Pharmacological Inhibition of MALT1 Reverses Activation-Induced Metabolic Reprogramming and Ameliorates Autoimmune Pathogenesis in Multiple Animal Models of Chronic Inflammation

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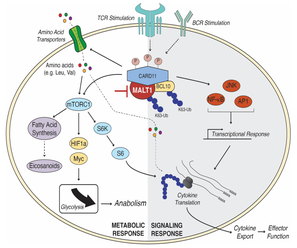
#ACR21 ACR Convergence

Poster Session D
Poster No. 1509

Introduction

Immune cells modulate their metabolism to develop effector functions and mediate immunity and inflammation[1]. Evaluating metabolic pathways can, therefore, provide mechanistic insights to support the development of novel therapeutics, select biomarkers, enable patient selection and, ultimately, transform the treatment of autoimmune and inflammatory diseases (A&ID).

MALT1 (Mucosa-associated lymphoid tissue lymphoma translocation protein-1) is a central node in immune cell signaling downstream of immunoreceptor tyrosine-based activation motif (ITAM)-containing immune cell receptors and has been implicated in the pathogenesis of A&ID[2]. While MALT1 signaling is known to control NF-kB activation, its key role in metabolic regulation is starting to be appreciated.[3,4]



Rheos is developing a MALT1 inhibitor for the treatment of A&ID. In this study, we show that ITAM-activated T cells, B cells, and macrophages engage a common set of metabolic pathways, reflecting their high metabolic demands, that we have termed the ITAM anabolic hub. MALT1 inhibition dampens the ITAM anabolic hub and associated proinflammatory effector function both *in vitro* and *in vivo* and leads to efficacy in multiple animal models of autoimmune diseases.

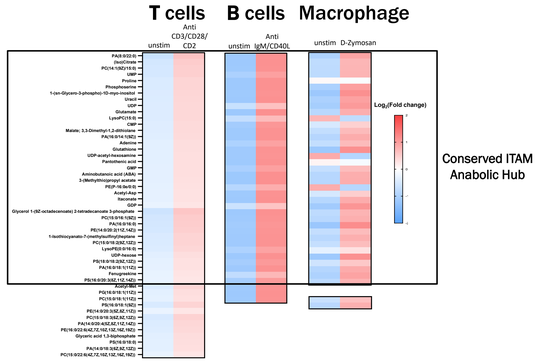
Table 1. Summary of activity of allosteric MALT1 inhibitors

		Potency (nM)	
Assay	Stimulus	Compound 1	Compound 2
Biochemical activity (paracaspase activity)	-	20	47
T cell IL-2 release (primary human memory T cells)	anti-CD3/CD28	20	31
T cell IFNg release (primary human memory T cells)	anti-CD3/CD28	43	48

References

1. Chimenti et al, 2015. Cell Death Dis. PMID: 26379192
2. Crotti et al, 2012. Arthritis Research and Therapy PMID 23146195
3. Hamilton et al. Sci Signaling 2014 PMID: 24917592
4. Zhu et al. 2019 Mo, Carcinog PMID 315569
5. Moore AR, 2003. Methods Mol Biol. PMID: 17172717
6. Constantinescu et al, 2011. Br J Pharmacol. PMID: 21371012
7. Strattan et al, 2019. Front Immunol. PMID: 31681336

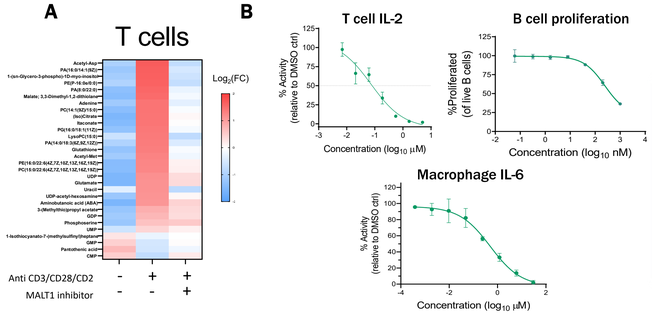
ITAM Activation Induces a Conserved Anabolic Phenotype Across Multiple Immune Cell Types



- Of the 37 metabolites found in all three cell types, 33 were coordinately induced upon ITAM stimulation.
- Conserved metabolites are enriched for biosynthetic precursors (amino acids, lipids and nucleic acids), TCA cycle intermediates, amino sugars, neurotransmitters, and several sulfur-containing species.
- Metabolite profile represent an ITAM-coupled anabolic phenotype or ITAM anabolic hub, consistent with proliferation, redox management, and cellular communication

Figure 1. **(A)** Intracellular metabolites were measured from primary human CD45RO$^+$ T cells activated with anti-CD3/anti-CD28/anti-CD2 (average of 25 independent experiments). Top 50 metabolites compared to unstimulated are represented. The impacts of ITAM stimulation on the same set of metabolites were assessed in **(B)** primary human B cells activated with anti-IgM/CD40L and **(C)** human monocyte derived macrophages activated with depleted Zymosan (D-Zymosan). Legend indicates level of induction (log2 fold change from median value). Red= increased, blue = decreased.

