BASICS TO UNDERSTAND HIV DRUG RESISTANCE

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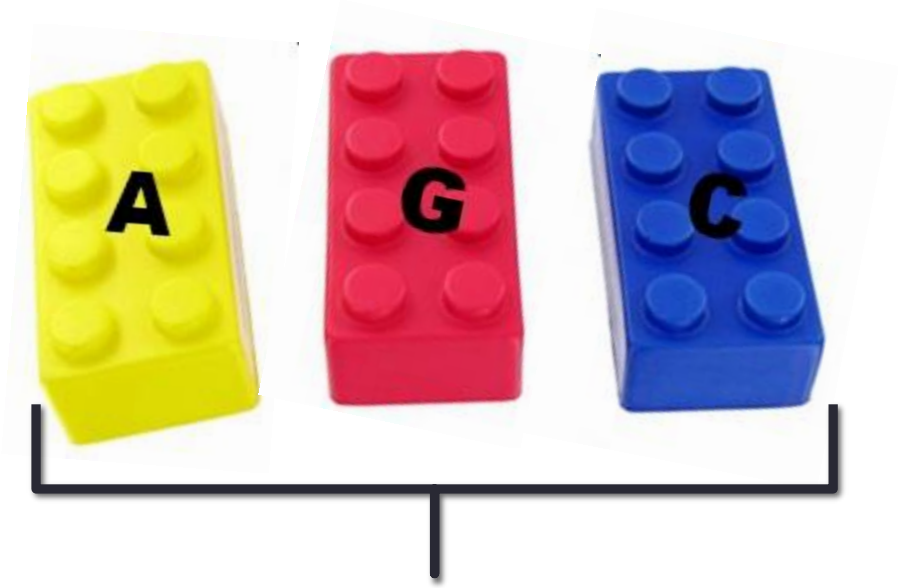
What makes up the HIV virus

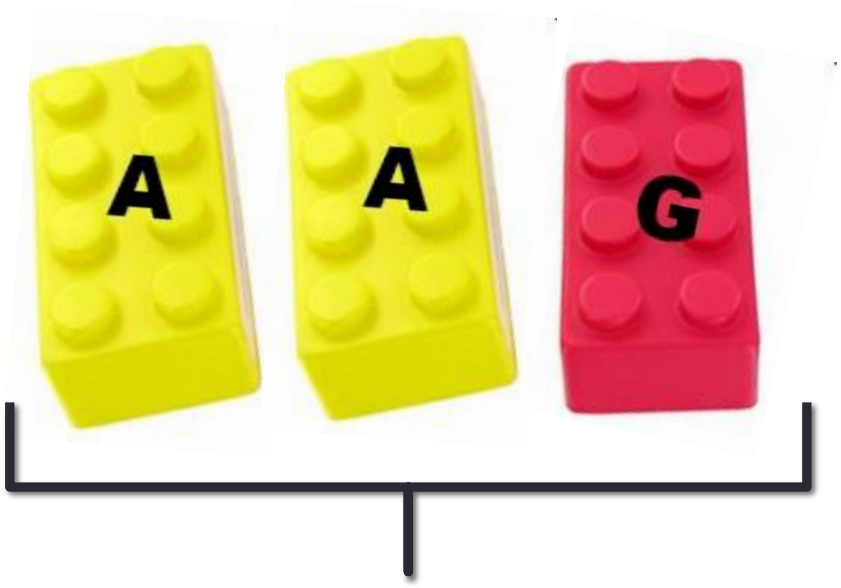
- HIV genome is made up of building blocks known as amino acids, each amino acid consists of three nucleotides.

