2\textsuperscript{nd} Line Treatment and Resistance

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Overview

• Basics of Resistance
• Treatment failure
• Strategies to manage treatment failure
Mutation

Definition: A change in nucleic acid sequence that results in a change in amino acid sequence

AAA     GAC     AGT
K     D     S
(Lys)  (Asp)  (Ser)

AAA     AAC     AGC
K     N     S
(Lys)  (Glu)  (Ser)
# Mutation

**Definition:** A change in nucleic acid sequence that results in a change in amino acid sequence.

<table>
<thead>
<tr>
<th>Codon</th>
<th>AAA</th>
<th>GAC</th>
<th>AGT</th>
<th>AAA</th>
<th>AAC</th>
<th>AGC</th>
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<tbody>
<tr>
<td><strong>Point Mutation</strong></td>
<td>K</td>
<td>D</td>
<td>S</td>
<td>K</td>
<td>N</td>
<td>S</td>
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<tr>
<td></td>
<td>(Lys)</td>
<td>(Asp)</td>
<td>(Ser)</td>
<td>(Lys)</td>
<td>(Glu)</td>
<td>(Ser)</td>
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<table>
<thead>
<tr>
<th>Amino Acid</th>
<th>AAA</th>
<th>GAC</th>
<th>AGT</th>
<th>AAA</th>
<th>GAC</th>
<th>AGC</th>
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<td><strong>Silent Mutation</strong></td>
<td>K</td>
<td>D</td>
<td>S</td>
<td>K</td>
<td>(Asp)</td>
<td>S</td>
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<td>(Lys)</td>
<td>(Asp)</td>
<td>(Ser)</td>
<td>(Lys)</td>
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<td>(Ser)</td>
</tr>
</tbody>
</table>

**Courtesy of Joseph Eron**
Nomenclature

Codon (position)
Protease = 1 - 99 amino acids
Reverse Transcriptase = 1 - 560 amino acids

M184V

Wild-type amino acid (Methionine)
Mutant amino acid (Valine)

Courtesy of Joseph Eron
Resistance Assays

• Genotype
  – Identify specific mutations
  – Mutations may precede phenotypic resistance
  – Mutations must be interpreted
  – Results in 7-14 days
  – Less expensive

• Phenotype
  – Culture based assay of growth in presence of drug
  – Direct measure of resistance (i.e., multiple mutation effects)
  – Resistance thresholds not defined for all drugs
  – Results in 21-28 days
  – More expensive
Importance of Antiretroviral History

- Can help to determine possible resistance even without genotype

- In agents with low threshold to resistance, a history of therapy with detectable viral load often means resistance to that agent
Antiretroviral History is Key Determinant of Resistance

• In general, once mutations have been selected for, they are archived in the body
• Removal of selective pressure may lead to outgrowth of wild type virus strains
• Reinstitution of ARV drugs will lead to rapid outgrowth of resistant strains
Mutations Occur During HIV Replication

- Virion half-life = 30 minutes
- Daily production = $10^9 - 10^{10}$ virions
- RT incorporates the wrong nucleotide once every $10,000 - 30,000$ nucleotides
- Approximately 1 mutation per viral copy (mutation rate of $10^{-4}$)
- Higher viral replication = more frequent mutations
- Every single point mutation occurs daily
How Quickly Resistance Can Occur Depends on the Viral Load

<table>
<thead>
<tr>
<th>Viral Load</th>
<th>Days Before Mutation Arises</th>
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<tbody>
<tr>
<td>300,000</td>
<td>0.1</td>
</tr>
<tr>
<td>30,000</td>
<td>1</td>
</tr>
<tr>
<td>3,000</td>
<td>10</td>
</tr>
<tr>
<td>300</td>
<td>100</td>
</tr>
<tr>
<td>30</td>
<td>1,000</td>
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</table>

Adapted from Siliciano, 2002
Different Drugs Have Different Genetic Barriers to Resistance

- **Non-Boosted PIs**
  - Small Change per Mutation
  - BUT
  - Low Drug Levels

- **NNRTIs**
  - High Drug Levels
  - BUT
  - Large Change per Mutation

- **Boosted PIs**
  - Small Change per Mutation
  - AND
  - High Drug Levels

Increasing Number of Mutations
Resistance Is a Matter of Degree

High-level resistance

Little or no virologic response

Low-level/intermediate resistance

Suboptimal virologic response

Susceptible

No reduced drug susceptibility
Nucleoside Resistance
Specific Mutations: AZT, d4T

