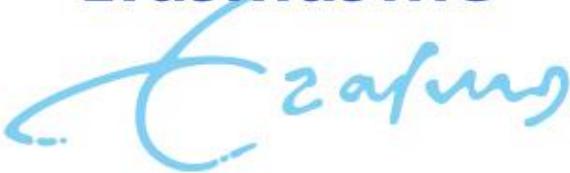


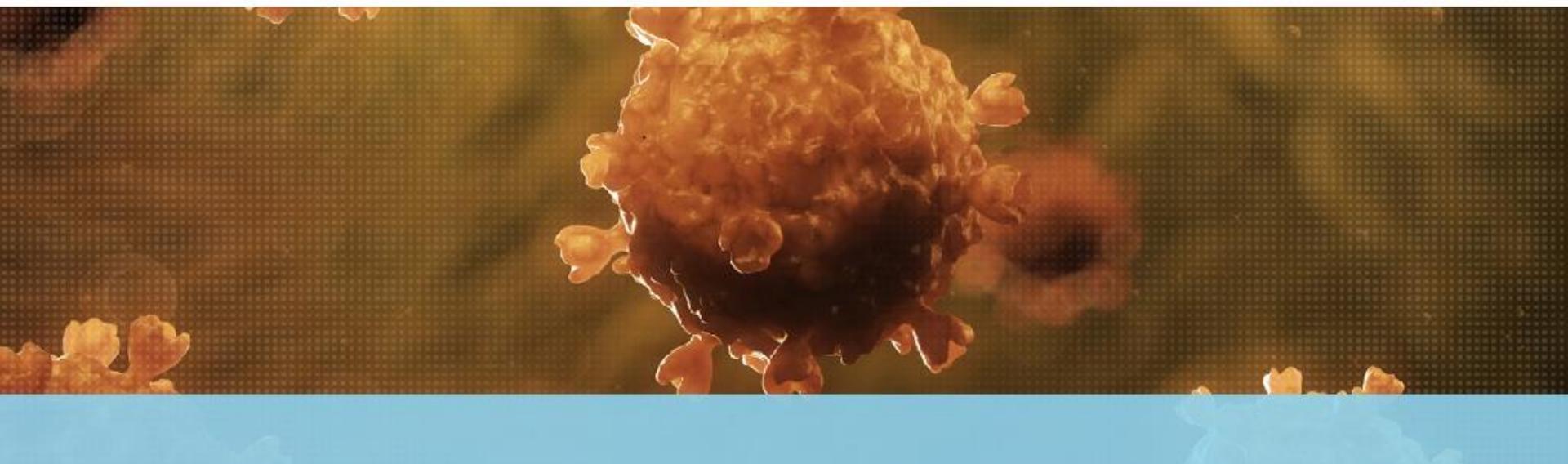
Erasmus MC



Vrije
Universiteit
Brussel

VIRGO
consortium

Viroscience lab
WHERE SKILLS MEET TO STUDY & PROTECT



MONITORING DC-BASED IMMUNOTHERAPY AGAINST HIV

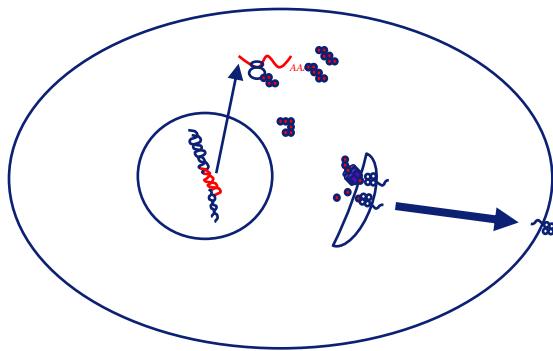
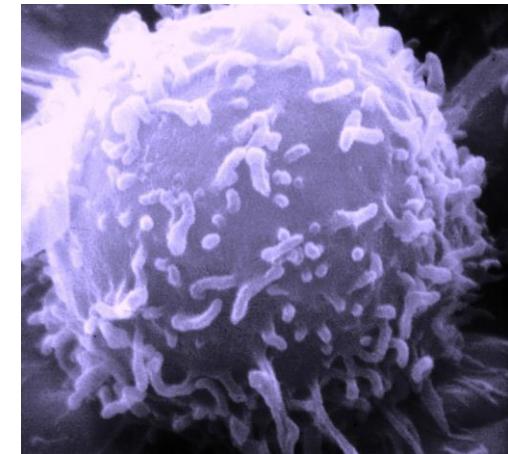


BREACH
BioRxiv Research Evaluation Assessment Consortium

Dr R.A. Gruters

Background: Cytotoxic T Lymphocytes (CTL)