Amino Acids

- Different Combinations of the nucleotides make up different amino acids



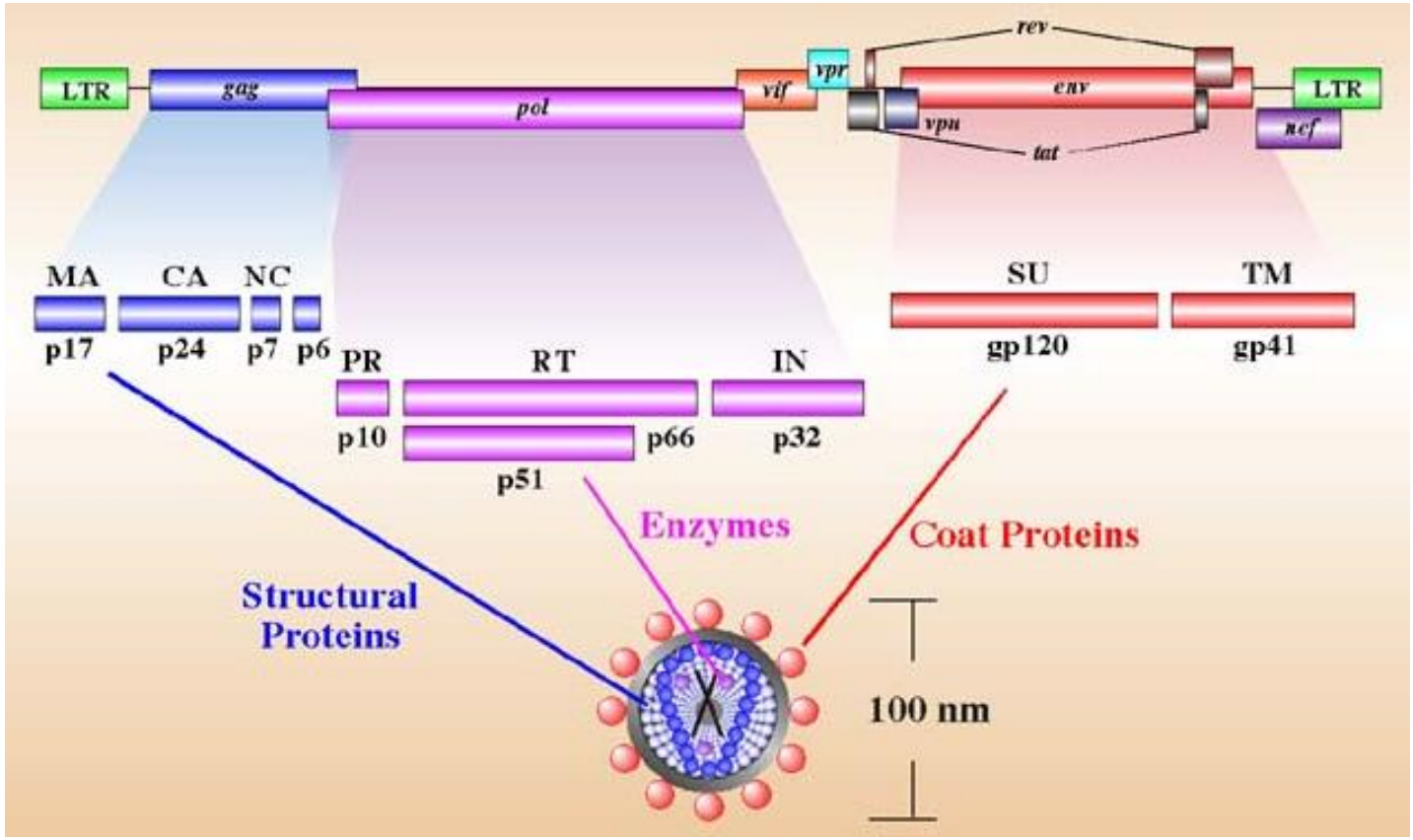
Serine (S)



Lysine (K)

