



# Prevalence of Neutralizing Antibody Responses in Chronic Clades A and D HIV-1 Infections



Njai HF<sup>1</sup>, Tomusange K<sup>1</sup>, Sokolik-Wolak B<sup>2</sup>, Montefiori D<sup>2</sup>, Balla S<sup>3</sup>, Vanham G<sup>3</sup>, Nakiyingi-Miiró J<sup>1</sup>, Levin J<sup>1</sup>, Pala P<sup>1</sup>, and Kaleebu P<sup>1</sup>

<sup>1</sup>MRC/UVRI Uganda Research Unit on AIDS, Entebbe, Uganda.

<sup>2</sup>Laboratory for AIDS Research & Development, Dept of Surgery, Div of Research Sciences, Duke University, Durham, USA.

<sup>3</sup>Institute of Tropical Medicine, Antwerp, Belgium.



## Background



- HIV-infected patients develop autologous Nabs within 19 weeks of infection
- Nab response reduces due to the emergence of Nab-escape variants
- Autologous Nabs effective against specific HIV isolates and its close relatives
- 7 broad human MAbs; 2G12, 2F5, 4E10, b12, 447-52D, PG9 and PG16 so far identified
- Few studies have used standardized assays to identify, characterize Nab responses in non B HIV-infections



# Objectives



- Identify and characterize Nab responses in HIV-1 subtypes A & D chronically infected individuals
- Develop env-pseudotype subtype A and D virus panels
- Document kinetics of Nab development and viral escape
- Establish relationship between *env* sequences, markers of disease progression and the development of broad Nabs in longitudinal samples



## Materials & methods



- Patient inclusion criteria:
  - infected for more than 5 years
  - shows no clinical symptoms of AIDS
  - known dates of seroconversion
  - infected with HIV-1 subtype A, D or A/D
- 45 out of 200 treatment naïve patients were randomly selected for Nab screening
- Two serum samples for each patient (total 90 samples); T1(early) and T2(late) selected for Nab activity screening



## Methods cont..



- Standardized TZM-bl assay was used following a 3 tier testing algorithm
- 3 Tier 1 viruses used for Nab screening:
  - SF162.LS (subtype B)
  - MW965.26 (subtype C)
  - SVA-MLV (ART screening)
- CD4 counting was done using flow cytometry (BD FACSCount™ REF 340167)
- Linear regression and Wilcoxon matched-pairs signed-ranks test (Wilcoxon 1945) used for Statistical analyses