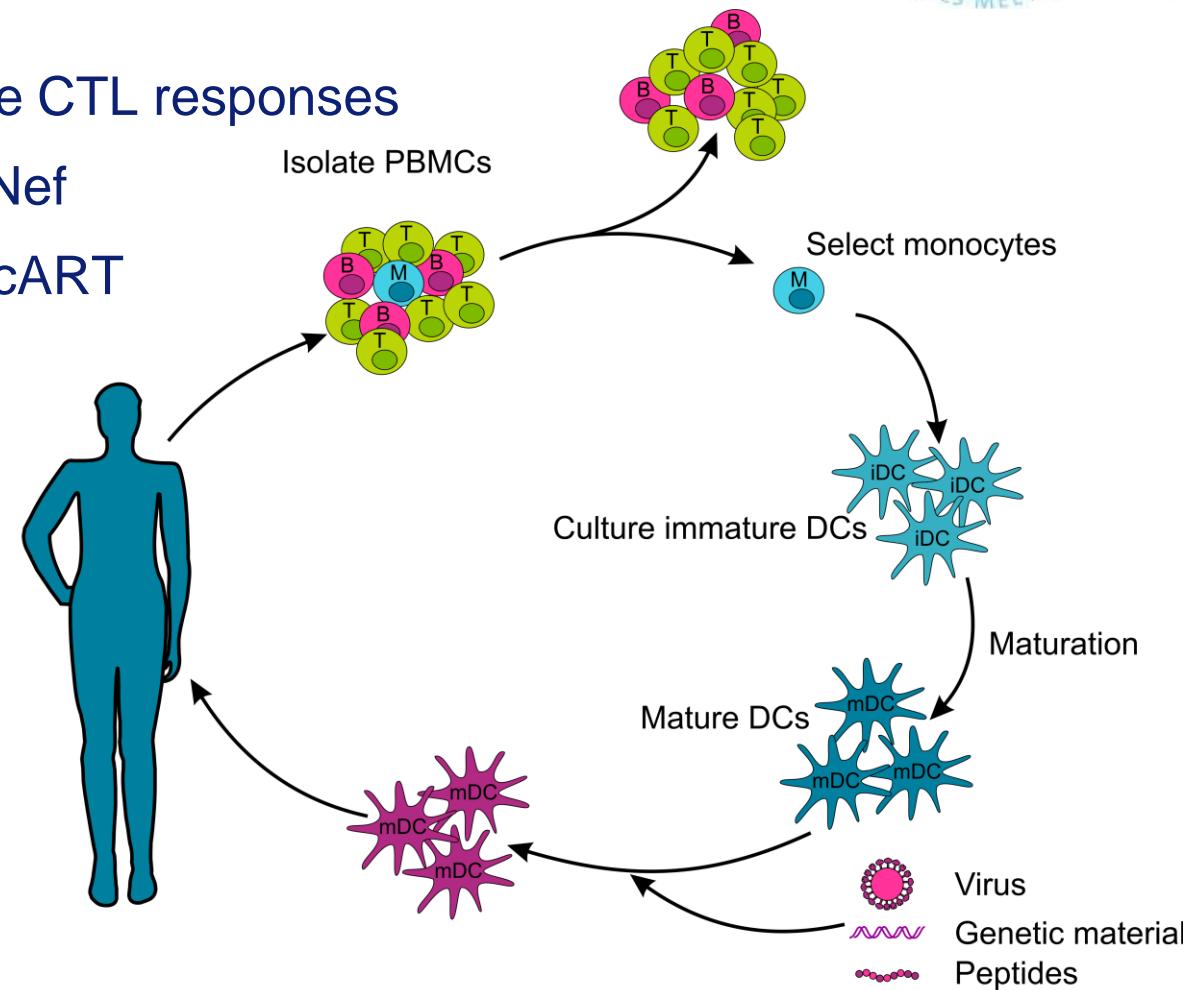
- Cellular immunity is important for the control of HIV
 - ✓ Appearance of CTL coincides with decrease in viremia
 - ✓ Escape from CTL pressure
 - ✓ CD8 depletion experiments in macaques
- Elite controllers have better/other CTL responses than progressors



DC immunotherapy for HIV

- Aim of the study:

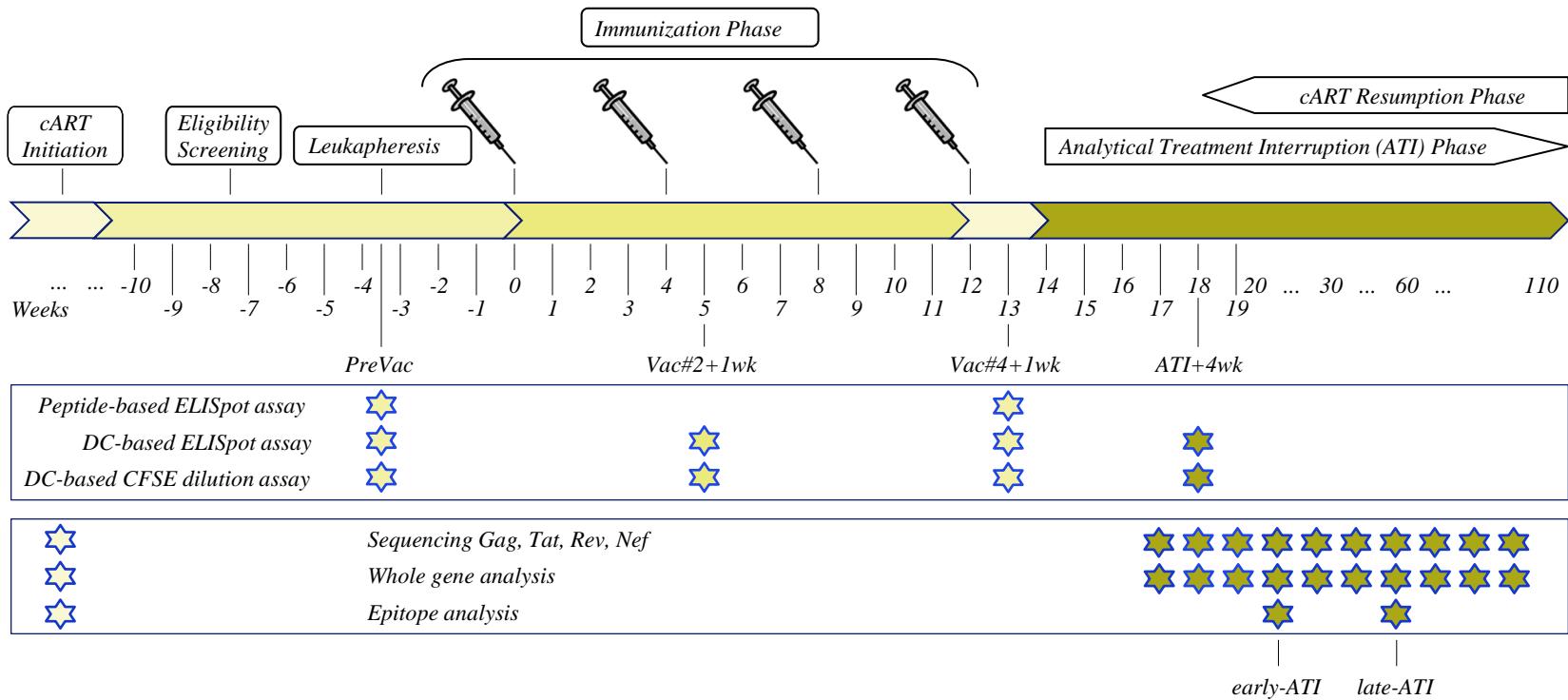
Induce and/or enhance CTL responses
against Tat, Rev and Nef
in HIV patients under cART



