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 10^9 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.**

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

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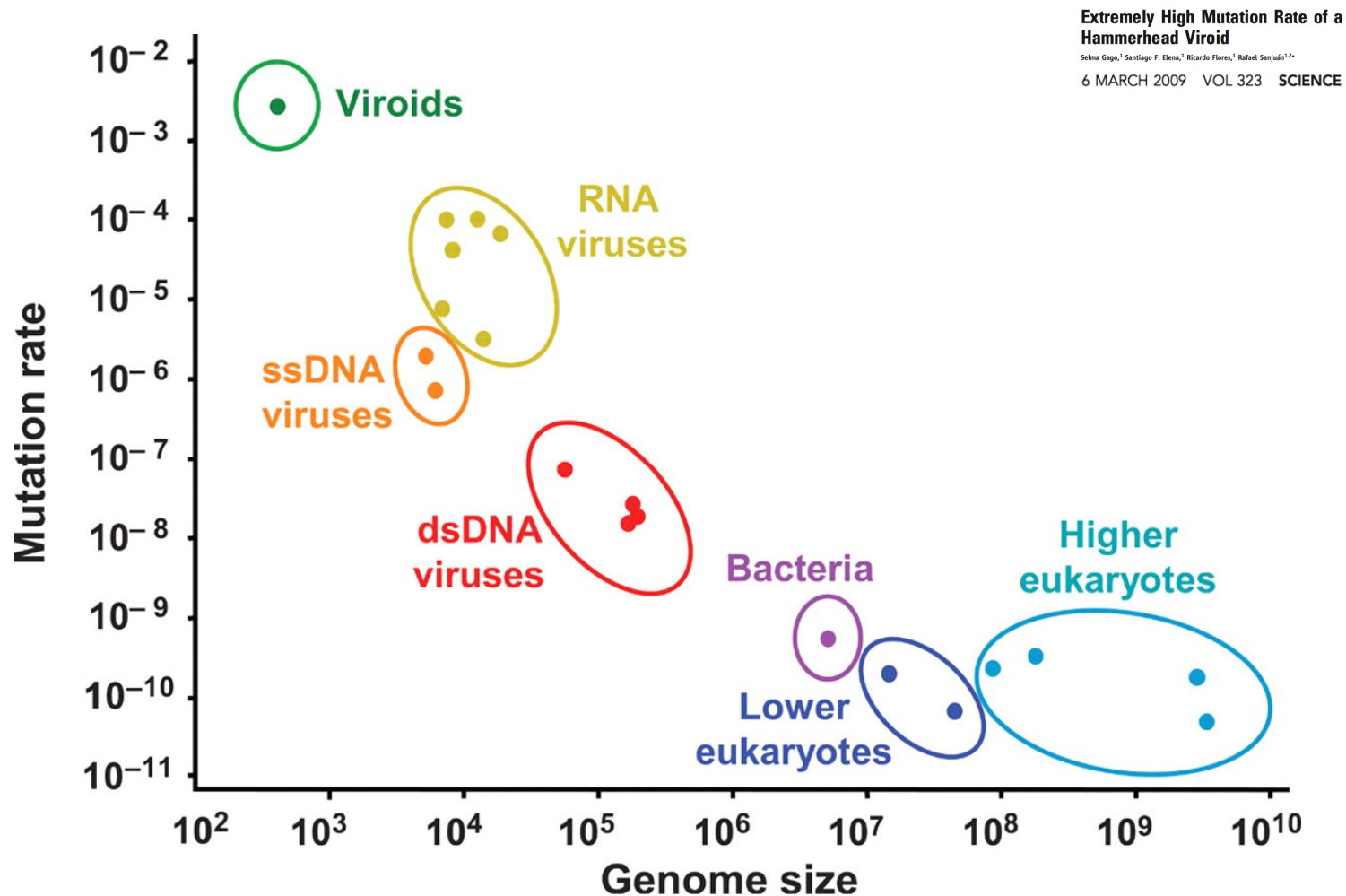
All organisms' genetic material changes

Causes are:
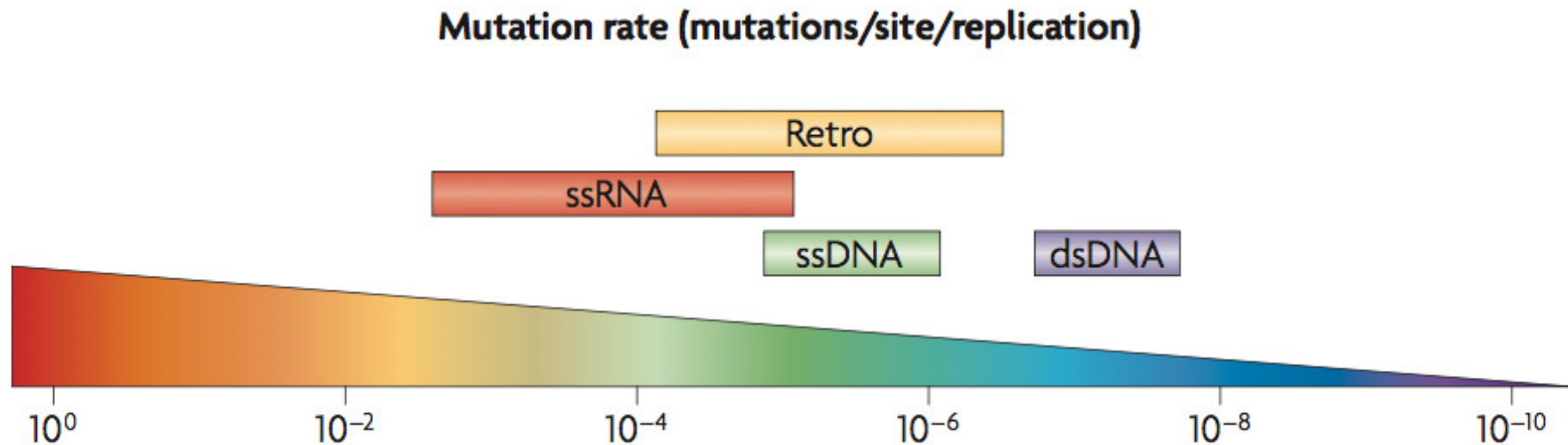- **Mutation**
- **Recombination**

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

Mutation rate (mutations/site/replication)