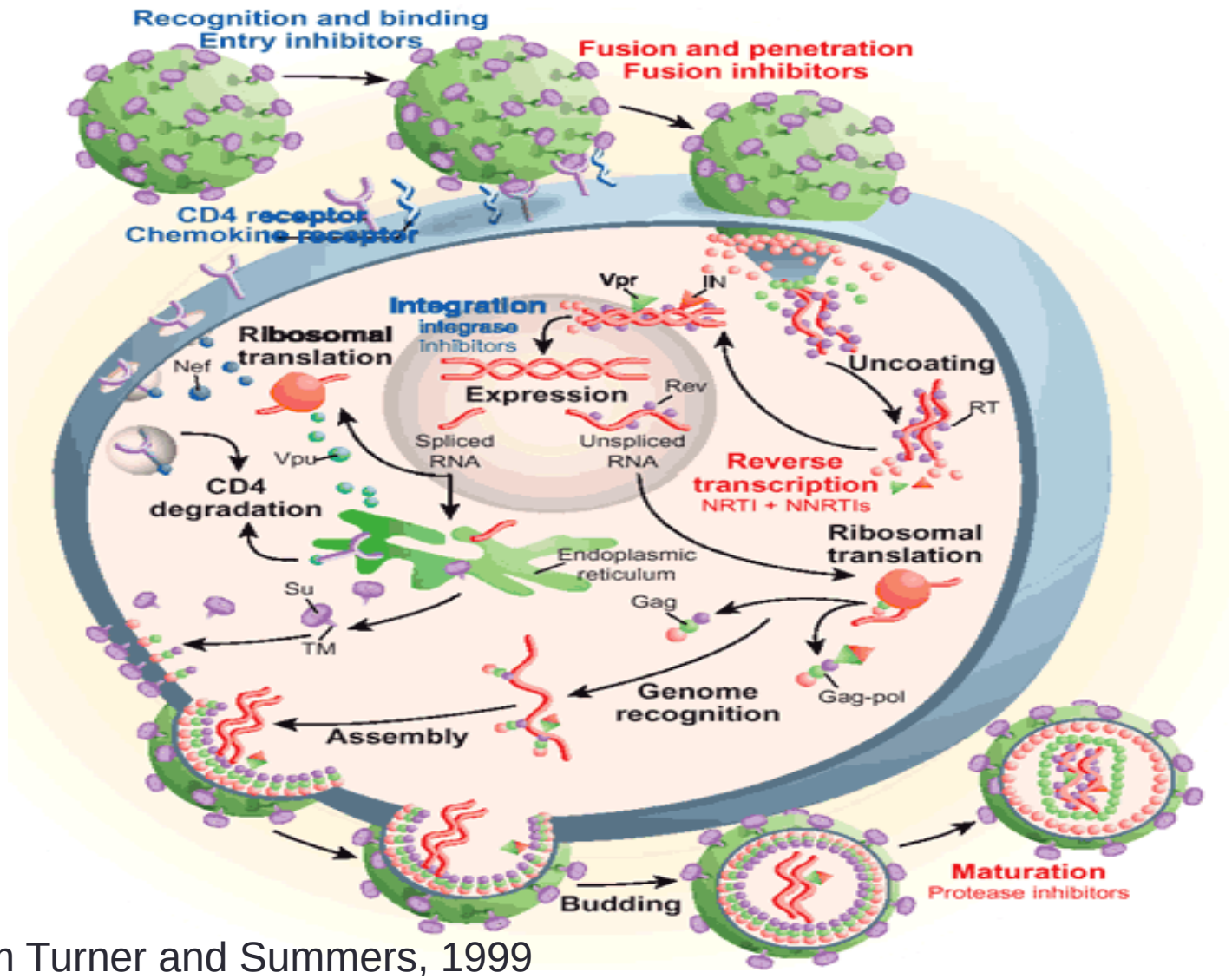
HIV Genome

- Amino Acids make up the HIV genes



http://www.stanford.edu/group/virus/retro/2005gongishmail/HIV-1b.jpg

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

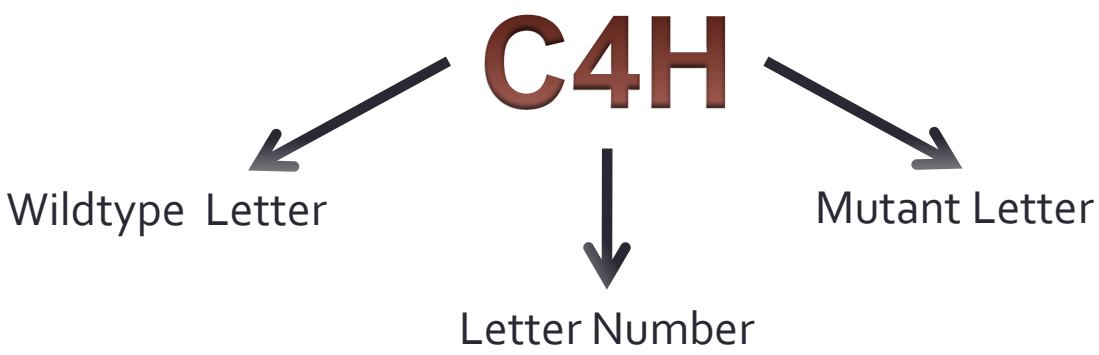
THE **H**AT SAT ON THE MAT

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

Naming a Mutation

THE CAT SAT ON THE MAT

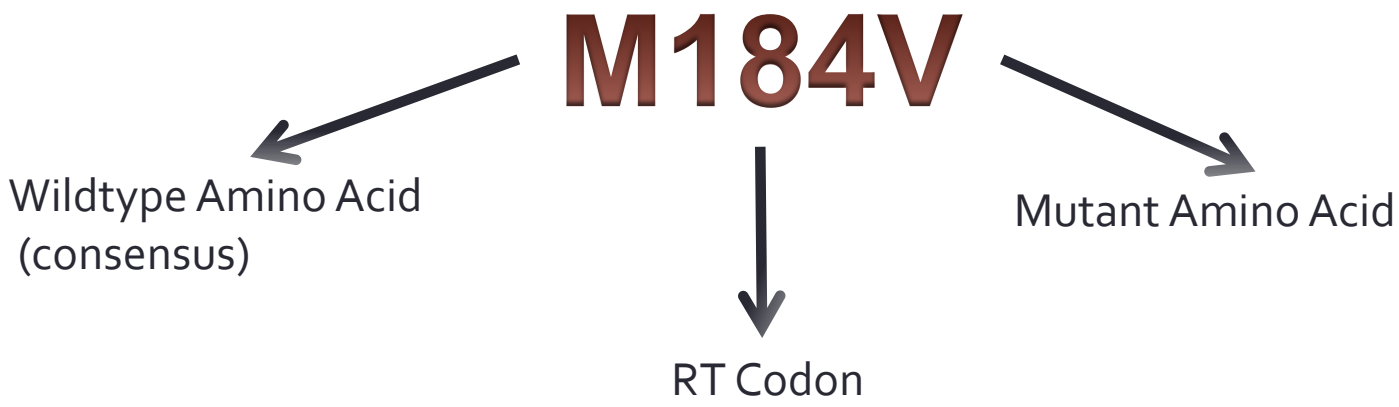
THE **H**AT SAT ON THE MAT



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.



