Clinical use of HIV-DNA quantity and resistance testing

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  - Abbott Molecular
  - Gilead Sciences
  - Janssen-Cilag
  - Merck Sharp and Dohme
  - ViiV Healthcare

- Research and educational grants
  - Abbott Molecular
  - Janssen-Cilag
  - Hologic
  - Merck Sharp and Dohme
  - ViiV Healthcare
Quantification of HIV DNA

Basics
Why to test
Caveats
Are we ready to test
Quantitation of HIV DNA vs. RNA following ART initiation

Threshold of sensitivity for HIV RNA

HIV RNA (copies/ml) spans 6 logs and responds timely to ART

>95% adherent patients have undetectable HIV RNA

Adapted from Strain & Richman, Curr Opin Virol 2013
Quantitation of HIV DNA vs. RNA following ART initiation

HIV DNA (copies/10^6 ells) spans 3 logs and responds slowly to ART

HIV RNA (copies/ml) spans 6 logs and responds timely to ART

>99% adherent patients have detectable HIV DNA

Threshold of sensitivity for HIV DNA

Threshold of sensitivity for HIV RNA

>95% adherent patients have undetectable HIV RNA

Adapted from Strain & Richman, Curr Opin Virol 2013
HIV-1 DNA Decay Dynamics in Blood During More Than a Decade of Suppressive Antiretroviral Therapy

- 30 patients studied during 7–12 years of effective ART
- HIV-1 DNA decreased significantly from years 0–1 and 1–4 of ART ($P < .001$)
- Decay was not significant for years 4–7 ($P = .17$)
- All participants had detectable HIV-1 DNA after 10 years (median 439 copies/$10^6$ CD4+ T-cells; range: 7–2074)

Besson, Clin Infect Dis 2014
Quantification of HIV DNA

Basics

Why to test

Caveats

Are we ready to test
Impact of HIV Type 1 DNA Levels on Spontaneous Disease Progression: A Meta-Analysis

- 1074 participants
- HIV-1 DNA was a strong predictive marker of AIDS [RR: 3.01, 95% CI: 1.88–4.82]
- HIV-1 DNA was a significantly better predictor than HIV-1 RNA of either AIDS alone (ratio of RRs = 1.47, 95% CI: 1.05–2.07) or of combined (AIDS or death) progression outcomes (ratio of RRs = 1.51, 95% CI: 1.11–2.05)

Tsiara, ARHR 2011
SPARTAC (Short Pulse Anti Retroviral Therapy at HIV Seroconversion)

The largest randomised controlled trial ever undertaken in primary (recent) HIV infection. The study ran between 2003 and 2011 across eight countries.

Participants with recent HIV infection (within 6 months)

Randomisation (like the roll of dice)

People who needed treatment straight away were not eligible to join and were treated outside of the trial.

Standard of Care (SOC)

12 weeks of treatment (ART-12)

48 weeks of treatment (ART-48)

CD4 immune cell count confirmed below 350 cells per mm³ blood, or started long-term treatment.
HIV-1 DNA predicts clinical progression (CD4 <350) in absence of ART. HIV-1 DNA data was divided into two ‘high’ and ‘low’ at the median level, which was 4.02 copies HIV-1 DNA per million CD4 T cells.
HIV-1 DNA and neurocognitive disorders

(a) HIV DNA of all individuals (n = 49) with normal cognition (NC) and HIV associated dementia (HAD). (b) Similar plot of HIV DNA of individuals with undetectable plasma viral levels (n = 24). Both plots demonstrate the skewed distribution of HIV DNA while showing the significant difference between the medians when comparing individuals with NC to those with HAD.
15 patients with CRF01_AE HIV with HAD and 15 without HAD

HIV RNA and CD4 counts not predictive of HAD

Baseline and longitudinal HIV DNA from monocytes correlated to cognitive performance irrespective of plasma HIV RNA and CD4 counts pre-HAART (P<0.001) and at 48 weeks post HAART (P<0.001)

