

ANTI-ALPHA-4-BETA-7 ANTIBODIES

1. SEQUENCE LISTING

[0001] The instant application contains a Sequence Listing which has been submitted electronically in ASCII format and is hereby incorporated by reference in its entirety. Said ASCII copy, created on July 14, 2021, is named 483WO_SL.txt and is 106,797 bytes in size.

2. TECHNICAL FIELD

[0002] The present application pertains to, among other things, novel anti- $\alpha 4\beta 7$ antibodies, polynucleotides encoding the antibodies, methods of making the same, and methods of use of these antibodies.

3. BACKGROUND

[0003] Over 37 million people are infected with human immunodeficiency virus (HIV) globally today and the prevalent population continues to grow. Significant progress has been made in the management of HIV with the advent of combination antiretroviral therapy (cART). cART needs to be taken consistently throughout the life of a person living with HIV. cART has risk benefit limitations and moreover, it does not decrease the HIV latent viral reservoir. There is a significant need for improved treatment that is capable of keeping the viral load below the level of detection without needing life-long treatment.

[0004] In acute human HIV infection, both high level viral replication and a profound depletion of infected CD4⁺ T cells are believed to play a central role in the development of immune deficiency associated with HIV infection. $\alpha 4\beta 7$ is a gut-homing integrin expressed on T cells (CD4⁺ or CD8⁺), B cells and other immune cells, and plays an important role in the pathogenesis of HIV infection. $\alpha 4\beta 7$ has also been reported to be incorporated into the envelope of HIV when the virions bud from the infected host cells (Guzzo et al., Sci. Immunol., 2017).

[0005] The $\alpha 4\beta 7$ integrin is a heterodimeric receptor expressed on T cell subsets, B cells, NK cells and other immune cells. This integrin mediates the lymphocyte trafficking into gut-associated lymphoid tissues (GALT) by binding to its ligand mucosal addressin cell adhesion molecule 1 (MAdCAM-1) expressed on endothelial venules of intestinal mucosa. High $\alpha 4\beta 7$ expressing CD4⁺ T cells are targets for HIV infection in vitro (Cicala et al., PNAS 2009), which are infected and therefore depleted during acute HIV infection (Sivro et al., Sci Transl Med 2018). $\alpha 4\beta 7$ present in

HIV-infected CD4⁺ cells as well as in HIV virions could mediate their trafficking to GALT (Guzzo et al., Sci Immunol 2017). CD4⁺ T cells that home to GALT account for the largest HIV reservoir in the body (Brenchley and Douek, Mucosal Immunol 2008) even during anti-retroviral therapy.

4. SUMMARY

[0006] The present disclosure provides anti- $\alpha 4\beta 7$ antibodies and binding fragments thereof that specifically bind to human $\alpha 4\beta 7$. The amino acid sequences of exemplary CDRs, as well as the amino acid sequence of the V_H and V_L regions of the heavy and light chains of exemplary anti- $\alpha 4\beta 7$ antibodies are provided in the Detailed Description below.

[0007] Polynucleotides comprising nucleotide sequences encoding the anti- $\alpha 4\beta 7$ antibodies of the disclosure are provided herein, as are vectors comprising polynucleotides. Additionally, prokaryotic cells transformed with and eukaryotic cells transfected with a vector comprising a nucleotide sequence encoding a disclosed anti- $\alpha 4\beta 7$ antibody are provided herein, as well as eukaryotic (such as mammalian) host cells engineered to express the nucleotide sequences. Methods of producing antibodies, by culturing host cells and recovering the antibodies are also provided.

[0008] The present disclosure provides methods of treating subjects, such as human subjects, diagnosed with HIV infection with an anti- $\alpha 4\beta 7$ antibody. The method generally involves administering to the subject an amount of an anti- $\alpha 4\beta 7$ antibody described herein effective to provide therapeutic benefit. The subject may be diagnosed with any clinical category of HIV infection.

[0009] Since the anti- $\alpha 4\beta 7$ antibodies described herein target human $\alpha 4\beta 7$ instead of a viral protein, they provide an advantageous therapeutic approach that does not induce HIV mutation-based resistance mechanisms, which frequently occur with treatments targeting viral proteins due to the high mutation frequency of HIV.

[0010] Based on data presented herein, it is expected that the anti- $\alpha 4\beta 7$ antibodies described herein will provide therapeutic benefit to subjects diagnosed with HIV infection.

5. BRIEF DESCRIPTION OF THE DRAWINGS

[0011] FIG. 1 shows the blockade of adhesion of HuT78 cells to MAdCAM-1 by murine antibody Ab-m1.

[0012] FIG. 2 shows the functional cynomolgus monkey cross-reactivity of Ab-m1 using CHOK1- $\alpha 4\beta 7$ (cyno $\alpha 4\beta 7$) cell adhesion assay to MAdCAM-1.

[0013] FIG. 3 shows Ab-m1 blocking of human MAdCAM-1 binding to primary human CD4+ memory T cells.

[0014] FIG. 4 shows binding of murine-human chimera Ab-c1 to human primary CD4+ memory T cells ($\alpha 4\beta 7$ +CD4+CD45RO+).

[0015] FIG. 5 shows the binding of liability engineered humanized anti- $\alpha 4\beta 7$ Ab-h1.9 scFv clones against human $\alpha 4\beta 7$ antigen displayed on yeast by flow cytometry. Clones Ab-h1.9 (a-e) had similar binding as parental Ab-h1.9.

[0016] FIGS. 6A-6F show $\alpha 4\beta 7$ expression analysis on samples from 45 HIV+ individuals and 10 healthy (HIV-) donors. Expression of $\alpha 4\beta 7$ (as % or as levels measured by MESF) on CD4+ and CD8+ T cells was compared among HIV+ and HIV- individuals. Only the comparisons that were significantly different by Mann-Whitney two-tailed test are shown in the figures. T cell populations are abbreviated as: N+ = naïve (CD28+CD45RO-), CM = central memory (CD28+CD45RO+CCR7+), TM = transient memory (CD28+CD45RO+CCR7-), EM = effector memory (CD28-CD45RO+), TE = terminal effector (CD28-CD45RO-); MESF = Molecules of Equivalent Soluble Fluorochrome.

[0017] FIGS. 7A-7G-2 show HIV virion capture with Ab-h1.9d-WT. All testing was done with a virion capture assay in a bead format. Ab-h1.9d-WT was tested with six laboratory grown HIV strains (FIGS. 7A-7F) at 5 nM and 15 nM, whereas the negative control antibody was tested at 15 nM only. Amount of HIV p24 gag in the captured samples is shown in pg/mL in 10 μ L assayed. Ab-h1.9d-WT was also tested with samples from two HIV-infected individuals with beads coated with 10 μ g of the antibody (FIGS. 7G-1 and 7G-2). Amount of HIV gag RNA in the input samples (FIG. 7G-1) and in captured samples (FIG. 7G-2) as detected by digital droplet PCR is shown in copies/mL.

[0018] FIGS. 8A-8E shows immune complexes (HIV virions with different antibodies) binding to Fc γ Rs. Immune complexes were first formed by incubating antibodies (Ab-h1.9d-WT, Ab-h1.9d with LALA mutations to significantly reduce Fc γ R binding, Ab-Vedo, and isotype negative control) with HIV NL4-3, and then captured on Fc γ Rs immobilized on a plate. Fc γ RI, and Fc γ RIIIa (V158) were captured on nickel plates, whereas Fc γ RIIa (H131), Fc γ RIIa (R131) and Fc γ RIIIa (F158) were captured on neutravidin plates to increase the sensitivity of the detection. Amount of HIV p24 gag detected is shown in pg/mL in 10 μ L assayed. Results of a representative experiment are shown.

[0019] FIGS. 9A-9C show $\alpha 4\beta 7$ - and Fc-dependence of Ab-h1.9d-WT-mediated uptake of $\alpha 4\beta 7$ -coated beads in THP-1 cells. Phagocytosis scores of immune complexes (containing $\alpha 4\beta 7$ coated beads and the indicated anti- $\alpha 4\beta 7$ or control antibody) in THP-1 cells treated with the complexes for 3 h are plotted. FIG. 9A shows data from one representative experiment. FIG. 9B shows normalized data from 3 independent experiments. Significance was determined using one-way ANOVA coupled to Tukey's multiple comparisons test. **** $p < 0.0001$, *** $p = 0.0001-0.001$, ** $p = 0.001-0.01$, * $p = 0.01-0.05$, ns ≥ 0.05 . FIG. 9C shows representative images of Ab-h1.9d-WT immune complex treated cell with 3 internalized $\alpha 4\beta 7$ -coated beads, acquired by imaging cytometry.

[0020] FIG. 10 shows binding of anti- $\alpha 4\beta 7$ antibodies to $\alpha 4\beta 7$ +GFP+VLPs (viral like particles). Binding of Abs to VLPs was determined using ELISA. Ab-h1.9d-WT, Ab-h1.9d-LALA and Ab-Vedo bound to $\alpha 4\beta 7$ +GFP+VLPs coated plated with similar EC50 values. Representative data from two independent experiments is shown.

[0021] FIGS. 11A-11B show $\alpha 4\beta 7$ - and Fc-dependence of Ab-h1.9d-WT-mediated $\alpha 4\beta 7$ +GFP+VLP (viral like particles) uptake by THP-1 cells. FIG. 11A shows $\alpha 4\beta 7$ +GFP+VLP uptake by THP-1 cells as a percentage of GFP+ cells measured by flow cytometry. FIG 11B shows inhibition of $\alpha 4\beta 7$ +GFP+VLP uptake by Latrunculin A (Lat A). For FIG. 11B, THP-1 cells were pretreated with Lat A for 2 h and then incubated with VLPs and antibodies as in FIG 11A. Mean $\pm$ s.d. presented in FIGS. 10A-10B were compiled from 4 and 3 independent experiments, respectively. Significance (**** $p = 5.4 \times 10^{-5}$) was calculated by two-tailed student's t-test, where $p < 0.05$ is considered significant.

[0022] FIGS. 12A-12B show inhibition of interaction of HIV gp120 with $\alpha 4\beta 7$ by different antibodies. FIG. 12A shows binding of RPMI 8866 cells to the HIV gp120-V2 WT peptide, but not the control peptide. RPMI 8866 cells constitutively express $\alpha 4\beta 7$ on the cell surface. HIV gp120 V2 WT peptide and HIV gp120 V2 control peptide were identical in sequence except four amino acids reported to mediate the binding between $\alpha 4\beta 7$ and gp120 were mutated in the control peptide. FIG. 12B shows inhibition of binding of HIV gp120 peptides to RPMI 8866 cells expressing $\alpha 4\beta 7$ by different antibodies. Ab-h1.9d-WT was more potent than Ab-Vedo in inhibiting the binding of HIV gp120-V2 WT peptide to RPMI 8866 cells expressing $\alpha 4\beta 7$.

[0023] FIG. 13 shows the percentage of human and cynomolgus CD4+ and CD8+ T subsets bound by Ab-h1.9d-WT. The binding of Ab-h1.9d-WT to human and cynomolgus CD4+ and CD8+ T cells

was assessed by flow cytometry analysis. The percentage of $\alpha 4\beta 7^+$ T cell subsets bound by Ab-h1.9d-WT were determined. The data were obtained from 3 human and 5 cynomolgus donors.

[0024] FIGS. 14A-14F show binding specificity of Ab-h1.9d-WT to various integrins. Binding specificity of Ab-h1.9d-WT was assessed on recombinant cells expressing human (14A, 14C and 14E) or cynomolgus integrins (14B, 14D and 14F). Binding to target integrin $\alpha 4\beta 7$ (FIG. 14A/14B) in comparison to $\alpha 4\beta 1$ (FIG. 14C/14D) and $\alpha E\beta 7$ (FIG. 14E/14F) integrins. Ab-Nata and etrolizumab-derived Ab-Etro were used as $\alpha 4$ and $\beta 7$ integrin specific positive controls, respectively and Ab-Ctet as an isotype control. FACS binding results are represented as MFI for titrated mAbs. N = 1.

[0025] FIG. 15 shows non-specific binding evaluation of Ab-h1.9d-WT in HEK293 cells. Non-specific binding of Ab-h1.9d-WT to HEK293 cells was assessed by flow cytometry analysis. A positive control showed high binding whereas negligible binding was observed for both Ab-h1.9d-WT and isotype control Ab-cTet at 100 $\mu\text{g/mL}$ test concentration. Percentage HEK293 cells bound to test mAbs (A) and MFI of binding intensity by test mAbs (B) are presented. N = 2.

[0026] FIGS. 16A-16B show internalization of $\alpha 4\beta 7$ complex with Ab-h1.9d-WT or Ab-Vedo on human primary cells. FIG. 16A shows internalization of Ab-h1.9d-WT on CD4⁺ T and CD8⁺ T naïve cells from peripheral blood human donors. Dot plots from two donors indicating the percentage of $\alpha 4\beta 7$ cells upon treatment with Ab-h1.9d-WT at 4 or 37°C at 18 hours post treatment. The remaining $\alpha 4\beta 7$ on the cell surface was detected with Alexa-647 labeled anti- $\beta 7$ Ab-Etro. FIG. 16B shows quantification of $\alpha 4\beta 7$ internalization (reduced surface $\alpha 4\beta 7$ expression related to expression observed after treatment at 4°C) in T cell subsets treated with Ab-h1.9d-WT. (N = two donors). Naive cells were defined as CD45RA⁺.

[0027] FIGS. 17A-17B show Ab-h1.9d-WT blocks MAdCAM-1 co-stimulation signal on human primary CD4⁺ T cells. For FIG. 17A, the activation of human primary CD4⁺ T cells by anti-CD3 and MAdCAM-1 in the presence of isotype control Ab and Ab-h1.9d-WT was measured as percentage of Ki67⁺CD25⁺ cells (Ki67⁺ on X axis, CD25⁺ on Y axis) by flow cytometry analysis. The data is from a representative donor. For FIG. 17B, the activation of human primary CD4⁺ T cells by anti-CD3 and MAdCAM-1 in the presence of isotype control Ab, Ab-h1.9d-WT and Ab-Vedo was measured as percentage of Ki67⁺CD25⁺ cells (on Y axis). Data is from 6 individual healthy donors. Statistical analysis was performed using two-tailed parametric paired t test: **P < 0.01, ****P < 0.0001).

[0028] FIG. 18 shows Ab-h1.9d-WT does not block VCAM-1 mediated cell adhesion. Cell adhesion blockade by Ab-h1.9d-WT to VCAM-1 was determined using HuT78 cell adhesion assay. Ab-Nata, an anti- $\alpha 4$ mAb, served as a positive control and Ab-cTet was used as a negative control. Representative data is shown where reduction in luminescence (RLU) indicates decreased cell binding due to ligand blockade. N = 3.

[0029] FIGS. 19A-19B show binding of Ab-h1.9d-WT to human Fc γ R-expressing cells in comparison to Ab-Vedo via flow cytometry. The binding of Ab-h1.9d-WT (FIG. 19A) and Ab-Vedo (FIG. 19B) to human Fc γ Rs was analyzed by using engineered CHO-K1 cells expressing various cell surface human Fc γ Rs and is represented by geometric mean of fluorescence. N = 1.

[0030] FIGS. 20A-20B show reporter-based ADCC and ADCP activity of Ab-h1.9d-WT. The ability of Ab-h1.9d-WT to induce Fc mediated in vitro ADCC and ADCP activities were assessed by reporter assays (FIG. 20A) ADCC activity using engineered Jurkat human Fc γ R IIIa V158+ effector reporter cells and RPMI8866 target cells; N = 2. (FIG. 20B) ADCP activity using engineered Jurkat human Fc γ RIIa H131+ effector reporter cells and RPMI8866 target cells; N = 2. Ab-Ritu was used as a positive control in the assays. Results are represented by luminescence (RLU); high signal indicates ADCC and ADCP activity.

[0031] FIG. 21 shows CDC Activity of Ab-h1.9d-WT. The ability of Ab-h1.9d-WT to induce Fc mediated in vitro CDC activity was assessed using RPMI8866 target cells and human serum as the source of complement factors; N = 2 donor serum tested. Ab-Ritu was used as a positive control. Results are represented as percentage cell killing; higher cell killing indicates CDC activity.

[0032] FIG. 22 shows cytotoxicity-based ADCC activity of Ab-h1.9d-WT. The ability of Ab-h1.9d-WT to induce Fc mediated in-vitro ADCC was assessed using HuT78 cells as target cells and human primary NK cells from two donors as effector cells in FACS-based cytotoxicity assay. Target cell killing (cytotoxicity) is represented by percentage ADCC for both donors.

6. DETAILED DESCRIPTION

[0033] Without being bound by theory, embodiments of the invention, are hypothesized to exert viral control against HIV infection via two major mechanisms of action: 1) Fab-dependent mechanism: blocking interaction of $\alpha 4\beta 7$ with its ligands such as MAdCAM-1 and HIV gp120, thus inhibiting the co-stimulation of CD4+ T cells mediated by the signaling of these ligands, and suppressing HIV replication in these stimulated cells (Nawaz et al., Mucosal Immunol. 2018, Livia et al., PNAS.

2020), HIV infection of gut tissues (Guzzo et al., Sci Immunol. 2017), and cell-to-cell viral transmission (Arthos et al. Nat. Immunol. 2008), respectively, and 2) Fc-dependent mechanism: inducing a "vaccination effect" wherein an anti- $\alpha 4\beta 7$ mAb binds to $\alpha 4\beta 7$ + HIV virions forming immune-complexes, which are internalized through the interaction of mAb Fc domain with Fc γ Rs on antigen presenting cells (APCs) and processed, and the resulting viral peptides are subsequently presented on the surface of the APCs to elicit new and durable HIV-specific immune responses to suppress viral replication (Parsons et al., Retrovirology 2018; Naranjo-Gomez et al. Curr. Opin. HIV AIDS 2019).

[0034] $\alpha 4\beta 7$ integrin is usually in a resting (inactive) state with low affinity for its ligands. Once it is activated, it can bind to its ligands (e.g MAdCAM-1 and gp120) with high-affinity (Ye et al., Blood, 2012; Lertjuthaporn et al., PloS One, 2018). During HIV infection, a motif in the V2 region of HIV gp120 mimics MAdCAM-1 and is capable of binding to $\alpha 4\beta 7$ (Peachman et al., PloS One, 2015). The interaction of $\alpha 4\beta 7$ with gp120 induces the activation of lymphocyte function-associated antigen-1 (LFA-1), potentially inducing the formation of virological synapses and thus enhancing HIV cell-to-cell transmission (Arthos et al. Nat Immunol 2008). Cell-to-cell transmission is critical for promoting viral spread in tissues, and is more important than cell-free virus for viral transmission. Upon binding to $\alpha 4\beta 7$, embodiments of the invention can reduce $\alpha 4\beta 7$ -mediated cell-to-cell transmission of HIV by disrupting the interaction of $\alpha 4\beta 7$ with gp120, inducing the internalization of the $\alpha 4\beta 7$ -antibody bound complex to the cells, or may inactivate $\alpha 4\beta 7$. Accordingly, the embodiments of the invention demonstrate the ability to inhibit HIV replication and viral spread in tissues.

[0035] By targeting the human protein of $\alpha 4\beta 7$ instead of a viral protein, embodiments of the invention do not induce the emergence of viral resistance mutations that are usually associated with a treatment targeting a viral protein due to the high mutation frequency of HIV.

6.1. Abbreviations

[0036] The antibodies described herein are, in many embodiments, described by way of their respective polypeptide sequences. Unless indicated otherwise, polypeptide sequences are provided in N→C orientation.

[0037] The polynucleotides described herein are, in many embodiments, described by way of their respective polynucleotide sequences. Unless indicated otherwise, polynucleotide sequences in 5'→3' orientation.

[0038] For polypeptide sequences, the conventional three or one-letter abbreviations for the genetically encoded amino acids may be used, as noted in TABLE 1, below.

TABLE 1 Encoded Amino Acid Abbreviations		
Amino Acid	Three Letter Abbreviation	One-Letter Abbreviation
Alanine	Ala	A
Arginine	Arg	R
Asparagine	Asn	N
Aspartic acid	Asp	D
Cysteine	Cys	C
Glutamic acid	Glu	E
Glutamine	Gln	Q
Glycine	Gly	G
Histidine	His	H
Isoleucine	Ile	I
Leucine	Leu	L
Lysine	Lys	K
Methionine	Met	M
Phenylalanine	Phe	F
Proline	Pro	P
Serine	Ser	S
Threonine	Thr	T
Tryptophan	Trp	W
Tyrosine	Tyr	Y
Valine	Val	V

[0039] Certain sequences are defined by structural formulae specifying amino acid residues belonging to certain classes (*e.g.*, aliphatic, hydrophobic, *etc.*). The various classes to which the genetically encoded amino acids belong as used herein are noted in TABLE 2, below. Some amino acids may belong to more than one class. Cysteine, which contains a sulfhydryl group, and proline, which is conformationally constrained, are not assigned classes.

TABLE 2 Encoded Amino Acid Classes	
Class	Amino Acids
Aliphatic	A, I, L, V
Aromatic	F, Y, W
Non-Polar	M, A, I, L, V
Polar	N, Q, S, T

TABLE 2 Encoded Amino Acid Classes	
Class	Amino Acids
Basic	H, K, R
Acidic	D, E
Small	A, G

[0040] Abbreviations used throughout the various exemplary embodiments include those provided in TABLE 3, below:

TABLE 3 Abbreviations	
Abbreviations	Definition
Ab-h1.9d-WT	Anti-human $\alpha 4\beta 7$ (AC-166667) hu IgG1/k WT mAb
Ab-h1.9d-LALA	Anti-human $\alpha 4\beta 7$ hu IgG1/k LALA mAb with reduced binding to human Fc γ Rs
$\alpha 4\beta 7$	Alpha 4 beta 7 integrin
$\alpha E\beta 7$	Alpha E beta 7 integrin
$\alpha 4\beta 1$	Alpha 4 beta 1 integrin
Ab or mAb	Antibody or monoclonal antibody
ADCC	Antibody dependent cellular cytotoxicity
ADCP	Antibody dependent cellular phagocytosis
AF647	Alexa fluorochrome 647 dye
ATI	Antiretroviral treatment interruption
APC	Allophycocyanin fluorochrome dye; or Antigen presenting cell
BSA	Bovine serum albumin
BV421	Brilliant violet fluorochrome dye
Ca	Calcium
CDB	Cell dissociation buffer
CDC	Complement dependent cytotoxicity
CellTrace™	Cell proliferation fluorescent dye(s)
CFSE	Carboxyfluorescein succinimidyl ester fluorochrome dye
CHOK1	Chinese hamster ovary cell line
CM5	Carboxymethyl-dextran sensor chip used in BIAcore
CMV	Cytomegalovirus
Conc	Concentration
Cyno	Cynomolgus
DMEM	Dulbecco's modified eagle's medium
DMSO	Dimethyl sulfoxide
DPBS	Dulbecco's phosphate buffered saline
E	Effector cells
EC ₅₀	Half maximal effective concentration
ECL	Electrochemiluminescent
EDTA	Ethylenediaminetetraacetic acid
FACS	Fluorescence activated cell sorting
FBS	Fetal bovine serum
Fc	Fragment crystallizable
FcRn	Neonatal Fc receptor

TABLE 3
Abbreviations

Fc γ	Fc gamma
Fc γ R	Fc gamma receptor
Fc γ RIIa	Fc gamma receptor IIa (H131 and R131)
Fc γ RIIb	Fc gamma receptor IIb
Fc γ RIIc	Fc gamma receptor IIc
Fc γ RIIIa	Fc gamma receptor IIIa (F158/176 or V158/176)
Fc γ RIIIb	Fc gamma receptor IIIb (NA1, NA2 and SH)
FITC	Fluorescein isothiocyanate fluorochrome dye
FVD	Fixable viability fluorochrome dye
FMO	Fluorescence Minus One
FR	Framework
G418	Geneticin (antibiotic used for stable cell line selection)
HBS	Hepes based buffer containing EDTA and NaCl for (HBS-EP)
HBSS	Hank's balanced salt solution
HEK293	Human embryonic kidney cell line
HER2	Human epidermal growth factor receptor 2
HI	Heat inactivated
hIL-2	Human interleukin 2
HIV	Human immunodeficiency virus
hr	Hour
hu Fc	Human Fragment crystallizable
HuT78	Human cutaneous t-cell lymphoma line endogenously expressing $\alpha 4\beta 7$
IC ₅₀	Half maximal inhibitory concentration
IgG	Immunoglobulin type G
IgG1	Immunoglobulin type G1
IgG1/ κ	Human immunoglobulin gamma 1 with kappa light chain
IMDM	Iscove's modified dulbecco's medium
K _D	Equilibrium dissociation constant
mAb	Monoclonal antibody
MES	MES buffer: 2-ethanesulfonic acid
MESF	Molecules of equivalent soluble fluorochrome
MAdCAM-1	Mucosal addressin cell adhesion molecule 1
MFI	Mean fluorescence intensity
μ g	Microgram
μ L	Microliter
mg	Milligram
Mg	Magnesium
mL	Milliliter
mM	Millimolar
Min	Minute
MnCl ₂	Manganese chloride
MOA	Mechanism of action
MSD	Meso Scale Discovery
N	Sample size
ND	Not determined
NaCl	Sodium chloride
nM	Nanomolar

TABLE 3 Abbreviations	
NFAT	Nuclear factor of activated T-cells
NK	Natural Killer
PBMCs	Peripheral blood mononuclear cells
PBS	Phosphate buffered saline
PE	Phycoerythrin fluorochrome dye
PFA	Paraformaldehyde
pg	Picogram
PHA	Phytohemagglutinin
Puro	Puromycin (antibiotic used for stable cell line selection)
RA	Retinoic acid
RBC	Research Blood Components
R _{Max}	Maximal response - analyte binding capacity by BIAcore
RPM	Revolutions per minute
RPMI8866	Human B-cell lymphoma line endogenously expressing $\alpha 4\beta 7$
RLU	Relative luminescence units
RT	Room temperature
RU	Response in binding used in BIAcore
SPR	Surface plasmon resonance allowing real-time binding via BIAcore
T	Target cells or T cells
VCAM-1	Vascular cell adhesion molecule 1
WT	Wild type

6.2. Definitions

[0041] Unless otherwise defined herein, scientific and technical terms used in connection with the present disclosure have the meanings that are commonly understood by those of ordinary skill in the art.

[0042] As used herein, numbering of antibody amino acid residues is done according to the EU numbering scheme, unless otherwise indicated.

6.3. Anti- $\alpha 4\beta 7$ Antibodies

[0043] In one aspect, the disclosure concerns antibodies that specifically bind $\alpha 4\beta 7$ heterodimeric integrin receptor (also known as $\alpha 4\beta 7$, LPAM-1, lymphocyte Peyer's patch adhesion molecule 1, and a dimer of Integrin $\alpha 4$ and Integrin $\beta 7$).

