



**HIV Gag-specific T cell responses in the female genital tract are detected in women with high magnitude blood responses during chronic HIV-1 infection**



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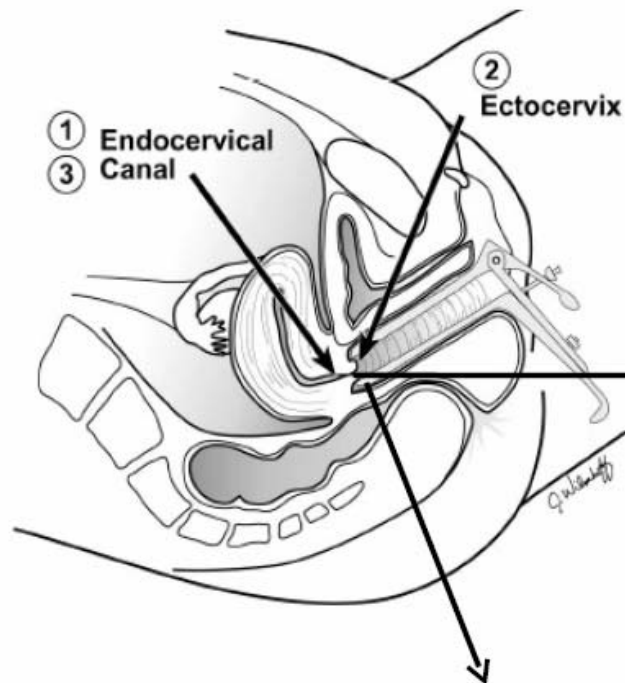
# Introduction

- Sexual transmission of HIV accounts for the **majority** of new infections worldwide, with the risk of becoming infected through heterosexual route being at least two times higher in women than men
- The **mucosal surface** of the female genital tract is **ultimately** the site of HIV transmission and shedding
- While there is **clear evidence** that **HIV-specific** cytotoxic T lymphocytes (CTLs) in **blood** play an important role in controlling HIV replication systemically, there is **comparatively** little known about CTL responses to HIV in the **genital tract** and factors that govern these
- Understanding the pathogenesis of **genital infection** and the role of **protective immunity** at the genital mucosa is of particular importance since it might provide insight into the development of an effective **mucosal HIV vaccine**

# Introduction

- There is an **urgent** need for **reliable** and **validated** methods for investigating mucosal immune responses in the **female** genital tract. Such methods would be particularly valuable in **HIV vaccine** trial settings where intensive **invasive** sampling is not an option
- HIV-specific mucosal CTL literature has been dominated by biopsy approaches to isolating lymphocytes from **rectal** and **gastrointestinal** mucosal tissue
- **Fewer** approaches are available for sampling **mucosal tissue** from the **female** genital tract (**cervical lavages** and **cervical cytobrushes**)
- The usefulness of both these methods are, however, constrained by the **low** numbers of *ex vivo* lymphocytes they yield

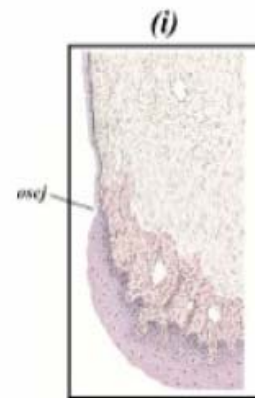
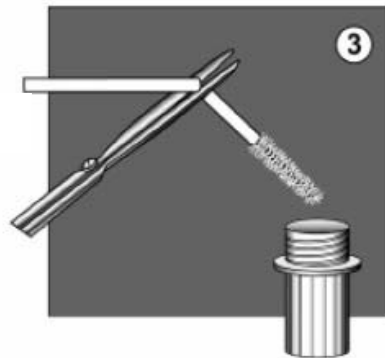
# How are these mucosal samples obtained ?



