RESISTANCE CHARACTERISTICS OF HIV INTEGRASE INHIBITORS

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HIV Clinical Fora Series Brazil: Integrase Inhibitors 2018
Integrase Inhibitors
Strand transfer

Trans-esterification reaction $\rightarrow$ direct nucleophilic attack of the 3’ OH group on the phosphodiester backbone of the host target DNA $\rightarrow$ covalent insertion of viral DNA into the cell DNA
Integrase

5 β-sheets + 6 α helices linked by flexible loops → allow conformational changes required for 3´ processing of the viral DNA and strand transfer

Two structural components are necessary for integrase binding:

- A **hydrophobic benzyl moiety** that buries into a highly hydrophobic pocket near the active site
- A **chelating triad that binds with two Mg$^{2+}$ ions** in a rather hydrophilic region

**All potent integrase inhibitors possess a substituted benzyl component that is critical for maintaining 3‘end joining potency.**

INSTIs: Mechanism of action

Mg\(^{2+}\) and Mn\(^{2+}\) are critical cofactors in the integration phase.

INSTIs bind to the active site of Mg\(^{2+}\) ions.

Functional impairment of integrase.
Stanford HIVdb

By Andrea Low, MD and Mark Muesing, PhD. Understanding and Inhibiting Integrase in the Treatment of HIV Disease Based on a presentation at PRN by Mark Muesing, PhD and Martin Markowitz, MD

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**Major Integrase Inhibitor (INI) Resistance Mutations**

<table>
<thead>
<tr>
<th>Position</th>
<th>Cons.</th>
<th>A1K</th>
<th>Q</th>
<th>KAT</th>
<th>SAC</th>
<th>RCH</th>
<th>HRK</th>
<th>H</th>
<th>K</th>
</tr>
</thead>
<tbody>
<tr>
<td>RAL</td>
<td>66</td>
<td>T</td>
<td>E</td>
<td>E</td>
<td>G</td>
<td>Y</td>
<td>S</td>
<td>Q</td>
<td>N</td>
</tr>
<tr>
<td>EVG</td>
<td>92</td>
<td>A1K</td>
<td>Q</td>
<td>KAT</td>
<td>SAC</td>
<td>RCH</td>
<td>HRK</td>
<td>H</td>
<td>K</td>
</tr>
<tr>
<td>DTG</td>
<td>138</td>
<td>K</td>
<td>Q</td>
<td>KAT</td>
<td>SAC</td>
<td>HRK</td>
<td>H</td>
<td>K</td>
<td></td>
</tr>
</tbody>
</table>

**Bold underline**: High-level reduced susceptibility or virological response.

**Bold**: Low-level reduced susceptibility or reduced susceptibility or virological response.

Plain text: Reduced susceptibility in combination with other INI-resistance mutations.

**Abbreviations**: Dolutegravir (DTG), elvitegravir (EVG), raltegravir (RAL).

**Additional mutations**: H51Y, L74M, Q95K, T97A, S153YF, E157Q, G163RK, and S230R are relatively nonpolyorphic RAL and/or EVG-selected accessory resistance mutations. E92GV, F121Y, Y143KSGA, P145S, Q146P, and N155ST are rare nonpolyorphic IN mutations that reduce RAL and/or EVG susceptibility. G118R is a rare nonpolyorphic mutation associated with resistance to each of the INSTIs.

**References**: hivdb.stanford.edu/s/instinotes
Integrase Inhibitors

First generation

- Raltegravir (RAL)
- Elvitegravir (EVG)

Second generation

![Raltegravir](image1)

![Elvitegravir](image2)
### RAL Resistance: Three Pathways

<table>
<thead>
<tr>
<th>N155H</th>
<th>Q148K/H/R</th>
<th>Y143C</th>
</tr>
</thead>
<tbody>
<tr>
<td>L74M</td>
<td>L74M</td>
<td>E92Q</td>
</tr>
<tr>
<td>E92Q</td>
<td>E92Q</td>
<td>T97A</td>
</tr>
<tr>
<td>T97A</td>
<td>T97A</td>
<td>V151I</td>
</tr>
<tr>
<td>V151I</td>
<td>E138A</td>
<td>G163R</td>
</tr>
<tr>
<td>G163R</td>
<td>E138K</td>
<td>S230R</td>
</tr>
<tr>
<td>G163K</td>
<td>G140A</td>
<td></td>
</tr>
<tr>
<td>S230R</td>
<td>G140S</td>
<td></td>
</tr>
<tr>
<td></td>
<td>G163R</td>
<td></td>
</tr>
</tbody>
</table>