Retro

ssRNA

ssDNA

dsDNA

10^0 10^{-2} 10^{-4} 10^{-6} 10^{-8} 10^{-10}

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: 3×10^{-5} / nucleotide base per replication cycle)
>
> This translates to roughly 0.2 errors per genome replicated
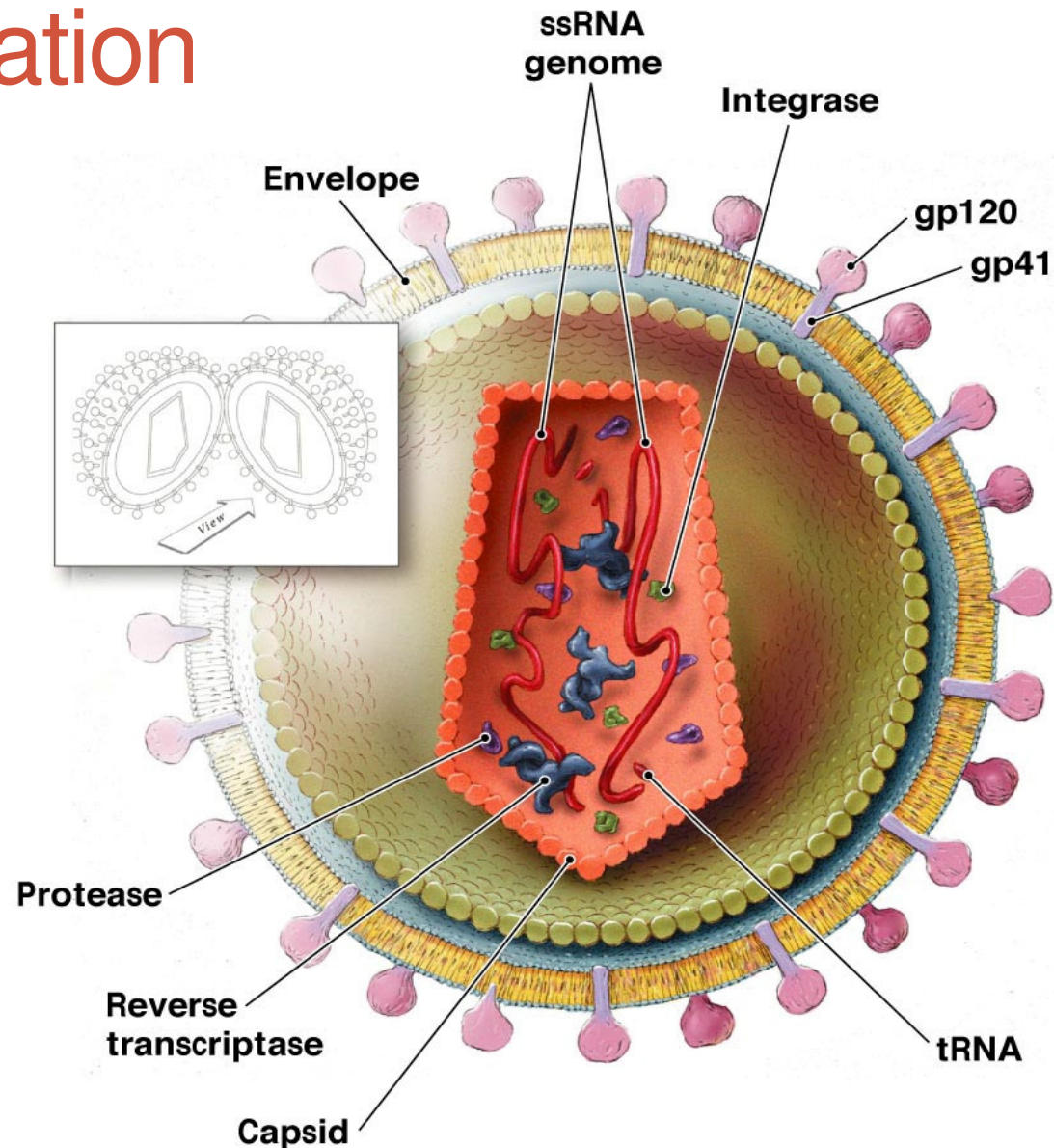
There are also subsequent mutations during transcription from DNA to RNA again
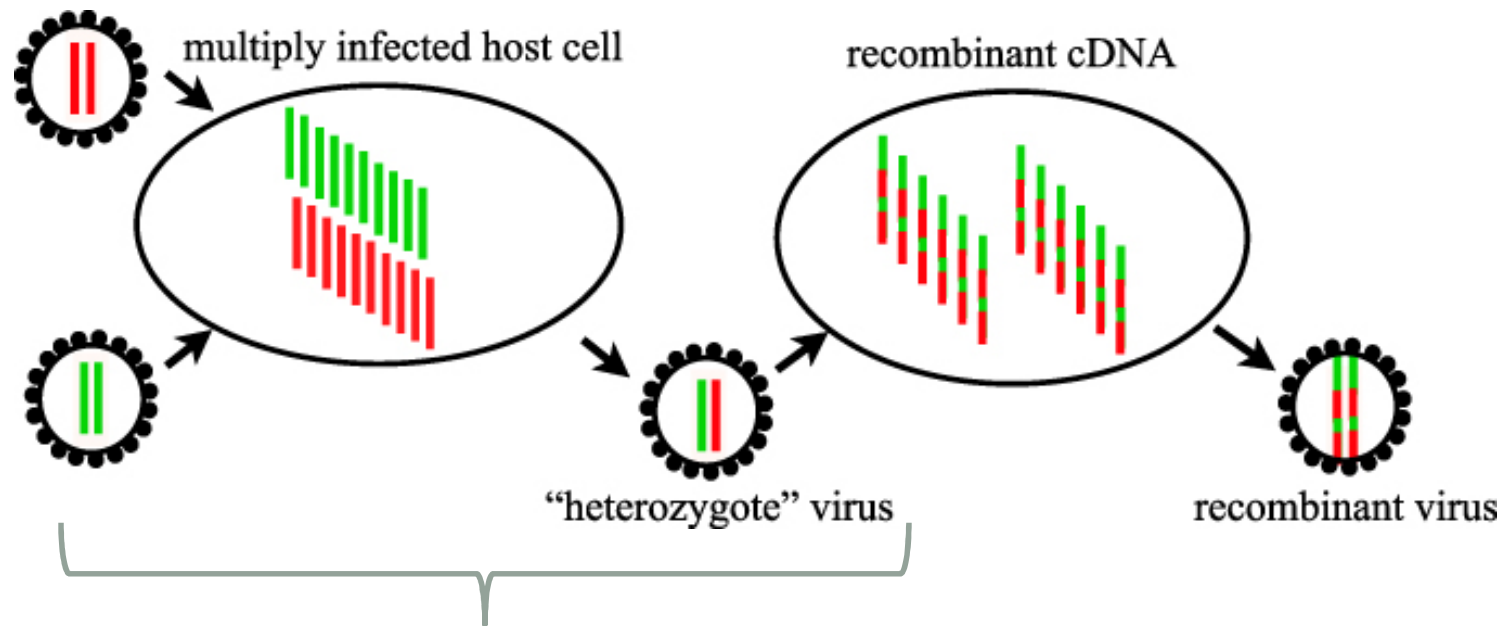
HIV recombination

