PN-943, an Oral α4β7 Integrin Antagonist, Inhibits MAdCAM1-mediated Proliferation and Cytokine Release from CD4+ T-cells Independent of Trafficking

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Introduction

- PN-943, an oral gastrointestinal (GI)-restricted peptide antagonist of α4β7 integrin, is being developed for the treatment of Inflammatory Bowel Disease (IBD).
- Blockade of α4β7 binding to mucosal addressin cell adhesion molecule-1 (MAdCAM1) expressed on endothelial venules is thought to treat IBD by preventing extravascular migration of blood T-cells into the inflamed GI mucosa.
- Recent evidence suggests involvement of α4β7 in trafficking-independent functions:
  1. Saturation of circulating T-cell α4β7 by vedolizumab is not sufficient for optimal efficacy (Ungar et al., Clin Gastroenterol Hepatol 2018).
  2. PTG-100, a PN-943 analog, showed evidence of clinical and histological remission in Ulcerative Colitis patients at sub-saturating blood receptor occupancy (%RO) (Sandborn et al., UEGW 2018).

Here, we assessed the potential of a local GI-acting function of α4β7 by evaluating the ability of PN-943 to inhibit MAdCAM1-mediated CD4+ T-cell proliferation and cytokine production.

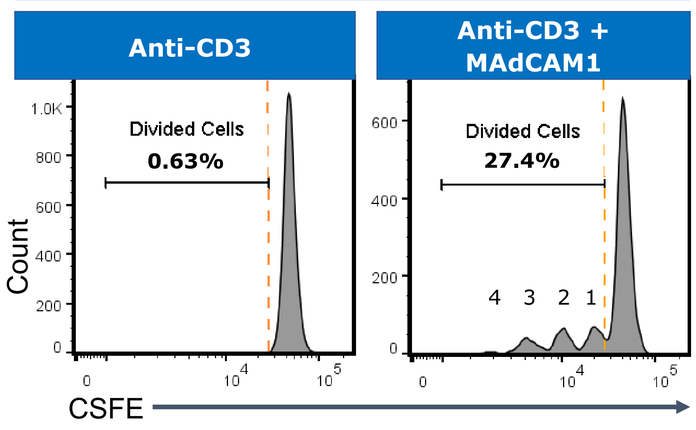
Methods

- Freshly isolated primary CD4+ T cells from multiple healthy donors were labeled with 5- (and 6-) carboxyfluorescein diacetate succinimidyl ester (CSFE) dye and incubated for three days with plate bound anti-CD3 monoclonal antibody (mAb) alone or together with plate-bound MAdCAM1 in the presence or absence of inhibitors.
- Immune phenotyping of the proliferated/divided cells (diluted CSFE) and undivided cells (undiluted CSFE) was performed by flow cytometry. Successive cycles of division are denoted by 1, 2, 3, and 4.
- The distribution of T helper (Th) Th1, Th2 and Th17 subsets was determined by phorbol 12-myristate 13-acetate and ionomycin (PMA/I) treatment followed by flow cytometry.
- The types and levels of cytokines released in the supernatants were quantified by multiplex assays.
- Drugs levels and %RO were measured from Peyer's Patches (PP), plasma or whole blood from mice (N=6) one hour after oral dosing.

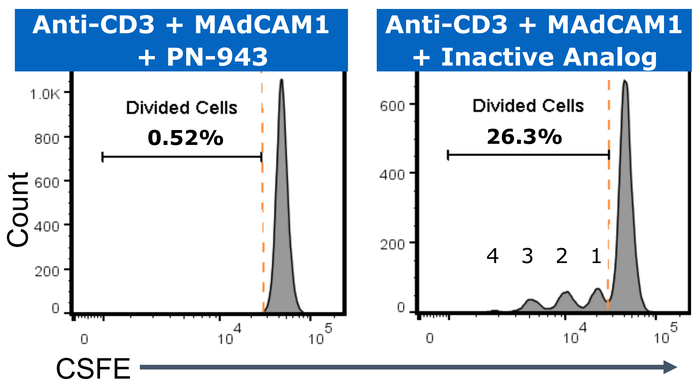
Results

1. PN-943 Inhibits MAdCAM1-mediated CD4+ T-cell Proliferation in a Concentration-dependent Manner

MAdCAM1 combined with anti-CD3 mAb markedly enhanced proliferation of CD4+ T cells compared to anti-CD3 mAb alone

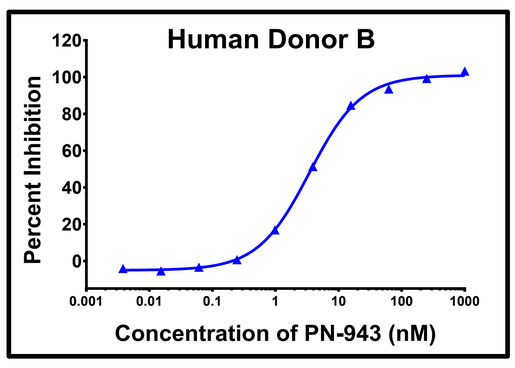


PN-943 at 1μM abolished MAdCAM1-mediated proliferation; inhibition is dependent on binding to α4β7



Inhibition by PN-943 is concentration dependent. The mean IC_{50} from four independent human donors was 4.4 nM.