• Multiple step-wise mutations are required for complete AZT, d4T resistance

• 1st: 41 →
  – 2nd: 210 →
    • 3rd: 215

• 1st: 67 →
  – 2nd: 70 →
    • 3rd: 219
Specific Mutations: AZT, d4T

• Multiple step-wise mutations are required for complete AZT, d4T resistance

• 1\textsuperscript{st}: 41 \rightarrow (mild AZT or d4T resistance)
  – 2\textsuperscript{nd}: 210 \rightarrow (moderate AZT or d4T resistance)
  • 3\textsuperscript{rd}: 215 (complete AZT or d4T resistance)
Specific Mutations: AZT, d4T

- Impact of TAMs on Other NRTIs:
  - ABC resistance is increased with increased number of thymidine analog mutations (TAMs)
  - The more TAMs a virus accumulates, the less likely any of the NRTIs are to be effective (cross-resistance)
Specific Mutations: 3TC/FTC

• Single point mutation: M184V
  – Confers high level resistance to 3TC/FTC
Specific Mutations: 3TC/FTC

• **Silver lining** of 3TC resistance:

• M184V reverses resistance to AZT and TDF
  
  – *i.e. increased susceptibility* to AZT and TDF

• Also causes decreased fitness of virus for replication
Specific Mutations: TDF

• Tenofovir
  – K65R
  – Increases susceptibility to AZT!
  – Impairs replication capacity (fitness) of virus.

• K65R
Specific Mutations: ABC

• Abacavir
  – L74V
    • (Also K65R sometimes)
  – L74V decreases susceptibility to ABC, ddi
  – But increases susceptibility to AZT, TDF
  – M184V + 2-3 TAMs decreases susceptibility to ABC
Specific Mutations: NRTIs

• Multinucleoside resistance mutations are selected by extensive NRTI therapy and confer broad resistance to all NRTIs
  – Q151M (TDF still susceptible)
  – T69 insertion (all nucleosides resistant)
Resistance Patterns after Failure of Typical NRTI Backbones

- **AZT/3TC**  \(\rightarrow\) M184V \(\rightarrow\) TAM 1 \(\rightarrow\) TAM 2 \(\rightarrow\) TAM 3 \(\rightarrow\) MULTI-RESISTANT

- **d4T/3TC**  \(\rightarrow\) M184V \(\rightarrow\) TAM 1 \(\rightarrow\) TAM 2 \(\rightarrow\) TAM 3 \(\rightarrow\) MULTI-RESISTANT

- **TDF/FTC**  \(\rightarrow\) M184V \(\rightarrow\) K65R

- **ABC/3TC**  \(\rightarrow\) M184V \(\rightarrow\) L74V (or K65R)
Nucleoside/tide Resistance Mutations

• The presence of multiple TAMs/NAMs confers resistance to most NRTI/NtRTIs
  – 41, 44, 67, 70, 118, 210, 215, 219

• Summary of NRTI Resistance:
  – Zidovudine 41, 44, 67, 70, 118, 210, 215, 219
  – Didanosine 65, 74, 75, 184
  – Stavudine 41, 44, 65, 67, 70, 75, 118, 210, 215, 219
  – Lamivudine 65, 184
  – Emtricitabine 65, 184
  – Abacavir 65, 74, 115, 184(+ 2-3 TAMs ↓, 4+ TAMs 0)
  – Tenofovir 65, ≥ 3 TAMS with 41 or 210
Predicted NRTI activity based on median phenotypes by genotype*

<table>
<thead>
<tr>
<th># Mutations</th>
<th>RT genotype</th>
<th>ZDV</th>
<th>d4T</th>
<th>ddI</th>
<th>3TC/FTC</th>
<th>ABC</th>
<th>TDF</th>
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<tr>
<td>1</td>
<td>184V/I</td>
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<tr>
<td></td>
<td>65R</td>
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<tr>
<td>2</td>
<td>65R + 184V/I</td>
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<tr>
<td></td>
<td>74V/I + 184V/I</td>
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</tr>
<tr>
<td></td>
<td>41L + 184V/I</td>
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<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>67N + 70R + 184V/I</td>
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<tr>
<td></td>
<td>215Y/F* + 184V/I</td>
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<tr>
<td>4</td>
<td>67N + 70R + 219E/Q + 184V/I</td>
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</tr>
<tr>
<td></td>
<td>41L + 215Y/F* + 184V/I</td>
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<tr>
<td>5</td>
<td>41L + 210W + 215Y/F* + 184V/I</td>
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</tbody>
</table>