Mixture

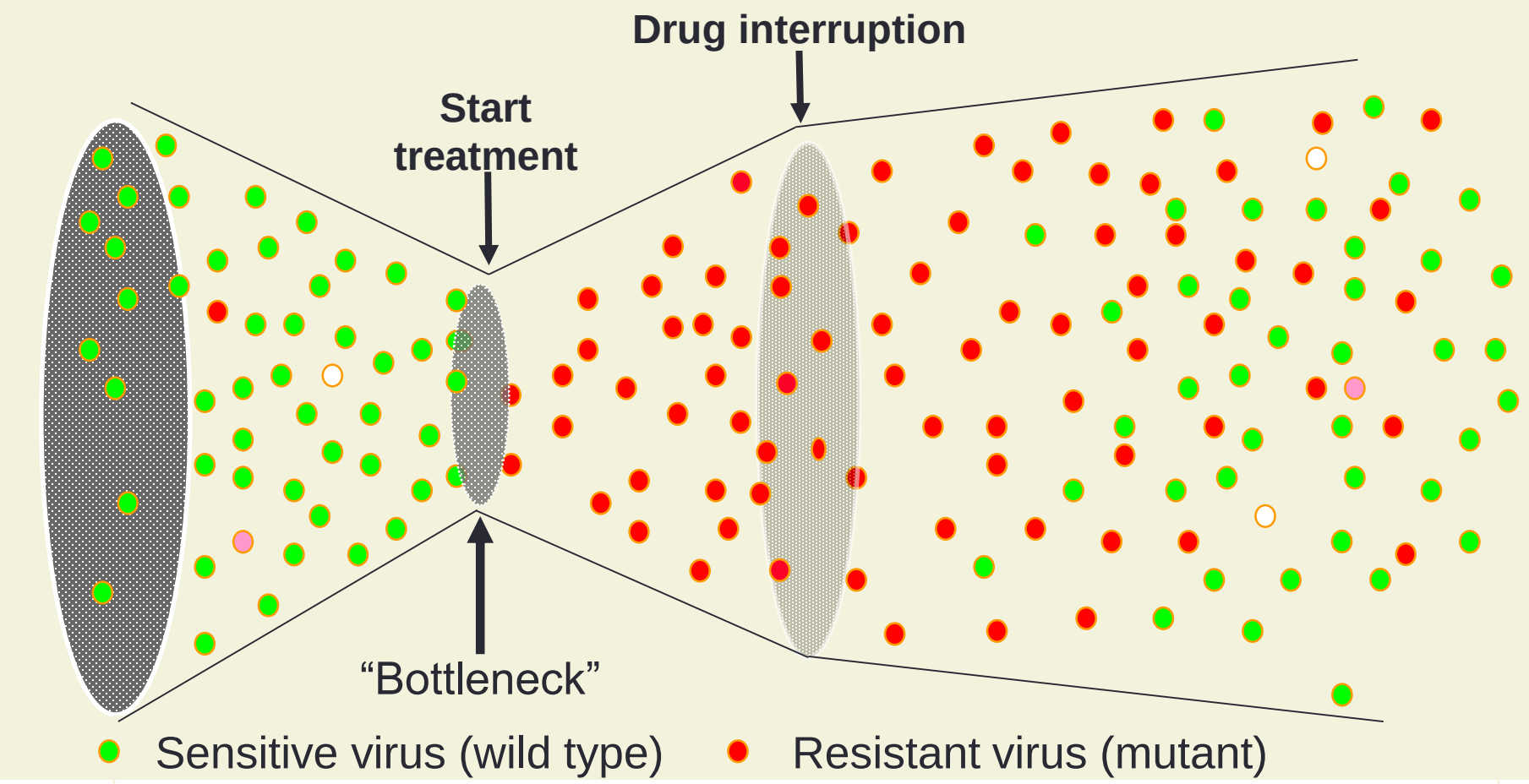
M184M/V

- 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



More about mutations...

