HIV RESISTANCE AND TREATMENT FAILURE

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Outline

• Why does HIV develop (so much) resistance?
• What is treatment failure?
• Reasons for virological failure
• Diagnosing virological failure
• Understanding HIV resistance testing
WHY DOES HIV DEVELOP (SO MUCH) RESISTANCE?
Just how much mutation is going on here?

Given in vivo replication kinetics with more than $10^8$ new cells infected every day, each and every mutation occurs between $10^4$ and $10^5$ times per day in an [untreated] HIV-infected individual.

Therefore, most drug resistance mutations are probably present before the start of therapy. However, naturally occurring mutations to multiple drugs are not thought to be present in previously untreated individuals with wild-type virus. This provides the basis for combination ART.
All organisms’ genetic material changes

Causes are:
- Mutation
- Recombination

Mutations can be caused by:
- **Extrinsic factors** — UV light, chemicals, cellular enzymes, etc.
- **Intrinsic factors** — errors during replication ± recombination

Source: Mayo Clinic
Mutation rate varies by organism type
Mutations vary by virus type

This corresponds roughly to the error rates of the various viral enzymes responsible for copying the genetic material (the polymerases):

- RNA viruses (which utilize RNA polymerases) mutate faster than retroviruses (which utilize reverse transcriptases), which mutate faster than DNA viruses (which utilize DNA polymerases)
Why does HIV develop so much resistance?

- **Reason 1**: it is a **retrovirus**, utilizing a copying method (*reverse transcriptase*) that is intrinsically more prone to errors than most other copying mechanisms.

  Roughly 3 errors for every 100,000 nucleotides copied
  (error rate: $3 \times 10^{-5}$/ nucleotide base per replication cycle)

  This translates to roughly 0.2 errors per genome replicated

There are also subsequent mutations during transcription from DNA to RNA again
HIV recombination

There are **two** copies of HIV’s genome in each virion.

The RT switches between them to generate DNA that’s a mixture of the two copies.

If a cell is infected with two different strains of HIV, this “recombination” can generate new strains of HIV within the cell.
HIV recombination

"If an infected cell simultaneously harbors two different proviruses, one RNA transcript from each provirus can be encapsidated into a single “heterozygous” virion."

Source: iopscience.iop.org
HIV recombination

The reverse transcriptase, while transcribing RNA into proviral DNA, can fall off its current RNA template and reattach to the other. If the infecting virion carries two distinct genomic RNA strands, then the process of template switching may lead to the production of a recombinant provirus.
Why does HIV develop so much resistance?

- **Reason 2:** In addition to mutating so fast, the viral RNA can change due to recombination.

HIV undergoes approximately 2-3 recombination events during the replication cycle.
HIV Replication Rate

HIV generates between 10 billion and 1 trillion new virions each day.
Why does HIV develop so much resistance?

- **Reason 3**: HIV has an extremely high replication rate.

  The more replication that occurs, the more chance there will be for errors to be made (i.e. for mutations to develop) and for recombination to occur.
Archived mutations

- There is a pool of cells that are infected with HIV, but are largely dormant.
  - Best described are memory CD4$^+$ T-cells, but other types are thought to exist (e.g. monocytes)
  - Anatomical reservoirs are also thought to play a role (lymph nodes, brain, genital tract, lungs, etc).
Archived mutations

• The dormancy is one reason why curing HIV is so hard (the drugs can’t target viruses that aren’t replicating).

• HIV integrated into cells there can be periodically released back into the circulation.
  • This is why the viral load rebounds quickly after stopping therapy.

• Numerous HIV strains from the blood – past and present – are “kept” by the reservoirs
Why does HIV develop so much resistance?

• **Reason 4**: Past mutations are *archived*, and thus an expanding pool of mutated viruses accumulates.

> “The latent reservoir is an archive, composed of a mixture of wild-type and drug-resistant strains. Archived variants are assumed to remain life-long, thereby precluding the successful recycling of any drug towards which resistance has arisen.”

*Turriziani et al.*  Resistant HIV variants in cellular reservoirs  
*Clinical Microbiology and Infection*, Volume 16 Number 10, October 2010
Just how much mutation is going on here?

Given in vivo replication kinetics with more than $10^8$ new cells infected every day, each and every mutation occurs between $10^4$ and $10^5$ times per day in an [untreated] HIV-infected individual.

Therefore, most drug resistance mutations are probably present before the start of therapy. However, naturally occurring mutations to multiple drugs are not thought to be present in previously untreated individuals with wild-type virus. This provides the basis for combination ART.

