ECCMID Symposium “CRISPR-Cas revolution”

CRISPR-Cas9 based therapy for persistent virus infections (HIV)

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The Netherlands
HIV in the Netherlands since 1983 (18 million inhabitants)

We have analyzed some 60,992 patient samples since start epidemic

End 2017: 19,582 HIV+ people in care (approx. 2300 don’t know their status)
  12,378 MSM, other men (3593) and women (3611)
  around 750 new cases in 2017 (PReP started in risk groups)
  90% diagnosed, 93% on ART, 95% viral suppression

Research towards a cure:
  Anti-HIV gene therapy (RNAi)
  CURE approaches (CRISPR-Cas)
Combination drug therapy saves lives: Durable HIV control possible

But HIV rebounds rapidly after stopping therapy > life-long therapy

Alternative: Gene therapy for durable therapeutic effect (single treatment)
- Protect cells against HIV, use lentiviral vector that stably integrates!
- Express antiviral molecules (RNAi)
- Direct attack on HIV DNA in the reservoir (CRISPR-Cas)
HIV drug therapy a success, but what about a cure?
Attack on the HIV reservoir

RNA → RT → RNA → DNA

RNAi

CRISPR-Cas9 → degradation

CRISPR-Cas9 → DNA repair

direct attack on reservoir
CRISPR-Cas9 molecule of the year 2015 (Science)

- bacterial origin
- dsDNA endonuclease
- RNA guided (guide RNA)
## RNAi versus CRISPR-Cas9

<table>
<thead>
<tr>
<th>Component</th>
<th>RNAi</th>
<th>CRISPR-Cas9</th>
</tr>
</thead>
<tbody>
<tr>
<td>Effector</td>
<td>short hairpin RNA</td>
<td>guide RNA</td>
</tr>
<tr>
<td>Target</td>
<td>RNA (sequence-specific)</td>
<td>dsDNA (sequence-specific)</td>
</tr>
<tr>
<td>Result</td>
<td>cleavage</td>
<td>cleavage</td>
</tr>
<tr>
<td>Components</td>
<td>1 (shRNA)</td>
<td>2 (gRNA + CRISPR-Cas9)</td>
</tr>
<tr>
<td>Viral escape</td>
<td>yes (point mutation in target)</td>
<td>no (initial literature)</td>
</tr>
</tbody>
</table>

**Bonus:**
- Direct attack on HIV DNA reservoir

Globally similar antiviral approaches
Yet many **surprising differences**
Cell culture infections: Potent inhibition by some shRNAs
But HIV escape, which can be blocked by a combinatorial attack (3 shRNAs)

Regular RT-induced mutations
Preference for silent mutations (25/27)
Targeting HIV DNA (conserved sites only) profound inhibition of viral gene expression by each gRNA (unlike shRNAs)

Transfect 293T cells with plasmids encoding CRISPR-Cas, gRNA and HIV
Transduce T cell line with LV-Cas-gRNA
Infect with HIV LAI isolate

Good inhibition of HIV replication
But sometimes immediate virus escape?
Unlike RT-induced pointmutations in RNAi escape, now **indels** (small insertions/deletions) very popular, but not in important ORFs (Gag): not compatible with HIV replication
Variable targets (high Shannon) = rapid escape by indels .......... *mechanism*?

Well-conserved targets (low Shannon) = slow escape by (regular) point mutations

indels not compatible with function
All occurs around the cleavage site: CRISPR-Cas9 mediates DNA cleavage, but subsequent DNA repair by the NHEJ pathway (non-homologous end joining) creates indels that allows viral escape.

HIV escapes with help from the host (NHEJ)
Combinatorial CRISPR attack

1. Better inhibition
2. Higher threshold for resistance

Possible bonus: **excision** of sequences between 2 cleavage sites (LTRs)
HIV replication: two combi’s prevent viral escape

gGag1+gTatRev and gGag1+gEnv2 (very conserved targets)

Time to “HIV breakthrough” (days, average 4 cultures)

< no escape at end experiment

HIV sterilization???
wild-type HIV proviruses disappear over time

<table>
<thead>
<tr>
<th>gRNAs</th>
<th>target region</th>
<th>wild-type sequence frequency (%)</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>day 12</td>
<td>day 110</td>
<td></td>
</tr>
<tr>
<td><strong>gGag1 + gTatRev</strong></td>
<td>gGag1</td>
<td>8 / 21 (38.1)</td>
<td>2 / 38 (5.3)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>gTatRev</td>
<td>7 / 28 (25.0)</td>
<td>0 / 39 (0.0)</td>
<td></td>
</tr>
<tr>
<td><strong>gGag1 + gEnv2</strong></td>
<td>gGag1</td>
<td>8 / 21 (38.1)</td>
<td>1 / 37 (2.7)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>gEnv2</td>
<td>0 / 21 (0.0)</td>
<td>0 / 43 (0.0)</td>
<td></td>
</tr>
</tbody>
</table>