Allard et al 2008 Vaccine 26: 3735-3741

DCTRN Trial set up and sampling

phase I/IIa, multicenter
open label, non controlled
 $n = 17$



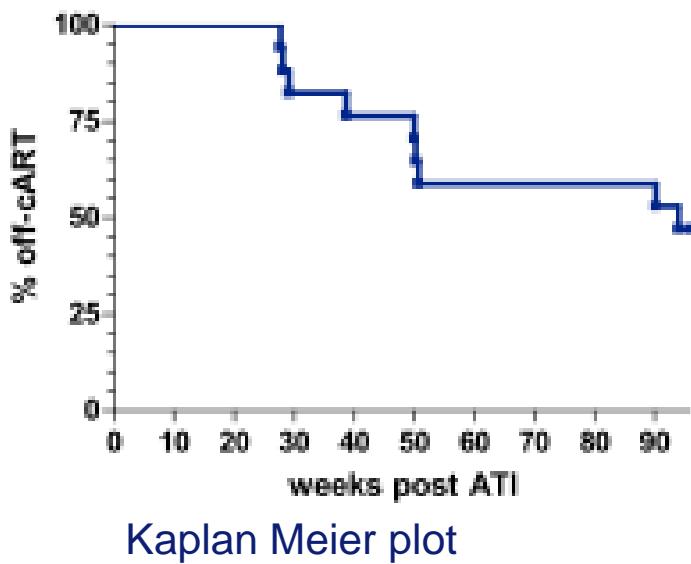
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Safety

- No serious adverse events related to vaccination
 - ✓ Most common events: pain and induration at injection site

- All participants interrupted cART
 - ✓ No severe adverse events related to treatment interruption



One patient remained 7 years off therapy!

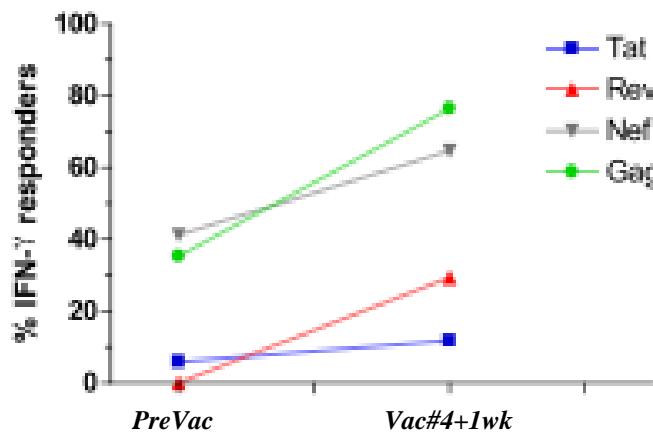


Immunogenicity

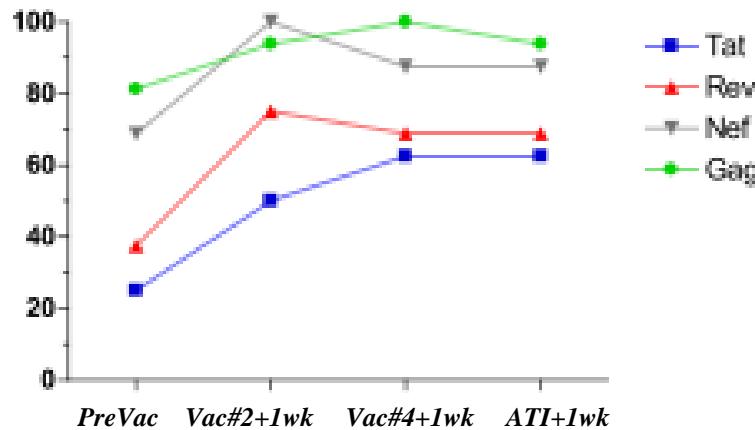
- Evaluate immune responses before and after vaccination
 - ✓ IFNy peptide ELIspot
 - ✓ New assay DC-ELIspot
 - ✓ Proliferation assay
- Effect on virus?
 - ✓ Sequencing for mutations
- Changes in the blood?
 - ✓ Genomics (mRNA expression profile PBMC)
 - ✓ Luminex plasma analyte analysis

Assay comparison

peptide-ELISpot



DC-ELISpot

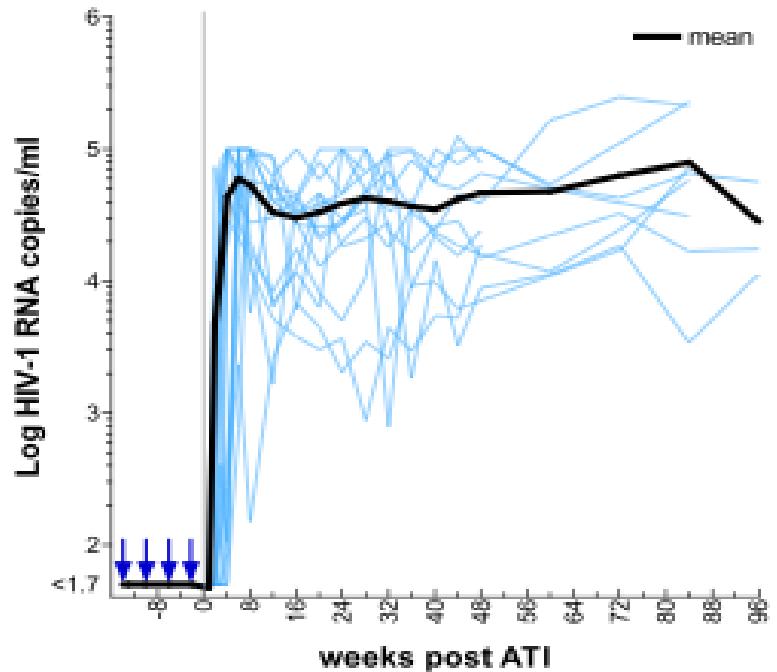


- DC-ELISpot more sensitive than peptide-ELISpot
- After vaccination, most subjects have IFN- γ responses to ≥ 1 antigen, either induction or enhancement
- Proliferation assay of CD4+ and CD8+ T-cells shows comparable results

Allard *et al* 2012 Clinical Immunology 142(3): 252-268

Sequencing (Sanger)