1 x **360°** Rotation

**Cell yield** =  $0.1 \times 10^6$  ( $\pm 0.7 \times 10^6$ ) cells

**>90%** with no red blood cell contamination



# Direct ex vivo analysis of mucosal samples

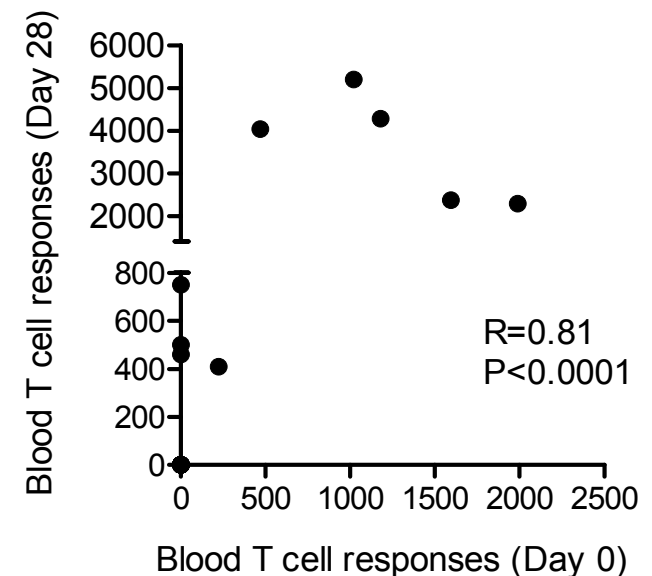
- Our laboratory (Gumbi et al., JVI, 2008) have shown that HIV Gag-specific T cell responses are **detectable** at the cervix immediately **ex vivo**
- Because of cervical yield constraints, this *ex vivo* study focused measuring a **single function** (IFN- $\gamma$  production) of CTLs to a **single antigen** (Gag)
- Clearly, **ex vivo** lymphocyte yields from a single cytobrush are **not sufficient** for thorough mapping of HIV Gag genome
- Short term *in vitro* polyclonal stimulation was explored to expand cervical CD3<sup>+</sup> T cells using anti-CD3 monoclonal antibodies and IL-2

## Aim

The aim of this study was to investigate the **feasibility** and **efficiency** of polyclonal ***in vitro* expansion** of cervical cytobrush-derived T cells to study HIV-specific T cell responses in the female genital tract to overcome the impact of low **cytobrush** yield

## Validation: Impact of expansion on HIV-specific T-cell responses in blood

- Initial experiments were conducted using PBMCs to investigate whether our **polyclonal expansion** protocol with anti-CD3 and IL-2 influenced the **regions** of Gag targeted by HIV specific T-cells
- PBMC from chronically HIV-infected individuals with well characterized *ex vivo* (day 0) Gag-specific ELISPOT responses were examined after 28 days of expansion for Gag-specific regions targeted
- In all individuals, **dominant ex vivo Gag regions** targeted were **maintained** following *in vitro* expansion ( $p < 0.0001$ ,  $Rho = 0.81$ )



# The Nyanga Day Hospital



Women were recruited from a cohort of chronically **HIV-infected** women enrolled in a longitudinal study of **cervical diseases** in relation to **HIV** infection attending the Nyanga Day Hospital

**~75% women** have CD4 counts **>300 cells/ $\mu$ l** have been enrolled in our study since July 2004

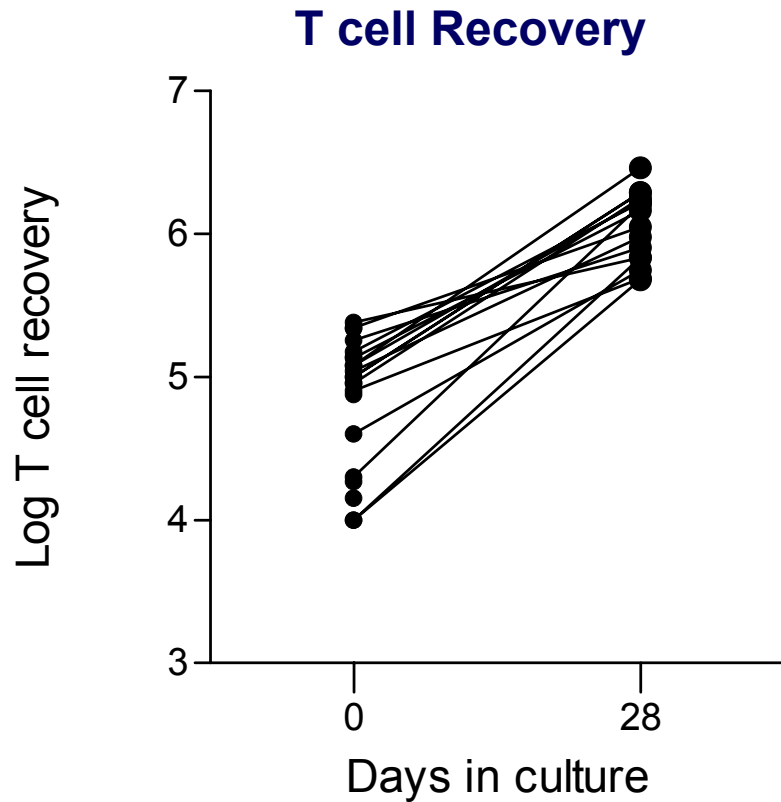
## Clinical description of HIV-infected women

