

PRECLINICAL CHARACTERIZATION OF AN ORAL SMALL MOLECULE INHIBITOR TARGETING THE INTEGRIN $\alpha4\beta7$ FOR THE TREATMENT OF INFLAMMATORY BOWEL DISEASES (IBD)

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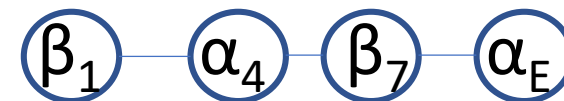
Abstract:

Leukocyte trafficking from the circulation to the bowel is a tightly regulated process through highly specific molecular interactions between leukocytes and gut tissues. A breakdown in the regulatory pathways contribute to the development of chronic inflammatory bowel diseases such as Crohn's disease (CD) and Ulcerative colitis (UC). Among the key players, the role of integrin $\alpha4\beta7$ and its interaction with mucosal addressin cell adhesion molecule 1 (MAdCAM-1) in the pathogenesis of IBD has been realized leading to the development of the life-changing therapy vedolizumab for patients with IBD. To overcome the limitations associated with biologics and to take advantage of the efficacy and safety profile of vedolizumab, Morphic has identified a novel, orally bioavailable, small molecule compound to perturb the $\alpha4\beta7$ integrin pathobiology. The compound has sub-nanomolar to single-digit nanomolar potency in a suite of biochemical and cell-based functional assays. It is highly selective over a broad panel of other integrin family members including $\alpha4\beta1$. The compound has favorable pharmacokinetic properties across preclinical species.

To demonstrate the mechanism of action of the $\alpha4\beta7$ inhibitor in vivo, the compound was evaluated in an acute pharmacodynamic assay in mice, in which the compound was able to retain gut trafficking lymphocytes in circulation with similar effectiveness as an anti- $\alpha4\beta7$ monoclonal antibody. When dosed in non-human primates (NHPs), compound treatment led to the accumulation of mucosal homing $\beta7$ high CD4+ T memory cells in the peripheral blood, similar to vedolizumab. A translational receptor occupancy assay was developed to determine the degree of receptor engagement required for efficacy on these cells, consistent with the effectiveness of the compound inhibitory activity. Finally, the oral small molecule compound was assessed in a lymphocytosis assay in mice by measuring the total lymphocytes and pre-B cell counts in the blood after compound treatment, confirming its high selectivity on $\alpha4\beta7$ over $\alpha4\beta1$.

Disclosures

- Jamie Wong, Matthew Bursavich, Natalia Blanco, Adam Camblin, Laura Cappellucci, Rhianna Cohen, Dan Cui, Kristopher Hahn, Megan Krumpoch, Dooyoung Lee, Fu-Yang Lin, Blaise Lippa, Alex Lugovskoy, Molly McShea, Maloy Mangada, Siavash Mostafavi, Terence Moy, Adrian Ray, Naresh Redhu, Allison Sang, Andrew Sullivan, Dawn Troast, Cheng Zhong, Liangsu Wang, and Bruce Rogers are/were paid employees of Morphic Therapeutic and owners of Morphic Therapeutic stock.
- Peter Traber is a paid consultant of Morphic Therapeutic

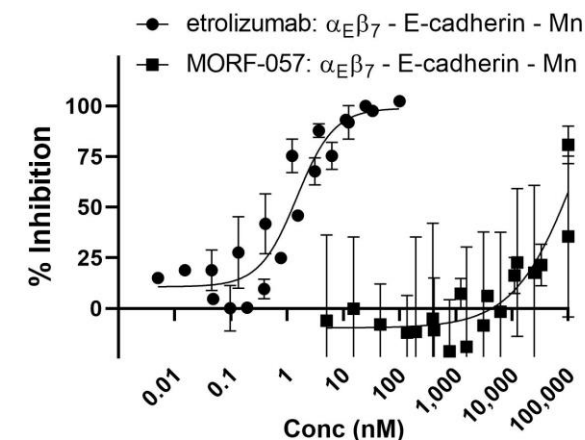
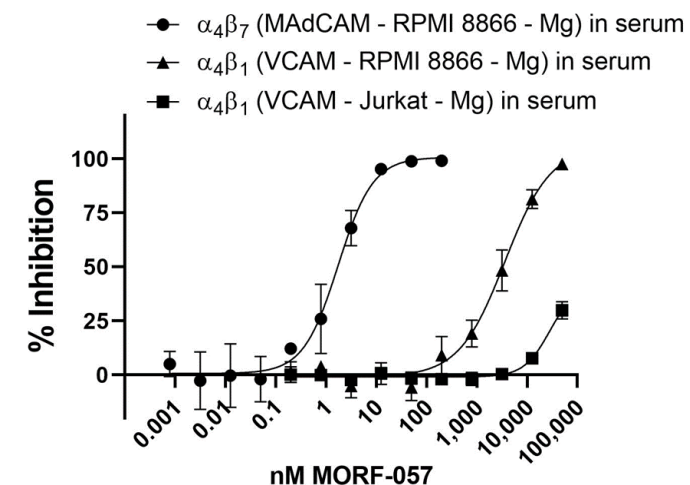