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.

ssRNA genome

Integrase

Envelope

gp120

gp41

Protease

Reverse transcriptase

Capsid

tRNA

View

HIV recombination

multiply infected host cell

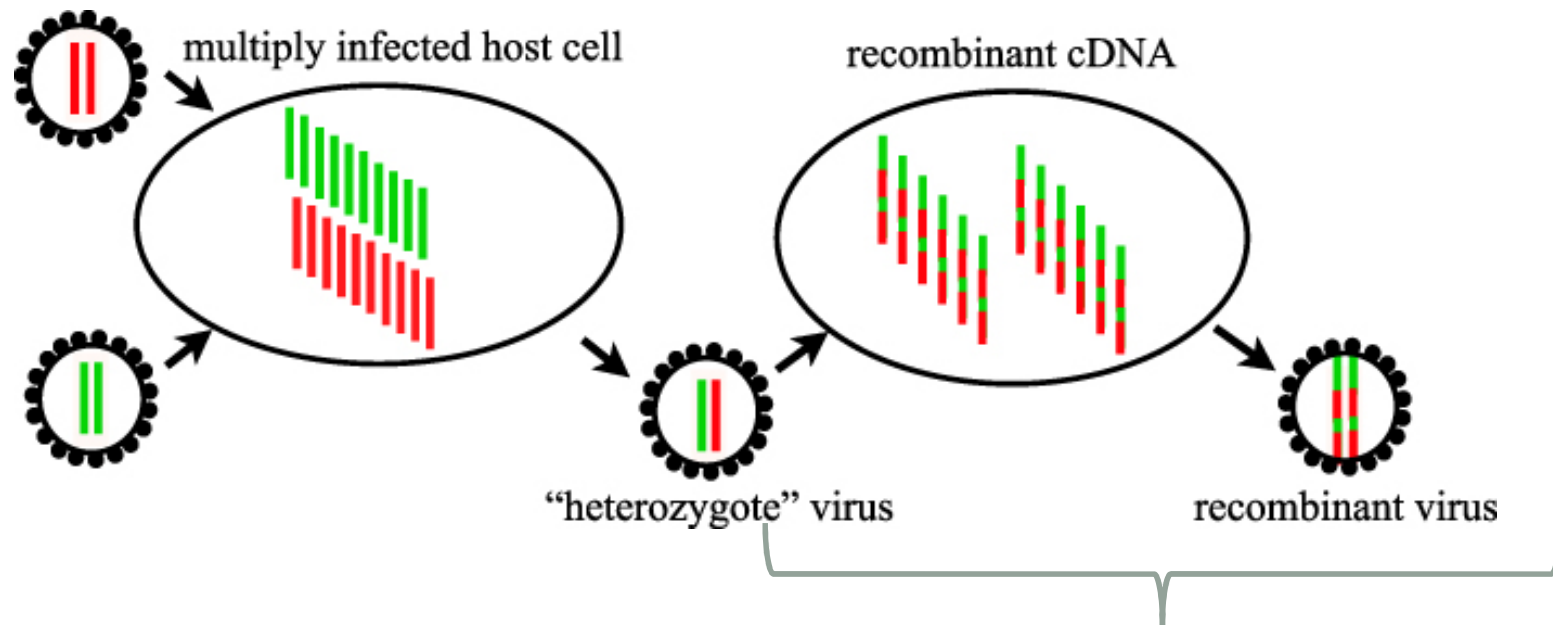recombinant cDNA

"heterozygote" virus

recombinant virus

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

Recombination in HIV: An Important Viral Evolutionary Strategy

Donald S. Burke

Emerging Infectious Diseases   *Vol. 3, No. 3, July–September 1997*

Source: iopscience.iop.org

HIV recombination

multiply infected host cell

recombinant cDNA

"heterozygote" virus

recombinant virus

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.
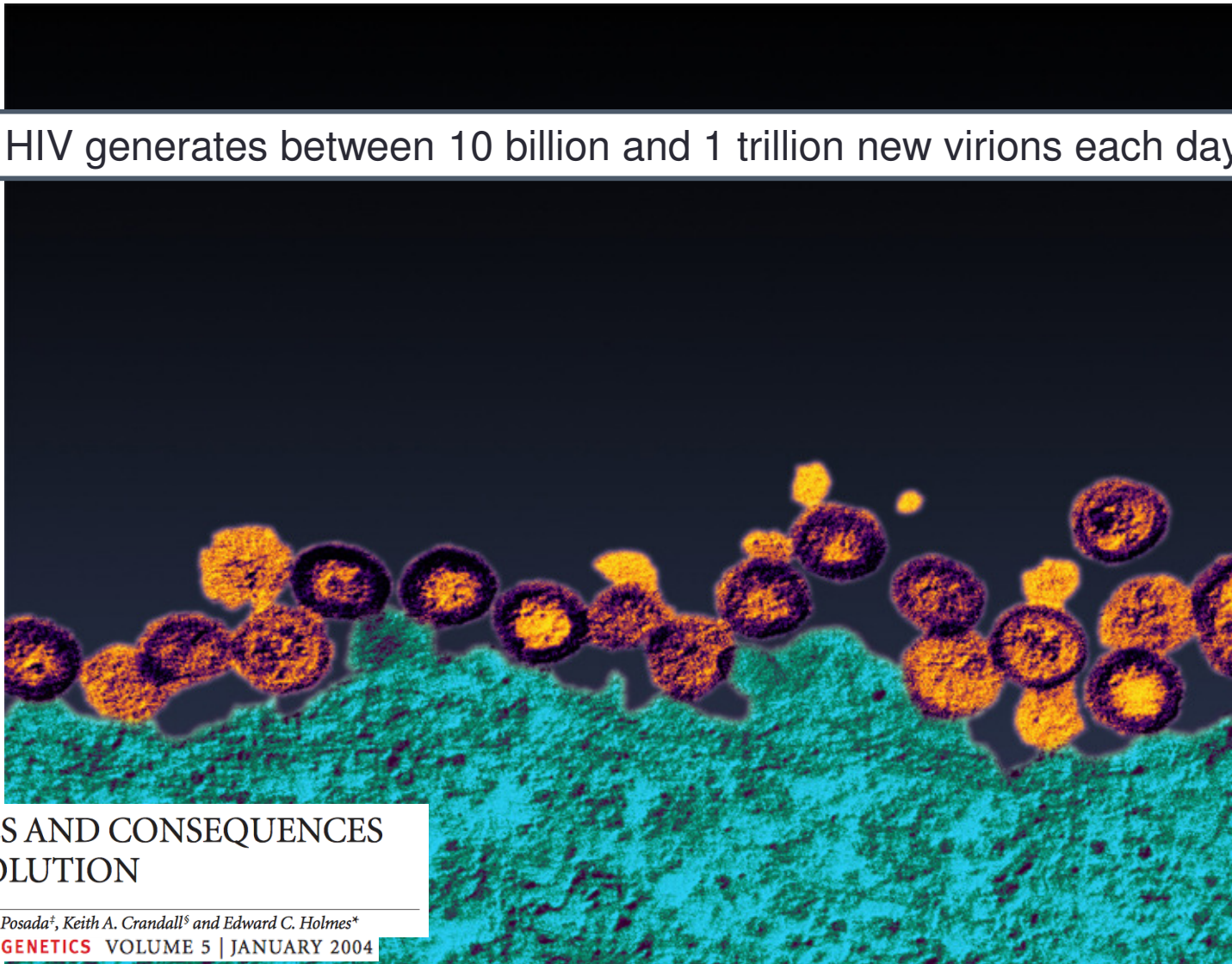
Why does HIV develop so much resistance?