Levels exceeding 3.5 log10 copies HIV DNA/10^6 monocyte at baseline distinguished all HAD and non-HAD cases (P<0.001)
### Predictors of VL rebound in MONO1 at week 96 (DRV monotherapy arm)

<table>
<thead>
<tr>
<th>Variables Associated with Rebound</th>
<th>Univariate Analysis</th>
<th>Multivariate Analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>OR (95%CI)</td>
<td>P</td>
</tr>
<tr>
<td>Difficulty in Adherence (&lt; 100% vs. 100% adherence)</td>
<td>2.36 (0.94, 5.92)</td>
<td>0.07</td>
</tr>
<tr>
<td>Duration of prior ART (per 5 years decrease)</td>
<td>2.38 (1.30, 4.38)</td>
<td>0.003</td>
</tr>
<tr>
<td>Baseline US HIV-1 RNA ( &lt;1 copy/mL vs. Others)</td>
<td>0.41 (0.16, 1.05)</td>
<td>0.06</td>
</tr>
<tr>
<td>HIV-1 DNA at D0 (per 1 log_{10} copies/10^6 cells increase)</td>
<td>2.45 (1.07, 5.61)</td>
<td>0.03</td>
</tr>
<tr>
<td>HIV-1 RNA at D0 (Blip vs. &lt;50 copies/mL)</td>
<td>4.05 (0.76, 21.5)</td>
<td>0.11</td>
</tr>
</tbody>
</table>
SPARTAC (Short Pulse Anti Retroviral Therapy at HIV Seroconversion)

HIV-1 DNA at ART interruption predicts time to viral rebound. Survival analyses of time to viral rebound (weeks) in participants undertaking TI after 48 weeks of ART. HIV-1 DNA levels are presented divided at the median level into high (red) and low (black).
HIV-1 DNA on ART (48 wks) predicts clinical progression (CD4 < 350) following treatment interruption. HIV-1 DNA data was divided into ‘high’ and ‘low’ at the median. DNA levels were measured at week 48, at the point of stopping ART.
TRIO Study: Combination of DRV/r, ETR and RAL in Heavily-Experienced Patients

**Combination of DRV/r, ETR and RAL ± NRTIs or ENF in Tx-Experienced Pts**

**Baseline Characteristics (n=103)**

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Median (IQR)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HIV RNA, log_{10}, copies/ml</td>
<td>4.0 (3.6 – 4.6)</td>
</tr>
<tr>
<td>CD4, cells/mm³</td>
<td>255 (132 – 350)</td>
</tr>
<tr>
<td>CD4 Nadir, cells/mm³</td>
<td>79 (25– 169)</td>
</tr>
<tr>
<td>Years ART prior to enrollment</td>
<td>13 (11 – 15)</td>
</tr>
<tr>
<td># mutations at screening</td>
<td></td>
</tr>
<tr>
<td>Major PI</td>
<td>4 (3 – 5)</td>
</tr>
<tr>
<td>NRTIs</td>
<td>5 (4 – 6)</td>
</tr>
<tr>
<td>NNRTIs</td>
<td>1 (0 – 2)</td>
</tr>
</tbody>
</table>

**Additional ARVs (OBR)**

<table>
<thead>
<tr>
<th>ARV</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>NRTIs</td>
<td>83%</td>
</tr>
<tr>
<td>Enfuvirtide</td>
<td>12%</td>
</tr>
</tbody>
</table>

**Virologic Response (ITT, M=F)**

- 90% (95% CI: 85% to 96%)
- 86% (95% CI: 85% to 96%)

**Conclusion:** High rate of suppression; unclear role of OBR given

Impact of baseline HIV DNA levels on response to salvage therapy (ANRS 139 - TRIO Study)

HIV-1 DNA level (log_{10} copies/10^6 PBMC) against Time (Weeks)

- Patients in virological failure (n = 11)
- Patients with viral blip (n = 20)
- Patients in virological success (n = 61)

P = 0.06

Charpentier, PLoS ONE 2013
Total HIV-1 DNA, a Marker of Viral Reservoir Dynamics with Clinical Implications