MORF-057 is a Potent, Selective Inhibitor of $\alpha_4\beta_7$

Cell Adhesion Assays (CAAs) mechanistically mimic the “adhesion” step immune cells undergo via integrins binding to ligand during the extravasation process. CAAs are the most sensitive potency assays that account for avidity interactions between cells expressing the target integrin and a ligand coated surface. The α_4 assays are run under physiologic conditions with Mg and are used to test selectivity with nearest $\alpha_4\beta_7$'s integrin heterodimers.

Inhibitor	$\alpha_4\beta_7$ IC ₅₀ ^a RPMI8866 MAdCAM in 50% serum	$\alpha_4\beta_1$ IC ₅₀ ^a Jurkat VCAM in 50% serum	$\alpha_4\beta_1$ IC ₅₀ ^a RPMI8866 VCAM in 50% serum	$\alpha_4\beta_7/\alpha_4\beta_1$ Fold selectivity	$\alpha_E\beta_7$ IC ₅₀ ^a K562- $\alpha_E\beta_7$ E- Cadherin	$\alpha_4\beta_7/\alpha_E\beta_7$ Fold selectivity
MORF-057	1.2 ± 0.8 nM	>50 μM	4,290 ± 670 nM	>3,000	52 μM	>143,000
Vedolizumab	0.035 ± 0.020 nM	>180 nM	>1,000 nM	>3,000	ND	--
Natalizumab	0.166 nM	1.8 nM	0.14 nM	1-12	ND	--
AJM300 ^b	93 ± 66 nM	4200 nM	779 ± 261 nM	8-45	ND	--
Etrolizumab	0.0185 nM	ND	>1,000 nM	>10 ⁶	1.2 nM	14

Cell line characteristics: Jurkat cells have been traditionally used for specifically assessing $\alpha_4\beta_1$ potency, as these cells do not express $\alpha_4\beta_7$. RPMI8866 cells have lower levels of $\alpha_4\beta_1$ that likely better approximate expression levels in human blood. ^aGeometric mean ± standard deviation for n = 3 to 8, no standard deviation is shown for n = 1 or 2. ^bactive metabolite of AJM300



Data points show mean values.
 Error bars show standard deviations.



MORF-057 is selective against other integrin family members

2nd tier Cell-Free Selectivity Assays

The Radioligand Competition Assays are cell-free and use purified proteins and probes that can be run under the physiologically relevant Mg concentration. This assay has greater sensitivity due to measurable $\alpha_4\beta_7$ potency, and greater contextual relevance using Mn-free conditions.

	MORF-057 Radioligand K_i^a (nM)	Fold selective vs $\alpha_4\beta_7$ in Mg
$\alpha_4\beta_7$	0.217 ± 0.142 ^a nM	1
$\alpha_v\beta_1$	8,645	39,800
$\alpha_v\beta_6$	> 19,400	>894,000
$\alpha_v\beta_8$	2,519	11,600