- <u>Reason 2:</u> 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

THE CAUSES AND CONSEQUENCES
OF HIV EVOLUTION

Andrew Rambaut*, David Posada‡, Keith A. Crandall§ and Edward C. Holmes*
NATURE REVIEWS | GENETICS VOLUME 5 | JANUARY 2004

Source: cell.com

Why does HIV develop so much resistance?

- <u>Reason 3:</u> 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.
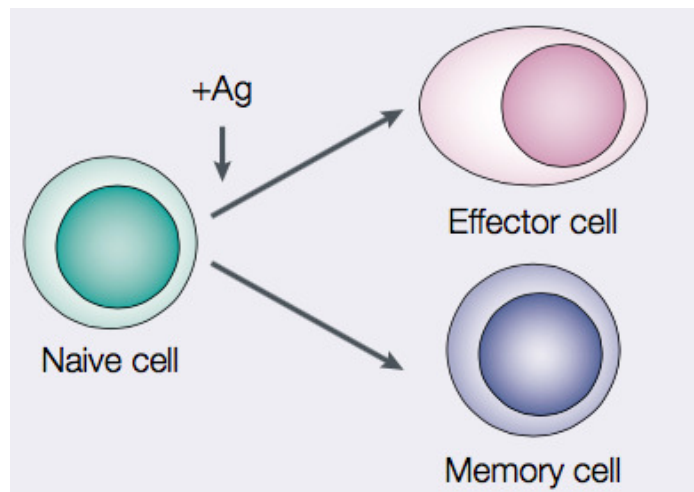
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Archived mutations

- 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).

EFFECTOR AND MEMORY T-CELL
DIFFERENTIATION: IMPLICATIONS
FOR VACCINE DEVELOPMENT
Susan M. Kaech, E. John Wherry and Rafi Ahmed

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?

- <u>Reason 4</u>: 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 10^9 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.**

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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.

WHAT IS TREATMENT FAILURE?

Definitions

Treatment failure

Clinical failure	**Adults and adolescents** New or recurrent clinical event indicating severe immunodeficiency (WHO clinical stage 4 condition)[a] after 6 months of effective treatment **Children** New or recurrent clinical event indicating advanced or severe immunodefiency (WHO clinical stage 3 and 4 clinical condition with exception of TB) after 6 months of effective treatment	The condition must be differentiated from immune reconstitution inflammatory syndrome[b] occurring after initiating ART For adults, certain WHO clinical stage 3 conditions (pulmonary TB and severe bacterial infections) may also indicate treatment failure[a]

Treatment failure

Immunological failure

Adults and adolescents

CD4 count falls to the baseline (or below)

or

Persistent CD4 levels below 100 cells/mm^3

Children

Younger than 5 years

Persistent CD4 levels below 200 cells/mm^3 or <10%

Older than 5 years

Persistent CD4 levels below 100 cells/mm^3

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; Phalguni 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 β_2-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 <u>potentially</u> 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?

Organisation	Virological failure threshold
US Department of Health and Human Services	200 copies/mL
European AIDS Clinical Society	Any detectable viral load
World Health Organization	1000 copies/mL
Southern African HIV Clinicians Society	1000 copies/mL
South African Department of Health	1000 copies/mL

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 <u>don't</u> 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

Justen Manasa,[1] David Katzenstein,[2] Sharon Cassol,[3] Marie-Louise Newell,[1]
and Tulio de Oliveira,[1] for the Southern Africa Treatment and Resistance Network (SATuRN)

AIDS RESEARCH AND HUMAN RETROVIRUSES
Volume 28, Number 6, 2012
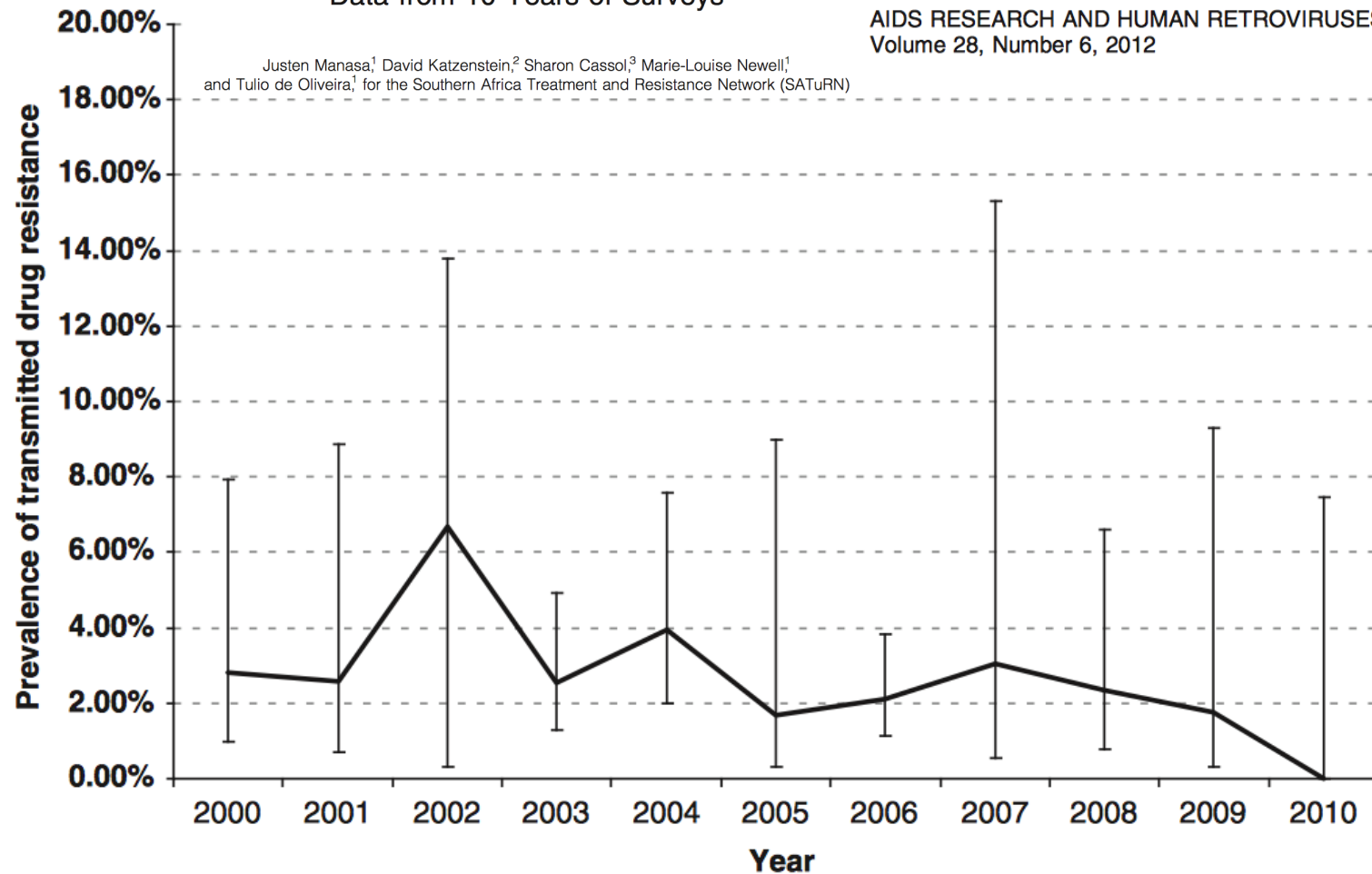
What can be done?