Véronique Avettand-Fenoël, Laurent Hocqueloux, Jade Ghosn, Antoine Cheret, Pierre Frange, Adeline Melard, Jean-Paul Viard, Christine Rouzioux

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FIG 3 Spectrum of total HIV-1 DNA levels in PBMC during HIV infection. The natural history data are from HIV DNA quantified for 552 adults at the time of the primary infection (PRIMO cohort, ANRS) (81), for 271 patients who had seroconverted 6 to 24 months previously (SEROCCO cohort, ANRS) (84), and for 121 perinatally infected children (median age, 6 years), of whom 46.6% and 20.3% were at CDC stage B and C, respectively (ANRS 1244/1278) (94). The data set for “during antiretroviral therapy” is for HIV DNA quantified during antiretroviral therapy initiated during the primary infection and continued for 2 years (90 patients; OPTIPRIM trial, ANRS) (109) or for a median of 3.6 years, with HIV RNA levels of <50 copies/ml for a median of 3.1 years (n = 35) (123). HIV DNA was quantified during antiretroviral therapy initiated early during the chronic phase in 116 adults (CD4 cell count, >350/mm³; plasma HIV RNA, <4.7 log copies/ml), with antiretroviral therapy for a median of 5.3 years (SALTO trial, ANRS) (220) and in 272 adults treated later (median CD4 cell nadir, 228/mm³; plasma HIV RNA, 5.3 log copies/ml) for a median of 7.3 years, with HIV RNA levels at <50 copies/ml for a median of 3.9 years (123). HIV DNA was quantified in 44 adults with advanced therapeutic failure and AIDS (CD4 count, ≤200/mm³; HIV RNA level, >4 log; genotypic score showing two or fewer active drugs) (ETOILE trial, ANRS) (197). For the HIV control data set, HIV DNA were quantified for patients who controlled the infection naturally (plasma HIV RNA undetectable for >10 years in the absence of antiretroviral treatment [15 patients]) (HIV controllers cohort, ANRS) (89) or after treatment interruption (14 patients) (VISCONTI study, ANRS) (92). The same standardized assay was used in the same laboratory. A broad range of HIV DNA levels was found. Medians and interquartile ranges are indicated.
How to use HIV DNA quantification?
*Personal view*

- **HIV diagnosis**
  - Risk of progression

- **First virosuppression**
  - How much HIV DNA decayed

- **Lower drug pressure?**
  - Good or bad candidate at what cutoff?

- **Follow-up**
  - Did lower drug pressure increase HIV DNA?
How to use HIV DNA quantification?

**Personal view**

- **HIV diagnosis**
  - Risk of progression
  - e.g. <2000

- **First virosuppression**
  - How much HIV DNA decayed
  - e.g. >10-fold

- **Lower drug pressure?**
  - Good or bad candidate at what cutoff?
  - e.g. <200

- **Follow-up**
  - Did lower drug pressure increase HIV DNA?

Figures to be integrated to have an ideal candidate to lower drug pressure (values expressed as copies/million CD4)
Quantification of HIV DNA

Basics

Why to test

Caveats

Are we ready to test
Total HIV DNA is associated with other easier-to-measure parameters in patients under successful therapy

- **Pre-therapy plasma HIV RNA**

- **Residual viremia, even when simply classified as detectable vs. undetectable**

- **Nadir CD4 counts**
  - Watanabe, BMCID 2011; Lambert-Niclot, PLoS ONE 2012

- **Duration of suppression of plasma HIV RNA**
  - Watanabe, BMCID 2011

- **Earlier treatment start**
  - Hocqueloux, JAC 2013
The various forms of HIV DNA

Is total HIV DNA a misleading surrogate?

[Diagram showing different forms of HIV DNA, including linear cDNA, auto-integration, recombination, host DNA repair, integration, truncated autointegrand, 1-LTR circle, 2-LTR circle, and integrated proviral DNA.]