[0044] As used herein, the term “antibody” (Ab) refers to an immunoglobulin molecule that specifically binds to a particular antigen, e.g., $\alpha 4\beta 7$. In some embodiments, the anti- $\alpha 4\beta 7$ antibodies of the disclosure bind to human $\alpha 4\beta 7$ and thereby modulate the immune system. Anti- $\alpha 4\beta 7$ antibodies of the disclosure comprise complementarity determining regions (CDRs), also known as hypervariable regions, in both the light chain and the heavy chain variable domains. The more highly

conserved portions of variable domains are called the framework (FR). As is known in the art, the amino acid position/boundary delineating a hypervariable region of an antibody can vary, depending on the context and the various definitions known in the art. Some positions within a variable domain may be viewed as hybrid hypervariable positions in that these positions can be deemed to be within a hypervariable region under one set of criteria while being deemed to be outside a hypervariable region under a different set of criteria. One or more of these positions can also be found in extended hypervariable regions. The disclosure provides antibodies comprising modifications in these hybrid hypervariable positions. The variable domains of native heavy and light chains each comprise four FR regions, largely by adopting a β -sheet configuration, connected by three CDRs, which form loops connecting, and in some cases forming part of, the β -sheet structure. The CDRs in each chain are held together in close proximity by the FR regions and, with the CDRs from the other chain, contribute to the formation of the target binding site of antibodies. *See* Kabat *et al.*, Sequences of Proteins of Immunological Interest (National Institute of Health, Bethesda, Md. 1987).

[0045] The antibodies of the disclosure may be polyclonal, monoclonal, genetically engineered, and/or otherwise modified in nature, including but not limited to chimeric antibodies, humanized antibodies, human antibodies, single chain antibodies, *etc.* In various embodiments, the antibodies comprise all or a portion of a constant region of an antibody. In some embodiments, the constant region is an isotype selected from: IgA (*e.g.*, IgA₁ or IgA₂), IgD, IgE, IgG (*e.g.*, IgG₁, IgG₂, IgG₃ or IgG₄), and IgM. In specific embodiments, the anti- $\alpha 4\beta 7$ antibodies described herein comprise an IgG₁. In other embodiments, the anti- $\alpha 4\beta 7$ antibodies comprise an IgG₂. In yet other embodiments, the anti- $\alpha 4\beta 7$ antibodies comprise an IgG₄. As used herein, the “constant region” of an antibody includes the natural constant region, allotypes or variants, such as any of T250Q, L234A, L235A, D356E, L358M, M428L, and/or A431G in human IgG₁.

[0046] The light constant region of an anti- $\alpha 4\beta 7$ antibody may be a kappa (κ) light region or a lambda (λ) region. A λ light region can be any one of the known subtypes, *e.g.*, λ_1 , λ_2 , λ_3 , or λ_4 . In some embodiments, an anti- $\alpha 4\beta 7$ antibody comprises a kappa (κ) light region.

[0047] The term “monoclonal antibody” as used herein is not limited to antibodies produced through hybridoma technology. A monoclonal antibody is derived from a single clone, including any eukaryotic, prokaryotic, or phage clone, by any means available or known in the art. Monoclonal antibodies useful with the present disclosure can be prepared using a wide variety of techniques

known in the art including the use of hybridoma, recombinant, and phage display technologies, or a combination thereof.

[0048] The term “chimeric” antibody as used herein refers to an antibody having variable sequences derived from a non-human immunoglobulin, such as a rat or a mouse antibody, and human immunoglobulin constant regions, typically chosen from a human immunoglobulin template.

[0049] “Humanized” forms of non-human (*e.g.*, murine) antibodies comprise substantially all of at least one, and typically two, variable domains, in which all or substantially all of the CDR regions correspond to those of a non-human immunoglobulin and all or substantially all of the FR regions are those of a human immunoglobulin sequence. The humanized antibody can also comprise at least a portion of an immunoglobulin constant region (Fc), typically that of a human immunoglobulin consensus sequence.

[0050] “Human antibodies” include antibodies having the amino acid sequence of a human immunoglobulin and include antibodies isolated from human immunoglobulin libraries or from animals transgenic for one or more human immunoglobulins and that do not express endogenous functional immunoglobulins. Human antibodies can be made by a variety of methods known in the art including phage display methods using antibody libraries derived from human immunoglobulin sequences.

[0051] Anti- $\alpha 4\beta 7$ antibodies of the disclosure include full-length (intact) antibody molecules.

[0052] The anti- $\alpha 4\beta 7$ antibodies may be antibodies whose sequences have been modified to alter at least one constant region-mediated biological effector function. For example, in some embodiments, an anti- $\alpha 4\beta 7$ antibody may be modified to reduce at least one constant region-mediated biological effector function relative to the unmodified antibody, *e.g.*, reduced binding to one or more of the Fc receptors (Fc γ R) such as Fc γ RI, Fc γ RIIa, Fc γ RIIb, Fc γ RIIIa and/or Fc γ RIIIb. Fc γ R binding can be reduced by mutating the immunoglobulin constant region segment of the antibody at particular regions necessary for Fc γ R interactions (*See, e.g.*, Canfield and Morrison, 1991, *J. Exp. Med.* 173:1483-1491; and Lund *et al.*, 1991, *J. Immunol.* 147:2657-2662). Reduction in Fc γ R binding ability of the antibody can also reduce other effector functions which rely on Fc γ R interactions, such as opsonization, phagocytosis and antigen-dependent cellular cytotoxicity (“ADCC”).

[0053] The anti- $\alpha 4\beta 7$ antibodies described herein include antibodies that have been modified to acquire or improve at least one constant region-mediated biological effector function relative to an

unmodified antibody, *e.g.*, to enhance Fc γ R interactions (*See, e.g.*, US Patent Appl. No. 2006/0134709). For example, an anti- $\alpha 4\beta 7$ antibody of the disclosure can have a constant region that binds Fc γ RI, Fc γ RIIa, Fc γ RIIb, Fc γ RIIIa and/or Fc γ RIIIb with greater affinity than the corresponding unmodified constant region.

[0054] Additional substitutions that can modify Fc γ R binding and/or ADCC effector function of an anti- $\alpha 4\beta 7$ antibody include the K322A substitution or the L234A and L235A double substitution in the Fc region. *See, e.g.*, Hezareh, et al. J. Virol., 75 (24): 12161-12168 (2001).

[0055] The anti- $\alpha 4\beta 7$ antibodies of the disclosure can comprise modified (or variant) CH2 domains or entire Fc domains that include amino acid substitutions that increase binding to Fc γ RIIb and/or reduced binding to Fc γ RIIIa as compared to the binding of a corresponding wild-type CH2 or Fc region. A variant CH2 or variant Fc domain may include one or more substitutions at position 263, position 266, position 273, and position 305. In some embodiments, the anti- $\alpha 4\beta 7$ antibodies comprise one or more substitutions selected from V263L, V266L, V273C, V273E, V273F, V273L, V273M, V273S, V273Y, V305K, and V305W, relative to the wild-type CH2 domain.

[0056] Other examples of variant CH2 or variant Fc domains that can afford increased binding to Fc γ RIIb and/or reduced binding to Fc γ RIIIa as compared to the binding of a corresponding wild-type CH2 or Fc region include those found in Vonderheide, et al. Clin. Cancer Res., 19(5), 1035-1043 (2013), such as S267E or S267E/L328F in human IgG₁.

[0057] Anti- $\alpha 4\beta 7$ antibodies that comprise a human IgG₄ constant region can comprise the S228P mutation, which has been reported to prevent Fab arm exchange. *See, e.g.*, Silva, JP et al. Journal of Biological Chemistry, 290(9), 5462-5469 (2015).

[0058] In some embodiments, the anti- $\alpha 4\beta 7$ antibodies include modifications that increase or decrease their binding affinities to the fetal Fc receptor, FcRn, for example, by mutating the immunoglobulin constant region segment at particular regions involved in FcRn interactions. In particular embodiments, an anti- $\alpha 4\beta 7$ antibody of the IgG class is mutated such that at least one of amino acid residues 250, 314, and 428 of the heavy chain constant region is substituted alone, or in any combinations thereof. For position 250, the substituting amino acid residue can be any amino acid residue other than threonine, including, but not limited to, alanine, cysteine, aspartic acid, glutamic acid, phenylalanine, glycine, histidine, isoleucine, lysine, leucine, methionine, asparagine, proline, glutamine, arginine, serine, valine, tryptophan, or tyrosine. For position 314, the substituting amino acid residue can be any amino acid residue other than leucine, including, but not limited to,

alanine, cysteine, aspartic acid, glutamic acid, phenylalanine, glycine, histidine, isoleucine, lysine, methionine, asparagine, proline, glutamine, arginine, serine, threonine, valine, tryptophan, or tyrosine. For position 428, the substituting amino acid residues can be any amino acid residue other than methionine, including, but not limited to, alanine, cysteine, aspartic acid, glutamic acid, phenylalanine, glycine, histidine, isoleucine, lysine, leucine, asparagine, proline, glutamine, arginine, serine, threonine, valine, tryptophan, or tyrosine. An exemplary substitution known to modify Fc effector function is the Fc substitution M428L, which can occur in combination with the Fc substitution T250Q. Additional specific combinations of suitable amino acid substitutions are identified in Table 1 of U.S. Patent No. 7,217,797. Such mutations increase binding to FcRn, which protects the antibody from degradation and increases its half-life.

[0059] Anti- $\alpha 4\beta 7$ antibodies with high affinity for human $\alpha 4\beta 7$ may be desirable for therapeutic and diagnostic uses. Accordingly, the present disclosure contemplates antibodies having a high binding affinity to human $\alpha 4\beta 7$. In specific embodiments, the anti- $\alpha 4\beta 7$ antibodies binds to human $\alpha 4\beta 7$ with an affinity of at least about 100 nM, but may exhibit higher affinity, for example, at least about 90 nM, 80 nM, 70 nM, 60 nM, 50 nM, 40 nM, 30 nM, 25 nM, 20 nM, 15 nM, 10 nM, 7 nM, 6 nM, 5 nM, 4 nM, 3 nM, 2 nM, 1 nM, 0.1 nM, 0.01 nM, or even higher. In some embodiments, the antibodies bind human $\alpha 4\beta 7$ with an affinity in the range of about 1 pM to about 10 nM, of about 100 pM to about 10 nM, about 100 pM to about 1 nM, or an affinity ranging between any of the foregoing values.

[0060] Affinity of anti- $\alpha 4\beta 7$ antibodies for human $\alpha 4\beta 7$ can be determined using techniques well known in the art or described herein, such as for example, but not by way of limitation, ELISA, isothermal titration calorimetry (ITC), surface plasmon resonance, or fluorescent polarization assay.

[0061] Anti- $\alpha 4\beta 7$ antibodies generally comprise a heavy chain comprising a variable region (V_H) having three complementarity determining regions (“CDRs”) referred to herein (in N→C order) as V_H CDR#1, V_H CDR#2, and V_H CDR#3, and a light chain comprising a variable region (V_L) having three complementarity determining regions referred to herein (in N→C order) as V_L CDR#1, V_L CDR#2, and V_L CDR#3. The amino acid sequences of exemplary CDRs, as well as the amino acid sequence of the V_H and V_L regions of the heavy and light chains of exemplary anti- $\alpha 4\beta 7$ are provided herein. Specific embodiments of anti- $\alpha 4\beta 7$ antibodies include these exemplary CDRs and/or V_H and/or V_L sequences, as well as antibodies that compete for binding human $\alpha 4\beta 7$ with such antibodies.

[0062] In some embodiments, an anti- $\alpha 4\beta 7$ antibody is suitable for administration to humans. In specific embodiments, the anti- $\alpha 4\beta 7$ antibody is humanized.

[0063] In some embodiments, the amino acid sequences of the CDRs of an anti- $\alpha 4\beta 7$ antibody are selected from the sequences of TABLE 4.

TABLE 4 CDR Sequences of Specific Embodiments		
CDR	Sequence (N→C)	Sequence Identifier
V _H CDR#1:	NTYMH	SEQ ID NO:12
	GFNIKNTYMH	SEQ ID NOS:32, 42, 52, 62, 72, 82
V _H CDR#2:	RIDPANGHTEYAP	SEQ ID NO:13
	RIDPANGHTEYAPKFQG	SEQ ID NO:33
	RIDPANKHTEYAPKFLG	SEQ ID NO:43
	RIDPARGHTEYAPKFSG	SEQ ID NO:53
	RIDPARGHTEYAPKFEG	SEQ ID NO:63
	RIDPAKGHTEYAPKFLG	SEQ ID NO:73
	RIDPAGGHTEYAPKFIG	SEQ ID NO:83
V _H CDR#3:	YYVDS	SEQ ID NO:14
	VDS	SEQ ID NO:34
	VAS	SEQ ID NOS:44, 64, 84
	VDQ	SEQ ID NO:54
	VDV	SEQ ID NO:74
V _L CDR#1:	HASQGISDNIG	SEQ ID NOS:15, 35
	HASQEISDNIG	SEQ ID NO:45, 85
	HASQDISDNIG	SEQ ID NO:55, 65, 75
V _L CDR#2:	HGTNLED	SEQ ID NOS:16, 36, 46, 56, 66, 76, 86
V _L CDR#3:	VQYAQFPWT	SEQ ID NOS:17, 37, 47, 57, 67, 77, 87

[0064] Specific exemplary embodiments of anti- $\alpha 4\beta 7$ antibodies with the above CDRs are described herein. In some embodiments, an anti- $\alpha 4\beta 7$ antibody has the CDRs of SEQ ID NOS:12, 13, 14, 15, 16, and 17. In some embodiments, an anti- $\alpha 4\beta 7$ antibody has the CDRs of SEQ ID NOS:32, 33, 34, 35, 36, and 37. In some embodiments, an anti- $\alpha 4\beta 7$ antibody has the CDRs of SEQ ID NOS:42, 43, 44, 45, 46, and 47. In some embodiments, an anti- $\alpha 4\beta 7$ antibody has the CDRs of SEQ ID NOS:52, 53, 54, 55, 56, and 57. In some embodiments, an anti- $\alpha 4\beta 7$ antibody has the CDRs of SEQ ID NOS:62, 63, 64, 65, 66, and 67. In some embodiments, an anti- $\alpha 4\beta 7$ antibody has the CDRs of SEQ ID NOS:72, 73, 74, 75, 76, and 77. In some embodiments, an anti- $\alpha 4\beta 7$ antibody has the CDRs of SEQ ID NOS:82, 83, 84, 85, 86, and 87.

[0065] In some embodiments, an anti- $\alpha 4\beta 7$ antibody comprises a V_H chain and a V_L chain selected from the sequences of TABLE 5:

TABLE 5 Variable Region Sequences of Specific Embodiments	
Type	Sequence ID
V_H :	SEQ ID NO:10
	SEQ ID NO:20
	SEQ ID NO:21
	SEQ ID NO:22
	SEQ ID NO:23
	SEQ ID NO:40
	SEQ ID NO:50
	SEQ ID NO:60
	SEQ ID NO:70
	SEQ ID NO:80
V_L :	SEQ ID NO:11
	SEQ ID NO:25
	SEQ ID NO:26
	SEQ ID NO:27
	SEQ ID NO:28
	SEQ ID NO:41
	SEQ ID NO:51
	SEQ ID NO:61
	SEQ ID NO:71
	SEQ ID NO:81

[0066] In some embodiments, an anti- $\alpha 4\beta 7$ antibody comprises a V_H chain corresponding in sequence to SEQ ID NO:10; and a V_L chain corresponding in sequence to SEQ ID NO:11. In some embodiments, an anti- $\alpha 4\beta 7$ antibody comprises a V_H chain corresponding in sequence to any one of SEQ ID NOS:20, or 22-23; and a V_L chain corresponding in sequence to any one of SEQ ID NOS:25, or 27-28. In some embodiments, an anti- $\alpha 4\beta 7$ antibody comprises a V_H chain corresponding in sequence to a variant of SEQ ID NO:21; and a V_L chain corresponding in sequence to a variant of SEQ ID NOS:26. In some embodiments, an anti- $\alpha 4\beta 7$ antibody comprises a V_H chain corresponding in sequence to SEQ ID NO:40; and a V_L chain corresponding in sequence to SEQ ID NO:41. In some embodiments, an anti- $\alpha 4\beta 7$ antibody comprises a V_H chain corresponding in sequence to SEQ ID NO:50; and a V_L chain corresponding in sequence to SEQ ID NO:51. In some embodiments, an anti- $\alpha 4\beta 7$ antibody comprises a V_H chain corresponding in sequence to SEQ ID NO:60; and a V_L

chain corresponding in sequence to SEQ ID NO:61. In some embodiments, an anti- $\alpha 4\beta 7$ antibody comprises a V_H chain corresponding in sequence to SEQ ID NO:70; and a V_L chain corresponding in sequence to SEQ ID NO:71. In some embodiments, an anti- $\alpha 4\beta 7$ antibody comprises a V_H chain corresponding in sequence to SEQ ID NO:80; and a V_L chain corresponding in sequence to SEQ ID NO:81.

[0067] Certain mutations of a V_H or V_L sequence in an anti- $\alpha 4\beta 7$ antibody described herein would be understood by a person of skill to afford anti- $\alpha 4\beta 7$ antibodies within the scope of the disclosure. Mutations may include amino acid substitutions, additions, or deletions from a V_H or V_L sequence as disclosed herein while retaining significant anti- $\alpha 4\beta 7$ activity. Accordingly, in some embodiments, an anti- $\alpha 4\beta 7$ antibody comprises a V_H sequence having at least 85%, at least 90%, at least 93%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity to the V_H sequence of any one of the antibodies shown in TABLE 5. An anti- $\alpha 4\beta 7$ antibody can comprise a V_H sequence having up to 8, up to 7, up to 6, up to 5, up to 4, up to 3, or up to 2 mutations compared with the V_H sequence of any one of the antibodies shown in TABLE 5. In some embodiments, an anti- $\alpha 4\beta 7$ antibody can comprise a V_H sequence having 5 or fewer, 4 or fewer, 3 or fewer, or 2 or fewer mutations compared with the V_H sequence of any one of the antibodies shown in TABLE 5. In some embodiments, an anti- $\alpha 4\beta 7$ antibody comprises a V_H sequence having a single amino acid substitution. In some embodiments, the mutation in the V_H sequence is located in V_H CDR#1, V_H CDR#2, or V_H CDR#3. In some embodiments, an anti- $\alpha 4\beta 7$ antibody comprises a V_L sequence having at least 85%, at least 90%, at least 93%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity to the V_L sequence of any one of the antibodies shown in TABLE 5. An anti- $\alpha 4\beta 7$ antibody can comprise a V_L sequence having up to 8, up to 7, up to 6, up to 5, up to 4, up to 3, or up to 2 mutations compared with the V_L sequence of any one of the antibodies shown in TABLE 5. In some embodiments, an anti- $\alpha 4\beta 7$ antibody can comprise a V_L sequence having 5 or fewer, 4 or fewer, 3 or fewer, or 2 or fewer mutations compared with the V_L sequence of any one of the antibodies shown in TABLE 5. In some embodiments, an anti- $\alpha 4\beta 7$ antibody comprises a V_L sequence having a single amino acid substitution. In some embodiments, the mutation in the V_L sequence is located in V_L CDR#1, V_L CDR#2, or V_L CDR#3.

[0068] In some embodiments, an anti- $\alpha 4\beta 7$ antibody comprises a heavy chain amino acid sequence, and/or a light chain amino acid sequence selected from the sequences of TABLE 6.

TABLE 6 Full Length Chains	
Chain	Sequence ID
Heavy Chain	SEQ ID NO:90
	SEQ ID NO:91
	SEQ ID NO:92
	SEQ ID NO:93
	SEQ ID NO:94
	SEQ ID NO:95
	SEQ ID NO:96
	SEQ ID NO:97
	SEQ ID NO:98
	SEQ ID NO:99
Light Chain	SEQ ID NO:100

[0069] In some embodiments, an anti- $\alpha 4\beta 7$ antibody comprises a heavy chain corresponding in sequence to SEQ ID NO:90; and a light chain corresponding in sequence to SEQ ID NO:100. In some embodiments, an anti- $\alpha 4\beta 7$ antibody comprises a heavy chain corresponding in sequence to SEQ ID NO:92; and a light chain corresponding in sequence to SEQ ID NO:100. In some embodiments, an anti- $\alpha 4\beta 7$ antibody comprises a heavy chain corresponding in sequence to SEQ ID NO:94; and a light chain corresponding in sequence to SEQ ID NO:100. In some embodiments, an anti- $\alpha 4\beta 7$ antibody comprises a heavy chain corresponding in sequence to SEQ ID NO:96; and a light chain corresponding in sequence to SEQ ID NO:100. In some embodiments, an anti- $\alpha 4\beta 7$ antibody comprises a heavy chain corresponding in sequence to SEQ ID NO:98; and a light chain corresponding in sequence to SEQ ID NO:100.

[0070] Post-translational modifications to the sequences of an anti- $\alpha 4\beta 7$ antibody may occur, such as cleavage of one or more (*e.g.*, 1, 2, 3, or more) amino acid residues on the C-terminal end of the antibody heavy chain, creating a truncated form.

[0071] In some embodiments, an anti- $\alpha 4\beta 7$ antibody comprises a heavy chain corresponding in sequence to SEQ ID NO:91; and a light chain corresponding in sequence to SEQ ID NO:100. In some embodiments, an anti- $\alpha 4\beta 7$ antibody comprises a heavy chain corresponding in sequence to SEQ ID NO:93; and a light chain corresponding in sequence to SEQ ID NO:100. In some embodiments, an anti- $\alpha 4\beta 7$ antibody comprises a heavy chain corresponding in sequence to SEQ ID NO:95; and a light chain corresponding in sequence to SEQ ID NO:100. In some

embodiments, an anti- $\alpha 4\beta 7$ antibody comprises a heavy chain corresponding in sequence to SEQ ID NO:97; and a light chain corresponding in sequence to SEQ ID NO:100. In some embodiments, an anti- $\alpha 4\beta 7$ antibody comprises a heavy chain corresponding in sequence to SEQ ID NO:99; and a light chain corresponding in sequence to SEQ ID NO:100.

[0072] In some embodiments, the anti- $\alpha 4\beta 7$ antibodies compete for binding human $\alpha 4\beta 7$ in *in vitro* assays with a reference antibody. In some embodiments, the anti- $\alpha 4\beta 7$ antibodies compete for binding human $\alpha 4\beta 7$ on cells expressing human $\alpha 4\beta 7$. The reference antibody may be any of the anti- $\alpha 4\beta 7$ antibodies described herein. In some embodiments, the reference antibody is an antibody provided for in TABLES 4-6. In some embodiments, the reference antibody is an antibody provided for in TABLE 8. In specific embodiments, the reference antibody is selected from a research grade antibody generated using amino acid sequences from an anti-human $\alpha 4\beta 7$ antibody or an antibody having an amino acid sequence equivalent thereto, such as vedolizumab.

[0073] In some embodiments, the anti- $\alpha 4\beta 7$ antibodies antagonize, *e.g.*, inhibit, human $\alpha 4\beta 7$ heterodimer of one $\alpha 4$ (SEQ ID NOS:1-2) and one $\beta 7$ (SEQ ID NOS:3-4). $\alpha 4\beta 7$ receptor antagonism can occur by a number of mechanisms, for example, by inhibiting binding of $\alpha 4\beta 7$ by at least one of its ligands, such as human MAdCAM-1 (SEQ ID NO:5) or human VCAM-1 (SEQ ID NO:6).

[0074] The anti- $\alpha 4\beta 7$ antibodies described herein bind to human $\alpha 4\beta 7$. Cross reactivity of the antibodies for binding to $\alpha 4\beta 7$ from other species, for example, from monkey, *e.g.*, cynomolgus monkey, may offer advantages, such as the ability to test in monkey animal models for biological activity. Such animal model testing may be used to screen anti- $\alpha 4\beta 7$ antibodies to select properties related to efficacy, *e.g.*, favorable pharmacokinetics, or those related to safety, *e.g.*, decreased hepatic toxicity. In some embodiments, the anti- $\alpha 4\beta 7$ antibodies bind to cynomolgus $\alpha 4\beta 7$ as well as human $\alpha 4\beta 7$.

[0075] Assays for competition include, but are not limited to, a radioactive material labeled immunoassay (RIA), an enzyme-linked immunosorbent assay (ELISA), a sandwich ELISA, fluorescence activated cell sorting (FACS) assays, and surface plasmon resonance assays.

[0076] In conducting an antibody competition assay between a reference antibody and a test antibody (irrespective of species or isotype), one may first label the reference with a detectable label, such as a fluorophore, biotin or an enzymatic (or even radioactive) label to enable subsequent identification. In this case, cells expressing human $\alpha 4\beta 7$ are incubated with unlabeled test antibody, labeled reference antibody is added, and the intensity of the bound label is measured. If the test

antibody competes with the labeled reference antibody by binding to an overlapping epitope, the intensity will be decreased relative to a control reaction carried out without test antibody.

[0077] In a specific embodiment of this assay, the concentration of labeled reference antibody that yields 80% of maximal binding (“ $\text{conc}_{80\%}$ ”) under the assay conditions (*e.g.*, a specified density of cells) is first determined, and a competition assay carried out with 10X $\text{conc}_{80\%}$ of unlabeled test antibody and $\text{conc}_{80\%}$ of labeled reference antibody.

[0078] The inhibition can be expressed as an inhibition constant, or K_i , which is calculated according to the following formula:

$$K_i = \text{IC}_{50} / (1 + [\text{reference Ab concentration}] / K_d),$$

where IC_{50} is the concentration of test antibody that yields a 50% reduction in binding of the reference antibody and K_d is the dissociation constant of the reference antibody, a measure of its affinity for human $\alpha 4\beta 7$. Antibodies that compete with anti- $\alpha 4\beta 7$ antibodies disclosed herein can have a K_i from 10 pM to 10 nM under assay conditions described herein.

[0079] In various embodiments, a test antibody is considered to compete with a reference antibody if it decreases binding of the reference antibody by at least about 20% or more, for example, by at least about 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95% or even more, or by a percentage ranging between any of the foregoing values, at a reference antibody concentration that is 80% of maximal binding under the specific assay conditions used, and a test antibody concentration that is 10-fold higher than the reference antibody concentration.