A shift from minor to major mutations (repeated Cas action)
**Ultra-sensitive screen for infectious HIV**

<table>
<thead>
<tr>
<th>gRNAs</th>
<th>Culture</th>
<th>Virus rescue</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>day 16</td>
</tr>
<tr>
<td>gGag1 + gTatRev</td>
<td>1</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>+</td>
</tr>
<tr>
<td>gGag1 + gEnv2</td>
<td>1</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>+</td>
</tr>
</tbody>
</table>

Cultures of cells were effectively **sterilized!**

**gGag1+gTatRev faster than gGag1+gEnv2**

**The host (NHEJ) helps to inactivate HIV-1**
Provirus excision only? (“HIV Excision” Meeting, NIH)
Prominent product in PCR, but in fact occurs surprisingly little

Instead, most HIV genomes are inactivated by NHEJ-hypermutation!

Why? DNA repair is simply too fast
1st break repaired before 2nd break occurs
no 2 breaks simultaneously > no excision

We can sterilize HIV .... but can we find the HIV reservoir in humans?
CD32a is a marker of a CD4 T-cell HIV reservoir harbouring replication-competent proviruses

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Receptor for IgG antibodies

Average HIV DNA enrichment of ~1000-fold

Descours et al, Nature 2017
Several groups have shown CD32 expression on a small subset of CD4+ T cells

Descours Nature 2017; Bertagnolli Nature 2018; Abdel-Mohsen Sci Transl Med 2018; Holgado Front Immunol 2018
So far nobody could confirm HIV DNA enrichment in CD32+ cells

CD32 is expressed on cells with transcriptionally active HIV but does not enrich for HIV DNA in resting T cells

Mohamed Abdel-Mohsen,1,2 Leticia Kuri-Cervantes,2,3 Judith Grou-Exposito,4,6 Adam M. Spirnak,5 Rachael A. Neil,7 Costin Teemescu,1 Surya Kumari Yadavelli,1 Leila B. Giron,8 Carla Serra-Peñalver,3 Meritxell Gené,3,9 Josep Castelví,2 Guoqin Wu,2 Perla M. Del Rio Estrada,3 Mauricio Gonzalez Navarro,1 Kownbok Ly,1,10 Carlos T. King,1 Sai Verma,9 Kara Coss,2 Yaming Wang,9 Qingsheng Li,9 Karam Moumen,10 Jay Kesterman,1 Ian Frank,7 Mikko Paajarvi,7 Davia Harada,1 Gustavo Reyes-Terés,7 Douglas Richman,1 Bonnie Howell,2 Pablo Tebas,3 Javier Martínez-Picado,12,13 Vicente Planas-Bou,4 Maria J. Bizerril,2 Michael R. Berke,1 Luís J. Montaner11

The Latent Human Immunodeficiency Virus (HIV) Reservoir Resides Primarily in CD32+CD4+ T Cells in Perinatally HIV-Infected Adolescents With Long-Term Virologic Suppression

All CD32+ cell purifications based on FACS

- Purity of the CD32 cell fraction a problem
Blood from HIV-infected individuals

PBMCs

Alternative purification scheme
2x negative selection (magnetic sorting)

CD4+ T cells

CD32+CD4+ T cells

CD32-CD4+ T cells

3x positive selection (magnetic sorting)

HIV-1 DNA and RNA measurements
Extra two rounds of CD32⁺ positive selection resulted in high (up to ~1000-fold) enrichment in HIV DNA in CD32⁺ fraction

Mean enrichment: 11.1x, 34.5x, 292.4x

HIV DNA load reached 0.53 copies per cell
No enrichment in HIV RNA was observed and HIV RNA/DNA ratio was significantly lower in the CD32+ fraction, indicating that HIV in these cells is mostly transcriptionally silent.

**CD32 remains a bona fide candidate marker of the HIV reservoir and a promising target for therapeutic cure strategies**
Human germline editing in the news

Jianku He (Shenzhen, China)
Out of the blue:
Not published
Not controlled by ethics committees
University employer did not know
Informed consent on vaccination

Protect the unborn child of a HIV-positive father during in vitro fertilization with his sperm and (?) later in life by germline removal of the main receptor for HIV: CCR5
- fertilized oocyte treated with anti-CCR5 CRISPR-Cas
- 9 months later the twins Lulu and Nana were born
- wt gene still present in one baby? (mosaicism)

Prevention?
No absolute protection (CXCR4-using strains)
Safe alternative means available: sperm washing!

Safe??
It is in germline!
Off target effects excluded?
R5 really not needed in life? (altered West Nile Virus susceptibility …….)