Cell-associated HIV: Antibody-based Strategies

Kevin J Whaley

Mapp Biopharmaceutical


Science
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ANTIBODIES
AAAS

Antibody-based Strategies for Cell-associated HIV

- Common Ab targets on cell free and cell-associated HIV
- Surface coating Ab targets derived from the male reproductive tract
- Translocation of Ab targets to plasma membrane of infected cells
- Cell-cell transmission and gp41 Abs (4E10, 2F5, 10E8)

SWOT Analysis

Transient Plant Production:

Strengths

Manufacturing speed
Fast gene to protein
Production yield
Low COGs
Safety benefits
Low regulatory risk

Weaknesses

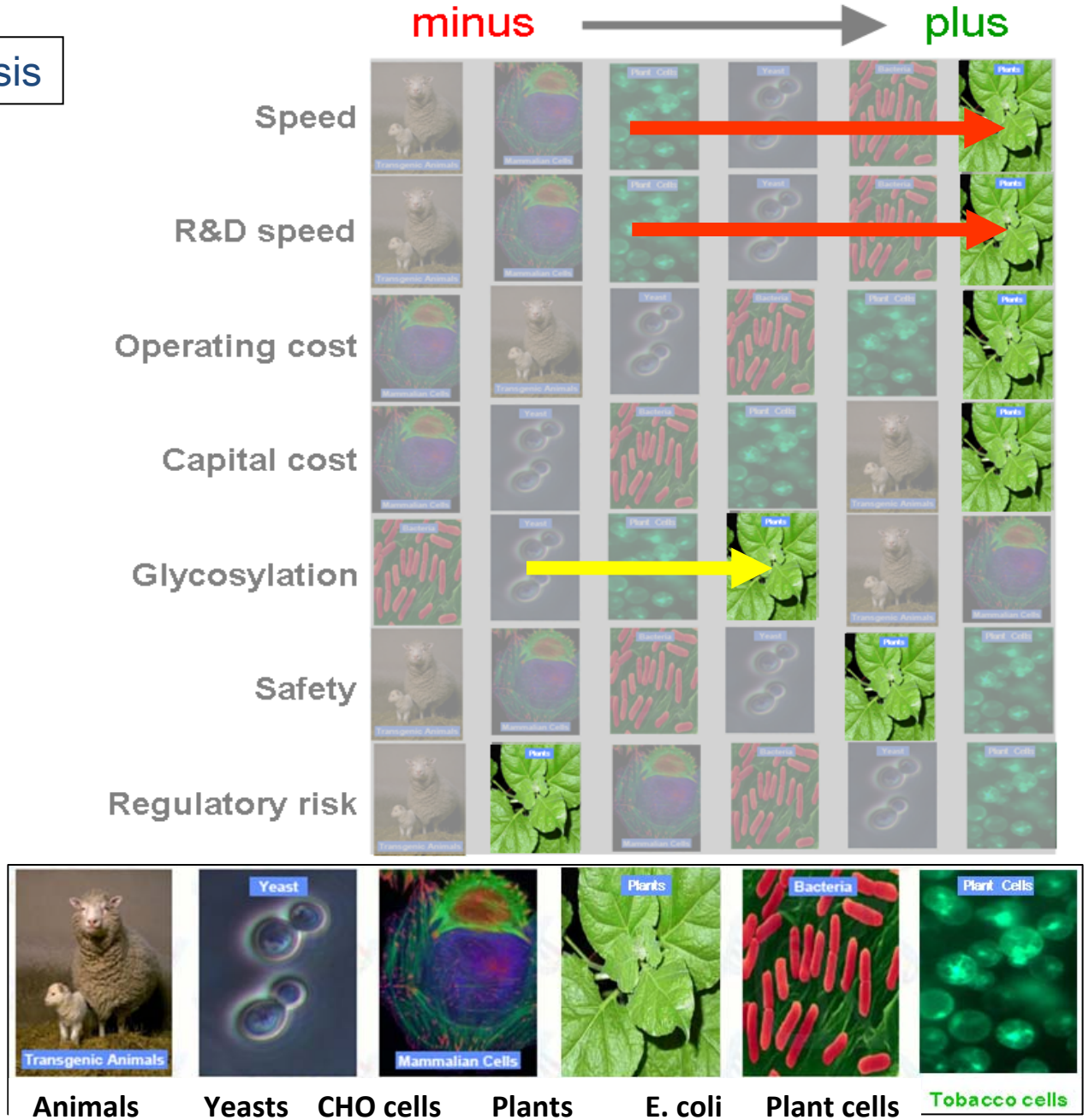
No approved product
No clear guidelines

Opportunities

Reduce COGs
Increase R&D&M speed
Increase flexibility

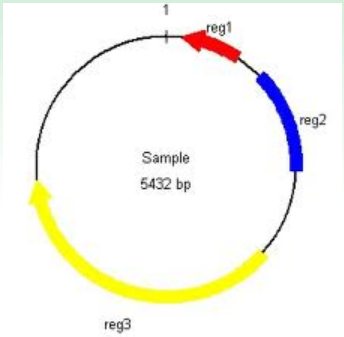
Threats

Regulatory burden

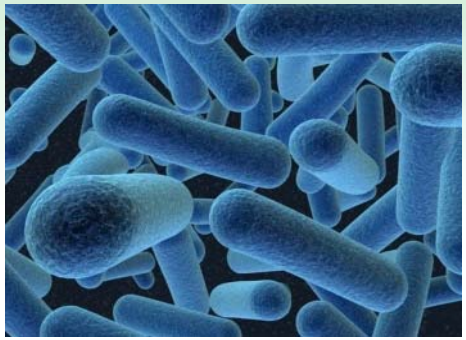


KBP
KENTUCKY BIOPROCESSING

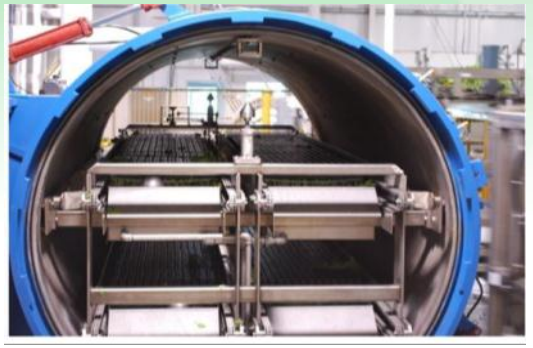
magnICON® Based Production



Plasmid Vector



Agrobacterium Strain Development



Infiltration Chamber

Plant AgroInfiltration

Extraction, Purification, Release and Stability Tasks

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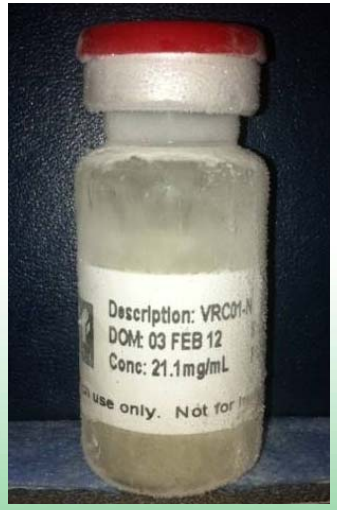
MB66 Large Scale Processing



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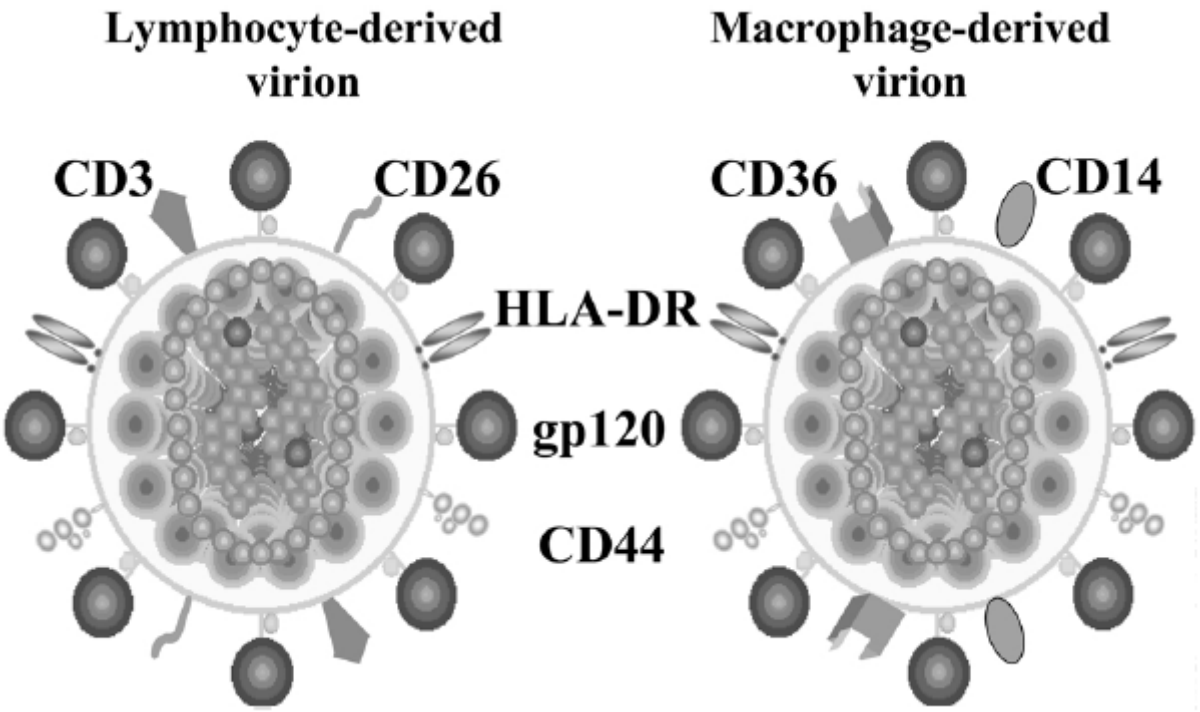
MB66 Downstream

- Extraction (complete cell rupture in presence of buffer)
- Clarification (removal of chlorophyll, cell debris, some host cell proteins)
- Protein A Affinity Chromatography
  - Slightly Alkaline Equilibration Conditions
  - Acidic elution conditions supplemented with Amino Acids
- Ion Exchange Q Resin (Negative Mode)
  - Intermediate Polish
  - Removes residual host cell proteins
  - Removes host cell DNA
  - Removes Endotoxin
- Ceramic Hydroxyapatite Type II 80 um or Type II 40 um
  - Final polish
  - Elution in Ion exchange mode with NaCl
  - Separate Aggregates and LMW species from Monomer
  - Reduce Endotoxin
- Ultrafiltration/Diafiltration
  - Concentration/Placement of mAb into appropriate stability buffer