[0080] Another aspect of the present disclosure includes anti- $\alpha 4\beta 7$ antibody binding fragments that are capable of specifically binding human $\alpha 4\beta 7$. In some embodiments, these anti- $\alpha 4\beta 7$ binding fragments comprise at least one and up to all CDRs of the anti- $\alpha 4\beta 7$ antibodies disclosed herein. Examples of antibody binding fragments include by way of example and not limitation, Fab, Fab', F(ab')₂, Fv fragments, single chain Fv fragments and single domain fragments.

[0081] An anti- $\alpha 4\beta 7$ antibody or binding fragment thereof may have one or more amino acids inserted into one or more of its CDRs, for example as described in Jung and Plückthun, 1997, Protein Engineering 10:9, 959-966; Yazaki *et al.*, 2004, Protein Eng. Des Sel. 17(5):481-9. Epub 2004 Aug 17; and U.S. Pat. Appl. No. 2007/0280931.

6.4. Polynucleotides Encoding the Anti- $\alpha 4\beta 7$ Antibodies, Expression Systems and Methods of Making the Antibodies

[0082] The present disclosure encompasses polynucleotide molecules encoding immunoglobulin light and heavy chain genes for anti- $\alpha 4\beta 7$ antibodies, vectors comprising such polynucleotides, and host cells capable of producing the anti- $\alpha 4\beta 7$ antibodies of the disclosure.

[0083] An anti- $\alpha 4\beta 7$ antibody of the disclosure can be prepared by recombinant expression of immunoglobulin light and heavy chain genes in a host cell. To express an antibody recombinantly, a host cell is transfected with one or more recombinant expression vectors carrying DNA fragments encoding the immunoglobulin light and heavy chains of the antibody such that the light and heavy chains are expressed in the host cell and, optionally, secreted into the medium in which the host cells are cultured, from which medium the antibodies can be recovered.

[0084] To generate polynucleotides encoding such anti- $\alpha 4\beta 7$ antibodies, DNA fragments encoding the light and heavy chain variable regions are first obtained. These DNAs can be obtained by amplification and modification of germline DNA or cDNA encoding light and heavy chain variable sequences, for example using the polymerase chain reaction (PCR).

[0085] Once DNA fragments encoding anti- $\alpha 4\beta 7$ antibody-related V_H and V_L segments are obtained, these DNA fragments can be further manipulated by standard recombinant DNA techniques, for example to convert the variable region genes to full-length antibody chain genes, to Fab fragment genes or to a scFv gene. In these manipulations, a V_L - or V_H -encoding DNA fragment is operatively linked to another DNA fragment encoding another protein, such as an antibody constant region or a flexible linker. The term "operatively linked," as used in this context, is intended to mean that the two DNA fragments are joined such that the amino acid sequences encoded by the two DNA fragments remain in-frame.

[0086] The isolated DNA encoding the V_H region can be converted to a full-length heavy chain gene by operatively linking the V_H -encoding DNA to another DNA molecule encoding heavy chain constant regions (CH1, CH2, CH3 and, optionally, CH4). The sequences of human heavy chain constant region genes are known in the art (*See, e.g.,* Kabat, E.A., *et al.*, 1991, Sequences of Proteins of Immunological Interest, Fifth Edition, U.S. Department of Health and Human Services, NIH Publication No. 91-3242) and DNA fragments encompassing these regions can be obtained by standard PCR amplification. The heavy chain constant region can be an IgG₁, IgG₂, IgG₃, IgG₄, IgA, IgE, IgM or IgD constant region, but in certain embodiments is an IgG₁ or IgG₄. For a Fab fragment

heavy chain gene, the V_H-encoding DNA can be operatively linked to another DNA molecule encoding only the heavy chain CH1 constant region.

[0087] The isolated DNA encoding the V_L region can be converted to a full-length light chain gene (as well as a Fab light chain gene) by operatively linking the V_L-encoding DNA to another DNA molecule encoding the light chain constant region, CL. The sequences of human light chain constant region genes are known in the art (*See, e.g.,* Kabat, *et al.*, 1991, Sequences of Proteins of Immunological Interest, Fifth Edition, U.S. Department of Health and Human Services, NIH Publication No. 91-3242) and DNA fragments encompassing these regions can be obtained by standard PCR amplification. The light chain constant region can be a kappa or lambda constant region, but in certain embodiments is a kappa constant region.

[0088] To express the anti- $\alpha 4\beta 7$ antibodies of the disclosure, DNAs encoding partial or full-length light and heavy chains, obtained as described above, are inserted into expression vectors such that the genes are operatively linked to transcriptional and translational control sequences. In this context, the term “operatively linked” is intended to mean that an antibody gene is ligated into a vector such that transcriptional and translational control sequences within the vector serve their intended function of regulating the transcription and translation of the antibody gene. The expression vector and expression control sequences are chosen to be compatible with the expression host cell used. The antibody light chain gene and the antibody heavy chain gene can be inserted into separate vectors or, more typically, both genes are inserted into the same expression vector.

[0089] The antibody genes are inserted into the expression vector by standard methods (*e.g.*, ligation of complementary restriction sites on the antibody gene fragment and vector, or blunt end ligation if no restriction sites are present). Prior to insertion of the anti- $\alpha 4\beta 7$ antibody-related light or heavy chain sequences, the expression vector can already carry antibody constant region sequences. For example, one approach to converting the anti- $\alpha 4\beta 7$ monoclonal antibody-related V_H and V_L sequences to full-length antibody genes is to insert them into expression vectors already encoding heavy chain constant and light chain constant regions, respectively, such that the V_H segment is operatively linked to the CH segment(s) within the vector and the V_L segment is operatively linked to the CL segment within the vector. Additionally or alternatively, the recombinant expression vector can encode a signal peptide that facilitates secretion of the antibody chain from a host cell. The antibody chain gene can be cloned into the vector such that the signal peptide is linked in-frame to the

amino terminus of the antibody chain gene. The signal peptide can be an immunoglobulin signal peptide or a heterologous signal peptide (*i.e.*, a signal peptide from a non-immunoglobulin protein).

[0090] In addition to the antibody chain genes, the recombinant expression vectors of the disclosure carry regulatory sequences that control the expression of the antibody chain genes in a host cell. The term “regulatory sequence” is intended to include promoters, enhancers and other expression control elements (*e.g.*, polyadenylation signals) that control the transcription or translation of the antibody chain genes.

[0091] In addition to the antibody chain genes and regulatory sequences, the recombinant expression vectors of the disclosure can carry additional sequences, such as sequences that regulate replication of the vector in host cells (*e.g.*, origins of replication) and selectable marker genes. The selectable marker gene facilitates selection of host cells into which the vector has been introduced. For expression of the light and heavy chains, the expression vector(s) encoding the heavy and light chains is transfected into a host cell by standard techniques. The various forms of the term “transfection” are intended to encompass a wide variety of techniques commonly used for the introduction of exogenous DNA into a prokaryotic or eukaryotic host cell, *e.g.*, electroporation, lipofection, calcium-phosphate precipitation, DEAE- dextran transfection and the like.

[0092] It is possible to express the antibodies of the disclosure in either prokaryotic or eukaryotic host cells. In certain embodiments, expression of antibodies is performed in eukaryotic cells, *e.g.*, mammalian host cells, of optimal secretion of a properly folded and immunologically active antibody. Exemplary mammalian host cells for expressing the recombinant antibodies of the disclosure include Chinese Hamster Ovary (CHO cells) (including DHFR⁻ CHO cells, described in Urlaub and Chasin, 1980, Proc. Natl. Acad. Sci. USA 77:4216-4220, used with a DHFR selectable marker, *e.g.*, as described in Kaufman and Sharp, 1982, Mol. Biol. 159:601-621), NSO myeloma cells, COS cells and SP2 cells. When recombinant expression vectors encoding antibody genes are introduced into mammalian host cells, the antibodies are produced by culturing the host cells for a period of time sufficient to allow for expression of the antibody in the host cells or secretion of the antibody into the culture medium in which the host cells are grown. Antibodies can be recovered from the culture medium using standard protein purification methods. Host cells can also be used to produce portions of intact antibodies, such as Fab fragments or scFv molecules. It is understood that variations on the above procedure are within the scope of the present disclosure. For example, it can be desirable to

transfect a host cell with DNA encoding either the light chain or the heavy chain (but not both) of an anti- $\alpha 4\beta 7$ antibody of this disclosure.

[0093] Recombinant DNA technology can also be used to remove some or all of the DNA encoding either or both of the light and heavy chains that is not necessary for binding to human $\alpha 4\beta 7$. The molecules expressed from such truncated DNA molecules are also encompassed by the antibodies of the disclosure.

[0094] For recombinant expression of an anti- $\alpha 4\beta 7$ antibody of the disclosure, the host cell can be co-transfected with two expression vectors of the disclosure, the first vector encoding a heavy chain derived polypeptide and the second vector encoding a light chain derived polypeptide. The two vectors can contain identical selectable markers, or they can each contain a separate selectable marker. Alternatively, a single vector can be used which encodes both heavy and light chain polypeptides.

[0095] Once a polynucleotide encoding one or more portions of an anti- $\alpha 4\beta 7$ antibody has been obtained, further alterations or mutations can be introduced into the coding sequence, for example to generate polynucleotides encoding antibodies with different CDR sequences, antibodies with reduced affinity to the Fc receptor, or antibodies of different subclasses.

[0096] The anti- $\alpha 4\beta 7$ antibodies of the disclosure can also be produced by chemical synthesis or by using a cell-free platform.

6.5. Purification of Anti- $\alpha 4\beta 7$ Antibodies

[0097] Once a polypeptide of the disclosure has been produced by recombinant expression, it can be purified by any method known in the art for purification of a protein.

[0098] The polypeptides may be purified as a monomer or as a dimer, *e.g.*, as an anti- $\alpha 4\beta 7$ antibody comprising two polypeptides.

[0099] Once isolated, an anti- $\alpha 4\beta 7$ antibody can be further purified.

6.6. Methods of Use

6.6.1. Therapeutic benefit

[00100] Data provided herein demonstrate that the disclosed anti-human $\alpha 4\beta 7$ antibodies demonstrate favorable binding affinity, specificity, and potency towards $\alpha 4\beta 7$, as well as favorable binding profiles on primary immune cells, Fc γ R binding, and lack of ADCC and ADCP activity on human

$\alpha 4\beta 7$ + cells. These anti-human $\alpha 4\beta 7$ antibodies were shown to bind to $\alpha 4\beta 7$ in the virions of different laboratory-grown HIV strains as well as from patients' HIV samples, and subsequently form immune complexes. These immune complexes can bind to different Fc γ Rs and taken up by a human monocytic cell line THP-1 by phagocytosis. These antibodies also block the interaction of $\alpha 4\beta 7$ with its ligands such as MadCAM-1 and HIV gp120 protein. Accordingly, the anti- $\alpha 4\beta 7$ antibodies, binding fragments, and/or pharmaceutical compositions comprising them may be used therapeutically to induce viral suppression of HIV infection or to reduce viral load in an HIV infected subject by Fc-dependent and Fab-dependent mechanisms. In some embodiments, viral suppression of HIV infection involves reducing function of the HIV virus and/or reducing replication of the HIV virus.

[00101] The disclosed anti-human $\alpha 4\beta 7$ antibodies may be used in a method of treating HIV infection in a subject in need thereof. In some embodiments, the method involves reducing viral load in the subject. In some embodiments, the viral load in the subject is reduced to undetectable levels. In some embodiments, the subject is a human subject infected with HIV.

[00102] In some embodiments, the method involves administering to a human subject having HIV infection an anti- $\alpha 4\beta 7$ antibody that antagonizes $\alpha 4\beta 7$ to provide therapeutic benefit. In some embodiments, the method involves administering to a human subject having HIV infection an anti- $\alpha 4\beta 7$ antibody which binds to HIV virions. In some embodiments, the method involves administering to a human subject having HIV infection an anti- $\alpha 4\beta 7$ antibody which binds to $\alpha 4\beta 7$ in HIV virions to form immune complexes. In some embodiments, the method involves administering to a human subject having HIV infection an anti- $\alpha 4\beta 7$ antibody forming immune complexes with HIV virions. In some embodiments, these immune complexes are taken up by APCs by phagocytosis. In some embodiments, the method involves administering to a human subject having HIV infection an anti- $\alpha 4\beta 7$ antibody which blocks the interaction of $\alpha 4\beta 7$ with its ligands such as MadCAM-1 and with HIV gp120. In some embodiments, the method involves administering to a human subject having HIV infection an anti- $\alpha 4\beta 7$ antibody that blocks the interaction of $\alpha 4\beta 7$ with HIV gp120, inhibiting the cell-to-cell HIV transmission. In some embodiments, the method involves administering to a human subject having HIV infection an anti- $\alpha 4\beta 7$ antibody that blocks CD4 T cell stimulation mediated by MadCAM-1 or HIV gp120. In some embodiments, the method involves administering to a human subject having HIV infection an anti- $\alpha 4\beta 7$ antibody which suppresses HIV replication in the CD4 T cells costimulated by MadCAM-1 or HIV gp120. In some embodiments, the method involves administering to a human subject having HIV infection an anti- $\alpha 4\beta 7$ antibody which

induces viral suppression of HIV replication. In some embodiments, the method involves administering to a human subject having HIV infection an anti- $\alpha 4\beta 7$ antibody which induces HIV suppression that is immune-mediated.

6.7. Table of Sequence Descriptions

[00103] A correlation of sequences disclosed in the incorporated Sequence Listing and their brief descriptions is shown in TABLE 7.

TABLE 7			
Brief Description of the Sequence Listing			
Sequence	Description	Sequence	Description
SEQ ID NO:1	human $\alpha 4$	SEQ ID NO:50	Ab-h1.9b VH
SEQ ID NO:2	human $\alpha 4$	SEQ ID NO:51	Ab-h1.9b VL
SEQ ID NO:3	human $\beta 7$	SEQ ID NO:52	Ab-h1.9b VH-CDR1
SEQ ID NO:4	human $\beta 7$	SEQ ID NO:53	Ab-h1.9b VH-CDR2
SEQ ID NO:5	MAdCAM-1	SEQ ID NO:54	Ab-h1.9b VH-CDR3
SEQ ID NO:6	VCAM-1	SEQ ID NO:55	Ab-h1.9b VL-CDR1
SEQ ID NO:7	gp120-V2 WT	SEQ ID NO:56	Ab-h1.9b VL-CDR2
SEQ ID NO:8	gp120-V2 control	SEQ ID NO:57	Ab-h1.9b VL-CDR3
SEQ ID NO:10	Ab-m1 VH	SEQ ID NO:60	Ab-h1.9c VH
SEQ ID NO:11	Ab-m1 VL	SEQ ID NO:61	Ab-h1.9c VL
SEQ ID NO:12	Ab-m1 VH-CDR1	SEQ ID NO:62	Ab-h1.9c VH-CDR1
SEQ ID NO:13	Ab-m1 VH-CDR2	SEQ ID NO:63	Ab-h1.9c VH-CDR2
SEQ ID NO:14	Ab-m1 VH-CDR3	SEQ ID NO:64	Ab-h1.9c VH-CDR3
SEQ ID NO:15	Ab-m1 VL-CDR1	SEQ ID NO:65	Ab-h1.9c VL-CDR1
SEQ ID NO:16	Ab-m1 VL-CDR2	SEQ ID NO:66	Ab-h1.9c VL-CDR2
SEQ ID NO:17	Ab-m1 VL-CDR3	SEQ ID NO:67	Ab-h1.9c VL-CDR3
SEQ ID NO:20	Ab-h1 VH.1	SEQ ID NO:70	Ab-h1.9d VH
SEQ ID NO:21	Ab-h1 VH.1 variable	SEQ ID NO:71	Ab-h1.9d VL
SEQ ID NO:22	Ab-h1 VH.1.a	SEQ ID NO:72	Ab-h1.9d VH-CDR1
SEQ ID NO:23	Ab-h1 VH.1.b	SEQ ID NO:73	Ab-h1.9d VH-CDR2
SEQ ID NO:25	Ab-h1 VL.1	SEQ ID NO:74	Ab-h1.9d VH-CDR3
SEQ ID NO:26	Ab-h1 VL.1 variable	SEQ ID NO:75	Ab-h1.9d VL-CDR1
SEQ ID NO:27	Ab-h1 VL.1.a	SEQ ID NO:76	Ab-h1.9d VL-CDR2
SEQ ID NO:28	Ab-h1 VL.1.b	SEQ ID NO:77	Ab-h1.9d VL-CDR3
SEQ ID NO:32	Ab-h1.9 VH-CDR1	SEQ ID NO:80	Ab-h1.9e VH
SEQ ID NO:33	Ab-h1.9 VH-CDR2	SEQ ID NO:81	Ab-h1.9e VL
SEQ ID NO:34	Ab-h1.9 VH-CDR3	SEQ ID NO:82	Ab-h1.9e VH-CDR1
SEQ ID NO:35	Ab-h1.9 VL-CDR1	SEQ ID NO:83	Ab-h1.9e VH-CDR2
SEQ ID NO:36	Ab-h1.9 VL-CDR2	SEQ ID NO:84	Ab-h1.9e VH-CDR3
SEQ ID NO:37	Ab-h1.9 VL-CDR3	SEQ ID NO:85	Ab-h1.9e VL-CDR1
SEQ ID NO:40	Ab-h1.9a VH	SEQ ID NO:86	Ab-h1.9e VL-CDR2
SEQ ID NO:41	Ab-h1.9a VL	SEQ ID NO:87	Ab-h1.9e VL-CDR3
SEQ ID NO:42	Ab-h1.9a VH-CDR1	SEQ ID NOS:90, 91*	Ab-h1.9d-hIgG1 (*terminal lysine trunc.)
SEQ ID NO:43	Ab-h1.9a VH-CDR2	SEQ ID NOS:92, 93*	Ab-h1.9d-WT HC (*K trunc.)
SEQ ID NO:44	Ab-h1.9a VH-CDR3	SEQ ID NOS:94, 95*	Ab-h1.9d-LALA HC (*K trunc.)
SEQ ID NO:45	Ab-h1.9a VL-CDR1	SEQ ID NOS:96, 97*	Ab-h1.9d-QL HC (*K trunc.)

TABLE 7 Brief Description of the Sequence Listing			
Sequence	Description	Sequence	Description
SEQ ID NO:46	Ab-h1.9a VL-CDR2	SEQ ID NOS:98, 99*	Ab-h1.9d-LALA/QL HC (*K trunc.)
SEQ ID NO:47	Ab-h1.9a VL-CDR3	SEQ ID NO:100	Ab-h1.9d LC (*K trunc.)

7. EXAMPLES

[00104] The following Examples, which highlight certain features and properties of the exemplary embodiments of the antibodies and binding fragments described herein are provided for purposes of illustration, and not limitation.

Antibodies

[00105] Test antibodies used through the examples including Ab-h1.9d-WT, positive controls and isotype controls are listed in TABLE 8.

TABLE 8 Partial List of Prepared Antibodies		
Name	Use	Description
Ab-h1.9d-WT	Exemplary Ab	Humanized anti-human $\alpha 4\beta 7$, liability engineered [hu IgG1/k, WT]
Ab-h1.9(x) series	Exemplary Ab	Humanized anti-human $\alpha 4\beta 7$, liability engineered [hu IgG1/k]
Ab-h1.(x) series	Exemplary Ab	Humanized anti-human $\alpha 4\beta 7$ [hu IgG1/k; WT]
Ab-h1	Exemplary Ab	Humanized anti-human $\alpha 4\beta 7$ [hu IgG1/k; WT]
Ab-c1	Exemplary Ab	Chimeric anti-human $\alpha 4\beta 7$ [hu IgG1/k; WT]
Ab-m1	Exemplary Ab	Mouse anti-human $\alpha 4\beta 7$ [mu IgG1/k; WT]
Ab-Vedo	Comparator	Humanized anti-human $\alpha 4\beta 7$ mAb [hu IgG1/k; LALA]; In-house version with vedolizumab (Entyvio®) variable domain
Ab-Abri	Comparator	Human anti-human $\alpha 4\beta 7$ mAb; [IgG2/k; WT]; In-house version with abrilumab (AMG181) variable domain
Ab-Etro	Comparator	Humanized anti-human $\beta 7$ mAb [hu IgG1/k; WT]; In-house version with etrolizumab (RG7413) variable domain
Ab-Nata	Comparator	Humanized anti-human $\alpha 4$ mAb [hu IgG4/k; WT] Natalizumab (Tysabri®)
Ab-Ritu	Comparator	Chimeric anti-human CD20 mAb [hu IgG1/k; WT] Rituximab (Rituxan®)
Ab-Tras	Comparator	Anti-human HER2 mAb Trastuzumab (HERCEPTIN®)
Ab-Ctet	Isotype control	Anti-tetanus toxoid (<i>Clostridium tetani</i>) mAb [hu IgG1/k; WT]
Ab-CMV	Isotype control	Anti-CMV gH MSL109 mAb [hu IgG1/k; WT]
Ab-Alem	ADCC control	Anti-human CD52 mAb (Campath-1H) [hu IgG1/k; WT]

Statistics

[00106] Half maximal inhibitory concentration (IC₅₀) and half maximal effective concentration (EC₅₀) values were determined by non-linear regression analysis of the concentration response curves using GraphPad Prism . Significance of comparisons were determined by Mann-Whitney two-tailed test.

All values were the average or standard deviation of results of at least three independent experiments, excepted noted.

7.1. Example 1: Generation of Anti-Human $\alpha 4\beta 7$ Antibodies

7.1.1. Hybridoma screening

[00107] Hybridoma-based techniques were utilized to generate an initial panel of mouse anti-human $\alpha 4\beta 7$ antibodies. Mice were immunized with HEK293, CHO-K1 or BaF3 recombinant cells expressing human $\alpha 4\beta 7$ in addition to adjuvant. The selection of candidate hybridoma derived antibodies was based on criteria of TABLE 9:

TABLE 9 Candidate Selection Criteria	
Binding	Bind to HuT78 cell, a human T lymphoma cell line expressing endogenous cell surface $\alpha 4\beta 7$
	Bind to human and cyno CD4+ and CD8+ T cells
Potency	Block the adhesion of HuT78 cells to plate-bound human MAdCAM-1-Fc protein
	Block the binding of MAdCAM-1-Fc to human and cyno CD4+ central memory T cells
Binding specificity	Bind to recombinant cells expressing human $\alpha 4\beta 7$
	Do not bind to human $\alpha 4\beta 1$
	Bind minimally or not at all to recombinant cells expressing human and cyno $\alpha E\beta 7$
Species cross-reactivity	Bind to recombinant cells expressing cyno $\alpha 4\beta 7$
	Bind to recombinant cells expressing rat and mouse $\alpha 4\beta 7$

[00108] A panel of functional hybridoma mAbs was identified from this screen, with all candidates displaying a favorable profile. However, none had rodent cross-reactivity, which may be explained by 1) human $\alpha 4\beta 7$ having 96%/97% amino acid sequence homology to cyno monkey $\alpha 4\beta 7$ but only 84%/85% sequence homology to rat and mouse $\alpha 4\beta 7$; and 2) all the selected functional hybridoma mAbs are highly selective for $\alpha 4\beta 7$, and may bind to conformational epitope(s) on the $\alpha 4\beta 7$ heterodimer.

7.1.2. Antibody Characterization

[00109] Small scale of antibody production by 200-300 ml roller bottle culture and Protein A affinity purification was performed on subcloned stable hybridoma cell lines. Purity of mAbs was verified by SDS-PAGE and mass spectrometry. Purified antibodies were characterized through binding and functional assays to determine isoform and species cross-reactivity.

Binding screening

[00110] Purified antibodies were characterized for isoform and species cross-reactivity by FACS with BaF3-h $\alpha 4\beta 7$, BaF3-h $\alpha E\beta 7$, BaF3-c $\alpha E\beta 7$, BaF3, CHOK1-c $\alpha 4\beta 7$, CHOK1-m $\alpha 4\beta 7$ and CHOK1-h $\alpha 4\beta 1$

cell lines at a concentration of 1 µg/ml. The FACS profiles, including that of exemplary mAb Ab-m1, is summarized in TABLE 10.

TABLE 10 Isoform and Species Cross-Reactivity								
Antibody	Isotype	FACS MFI						
		BaF3	BaF3- α 4 β 7	BaF3- α E β 7	BaF3- α E β 7	CHOK1- α 4 β 7	CHOK1- α 4 β 7	CHOK1- α 4 β 1
Ab-m1	IgG1/k	3.97	453.71	17.35	54.31	45.14	3.6	3.72
Ab-Vedo	hIgG1/k	3.53	706.72	11.60	57.21	100.88	2.91	4.23
Ab-Abri	hIgG2/k	3.54	623.52	9.81	68.35	89.85	2.87	4.25
Ab-Etro	hIgG1/k	5.78	439.75	73.21	247.12	97.21	92.16	4.53

Functional validation

[00111] Purified antibody was characterized by adhesion assay to MadCAM-1 in HuT78 cells. Ab-m1 showed potential blocking potency in the MadCAM-1 assay and was therefore functionally validated. Data from three independent experiments for Ab-m1 is summarized in TABLE 11. Representative data is shown in FIG. 1.

TABLE 11 Functional Assays				
Antibody	Hut78 cell adhesion assay		CHOK1- α 4 β 7 adhesion assay	
	IC50/MadCAM-1 (pM)	Max inhibition (%)	IC50/MadCAM-1 (pM)	Max inhibition (%)
Ab-m1	55.5±6.3	88.9±3.0	201.1±51.2	84.6±15.6
Ab-Vedo	94.9±4.5	86.5±13.5	562.3±249.5	76.0±12.6
Ab-Abri	12.87	103.28	99.40	95.94
Ab-Etro	17.83	103.5	241.5±38.6	91.5±7.0

Functional characterization

[00112] Further functional characterization of Ab-m1 was performed using an adhesion assay to MadCAM-1 in CHOK1- α 4 β 7, to check for cyno cross-reactivity. The adhesion assay data in CHOK1- α 4 β 7 is summarized in TABLE 11 above. Representative data is shown in FIG. 2.

[00113] To determine the affinity of Ab-m1 binding to human α 4 β 7, FACS-based titrations were performed in BaF3- α 4 β 7. EC50 was determined, as summarized in TABLE 12.

TABLE 12 Affinity to Human α 4 β 7			
Antibody	Isotype	BaF3- α 4 β 7 FACS titration	
		EC50 (pM)	Max MFI
Ab-m1	IgG1/k	474	255.6

TABLE 12 Affinity to Human $\alpha 4\beta 7$			
Ab-Vedo	hIgG1/k	713.2 $\pm$ 100.9	848.0 $\pm$ 289.4
Ab-Abri	hIgG2/k	679	775.3
Ab-Etro	hIgG1/k	704.8 $\pm$ 66.8	950.7 $\pm$ 202.0

[00114] Epitope binning was performed by competitive FACS using comparator antibodies conjugated with Alexa488 fluorophore in the presence of 50x excess of non-labeled Abs indicated in the TABLE 13. Ab-m1 was characterized as belonging to a vedolizumab (Ab-Vedo) like group based on the percentage of binding inhibition of Alexa488-labeled Abs by the non-labeled Abs (TABLE 13).

