ARV resistance and why it matters (especially for tenofovir)

Dr Edmund Wilkins
The aim of this session

• What is resistance?
• Why does it occur?
• Why are we so bothered?
• The resistance test – when, why and how?
• The virological benefits and concerns of using TDF 1st line
• How do we minimise the risk of resistance, particularly to tenofovir?
What is resistance?
Basic Theory – what is resistance

- Mutations of the viral genetic material that result in the drugs no longer being able to block viral replication
What is happening at the molecular level?

**NRTI**
- RNase-H
- Viral RNA
- DNA chain produced by reverse transcriptase

**NNRTI**
- Inhibitor binds to reverse transcriptase and denatures it
- Non-nucleoside reverse transcriptase inhibitor
- Reverse transcriptase
- Viral RNA
- Enzyme cannot produce viral DNA
Why Does it Occur?
Because of the virus...

- HIV has a high mutation rate
  - Makes mistakes when replicating itself
  - Therefore lots of potential to develop resistance
    - >1 billion viral particles made/day
    - $\rightarrow 1-10^6$ mutations/day
    - Every single mutation is possible every single day...
Because of the virus...

- Low barrier to resistance
  - It doesn’t take many resistance mutations to knock out a drug
  - These mutations not ‘lethal’
  - Limited effect on virulence
Because of the drugs..

- Viral replication in presence of detectable drug(s) because they are not potent enough
Because of the drugs...

• Viral replication in presence of detectable drug(s) when there is pre-existing/emergent resistance
Often because of past mistakes

- Drugs added one by one as they became available
- Temporary CD4 improvement and VL fall with each change
- No resistance test available
- Increasing/overlapping toxicity
- Doctors/patients trying everything
The consequence!

<table>
<thead>
<tr>
<th>Trade Name</th>
<th>Generic Name</th>
<th>Mutations</th>
<th>Interpretation</th>
<th>Additional Information</th>
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<tbody>
<tr>
<td>Retrovir®</td>
<td>Zidovudine</td>
<td>✓</td>
<td>Evidence of Resistance</td>
<td>AZT resistance may be reversed</td>
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<tr>
<td>Epivir®</td>
<td>Lamivudine</td>
<td>✓</td>
<td>Evidence of Resistance</td>
<td>184: characteristic mutation</td>
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<tr>
<td>Videx®</td>
<td>Didanosine</td>
<td>✓</td>
<td>Evidence of Resistance</td>
<td></td>
</tr>
<tr>
<td>Hivid®</td>
<td>Zalcitabine</td>
<td>✓</td>
<td>Evidence of Resistance</td>
<td></td>
</tr>
<tr>
<td>Zerit®</td>
<td>Stavudine</td>
<td></td>
<td>No Evidence of Resistance</td>
<td></td>
</tr>
<tr>
<td>Ziagen®</td>
<td>Abacavir</td>
<td>✓</td>
<td>Evidence of Resistance</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Adefovir</td>
<td></td>
<td>No Evidence of Resistance</td>
<td></td>
</tr>
<tr>
<td>Viramune®</td>
<td>Nevirapine</td>
<td>✓</td>
<td>Evidence of Resistance</td>
<td></td>
</tr>
<tr>
<td>Rescriptor®</td>
<td>Delavirdine</td>
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<td>Evidence of Resistance</td>
<td></td>
</tr>
<tr>
<td>Sustiva®</td>
<td>Efavirenz</td>
<td>✓</td>
<td>Evidence of Resistance</td>
<td></td>
</tr>
<tr>
<td>Crixivan®</td>
<td>Indinavir</td>
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<tr>
<td>Norvir®</td>
<td>Ritonavir</td>
<td>✓</td>
<td>Evidence of Resistance</td>
<td></td>
</tr>
<tr>
<td>Viracept®</td>
<td>Nelfinavir</td>
<td>✓</td>
<td>Evidence of Resistance</td>
<td></td>
</tr>
<tr>
<td>Invirase®/Fortovase®</td>
<td>Saquinavir</td>
<td>✓</td>
<td>Evidence of Resistance</td>
<td></td>
</tr>
<tr>
<td>Agenerase®</td>
<td>Amprenavir</td>
<td>✓</td>
<td>Evidence of Resistance</td>
<td></td>
</tr>
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</table>
Because of the patient...

• Viral replication in presence of detectable drug(s) where poor adherence or drug-drug interactions
Why Are We So Bothered?
Once resistance develops it is always there – archived
Archived NNRTI Resistance Markedly Reduces Treatment Response

One mutation may be all that is needed

• A single mutation may wipe out activity…..
  • M184V - lamivudine or emtricitabine
  • K103N – nevirapine
One mutation may mean that other drugs have no/reduced activity.