*Witmer et al, ICAAC 2008*
Key Raltegravir Mutations
Emerging RAL-resistant mutants originate from pre-existing viruses

Codoñer et al, Antiv Res 2010; Armenia D et al., JID 2012
Genotype – Phenotype Correlations of RAL Mutations

Q148 Pathway

Fold-Change IC₅₀

- Q148H
- Q148H/G140S
- Q148K
- Q148K/E138A
- Q148K/G140A
- Q148K/E138A/G140A
- Q148R
- Q148R/G140S
Integrase Inhibitors

First generation

- Raltegravir (RAL)
- Elvitegravir (EVG)

Second generation
Integrase Inhibitors

**First generation**
- Raltegravir (RAL)
- Elvitegravir (EVG)

**Second generation**
- Dolutegravir (DTG)
- Bictarvy (BIC/FTC/TAF) – FDA approved Feb 2018
- Cabotegravir - Advanced development
DTG versus RAL alignment in active site
# Optimizing the Scaffold Against a Key Mutant

<table>
<thead>
<tr>
<th>Series</th>
<th>Structure</th>
<th>IC$_{50}$ (nM) wt</th>
<th>Q148K Resistance (fold)</th>
<th>Protein Shift (fold)</th>
</tr>
</thead>
<tbody>
<tr>
<td>B-1.</td>
<td><img src="image1.png" alt="Structure B-1" /></td>
<td>3</td>
<td>48x</td>
<td>16.8x</td>
</tr>
<tr>
<td>B-2.</td>
<td><img src="image2.png" alt="Structure B-2" /></td>
<td>5</td>
<td>29x</td>
<td>3.1x</td>
</tr>
<tr>
<td>C-1.</td>
<td><img src="image3.png" alt="Structure C-1" /></td>
<td>&lt;2</td>
<td>3.8x</td>
<td>3.7x</td>
</tr>
<tr>
<td>D-1.</td>
<td><img src="image4.png" alt="Structure D-1" /></td>
<td>5</td>
<td>4.3x</td>
<td>7.9x</td>
</tr>
</tbody>
</table>
Bictegravir: Optimization of Structural A-and D-rings

Structure of Bictegravir

A-Ring

D-Ring

Lazerwith et al. ASM Microbe 2016; June 16-20, 2016; Boston, MA. Poster 414.
### Bictegravir Characteristics After Optimizing Both the A & D-ring

<table>
<thead>
<tr>
<th></th>
<th>RAL</th>
<th>EVG</th>
<th>DTG</th>
<th>BIC</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>EC$_{50}$ (nM, MT4)</strong></td>
<td>8.9</td>
<td>1.8</td>
<td>1.7</td>
<td>1.9</td>
</tr>
<tr>
<td>Plasma Adjusted EC$_{50}$ (nM, MT4)</td>
<td>21</td>
<td>43</td>
<td>47</td>
<td>83</td>
</tr>
<tr>
<td><strong>human liver microsomal Cl (L/h/kg)</strong></td>
<td>0.24</td>
<td>0.43</td>
<td>0.16</td>
<td>0.17</td>
</tr>
<tr>
<td><strong>%free human plasma</strong></td>
<td>12.2</td>
<td>0.5</td>
<td>0.7</td>
<td>0.30</td>
</tr>
<tr>
<td><strong>OCT-2 IC$_{50}$ (µM)</strong></td>
<td></td>
<td>0.13</td>
<td>0.49</td>
<td></td>
</tr>
<tr>
<td><strong>PXR %Emax@ 15 µM</strong></td>
<td></td>
<td>51</td>
<td>18</td>
<td></td>
</tr>
<tr>
<td><strong>Solubility (pH = 7, µg/mL)</strong></td>
<td>1906</td>
<td>2</td>
<td>53</td>
<td>119</td>
</tr>
<tr>
<td><strong>G140S/Q148R fold shift</strong></td>
<td>249</td>
<td>297</td>
<td>4.8</td>
<td>2.0</td>
</tr>
</tbody>
</table>