N= 22

ARV Naïve

	Mean ( $\pm$ SD)	Range
Age	32 ( $\pm$ 5.7)	22-39
Absolute CD4 Count (cell/ $\mu$ l)	501 ( $\pm$ 223)	300 -1300
HIV Viral Load (RNA copies/ml)	90308 ( $\pm$ 294363)	970 -1300000

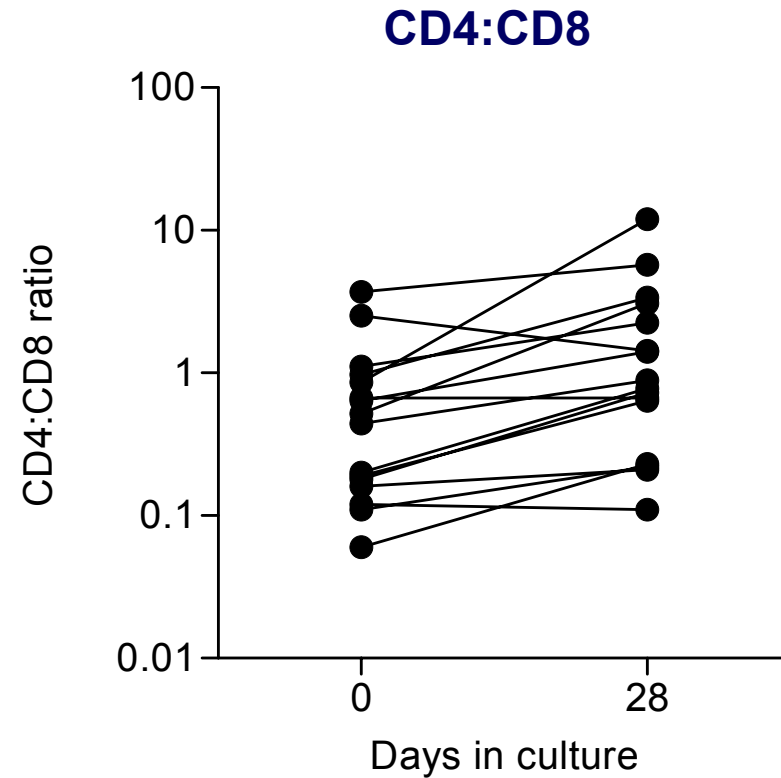
# Cervical expansion kinetics and efficiency



