Blips e low level viremia, come influiscono nella gestione del paziente

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Massimo Andreoni
Cattedra di Malattie Infettive
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- Janssen-Cilag
- Viiv Healthcare
- Merck Sharp and Dohme
- Abbvie
- GSK
- Angelini
- Pfizer
VIREMIA RESIDUA E VIRAL BLIPS

DEFINIZIONE
Low level viremia

Longer lived cells
Macrophage?

Activated lymphocytes

Very low level viremia

Lymphatic tissue

Residual viremia

Viral blips

Viral eradication (?)
VIREMIA RESIDUA E VIRAL BLIPS
Long lived cells containing non-defective HIV are a very small fraction of total DNA positive-cells (<1%).

Andreoni Current HIV Medicine 2015
How HIV persists during therapy?

- Reservoir cells, like other memory T cells, divide very slowly to maintain the memory of the immune system (A. Bosque and V. Planelles)
HIV integrates its DNA into many sites in the host genome; 2,410 integration sites in peripheral blood lymphocytes of five infected individuals on cART were identified.

Some infected cells clonally expanded; a significant fraction of the infected cells in patients on cART are from expanded clones.

Genes in which we isolated a particular integration site seven or more times are shown.
### Table: Clones Persist for >11 years in Patient 1

<table>
<thead>
<tr>
<th>Gene</th>
<th>Site</th>
<th>Detected in year</th>
<th>When seen</th>
</tr>
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<tr>
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<td>CHD7</td>
<td>chr8:61594573</td>
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</tr>
</tbody>
</table>

- **Seen at all times**
- **Seen at 4.8 and 11.4 years of therapy**
- **Seen at 0.2 and 11.4 years of therapy**

F. Maldarelli, Science 2014
The Persistent Steady State

The number of infected cells remains about the same, but the clonal populations appear...
Clonal expansion and immunological memory
Relationship Between Residual Plasma Viremia and the Size of HIV Proviral DNA Reservoirs in Infected Individuals Receiving Effective Antiretroviral Therapy

- 127 ART treated patients
- Med Duration: 6.5 y
- CD4 med: 580 /mm³
- HIV RNA < 50 cp: 100%
- CD4/CD8: 0.8
- HIV RNA us < 1 cp/ml: 37%
- CV median: 2.6 cp/ml

Correlation between residual viremia and HIV-DNA

Chun, Kovac, Fauci et al JID 2011; 204:135
VIREMIA RESIDUA E VIRAL BLIPS

DEFINIZIONE

ORIGINE

FREQUENZA
Evidence of persistent low-level viremia in long-term HAART-suppressed, HIV-infected individuals

In the first year RNA levels declined at a rate of -0.25 S/Co per month (p=0.001) but after month 12 there was no evidence for a continued decline (p=0.383).
Detection of viral load by ultrasensitive method in 420 patients with <50 HIV-RNA copies/ml
VIREMIA RESIDUA E VIRAL BLIPS

DEFINIZIONE

ORIGINE

FREQUENZA

SIGNIFICATO VIROLOGICO
Identifying the source of HIV viremia during suppressive antiretroviral therapy is essential to eradication.

**Active replication cycles**
- Infected cell
- Uninfected cell
- HIV production from active replication is blocked by ARV therapy

**Stable reservoirs**
- HIV production from reservoirs is NOT blocked by ARV therapy

IMPROVED ARV NEEDED

NEW STRATEGIES NEEDED
The compartmentalization of the viruses between the plasma and the blood monocytes suggests at least two origins of residual virus production during effective cART. CXCR4-using viruses might be produced preferentially in patients on cART.

Mavigner PLOS One 2009
HIV-DNA value in resting CD4 is higher than viral outgrowth assay value (186 vs. 0.62, P=0.0001).

High fraction of proviruses are defective.
Rapid accumulation of defective proviruses…

as early as two to three weeks after infection to make up over 93% of all proviruses, regardless of how early ART is initiated

Bruner et al, Nat Med 2016
Patients with equal HIV-DNA copies number but different probability of plasma viremia rebound at cessation of ART.
Plasma HIV-1 RNA Detection Below 50 Copies/mL and Risk of Virologic Rebound in Patients Receiving Highly Active Antiretroviral Therapy

Tomas Doyle,1,3 Colette Smith,4 Paola Vitiello,3 Valentina Cambiano,6 Margaret Johnson,2,5 Andrew Owen,6 Andrew N. Phillips,4 and Anna Maria Geretti1,3,7

[Graph showing the probability of virologic rebound over time with different VL levels.]
Higher pre-treatment interruption CA-RNA levels are significantly associated with shorter time to viral rebound to a threshold of either 200 HIV-1 RNA copies/ml or 1000 HIV-1 RNA copies/ml.

