



# Pattern of Interferon Gamma T-cell Responses to HIV-1 and their Association with CD4 Counts and Viral Load Trajectory in Early Infection



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# Objectives



- To identify 30 individuals with new HIV-1 infections close to time of viral transmission
- To prospectively determine HIV-1 plasma viral load trajectory vis a vis HLA class I alleles through viral set point
- To measure the pattern of epitope recognition and size of HIV-1-specific CD8+ responses longitudinally post HIV-1 infection



# Methods



- 4 acute (P24<sup>+</sup>EIA<sup>-</sup>) and 10 early (P24<sup>+/-</sup> EIA<sup>+</sup>) infections from 156 Ugandan HIV-1 discordant couples
- Cryopreserved PBMCs; CD4 counts - Coulter Act5; PVL/ml - Amplicor V1.5 Roche
- Peptides; A1 / D consensus proteome (18mers overlapping by 10aa - NMI Technology, Germany
- IFN- $\gamma$  culture ELISPOT responses to peptide pools
- Epitopes mapping; ex-vivo IFN- $\gamma$  ELISPOT
- HLA class I alleles; PCR - Reference strand conformation Analysis [Arguello et al., 1998]

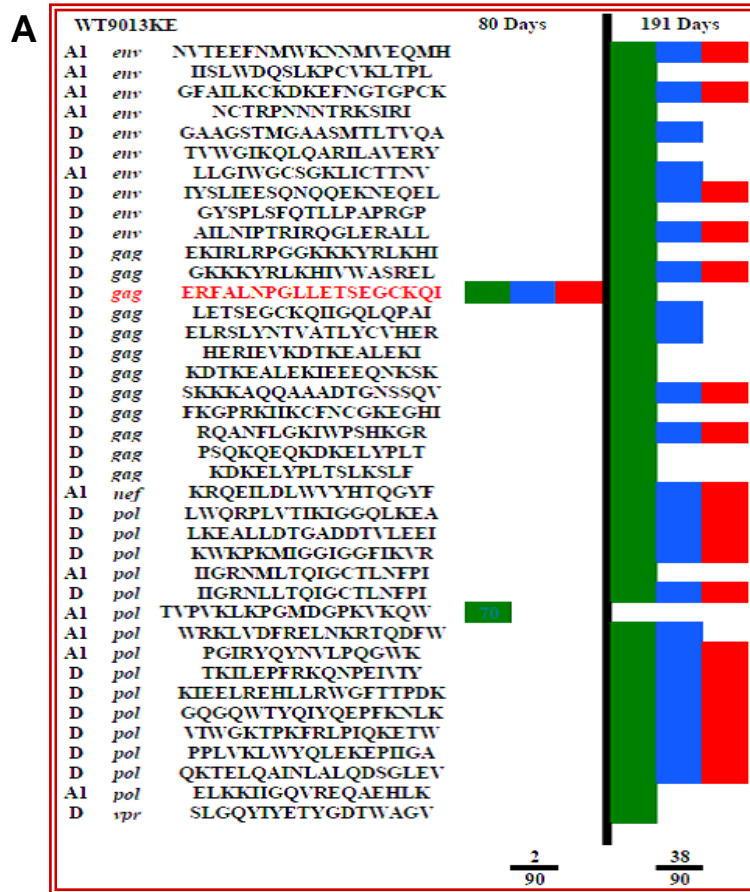


EDCTP

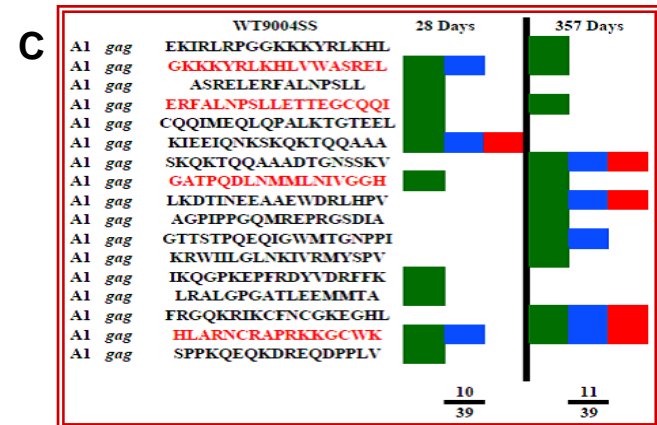
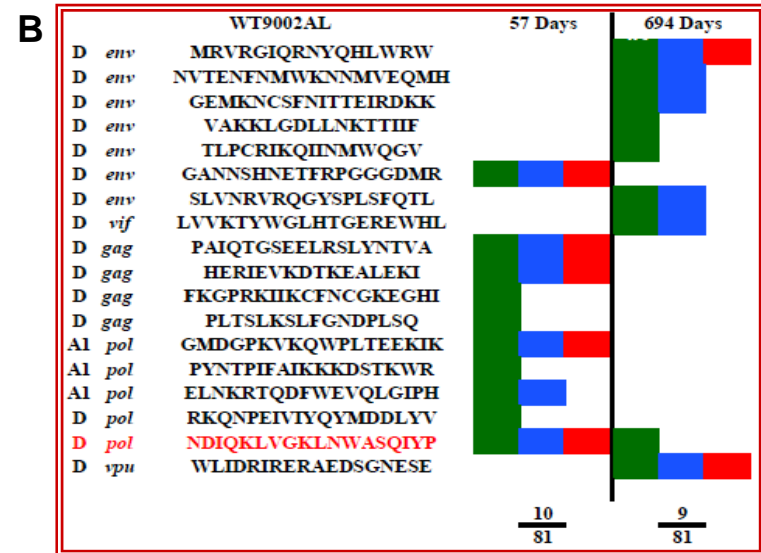
# Results



## Magnitude and Breadth of Epitope Recognition for 3 Patients



■ 3\*DMSO (mock)  
■ 4\*DMSO (mock)  
■ 5\*DMSO (mock)