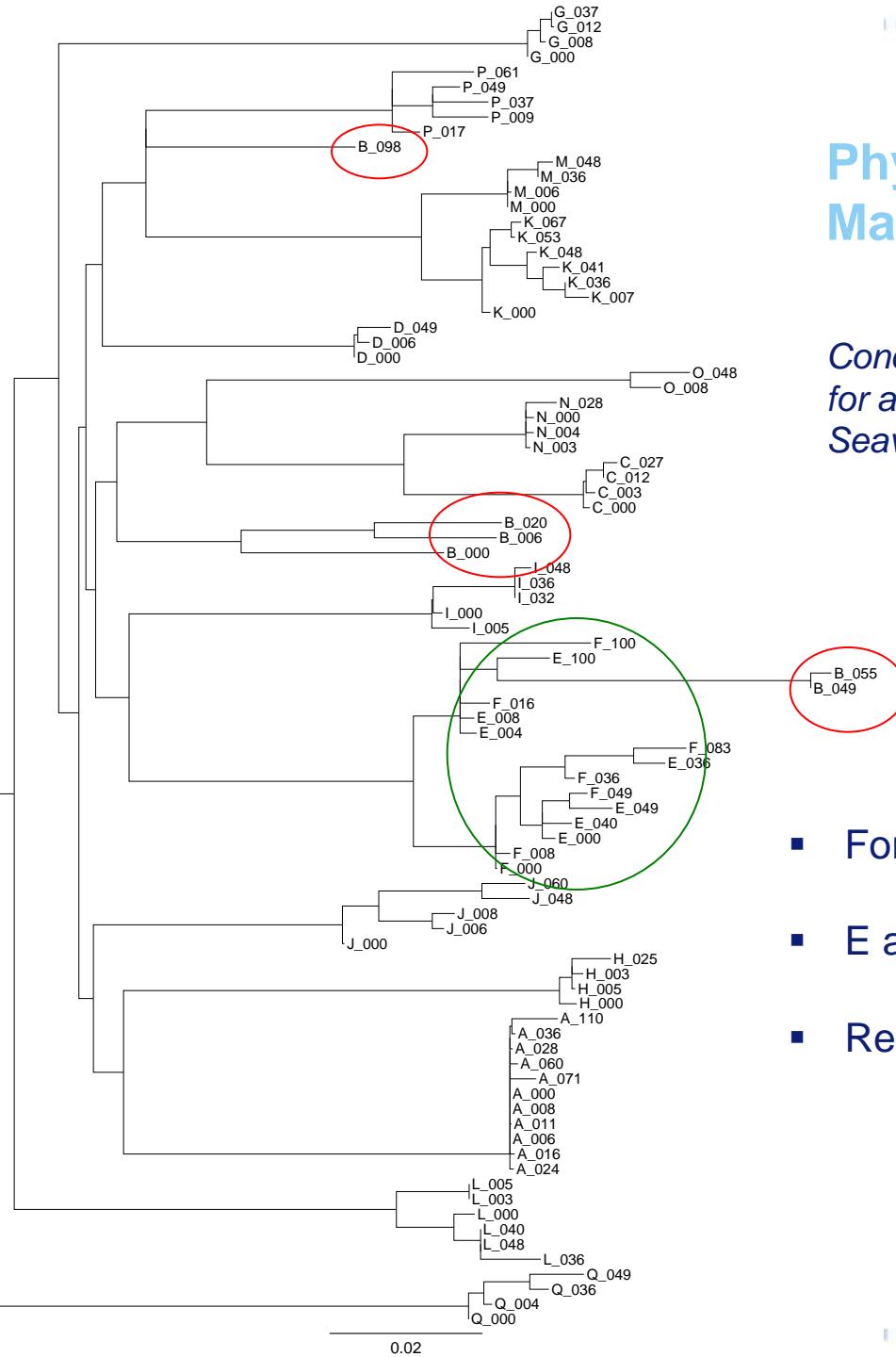
- Pre-cART sample
- Plasma viral load rebound after ATI
- First plasma viral load $>10^4$ copies/ml
- Later samples
 - ✓ 1 year after ATI
 - ✓ at re-start cART





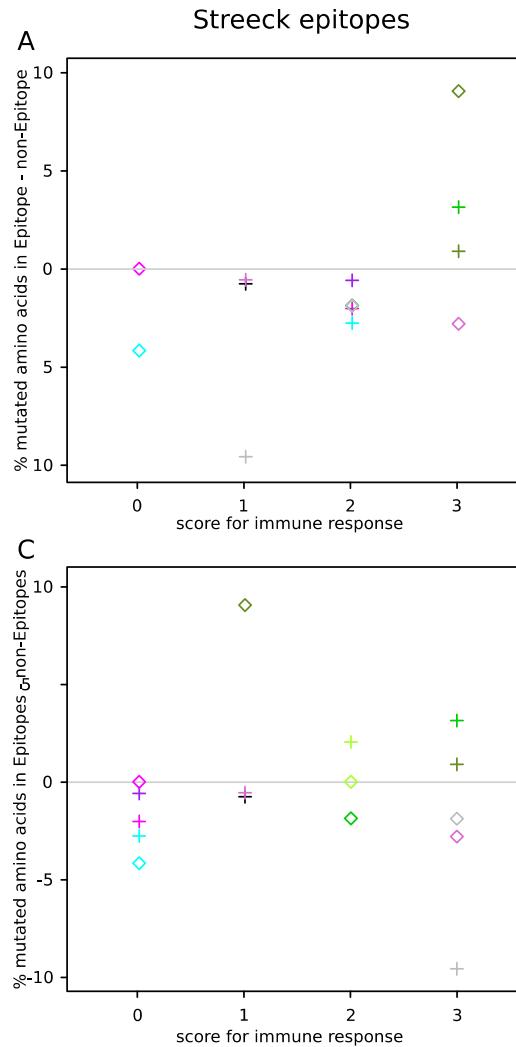
Phylogenetic tree: Maximum-likelihood

*Concatenated nucleotide sequences of the ORFs
for all time points
Seaview version 4.2.6*

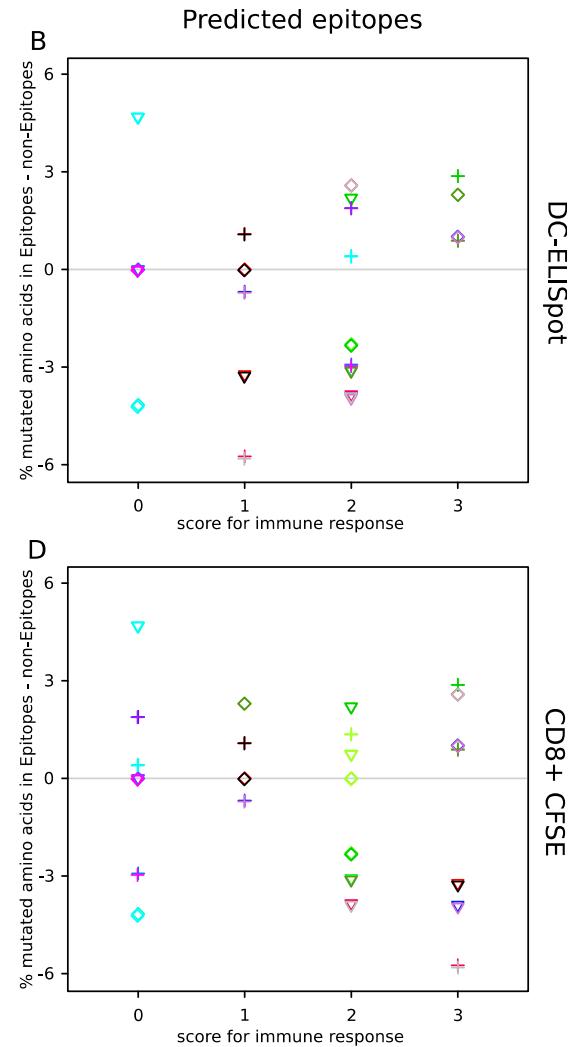


- For each subject sequences within one cluster
- E and F cluster together (continuous exchange)
- Recombination event B (superinfection?)

