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Predictive Translational Blood Biomarker Dynamics of Small Molecule α4β7 Inhibition in Nonhuman Primate

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MORPHIC

Background

α4β7 integrin inhibition is a proven mechanism for treating IBD patients. To study novel α4β7 small molecule inhibitors, measurements of target engagement, cellular compositional changes, and additional molecular markers from blood samples are important to better understand their mechanistic connection with: 1) exposure to an inhibitor, and 2) drivers of disease. Potentially, data related to these biomarkers can provide reference points for benchmarking drug exposures leading to intended biological changes such as long-term disease responses in IBD patients.

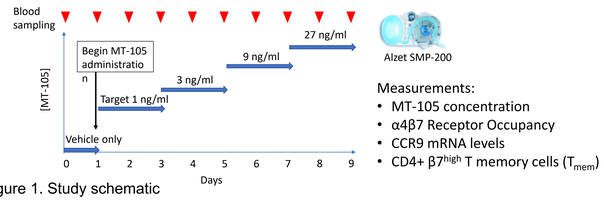
Nonhuman primate (NHP) immune systems mirror those of humans in several aspects of α4β7 biology which make them instrumental for exploring both known and novel biomarkers driven by inhibition of the pathway. Previously, several monoclonal antibodies, including vedolizumab, that target the α4β7:MAdCAM pathway have used NHP as a model to demonstrate proof-of-mechanism changes [1-4]). Additionally, pre-clinical validation of targeting α4β7 inhibition for IBD was demonstrated in part using colitis-bearing cotton top tamarins, an endangered primate [5].

Currently, novel, orally bioavailable, small molecule inhibitors of α4β7 integrin are being tested in clinical trials for UC. Here we show data from testing α4β7-inhibiting compound MT-105 in a preclinical NHP model examining blood biomarker changes associated with inhibition of the pathway. Biomarker changes were measured employing a variety of methods including: flow cytometry, quantitation of circulating mRNA, and scRNAseq of CD45+ cells. Peripheral blood biomarker changes were consistent with changes reported in vedolizumab-related studies suggesting a small molecule inhibitor of α4β7 is impacting the downstream biology of the integrin similarly.

Study Design and Methods

Naïve, male cynomolgus macaques were used in this study (purchased from Hainan Jingang Biotech Co. LTD). The animals ranged in age from 2-3 years, and 2.5-3.5 kg in weight. Animals were cared for in the AAALAC accredited, Large Animal Center of Shanghai ChemPartner Co., Ltd. This experimental design, all study protocols, and experimental procedures were reviewed and approved by the Institutional Animal Care and Use Committee of Shanghai ChemPartner.

Study events are depicted in Figure 1. Programmable minipumps (iPRECIO SMP-200) were implanted subcutaneously on Day -3. Minipump infusion was initiated on Day 0 beginning with vehicle (DMSO 50%/ water 50%) for 24 h, prior to switching the infusion to test article MT-105 (also formulated in DMSO 50%/ water 50%) for the remainder of the study. Test article was introduced into animals via the minipump on Day 1 continuing the initial infusion rate for a duration of 48 h. Every 48 h from Day 1 through Day 9, the minipump infusion rate was increased 3-fold in order to escalate the compound exposure for 4 discrete intervals. Blood samples were collected every 24 h for measurements prior to any scheduled pump rate adjustments. No obvious clinical signs were observed during cage-side observations. Two consecutive studies were run in separate animal cohorts (A and B) using 12 total animals.



Measurements:
- MT-105 concentration
- α4β7 Receptor Occupancy
- CCR9 mRNA levels
- CD4+ β7^{high} T memory cells (T_{mem})

Figure 1. Study schematic

Plasma exposures were measured in plasma by LC-MS

Flow cytometry: PBMCs were stained using standard methods along with viability dye. Antibodies specific for human or primate CD45, CD45RA, CD3, CD4, CD8, CD20, integrin β7. CD4$^+$ T_{mem} cells defined by: CD45$^+$CD3$^+$CD20$^-$CD4$^+$CD8$^-$CD45RA$^-$. Receptor Occupancy (RO) for α4β7 was measured using a Mucosal Vascular Addressin Cell Adhesion Molecule 1 (MAdCAM-1) based probe in whole blood, Mn-free.