- **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?

	SA Dept. Health	SA HIV Clin. Soc.	DHHS (USA)
At initiation	✗	✓	✓
Before 6 months	✗	3 months	At 2-8 weeks, then every 4-8 weeks until suppressed
6 months	✓	✓	✓
12 months	✓	✓	✓
Thereafter	Every 12 months	Every 6-12 months	Every 3-6 months

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

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

SA HIV Clinicians Society

- <u>> 50</u>: adherence counselling & repeat VL in 2-3 months
- <u>> 1000</u> on 2 occasions 2-3 months apart: switch therapy
- <u>> 200</u> for more than 1 year: switch therapy

When should you do an HIV resistance test?

SA National Department of Health

- Failing a 2nd line PI-based regimen for at least 1 year

SA HIV Clinicians Society

- If the patient has confirmed virological failure (on any regimen, even 1st line)
  - PI-based regimens: only if on regimen for > 1 year.

Why would you do resistance testing after 1st line failure?

1. Avoids switching regimens purely due to adherence problems (if no significant mutations are found)

2. Identifies which 1st line drugs may be reused (esp. TDF – K65R mutation often not present at time of switch).

3. Guarantees a working 2nd 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 2nd line failure (some "1st 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
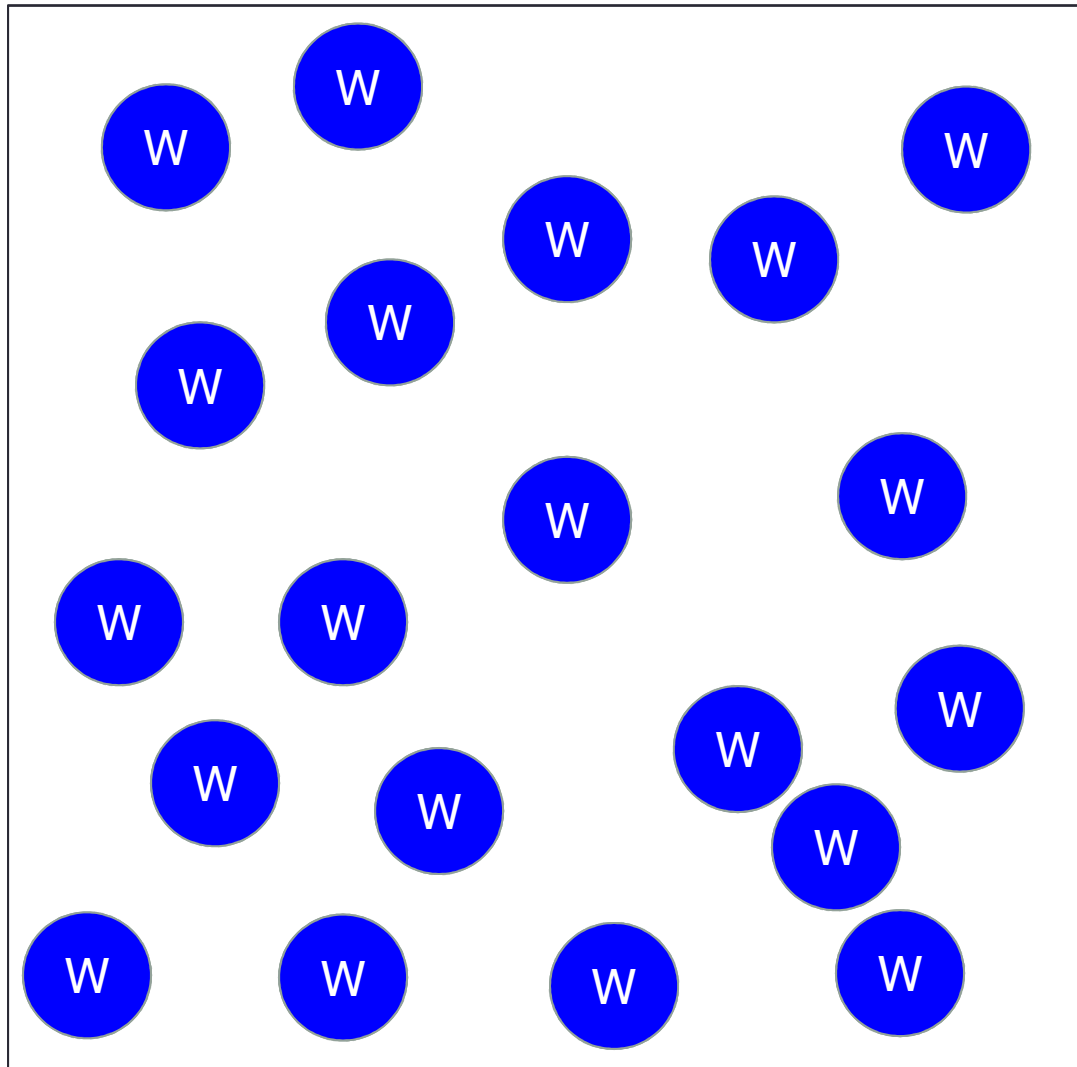
Viral load = 250 000

