Selective Suppression of Regulatory T-cell Development with Small Molecule Inhibitors of Mucosa-Associated Lymphoid Tissue Lymphoma Translocation Protein 1 (MALT1)

Niklas Finnberg[1], Fredrik Öberg[1], Anders Kallin[1], Agni Christodoulidou[1], Susanne Sedig[1], Anna-Karin Sternbeck[1], Johan Bylund[1], Sara Moses[1], Lourdes Salvador Oden[1], Mari Kullman-Magnusson[1], Ian Henderson[1], Sara Mangsbo[2], Hans-Georg Beisel[1], Karolina Ersmark[1], Sofia Karlström[1], Richard Bethell[1] and Mark Albertella[1]

[1]*Medivir AB, Huddinge, Sweden 141 22*

[2]*Department of Pharmaceutical Biosciences, Science for Life Laboratory, 751 24 Uppsala University, Uppsala, Sweden*

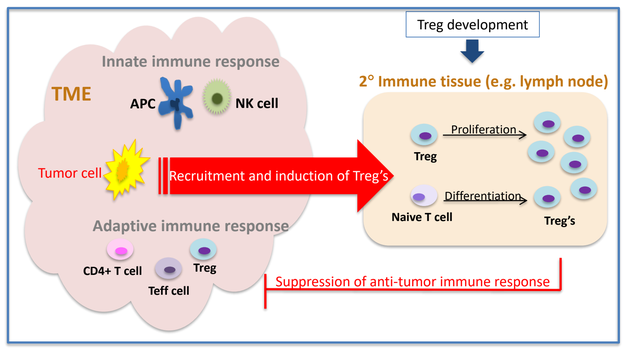
Introduction

- Mucosa-associated lymphoid tissue lymphoma translocation protein 1 (MALT1) is a protease and scaffold protein that mediates NF-kappaB signaling downstream of the T cell receptors (TCR)
- MALT1 is a molecular target in hematologic malignancies with constitutively active MALT1-signaling and Th_{17}-driven autoimmune disease indications. Pharmacologic inhibition of MALT1 has shown efficacy in preclinical disease models
- Genetic evidence from MALT1-deficient mice suggest that MALT1 promotes development of immune suppressive regulatory T cells (Treg) in vivo. We hypothesize that this could be a novel target for small molecules in the context of cancer immune therapy applicable to multiple cancer indications[1]
- Here we describe our chemistry program that explores two distinct chemical series with unique inhibition profiles of MALT1's protease (**Series 1** and **2**) and scaffold functions (**Series 2**)
- MALT1-inhibitors (MALT1i) of **Series 1** and **2** inhibit Treg differentiation from human CD4+ naïve T cells in vitro

Scientific rationale

Suppression of regulatory T-cells (Treg) to enhance anti-tumor immune response

- Immune system plays a key role in tumor development
  - Treg cells inhibit several types of immune cells and thereby suppress the anti-tumor immune response
  - Tumor cells recruit and induce development of Treg cells
- Selective suppression of Treg cells, without negatively affecting Teff and other immune cells, is predicted to enhance the anti-tumor immune response in cancer patients and possibly potentiate other immunotherapies[2-5]

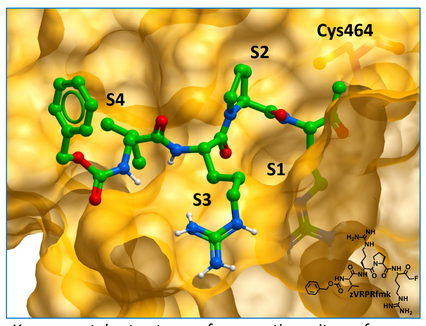


Tregs in the tumor microenvironment (TME)

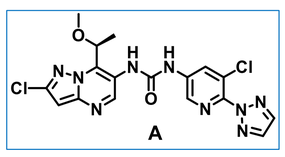
Chemistry

Two distinct chemical series with different inhibition profiles have been identified

