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Translational biomarkers for selective, oral, small molecule $\alpha_4\beta_7$ inhibitor MORF-057

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Introduction

Inhibition of $\alpha_4\beta_7$ is a clinically validated mechanism for the effective treatment of inflammatory bowel disease (IBD) and MORF-057 is an orally bioavailable small molecule inhibitor of $\alpha_4\beta_7$. Nonclinical pharmacology studies of MORF-057 have demonstrated potent and selective inhibition of $\alpha_4\beta_7$ in biochemical and cell-based assays, inhibition of gut homing of inflammatory cells in mice, and an increase in $\alpha_4\beta_7$ central memory T cells in monkeys (Wong et al. DDW, 2020). The current studies establish translational biomarker assays ex vivo in human whole blood and in vivo in animals to support the clinical development of MORF-057.

Methods

Mn-FREE RECEPTOR OCCUPANCY ASSAY:

Flow cytometry-based receptor occupancy (RO) assays for $\alpha_4\beta_7$ and $\alpha_4\beta_1$ were established under physiologic conditions including natural ligands Mucosal Vascular Addressin Cell Adhesion Molecule 1 (MAdCAM-1) for $\alpha_4\beta_7$ and Leucine-Aspartic Acid-Valine (LDV) peptide for $\alpha_4\beta_1$ in the absence of manganese. MORF-057 was assessed for its 50% and 90% inhibition constants (IC_{50} and IC_{90}) in fresh human blood treated ex vivo with varying concentrations of MORF-057 and stained with CD45RA, CD3, CD4, integrin α_4 ,integrin β_1, integrin β_7, viability, Free and Total $\alpha_4\beta_1$ and $\alpha_4\beta_7$ probes. The cells were acquired using BD FACSCanto II flow cytometer and the data analyzed using FlowJo. %RO vs MORF-057 concentration was measured in the $\alpha_4\beta_7$ and $\alpha_4\beta_1$ subpopulations from whole blood from normal healthy volunteers (NHV) and Ulcerative Colitis (UC) patients.

β7 LYMPHOCYTE SUBSET IMMUNOPHENOTYPING ASSAY AND CCR9 mRNA qRT-PCR ASSAY:

Whole blood from naïve Cynomolgus monkeys administered by oral gavage a MORF-057 analog as a surrogate or healthy human donors were used as samples in the following assays.

The presence of β_7 high effector, and memory T cells were characterized by flow cytometry. Whole blood was collected in heparin vacutainers and the PBMC isolated and stained for CD45RA, CD3, CD4, CD8, CD14, CD20, CD45, integrin β_7, and viability. The cells were acquired using an Attune Nxt flow cytometer and the data analyzed using FlowJo.

CCR9 mRNA was characterized at different time points. Whole blood was collected in Paxgene tubes, allowed to sit at room temperature for at least two hours and then shipped and stored frozen until RNA isolation. RNA isolation was performed using the Paxgene isolation kit from Qiagen. cDNA was generated using the qScript cDNA Supermix from Quanta Biosciences. Taqman reactions were run using gene expression assays from ThermoFisher and PerfeCTa FastMix II from Quanta Biosciences on a QuantStudio 5 from ThermoFisher.

PEYER'S PATCH DECELLULARIZATION ASSAY: To determine the effect of $\alpha_4\beta_7$ inhibition on steady state immune cell trafficking to the gastrointestinal tract, an acute Peyer's Patch (PP) decellularization assay was developed. First, the cellular composition of PP and the target cells expressing α_4 and β_7 integrins were identified by performing single cell RNA sequencing (scRNAseq) on PPs isolated from naïve C57BL/6 mice using 10X Genomics' Chromium 3' kit. We then investigated the effect of anti-$\alpha_4\beta_7$ blocking antibody (DATK32) or rat IgG2a isotype antibody on PP cellularity enumerated by multicolor flow cytometry following a single intraperitoneal injection for 48 hours at doses of 100 and 300 µg.

Results

RECEPTOR OCCUPANCY: Free $\alpha_4\beta_7$ and $\alpha_4\beta_1$ signal intensities were inhibited by increasing concentrations of MORF-057. The results also show that MORF-057 is highly potent and selective for $\alpha_4\beta_7$. The RO assay exhibits almost identical performance between healthy subjects and UC patients.

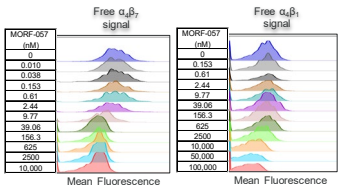

Figure 1. Free $\alpha_4\beta_7$ and $\alpha_4\beta_1$ signal is specifically inhibited with increasing MORF-057 concentration.

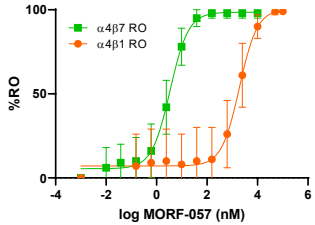

Figure 2. Calculated percentage $\alpha_4\beta_7$ and $\alpha_4\beta_1$ occupancy at varying MORF-057 concentrations. Data are mean ± SD of 26 donors.

