MALT1 protease inhibition overcomes BTK inhibitor resistance and shows synergistic activity with venetoclax in models of B cell lymphoma and leukemia

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Abstract

Introduction: Specific B cell malignancies, including CLL and the aggressive non-GCB subtype of DLBCL, are driven by constitutive activation of the B cell receptor (BCR) pathway and the transcription factor NF-κB. Pharmacological inhibition of MALT1 protease, a key mediator of the BCR/NF-κB signal transduction pathway, may therefore provide an attractive treatment option for patients with these cancers. Further, as combination therapy is often required for the treatment of aggressive B cell malignancies, the identification of therapies that synergistically combine with MALT1 inhibitors could afford additional and promising treatment options.

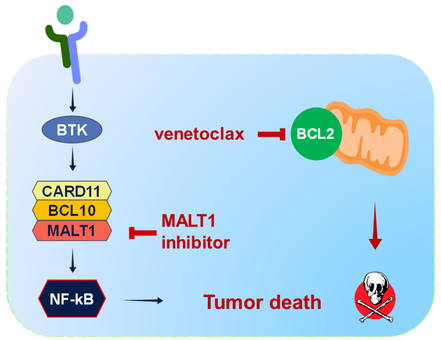
Experimental Procedures: A highly potent and orally bioavailable MALT1 protease inhibitor (ABBV-MALT1) was used to test the hypothesis that MALT1 inhibition will abrogate the proliferation of preclinical models of B cell malignancies in vitro and in vivo. Tumors treated with ABBV-MALT1 were subjected to transcriptomic and functional proteomic assays to elucidate molecular mechanisms of action and rational combination partners.

Results: Mechanistic studies reveal that ABBV-MALT1 effectively inhibits signal transduction of the BCR pathway and reduces NF-κB gene activation in non-GCB DLBCL cell lines resulting in cell cycle arrest and diminished viability. In vivo, oral administration of this compound demonstrates robust tumor growth inhibition in several models of B cell tumors, including non-GCB DLBCL models that are resistant to Bruton's tyrosine kinase (BTK) inhibitors. NF-κB target genes include the pro-survival family members BCL-X_L and BCL2-A1, which aid in regulation of the intrinsic apoptosis pathway. As ABBV-MALT1-induced inhibition of the NF-κB pathway resulted in downregulation of these genes, we hypothesized that the associated tumor models would become increasingly dependent on the pro-survival family member BCL-2. To test this hypothesis, combination studies of ABBV-MALT1 and the selective BCL-2 inhibitor venetoclax were performed in both cell line and patient-derived xenograft models of DLBCL. Herein we show that concomitant administration of ABBV-MALT1 and venetoclax results in dramatic antitumor activity in all models tested in vivo. This efficacy also translates to primary patient CLL cells in vitro where the combination confers greater levels of apoptosis compared to either agent alone.

Conclusion: ABBV-MALT1 demonstrates robust single agent anti-tumor activity in malignant B cell models that are resistant to BTK inhibitors. Moreover, combination of ABBV-MALT1 with the BCL-2 inhibitor venetoclax shows synergistic cell killing of B cell tumors in vitro and dramatic tumor regression in vivo. Together, these data indicate that MALT1 inhibition may overcome BTK inhibitor resistance and combine with venetoclax to effectively treat patients with B cell malignancies.

Graphical Abstract

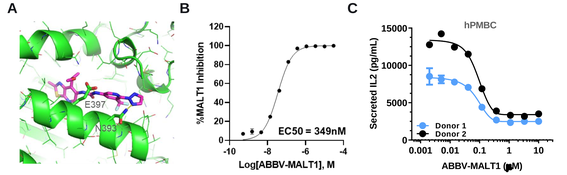
Inhibit both MALT1 and BCL-2 to drive **deep responses in B-cell tumors**



1) Shut-down pro-survival NF-κB expression programs by MALT1 inhibition.
2) Augment cell death via BCL2:BIM complex inhibition

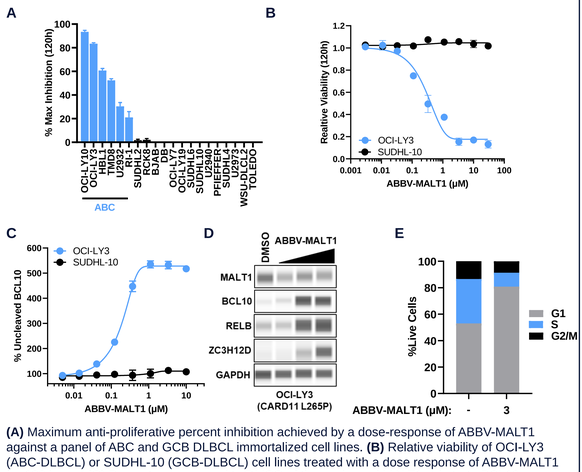
Results

Figure 1. ABBV-MALT1 inhibits MALT1 protease function through an allosteric mechanism