# Results



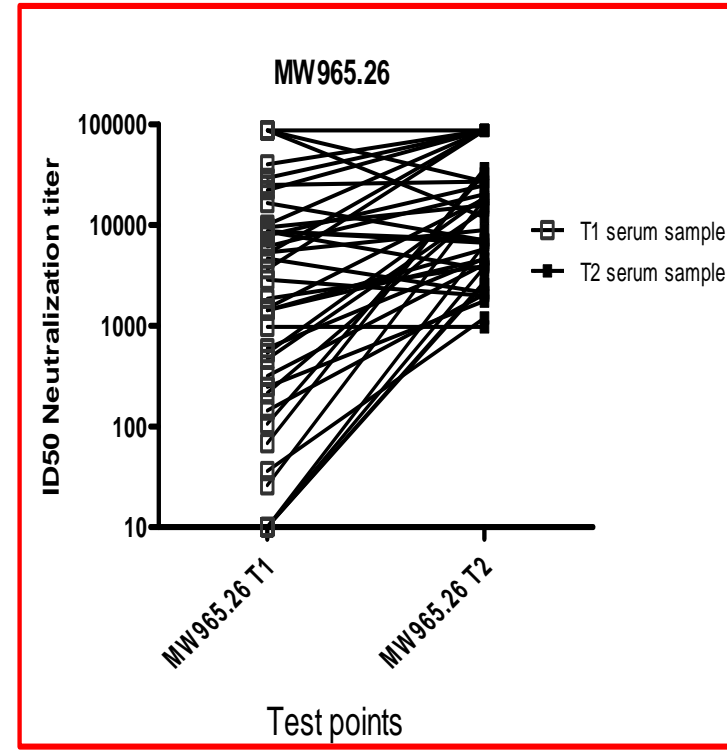
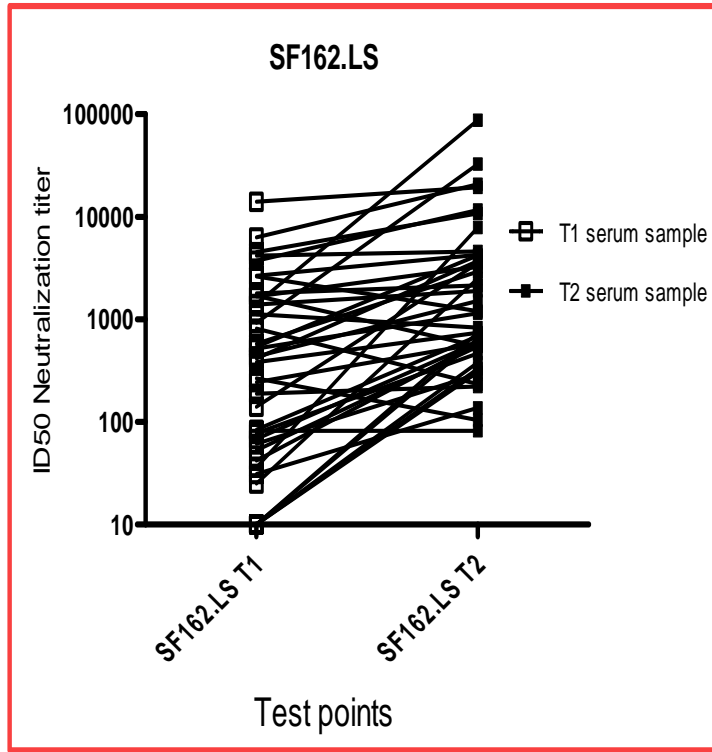
Table 1. Summary of serum Nab activity (RLUs)

Viruses	No.	mean	Sd.	Min	median	Max	iqr
SF162.LS	78*	3710.269	10937.2	10	668.5	87480	2428
MW965.26	78*	18715.45	28106.92	10	6264.5	87480	18597

## Notes:

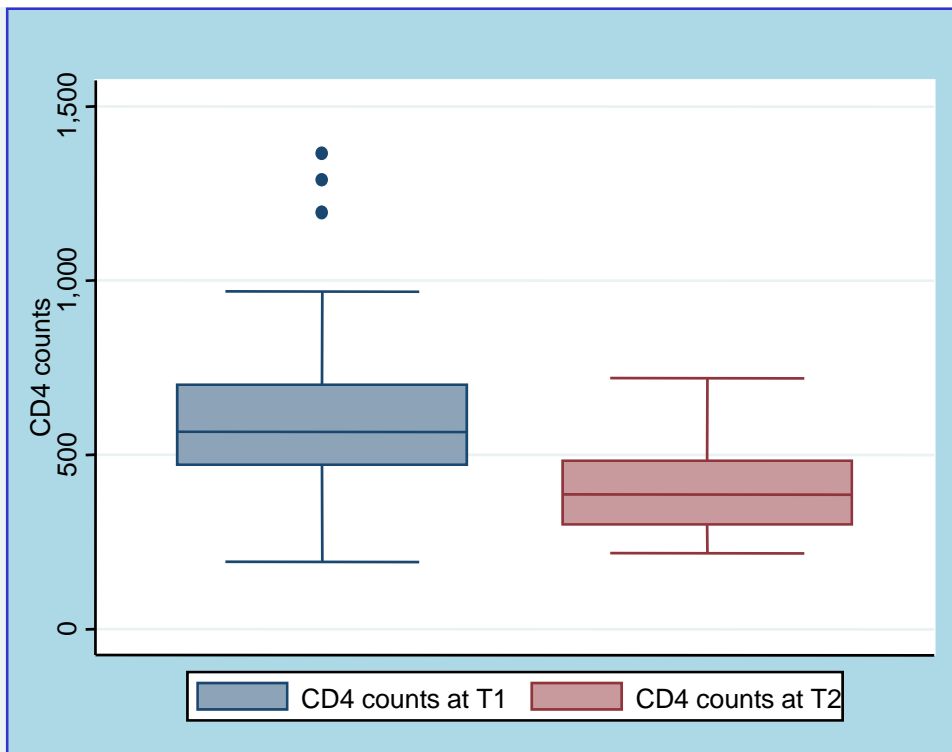
\* 12 of the 90 samples were excluded from the analysis: 8 showed activity against SVA-MLV, 4 were duplicated samples

