Hacking HIV: Creating and Assessing a novel CTL-based HIV-1 vaccine

Craig Rouskey
Immunity Project
Waag Society, Amsterdam, NL
September 9, 2014

Immunity Project

Immunity Project is a non-profit initiative dedicated to developing an **Open-Access** HIV vaccine.

We are a non-profit initiative of Flow Pharma, Inc. (Business hack)

EPIDEMIOLOGY

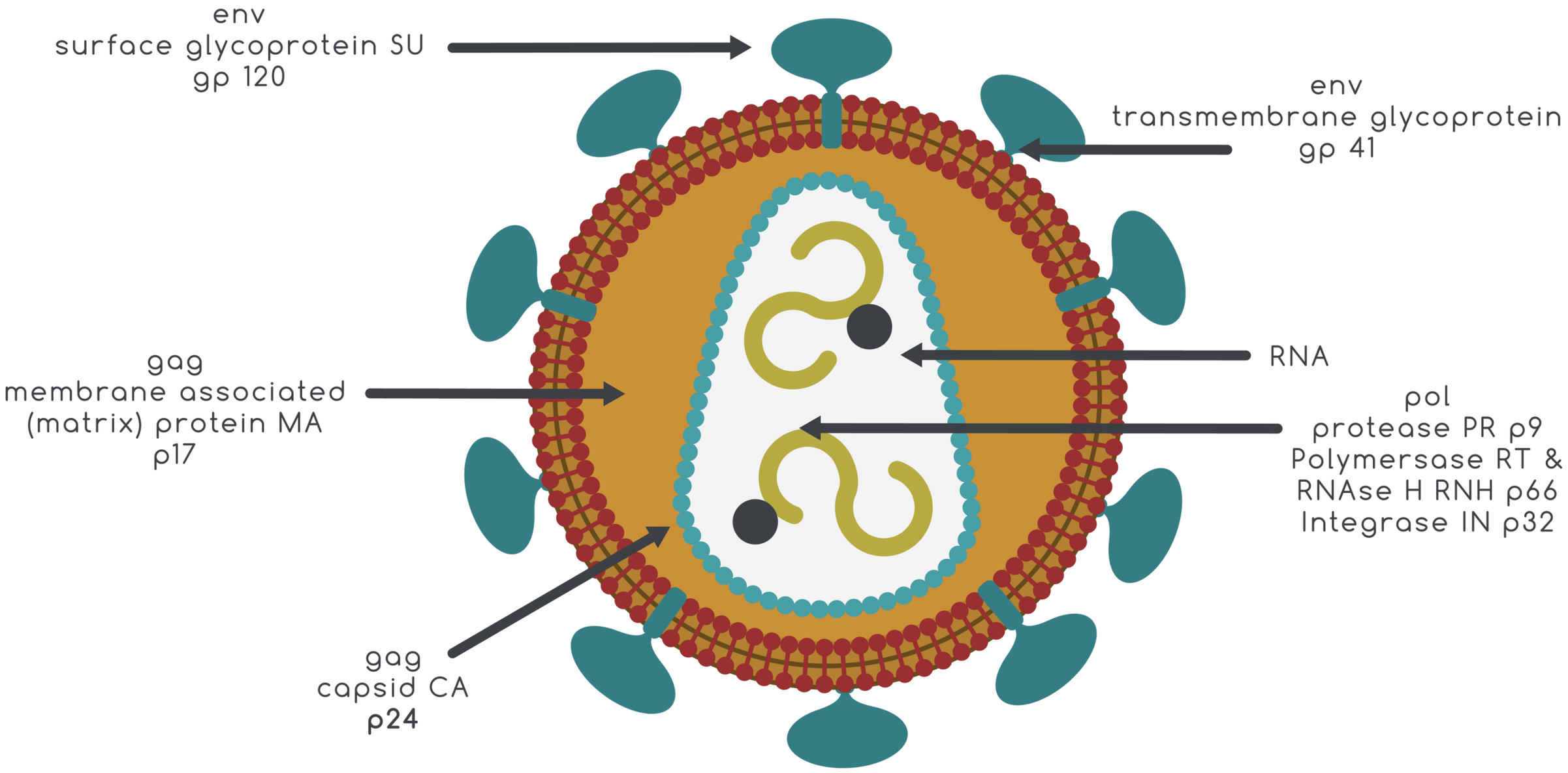
Global summary of the AIDS epidemic | 2013

Number of people living with HIV in 2013	**Total**	35.0 million	[33.1 million – 37.2 million]
	Adults	31.8 million	[30.1 million – 33.7 million]
	Women	16.0 million	[15.2 million – 16.9 million]
	Children (<15 years)	3.2 million	[2.9 million – 3.5 million]
People newly infected with HIV in 2013	**Total**	2.1 million	[1.9 million – 2.4 million]
	Adults	1.9 million	[1.7 million – 2.1 million]
	Children (<15 years)	240 000	[210 000 – 280 000]
AIDS deaths in 2013	**Total**	1.5 million	[1.4 million – 1.7 million]
	Adults	1.3 million	[1.2 million – 1.5 million]
	Children (<15 years)	190 000	[170 000 – 220 000]

*Data from WHO, UNAIDS and UNICEF

More than 1.1 million people in the United States are living with HIV infection, and almost 1 in 6 (15.8%) are unaware of their infection.

THE VIRUS


env
surface glycoprotein SU
gp 120
env
transmembrane glycoprotein
gp 41
RNA
gag
membrane associated
(matrix) protein MA
p17
pol
protease PR p9
Polymersase RT &
RNAse H RNH p66
Integrase IN p32
gag
capsid CA
p24

Immune Response to HIV

- **Humoral** - antibody responses to virus are difficult due to high mutation frequency (old vaccines)

- **Cellular** - the infected body makes CD8+ CTL responses to viral infection, but these cells are down regulated during infection.

- Unless you're a **controller**!

Controller

- **Controllers** have the natural ability to prevent advancement of HIV into AIDS.

- This is accomplished by the production of T cells with the ability to kill HIV infected cells (Killer T Cells).

