Resistance

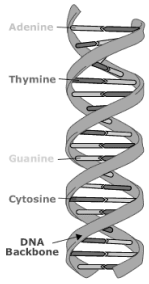
- Political resistance is a good thing - to change something that is wrong - some people in society resistance the system
- Drug resistance is a bad thing - parts of the HIV virus have learnt how to get round the drugs

Definitions & questions

- What is resistance?
- How does resistance develop?
- How can you stop resistance?
- How is resistance measured?
- What are the implications of resistance?

What is resistance

- genetic code carries all information about every living organism
- It is in the form of a chemical package called DNA (RNA for HIV)
- This is like an instruction manual or recipe book



What is resistance

- There are only 4 bases:
  Adenine (A), Thymine (T), Guanine (G) and Cytosine (C)
- DNA (the recipe book) can REPLICATE (photocopy itself)
- This is because with the 4 bases:
  A binds with T and G binds with C.

What is resistance

- REPLICATION

What is resistance

Each amino acid is a 'codon'

A A T G C G T A A T A T T G G T T T A A A etc
| | | | |
T T A C G C A T T A T A A C C t A A T T T etc

T T G = Leucine (amino acid)

G T T = Valine (amino acid)

A A C = Asparagine

Ile A A T G C A A T G C G T A A T A T T G G T T T A A A A A T G C
G T A A T A T T G G T T T A A A A A T G C G T A A T A T T G G
T T T A A A A A T G C G T A A T A T T G G T T T A A A A A T G
C G T A A T A T T G G T T T A A A A A T G C G T A A T A T T G
G T T T A A A A A T G C G T A A T A T T G G T T T A A A A A T
G C G T A A T A T T G G T T T A A A A A T G C G T A A T A T T
G G T T T A A A A A T G C G T A A T A T T G G T T T A A A A A
T G C G T A A T A T T G G T T T A A A A A T G C G T A A T A T
T G G T T T A A A A A T G C G T A A T A T T G G T T T A A A A
A T G C G T A A T A T T G G T T T A A A A A T G C G T A A T A
T T G G T T T A A A A A T G C G T A A T A T T G G T T T A A A
A A T G C G T A A T A T T G G T T T A A A A A T G C G T A A T
A T T G G T T T A A A A A T G A A T G C G T A A T A T T G G T
T T A A A A A T G C G T A A T A T T G G T T T A A A A A T G C
G T A A T A T T G G T T T A A A A A T G C G T A A T A T T G G
T T T A A A A A T G C G T A A T A T T G G T T T A A A C G T A
A T A T T G G T T T A A A A A T G C G T A A T A T T G G T T T
A A A A A T G C G T A A T A T T G G T T T A A A A A T G C G T
A A T A T T G G T T T A A A A A T G C G T A A T A T T G G T T
T A A A A A T G C G T A A T A T T G G T T T A A A A A T G C G
T A A T A T T G G T T T A A A G T A A T A T T G G T T T A A A
etc

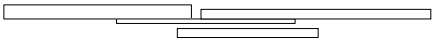
- One of most important scientific achievements is this book of 3,000,000,000,000 letters in code
- Only just finding out which bits are important
- Much of it appears to be rubbish

How does resistance develop

- 3 base-pairs = an amino acid (codon) - bases are letters and amino acids are like words
- Groups of amino acids are called genomes - like paragraphs
- For human DNA this is like a recipe with 1,000,000,000,000 words
- For HIV it is like 9,200 words
- If one of the words change, it can change the recipe
- Some of this recipe changes are like resistance

HIV genetic code

- 9,200 amino acids



- Sometimes shown as overlapping block - a diagram to show the virus structure
- One part is responsible for one part of the recipe - for example - reverse transcriptase - a section of 400 amino acids (codon)
- These are numbered 1 to 400 in the RT gene

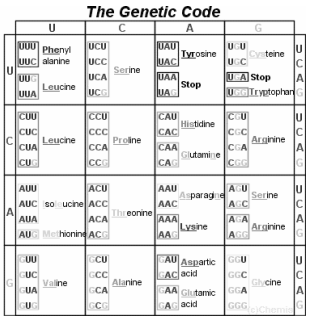
How does resistance develop?

- If one base pair changes, this can change the amino acid at one codon into a different amino acid
- For some drugs, one mutation is enough to stop the drug working:

Ile M184V in RT (methionine to valine) stops 3TC from working

K103N - for NNRTIs

Bases and amino acids



Recipe changes

- In a large mixing bowl - make well in 1 lb (450g) stoneground flour and add 1 1/2 level tspns fast action dried yeast. Mix with 13/14 fl oz (360 ml) water at body heat by hand until dough feels elastic and leaves sides of bowl clean.
- Put in warmed greased 2lb loaf tin and then leave covered in a warm place for approx 20 mins and dough will have risen to just below rim of tin. Bake in hot oven 400F/200 C for 35 mins. Bread will sound hollow when tapped.
- Variations: Sprinkle poppy seeds thickly and press down gently on dough