TABLE 13 Epitope Binning				
Antibody	Ab-m1	Ab-Vedo	Ab-Abri	Ab-Etro
Ab-Vedo-Alexa488	76	88	89	18
Ab-Abri-Alexa488	73	88	89	80
Ab-Etro-Alexa488	-1	6	60	93
IC50(pM) HuT78	44.7 $\pm$ 19.3	89 $\pm$ 10.7	12.9	17.8

Candidate Profiling

[00115] The affinity of mAbs binding to human $\alpha 4\beta 7$ was confirmed in primary human CD4+memory T cells. Ab-m1 was also capable of blocking human MadCAM-1 binding to primary human CD4+ memory T cells with high potency (IC50 = 63.7 pM, max inhibition 100%; FIG. 3).

7.1.3. VH/VL sequencing, chimeric antibody generation and characterization

[00116] Hybridoma clones were recovered and expanded in complete hybridoma culture medium (DMEM with 10% FBS). Cells were harvested by centrifuging at 1000 rpm for 5 minutes at room temperature and washed twice with PBS, pH 7.4. RNA was extracted from the cell pellets (approximately 1×10^7 cells) with Trizol.

[00117] Antibody VH and VL fragments were separately amplified from their hybridoma total RNA by RT-PCR using mouse Ig primer set (Novagen, 69831-3). Positive PCR products of appropriate size were inserted to T-vector for sequencing and identification of VH and VL regions (TABLE 14). Full length antibody sequences were built using combinations of VH/VL sequences together with theoretical constant region sequences.

[00118] After primer designing, VL/VHs were individually amplified and cloned into expression vectors to make chimeric antibody constructs via homologous recombination. The amino acid sequences of CDRs in VH and VL are shown in TABLE 14.

TABLE 14				
Exemplary Murine Variable Chains				
Ab-m1	Heavy Chain			
	CDR1	CDR2	CDR3	VH
	NTYMH SEQ ID NO:12	RIDPANGHTEYAP SEQ ID NO:13	VDS SEQ ID NO:14	SEQ ID NO:10
	Light Chain			
	CDR1	CDR2	CDR3	VL
	HASQGSDNIG SEQ ID NO:15	HGTNLED SEQ ID NO:16	VQYAQFPWT SEQ ID NO:17	SEQ ID NO:11

[00119] Chimeric antibodies were produced by transient transfection in HEK293 cells. After expression, proteins were purified. A high percentage monomer for the chimeric antibodies was confirmed by SEC-HPLC.

[00120] Ab-c1 is a chimeric antibody having Ab-m1's variable regions and human IgG1/k constant regions. The affinity of Ab-c1 binding to human primary CD4⁺CD45RO⁺ T cells was determined by FACS with the binding EC₅₀ value of 3.9 pM from one representative human whole blood (shown in FIG. 4 and TABLE 15). The ability of Ab-c1 to block $\alpha 4\beta 7$ integrin binding to MadCAM-1 was also evaluated in HuT78 cells and CHOK1- $\alpha 4\beta 7$ cells (TABLE 15).

Antibody	Primary CD4⁺ memory T cell mAb binding EC₅₀ (pM)
Ab-m1	9.8
Ab-c1	3.9
Ab-Vedo	43.2±4.5
Ab-Etro	5.4±0.2
Ab-Abri	4.9±1.6

[00121] Purified chimeric antibody Ab-c1 was further characterized by FACS with BaF3- $\alpha 4\beta 7$, BaF3- $\alpha E\beta 7$, BaF3- $\alpha E\beta 7$, BaF3, CHOK1- $\alpha 4\beta 7$ and CHOK1- $\alpha 4\beta 1$ cell lines at one concentration (1 μ g/ml) to confirm its binding specificity and cynomolgus cross-reactivity. The FACS profile of tested chimera is summarized in TABLE 16.

TABLE 16 FACS Profiles							
Antibody code	Isotype	FACS MFI					
		BaF3	BaF3- $\alpha 4\beta 7$	BaF3- $\alpha E\beta 7$	BaF3- $\alpha E\beta 7$	CHOK1- $\alpha 4\beta 7$	CHOK1- $\alpha 4\beta 1$
Ab-m1	mIgG1/k	1.37	153.69	7.35	38.09	34.36	2.96
Ab-c1	hIgG1/k	2.85	357.77	20.65	67.81	49.40	2.95
Ab-Vedo	hIgG1/k	2.78	153.43	19.5	60.87	46.47	2.87

[00122] HuT78 was used to investigate whether chimeric Ab-c1 was capable of blocking MadCAM-1 mediated adhesion to $\alpha 4\beta 7$ integrin. As shown in TABLE 17, Ab-c1 demonstrates the capacity to inhibit HuT78 cell adhesion to MadCAM-1.

[00123] CHOK1- $\alpha 4\beta 7$ was used to investigate whether chimeric antibodies could inhibit MadCAM-1 mediated adhesion to $\alpha 4\beta 7$. As shown in TABLE 17, the Ab-c1 demonstrates the capacity to inhibit CHOK1- $\alpha 4\beta 7$ cell adhesion to MadCAM-1.

TABLE 17 Adhesion Assays				
Antibody code	MadCAM-1/HuT78 ($\alpha 4\beta 7$)		MadCAM-1/CHOK1 ($\alpha 4\beta 7$)	
	IC50 (pM)	Max Inhibition (%)	IC50 (pM)	Max Inhibition (%)
Ab-m1	22.8	99.44%	65.8	92.78%
Ab-c1	13.7	98.06%	86.4	85.54%
Ab-Vedo	57.4	94.67%	540.8 $\pm$ 336.5	94.41%

7.1.4. Humanization

[00124] Antibody Ab-m1 was selected based on binding and potency on both HuT78 cells and human primary CD4⁺ T memory cells, cyno cross-reactivity, CDR sequence diversity, binding selectivity, sequence liability (i.e. glycosylation), and epitope group. See TABLE 18 for specified characteristics.

TABLE 18 Ab-m1 Characterization					
Antibody	MadCAM-1/HuT78 Potency (pM)	MadCAM-1/ $\alpha 4\beta 7$ Potency (pM)	Cyno x-reactivity	Human CD4 ⁺ CD45RO ⁺	
				binding EC50 (pM)	MadCAM-1 inh. IC50 (pM)
Ab-m1	55.5 $\pm$ 6.3	201.1 $\pm$ 51.2	4x	9.8	63.7

[00125] Humanized antibodies were designed from assembling VH and VL fragments in accordance with TABLE 19, and incorporating human IgG1 and kappa constant regions.

Table 19 VH and VL Chains of Humanized Ab-h1			
Rodent Parental	Humanized Ab	VH	VL
Ab-m1	Ab-h1.1	Ab-h1VH.1 (SEQ ID NO:20)	Ab-h1VL.1 (SEQ ID NO:25)
	Ab-h1.2	Ab-h1VH.1 (SEQ ID NO:20)	Ab-h1VL.1a (SEQ ID NO:27)
	Ab-h1.3	Ab-h1VH.1 (SEQ ID NO:20)	Ab-h1VL.1b (SEQ ID NO:28)
	Ab-h1.4	Ab-h1VH.1a (SEQ ID NO:22)	Ab-h1VL.1 (SEQ ID NO:25)
	Ab-h1.5	Ab-h1VH.1a (SEQ ID NO:22)	Ab-h1VL.1a (SEQ ID NO:27)
	Ab-h1.6	Ab-h1VH.1a (SEQ ID NO:22)	Ab-h1VL.1b (SEQ ID NO:28)
	Ab-h1.7	Ab-h1VH.1b (SEQ ID NO:23)	Ab-h1VL.1 (SEQ ID NO:25)
	Ab-h1.8	Ab-h1VH.1b (SEQ ID NO:23)	Ab-h1VL.1a (SEQ ID NO:27)
	Ab-h1.9	Ab-h1VH.1b (SEQ ID NO:23)	Ab-h1VL.1b (SEQ ID NO:28)

[00126] These humanized anti- $\alpha 4\beta 7$ mAbs were produced, having good expression, showing >95% monomers by SEC. Identity was confirmed by MS, and stability of the humanized anti- $\alpha 4\beta 7$ was measured by DSC. Four of the humanized Ab-h1 were evaluated for their ability to block MAdCAM-1 mediated HuT78 cell adhesion and they all displayed strong potency with IC50 values of single digit or low double-digit pM range (TABLE 20).

TABLE 20 Humanized $\alpha 4\beta 7$ mAb properties		
Humanized Ab	HuT78 cell adhesion potency IC50 (pM)	Cyno X-reactivity CHOK1/ $\alpha 4\beta 7$ adhesion to MAdCAM-1 IC50 (pM)
Ab-h1.5	9.1	
Ab-h1.6	8.1	
Ab-h1.8	13.8	
Ab-h1.9	11.4	36.5
Ab-Vedo	31; 60	120
Ab-Abri		50.9
Ab-Etro		49.9

7.1.5. Liability Engineering of Ab-h1.9

[00127] Antibody Ab-h1.9 was selected for generating variant VH and VL chains having reduced chemical liability with respect to deamidation, isomerization, oxidation, glycosylation, hydrolysis and cleavage.

The sequence of Ab-h1.9 VH is:

EVQLVQSGAEVKKPGSSVKVSCKASGFNIKNTYMHWRQAPGQGLEWIGRIDPAN
 GHTEYAPK**FQGR**VTITADESTNTAYMELSSLRSEDTAVYYCYY**VDS**WGQGTTVTV
 SS (SEQ ID NO:23)

and the sequence of Ab-h1.9 VL is:

DIQMTQSPSSLSASVGDRVITITCHASQGISDNIGWLQQKPGKSFKLLIYHGTNLEDG
VPSRFGSGSGTDYTLTISSLPEDFATYYCVQYAQFPWTFGGGKVEIKR
(SEQ ID NO:28)

wherein selected liability mutation sites are underlined and CDRs bolded. Liabilities are present in VH-CDR2, VH-CDR3, and VL-CDR1.

[00128] Two rounds of liability free anti- $\alpha 4\beta 7$ clone selection using biotinylated human $\alpha 4\beta 7$ extracellular domain protein were performed. Colonies from each library were sequenced, and those having additional liabilities in any CDR were removed. Clones from each library were screened for binding to surface $\alpha 4\beta 7$ antigen on yeast by FACS, in comparison to parental Ab-h1.9.

[00129] Liability-engineered clones that were identified as binding similarly as parental (FIG. 5) are presented in TABLE 21. These liability-engineered clones along with their variable regions were converted to IgG format.

TABLE 21 Sequences of Selected Clones from Liability Engineering of Ab-H1.9				
Antibody	Heavy Chain			
	CDR1	CDR2	CDR3	VH
Ab-H1.9 (parental)	GFNIKNTYMH SEQ ID NO:32	RIDPANGHTEYAPKFQG SEQ ID NO:33	VDS SEQ ID NO:34	SEQ ID NO:30
Ab-h1.9a	GFNIKNTYMH SEQ ID NO:42	RIDPAN <u>K</u> HTEYAPKF <u>L</u> G SEQ ID NO:43	V <u>A</u> S SEQ ID NO:44	SEQ ID NO:40
Ab-h1.9b	GFNIKNTYMH SEQ ID NO:52	RIDPA <u>R</u> GHTTEYAPKF <u>S</u> G SEQ ID NO:53	VD <u>Q</u> SEQ ID NO:54	SEQ ID NO:50
Ab-h1.9c	GFNIKNTYMH SEQ ID NO:62	RIDPA <u>R</u> GHTTEYAPKF <u>E</u> G SEQ ID NO:63	V <u>A</u> S SEQ ID NO:64	SEQ ID NO:60
Ab-h1.9d	GFNIKNTYMH SEQ ID NO:72	RIDPA <u>K</u> GHTTEYAPKF <u>L</u> G SEQ ID NO:73	VD <u>V</u> SEQ ID NO:74	SEQ ID NO:70
Ab-h1.9e	GFNIKNTYMH SEQ ID NO:82	RIDPA <u>G</u> GHTTEYAPKF <u>I</u> G SEQ ID NO:83	V <u>A</u> S SEQ ID NO:84	SEQ ID NO:80
	Light Chain			
	CDR1	CDR2	CDR3	VL
Ab-H1.9 (parental)	HASQGISDNIG SEQ ID NO:35	HGTNLED SEQ ID NO:36	VQYAQFPWT SEQ ID NO:37	SEQ ID NO:31
Ab-h1.9a	HASQ <u>E</u> ISDNIG SEQ ID NO:45	HGTNLED SEQ ID NO:46	VQYAQFPWT SEQ ID NO:47	SEQ ID NO:41
Ab-h1.9b	HASQ <u>D</u> ISDNIG SEQ ID NO:55	HGTNLED SEQ ID NO:56	VQYAQFPWT SEQ ID NO:57	SEQ ID NO:51
Ab-h1.9c	HASQ <u>D</u> ISDNIG	HGTNLED	VQYAQFPWT	SEQ ID NO:61

TABLE 21 Sequences of Selected Clones from Liability Engineering of Ab-H1.9				
	SEQ ID NO:65	SEQ ID NO:66	SEQ ID NO:67	
Ab-h1.9d	HASQD I SDNIG SEQ ID NO:75	HGTNLED SEQ ID NO:76	VQYAQFPWT SEQ ID NO:77	SEQ ID NO:71
Ab-h1.9e	HASQ E ISDNIG SEQ ID NO:85	HGTNLED SEQ ID NO:86	VQYAQFPWT SEQ ID NO:87	SEQ ID NO:81

[00130] Liability-engineered mAbs Ab-h1.9a through Ab-h1.9e were tested for binding to $\alpha\beta 7$ -expressing HuT78 cells by FACS. These antibodies retained binding activity, having EC₅₀ values ranging from 253 pM to 702 pM. This retention of $\alpha\beta 7$ binding was surprising, given that a large portion of VH-CDR3 (e.g., one third of the amino acid residues in the CDR) was mutated in the liability-engineered mAbs. Ab-h1.9d showed the strongest binding affinity (253 pM) and exhibited good drug-like properties.

[00131] Protein production properties of antibodies Ab-h1.9(a)-(e) in human IgG1 Fc LALA format were also tested, and Ab-h1.9d demonstrated superior properties.

7.1.6. Antibodies

[00132] Exemplary Ab-h1.9d derived IgG1 antibody conversions are shown in TABLE 22.

Ab-h1.9d-HuIgG1 is human IgG1/kappa type antibody having a HC of SEQ ID NO:90 featuring a canonical human heavy chain constant region, and a LC of SEQ ID NO:100 featuring a canonical human kappa light chain constant region. Ab-h1.9d-HuIgG1 may also have a C-terminal lysine truncated HC of SEQ ID NO:91. Ab-h1.9d-WT is an IgG1/kappa type antibody having a HC of SEQ ID NO:92 featuring variant CH3 substitutions D356E and L358M, and a LC of SEQ ID NO:100. Ab-h1.9d-WT may also have a C-terminal lysine truncated HC of SEQ ID NO:93. Ab-h1.9d-LALA is an IgG1/kappa type antibody having a HC of SEQ ID NO:94 featuring variant CH2 substitutions L234A and L235A, and variant CH3 substitutions D356E and L358M, and a LC of SEQ ID NO:100. Ab-h1.9d-LALA may also have a C-terminal lysine truncated HC of SEQ ID NO:95. Ab-h1.9d-QL is an IgG1/kappa type antibody having a HC of SEQ ID NO:96 featuring variant CH2 substitution T250Q, and variant CH3 substitutions D356E, L358M, and M428L, and a LC of SEQ ID NO:100. Ab-h1.9d-QL may also have a C-terminal lysine truncated HC of SEQ ID NO:97. Ab-h1.9d-LALA/QL is an IgG1/kappa type antibody having a HC of SEQ ID NO:98 featuring variant CH2 substitution T250Q, and variant CH3 substitutions L234A,

L235A, D356E, L358M, and M428L, and a LC of SEQ ID NO:100. Ab-h1.9d-LALA/QL may also have a C-terminal lysine truncated HC of SEQ ID NO:99.

TABLE 22
Exemplary Selected Antibodies

Antibody	Features	HC	LC (kappa)
Ab-h1.9d-hIgG1	IgG1/k	90 or 91	100
Ab-h1.9d-WT	IgG1/k with D356E and L358M	92 or 93	100
Ab-h1.9d-LALA	IgG1/k with L234A, L235A, D356E, and L358M	94 or 95	100
Ab-h1.9d-QL	IgG1/k with T250Q, D356E, L358M, and M428L	96 or 97	100
Ab-h1.9d-LALA/QL	IgG1/k with L234A, L235A, T250Q, D356E, L358M, and M428L M428L	98 or 99	100

EVQLVQSGAEVKKPGSSVKVCKASGFNIKNTYMHWRQAPGGGLEWIGRIDPAKGHTEYAPKFLGRVTITADESTNTAYMELSSLRSEDAVYYCYVVDVWGQGTITVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTYICNVN HKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRV VSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSD GSFFLYSKLTVDKSRWQQGNVFSCSVMEALHNHYTQKSLSLSPGK (SEQ ID NO:90)

EVQLVQSGAEVKKPGSSVKVCKASGFNIKNTYMHWRQAPGGGLEWIGRIDPAKGHTEYAPKFLGRVTITADESTNTAYMELSSLRSEDAVYYCYVVDVWGQGTITVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTYICNVN HKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRV VSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSD GSFFLYSKLTVDKSRWQQGNVFSCSVMEALHNHYTQKSLSLSPGK (SEQ ID NO:91)

EVQLVQSGAEVKKPGSSVKVCKASGFNIKNTYMHWRQAPGGGLEWIGRIDPAKGHTEYAPKFLGRVTITADESTNTAYMELSSLRSEDAVYYCYVVDVWGQGTITVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTYICNVN HKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRV VSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSD GSFFLYSKLTVDKSRWQQGNVFSCSVMEALHNHYTQKSLSLSPGK (SEQ ID NO:92)

EVQLVQSGAEVKKPGSSVKVCKASGFNIKNTYMHWRQAPGGGLEWIGRIDPAKGHTEYAPKFLGRVTITADESTNTAYMELSSLRSEDAVYYCYVVDVWGQGTITVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTYICNVN HKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRV VSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSD GSFFLYSKLTVDKSRWQQGNVFSCSVMEALHNHYTQKSLSLSPGK (SEQ ID NO:93)

EVQLVQSGAEVKKPGSSVKVCKASGFNIKNTYMHWRQAPGGGLEWIGRIDPAKGHTEYAPKFLGRVTITADESTNTAYMELSSLRSEDAVYYCYVVDVWGQGTITVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTYICNVN HKPSNTKVDKKVEPKSCDKTHTCPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRV VSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSD GSFFLYSKLTVDKSRWQQGNVFSCSVMEALHNHYTQKSLSLSPGK (SEQ ID NO:94)

EVQLVQSGAEVKKPGSSVKVCKASGFNIKNTYMHWRQAPGGGLEWIGRIDPAKGHTEYAPKFLGRVTITADESTNTAYMELSSLRSEDAVYYCYVVDVWGQGTITVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTYICNVN HKPSNTKVDKKVEPKSCDKTHTCPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRV VSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSD GSFFLYSKLTVDKSRWQQGNVFSCSVMEALHNHYTQKSLSLSPGK (SEQ ID NO:95)

EVQLVQSGAEVKKPGSSVKVCKASGFNIKNTYMHWRQAPGGGLEWIGRIDPAKGHTEYAPKFLGRVTITADESTNTAYMELSSLRSEDAVYYCYVVDVWGQGTITVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTYICNVN HKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRV VSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSD GSFFLYSKLTVDKSRWQQGNVFSCSVLMEALHNHYTQKSLSLSPGK (SEQ ID NO:96)

EVQLVQSGAEVKKPGSSVKVCKASGFNIKNTYMHWRQAPGGGLEWIGRIDPAKGHTEYAPKFLGRVTITADESTNTAYMELSSLRSEDAVYYCYVVDVWGQGTITVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTYICNVN

TABLE 22 Exemplary Selected Antibodies			
Antibody	Features	HC	LC (kappa)
HKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDQLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRV VSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSD GSFFLYSKLTVDKSRWQQGNVFCFCSVLHEALHNHYTQKSLSLSPG (SEQ ID NO:97)			
EVQLVQSGAEVKKPGSSVKVSKASGFNIKNTYMHWRQAPGGLEWIGRIDPAKGHTEYAPKFLGRVTITADESTNTAYMELSSLRSEDTAVYYCYVY DVWGQGTTTVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSVVTVPSSSLGTQTYICNVN HKPSNTKVDKKVEPKSCDKTHTCPPCPAPEAAGGPSVFLFPPKPKDQLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRV VSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSD GSFFLYSKLTVDKSRWQQGNVFCFCSVLHEALHNHYTQKSLSLSPG (SEQ ID NO:98)			
EVQLVQSGAEVKKPGSSVKVSKASGFNIKNTYMHWRQAPGGLEWIGRIDPAKGHTEYAPKFLGRVTITADESTNTAYMELSSLRSEDTAVYYCYVY DVWGQGTTTVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSVVTVPSSSLGTQTYICNVN HKPSNTKVDKKVEPKSCDKTHTCPPCPAPEAAGGPSVFLFPPKPKDQLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRV VSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSD GSFFLYSKLTVDKSRWQQGNVFCFCSVLHEALHNHYTQKSLSLSPG (SEQ ID NO:99)			
DIQMTQSPSSLSASVGDRVTITCHASQDISNIGWQQKPGKSKFLIYHGTNLEDGVPVSFSGSGSGTDYTLTISSLQPEDFATYYCVQYAFPTWFG GGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSYSTLSSTLTLSKADYEKHKVYACEVTH GGLSSPVTKSFNRGEC (SEQ ID NO:100)			

[00133] After production, Ab-h1.9d-WT showed superior properties for an anti-human $\alpha 4\beta 7$ antibody, demonstrating high specificity (low binding to $\alpha E\beta 7$), and desirable PK/PD (low ADA titers and high plasma exposure).

7.2. Example 2: $\alpha 4\beta 7$ Expression on CD4+ and CD8+ T Cell Subsets from Healthy Donors or HIV+ Individuals

7.2.1. Materials and Methods

[00134] During acute HIV infection, $\alpha 4\beta 7$ expressing CD4+ T cells are preferentially infected and therefore depleted (Sivro et al., Sci Transl Med 2018). To investigate whether peripheral $\alpha 4\beta 7$ -expressing cells recover over time in HIV+ individuals receiving cART, the percentage and expression levels of $\alpha 4\beta 7$ on CD4+ and CD8+ T cell subsets from PBMCs of 10 healthy (HIV-) donors and 45 HIV+ individuals receiving cART (age: 26 to 66 years, median 42 years; duration of HIV infection: 1 to 36 years, median 12 years) were compared by flow cytometry analysis using a cocktail of antibodies for CD3, CD4, CD8, CD28, CD45RO, CCR7, and $\alpha 4\beta 7$. Both percentage (%) and expression level (MESF) of $\alpha 4\beta 7$ in CD4+ and CD8+ T cell populations were analyzed. CD4+ and CD8+ T cell subsets were defined as: CD28+CD45RO- naïve cells, CD28-CD45RO- terminal effector cells, CD28-CD45RO+ effector memory cells, CD28+CD45RO+CCR7+ central memory cells, and CD28+CD45RO+CCR7- transient memory cells.

7.2.2. Results

[00135] For both HIV- and HIV+ individuals receiving cART, the percentage of cells expressing $\alpha 4\beta 7$ is higher for naïve CD4+ or CD8+ T cells as compared with memory T cell subsets, while $\alpha 4\beta 7$ expression level (as measured by MESF) was higher on central memory, transient memory, and effector memory cells than naïve cells (FIGS. 6A-6F). Both peripheral CD4+ and CD8+ T cells expressed similar levels of $\alpha 4\beta 7$ in HIV- and HIV+ individuals (MESF measurement). This analysis demonstrated that $\alpha 4\beta 7$ expression on peripheral CD4+ or CD8+ T cells among HIV+ individuals receiving cART, though highly variable, was not different with that from the uninfected individuals. These results suggested that the $\alpha 4\beta 7$ expression in peripheral CD4+ T cells in HIV+ individuals could support the incorporation of $\alpha 4\beta 7$ into the budding HIV virions.

7.3. Example 3: HIV Virion Capture with Ab-h1.9d-WT

7.3.1. Materials and Methods

[00136] To confirm $\alpha 4\beta 7$ is present in the envelope of HIV virions, and to test if Ab-h1.9d-WT can bind to $\alpha 4\beta 7$ incorporated into the envelope of HIV virions to form immune complexes, Ab-h1.9d-WT was first tested in a virion capture assay (bead format) using laboratory-grown HIV-1 strains or samples from viremic HIV+ individuals according to a published method (Guzzo et al., Sci Immunol 2017). For laboratory-grown HIV-1 strains, a panel of six strains (BCF06, CMU08, NL4-3, RU507, YBF30, and IIIB) representing different groups, genetic subtypes, and co-receptor usage (TABLE 23), were produced in activated primary human PBMCs [e.g. activated by OKT3 antibody or phytohemagglutinin (PHA)] in the presence of retinoic acid (RA) to induce the expression of $\alpha 4\beta 7$ in the cells. Protein G-conjugated immunomagnetic beads (Dynabeads, Thermo Fisher) were armed with the appropriate antibody and then incubated with virus stock of each HIV strain (~2 ng p24 gag/reaction). After incubation, the armed beads were washed to remove unbound virus particles, and subsequently treated with Triton X-100 to lyse the captured virions for p24 gag quantification. For HIV patients' samples, the viral titer (copies/mL) of the samples were determined by the COBAS Taqman 2.0 assay. Protein G-conjugated immunomagnetic beads armed with 10 μ g of the appropriate antibody were incubated with 400 μ L of patient sera for 2 hours. Beads were then washed to remove unbound virus particles, and RNA of the bound virions was subsequently extracted using Qiagen's viral RNA extraction kit per manufacturer's instructions. Copy number of the captured virions from patients' samples was subsequently quantified by digital droplet PCR using primers and probe targeting conserved region in LTR-gag for HIV subtype B.

[00137] To determine the EC₅₀ values of antibody for capturing HIV virions, Ab-h1.9d-WT was tested in a virion capture assay modified to a 96-well plate format. Serially diluted antibody was added to a prewashed Pierce™ Protein G coated plate (Thermo Fisher). After incubation, the plates were washed to remove unbound antibodies. Viral stock of each HIV strain (approximately 2 ng p24 gag) was added to each well and incubated. Plates were washed to remove unbound viral particles, and then treated with Triton X-100 to lyse the captured virions for p24 gag quantification. HIV p24 gag was detected by high-sensitivity AlphaLISA p24 gag detection kit (Perkin Elmer).

