A HIV-infected man took antiretroviral treatment (ART) for 3 years. At times he did not take his medicine because he did not have money to buy it. Now he is sick, his CD4 cells have dropped, and his plasma HIV RNA is high.

- What is his problem?
- What caused it?

Overview of HIV-1 Drug Resistance

- How is drug-resistant HIV-1 defined?
- What phenomena regulate the selection of drug resistant viruses?
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Definition of HIV-1 Drug Resistance

- Phenotypic
  - Drug concentration that suppresses viral replication by half (IC_{50}) or 90% (IC_{90})
  - Generally performed by inserting PCR-amplified segment of "patient’s" virus (polymerase, protease or envelope gene) into a pseudotyped virus, with results expressed as "fold-resistant" compared to the lab strain
  - Cutoff for susceptible/resistant:
    - Limitations of the assay - outside the variability of the controls (i.e. <2-fold = susceptible; 2-10-fold = moderate; >10-fold = high-level)
    - Biologic - outside of range found to suppress replication of virus from untreated individuals
    - Clinical - values associated with failure to suppress viral replication in clinical studies

- Genotypic
  - Mutations selected during virus passage in cell culture, or associated with drug not suppressing viral replication in vivo
  - Multiple algorithms to interpret mutations:
    - Generally by rules or machine learning
    - Example: Stanford HIV database (UW uses Stanford website)
      - Each mutation is given a drug penalty score; the sum determines the level of resistance
        - 0-9 = susceptible; 10-14= potential low-level; 15-29= low-level; 30-59= intermediate; >60= high-level resistance
        - Genotypic susceptibility score (GSS)
          - Calculated for each drug as \([1 - (drug\ score/60)]\) and when negative assigned "0"
          - Sum provided for each drug in regimen or for all available drugs
Mutations in HIV-1

- Resistance to antiretrovirals
  - Decrease substrate binding (PI, NRTI, NNRTI, EI)
  - Increase phosphorolysis
    - ATP or pyrophosphate mediated excision of chain terminator and compensatory increases in polymerase processivity
  - Viral mutation interact differently with host (e.g., CCR5 antagonist resistance or switch to CXCR4 co-receptor)

- Decrease/increase viral replication capacity

- Increase susceptibility to other antiretrovirals
  - Example: K65R & TAM (thymidine analogue mutations), respectively, increase susceptibility to zidovudine and tenofovir

- Modify immunogenic epitopes

- Encode defective virus

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Drug-resistant HIV is generally less fit than wild-type, thus, resistant viruses become undetectable after stopping ART (shifts in viral population)

S Deeks et al. NEJM 2001;344:472-80

Axioms of HIV-1 Drug Resistance

- High rate of HIV-1 replication, \( \sim 10^{10} \) virions/day
- Misincorporation of nucleotides (each base mutates daily)
  - HIV-1 polymerase lacks proofreading activity
  - \( \sim 5 \times 10^{-9} \) mutations/site/generation
  - Results in presence of “all” single mutants (prior to therapy)
  - 1 to 3 mutants required for high-level resistance to a drug
  - \( \geq 3 \) drug therapy is effective due to the low probability of 3 mutations in a single virus (Perelson AS. AIDS 1997;11suplA:517-24)
- Replication capacity of drug-resistant virus is generally less than wild-type virus
- Mutants and wild-type persist in long-lived cells

Viral-Host-Drug Factors Impact on Selection of Drug Resistant HIV-1

Host:
- Adherence to ART
- Polymorphisms: enzyme isotypes
- CD4 & co-receptor levels

Virus:
- Level of replication
- Replication capacity
- Reservoirs of mutants

Drugs:
- Genetic barrier of ART
- Absorption/excretion
- Tissue penetration
- Drug activation

HIV-1 Drug Resistance: Viral dynamics, cell turnover & drug pressure

Productively Infected CD4+ repopulated with wild-type virus

Activated CD4+ lymphocytes

CD4+ lymphocytes infected with defective virus

Macrophages

virions \( 10^{10}/\)day \( t_{1/2} = 6 \) hrs

Reservoirs of drug resistant virus &

“Latently” Infected CD4+ lymphocytes, populated primarily during 1st infection

\( t_{1/2} = 70 \) years

Selection of Drug Resistant HIV-1 as Related to Adherence to ART

Selection of mutants dependent on viral replication
i.e., NOT selected when not adherent to ART (no drug pressure), and
NOT selected when adherent to ART (as little viral replication)
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Transmission of Drug-Resistant HIV-1

- Incidence varies from 5-25%, as detected by consensus DNA sequencing
  - Evaluation of minority variants suggests even higher rates (~50%)
- Majority variants
  - Diminish efficacy of ART
  - Generally persist for years, infrequently “reverts” to wild-type virus
    - Superinfection with wild-type virus
    - Mutation to wild-type virus
    - Small population of wild-type can outgrow mutant
- Minority variants
  - Variable effect on ART
  - Not all changes have equal clinical effects (e.g., K103N vs. Y181C)
  - Threshold concentration may determine clinical relevance

Efficacy of ART (ZDV+3TC+NVP) in Women Previously Given Single-dose Nevirapine (Mashi Trial)


![Graph showing time to virologic failure in women starting ART with or without nevirapine.]