Some DNA code is rubbish

- In a large xxxxx mixing xxxxx bowl - make well in 1 lb (450g) stoneground xxxxx xxxxx xxxxx flour and add 1 1/2 level tspns fast xxxxx action dried yeast. Mix with 13/14 fl oz (360 ml) water at body heat by hand until dough feels elastic and leaves sides of bowl clean. xxxxx xxxxx xxxxx xxxxx xxxxx xxxxx xxxxx xxxxx xxxxx xxxxx xxxxx xxxxx
- Put in warmed xxxxx xxxxx xxxxx xxxxx greased 2lb loaf tin and then leave covered in a warm place for approx 20 mins xxxxx xxxxx xxxxx xxxxx xxxxx xxxxx xxxxx and dough will have risen to just below rim of tin. xxxxx xxxxx xxxxx xxxxx xxxxx xxxxx etc

Recipe changes

- Some change make no difference:
  "Mix with 13/14 fl oz (360 ml) water at body heat by hand until dough feels elastic and leaves sides of bowl clean. xxxxx xandx xxxxx xxxxx xxxxx xxxxx xxxxx xxxxx xxxxx xxxxx xxxxx xxxxx xxxxx"
- Some change make a big difference:
  "Put in warmed xxxxx xxxxx xxxxx xxxxx greased 2lb t-shirt and then leave covered in a wet place for approx 20 mins xxxxx xxxxx xxxxx"

and
blue
bowl
glue
t-shirt
always
wet
hello
cold
radio
car

What is resistance?

- Some mutations have no effect
- Some may improve the virus
- Some make it less efficient
- Some mutations stop a drug from working

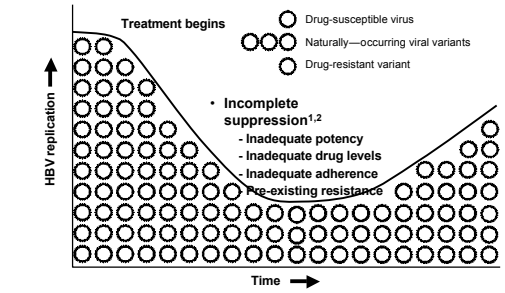
What is resistance?

- Virus with no mutations that cause drug resistance = Wild-Type
- Virus with no mutations that cause drug resistance = Resistance

Drug pressure

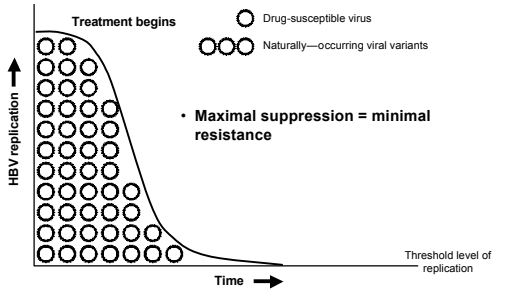
- Single / double mutations pre-exist in HIV from patients prior to therapy:
  WHY 1-2 drug THERAPIES FAIL
- Triple / quadruple mutations require replication in the presence of selection pressure, thus their preexistence of antiviral therapy is very rare/absent:
  WHY COMBINATION THERAPY WORKS

Incomplete suppression of viral load



1. Fung SK & Lok ASF. *Antivir Ther* 2004; 9:1013–1026
2. Locarnini S, et al. *Antivir Ther* 2004; 9:679–693

Maximal suppression (<50 copies/mL



Locarnini S, et al. *Antivir Ther* 2004; 9:679–693

Viral fitness

- Viral fitness = how well it reproduces
- High HIV turnover means every mutation is produced
- But not all on the same single virus
- ie each quasispecies may have only one mutation
- Using 3 drugs makes sure every there is at least two drugs for each mutation

Viral fitness

- When the drug is there - drug pressure - the mutation stays around
- When the drug goes away - the relative advantage that the resistant virus has over wild-type has gone - so wild-type comes back
- But resistance is still there even though the test doesn't show it
- To work out resistance, someone's treatment history is as important as a resistance test

How does resistance occur?

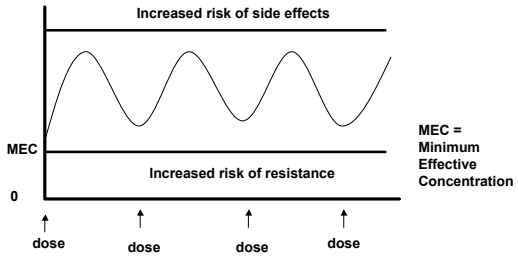
- Resistance only develops when there is ongoing replication (detectable viral load) AND someone is taking drugs
- Mutations have no reason to keep reproducing because they are usually less 'fit'

How can you stop resistance?