Viral escape after vaccination?



Subject colour code:
 A C D E F G H I J K L M N O P Q
 Protein symbols:
 ▽ Tat ◊ Rev +Nef



de Goede et al 2013 AIDS 27: 2679-2689

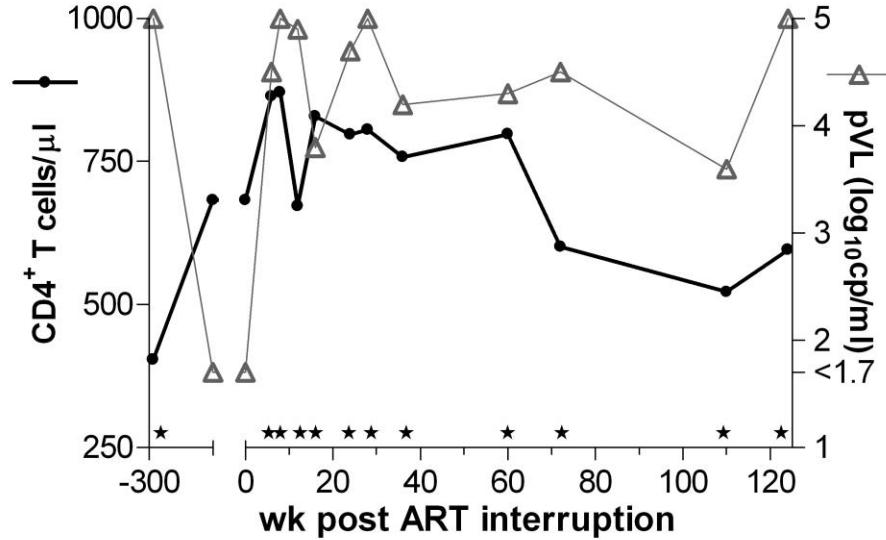
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Sequence variation within novel B*4403 Rev epitope EELLKTVRL (EL9)

