Novel mutations located outside the integrase gene confer HIV-1 integrase strand-transfer inhibitors resistance.

Dr Olivier Delelis
Pr Anne-Geneviève Marcelin
Resistance to Anti-Integrases

• Raltegravir (RAL) / Elvitegravir (FDA. 2007/2012) 1st generation molecules
  • Several pathways of resistance characterized (G148, N155, Y143)

• Dolutegravir (DTG) (2014) 2nd Generation
  • No pathways of resistance
  • Some patients have become non-responsive to DTG regimen

• Characterization achieved by:
  • in vitro recombinant Integrase assays
  • Virological assays mainly based on quantitative PCR
SELECTION OF VIRUS RESISTANT TO DTG?

HIV-1

Dolutegravir
High concentration
Highly Resistant Mutant

Further selection demonstrates an increasing resistance and thereby an enrichment of a “selected” virus resistant to DTG.
MUTANT IS INHIBITED BY RT INHIBITORS AS WELL AS THE WT
No mutation in the integrase
Mechanism of replication?

**Total Viral DNA PPT Mutant**

- Mutant +DTG
- Mutant -DTG
- Mutant +CBT
- WT

**Increasing Specificity of Integrated Forms Upon Dilution**

- Mutant DTG J11
- Mutant DTG J13
- WT 0 J5
Mechanism of replication?

- qPCR data indicate the profile of a replicating mutant which bypasses integration

- No Integration

<table>
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<tr>
<th>Sample</th>
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<th>Gene</th>
<th>Non Gene</th>
</tr>
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<tbody>
<tr>
<td>WT</td>
<td>8000</td>
<td>70</td>
<td>30</td>
</tr>
<tr>
<td>100 J10</td>
<td>5000</td>
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<tr>
<td>S105 500 J7</td>
<td>125</td>
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</table>
1-LTR circles ratio during WT and PPT infection

2-LTR circles: no accumulation
Unintegrated Viral DNA forms are accumulated by INSTI treatment.

Function of episomes: synthesis of viral proteins.

Could they involved in HIV-1 replication?
An alternative pathway of replication?
1-LTR circle Accumulation

- 3’ PPT offers (+) strand synthesis and secondary LTR in viral DNA
- Augmented 1-LTR circle levels highlight the increased importance of this pathway
- PPT mutation leads to abnormal degradation 3’ PPT
Conclusions:

- A virus has been obtained by DTG selection.
- This virus is able to replicate.
- This virus is resistant to DTG and all INSTIs.
- No mutation in the integrase gene.

- No integrated viral DNA has been detected by 2 different methods.
- Accumulation of 1-LTR circles.

Expression of Unintegrated viral DNA?

Experiments are ongoing but expression from this virus seems to be stronger than the WT
Acknowledgements

LBPA, Eric Deprez and the Biophotonic Team

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- Dr Hervé Leh
- Béatrice Herrmann

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- Dr Gilles Collin
- Pr Vincent Calvez
- Pr Anne-Geneviève Marcelin
- Dr Charlotte Charpentier
- Pr Diane Descamps

Funding

anRS

Agence nationale de recherches sur le sida et les hépatites virales
Sequencing of the integration sites?

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qPCR experiments: no integrated DNA
Confirmed by integration sites sequencing?
### qPCR sensitivity

#### Dilution of WT integrated DNA

![Graph showing dilution of WT integrated DNA with alu+ and alu- ratios.]

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<tr>
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<tr>
<td>WT 500nM DTG 5 p.i.</td>
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<tr>
<td>Mutant 500nM DTG 7 p.i.</td>
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<td>nul</td>
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Unintegrated viral DNA expression
Day 6 post-infection

Uninfected cells

WT

WT+DTG

5% of GFP+ cells

0.035% of GFP+ cells
4.17% of GFP+ cells

0.09% of GFP+ cells
8.7% of GFP+ cells

5.86% of GFP+ cells
% of GFP+ cells normalised by the WT (without DTG)
Escaping Dolutegravir

Classical HIV-1 Replication Cycle

Proposed Non-Integrative HIV-1 Replication Cycle
Escaping Dolutegravir

Classical HIV-1 Replication Cycle

Proposed Non-Integrative HIV-1 Replication Cycle
Conclusions:

- A replicative virus, without mutation in the integrase gene has been obtained.
- «Stable mutations» were observed in the 3′-PPT.
- The «selected» virus is resistant to all known INSTIs inhibitors.
- No integrated viral DNA has been observed by 2 different approaches.

Relevance of this study: patient failing DTG treatment was identified with similar mutations in the 3′-PPT but no mutation in IN

Future directions:

- Cloning of the «selected virus».
- Site directed mutagenesis in the catalytic site of IN.
- Check action of other IN inhibitors.
- Localisation of viral DNA.
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- Dr Marc Ruff