HIV/AIDS and Cancer cure: Is it the same battle?

Monsef Benkirane
Molecular Virology Laboratory
monsef.benkirane@igh.cnrs.fr
Dynamic of imatinib-treated Chronic Myeloid Leukemia

Mature cancer cells
Highly dividing

Cancer Stem cells
Persistence and resistance are the Barriers to Cure

<table>
<thead>
<tr>
<th>HIV/AIDS</th>
<th>Cancer</th>
</tr>
</thead>
<tbody>
<tr>
<td>- Latent provirus</td>
<td>- Quiescent / slow-cycling</td>
</tr>
<tr>
<td>- Quiescent/ slow-cycling</td>
<td>- Long-lived</td>
</tr>
<tr>
<td>- Long-lived</td>
<td>- Indefinite proliferative potential</td>
</tr>
<tr>
<td>- Non sensitive to ART</td>
<td>- Enhanced repair capacity</td>
</tr>
<tr>
<td>- Not visible to the immune system</td>
<td>- Highly resistant to DNA damage/Tolerance</td>
</tr>
<tr>
<td>- HIV-specific CD8 T cell response decreases with cART</td>
<td>- Enhanced checkpoint kinase expression</td>
</tr>
<tr>
<td></td>
<td>- drug efflux transporter</td>
</tr>
<tr>
<td></td>
<td>- Renewed by dedifferentiation of proliferating cancer</td>
</tr>
</tbody>
</table>

HIV/AIDS and cancer are Residual diseases

Although their molecular bases are different, similar cure strategies are being developed
Two main strategies are being pursued

Eradication, which will require a complete elimination of infected cells, for which the Berlin patient represents a proof of concept.
Considering the Boston patients, eradication will require elimination of HIV infected cells and replacement with HIV resistant cells.

Remission: HIV controllers and the VISCONTI patients suggest that remission is achievable.
This strategy should include monitoring of inflammatory and procoagulant indices.

- Find and diminish size of the reservoir (LRAs, bNAbs, CAR-T cell)
- Reduce seeding of latent pool with early/more ART
- Reverse latency (LRAs, TLR7)
- Increase HIV-specific immune function (vaccines or anti PD-L1)
- Reduce immune activation
- Gene therapy targeting the virus and the host
- Allogeneic stem cell transplantation

Combination therapy may be necessary

While proof-of-concepts are there, we still have to gain important knowledge to achieve HIV eradication or remission
Strategies to eradicate latent reservoirs.

– shock and kill strategy –

1- Purge of reservoirs.
   (TCR and PKC agonists, Latency Reverting Agents, Cytokines)

Required a deep understanding of the mechanisms of latency maintenance.

2- Killing of reactivated reservoirs.
   (cytopathic effects, therapeutic vaccines, bNAbs)

– kill strategy –

Direct targeting of the reservoirs.
conditioned by the identification of specific marker(s) that differentiate latently infected cells from their non-infected counterparts.
Mechanisms of HIV-1 transcriptional silencing

Two Rate limiting steps:
* Chromatin repression
* Transcription elongation block
Tanscription factors-mediated reversion of transcriptional latency from the HIV promoter

- Tat or NF-kB
  - Co-Activators
  - Chromatin modifiers
  - Chromatin derepression
  - pTEFb/SEC
  - Processive elongation
Disrupting transcriptional latency ex vivo using Latency Reversing Agents

but do not significantly reduce the size of the latent reservoir when LRAs were used in vivo

How to improve and achieve efficient HIV reactivation?
Spatial Organization of Chromatin and its Impact on HIV integration site selection on Gene Expression

- Chromatin is organized into unit blocks termed topologically associated domains (TADs)
- TADs can be classified into two types of compartments:
  - A-type, which are active domains (here in blue and green)
  - B-type, which are inactive (here in orange, red and yellow)
- Inside TADs, long-range chromatin interactions can be detected between promoters and enhancers (*).
   Activation or repression of gene transcription
- TADs associated with the nuclear lamina have also been defined as LADs.
  (one LAD can consist of few TADs or a TAD can contain one or more LADs)
Applying 3C technologies to understand HIV integration site selection and transcription regulation

The goal of the technology is to identify distal sequences that significantly interact with gene promoters.