Common Targets on the Surface of Cell-free and Cell-associated HIV

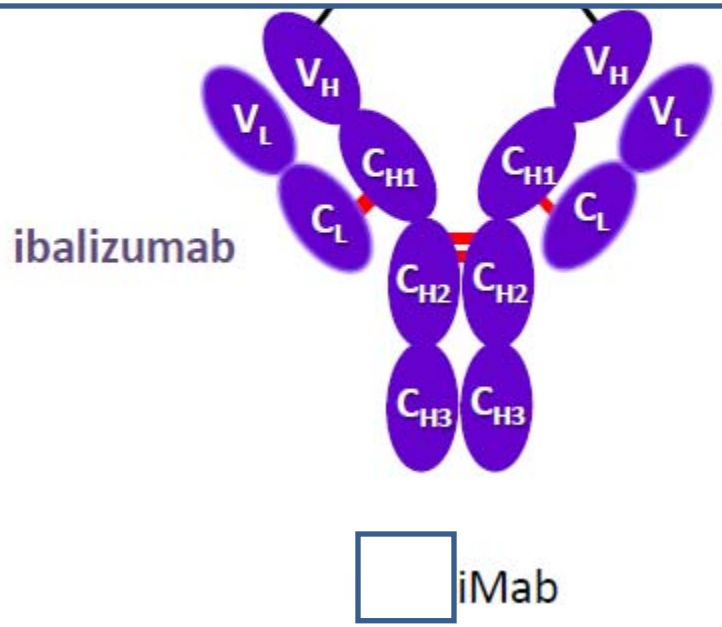


Lawn, J. Inf. 2004

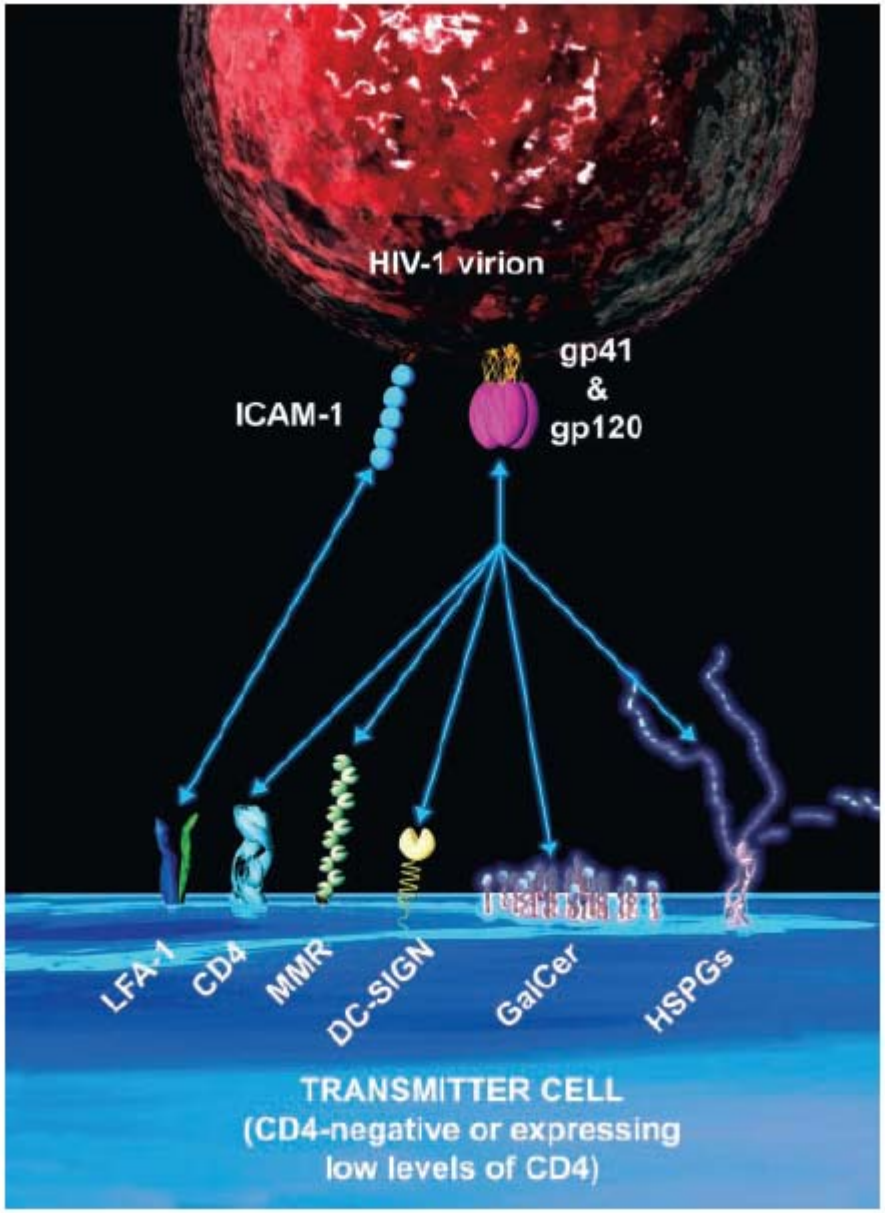
Ibalizumab

- Humanized anti-CD4 domain 2 mAb
- Safe and effective *in vivo*
- Recently completed Phase IIb and Phase 1 trials in HIV^{+} and HIV^{-} individuals, respectively

Not evaluated topically to prevent cell-associated HIV transmission



Pace, 2012



Bounou et al.,
FASEB J 2004

Table I. *Anti-mouse ICAM-1 blocks transmission of cell-associated HIV-1 in vivo*

Treatment	HIV-1-positive Mice/Total (%)
Experiment A	
PBS plus 1% BSA	4/5 (80)
Anti-human ICAM-1	7/7 (100)
Isotype control Ab	6/7 (86)
Experiment B	
PBS plus 1% BSA	5/6 (83)[a]
Anti-human ICAM-1 plus anti-mouse ICAM-1	1/9 (11)
Isotype control Ab	5/7 (71)[a]
Experiment C	
PBS plus 1% BSA	7/10 (70)
Anti-mouse ICAM-1	2/7 (29)[b]
Isotype control Ab	10/10 (100)[a]