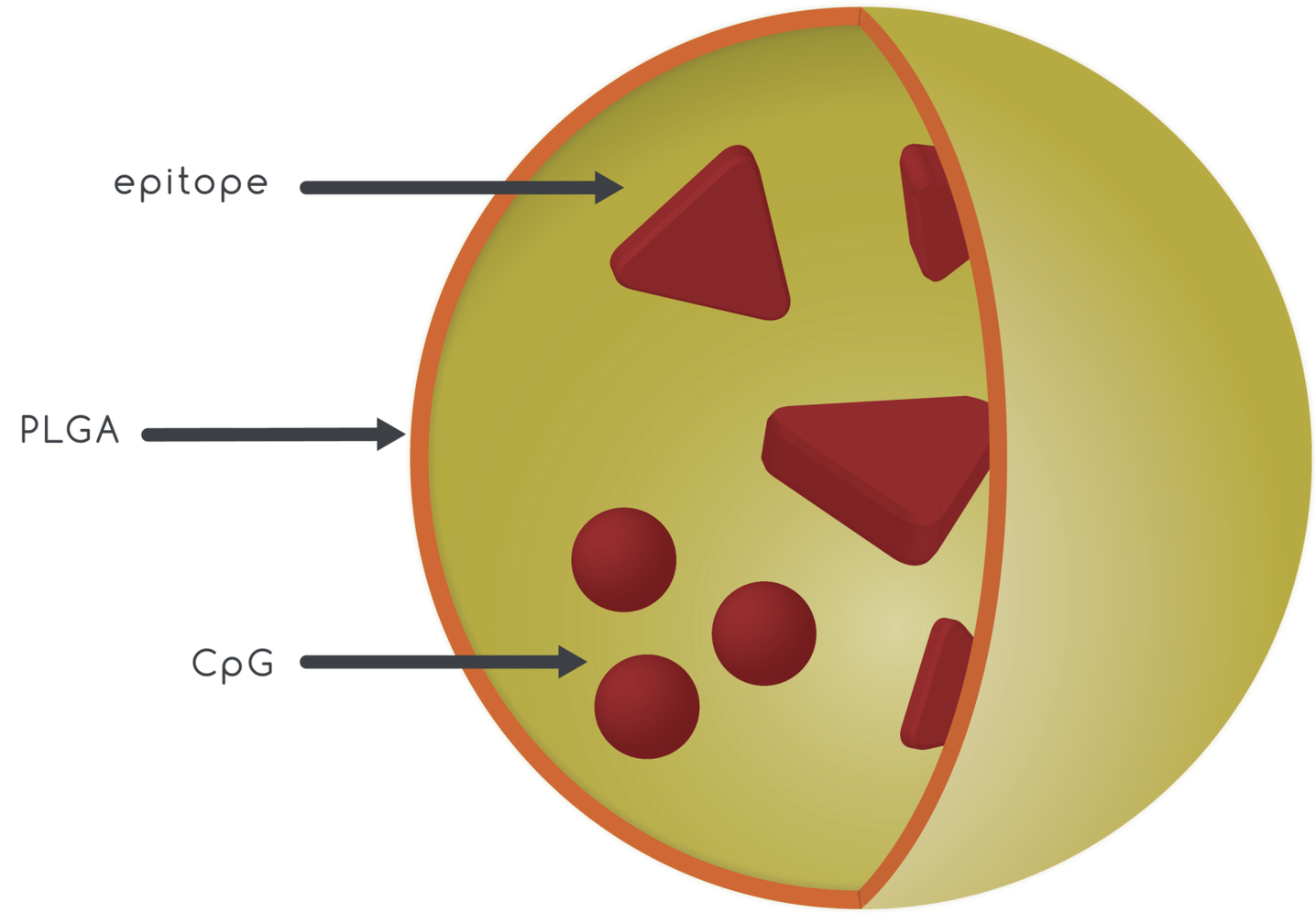
- All people with HIV produce killer T cells, but in **non-controllers**, these cells are deleted by HIV-programmed cells.

- Different from **slow progressors**.

- We want to turn everyone into controllers!

THE VACCINE

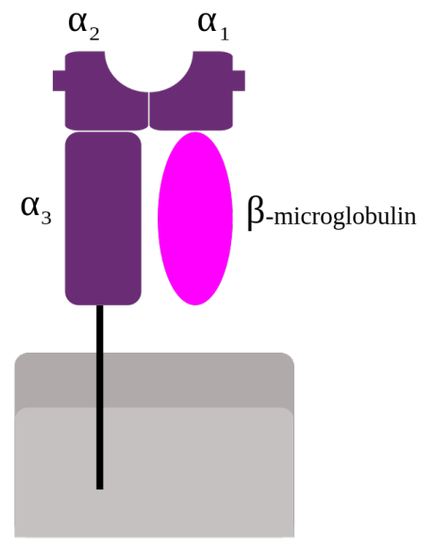
Component	Quantity
PLGA	98.5%
CpG	0.25%
Peptide	0.05% (100ng/mL)
Mannose	1.25%

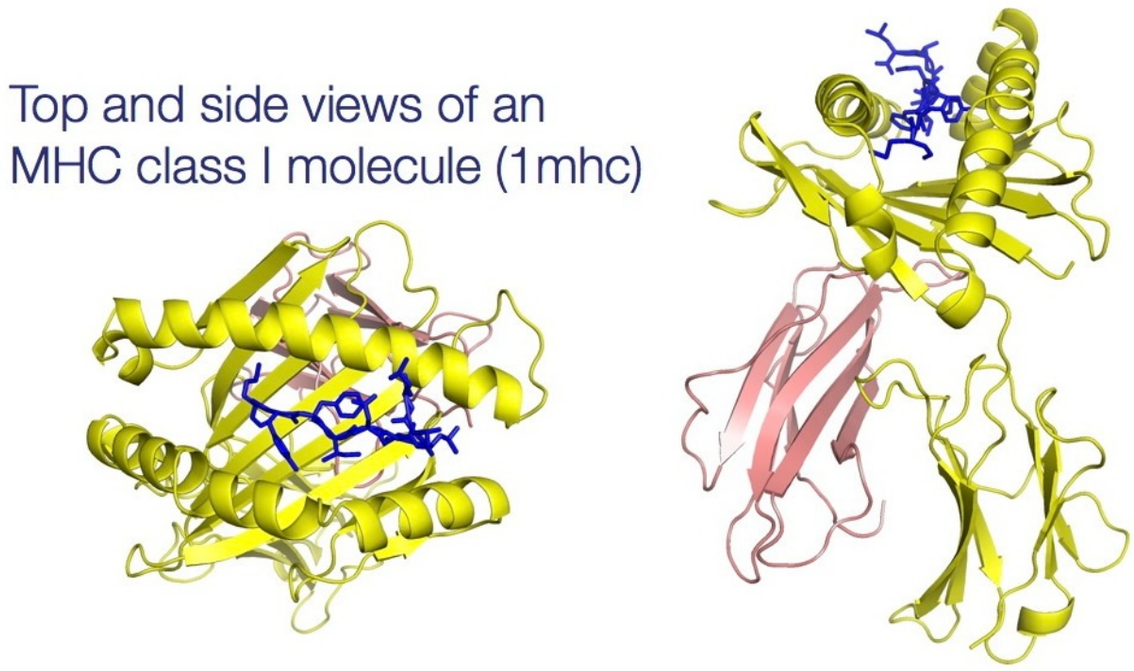


poly(lactic-co-glycolic acid)

Hacking CTL Peptides

- Identification of immunogenic proteins
- Identification of peptides that possibly bind to Class I molecules (based on HLA restriction)
- Creating databases of peptide binding (syfpeithi.de)
- Designing peptides for inclusion in our vaccine





A2, B57 AND B44

- Based on these databases, we chose two peptides specific for class I HLA: SL09 (SLYNTVATL) and KF11 (KAFSPEVIPMF)

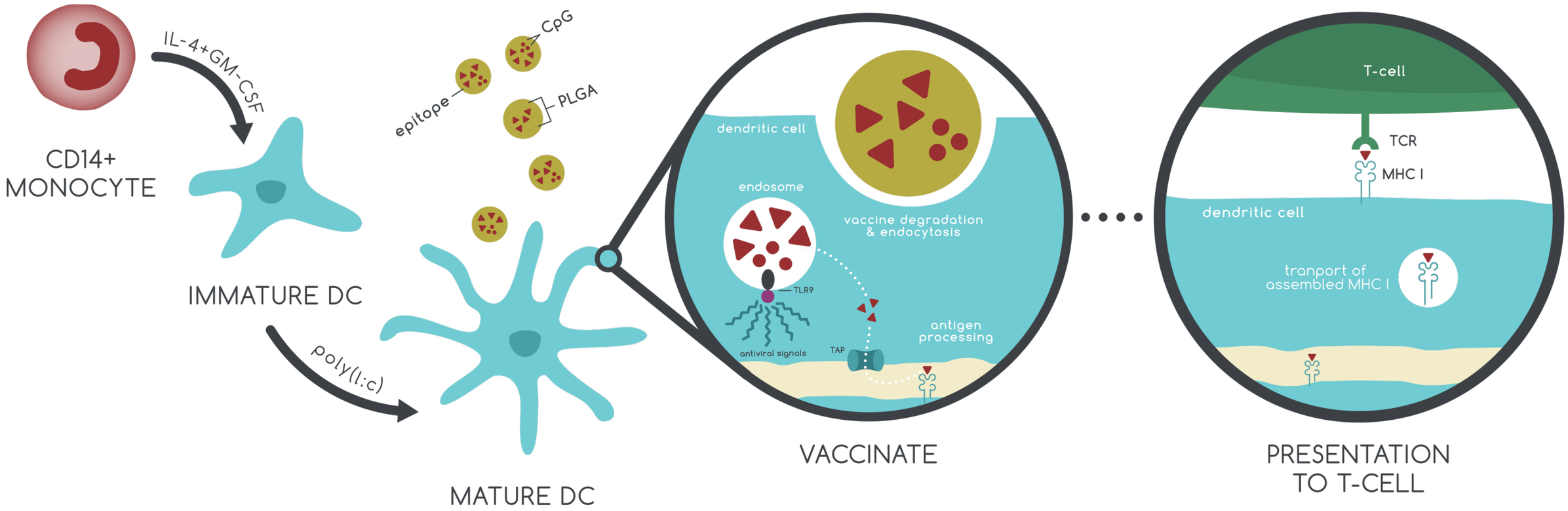
- We performed a SYFPEITHI analysis to determine the binding probability of HIV-1derived epitopes to class I molecules used in our studies.

