Understanding HIV resistance

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Objectives

• Pass the DipHIV
  - Background to ARV resistance
  - Practice OSCE cases
  - This focuses specifically on the OSCE, it’s not everything you need to know about resistance in clinical practice!
Resistance in Dip HIV

- BHIVA 2016 Monitoring Guidelines
  - Mainly: 3.11; 3.3; 4.3.3.3; 4.5.2.3; 5.10; Appendix 1
- BHIVA 2015 Antiretroviral Guidelines
  - Mainly: 7.0-7.5
- Beware of straying away from the guidelines, regardless of your individual clinic’s policy
- Other resources: www.iasusa.org Stanford
Basics
Basics (1)

- HIV exists as a quasispecies
- High replication rate and error prone
- Mutations arise
- Mutation → amino acid substitution → altered protein structure, e.g. M184V
- Inadequate drug pressure → DRM selected for → overt virological failure OR gradual accumulation of additional resistance mutations.
Basics (2)

• Ongoing virus replication under drug pressure → evolution of resistance

• Once drug pressure removed, resistant mutants outgrown by fitter wild-type virus → become undetectable by routine tests

• Resistant mutants persist at low frequency in plasma & are “archived” in latently infected cells
Key Terminology

- **Viral fitness**: ability of a virus to out-compete another
- **Replication capacity**: ability of the virus to replicate in comparison to another virus
- **Signature Mutation**: mutation typically associated with resistance to a particular drug
- **Cross resistance**: resistance to drugs other than the drug that selected the mutation
- **Hypersusceptibility**: resistance to 1 drug can lead to ↑ susceptibility to another
Types of resistance

• **Transmitted resistance:** ARV resistance of HIV in individuals who have never received treatment
  – Takes time to revert back to WT: M184V lost rapidly (1yr), NNRTI/PI mutations (2.7-5.8yrs), most TAMs lost slowly
  – Most is TAMs, esp T125rev
  – UK TDR stable at 10%

• **Acquired drug resistance:** resistance of HIV to drugs in individuals on treatment
Guidelines for starting ART with TDR/no resistance test yet

• **BHIVA monitoring:** regimen with high barrier to resistance (e.g. PI/b) should probably be selected when low minority variants compromising susceptibility to NNRTIs detected

• **EACS 2016:** If ART has to start before the resistance test results are available, include a PI/b in order to increase the barrier to resistance of the regimen – evolving evidence - TDF (or TAF) + FTC (or 3TC) + DRV/b + integrase inhibitor

• **DHHS 2016:** Avoid NNRTI-based regimens if need to start ART before RT available; consider DRV/r or Dolutegravir 3rd agent
Tests for Drug Resistance
Tests for Drug Resistance

• Genotype
  – *In vitro* MOLECULAR assessment of changes in the HIV nucleoside sequence associated with resistance

• Phenotype
  – *In vitro* BIOLOGICAL assessment of HIV growth in the presence of drug (IC$_{50}$ = conc$^n$ of drug to impair growth by 50%). Threshold for clinical significance varies (2.5-5 fold)
### NRTI Resistance Mutations:

### NNRTI Resistance Mutations:
- Y181C

### Other Mutations:
- None

### Nucleoside RTI

<table>
<thead>
<tr>
<th>Drug</th>
<th>Resistance</th>
</tr>
</thead>
<tbody>
<tr>
<td>lamivudine (3TC)</td>
<td>High-level resistance</td>
</tr>
<tr>
<td>abacavir (ABC)</td>
<td>High-level resistance</td>
</tr>
<tr>
<td>zidovudine (AZT)</td>
<td>High-level resistance</td>
</tr>
<tr>
<td>stavudine (D4T)</td>
<td>High-level resistance</td>
</tr>
<tr>
<td>didanosine (DDI)</td>
<td>High-level resistance</td>
</tr>
<tr>
<td>emtricitabine (FTC)</td>
<td>High-level resistance</td>
</tr>
<tr>
<td>tenofovir (TDF)</td>
<td>Intermediate resistance</td>
</tr>
</tbody>
</table>

