BASICS TO UNDERSTAND HIV DRUG RESISTANCE

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What makes up the HIV virus

- HIV genome is made up of building blocks known as amino acids, each amino acid consists of three nucleotides.
Amino Acids

- Different Combinations of the nucleotides make up different amino acids

Serine (S)

Lysine (K)
HIV Genome

• Amino Acids make up the HIV genes

HIV Life Cycle & Drug Targets

Modified from Turner and Summers, 1999
How do mutations arise in the HIV genome

- When the HIV replicates it makes mistakes.

- Why does the virus make mistakes:
  - It doesn’t check what it is doing (high error rate of the reverse transcriptase [RT] enzyme);
  - Replicates very fast (high HIV replication rate).

- Mistake=Mutation
Example of what a mutation does

THE CAT SAT ON THE MAT

THE HAT SAT ON THE MAT

It changes the sentence (gene) so it still makes sense; but says something different.
Naming a Mutation

THE CAT SAT ON THE MAT

THE HAT SAT ON THE MAT

C4H

Wildtype Letter

Mutant Letter

Letter Number
Naming of an HIV Mutation

The length of the gene:
- Protease Region of Polymerase Gene is from Amino Acid 1 to Amino Acid 99
- Reverse Transcriptase Region of Polymerase Gene is from Amino Acid 1 to Amino Acid 540
- Anywhere along these amino acids you can get a change in the sentence and a mutation.

M184V

Wildtype Amino Acid (consensus)  Mutant Amino Acid

RT Codon
Mixture

M184M/V

- Means there is both wild-type and mutant viruses present
- Treat as if it were a mutation.
Viral dynamics and resistance

What happens when the virus makes changes to its genes that the antiretroviral are targeting?

- The antiretroviral no longer ‘understands’ the sentence
- This allows the HIV virus to grow
- So you see an increase in HIV Viral Load

Sensitive virus (wild type)  
Resistant virus (mutant)
More about mutations...

- Mutation can be specific to one ARV.

- Mutation can be specific to several ARVs (cross-resistance).

- Strength of resistance of a mutation can be different
  - Some mutations can be weak;
  - Some mutations can be very strong.

- How easy to get resistance
  - Often dependent on the ARV;
  - One mutation to give resistance (low genetic barrier drugs);
  - Lots of mutations to give resistance (high genetic barrier drugs);
  - Resistance can get worse overtime because mutations keep accumulating.
Reverse Transcriptase

- HIV Enzyme

- Transcribes single stranded viral RNA into viral cDNA in the cytoplasm.

- The RT crystal structure looks like a right hand→ fingers, palm and thumb.
Reverse Transcriptase cont…

- The thumb and the fingers hold the nucleic acid in place over the palm.

- Palm is the active site of the enzyme.
Mutations that give resistance to NRTIs
How do NRTIs work?

• To replicate HIV uses nucleotides to make copies of itself.

• NRTIs are nucleoside analogues → “artificial nucleotides” modified to cause chain termination/stop replication.

• During replication NRTIs competitively inhibit RT activity.

• When the virus is replicating it inserts an “artificial nucleotide” rather than a naturally occurring nucleotide, results in replication stopping.

• During ARV drug pressure the HIV-1 RT is able to develop resistance to these drugs by generating mutations.
Mechanism 1 NRTI Resistance

- RT-residues that encode amino acids on the tips of the fingers that come into direct contact with the dNTPs or NRTIs can mutate.

- These mutations affect the rate of binding and incorporation of nucleotides.

- Primary mutations are amino acid substitutions in critical positions of the enzyme that cause an immediate decrease in susceptibility to the drug, ultimately leading to virological failure.

Mechanism 2 NRTI Resistance

• Increased rate of excision of the NRTIs.

• This process is driven by adenosine triphosphate (ATP) and is caused by thymidine analogue mutations (TAMs) that occur close to the triphosphate binding site.

• As the number of TAMs such as M41L, D67N, K70E, L210W, T215Y/F, K219Q/E/N/K increase in the RT, the level of resistance increases.
Mutations that give resistance to NNRTIs
Reverse Transcriptase

- Thumb and Fingers linked to NRTI resistance
- Palm is the active site of the enzyme and a hydrophobic pocket linked to NNRTI resistance.
How do NNRTIs work?

- The NNRTIs are molecules which have a high affinity for the hydrophobic pocket of the RT enzyme.

- This results in the NNRTIs binding irreversibly to the pocket (palm of the RT).

- When they bind to the palm this inhibits replication of HIV.
Mechanism of NNRTI resistance

• Resistance to EFV and NVP develops when mutations occur in the hydrophobic pocket.

• These changes, change the charge of the palm.

• This decreases the ability of the NNRTIs to bind.

• The mutations that develop in the hydrophobic pocket result in cross-resistance to all first-generation NNRTIs (EFV and NVP).
How do second-generation NNRTIs work?

- Etravirine (ETR):
  - A highly flexible molecule resulting in a high genetic barrier to resistance.
  - ETR is susceptible to viruses with the K103N mutation, which results in cross resistance to both EFV and NVP.
  - The level of susceptibility is determined using a weighted scoring system for each mutation.
Mutations that give resistance to PIs
Protease is like a bowl with a lid
How do PIs work?

• PIs are a powerful class of drugs which bind more tightly to the active site of the PR enzyme than the natural substrates (polyproteins) and act as preferred substrates.

• Polyproteins and PIs are competitive.

• When the PI binds protease is unable to cleave polyproteins.

• Reduction of mature HIV virions that are produced.
Mechanism of PI resistance

- Mutations occur in the active site or the flap (glycine tips).

- Mutations prohibit the binding of the PIs.

- PIs have a high genetic barrier for resistance, and require an accumulation of major mutations to lose complete susceptibility to the PIs.
More about PI resistance...

- Some mutations make big change to the protease enzyme → Major Mutation.

- Some mutations make small change to the protease enzyme → Minor Mutation.

- Depending on the protease inhibitor and because you add ritonavir you need MORE than one Major Mutation to give you HIGH resistance.
Mutations that give resistance to Integrase Inhibitors
Integrase

- Integrates HIV into host DNA so it can be replicated.

- HIV cDNA integrase cuts ends → moves into nucleus → cuts host DNA → integrate the HIV cDNA
Raltegravir and Dolutegravir

- Inhibit the integrase enzyme from performing strand transfer by binding to the active site of integrase.

- Results in no integration of HIV cDNA into the host DNA.

- Therefore no replication of HIV.
Integrase Inhibitor Resistance

- Changes are at the active site of the integrase enzyme.
- Dolutegravir resistance is rare;
- Most mutations that arise to reduce the susceptibility to dolutegravir; result in a non-viable virus; however, if mutations are already present in integrase when DTG is initiated this can compromise treatment outcome.
- Integrase resistance testing needs to be ordered as a separate test.

### Major Integrase Inhibitor (INSTI) Resistance Mutations

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Summary

• Mutations to an antiretroviral develop in the target gene.

• Mutations can give resistance to other drugs in the same class.

• Longer a patient is on a failing regimen the more mutations will develop and the more resistance the patient will have.