	$\alpha_4\beta_7$ IC_{50} (nM)	$\alpha_4\beta_1$ IC_{50} (nM)	Selectivity Index (Average)
Healthy (n = 19)	3.44 ± 1.74	1,560 ± 540	717
UC (n = 7)	2.00 ± 0.93	2,810 ± 840	1939

Table 1. MORF-057 is a potent and selective inhibitor of $\alpha_4\beta_7$ over $\alpha_4\beta_1$ in human whole blood ex vivo in both normal healthy volunteers and UC patients. Values are the mean ± standard deviation.

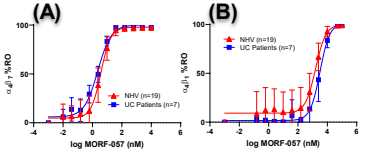

Figure 3. The performance of the RO assay for $\alpha_4\beta_7$ (A) and $\alpha_4\beta_1$ (B) is similar between NHV and UC patients. Data are mean ± SD

β7 LYMPHOCYTE SUBSET AND CCR9 mRNA MEASUREMENTS IN BLOOD FROM NON-HUMAN PRIMATES AND HEALTHY HUMAN DONORS: A general trend of increasing frequencies of β_7 memory T cells up to 50% over baseline was observed during the course of the study. MORF-057 analog treatment also shows a trend of increased CCR9 signal in non-human primate blood. There is a significant correlation between the fold change of CCR9 Taqman signal and the fold change of %$\alpha_4\beta_7$+ T memory cells from baseline. β_7 memory T cells from human whole blood were quantified using a similar flow cytometry panel (data now shown). No differences were observed in CCR9 mRNA measured in whole blood from healthy volunteers and UC patients (data now shown).

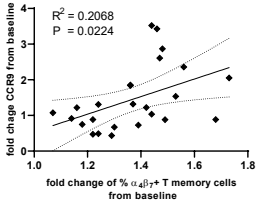

Figure 4. Correlation of CCR9 mRNA and β_7 memory T cells in non-human primate blood after 6 days. Monkeys were given twice daily treatment of MORF-057 surrogate $\alpha_4\beta_7$ inhibitor

DECELLULARIZATION ASSAY: Several cellular populations (e.g. total, naïve and immature B cells, T cells) in the PP were identified as potential target cells by their $\alpha_4\beta_7$ expression. Naïve mice treated with anti-$\alpha_4\beta_7$ for 48 hours showed robust inhibition of trafficking of B cells, specifically naïve B cells to PP, whereas the frequency of non-B cells and $IgM^{lo}IgD^{-}$ (immature) B cells was reciprocally increased. $CD4^{+}$ and $CD8^{+}$ T cells showed relatively higher expression of integrin β_7 and were slightly reduced in this acute experiment (data not shown).

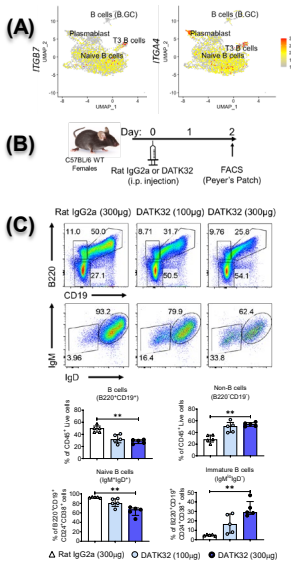

Figure 5. Effect of $\alpha_4\beta_7$ blockade on Peyer's Patch (PP) cellularity. (A) Feature plots showing the expression of integrin β_7 and α4 in B cell subsets in PP. (B) Schematic showing the treatment of naïve C57BL/6 mice with isotype or anti-$\alpha_4\beta_7$ antibody. (C) Effect of $\alpha_4\beta_7$ blocking on total, naïve, and immature B lymphocyte populations is shown as representative flow cytometry plots (left) and the summary graphs of frequencies from PP of 5 mice (right). **p<0.01, Kruskal-Wallis test, data in (A-B) is from 2 mice, in (B-C) is representative of 2 independent experiments.

Conclusions

Consistent with results in cell lines, MORF-057 is a potent and selective inhibitor of $\alpha_4\beta_7$ in human whole blood using a receptor occupancy assay conducted under physiologically relevant conditions. Receptor occupancy did not differ between healthy donors and UC patients. This $\alpha_4\beta_7$ RO assay gives similar results and may be used to determine MORF-057 occupancy in UC patients. Potential blood and tissue biomarkers were characterized in animal models and human blood.

References: PRECLINICAL CHARACTERIZATION OF AN ORAL SMALL MOLECULE INHIBITOR TARGETING THE INTEGRIN $\alpha_4\beta_7$ FOR THE TREATMENT OF INFLAMMATORY BOWEL DISEASES (IBD). Jamie Wong et al. Digestive Disease Week ePoster Tu1283

Conflict of Interest statement: All authors received salary or stock as part of Morphic Therapeutic