### Non-Nucleoside RTI

<table>
<thead>
<tr>
<th>Drug</th>
<th>Resistance</th>
</tr>
</thead>
<tbody>
<tr>
<td>efavirenz (EFV)</td>
<td>Intermediate resistance</td>
</tr>
<tr>
<td>etravirine (ETR)</td>
<td>Intermediate resistance</td>
</tr>
<tr>
<td>nevirapine (NVP)</td>
<td>High-level resistance</td>
</tr>
<tr>
<td>rilpivirine (RPV)</td>
<td>Intermediate resistance</td>
</tr>
</tbody>
</table>

### RT Comments

**NRTI**
- M41L is a TAM that usually occurs with T215Y. Together, M41L and T215Y confer high-level resistance to AZT and d4T and intermediate-level resistance to ddi, ABC and TDF. However, viruses with M41L + T215Y + M184V will exhibit intermediate-level resistance to AZT and d4T and low-level resistance to TDF.
- D67N is a nonpolymorphic TAM associated with low-level resistance to AZT and d4T. When present with other TAMs, it reduces susceptibility to ABC, TDF and ddi.
- T69N is a relatively non-polymorphic mutation weakly selected in patients receiving NRTIs. Their effects on NRTI susceptibility have not been well studied.
- K70R causes intermediate-level resistance to AZT and possibly low-level resistance to d4T and TDF.
- M184V/I cause high-level resistance to 3TC and FTC and low-level resistance to ddi and ABC. However, M184V/I are not contraindications to continued treatment with 3TC or FTC because they increase susceptibility to AZT, TDF and d4T and are associated with clinically significant reductions in HIV-1 replication. In combination with K101E or E138K, M184I synergistically reduces RPV susceptibility.
- T215F is a TAM that causes intermediate/high-level resistance to AZT and d4T and low-level resistance to ABC, ddi and TDF. Compared with T215Y, T215F occurs more commonly with the Type II TAMs (D67N, K70R, and/or K219E) and in this context, it affects susceptibility to TDF, ABC, and ddi less markedly than T215Y.
- K219Q/E are accessory TAMS associated with reduced susceptibility to AZT and possibly d4T.

**NNRTI**
- Y181C is a nonpolymorphic mutation selected in patients receiving NVP, ETR and RPV. It reduces susceptibility to NVP, ETR, RPV, and EFV by >50-fold, 5-fold, 3-fold, and 2-fold, respectively. Although Y181C itself reduces EFV susceptibility by only 2-fold, it is associated with a reduced response to an EFV-containing regimen because viruses with this mutation often harbor additional minority variant NNRTI-resistance mutations. Y181C has a weight of 2.5 in the Tibotec ETR GSS.
Advantages & Limitations of Genotypic Resistance Testing

- Cost
- Reliability with lower viral loads (<200 copies/mL)
- Ability to detect minority populations of resistant virus if < 20% of the sample (common after drug discontinuation)
- Resistant strains that are in sanctuary sites may not be detected
- Role of Next Generation/Deep Sequencing
Baseline resistance test

• Genotypic resistance test: RT & protease
• Sample closest to diagnosis
• Repeat test prior to ART only if ?ART exposure or superinfection
• Integrase only if other baseline TDR mutations or ?transmitted INSTI resistance
• Genotypic tropism test performed just prior to starting MVC
Resistance test for failure/suboptimal response to initiation

- All genes encoding proteins are targeted by current & future treatment agents
- Review all previous drug resistance reports
- Cumulative Stanford HIV drug resistance report
- Repeat tropism test if fail on MVC
- Lower threshold in pregnancy
- Sample whilst on failing regimen or within 2-4/52 of discontinuation
HIV Tropism
HIV Tropism Patterns

- **CCR5 tropic (R5)**
- **CXCR4 tropic (X4)**
- **Dual tropic**
- **Mixed tropism**
- **Dual/Mixed (D/M)**
Tropism test

