Advances in HIV drug resistance to obtain optimal care and to avoid treatment failures

Anti- HIV Drug Targets

Five classes of drugs are currently in clinical use:
1. nucleoside and nucleotide reverse transcriptase (RT) inhibitors
2. non-nucleoside reverse transcriptase inhibitors
3. protease inhibitors (Pis)
4. Entry inhibitors (Eis)
5. Integrase inhibitors (INIs)
What Treatment to Start: IAS-USA Recommendations 2014

- ART is considered lifelong; sustained viral suppression is foundation for immune recovery, optimal health, and prevention of resistance and transmission.
- Maximize adherence and minimize toxicity: Goal is to treat with effective, well-tolerated therapy, with limited drug interactions and effects on comorbid conditions.
- Base selection on baseline resistance testing and patient characteristics and preferences.

Recommended Initial ART Regimens: INSTI plus 2 nRTIs

<table>
<thead>
<tr>
<th>INSTI plus 2 nRTI Combinations</th>
<th>Rating</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>DTG plus TDF/FTC</td>
<td>Ala</td>
<td>DTG is dosed once daily. Associated with modest increases in creatinine level due to inhibition of creatinine secretion.</td>
</tr>
<tr>
<td>DTG plus ABC/3TC</td>
<td>Ala</td>
<td>No evidence that ABC/3TC performs less well at HIV-1 RNA levels &gt;100,000 copies/mL when given with DTG. A fixed-dose combination is in late-stage development.</td>
</tr>
<tr>
<td>EVG/cobi/TDF/FTC</td>
<td>Ala</td>
<td>Once-daily fixed-dose combination. Cobi is associated with modest increases in creatinine level; has drug interactions similar to RTV.</td>
</tr>
<tr>
<td>RAL plus TDF/FTC</td>
<td>Ala</td>
<td>RAL is taken twice daily.</td>
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</table>

Recommended Initial ART Regimens: NNRTI plus 2 nRTIs

<table>
<thead>
<tr>
<th>NNRTI plus 2 nRTI Combinations</th>
<th>Rating</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>EFV/TDF/FTC</td>
<td>Ala</td>
<td>EFV central nervous symptoms may persist beyond 2-4 weeks, but is no longer contraindicated for use in pregnant women.</td>
</tr>
<tr>
<td>EFV plus ABC/3TC</td>
<td>Ala</td>
<td>EFV central nervous symptoms may persist beyond 2-4 weeks, but is no longer contraindicated for use in pregnant women.</td>
</tr>
<tr>
<td>RPV/TDF/FTC</td>
<td>Ala</td>
<td>Once-daily fixed-dose combination. RPV-based therapy is not recommended in patients with baseline HIV-1 RNA levels &gt;100,000 copies/mL.</td>
</tr>
</tbody>
</table>

Alternatives to Recommended Initial Regimens

<table>
<thead>
<tr>
<th>Type of Regimen</th>
<th>Alternative ARV Drug Combinations</th>
<th>Rating</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>INSTI plus 2 nRTIs</td>
<td>RAL plus ABC/3TC</td>
<td>B1a</td>
<td>No evidence that ABC/3TC performs less well at HIV-1 RNA levels &gt;100,000 copies/mL when taken with RAL.</td>
</tr>
<tr>
<td>NNRTI plus 2 nRTIs</td>
<td>NVP plus 2 nRTIs</td>
<td>B1a</td>
<td>Severe hepatotoxicity may occur in initial therapy when CD4 cell count is &gt;250 µL in women and &gt;400 µL in men. Severe rash is more common than with other NNRTIs.</td>
</tr>
<tr>
<td>NNRTI plus 2 nRTIs</td>
<td>RPV plus 2 nRTIs</td>
<td>B1a</td>
<td>RPV-based therapy is not recommended in patients with baseline HIV-1 RNA levels &gt;100,000 copies/mL.</td>
</tr>
</tbody>
</table>
Why Does HIV Resistance Occur?

- Patient nonadherence to ARVs
- Suboptimal dosing of drugs
- Spontaneous mutation of the HIV genome
- Transmission of drug-resistant virus

What Causes Resistance Mutations?

- Viral dynamics, mutation rate, and genome size predict that HIV will develop mutations at every position numerous times per day
  - $10^9$ new virions/day
  - $3.4 \times 10^{-5}$ mutations/replication cycle
  - $10^4$ bases in the genome
  - $10^4$ to $10^5$ mutations at every site/day
- Under drug selection pressures, complete replacement of wild-type (WT) virus by drug-resistant virus can occur in 14-28 days
Screening for optimal treatment regimens and monitoring treatment in HIV patients: The Genotypic Assays

HIV-1 Population Detection of Minority HIV-1 Variants

**HIV Research Studies: Sanger vs. Deep Sequencing**

<table>
<thead>
<tr>
<th>PLATFORM</th>
<th>OUTPUT</th>
<th>ANALYSIS</th>
<th>OUTCOME</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sanger Sequencing (1996)</td>
<td></td>
<td></td>
<td><em>Dozens of gels</em></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td><em>145,000 nt</em></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td><em>3½ years</em></td>
</tr>
</tbody>
</table>

Deep Sequencing (2013) | | | *One run* |
| | | | *630 million nt* |
| | | | *5 days* |

**DEEPGEN™HIV: Rationale**

To develop a test that could select the right antiretroviral treatment with a single assay!
**DEEPGEN™HIV: Characterization, Verification & Validation**

