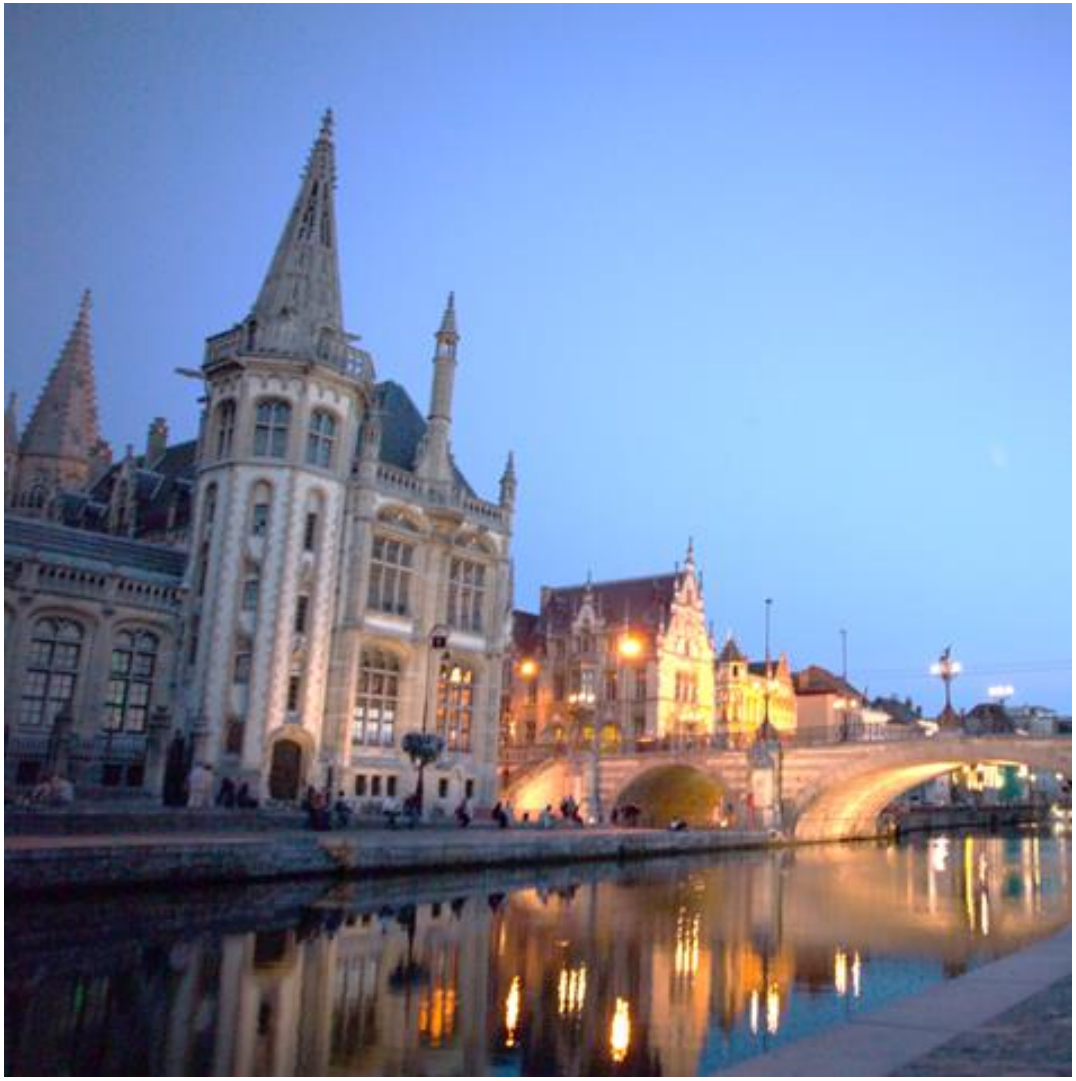


Quantifying and characterizing HIV reservoirs in an HIV cure setting

One day symposium

Latest news on European HIV Cure trials

Optional three day workshop
20-22 Sept 2016

19-Sept-2016

HCRC
Ghent University and
Ghent University Hospital

HIV Cure Research Center

Empowered by patients, driven by science

Ghent University Hospital, Entrance 69

Ghent University: MRB II

Country list of attendees 2016

Togo, Cameroon ,Italy ,
Germany, United Kingdom,
Turkey, France, Luxembourg ,
the Netherlands, Germany,
Spain, USA, Israel, Austria,
Denmark, Sweden

Current HIV (reservoir) studies in Belgium

- ISALA: do some patients that harbour a low reservoir control the virus in the absense of cART
- HIV STAR: where does the rebounding virus come from
- ABIVAX: does ABX464 suppress HIV viral load in the absence of cART
- EPISTEM

UNIVERSITEIT GENT

Isala Trial

"Analytical treatment interruption in HIV positive patients with low viral reservoir to evaluate the potential of a functional cure"

ISALA trial

Single arm – non-randomized - multi-centric – prospective trial

Intervention: Treatment interruption among pts with low viral reservoir

Funding through TBM program from IWT

Research consortium

Eric Florence
Guido Vanham
Natacha Herssens

UMC Sint-Pieter

Stéphane De Wit
Coca Necsoi

Universitair Ziekenhuis Gent

Linos Vandekerckhove
Ward Despiegelaere

Vrije Universiteit Brussel

Sabine Allard
Joeri Aerts

Innovative goal : Viral reservoir assessment

Reservoir measurements:

- UsRNA
- Total integrated DNA

Viral Outgrowth Assay (VOA)

Endpoints

Primary:

Proportion of Post Treatment Controllers (PTC)

Proportion of patients with undetectable pVL (<50 HIV RNA copies/ml plasma) at 48 weeks post treatment interruption.

Secondary:

- Viral Outgrowth Assay as predictor for PTC
- Safety of the intervention
- Reservoir replenishment at viral rebound

+ Substudies

Study in two phases (status 15 september)

- **First phase**= reservoir measurement

  65 participants

- **Second phase**= treatment interruption

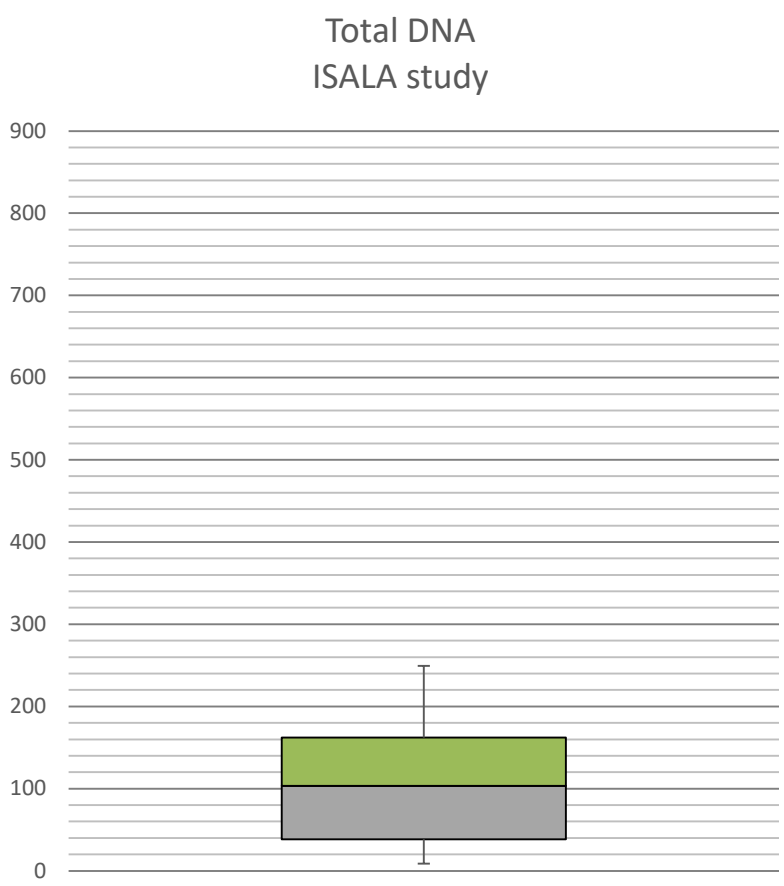
  (Cytapheresis before interruption)

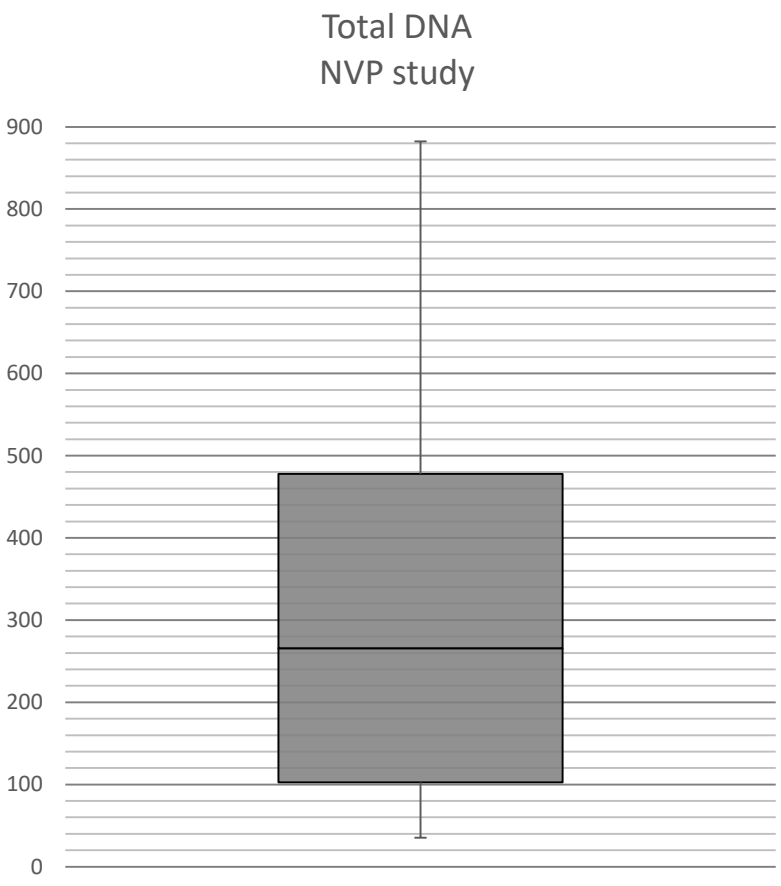
2 interrupted treatment already

(rebound at 4 & 6 weeks)