• Just prior to starting/switching to MVC
• Genotypic test (phenotypic not in UK)
• Can do on PBMCs if VL<500 copies/mL
  – Technical difficulties of working with PBMCs
  – Sensitivity of PBMCs picking up minority X4 virus
• At failure, 2/3 have tropism switch to X4
• If remain R5 tropic, 1/3 have MVC-specific resistance - no genotypic signature mutations (phenotypic resistance) so can’t test for
Important mutations
Thymidine Analogue Mutations (TAMs)

- **d4T or AZT**
  - **COMMONER**
    - Common with dual therapy (incl AZT/3TC)
      - 41L
      - 210W
      - 215Y
    - Higher-level ZDV and d4T resistance
      - More NRTI cross-resistance
        - Including TDF
      - Less reversal with M184V
  - 67N
    - 70R
    - 219Q/E
    - Lower-level ZDV and d4T resistance
      - Less NRTI cross-resistance
      - More reversal with M184V

- Common with AZT monotherapy
What does it mean?

• TAMs still commonest *transmitted* mutations in UK
• In treated individuals, if there are TAMs there may be other mutations lurking around
• 2 NRTI may not be robust enough and therefore will not support:
  – NNRTI (TMC-C227)
  – Integrase inhibitor (SWITCHMRK-1 & -2)
NRTI: M184V/I

• Selected by 3TC/FTC & to a lesser extent ABC
• High level resistance (>100 fold loss of susceptibility) to 3TC/FTC
• With 1+ TAM – ABC resistance
• Increases susceptibility to ZDV, d4T and TDF especially in presence of TAMs (especially 215)
• Appears to delay development of TAMs
• Reduces viral replication fitness
Other important NRTI mutations

• **K65R**
  – Selected by TDF and ABC, also d4T and ddI
  – Reduced susceptibility to all non-thymidine NRTIs
  – Increased susceptibility to AZT & d4T (especially in presence of TAMs)
  – Usually preceded by M184V: increases R to ABC but reduces to TDF

• **L74V**
  – Selected by & causes resistance to DDI & ABC
  – Increased susceptibility to AZT & d4T, especially in presence of TAMs

• **Others:** Q151M complex a/w resistance to all NRTIs (least effect on Tenofovir), T69 same when accompanied by TAMs 41, 210, 215
Key NNRTI mutations

50-70% of NNRTI failures have these 2 mutations:

• **K103N**
  – high level resistance to NVP and EFV (1\textsuperscript{st} gen NNRTI)
  – Does not cause ETV or RPV resistance (2\textsuperscript{nd} gen NNRTI)
  – Most common mutation in Europe

• **Y181C**
  – high level resistance to NVP and intermediate to EFV and Etravirine
  – Can cause \textit{hyper-susceptibility to AZT and TDF} esp in presence of TAMs
Resistance to 1\textsuperscript{st} generation NNRTIs

- Prior NNRTI resistance reduces subsequent response to re-introduction of NNRTIs
- Low level K103N may be archived
- Switch quickly to prevent selection of other mutations that may impair response to 2\textsuperscript{nd} gen NNRTI (etravirine)
- Most will be susceptible to ETR; 62\% to Rilpivirine
- Rilpivirine: E138K
### Weighting mutation system of ETR RAMs (from DUET Studies)

#### Relative weight* for individual ETR RAMs

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<thead>
<tr>
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<th>1</th>
<th>1.5</th>
<th>2.5</th>
<th>3</th>
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<tbody>
<tr>
<td>V901</td>
<td>V106I</td>
<td>L100I</td>
<td>Y181I</td>
<td></td>
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<tr>
<td>A98G</td>
<td>E138A</td>
<td>K101P</td>
<td>Y181V</td>
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<td>K101E</td>
<td>V179F</td>
<td>Y181C</td>
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<tr>
<td>K101H</td>
<td>G190S</td>
<td>M230L</td>
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<tr>
<td>G190A</td>
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#### Total weighted genotypic score