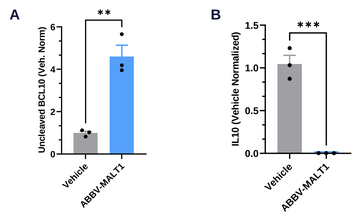
(A) Close up view of ABBV-MALT1 binding mode making mostly hydrophobic interactions with the surrounding protein residues but also makes hydrogen bonds with E397 and N393. **(B)** Biochemical assay measuring inhibition of WT MALT1 protease activity by a dose-response of ABBV-MALT1 **(C)** Secreted human IL-2 from PBMC samples from two independent donor sources (Donor 1 and Donor 2) with PMA stimulation and/or a dose response pretreatment of ABBV-MALT1. IL-2 values calculated as pg/mL and error shown as standard error of the mean of three independent experiments

Figure 2. Potent and selective inhibition of ABC subtype diffuse large B-cell lymphoma models *in vitro*



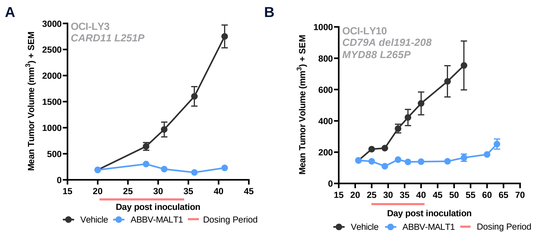
(A) Maximum anti-proliferative percent inhibition achieved by a dose-response of ABBV-MALT1 against a panel of ABC and GCB DLBCL immortalized cell lines. **(B)** Relative viability of OCI-LY3 (ABC-DLBCL) or SUDHL-10 (GCB-DLBCL) cell lines treated with a dose response of ABBV-MALT1 for 120 hours. **(C)** Percent as compared to DMSO of the uncleaved MALT1 substrate BCL10 as determined by MSD after 12-hour treatment with a dose response of ABBV-MALT1 in OCI-LY3 (ABC) and SUDHL-10 (GCB) cell lines. **(D)** Protein blot demonstrating dose-dependent upregulation of the MALT1 protease substrates BCL10, RELB, and ZC3H12D after 12-hour treatment of OCI-LY3 cells with ABBV-MALT1. **(E)** Flow based cell cycle analysis of OCI-LY3 cells treated with 3μM ABBV-MALT1 for 120 hours. Grey, G1 phase. Blue, S phase. Black, G2/M phase. Remaining percentage of cells were determined to be debris.

Figure 3. Potent and selective inhibition of MALT1 activity *in vivo*



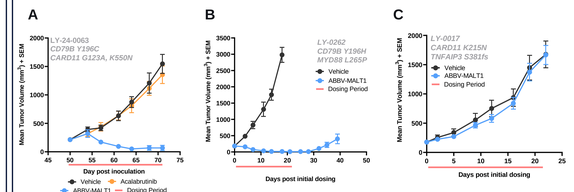
(A) Uncleaved BCL10 as determined by MSD from OCI-LY3 CDX tumors 12 hours after single 30m/kg dose of ABBV-MALT1. **(B)** Secreted human IL-10 in from plasma of mice implanted with OCI-LY3 CDX tumors 12 hours after single 30m/kg dose of ABBV-MALT1. Paired t test: ns, not significant (P > 0.05); *, P ≤ 0.05; **, P ≤ 0.01 compared with DMSO control.

Figure 4. Potent and selective inhibition of cell-line derived ABC subtype diffuse large B-cell lymphoma xenograft (CDX) models *in vivo*



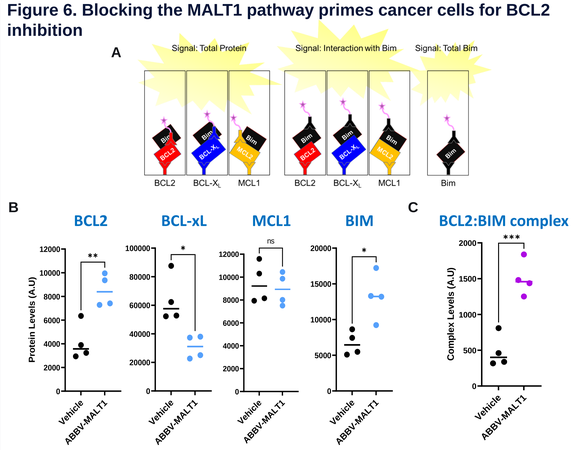
(A) ABBV-MALT1 is efficacious in the OCI-LY3 xenograft model. ABBV-MALT1 was dosed orally at 30 mg/kg. Error bars represent the standard error of the mean of n = 8 animals per group. **(B)** ABBV-MALT1 is efficacious in the OCI-LY10 xenograft model. ABBV-MALT1 was dosed orally at 30 mg/kg. Error bars represent the standard error of the mean of n = 8 animals per group.