# Fiebig Staging and Phylogeny of HIV-1 Early Infections



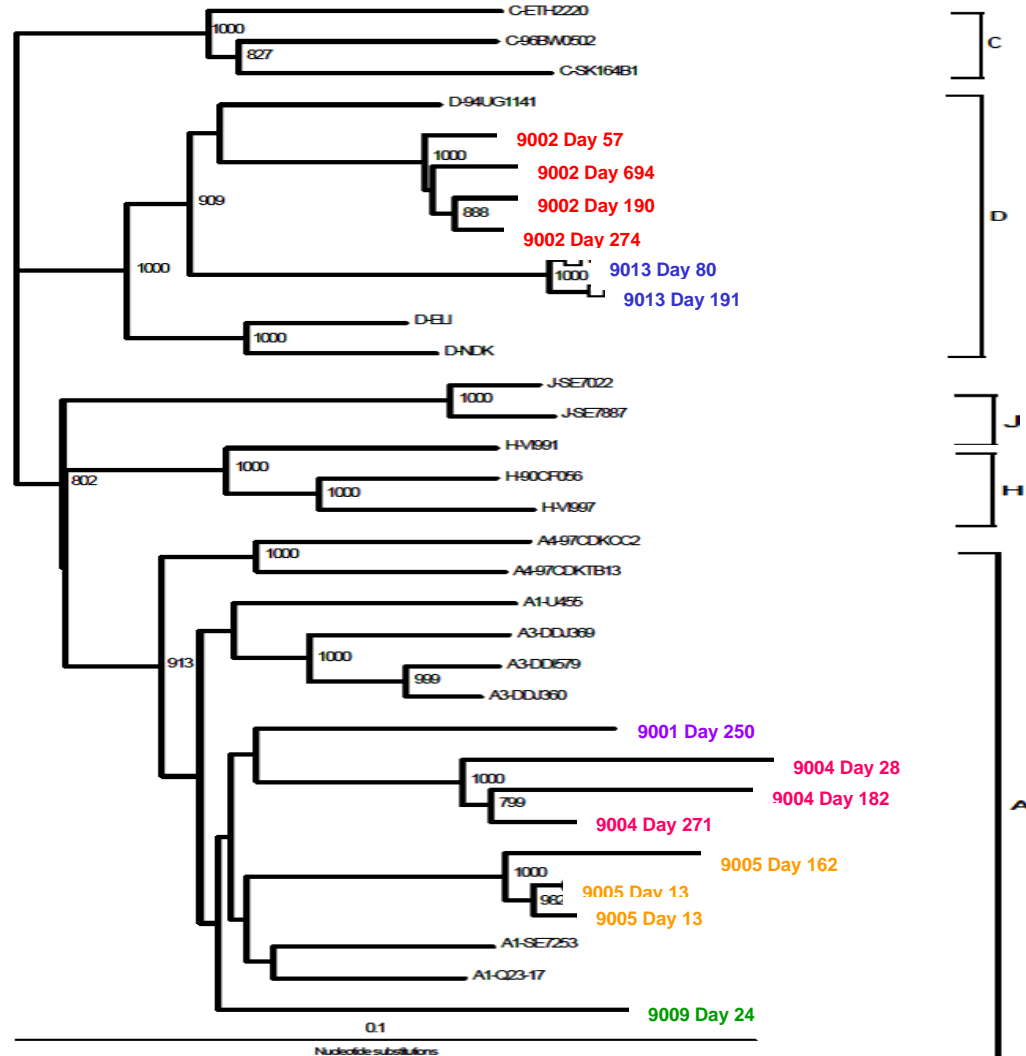
Fig 1

PTID	Screening		Enrolment		
	P24 Antigen	Antibody EIA	Antibody EIA	Western Blot	Fiebig Stage
9001SE		+	+	+	VI
9002AL		+	+	+	VI
9003SJ	-	+	+	+	VI
9004SS	+	-	+	+ <sup>P31</sup>	V
9005MC		+	+	+	VI
9006JG		+	+	+	VI
9007NM		+	+	+	VI
9009SA	+	-	+		IV
9011NM	-	+	+	+ <sup>P31</sup>	V
9012KM		+	+	+	VI