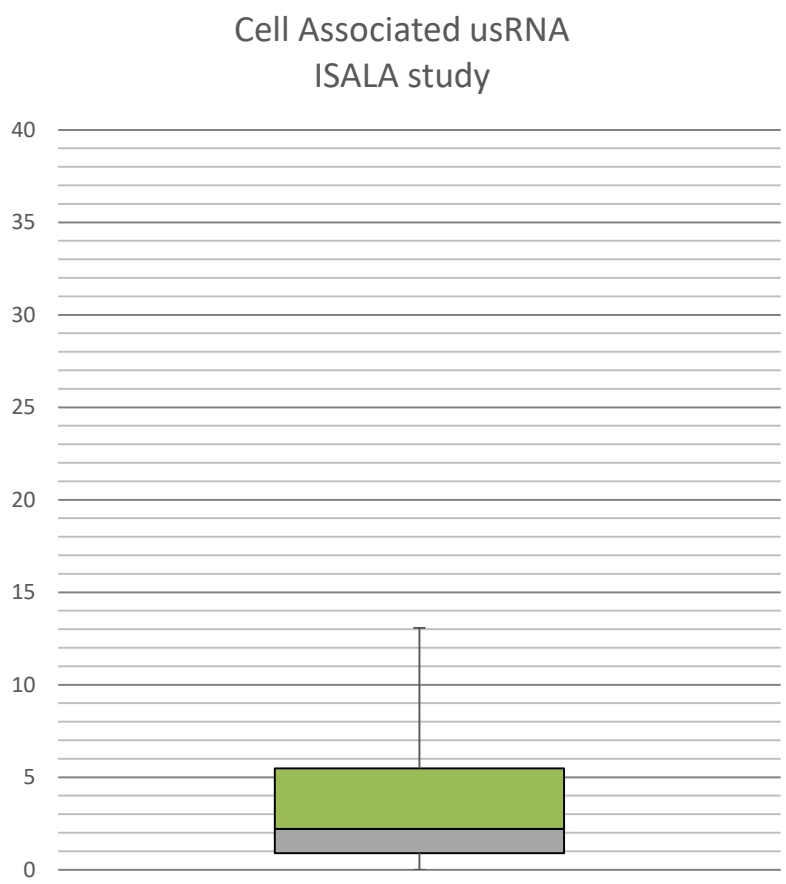
2 planned to interrupt soon (cytapheresis planned)

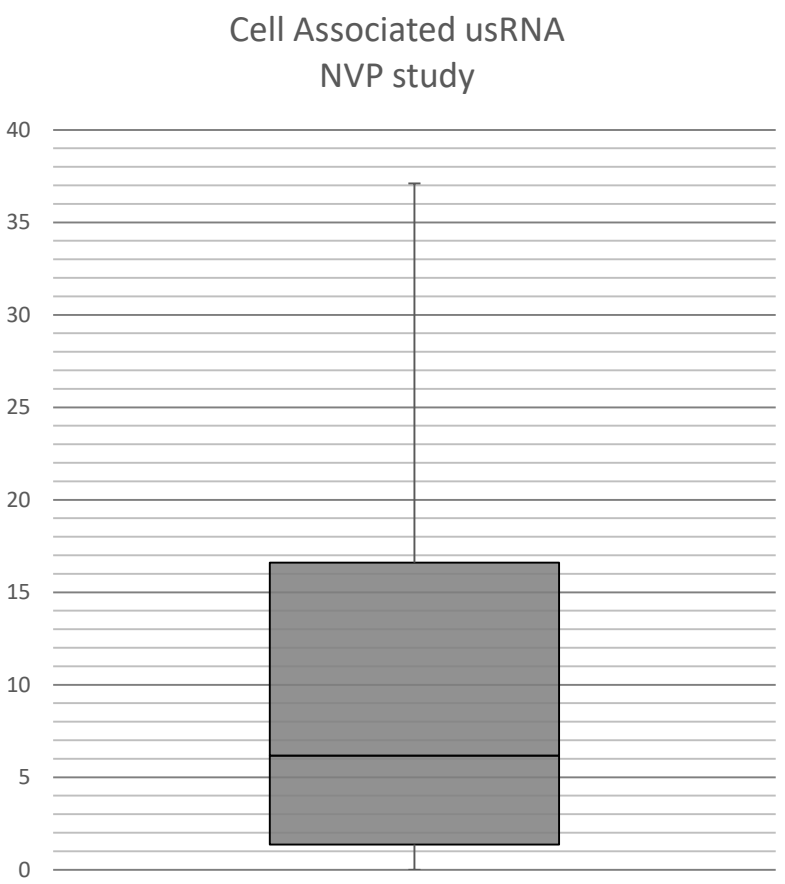
Screening results



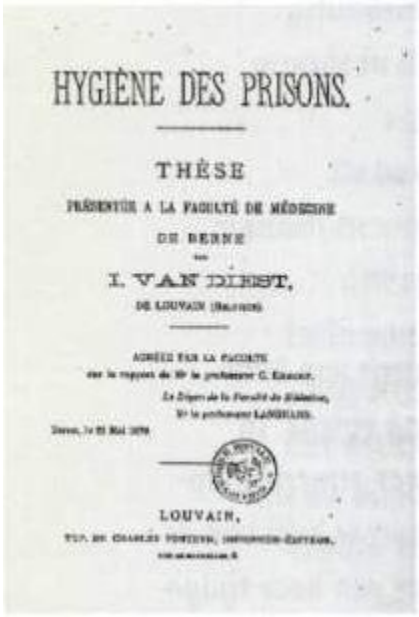


Screening results





What is in a name?



HYGIÈNE DES PRISONS.

THÈSE

PRÉSENTÉE À LA FACULTÉ DE MÉDECINE

DE BERNE

PAR

I. VAN DIEST,

DE LOUVAIN (BELGIQUE)

AGRÉÉE SUR LA FACULTÉ

sur le rapport de Mr le professeur G. Eberth.

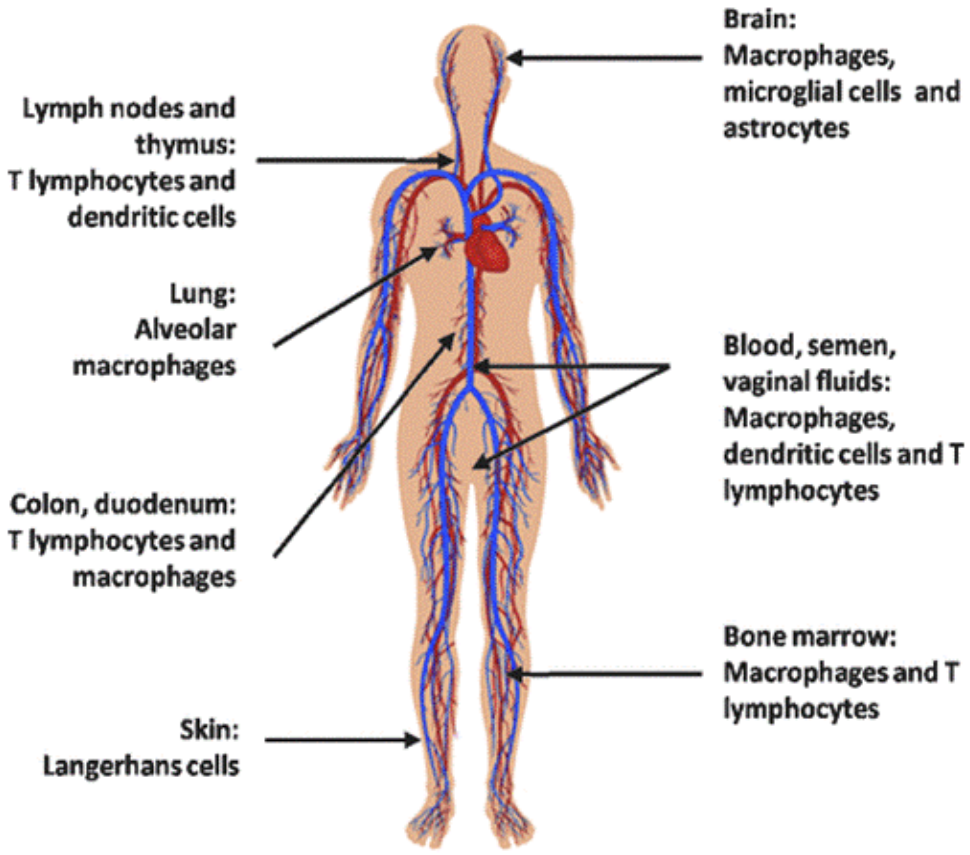
Le Doyen de la Faculté de Médecine,

Mr le professeur LANGHANS.