7.3.2. Results

[00138] Ab-h1.9d-WT was able to capture virions from all of the six laboratory-grown HIV strains tested, whether they were clinical or laboratory-adapted strains, as indicated by the viral p24 gag protein in the virions captured (FIGS. 7A-7F). The amount of p24 gag protein from captured virions demonstrated a dose response to the antibody concentrations (5 and 15 nM) used in the assay. Ab-h1.9d-WT was also able to capture virions from HIV-1 patients' samples (viral input shown in FIG. 7G-1), as indicated by the higher number of HIV RNA copy number in samples reacted with Ab-h1.9d-WT compared to those with the negative control antibody (FIG. 7G-2).

[00139] Ab-h1.9d-WT was next tested in a virion capture assay modified to a 96-well plate format to determine its EC₅₀ values for capturing HIV virions. The EC₅₀ values were similar for all the viruses tested and ranged from 0.12 nM (0.019 µg/mL) to 0.25 nM (0.038 µg/mL) (TABLE 23). When tested against the same panel of viruses, Ab-Vedo was less potent than Ab-h1.9d-WT in capturing HIV virions, with EC₅₀ values ~2–3-fold higher than those of Ab-h1.9d-WT for each individual virus (TABLE 23). HIV IIIB strain could be captured by Ab-h1.9d-WT (FIG. 7F) and Ab-Vedo (data not shown) using the bead format assay, but its EC₅₀ values could not be determined by the plate format assay due to the low titer of the viral stock.

TABLE 23 HIV Virion Capture and Neutralization with Ab-h1.9d-WT In Vitro								
HIV Strain	Group	Sub-type	Co-Receptor	Virion Capture, EC ₅₀				Viral Neutralization, IC ₅₀
				Ab-h1.9d-WT		Ab-Vedo		Ab-h1.9d-WT
				nM	μg/mL	nM	μg/mL	
BCF06	O	-	X4/R5	0.18 ± 0.03	0.027 ± 0.005	0.37 ± 0.07	0.056 ± 0.010	> 50
CMU08	M	AE	X4	0.25 ± 0.02	0.038 ± 0.003	0.50 ± 0.08	0.075 ± 0.012	> 50
NL4-3	M	B	X4	0.16 ± 0.03	0.024 ± 0.005	0.34 ± 0.02	0.050 ± 0.002	> 50
RU570	M	G	R5	0.12 ± 0.06	0.019 ± 0.010	0.32 ± 0.03	0.048 ± 0.005	> 50
YBF30	N	-	R5	0.14 ± 0.06	0.021 ± 0.009	0.48 ± 0.15	0.072 ± 0.022	> 50
IIIB	M	B	X4	ND ^a	ND ^a	ND ^a	ND ^a	ND

EC₅₀ = half maximal effective concentration; IC₅₀ = half maximal inhibitory concentration; ND = not determined

a. IIIB strain could be captured by Ab-h1.9d-WT and Ab-Vedo in bead assay format, but its EC₅₀ values could not be determined in plate assay format due to the low titer of the viral stock.

[00140] Taken together, these data confirmed that α4β7 was present in virions of all laboratory-grown HIV strains and HIV-1 patients' samples tested, and demonstrated that Ab-h1.9d-WT was more potent than Ab-Vedo in binding to α4β7 on HIV virions forming immune complexes, the first of a series of steps required for the induction of the proposed "vaccination effect" for durable HIV viral control.

[00141] Ab-h1.9d-WT is a potent anti-α4β7 antibody that can bind to α4β7 on the envelope of virions of all the HIV strains and HIV-1 patients' samples tested.

7.4. Example 4: Binding of Immune complexes of Ab-h1.9d-WT and HIV virions to FcγRs

7.4.1. Materials and Methods

[00142] To test if the immune complexes formed by Ab-h1.9d-WT and HIV virions could bind to FcγRs in vitro, the appropriate antibody was first mixed with HIV NL4-3 virus (prepared in activated human PBMC in the presence of RA to induce the expression of α4β7) to form immune complexes, which were subsequently incubated with His-tagged FcγRs immobilized on nickel coated plates [for FcγRI and FcγRIIIa (V158), which have relatively higher affinity to Ab-h1.9d-WT, as shown in TABLE 32], or biotinylated FcγRs immobilized on neutravidin coated plates to increase the sensitivity of the detection [for FcγRIIa (H131 or R131) and FcγRIIIa (F158), which have relatively lower affinity to Ab-h1.9d-WT, as shown in TABLE 32]. After incubation, plates were washed to

remove immune complexes not bound to FcγRs, and then treated with lysis buffer to lyse the captured immune complexes to release viral proteins for p24 gag quantification.

7.4.2. Results

[00143] The immune complexes formed by HIV virions and Ab-h1.9d-WT, which has a WT Fc domain, were able to bind to different FcγRs as shown by the presence of HIV p24 capsid protein in the protein complex bound to the FcγRs (FIGS. 8A-8E). HIV virion by itself did not bind to the FcγRs (data not shown), nor immune complexes formed by HIV virions and Ab-h1.9d-LALA, an antibody identical to Ab-h1.9d-WT except it has engineered LALA mutations in its Fc domain to significantly reduce its binding to FcγRs (FIGS. 8A-8E). In addition, the immune complexes formed by HIV virions and Ab-Vedo demonstrated minimal binding to FcγRs (FIGS. 8A-8E). This is consistent with the significantly lower binding affinity of Ab-Vedo to different FcγRs than Ab-h1.9d-WT due to the engineered mutations in the Fc domain of Ab-Vedo. Due to the lower binding affinity of Ab-h1.9d-WT to human FcγRIIa (H131 or R131) and FcγRIIIa (F158, also known as F176) than FcγRI and FcγRIIIa (V158, also known as V176) (FIG. 19A), biotinylated FcγRIIa (H131 or R131) and FcγRIIIa (F158) were immobilized on neutravidin coated plates to enhance the capture of the immune complexes of Ab-h1.9d-WT by these FcγRs instead of using the corresponding His-tagged FcγRs immobilized on nickel plates.

[00144] The immune complexes formed by Ab-h1.9d-WT and HIV virions could bind to different FcγRs including FcγRIIa (responsible for ADCP), a step that could enable the complexes to be taken up by APCs to induce the proposed "vaccination effect" for HIV control.

7.5. Example 5: Ab-h1.9d-WT mediates Fc-dependent uptake of α4β7-coated beads in THP-1 cells

7.5.1. Materials and Methods

Cell culture

[00145] THP-1 cells were cultured at 37°C, 5% CO₂ in RPMI media (Gibco) supplemented with 10% FBS (Sigma). Cells were passaged every two to three days and maintained at a density of 500,000 – 1,000,000 cells/ml.

Bead preparation

[00146] Recombinant human $\alpha 4\beta 7$ protein (R & D systems) was dialyzed overnight at 4°C in 1x PBS using a 3.5 kDa MWCO dialysis device (Thermo Fisher Scientific Inc.). The resulting $\alpha 4\beta 7$ was then biotinylated at 100x molar excess NHS-Biotin (Thermo Fisher Scientific Inc.) for 2 h at 4°C. Excess biotin was removed by overnight dialysis at 4°C. For $\alpha 4\beta 7$ coated beads, NeutrAvidin-labeled fluorescent beads (Thermo Fisher Scientific Inc.) were incubated with biotinylated $\alpha 4\beta 7$ for 1-24 h at 4°C. Bead-protein conjugation reactions occurred at a ratio of 2 mg $\alpha 4\beta 7$ protein per 1 ml of stock beads, unless otherwise stated. Protein conjugated beads were washed twice with 1x PBS containing 1% BSA (Sigma), then diluted 100x prior to use. Successful conjugation of protein to beads was confirmed in phagocytosis assays comparing anti- $\alpha 4\beta 7$ with isotype control conditions.

Phagocytosis assay

[00147] Phagocytosis assays using protein coated beads and THP-1 cells were adapted from a previously described study (Ackerman et al 2011). Assays were performed in 96 well plates. Immune complexes were formed by combining 10 μ l of prepared $\alpha 4\beta 7$ coated beads with 10 μ l of the indicated antibody (10 μ g/ml). These were incubated for 1-2 h at 37°C, 5% CO₂. THP-1 cells (100,000/well) were then incubated with immune complexes for the indicated amount of time in a final volume of 200 μ l. Following incubation, cells were stained with a fixable live/dead stain (ThermoFisher), followed by washing with FACS buffer and fixation. Data on resulting cells were collected using a LSR Fortessa X20 from BD. Fluorescent beads and live/dead stain fluorescence were detected with the PE-CF594 and BV510 settings, respectively. Data were then analyzed using FlowJo software. Phagocytosis score was calculated as follows: **phagocytosis score** = (MFI x percent bead positive cells)/1000. **Normalization:** Data from 3 independent experiments were normalized and plotted on one graph. To normalize, one replicate in the “beads only” condition was set to 1 by dividing this value by the same value (done for each experiment). All other values were divided by this normalization value to display the normalized phagocytosis score, which represents fold change from the “beads only” condition.

Imaging Flow Cytometry

[00148] Internalization of beads were confirmed using imaging flow cytometry. Fixed cells were analyzed on ImagestreamX Mark II imaging flow cytometer (Luminex Corp.) at 40x magnification and medium sensitivity and medium speed setting. Fluorescent beads were imaged using 488 nm (5 mW)/560-595nm (excitation/emission). Live/dead stain was imaged using 405 nm (5mW)/ 430-480nm (excitation/emission). Brightfield image was collected in channel 2 (camera 1). Data was

analyzed using IDEAS analysis software v6.1 (Luminex Corp.). Standard gating strategy was used to find appropriate cell populations. Briefly, focused cells were identified using high (>40) gradient RMS (root mean square) for brightfield image sharpness. Single cells were identified by high aspect ratio and low object area of brightfield image. Cellular object area was identified in the brightfield image and 4 pixels were eroded from the cell boundary to define an 'intracellular mask'. Cells with positive fluorescence signal in the intracellular mask were identified as true internalization events. Spot count feature was used to count the number of internalized beads in cells with true internalization events. At least 2000 cells with true internalization events were analyzed per sample.

Statistical Analysis

[00149] Data were plotted using GraphPad Prism. Significance was determined using one-way ANOVA coupled to Tukey's multiple comparisons test. **** $p < 0.0001$, *** $p = 0.0001-0.001$, ** $p = 0.001-0.01$, * $p = 0.01-0.05$.

7.5.2. Results

[00150] Uptake of immune complexes by antigen presenting cells is critical to initiate downstream cellular and humoral immunity. THP-1 cells have been reported to phagocytose antibody-fluorescent bead immune complexes in an Fc/FcγR-dependent manner (Ackerman et al., 2011). Therefore, THP-1 cells were used to investigate whether Ab-h1.9d-WT mediates phagocytosis of α4β7-coated fluorescent beads. Cells treated for 3 h with α4β7-beads/Ab-h1.9d-WT antibody immune complexes displayed significant uptake of fluorescent beads relevant to Ab-h1.9d-LALA and Ab-Vedo (featuring LALA) by flow cytometry (FIGS. 9A-9B). LALA mutations are known to drastically reduce IgG Fc binding to FcγRs. These data suggest that Ab-h1.9d-WT mediates robust Fc/FcγR-dependent phagocytosis of immune complexes containing α4β7-coated fluorescent beads.

[00151] Imaging flow cytometry with the Amnis Imagestream was performed to confirm that Ab-h1.9d-WT/α4β7-bead immune complexes were internalized in THP-1 cells. As expected, fluorescent beads were localized within THP-1 cells after Ab-h1.9d-WT immune complex treatment (FIG. 9C

7.6. Example 6: Ab-h1.9d-WT-induced uptake of α4β7+GFP+VLPs/Ab immune complex in THP-1 cells is α4β7- and Fc-dependent

7.6.1. Materials and Methods

Antibody binding to α4β7-expressing GFP+ viral like particles (VLPs) by ELISA

[00152] $\alpha 4\beta 7$ + GFP+VLPs were generated from HEK293 cells sequentially transfected with $\alpha 4\beta 7$ cDNA construct and HIV gag-GFP DNA construct, followed by the purification of VLPs from the cell culture supernatants. ELISA was performed to confirm the expression of $\alpha 4\beta 7$ on the GFP+VLP surface and the binding specificity by various testing Abs. Briefly, each well of a high binding flat-bottomed 96-well plate was coated with 50 μ L of $\alpha 4\beta 7$ +GFP+VLPs at a concentration of 7.5×10^7 particles/mL in PBS and incubated overnight at 4°C. The wells were washed with PBS+1% FBS and blocked with 100 μ L of a superbloc solution for 30 min at room temperature (RT). After three washes, each well was incubated with 50 μ L of 4-fold serially diluted primary antibody in PBS+1% FBS for 1 h at RT, followed by washing and incubation with 50 μ L of an HRP-conjugated donkey anti-human IgG-Fc γ -specific secondary antibody in PBS+1% FBS for 1 h at RT. After final washes, TMB substrate was added to each well for color development, and the reaction was stopped by adding 2N H₂SO₄. The optical density (OD) for each well was measured by a plate reader at 450 nm.

Internalization of $\alpha 4\beta 7$ +GFP+VLPs/anti- $\alpha 4\beta 7$ immunocomplex in THP-1 cells

[00153] For $\alpha 4\beta 7$ +GFP+VLP uptake experiments, 5×10^4 THP-1 cells were mixed in a flat-bottomed 96-well plate without or with an antibody at the final concentration of 1 μ g/ml and $\alpha 4\beta 7$ + GFP+VLPs at the cell-to-particle ratio of 1:100 in 100 μ L volume of RPMI, 10% FBS. The plate was incubated for 16 h at 37°C in a CO₂ incubator. Cells were then resuspended and transferred to a V-bottom 96-well plate to wash once with 200 μ L of PBS, 2% FBS through centrifugation. Cell pellet was resuspended in 200 μ L of PBS, 0.5% paraformaldehyde and subjected to the determination of percent GFP+ cells by flow cytometry.

[00154] For the inhibition of VLP uptake, THP-1 cells (0.5×10^6 /mL) were pretreated with or without Latrunculin A (Lat A) at a final concentration of 240 nM or with 0.1% DMSO (control) for 2 h at 37°C in a CO₂ incubator, followed by the incubation with or without antibody and $\alpha 4\beta 7$ +GFP+VLPs, as indicated above.

7.6.2. Results

Ab-h1.9d-WT-induced uptake of $\alpha 4\beta 7$ +GFP+VLPs/Ab immune complex in THP-1 cells is $\alpha 4\beta 7$ - and Fc-dependent

[00155] Recombinant viral like particles (VLPs) generated from mammalian cells have dynamic sizes ranging from 0.1 to 0.2 microns. Thus, they are more similar in size to virions than the fluorescence-labeled beads used in the previous experiments and were utilized as a tool to model the

internalization/uptake of $\alpha 4\beta 7$ +virions/Ab immune complex by THP-1 cells. The purified $\alpha 4\beta 7$ -expressing GFP+VLPs was evaluated for their ability to bind anti- $\alpha 4\beta 7$ Abs via ELISA. As shown in FIG. 10, Ab-h1.9d-WT, Ab-h1.9d-LALA and Ab-Vedo bound to the $\alpha 4\beta 7$ +GFP+VLPs comparably with the similar binding EC50 values (0.050 nM, 0.048 nM and 0.058 nM, respectively), while an isotype control Ab (anti-CMV IgG) did not bind at all to the $\alpha 4\beta 7$ +GFP+VLPs. This data suggests that various anti- $\alpha 4\beta 7$ Abs can bind $\alpha 4\beta 7$ +GFP+VLPs with good binding affinity and specificity.

[00156] Next, the uptake of $\alpha 4\beta 7$ +GFP+VLPs by THP-1 cells was evaluated in the presence of various anti- $\alpha 4\beta 7$ Abs with or without the functional Fc. As shown in FIG. 11A, Ab-h1.9d-WT treatment induced the detection of more than 60% GFP+ THP-1 cells compared to 1.2% GFP+ cells by an isotype Ab control treatment representing a 50-fold induction of GFP+ cells. This suggests that $\alpha 4\beta 7$ +GFP+VLP uptake by THP-1 cells is Ab-h1.9d-WT-specific and depends on $\alpha 4\beta 7$ expression. However, Ab-h1.9d-LALA or Ab-Vedo (LALA) showed only minimal levels of VLP uptake in THP-1 cells, suggesting that anti- $\alpha 4\beta 7$ Ab-induced $\alpha 4\beta 7$ +GFP+VLP uptake by these cells requires functional Fc domain of the antibody to enable Fc/Fc γ R mediated internalization of immune complex, in addition to its $\alpha 4\beta 7$ -binding Fab domain.

[00157] To confirm that Ab-h1.9d-WT mediated uptake of $\alpha 4\beta 7$ +GFP+VLPs is truly due to the internalization, not merely binding to the cell surface Fc γ Rs, a known internalization inhibitor, Latrunculin A (Lat-A), was used to pre-treat THP-1 cells prior to their incubation with Ab/VLP immune complex. Shown in FIG. 11B, there was a 40% reduction of GFP+ cells in Lat A-treated cells compared to untreated cells. This suggests that at least 40% of the total GFP signals observed in untreated (mock) or DMSO-treated control resulted from Ab-h1.9d-WT dependent VLP internalization in THP-1 cells.

7.7. Example 7: Ab-h1.9d-WT Demonstrated No Neutralization Activity Against HIV

7.7.1. Materials and Methods

[00158] Some Abs that can bind to HIV virions, e.g. HIV broad neutralizing antibodies, are capable of blocking viral infection. To test if the binding of Ab-h1.9d-WT to $\alpha 4\beta 7$ on HIV virions could block HIV infection of the host cells, it was evaluated in a viral neutralization assay using the TZM-bl indicator cell line (Arrildt et al., J Virol 2015).

HIV Neutralization Assay

[00159] HIV virus (prepared with PHA and RA) corresponding to approximately 150,000 RLU (previously determined by viral titration on TZM-bl cells) was preincubated with serially diluted antibodies. TZM-bl cells containing DEAE-dextran were added to the mixture containing pre-incubated HIV and antibodies, and then incubated for 48 hours at 37°C. The cells were treated with Bright-Glo (Promega), and luciferase signal was measured.

7.7.2. Results

[00160] When tested against the panel of HIV strains shown in TABLE 23, Ab-h1.9d-WT did not demonstrate any neutralization activity at a concentration up to 50 µg/mL, whereas a HIV broad neutralizing antibody targeting the CD4+ binding site had neutralization IC₅₀ values of approximately 0.1 µg/mL against all of the Group M HIV strains tested (i.e., CMU08, NL4-3, and RU570) (data not shown).

[00161] Although Ab-h1.9d-WT binds HIV virions, it does not neutralize HIV infection, which is consistent with the notion that α4β7 is not a viral receptor on host cells. This observation supports the hypothesis that targeting α4β7 and not a virally encoded glycoprotein may avoid viral resistance mechanisms.

7.8. Example 8: Inhibition of the Interaction of α4β7 with HIV gp120 by Different Antibodies

7.8.1. Materials and Methods

[00162] The interaction between α4β7 and HIV gp120 has been reported to activate LFA-1, potentially facilitating cell-to-cell transmission of HIV (Arthos et al. Nat Immunol 2008). To test if Ab-h1.9d-WT could disrupt this interaction, an α4β7/gp120 binding assay was set up using α4β7-expressing RPMI 8866 cells binding to a HIV gp120-V2 peptide immobilized on a plate according to a published method (Peachman et al., PloS One). NeutrAvidin coated high capacity 96-well plate (Thermo Fisher) was coated with biotinylated HIV gp120-V2 WT or gp120-V2 control peptide (TABLE 24). The sequences of the two biotinylated peptides were identical except four amino acids reported to mediate binding between α4β7 and gp120 were mutated in the control peptide.

[00163]

TABLE 24 Biotinylated HIV gp120-V2 Peptides	
Peptide	Amino Acid Sequence*
gp120-V2 WT	Biotin-Ttds-CSFNMTTEL RDKKQKVHALFYK L DIVPIEDNTSSSEYRLINC-NH ₂ (SEQ ID NO:7)
gp120-V2 control	Biotin-Ttds-CSFNMTTEL RDKKQKVHALFYK AAA PIEDNTSSSEYRLINC-NH ₂ (SEQ ID NO:8)

* Amino acids different between the two peptides are shown in bold.

[00164] Before adding the antibody-cell mixture, the peptide-coated plates were washed to remove the unbound peptides. To generate the antibody-cell mixture stock, RPMI 8866 cells (8×10^6 cells/mL) were resuspended in a cold blocking buffer supplemented with MnCl_2 at a final concentration of 2 mM to activate the conformation of $\alpha 4\beta 7$. Serially diluted Ab-h1.9d-WT, Ab-Vedo, or isotype negative control antibody was mixed with the MnCl_2 -treated cells, and the mixture was incubated. The antibody-cell mixture (2×10^5 cells in 50 μL per well) was added to each well of the plate coated with the gp120 peptides and incubated. The plate was washed, and viable cells attached to the peptide-coated plate were determined by CellTiter-Glo 2.0 reagent (Promega).

7.8.2. Results

[00165] The specificity of this assay was demonstrated by the binding of $\alpha 4\beta 7$ -expressing RPMI 8866 cells to a HIV gp120-V2 WT peptide immobilized on a plate, but not to an immobilized gp120-V2 control peptide (FIG. 12A) which harbored mutations at 4 amino acids reported to mediate the binding of gp120 to $\alpha 4\beta 7$ (TABLE 24). When tested in this assay format, Ab-h1.9d-WT blocked the binding of RPMI 8866 cells to the HIV gp120-V2 WT peptide with an IC_{50} value of 0.022 ± 0.016 $\mu\text{g/mL}$ (FIG. 12B). Ab-Vedo had an IC_{50} value (0.042 ± 0.023 $\mu\text{g/mL}$) higher than that of Ab-h1.9d-WT when tested in the same assay, indicating that it was less potent than Ab-h1.9d-WT in blocking this interaction (FIG 12B). Taken together, these results indicate that Ab-h1.9d-WT can effectively disrupt the interaction of $\alpha 4\beta 7$ with gp120, potentially inhibiting the cell-to-cell viral spread.

[00166] When tested in this assay format, Ab-h1.9d-WT blocked the binding of RPMI 8866 cells to the HIV gp120-V2 WT peptide with an IC_{50} value of 0.022 ± 0.016 $\mu\text{g/mL}$ (FIG. 12B). These results indicate that Ab-h1.9d-WT can effectively disrupt the interaction of $\alpha 4\beta 7$ with gp120, potentially inhibiting the cell-to-cell viral spread.

7.9. Example 9: Binding of Ab-h1.9d-WT to Human and Cynomolgus Monkey Cells Expressing $\alpha 4\beta 7$ Integrin

7.9.1. Materials and Methods

[00167] The binding of Ab-h1.9d-WT was evaluated on HuT78 cells (human T lymphoma cells expressing endogenous $\alpha 4\beta 7$) using ECL binding assay and on human and cynomolgus monkey peripheral lymphocytes using flow cytometry.

[00168] Additionally, the binding EC_{50} values of Ab-h1.9d-WT were determined and compared for both human and cynomolgus blood-derived total, naive, and memory CD4+ and CD8+ T cells.

ECL Cell Binding Assay

[00169] HuT78 cells expressing endogenous $\alpha 4\beta 7$ were cultured in IMDM media containing 20% FBS, Penicillin (50 units/mL)/Streptomycin (50 μ g/mL). HuT78 cells were harvested, washed 1x and resuspended in DPBS at 1.5×10^6 cells/mL. Cells (7.5×10^4 in 50 μ L) were added to each well of MSD high binding plate(s). Fetal bovine serum at 6.7% (diluted in DPBS) was added and plate(s) were incubated at 37°C for one hour. Supernatant was removed and 25 μ L of titrated Ab-h1.9d-WT antibody or isotype control prepared through 1:4 fold 8-point dilutions ranging from 1.5 μ g/mL to 0.000091 μ g/mL (in DPBS buffer containing 5% FBS, and 1mM $MnCl_2$) were added to each well and then plates were incubated at 37°C for one hour. Plates were washed 2x with DPBS and 25 μ L of goat anti-human Ab sulfo tag at a 1:500 dilution in 5% FBS/DPBS/1 mM $MnCl_2$ was added to each well, followed by incubation at 37°C for 30 minutes. Cells were washed twice with DPBS and then 150 μ L of 2x MSD read buffer T was added to each well. Plate(s) were read on Sector Imager 6000 reader and binding curves and binding EC_{50} values were generated using GraphPad Prism 7.0 software.

Human and Cynomolgus Monkey Peripheral T Cell Binding Assay

[00170] Frozen human or cynomolgus PBMCs (isolated from blood donors using standard Ficoll Paque isolation method) were thawed in RPMI1640/10% FBS media, washed 1x with FACS buffer (DPBS, w/o Ca^{+2}/Mg^{+2} , 1% BSA) and resuspended in FACS buffer containing 5% goat serum. Cells at $\sim 1-2 \times 10^5$ (in 100 μ L) were added to a 96-well U-bottom plate and incubated on ice for 30 minutes. Plate was centrifuged and supernatants were removed. Titrated Ab-h1.9d-WT or isotype control (25 μ L) prepared through 1:5 fold dilution with final concentrations ranging from 5 to 0.000016 μ g/mL diluted in FACS buffer containing 5% goat serum and fluorochrome-labeled

antibody cocktail (25 μ L) were added to each well and then plate was incubated on ice for one hour. At the same time, compensation and FMO controls were also prepared. Following incubation, cells were centrifuged and washed 2x with FACS buffer. Secondary antibody (PE-conjugated) was diluted 1:2,000 in FACS buffer containing 5% goat serum and 50 μ L was added to each well. Plate was incubated on ice for one hour. Following incubation, cells were washed 2x with FACS buffer, resuspended in 200 μ L of 0.5% PFA in PBS. The plate was read on FACS (Canto II, BD) and live cells were gated based on forward and side scatters. Flow data (FCS 3.0 files) was analyzed using FlowJo Version 10 software and binding curves and binding EC₅₀ values were generated using GraphPad Prism 7.0 software.

7.9.2. Results

ECL Cell Binding

[00171] As summarized in TABLE 25, Ab-h1.9d-WT displayed binding EC₅₀ values of 26 pM, 130 pM, 62 pM for HuT78 cells, human and cynomolgous monkey blood derived lymphocytes, respectively.