Haplotype	SL09 score	KF11 score	Positive Control Score
A2	31	9	31
B44	14	20	17 (228-239, vimentin)

IN VITRO ASSAY (PART I)

DAY

0 ..... 5 ..... 6 ..... 7 8



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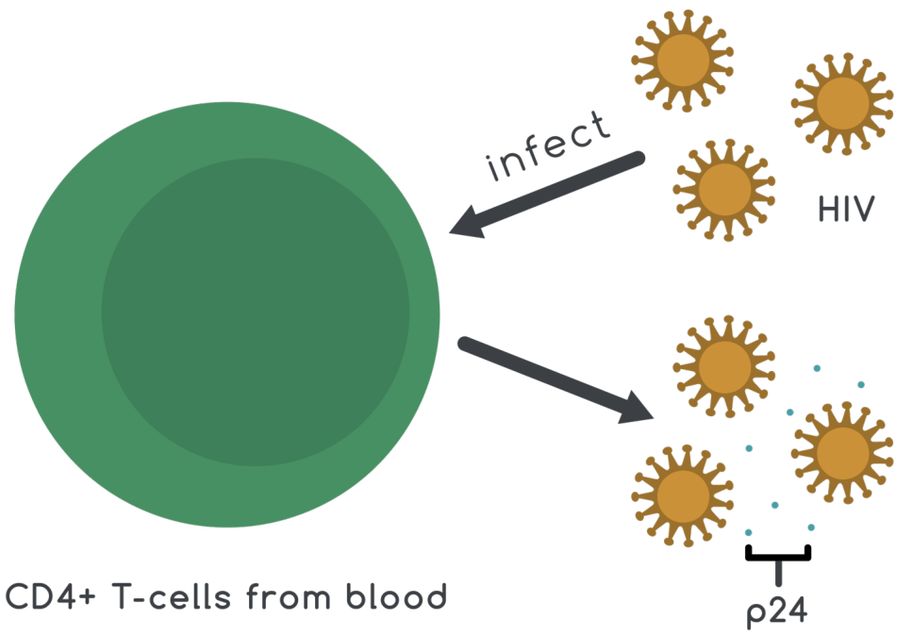
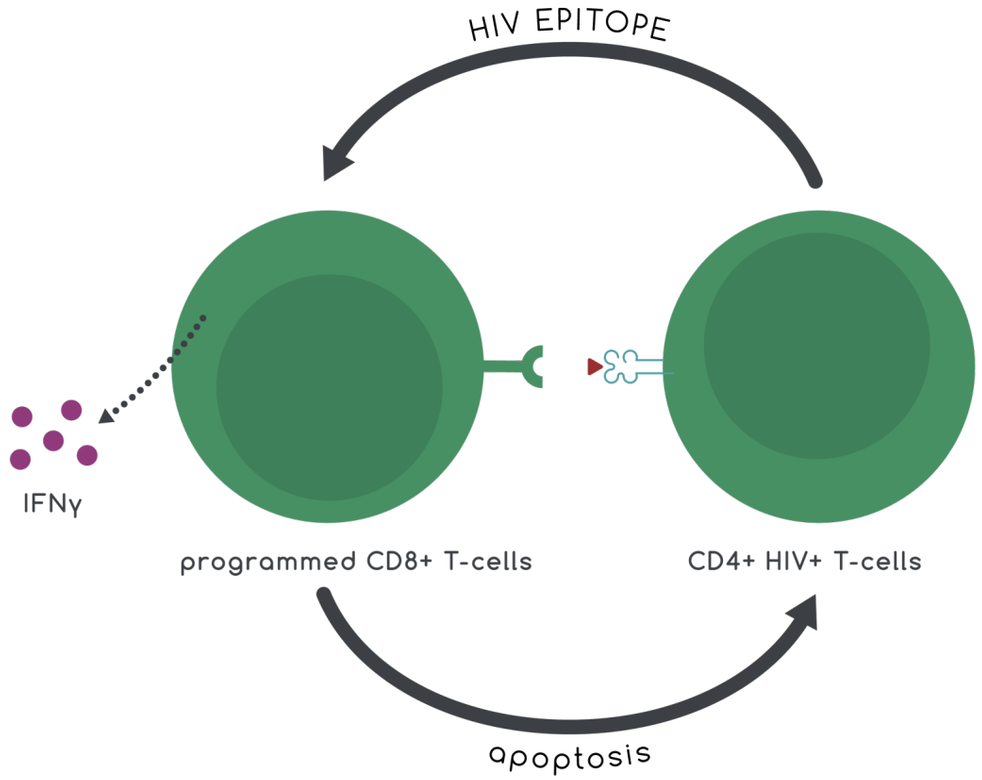
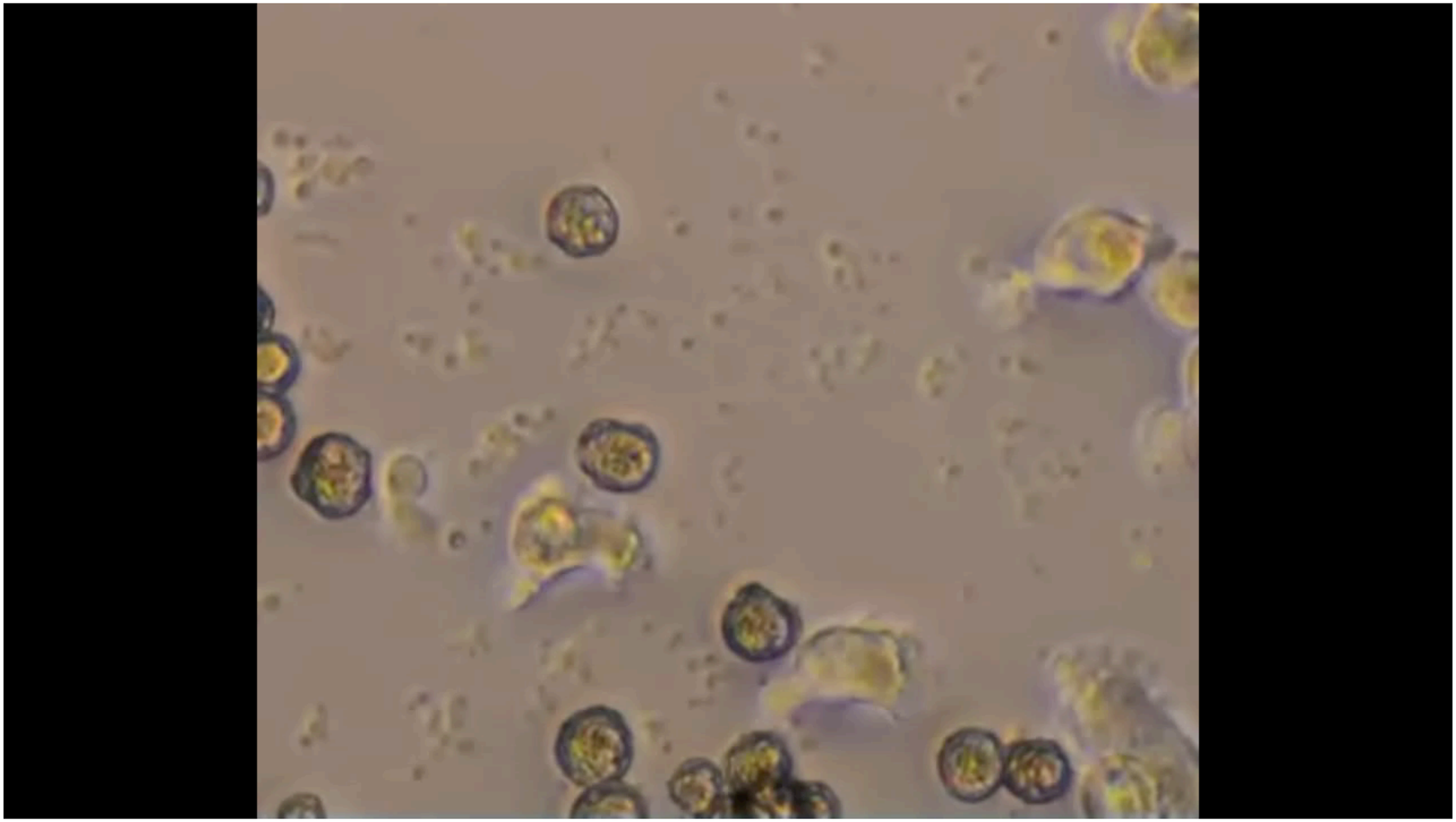
IN VITRO ASSAY (PART II)

