19-Sept-2016

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

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 absence of cART

- HIV STAR: where does the rebounding virus come from

- ABIVAX: does ABX464 suppress HIV viral load in the absence of cART

- EPISTEM
Isala Trial

“Analytical treatment interruption in HIV positive patients with low viral reservoir to evaluate the potential of a functional cure”
Single arm – non-randomized - multi-centric – prospective trial

**Intervention:** Treatment interruption among pts with low viral reservoir

**Funding** through TBM program from IWT
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

Total DNA
ISALA study

Total DNA
NVP study
Screening results

Cell Associated usRNA
ISALA study

Cell Associated usRNA
NVP study
What is in a name?
HIV STAR study:
HIV Sequencing after Treatment Interruption to identify the clinically relevant Anatomical Reservoir
Anatomical compartments involved in the HIV latent reservoir

- **Brain:** Macrophages, microglial cells, and astrocytes
- **Lung:** Alveolar macrophages
- **Colon, duodenum:** T lymphocytes and macrophages
- **Skin:** Langerhans cells
- **Blood, semen, vaginal fluids:** Macrophages, dendritic cells, and T lymphocytes
- **Bone marrow:** Macrophages and T lymphocytes
Treatment interruption trial to link the rebounding virus to a specific compartment

1. Peritracheal lymph nodes
   - Bronchoalveolar lavage
   - Blood cells
   - Ileal GALT
   - Colon GALT
   - Rectal GALT
   - Bone marrow
   - Inguinal lymph nodes

2. Viral rebound off cART

3. Suppressive cART
   - Sequencing
   - Free virus
   - ?
   - Sequencing
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)

Guided Therapy Interruption: N=10

Virological rebound: N=9

Hospital admission for lumbar puncture, gastro-intestinal biopts, bone marrow biopsy, lymph node biopsy and bronchoscopy

3 months interval

Ambulatory leucapheresis, semen/vaginal sample

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
Digital PCR in HIV reservoir quantification
Markers for HIV reservoir

- Spliced vs unspliced cellular RNA
- Plasma RNA
- Total HIV DNA
- Integrated HIV DNA
- 2LTR circles
- Viral RNA
- Unspliced RNA
- Spliced RNA
- Integrated DNA
- Reverse transcription
- Episomal DNA
- 1LTR
- 2LTR
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

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
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
**Immunocompromised (NSG) mice lack B, T and NK cells, engraft with human T cells but develop GVHD**

- Inject 10-50 million PBMCs or CD4+ T cells from HIV-infected participants into each mouse intraperitoneally

**+/− activation with anti-CD3**

**+/− depletion of CD8+ T cells**

**Measure plasma viremia in mice through weekly bleeds and in terminal bleed**

**Murine Quantitative Viral Outgrowth Assay (mVOA)**

Kelly A. Metcalf, JID 2015
Flow Cytometry-based Assay for Quantification of the Inducible Reservoir

**Cell Host & Microbe**

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

Graphical Abstract

Uninfected Control → HIV mRNA → HIV Protein → Low false positive rate

HIV mRNA

Untreated Viremic → Characteristics of virus-replicating cells in primary human samples

HIV mRNAA

HIV Protein

Treated Aviremic

HIV mRNA

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 suppressed, treated patients.

Authors

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Baxter, Cell Host and Microbe 2016
The only way to know if a patient is cured is to stop cART...
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
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 Oncogene-Activation
Closer analysis of integrations in the most interesting genes

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

<table>
<thead>
<tr>
<th>Unique ISs</th>
<th>Wagner</th>
<th>Maldarelli</th>
<th>DKFZ</th>
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<tbody>
<tr>
<td></td>
<td>Unique IS n</td>
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<tr>
<td>BACH2</td>
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<tr>
<td>STAT5B</td>
<td>4</td>
<td>0,75</td>
<td>10</td>
</tr>
</tbody>
</table>

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

Rectal Tissue: 394 IS
PBMC: 486 IS
Intersection: 4 IS
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 status cannot be confirmed
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
days following ART discontinuation

log SIV RNA copies / ml

- sham
- TLR7
- Ad26/MVA
- Ad26/MVA+TLR7

days following ART discontinuation