CCR9 mRNA quantification: Whole blood was collected prepared using QuantiGene Sample Processing Kit was used per manufacturer's instructions (QS0110). QuantiGene bDNA assays measured target gene CCR9 and 4 housekeeping genes (B2M, IPO8, TBP, ACTB) for comparison.

scRNA seq was performed on CD45+ sorted lymphocytes from blood sampled at timepoints Day 1, 3, 5, and 9 from two animals. Library preparation was performed on the 10x Chromium device following the manufacturer's protocol.

Stepwise MT-105 exposures and saturation of α4β7 achieved

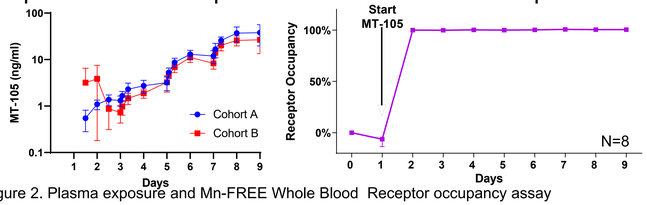


Figure 2. Plasma exposure and Mn-FREE Whole Blood Receptor occupancy assay

MRT-105 Induces Time and Dose-dependent Changes in T_{mem}

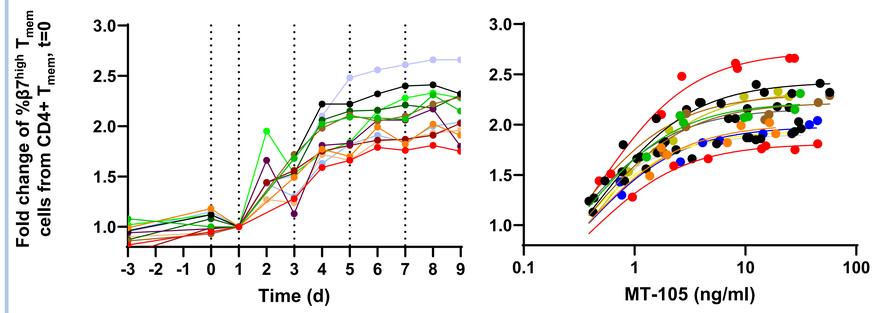
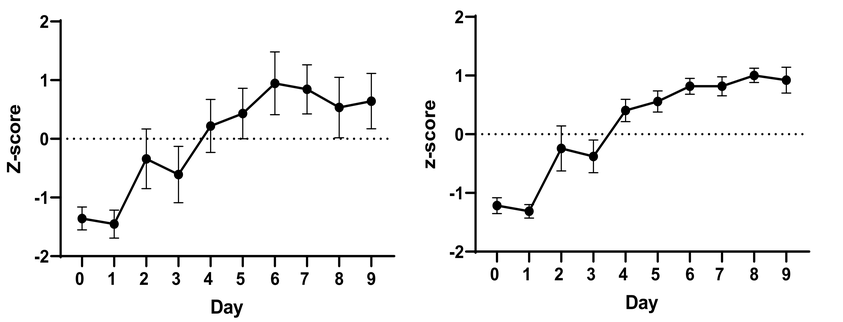


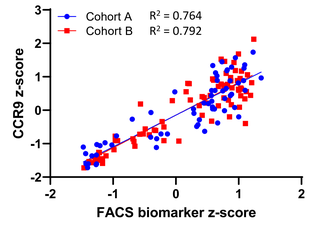
Figure 3. β7 lymphocyte subset immunophenotyping assay
The presence of β7^{high} T_{mem} cells were characterized at different timepoints by flow cytometry (left). Data are expressed as fold change, normalized to "vehicle" exposure of the Day 1. Relationship of MT-105 exposure and T_{mem} biomarker changes (right) where each color series represents one animal's data. Trendlines were drawn using non-linear least squares fit.