DAY

24 25







DENDRITIC CELL UPTAKE

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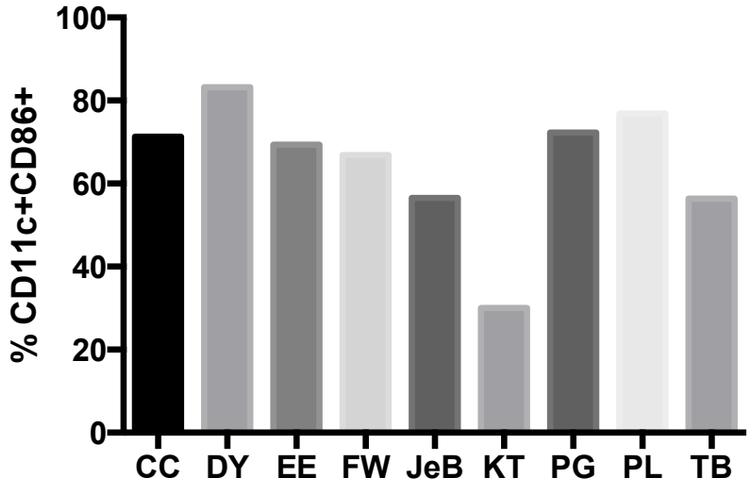
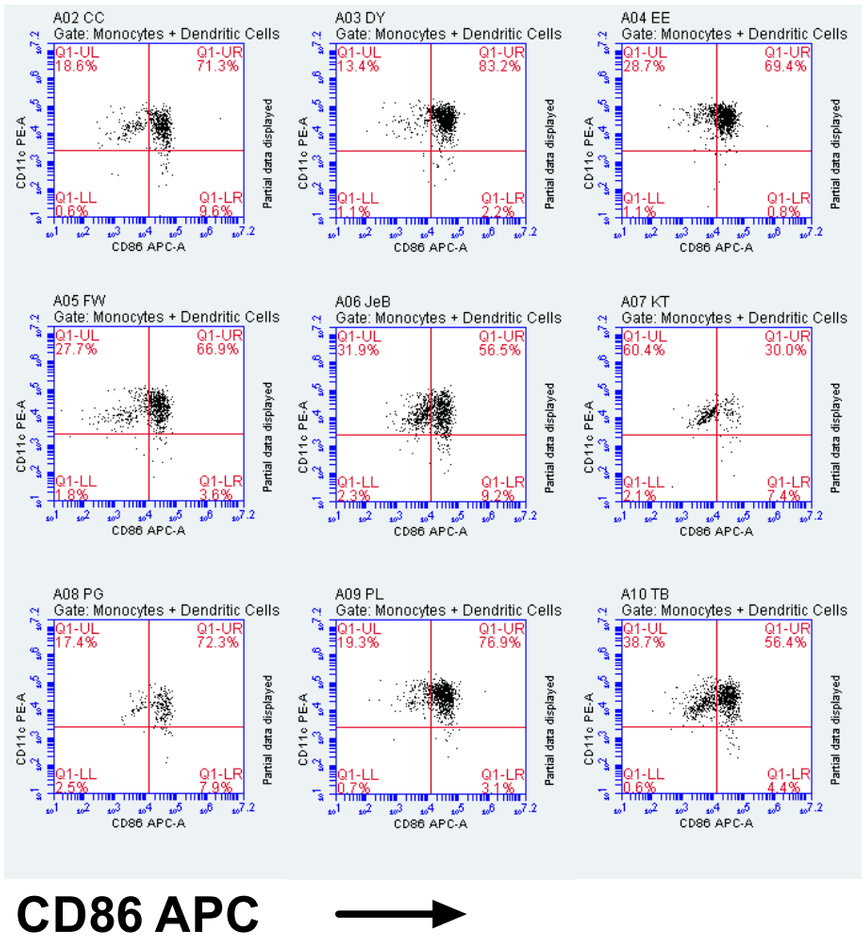
Dendritic cell maturation