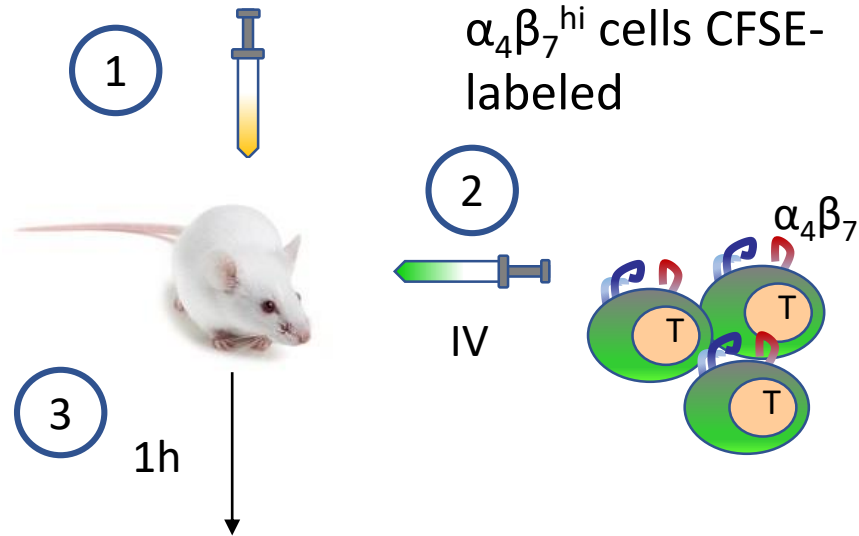
^a Geometric mean ± standard deviation with n = 3, no standard deviation is shown for n = 1 or 2 or for values with > or < qualifiers

Fluorescence Polarization Competition Assays are cell-free, high-throughput potency assays that must be run under Mn conditions. The presence Mn in the assays is not physiologically relevant and is known to increase the affinity of Morphic inhibitors to the target.

	MORF-057 FP-Mn IC_{50}^a (nM)	Fold selective vs $\alpha_4\beta_7$ in Mn
$\alpha_4\beta_7$	BLLOQ (< 3.3)	1
$\alpha_2\beta_1$	1,910	> 578
$\alpha_3\beta_1$	> 200,000	> 60,600
$\alpha_5\beta_1$	7,840	> 2,370
$\alpha_v\beta_3$	> 200,000	> 60,600
$\alpha_v\beta_5$	> 200,000	> 60,600
$\alpha_{IIb}\beta_3$	> 200,000	> 60,600

Gut Homing Assay Schematic

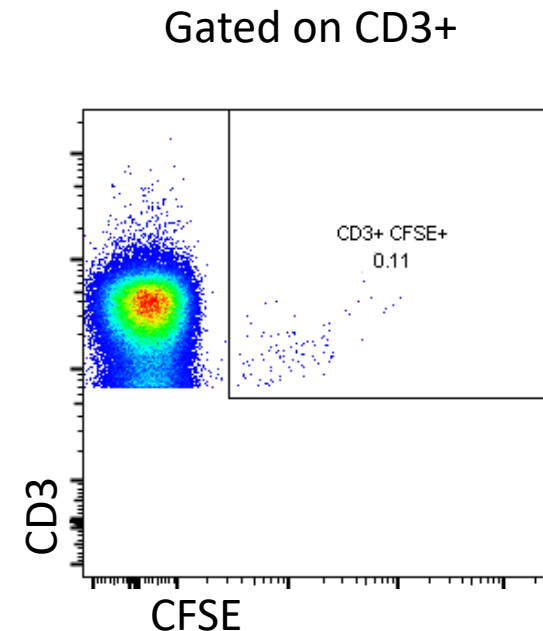
1' Drug (S.C.)