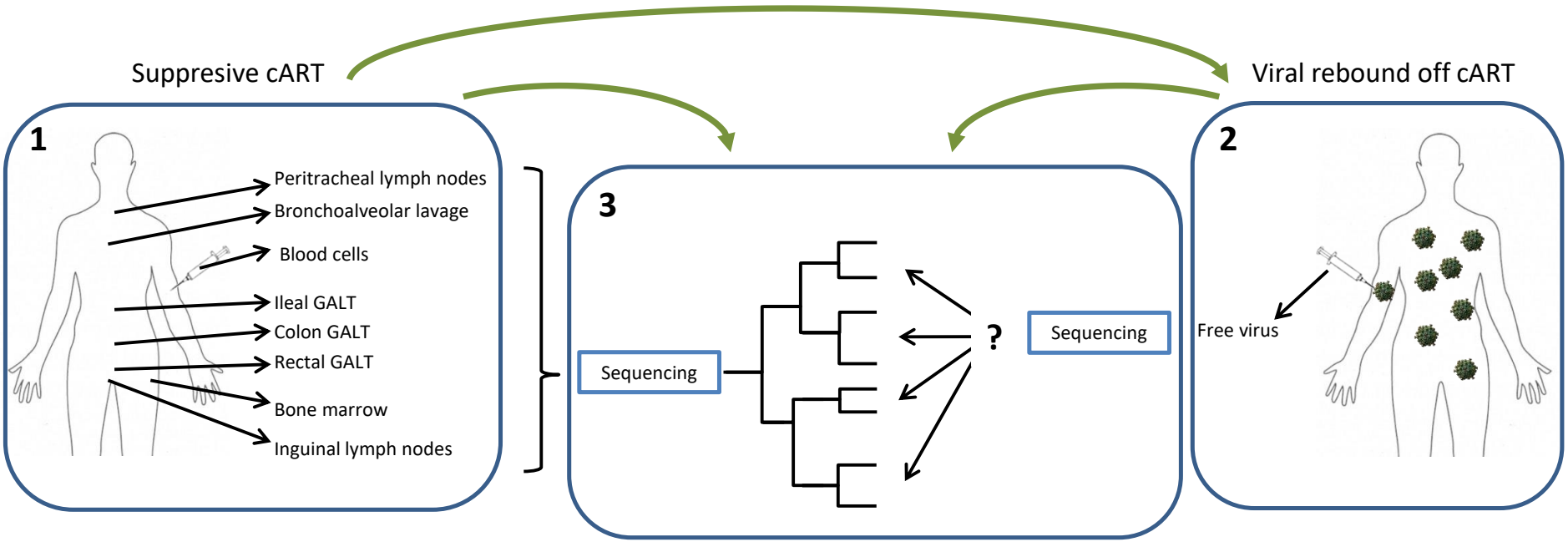
LOUVAIN,

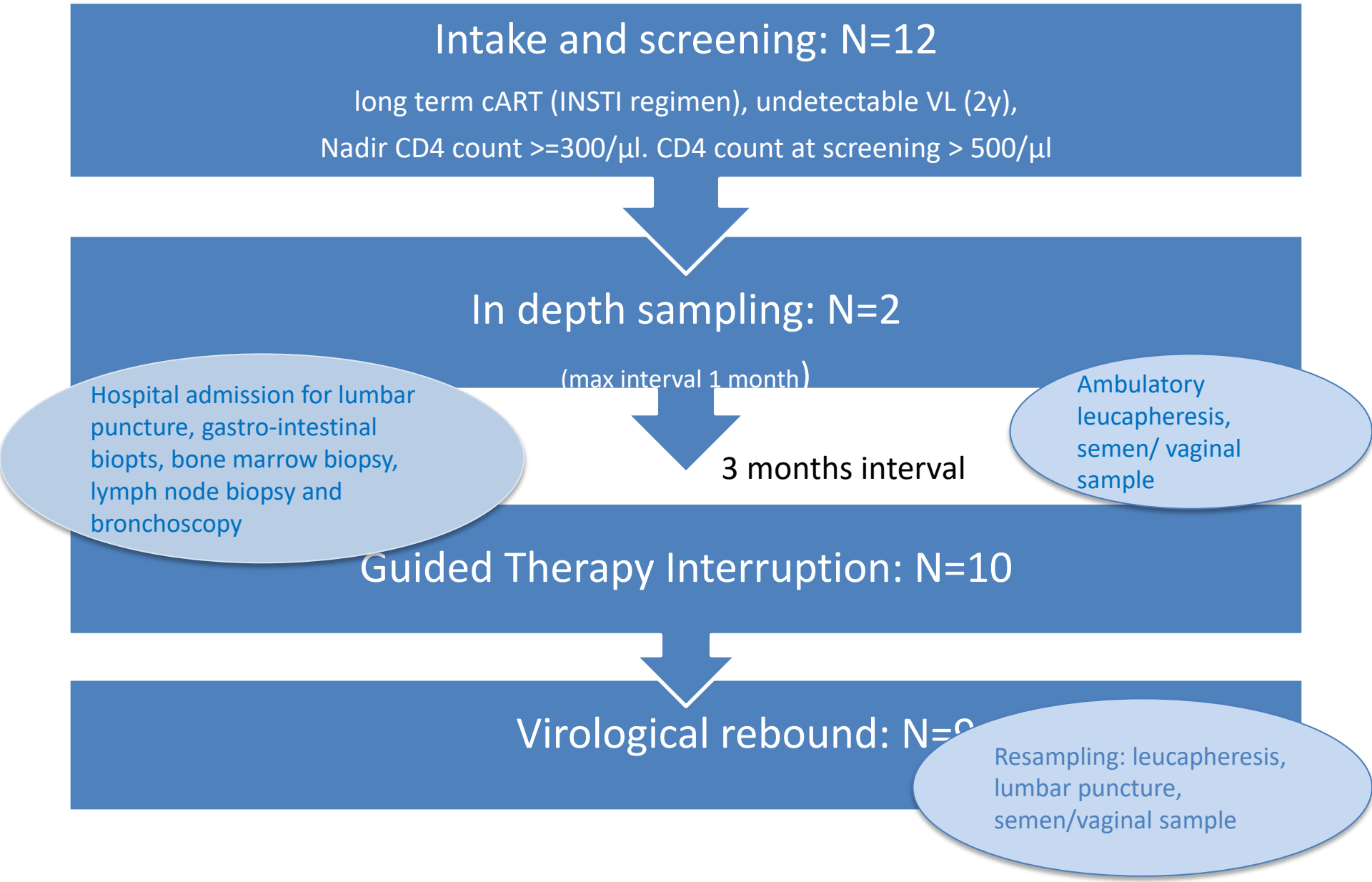
HIV STAR study:
HIV Sequencing after Treatment Interruption to identify the clinically relevant Anatomical Reservoir

Anatomical compartments involved in the HIV latent reservoir



Treatment interruption trial to link the rebounding virus to a specific compartment




Intake and screening: N=12
long term cART (INSTI regimen), undetectable VL (2y),
Nadir CD4 count >=300/µl. CD4 count at screening > 500/µl
In depth sampling: N=2
(max interval 1 month)
Hospital admission for lumbar puncture, gastro-intestinal biopts, bone marrow biopsy, lymph node biopsy and bronchoscopy
Ambulatory leucapheresis, semen/ vaginal sample
3 months interval
Guided Therapy Interruption: N=10
Virological rebound: N=
Resampling: leucapheresis, lumbar puncture, semen/vaginal sample

Phylogenetic analysis to link the rebounding virus to a specific compartment

Characterising the HIV reservoir

Sorting different T cell populations in different tissues

Quantitative analysis by PCR
- Total HIV DNA
- Integrated HIV DNA
- 2LTR
- Ca-RNA