Fig. 3. Association of pre-ATI levels of CA-RNA with timing of viral rebound. Levels of pre-ATI CA-RNA categorized by timing of viral rebound to the (a) 200 HIV RNA copies/ml and (b) 1000 HIV RNA copies/ml thresholds. Open symbols represent values from participants treated during chronic infection and closed symbols represent values from participants treated during acute infection.

Li et al., AIDS 2016
2,795 patients with virologic suppression (<50 on two consecutive viral loads) were enrolled into the HIV Research Network between 2005 and 2015. Patients were categorized into three categories: no blips or LLV, 51–200, 201–500 and virologic failure (two consecutive >500).

**Results:**
- 283 (10.1%) patients experienced virologic failure
- 152 (5.4%) patients experienced LLV to 51–200
- 110 (3.9%) patients experienced LLV to 201–500
Both LLV 51–200 [adjusted hazard ratio (aHR) 1.83 (1.10, 3.04)] and LLV 201–500 [aHR 4.26 (2.65, 6.86)] were associated with virologic failure.
Low-level viremia and virologic failure in persons with HIV infection treated with antiretroviral therapy


Failure rates per blip category
Low level viremias have been associated with selection of drug-resistant virus in several (but not all) studies

- **Cohen Stuart, et al.** Transient relapses (“blips”) of plasma HIV RNA levels during HAART are associated with drug resistance. *JAIDS 2001.*
- **Greub, et al.** Intermittent and sustained low-level HIV viral rebound in patients receiving potent antiretroviral therapy. *AIDS 2002*
- **Hermankova, et al.** HIV-1 drug resistance profiles in children and adults with viral load of <50 copies/ml receiving combination therapy. *JAMA 2001*
- **Nettles, et al.** Intermittent HIV-1 viremia (blips) and drug resistance in patients receiving HAART. *JAMA 2005.*
- **Santoro MM et al.** Reliability and Clinical Relevance of the HIV-1 Drug-Resistance Test in Patients
VIREMIA RESIDUA E VIRAL BLIPS

DEFINIZIONE
ORIGINE
FREQUENZA
SIGNIFICATO VIROLOGICO
SIGNIFICATO CLINICO
STRATEGIE TERAPEUTICHE
Incomplete ART adherence is associated with higher levels of residual HIV-1 viremia, but detectable residual viremia can be present despite 100% measured ART adherence.
Treatment intensification does not reduce residual HIV-1 viremia in patients on highly active antiretroviral therapy

J. B. Dinoso

PNAS

June 9, 2009 vol. 106 no. 23 9403–9408
HIV-1 replication and immune dynamics are affected by raltegravir intensification of HAART-suppressed subjects

Maria J Buzón, Marta Massanella, Josep M Llibre, Anna Esteve, Viktor Dahl, Maria C Puertas, Josep M Gatell, Pere Domingo, Roger Paredes, Mark Sharkey, Sarah Palmer, Mario Stevenson, Bonaventura Clotet, Julià Blanco & Javier Martinez-Picado
RAL intensification of HAART resulted in a specific and transient increase in episomal DNAs. Patients on HAART <50 HIV-RNAcp/ml for at least 1 year.

**INTENSIFICATION**

HAART + RAL  
$n = 45$

**CONTROL**

Continue HAART  
$n = 24$

- 2-LTR+  
$n = 13$
- 2-LTR-  
$n = 32$

Pts with HIV replication.

**Buzon 2010**

*Intensified*  
*Control*
# HIV Type 1 (HIV-1) Proviral Reservoirs Decay Continuously Under Sustained Virologic Control in HIV-1–Infected Children Who Received Early Treatment