Mean  $\pm$  SD

Day 0 =  $0.11 \times 10^6 (\pm 0.69 \times 10^6)$  cells

Day 28 =  $1.38 \times 10^6 (\pm 0.63 \times 10^6)$  cells **22.9-fold**



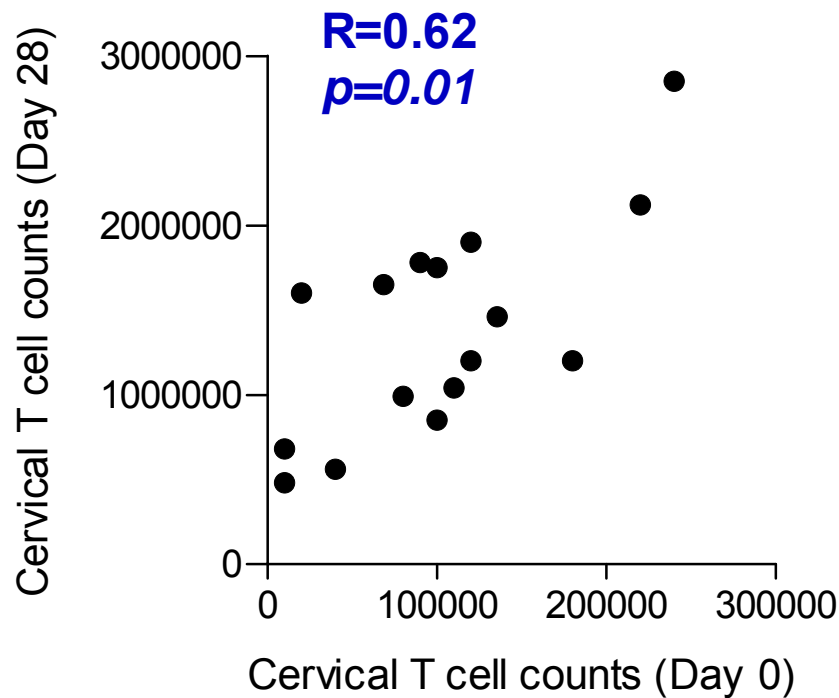
Median CD4:CD8

Day 0 = 0.48