- Best surrogate for HIV reservoir Marker in eradication studies
- Marker of new infection cycles? Useful in eradication studies?
Status of HIV DNA

Is total HIV DNA a misleading surrogate?
Integrated and Total HIV-1 DNA Predict Ex Vivo Viral Outgrowth

Integrated HIV DNA and total HIV DNA are associated with infectious units per million CD4 T cells

P = 0.041

P = 0.039
Quantification of HIV DNA

Basics

Why to test

Caveats

Are we ready to test
Quantification of HIV DNA Assays

- **Homebrew technology** available
  - Many different methods developed in different years

- **Commercial assays** developed by small companies available
  - DIATHEVA, Italy
  - Biocentric, France

- **Commercial assays** under development by large companies as an adaptation of HIV RNA assays
  - Just omit the RT step and go...
Homebrew quantitative HIV DNA assay

Current protocol at the University of Siena

Certified HIV DNA standards

Certified human DNA standards

Normalize HIV DNA amount per DNA input and express result as:

- Copies/10^6 WBC
- Copies/10^6 PBMC
- Copies/10^6 CD4 cells
Potential issues (and aberrant results) in homebrew quantification of HIV DNA

- DNA input based on spectrophotometric measurement
  - Error-prone for several reasons, better to use an internal quantitation control

- Maximum DNA allowed in the reaction
  - Dependent on master mix

- Recovery of unintegrated HIV DNA
  - Dependent on extraction method

- Accuracy and precision
  - Test thoroughly with international standards and dilution series

- Careful choice of standards
  - Plasmid or cell lines, be ready to challenge previous knowledge
Effect of culture passage on HIV DNA content per cell for 8E5 Standard 3 measured using the RainDrop® dPCR platform. Mean values with standard deviations are plotted.
Pitfalls in using latently HI-infected cell lines as a source for HIV DNA standards

Change in frequency of HIV integration sites following passaging of cell lines infected with replication-competent HIV.

The frequency of unique integration sites per 150,000 cells is shown following passaging of the U1 (green), ACH2 (blue) and J1.1 (red) cell lines. Linear regression was used to determine if the change was statistically significant.
HIV DNA at lower limit of detection

Rare objects fluctuate...

Poisson distribution describes the probability to detect an object (HIV DNA target) in a volume (specimen) at any given concentration of the object in that volume.

Below a certain threshold of concentration, replicate analysis is required to obtain an acceptable estimate of the object concentration.
Digital PCR helps improve accuracy of quantitation of low-copy targets

1. **Partitioning.** The PCR reaction mixture is partitioned into 20,000 water in oil droplets with target and background DNA randomly distributed among the reactions.

2. **Amplification.** Target DNA is amplified by PCR using standard thermal cycling.

3. **Detection.** Each reaction provides a fluorescent positive or negative signal indicating the target DNA was present or absent after partitioning. The fraction of positive droplets is used to calculate the target DNA concentration.

- Reaction volume partitioned (dispersed phase of an emulsion or arrays of miniaturized chambers)
- Fraction of positive and negative reactions analysed via Poisson distribution
- Accuracy and precision increased up to 10-fold with respect to RealTime PCR at low target copy numbers (i.e. total HIV DNA or 2-LTR circles in patients with prolonged suppression of plasma HIV RNA)
The digital PCR concept applied to HIV DNA quantification in 1990...

Human Immunodeficiency Virus-Infected Individuals Contain Provirus in Small Numbers of Peripheral Mononuclear Cells and at Low Copy Numbers

PETER SIMMONDS,¹ PETER BALFE,¹ JOHN F. PEUTHERER,² CHRISTOPHER A. LUDLAM,³ JOHN O. BISHOP,¹* AND ANDREW J. LEIGH BROWN¹

Department of Genetics, University of Edinburgh, Kings Buildings, Edinburgh EH9 3JN,¹ Department of Bacteriology, University of Edinburgh, Teviot Place, Edinburgh EH3 9AG,² and Department of Haematology, Royal Infirmary, Edinburgh EH3 9YW,³ Scotland

- Single-copy sensitivity
- Limiting dilution
- Poisson statistics
HIV DNA for drug resistance testing

Basics

Why to test

Caveats

Are we ready to test
Kinetics of drug resistant mutants in plasma HIV RNA and cellular HIV DNA

Different HIV RNA species detected at different times depending on drug pressure and resistance & fitness levels.

