Cyclic Panobinostat (LBH589) dosing in HIV-1 patients: Findings from the CLEAR trial

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Background Viral Reactivation

• Part of “kick and kill” strategy Histone Deacetylase Inhibitors (HDACi) have been subject of great interest

• HDACi influence chromatin condensation equilibrium resulting in enhanced transcription of latent virus.

Proof that HIV-1 transcription can be turned on by HDACi treatment (the kick).

...Yet to prove production of viral proteins which is believed to be a necessity (to facilitate the kill)

• Single dose Vorinostat led to 4.8-fold induction of HIV RNA
  

• Multiple doses of Vorinostat led to 2.6-fold induction of HIV RNA
  
  Elliott et al CROI 2013
Comparison of a number of HDACi either in development or licensed
We found that panobinostat was able to potently activate latent HIV in both cell lines as well as in a primary T cell model

$C_{\text{max/EC}_{50}}$ dose of 20 mg Panobinostat 3-5 fold higher than 400 mg Vorinostat

Rasmussen et al 2013. Human Vac Immunotherapeutics
Panobinostat (LBH589)

- Panobinostat is a cinnamic hydroxamic acid analogue very similar to Vorinostat
- Panobinostat is being developed by Novartis for treatment of Multiple myeloma (Phase III) and Acute Myeloid Leukemia (Phase II)
- Panobinostat is described as a Pan-HDAC inhibitor with inhibitory activity in the lower nM range
- Considerable inhibitory activity against HDAC 1, 2 and 3 which appear important for disruption of HIV latency

Archin et al 2009. AIDS
Hypothesis

Would 20 mg oral Panobinostat dosing lead to viral reactivation in virological suppressed HIV-1 patients?

Could a cyclic dosing pattern lead to more efficient viral “kick” and reduce side effects especially thrombocytopenia?
Study design Overall

- Oral tablet 20 mg three times per week every other week
- Repeated 4 times for a total of 8 weeks.
- Total of 12 doses

Panobinostat dosing 20 mg/3 times per week

Weeks:
- Screening
- Treatment
- Follow-up

Optional ART interruption

Extensive sampling
Sampling

- Extensive sampling twice per treatment cycle and once every pause week.
- Optional lumbar puncture at baseline and during last treatment cycle.
- Optional sigmoidal biopsy at baseline and during last treatment cycle.
Primary endpoint:
• Change from baseline in cell-associated HIV-RNA (unspliced gag HIV-RNA)/10^6 CD4+ T cells

Secondary endpoints:
• HIV-DNA
  – Dynamics of integrated HIV-DNA/10^6 CD4+ T cells
  – Total HIV-DNA/10^6 CD4+ T cell subsets
  – Dynamics of 1- and 2-LTR circles in CD4+ T cell subsets
• Viral reservoir (using the viral outgrowth assay)
  – Baseline, 12 and 32 weeks after initiation of panobinostat
• Low-level viremia (single copy assay and TMA based)
• Viral rebound kinetics during optional treatment interruption
Criteria

• **Inclusion**
  • Age ≥ 18 years
  • Continuous cART for > 2 years prior to enrollment
  • CD4 count > 500 cells/mm³ at last measurement
  • Suppression of plasma HIV-RNA levels < 50 copies/ml for > 2 years

• **Exclusion**
  • Co-infection with HBV or HCV
  • Use of Protease inhibitors as part of backbone cART
  • Clinically significant cardiac disease
Patient Characteristics

- Very dedicated patient population
- Total of 15 scheduled outpatient visits (14 visits over a 16 week period)
- 11 consented to additional 2 visits for lumbar puncture
- 9 consented to additional 2 visits for gut biopsies

### Baseline Characteristics

<table>
<thead>
<tr>
<th></th>
<th>Median (range)</th>
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<tbody>
<tr>
<td>Total inclusions</td>
<td>15</td>
</tr>
<tr>
<td>Gender (% Male)</td>
<td>100</td>
</tr>
<tr>
<td>Race (% Caucasian)</td>
<td>100</td>
</tr>
<tr>
<td>Age (years)</td>
<td>47 (28–53)</td>
</tr>
<tr>
<td>Time since HIV-diagnosis (months)</td>
<td>81.4 (33.4–340)</td>
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<tr>
<td>Time on HAART (months)</td>
<td>43.4 (30.5–191.7)</td>
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<tr>
<td>Time with HIV-RNA &lt;50/ml (months)</td>
<td>38 (26.3–169.6)</td>
</tr>
<tr>
<td>Nadir CD4+ T-cell count (10⁶/mL)</td>
<td>350 (130–710)</td>
</tr>
<tr>
<td>Baseline CD4+ T-cell count (10⁶/mL)</td>
<td>935 (615–1990)</td>
</tr>
<tr>
<td>HIV diagnosis to HAART initiation (days)</td>
<td>540 (0–6574)</td>
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</tbody>
</table>
Safety - Adverse Events

- Fatigue most commonly reported
- All side-effects self-limiting
- All patients completed full dosing schedule