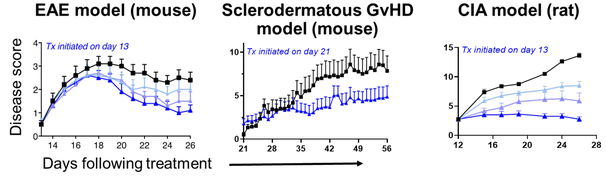
MALT1 Inhibition Reverses ITAM-Induced Anabolic Hub and Effector Function *In Vitro*



- MALT1 inhibition reverses engagement of ITAM- coupled anabolic metabolites in T cells
- MALT1 inhibition blocks multiple effector functions across multiple cell types

Figure 2. **(A)** Treatment with a MALT1 inhibitor (MALT1i, compound 1) reversed activated T cell associated anabolic phenotype. Heatmap displays 30 metabolites from the ITAM anabolic hub shown as the average Log2 fold change from the median value across 2 donors. Red = increased, blue = decreased. **(B)** MALT1 inhibition (compound 1) blocked ITAM-mediated effector cytokine production in a concentration dependent manner. MALT1 inhibition reduced IL-2 production from anti-CD3/CD28/CD2 activated CD45RO memory T cells, IgM/CD40L induced B cell proliferation and IL-6 production from D-Zymosan activated macrophages.

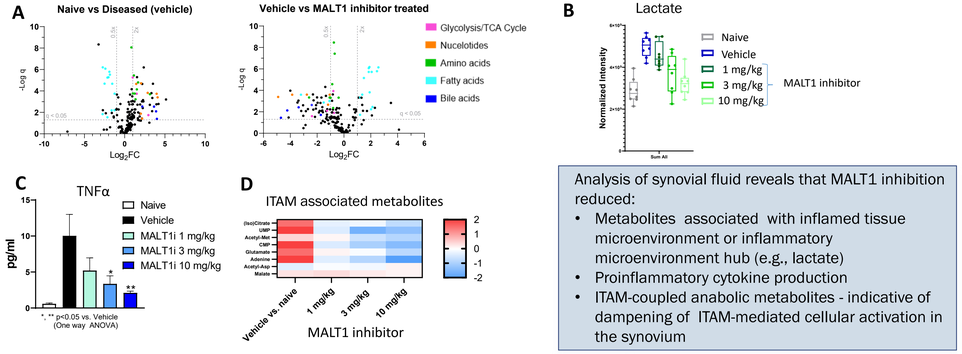
MALT1 Inhibition is Efficacious in Multiple Preclinical Models of Autoimmune and Inflammatory Diseases



MALT1 inhibition decreased disease activity across multiple pre-clinical models representing pathogenic drivers of human autoimmune and inflammatory diseases

Figure 3. MALT1 inhibition is efficacious in multiple animal models of chronic autoimmune and inflammatory diseases. MALT1 inhibitors were administered orally once per day. Treatment in all models was initiated at first signs of disease (as indicated). Black line = Vehicle, Blue lines = MALT1 inhibitor with increasing dose represented by increasing darkness. Each model was performed as previously described.[5,6,7] Dosing scheme for each model: EAE (cmpd 1) = 0.1, 1, 10 mg/kg; scGVHD (cmpd 1) = 100 mg/kg; rat CIA (cmpd 2) = 1, 3, 10 mg/kg.

MALT1 Inhibition Normalizes the Metabolic Microenvironment in the Joints of Rats with CIA



Analysis of synovial fluid reveals that MALT1 inhibition reduced:

- Metabolites associated with inflamed tissue microenvironment or inflammatory microenvironment hub (e.g., lactate)
- Proinflammatory cytokine production
- ITAM-coupled anabolic metabolites - indicative of dampening of ITAM-mediated cellular activation in the synovium

Figure 4. Synovial fluid was collected from arthritic knees of rats in the CIA model by lavage (50 µl PBS/joint) and analyzed via pHILIC chromatography (LCMS). **(A)** Shown are a subset of metabolites induced in disease (left panel) and were reversed upon MALT1 inhibitor treatment (right panel) **(B)** Lactate levels were reduced after MALT1 inhibitor treatment. **(C)** Proinflammatory cytokines were reduced in synovial fluid after MALT1 inhibitor treatment as exemplified by the dose dependent decrease in TNFα. **(D)** ITAM activation associated metabolites were detected in synovial fluid and modulated by MALT1 inhibition

CONCLUSIONS

1. MALT1 is a promising target for the treatment of autoimmune and inflammatory diseases.

2. Metabolic analyses provide insight into both the cellular physiology of ITAM-mediated activation as well as the mechanisms of MALT1 inhibition *in vitro* and *in vivo*.

- ITAM activation induced a conserved anabolic phenotype (anabolic hub) in multiple immune cell types, reflecting their metabolic demands. MALT1 inhibition dampens the engagement of the anabolic hub that is associated with decreased effector function.
- A subset of ITAM-coupled anabolic metabolites are present in synovial fluid of rat CIA, indicating ITAM mediated cellular activation. These metabolites are dose dependently inhibited via MALT1 inhibition consistent with *in vitro* findings.