Different HIV DNA species present at different levels depending on fitness, duration as prevalent species, time elapsed since peaking.

Viral load

Time

Ongoing viremia
Population sequencing detects resistance mutations in *RNA* but not in *DNA* at this time.
Kinetics of drug resistance mutations in plasma RNA vs. PBMC DNA Following drug removal

Population sequencing detects resistance mutations in DNA but not in RNA at this time
Kinetics of drug resistance mutations in plasma RNA vs. PBMC DNA

**OFF vs. ON therapy**

A. 58 patients failing their first ART while still on therapy

- More drug resistance in **plasma RNA** than in PBMC DNA in the **on-therapy** group (P=0.004)

B. 50 patients after a median of 18.6 weeks after treatment interruption following multiple treatment failures

- More drug resistance in **PBMC DNA** than in plasma RNA in the **off-therapy** group (P=0.04)

Venturi, Antivir Ther 2002
Use of HIV DNA rather than HIV RNA for drug resistance genotyping in drug-naïve patients?

<table>
<thead>
<tr>
<th>Study*</th>
<th>Patients</th>
<th>No. DRM in RNA</th>
<th>No. DRM in DNA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lubke 2015</td>
<td>48</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>Steegen 2007</td>
<td>10</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Vicenti 2007</td>
<td>169</td>
<td>58</td>
<td>62</td>
</tr>
<tr>
<td>Parisi 2007</td>
<td>288</td>
<td>108</td>
<td>131</td>
</tr>
<tr>
<td>Bon 2007</td>
<td>29</td>
<td>16</td>
<td>27</td>
</tr>
<tr>
<td>Chew 2005</td>
<td>11</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>555</strong></td>
<td><strong>188</strong></td>
<td><strong>230</strong></td>
</tr>
</tbody>
</table>

- More DRMs consistently detected in DNA than in RNA
- However, cases with resistance detected only in plasma RNA or only in PBMC DNA were documented
- Potential predictors of DNA vs. RNA discrepancy not investigated but likely include time from diagnosis, viral load, type of mutation
- PBMC DNA could better integrate, rather than replace, plasma RNA testing in drug-naïve patients

*Reference drug resistance mutation lists subject to changes over time
HIV DNA for drug resistance testing

- Basics
- Why to test
- Caveats
- Are we ready to test
Potential benefits of HIV DNA resistance testing in patients with suppressed plasma HIV RNA

- Can I see complete drug resistance history?
- Have undetected drug resistance mutations actually vanished?
Genotypic resistance test in proviral DNA can identify resistance mutations never detected in historical genotypic test in patients with low level or undetectable HIV-RNA.

Proportion of Patients with major DRM in PBMCs and Cumulative Plasma (149 Patients with DNA GRT and ≥2 Plasma GRTs)

- Mutations detected only in cumulative plasma
- Mutations detected in PBMCs and cumulative plasma
- Mutations detected only in PBMCs

Resistance prevalence (%)

- NRTI: 50.3, 38.3, 11.4
- NNRTI: 43, 28.2, 10.1
- PI: 30.2, 16.8, 1.3
- INSTI: 11.1, 11.1, 0.3
- Overall (PI/NNRTI/NRTI): 61.1, 51, 20.1

Zaccarelli, J Clin Virol 2016
Genotypic resistance test in proviral DNA can identify resistance mutations never detected in historical genotypic test in patients with low level or undetectable HIV-RNA

Table 2
Factors associated with detection of resistance in PBMCs.