TABLE 25 Binding of Ab-h1.9d-WT to HuT78 Cells and Lymphocytes		
*EC ₅₀ (pM) (Mean $\pm$ SD)		
ECL	FACS	
HuT78	Human Lymphocytes	Cynomolgus lymphocytes
26 $\pm$ 2	130 $\pm$ 85	62 $\pm$ 46

* Average $\pm$ SD; N = 3 (Human) or N = 5 (Cynomolgus)

Human and Cynomolgus Monkey Peripheral T Cell Binding Assay

[00172] The binding EC₅₀ values of Ab-h1.9d-WT for both human and cynomolgus blood-derived total, naïve, and memory CD4⁺ and CD8⁺ T cells are shown in TABLE 26.

TABLE 26 Binding EC ₅₀ of Ab-h1.9d-WT to Human and Cynomolgus Monkey T Cell Subsets						
EC ₅₀ (pM) (Mean $\pm$ SD)						
Species	Total CD4 ⁺ T Cells	CD4 ⁺ Naïve T Cells	CD4 ⁺ Memory T Cells	Total CD8 ⁺ T Cells	CD8 ⁺ Naïve T Cells	CD8 ⁺ Memory T Cells
Human ^a	63 $\pm$ 38	84 $\pm$ 32	20 $\pm$ 12	165 $\pm$ 121	144 $\pm$ 120	52 $\pm$ 51
Cyno monkey ^b	30 $\pm$ 16	45 $\pm$ 27	10 $\pm$ 12	35 $\pm$ 25	42 $\pm$ 26	36 $\pm$ 30

CD4⁺ naïve = CD4⁺CD45RA⁺CCR7⁺ cells, CD4⁺ memory = CD4⁺CD45RA⁺ cells
CD8⁺ naïve = CD8⁺CD45RA⁺CCR7⁺ cells, CD8⁺ memory = CD8⁺CD45RA⁺ cells

- a. Human PBMC = 3 donors
- b. Cynomolgus PBMC = 5 donors

[00173] The average binding EC_{50} values range from 10 to 165 pM on all the T cell subsets evaluated. Ab-h1.9d-WT binds very similarly to human and cynomolgus CD4⁺ and CD8⁺ T cells or their subsets, since the mean EC_{50} values for each corresponding cell-type vary only by 2- to 3-fold between these two species.

[00174] The binding of Ab-h1.9d-WT to human and cynomolgus monkey CD4⁺ and CD8⁺ T subsets was also analyzed to compare percentage of Ab-h1.9d-WT bound T cell subsets, shown in FIG. 13. Overall, the percentage of Ab-h1.9d-WT bound cells in each T cell subset are comparable between human and cynomolgus monkey. These findings were not surprising due to the fact that both $\alpha 4$ and $\beta 7$ from human and cynomolgus are highly homologous (97% amino acid identity, see TABLE 27).

TABLE 27 Amino Acid Identity (%) of $\alpha 4$ and $\beta 7$ Amongst Species				
Integrin	Cynomolgus Monkey	Rabbit	Rat	Mouse
Human $\alpha 4$	97	89	84	84
Human $\beta 7$	97	88	86	86

[00175] Furthermore, functional cross-reactivity of Ab-h1.9d-WT to cynomolgus monkey was confirmed in an in vivo study. Repeated dosing of Ab-h1.9d-WT in cynomolgus monkeys resulted in increased peripheral blood CD4⁺ T cell counts when the $\alpha 4\beta 7$ receptors were fully occupied. These data demonstrated the on-target functional pharmacodynamic effect of Ab-h1.9d-WT and supported cynomolgus monkey as a pharmacologically relevant species for toxicological evaluation of Ab-h1.9d-WT.

[00176] In summary, Ab-h1.9d-WT binds strongly to both human and cynomolgus monkey CD4⁺ and CD8⁺ T subsets, demonstrating excellent cynomolgus binding cross-reactivity.

7.10. Example 10: Integrin Binding Specificity of Ab-h1.9d-WT

7.10.1. Materials and Methods

[00177] Recombinant cells expressing individual human and cynomolgus monkey heterodimeric integrin $\alpha 4\beta 7$, $\alpha 4\beta 1$ and $\alpha E\beta 7$ enabled the evaluation of binding specificity of Ab-h1.9d-WT.

[00178] Ab-h1.9d-WT was also evaluated for non-specific binding to human epithelial HEK293 cells.

Integrin Binding Specificity Assay

[00179] Various human and cynomolgus integrins ($\alpha 4\beta 7$, $\alpha 4\beta 1$ or $\alpha E\beta 7$) expressing CHO-K1 or BAF3 cells, except for cynomolgus $\alpha 4\beta 1$ were harvested, counted and prepared in FACS buffer (DPBS, w/o $\text{Ca}^{+2}/\text{Mg}^{+2}$, 1% BSA) at a density of 1.5×10^6 cells per milliliter. A 100 μL containing 1.5×10^5 cells was added to each well of a 96-well U-bottom plate and centrifuged to remove supernatant. Titrated Ab-h1.9d-WT, assay control or isotype control (100 μL each) in FACS buffer were added to reconstitute cell pellets. Well contents were mixed, followed by a one-hour incubation on ice. Cells were washed 2x with FACS buffer and then 100 μL of 1:600 diluted secondary antibody (goat anti-hu IgG Fc γ specific Alexa Fluor 488) in FACS buffer was added to each well and mixed. Plate was incubated for 45 minutes on ice. Subsequently, cells were washed 2x with FACS buffer and resuspended in 200 μL of 0.5% PFA in PBS. The plate was read on FACS (Canto-II, BD) and live cells were determined based on forward and side scatters. Median fluorescence intensities for cell-bound antibodies were generated using GraphPad Prism 7.0 software.

[00180] For CHO-K1 cells expressing cynomolgus $\alpha 4\beta 1$, an antibody staining cocktail containing 25 μL of CD29-APC and CD49d-BV421 mixture each at 1:25 dilution, and 25 μL of Ab-h1.9d-WT, assay control or isotype control prepared by 1:5 fold serial dilutions in FACS buffer were added to each well of a 96-well U-bottom to reconstitute cynomolgus $\alpha 4\beta 1$ cell pellets. Well contents were mixed, followed by a 45 minute incubation on ice. In conjunction, staining controls were also prepared. Cells were washed 2x with FACS buffer and then 50 μL of 1:600 diluted secondary antibody (goat anti-hu IgG Fc γ specific PE) in FACS buffer was added to each well and mixed. Plate was incubated for another 45 minutes on ice. Subsequently, cells were washed 2x with FACS buffer and resuspended in 200 μL of 0.5% PFA in PBS. The plate was read on FACS (Canto-II, BD) and live cells were gated based on forward and side scatters. CD29+CD49d+ cells were then gated to determine median fluorescence intensities for cell-bound test antibodies using FlowJo Version 10 software. The data were plotted using GraphPad Prism 7.0 software.

Non-Specific HEK293 Cell Binding Assay

[00181] HEK293G cells were cultured in complete DMEM (DMEM + 10% FBS + 1% Na Pyruvate). Cells were harvested using non-enzymatic dissociation buffer (Gibco, Cat 13151-014), counted and resuspended at 1.5×10^6 cells/mL in FACS buffer (2% BSA/PBS). To each well of a 96-well U-bottom plate, 7.5×10^4 cells were dispersed. Ab-h1.9d-WT, positive control antibody or isotype control antibody was added to each well at 100 $\mu\text{g}/\text{mL}$ and incubated on ice for one hour. Following incubation, cells were washed 2x with FACS buffer and further incubated with 100 μL of 1:100

diluted goat anti-huIgG Fc-PE (Jackson, Cat 109-116-098) in FACS buffer for 30 minutes on ice. Subsequently, cells were washed 2x with FACS buffer and resuspended in 200 μ L FACS buffer. The plate was read by FACS (Canto II, BD). Binding data was analyzed using GraphPad Prism 7.0 software.

7.10.2. Results

Integrin Binding Specificity

[00182] As shown in FIGS. 14A-14C Ab-h1.9d-WT bound specifically to human $\alpha 4\beta 7$ -expressing cells and did not bind to human $\alpha 4\beta 1$ expressing cells, whereas an anti- $\alpha 4$ mAb, Ab-nata, did bind to both integrins. Ab-h1.9d-WT bound minimally to human $\alpha E\beta 7$ expressing cells in comparison to the much stronger binding observed for an anti- $\beta 7$ mAb, research grade Etrolizumab. Ab-h1.9d-WT displayed a similar binding specificity profile to cynomolgus integrins. These data demonstrated that Ab-h1.9d-WT has binding specificity for both human and cynomolgus $\alpha 4\beta 7$.

Non-Specific HEK293 Cell Binding

[00183] Ab-h1.9d-WT was also evaluated for non-specific binding to human epithelial HEK293 cells. As demonstrated in FIG. 15, Ab-h1.9d-WT did not exhibit any non-specific binding to HEK293 cells at high concentration (100 μ g/mL), comparable to a control antibody, while a positive control mAb displayed strong non-specific binding to these cells.

7.11. Example 11: Rabbit and Rodent Binding Cross-Reactivity of Ab-h1.9d-WT

7.11.1. Materials and Methods

[00184] The binding of Ab-h1.9d-WT to PBMCs isolated from rabbit, rat and mouse was evaluated via flow cytometry.

Rabbit and Rodent Cross-Reactivity Binding Assay

[00185] Rabbit PBMCs were thawed, placed in RPMI1640/10% FBS media, washed 1x with FACS buffer (DPBS, w/o $\text{Ca}^{+2}/\text{Mg}^{+2}$, 1% BSA) and resuspended in FACS buffer containing 5% goat serum. Cells (1×10^5 in 100 μ L) were dispensed into a 96-well U-bottom plate and incubated on ice for 30 minutes. Plates were centrifuged to remove supernatant and 25 μ L of titrated Ab-h1.9d-WT or isotype control plus 25 μ L of 1:10 diluted CD4-FITC antibody (all Abs diluted in FACS buffer) were added to each well to reconstitute cell pellets. Well contents were mixed, followed by a 45 minute incubation on ice. In conjunction, appropriate staining controls were also prepared. Subsequently,

cells were washed 2x with FACS buffer and 50 μ L of a secondary antibody-PE was added to cell pellets at 1:2000 dilutions in FACS buffer containing 5% goat serum. Plate was further incubated on ice for 45 minutes. Cells were washed 2x with FACS buffer and resuspended in 200 μ L of 0.5% PFA in PBS. The plate was read on FACS (Canto II, BD) and live cells were gated based on forward and side scatters. Percentage antibody-bound cells were determined by using FlowJo version 10 software, binding curves and binding EC_{50} values were generated using GraphPad Prism 7.0 software.

[00186] Female C57BL/6N mice and female Lewis rats were received from Taconic Laboratories and Charles River Laboratories, respectively. PBMCs were isolated from pooled mouse and rat blood from the animals. Red blood cells were lysed using RBC lysis buffer (eBioscience). Cells were washed 1x in PBS and resuspended in FACS buffer (DPBS, w/o Ca^{+2}/Mg^{+2} , 1% BSA) containing 5% goat serum. Approximately 2.5×10^5 cells (100 μ L) were added to a 96-well U-bottom plate then incubated on ice for 30 minutes. Appropriate fluorochrome-conjugated antibodies were added to mouse cells (CD3-APC, $\alpha 4\beta 7$ -PE or IgG2a-FITC control) or rat cells (CD3-APC, $\alpha 4$ -FITC or IgG2a-PE control) at a 1:50 dilution in addition to Ab-h1.9d-WT or isotype control. Well contents were mixed thoroughly, and plate was incubated on ice for one hour. Final concentrations were 10 and 1 μ g/mL for isotype controls and Ab-h1.9d-WT. Subsequently, cells were washed 2x with FACS buffer and 100 μ L of secondary antibody-PE or secondary antibody-AF488 was added at 1:800 dilution to wells and plate was incubated on ice for 45 minutes. Following incubation, cells were washed 2x with FACS buffer, resuspended in 200 μ L of 0.5% PFA in PBS. The plate was read on FACS (Canto II, BD) and live cells were gated based on forward and side scatters. Percentage CD3+ cells bound to test antibody or isotype control were determined by using FlowJo version 10 software and graphed using GraphPad Prism 7.0 software.

7.11.2. Results

[00187] As summarized in TABLE 28, Ab-h1.9d-WT displayed similar binding EC_{50} values for rabbit lymphocytes and CD4+ T cells in comparison to the human and cynomolgus counterparts, whereas Ab-h1.9d-WT did not show any measurable binding to rat or mouse PBMCs (lymphocytes and CD4+ T cells).

TABLE 28		
Binding of Ab-h1.9d-WT to Rabbit and Rodent Blood Lymphocytes and CD4+ T Cells in Comparison to Human and Cynomolgus		
	EC ₅₀ (pM) (Mean ± SD)	
Species	Lymphocytes	CD4+ T Cells
Human ^a	130 ± 85	63 ± 38
Cynomolgus ^b	62 ± 45	30 ± 16
Rabbit ^a	89 ± 34	97 ± 11
Rat ^c	No binding	No binding
Mouse ^c	No binding	No binding

EC50 values determined using % of lymphocytes and CD4+ cells expressing α4β7

a. Human/Rabbit PBMC = 3 donors

b. Cynomolgus PBMC = 5 donors

c. Rodent PBMC from pooled blood

7.12. Example 12: Ab-h1.9d-WT-induced α4β7 Internalization

7.12.1. Materials and Methods

[00188] Ab-h1.9d-WT was investigated for its ability to induce internalization of cell surface α4β7 on human primary CD4+ and CD8+ naïve T cells from two PBMC donors.

Internalization Assay

[00189] Ab-h1.9d-WT internalization was investigated and quantified using a similar FACS protocol used to determine cellular mechanisms of Etrolizumab (Lichnog et al., Front Pharmacol). Peripheral blood mononuclear cells (PBMCs) were isolated from two healthy blood donors (RBC, Donor KP58219 and KP58239) using Ficoll Paque (GE 17-1440-03) and SepMate tubes (StemCell 85450), resuspended in FBS (Gibco 10438-026) containing 5% DMSO and cryopreserved in liquid nitrogen. Frozen PBMCs were thawed, counted and reconstituted at 1×10^6 cells/mL in RPMI media + 10% FBS and 100 μL of cells were plated at 1×10^5 cells per well. The plated cells were then incubated at 4°C or placed in 37°C, 5% CO₂ incubator for 30 minutes to acclimate plate temperature. Human PBMCs were pre-incubated with 100 μL of 2x conc. unlabeled Ab-h1.9d-WT antibody at 1.25 μg/mL (final 0.625 μg/mL) for one hour at 4°C. Cells were centrifuged, washed and resuspended in 200 μL of RPMI + 10% FBS and incubated at 4°C or 37°C, 5% CO₂ for 18 hours. Following incubation, cells were washed 2x with FACS buffer (PBS + 1% FBS), and then stained with CD4+ (Biolegend 317410), CD8+ (Biolegend 344710), and CD45RA (Biolegend 304130) with or without the AF647 labeled noncompeting anti-β7 antibody for 30 minutes. Cell fluorescence was acquired by flow cytometry (LSR-Fortessa). The data was analyzed using FlowJo 10 software and percentage internalization was calculated by $100 \times [\text{Total cell surface } \alpha 4\beta 7 \text{ expression before}]$

internalization (MFI at 4°C) - Remaining cell surface $\alpha 4\beta 7$ expression after internalization (MFI at 37°C)/Total cell surface $\alpha 4\beta 7$ expression before internalization (MFI at 4°C)].

7.12.2. Results

[00190] As shown in FIG. 16A, when evaluated 18 hours after Ab-h1.9d-WT bound to human PBMCs at 4°C to minimize internalization, 73% of CD4⁺ naïve T cells (CD4⁺CD45RA⁺) and 49% of CD8⁺ naïve T cells (CD8⁺CD45RA⁺) from donor 1 remained $\beta 7$ -positive. However, after Ab-h1.9d-WT incubation under the same protocol but at 37°C to promote internalization, only 17% of CD4⁺ naïve T cells and 7% of CD8⁺ naïve T cells were found to be $\beta 7$ -positive. These data indicate that significant internalization of surface $\alpha 4\beta 7$ bound to Ab-h1.9d-WT occurred at 37°C. Similar results were observed with PBMCs from donor 2. Based on the quantification of $\alpha 4\beta 7$ internalization at 37°C (reduced surface $\alpha 4\beta 7$ expression relative to the expression observed after treatment at 4°C) in FIG. 16B, Ab-h1.9d-WT was able to induce $\alpha 4\beta 7$ internalization by ~80% on both $\alpha 4\beta 7^+$ naïve CD4⁺ and CD8⁺ T cells.

[00191] Ab-h1.9d-WT is more potent in inducing $\alpha 4\beta 7$ internalization in comparison to Ab-Vedo.

7.13. Example 13: MAdCAM-1 Ligand Blockade By Ab-h1.9d-WT

7.13.1. Materials and Methods

MAdCAM-1 Ligand Blockade Assay

[00192] For FACS-based assay, HuT78 cells or human PBMCs were harvested, washed 1x with DPBS, adjusted to a density of 1.5×10^6 cells/mL and resuspended in FACS buffer (DPBS, w/o $\text{Ca}^{+2}/\text{Mg}^{+2}$, 1% BSA/1mM MnCl_2). Cells at 1×10^5 (100 μL)/well were dispensed into a 96-well U-bottom plate and centrifuged to remove supernatant. Titrated Ab-h1.9d-WT or isotype control (50 μL) plus 50 μL mixture containing 0.3 $\mu\text{g}/\text{mL}$ MAdCAM-1-mFc, 2 mM MnCl_2 , and 1:50 diluted (30 $\mu\text{g}/\text{mL}$) Alexa488 conjugated detection Ab in FACS buffer were added to each well. The plate was incubated on ice for one hour then centrifuged. The plate was gently washed 1x with 200 μL FACS buffer and cells were reconstituted in same buffer. The plate was read by FACS (Canto II) and live cells were determined by forward and side scatter gating. Flow data (FCS 3.0 files) was analyzed using FlowJo Version 10 software, binding curves and inhibition IC_{50} values were generated using GraphPad Prism 7.0 software.

[00193] For plate-based assay, 96-well flat-bottom plates (Greiner, Cat 655077) were coated with 100 μ L of 20 μ g/mL MAdCAM-1 hFc or isotype control (final concentration 2 μ g/well) using coating buffer (PBS w/o $\text{Ca}^{+2}/\text{Mg}^{+2}$, 0.1% BSA) at 4°C for overnight. Next day, plate(s) were washed 2x with 200 μ L of wash buffer (PBS w/ $\text{Ca}^{+2}/\text{Mg}^{+2}$, 0.1% BSA) then blocked at 37°C for one hour using blocking buffer (PBS w/ $\text{Ca}^{+2}/\text{Mg}^{+2}$, 1% BSA). During blocking incubation, dilutions of Ab-h1.9d-WT and isotype control were prepared and HuT78 cells harvested. A 2x initial concentration was prepared at 5 μ g/mL and then serial 1:4.5 7-point dilutions (final concentrations ranging from 2.5 to 0.0003 μ g/mL) were performed in duplicate or triplicate. The cells were counted, washed and resuspended at a 2×10^6 cells/mL in assay media (IMDM, 1% BSA) to which a final concentration of 2 mM MnCl_2 was added and 100,000 cells were dispensed to each well of 96-well plates. The diluted antibodies were then added to the cells and the mixture was incubated at 37°C, 5% CO_2 for 30 minutes. The blocking solution from MAdCAM-1 hFc coated plates was decanted and 100 μ L/well of pre-incubated HuT78 cells and mAb mixture was distributed into each well of MAdCAM-1 hFc coated plates. Plate(s) were spun at 1,000 rpm for one minute and incubated at 37°C, 5% CO_2 for 30 minutes. Adhesion plates were decanted and washed gently 4x with 150 μ L of wash buffer. Post-washing, 100 μ L/well of a mixture (containing 50 μ L of CellTiter-Glo reagent and 50 μ L assay medium) was added to each well. Plate(s) were placed on orbital shaker for two minutes then incubated at RT for 10 minutes. Plate luminescence was read on a luminescence plate reader (Topcount, Perkin Elmer). Luminescence signal was plotted and IC_{50} values were determined in GraphPad Prism 7.0 using 4-parameter curve fit analysis.

7.13.2. Results

[00194] Ab-h1.9d-WT was tested for its ability to block the binding of recombinant extracellular domain of MAdCAM-1 protein to $\alpha 4\beta 7$ -expressing HuT78 cells and human lymphocytes (PBMCs) using both plate-based and FACS-based assays.

[00195] IC_{50} values of 50 pM and 25 pM were obtained by using FACS- and plate-based assays, respectively.

[00196] In addition, the blockade of MAdCAM-1 binding to human blood-derived lymphocytes by Ab-h1.9d-WT was observed at IC_{50} of 223 pM (TABLE 29).

TABLE 29		
MAdCAM-1 Blockade Potency IC ₅₀ of Ab-h1.9d-WT		
*IC ₅₀ (pM)		
FACS		Luminescence
HuT78	Human Lymphocytes	HuT78
50 ± 11	223 ± 86	25 ± 4

* Average ± SD; N = 3

[00197] These data demonstrated the strong inhibitory effect of Ab-h1.9d-WT on MAdCAM-1/α4β7 interaction.

7.14. Example 14: Ab-h1.9d-WT blocks MAdCAM-1 co-stimulation on human primary CD4⁺ T cells

7.14.1. Materials and Methods

Human PBMCs and CD4⁺ T cell isolation

[00198] Human peripheral blood mononuclear cells (PBMCs) were isolated from fresh blood collected from healthy donors. Human CD4⁺ T cells were then isolated from PBMCs using a CD4 negative selection kit (Stem Cell Technologies).

CD4⁺ T cell activation and proliferation assay

[00199] 96-well flat bottom tissue culture plates were coated with 200 ng/well anti-CD3 antibody (Biolegend) in HBSS at 4°C overnight. On the following day, the anti-CD3 coated plates were washed once with HBSS and incubated with 200 ng/well MAdCAM-1 (R&D systems) for 1 hour at 37°C. Following the incubation, the plates were washed once with 200 ul of HBSS and 50,000 CD4⁺ T cells were added to each well in the presence or absence of 1 µg/ml testing antibodies. After culturing the cells at 37°C, 5% CO₂ for 96 hours, the cells were washed and stained with Live-Dead Aqua viability dye (ThermoFisher) and subsequently stained with the cell activation markers anti-CD25 FITC (Clone MA0251 BD bioscience) and anti-Ki67 APC (Biolegend). Cells were analyzed on a flow cytometer and the data were analyzed with FlowJo software. Data from multiple donors were plotted and the statistical analysis was performed using GraphPad Prism. Significance was determined using one-way ANOVA coupled to Tukey's multiple comparisons test. **** p<0.0001, ** p=0.001-0.01.

7.14.2. Results

Ab-h1.9d-WT blocks MAdCAM-1 co-stimulation on human primary CD4⁺ T cells

[00200] MAdCAM-1-mediated gut-homing of $\alpha 4\beta 7$ +CD4+ T cells plays a central role in HIV infection of GALT (gut-associated lymphoid tissues). In addition to this role, MAdCAM-1 has also been reported to deliver a co-stimulation signal to human primary CD4+ T cells and promote HIV replication (Nawaz et. al., Mucosal Immunology 2018). Given that HIV infection and replication require metabolic activation of these cells and Ab-h1.9d-WT can block MAdCAM-1 binding to human lymphocytes at IC50 value of 223 pM (TABLE 35), we evaluated whether Ab-h1.9d-WT is capable of blocking MAdCAM-1 co-stimulation signal on human primary CD4+ T cells, which would in turn inhibit the MAdCAM-1-mediated viral replication in these cells.

[00201] When human primary CD4+ T cells from one representative healthy donor were incubated with plate bound anti-CD3 alone for 96 hours, 22.6% of the cells were activated displaying Ki67+CD25+ phenotype (FIG. 17A). When the cells were incubated with the plate bound anti-CD3 and MAdCAM-1 together, 56.6% of the CD4+ T cells were activated. This indicates that MAdCAM-1 delivered a co-stimulation signal to CD4+ T cells mediated by interacting to the cell surface $\alpha 4\beta 7$. Addition of Ab-h1.9d-WT to the cells during the 96-hour incubation reduced the cell activation to 20.8%, a level comparable to the anti-CD3 alone, while an isotype control Ab did not have any inhibitory effect on the cell activation. The data suggest that Ab-h1.9d-WT effectively and completely blocked the co-stimulation signal exerted by MAdCAM-1. The assay was repeated with the primary CD4 T cells from five additional individual donors and the data is summarized in FIG. 17B. Consistent with the data obtained from one representative donor in FIG. 17A, Ab-h1.9d-WT almost completely blocked MAdCAM-1/ $\alpha 4\beta 7$ mediated co-stimulation of CD4+ T cells while the isotype control Ab did not have any inhibitory effect.

7.15. Example 15: VCAM-1 Ligand Blockade Specificity of Ab-h1.9d-WT

7.15.1. Materials and Methods

[00202] The effect Ab-h1.9d-WT on the $\alpha 4\beta 7$ /VCAM-1 interaction in a VCAM-1 mediated cell adhesion assay was assessed.

VCAM-1 Ligand Blockade Assay

[00203] Plates (96-well flat-bottom, Greiner, Cat 655077) were coated on Day 1 with 100 μ L of 20 μ g/mL VCAM-1 hFc or isotype control (final concentration 2 μ g/well) using coating buffer (PBS w/o Ca^{+2} / Mg^{+2} , 0.1% BSA) at 4°C overnight. On Day 2, plate(s) were washed 3x with 200 μ L of wash buffer (PBS w/ Ca^{+2} / Mg^{+2} , 0.1% BSA) then blocked at 37°C for 1h or longer using blocking buffer

(PBS w/ $\text{Ca}^{+2}/\text{Mg}^{+2}$, 1% BSA). During blocking incubation, dilutions of Ab-h1.9d-WT, Ab-nata and isotype control were prepared and HuT78 cells were harvested. A 2x initial concentration of antibody was prepared at 4 $\mu\text{g/mL}$ and then 1:4 fold serial dilutions in assay medium (IMDM, 1% BSA) were made. HuT78 cells were counted, washed and resuspended at a 2×10^6 cells/mL in assay medium to which a final concentration of 2 mM MnCl_2 was added and 100,000 cells were dispensed to each well of 96-well plates. The diluted antibodies were then added to the cells and the mixture was incubated at 37°C, 5% CO_2 for 30 minutes. The blocking solution from VCAM-1 hFc coated plates was decanted, and 100 μL of pre-incubated HuT78 cells and mAb mixture were distributed into each well of VCAM-1 hFc coated plates. Plate(s) were incubated at 37°C, 5% CO_2 for 30 minutes and then were washed gently 3x using washing buffer. Post-washing, 100 μL /well of a mixture (containing 50 μL of CellTiter-Glo reagent and 50 μL assay medium) was added to each well. Plate(s) were placed on orbital shaker for two minutes then incubated at RT for 10 minutes. Plate luminescence was read on luminescence plate reader (Topcount, Perkin Elmer). Luminescence signals were plotted to determine IC_{50} values in GraphPad Prism 7.0 using 4-parameter curve fit analysis.