W = wild type

Resistance testing:
no mutations detected

On treatment

TDF

Viral load < 50

W = wild type

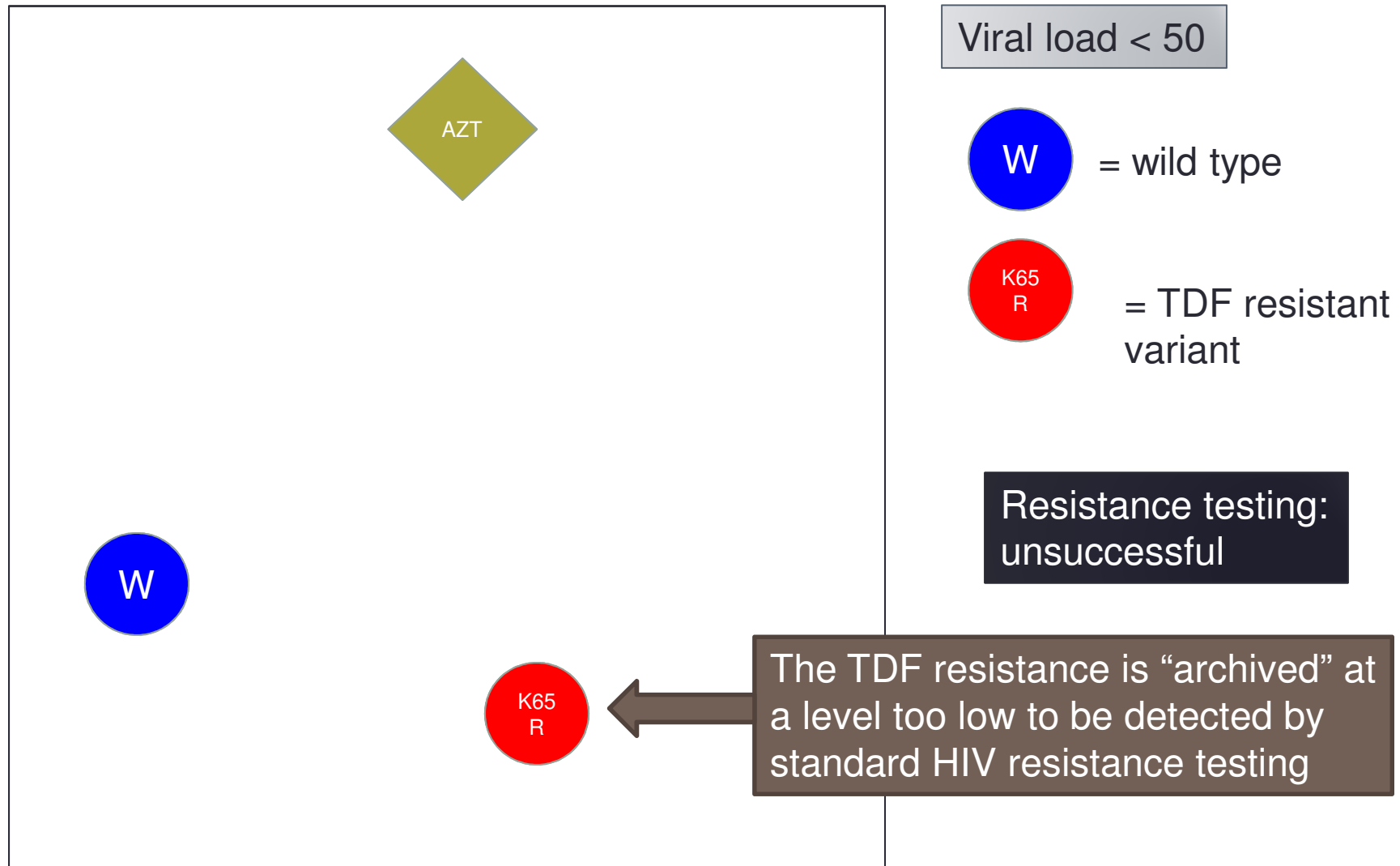Resistance testing: unsuccessful