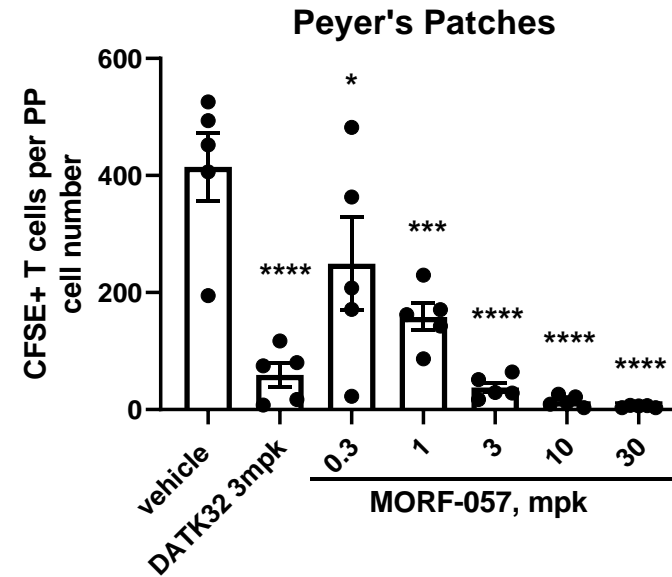
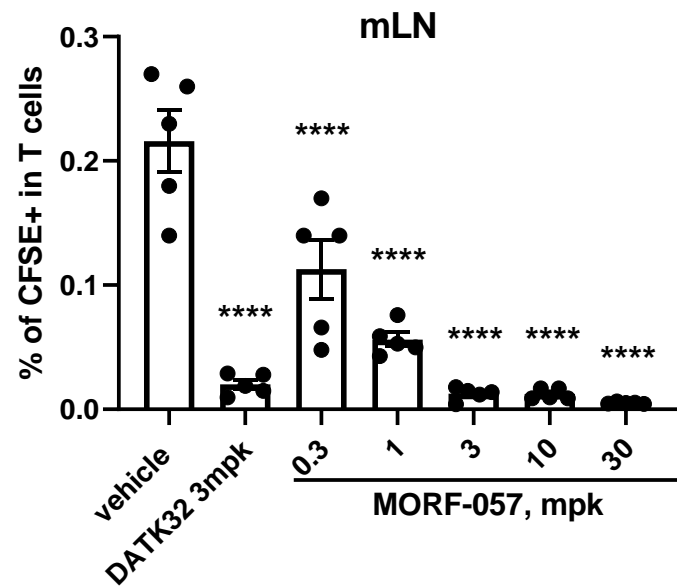
FACS analysis:

1. mLN/PP = mesenteric LN/Peyer's Patches = MAdCAM-dependent homing
2. pLN = peripheral LN
3. BL = blood (PK and RO)
4. SP=spleen

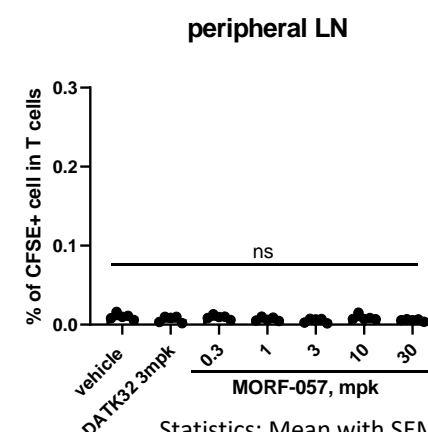
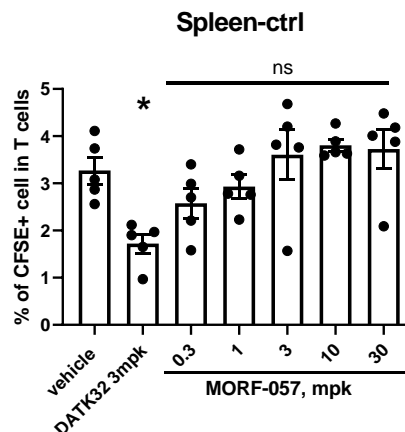
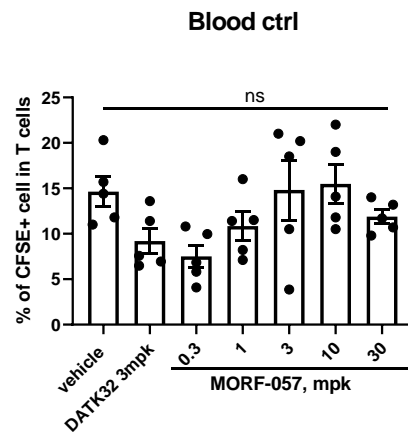
The Gut Homing Assay provides a method to examine $\alpha_4\beta_7$ -specific inhibition in vivo. The T cells used in this assay only have integrin $\alpha_4\beta_7$ for homing and do not express other adhesion molecules.



