Introduction to ARV Drug Resistance
New Clinicians’ Workshop

Susa Coffey, MD
Division of HIV, ID and Global Medicine

Disclosures
I have no disclosures

ARS Question
Which resistance test do you order for ART-naïve patients?
1. Standard genotype (RT and PR)
2. Standard phenotype (RT and PR)
3. Genotype + phenotype (RT and PR)
4. Integrase phenotype
5. RT/PR/IN genotype

Introduction
What this is:
• For new clinicians (not experts)
• Focus on
  • Currently-used ARVs
  • Common resistance scenarios
  • Genotype tests
  • Approaches to interpreting R test results
• Proviral DNA genotype: a few words
Mechanisms of HIV Drug Resistance

- High rate of HIV replication - $10^9$ virions per day
- Many mutations and quasi species
  - RT: error-prone, no copyediting
- In setting of drug pressure with viral replication, selection of resistant viruses
  - <- Inadequate adherence to ART
  - <- Wrong ARVs (potency), wrong doses (drug levels), drug-drug interactions, absorption issues, etc.
  - (Corollary: in absence of drug pressure, possible “disappearance” of mutations [minority populations])

**Bottom Line:** Assume that once there, always there (archived)

Mechanisms of HIV Drug Resistance

- Mutations may decrease susceptibility to ARVs, and cause or contribute to virologic failure
  - (a few mutations may increase susceptibility)
- Some mutations may impair viral fitness
- Some single mutations severely impact certain ARVs (eg, M184I/V)
- In some cases, several mutations are needed to cause a significant impact, esp. with NRTIs and PIs (eg, TAMs)
- Some ARVs may retain residual activity (eg, 3TC/FTC)
- Cross-resistance within an ARV class is common

When to do Resistance Test:
DHHS Recommendations

- Before ART -- at first visit (as close to the time of infection as possible)
  - Resistance mutations more likely to be detected earlier in the course of HIV infection
  - GT: RT, PR; IN if concern for transmitted INI resistance
- Virologic failure -- do on failing ARVs or shortly after d/c (w/in 4 weeks)
  - GT for 1st or 2nd ART, add PT if “known or suspected complex drug resistance pattern”
  - IN GT if virologic failure on INI
- Suboptimal virologic response on ART
- Pregnancy

Case: Transmitted Drug Resistance

- 31 yo man with new diagnosis of HIV, chronicity unknown (no previous HIV test)
- CD4: 525, HIV RNA: 93,000 c/mL
- GT: RT - M41L, K103N; PR – L63P

Genotypic analysis of samples from newly diagnosed patients in CDC National HIV Surveillance System (N = 12,668)

- All cases with sequences
- Cases classified as recent infections
- Cases classified as long-standing infections


INSTI: case reports, but no significant transmitted resistance—0.2% in one study in N. Carolina

Transmitted HIV Drug Resistance in MSM, 2010-2012

Newly diagnosed MSM patients age ≥13 in CDC National HIV Surveillance System, 8 jurisdictions (N = 9,629)

Genotype Test

- Standard GT examines RT and PR
- For IN, must order special test
- With GT, often possible to predict/anticipate resistance depending on the specific mutations, but is not a direct measure of resistance
Phenotype Test

- Measures inhibition of viral replication by individual ARVs in vitro
- IC\textsubscript{50} = concentration of drug required to inhibit viral replication by 50%
- Compares patient virus IC\textsubscript{50} to that of a reference strain, -> fold-change in IC\textsubscript{50} relative to the reference strain
  \[ FC = \frac{IC_{50\text{ patient}}}{IC_{50\text{ reference}}} \]

Limitations of GT and PT

- Conventional tests reliably detect mutant virus that comprises about 20% of the circulating viral quasispecies
- May miss minority populations
- HIV RNA must be >500-1,000 c/mL
- Do not assess interaction of ARVs