- **0–2**: Highest (74.4%)
- **2.5–3.5**: Intermediate (52%)
- **≥ 4**: Reduced (37.7%)

#### Example: K101H + G190A = weighted score of 2 = highest response

*When the genotype report shows a mixture of two or more different substitutions at the same position, only the highest of the individual weight factors for these substitutions is counted when calculating the weighted genotypic score.*

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Protease Resistance

- Too many to remember!
- D30N, N88D, L90M – ‘historic’ (often unboosted) PIs
- I50L signature mutation of unboosted Atazanavir
- **What’s important**
  - Darunavir usually best option in setting of PI resistance
  - If ≥2 DRV RAMs use BD darunavir rather than OD darunavir
  - DRV RAMs include: I54L/M, G73S,T74P,L76V,V82F,I84A/C/V,L89V
**PI mutations**

<table>
<thead>
<tr>
<th>MUTATIONS IN THE PROTEASE GENE ASSOCIATED WITH RESISTANCE TO PROTEASE INHIBITORS$^{p,q,r}$</th>
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</thead>
<tbody>
<tr>
<td><strong>Atazanavir</strong></td>
</tr>
<tr>
<td>L 10  16  20  24  32  33  34  35  45  48  50  53  54  60  62  64  71  73  82  84  85  88  90  93</td>
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<tr>
<td>+/- ritonavir$^i$</td>
</tr>
<tr>
<td>I  E  R  I  I  I  Q  I  I  Y  L  L  L  E  V  L  L  C  A  Y  V  S  M  L</td>
</tr>
<tr>
<td>F  M  F  L  L  Y  V  M  I  S  T  M  V  T  T  F</td>
</tr>
<tr>
<td>V  I  V  V  C  T  T  L  A  I</td>
</tr>
<tr>
<td>C  T  V</td>
</tr>
<tr>
<td><strong>Darunavir/ritonavir$^i$</strong></td>
</tr>
<tr>
<td>V  1  11  32  33  47  50  54  74  76  84  89</td>
</tr>
<tr>
<td>I  I  I  V  V  M  P  V  Y  V</td>
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Roughly...

45-55

75-85
Key integrase resistance mutations

• N155H : 10x ↓ RAL susceptibility

• Q148H/R/K: 25x ↓ RAL susceptibility
  • Most common pattern, Q148H + G140S, > 100x ↓ susceptibility to raltegravir
  • Extensive cross resistance between RAL & EVG: mutations at position 155 and 148

• Y143R

• Q148 alone doesn’t affect DTG much but in combination with E138K it does

• Switch off failing RAL/EVG early!
Dolutegravir

• No resistance in Tx naïve studies

• Tx experienced:
  – SAILING: DTG 50 mg OD statistically superior to RAL BD in the proportion of subjects achieving HIV-1 RNA <50 c/mL - lower virologic failure rate
  – VIKING: DTG 50mg BD effective in subjects with RAL &/or EVG resistance & multiple class ARV resistance – virological suppression in 68% at 24/52 & 56% at 48/52

• DTG 50mg **BD** with known INSTI resistance
### INSTI mutations

#### Mutations in the Integrase Gene Associated with Resistance to Integrase Strand Transfer Inhibitors

<table>
<thead>
<tr>
<th></th>
<th>Dolutegravir&lt;sup&gt;aa&lt;/sup&gt;</th>
<th>Elvitegravir&lt;sup&gt;bb&lt;/sup&gt;</th>
<th>Raltegravir&lt;sup&gt;cc&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Position</strong></td>
<td><strong>F</strong> 121 <strong>E</strong> 138 <strong>G</strong> 140</td>
<td><strong>T</strong> 92 <strong>F</strong> 121 <strong>S</strong> 147</td>
<td><strong>E</strong> 92 <strong>T</strong> 121 <strong>G</strong> 148</td>
</tr>
<tr>
<td><strong>Mutations</strong></td>
<td><strong>Y</strong> A <strong>K</strong> A <strong>A</strong> S</td>
<td><strong>Q</strong> G <strong>Q</strong> G <strong>H</strong> K</td>
<td><strong>Y</strong> <strong>H</strong> <strong>R</strong> <strong>H</strong> <strong>C</strong></td>
</tr>
<tr>
<td><strong>Residues</strong></td>
<td><strong>R</strong> 263</td>
<td><strong>R</strong> 263</td>
<td><strong>R</strong> 263</td>
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<sup>aa</sup> Data from the INSTI resistance database.