Single point mutations in the NNRTI binding pocket (e.g. K103N) lead to VF

- As EFV and NVP share similar binding sites, mutations often lead to cross resistance to the other agent\(^2\)
- NNRTI resistance accumulation can compromise the efficacy of second-generation NNRTIs\(^3\)

The Resistance Test – when?
Case 1

• 33 year old heterosexual male

• Presents with oropharyngeal and oesophageal candidiasis

• CD4 142 cells/mL

• Viral load 37,567 copies/ml

• Started on Septrin

Options:
- ZDV or TDF
- 3TC or FTC
- EFV or NVP
He receives EFV/AZT/3TC

- 33 year old heterosexual male
- Presents with oropharyngeal and oesophageal candidiasis
- CD4 142 cells/mL
- Viral load 37,567 copies/ml
- Started on Septrin

Options:
- ZDV or TDF
- 3TC
- EFV or NVP
Response to therapy EFV/AZT/3TC
Response to therapy – 6m later

Viral Load

Time
Resistance test at failure: VL 800

Viral Load

Time

VL 800

K103N/Y181C=NNRTIs

M184V=3TC
Resistance test of baseline sample
Response to therapy – 12m on

Viral Load

Time

- TAMS=AZT
- K103N/Y181C=NNRTIs
- M184V=3TC

400
50
Evolution of resistance

- Increasing number of mutations
- Accumulation of mutations on the same viral genome
- INCREASING RESISTANCE
The resistance test – why?
For many drugs the more mutations the more resistance...

Accumulation of TAMs:


Susceptible  Partial Resistance  Resistance

Number of TAMs present
The more AZT resistance, the more abacavir resistance

- WT (n=15): 40% <400 c/mL, 80% <400 c/mL or 0.5 log$_{10}$ decrease
- 184V (n=75): 64% <400 c/mL, 88% <400 c/mL or 0.5 log$_{10}$ decrease
- 1-2 Muts (n=29): 41% <400 c/mL, 72% <400 c/mL or 0.5 log$_{10}$ decrease
- 3 Muts (n=19): 5% <400 c/mL, 37% <400 c/mL or 0.5 log$_{10}$ decrease
- 4+ Muts (n=28): 11% <400 c/mL, 25% <400 c/mL or 0.5 log$_{10}$ decrease

Data on file, GlaxoSmithKline
The more AZT resistance the more tenofovir resistance

<table>
<thead>
<tr>
<th>Group</th>
<th>Tenofovir DF</th>
<th>Placebo</th>
</tr>
</thead>
<tbody>
<tr>
<td>All patients</td>
<td>222</td>
<td>110</td>
</tr>
<tr>
<td>No TAMs</td>
<td>68</td>
<td>29</td>
</tr>
<tr>
<td>1 or 2 TAMs</td>
<td>55</td>
<td>33</td>
</tr>
<tr>
<td>3 or more TAMs + M41L or L210W</td>
<td>57</td>
<td>42</td>
</tr>
<tr>
<td>3 or more TAMs / No M41L or L210W</td>
<td>19</td>
<td></td>
</tr>
</tbody>
</table>

n = All responses were statistically significant versus placebo.
The resistance test – how?
How does the resistance work?
What do these mean.....
Before 3TC

“M” is the “wild type” amino acid

“184” is the codon position

“M” is the wild type amino acid
After 3TC and resistance...

- How do we identify a resistance mutation?

“M” is the “wild type” amino acid

“184” is the codon position

“V” is the mutant amino acid
A prediction of phenotype is then used to give the report.
Limitations of resistance testing

• Population sequencing
  • Standard resistance testing will only detect mutations that are in >20% of the circulating virus

• Archived resistance
  • May be so low they cannot be detected.... But they are still there.... and will rapidly re-emerge under drug pressure
  • So you need to look at all ART when VL failure
  • And maybe make a guess
The virological benefits of using TDF 1st line?
What if with case 1..

- 33 year old heterosexual male
- Presents with oropharyngeal and oesophageal candidiasis
- CD4 142 cells/mL
- Viral load 37,567 copies/ml
- Started on Septrin

Options:
- ZDV or TDF
- 3TC/FTC
- EFV or NVP
Case 1 with EFV/TDF/3TC: VL 800

Viral Load

K103N/Y181C=NNRTIs
M184V=3TC

Time
Case 1 with EFV/TDF/3TC: VL 1000

Viral Load

K65R

K103N=NNRTIs

K103N/Y181C=NNRTI

M184V=3TC
Susceptibility to NRTIs if K65R and M184V develop

PhenoSense Results for K65R + M184V (n=58)

<table>
<thead>
<tr>
<th>Nucleoside Inhibitor</th>
<th>% of Viruses</th>
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<tbody>
<tr>
<td>AZT</td>
<td>100%</td>
</tr>
<tr>
<td>d4T</td>
<td>100%</td>
</tr>
<tr>
<td>TDF</td>
<td>90%</td>
</tr>
<tr>
<td>ABC</td>
<td>55%</td>
</tr>
<tr>
<td>ddl</td>
<td>42%</td>
</tr>
<tr>
<td>ddC</td>
<td>18%</td>
</tr>
<tr>
<td>3TC</td>
<td>3%</td>
</tr>
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Miller et al (2003) 43rd ICAAC #H-904 and presentation
Hence sequencing Options: PI AND....