- **Series 1:** Active site non-covalent binders inhibiting mainly the protease function
  - Design based on protease substrate preferences and co-crystallization studies of ligands with MALT1 protein. A docking platform is available for design
  - Selective compounds with high MALT1 inhibitory activity and good solubility
- **Series 2:** Allosteric non-covalent binders[6] inhibiting both the protease and some activities associated with the scaffold function
  - "Literature-to-lead" approach based on known MALT1 inhibitors
  - Selective compounds with high potency and promising DMPK profiles
  - Reference **A** from the patent literature[7] was used as tool compound in different assays



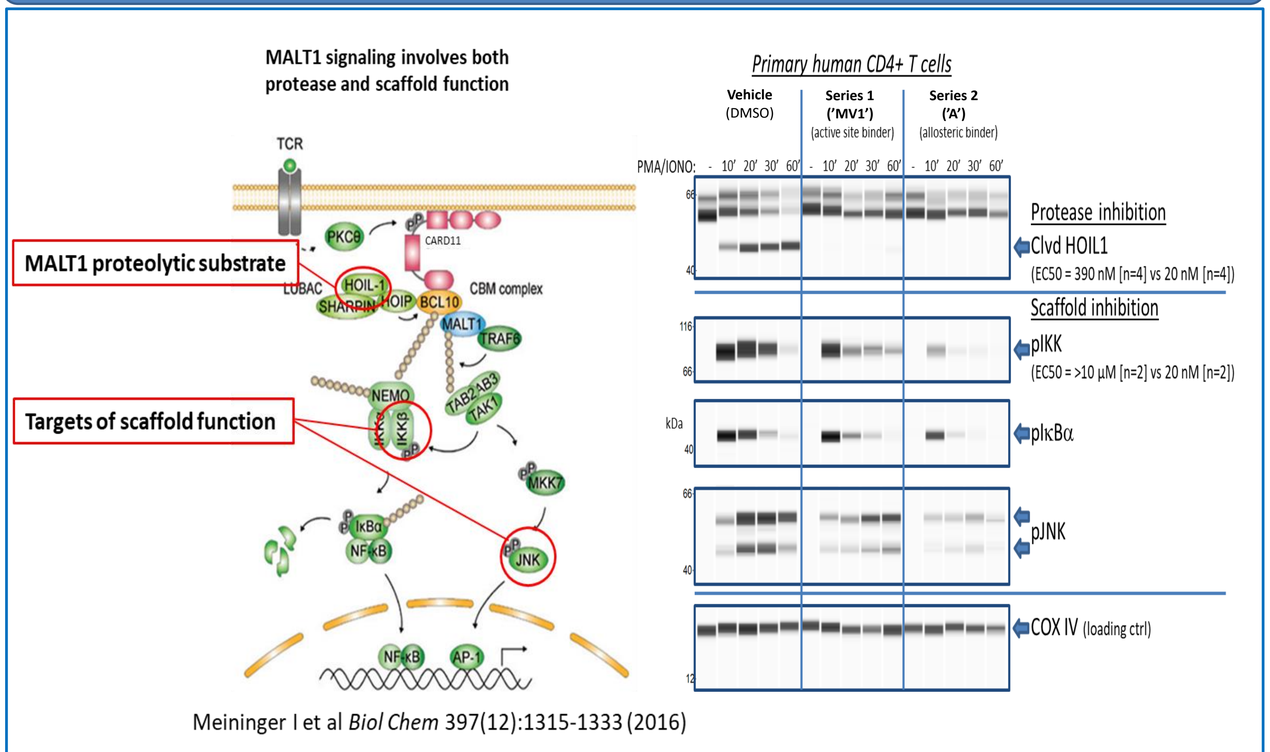
*X-ray crystal structure of an active site reference compound (**zVRPRfmk,** MALT1 K_i = 2 nM) bound to the active site of MALT1. Interactions with S1 and S4 subsites turned out to be important for potency*