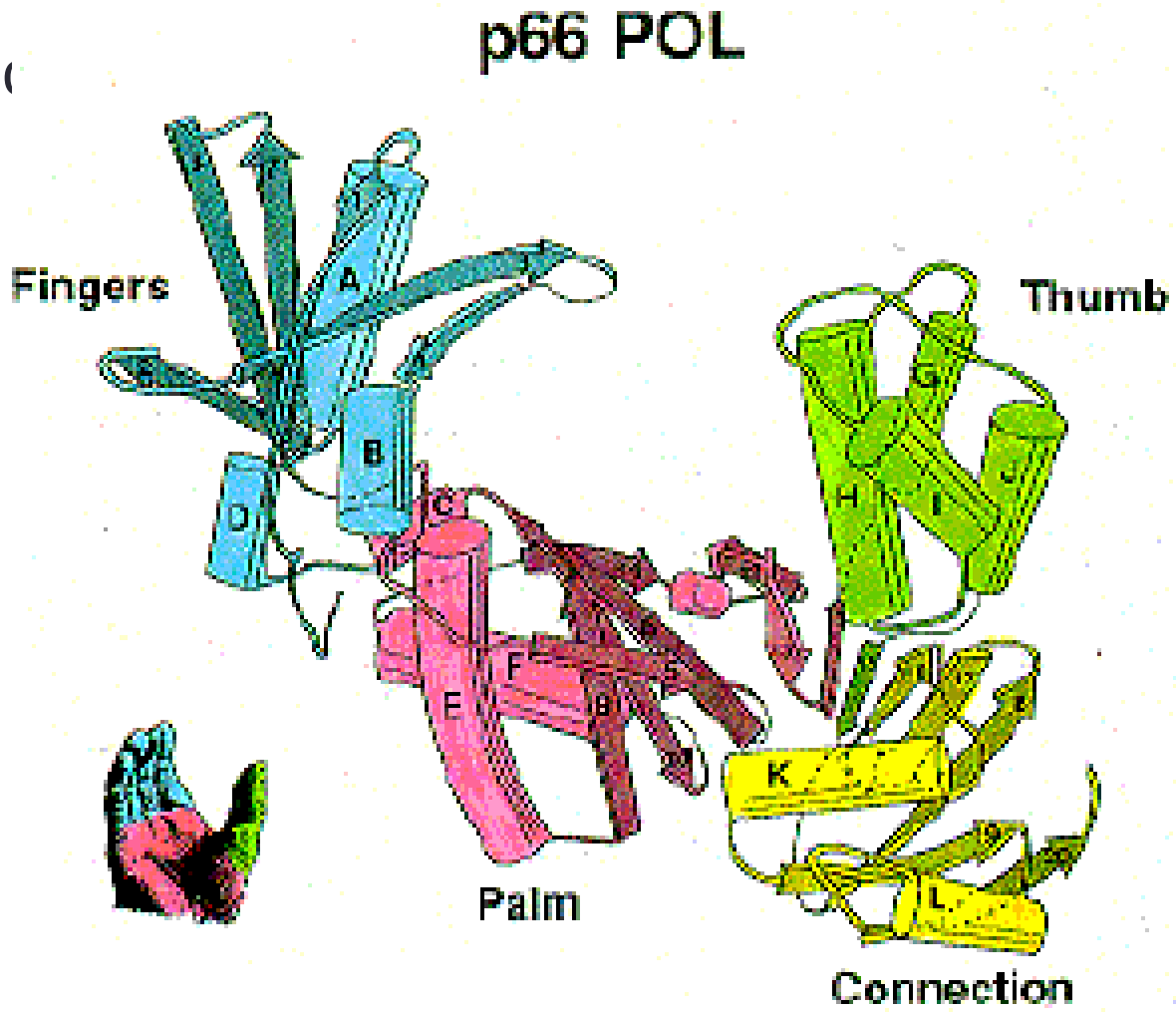
- Mutation can be specific to one ARV.
- Mutation can be specific to several ARVs (cross-resistance).
- Strength of resistance of a mutation can be different
  - Some mutations can be weak;
  - Some mutations can be very strong.
- How easy to get resistance
  - Often dependent on the ARV;
  - One mutation 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.
- K65R, L74V, Y115F, M184V/I and Q151M and its associated mutations.