<sup>bb</sup> Data from the INSTI resistance database.

<sup>cc</sup> Data from the INSTI resistance database.
What does BHIVA say? No/Limited resistance

### Failure Type
- WT at baseline & no emergent mutation at failure
- WT at baseline & limited emergent resistance (two class NRTI/NNRTI)
- On first line PI/b + 2 NRTI, limited major PI mutation
- Historical NRTI/NNRTI resistance on PI/b regimen

### Recommendation
- PI/b based combination. *Consider* restarting existing regimen
- New PI/b + 1, pref. 2 active drugs
- New active PI/b + 1, pref 2 active drugs (1 novel M.O.A)
- Avoid switching the PI/b to INSTI or NNRTI
What does BHIVA say? Multiple Class Failure

**Failure Type**
- Extensive drug resistance
- Extensive drug resistance + DRV resistance
- Limited/no therapeutic options; fully suppressive regimen cannot be constructed

**Recommendation**
- PI/b (e.g. DRV/r BD) + 2 (pref 3) fully active agents, 1 with novel MOA
- DTG = INSTI of choice
- Newer agents through research trials
  - Consider recycling NRTIs
  - Avoid discontinuing/interrupting ART
  - If triple class resistance and RAL/ETG resistance – BD DTG.
OSCE cases
Case 1

- David is a 39 year old MSM who was diagnosed with HIV 5 years ago and started on Eviplera. He had wild type virus at baseline and was undetectable for a number of years. He was made redundant from his job 3/12 ago. At his routine clinic appointment his viral load was 4200 copies/mL. Resistance screen shows wild type virus. David is very concerned about his VL and wishes to discuss his ART regimen.

- Please discuss ongoing management with David.
What are you going to ask David?

Tolerability
Adherence
Resistance test
Medication
Absorption
Comorbidities

• Does David have any concerns or specific questions at this point?
What are you going to tell David?

- Reason for failure - poor treatment adherence (DDIs/food interactions/resistance)
- Risks of failure
- Review past ART & resistance tests
- Reason for new regimen being chosen
- Starting new regimen discussion
- Transmission risk with detectable VL
- Adherence support (dosette, timers, apps)
- MDT discussion
- Follow up – adherence nurse, pharmacist, PIL, bloods
- Does David have any questions?
Which regimen?

- BHIVA: virological failure on first-line ART with wild-type virus at baseline & without emergent resistance mutations at failure - switch to a PI/b-based combination ART regimen
Case 1 continued

• David’s friend is on ART including darunavir/b and has problems with diarrhoea. David really doesn’t want to take this – he is concerned about side effects and wants to start back on Eviplera which he has tolerated well for 5 years. Please discuss David’s concerns.
What are you going to tell David about restarting Evipleria?

- Side effects of Darunavir, time for these to settle, help controlling any side effects
- BHIVA: Restarting the previous failing regimen is an alternative option, especially where poor adherence has been identified as the likely cause and has been addressed
- Monitor carefully & repeat VL after 4/52
- If inadequate virological response - resistance testing to detect any archived resistance
Case 2

Sandra is 48 years old and was diagnosed with HIV 10 years ago. Baseline resistance test was wild type. She started on TDF/FTC and Efavirenz 8 years ago and has had a suppressed viral load for a number of years. She was lost to follow up 6 months ago and reattended 3 weeks ago. HIV viral load is 8,800 copies/mL, CD4 540 cell/mm³. Please discuss restarting ART with Sandra.

Resistance test
- NRTI: M184V
- NNRTI: K103N
- PI: No resistance mutations
What will you discuss with Sandra?