Identification of the origen of viral rebound

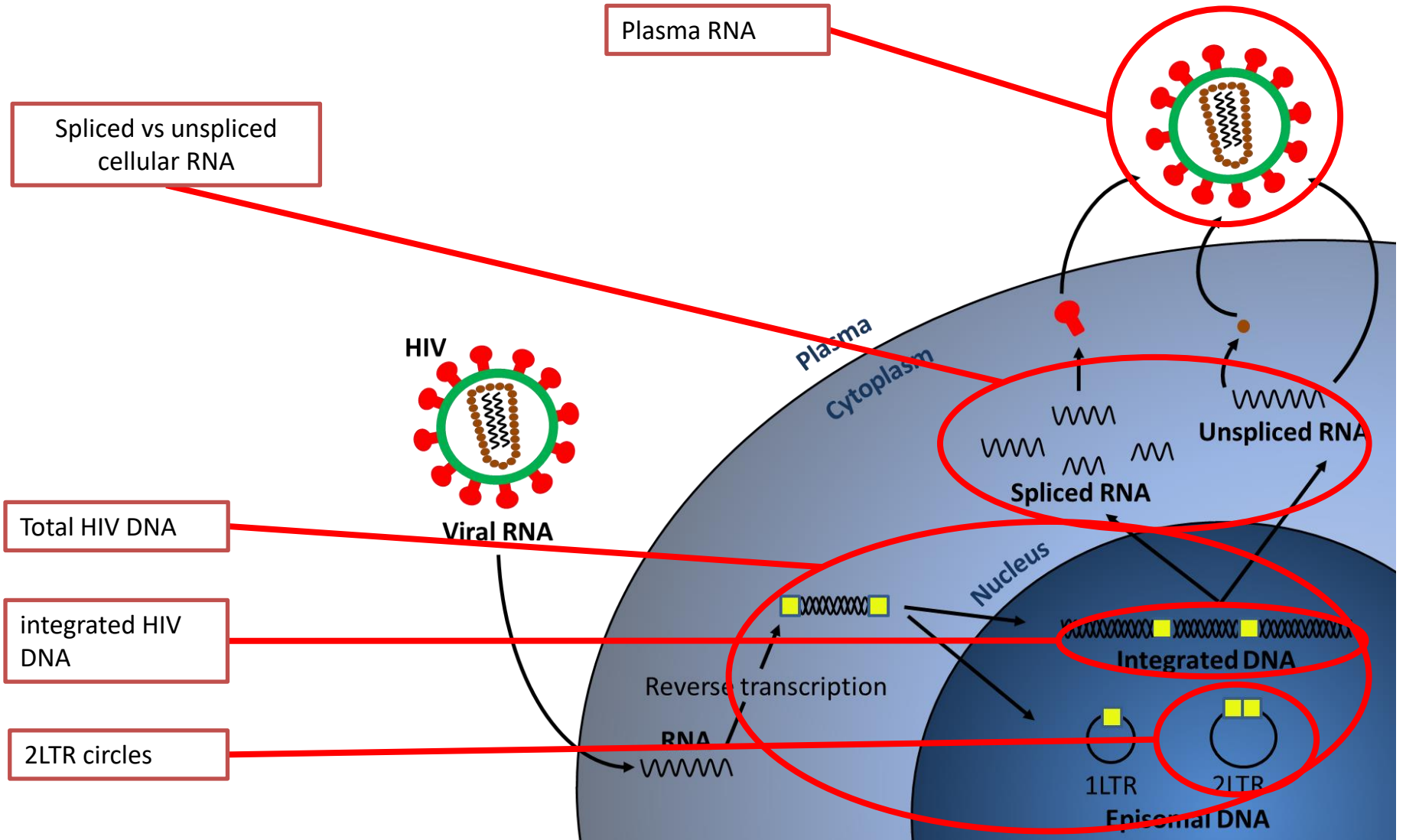
Sequencing and phylogenetic analysis of the virus found in different compartments

Phylogenetic comparison of the rebounding virus in plasma to the viruses found during phase 1

Ward De Spiegelaere
HIV Cure Research Center
Ghent, Belgium

Digital PCR in HIV reservoir quantification

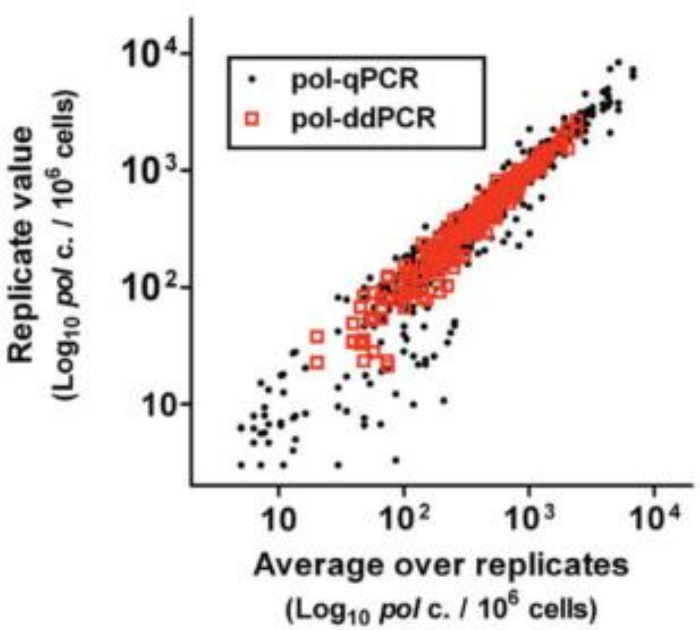
Markers for HIV reservoir

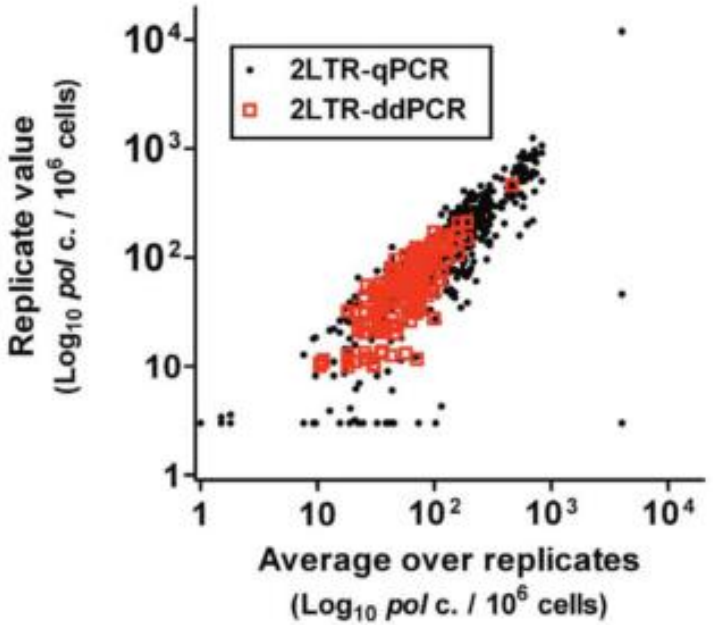


dPCR is more precise

Precision:

- ddPCR is superior when compared to qPCR
- CV 4 to 20-fold lower in ddPCR compared to qPCR



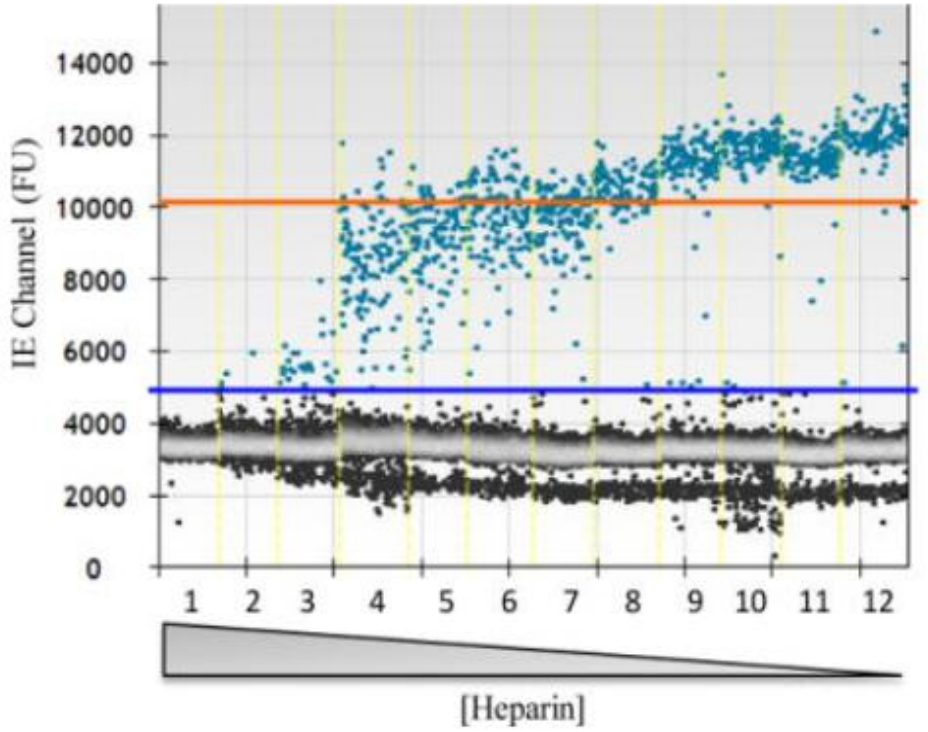


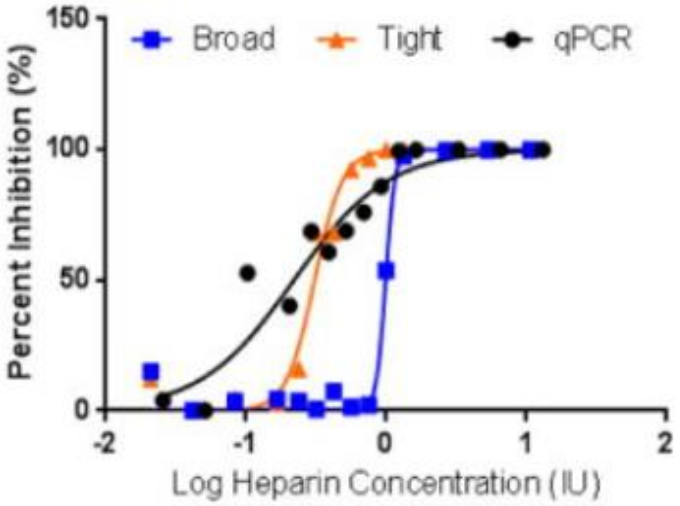
Strain et al., Plos One (2013) 8: e55943

dPCR and inhibition

Inhibition

- dPCR outperforms qPCR comparing inhibitory substances (SDS, EDTA and Heparin)

