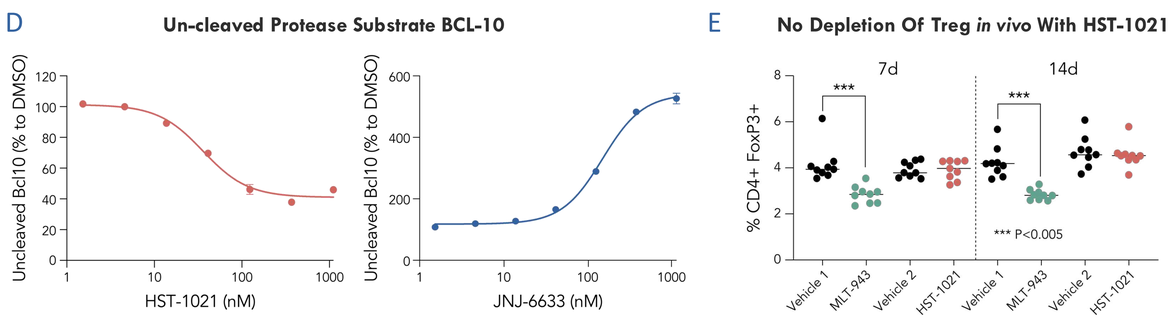
Results

Figure 1. MALT1 Glue Inhibited CBM Signalosome Activity Without Affecting Protease Activity, Whereas Protease Inhibitor Did Not Affect CBM Activities

HST-1021 Inhibited CBM Signalosome Mediated NFκB, AP1 and mTORC1 Activity



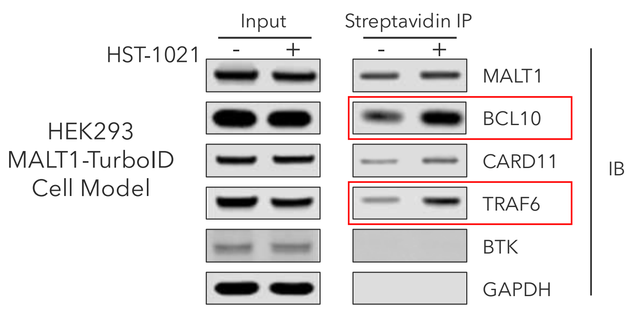
HST-1021 Did Not Affect MALT1 Protease Activity, Therefore No Systemic Impact on Treg Population *in vivo*



(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 1 hr, followed by stimulation with 10 nM PMA for 6 hr, and AP1 reporter activity was measured using a luciferase assay. (C) OCI-LY3 were treated with HST-1021 (0.3, 1, 3 μM), JnJ-6633 (0.3, 1, 3 μM), and rapamycin (10 nM) for 24hrs. The phosphorylated ribosomal protein S6 (Ser240) was analyzed by flow cytometry. (D) MALT1 protease activity was measured using an uncleaved BCL10 MSD assay. OCI-LY3 cells were treated with HST-1021 or JNJ-6633 for 24 hr. (E) 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.