Sensitive Deep-Sequencing-Based HIV-1 Genotyping Assay To Simultaneously Determine Susceptibility to Protease, Reverse Transcriptase, Integrase, and Maturation Inhibitors, as Well as HIV-1 Coreceptor Tropism

**DEEPGEN™HIV: Bioinformatic Analysis**

**DEEPGEN™HIV: HIV-1 Genotyping - Comparing with Sanger Sequencing**
The numbers so far.....and general observations

- ~2000 drug resistance tests in Northeast Ohio
- 10,000 drug resistance tests in Uganda and East Africa

The so-called A1a treatment regimens are incredibly successful in first line cART with a drop to undetectable levels by 3-6 months in >95% of treated patients

Treatment failures are typically less than 5% per year and even higher with strong adherence

In cases of treatment failure, very high treatment success of salvage regimens involving a ritonavir-boosted regimen or with regimens where the “backbone” can be changed out to avoid drug class resistance

Persistent problems:

CD4 cell counts and even viral load is lag indicator of treatment failure. Delays in drug resistance testing could result in loss of active antiretroviral drugs in treatment regimen and eventually lead to drug class failures.

Never trust general observations....

Kaplan-Meier plot of time to protocol-defined virologic failure among subjects in the randomly selected subcohort with and without virus resistant to nonnucleoside reverse transcriptase inhibitors at baseline.


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Dominant, pre-existing NNRTI drug resistance mutations impacts treatment outcomes with NNRTI-based treatment regimens

OCTANE – Epic failure in Africa.

Despite the A5095 trial and clear data showing that pre-existing NNRTI resistance led to failure of NNRTI-based treatment regimens. ACTG proceed with a trial to treat with a LPVr versus NVP-based regimens in women previously receiving single dose NVP to prevent mother-to-infant transmission. All drug resistance genotypes on women prior to treatment were performed retrospectively.
The risk of virologic failure on NVP-based cART from NVP-resistant variants differs between single dose NVP-exposed and -unexposed women. This difference may be driven by drug-resistance mutations emerging after single dose NVP exposure that are linked on the same viral genome.

### Table 1: Significant Risk of Endpoints Associated With NVP and FTC Mutations Among Women With Prior Single-Drug NVP/PF Exposure and Women With No NVP/PF Exposure

<table>
<thead>
<tr>
<th>Endpoint</th>
<th>NVP Exposure</th>
<th>FTC Exposure</th>
<th>Risk Ratio</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yes</td>
<td>120</td>
<td>120</td>
<td>1.23</td>
<td>0.03</td>
</tr>
<tr>
<td>No</td>
<td>120</td>
<td>120</td>
<td>0.85</td>
<td>0.05</td>
</tr>
<tr>
<td>NVP</td>
<td>120</td>
<td>120</td>
<td>1.00</td>
<td>0.01</td>
</tr>
<tr>
<td>FTC</td>
<td>120</td>
<td>120</td>
<td>0.00</td>
<td>0.00</td>
</tr>
</tbody>
</table>

**Drug resistance in Uganda: a ten year perspective**

Joint Clinical Research Centre in Uganda treats ~60,000 people living with HIV/AIDS.

**How resistance testing impacts treatment decisions in Uganda**

A. Viral load

B. CD4 cell counts

Treatment failures without drug resistance

~25% of all treatment failures in Uganda have no detectable drug resistance mutations by Sanger sequencing.
The ~25% that are failing the current treatment regimen but with no detectable drug resistance mutation are typically maintained on their treatment regimen especially if there is history or possibility of poor adherence. Failures after 1 year post resistance testing is >80%.

HIV-1 Minority Variants: Contributing to treatment failure in Uganda?

Identification of low frequency drug resistant variants of HIV-1 Conferring Drug resistance in a Cohort of HIV infected Patients Failing ART but having a Drug Susceptible Genotype

Aim
• To investigate causes of treatment failure in genotypically-susceptible viruses from Ugandan patients failing ART treatment.
• To determine the presence and distribution of minority HIV-1 drug resistant variants in Ugandan patients failing treatment but with drug susceptible viral genotypes (Sanger sequencing)

Hypothesis
• Patients who are failing treatment with a susceptible genotype carry minority drug resistant variants that are not detected by the conventional Sanger sequencing.

HIV-1 Drug Resistance in Uganda: HIV-1 drug resistance interpretation

Patients failing in the absence of mutations (detected by Sanger) have an RT resistance profile similar to patients failing with mutations detected by Sanger sequencing when minority mutations are used in the interpretation.
How about partial resistance?

Hidden AZT and d4T resistance
Impact of low frequency drug resistance mutations in Ontario

There is low percentage of people living with HIV and treated with cART that have strong immunologic control but maintain a low level but persistent viral load (<200 copies/ml).

Many of these patients have been on multiple treatment regimes with evidence of past treatment failures but many are also on first line treatment.

With Dr. Colin Kovacs as a lead and in collaboration with Maple Leaf Clinic, Sunnybrook, and TGH, we have obtained and analyzed samples from 20 patients showing this low, persistent viral loads. This pilot study will be expanded to ~50 patients in early 2017.

This is currently an experimental study, and despite the heavy use of the CLIA-certified DEEPGEN™ in the US and Uganda for drug resistance testing, we are not yet providing the assay under OLA-certification in Ontario.

OVERALL CONCLUSION:

Low frequency drug resistance does matter to treatment outcome without careful follow-up, treatment intensification, and ongoing resistance monitoring.

HIV drug resistance testing

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