CCR9 mRNA levels in blood increase with MT-105 exposure



	Consecutive days compared	CCR9 (mRNA) Summary	CCR9 (mRNA) Adjusted P-Value	CD4 T_{mem} (FACS) Summary	CD4 T_{mem} (FACS) Adjusted P-Value
Days with increased pump rate	1 vs. 2	*	0.0179	**	0.0057
	3 vs. 4	ns	0.0526	****	<0.0001
	5 vs. 6	*	0.0261	ns	0.0894
	7 vs. 8	ns	0.8423	ns	0.1035
Days with no change in rate	0 vs. 1	ns	0.9859	ns	0.7985
	2 vs. 3	ns	0.9699	ns	0.9969
	4 vs. 5	ns	0.9211	ns	0.3368
	6 vs. 7	ns	>0.9999	ns	>0.9999
	8 vs. 9	ns	0.9999	ns	0.9849

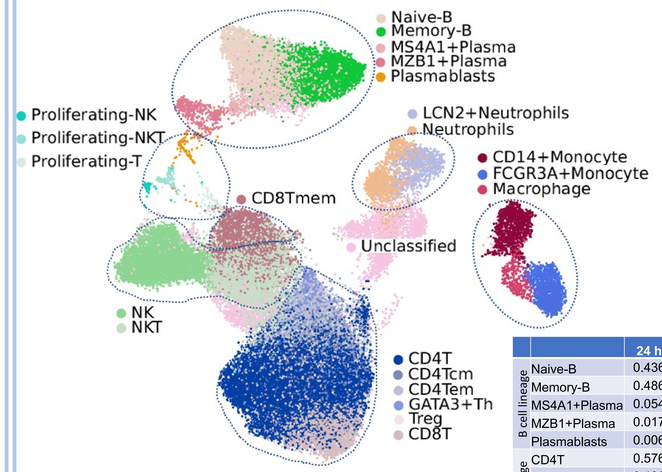
Tukey's multiple comparisons test



Greatest biomarker changes occurred during the first two increases of MT-105 exposure (1 vs. 2 and 3 vs.4)

Figure 4. CCR9 mRNA assay and cross-assay correlation
CCR9 expression and T_{mem} biomarker values were transformed to z-score for plotting purpose (top). Table examines comparison of data from consecutive days using Tukey's multiple comparisons test. Cross-comparison of z-scores of all datapoints (bottom right); trendlines drawn using simple linear regression.

Plasma cells and plasma blasts increase with MT-105 exposure



		24 h	72 h	120 h	216 h
B cell lineage	Naive-B	0.436	0.380	0.385	0.276
	Memory-B	0.486	0.481	0.443	0.469
	MS4A1+Plasma	0.054	0.103	0.078	0.159
	MZB1+Plasma	0.017	0.027	0.076	0.085
	Plasmablasts	0.006	0.008	0.018	0.012
T cell lineage	CD4T	0.576	0.663	0.700	0.465
	CD4Tcm	0.199	0.184	0.128	0.277
	CD4Tem	0.140	0.088	0.098	0.169
	GATA3+Th	0.069	0.054	0.061	0.076
	Treg	0.016	0.011	0.013	0.014

Figure 5. scRNAseq analyses of >55,000 CD45+ sorted blood cells followed longitudinally
UMAP representation and annotation of major immune populations identified in NHP blood. Tabulated data are from both animals.

Conclusions

- α4β7 inhibition in NHP using small molecule inhibitor MT-105 evokes similar biomarker changes as those seen in humans using vedolizumab, ontamalimab, or MORF-057, namely increases of circulating CD4+β7^{high} T_{mem} cells and CCR9 mRNA [8-13].
- The individual exposure response curves seen in the T_{mem} biomarker show that each subject has a similar response to α4β7 inhibition with its own starting and maximal response levels.
- scRNAseq analyses demonstrated increasing proportions of total circulating plasma and plasmablasts among all blood leukocytes with increasing exposure of MT-105 suggesting α4β7 inhibition impacts antibody secretion related to gut tissues.
- Our scRNAseq cell atlas also serves as a valuable resource for investigating the heterogeneity of immune cells and the biological role of α4β7 in NHPs and humans.

Following this collection of biomarkers in patients will be important to understand the translation of these findings and what implications different degrees of inhibiting the pathway have on disease resolution.

References: [1]Fedyk et al. 2012, [2]Pan et al. 2013, [3]Stefanich et al 2011, [4]Pullen et al. 2009, [5]Hesterberg et al. 1996, [6]Redhu et al. 2021, [7]Mangada et al. 2020, [8]Uzzan et al. 2018, [9]Boden et al. 2018, [10]Veny et al. 2021, [11]D'Haens et al 2018, [12]Ray et al. 2020, [13]Hussain et al. 2022

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