Christos J Petropoulos is:

- The Chief Scientific Officer of Monogram Biosciences, a member of the LabCorp Specialty Testing Group
  - Monogram Biosciences markets services for the diagnosis and treatment of viral diseases including HIV and HCV infection
- An Officer (Vice President) of Laboratory Corporation of America
- A shareholder of Laboratory Corporation Holdings (LH)
• HIV Suppression Management
• HIV DNA Drug Resistance Assay (GenoSure Archive)
  – Technical challenges and solutions
  – Analytical challenges and solutions
  – Sample limitations and improvements
• Survey of routine patient sample submissions
  – Prevalence of drug resistance associated mutations
  – Hyper-mutation statistics
• Application to HIV “Shock and Kill” Cure therapy
Advancements in HIV therapy enable most patients to achieve and maintain full suppression of virus replication.

Increasingly, there are needs and desires to “fine tune” regimens while the patient’s plasma virus is undetectable.

- Improve quality of life: reduce side affects and adverse events
- Simplify regimen (reduce pill burden and/or dosing frequency) to improve adherence
- Minimize or address drug-drug interactions
- Decrease long term toxicity and enhance tolerability
- Reduce healthcare expenses

Originally planned to validate GenoSure Archive using conventional sequencing

During development it became clear that GenoSure Archive samples often could not be analyzed due to poor sequence quality:

- The cause was determined to be the presence of mixtures of APOBEC-induced hypermutated and non-hypermutated HIV sequences
  - Hypermutated (HM) sequences contain a unusually high percentage of adenine bases which results in differential electrophoretic mobility during capillary electrophoreses leading to poor sequence quality
  - HM results in an overabundance of mutations resulting in stop codons
    - TGG \(\rightarrow\) TAG or TGA or TAA)
- Next generation (clonal) sequencing overcame this technical limitation
G to A Hypermutation Reduces HIV DNA Sequence Quality

Clone 1: 50%
Clone 2: 50%

Clone 1: 80%
Clone 2: 20%

Clone 1: 90%
Clone 2: 10%

Clone 1: 95%
Clone 2: 5%
APOBEC Cytidine Deamination: Innate Defense Against HIV-1
APOBEC 3G Cytodine De-amination: G to A Hypermutation

---L-------Q-------W-------H-------L---
---L-------Q-------*-------H-------L---

TGG > TAG : TGG > TGA : TGG > TAA
# APOBEC 3G/3F Hypermutation: Potential Amino Acid Substitutions

<table>
<thead>
<tr>
<th>WT AA</th>
<th>MT AA</th>
<th>NRTI</th>
<th>NNRTI</th>
<th>PI</th>
<th>INI</th>
<th>AI</th>
<th>El</th>
<th>Tropism</th>
</tr>
</thead>
<tbody>
<tr>
<td>G (Gly)</td>
<td>S (Ser)</td>
<td></td>
<td>G190S</td>
<td>G73S</td>
<td>G140S, G163S</td>
<td>G357S</td>
<td>G36S</td>
<td>G11S</td>
</tr>
<tr>
<td>G (Gly)</td>
<td>E (Glu)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>G190E, G16E, G163E</td>
<td></td>
</tr>
<tr>
<td>D (Asp)</td>
<td>N (Asn)</td>
<td></td>
<td>D67N</td>
<td>D30N</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>E (Glu)</td>
<td>K (Lys)</td>
<td></td>
<td>E138K</td>
<td></td>
<td>E138K</td>
<td></td>
<td>E25K</td>
<td></td>
</tr>
<tr>
<td>R (Arg)</td>
<td>Q (Gln)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>R (Arg)</td>
<td>K (Lys)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M (Met)</td>
<td>I (Ile)</td>
<td>M184I</td>
<td>M184I, M230I</td>
<td>M36I, M46I</td>
<td>M154I</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
G to A Hypermutation Classifiers for Bayesian Model

- **Stop burden atypical preference**

  - **Stop**
    - Number of stop codons
    - Density
    - Chart showing distribution of stop codons for non-hypermutated and hypermutated groups.