NRTI Resistance

- A number of key mutations for 3TC/FTC, TDF/TAF, ABC, + numerous others
- Some single mutations severely impact viral replication, but with others an accumulation of mutations usually required for high-level resistance
  - (More = worse)

NRTI Strategy:

- Know key mutations (M184V, K65R, TAMs)
- Look up others
NRTI Resistance: M184I/V
- Selected by 3TC, FTC (sometimes ABC), cause resistance to 3TC, FTC
- Very common -- 3TC/FTC have low genetic barrier to resistance
- Cross resistance: decreases susceptibility to ABC, ddl (especially if TAMs)
- Increases susceptibility to TDF/TAF, AZT, d4T
  - Partially restores activity if TAMs present
- Decreases viral fitness

NRTI Resistance: K65R
- Selected by TDF/TAF, ABC, ddl
- Causes resistance to TDF/TAF, all other NRTIs except AZT
- Increases susceptibility to AZT
- Decreases viral fitness

NRTI Resistance: TAMs
Thymidine-associated mutations (TAMs)
- Selected by AZT, d4T
- Decrease susceptibility to AZT, d4T, TDF/TAF, ABC; to all NRTIs if numerous
- Cross resistance: increases with increasing number of TAMs
  - (More = worse)
- 2 pathways:
  - M41L, L210W, T215Y (more resistance; incl. more impact on TDF)
  - D67N, K70R, T215F, K219Q/E

NRTI Resistance: L74V/I
- Selected by ABC and ddl
  - (L74V sometimes selected by TDF)
- Resistance to ABC and ddl
- Increases susceptibility to TDF/TAF, AZT
Case 1: PrEP Failure

- 31 yo MSM on PrEP (TDF/FTC, Truvada), presents to clinic after absence of 6 months, reports spotty adherence to his PrEP, but continues to take.
  - HIV Ab+, HIV RNA 65,000 c/mL.
  - HIV genotype: RT – K65R, M184V; PR – WT.

NRTI Strategy:
- Know key mutations (M184V, K65R, TAMS)
- Look up others

Case 2: Audience Response

Which ART regimen would be most likely to be effective?
1. Rilpivirine (RPV)/TAF/FTC (Odefsey)
2. EVG/cobi/TAF/FTC (Genvoya)
3. DTG/ABC/3TC (Tivicay)
4. DRV/r + rilpivirine/TAF/FTC

Bottom Line: need 3 active ARVs, if possible (especially if ARVs have low genetic barrier to resistance).

NNRTI Resistance

- Several single mutations confer high-level resistance to certain NNRTIs; numerous others contribute to resistance
- Low genetic barrier to resistance (except etravirine, doravirine)
- Cross resistance is common

NNRTI Strategy:
- Know several key mutations (K103N, Y181///, E138K [V106])
- Look up others
- Be aware of mutation scoring system for etravirine (ETR)
- (Be cautious about DOR – little is known)

NNRTI Resistance: Important Mutations

- **K103N**
  - Commonly selected by EFV
  - Emerges early in VF (low genetic barrier to resistance), commonly transmitted
  - Resistance to EFV, NVP
  - By itself, does not decrease susceptibility to ETR, RPV, DOR
- **Y181I/V/C/F/G/S**
  - Selected by NNRTIs
  - Cross resistance to all NNRTIs (except DOR?)
- **E138K**
  - Selected by RPV
  - Usually occurs with M184I or M184V; this enhances resistance to RPV
  - Resistance to RPV, cross resistance to EFV, ETR
- **V106I//**
  - Frequently seen after DOR failure, with other mutations
Doravirine

- **In vitro**, active against common NNRTI resistance mutations (incl. K103N, E138K, Y181C, G190A)
- **In vivo**, emergent resistance:
  - Treatment-naive trials: V106I, Y188L, H221Y, P225H, F227C
  - ALSO NRTI resistance mutations – eg, M184V, K65R, M41L
  - Switch study (DRIVE-SHIFT): none
- NO clinical data in salvage settings

---

**Case 2: NNRTI Resistance**

- 53 yo man with VF years ago on EFV/TDF/FTC (Atripla), now on dolutegravir/ABC/3TC (Triumeq); VL <40 c/mL x 1 year. He complains of headache and GI symptoms and wants to change ARVs.
- Previous GT (while on EFV/TDF/FTC):

> Could we use rilpivirine (or doravirine) + 2 NRTIs as his next ART regimen?