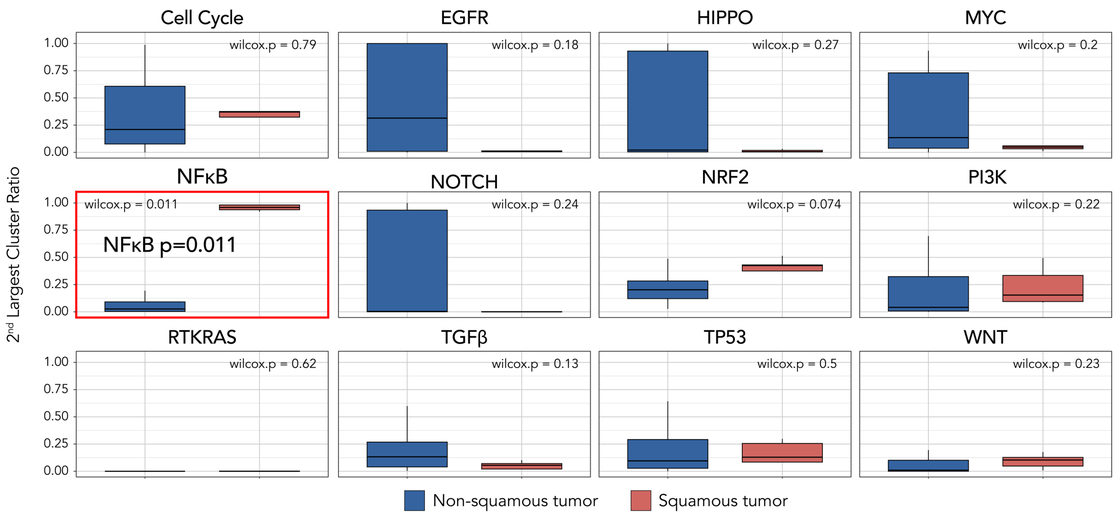
HST-1021: MALT1 Signalosome glue, development compound; JnJ-6633: protease inhibitor (Janssen); MLT-943: protease inhibitor (Novartis)

Figure 2. MALT1 Signalosome Glue Secured CBM Complex in an Inactive Form



A recombinant fusion protein, TurboID-MALT1, was transduced into HEK293 cells to generate a stable cell model. After treatment with 1 μM HST-1021 for 72 hrs, the cells were lysed and immunoprecipitated (IP) with streptavidin beads. Proteins interacting with MALT1 were detected by Western blot.

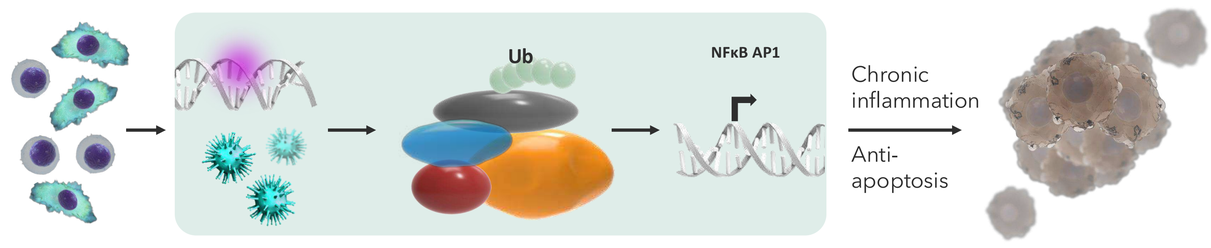
Figure 3. NFκB as a Potential Oncogenic Driver of Squamous Cancer through Genetic Mutations and/or Oncogenic Viral Infection



Unsupervised consensus clustering of 9125 patient samples from 33 cancer types in the TCGA pan-cancer atlas was performed using 11 known oncogenic pathways and the NFκB pathway.

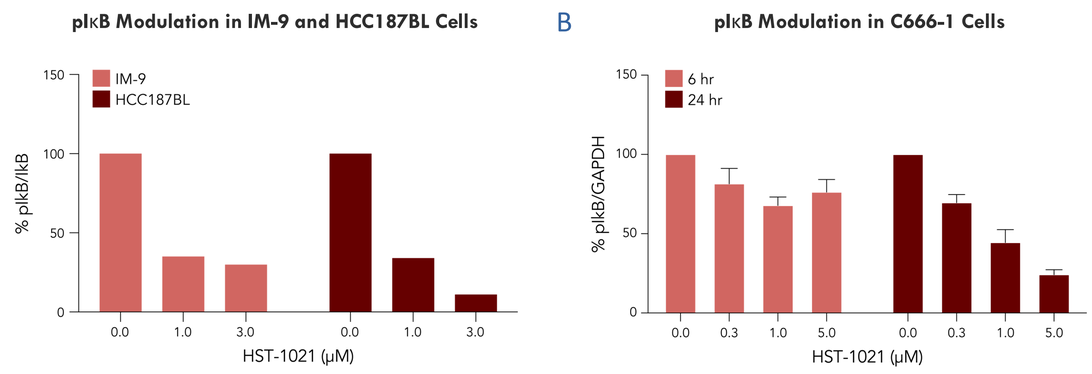
Oncogenic Signaling Pathways in TCGA, Cell 2018, 10.1016/j.cell.2018.03.035
Comprehensive Molecular Characterization of the Hippo Signaling Pathway in Cancer, Cell Reports 2018, 10.1016/j.celrep.2018.10.001