<table>
<thead>
<tr>
<th>Adverse event</th>
<th>AE (any grade)</th>
<th>CTCAE I</th>
<th>CTCAE II</th>
<th>SAE</th>
<th>Related to study drug</th>
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<tbody>
<tr>
<td>Fatigue</td>
<td>7</td>
<td>7</td>
<td></td>
<td>No</td>
<td>Presumed</td>
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<tr>
<td>Rash</td>
<td>2</td>
<td>2</td>
<td></td>
<td>No</td>
<td>Presumed</td>
</tr>
<tr>
<td>Diarrhea</td>
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<td>No</td>
<td>Presumed</td>
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<tr>
<td>Nausea</td>
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<td>2</td>
<td></td>
<td>No</td>
<td>Presumed</td>
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<tr>
<td>Palpitations (normal cardiac examination)</td>
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<td>1</td>
<td></td>
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<td>Stomach ache</td>
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<td>Vomiting</td>
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<td>Presumed</td>
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<tr>
<td>Aphthous stomatitis</td>
<td>1</td>
<td>1</td>
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<td>No</td>
<td>Presumed</td>
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<tr>
<td>Sleeplessness</td>
<td>1</td>
<td>1</td>
<td></td>
<td>No</td>
<td>Presumed</td>
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<tr>
<td><strong>Total</strong></td>
<td><strong>18</strong></td>
<td><strong>18</strong></td>
<td><strong>0</strong></td>
<td></td>
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</table>
Safety - Biochemistry

- No adverse effect on CD4+ T cells

- Initial drop in Neutrophils. No difference after 12 doses

- Trend to a decline in thrombocytes although well within the normal limits

- 20 mg panobinostat was very well tolerated without any need for dose modifications
Histone acetylation - first week

• Determination of H3 acetylation by flow cytometry on fresh samples

• Rapid increase in Histone H3 acetylation levels only 2 hrs after 20 mg oral dosing

Rigby L et al 2012. Epigenetics
Histone Acetylation - cyclic dosing

- Very clear acetylation/deacetylation pattern following treatment weeks
Low-level viremia

• Evaluation of plasma HIV RNA as determined by the qualitative NAT-screening system (PROCLEIX ULTRIO Plus, Genprobe)
  • Transcription Mediated Amplification (TMA) detection of HIV RNA
  • 50% analytic sensitivity to detect 3.8 copies/mL
  • 95% analytic sensitivity to detect 12 copies/mL

• Fully automated certified detection method
• Only get a yes/no answer - no quantification

Stramer et al 2013. Transfusion
Low level viremia - First treatment week

- Highly statistical significant increase in plasma virus detection during the first treatment cycle ($p=0.009$, $x^2$-test)

<table>
<thead>
<tr>
<th></th>
<th>Positive</th>
<th>Negative</th>
<th>Total</th>
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</thead>
<tbody>
<tr>
<td>Baseline</td>
<td>8</td>
<td>22</td>
<td>30</td>
</tr>
<tr>
<td>First week</td>
<td>18</td>
<td>12</td>
<td>30</td>
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</tbody>
</table>

15 patients
Day -30 and 0
N=30

15 patients
Day 1 and 4
N=30
Low level viremia

- Only 1/15 remain undetectable at all time-points during panobinostat dosing
- 3 patients positive at all time-points (make up 6/8 positive baseline values)
- The proportion of 3/15 detectable (all time-points) is in line with earlier reports

<table>
<thead>
<tr>
<th></th>
<th>Positive</th>
<th>Negative</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline</td>
<td>8 (26%)</td>
<td>22 (74%)</td>
</tr>
<tr>
<td>Panobinostat</td>
<td>85 (55%)</td>
<td>69 (45%)</td>
</tr>
</tbody>
</table>

\( p=0.004, \chi^2\)-test for proportions

References:
Palmer et al. 2008. PNAS
Dinoso et al. 2009. PNAS
McMahon et al. 2010 CID
Gandhi et al. 2010 PLOS Medicine
Low-level viremia dynamics

- Cyclic dynamics of plasma HIV RNA detection coinciding with on/off panobinostat
- Prolonged robust viral response upon initiation of panobinostat
- Difference in acetylation dynamics
Conclusions

- Panobinostat cyclic dosing (20 mg/TIW) in HIV-1 patients were very well tolerated and conserved thrombocytes and neutrophils levels within the normal limits.

- Histone acetylation data follow the cyclic dosing pattern with no waining effect over the 4 cycles

- A highly statistical significant increase in HIV-RNA plasma detection following panobinostat dosing ($p= 0.004$, $x^2$-test)

- Only 1 of the 15 treated patients remained HIV-RNA negative in plasma following panobinostat dosing

- First proof of a viral “kick” leading to consistent plasma release of viral particles
Further directions

To

• Analyse the complete data set for cell-associated unspliced HIV-RNA
• Perform the HIV DNA measurements and the viral outgrowth assay
• Quantify the low-level viremia data with the single copy assay
• Quantify both the HIV-specific and unspecific immune dynamics during cyclic treatment
• Evaluate impact on the CNS compartment with regard to neuro-inflammation as well as viral dynamics single copy assay HIV quantification
• Evaluate cell-associated unspliced HIV-RNA and HIV DNA in CD4+ cells from the rectal biopsies
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