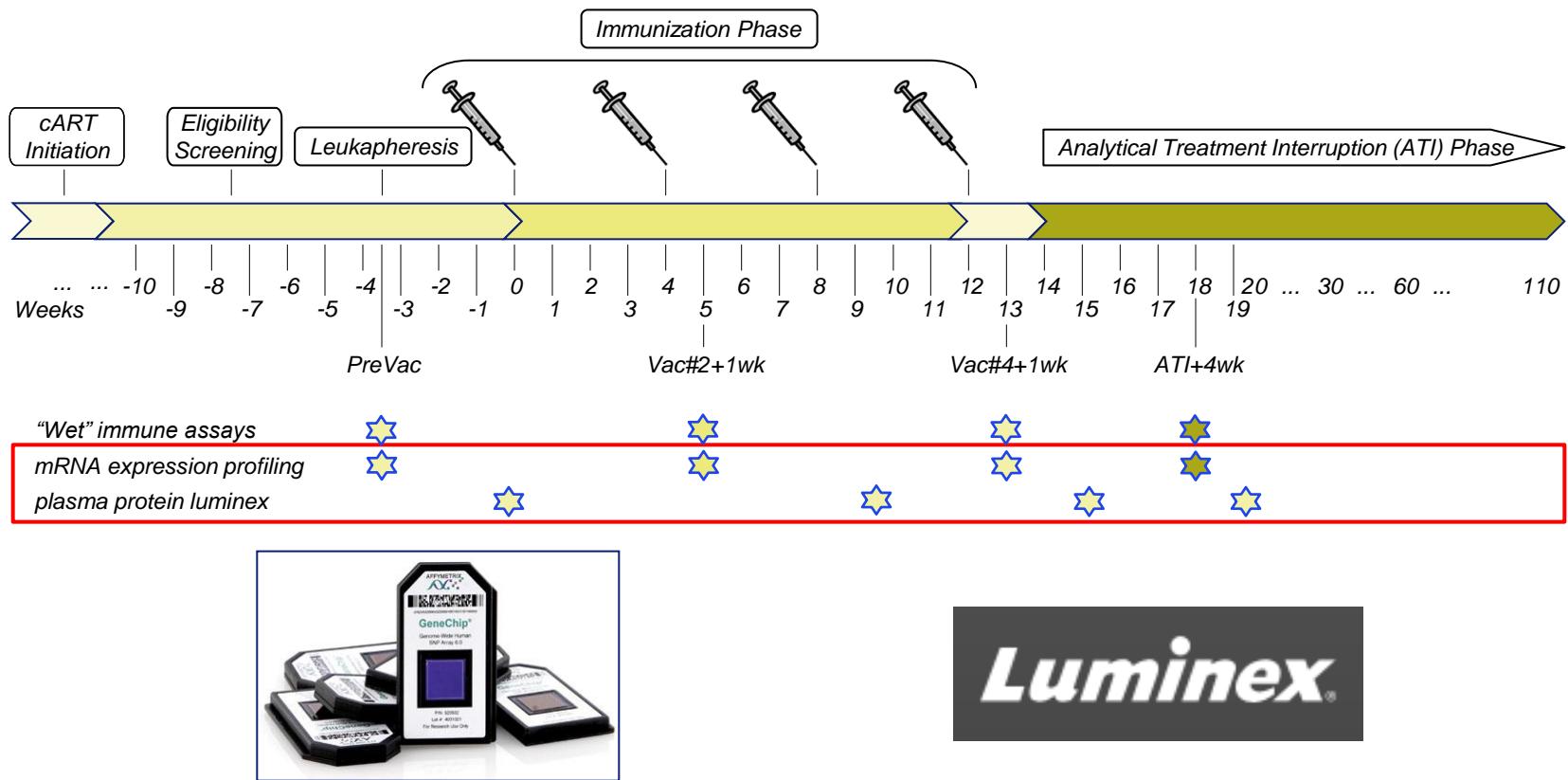


FIGURE 3. EL9 sequence evolved following ART interruption



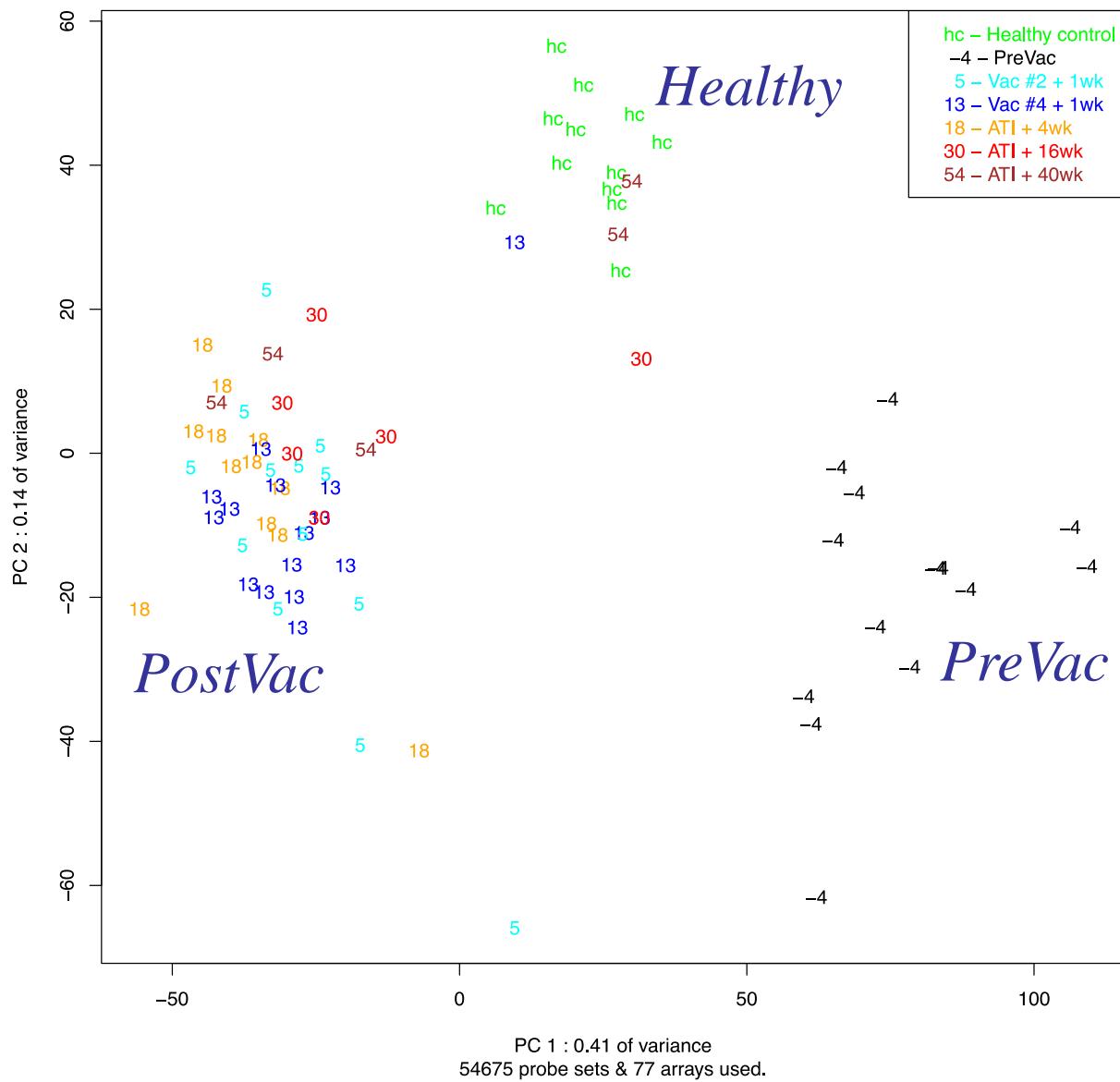
WT EL9 +	+++	++	+
E10D			+
E11D			+
E11D,K14Q	+	+	++

Host response analysis continued



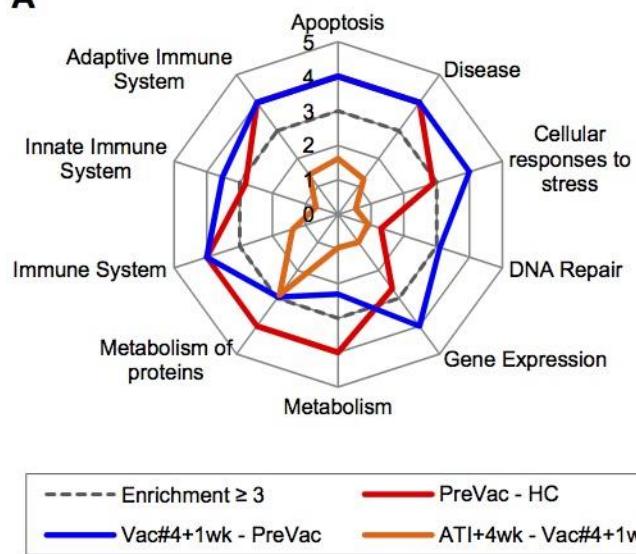
- **Characterize vaccine-induced host-responses by mRNA and protein profiling**
 - ✓ mRNA expression changes in PBMC: 50.000 probes, 19.000 genes
 - ✓ Protein/hormone expression changes in plasma: 243 markers