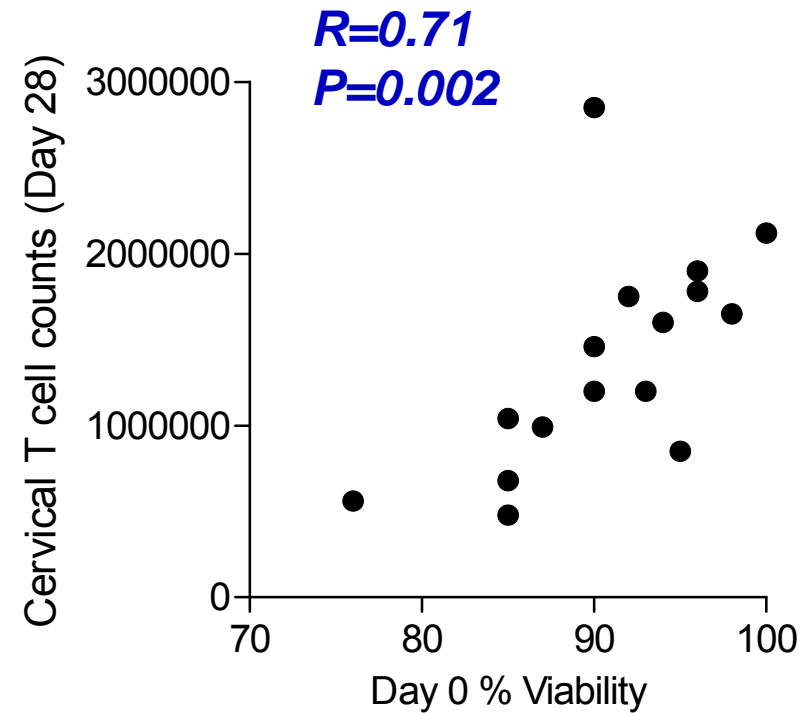
Day 28 = 0.83

## Impact of *ex vivo* yield and viability on expansion of cervical cytobrush-derived CD3+ T cells

### Input cell yield

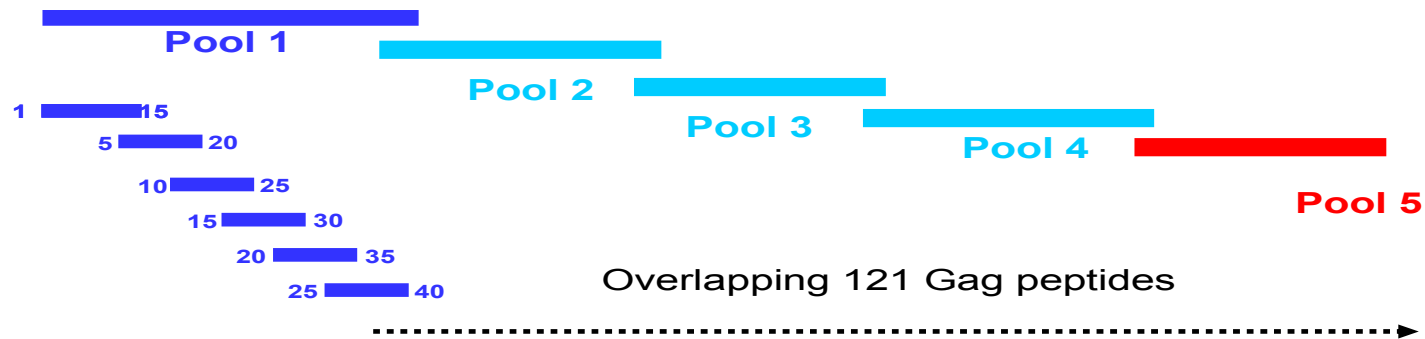