  - **Atypical**
    - Number of atypical G-to-A mutations
    - Density
    - Chart showing distribution of atypical mutations for non-hypermutated and hypermutated groups.

  - **Burden**
    - Number of G-to-A mutations/number of G nucleotides
    - Density
    - Chart showing distribution of G-to-A mutations for non-hypermutated and hypermutated groups.

  - **Preference**
    - Number of G-to-A substitutions/number of all substitutions
    - Density
    - Chart showing distribution of G-to-A substitutions for non-hypermutated and hypermutated groups.
Filtering Hypermutated Sequences from Viral DNA Samples

Cell vDNA Before Filtering
32% hypermutated

Cell vDNA After Filtering

Plasma vRNA Before Filtering
3% hypermutated

Plasma vRNA After Filtering
Filtering Hypermutated Sequences from Viral DNA Samples

Cell vDNA Before Filtering
2% hypermutated

Cell vDNA After Filtering

Plasma vRNA Before Filtering
1.7% hypermutated

Plasma vRNA After Filtering
Hypermutation Reads = 8%
Hypermutation Reads = 63%
Hypermutation Reads = 82%
## Amplification Sensitivity: 8E5 Cell Titration

<table>
<thead>
<tr>
<th>HIV-1 DNA copies per amplification</th>
<th># tested</th>
<th># pass</th>
<th># fail</th>
<th>Amplification success</th>
</tr>
</thead>
<tbody>
<tr>
<td>300</td>
<td>5</td>
<td>5</td>
<td>0</td>
<td>100%</td>
</tr>
<tr>
<td>30</td>
<td>5</td>
<td>5</td>
<td>0</td>
<td>100%</td>
</tr>
<tr>
<td>22.5</td>
<td>5</td>
<td>5</td>
<td>0</td>
<td>100%</td>
</tr>
<tr>
<td>15</td>
<td>5</td>
<td>5</td>
<td>0</td>
<td>100%</td>
</tr>
<tr>
<td>7.5</td>
<td>5</td>
<td>5</td>
<td>0</td>
<td>100%</td>
</tr>
<tr>
<td>3</td>
<td>5</td>
<td>5</td>
<td>0</td>
<td>100%</td>
</tr>
<tr>
<td>1.5</td>
<td>5</td>
<td>4</td>
<td>1</td>
<td>80%</td>
</tr>
<tr>
<td>0.3</td>
<td>5</td>
<td>2</td>
<td>3</td>
<td>40%</td>
</tr>
</tbody>
</table>

- **Positive:** Low non-reportable rate
- **Negative:** Susceptible to founder effect and under representation
DNA Template # and Founder Effect

- **R5 = 75% of total**
  - X4 = 25%

- **R5 = 80%**
  - X4 = 20%

- **R5 = 100%**
  - X4 = 0%
Minimizing Sample Bias
Maximizing Reproducibility

- Increase sample extraction volume from 0.2 mL to 1.0 mL
- Perform PCR in triplicate and pool amplification products
- Set high read count threshold (deep coverage)
Resistance Profiles in the Archive Sequence Database
Bridging NGS and Sanger Data: NGS Reporting Threshold

correlation between Sanger and NGS mut lists, 38 identical-aliquot plasma samples, 50 NGS pct thresholds

10% report threshold
Percent of Samples with RAMs by Drug Class

- NRTI: 33.96%
- NNRTI: 29.46%
- PI: 28.77%
- IN: 2.1%
- NRTI only: 7.42%
- NNRTI only: 8.26%
- PI only: 8.28%
- Any class: 52.95%
- All classes: 0.57%
# Top Five RAMs within Each Drug Class