<table>
<thead>
<tr>
<th>Variables</th>
<th>OR(^a) (95% C.I.)</th>
<th>P value</th>
<th>AOR(^b) (95% C.I.)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (by year)</td>
<td>1.03 (1.01–1.05)</td>
<td>0.005</td>
<td>1.01 (0.99–1.04)</td>
<td>0.337</td>
</tr>
<tr>
<td>Gender (female vs. male)</td>
<td>0.72 (0.48–1.08)</td>
<td>0.111</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IDU as HIV Risk Factor</td>
<td><strong>1.32 (1.05–1.67)</strong></td>
<td><strong>0.016</strong></td>
<td><strong>1.36 (1.06–1.75)</strong></td>
<td><strong>0.016</strong></td>
</tr>
<tr>
<td>HIV-RNA &lt; 50 copies/mL at PBMC genotyping</td>
<td>0.91 (0.62–1.33)</td>
<td>0.613</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CD4 count at PBMC genotyping (per 50 cell/mm(^3) increase)</td>
<td><strong>0.96 (0.93–0.99)</strong></td>
<td><strong>0.012</strong></td>
<td><strong>0.96 (0.92–0.99)</strong></td>
<td><strong>0.033</strong></td>
</tr>
<tr>
<td>CD4 count nadir (per 50 cell/mm(^3) increase)</td>
<td>0.96 (0.91–1.00)</td>
<td>0.070</td>
<td>1.04 (0.98–1.11)</td>
<td>0.203</td>
</tr>
<tr>
<td>No. of previous regimens (each)</td>
<td><strong>1.18 (1.12–1.24)</strong></td>
<td>&lt;0.001</td>
<td><strong>1.18 (1.04–1.33)</strong></td>
<td><strong>0.008</strong></td>
</tr>
<tr>
<td>Years of cART exposure (each)</td>
<td>1.05 (1.02–1.09)</td>
<td>&lt;0.001</td>
<td>0.99 (0.97–1.03)</td>
<td>0.958</td>
</tr>
<tr>
<td>No. of drugs used (each)</td>
<td><strong>1.18 (1.12–1.24)</strong></td>
<td>&lt;0.001</td>
<td><strong>1.00 (0.87–1.14)</strong></td>
<td>0.950</td>
</tr>
</tbody>
</table>

AOR: adjusted odd ratio; C.I.: Confidence interval; cART: combined antiretroviral therapy; IDU: injection drug user; OR: odd ratio.

\(^a\) Univariable logistic regression.

\(^b\) Multivariable logistic regression. Variables with a p value < 0.1 at univariable analysis were retained in multivariable model. In bold factors significantly associated to resistance detection by uni-multi variable logistic regression.
HIV DNA for drug resistance testing

Basics

Why to test

Caveats

Are we ready to test
RT: 134 current DNA compared with 443 past RNA

PR: 141 DNA compared with 462 past RNA

Mutations detected more in past RNA genotypes than in current DNA genotype

DNA has more mixtures with respect to RNA

Historical HIV-RNA resistance test results are more informative than proviral DNA genotyping in cases of suppressed or residual viraemia
169 patients from the EASIER trial (RAL replacing T20)

Median 4 earlier RNA genotypes vs. 1 DNA genotype

Median RAMs in RNA and DNA:
- 5 and 4 for NRTIs (P < 0.001)
- 2 and 1 for NNRTIs (P < 0.001)
- 10 and 8 for PIs (P < 0.001)

Resistance exclusively in RNA or in DNA:
- 63% and 13% NRTI
- 47% and 1% NNRTI
- 50% and 7% PI

Historical HIV-RNA resistance test results are more informative than proviral DNA genotyping in cases of suppressed or residual viraemia.
Usefulness of an HIV DNA resistance genotypic test in patients who are candidates for a switch to the TDF/FTC/RPV combination

- 130 patients without previous VF and 114 patients with at least one previous VF
- DNA genotype after >=1 yr virosuppression compared with latest RNA genotype
- At least one mutation for TDF or FTC or RPV
  - Comparable in non-VF, 8% RNA vs. 9% DNA
  - Different in VF, 60% RNA vs. 45% DNA

Lambert-Niclot, JAC 2016
Dynamics of INSTI resistance mutations

Four heavily treatment-experienced subjects monitored over a median of 1.2 years since the initiation of raltegravir-containing regimens

