ABBV-382, an Anti-α4β7 Ab That Enhances HIV-1 Antigen Presentation for Immune-Mediated Viral Control

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BACKGROUND

- The α4β7 integrin is a heterodimeric receptor expressed on T cell subsets, B cells, NK cells and other immune cells
- A recent study showed that α4β7 is present on the envelope of HIV-1 virions[1], suggesting that α4β7 could be a highly conserved target for HIV-1 treatment
- ABBV-382 is a novel anti-human α4β7 monoclonal antibody (mAb) with preserved Fc functionality
- Objective: To characterize the biological characteristics and mechanisms of action of ABBV-382 as a potential agent for immune-mediated HIV-1 control

MATERIALS AND METHODS

Table 1. Antibodies*

Name	Description
ABBV-382	Human anti-α4β7 [hu IgG1/k]
ABBV-382 LALA	ABBV-382 with LALA substitutions in the Fc domain
Anti-α4β7 Ctrl	Human anti-α4β7 [hu IgG1/k] control mAb# with LALA substitutions in the Fc domain

*Research grade mAbs generated internally
Control mAb generated from published sequences for comparative purposes

- α4β7 virus-like particles (VLPs): α4β7-expressing VLPs encapsulated with GFP and HIV-1 Gag proteins; serve as surrogates of HIV-1 virions
- ABBV-382 was evaluated in vitro in biochemical, virological, immunosafety, and immunopeptidomics studies to characterize its properties and determine its mechanisms of action for immune-mediated HIV-1 control

RESULTS

ABBV-382 demonstrates high binding affinity and specificity to α4β7

Table 2. Key attributes of ABBV-382 in vitro (mean ± SD)

Attributes	ABBV-382	Anti-α4β7 Ctrl
Binding EC_{50} to HuT78 cells	199 ± 62 pM	911 ± 116 pM
Binding EC_{50} to human lymphocytes	130 ± 85 pM	502 ± 212 pM
Binding EC_{50} to human CD4+ Tm cells	20 ± 12 pM	270 ± 209 pM
Binding EC_{50} to cynomolgus CD4+ Tm cells	10 ± 12 pM	148 ± 229 pM
Potency IC_{50} on HuT78 cells (FACS-based blockade of MAdCAM-1 binding)	50 ± 11 pM	193 ± 46 pM
Potency IC_{50} on human lymphocytes (FACS-based blockade of MAdCAM-1 binding)	223 ± 86 pM	630 ± 193 pM
Binding specificity (recombinant cells expressing α4β7, α4β1 or αEβ7)	Binds to α4β7 Does not bind to α4β1	Binds to α4β7 Does not bind to α4β1
Ab-mediated α4β7 internalization in cells	Yes	Yes
Human and cynomolgus FcγR binding	Yes	No significant binding
In vitro Fc mediated ADCC, ADCP and CDC activities	No activity	No activity

- ABBV-382 blocks the interaction of α4β7 with its ligand MAdCAM-1
- ABBV-382 binds to FcγR, but does not elicit ADCC, ADCP or CDC

ABBV-382 blocks MAdCAM-1-dependent co-stimulation of human primary CD4+ T cells and HIV-1 replication in these cells

Co-stimulation of CD4+ T cells

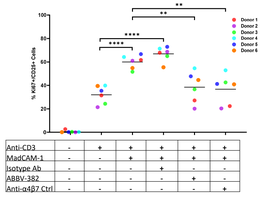


Fig. 1A. Activation of human primary CD4+ T cells by an anti-CD3 Ab and MAdCAM-1 in the presence of an isotype control Ab, ABBV-382 or Anti-α4β7 Ctrl mAb was measured as percentage of Ki67+CD25+ cells by flow cytometry analysis[2]

HIV-1 replication

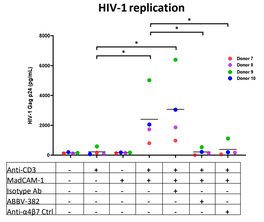


Fig. 1B. HIV-1 replication in human primary CD4+ T cells activated with anti-CD3 Ab and MAdCAM-1 in the presence of an isotype control Ab, ABBV-382 or Anti-α4β7 Ctrl mAb was measured by HIV-1 Gag p24 assay[2]

ABBV-382, a novel anti-human α4β7 monoclonal antibody, inhibits HIV-1 replication/cell-to-cell spread via direct antagonism of the interaction of α4β7 with its cognate ligand MAdCAM-1 or HIV-1 gp120. Additionally, ABBV-382 can bind to α4β7 incorporated in the HIV-1 virions to form immune-complexes (ICs). These ICs can bind to FcγRs expressed on antigen-presenting cells and enhance viral antigen presentation to T cells, potentially inducing immune responses to control viral replication.

