Consequences of sequence variation on treatment response in different HIV-1 subtypes

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Conférence Fondation Méridien,
Veyrier-du-Lac, France,
April 1st, 2009
Global View of HIV Infection
Approximately 39.5 million people are living with HIV
Driving Forces of the HIV/AIDS Pandemic

Transmission

Engine
RT, Integrase & Protease

Brakes
HAART Prevention Immune & Host Factors

Model
Subtypes & Recombinant Forms (CRFs)
10-40% Diversity

The West
MSM/IDU
Subtype B
HAART
Culture, War, Economics

Africa/Asia
Heterosexual
Non-B Subtype
Suboptimal therapy
Genetic diversity of HIV

HIV

HIV-1

M

N

O

HIV-2

A

B

CRF1-43

URFs

B A C D F G H J K
Epicenters of the African pandemic
Global Distribution of HIV-1 Subtypes

Subtype B = 10%
Subtype C = 52%

Evolution of the subtype C epidemic in Africa

A. Subtype C in 1980-1985

'80-85

B. Subtype C in 1986-1990

'86-90

C. Subtype C in 1991-1996

'91-96

D. HIV-1 Subtype C percentage per Country 1996-2001

'96-'01
HIV Transmission Dynamics

Viral load - Infectivity

Non-diagnosed ~23%

1/30-1/200

1/1000-1/10,000

1/100-1/1,000

0         6 mo       2 yrs                     5 yrs            10 yrs   > 20 yrs

HAART

PHI/ RECENT INFECTION

CHRONIC NON-TREATED

CHRONIC TREATED

Regroupments « Clustering »

>50%  16%  <10%
Clonal Transmission of HIV-1

Donor

Mucosa

Recipient

>10^6 virions/ml plasma
(t_{1/2} <1 day)

1 clone: 75% des infections
2-5 (median 3) quasispecies: 23% of PHI

~10^{11} infections

Abortive

Abortive

Less fit epidemic

Viral Diversification

CTL/Immune/Host

Drug Pressure

Temps (jours)

0 7 14 21 28

Keele et al., PNAS 2008
HIV Diversity and Drug Resistance

• RT Properties increasing its propensity for Resistance Development
  • $10^{-5}$ mutational rate (vs. host polymerases $10^{-9}$ – $10^{-11}$)
  • Lack of exonuclease proofreading activity
  • One mutation introduced per replication cycle

• Most of the genetic diversity (40-50%) between subtypes lies in the *env* genes.

• Highly active antiretroviral therapy targets the key enzymes (reverse transcriptase, protease and integrase) with lower (10-15% diversity) between subtypes
Non-B Subtype Diversity in Quebec reflects the diversity at the epicenter of the pandemic.

10-15% Pol diversity
HIV Replication Cycle and Sites of Drug Activity

**Capsid** proteins

**Viral** RNA

**CD4** Receptor

**CDR5** or **CXCR4** co-receptor

**Viral** RNA

**New HIV particles**

**Unintegrated double stranded Viral DNA**

**Integrated viral DNA**

**Viral mRNA**

**gag-pol polyprotein**

**Attachment**

**Uncoating**

**Reverse Transcription**

**Integration**

**Transcription**

**Translation**

**Assembly and Release**

**NRTIs**
- AZT (Zidovudine-Retrovir)
- ddl (Didanosine-Videx)
- ddC (Zalcitabine-Hivid)
- d4T (Stavudine-Zerit)
- 3TC (Lamivudine-Epivir)
- ABC (Abacavir-Ziagen)
- TDF (Tenofovir-Viread)

**NNRTIs**
- Efavirenz (Sustiva)
- Delavirdine (Rescriptor)
- Nevirapine (Viramune)

**INls**
- Raltegravir

**Protease Inhibitors**
- Indinavir (Crixivan)
- Ritonavir (Norvir)
- Saquinavir (Fortovase)
- Nelfinavir (Viracept)
- Amprenavir (Agenerase)
- Lopinavir/r (Kaletra)