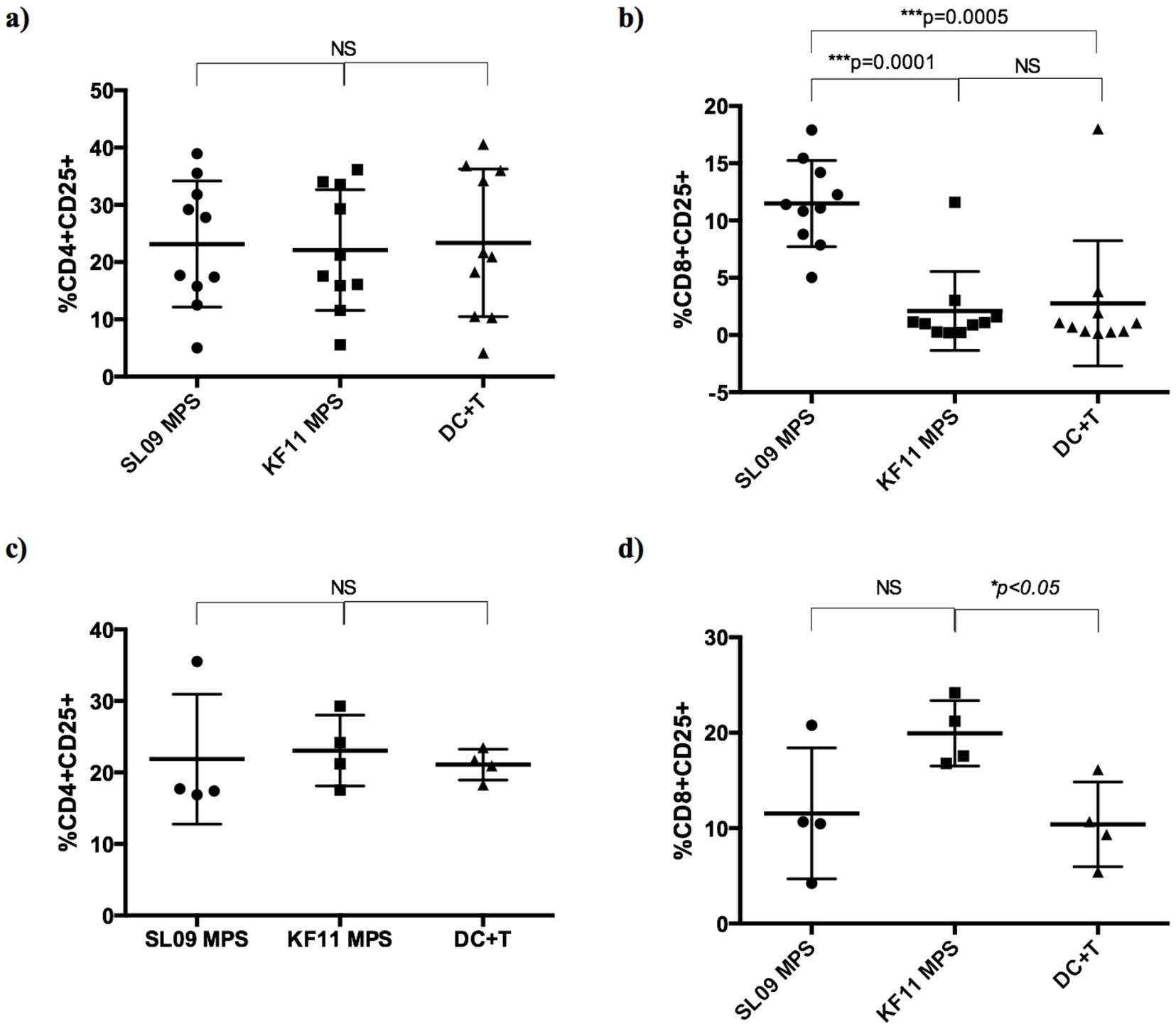


	CC	DY	EE	FW	JeB	KT	PG	PL	TB
CD11c+CD86+	71.3	83.2	69.4	66.9	56.5	30.0	72.3	76.9	56.4

- Flow Cytometry

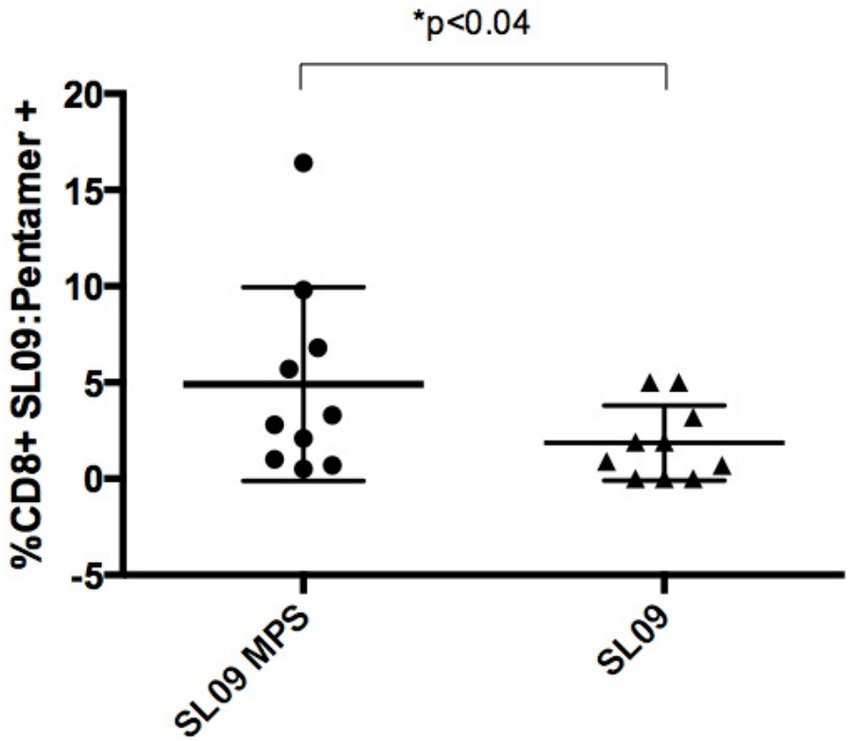


ACTIVATION


a)
NS
%CD4+CD25+
50
40
30
20
10
0
SL09 MPS
KF11 MPS
DC+T
b)
***p=0.0005
***p=0.0001
NS
%CD8+CD25+
20
15
10
5
0
-5
SL09 MPS
KF11 MPS
DC+T
c)
NS
%CD4+CD25+
40
30
20
10
0
SL09 MPS
KF11 MPS
DC+T
d)
NS
*p<0.05
%CD8+CD25+
30
20
10
0
SL09 MPS
KF11 MPS
DC+T

TCR UPREGULATION

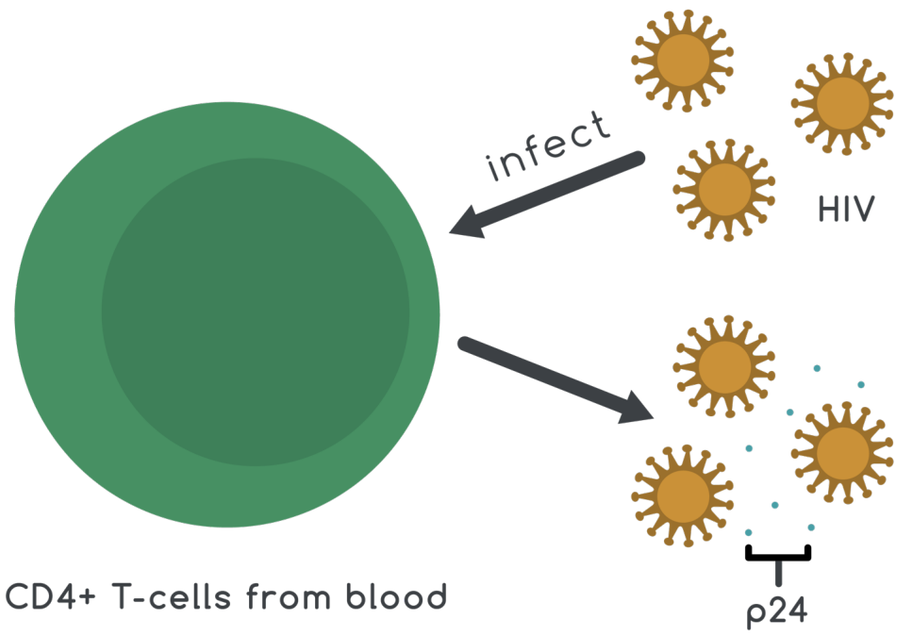
- On day 15, programmed CD8+ T cells were assessed for their expression of TCR that recognize SL09 in the context of HLA A*02 using A2:SL09 Pentamer (ProImmune).