7.15.2. Results

[00204] In addition to $\alpha 4\beta 7$ binding to MAdCAM-1 enabling gut homing of blood lymphocytes, $\alpha 4\beta 7$ can also bind to VCAM-1 expressed on endothelial cells (TABLE 30).

TABLE 30				
VCAM-1 Blockade Selectivity of Ab-h1.9d-WT				
mAb	Experiment 1	Experiment 2	Experiment 3	Average (N = 3)
	IC_{50} (pM)			IC_{50} (pM) $\pm$ SD
Ab-h1.9d-WT	no inhibition	no inhibition	no inhibition	no inhibition
Ab-nata	38	54	77	56 ± 20

[00205] Natalizumab, Ab-nata an anti- $\alpha 4$ mAb, capable of blocking $\alpha 4\beta 7$ and $\alpha 4\beta 1$ binding to VCAM-1 caused progressive multifocal leukoencephalopathy (PML) due to its blockade of trafficking of circulating lymphocytes to the brain. Therefore, assessment of the effect Ab-h1.9d-WT on the $\alpha 4\beta 7$ /VCAM-1 interaction in a VCAM-1 mediated cell adhesion assay was an essential safety parameter to investigate using Ab-nata as a positive control. As expected, Ab-nata blocked VCAM-1-mediated HuT78 cell adhesion with mean IC_{50} value of 56 pM. In contrast, Ab-h1.9d-WT showed no detectable blockade of HuT78 cell adhesion mediated by VCAM-1 (FIG. 18).

[00206] Despite Ab-h1.9d-WT selectively blocking $\alpha 4\beta 7$ /MAdCAM-1 interaction with high potency, it shows no inhibition of $\alpha 4\beta 7$ /VCAM-1 interaction.

7.16. Example 16: Ab-h1.9d-WT Binding Affinity to Human and Cynomolgus Monkey FcγRI, FcγRIIa, FcγRIIb, FcγRIIIa and FcRn

7.16.1. Materials and Methods

[00207] Ab-h1.9d-WT was evaluated by BIAcore for its binding affinity to a panel of recombinant human and cynomolgus monkey FcγR extracellular domain (ECD) proteins in comparison to antibody IgG1 control, Trastuzumab.

[00208] Ab-h1.9d-WT binding to human FcγRs was also evaluated via flow cytometry by using CHO-K1 cells engineered to express various cell surface human FcγRs.

[00209] Binding of Ab-h1.9d-WT to human and cynomolgus FcRn was evaluated by BIAcore at pH 6.0 and pH 7.4 using recombinant human and cynomolgus FcRn ECD protein (TABLE 31).

TABLE 31 Binding Affinity of Ab-h1.9d-WT to Human and Cynomolgus FcRn via BIAcore				
Antibody	Human FcRn K_D (M) (Mean $\pm$ SD)		Cynomolgus FcRn K_D (M) (Mean $\pm$ SD)	
	pH 6.0	pH 7.4	pH 6.0	pH 7.4
Ab-h1.9d-WT	$3.3 \times 10^{-6} \pm 0.2 \times 10^{-6}$	No significant binding	$2.2 \times 10^{-6} \pm 0.3 \times 10^{-6}$	No significant binding
Trastuzumab	$3.2 \times 10^{-6} \pm 0.1 \times 10^{-6}$	No significant binding	$2.2 \times 10^{-6} \pm 0.4 \times 10^{-6}$	No significant binding

N = 3

Human and Cynomolgus Monkey FcγRI, FcγRIIa, FcγRIIb, and FcγRIIIa Surface Plasmon Resonance (SPR) Binding Assay

[00210] Binding kinetics of Ab-h1.9d-WT for His tagged human FcγRs were determined by SPR measurements made on Biacore T200 instrument (GE Healthcare) at 25°C using anti-His capture. Approximately 10000 RU of mouse anti-His antibody (R&D) diluted to 25 µg/mL in 10 mM sodium acetate (pH 4.5) was immobilized across a CM5 biosensor chip using a standard amine coupling kit according to manufacturer's instructions. Unreacted moieties on the biosensor surface were blocked with 1M ethanolamine. Activated and deactivated surface on flow cell 1 were used as a reference. Chip preparation and binding kinetic measurements were made in assay running buffer, HBS-EP+ (10 mM Hepes, pH 7.4, 150 mM NaCl, 3 mM EDTA, 0.05% Tween 20). Human and cynomolgus FcγRs were then captured on flow cells 2 to achieve capture level of 250 to 500 RU. Ab-h1.9d-WT samples were injected over all flow cells at a flow rate of 50 µL/min for one to five minutes (one minute for hu and cynomolgus FcγRIIb and FcγRIIa, two minutes for hu FcγRIIIa and five minutes for hu and

cynomolgus FcγRI & cynomolgus FcγRIII). Analyte concentrations ranged from 0.78 to 200 nM for hu and cynomolgus FcγRI cynomolgus FcγRIII, 46.9 to 12000 nM for hu and cynomolgus FcγRII and 7.8 to 4000 nM for FcγRIII (2-fold serial dilution). A buffer only injection was included for double referencing. Bound FcγRs dissociation was monitored for one to five minutes (one minute for FcγRIIb, FcγRIIa, three minutes for FcγRIIIa and five minutes for FcγRI). The chip surface was regenerated with injected 100 mM HCl at a flow rate of 100 μL/min for two seconds across all eight channels. Three experiments using the same CM5 chip were run for each sample. The results of these three experiments were averaged.

Human FcγR1, FcγRIIIa, FcγRIIb, FcγRIIIa Cell Binding Assay

[00211] CHO-K1 expressing hFcγR cells were grown in 150 cm² culture flasks and loaded with a specific amount of fluorophore, CellTrace CFSETM and CellTrace VioletTM (Molecular Probes) according to manufacturer instructions to establish a unique fluorescence footprint (barcoding method) for each line. Lines were mixed and incubated at 4°C for one hour with monomeric Ab-h1.9d-WT at different concentrations (0, 0.01, 0.1, 1, 10, 50, 100, and 250 μg/mL) in RPMI1640/2 mM L-glutamine/10% Ultra Low IgG heat inactivated FBS (binding media). Following incubation, cells were washed 2x in PBS, pH7.4 (w/o Ca⁺²/Mg⁺²) and incubated for 15 minutes at 4°C with secondary antibody (F(ab')₂ goat anti-human IgG (H+L) coupled to AF647 in binding media to detect cell bound Ab-h1.9d-WT. Following incubation, cells were further washed 2x with PBS, pH7.4 (w/o Ca⁺²/Mg⁺²). Cell surface fluorescence was detected and recorded using a flow cytometry analyzer (LSR-Fortessa). The recorded fluorescence data were analyzed using FlowJo software Version 10 (Tristar) and Ab-h1.9d-WT binding to CHO-K1 hFcγR cells was reported as the geometric mean of fluorescence of AF647 (binding curves of gMFI as a function of Ab-h1.9d-WT concentration were generated).

Human and Cynomolgus Monkey FcRn Surface Plasmon Binding Assay

[00212] For FcRn binding analysis, Ab-h1.9d-WT was directly immobilized on a CM5 chip amine coupling according to manufacturer's protocol to a density of 750 RU. Human and cynomolgus FcRn recombinant proteins were injected across all flow cells at a flow rate of 50 μL/min for one minute at concentrations ranging from 5.5 to 12000 nM (3-fold serial dilution), followed by a one minute dissociation. The surface was regenerated with an injection of HBS-EP + pH 7.4 for 15 seconds. Samples were prepared and run in two running buffers, MES EP + pH 6.0 and HBS-EP + pH 7.4. Three experiments with the use of the different CM5 chips were run for each sample (each in

duplicate). The results of these three experiments were averaged. Data from human FcγRI, FcγRIIIa (F158), FcγRIIIa (V158) and cynomolgus FcγRI, FcγRIII binding to all samples were fitted to a 1:1 global kinetics model with fixed R_{max} . Data from human, FcγRIIb, FcγRIIa (H131), FcγRIIa (R131), cynomolgus FcγRIIa, FcγRIIb and FcRn binding to all samples were fitted to a steady state affinity model. Biacore T200 Evaluation Software Version 2.0 was used to fit FcγR and FcRn data.

7.16.2. Results

Human and Cynomolgus Monkey FcγRI, FcγRIIa, FcγRIIb, and FcγRIIIa Binding

[00213] The binding kinetic parameters are summarized in TABLE 32 (for human) and TABLE 33 (for cynomolgus).

TABLE 32 Binding Affinity of Ab-h1.9d-WT to Human FcγRs via BIAcore						
Antibody	Captured FcγRs					
	$K_D(M)$ (Mean $\pm$ SD)					
	hFcγRI	hFcγRIIa (H131)	hFcγRIIa (R131)	hFcγRIIb	hFcγRIIIa (F158)	hFcγRIIIa (V158)
Ab-h1.9d-WT	$1.6 \times 10^{-8} \pm 0.2 \times 10^{-8}$	$5.6 \times 10^{-6} \pm 0.8 \times 10^{-6}$	binds but too weak to determine	binds but too weak to determine	$4.6 \times 10^{-6} \pm 2.1 \times 10^{-6}$	$4.8 \times 10^{-7} \pm 3.1 \times 10^{-7}$
Trastuzumab	$1.5 \times 10^{-8} \pm 0.3 \times 10^{-8}$	$6.1 \times 10^{-6} \pm 0.3 \times 10^{-6}$	$8.9 \times 10^{-6} \pm 0.3 \times 10^{-6}$	binds but too weak to determine	$9.5 \times 10^{-7} \pm 0.3 \times 10^{-7}$	$9.4 \times 10^{-8} \pm 0.1 \times 10^{-8}$

N = 3

[00214] Ab-h1.9d-WT and Trastuzumab had similar measurable binding affinities to both hFcγRI and hFcγRII (H131), weaker binding to hFcγRII (R131) and hFcγRIIb receptors. Ab-h1.9d-WT and Trastuzumab had measurable binding affinities to hFcγRIIIa (F158/V158) with higher affinity for the V158 polymorphic variant as expected.

TABLE 33 Binding Affinity of Ab-h1.9d-WT to Cynomolgus FcγRs via BIAcore				
Antibody	Captured FcγRs			
	$K_D(M)$ (Mean $\pm$ SD)			
	Cynomolgus FcγRI	Cynomolgus FcγRIIa	Cynomolgus FcγRIIb	Cynomolgus FcγRIII
Ab-h1.9d-WT	$1.2 \times 10^{-8} \pm 0.1 \times 10^{-8}$	binds but too weak to determine	binds but too weak to determine	$2.8 \times 10^{-7} \pm 4.6 \times 10^{-7}$
Trastuzumab	$1.3 \times 10^{-8} \pm 0.2 \times 10^{-8}$	binds but too weak to determine	binds but too weak to determine	$8.0 \times 10^{-8} \pm 4.6 \times 10^{-8}$

N = 3

[00215] While both Ab-h1.9d-WT and trastuzumab exhibited binding to both cynomolgus FcγRI and cynomolgus FcγRIII, no measurable binding parameters could be determined for cynomolgus FcγRIIIa and cynomolgus FcγRII receptors likely due to their weak binding to these receptors.

Human FcγRI, FcγRIIIa, FcγRIIb, FcγRIIIa Cell Binding Assay

[00216] Ab-h1.9d-WT displayed the highest binding to human FcγRI and FcγRIIIa (V176) polymorphic variant, but relatively low binding to other human FcγRs [FcγRIIIa, FcγRIIb, FcγRIIc, FcγRIIIa (F176) and FcγRIIIb] (FIGS. 19A-19B).

Human and Cynomolgus Monkey FcRn Surface Plasmon Binding Assay

[00217] Ab-h1.9d-WT exhibited measurable binding to human and cynomolgus FcRn under acidic conditions (pH 6.0), but no measurable binding at neutral pH (pH 7.4) (TABLE 34). FcRn binding properties were comparable to control (Trastuzumab).

TABLE 34				
Binding Affinity of Ab-h1.9d-WT to Human and Cynomolgus FcRn via BIAcore				
Antibody	Human FcRn K_D (M) (Mean $\pm$ SD)		Cynomolgus FcRn K_D (M) (Mean $\pm$ SD)	
	pH 6.0	pH 7.4	pH 6.0	pH 7.4
Ab-h1.9d-WT	$3.3 \times 10^{-6} \pm 0.2 \times 10^{-6}$	No significant binding	$2.2 \times 10^{-6} \pm 0.3 \times 10^{-6}$	No significant binding
Trastuzumab	$3.2 \times 10^{-6} \pm 0.1 \times 10^{-6}$	No significant binding	$2.2 \times 10^{-6} \pm 0.4 \times 10^{-6}$	No significant binding

N = 3

7.17. Example 17: Ab-h1.9d-WT In vitro ADCC, ADCP and CDC Activity

7.17.1. Materials and Methods

[00218] Ab-h1.9d-WT was evaluated for in vitro ADCC and ADCP activities using native $\alpha 4\beta 7$ /CD20-expressing RPMI8866 cells as target cells and the reporter Jurkat cells expressing either hFcγRIIIa (V158) or hFcγRIIIa (H131) as effector cells. This Jurkat cell line had an NFAT response element that drives the expression of firefly luciferase as reporter. Ab-Ritu, an anti-CD20 mAb was used as a positive control in these assays.

[00219] Furthermore, Ab-h1.9d-WT was evaluated in a cell cytotoxicity-based ADCC assay using $\alpha 4\beta 7$ /CD52 expressing HuT78 cells as targets, human primary NK cells as effectors and Campath (anti-CD52) as a positive control antibody.

ADCC Reporter Assay

[00220] Target $\alpha 4\beta 7$ expressing RPMI8866 cells were grown and maintained in culture media (RPMI1640, 2 mM L-glutamine and 10% FBS). Log phase cells were harvested, counted, washed and resuspended at a 6×10^5 cells/mL stock in assay medium (RPMI1640 containing low IgG serum) and placed at 37°C, 5% CO₂ until ready for use. Ab-h1.9d-WT and control mAbs were prepared at a 3x initial concentration of 30 μ g/mL then serially (2 pt.) diluted at 1:100 in Costar 3956 dilution plates. Antibodies (25 μ L) were mixed with an equal volume of target RPMI8866 cells (25 μ L) in duplicate according to plate layout(s). Effector Jurkat cells (stably expressing the human Fc γ RIIIa V158 variant and an NFAT response element driving expression of firefly luciferase from Promega™) were thawed rapidly and adjusted to 3×10^6 cells/mL in assay media. Jurkat effectors (25 μ L) were then added to the 96-well assay plates containing RPMI8866 targets and antibody. Control wells included media alone, Effector (E) and Target (T) alone, E + T, E + mAb (30 μ g /mL) or T + mAb (30 μ g/mL). All wells were adjusted to 75 μ L per well according to plate layout(s). The final cell numbers were 75,000 Jurkat effector cells/well and 15,000 RPMI8866 target cells/well corresponding to an E:T ratio of 5:1. The final antibody concentrations per well were 67 nM (10 μ g/mL), 0.67 nM (0.1 μ g/mL) and 0.0067 nM (0.001 μ g/mL). Plates were incubated at 37°C, 5% CO₂ for six hours during which Bio-Glo™ buffer and substrate (Cat 7110) was equilibrated to RT prior to use. At the end of the incubation, Bio-Glo substrate was reconstituted with buffer to form enzyme/substrate solution (Bio-Glo reagent). An equal volume of 75 μ L/well Bio-Glo was added to all wells. Plates were then incubated at RT for 10 minutes. Plate luminescence was read on a luminescence plate reader (Topcount, Perkin Elmer). Luminescence signal was plotted as RLU using GraphPad Prism 7.0 software.

ADCP Reporter Assay

[00221] Target $\alpha 4\beta 7$ expressing RPMI8866 cells were grown and maintained in culture media (RPMI1640, 2 mM L-glutamine and 10% FBS). Log phase cells were harvested, counted, washed and resuspended at a 2×10^5 cells/mL stock in assay medium (RPMI1640 containing low IgG serum) and placed at 37°C, 5% CO₂ until ready for use. Ab-h1.9d-WT and control mAbs, were prepared at a 3x initial concentration of 30 μ g/mL then serially (2 pt.) diluted at 1:100 in Costar 3956 dilution plates. Antibodies (25 μ L) were mixed with an equal volume of target cells (25 μ L) in duplicate according to plate layout(s). Effector Jurkat cells (stably expressing the human Fc γ RIIIa H131 variant and an NFAT response element driving expression of firefly luciferase from Promega™) were thawed rapidly and adjusted to 1×10^6 cells/mL in assay media. Jurkat effectors (25 μ L) were then

added to the 96-well assay plates containing RPMI8866 targets and antibody. Control wells included media alone, Effector (E) and Target (T) alone, E + T, E + mAb (30 $\mu\text{g}/\text{mL}$) or T + mAb (30 $\mu\text{g}/\text{mL}$). All wells were adjusted to 75 μL per well according to plate layout(s). The final cell numbers were 25,000 Jurkat effector cells/well and 5,000 target cells/well corresponding to an E:T ratio of 5:1. The final antibody concentrations per well were 67 nM (10 $\mu\text{g}/\text{mL}$), 0.67 nM (0.1 $\mu\text{g}/\text{mL}$) and 0.0067 nM (0.001 $\mu\text{g}/\text{mL}$). Plates were incubated at 37°C, 5% CO₂ for six hours during which Bio-Glo™ buffer and substrate (Cat 7110) was equilibrated to RT prior to use. At the end of the incubation, Bio-Glo substrate was reconstituted with buffer to form enzyme/substrate solution (Bio-Glo reagent). An equal volume of 75 μL /well Bio-Glo was added to all wells. Plates were then incubated at RT for 10 minutes. Plate luminescence was read on a luminescence plate reader (Topcount, Perkin Elmer). Luminescence signal was plotted as RLU using GraphPad Prism 7.0 software.

CDC Assay

[00222] RPMI8866 cells were grown and maintained in culture media (RPMI1640, 2 mM L-glutamine and 10% FBS). Log phase cells were harvested, counted, washed and resuspended in assay medium (RPMI1640 minus phenol red, Cat 11835-030) at a 4×10^6 cells/mL stock and placed at 37°C, 5% CO₂ until ready for use. Ab-h1.9d-WT and control mAbs were prepared at a 3x initial concentration of 45 $\mu\text{g}/\text{mL}$ and 0.45 $\mu\text{g}/\text{mL}$ in Costar 3956 dilution plates. Human donor serum (HMN19169 and HMN19170) was thawed using cold running water and immediately placed on ice. Antibodies, controls (25 μL) and media were added to assay plates (Costar 3599). Donor serum (25 μL each), the target cells (25 μL) and diluted mAbs (25 μL) were mixed at the final volume of 75 μL per well containing 33% serum complement, 1×10^5 cells and 15 $\mu\text{g}/\text{mL}$ mAb. Plate(s) were then incubated at 37°C, 5% CO₂ for two hours. After the incubation cell permeable dye (Sigma; Resazurin Sodium Salt) at a 5x stock solution of 1.5 mg/mL was diluted 1:5 in DPBS and 25 μL of dye was added to each well. Plates were incubated for additional 16 hours and then absorbance (545/600) was read using Clariostar plate reader. Percentage target-specific cell lysis was calculated in Excel using formula: $100 - 100 \times [(\text{absorbance with mAb incubation})/(\text{control absorbance})]$; and the results were plotted using GraphPad Prism 7.0 software.

ADCC Cytotoxicity Assay

[00223] Target $\alpha 4\beta 7$ expressing HuT78 cells were harvested, washed 2x with PBS (w/o Ca⁺²/Mg⁺², 1% BSA) and resuspended in PBS at 1×10^7 cells/mL then labeled with CFSE at RT for eight

minutes at a final concentration of 2 μ M. After incubation, FBS was added at a 10% final concentration to quench labeling. Cells were washed 2x with RPMI + 10% FBS media and then CFSE labeled HuT78 cells were incubated with 100 μ L of a 2x conc. of Ab-h1.9d-WT or control antibodies at 10 μ g/mL for 30 minutes at 37°C, 5% CO₂ in a 96-well V-bottom plate. Subsequently, 100 μ L of 2.5×10^5 NK (Fc γ RIIIa V158+) effector cells (preincubated with IL-2 at 200 U/mL) were added to target cells at a 5:1 ratio. Well contents were mixed thoroughly, followed by a five hour incubation at 37°C in CO₂ incubator. NK and HuT78 cell mixtures were washed 2x with PBS and resuspended at 1×10^6 cells/mL with azide-free and protein-free PBS containing 1 μ L of FVD dye/mL and incubated at RT for 20 minutes. Cells were washed 2x with FACS buffer, resuspended in 200 μ L of 0.5% PFA in PBS and plate was read on FACS (Canto II, BD). Flow data (FCS 3.0 files) was analyzed by using FlowJo Version 10 software. All HuT78 target cells (live and dead) were gated to determine % dead targets within the total target cell population. The formula used for % ADCC calculation was % ADCC = $100 \times [\% \text{ dead targets in (E + T + Ab) mix} - \% \text{ dead targets in (E + T) mix}] / [100 - \% \text{ dead targets in (E + T) mix}]$. Percentage ADCC was graphed using GraphPad Prism 7.0 software.

7.17.2. Results

ADCC Reporter Activity, ADCP Reporter Activity, and CDC

[00224] As expected, Ab-Ritu demonstrated strong concentration-dependent in vitro ADCC and ADCP activity; however, Ab-h1.9d-WT and isotype control did not show any of these activities at three concentrations (10, 0.1, 0.001 μ g/mL) tested (FIGS. 20A and 20B). Only minimal signals were detected for three negative control assay conditions namely targets and effectors alone, targets plus antibody, and effectors plus antibody (data not shown). Ab-Ritu also demonstrated strong in vitro CDC activity on RPMI8866 cells at 0.15 and 15 μ g/mL (100 nM) whereas Ab-h1.9d-WT and isotype control showed no CDC signal at corresponding concentrations using two human serum donors (FIG. 21).

ADCC Cytotoxicity

[00225] In this in vitro assay, Campath (Ab-Alem) displayed strong cell cytotoxicity against the target cells. In contrast, Ab-h1.9d-WT did not induce any in vitro cell cytotoxicity at 10 μ g/mL (67 nM) concentration when the assay was performed using NK effector cells isolated from two different donors with Fc γ RIIIa V158 genotype (FIG. 22). Thus, although Ab-h1.9d-WT binds to human Fc γ Rs

as shown above (Example 12), it does not induce undesired Fc-mediated ADCC, ADCP and CDC activities against uninfected $\alpha 4\beta 7^{+}$ cells in vitro.

7.18. Example 18: Homology modeling of Ab-h1.9d-WT binding site on the target

7.18.1. Materials and Methods

[00226] The binding mode of Ab-h1.9d-WT to $\alpha 4\beta 7$ was explored by homology modeling. The crystal structure of recombinant human $\alpha 4\beta 7$ extracellular domain protein in complex with another anti- $\alpha 4\beta 7$ antibody, vedolizumab, has been published (Yu et al., J Cell Biol 2012). Based on that data and the unique sequence of the Fab region of Ab-h1.9d-WT, modeling of the binding modes of the candidate antibody in comparison to benchmark antibodies vedolizumab and AMG181 was undertaken.

7.18.2. Results

[00227] In agreement with the in vitro characterization data, Ab-h1.9 which is a humanized variant of hybridoma Ab-m1 and the parent of Ab-h1.9d-WT, and the two benchmark mAbs bind to $\alpha 4\beta 7$ primarily through interactions with $\beta 7$ subunit but also interacting, albeit slightly, with the $\alpha 4$ subunit. This model explains their lack of binding to $\alpha 4\beta 1$ and very low binding to $\alpha E\beta 7$. Interestingly, the model suggests a subtle difference between Ab-h1.9 and the two benchmark mAbs in that Ab-h1.9 binds slightly more residues on the $\alpha 4$ subunit. The overlapping epitopes evident in the model predicts that Ab-h1.9 and vedolizumab should compete for binding to their target. Indeed, in a FACS-based binding competition study, Ab-m1 was able to compete with Ab-Vedo binding to $\alpha 4\beta 7^{+}$ cells, indicating they bind to a similar binding epitope (TABLE 13).

7.19. Discussion

[00228] The expression levels of $\alpha 4\beta 7$ on peripheral human CD4⁺ and CD8⁺ T cell subsets from HIV⁺ and HIV⁻ individuals are comparable, suggesting that the $\alpha 4\beta 7$ expression in CD4⁺ T cells from HIV⁺ individuals could support the incorporation of $\alpha 4\beta 7$ into the budding HIV virions.

[00229] Ab-h1.9d-WT is a potent anti- $\alpha 4\beta 7$ antibody that can bind to $\alpha 4\beta 7$ on the envelope of virions of all laboratory grown HIV strains and HIV patients' samples tested. The immune complexes formed by Ab-h1.9d-WT and HIV virions could bind to different Fc γ Rs through its Fc domain, a step that could enable it to be taken up by APCs by phagocytosis to induce the proposed "vaccination effect" for HIV control.

[00230] Although Ab-h1.9d-WT binds HIV virions, it does not neutralize HIV infection, which is consistent with the notion that $\alpha 4\beta 7$ is not a viral receptor on host cells. By targeting $\alpha 4\beta 7$ integrin, a host protein, on the HIV viral envelope, Ab-h1.9d-WT may exhibit a higher barrier to resistance compared to other antibodies targeting the HIV virally encoded gp120/41 glycoprotein in the viral envelope such as HIV broadly neutralizing antibodies.

[00231] Ab-h1.9d-WT can disrupt the interaction between $\alpha 4\beta 7$ and its ligands such as MadCAM-1 or HIV gp120 through an Fab-dependent mechanism, inhibiting the CD4 T cell co-stimulation mediated by MadCAM-1 and gp120, and potentially inhibiting HIV replication in these stimulated cells.

[00232] Ab-h1.9d-WT can potentially inhibit cell-to-cell HIV viral transmission by an Fab-mediated mechanism through its ability to disrupt the interaction between $\alpha 4\beta 7$ and HIV gp120.

[00233] When compared with Ab-h1.9d-WT, Ab-Vedo demonstrated lower activity in capturing HIV virions and disruption of the interaction between $\alpha 4\beta 7$ and HIV gp120. Furthermore, although Ab-Vedo was capable of binding HIV virions to form immune complexes, these immune complexes bound to Fc γ Rs with a much lower affinity than complexes formed by Ab-h1.9d-WT due to the engineered mutations in Ab-Vedo Fc domain to reduce Fc functions.