Representative Dose Response Curve and IC_{50} Values from PN-943 Inhibition

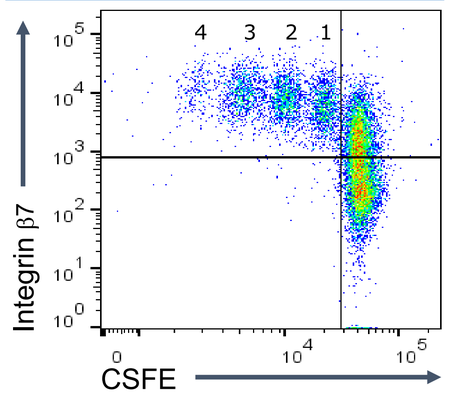


Human Donor	IC_{50} (nM)
A	1.5
B	3.5
C	4.9
D	7.7
Mean (SD)	**4.4 (2.6)**

2. PN-943 Downregulates Surface Expression of Integrin β7 in MAdCAM1-costimulated CD4+ T-cells

MAdCAM1 mediated proliferation was restricted to the β7+ population with successive cycles of proliferation showing increased surface expression in β7. Data shown are from human donors A-E.

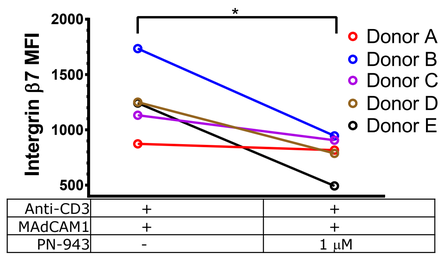
Anti-CD3 + MAdCAM1



	Divided CD4+ T-Cells				
	A	B	C	D	E
% β7+	98.9	94.5	99.9	99.9	99.7
β7 MFI-1	5,692	5,549	4,299	3,671	5,441
β7 MFI-2	7,312	7,137	5,773	5,628	7,641
β7 MFI-3	7,742	7,537	6,599	6,973	9,699
β7 MFI-4	6,807	6,990	6,949	8,038	11,141

Surface expression of β7 is lowered in undivided CD4+ T-cells in the presence of PN-943, indicative of internalization by PN-943. Data shown are from human donors A-E.

Undivided β7+ CD4+ T-Cells



Statistical significance assessed by Wilcoxon matched pairs signed rank test; *p≤0.05

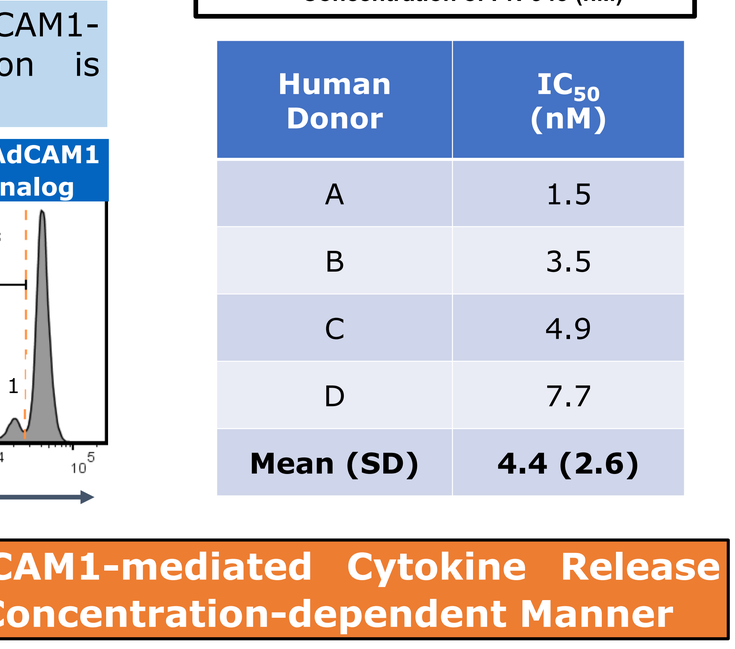
β7 MFI	Undivided β7+CD4+ T-Cells				
	A	B	C	D	E
0 nM PN-943	873	1735	1132	1250	1242
1 μM PN-943	816	945	906	787	493
Percent Change	-6.5%	-46%	-20%	-37%	-60%

3. PN-943 Inhibits MAdCAM1-mediated Cytokine Release from CD4+ T-cells in a Concentration-dependent Manner

Amongst the proliferated CD4+ T-cells, the percentage of the IFNγ-producing Th1 cells was higher than IL-17A-producing Th17 and IL-4-producing Th2 subsets.

Human Donor D	CD4+ T-Cells		
	Th1-IFNγ	Th2-IL-4	Th17-IL-17A
No Activation	7.4%	0.3%	0.5%
Anti-CD3	47%	2.2%	2.8%
Anti-CD3 +MAdCAM1	15%	0.6%	1.1%
Anti-CD3 +MAdCAM1 + 1 μM PN-943	15%	0.7%	0.6%

MAdCAM1 promoted release of IFNγ, IL-13, GM-CSF, IL-10, IL-5 and TNFα were inhibited by PN-943.



4. Higher *In Vivo* Exposure and Pharmacodynamic Activity in GI Tissues as Compared to the Periphery

Oral dosing of PTG-100 (a PN-943 analog) at 30 mg/kg in mice demonstrated high exposure (37 nM) and occupancy of T-cell α4β7 (92% RO) in the Peyer's Patches compared to in the blood.

Statistical significance assessed by Mann-Whitney non-parametric test; **p≤0.01

Conclusions

- Previous preclinical studies have shown PN-943 affects trafficking of CD4+ T-cells (Mattheakis et al., DDW 2019).
- Here, we demonstrate the ability of PN-943 to inhibit MAdCAM1-mediated proliferation and cytokine release as an additional beneficial mechanism which may control chronic inflammation occurring in the gut of IBD patients.
- Consistent with the requirement for a locally acting drug, the drug levels and occupancy of α4β7-expressing target cells were higher in the GI tissues than in the blood.
- These findings support an intervention strategy that is best exploited by an oral GI-restricted approach, whereby PN-943 is delivered locally to directly block α4β7 function in the GI.

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