John M. Coffin.  
*HIV Population Dynamics in Vivo: Implications for Genetic Variation, Pathogenesis, and Therapy*  
Science. 1995 Jan;267(5197):483-9
WHAT IS TREATMENT FAILURE?

Definitions
## Treatment failure

### WHO definitions

<table>
<thead>
<tr>
<th>Clinical failure</th>
<th>Adults and adolescents</th>
<th>Children</th>
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<tbody>
<tr>
<td></td>
<td>New or recurrent clinical event indicating severe immunodeficiency (WHO clinical stage 4 condition)(^\text{a}) after 6 months of effective treatment</td>
<td>New or recurrent clinical event indicating advanced or severe immunodeficiency (WHO clinical stage 3 and 4 clinical condition with exception of TB) after 6 months of effective treatment</td>
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\(^\text{a}\) Indicates patients for whom ART is indicated.

\(^\text{b}\) Indicates patients for whom ART is not indicated.
# Treatment failure

<table>
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<tr>
<th>Immunological failure</th>
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<tr>
<td><strong>Adults and adolescents</strong></td>
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<tr>
<td>CD4 count falls to the baseline (or below)</td>
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<tr>
<td>or</td>
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<tr>
<td>Persistent CD4 levels below 100 cells/mm$^3$</td>
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<tr>
<td><strong>Children</strong></td>
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<tr>
<td>Younger than 5 years</td>
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<tr>
<td>Persistent CD4 levels below 200 cells/mm$^3$ or $&lt;10%$</td>
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<tr>
<td>Older than 5 years</td>
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<tr>
<td>Persistent CD4 levels below 100 cells/mm$^3$</td>
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Without concomitant or recent infection to cause a transient decline in the CD4 cell count

A systematic review found that current WHO clinical and immunological criteria have low sensitivity and positive predictive value for identifying individuals with virological failure (182). The predicted value would be expected to be even lower with earlier ART initiation and treatment failure at higher CD4 cell counts. There is currently no proposed alternative definition of treatment failure and no validated alternative definition of immunological failure.
Treatment failure

Virological failure

Plasma viral load above 1000 copies/ml based on two consecutive viral load measurements after 3 months, with adherence support

The optimal threshold for defining virological failure and the need for switching ART regimen has not been determined

An individual must be taking ART for at least 6 months before it can be determined that a regimen has failed

Assessment of viral load using DBS and point-of-care technologies should use a higher threshold
How good are the clinical and immunological criteria?

- Not as good as viral load:

**Plasma Viral Load and CD4⁺ Lymphocytes as Prognostic Markers of HIV-1 Infection**

John W. Mellors, MD; Alvaro Muñoz, PhD; Janis V. Giorgi, PhD; Joseph B. Margolick, MD, PhD; Charles J. Tassoni, PhD; Phalgungi Gupta, PhD; Lawrence A. Kingsley, DrPH; John A. Todd, PhD; Alfred J. Saah, MD; Roger Detels, MD; John P. Phair, MD; and Charles R. Rinaldo Jr., PhD


**Results:** Plasma viral load was the single best predictor of progression to AIDS and death, followed (in order of predictive strength) by CD4⁺ lymphocyte count and serum neopterin levels, serum β₂-microglobulin levels, and thrush or fever. Plasma viral load discriminated risk at all levels of CD4⁺ lymphocyte counts and predicted their subsequent rate of decline.
How good are the clinical and immunological criteria?

Methods. We assessed the performance of CD4 cell criteria to predict virologic outcomes in a large ART program in Nigeria. Laboratory monitoring consists of CD4 cell count and VL at baseline, then every 6 months. Failure was defined as 2 consecutive VLs >1000 copies/mL after at least 6 months of ART. Virologic outcomes were compared with the 3 WHO-defined immunologic failure criteria.

Results. A total of 9690 patients were included in the analysis (median follow-up, 33.2 months). A total of 1225 patients experienced failure by both immunologic and virologic criteria, 872 by virologic criteria only, and 1897 by immunologic criteria only. The sensitivity of CD4 cell criteria to detect viral failure was 58%, specificity was 75%, and the positive-predictive value was 39%. For patients with both virologic and immunologic failure, VL criteria identified failure significantly earlier than CD4 cell criteria (median: 10.4 vs 15.6 months; P < .0001).

Conclusions. Because of the low sensitivity of immunologic criteria, a substantial number of failures are missed, potentially resulting in accumulation of resistance mutations. In addition, specificity and predictive values are low, which may result in large numbers of unnecessary ART switches. Monitoring solely by immunologic criteria may result in increased costs because of excess switches to more expensive ART and development of drug-resistant virus.
Virological failure: what level?