- K103N alone should not affect RPV, DOR, or ETR

**Issues:**
- Accuracy of GT?
- Mutations may fade from view with time and removal of selective drug pressure
- GT captures strains that comprise >5-20% of the circulating viruses
- Once there, always there: “archived” mutations

**Bottom Line:** caution in interpreting GTs – consider what may be hidden from view

---

**Case 3: 1st ART Failure**

- 35 yo woman on RPV/TAF/FTC (Odefsey). HIV RNA suppressed x 2 years, then increases to >5,000 c/mL in setting of several months of OTC PPI use.
- Genotype:
  - RT - K101E, E138K, Y181C, M184I
  - PR - no mutations
**Case 3: Audience Response**

Could you use etravirine (ETR) or doravirine (DOR) in her next regimen?

1. Yes – should have full activity
2. No – not effective after rilpivirine failure
3. I need more information

**RT:** K101E, E138K, Y181C, M184I  
**PR:** none

**NNRTI Strategy:**
- Know several key mutations (K103N, Y181///, E138K [V106])
- Look up others
- Mutation scoring system for ETR
- Caution re DOR (little is known)

**Etravirine Resistance**

<table>
<thead>
<tr>
<th>Mutation Weight Factor</th>
<th>Response by ETR weight</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Genotypic score</td>
</tr>
<tr>
<td>1.0</td>
<td>V90I A98G</td>
</tr>
<tr>
<td></td>
<td>V101I/157A</td>
</tr>
<tr>
<td></td>
<td>176R/207G</td>
</tr>
<tr>
<td></td>
<td>G190A</td>
</tr>
<tr>
<td>1.5</td>
<td>V106I E138A</td>
</tr>
<tr>
<td></td>
<td>V179F G190S</td>
</tr>
<tr>
<td>2.5</td>
<td>Y181C M230L</td>
</tr>
<tr>
<td>3</td>
<td>Y181I Y181V</td>
</tr>
</tbody>
</table>

**Bottom line:** ETR may not be effective if ETR score >2.

**Integrase Inhibitor Resistance**

- Raltegravir and elvitegravir have low-ish barriers to resistance  
  - Several possible mutation pathways  
  - Different effects on dolutegravir, bictegravir
- Dolutegravir and bictegravir primary resistance is rare, not well understood
- Order integrase *genotype* (not phenotype) = special order  
  - Important for predicting sensitivity to DTG, BIC

**INSTI Strategy:**
- Assume cross-resistance between RAL and EVG
- Recognize Q148/// (most damaging to DTG)
- Look up all others
- (Be very cautious about BIC – little is known)

---

Integrase Inhibitor Resistance

<table>
<thead>
<tr>
<th>Pathway/mutations</th>
<th>Resistance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Raltegravir</td>
<td>All INIs (for DTG, Q148 + at least one other)</td>
</tr>
<tr>
<td>E92Q</td>
<td>RAL, EVG</td>
</tr>
<tr>
<td>Q148H/K/R, G140S/A</td>
<td>RAL</td>
</tr>
<tr>
<td>N155H</td>
<td>EVG, RAL, low level DTG</td>
</tr>
<tr>
<td>Y143R/H/C</td>
<td>All INIs (for DTG, Q148 + at least one other)</td>
</tr>
<tr>
<td>Elvitegravir</td>
<td>EVG</td>
</tr>
<tr>
<td>E92Q</td>
<td>RAL, EVG</td>
</tr>
<tr>
<td>Q148H/K/R, G140S/A</td>
<td>RAL</td>
</tr>
<tr>
<td>T66I</td>
<td>EVG</td>
</tr>
<tr>
<td>N155H</td>
<td>RAL, EVG</td>
</tr>
</tbody>
</table>