Figure 5. Potent and selective inhibition of primary patient derived ABC subtype diffuse large B-cell lymphoma xenograft (PDX) models containing upstream activating mutations *in vivo*



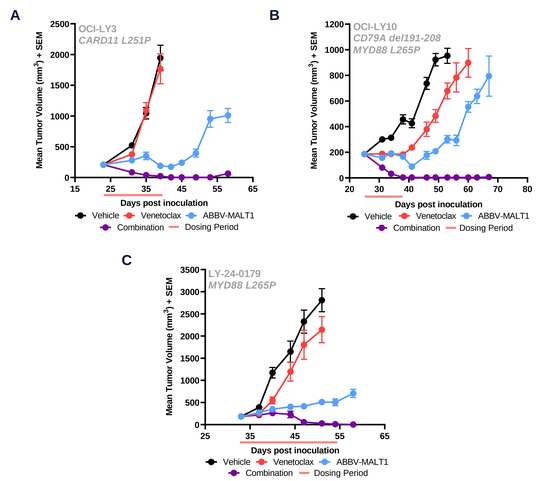
(A) ABBV-MALT1 is efficacious in the LY-0063 PDX mode whereas a covalent BTK inhibitor is inactive as predicted based on the CARD11 activating mutation. **(B)** ABBV-MALT1 is efficacious in the LY-0262 PDX model. **(C)** ABBV-MALT1 is not efficacious in the LY-0017 PDX model as predicted based on a downstream inactivating frameshift mutation of TNFAIP3. **(A-C)** ABBV-MALT1 was dosed orally at 30 mg/kg. Error bars represent the standard error of the mean of n = 8 animals per group.

Figure 6. Blocking the MALT1 pathway primes cancer cells for BCL2 inhibition



(A) Schematic of MSD assay to measure BCL2 family member proteins and the quantification of BIM complex formation. **(B)** Protein levels of BCL family member proteins (BCL2, BCL-xL, MCL1, BIM) determined by MSD in OCI-LY3 xenograft lysates after 3 days treatment with ABBV-MALT1. Paired t test: ns, not significant (P > 0.05); *, P ≤ 0.05; **, P ≤ 0.01; ***, P ≤ 0.001 compared with Vehicle control. **(C)** Protein-Protein interaction levels of BCL2 and BIM in OCI-LY3 xenograft lysate after 3 days treatment with ABBV-MALT1. Paired t test: ns, not significant (P > 0.05); *, P ≤ 0.05; **, P ≤ 0.01; ***, P ≤ 0.001 compared with Vehicle control.

Figure 7. Combination of MALT1 and BCL2 inhibition produces synergy and is highly efficacious in both CDX and PDX diffuse large B-cell lymphoma models *in vivo*



(A) ABBV-MALT1 combination with venetoclax is efficacious in the OCI-LY3 xenograft model. Compounds dosed for 14 days (Day 24-37). ABBV-MALT1 was dosed orally at 30 mg/kg and venetoclax was dosed orally at 50 mg/kg. **(B)** ABBV-MALT1 combination with venetoclax is efficacious in the OCI-LY10 xenograft model. Compounds dosed for 14 days and tumor measurements carried until study termination. ABBV-MALT1 was dosed orally at 30 mg/kg and venetoclax was dosed orally at 50 mg/kg. **(C)** ABBV-MALT1 combination with venetoclax is efficacious in the LY-24-0179 FP5 PDX model. Compounds dosed for 21 days, and tumor measurements carried until study termination. ABBV-MALT1 was dosed orally at 30 mg/kg and venetoclax was dosed orally at 50 mg/kg. **(A-C)** Error bars represent the standard error of the mean of n = 8 animals per group

Conclusions

- ABC-DLBCL represents a clinically challenging subtype of non-Hodgkin lymphoma and new treatment regimens may require combination therapy to provide meaningful patient benefit.
- ABBV-MALT1 is an allosteric inhibitor of MALT1 protease activity with an outstanding selectivity profile across lymphoma models.
- *In vitro,* ABBV-MALT1 can inhibit signal transduction downstream of cognate antigen receptors in primary immune and tumor cells. Pathway inhibition results in down-regulation of NF-κB activity making ABC-DLBCL particularly vulnerable to MALT1 inhibition.
- *In vivo*, ABBV-MALT1 monotherapy is efficacious in both CDX and PDX models of disease in a predictable manner where mutations at or upstream of the CBM complex are sensitive whereas mutations downstream of the CBM complex do not respond.
- BCR pathway inhibition by ABBV-MALT1 confers a heightened dependency on BCL-2 for tumor cell survival.
- The combination of ABBV-MALT1 with the BCL-2 inhibitor venetoclax demonstrates synergistic anti-tumor activity in preclinical DLBCL models suggesting utility in therapeutic use for the treatment of B cell lymphoma and leukemia.

Disclosures and QR Code

All authors are employees of AbbVie. The design, study conduct, and financial support for this research were provided by AbbVie. AbbVie participated in the interpretation of data, review, and approval of the publication.

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