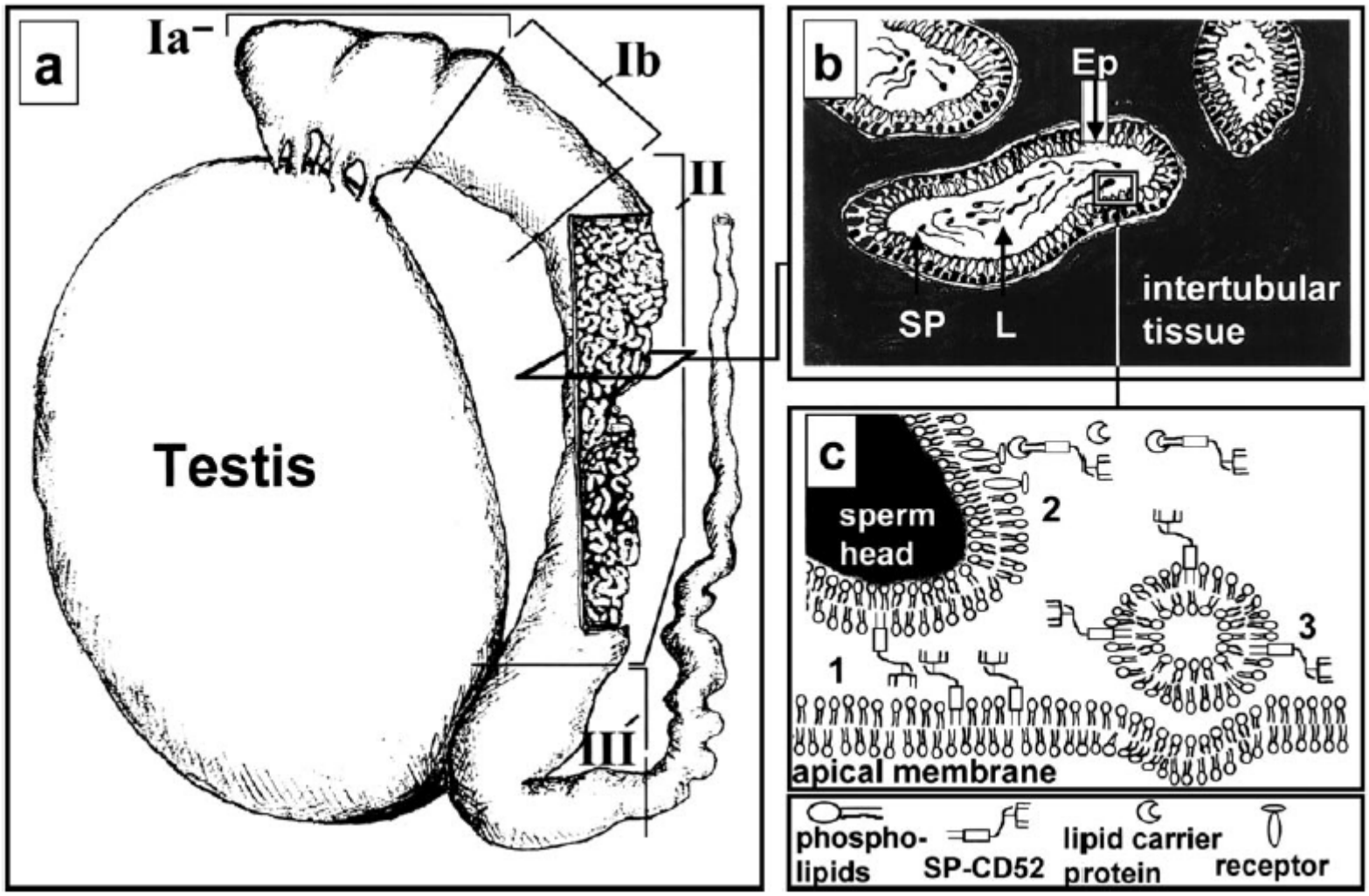
[a] $p < 0.05$ by Fisher's exact analysis, compared to anti-ICAM-1 treatment in corresponding experiment.

[b] Two to three mice were excluded for engraftment failure.

Khanna et al., J Immunol. 2006

ICAM1-N

- 1A6 humanized Ab
- Expresses >200mg/kg in Nicotiana (post-protein A)
- Pending evaluation in cell-associated models at BU



