Principles of Viral heterogeneity

- Enormous viral population
- Extreme genetic diversity
- Every possible point mutations is present
  2-20 billion mutations a day

Infection is characterized by a swarm of viruses: Quasispecies

Evolution of RNA viruses

The amount of mutations in a year are comparable with the amount of mutations in the human genome per 1 million year

Effective population size (Ne)

- Not all viruses contribute to the next generation of infectious virus
- Effective population size: Number of viral variants that contribute to the next generation: $10^3$ - $10^4$
- All single mutants are present, rarely double mutants

Nijhuis et al. PNAS 1998
Viral fitness – replication capacity

- Fitness: the ability of a virus to replicate in a certain environment
- Wild type is the virus that has the best replication capacity (is most fit) within the quasispecies of a particular host
- Wildtype is the most dominant variant in the quasispecies
- However, most variants in the viral population differ one mutation from wildtype

Antiviral Resistance

When viral replication is insufficiently suppressed variants with single mutations which are less susceptible for the therapy are selected and will dominate the quasispecies.

These resistant variants are less fit than wildtype.

Selection of mutations

- A single mutant may result in high level resistance to some drugs
- For other drugs or combinations of drugs more mutations are needed for high level resistance
- HIV will continue to adapt after therapeutic failure by selection of mutations that improve the viral fitness: compensatory mutations

Viral replication capacity

Fraction of original ratio to wildtype

<table>
<thead>
<tr>
<th>Weeks</th>
<th>Pre-therapy virus (day 0)</th>
<th>36I+54V+82T (day 28)</th>
<th>38I+54V+71V+82T (day 52)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>10</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>2</td>
<td>10</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>4</td>
<td>10</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>6</td>
<td>10</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>

Genetic barrier to Resistance

- For successful therapy
  - the number of mutations required for resistance to develop should be at least 3
  - Lopinavir/r: genetic barrier is \( \geq 2 \)
  - Integrase inhibitor: genetic barrier is 1

Genetic barrier to Resistance

- the number of mutations required for resistance to develop
  AND
  - the likelihood that such mutations indeed occur
    - Level of resistance
    - Replication Capacity
    - Drug level
Genetic barrier to Resistance

The likelihood of selection of a specific mutation is better indicated by differences in fitness than the level of drug resistance.

- 70R in RT gives less resistance to zidovudine than 215Y but has a lower effect on RC and appears first.
- TDF + FTC + NNRTI: loss of viral inhibition with 65R and NNRTI-mutation.
- In practice: 184V + NNRTI → 65R.

Use of Drug Resistance tests in clinical Practice

VIRADAPT: GT-geleide switch

Optimal Choice of HAART

Important information to start

- Viral load + CD4-cell count: baseline, dynamics
- Antiretroviral History: reason therapy changes
- Estimation of adherence
- Drug levels
- Toxicity, Co-morbidity and co-medication
- Preference patient
- Future options options
Study the history!

B. Resistance determination
- Recent test; also by low hiv-loads
- Cumulative resistance profile
- Study moments without resistance determinations
  - Estimate based on dynamics and therapy history
  - Retrospective determination

Study failure moments

C. Use of active drugs
- Cross resistance
- Interpretation algorithm/Consult an expert
- New HIV-drugs:
  - Integrase inhibitor Raltegravir
  - CCR5-inhibitor: Maraviroc
  - NNRTI: Etravirine
  - PI: Darunavir/r

Interpretation tools

Identification relevant mutations: Tables:
- www.iasusa.org
- www.hivresistanceweb.com

Interpretation systems: Algorithms:
- www.hivdb.stanford.edu
- www.hiv-grade.de

Interpretation evolution of resistance patterns
- Consultation

Optimal Choice for therapy changes

C2. Choose an optimal backbone
- Genotypic Sensitivity Score
- Interactions between mutations
- Insight in evolution patterns (Subtype)
- Calculate genetic barrier
Is drug Resistance still an issue?

Increase suppression in HIV-infected population

Trends in incidence of HIV resistance

Switch from boosted PI to integrase inhibitor in pretreated individuals

- What is the genetic barrier of recently approved drugs?

TMC-125 and TMC 278: 2nd generation NNRTIs
Maraviroc: mechanism of failure in Motivate study

Pretreated patients with virological failure at week 24:
- 2/3 switch to X4 virus from pre-therapy reservoir
- MVC resistance was only observed in <50% of patients without receptor switch (R5-virus):
- gp120 mutations, no clear correlation between mutations and resistance
- Genetic barrier?

Darunavir: new boosted PI genetic barrier >3

![Graph showing efficacy analysis](Arribas et al. AIDS 2010, 24:223-230)

MPC-4326 (bevirimat)

- Maturation inhibitor: Interferes with the last step in the processing of the HIV-1 Gag protein.