*215Y and 215F both require 2 mutations from wild type

Lanier R, et al. 10th CROI, Boston 2003, #586
NNRTI
Mechanism of Action
Non-Nucleoside RT Inhibitors

• Non-competitive inhibitors of RT enzyme

• Bind at different site from NTP binding site

• All NNRTIs bind to the same site
Resistance Points
Non-Nucleoside RT Inhibitors

• Resistance to NNRTIs develops rapidly with single point mutations

• HIV-2 intrinsically resistant to NNRTIs
NNRTI Resistance Mutations

- **Low Mutation Threshold**
- **Nevirapine**: 100, 103, 106, 108, 181, 188, 190
- **Delavirdine**: 103, 106, 181, 188, 236
- **Efavirenz**: 100, 103, 106, 108, 181, 188, 190, 225
- **Etravirine**: 90, 98, 100, 101, 106, 179, 181, 190

**Multi-NNRTI Resistance**

- Reduced Activity with any 1 mutation
  - 103, 106, 188
- Reduced activity with ≥ 2 mutations
  - 100, 106, 181, 190, 230
Etravirine - Number of NNRTI Mutations and Virologic Response at Week 24

All subjects had NNRTI mutations from prior genotyping

Mean change in viral load (log_{10} c/ml)

Baseline NNRTI mutations in TMC125 800 mg bid

- 0*
- 1
- 2
- ≥3

TMC125 800 mg bid
Active control

Mean change in viral load:

N=79: -1.18
N=40: -0.19
N=15: -1.82
N=18: -1.65
N=17: -1.00
N=29: -0.66

Relevant NNRTI Mutations:

*All subjects had NNRTI mutations from prior genotyping

Mutation Pathways Leading to Resistance to RAL Identified

- Mutations observed upon RAL treatment
  - Near active site
  - Similar to mutations selected with other integrase inhibitors in cell culture

- 2 genetic pathways appear to confer resistance to RAL
  - Catalytic residues
  - 155H pathway
  - 148H/K/R pathway

Protease Resistance Mutations

- **Atazanavir**: 10, 16, 20, 24, 32, 33, 34, 36, 46, 48, 50L, 53, 54, 60, 62, 64, 71, 73, 82, 84, 85, 88, 90, 93
- **Darunavir/r**: 11, 32, 33, 47, 50, 54, 74, 76, 84, 89
- **Fosamprenavir**: 10, 32, 46, 47, 50V, 54, 73, 76, 82, 84, 90
- **Indinavir**: 10, 20, 24, 32, 36, 46, 54, 71, 73, 76, 77, 82, 84, 90
- **Lopinavir/r**: 10, 20, 24, 32, 33, 46, 47, 50, 53, 54, 63, 71, 73, 76, 82, 84, 90
- **Nelfinavir**: 10, 30, 36, 46, 71, 77, 82, 84, 88, 90
- **Ritonavir**: 10, 20, 32, 33, 36, 46, 54, 71, 77, 82, 84, 90
- **Saquinavir**: 10, 24, 30, 46, 48, 54, 63, 64, 71, 73, 77, 82, 84, 88, 90
- **Tipranavir/r**: 10, 13, 20, 33, 35, 36, 43, 46, 47, 54, 58, 69, 74, 82, 83, 84, 90
Resistance after Treatment Failure

Adapted from Gallant, 2007
Resistance after Treatment Failure

Adapted from Gallant, 2007
Resistance after Treatment Failure

Adapted from Gallant, 2007
Selective Pressure of Antiretroviral Therapy Selects for Resistance

Incomplete suppression
- Inadequate potency
- Inadequate drug levels
- Inadequate adherence
- Pre-existing resistance

Treatment begins

Selection of resistant quasispecies

Drug-susceptible quasispecies
Drug-resistant quasispecies
Resistance after Treatment Failure