- The goal of ART is to suppress viral replication. ANY detectable viral load therefore potentially represents viral failure.

**Viral blips** are transient and low-level increases in the viral load in a patient who had previously demonstrated viral suppression.

- Typically between 50-200 copies/mL. Never more than 1000.
- Can represent laboratory error, intermittent poor adherence, or transient bursts of HIV replication
- Not usually associated with subsequent virological failure.
Virological failure: what level?

<table>
<thead>
<tr>
<th>Organisation</th>
<th>Virological failure threshold</th>
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<tbody>
<tr>
<td>US Department of Health and Human Services</td>
<td>200 copies/mL</td>
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<tr>
<td>European AIDS Clinical Society</td>
<td>Any detectable viral load</td>
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<tr>
<td>World Health Organization</td>
<td>1000 copies/mL</td>
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<tr>
<td>Southern African HIV Clinicians Society</td>
<td>1000 copies/mL</td>
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<tr>
<td>South African Department of Health</td>
<td>1000 copies/mL</td>
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Note: all guidelines emphasize that a viral load should be repeated after 2-3 months, before virological failure is definitively diagnosed.
Immune non-responders

- Immune non-responders are patients whose CD4 counts fail to rise appropriately on therapy, despite a suppressed viral load – “immunological discordance”.

- Such patients don’t benefit from a change in ART regimen.
  - Prognosis somewhere between those whose CD4 counts do rise appropriately, and those with an equivalent CD4 count but an unsuppressed viral load.

- Consider other causes for a decreased CD4 count:
  - Drugs (e.g. steroids)
  - Infections (e.g. TB)
  - Malignancies (e.g. lymphomas)
REASONS FOR VIROLOGICAL FAILURE
Reasons for Virological Failure

**Patient factors**
- Resistance – transmitted or acquired
- Poverty
- Difficulty with clinic attendance during work hours
- Psychiatric diseases
- Substance abuse

**ART regimen factors**
- Adverse effects
- Food requirements
- High pill burden and/or dosing frequency
- Suboptimal pharmacokinetics
- Prescription errors, stock-outs

Modified from DHHS ART Guidelines (2015)
Transmitted resistance in SA

Primary Drug Resistance in South Africa: Data from 10 Years of Surveys

Prevalence of transmitted drug resistance

Year

2000 2001 2002 2003 2004 2005 2006 2007 2008 2009 2010
What can be done?

- **Monitor adherence**
  - Self-reporting
  - Pharmacy refill checks
- **Notice if visits missed**
- **Simplify regimens**
  - Once-daily regimens
  - Fixed-dose combinations

- **Specialised counselling**
  - Peer support groups may be considered
- **Reminder devices**
  - Pill boxes
  - Cellphone alarm reminders
- **Food supplementation packages**
DIAGNOSING VIROLOGICAL FAILURE
and interpreting HIV resistance tests
When should you check a viral load?

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<tr>
<th></th>
<th>SA Dept. Health</th>
<th>SA HIV Clin. Soc.</th>
<th>DHHS (USA)</th>
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<tbody>
<tr>
<td>At initiation</td>
<td>X</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>Before 6 months</td>
<td>X</td>
<td>✓</td>
<td>At 2-8 weeks, 3 months, then every 4-8 weeks until suppressed</td>
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<tr>
<td>6 months</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
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<tr>
<td>12 months</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>Thereafter</td>
<td>Every 12 months</td>
<td>Every 6-12 months</td>
<td>Every 3-6 months</td>
</tr>
</tbody>
</table>

Why check viral loads before 6 months?
- Enables early detection of virological failure (usually due to poor adherence), before resistance develops, or worsens.
- At 3 months, most patients will be virally suppressed, but a small group of people who started with a very high viral load may still have detectable viraemia… although they’ll still show at least a $2 \log_{10}$ drop from their initiation viral loads.
What should you do if you find a high viral load?

**SA National Department of Health**

- **< 400**: no specific action
- **400-1000**: adherence counselling & repeat VL 6 monthly
- **> 1000**: adherence counselling, repeat VL 2-3 months
  - If repeat < 1000, repeat VL in 6 months
  - If repeat > 1000, switch therapy

**SA HIV Clinicians Society**

- **> 50**: adherence counselling & repeat VL in 2-3 months
- **> 1000** on 2 occasions 2-3 months apart: switch therapy
- **> 200** for more than 1 year: switch therapy
When should you do an HIV resistance test?