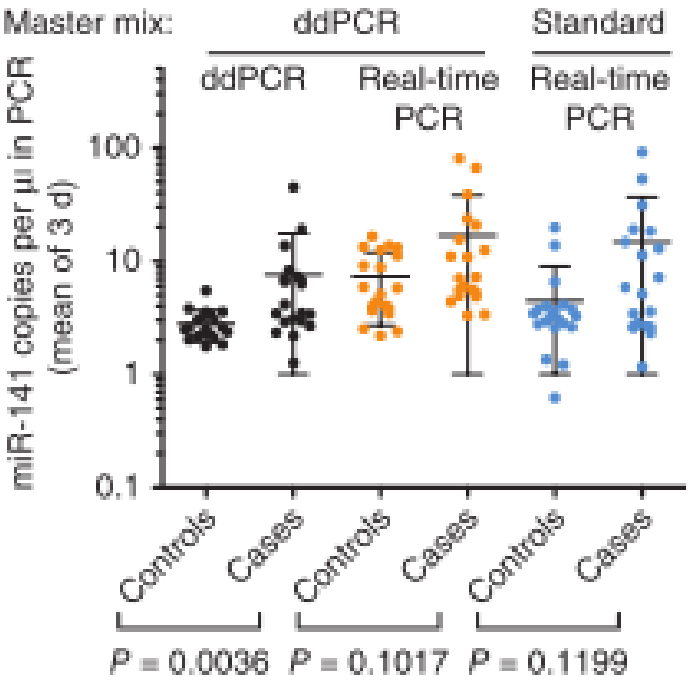




Dingle et al., Clin Chem (2013)59:1670-2

Reproducibility

- Replicate experiments on 3 consecutive days



- Factor of 7 more reproducible compared to qPCR

Hindson et al., Nature Methods (2013) 10:1003-5

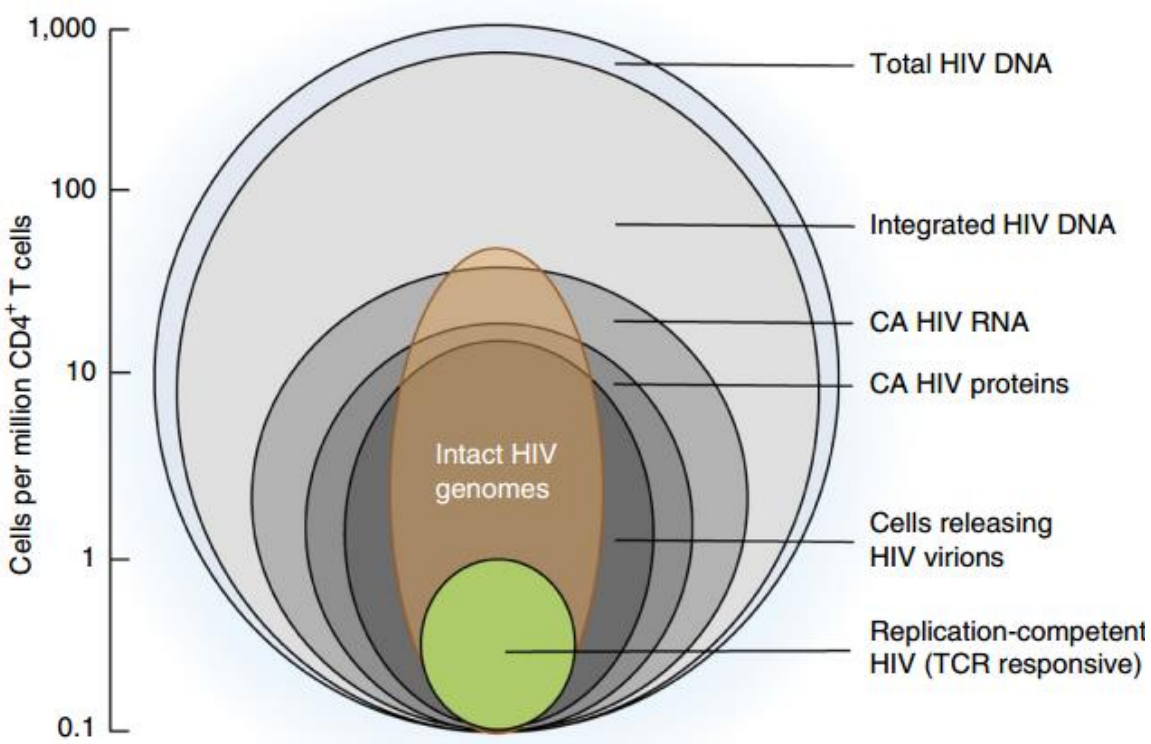
Conclusions

- dPCR: optimal tool to quantify needles in a haystack
- Diverse applications in HIV research
- Some issues, related to the technology and data analysis should be overcome to further enhance precision and accuracy

Maria Buzon
Vall d'Hebron Institute of Research / AIDS Research Institute, IrsiCaixa
Barcelona, Spain

Ex-vivo assays to measure the latent HIV reservoir

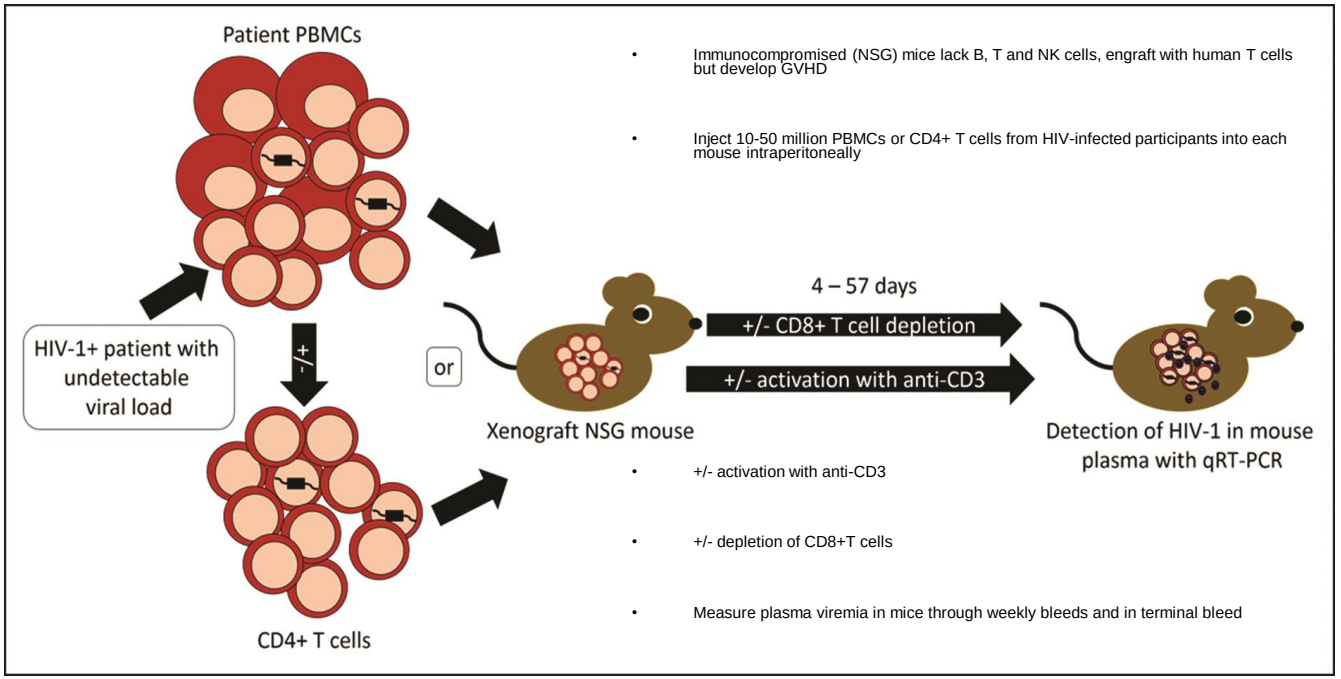
Measuring HIV Persistence



Deeks, Nat Med 2016

New/Non-conventional Assays to Measure the latent HIV Reservoir

Murine Quantitative Viral Outgrowth Assay (mVOA)



Kelly A. Metcalf, JID 2015

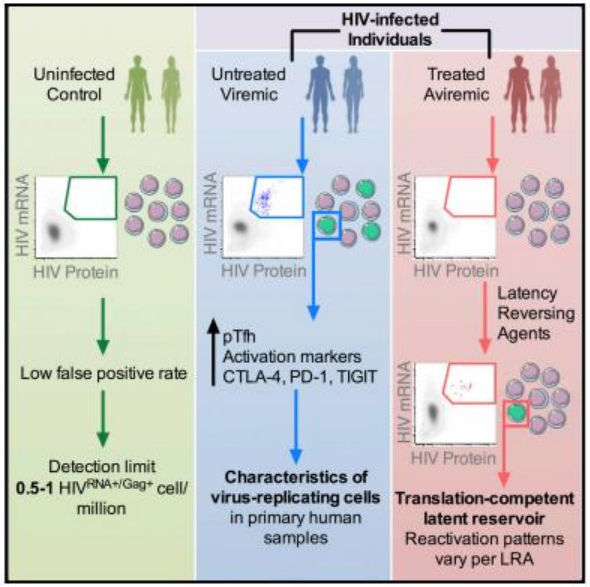
Flow Cytometry-based Assay for Quantification of the Inducible Reservoir

Article

Cell Host & Microbe

Single-Cell Characterization of Viral Translation-Competent Reservoirs in HIV-Infected Individuals

Graphical Abstract



Authors

Amy E. Baxter, Julia Niessl, Rémi Fromentin, ..., Andrés Finzi, Nicolas Chomont, Daniel E. Kaufmann