W
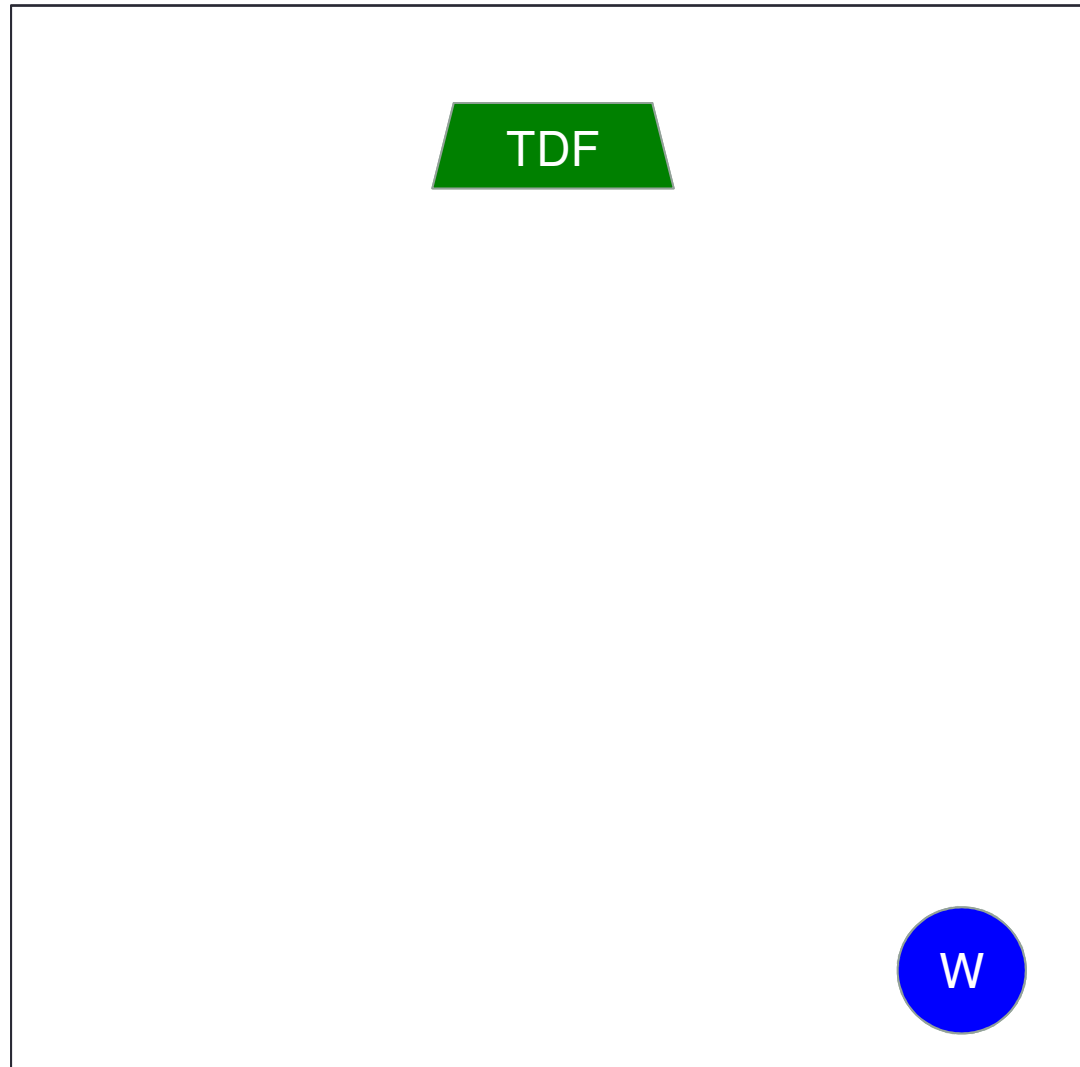
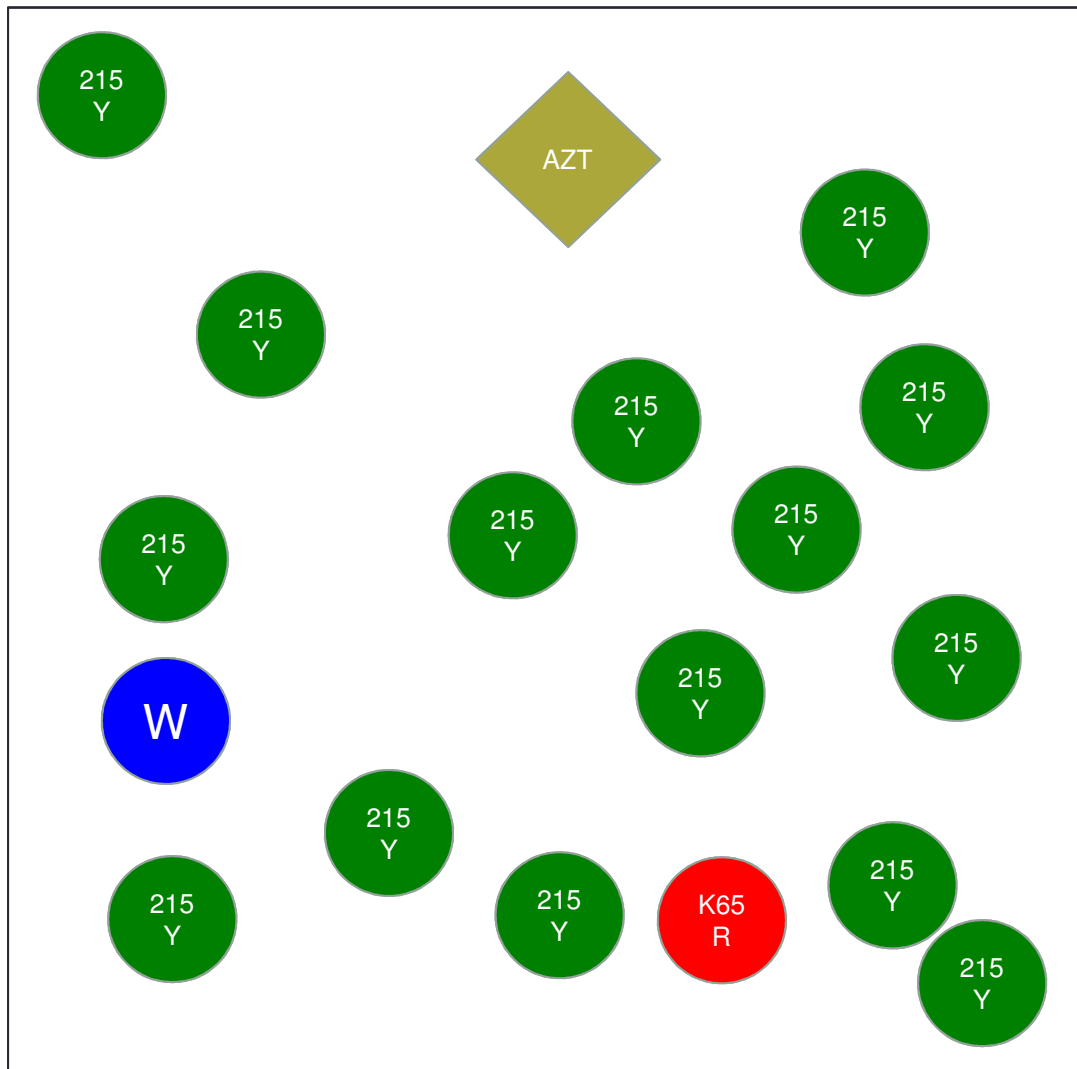# Development of TDF resistance
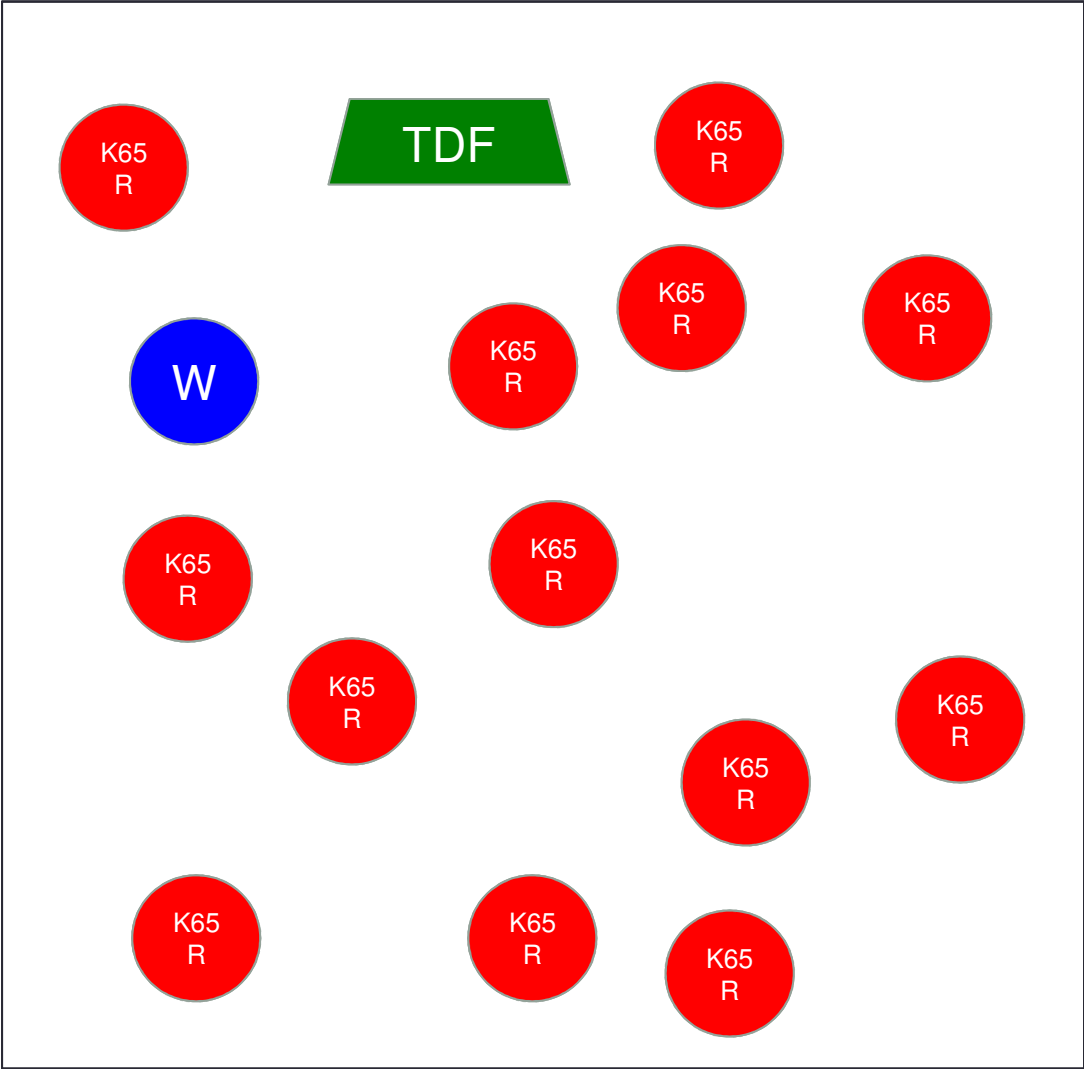
Viral load = 110 000