Kirchoff and Schroter, Cells, Tissues, Organs 2001

Antibody-based Contraception

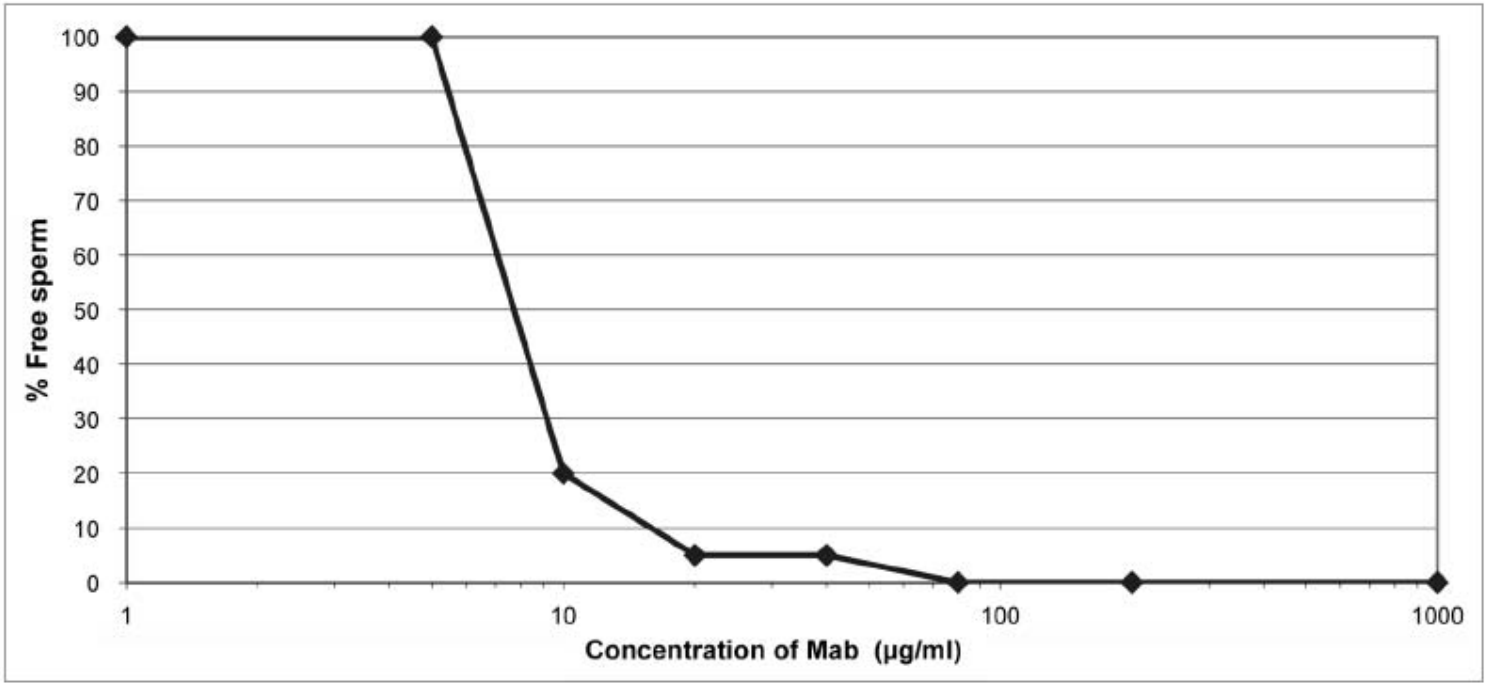