Correspondence

daniel.kaufmann@umontreal.ca

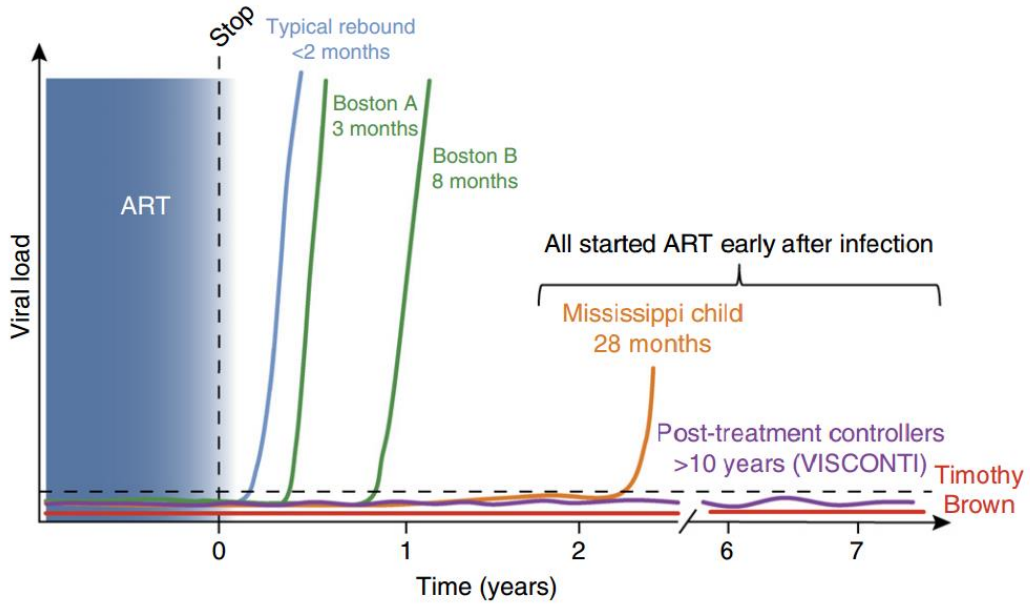
In Brief

Technological limitations hamper characterization of CD4 T cells supporting ongoing HIV infection and quantification of the latent reservoir. Baxter et al. (2016) use simultaneous detection of viral protein and mRNA to quantify and phenotype both the ongoing infection during viremia and the translation-competent inducible reservoir in virally supressed, treated patients.

Baxter, Cell Host and Microbe 2016

Ultimate Assay → Analytical Treatment Interruption

The only way to know if a patient is cured is to stop cART…



Deeks, Nat Med 2016

Christine Rouzioux
Virologie - Hôpital Necker - Université Paris Descartes
Paris, France

Total HIV-1 DNA as a marker of Reservoir Dynamics: A Cure Biomarker?

In the context of Cure/Remission of clinical trials, we will have to explore the impact of Latency Reversal Agents on HIV reservoirs.

There are many methods to quantify reservoirs. There is no well accepted assay to measure the total burden of HIV reservoir.

There is no consensus on which are the best markers for clinical trial endpoints.

We propose a broader definition of HIV reservoirs:

" HIV reservoirs include all infected cells and tissues containing all forms of HIV persistence that can participate in HIV pathogenesis."

All type of infected cells should be

Considered in the context of Cure

Avettand-Fenoel et al,
Clinical Microbiological Review 2016

Conclusions

In the context of HIV Cure/Remission clinical trials, we will need to choose markers:

- to estimate the level of HIV reservoirs
- to estimate their activity and capacity to produce virus

There won't be a magic marker. We will need a more integrative approach. We will certainly need a combination of markers which would be more helpful. That will include not only

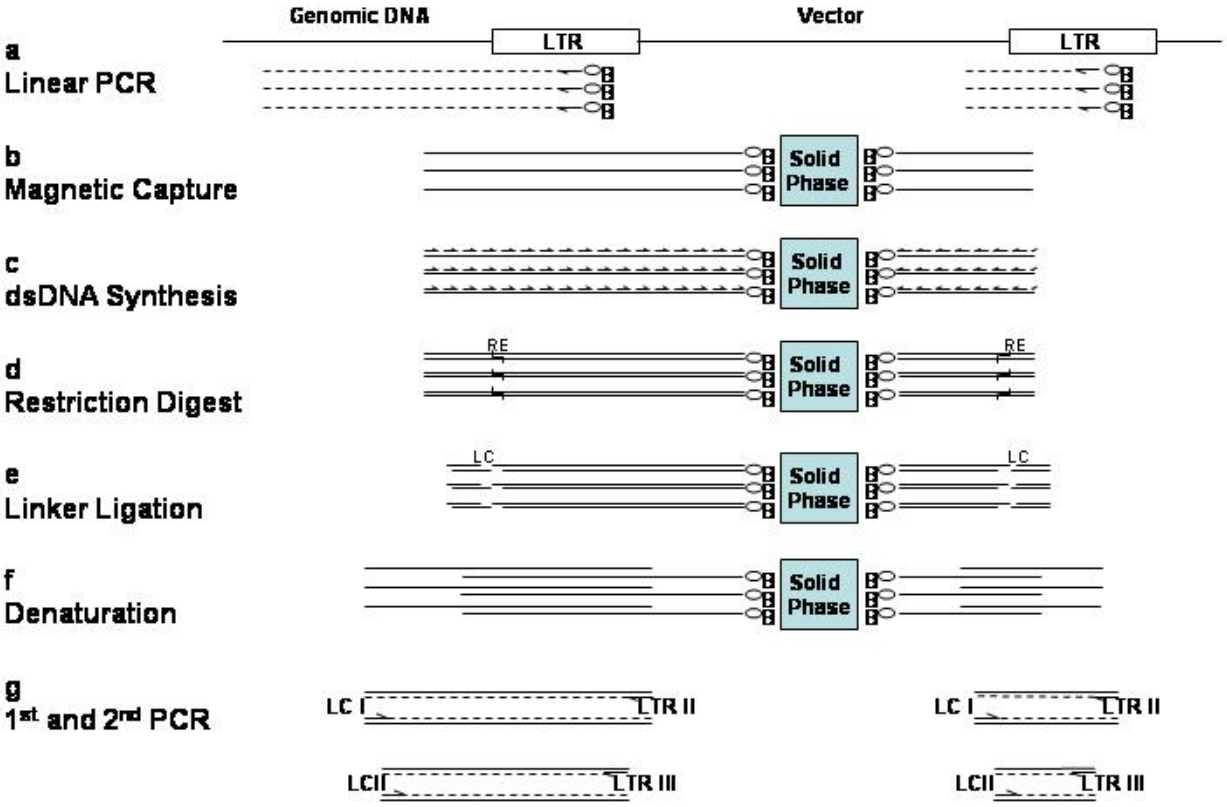
markers of HIV reservoirs (quantification and functionality)
markers of activation/inflammation
Immunological markers
Cellular markers

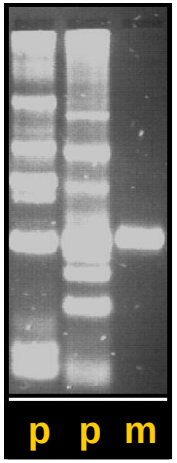
The objective is To best define the group of patients that might respond and beneficiate of the therapy

Manfred Schmidt
National Center for Tumor Diseases - German Cancer Research Center
Heidelberg, Germany