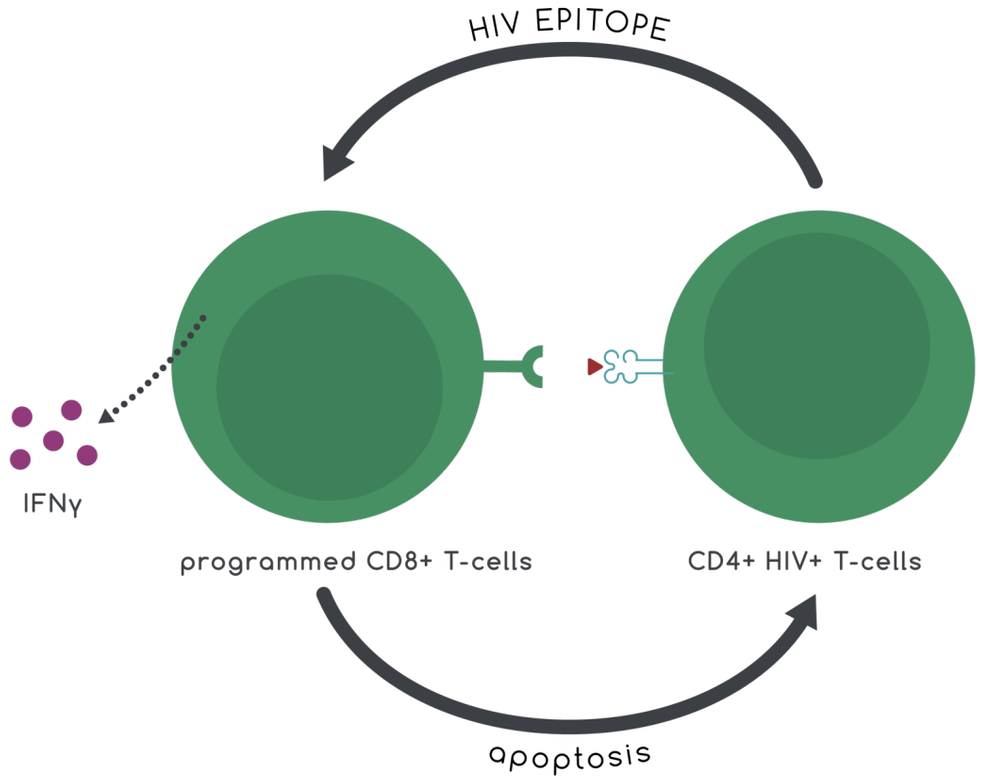


IN VITRO ASSAY (PART II)

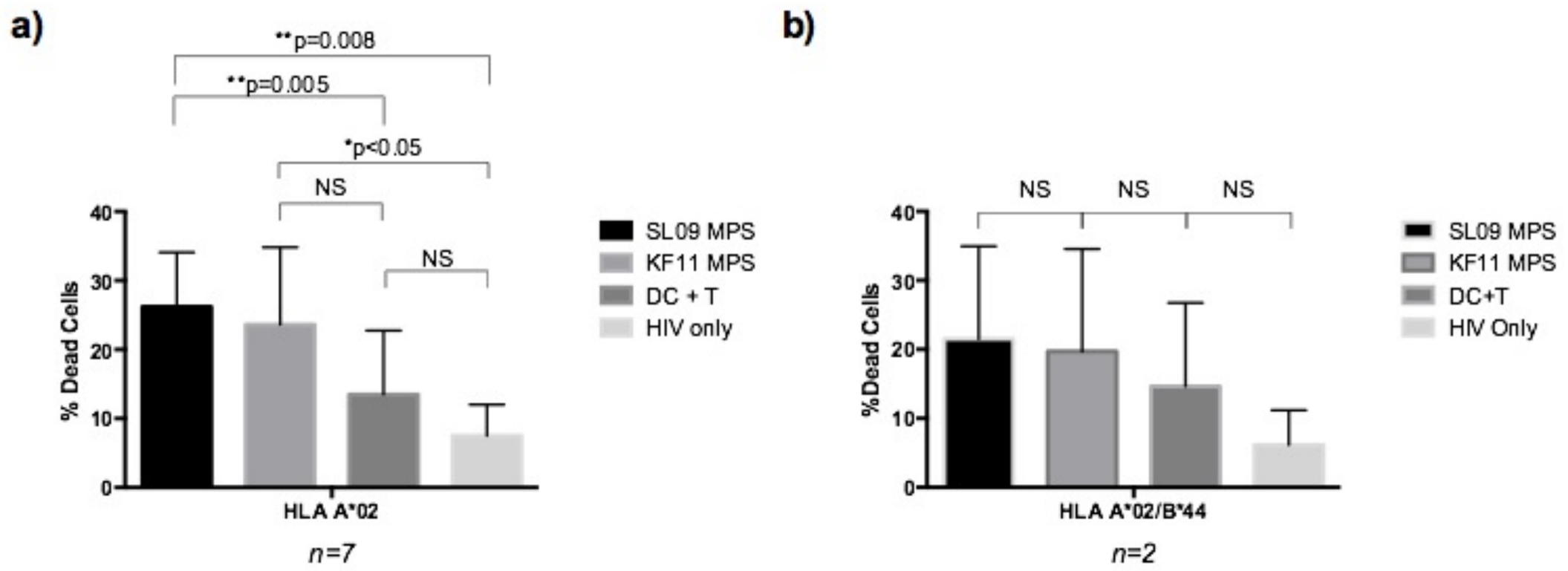
DAY

24 25



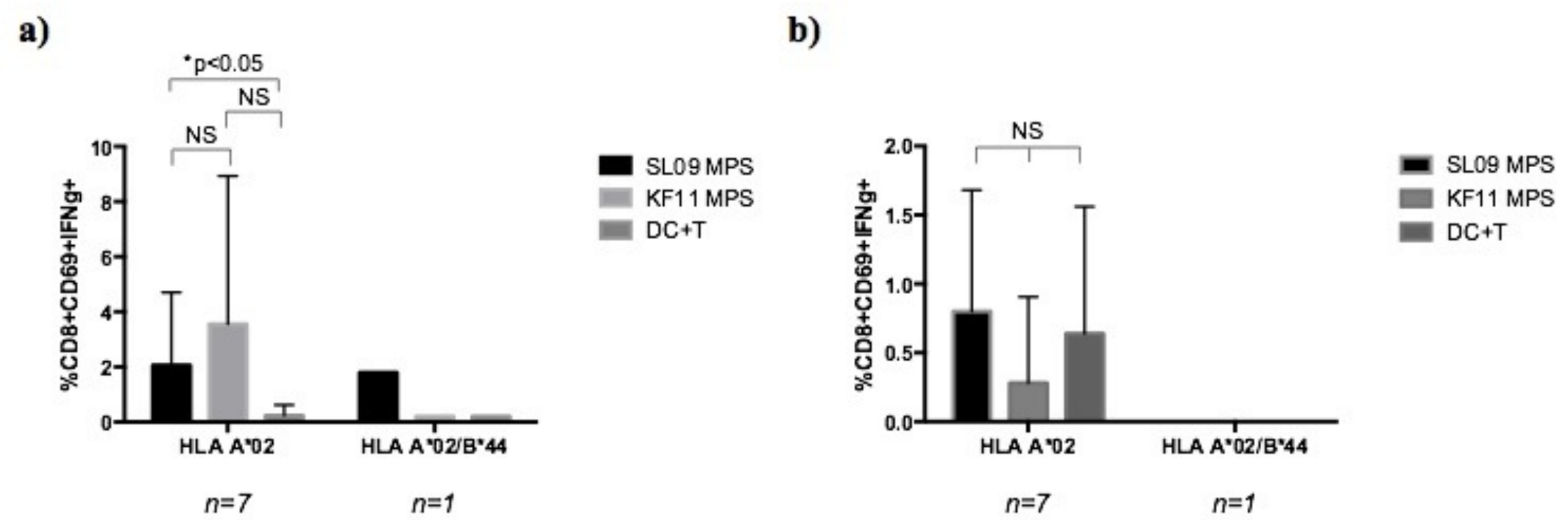


CTL-MEDIATED KILLING



- HIV-infected autologous CD4+ T cells were co-cultured with CD8+ T cells 5:1 (E:T)
- CD4+ T cells were assessed for apoptosis and death.
- CD8+ T cells were assessed for their ability to produce IFNg in the presence of targets.