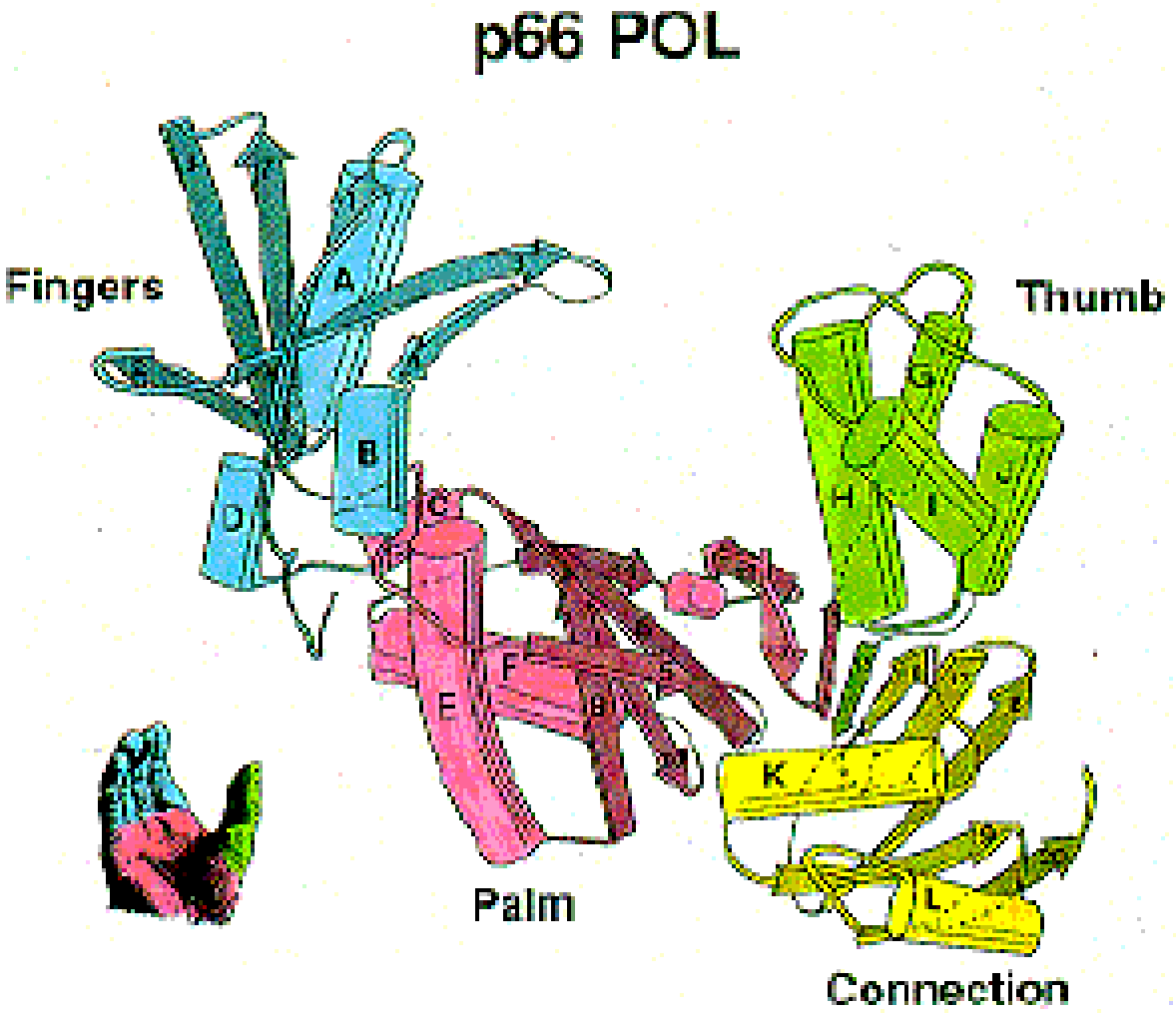
Mechanism 2 NRTI Resistance

- 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.