MORF-057 Specifically Inhibits Gut Homing T cells in Mice



- Treatment with MORF-057 blocks gut homing of $\alpha_4\beta_7^+$ cells in mice
- MORF-057 achieves inhibition of gut trafficking equivalent to antibody treatment (DATK32)



DATK32 = anti- $\alpha_4\beta_7$ mouse mAb
(Andrew et al, J. Immunol. 1994)

Statistics: Mean with SEM, treatment groups compared to vehicle, One-way analysis of variance (ANOVA), followed by Bonferroni's multiple-comparison test,* P<0.05, ***P<0.001, ****P<0.0001

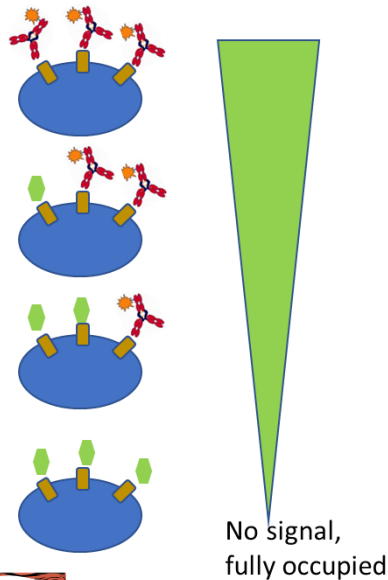
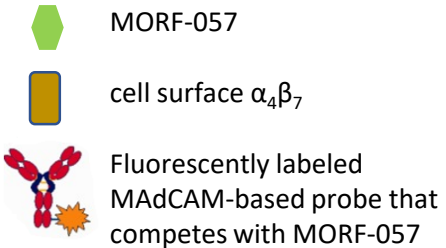


$\alpha_4\beta_7$ Receptor Occupancy (RO) in NHP Dosed with $\alpha_4\beta_7$ selective inhibitor, MR-5288, is >90% at C_{trough}

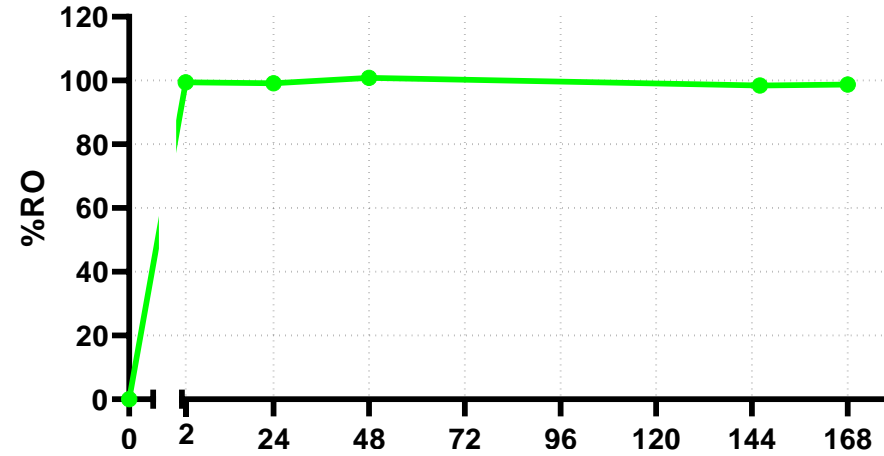
Cynomolgus monkeys were dosed orally, twice daily with MORF-057^a or close analog MR-5288^b. Receptor occupancy and changes in lymphocyte populations were examined in the peripheral blood.

^alymphocyte population data on following slide

^bMR-5288 has a cell adhesion assay IC_{50} potency (RPMI8866/MAdCAM, with serum) of 0.34 ± 0.17 nM