Principal component analysis of mRNA expression profiles

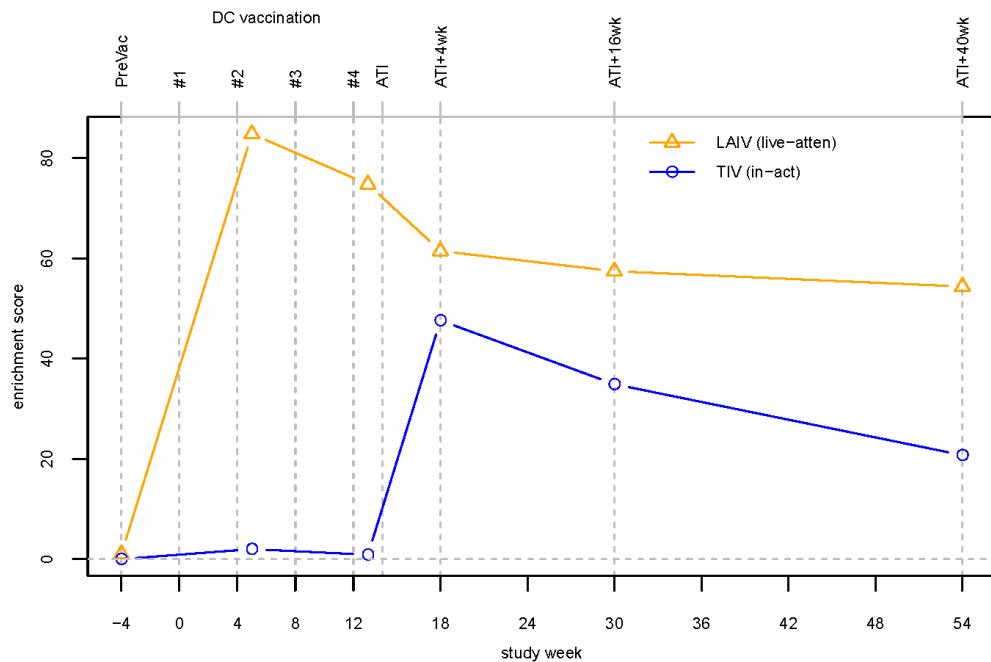
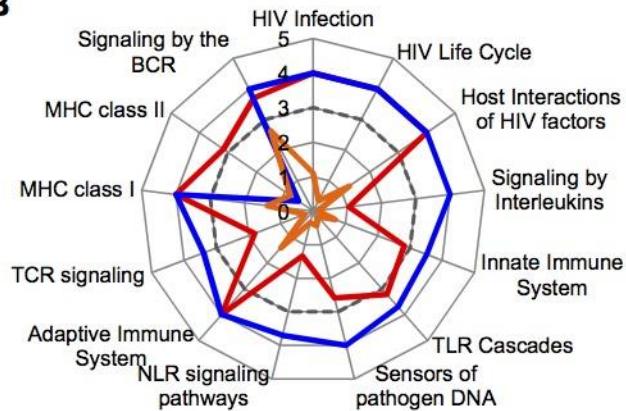


Pathway analysis

A

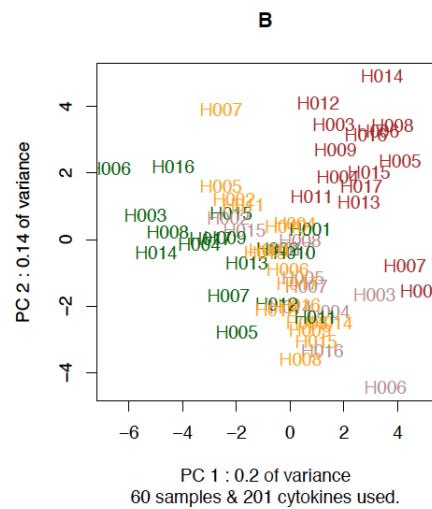
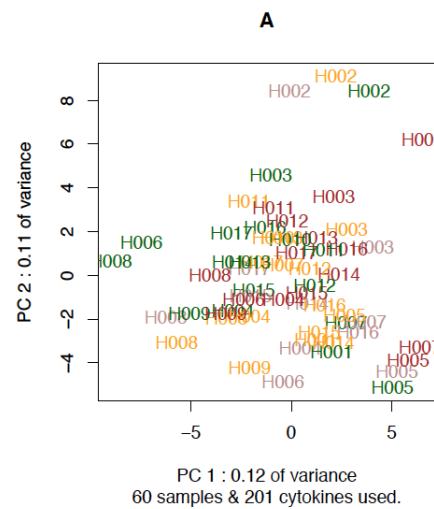
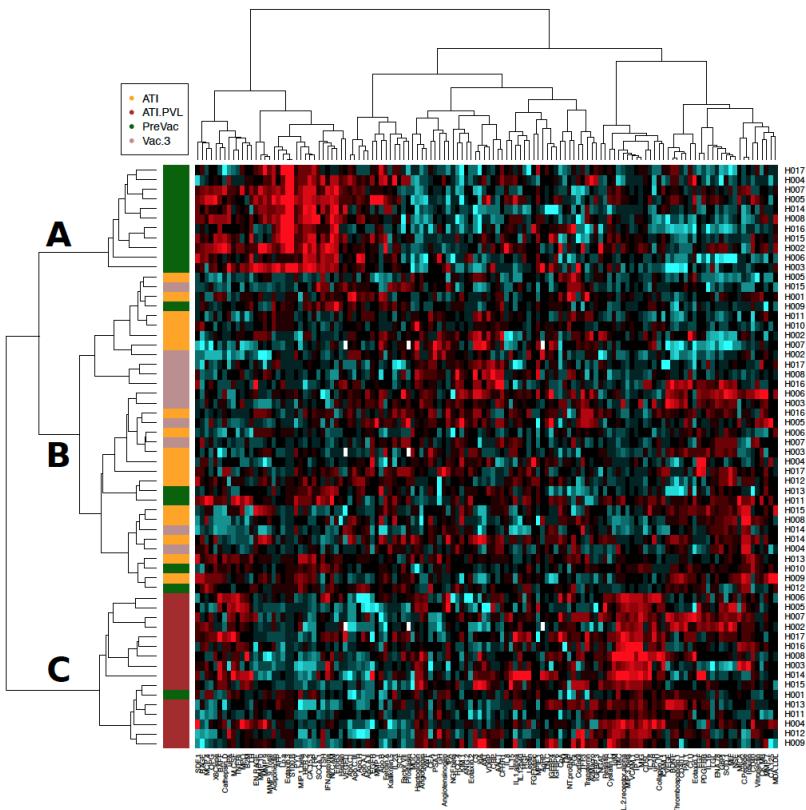