Dolutegravir (DTG)
- No significant reported emergent mutations if used in 3-drug regimen for initial therapy
- Various mutations if used as monotherapy
- Various mutations if used after RAL or EVG
  - Q148 H/K/R + others, N155H + others, R263K

Bictegravir (BIC)
- No reported emergent mutations if used in 3-drug regimen for initial therapy
- No data for use in salvage therapy

Case 4: INSTI Resistance
- 50 yo man, on ATV/r/TDF/FTC for years, switched to EVG/cobi/TAF/FTC (Genvoya) to simplify his ART
- VL remained <40 c/mL x 9 months, then increased to 7,200 c/mL
- GT (incl. integrase) done:
  - IN: G140S, Q148H
  - Elvitegravir
  - Raltegravir

IN: G140S, Q148R

Insti Strategy:
- Assume cross-resistance between RAL and EVG
- Recognize Q148H (most damaging to DTG)
- Look up all others
- Caution re BIC – little is known
Dolutegravir Resistance

Viking-3 Study: Virologic response lowest in patients with Q148 + ≥2 secondary mutations

Bottom Line:
- If Q148 + 0 or 1 other mutation, DTG may be effective (with other active ARVs)
- Use BID dosing

PI Resistance

- Primary/Major mutations
  - Emerge first, decrease antiviral effect
- Secondary/Minor mutations
  - Emerge later, increase fitness of strains with primary mutations or further decrease antiviral effect
- Specific primary and secondary PI mutations, depending upon PI
- **Accumulation of mutations usually required for high-level resistance
- Cross resistance is complex

Bictegravir: in vitro activity against resistant virus

Bottom Line: BIC very little information on resistance

PI Resistance

- PI Strategy:
  - Be aware of (but don’t memorize) list of DRV resistance-associated mutations (RAMs)
  - Look up all others
DRV Resistance-Associated Mutations (DRV-RAMs)

- 11 mutations associated with resistance to DRV:
  - V11I, V32I, L33F, I47V, I50V, I54L/M, T74P, L76V, I84V, L89V
- Once-daily dosing possible if no DRV-RAMs (ODIN study)
- Twice-daily dosing recommended if ≥1 DRV-RAM
- DRV response diminished with ≥3 DRV-RAMs

Cahn et al, AIDS. 2011;25:929-39

Case 5: PI (and other) Resistance

- 48 yo man, reports that he has been on “everything” in the past (except INSTIs), sometimes with poor adherence.
- Historic genotype(s):
  - PR -- L10I, L33F, M36I/M, M46L, I54V, L63S, I64V, V82A

Assess PI resistance:
- Check for DRV resistance-mutations
  - V11I, V32I, L33F, I47V, I50V, I54L/M, T74P, L76V, I84V, L89V
  - One DRV-RAM: L33F -> DRV/r likely to be effective but should be given BID
- Look up all other mutations

Case: RT -- M41L, D67N, L210W, T215Y; V106I, Y181V
PR -- L10I, L33F, M36I/M, M46L, I54V, L63S, I64V, V82A

Case 5: Multiclass Resistance, use strategies

- 48 yo man, reports that he has been on “everything” in the past (except INSTIs), sometimes with poor adherence.
- Historic genotype(s):
  - RT -- M41L, D67N, L210W, T215Y; V106I, Y181V
  - PR -- L10I, L33F, M36I/M, M46L, I54V, L63S, I64V, V82A

Approaching this: be methodical, apply strategies
- NRTI
- NNRTI
- PI
- (IN)
Case 5: Multiclass Resistance (2)

- Historic genotype(s):
  - PR: L10I, L33F, M36I/M, M46L, I54V, L63S, I64V, V82A

Assess NRTI resistance:
- 4 mutations (all TAMs) - affect TDF/TAF, ABC
- Anything missing?
  - No M184 V/I – why???