Fig 1. Serum Nab activity of 78 serum samples at T1 and T2



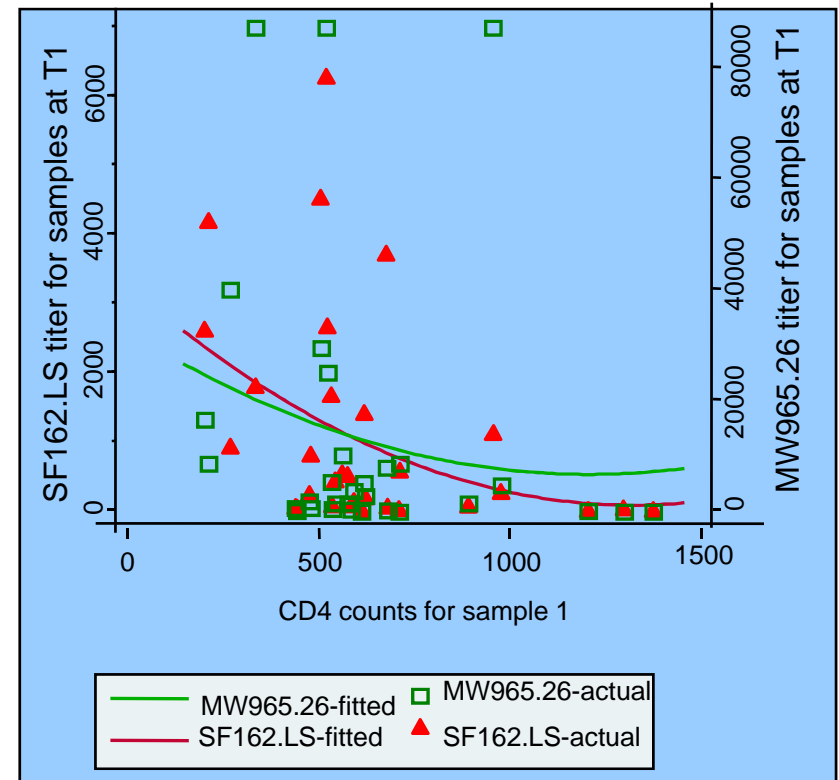
**Notes:** Higher Nab. Activity at T2 than at T1 against both SF162.LS and MW965.26

Figure 2: CD4 counts for first (T1) and second (T2) samples



T1 CD4 counts higher than T2 CD4 counts.

Figure 3. Nabs versus CD4 counts for samples at T1



Higher CD4 counts were associated with low Nab responses



## Discussion/conclusions



- 78 samples infected with subtypes A and D or A /D readily neutralized both SF162.LS and MW965.26 tier 1 viruses as demonstrated by several other studies
- Samples neutralized MW965.26 more than SF162.LS with a 9-fold median IC<sub>50</sub> titre difference
- Higher CD4 counts, were associated with low Nab responses
- Serum samples from chronically HIV-1 infected Ugandans possess Nabs against tier 1 viruses



## Future perspectives



- Characterize broad Nab activity with Tier 2 viruses and later with Tier 3 viruses
- Sequence samples to determine their subtypes
- Develop env-pseudoviruses for subtype A and D
- Map antibody specificity to precisely known env sequence
- Relate Nab characteristics with markers of disease progression