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<thead>
<tr>
<th>NNRTI +</th>
<th>TDF + 3TC/FTC</th>
<th>K65R +184V</th>
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<tr>
<td>AZT/d4T + 3TC/FTC</td>
<td>184V +/- TAMs</td>
<td></td>
</tr>
<tr>
<td>3TC/FTC</td>
<td>184V</td>
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Hence sequencing Options: PI AND....

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<th>NNRTI +</th>
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<th>Resistance to TDF/3TC/FTC/ddI and ABC</th>
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<th>AZT/d4T + 3TC/FTC</th>
<th>Resistance to 3TC/FTC Maybe Broader NRTI-class Resistance</th>
<th>Options?</th>
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<th>Resistance to 3TC/FTC</th>
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<td>184V</td>
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Hence sequencing Options: PI AND….

**NNRTI +**

- **TDF + 3TC/FTC**
  - K65R +184V
  - Resistance to TDF/3TC/FTC/ddi and ABC
  - Options?

**AZT/d4T + 3TC/FTC**

- 184V +/- TAMs
  - Resistance to 3TC/FTC
  - Maybe Broader NRTI-class Resistance
  - Options?

**3TC/FTC**

- 184V
  - Resistance to 3TC/FTC
  - Options?

**Boosted PI +**

- AZT (yes, ↑ activity)
- d4T (yes)
- TDF (maybe)

TDF, ABC (if <3-4 TAMs) and depending on pattern

AZT (yes, ↑ activity)
ABC, d4T, ddl (yes)
TDF (yes, ↑ activity)
The virological concerns of using TDF 1st line?
What about tenofovir?

- Recommended with FTC/3TC as 1\textsuperscript{st} line NRTI backbone in all guidelines

- Over 15 million globally receiving ART

- Increasingly used around the world in treatment and prophylaxis
  - AZT and NVP being phased out
  - EFV preferred NNRTI
Resistance rate to TDF

- Signature mutation is K65R
- Resistance rare in high income countries but much commoner in low/middle
TDF resistance

• 20% in Europe to >50% of isolates in sub-Saharan Africa - 39% in Asia

• Estimated that 15-35% of patients on ART in SSA have ARV resistant virus by 12 months

  • Likely that 5-10% will develop TDF resistance WITHOUT VL monitoring

• Commoner with subtype C (also D4T may generate)

• Associated with:
  • Lower CD4 <100
  • 3TC use vs. FTC
  • NVP use vs. EFV
But TDF is well tolerated with minimal long-term toxicity
So how do we minimise risk of resistance particularly tenofovir?
Follow national Guidelines

**Recommended**
- ZDV
- TDF
- 3TC/FTC
- EFV
- NVP

**Alternative**
- ZDV or TDF
- and
- Dolutegravir ABC/3TC
- and
- 3TC
- and
- EFV or NVP

70% 25%
Viral load measurements and resistance

• More frequent routine VL testing will detect virological failure earlier:
  
  • Identifies patients before resistance has developed
  
  • Allows earlier switch
  
  • New regimen more likely to be active
  
  • More valuable than basing solely on clinical, CD4 markers
Viral load measurements and resistance

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  • Identifies patients before resistance has developed
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  • More valuable than basing solely on clinical, CD4 markers

• Viral load levels at which resistance likely to develop
  • 300 – 500: 3TC, EFV and NVP
  • 500 – 1000: AZT and ABC
  • 1000: TDF
  • Rare irrespective of VL: boosted PIs
Your reality!

• Financial constraint and human resources
• Technical expertise
• Transportation of sample from remote areas to the PCR sites
• Infrastructure (electricity etc.)
• Coordination between national lab and township level, within laboratory, and within service provider
• Quality control and trouble shooting
Naïve patient

• Follow National ART guidelines = potent

• TDF with FTC/3TC preferable for sequencing options

• ? Any national resistance surveillance data

• ?? Baseline resistance test

• Check on adherence/tolerability etc.

• If VL fails to fall OR rebounds further resistance test

• Switch early <1000 c/ml
Experienced patient

• Know your patient’s ART history and:
  • What he was on when his VL increased OR his CD4 fell
  • What he wasn’t taking properly/was suboptimal treatment

• Resistance test before new regimen
• If none
  • Assume NNRTI/3TC resistance
  • Boosted PI based new combination
  • NRTI choice dependent on 1st line treatment
Targeted resistance testing

• Baseline resistance testing:
  • Prevents use of partially resistant combinations which may lead to selection of resistance
  • Early virological failure
  • Restricted options

• Resistance testing at first VL >400
  • By definition NOT a blip
  • Early detection of NNRTI and 3TC resistance
  • Preservation of TDF sensitivity
  • Allows continuing use of TDF
Management of suspected failure

VL RESULT

VL > 1000
Viral blip or virological failure

Screen and treat OI
Screen and treat STI
Reassess adherence
Repeat VL after 3-6 months

VL > 1000
Switch to Second Line

VL < 1000
Maintain First line RX
The aim of this session

- What is resistance?
- Why does it occur?
- Why are we so bothered?
- The resistance test – when, why and how?
- The virological benefits and concerns of using TDF 1\textsuperscript{st} line
- How do we minimise the risk of resistance, particularly to tenofovir?
Discussion and questions?