<table>
<thead>
<tr>
<th>Drug class</th>
<th>Mutation</th>
<th>% Samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>NRTI</td>
<td>M184V</td>
<td>23.90</td>
</tr>
<tr>
<td>NRTI</td>
<td>M41L</td>
<td>15.46</td>
</tr>
<tr>
<td>NRTI</td>
<td>D67N</td>
<td>13.90</td>
</tr>
<tr>
<td>NRTI</td>
<td>K70R</td>
<td>13.26</td>
</tr>
<tr>
<td>NRTI</td>
<td>T215Y</td>
<td>13.24</td>
</tr>
<tr>
<td>NNRTI</td>
<td>K103N</td>
<td>17.17</td>
</tr>
<tr>
<td>NNRTI</td>
<td>Y181C</td>
<td>7.45</td>
</tr>
<tr>
<td>NNRTI</td>
<td>V108I</td>
<td>5.64</td>
</tr>
<tr>
<td>NNRTI</td>
<td>G190A</td>
<td>4.40</td>
</tr>
<tr>
<td>NNRTI</td>
<td>K101E</td>
<td>3.54</td>
</tr>
<tr>
<td>PI</td>
<td>L90M</td>
<td>11.14</td>
</tr>
<tr>
<td>PI</td>
<td>M46I</td>
<td>8.63</td>
</tr>
<tr>
<td>PI</td>
<td>V82A</td>
<td>6.51</td>
</tr>
<tr>
<td>PI</td>
<td>I54V</td>
<td>5.72</td>
</tr>
<tr>
<td>PI</td>
<td>G73S</td>
<td>5.59</td>
</tr>
<tr>
<td>INI</td>
<td>N155H</td>
<td>0.82</td>
</tr>
<tr>
<td>INI</td>
<td>G140S</td>
<td>0.52</td>
</tr>
<tr>
<td>INI</td>
<td>Q148H</td>
<td>0.39</td>
</tr>
<tr>
<td>INI</td>
<td>E138K</td>
<td>0.32</td>
</tr>
<tr>
<td>INI</td>
<td>S147G</td>
<td>0.27</td>
</tr>
</tbody>
</table>
Hypermutation Profiles in the Archive Sequence Database
HM Statistics for Archive Samples

- Reportable archive samples
  - lowest % HM: 1.2%
  - highest % HM: 65.9%
  - median % HM: 14.1%
  - Samples with HM percent ≥70% are typically failed

- Non-reportable archive samples
  - lowest % HM: 1.5%
  - highest % HM: 95.9%
  - median % HM: 61.25%

- Reportable and non-reportable archive samples
  - lowest % HM: 1.2%
  - highest % HM: 95.9%
  - median % HM: 14.8%
HM Distribution: Reportable Plus Non-Reportable
Distribution of Stop Codons among Non-reportable Samples before HM Filtering
Distribution of TGA, TAA & TAG Codons among Non-reportable Samples

<table>
<thead>
<tr>
<th>Codon</th>
<th>Expected</th>
<th>Observed</th>
</tr>
</thead>
<tbody>
<tr>
<td>TAG</td>
<td>(4) AP3G</td>
<td>(4) AP3G</td>
</tr>
<tr>
<td>TAA</td>
<td>(1) AP3G</td>
<td>(5) AP3F</td>
</tr>
<tr>
<td>TGA</td>
<td>(1) AP3G</td>
<td>(1) AP3F</td>
</tr>
</tbody>
</table>

AP3G >>> AP3F

Expected
TAG > TAA > TGA

Observed
TAG > TAA > TGA
Can the degree of APOBEC-mediated G to A hypermutation of HIV-1 DNA be used as a marker for latent reservoir viability?

Quantitative Viral Outgrowth Assays (Q-VOA) under-estimate reservoir size
- **Pro**: current “gold standard”; estimates reservoir virus viability
- **Con**: extremely time and labor intensive

PCR assays over-estimate reservoir size
- **Pro**: convenient, rapid, amenable to automation
- **Con**: does not assess reservoir virus viability (all sequences amplify)

Perform quantitative PCR followed by NGS and HM analysis
- Look for incremental shift from low HM to high HM following repeated cycles of “shock and kill” therapy
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