Interferon Gamma

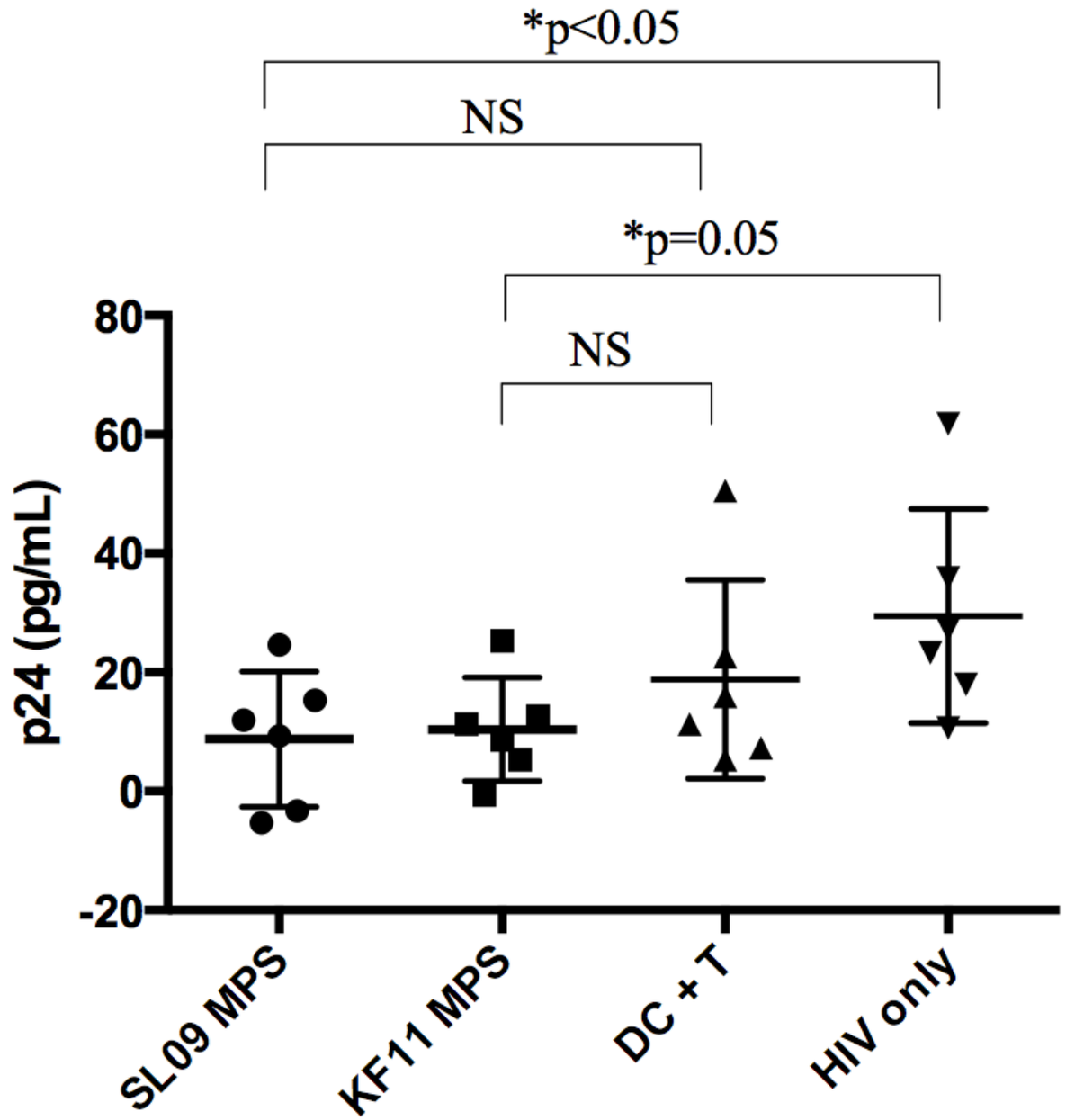


- CD8+ Effector T cells were co-cultured with autologous HIV+ CD4+ T cell targets and assessed for their production of Interferon gamma (IFNg) on day 30.

P24 SUPPRESSION

- p24 ELISA was performed to assess the amount of HIV in the supernatant of CTL assay co-cultures.

- A decrease in p24 in tissue culture supernatant means that the T cells are effective at inhibiting HIV.



Future Directions

- We are designing pre-clinical experiments in Monkeys.
- We are working on clinical trials…
- We need a High Throughput Assay (that doesn't take 30 days to perform).

Dank Je Wel!

- Dank je wel Pieter van Boheemen en de Waag Society!
- Co-Founders:
  - Dr. Reid Rubsamen
  - Naveen Jain
- Scientists
  - Charlie Herst
  - Vikram Paranjpe (former Intern)
- Interns:
  - Rohun Patel
  - Hannah Hoban
- **COMMUNITY!**

Todays Experiment

- Ouchterlony (Double Diffusion)
  - A test for reactivity of an **antigen** (protein) and **antibody.**
  - Precipitation reaction occurs and determines whether or not the antibody recognizes antigen.
  - This assay can be used in the field to determine whether or not a patient has come into contact with a virus containing a **known** antigen.

Precipitation

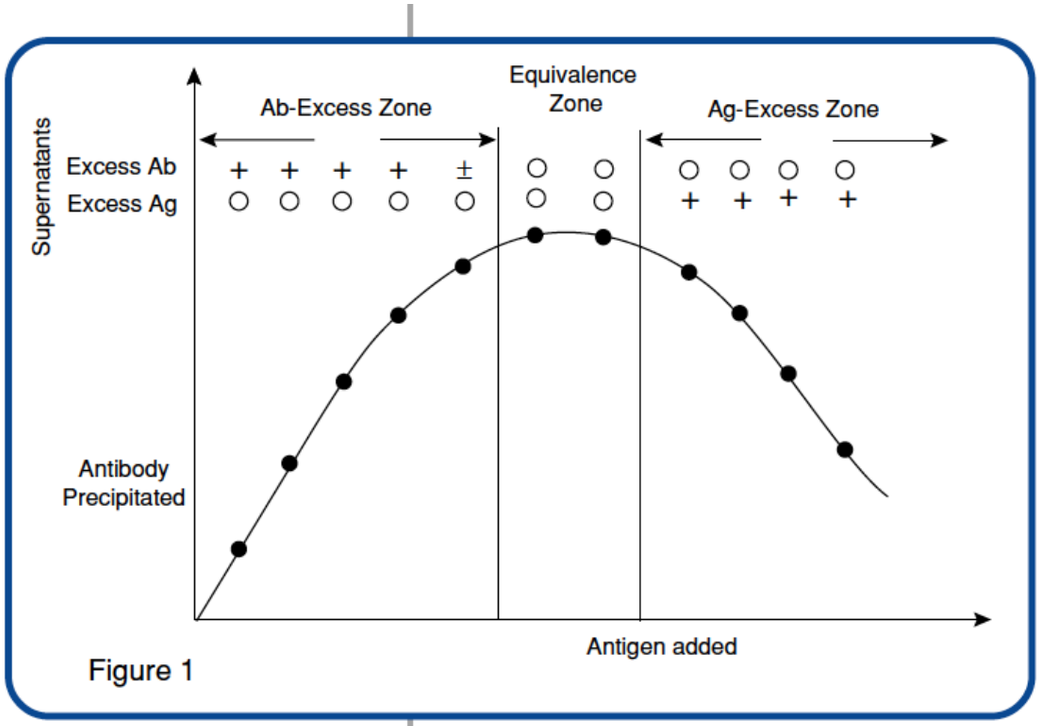
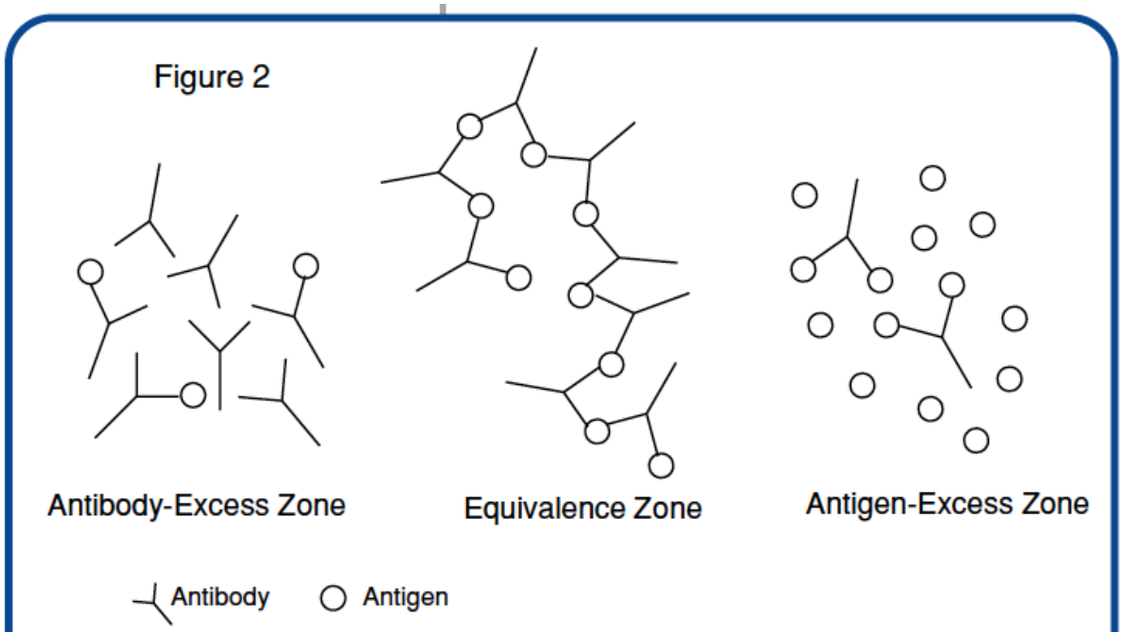