HIV-1 drug resistance assays

- **Phenotype:**
  - Recombinant viral constructs tested for Inhibitory Concentration 50% (IC50) compared to laboratory standard
    - Replication capacity: virus production after single-cycle of replication (Monogram Biosciences, So. San Francisco, CA)
      - Cost >$1000/specimen
- **Genotype (consensus):**
  - RNA sequence
    - Cost >$500/specimen
  - Virtual phenotype: genotype compared to database of paired data

Vector-based Recombinant Virus Assay (ViroLogic PhenoSense™)

- Costs ~$1000/specimen
- Test virus is constructed with patient’s HIV pol, inserted in lab strain of HIV with indicator gene (luciferase) minus env gene
- Co-transfect cells with plasmid containing env of amphotropic murine leukemia virus
- Pseudotyped virus particles are tested for susceptibility to dilutions of drug
- IC50 or IC95 of patient’s construct compared to lab viral strain
Detection of Drug-Resistant Virus by Consensus Genotype or Phenotype

A subset of the billions of virions in a person’s blood enters assay en masse:

Sequence virus population

Assay result suggests one genotype: i.e., the majority viral variant

Multiple rounds of PCR amplification

Methods for HIV-1 Genotyping

Commercial:
- Dideoxynucleotide terminator cycle sequencing
  - Kits – reagents cost ~$120/specimen
  - "in house" – reagents cost ~$30/specimen

Research:
- “Ultra deep”, 454 Sequencing
- Oligonucleotide array hybridization (Affymetrix)
  - Not readily adaptable to the detection of "novel" mutations
- Assays to detect point mutations
  - Line Probe Assay (LPA)
  - Allele-specific PCR (ASPCR)
- Oligonucleotide ligation assay (OLA)
- Ligation amplification (LigAmp)

HIV-1 Genome & Resistance Notation

UW Clinical lab uses Stanford Database to assess consensus genotypes http://www.hivdb.standford.edu/

Interpretation of HIV-1 drug-resistance tests are dissimilar to those that assess bacterial or fungal resistance, due to:

- Integration of HIV-1 in host’s genome, where it persists in long-lived cells
- Drug-resistant viruses, either transmitted or selected, enter and generally persist in cells
- Current commercial assays are not sensitive in detecting low-levels of drug-resistant virus that can be clinically important

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Summary of Prospective Randomized Trials of Drug Resistance Testing After Virologic Failure

<table>
<thead>
<tr>
<th>Study</th>
<th># Enrolled</th>
<th>Study Duration (months)</th>
<th>Study arm</th>
<th>Change in plasma HIV RNA</th>
<th>Plasma HIV RNA, % Below Detection</th>
<th>Change CD4 Count Cellular</th>
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<tbody>
<tr>
<td>VIRADAPT</td>
<td>108</td>
<td>Genotype</td>
<td>-1.19</td>
<td>32</td>
<td>14 (P=0.07)</td>
<td>+21</td>
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<tr>
<td>GART</td>
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<td>Genotype</td>
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<td>55</td>
<td>25 (P=0.001)</td>
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<tr>
<td>Novena</td>
<td>328</td>
<td>Genotype</td>
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<td>49</td>
<td>36 (P=0.03)</td>
<td>Not reported</td>
</tr>
<tr>
<td>USA-3001</td>
<td>272</td>
<td>Phenotype</td>
<td>-1.23</td>
<td>46</td>
<td>34 (P=0.08)</td>
<td>+27</td>
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<tr>
<td>ARGENTIA</td>
<td>174</td>
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<td>+15</td>
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<tr>
<td>NATRAL</td>
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<tr>
<td>CCTG-STS</td>
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<td>GERT</td>
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<td>46 (P=0.02)</td>
<td>Not reported</td>
</tr>
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</table>
Summary of Randomized Trails of Resistance Testing Following “Virologic Failure” of ART

- Resistance testing associated with short-term viral suppression, especially when:
  - Individual is less “drug-experienced”
  - When unused classes of ART are given
  - When higher drug-levels are achieved

- Immunologic benefits have NOT been associated with drug-resistance testing

- Genotypic tests are equal or superior to phenotypic tests

Why Doesn’t Resistance Testing After Virologic Failure of HAART Improve Outcome?