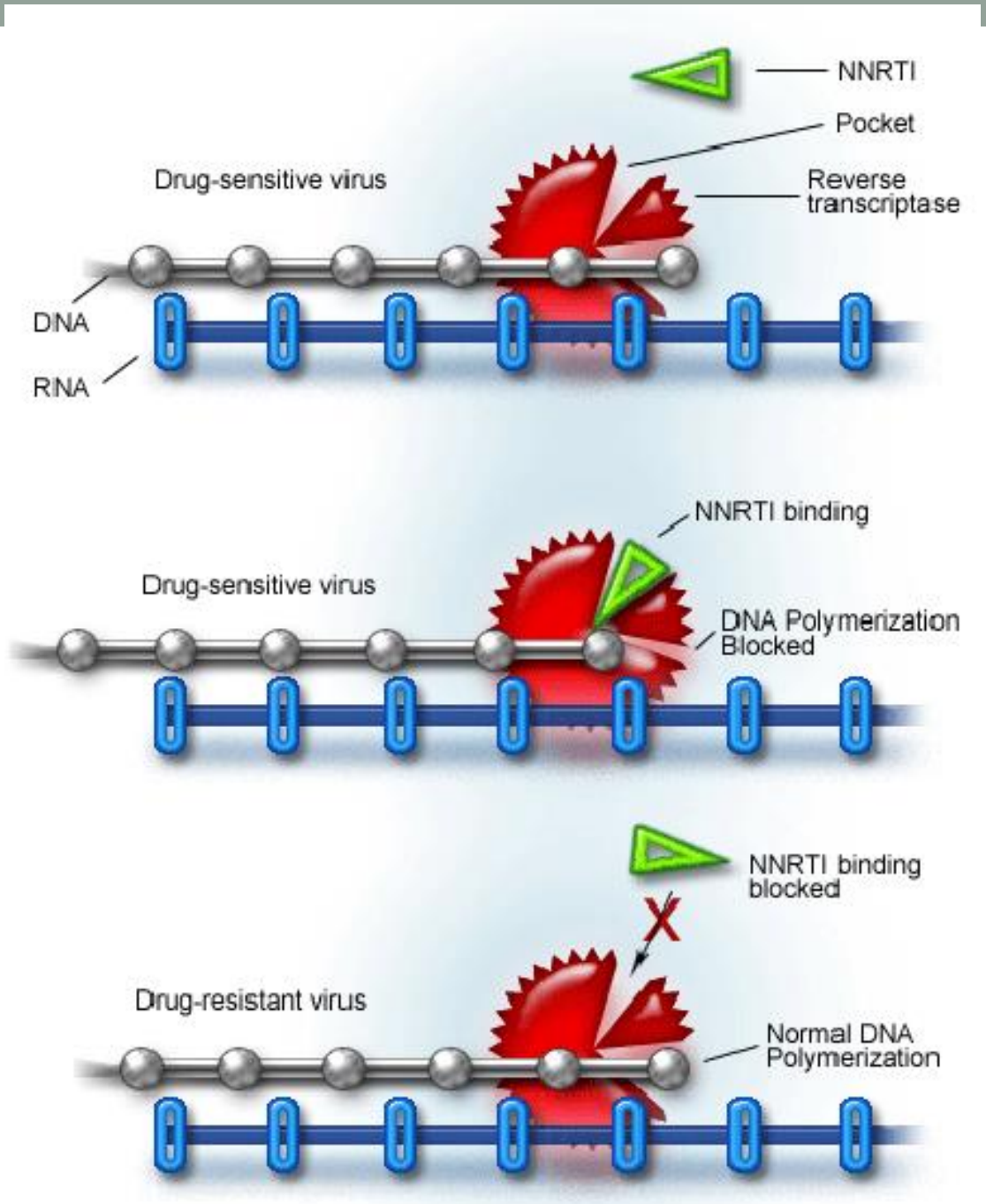


How do NNRTIs work?

- 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).

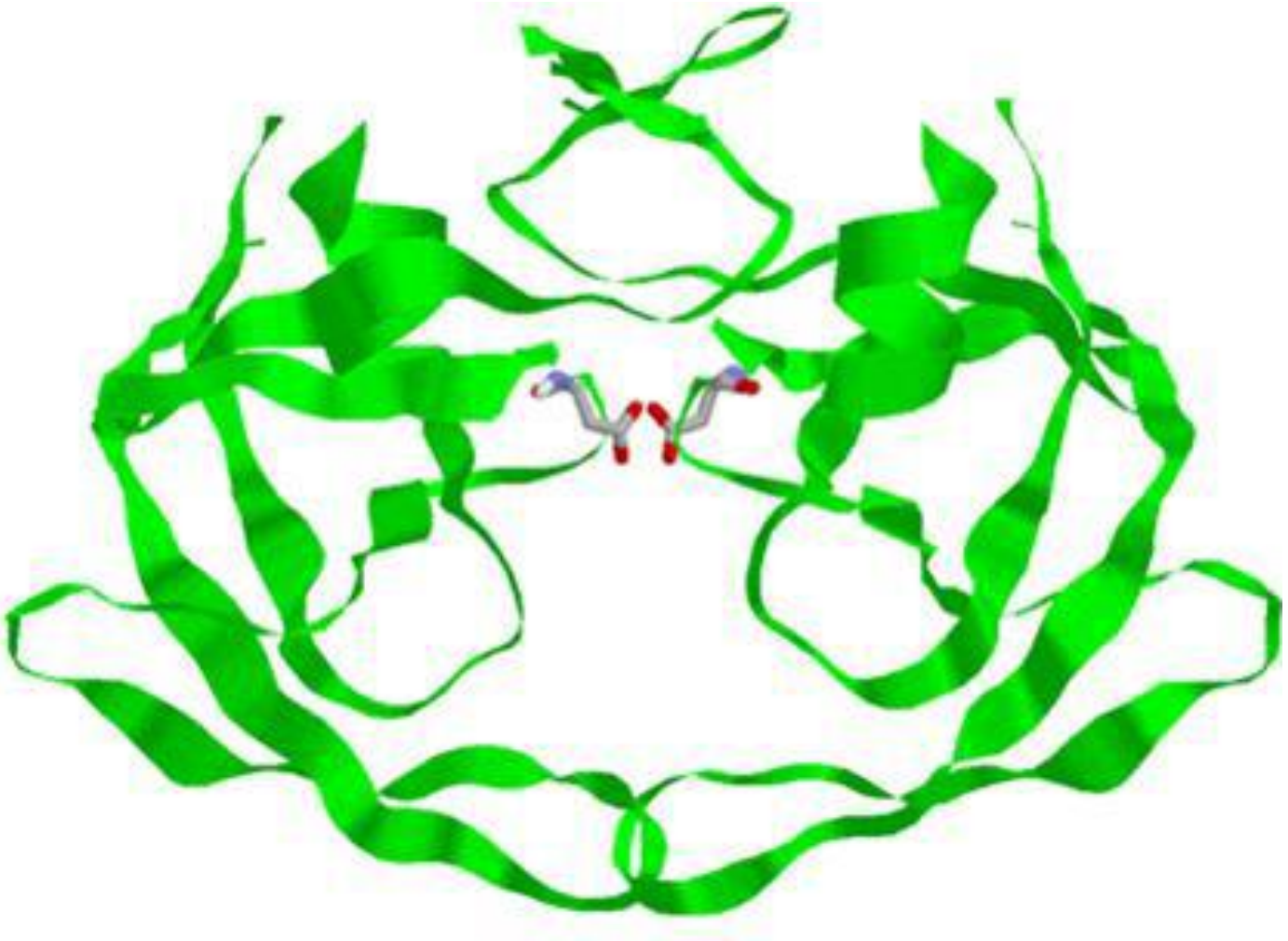

NNRTI
Pocket
Drug-sensitive virus
Reverse transcriptase
DNA
RNA
NNRTI binding
Drug-sensitive virus
DNA Polymerization Blocked
NNRTI binding blocked
Drug-resistant virus
Normal DNA Polymerization