B

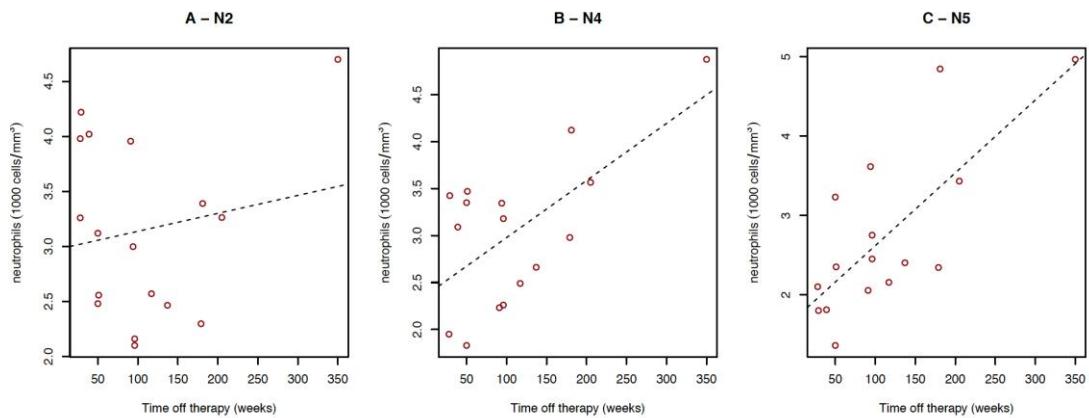
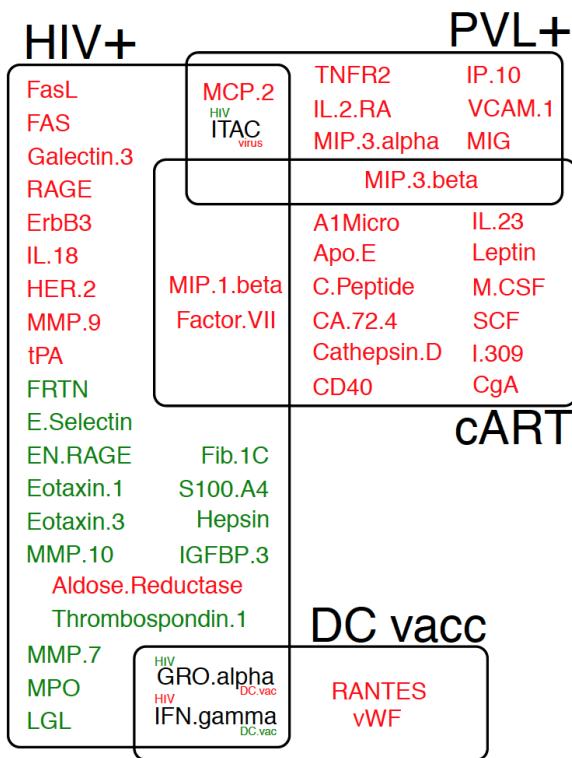




Luminex: removal of patient effect



Luminex results



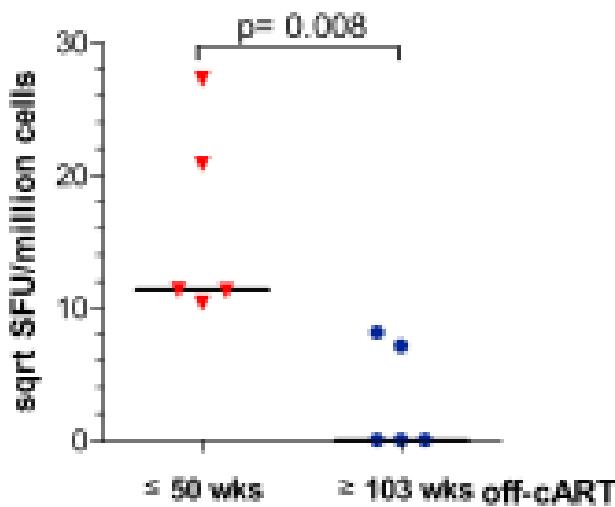
Conclusions

- DC immunotherapy for HIV safe and well tolerated
- HIV-specific CD4+ and CD8+ T-cell responses induced and/or enhanced
- Anecdotal but not overall effect on sequence evolution (Sanger)
- Major shift in mRNA expression profile after vaccination
- Shift plasma proteins when plasma virus rebounds



Discussion

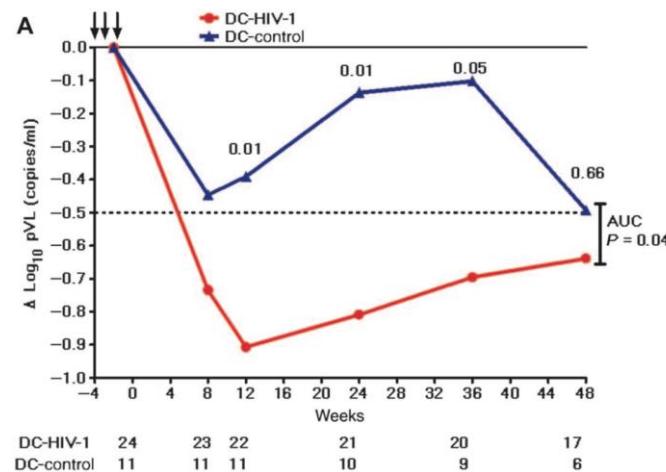
- Transcriptomics: signature of T-cell skewed immune response
- Luminex: too low patient numbers for firm conclusions (TBG)
- Specific immune responses against HIV counteracted by general immune activation?





Future plans

DCV2 study

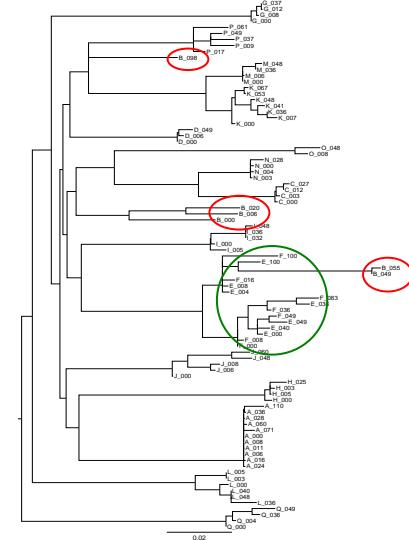


Transcriptomics

iHIVARN^A

Next Gen Seq

DC-TRN



40 vaccinees,
15 adjuvants controls
15 placebo controls

24 vaccinees*
12 controls

*half of these responded well

Start January 2017

Ongoing

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People



EMC Viroscience

AL de Goede
HJ van den Ham
P Boers
M Bijl
Fatiha Zaaraoui
J Koetsveld
M Reedijk
E Verschuren-de Winter
C Burghoorn-Maas
M Schutten
AC Andeweg
CA van Baalen
ADME Osterhaus

EMC Internal Medicine

ME van der Ende

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S Allard
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P Lacor
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K Thielemans

Rega Institute Leuven

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Utrecht University

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