- Extensive cross resistance between drugs within each class
- Continued pattern of non-adherence
- Inadequate drug-levels
- Inadequate “genetic bottleneck”
- Resistant variants not detected due to low concentrations in viral population

Summary of ART Management

- Use the most potent, convenient, and well-tolerated regimen for a given patient
- Prepare for eventual treatment failure with drug-resistant virus
- Switch ART shortly after virologic failure to minimize selection of mutations
- While not adequately evaluated, testing for resistance testing may be most useful:
  - Prior to initiation of ART
  - Early in the time course of virologic failure
  - Viral load is slow to decrease after initiation of a new ART regimen, as this can occur with rekindling of low-level drug-resistance

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Panel Recommendations for Use of Drug-Resistance Assays and Sites for Interpreting Genotypes

Panel Recommendations

- International AIDS Society-USA panel: www.iasociety.org

Interpreting Genotypes

- http://www.hivdb.standford.edu/ (used for UW HIV Drug Resistance Testing)
- http://www.hivinvite.ucsf.edu/hnSite
- http://www.hivfrenchresistance.org

Current HIV Resistance Testing Guidelines

<table>
<thead>
<tr>
<th></th>
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</thead>
<tbody>
<tr>
<td>Primary Infection</td>
<td>Recommend</td>
<td>Recommend</td>
<td>Recommend</td>
</tr>
<tr>
<td>PEP (Source Patient)</td>
<td>—</td>
<td>Recommend</td>
<td>Recommend</td>
</tr>
<tr>
<td>Chronic</td>
<td>Recommend</td>
<td>Recommend</td>
<td>Recommend**</td>
</tr>
<tr>
<td>Treatment Failure or Suboptimal Response</td>
<td>Recommend*</td>
<td>Recommend</td>
<td>Recommend</td>
</tr>
<tr>
<td>Pregnancy</td>
<td>Recommend*</td>
<td>Recommend*</td>
<td>Recommend*</td>
</tr>
<tr>
<td>Pediatric</td>
<td>Recommend*</td>
<td>—</td>
<td>Recommend**</td>
</tr>
</tbody>
</table>

* Test earliest specimen
** Genotypic 1st or 2nd ART failure (AII); phenotype too, suspect complex resistance (BIII)
* When detectable plasma HIV-1 RNA and treatment initiated or changed
** When mother was viremic and on treatment at time of delivery

1. DHHS. Guidelines – Adults, updated Dec 2009; Pediatric, Feb 2009
Summary of HIV-1 Drug-resistance

- Acquired at the time of infection, or selected by inadequate ART
- Generally, persists whether transmitted or selected
  - Exceptions:
    - Very brief selection (e.g., single-dose nevirapine)
    - May decay during long-term suppressive ART
- HIV-1 readily mutates; virions $t_{1/2} < 1/4$ day; latent for decades; wild-type viruses more fit (outgrows mutants)
- Current assays evaluate “consensus” of virus population; research assays are more sensitive, although clinical relevance uncertain
- Value of resistance testing to clinical management of ART failure is uncertain; expert clinician can anticipate effective ART regimens
- While not adequately evaluated, testing for resistance testing may be most useful:
  - Prior to initiation of ART
  - Early virologic failure to evaluate if resistant to some or all ARV in regimen
  - Viral load is slow to fall after initiation of a new ART regimen

Ultra Deep Sequencing (454 Life Sciences™)
- DNA fractionated in to 300-500 bp fragments that are blunted
- Adaptors A (sequencing) & B-linked to biotin are ligated to DNA fragments and purified with Strepavidin beads
- DNA nicked from bead to serve as single-strand DNA PCR template after quantification by titration
- A single DNA is bound to a 2nd bead, captured by microreactor for PCR generating a bead with clonal transcripts

Ultra Deep Sequencing (454 Life Sciences™)
- A single bead loaded into each well of PicoTiterPlate by centrifugation, packed into place with enzyme beads
- Sequencing reagents flow into wells, if polymerase adds one or more bases a light signal is generated and recorded
- Thousands of beads sequenced at once
- Reagent costs for a single assay about $7K

Oligonucleotide Ligation Assay (OLA): Detects Point Mutations

Combines:
- PCR amplification
- Oligonucleotide ligation
- ELISA

Detects:
- Single base changes in PCR-amplified nucleic acid templates