Figure 4. Constitutive NFκB Activation Induced by Pathway Genetic Alteration or Disease Pathogenesis in Nasopharyngeal Carcinoma (NPC), a Type of Squamous Cell Carcinoma



- Normal cells
- Latent virus infection
- Genetic alteration
- CBM signalosome activation
- NFκB constitutive activation
- NFκB dependent tumor progression/ metastasis

Figure 5. HST-1021 Inhibited pIκB in EBV-Transformed B Lymphoblastoid Cells and EBV+ NPC Cells



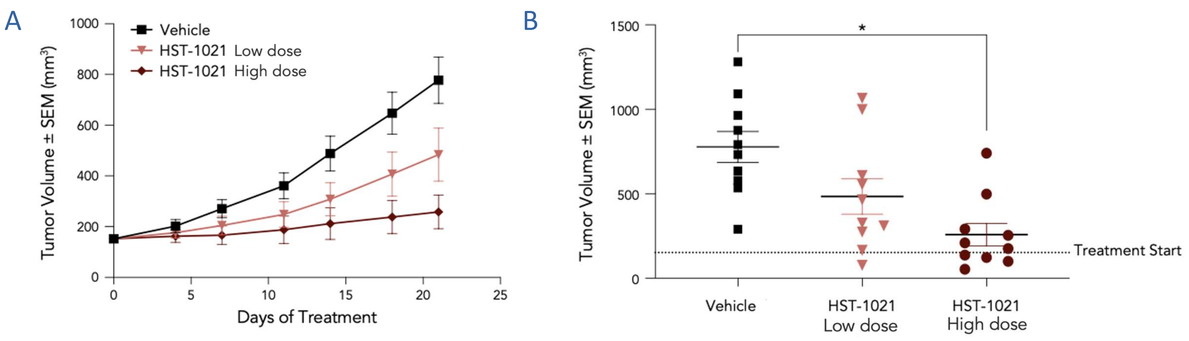
IM-9 and HCC187BL
- EBV-transformed B lymphoblastoid cells

Genetic background of C666-1
- EBV+
- NFKBIA, frameshift deletion, homozygous
- TRAF3, frameshift deletion, homozygous

(A) EBV-transformed B lymphoblastoid cells (IM-9 and HCC187BL) were treated with HST-1021 for 24 hr in the presence of 5 μM MG-132 for 90 min. pIκB modulation was monitored by Western blot, and the protein bands were quantified and presented. (B) EBV+ NPC cells (C666-1) were treated with HST-1021 for 6 or 24 hr. pIκB modulation was monitored by Western blot, and the protein bands were quantified and presented.

Figure 6. HST-1021 Exerted Profound Anti-tumor Activity in an NPC PDX Model Containing NFκB Activating Mutations

LD1-0023-361686 PDX Model: NFKBIA and TRAF3 Mutations



HST-1021 was dosed orally for 21 days. The tumor growth inhibition (A) and tumor volume at the end of the study (B) are presented.

Conclusions

- The bioinformatics analysis provided the first evidence that NFκB is a potential driver in squamous cancers.
- As a proof of principle, the MALT1 signalosome glue demonstrated pre-clinical activity against NFκB driven NPC, highlighting its potential as a precision oncology approach for NFκB driven solid tumors.