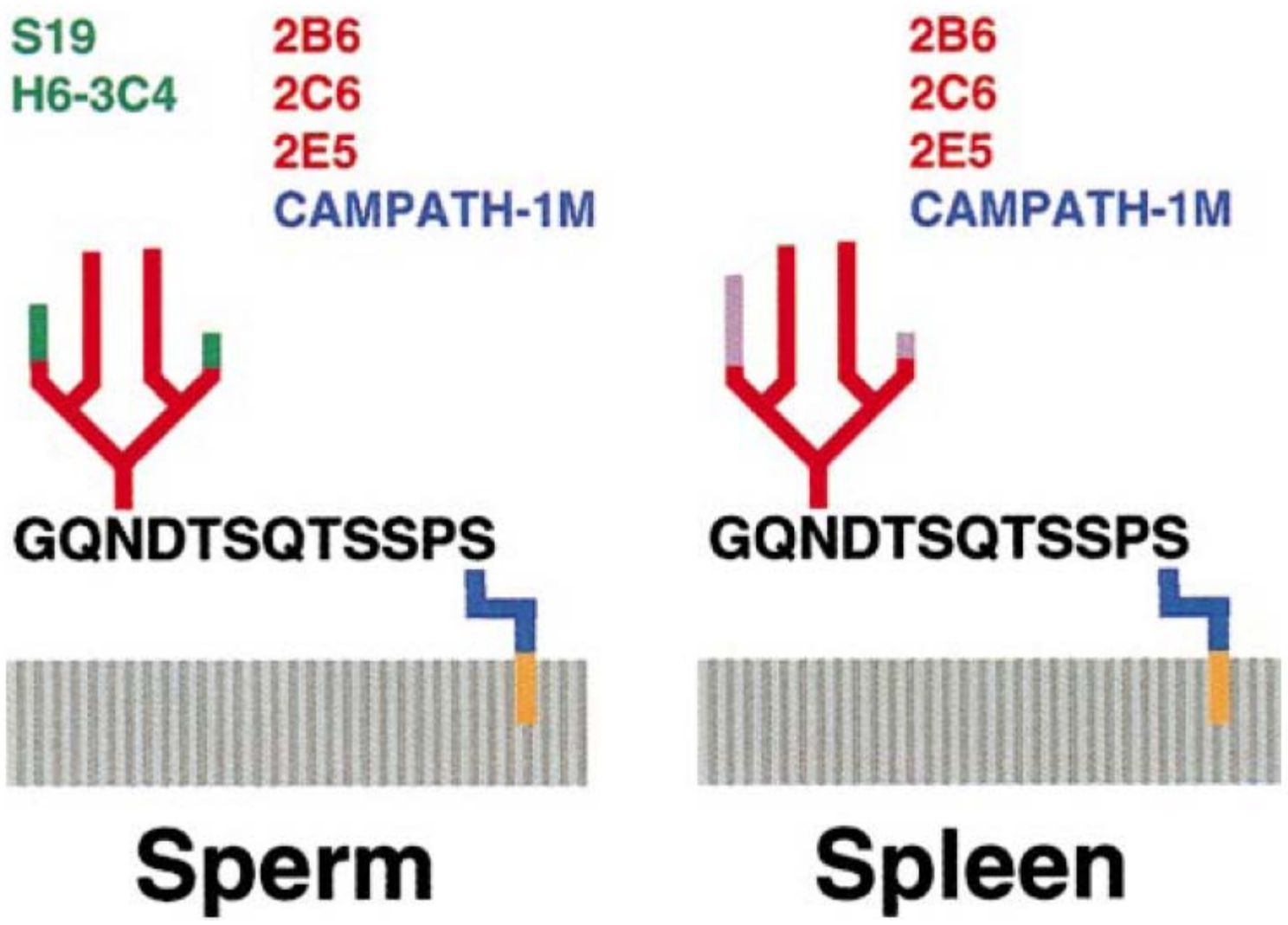
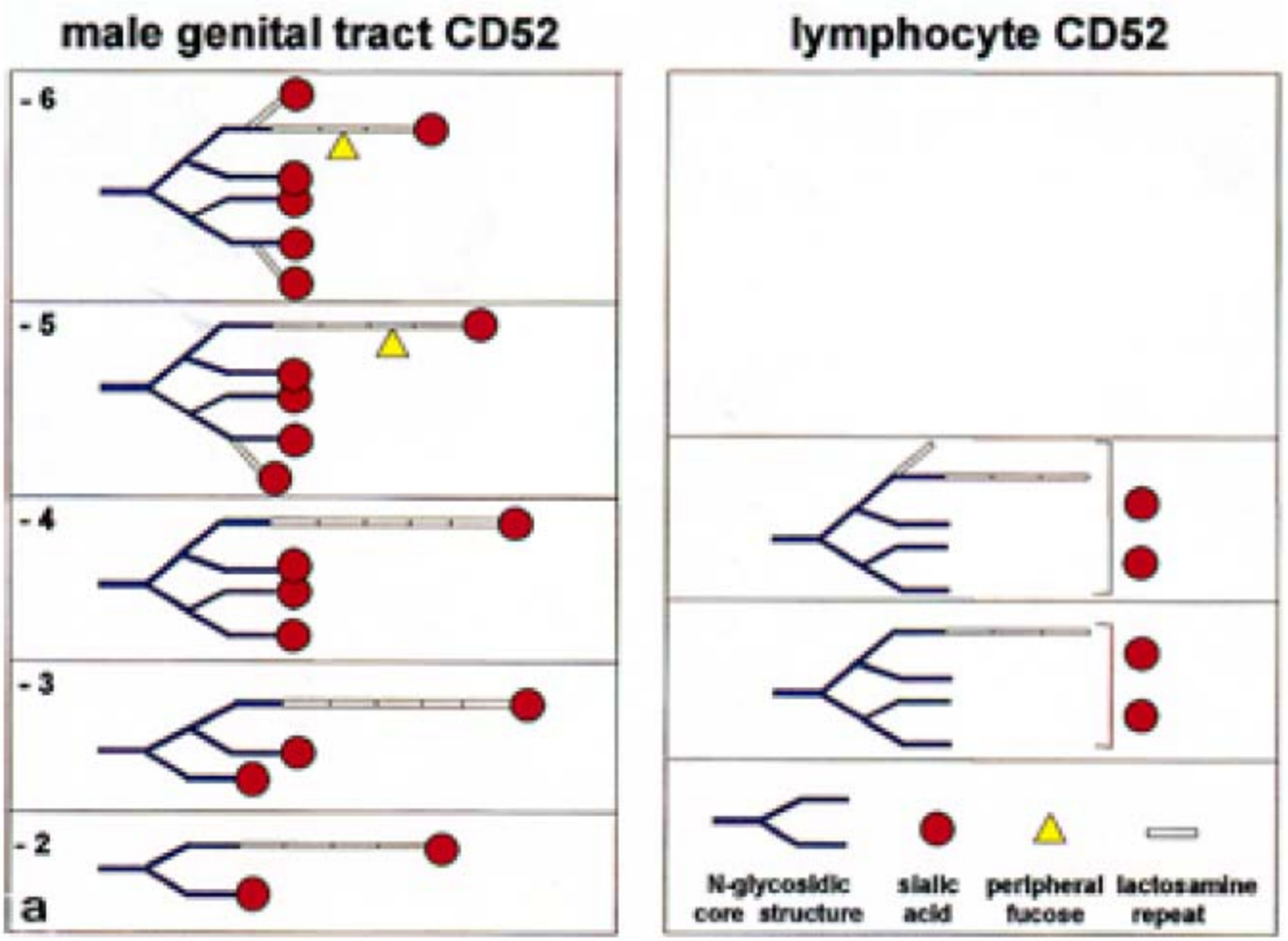