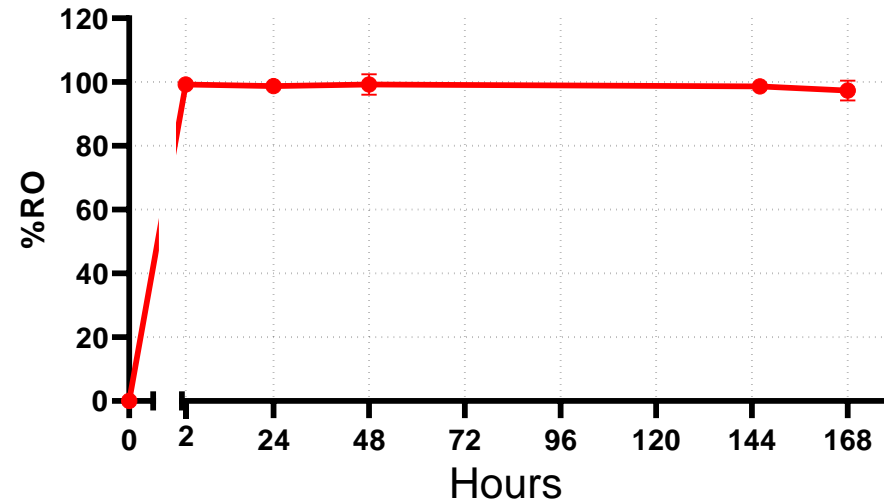


MR-5288 50 mpk (Mean \pm SD), N=8

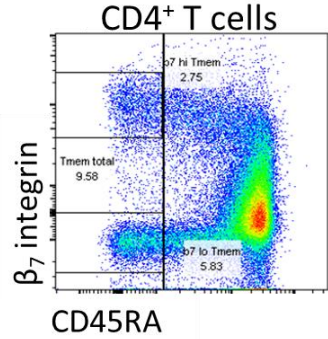


- Mn-free RO assay (ex vivo whole blood) using a multimeric, MAdCAM-based probe enables physiologically relevant RO measurements
- Range of % RO for all animals at all timepoints post first dose is 90.4 to 100%.