Integration site analysis in HIV infected patients

Integration Site Analysis by LAM-PCR



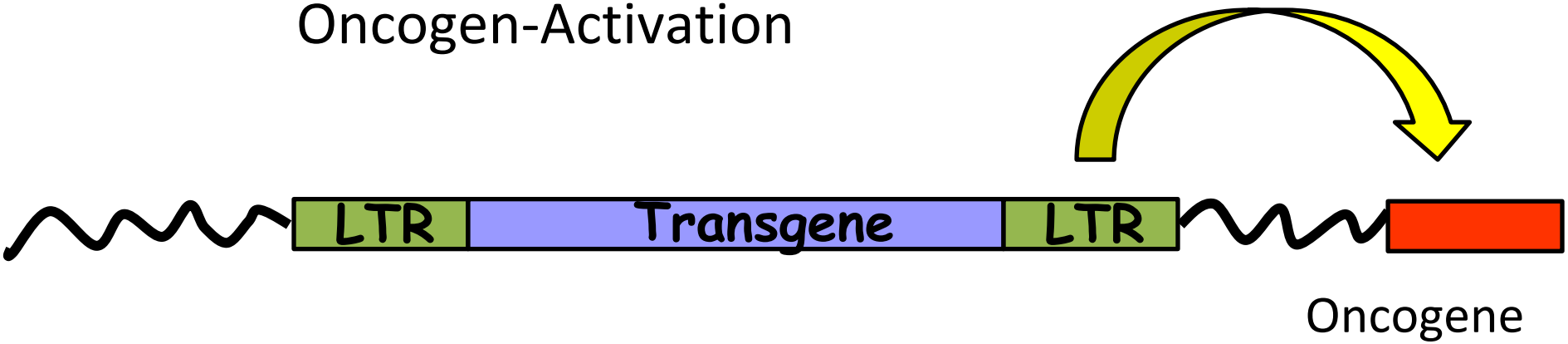


Schmidt *et al*., Nat Methods 2007

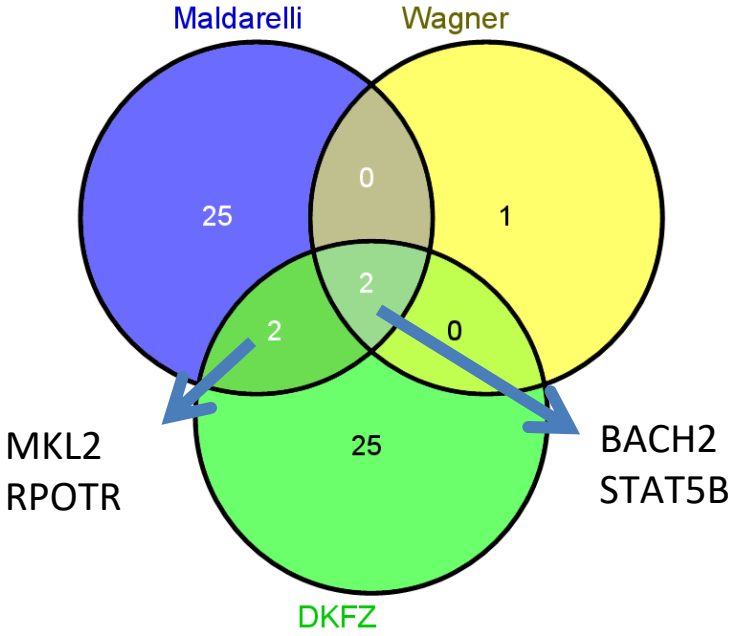
High Genotoxic Risks of Full-LTR Driven Gammaretroviral Vectors

X-SCID: Lymphopoiesis, LMO2!!
CGD: Myelopoiesis, MDS1-EVI1!!
WAS: LMO2 and MDS1-EVI1!!

Insertional Mutagenesis Mediates Oncogen-Activation



Closer analysis of integrations in the most interesting genes



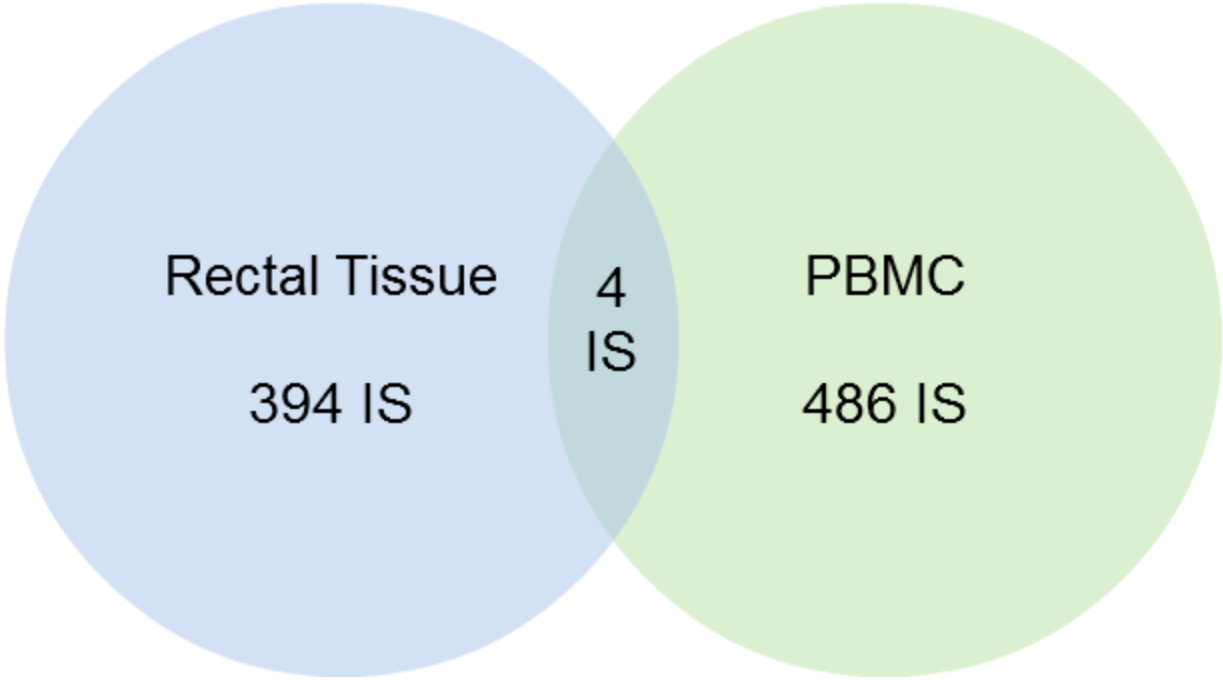
Multiply hit genes (3 or more different IS) in the different data sets.

Unique ISs	534		1632		2315	
	Wagner		Maldarelli		DKFZ	
	Unique IS n	**IS%**	Unique IS n	**IS%**	Unique IS n	**IS%**
BACH2	9	**1,69**	17	**1,04**	3	**0,13**
MKL2	4	**0,75**	23	**1,41**	4	**0,17**
STAT5B	4	**0,75**	10	**0,61**	7	**0,30**

Frequency of unique ISs in the „integration hot spot“ genes

Laufs, S., Schenkwein D., *et al.* Manuscript in submission

IS number retrieved by GENE-IS



Annemarie Wensing
University Medical Center Utrecht
Utrecht, the Netherlands

IciStem: International Collaboration to guide and investigate the potential for HIV cure by Stem Cell Transplantation

IciStem objectives

- Observational study
  - Investigate HIV cure potential of allogeneic stem cell transplant
- Identify cases of remission
- Understand mechanisms of reservoir reduction

IciStem progress

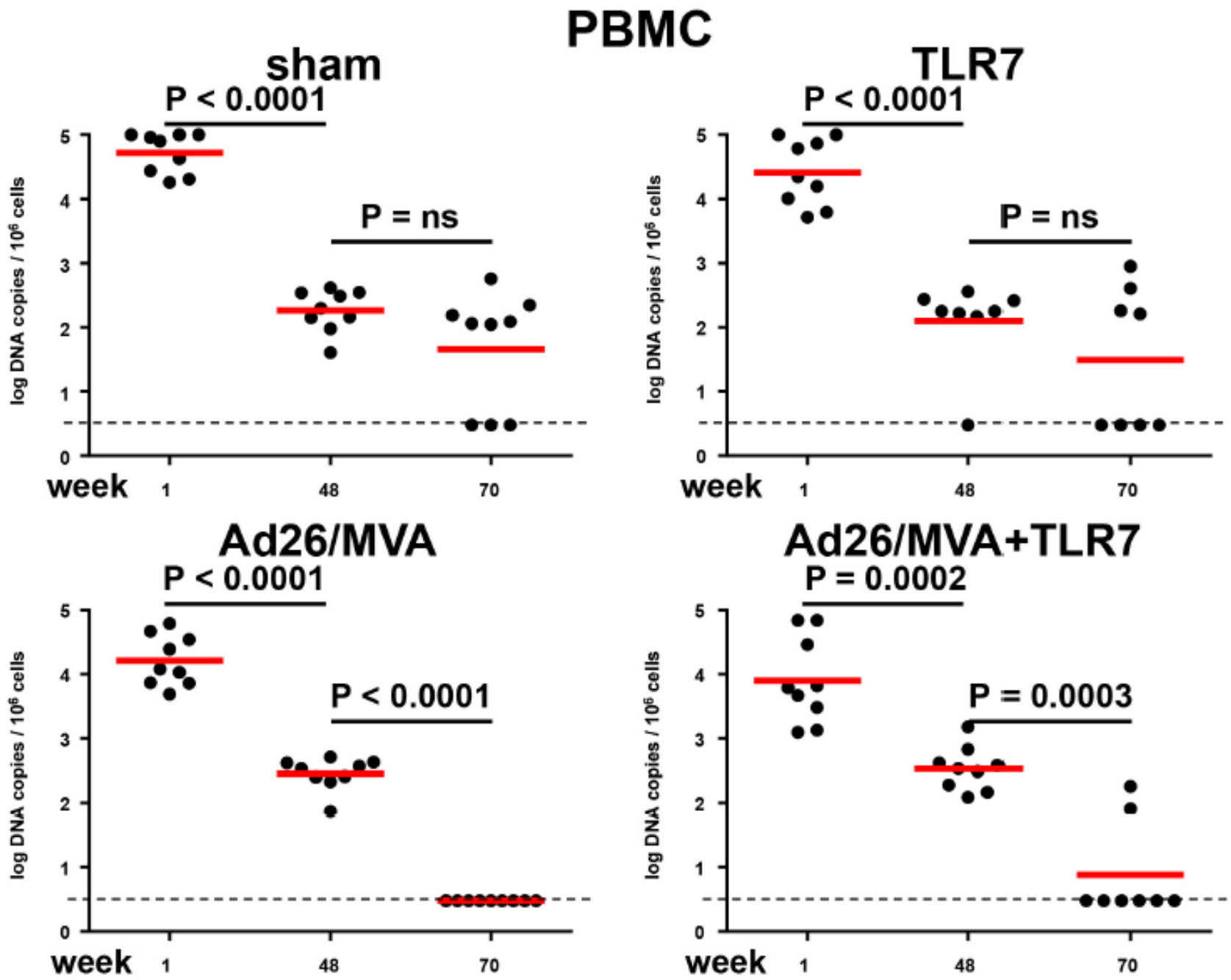
- 22 patients have received a SCT so far
- 4 cases were presented in detail
  - 3 out of 4 had a successful transplant
  - 2 out of these 3 have undetectable HIV DNA
  - Though, these patients remain on cART
  - Definitive cure stauts cannot be confirmed