<table>
<thead>
<tr>
<th>Patient</th>
<th>INI DRM</th>
<th>Days on RAL</th>
<th>% in RNA</th>
<th>% in DNA</th>
</tr>
</thead>
<tbody>
<tr>
<td>3180</td>
<td>Q148H/G140S</td>
<td>177</td>
<td>100</td>
<td>1</td>
</tr>
<tr>
<td>3242</td>
<td>N155H</td>
<td>224</td>
<td>100</td>
<td>36</td>
</tr>
<tr>
<td>3501</td>
<td>Q148H/G140S</td>
<td>338</td>
<td>78</td>
<td>11</td>
</tr>
<tr>
<td>3508</td>
<td>Y143R</td>
<td>197</td>
<td>100</td>
<td>0</td>
</tr>
</tbody>
</table>

Lee, PLoS ONE 2012
Dynamics of PI resistance mutations

[Graph showing changes in CD4 cells, plasma RNA, PBMC DNA, and PBMC RNA over time (in months) with various symbols representing medications such as Zidovudine, Didanosine, Lamivudine, Stavudine, Ritonavir, Saquinavir, Nelfinavir, and Indinavir.]
Dynamics of PI resistance mutations

![Graph showing dynamics of PI resistance mutations with CD4 cells/mm³, Log HIV-1 DNA/10⁶ CD4, Log HIV-1 RNA/ml, and CD4 plasma RNA, PBMC DNA, PBMC RNA, PLASMA RNA, PBMC DNA, and PBMC RNA. The graph includes lines for Wild type and Resistant.](image-url)
Dynamics of PI resistance mutations

Emergence of Protease Inhibitor Resistance–Associated Mutations in Plasma HIV-1 Precedes That in Proviruses of Peripheral Blood Mononuclear Cells by More Than a Year

Xiuqiong Bi, Hiroyuki Gatanaga, Setsuko Ida, Kiyoto Tsuchiya, Saori Matsuoka-Aizawa, Satoshi Kimura, and Shinichi Oka

- 275 plasma RNA and 211 PBMC DNA HIV-1 PR sequences from 22 patients on PI therapy
- Plasma viruses had more PI RAMs than PBMC proviruses (P = 0.0004)
- PI RAMs detected about 425 days earlier than PBMC RAMs when VL <10^4 copies/mL
PBMC DNA HIV genotype vs. inducible HIV RNA genotype

Incidental observation during preliminary latency reversal experiments (unpublished)

5 ml of blood

Single culture with HIV inducers

HIV RNA detection/sequencing in supernatant

Wk 1  Wk 2  Wk 3  Wk 4
## HIV RNA detection *in vitro* following HIV induction

<table>
<thead>
<tr>
<th>Patient (months undetectable HIV RNA)</th>
<th>PBMC HIV DNA at baseline</th>
<th>Supernatant HIV RNA at week 1</th>
<th>Supernatant HIV RNA at week 2</th>
<th>Supernatant HIV RNA at week 3</th>
<th>Supernatant HIV RNA at week 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>SI-9081 (5)</td>
<td>Positive</td>
<td>Positive</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>SI-338 (24)</td>
<td>Positive</td>
<td>Positive</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>SI-364 (12)</td>
<td>Positive</td>
<td>Negative</td>
<td>Positive</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>SI-5157 (69)</td>
<td>Positive</td>
<td>Negative</td>
<td>Negative</td>
<td>Negative</td>
<td>Negative</td>
</tr>
<tr>
<td>SI-3389 (59)</td>
<td>Positive</td>
<td>Positive</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>SI-9850 (33)</td>
<td>Positive</td>
<td>Negative</td>
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<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>SI-9991 (8)</td>
<td>Positive</td>
<td>Negative</td>
<td>Negative</td>
<td>Negative</td>
<td>Negative</td>
</tr>
<tr>
<td>SI-3682 (21)</td>
<td>Positive</td>
<td>Negative</td>
<td>Positive</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>SI-665 (24)</td>
<td>Positive</td>
<td>Negative</td>
<td>Positive</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>SI-10161 (41)</td>
<td>Positive</td>
<td>Negative</td>
<td>Positive</td>
<td>ND</td>
<td>ND</td>
</tr>
</tbody>
</table>
**HIV drug resistance in HIV DNA before induction and HIV RNA following HIV induction**