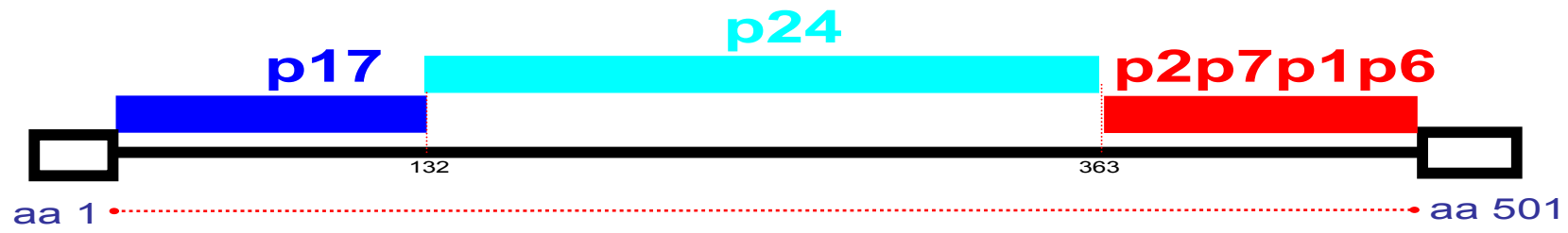


### Viability



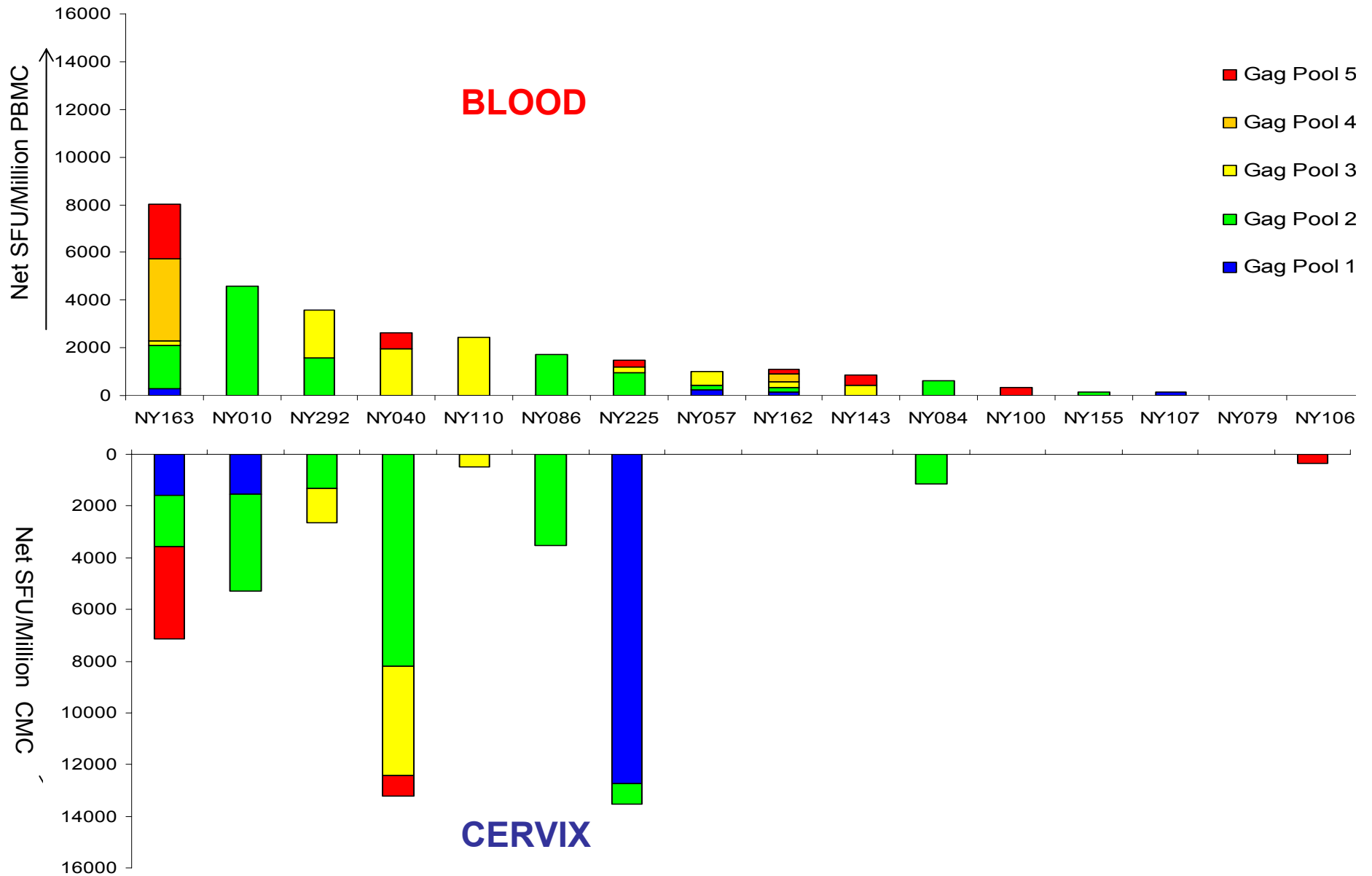
*Ex vivo* cervical samples will (1) yields of  $\leq 100,000$  cells and/or (2) viability of  $\leq 85\%$  are unlikely to expand in culture