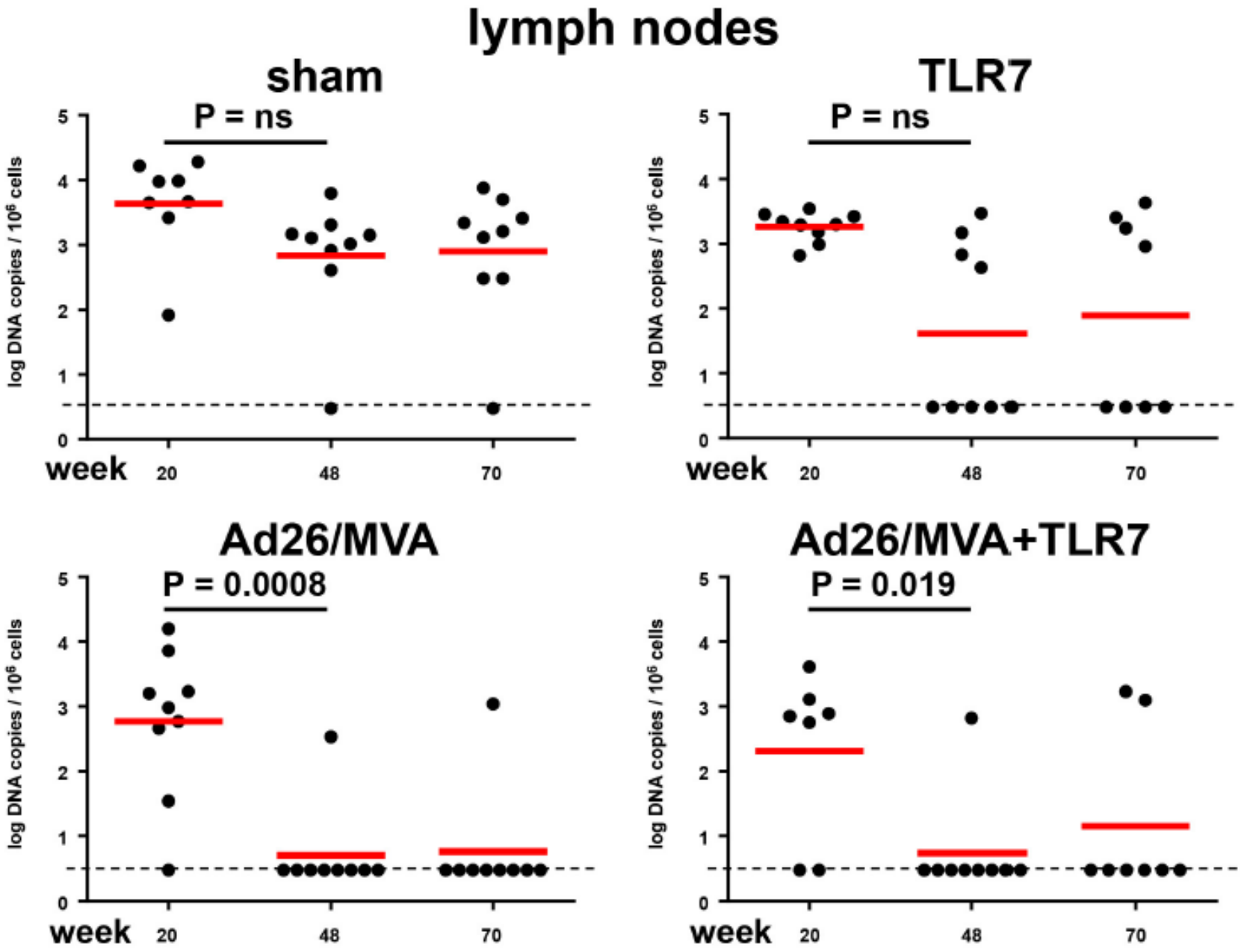
nature Accelerated Article Preview

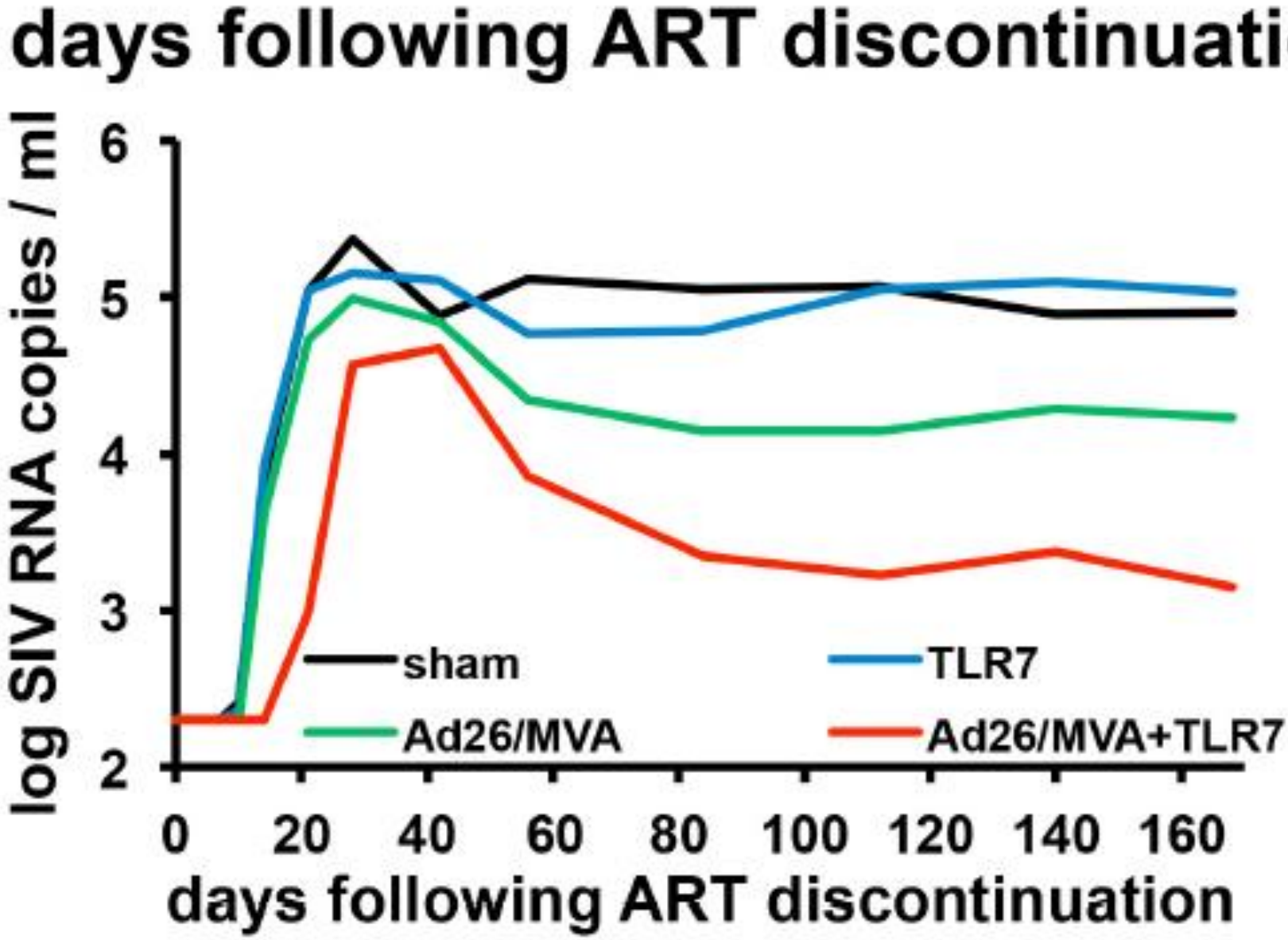
LETTER
doi:10.1038/nature20583

Ad26/MVA Therapeutic Vaccination with TLR7 Stimulation in SIV-Infected Rhesus Monkeys

Erica N. Borducchi, Crystal Cabral, Kathryn E. Stephenson, Jinyan Liu, Peter Abbink, David Ng'ang'a, Joseph P. Nkolola, Amanda L. Brinkman, Lauren Peter, Benjamin C. Lee, Jessica Jimenez, David Jetton, Jade Mondesir, Shanell Mojta, Abishek Chandrashekar, Katherine Molloy, Galit Alter, Jeff M. Gerold, Alison L. Hill, Mark G. Lewis, Maria G. Pau, Hanneke Schuitemaker, Joseph Hesselgesser, Romas Geleziunas, Jerome H. Kim, Merlin L. Robb, Nelson L. Michael and Dan H. Barouch


PBMC
sham
P < 0.0001
P = ns
log DNA copies / 10^6 cells
week
1
48
70
TLR7
P < 0.0001
P = ns
Ad26/MVA
P < 0.0001
P < 0.0001
Ad26/MVA+TLR7
P = 0.0002
P = 0.0003


lymph nodes
sham
P = ns
log DNA copies / 10^6 cells
week
20
48
70
TLR7
P = ns
Ad26/MVA
P = 0.0008
Ad26/MVA+TLR7
P = 0.019


days following ART discontinuation
log SIV RNA copies / ml
6
5
4
3
2
sham
TLR7
Ad26/MVA
Ad26/MVA+TLR7
0
20
40
60
80
100
120
140
160
days following ART discontinuation


www.scip.be