SA National Department of Health

• Failing a 2\textsuperscript{nd} line PI-based regimen for at least 1 year

SA HIV Clinicians Society

• If the patient has confirmed virological failure (on any regimen, even 1\textsuperscript{st} line)
  • PI-based regimens: only if on regimen for > 1 year.
Why would you do resistance testing after 1\textsuperscript{st} line failure?

1. Avoids switching regimens purely due to adherence problems (if no significant mutations are found).
2. Identifies which 1\textsuperscript{st} line drugs may be reused (esp. TDF – K65R mutation often not present at time of switch).
3. Guarantees a working 2\textsuperscript{nd} line regimen (e.g. if AZT contraindicated, but patient failing FTC/TDF/EFV).
4. Gives fuller picture of which mutations will be archived than if resistance testing only done after 2\textsuperscript{nd} line failure (some “1\textsuperscript{st} line” mutations may not be identified at this point).
Problems with HIV resistance testing

- **Often unsuccessful if viral load < 1000 copies/mL.**
  - BUT: the inability to get a successful resistance test does not mean you can’t switch therapy if required. Just need expert advice.

- **Only picks up mutations present in >10-20% of the circulating HIV population.**
  - Archived mutations that confer a loss of viral fitness often won’t show up (*minority drug-resistant variants*)
  - Practically, this means that you often only detect the mutations to the drugs the patient is currently on (even if mutations to previous regimens are archived).
Before treatment initiation

Viral load = 250 000

Resistance testing: no mutations detected

W = wild type
On treatment

Viral load < 50

TDF

W = wild type

Resistance testing: unsuccessful
Development of TDF resistance

Viral load = 110 000

W = wild type
K65 R = TDF resistant variant

Resistance testing: TDF resistance
Switch to AZT

The TDF resistance is “archived” at a level too low to be detected by standard HIV resistance testing.

Viral load < 50

W = wild type

K65 R = TDF resistant variant

Resistance testing: unsuccessful
Development of AZT resistance

Viral load = 130 000

- W = wild type
- K65 R = TDF resistant variant
- = AZT resistant variant

Resistance testing:
AZT resistance detected.
No TDF resistance detected
If TDF reintroduced...

- $W = $ wild type
- $K65R = $ TDF resistant variant
- $215Y = $ AZT resistant variant

Viral load = 120 000
How to interpret HIV resistance tests

• Report format varies according to lab.
• If unsure, enter the mutations into the Stanford University HIV drug resistance database program:
  • [http://hivdb.stanford.edu](http://hivdb.stanford.edu) (navigate to HIVdb Program)
How to interpret HIV resistance tests

- Report generated will usually mark each potential antiretroviral medication as:
  - Susceptible, or low-, intermediate- or high-level resistance

<table>
<thead>
<tr>
<th>NRTI Resistance Mutations:</th>
<th>K65R, M184V</th>
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<tr>
<td>NNRTI Resistance Mutations:</td>
<td>K103N</td>
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<tr>
<td>Other Mutations:</td>
<td>None</td>
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<thead>
<tr>
<th>Nucleoside RTI</th>
<th>High-level resistance</th>
<th>Intermediate resistance</th>
<th>High-level resistance</th>
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<tbody>
<tr>
<td>lamivudine (3TC)</td>
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<td>abacavir (ABC)</td>
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<td>zidovudine (AZT)</td>
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<td>stavudine (D4T)</td>
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<td>didanosine (DDI)</td>
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<td>emtricitabine (FTC)</td>
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<td>tenofovir (TDF)</td>
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<th>Non-Nucleoside RTI</th>
<th>High-level resistance</th>
<th>Susceptible</th>
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<tr>
<td>efavirenz (EFV)</td>
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<td>etravirine (ETR)</td>
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<tr>
<td>nevirapine (NVP)</td>
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<tr>
<td>rilpivirine (RPV)</td>
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Interpretation can be hard...!

- Certain drugs can be used despite resistance
  - e.g. 3TC with M184V mutation

- Certain drugs may have hidden “archived” mutations to them – likelihood varies according to ART history and particular mutation.

- Certain drug combinations can’t be used, even if all are susceptible
  - e.g. TDF + ddl (paradoxical drop in CD4 with good viral control)
Resources

• Adult HIV Treatment Failure Discussion Group
  • https://groups.google.com/forum/#!forum/adult-hiv-treatment-failure

• Southern African HIV Clinicians Society: Adult antiretroviral therapy guidelines 2014
  • http://sahivsoc.org/practise-guidelines/sa-hiv-clinicians-society-guidelines

• Stanford University HIV drug resistance database program
  • http://hivdb.stanford.edu