9013KE		+	+	+	VI
9014NA	+	-	+		IV
9015KF		+	+	+	VI
9016SM	+	-	+	+ <sup>P31</sup>	V

+<sup>P31</sup> Positive western blot without p31 band

| Indeterminate western blot

Fig 2



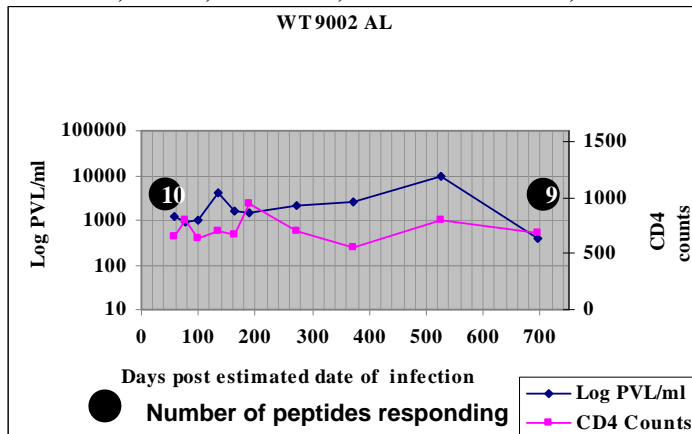


# Plasma Viral Load Trajectories, CD4 Counts and Epitope Recognition



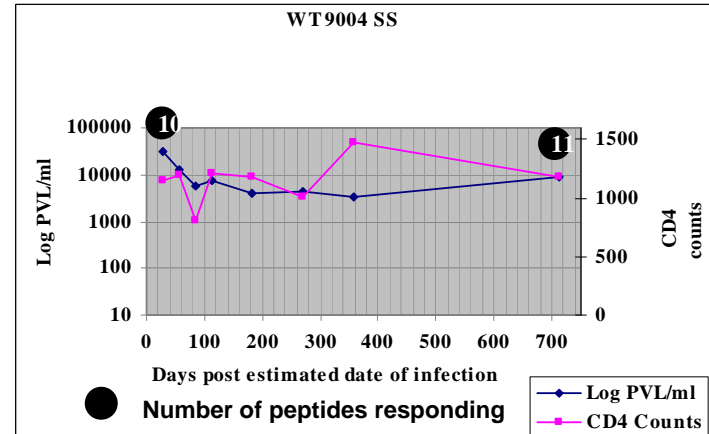
A\*0201, \*3002; B\*4403, \*1402 Cw\*0401, \*0802