Same virus but in 4/8 cases different drug resistance mutations in supernatant RNA compared to starting PBMC DNA

<table>
<thead>
<tr>
<th>Patient ID</th>
<th>PBMC DNA</th>
<th>Supernatant RNA</th>
</tr>
</thead>
<tbody>
<tr>
<td>SI-9081</td>
<td>PR: none</td>
<td>PR: none</td>
</tr>
<tr>
<td></td>
<td>RT: V106I</td>
<td>RT: none</td>
</tr>
<tr>
<td>SI-338</td>
<td>PR: none</td>
<td>PR: none</td>
</tr>
<tr>
<td></td>
<td>RT: M41LM, T215NSTY</td>
<td>RT: none</td>
</tr>
<tr>
<td>SI-364</td>
<td>PR: none</td>
<td>PR: none</td>
</tr>
<tr>
<td>SI-9991</td>
<td>PR: none</td>
<td>PR: none</td>
</tr>
<tr>
<td></td>
<td>RT: none</td>
<td>RT: V108I</td>
</tr>
</tbody>
</table>
Phylogenetic analysis confirms high relatedness between HIV DNA before induction and HIV RNA following induction

- Same patient virus when "baseline" DNA vs. "induced" RNA DRMs differ
- Extra DNA DRMs compatible with previous RNA DRMs and/or drug exposure
- Is HIV DNA testing misleading in patients with prolonged control of HIV?
- Further studies of this model warranted

Red patient ID, sequencing of matched sample pending
Potential benefits of HIV DNA resistance testing in patients with suppressed plasma HIV RNA

- Past signatures washed out by time yet still present and selectable
- Stochastic pick up of a non-functional quasispecies
| Pretreated patient with unknown treatment history | • Increased detection of DRMs in addition to HIV RNA DR testing |
| Drug-naive patient (especially if old infection) | • Increased detection of DRMs in addition to HIV RNA DR testing? |
| Virosuppressed patient with previous failures or LLV patient considered for switching to another therapy | • Negative finding not to be trusted completely! |
| HTE patient with MDR virus | • Checking what mutants do not appear in DNA (less pathogenic?) |
HIV DNA for drug resistance testing

Basics

Why to test

Caveats

Are we ready to test
HIV DNA for drug resistance testing

Assays

- **Homebrew assays**
  - No problem, just extract DNA and go with PCR

- **Commercial systems** (only Viroseq remains)
  - Not directly feasible (but workaround possible)

- **Sequence reading issues**
  - Usually, larger prevalence of mixtures compared to plasma RNA (e.g. ARCA: 2-base degenerations in 23% DNA muts vs. 17% RNA muts; P <0.0001)
  - Sometimes, hypermutated sequences with stop codons (surrogate of excellent treatment response?)
Picking up hypermutated sequences during HIV DNA genotypic resistance testing

Stanford HIVdb report for an RT sequence with evidence of APOBEC induced hypermutation

Sequence Summary

Sequence includes RT: codons 1 - 246
Subtype: B (3.52%)
RT SDRMs: M184I

Sequence Quality Assessment

- **Severe warning:** There are 3 stop codons in RT: W88*, W153*, W229*.
- **Warning:** The following 3 APOBEC muts were present in the sequence: RT: W88*, W153*, W229*. The following 1 DRMs in this sequence could reflect APOBEC activity: RT: M184I.
Picking up hypermutated sequences during HIV DNA genotypic resistance testing

- Very rare occurrence (~1% of 2,238 patients in Siena HIV & Hepatitis Monitoring Laboratory)
  - Do labs store this data?

- Most often involving TGG → TGA/TAG changes (particularly at RT codons 71, 88, 153, 212, 229)
  - Preferential GG dinucleotide target for APOBEC

- Usually not confirmed at second or third run when restarting from DNA extract
  - Suggests stochastic pick up of non-homogeneous population

- Factors associated with detection and significance still obscure
  - Surrogate of excellent treatment response?
Clinical use of HIV-DNA quantity and resistance testing