Figure 2. Agglutination of human sperm with Mab HC4 produced in *N. benthamiana*. Purified Mab was added to undiluted human semen and observed within 30 seconds via light microscopy.

(Whaley, Hiatt, Zeitlin,
Human Vaccines, 2011)



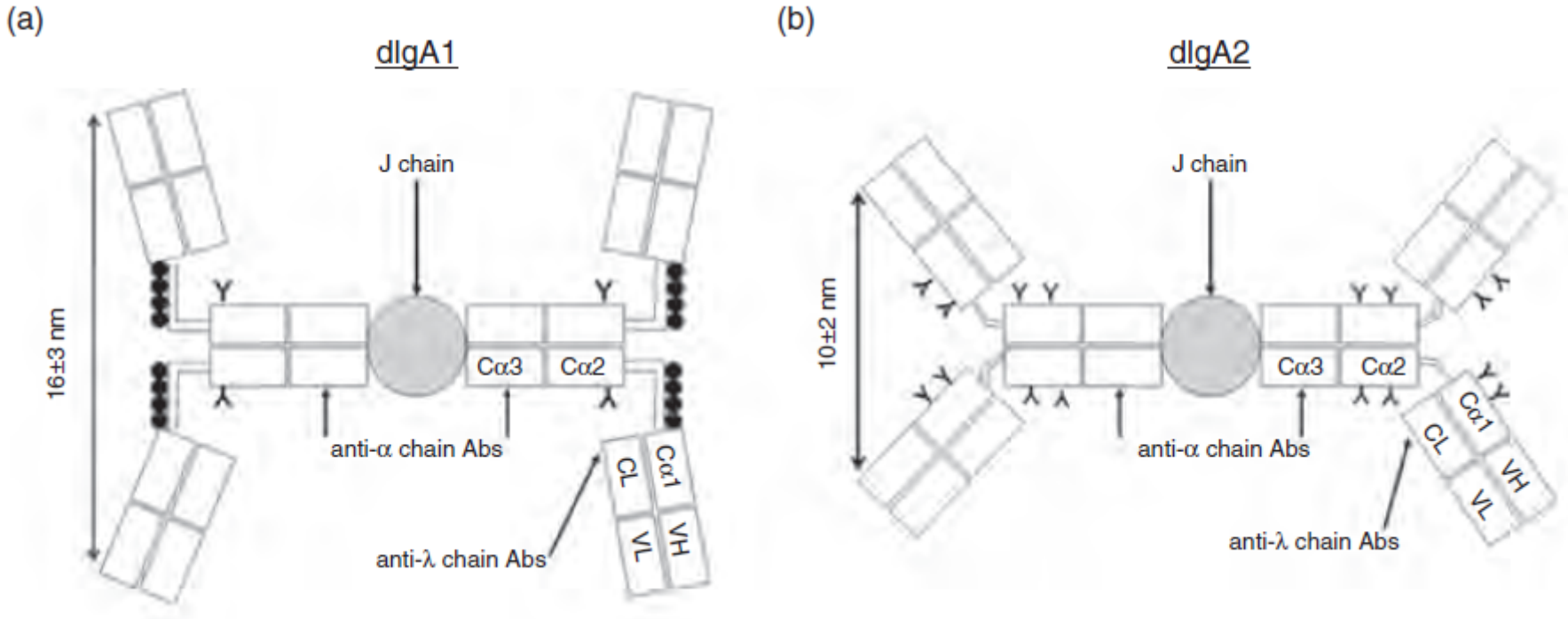
Diekman et al., FASEB J 1999


male genital tract CD52
- 6
- 5
- 4
- 3
- 2
a
lymphocyte CD52
N-glycosidic core structure
sialic acid
peripheral fucose
lactosamine repeat