ABBV-382 inhibits the interaction of α4β7 with HIV-1 gp120

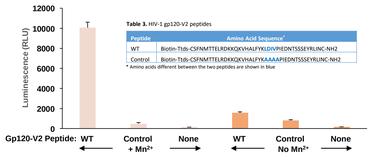


Fig. 2A. α4β7/gp120 binding assay. α4β7-expressing RPMI 8866 cells were incubated with HIV-1 gp120-V2 (WT or Control) peptides immobilized on a plate; bound cells were detected by CellTiter-Glo (luminescence assay)[3]

- HIV-1 gp120-V2 "WT" but not "Control" peptide binds to RPMI 8866 cells which constitutively express α4β7 on their cell surfaces
  - HIV-1 gp120-V2 "Control" peptide has substitutions in 4 amino acids reported to mediate the binding between α4β7 and gp120 (Table 3)
- Binding is greater when cells are treated with Mn^{2+} to activate α4β7 on the cell surface

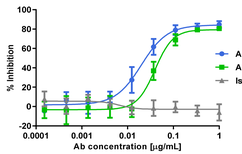


Fig. 2B. ABBV-382 and Anti-α4β7 Ctrl mAbs inhibit the binding of HIV-1 gp120-V2 WT peptide to α4β7 expressed on RPMI 8866 cells in a dose-responsive manner

- ABBV-382 inhibits the interaction of α4β7 with HIV-1 gp120, and thus is proposed to inhibit the cell-to-cell viral spread mediated by this interaction[4]

ABBV-382 can bind to virions from different HIV-1 strains/isolates

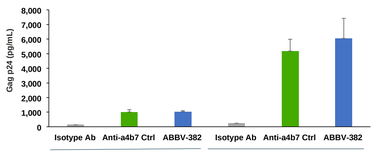


Fig. 3. Virion capture assay. HIV-1 (NL4-3) stocks were prepared with or without retinoic acid (RA, an enhancer of α4β7 expression). Viral stocks were incubated with ABBV-382 or control mAbs (15 nM) immobilized on magnetic beads. Quantity of HIV-1 virions captured by each mAb on beads was measured by the viral Gag p24 protein

- ABBV-382 can bind to and capture HIV-1 virions; it captures a larger quantity of virions from a viral stock prepared with than without RA

Table 4. HIV-1 virion capture with different HIV-1 strains/isolates (mean ± SD)

HIV-1				Virion Capture, EC_{50}	
Strain/ Isolate	Group	Subtype	Co-Receptor	ABBV-382 (nM)	Anti-α4β7 Ctrl (nM)
BCF06	O	-	X4/R5	0.18 ± 0.03	0.37 ± 0.07
CMU08	M	AE	X4	0.25 ± 0.02	0.50 ± 0.08
NL4-3	M	B	X4	0.16 ± 0.03	0.34 ± 0.02
RU570	M	G	R5	0.12 ± 0.06	0.32 ± 0.03
YBF30	N	-	R5	0.14 ± 0.06	0.48 ± 0.15

- ABBV-382 can bind to virions from different HIV-1 strains/isolates to form immune complexes (ICs)
- ABBV-382 does not neutralize these HIV-1 strains/isolates (data not shown)

ABBV-382 mediates uptake of α4β7 virus-like particles (VLPs)* in THP-1 cells

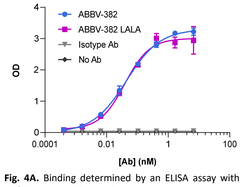


Fig. 4A. Binding determined by an ELISA assay with α4β7 VLPs coated on a plate

Table 5. Binding of mAbs to α4β7 VLPs

ELISA Assay	ABBV-382	ABBV-382 LALA
EC_{50} (nM)	0.050	0.048

- ABBV-382 and ABBV-382 LALA bind with similar EC_{50} values to α4β7 VLPs

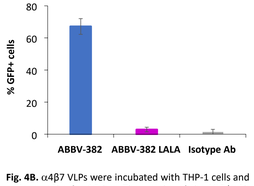


Fig. 4B. α4β7 VLPs were incubated with THP-1 cells and various mAbs for 16 hrs. The uptake of the VLP/mAb immune complexes in THP-1 cells was measured by percentage of GFP+ cells using flow cytometry

*α4β7 VLPs: α4β7-expressing VLPs encapsulated with GFP and HIV-1 Gag proteins; serve as surrogates of HIV-1 virions

- α4β7 VLP uptake by THP-1 cells is α4β7- and Fc-dependent

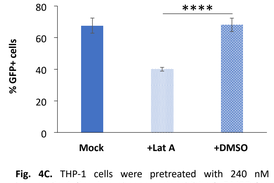


Fig. 4C. THP-1 cells were pretreated with 240 nM latrunculin A (Lat A, a phagocytosis inhibitor), DMSO (no Lat A) or no chemical (mock) for 2 hrs, and then incubated with α4β7 VLPs/ABBV-382 immune complexes. The uptake of the immune complexes was measured by percentage GFP+ cells using flow cytometry