# Comparison of cervical and blood HIV Gag-specific IFN- $\gamma$ responses in women with chronic HIV-1-infection.

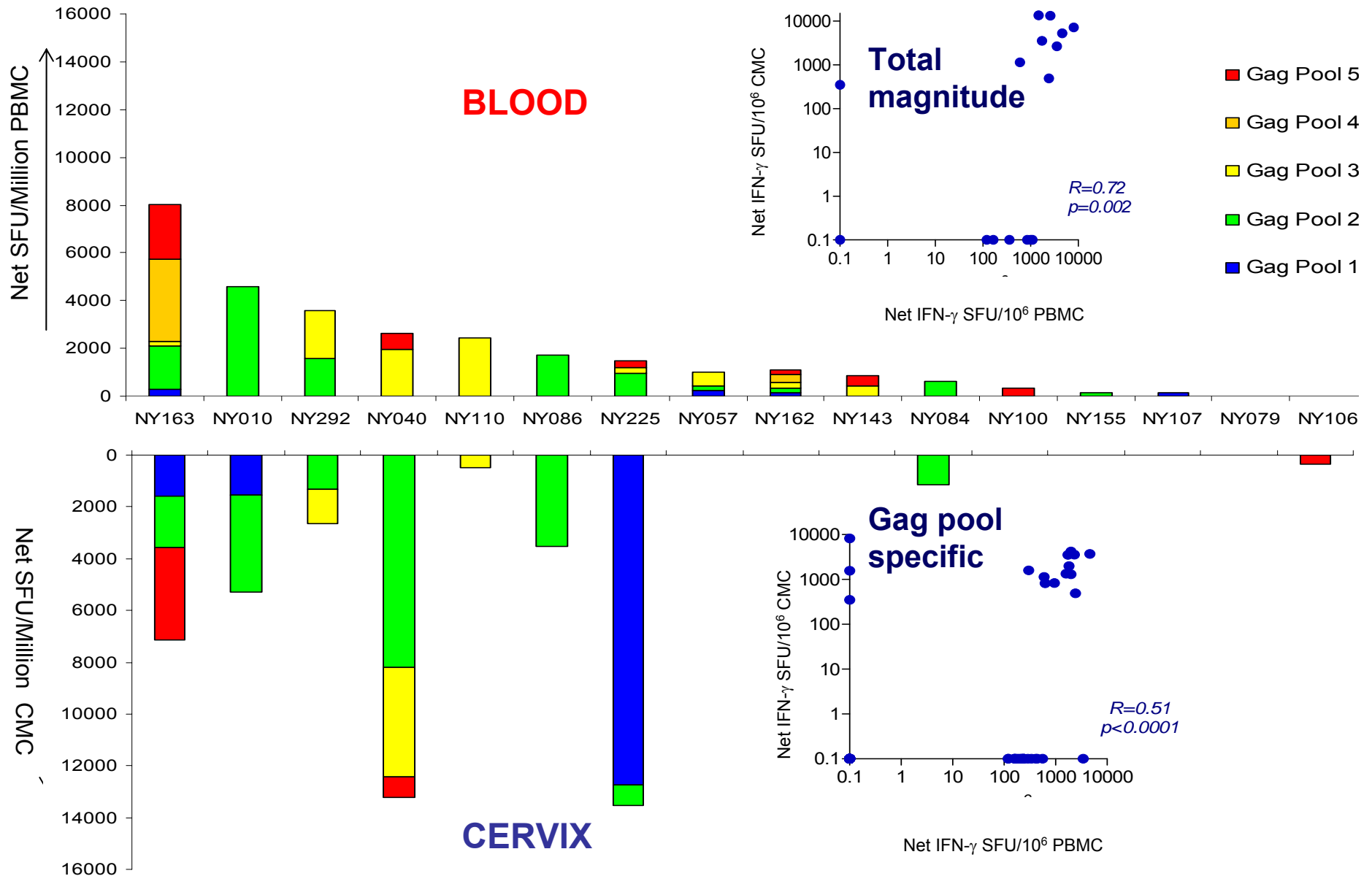


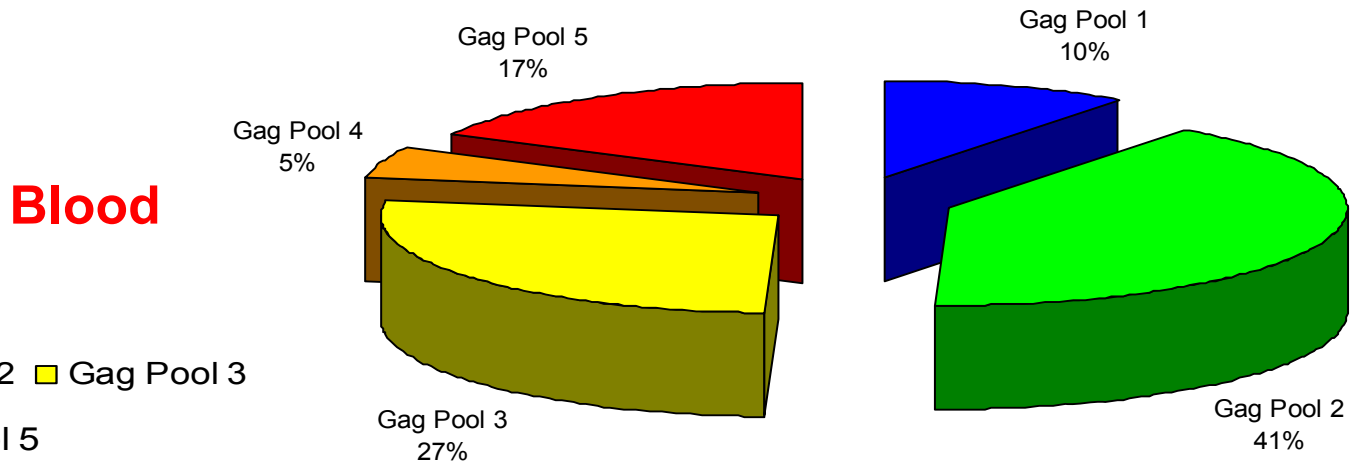
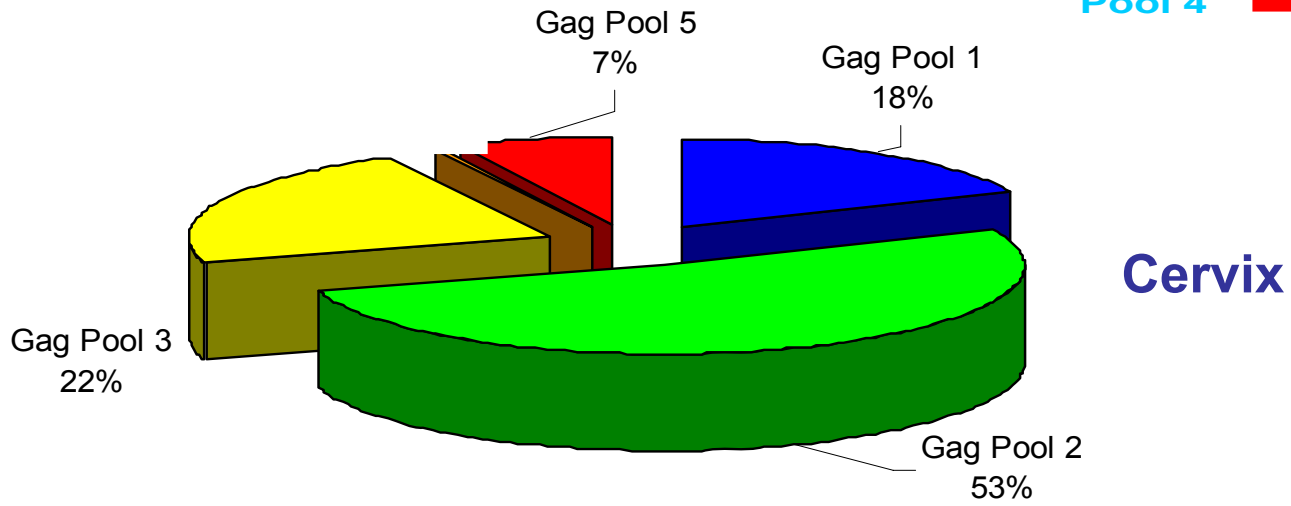
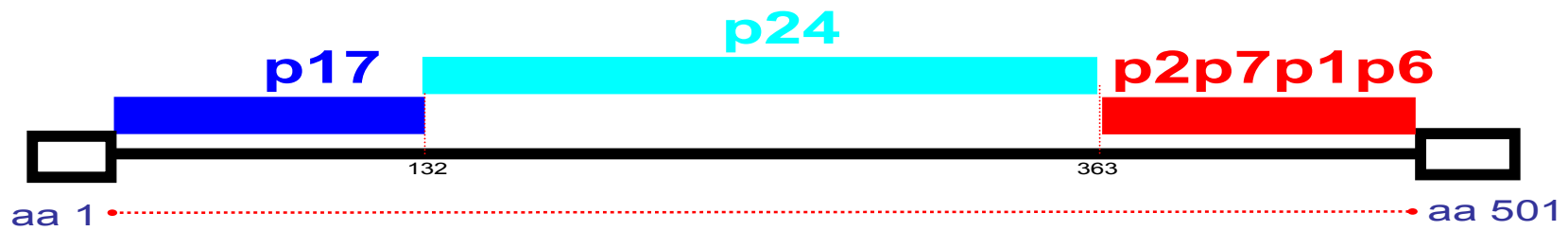
HIV-1 subtype C Du422 Gag overlapping peptides spanning the entire Gag sequence

# Comparison of cervical and blood HIV Gag-specific IFN- $\gamma$ responses in 16 women with chronic HIV-1-infection.



# Comparison of cervical and blood HIV Gag-specific IFN- $\gamma$ responses in 16 women with chronic HIV-1-infection.





■ Gag Pool 1 ■ Gag Pool 2 ■ Gag Pool 3  
 ■ Gag Pool 4 ■ Gag Pool 5

# Conclusions

- We found that *ex vivo* cervical cell numbers and **viability** are important **determinants** in the efficiency of cervical T cell expansion
- The **magnitude** and **specificity** of cervical T cell responses to HIV Gag **correlate** significantly with those detected in blood
- Women were more likely to have detectable cervical responses to Gag if they had **matching** but relatively **high** magnitude blood responses

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