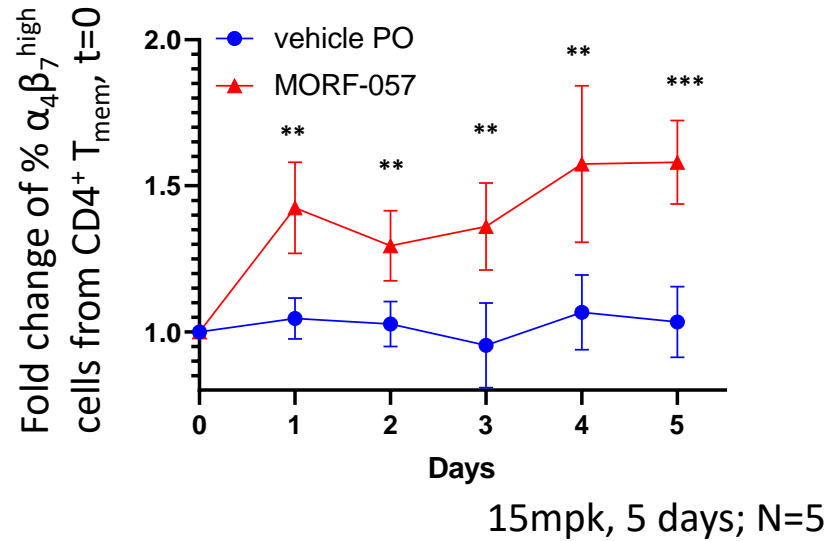
MR-5288 30 mpk (Mean \pm SD), N=8



Morphic Inhibitors Impact the T_{mem} Biomarker in a Dose-Responsive Manner

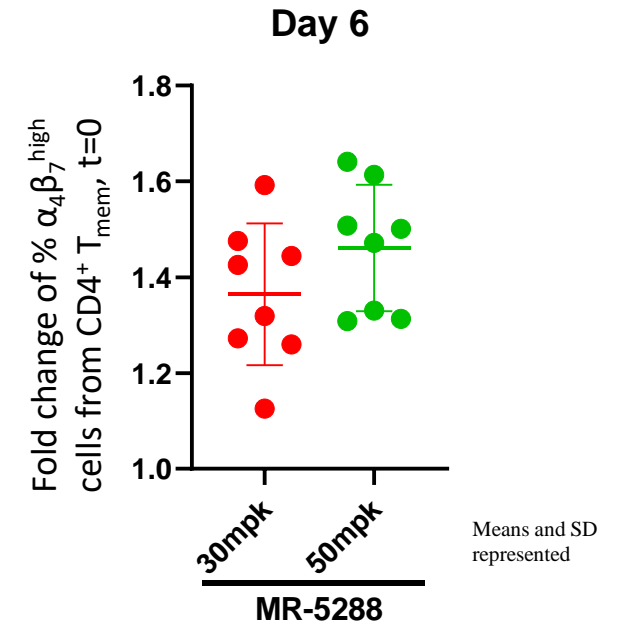
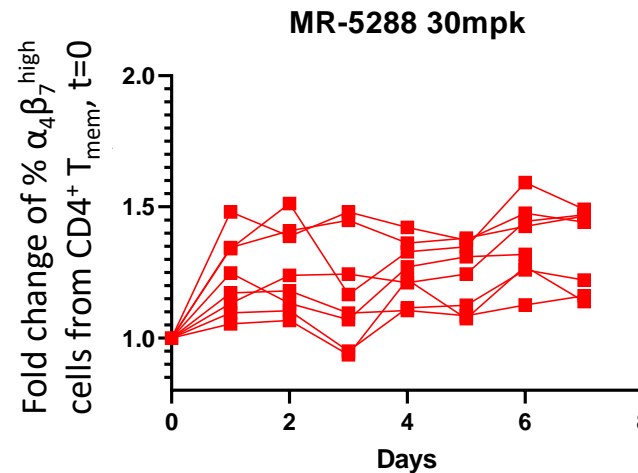
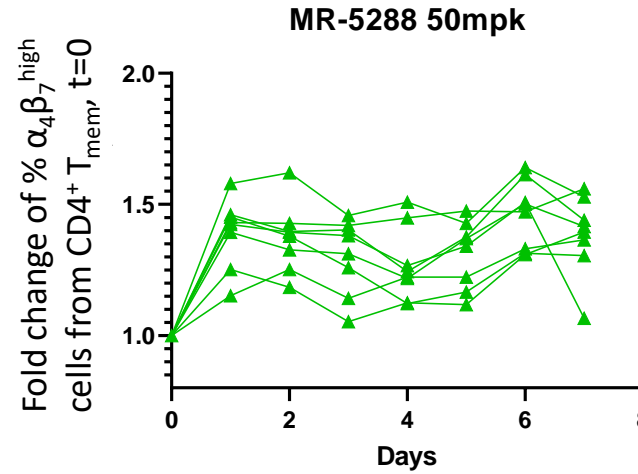


- MORF-057 inhibits $\alpha_4\beta_7^+$ CD4⁺ T cell trafficking to mucosal sites in NHP



Means and SD of data normalized per individual at timepoint 0h (first dose administration). T test analysis was performed at each timepoint. Statistical significance determined using the Holm-Sidak method, with alpha = 0.05. ** p < 0.01, *** p < 0.001

MR-5288 Dose Response, N=8

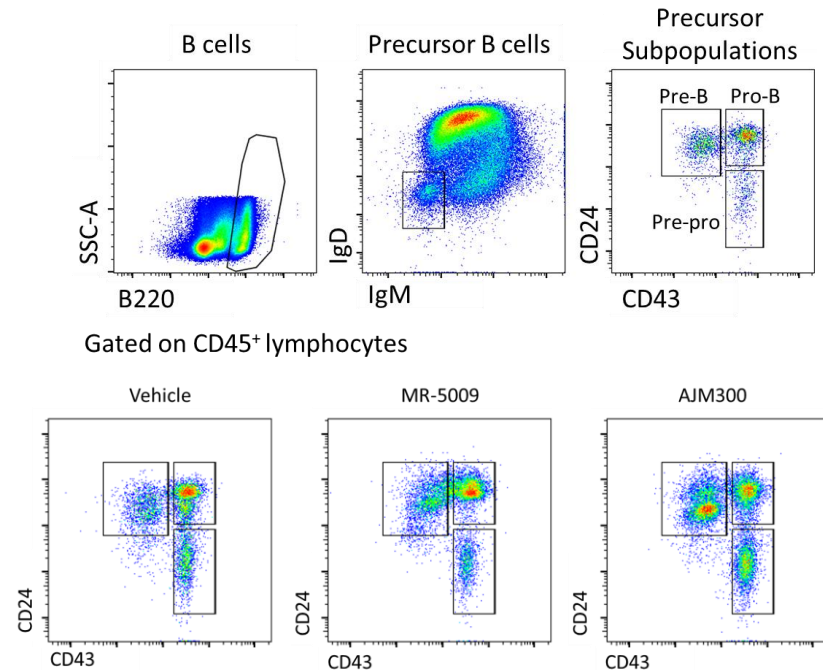
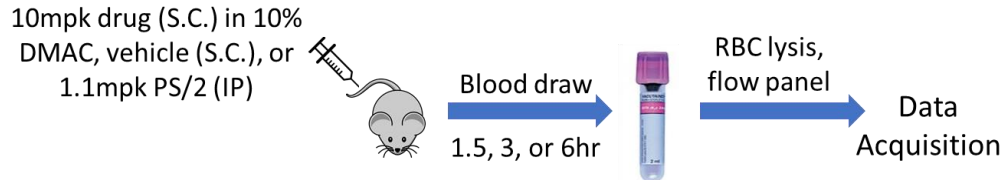