- The uptake of α4β7 VLP/ABBV-382 immune complexes is mediated by ABBV-382-dependent phagocytosis of the complexes

Enhanced presentation of HIV-1 peptides in MHC Class II in THP-1 cells treated with immune complexes of ABBV-382/α4β7 VLPs

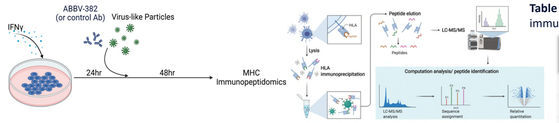


Fig. 5A. Schematic describing cell treatment and immunopeptidomics workflow

Table 6A. Detection statistics from a representative immunopeptidomics experiment with ABBV-382

MHC Class II Immunopeptidomics (FDR* 1%)	
No. of unique peptides	5,640
No. of proteins	1,052
HIV-1 peptides detected?	Yes

*FDR: False discovery rate

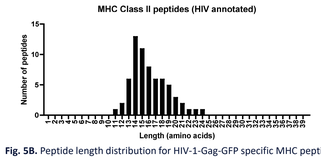


Fig. 5B. Peptide length distribution for HIV-1-Gag-GFP specific MHC peptides

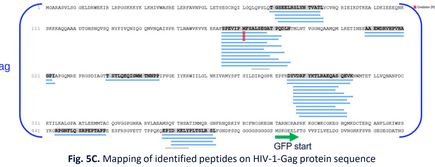


Fig. 5C. Mapping of identified peptides on HIV-1-Gag protein sequence

Table 6B. HIV-1-Gag-GFP protein and peptide detection statistics

Statistics of HIV-1 Gag-GFP Peptides Identified	
Protein length	759 amino acids
Peptide coverage	179 / 759 = 24%

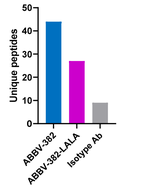


Fig. 5D. Number of identified unique peptides mapping to HIV-1-Gag-GFP in the indicated samples

- ABBV-382 treated cells presented more unique HIV-1 peptides in MHC class II complex than those treated with ABBV-382 LALA or isotype control Ab

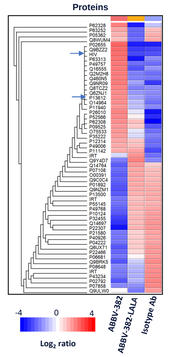


Fig. 5E. Heatmap displaying proteins with >3-fold change across ABBV-382, ABBV-382 LALA, and isotype Ab treated samples

- HIV-1-Gag (HIV) and α4 protein (P13612) are among the proteins with higher MHC Class II presentation with ABBV-382 treatment

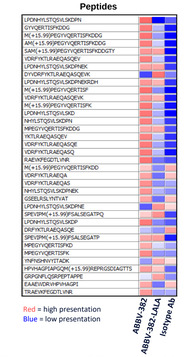
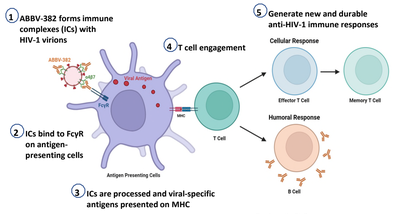


Fig. 5F. Higher MHC Class II presentation of peptides mapping to HIV-1-Gag-GFP in ABBV-382 treated samples

- 36 differentially presented peptides mapped to HIV-1-Gag-GFP, covering 23% of this protein
- Most of the 36 differentially presented HIV-1-Gag-GFP peptides were increased in ABBV-382 treated cells

Proposed model for the immune modulation mediated by ABBV-382



CONCLUSIONS

- ABBV-382 not only can block CD4+ T cell stimulation and HIV-1 infection by disrupting the interaction between α4β7 and its ligands MAdCAM-1 and HIV-1 gp120, but also can enhance antigen presentation of immune complexes formed by ABBV-382 and HIV-1 α4β7 VLPs in vitro, potentially inducing HIV-1-specific immune responses
- ABBV-382, in combination with budigalimab, an anti-PD1 mAb, (Late Breaker oral presentation, Monday, Abstract ID: 3325) is being studied in a Phase 2 study (NCT06032546) examining ART-free viral control

References:
1.Guzzo C et al. Sci Immunol. 2017;12;2(11)
2.Nawaz F et al. Mucosal Immunol. 2018;11(5)
3.Peachman KK et al. PLoS One. 2015;1;10(12)
4.Arthos J et al. Nat Immunol. 2008;9(3)

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