HC4-N/S19-N Abs

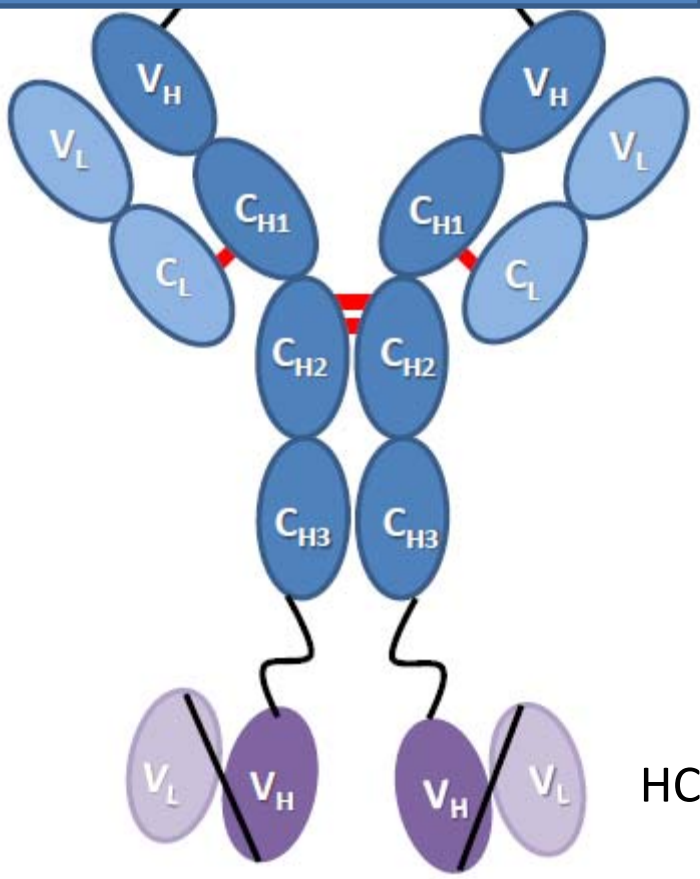
- Coagglutination: sperm, cells, vesicles
- ~150 mg/kg (post protein A)
- Undergoing evaluation at BU

IgM is more potent (100-1000X) than IgG at coagglutination



Watkins et al., AIDS 2013

HC4/HC4 bispecific and
Cell-associated HIV



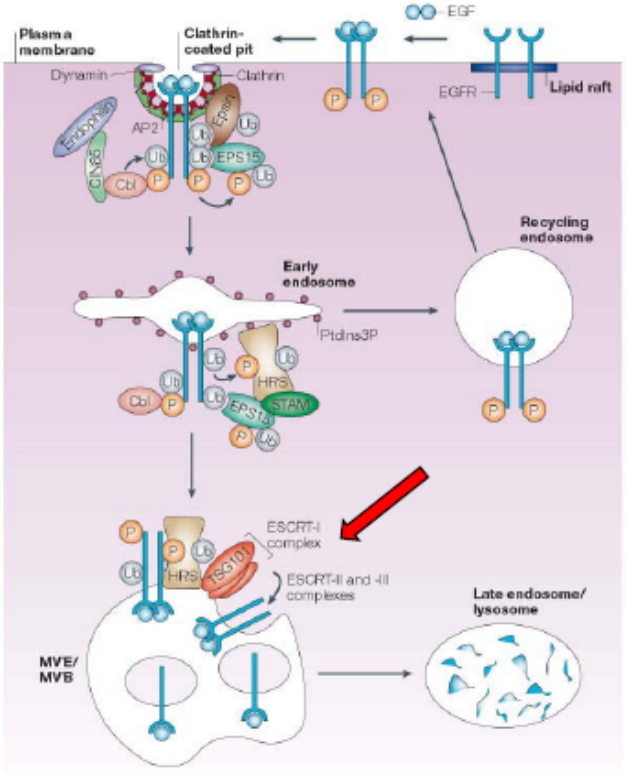
HC4 (SCA)

HC4 (SCA)

Functional Genetics

TSG101 is Translocated to Plasma Membrane Upon Viral Infection

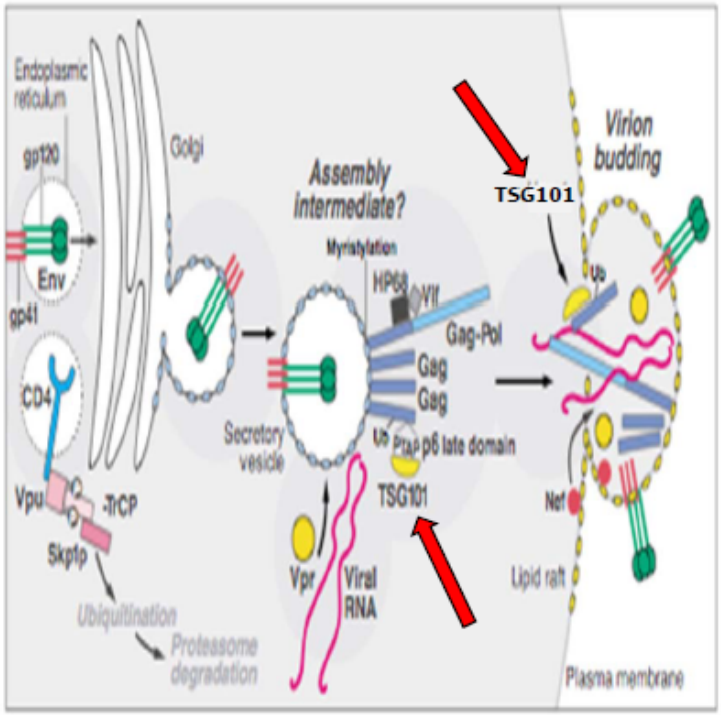
TSG101* regulates protein transport to MultiVesicular Body (MVB)