Lazerwith et al. ASM Microbe 2016; June 16-20, 2016; Boston, MA. Poster 414.
Integrase Inhibitor Resistance to Mutations and Combinations

Adapted from White K et al, 14th Euro Workshop, May 2016, Rome
BIC vs. DTG: clinically meaningful?

BIC has Potent Activity Against INSTI-Resistant HIV-1 (n=47)

<table>
<thead>
<tr>
<th>INSTI</th>
<th>≤2.5</th>
<th>&gt;2.5-≤10</th>
<th>&gt;10-fold</th>
</tr>
</thead>
<tbody>
<tr>
<td>BIC</td>
<td>70%</td>
<td>28%</td>
<td>2%</td>
</tr>
<tr>
<td>DTG</td>
<td>49%</td>
<td>34%</td>
<td>17%</td>
</tr>
<tr>
<td>EVG</td>
<td>6%</td>
<td>2%</td>
<td>92%</td>
</tr>
<tr>
<td>RAL</td>
<td>2%</td>
<td>8%</td>
<td>89%</td>
</tr>
</tbody>
</table>

a. Phenotype of INSTI against 47 patient-derived isolates with INSTI resistance in the PhenoSenseIN assay (Monogram Biosciences)

### BIC resistance *ex vivo*

**Two patterns of resistance substitutions in IN after extended culture with BIC**

- **R263K ± M50I** (<3-fold reduced susceptibility)
- **S153F or S153Y** (≤2-fold reduced susceptibility)

Selected variants remained sensitive or had low-level reduced susceptibility to BIC

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**Selecting Drug** | **Key IN Substitutions at Select Passages by Deep Sequencing (Frequency)a** | **IN Genotype of Representative SDM** | **EC_{50} Fold Change Compared to HIV-1 xLAI WT**<br>**RC**<br>| **BIC** | **DTG** | **EVG** | **RAL** | **Drug Concentration (nM)** | **Days in Culture**
| --- | --- | --- | --- | --- | --- | --- |
| BIC | None | T66I (8.0%)<br>S153F (2.4%) | T66I (3.1%)<br>S153F (38%)<br>E157K (3.4%) | S153F (97%)<br>E157K (3.2%)<br>S24G (32%) | S153F (>99%)<br>S24G (11%) | T66I<br>S153F<br>T66I/S153F<br>E157K<br>S153Y/L234F | 0.4<br>1.4<br>0.6<br>1.0<br>2.0<br>1.2<br>2.2<br>0.8<br>0.8<br>1.7<br>1.2<br>1.1<br>1.3<br>3.0<br>1.4<br>3.4<br>1.4<br>1.3<br>2.4<br>1.3<br>0.8<br>1.1<br>0.8<br>0.7<br>76%<br>52%<br>12%<br>16%<br>45%<br>120%<br>33% | 0.1<br>0.5<br>1<br>1.0<br>10<br>100<br>1000<br>10000<br>100000 | 0<br>50<br>100<br>150<br>200<br>250 | BIC (Final = P13, 207 days)<br>DTG (Final = P13, 229 days)<br>EVG (Final = P13, 138 days)<br>RAL (Final = P13, 166 days)

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*Andreatta, et al. CROI 2018*
No DTG resistance after 1st-line DTG VF in RCTs

<table>
<thead>
<tr>
<th>Study</th>
<th>Summary efficacy</th>
<th>PDVF in DTG arm</th>
<th>INSTI resistance</th>
</tr>
</thead>
<tbody>
<tr>
<td>FLAMINGO</td>
<td>DTG &gt; DRV/r</td>
<td>2 / 242</td>
<td>0</td>
</tr>
<tr>
<td>ARIA</td>
<td>DTG &gt; ATV/r</td>
<td>1 / 248</td>
<td>0 (1 K219K/Q + E138E/G)</td>
</tr>
<tr>
<td>SINGLE</td>
<td>DTG &gt; EFV</td>
<td>18 / 422</td>
<td>1 E157Q/P (no emergent INSTI DR)</td>
</tr>
<tr>
<td>SPRING-2</td>
<td>DTG = RAL</td>
<td>16 / 411</td>
<td>0</td>
</tr>
</tbody>
</table>