**Bevirimat**
Subtype C Signature
V106M
NNRTI Resistance Pathway
### V106M: Subtype C signature mutation

<table>
<thead>
<tr>
<th>Selection Pressure</th>
<th>B, Non-C Subtypes</th>
<th>Sous-type C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment naive</td>
<td>V(GTA)</td>
<td>V(GTG)</td>
</tr>
<tr>
<td>WT- codon 106</td>
<td></td>
<td></td>
</tr>
<tr>
<td>EFV resistance subtype C</td>
<td></td>
<td>M(ATG)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>30-50% patients</td>
</tr>
<tr>
<td>NVP resistance subtype</td>
<td>A(GCA)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>&gt;5% patients</td>
<td></td>
</tr>
<tr>
<td>V106A: Resistance to NVP</td>
<td></td>
<td></td>
</tr>
<tr>
<td>V106M Cross-resistance</td>
<td></td>
<td></td>
</tr>
<tr>
<td>NVP/EFV/DLV</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Prevalence of V106M in patients failing EFV regimens

Stanford Database
Marconi & Kuritkes Clin Infect Dis 2008
Subtype C Signature
K65R
NRTI Resistance Pathway
Discrimination Mutations
(M184V and K65R)
3TC, FTC, TFV Resistance

Excision mutations
(TAMs: M41L, D67N, K70R
L210W, L215F/Y, K219Q/E)
AZT, d4T, ddI Resistance

Nucleos(t)ide RT Inhibitor (NRTI) Pathways

Subtype C
K65R: ddl, d4T, TDF, ABC

TDF:K65R

Infrequent
Low fitness
K65R-TAM bidirectional antagonism
Hypersensitizes virus to AZT
K65R-M184V genomic antagonism

Bidirectional antagonism

AZT/d4T:TAM-2
(70R,215F)

Frequent
K65R bidirectional antagonism
K65R-M184V genomic antagonism

AZT/d4T:TAM-1
(41L,210W, 215Y)

3TC/FTC:184V

Low fitness
Increases fidelity
Decreases processivity
K65R genomic antagonism
Reduces emergent resistance to NNRTI & PI
Sensitizes TAM/215Y/F resistant viruses to AZT/d4T

ABC: L74V, Y115I, M184I,K65R
ddl: L74V

Infrequent
Low fitness
Rapid Selection of K65R Resistance in Subtype C Isolates

K65R (wk 10-12)

wt (wk 34-78)

[TDF] (µM)

0.0 2.5 5.0 7.5 10.0 12.5 15.0 17.5 20.0

Week

Subtype C

Subtype B
Facilitated Development of K65R Pathway in Subtype C

Quebec (n=1679)

Malawi (n=96)

Few Active Second-line NRTIs in 94 Pts Failing First-line Regimes in Malawi

- Many patients predicted to have no fully active NRTIs for second-line regimen based on resistance selected during first-line failure

<table>
<thead>
<tr>
<th>Potential NRTI Backbones for Second-Line Therapy, %</th>
<th>2 Fully Active NRTIs*</th>
<th>No Fully Active NRTIs</th>
</tr>
</thead>
<tbody>
<tr>
<td>FTC/TDF</td>
<td>2</td>
<td>29</td>
</tr>
<tr>
<td>ABC/ddI</td>
<td>1</td>
<td>50</td>
</tr>
<tr>
<td>ZDV/3TC + TDF</td>
<td>21</td>
<td>22</td>
</tr>
</tbody>
</table>

*Based on lower limit of Monogram assay

Phenotype of K65R Resistant Viruses

- **Recombinant B-K65R**
- **Clade C-K65R**
- **Clade AE-K65R**

Subtypes: TDF, ZDV, ABC, 3TC, ddI

Fold Resistance

0 5 10 15 20 25
Site-directed mutagenesis validation of cell culture results

What is the propensity of different recombinant viruses to develop the K65R resistance mutation under N(t)RTI treatment?

Infections of MT-2 cells and CBMCs with these viruses followed by treatment with different N(t)RTIs (single drugs or in combination).
# Mutations in MT-2 Cells after 10 Weeks