		Series 1 (active site binders)		Series 2 (allosteric binders)		
		MV1	MV2	A	MV3	MV4
PhysChem properties	Mw	569	514	448	414	409
	Solubility (Kin. @ pH 7.4) (µM)	98	99	1	33	4
In vitro properties	MALT1 Human/Mouse K_i (nM)	5/22	20/ND	11/5	18/ND	37/ND
	Jurkat HOIL1/IL2 IC_{50} (nM)	340/430	480/540	13/12	41/20	46/9
	CC_{50} A549/CEM/MOLT-4/RAJI (µM)	>50/>50/19/>50		>100/>50/20/>50		
	Selectivity Thrombin/Trypsin/Cat S	>40-fold*		>40-fold*	>40-fold	
	HLM/MLM (µL/min/mg)	250/>300	43/60	7/10	37/45	18/9
	Caco-2 Papp -/+ GF120918 (10^{-6} cm/s)	3/10	0.4/5	20/28	5/15	**/19
	fu plasma Human/Mouse (%)	1.2/9.4		1.1/2.6	ND/12	
In vivo properties	F Mouse (%)	5		93	65	
	CL Mouse (mL/min/kg)	87		2	7.4	
	Vss Mouse (L/kg)	1.7		0.9	0.8	

* Also including Caspases 1-10, EGFR and BTK. **Recovery <70%. ND=Not determined

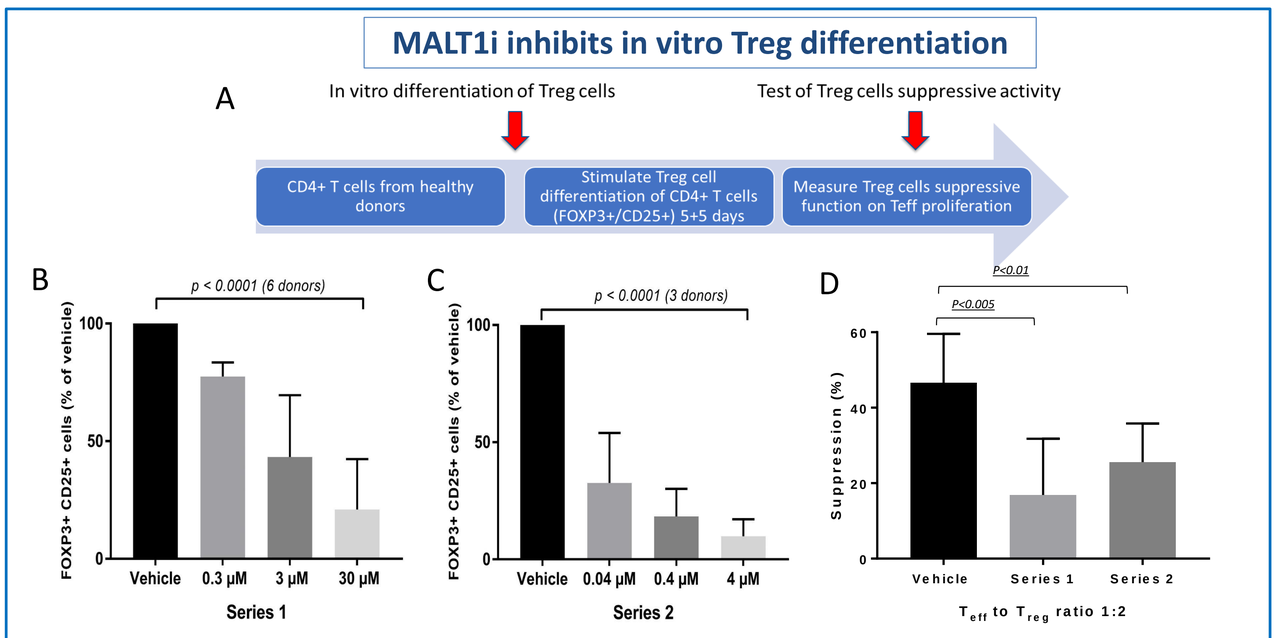
Biology



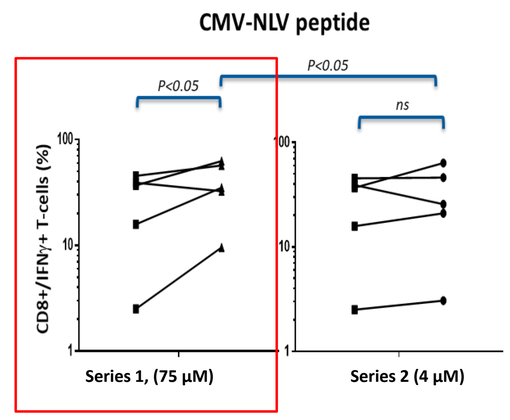
Meininger I et al *Biol Chem* 397(12):1315-1333 (2016)

Allosteric MALT1i disrupt the scaffold function of MALT1 The capacity of the active site inhibitor '**MV1**' and the allosteric reference compound '**A**' to inhibit MALT1's scaffold and protease function was assessed by Simple Western of lysates from human CD4+ T cells following activation with PMA and ionomycin

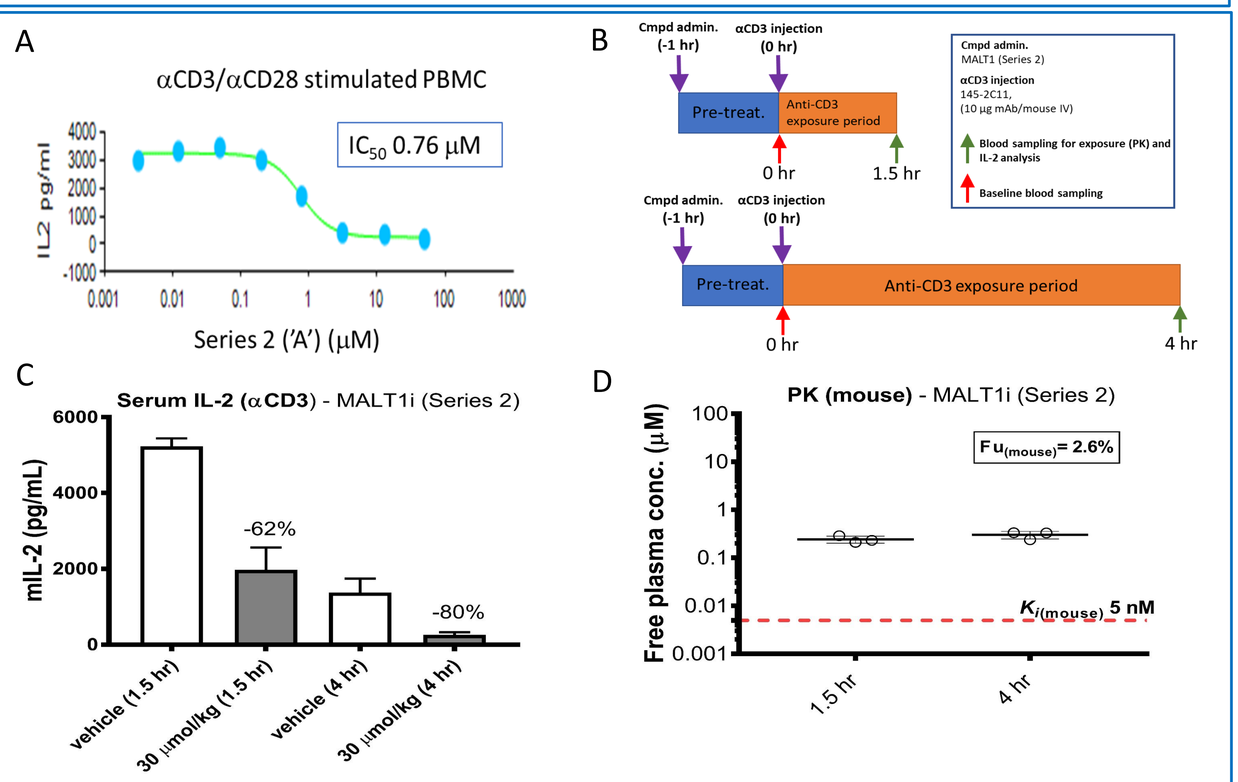
MALT1i inhibits in vitro Treg differentiation