- DTG better than non-INSTIs, non-inferior to RAL
- No INSTI resistance emergence in ideal conditions
  - ART-naive
  - WT virus → Active backbone
  - Early ART switch after PDFV
Randomized, double-blind, multicenter, active-controlled, Phase 3 studies of treatment-naïve HIV-1 infected participants: GS-US-380-1489 (NCT02607930) and GS-US-380-1490 (NCT02607956)

Primary endpoint: proportion with HIV-1 RNA < 50 copies/mL at Week 48 by US FDA-defined Snapshot Algorithm²,³

Figure 2. Rapid Suppression of HIV-1 RNA to < 50 copies/mL through Week 48 (Missing = Excluded Approach)

B/F/TAF vs. DTG/ABC/3TC or vs. DTG + F/TAF: displayed rapid viral suppression and non-inferior efficacy at Week 48

AE=adverse event; DC=discontinuation; Other reasons= lost to follow-up, withdrew consent, investigator discretion, noncompliance, etc.)

White K, CROI, 2018, #532
Figure 4. Week 48 Virologic Outcome of B/F/TAF-treated Participants Stratified by Baseline Resistance Category

1º=Primary Resistance Substitution; 2º=Secondary Resistance Substitution

White K, CROI, 2018, #532
Table 7. Resistance Analysis Population through Week 48 and Resistance Summary

<table>
<thead>
<tr>
<th>Resistance Category</th>
<th>Number of Participants, n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>B/F/TAF (N=634)</td>
</tr>
<tr>
<td>Resistance Analysis Population (RAP) (% of FAS)</td>
<td>8 (1.3%)</td>
</tr>
<tr>
<td>Subjects with Data for Any Gene (% of RAP)</td>
<td>8 (100%)</td>
</tr>
<tr>
<td>Subjects with RT Data</td>
<td>8 (100%)</td>
</tr>
<tr>
<td>Subjects with IN Data</td>
<td>8 (100%)</td>
</tr>
<tr>
<td>Developed Resistance Substitutions to Study Drugs</td>
<td>0</td>
</tr>
</tbody>
</table>

PI = protease inhibitor; PR = protease; R = resistance
Slow resistance development and transmission in resource-rich settings

SAILING: Subjects 4 & 3

<table>
<thead>
<tr>
<th>HIV-1 RNA</th>
<th>Day 1</th>
<th>PDVF</th>
</tr>
</thead>
<tbody>
<tr>
<td>A49G, S230R, R263K</td>
<td>0.73</td>
<td>3.82</td>
</tr>
<tr>
<td>DTG FC</td>
<td>0.54</td>
<td>2.39</td>
</tr>
<tr>
<td>RAL FC</td>
<td>NR</td>
<td>7.1%</td>
</tr>
<tr>
<td>IN RC*</td>
<td>20%</td>
<td>7.1%</td>
</tr>
</tbody>
</table>

PDVF BR: No emergent resistance, and no NRTI resistance at any time points

Underwood, et al. Abs#85. IDRW June 4-8, 2013. Toronto, Canada
DTG monotherapy

10 years on NNRTI 3-drug ART

Blanco JL, et al EACS 2017
Viral dynamics during DTG monotherapy maintenance failure

Slide Courtesy of Charles Boucher, Erasmus University
Conclusions

• INSTIs are the current mainstay of ART
• Genetic barrier
  • RAL, ELV: Low
  • DTG, BIC: Higher *(but not as high as boosted PIs)*
    • Failure to DTG monotherapy with “RAL-like” resistance profiles

• Currently no need for pre-ART genotyping for clinical management
• Surveillance is warranted

• **NEVER** monotherapy
Gràcies!

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UVIC-UCC: Malu Calle

Jonathan Shapiro, Slides

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