## Table 1: Characteristics of Early and Late Treated Patients

<table>
<thead>
<tr>
<th>Cohort, Patient</th>
<th>Age at Study, y</th>
<th>Current cART Regimen</th>
<th>cART Duration, y</th>
<th>CD4+ T-Cell Percentage</th>
<th>CD4+ T-Cell Count</th>
<th>CD4+ T-Cell: CD8+ T-Cell ratio</th>
<th>HLA Types</th>
</tr>
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<tbody>
<tr>
<td><strong>Early treated</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>P-1042</td>
<td>17.7</td>
<td>2 NRTIs + 1 PI/boosted</td>
<td>17.5</td>
<td>53</td>
<td>935</td>
<td>2.8</td>
<td>A26, A30, B18, B39, Cw5, Cw12</td>
</tr>
<tr>
<td>P-1043</td>
<td>17.7</td>
<td>2 NRTIs + 1 PI/boosted</td>
<td>17.5</td>
<td>58</td>
<td>951</td>
<td>2.8</td>
<td>A11, A26, B39, B51, Cw3, Cw12</td>
</tr>
<tr>
<td>P-1048</td>
<td>16</td>
<td>2 NRTIs + 1 NNRTI</td>
<td>15.8</td>
<td>39</td>
<td>1328</td>
<td>1.4</td>
<td>A29, A30, B44, Cw4, Cw16</td>
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<tr>
<td>P-1049</td>
<td>14</td>
<td>2 NRTIs + 1 PI</td>
<td>13.9</td>
<td>38</td>
<td>689</td>
<td>1.4</td>
<td>A1, A2, B7, B50, Cw6, Cw7</td>
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<tr>
<td>Overall, median (IQR)</td>
<td></td>
<td></td>
<td>16.9 (14.5–17.7)</td>
<td>16.7 (14.4–17.5)</td>
<td>46 (38–57)</td>
<td>943 (751–1234)</td>
<td>2.1 (1.4–2.8)</td>
</tr>
</tbody>
</table>

| **Late treated** | | | | | | | |
| P-1376          | 22             | 3 NRTIs + 1 PI/boosted | 9.4             | 32                     | 452              | 0.6                           | A1, A2, B7, B35, Cw4         |
| P-1377          | 23             | 2 NRTIs + 1 PI/boosted | 9.8             | 23                     | 500              | 0.5                           | A30, A68, B7, B35, Cw7       |
| P-1378          | 19             | 1 NRTI + 1 NNRTI + 1 PI | 4.5             | 30                     | 1008             | 0.8                           | A23, A30, B7, B15, Cw3, Cw7  |
| P-1379          | 23             | 1 NRTI + 1 NNRTI + 1 PI/boosted | 10.7            | 18                     | 397              | 0.3                           | A2, A68, B15, B48, Cw1, Cw8  |

K. Luzuriaga
Plasma viremia was not detected in any ET youth but was detected in all LT youth (median, 8 copies/mL; P = .03).

<table>
<thead>
<tr>
<th>Cohort, Patient</th>
<th>Age at Study, y</th>
<th>Proviral DNA Load, Copies/10⁶ PBMCs</th>
<th>Replication-Competent Virus</th>
<th>Resting CD4⁺ T Cells Cultured, No., ×10⁶</th>
<th>Residual Viremia, RNA Copies/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Early treated</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>P-1042</td>
<td></td>
<td>V1 16.3</td>
<td>ND</td>
<td>&lt;.01</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td></td>
<td>V2 16.6</td>
<td>8.9</td>
<td>&lt;.01</td>
<td>14.5</td>
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<tr>
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<td></td>
<td>V3 17.7</td>
<td>12.0</td>
<td>&lt;.01</td>
<td>15</td>
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<tr>
<td>P-1043</td>
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<td>V1 16.3</td>
<td>ND</td>
<td>&lt;.01</td>
<td>12.5</td>
</tr>
<tr>
<td></td>
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<td>V2 16.6</td>
<td>&lt;4</td>
<td>&lt;.01</td>
<td>17</td>
</tr>
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<td>V3 17.7</td>
<td>6.6</td>
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<td>24</td>
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<tr>
<td>P-1048</td>
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<td>V1 14.5</td>
<td>&lt;4</td>
<td>&lt;.01</td>
<td>21</td>
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<tr>
<td></td>
<td></td>
<td>V2 15.9</td>
<td>&lt;4</td>
<td>0.05</td>
<td>19.5</td>
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<tr>
<td>P-1049</td>
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<td>V1 13.1</td>
<td>5.5</td>
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<tr>
<td></td>
<td></td>
<td>V2 14.0</td>
<td>8.3</td>
<td>ND</td>
<td>ND</td>
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<td>P-1376</td>
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<td>V1 22.0</td>
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<td>0.51</td>
<td>12.5</td>
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<td></td>
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<td>P-1377</td>
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<td>V1 23.0</td>
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<td>1.83</td>
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<td>0.51</td>
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<td></td>
<td></td>
<td>V2 23.0</td>
<td>68.0</td>
<td>0.22</td>
<td>12.5</td>
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</table>
Replication-competent virus was recovered from all LT youth but only 1 ET youth.
Dual Therapy: reaching clinical evidence