Assessment of the impact of MALT1i on Treg differentiation in vitro. (**A**) Schematic of the Treg in vitro differentiation protocol. (**B**) Series 1 ('**MV1'**) and (**C**) Series 2 ('**A'**) compounds are potent inhibitors of TGF-beta/IL2-dependent in vitro Treg differentiation following CD3/CD28 bead stimulation. No general inhibitor effect on CD4+ T cell activation and proliferation was observed (data not shown). (**D**) Suppression of CD3/CD28 stimulated Teff cells (CD4+/CD25-) by in vitro differentiated Treg cells was assessed after 4 days. Statistical assessment by Students *t* test. Mean percentage +/-SEM is shown



Series 1 MALT1i enhance activation of CD8+ memory T cells following CMV re-challenge ex vivo. Whole blood from four to five different healthy donors was used as an ex vivo ('blood loop') system to measure memory CD8+ T cell activation (percentage of CD8+/IFN+ T cells) following CMV peptide re-challenge in the absence and presence of MALT1i[8]. Series 1 MALT1i ('**MV1'**) but not MALT1i of series 2 ('**A'**) generated improved recall responses that were blood donor-independent and statistically significant (P<0.05, Students t test) in whole-blood system with dosing adjusted for differences in protein plasma binding



Series 2 ('A') MALT1i blocks serum IL-2 release in vitro and in vivo following anti-CD3 challenge. Administration of 30 µmol/kg PO of compound A to mice was well-tolerated and gave an average 24 hr free plasma exposure twenty-one times greater than the mouse K_i (data not shown). (**A**) IL-2 secretion in human PBMC stimulated with anti-CD3/anti-CD28 beads for 24 hr. (**B**) Schematic of mouse in vivo experiment. (**C**) Mouse serum IL-2 levels 1.5 and 4 hrs after anti-CD3 treatment in the presence and absence (vehicle) of MALT1i. Average of N=3 is shown +/-SEM. (**D**) Free plasma concentration of 'A' in mouse plasma at 1.5 and 4 hrs following anti-CD3 in relation to K_i. Median of an N=3/time point is shown. Fu indicates fraction unbound (%) drug

Conclusions

- Two distinct chemical series of MALT1i have been identified, **Series 1** and **Series 2,** with different capacities to disrupt the MALT1 scaffold function
- Both MALT1i series show selective inhibition of human CD25+/FoxP3+ Treg differentiation in vitro without inhibition of activation-induced proliferation of other T cell populations or apparent cytotoxicity
- This novel small molecule approach to Treg-targeting may improve the response to immune therapy for multiple cancer indications without additive/synergistic toxicities
- In vivo TGI and Treg data is currently being validated
- A chemistry program is in progress with the aim to select a final molecule for clinical development
- ***Medivir is seeking a collaboration partner***

References

1. Albertella, M., Öberg, F. 2018 WO 2018/141749.
2. Jaworski, M. and Thome, M. Cell Mol Life Sci. 2016. 73(3): 459-73.
3. Hachmann, J. and Salvesen G. Biochimie 2016. 122: 324-338.
4. Rebeaud, F. et al. Nat Immunol 2008. 9 (3): 272-81.
5. Meininger I et al Biol Chem 2016. 397 (12):1315-1333.
6. Surface plasmon resonance (SPR) and enzyme kinetics studies are in support of an allosteric binding site (data not shown).
7. Pissot-Sodermann, C. et al. 2015 WO 2015/181747
8. Fletcher, E. A. K. et al. J Immunol 2018. 201 (1): 87-97.