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…

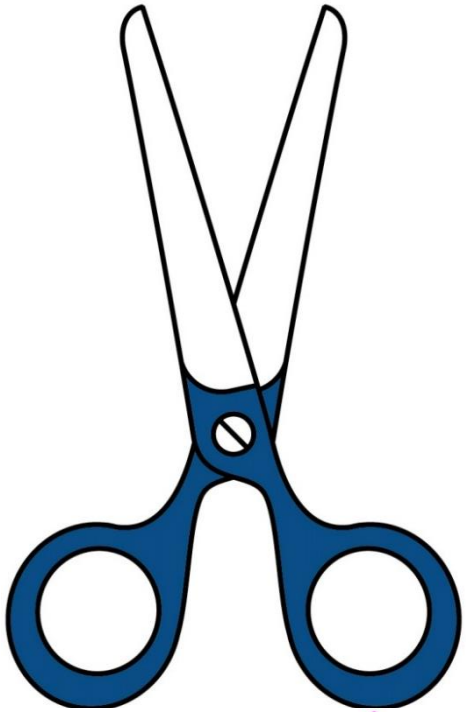
- Some mutations make big change to the protease enzyme→ Major Mutation.
- Some mutations make small change to the protease enzyme→ Minor Mutation.
- Depending on the protease inhibitor and because you add ritonavir you need MORE than one Major Mutation to give you HIGH resistance.

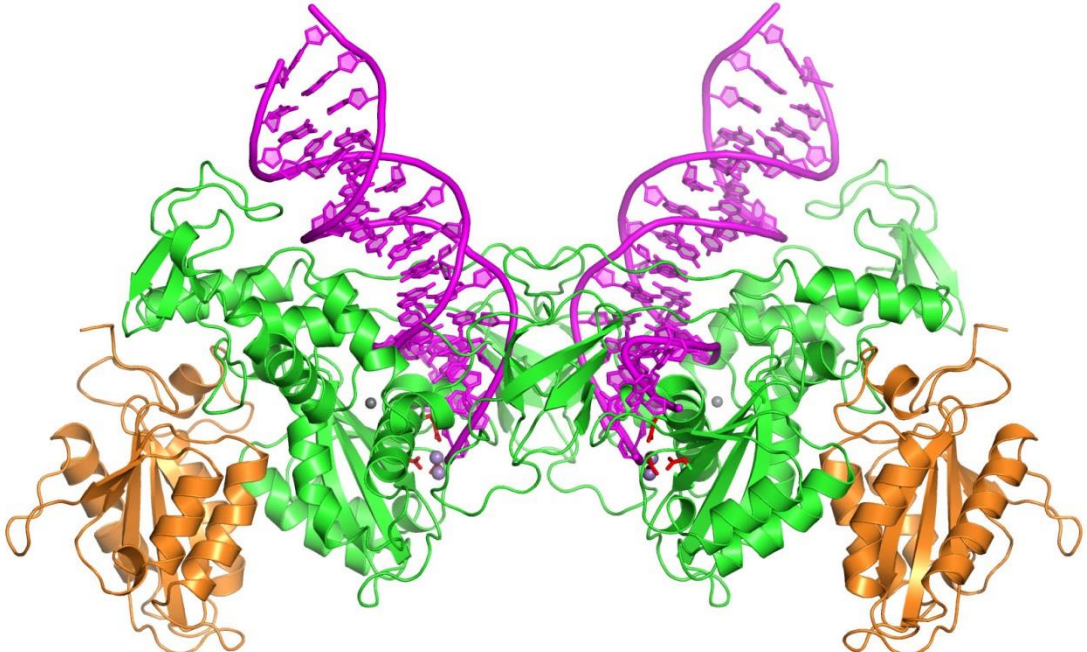
Mutations that give resistance to Integrase Inhibitors

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

Major Integrase Inhibitor (INSTI) Resistance Mutations

	66	92	118	138	140	143	147	148	155	263
Consensus	T	E	G	E	G	Y	S	Q	N	R
Bictegravir (BIC)	K	Q	R	KAT	SAC			HRK	H	K
Dolutegravir (DTG)	K	Q	R	KAT	SAC			HRK	H	K
Elvitegravir (EVG)	AIK	Q	R	KAT	SAC		G	HRK	H	K
Raltegravir (RAL)	AIK	Q	R	KAT	SAC	RCH		HRK	H	K

- 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.

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.