Mifsud et al., 2015

IAS HIV Cure & Cancer Forum
Paris, France
Analyses of HIV integration site based on 3D genome organization

p-value associated with the overlapping of our two set of data*

M/W captured fragment

* IS obtained from Maldarelli et al. and Wagner et al. – Science 2014
Analyses of HIV integration site based on 3D genome organization

p-value associated with the overlapping of our two set of data*

M/W whole graph (level 1)

* IS obtained from Maldarelli et al and Wagner et al – Science 2014
Analyses of HIV integration site based on 3D genome organization

HIV-1 integration sites overlap more frequently when analyzed in the context of 3D genome organization

*p-value associated with the overlapping of our two set of data*

*IS obtained from Maldarelli et al and Wagner et al – Science 2014*
HIV-1 targets specific 3D clusters for its integration

- Defining 3D cluster of fragments highlight specific enrichment in HIV-1 integration site selection.
Identification of 3D gene clusters targeted for HIV integration
HIV-1 integrates preferentially in Enhancer region
• Analyses of HIV-1 integration sites in the context of 3D genome revealed specific targeting of enhancer element associated with specific 3D gene clusters
– kill strategy –

**Direct targeting of the reservoirs.**

conditioned by the identification of specific marker(s) that differentiate latently infected cells from their non-infected counterparts.
**In vitro** model of HIV-infected quiescent CD4 T cells to study viral latency

Model that recapitulates 2 features of in vivo persisting reservoirs: transcriptionally silent virus in a quiescent environment
We hypothesized that latently infected quiescent CD4 T cells express markers that distinguish them from their non-infected counterparts.

\[ \text{⇒ Cellular response to viral infection} \]

\[ \text{⇒ Virus induced signature} \]
CD32a is a highly specific marker of HIV-1 infected quiescent CD4 T cells in vitro.

CD32a is an activating receptor of low affinity for Fc fragment of several IgGs.
Can CD32a be used to isolate HIV reservoir cells from blood of infected individuals?

Participant 489:

<table>
<thead>
<tr>
<th>Total Lymphocytes</th>
<th>T Lymphocytes</th>
<th>CD4 T Lymphocytes</th>
</tr>
</thead>
<tbody>
<tr>
<td>77,7</td>
<td>59,5</td>
<td>0,026</td>
</tr>
<tr>
<td>73,8</td>
<td>55,9</td>
<td>0,001</td>
</tr>
</tbody>
</table>

FACS Sorting
~ 1,000 CD32a<sup>hi</sup> cells/patients

Total HIV-DNA qPCR quantification

98,4
99,8
CD32a CD4 T cells are highly enriched in HIV DNA

*CD32a expression level correlates with infection frequencies.

*CD32a contribution to total HIV-DNA ≈ 50% (27-86%).
Determining the contribution of CD32a cells to the inducible CD4 T cell reservoir.

Depletion of CD32a CD4 T cells (500 cells per well) resulted in a dramatic delay (>10 days) in virus production and spreading. CD32a CD4 T cells make a dominant contribution to the pool of inducible CD4 reservoirs.
Concluding remarks

From ex vivo results
1. CD32a fulfills the necessary criteria of a biomarker for CD4 T cells HIV-1 reservoirs.

Combining in vitro and ex vivo results
1. Strengthen the idea that direct infection of quiescent cells accounts for latent reservoirs establishment in vivo.
2. And that the regulation of SAMHD1 activity is pivotal for latency establishment.

Complete the mapping of the blood reservoir.
In vitro model gave 6 other putative candidates that we will study for their ability to stain in vivo reservoirs.
MOLECULAR VIROLOGY LAB
Benjamin DESCOURS
Thomas GAYRAUD
Gael PETITJEAN
Raoul RAFFEL

William Ritchie (IGH)
Patrick Cramer (Max-Planck)

Peter Fraser (Babraham Institute, UK)
Mickhail Spivakov(Babraham Institute, UK)

Participants:
More than 20, with an exceptional compliance.

Collaborators:

CHU Créteil Henri Mondor
Jean-Daniel LELIEVRE
José-Luis LOPEZ ZARAGOZA
Christine LACABARATZ
Yves LEVY

Virus et Immunité LAB
Pasteur Institute
Olivier SCHWARTZ
Timothée BRUEL

CHU Montpellier Gui de Chauillac
Jacques REYNES
Christina PSOMAS
Phenotypical and functional analysis of CD32a CD4 T cells
Cluster Dendrogram

dist.all
hclust(*, "complete")
which cure and how to achieve it

In principle, HIV replication can be contained, allowing preservation or restoration of Functional immune competence in most infected persons.

HIV cure has become a priority since the report of an HIV infected person undergoing treatment for leukemia in Berlin was cure after allogeneic hematopoietic progenitor cell transplantation from donor homozygote for CCR5D32 deletion.