A



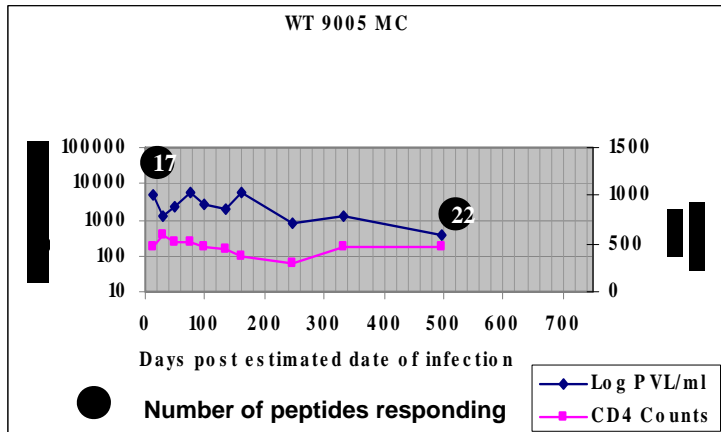
A\*3402, \*7401; B\*4403, \*5802; Cw\*0401, \*0602

B



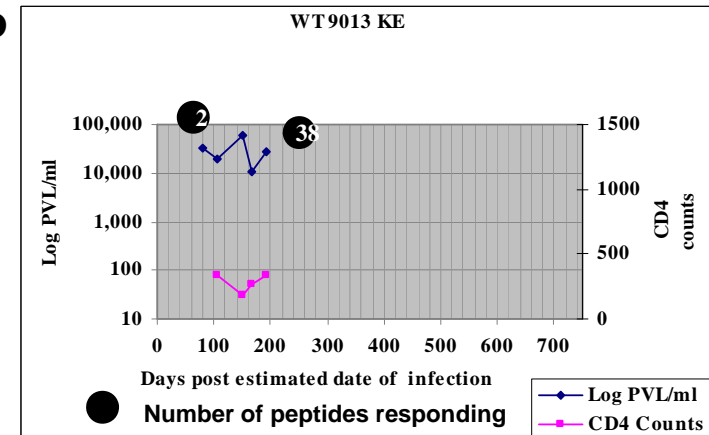
A\*2301, \*7401; B\*4403, \*1510; Cw\*0401, \*1601

C



A\*0201, \*3402; B\*4501, \*4701; Cw\*0602, \*1601

D





# Discussion & Conclusions



- No association has been observed between T-cell responses elicited by founder HIV-1 virus and viral set point hitherto
- A caveat exists since consensus peptides were used; it is unknown how the virus sequences match the peptides
- Early T-cell responses displayed narrow breadth but later widened although some of the later responses were low in magnitude
- HLA allele B\*5802 has been associated with faster progression to AIDS however in patient WT9004SS we find conserved CD4 counts and low PVL copies/ml



# Future Work



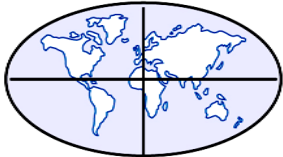
- Phenotypic and functional characterization of HIV-1 epitope specific T-cell responses by multi-parameter flow cytometry
- Phenotypic markers: CD3, CD4, CD8, CD57, CD27, CD28.
- Functional markers: IFN- $\gamma$ , TNF- $\alpha$ , IL-2, CD107a, perforin and CFSE for proliferation
- Sequence the viruses to find out how they match / relate to the peptides used.



# Acknowledgement

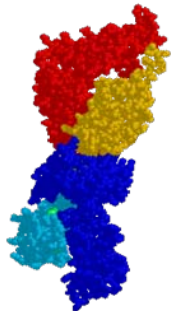
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