NRTI Strategy:
- Know key mutations (M184V, K65R, TAM5)
- Look up others

Assess PI resistance:
- 8 PI mutations (1 DRV mutation)

PI Strategy:
- Be aware of list of DRV resistance-associated mutations (RAMs)
- Look up all others

Case 5: Multiclass Resistance (3)

- Historic genotype(s):
  - PR: L10I, L33F, M36I/M, M46L, I54V, L63S, I64V, V82A

Assess NNRTI resistance:
- 2 NNRTI mutations (incl. Y181)
- ETR score:
  - 4.5
- DOR: little known (caution: V106I)

NNRTI Strategy:
- Know several key mutations (K103N, Y181///, E138K [V106])
- Look up others
- Mutation scoring system for ETR
- Caution re DOR – little is known

Case 5: Multiclass Resistance (4)

- Historic genotype(s):
  - PR: L10I, L33F, M36I/M, M46L, I54V, L63S, I64V, V82A

Assess PI resistance:
- 8 PI mutations (1 DRV mutation)

Case 5: Multiclass Resistance (5): “Look up other mutations”

- Conclusion: likely resistance to all NNRTIs (??DOR), and to all PIs except DRV – use BID

Case 5, continued

• Summary:
  • 3-class resistance:
    • NRTI: 4 mutations (TAMs): M41L, D67N, 210W, T215Y
    • Cross resistance to TDF, ABC
    • No M184V/I but we will assume it is there
      • 3TC/FTC resistance
    • NNRTI: 2 mutations: V106I, Y181V
      • Likely resistance to ETR, DOR
    • PI: 8 mutations (1 DRV RAM)
  • Integrase inhibitors: never exposed
  • What ARVs should we use in the next regimen?

What ARVs should we use in the next regimen?

• Integrase inhibitor?
• Darunavir/r?
• NNRTI?
• NRTIs?
• CCR5 antagonist?

Proviral DNA Genotype

Background:
• Standard resistance test requires plasma HIV RNA of >500-1,000 c/mL
• Many patients currently on ART with VL <40 may have resistance from previous regimens but have no available resistance tests to guide ART changes (eg, simplification)
• Can we do resistance testing on archived HIV?

Proviral DNA Genotype

• Amplifies cell-associated HIV-1 proviral DNA from infected cells (PBMCs)
  • Whole blood samples
• Analyzes HIV-1 polymerase region by new generation sequencing technology
Case 6

- 55 yo man HIV+ 1993, on ART x years
- Previous ART history unclear – remembers AZT, D4T, 3TC, ABC, DDI, EFV, NVP, IDV, SQV, LVP-r
- Transferred to this clinic on ATV-r + AZT + TDF/FTC, HIV RNA = ND
- Changed to ATV-r + RAL + TDF/FTC x 5 years, HIV RNA = ND
- Proviral DNA GT:
  - RT: M184V, K103K/N
  - IN: None
  - PR: L90I/M
  - Changed to EVG/cobi/TAF/FTC + DRV

Proviral DNA Genotyping – How Accurate?

DNA Genotype

Bottom Line:
- Can be helpful if shows resistance mutations.
- May miss some or all existing resistance mutations: caution re negative results
- No clinical data

Summary

- Do resistance tests at time of diagnosis and if virologic failure + suspected resistance (usually GT)
- Resistance: once there always there (archived)
- Beware of what you don’t see (minority populations)
- Look at old resistance reports, treatment history
- Read resistance reports methodically
- Caution re ARVs with scanty data (eg, BIC, DOR)
- **Seek expert advice**
- Goal of treatment: suppressed HIV RNA for all
Resources

- Stanford HIV Drug Resistance Database
  - http://hivdb.stanford.edu

- IAS-USA HIV Drug Resistance Mutations Figures and User Notes
  - https://www.iasusa.org

- Clinician Consultation Center
  - (800) 933-3413
  - Monday – Friday, 9 a.m. – 8 p.m. EST
  - (Free!)