Figure 1



Precipitation in two dimensions

- Add antigen and antibody to wells and assess precipitation…

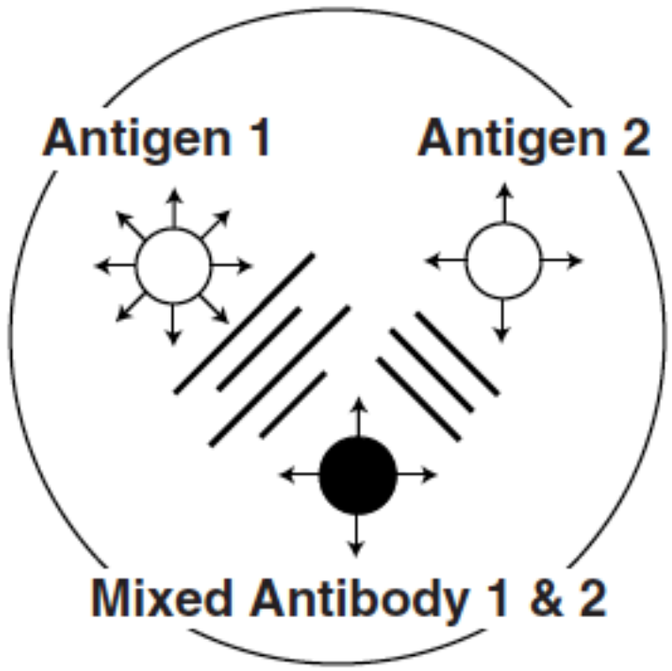


Figure 3

Today's Experiment (Cont.)

- The Ouchterlony procedure allows us to measure different **reactivities**.

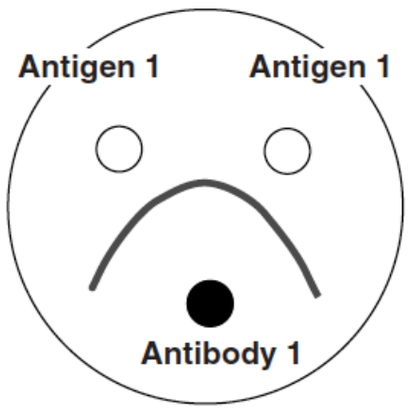


Figure 4:
Reaction of Identity

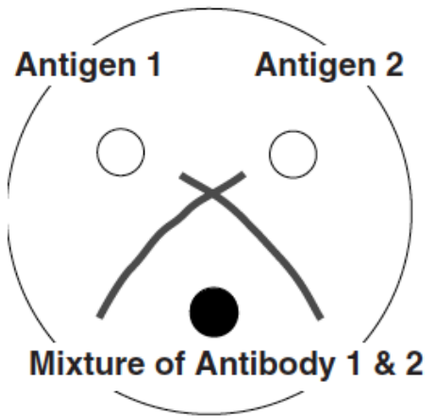


Figure 5
Reaction of Non-identity

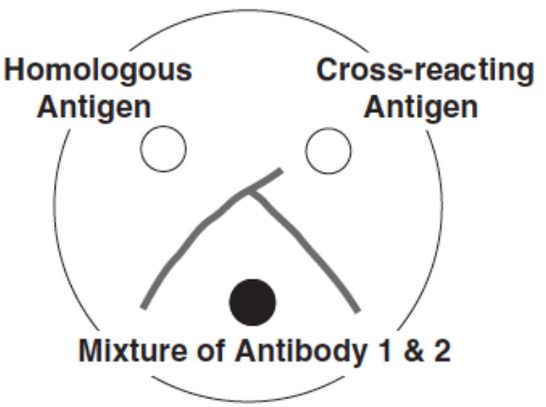


Figure 6
Reaction of Partial Identity

Procedure

- Pour plates (5mL of molten 1% agarose in Borate Buffer, pH 7.4)
- Allow to cool (5-10min)
- Punch holes in each plate (x3) and remove plug.
- Add antigen and antibody to each plate.
- Incubate overnight at 37C, in humid chamber.

Plate Setup

Tiny Holes

10uL of antibody
10ul of antigen

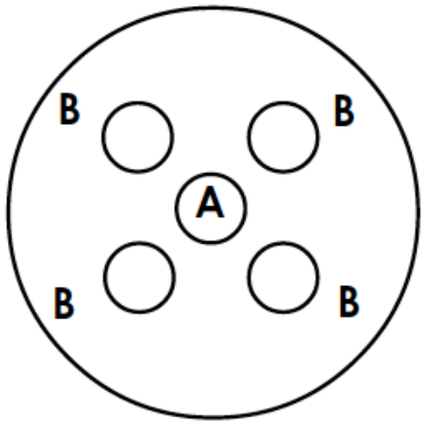


Plate 1 Center well: antiserum to the fluid containing antibodies (Tube A)
Left upper well: Whole serum (Tube B)
Right upper well: Whole serum (Tube B)
Left lower well: Whole serum (Tube B)
Right lower well: Whole serum (Tube B)

Large Holes
30uL antibody
30uL antigen

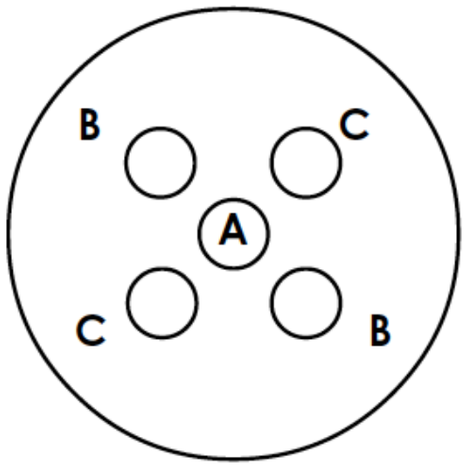


Plate 2 Center well: antiserum to the fluid containing antibodies (Tube A)
Left upper well: Whole serum (Tube B)
Right upper well: albumin (Tube C)
Left lower well: albumin (Tube C)
Right lower well: Whole serum (Tube B)

Plate 3 Center well: antiserum to the fluid containing antibodies (Tube A)
Left upper well: IgG (Tube D)
Right upper well: albumin (Tube C)
Left lower well: albumin (Tube C)
Right lower well: IgG (Tube D)