<table>
<thead>
<tr>
<th>DRUG</th>
<th>VIRUS</th>
<th>VIRUS</th>
<th>VIRUS</th>
<th>VIRUS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>NL4-3 (wt)</td>
<td>NL4-3 (64)</td>
<td>NL4-3 (65)</td>
<td>NL4-3 (64/65)</td>
</tr>
<tr>
<td>3TC</td>
<td>M184I</td>
<td>Not done</td>
<td>Not done</td>
<td>M184I</td>
</tr>
<tr>
<td>FTC</td>
<td>M184I</td>
<td>M184I</td>
<td>M184I</td>
<td>M184I</td>
</tr>
<tr>
<td>ABC</td>
<td>M184I</td>
<td>M184I</td>
<td>M184I</td>
<td><strong>K65R</strong></td>
</tr>
<tr>
<td>ddI</td>
<td>L74V</td>
<td>M184I</td>
<td>V75I</td>
<td><strong>K65R</strong></td>
</tr>
<tr>
<td>d4T</td>
<td>None</td>
<td>None</td>
<td>None</td>
<td><strong>K65R</strong></td>
</tr>
<tr>
<td>TNF</td>
<td>None</td>
<td>None</td>
<td>None</td>
<td><strong>K65R</strong></td>
</tr>
</tbody>
</table>
K65R Acquisition: Cell Culture

- Subtype B viruses with 2 point mutations (codons 64/65) used to infect MT2 cells

<table>
<thead>
<tr>
<th>Drugs</th>
<th>Virus</th>
<th>Virus</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>NL4-3 (Wild-Type - Subtype B)</td>
<td>NL4-3 (64/65 – Subtype B)</td>
</tr>
<tr>
<td>3TC</td>
<td>M184I</td>
<td>M184I</td>
</tr>
<tr>
<td>FTC</td>
<td>M184I</td>
<td>M184I</td>
</tr>
<tr>
<td>ABC</td>
<td>M184I</td>
<td>K65R</td>
</tr>
<tr>
<td>ddI</td>
<td>L74V</td>
<td>K65R</td>
</tr>
<tr>
<td>d4T</td>
<td>None</td>
<td>K65R</td>
</tr>
<tr>
<td>TFV</td>
<td>K65R</td>
<td>K65R</td>
</tr>
<tr>
<td>ABC + 3TC</td>
<td>M184I</td>
<td>M184I</td>
</tr>
<tr>
<td>ABC + FTC</td>
<td>M184I</td>
<td>K65R</td>
</tr>
<tr>
<td>d4T + ddI</td>
<td>V75I</td>
<td>K65R</td>
</tr>
<tr>
<td>TFV + 3TC</td>
<td>K65R/M184I/T215F</td>
<td>K65R</td>
</tr>
<tr>
<td>TFV + FTC</td>
<td>K65R</td>
<td>K65R</td>
</tr>
</tbody>
</table>

Duration of study: 20 passages
(Invernizzi CF, Coutsinos D et al. 15th CROI, Boston, 2008 [Poster no.849])
Reverse Transcription Leading to K65R in Subtype C and B

(+)RNA

(Subtype C) 5’- … GCU AUA AAA AAG AAA GAC AGC … -3’

(Subtype B) 5’- … GCC AUA AAA AAG AAA GAC AGC … -3’

(-)ssDNA

(Subtype C) 5’- … ACT GTC TTT CTT TTT TAT AGC … -3’

(Subtype B) 5’- … ACT GTC TTT CTT TTT TAT GGC … -3’

(+)dsDNA

(Subtype C) 5’- … GCC ATA AAA AAG AAA GAC AGT … -3’

(Subtype B) 5’- … GCC ATA AAA AAG AAA GAC AGT … -3’

Why does K65R develop more rapidly in Subtype C HIV?
No differences that explain distinct subtype patterns of K65R acquisition

Step 2: (+)dsDNA Synthesis from (-)ssDNA

In subtype C, pausing is seen at the exact nucleotide responsible for the AAG to AGG mutation which gives rise to K65R

K65R Acquisition: DNA Synthesis with Repeats

Aim 1
Conclusion: K65R Acquisition in Subtype C

- The mechanism is template-specific and enzyme-independent.
- Pausing is observed during the synthesis of dsDNA from the ssDNA intermediate.
- RT has difficulty synthesizing homopolymeric stretches.
- K65 pausing is only seen on the subtype C template during dsDNA synthesis.
- D67 pausing is more stable on the subtype B template during dsDNA synthesis.
Acknowledgements

• Dr. Mark Wainberg
• Dimitri Coutsinos
• Dr. Cédric Invernizzi
• Dr. Hongtao Xu
• Dr. Hugues Loemba
• Dr. Florence Doualla-Bell
• Dr. Max Essex
• Daniela Moisi
• Maureen Oliveira