- Tolerability
- Adherence
- Resistance test
- Medication
- Absorption
- Comorbidities

- Does Sandra have any questions/concerns?
Which regimen (NNRTI resistance)?

- BHIVA: switch to a new PI/b-based regimen with the addition of at least 1, preferably 2, active drugs
- NRTI resistance documented/likely: consider adding new active NRTIs/ARV(s) + PI/b
- Only M184V: recycling of NRTIs may be feasible
- RTG + PI/b as efficacious as PI/r regimen + at least 2 new/recycled NRTIs
Which regimen?

- Don’t go from EFV/NVP to ETR unless new combination including PI/b
- Switching to an INI (raltegravir, elvitegravir or dolutegravir) or maraviroc with two active NRTIs is an option but not recommended if RT mutations/previous NRTI virological failure
Case 3

• Mary is an 22 year old from Zimbabwe who has arrived in the UK 3 weeks ago. She was diagnosed aged 12 years and has received the following treatment regimens:

• D4T/3TC/EFV
• AZT/DDI/LPV/r
• TDF/FTC/DRV/r/ETV/RTG
• ABC/FTC/TDF/AZT (current regimen)
Case 3 continued

• After each switch Mary initially suppressed her viral load, prior to developing virological failure. Her current viral load is 6,490 copies/mL, CD4 count 120 cells/mm$^3$. Mary says she has always missed multiple doses of ART as she has had trouble coming to terms with her diagnosis and does not like taking tablets. Her mother died 5 years ago from an HIV related infection.

• Resistance test results (including historical):
  – NRTI: D67N, T69AD, K70R, M184I/V/M, T215Y, K219Q
  – NNRTI: K103N, Y181C, E138Q/E, V179I/N/D/V

• Tropism: R5
What will you discuss with Mary?

- Tolerability
- Adherence
- Resistance test
- Medication
- Absorption
- Comorbidities

- Does Mary have any questions?
What will you discuss with Mary?

- Resistance MDT/virtual clinic/Stanford
- Co-trimoxazole prophylaxis & risk of OIs
- Psychological support
- Intensive adherence support: nurses/?DOT
- Newer agents: research trials, expanded access & named individual programmes
- Not to discontinue/interrupt ART: reduce risk of disease progression
Which regimen will you choose?

• NRTIs  High level res: ABC/AZT/FTC/3TC
  Intermediate res: TDF

• NNRTIs  High level res: EFV/NVP/RPV
  Intermediate res: ETR

• PIs  High level res: ATZ/r, LPV/r
  Intermediate res: DAR/r

• INSTIs  High level res: EVG/RTG
  Potential low level res: DTG
• Switch to a new regimen with at least 2 & preferably 3 fully active agents with at least 1 active PI/r (e.g. BD DRV/r, OD if no DRV mutations) & 1 one agent with a novel mechanism (INSTI/MVC/T20, ETR also an option based on viral susceptibility)

• Consider inclusion of NRTIs with reduced activity on genotypic testing will provide additional antiviral activity

• Not to add a single, fully active ARV

• With triple-class failure & raltegravir/elvitegravir selected integrase resistance, BD DTG should be included as part of a new regimen where there is at least one fully active agent in the background regimen
You don’t have Stanford in the exam-

- Most robust PI: BD DAR/r
- Most robust INSTI: BD DTG
- ? Recycle most robust NRTI: Truvada
- New agents: MVC (check tropism), T20

“The decision on the best drug regimen for you will need to be discussed at our resistance MDT but this is the regimen that I think that we are likely to advise you to start on…”
Top Tips

• Rehearse a ‘template’ for the station, use a mnemonic if it helps!
• Discuss: potential causes, potential implications, action and follow up.
• Always check patient concerns
• Every resistance station therefore virtually identical in what you need to do
• If you go blank, think what you’d do in real life: d/w consultant, MDT, Stanford
• If all else fails, give Darunavir/b!!
Acknowledgements

• Dr Emily Clarke
• Prof Ravinda Gupta

Good Luck!