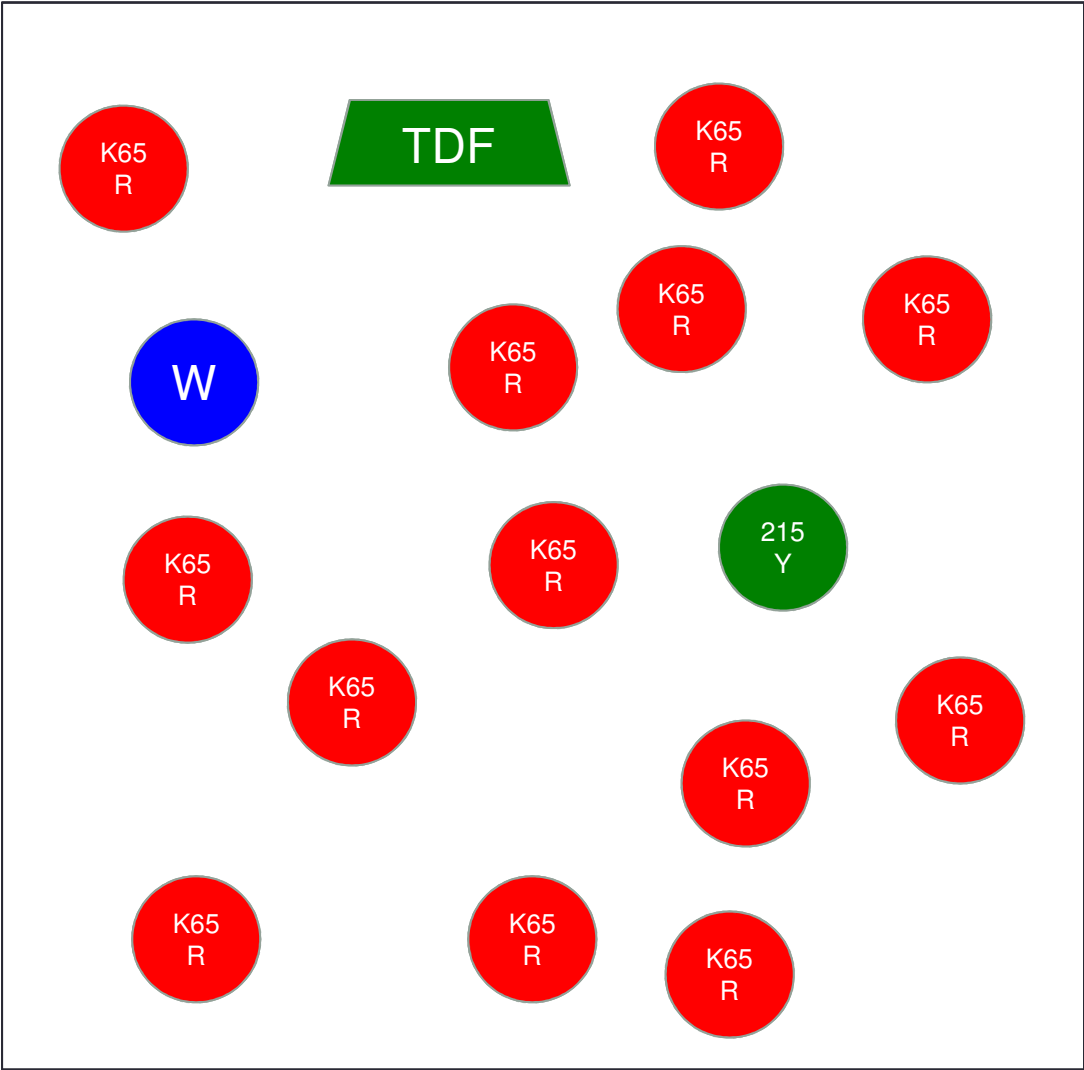W = wild type

K65 R = TDF resistant variant

Resistance testing:
TDF resistance

Switch to AZT

Viral load < 50

W = wild type

K65R = TDF resistant variant

Resistance testing: unsuccessful

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

Development of AZT resistance

If TDF reintroduced…

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 (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

NRTI Resistance Mutations:	**K65R, M184V**		
NNRTI Resistance Mutations:	**K103N**		
Other Mutations:	None		

Nucleoside RTI		Non-Nucleoside RTI	
lamivudine (3TC)	High-level resistance	**efavirenz (EFV)**	High-level resistance
abacavir (ABC)	High-level resistance	**etravirine (ETR)**	Susceptible
zidovudine (AZT)	Susceptible	**nevirapine (NVP)**	High-level resistance
stavudine (D4T)	Intermediate resistance	**rilpivirine (RPV)**	Susceptible
didanosine (DDI)	High-level resistance		
emtricitabine (FTC)	High-level resistance		
tenofovir (TDF)	High-level resistance		

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 + ddI (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