- My adherence which gives you the best drug levels
- This will minimise your risk of drug levels dropping below the MEC for all 3 drugs
- If your viral load gets to less than 50 copies/mL, then resistance is very unlikely - because there is not enough HIV replication

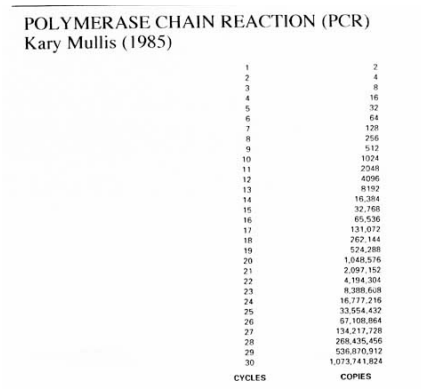
Drug levels and resistance

The target drug level needs to be above the MEC to avoid resistance and not so high as to cause side effects



How is resistance measured?

- There are two main ways to measure and describe resistance
- GENOTYPIC resistance looks for changes in the structure of HIV - results as given as specific mutations ie M184V
- PHENOTYPIC resistance measures how effect a drug in against a sample of virus in a test tube - results as given as 'fold-changes' in a drugs activity



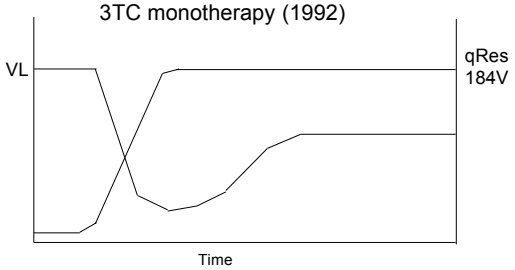
Evidence - *In Vitro (recombinant virus technology)*

Drug	Codon Change	Change Sensitivity
AZT	M41L (ATG-TTG)	4 fold decrease
	D67N (GAC-AAC)	-
	K70R (AAA-AGA)	8 fold decrease
	L210W (TTG-TGG)	2 fold decrease
	T215Y (ACC-TAT)	60-70 fold decrease
	K219Q (AAA-CAA)	5 fold decrease
Combinations	60 + 70 + 215	180 fold decrease
	41 + 70 + 215	180 fold decrease
3TC	M184V	1000 fold decrease

Evidence - *In Vivo*

Failure of virological suppression is associated with evolving resistance

3TC monotherapy (1992)



Transmitted drug resistance

- Studies report prevalence of drug resistance in ARV-naïve patients:
  - Newly infected (11–15%)
  - Newly diagnosed (7–11%)
- Persistence of transmitted resistant virus (median follow-up 2.1 years)
  - NNRTI resistance in 10/14 patients
  - Resistant virus persistently detectable in 13/14 patients
  - Mean time to first detectable wt/resistant mixture was 103 weeks (95% CI: 49–216)
- **Response to therapy in patients with transmitted resistance (AIEDRP)**
  - **NNRTI (n=67), PI (n=18), NRTI (n=25): some with MDR virus**
  - **45% (38/84) failed to suppress, best response in those receiving >2 active drugs (p=0.01)**

Little S, *et al. 14th CROI*, Los Angeles 2007, #60

ACTG 5095: Baseline resistance

- A5095: patients treated with EFV and ZDV/3TC or ZDV/3TC/ABC: Selected failures (n=191) and non-failures (n=162)
- Looked at baseline drug resistance for each group

Kuritzkes D, *et al. 14th CROI*, Los Angeles 2007, #665

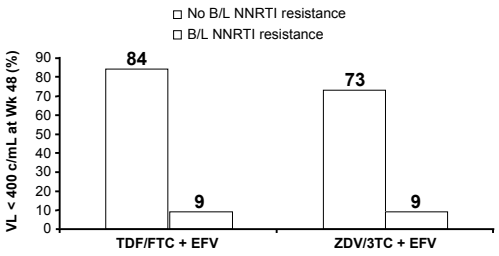
ACTG 5095: Baseline resistance

Drug	Failures	Controlled
EFV	**16 (8%)**	**3 (2%)**
3TC	1 (1%)	0 (0%)
PI	6 (3%)	3 (2%)

- **NNRTI-resistance was associated with a 2.3-fold increased risk of failure (3-fold increased risk when adjusted for race/ethnicity and adherence)**

Kuritzkes D, *et al. 14th CROI*, Los Angeles 2007, #665

GS934: Baseline NNRTI Resistance Markedly Reduces Virologic Response



Gallant JE, et al. N Engl J Med. 2006;354:251-260.

Cross resistance?

- Resistance to one drug can be cross-resistant to others in the same family
  ie K103N stops nevirapine and efavirenz
- Nukes and PIs have more complication resistance - ie need several steps and mutations to stop working
- Tenofovir ans AZT cross-resistance
- New drugs are developed to work against resistance

What are the implications of resistance?

- Once you get resistance, it doesn't disappear
- Resistance can only be detected while you are on treatment
- Resistance to one drug can be cross-resistant to others in the same family
- When you stop treatment, those mutations drop to a small minority until eventually they are so rare that the test cannot find them.
- BUT, when you restart treatment the mutations come back - survival of the fittest!

Stopping drugs with different half lives

S. Taylor et al. 11th CROI Abs 131