Healthy cells

From Le Roy and Wrana,, *Nature Rev Mol Cell Biol* 6, 112

TSG101 is translocated to surface of cells upon viral infection

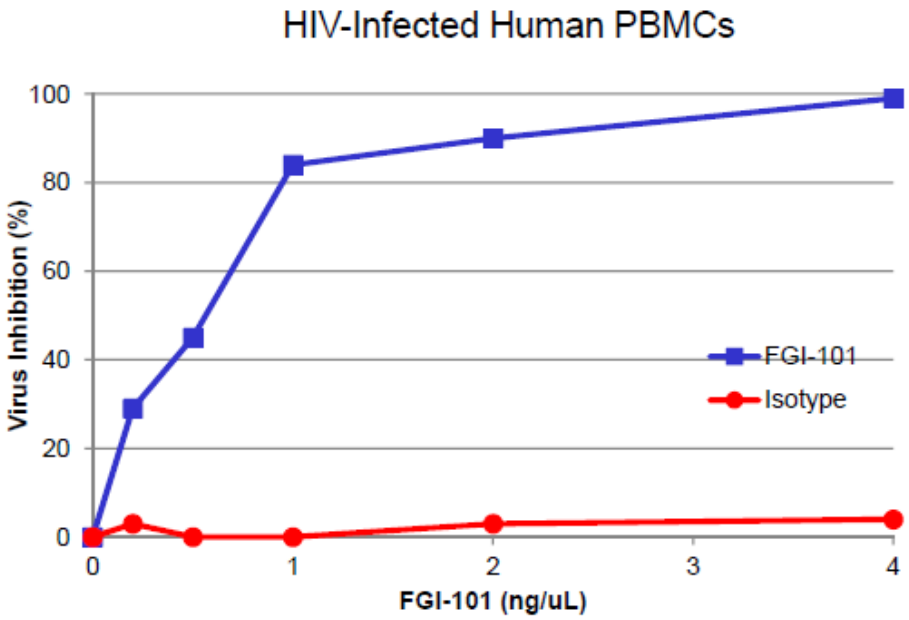


Infected cells

Adapted from Greene and Peterlin, *Nature Medicine*, 8, 673

FGI-101 Targeting of HIV *ex vivo*

- Elimination of Infected Cells
- No Toxicity to Uninfected Cells at Any Dose



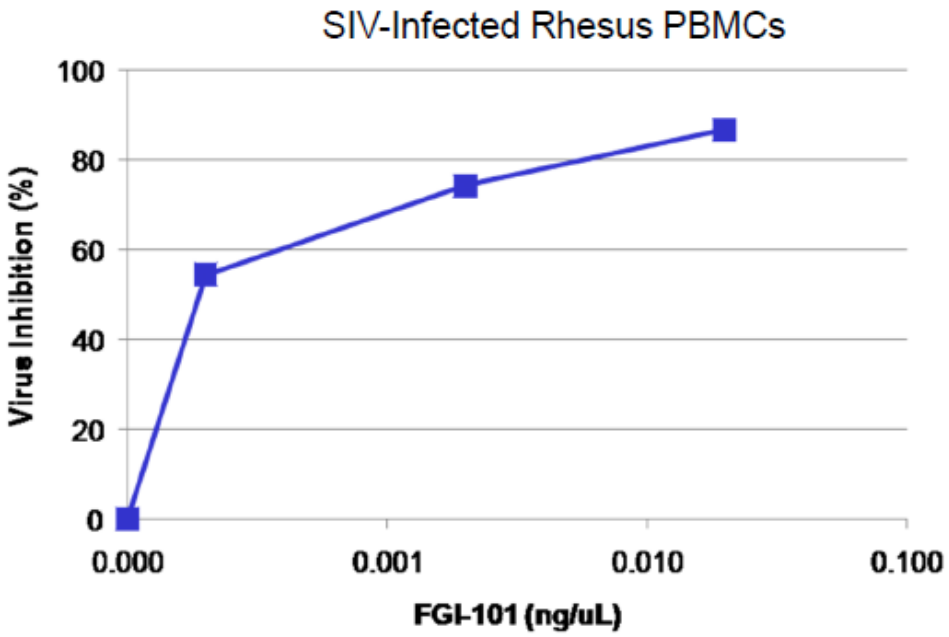
	EC_{50}
HIV-1	4 nM

FGI-101 Selectively Eliminates HIV-Infected Cells

Experimental Design:
- **TSG101 mAb:** CB8-2
- **Host:** Human PBMC Infection Assay (incl. NK cells)
- **Constants:** t=8 days post infection

FGI-101 Targeting
Inhibition of SIV via ADCC *ex vivo*

- FGI-101 Selectively Eliminates SIV-Infected Cells
- No Killing of Uninfected Cells
- Broad Window for Activity



SIV Similarity to HIV-2 Suggests Potential for Relevance to All Lentiviruses

Experimental Design:
- **TSG101 mAb:** FGI-101-1A6
- **Host:** Rhesus PBMC
- **Readout:** p27 ELISA
- **Constants:** t=3 days

gp41 Abs and Cell-cell transmission

Table 5. Antiviral efficacy of m9 in CCR5-dependent cell-to-cell transmission assay

Compound	IC_{50} (nM)	IC_{90} (nM)
AMD3100[a]	>10,000	>10,000
TAK779[b]	3.0	6,420
enfuvirtide	35	>1,000
m9	0.066	2.3
4E10	1.4	9.1
2F5	1.5	6.4

(Zhang…Dimitrov, mAbs 2010)

Status

1.10E8-N and 4E10-N have been GMP manufactured at KBP for NHP studies

2.10E8-N and 4E10-N are being evaluated at BU