- Inhibitory quotient
- Time

Viral quasispecies

- Triple therapy
- Dual therapy
- Monotherapy

Spontaneous mutants with reduced drug-sensitivity
HIV RNA <50 copies/mL at Week 144 by baseline HIV RNA (Per Protocol, TLOVR, Switch=Failure) MONET study

Viral blips & virological failure in dual therapy

Blips by treatment arm

<table>
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<tr>
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<th>SALT</th>
<th>OLE’</th>
<th>ATLAS-M</th>
<th>DUAL</th>
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<td>15</td>
<td>10</td>
<td>7.5</td>
<td>11</td>
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<tr>
<td>Triple</td>
<td>17</td>
<td>9.9</td>
<td>12</td>
<td>14</td>
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Virological failure by arm

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<td>4.5</td>
<td>3</td>
<td>1 (V10I)</td>
<td>3</td>
</tr>
<tr>
<td>Triple</td>
<td>2.5</td>
<td>2.4</td>
<td>1 (V10I, W71T, D76W)</td>
<td>2</td>
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</table>

3. DUAL GESIDA, CID 2017
Blip Frequencies and Number by Visit Week

- Similar ‘blip’ frequencies were seen across arms by visit week
- Cumulative occurrences: DTG + 3TC (N=87); DTG + TDF/FTC (N=109)

CVWs were not associated with prior VL blips

CVW: Confirmed virologic withdrawal

*Percentages were calculated from number of blips using previously suppressed (<50 c/mL) participant Ns, respectively, for DTG + 3TC and DTG + TDF/FTC at Wk 8 (N=517) and (N=496); Wk 12 (N=625) and (N=632); Wk 16 (N=657) and (N=659); Wk 24 (N=714) and (N=726); Wk 36 (N=674) and (N=683); and Wk 48 (N=678) and (N=691). Bold numbers on chart are # of blips at given week visits. Individual participants can have had more than one blip.

Underwood et al. IAS 2019; Mexico City, Mexico. MOPEB231.

Conference on Retroviruses and Opportunistic Infections; March 4-7, 2019; Seattle, WA
Proportion of Subjects with Plasma HIV-1 RNA <40 c/mL and TND Status by Visit - Snapshot Analysis

- At Week 48 similar proportion of subjects had snapshot TND (target not detected) in the 2DR and 3DR arms (77% [553/716] vs 73% [525/717], adjusted difference 3.8%, 95% CI -0.6%, 8.2%)
- Proportions were also similar at Weeks 4 (34% vs 32%), 8 (52% vs 49%), 12 (60% vs 57%), 16 (59% vs 56%), 24 (65% vs 63%), and 36 (65% vs 68%)
Residual Viremia Substudy
Proportions of TND by Week for Participants With Baseline TND

Underwood et al. HIV Glasgow; Glasgow, UK. Poster P311.
VIREMIA RESIDUA E VIRAL BLIPS

DEFINIZIONE

ORIGINE

FREQUENZA

SIGNIFICATO VIROLOGICO

SIGNIFICATO CLINICO

STRATEGIE TERAPEUTICHE

INFIAMMAZIONE IMMUNOATTIVAZIONE
Inflammation

- ↑ Endothelium adhesion
- ↑ Monocyte activation
- Dyslipidemia
- Hypercoagulation/thrombotic events
- Endothelial dysfunction

Increased incidence of comorbidities and clinical disease

HIV-mediated loss of regulatory cells (Tregs)

HIV production
HIV replication

HIV-associated fat Metabolic syndrome

CMV-HCV Excess pathogens

Microbial translocation
In 190 subjects with persistently HIV-RNA <50 cp/ml during ART, cell-associated RNA and proviral DNA levels were significantly higher in the low (<350 cells/mm³) CD4⁺ T-cell count group.

Hatano H, 2013;208:50–6
Non-Suppressible Viremia

• Can be caused by clonal proliferation of CD4+ T-cells carrying replication-competent proviruses: “Repliclones”
  – Some cells within the clones are producing virions
  – Clones are large ($10^7$-$10^8$ cells) but overall are rare integrants (0.03 -1%)
  – Intact proviruses are intragenic, within introns and in either orientation to gene
• Clinical Implications
  – Clinically-detectable viremia may not be due to non-adherence or drug resistance
• Cure Implications
  – Smaller clones may be producing infectious virus throughout lymphoid organs
  – May fuel rapid viremia rebound off ART
  – Need to eliminate or suppress repliclones!
  – Have the potential to regrow.
• Unanswered Questions
  – Mechanisms of clonal escape?
  – Latency? CTL or antibody resistance?