Adapted from Gallant, 2007
Resistance after Treatment Failure

Adapted from Gallant, 2007
Resistance after Treatment Failure

Adapted from Gallant, 2007
Resistance after Treatment Failure

AZT/3TC/NVP

Virologic Failure

Immunologic Failure

Clinical Failure

CD4

Viral Load

Threshold

M184V

Adapted from Gallant, 2007
Resistance after Treatment Failure

- AZT/3TC/NVP
- Virologic Failure
- Immunologic Failure
- Clinical Failure

Genetic Resistance Markers:
- K103N
- M184V

Adapted from Gallant, 2007
Resistance after Treatment Failure

Adapted from Gallant, 2007
Resistance after Treatment Failure

Adapted from Gallant, 2007
Resistance after Treatment Failure

AZT/3TC/NVP

Virologic Failure

Immunologic Failure

Clinical Failure

CD4

K103N
M184V

TAM 1
TAM 2
TAM 3

Viral Load

Adapted from Gallant, 2007
Resistance after Treatment Failure

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TDF/3TC/NVP

Virologic Failure

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CD4

Viral Load

M184V

K103N

K65R
Resistance after Treatment Failure

Adapted from Gallant, 2007
Resistance after Treatment Failure

Adapted from Gallant, 2007
Resistance after Treatment Failure

Adapted from Gallant, 2007

AZT/3TC/NVP

Virologic Failure  Immunologic Failure  Clinical Failure

CD4  Viral Load

M184V  K103N  TAM 1  TAM 2  TAM 3

2nd Line Regimen?

Kaletra

Adapted from Gallant, 2007
Resistance after Treatment Failure

Adapted from Gallant, 2007
Implications of Stages of Treatment Failure

Remaining Treatment Options

NRTIs: ABC, AZT, ddl, d4T, TDF

NNRTIs: None

Pls: All

NRTIs: None

NNRTIs: None

Pls: All

Clinical Failure (Late Virologic Failure)

Early Virologic Failure

d4T, 3TC, Nevirapine

TDF, 3TC, Nevirapine
Definitions of Failure (WHO 2009)

• Clinical
  – New or recurrent WHO stage 4 condition

• Immunological
  – Fall of CD4 count to baseline (or below) OR
  – 50% fall from on-treatment peak value OR
  – Persistent CD4 levels below 100 cells/mm$^3$

• Virological
  – persistent VL of >5000 copies/ml confirms treatment failure
Timing of Failure

• After initiating ART, inability to maintain viral suppression can occur very early:
  – Viral resistance to NNRTI and 3TC/FTC can occur very early
  – Viral suppression at 6 months is a good predictor of viral suppression at 48 weeks and decreases in mortality

• Viral failure will often occur many months prior to the onset of recognizable immunological or clinical failure

• The timing of onset of clinical and immunological failure after viral failure has occurred will depend on:
  – CD4 nadir prior to stating ART
  – Previous OI history
  – Viral load set point
  – ART regimen, mutational pattern which develops (Replicative Capacity)
Immunological Failure

- Depending on the study, 8% to 40% of individuals who present with evidence of immunological failure have virological suppression and risk being unnecessarily switched to second-line ART.
What leads to Regimen Failure and Resistance?

• Sequential monotherapy
• Pharmacokinetics
  – Drug-drug interactions
  – Half-life
• Characteristics of the virus
  – Pre-existing resistance
  – Pathogenicity

• Poor adherence
  – Social situation
  – Access to care
  – Substance abuse
  – Medication side effects/toxicity
  – Co-morbid illness (i.e., mental disorders)
Basic Rules for Switching to 2\textsuperscript{nd} Line ART

1. Inability to obtain viral suppression 4-6 months after initiation of therapy

2. Reappearance of detectable viremia after suppression to undetectable levels

3. Persistently declining CD4 in combination with other clinical “clues” (in absence of intercurrent illness)

4. Reconfirm viral loads and CD4 counts before changing therapy. Evaluate CD4% if possible
Basic Rules for Switching to 2\textsuperscript{nd} Line ART

   a. Inability to adhere
   b. Probable Drug resistance

6. In general do not change or add a single drug to a failing regimen. (AZT for D4T, ABC or DDI for TDF)

7. In a patient with clinical failure, assume multiple drug resistance, and cross resistance