[00234] Vedolizumab demonstrated modest efficacy in two clinical studies for HIV studies. This efficacy can be attributable to Fab-dependent (e.g., antibody binding to $\alpha 4\beta 7$ disrupting its interactions with its ligands such as MADCAM-1 and HIV gp120, thus inhibiting the CD4 T cell co-stimulation and the HIV replication in these stimulated cells, and cell-to-cell viral transmission), but not Fc-dependent mechanism of actions. The reduced binding affinity of vedolizumab to Fc γ Rs renders it deficient in mediating Fc-dependent mechanisms. In contrast, Ab-h1.9d-WT, which has intact Fc functionality, may be positively differentiated from vedolizumab for its ability to induce sustained HIV viral suppression through its Fc-dependent mechanisms of action. The binding of the immune complexes formed by HIV virions and anti- $\alpha 4\beta 7$ antibodies (with intact Fc domain) to Fc γ Rs on APCs is required to induce new and durable HIV-specific immune responses (vaccination effect). Indeed, Ab-h1.9d-WT can mediate the uptake of $\alpha 4\beta 7$ -coated beads or $\alpha 4\beta 7$ -expressing GFP+VLPs (viral like particles) in an $\alpha 4\beta 7$ - and Fc-dependent manner in THP-1 cells. In summary, Ab-h1.9d-WT demonstrates activity in several proposed mechanisms of action for HIV control, including those that are Fc-dependent or Fab-dependent. Therefore, Ab-h1.9d-WT is predicted to be a more potent

agent than vedolizumab for sustained reduction of HIV viral load due to its higher affinity to $\alpha 4\beta 7$ and its intact Fc functionality to induce “vaccination effect”.

[00235] Key attributes of Ab-h1.9d-WT from a comprehensive in vitro characterization are summarized in TABLE 35.

TABLE 35	
Ab-h1.9d-WT In Vitro Characterization Summary	
Binding EC₅₀ (pM)	
HuT78 T cells	26
Human/Cynomolgus lymphocytes	130/62
Human/Cynomolgus CD4+ memory T cells	20/10
Human/Cynomolgus CD8+ memory T cells	52/36
MAdCAM-1 Blockade Potency IC₅₀ (pM)	
HuT78 T cells	50
Human lymphocytes	223
Binding Specificity	
$\alpha 4\beta 7$	Yes
$\alpha 4\beta 1$	No
Non-specific Binding	
HEK293	No
Human and Cynomolgus FcγR Binding	
Expected WT IgG1 binding	
Human and Cynomolgus FcRn Binding	
Expected binding at pH 6.0, no binding at pH 7.4	
In Vitro Fc Mediated Effector Activities	
ADCC	No
ADCC	No
CDC	No

[00236] Ab-h1.9d-WT is an antagonistic anti- $\alpha 4\beta 7$ human IgG1/k monoclonal antibody that binds to $\alpha 4\beta 7$ but not to $\alpha 4\beta 1$ and minimally to $\alpha E\beta 7$. Ab-h1.9d-WT binds strongly to both human and cynomolgus monkey CD4+ and CD8+ T subsets, demonstrating excellent cynomolgus binding cross-reactivity. However, Ab-h1.9d-WT does not bind to rodent PBMCs. Ab-h1.9d-WT selectively blocks $\alpha 4\beta 7$ /MAdCAM-1 interaction with high potency without inhibiting $\alpha 4\beta 7$ /VCAM-1 interaction. Ab-h1.9d-WT is capable of blocking MAdCAM-1-mediated co-stimulation of human primary CD4+ T cells. As expected for a human IgG1, Ab-h1.9d-WT binds to human Fc γ Rs (a necessary prerequisite for Fc-mediated "vaccination effect" in vivo) without triggering ADCC, ADCP and CDC activities against uninfected $\alpha 4\beta 7$ + cells in vitro. Lack of in vitro Fc effector activities by Ab-h1.9d-WT may be partly explained by the reduced cell surface $\alpha 4\beta 7$ expression due to antibody-induced target internalization. Additionally, Ab-h1.9d-WT can mediate the uptake of $\alpha 4\beta 7$ -coated

beads or $\alpha 4\beta 7$ -expressing VLPs (viral like particles) in an $\alpha 4\beta 7$ -dependent and Fc-dependent manner in THP-1 cells. Ab-h1.9d-WT exhibits the intended in vitro pharmacological properties necessary for clinical candidacy.

[00237] As provided in the disclosure, Ab-h1.9d-WT is a potent $\alpha 4\beta 7$ -selective antagonist that is differentiated and improved from vedolizumab. The key attributes of Ab-h1.9d-WT in comparison to Ab-Vedo are shown in TABLES 36-40 and summarized in Table 41.

TABLE 36 Ab-h1.9d-WT binding EC ₅₀ to human and cynomolgus monkey blood derived CD4+ and CD8+ T subsets in comparison to Ab-Vedo							
		CD4+ Binding EC ₅₀ pM*			CD8+ Binding EC ₅₀ pM*		
		CD4+ total	CD4+ naïve	CD4+ memory	CD8+ total	CD8+ naïve	CD8+ memory
Ab-h1.9d-WT	Human	63 ± 38	84 ± 32	20 ± 12	165 ± 121	144 ± 120	52 ± 51
	Cyno	30 ± 16	45 ± 27	10 ± 12	35 ± 25	42 ± 26	36 ± 30
Ab-Vedo	Human	381 ± 61	381 ± 101	270 ± 209	658 ± 438	727 ± 725	258 ± 203
	Cyno	202 ± 149	228 ± 229	148 ± 229	199 ± 101	256 ± 189	204 ± 125

TABLE 37 Ab-h1.9d-WT binding EC ₅₀ to human blood derived CD4+ and CD8+ T subsets in comparison to Ab-Vedo							
		CD4+ Binding EC ₅₀ pM*			CD8+ Binding EC ₅₀ pM*		
		CD4+ total	CD4+ naïve	CD4+ memory	CD8+ total	CD8+ naïve	CD8+ memory
Ab-h1.9d-WT	Human	63 ± 38	84 ± 32	20 ± 12	165 ± 121	144 ± 120	52 ± 51
Ab-Vedo	Human	381 ± 61	381 ± 101	270 ± 209	658 ± 438	727 ± 725	258 ± 203

Human PBMC = 3 donors

TABLE 38 Ab-h1.9d-WT binding EC ₅₀ to cynomolgus monkey blood derived CD4+ and CD8+ T subsets in comparison to Ab-Vedo							
		CD4+ Binding EC ₅₀ pM*			CD8+ Binding EC ₅₀ pM*		
		CD4+ total	CD4+ naïve	CD4+ memory	CD8+ total	CD8+ naïve	CD8+ memory
Ab-h1.9d-WT	Cyno	30 ± 16	45 ± 27	10 ± 12	35 ± 25	42 ± 26	36 ± 30
Ab-Vedo	Cyno	202 ± 149	228 ± 229	148 ± 229	199 ± 101	256 ± 189	204 ± 125

Cynomolgus PBMC = 5 donors

CD4+ naïve = CD4+CD45RA+CCR7+ cells, CD4+ memory = CD4+CD45RA-cells

CD8+ naïve = CD8+CD45RA+CCR7+ cells, CD8+ memory = CD8+CD45RA-cells

[00238] TABLE 39 – Binding Affinity of Ab-h1.9d-WT to Human FcγRs in comparison to Ab-Vedo via BIAcore.

TABLE 39						
Binding Affinity of Ab-h1.9d-WT to Human FcγRs in comparison to Ab-Vedo via BIAcore						
	FcγRI	FcγRIIb	FcγRIIa 131R	FcγRIIa 131H	FcγRIIIa158F	FcγRIIIa158V
Ab-h1.9d-WT	7.0E-09	1.2E-05	5.2E-06	4.0E-06	3.1E-06	1.0E-07
Ab-Vedo	No significant binding					

N=1

[00239] TABLE 40 – Binding Affinity of Ab-h1.9d-WT to cynomolgus monkey FcγRs in comparison to Ab-Vedo via BIAcore.

TABLE 40				
Binding Affinity of Ab-h1.9d-WT to cynomolgus monkey FcγRs in comparison to Ab-Vedo via BIAcore				
	Cyno FcγRI	Cyno FcγRIIa	Cyno FcγRIIb	Cyno FcγRIII
Ab-h1.9d-WT	1.4E-09	8.2E-06	7.1E-06	1.6E-07
Ab-Vedo	Binds but too weak to measure			

N=1

[00240] TABLE 41 – Key attributes of Ab-h1.9d-WT in comparison to Ab-Vedo.

TABLE 41		
Key attributes of Ab-h1.9d-WT in comparison to Ab-Vedo		
	Ab-h1.9d-WT	Ab-Vedo
CDR sequences	Unique	
¹ Binding EC50 to HuT78 cells (Flow cytometry)	199 pM	911 pM
² Binding EC50 to human lymphocytes	130 ± 85 pM	502 ± 212 pM
² Binding EC50 to human CD4+ Tm cells	20 ± 12 pM	270 ± 209 pM
² Binding EC50 to cyno CD4+ Tm cells	10 ± 12 pM	148 ± 229 pM
¹ Potency IC50 on HuT 78 cells (FACS-based blockade of MAdCAM-1 binding)	50 ± 11 pM	193 ± 46 pM
² Potency IC50 on human lymphocytes (Plate-based cell adhesion blockade to MAdCAM-1)	223 ± 86 pM	630 ± 193 pM
Binding specificity	Binds to α4β7 No binding to α4β1	Binds to α4β7 No binding to α4β1
Human and cyno FcγR binding	Binds as expected for human IgG1	No significant binding
In vitro Fc mediated ADCC, ADCP and CDC activities	No activity	No activity
Human and cyno FcRn binding	Binds at pH 6	Binds at pH 6

¹EC50 and IC50 values are based on ≥3 independent experiments

²Binding EC50 values are based on the PBMC isolated from ≥ 3 donors

[00241] The major parameters of Ab-h1.9d-WT, such as its better binding affinity to $\alpha 4\beta 7$ and its ability to bind human Fc γ Rs without triggering Fc mediated effector functions, are the key differentiation factors from vedolizumab and these characteristics are anticipated to drive the improved efficacy over vedolizumab. Ab-h1.9d-WT also possesses the *in vitro* pharmacological attributes required for an anti- $\alpha 4\beta 7$ clinical candidate.

8. EXEMPLARY EMBODIMENTS

[00242] While various specific embodiments have been illustrated and described, and some are represented below, it will be appreciated that various changes can be made without departing from the spirit and scope of the inventions(s).

1. An anti-human $\alpha 4\beta 7$ antibody which comprises (i) a VH chain region comprising three CDRs; and (ii) a VL chain region comprising three CDRs, wherein:
 - VH CDR#1 is GFNIKNTYMH (SEQ ID NO:72);
 - VH CDR#2 is RIDPAKGHTEYAPKFLG (SEQ ID NO:73);
 - VH CDR#3 is VDV (SEQ ID NO:74);
 - VL CDR#1 is HASQDISDNIG (SEQ ID NO:75);
 - VL CDR#2 is HGTNLED (SEQ ID NO:76); and
 - VL CDR#3 is VQYAQFPWT (SEQ ID NO:77).
2. The anti-human $\alpha 4\beta 7$ antibody of embodiment 1, which comprises
 - a VH chain region of:

EVQLVQSGAEVKKPGSSVKVSCKASGFNIKNTYMHWRQAPGQGLEWIGRIDP
AKGHTEYAPKFLGRVTITADESTNTAYMELSSLRSEDTAVYYCYVVDVWGQGT
TVTVSS (SEQ ID NO:70); and
 - a VL chain region of:

DIQMTQSPSSLSASVGDRVTITCHASQDISDNIGWLQKPKGSKFKLLIYHGTNLED
GVPSRFSGSGSGTDYTLTISSLQPEDFATYYCVQYAQFPWTFGGGTKVEIK
(SEQ ID NO:71).
3. The anti-human $\alpha 4\beta 7$ antibody of embodiment 1 or 2, which is humanized.
4. The anti-human $\alpha 4\beta 7$ antibody of any of embodiments 1-3, which is an IgG.

5. The anti-human $\alpha 4\beta 7$ antibody of any of embodiments 1-4, comprising a kappa light constant region.
6. The anti-human $\alpha 4\beta 7$ antibody of any one of embodiments 1-5, which is an IgG₁.
7. The anti-human $\alpha 4\beta 7$ antibody of any one of embodiments 1-6, comprising a variant CH3 domain having amino acid substitutions D356E and L358M.
8. The anti-human $\alpha 4\beta 7$ antibody of any one of embodiments 1-7, which comprises a heavy chain having an amino acid sequence of SEQ ID NO:92 or SEQ ID NO:93, and a light chain having an amino acid sequence of SEQ ID NO:100.
9. The anti-human $\alpha 4\beta 7$ antibody of any one of embodiments 1-8, comprising a variant CH2 domain having amino acid substitutions L234A and/or L235A.
10. The anti-human $\alpha 4\beta 7$ antibody of any one of embodiments 1-9, comprising a variant CH2 domain having an amino acid substitution T250Q, and/or a variant CH3 domain having an amino acid substitution M428L.
11. A polynucleotide comprising a nucleotide sequence encoding an anti-human $\alpha 4\beta 7$ antibody, wherein the antibody comprises (i) a VH chain region comprising three CDRs; and (ii) a VL chain region comprising three CDRs, wherein:
 - VH CDR#1 is GFNIKNTYMH (SEQ ID NO:72);
 - VH CDR#2 is RIDPAKGHTEYAPKFLG (SEQ ID NO:73);
 - VH CDR#3 is VDV (SEQ ID NO:74);
 - VL CDR#1 is HASQDISDNIG (SEQ ID NO:75);
 - VL CDR#2 is HGTNLED (SEQ ID NO:76); and
 - VL CDR#3 is VQYAQFPWT (SEQ ID NO:77).
12. An expression vector comprising the polynucleotide of embodiment 11.
13. A eukaryotic host cell transfected with the vector of embodiment 12.
14. A eukaryotic host cell engineered to express the polynucleotide of embodiment 11.
15. The eukaryotic host cell of embodiment 13 or 14, which is a mammalian host cell.

16. A method of producing an anti-human $\alpha 4\beta 7$ antibody, comprising: (a) culturing the eukaryotic host cell of embodiment 15 and (b) recovering the anti-human $\alpha 4\beta 7$ antibody.
17. A prokaryotic host cell transformed with the vector of embodiment 12.
18. A prokaryotic host cell engineered to express the polynucleotide of embodiment 17.
19. The prokaryotic host cell of embodiment 18, which is a bacterial host cell.
20. A method of producing an anti-human $\alpha 4\beta 7$ antibody, comprising: (a) culturing the prokaryotic host cell of embodiment 19 and (b) recovering the anti-human $\alpha 4\beta 7$ antibody.
21. A method of inducing viral suppression of HIV infection in an HIV-infected subject, comprising administering to the subject an amount of the anti-human $\alpha 4\beta 7$ antibody of any one of embodiments 1-10.
22. The method of embodiment 21, wherein the viral suppression is immune-mediated.
23. A method of treating HIV infection in an HIV-infected subject, comprising administering to the subject an amount of the anti-human $\alpha 4\beta 7$ antibody of any one of embodiments 1-10.
24. An anti-human $\alpha 4\beta 7$ antibody, which suppresses HIV.
25. The anti-human $\alpha 4\beta 7$ antibody of embodiment 24, which inhibits HIV replication and/or HIV infection.
26. The anti-human $\alpha 4\beta 7$ antibody of embodiment 24, which inactivates and/or reduces activation of human $\alpha 4\beta 7$ on cells expressing human $\alpha 4\beta 7$.
27. The anti-human $\alpha 4\beta 7$ antibody of embodiment 24, which induces internalization of human $\alpha 4\beta 7$ on cells expressing human $\alpha 4\beta 7$.
28. The anti-human $\alpha 4\beta 7$ antibody of embodiment 24, which inhibits CD4 T cell co-stimulation.
29. The anti-human $\alpha 4\beta 7$ antibody of embodiment 28, wherein CD4 T cell co-stimulation is mediated by MAdCAM-1 or HIV gp120.

30. The anti-human $\alpha 4\beta 7$ antibody of embodiment 25, which inhibits HIV replication in CD4 T cells.
31. The anti-human $\alpha 4\beta 7$ antibody of embodiment 30, wherein the CD4 T cells are MAdCAM-1 stimulated CD4 T cells or HIV gp120 stimulated CD4 T cells.
32. The anti-human $\alpha 4\beta 7$ antibody of embodiment 24, which disrupts interaction of $\alpha 4\beta 7$ with at least one of its ligands.
33. The anti-human $\alpha 4\beta 7$ antibody of embodiment 32, wherein the $\alpha 4\beta 7$ ligand is MAdCAM-1.
34. The anti-human $\alpha 4\beta 7$ antibody of embodiment 32, wherein the $\alpha 4\beta 7$ ligand is HIV gp120.
35. The anti-human $\alpha 4\beta 7$ antibody of embodiment 34, which inhibits gp120-mediated cell-to-cell transmission of HIV.
36. The anti-human $\alpha 4\beta 7$ antibody of embodiment 24, which binds to an HIV virion.
37. The anti-human $\alpha 4\beta 7$ antibody of embodiment 36, wherein the antibody binding to the HIV virion forms an immune complex.
38. The anti-human $\alpha 4\beta 7$ antibody of embodiment 37, wherein the immune complex binds to a Fc γ R on a cell.
39. The anti-human $\alpha 4\beta 7$ antibody of embodiment 37, wherein the immune complex binds to a Fc γ R on an antigen presenting cell (APC).
40. The anti-human $\alpha 4\beta 7$ antibody of embodiment 39, wherein the immune complex is taken up by the APC by phagocytosis.
41. The anti-human $\alpha 4\beta 7$ antibody of embodiment 40, which induces an HIV-specific immune response.
42. The anti-human $\alpha 4\beta 7$ antibody of embodiment 40 or 41, which induces a vaccination effect against HIV.

43. The anti-human $\alpha 4\beta 7$ antibody of embodiment 41, wherein the HIV-specific immune response results in viral control of HIV in a HIV-infected individual.
44. The anti-human $\alpha 4\beta 7$ antibody of embodiment 43, wherein the viral control results in reduced viral load
45. The anti-human $\alpha 4\beta 7$ antibody of embodiment 43, wherein the viral control results in lower viral setpoint
46. The anti-human $\alpha 4\beta 7$ antibody of embodiment 43, wherein the viral control results in delay in viral rebound after an antiretroviral treatment interruption (ATI).
47. The anti-human $\alpha 4\beta 7$ antibody of any one of embodiments 24-46, having a set of six complementary determining regions (CDRs) or the variable heavy chain region and variable light chain region from an antibody selected from Ab-m1, Ab-c1, Ab-h1.1, Ab-h1.2, Ab-h1.3, Ab-h1.4, Ab-h1.5, Ab-h1.6, Ab-h1.7, Ab-h1.8, Ab-h1.9, Ab-h1.9a, Ab-h1.9b, Ab-h1.9c, Ab-h1.9d, or Ab-h1.9e.
48. The anti-human $\alpha 4\beta 7$ antibody of any one of embodiments 1-10, which suppresses HIV.
49. The anti-human $\alpha 4\beta 7$ antibody of embodiment 48, which inhibits HIV replication and/or HIV infection.
50. The anti-human $\alpha 4\beta 7$ antibody of embodiment 48, which inactivates and/or reduces activation of human $\alpha 4\beta 7$ on cells expressing human $\alpha 4\beta 7$.
51. The anti-human $\alpha 4\beta 7$ antibody of embodiment 48, which induces internalization of human $\alpha 4\beta 7$ on cells expressing human $\alpha 4\beta 7$.
52. The anti-human $\alpha 4\beta 7$ antibody of embodiment 48, which inhibits CD4 T cell co-stimulation.
53. The anti-human $\alpha 4\beta 7$ antibody of embodiment 52, wherein CD4 T cell co-stimulation is mediated by MAdCAM-1 or HIV gp120.
54. The anti-human $\alpha 4\beta 7$ antibody of embodiment 49, which inhibits HIV replication in CD4 T cells.

55. The anti-human $\alpha 4\beta 7$ antibody of embodiment 54, wherein the CD4 T cells are MAdCAM-1 stimulated CD4 T cells or HIV gp120 stimulated CD4 T cells.
56. The anti-human $\alpha 4\beta 7$ antibody of embodiment 48, which disrupts interaction of $\alpha 4\beta 7$ with at least one of its ligands.
57. The anti-human $\alpha 4\beta 7$ antibody of embodiment 56, wherein the $\alpha 4\beta 7$ ligand is MAdCAM-1.
58. The anti-human $\alpha 4\beta 7$ antibody of embodiment 56, wherein the $\alpha 4\beta 7$ ligand is HIV gp120.
59. The anti-human $\alpha 4\beta 7$ antibody of embodiment 58, which inhibits gp120-mediated cell-to-cell transmission of HIV.
60. The anti-human $\alpha 4\beta 7$ antibody of embodiment 48, which binds to an HIV virion.
61. The anti-human $\alpha 4\beta 7$ antibody of embodiment 60, wherein the antibody binding to the HIV virion forms an immune complex.
62. The anti-human $\alpha 4\beta 7$ antibody of embodiment 61, wherein the immune complex binds to a Fc γ R on a cell.
63. The anti-human $\alpha 4\beta 7$ antibody of embodiment 61, wherein the immune complex binds to a Fc γ R on an antigen presenting cell (APC).
64. The anti-human $\alpha 4\beta 7$ antibody of embodiment 63, wherein the immune complex is taken up by the APC by phagocytosis.
65. The anti-human $\alpha 4\beta 7$ antibody of embodiment 64, which induces an HIV-specific immune response.
66. The anti-human $\alpha 4\beta 7$ antibody of embodiment 64 or 65, which induces a vaccination effect against HIV.
67. The anti-human $\alpha 4\beta 7$ antibody of embodiment 64 or 65, wherein the HIV-specific immune response results in viral control of HIV in a HIV-infected individual.

68. The anti-human $\alpha 4\beta 7$ antibody of embodiment 67, wherein the viral control results in reduced viral load.
69. The anti-human $\alpha 4\beta 7$ antibody of embodiment 67, wherein the viral control results in lower viral setpoint.
70. The anti-human $\alpha 4\beta 7$ antibody of embodiment 67, wherein the viral control results in delay in viral rebound after an antiretroviral treatment interruption (ATI).
71. A pharmaceutical composition comprising an antibody of any one of the preceding embodiments.

WHAT IS CLAIMED:

1. An anti-human $\alpha 4\beta 7$ antibody which comprises (i) a VH chain region comprising three CDRs; and (ii) a VL chain region comprising three CDRs, wherein:
VH CDR#1 is GFNIKNTYMH (SEQ ID NO:72);
VH CDR#2 is RIDPAKGHTEYAPKFLG (SEQ ID NO:73);
VH CDR#3 is VDV (SEQ ID NO:74);
VL CDR#1 is HASQDISDNIG (SEQ ID NO:75);
VL CDR#2 is HGTNLED (SEQ ID NO:76); and
VL CDR#3 is VQYAQFPWT (SEQ ID NO:77).
2. The anti-human $\alpha 4\beta 7$ antibody of claim 1, which comprises
a VH chain region of:
EVQLVQSGAEVKKPGSSVKVSCKASGFNIKNTYMHWVRQAPGQGLEWIGRIDP
AKGHTEYAPKFLGRVTITADESTNTAYMELSSLRSEDTAVYYCYVVDVWGQGT
TVTVSS (SEQ ID NO:70); and
a VL chain region of:
DIQMTQSPSSLSASVGDRVTITCHASQDISDNIGWLQKPKGKSFKLLIYHGTNLED
GVPSRFSGSGSGTDYTLTISSLQPEDFATYYCVQYAQFPWTFGGGTKVEIK
(SEQ ID NO:71).
3. The anti-human $\alpha 4\beta 7$ antibody of claim 2, which is an IgG.
4. The anti-human $\alpha 4\beta 7$ antibody of claim 3, comprising a kappa light constant region.
5. The anti-human $\alpha 4\beta 7$ antibody of claim 4, which is an IgG₁.
6. The anti-human $\alpha 4\beta 7$ antibody of claim 5, comprising a variant CH3 domain having amino acid substitutions D356E and L358M.
7. The anti-human $\alpha 4\beta 7$ antibody of claim 1, which comprises a heavy chain having an amino acid sequence of SEQ ID NO:92 or SEQ ID NO:93, and a light chain having an amino acid sequence of SEQ ID NO:100.

8. The anti-human $\alpha 4\beta 7$ antibody of claim 1, which is an IgG1 antibody comprising heavy chains each consisting of the amino acid sequence of SEQ ID NO:92, and light chains each consisting of the amino acid sequence of SEQ ID NO:100.
9. The anti-human $\alpha 4\beta 7$ antibody of claim 1, which is an IgG1 antibody comprising heavy chains each consisting of the amino acid sequence of SEQ ID NO:93, and light chains each consisting of the amino acid sequence of SEQ ID NO:100.
10. A polynucleotide comprising a nucleotide sequence encoding an anti-human $\alpha 4\beta 7$ antibody, wherein the antibody comprises (i) a VH chain region comprising three CDRs; and (ii) a VL chain region comprising three CDRs, wherein:
 - VH CDR#1 is GFNIKNTYMH (SEQ ID NO:72);
 - VH CDR#2 is RIDPAKGHTEYAPKFLG (SEQ ID NO:73);
 - VH CDR#3 is VDV (SEQ ID NO:74);
 - VL CDR#1 is HASQDISDNIG (SEQ ID NO:75);
 - VL CDR#2 is HGTNLED (SEQ ID NO:76); and
 - VL CDR#3 is VQYAQFPWT (SEQ ID NO:77).
11. An expression vector comprising the polynucleotide of claim 10.
12. A eukaryotic host cell transfected with the vector of claim 11.
13. The eukaryotic host cell of claim 12, which is a mammalian host cell.
14. A method of producing an anti-human $\alpha 4\beta 7$ antibody, comprising: (a) culturing the eukaryotic host cell of claim 13 and (b) recovering the anti-human $\alpha 4\beta 7$ antibody.
15. A method of inducing viral suppression of HIV infection in an HIV-infected subject, comprising administering to the subject an amount of the anti-human $\alpha 4\beta 7$ antibody of any one of claims 1-9.
16. A method of treating HIV infection in an HIV-infected subject, comprising administering to the subject an amount of the anti-human $\alpha 4\beta 7$ antibody of any one of claims 1-9.
17. A pharmaceutical composition comprising an antibody of any one of the claims 1-9.