- The biomarker response with MR-5288 (close analogue of MORF-057) is dose responsive

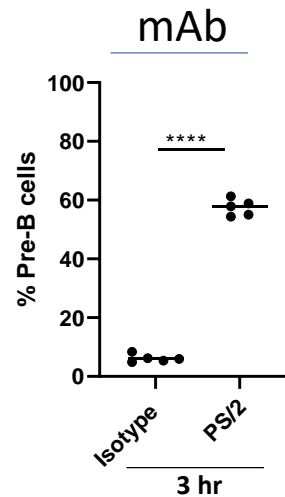
Morphic Inhibitor Does Not Increase the Pre-B Cell Frequencies in Mouse Peripheral Blood at 1.5 Hours

Disruption of $\alpha_4\beta_1$ binding to VCAM displaces pre-B cells from the bone marrow niche into the circulation.

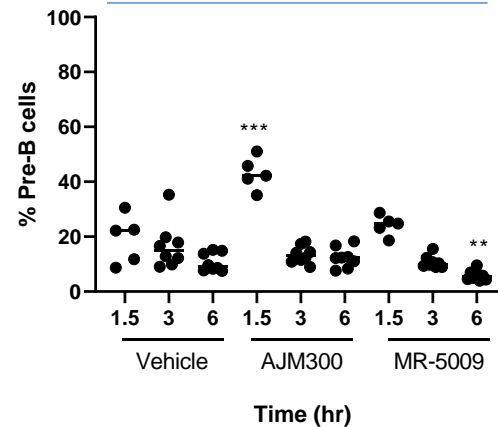
- Treatment with a close analog of MORF-057 (MR-5009^a) shows no increase of pre-B cell frequencies in the peripheral blood after 1.5hr.
- On the contrary, α_4 inhibitors (AJM300, PS/2) significantly increase the frequency of pre-B cells in the periphery.



Pre-B cells^b



Small molecule inhibitors



PS/2 = anti- α_4 mouse mAb (1.1mpk)

AJM300^c = small molecule α_4 antagonist (10mpk)

MR-5009 = MORF-057 close analog (10mpk)

^aMR-5009 has a cell adhesion assay IC₅₀ potency (RPMI8866/MAdCAM, no serum) of 1.5 nM, and MORF-057 and MR5009 have K_i selectivity of 227- vs 114-fold for $\alpha_4\beta_7$ over $\alpha_4\beta_1$, respectively. ^bPre-B cells: CD24⁺ CD43^{lo} B cells. ^cactive metabolite of AJM300

Statistics: Mean with SEM, treatment groups compared to vehicle or isotype, data combined from three studies, significance was assessed by Student's t- or one-way ANOVA, followed by Bonferroni's multiple-comparison test; ** P<0.01, *** P<0.001, ****P<0.0001

Conclusions

- In *in vitro* cell adhesion assays, MORF-057 (and other selective $\alpha_4\beta_7$ inhibiting analog compounds MR-5288 and MR-5009) are highly selective for $\alpha_4\beta_7$ over the nearest family members $\alpha_4\beta_1$ and $\alpha_E\beta_7$ (>3,000 fold)
- In mouse gut homing assay, MORF-057 inhibits T cell homing with equivalent potency as an $\alpha_4\beta_7$ antibody
- Oral administration to monkeys of MORF-057 analog (MR-5288) saturates $\alpha_4\beta_7$ receptor occupancy over the dosing interval and causes a dose dependent increase in circulating $\alpha_4\beta_7^{\text{high}}$ CD4⁺ memory T cells
- In mice, a MORF-057 analog (MR-5009) does not cause $\alpha_4\beta_1$ driven lymphocytosis of pre-B cells unlike α_4 mAb or non-selective α_4 small molecule antagonist AJM300
- The mechanistic data presented here coupled with additional safety, efficacy and pharmacokinetic profiling of MORF-057 will inform initial clinical development.*
- MORF-057 is projected to begin Phase 1 clinical studies in the third quarter of 2020

*as human clinical dosing is difficult to predict, it will be further informed by clinical safety, efficacy and evaluation of human clinical trial data