![Immature versus mature Gag](IMMATURE MATURE)

MPC-4326 (bevirimat): GB differs per individual

- Accumulation of bevirimat mutations in gag in isolates that already contain protease mutations
- A shift to a genetic gag motif favoring resistance to bevirimat in people with mutations conferring resistance to PIs
- A high rate of bevirimat mutations in gag in untreated people with HIV-1 subtypes other than B

![Graph showing GB differences](Van Baelen Antimicrob Agents Chemother. 2009; Verheyen J, IDRW 2009)

Virological efficacy and emergence of drug resistance in rural Tanzania Johannessen et al. BMC 2009

Overall the reported resistance rate does not appear to exceed earlier rates reported in industrialised countries (9% after 2 yrs of HAART Phillips et al. AIDS 2005)

![Resistance rates over years on ART](Phillips et al. AIDS 2005)

Dual-class resistance, (NRTIs and NNRTIs) was found in 64%, raising concerns about exhaustion of future antiretroviral drug options
HAART in a rural district of Malawi: Ferradini et al. Lancet 2006

- Programme in Chiradzulu: Cross-sectional study
- 84% (334/398) HIV-RNA <400 cp/mL
- Self-reported poor adherence (<80%) in the past 4 days best predictor of detectable VL
- GT analysis: subtype C
- 6% no mutations.
- 84% NRTI: 75% 184V, 10% 65R
- 94% NNRTI:
- 84% of patients with HIV-RNA >1000 double

**Subtype C**
Mean 9 months on therapy, first VL determination: 10% mutation 65R

Resistance data from Elanddoorn: Subtype C

- Viral suppression was achieved in 73% (230/313) of pts

<table>
<thead>
<tr>
<th>ART regimen</th>
<th>Resistance mutations</th>
<th>Time on ART</th>
<th>NRTI resistance mutations</th>
<th>Viral</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 D4T/3TC/3TC</td>
<td>None</td>
<td>12</td>
<td>None</td>
<td>100% 3TC</td>
</tr>
<tr>
<td>2 D4T/3TC</td>
<td>154V</td>
<td>12</td>
<td>100% 3TC</td>
<td>100% 3TC</td>
</tr>
<tr>
<td>3 D4T/3TC</td>
<td>154V</td>
<td>12</td>
<td>100% 3TC</td>
<td>100% 3TC</td>
</tr>
<tr>
<td>4 D4T/3TC</td>
<td>154V</td>
<td>12</td>
<td>100% 3TC</td>
<td>100% 3TC</td>
</tr>
<tr>
<td>5 D4T/3TC</td>
<td>154V</td>
<td>12</td>
<td>100% 3TC</td>
<td>100% 3TC</td>
</tr>
<tr>
<td>6 D4T/3TC</td>
<td>154V</td>
<td>12</td>
<td>100% 3TC</td>
<td>100% 3TC</td>
</tr>
<tr>
<td>7 D4T/3TC</td>
<td>154V</td>
<td>12</td>
<td>100% 3TC</td>
<td>100% 3TC</td>
</tr>
<tr>
<td>8 D4T/3TC</td>
<td>154V</td>
<td>12</td>
<td>100% 3TC</td>
<td>100% 3TC</td>
</tr>
<tr>
<td>9 D4T/3TC</td>
<td>154V</td>
<td>12</td>
<td>100% 3TC</td>
<td>100% 3TC</td>
</tr>
<tr>
<td>10 D4T/3TC</td>
<td>154V</td>
<td>12</td>
<td>100% 3TC</td>
<td>100% 3TC</td>
</tr>
<tr>
<td>11 D4T/3TC</td>
<td>154V</td>
<td>12</td>
<td>100% 3TC</td>
<td>100% 3TC</td>
</tr>
<tr>
<td>12 D4T/3TC</td>
<td>154V</td>
<td>12</td>
<td>100% 3TC</td>
<td>100% 3TC</td>
</tr>
<tr>
<td>13 D4T/3TC</td>
<td>154V</td>
<td>12</td>
<td>100% 3TC</td>
<td>100% 3TC</td>
</tr>
<tr>
<td>14 D4T/3TC</td>
<td>154V</td>
<td>12</td>
<td>100% 3TC</td>
<td>100% 3TC</td>
</tr>
<tr>
<td>15 D4T/3TC</td>
<td>154V</td>
<td>12</td>
<td>100% 3TC</td>
<td>100% 3TC</td>
</tr>
<tr>
<td>16 D4T/3TC</td>
<td>154V</td>
<td>12</td>
<td>100% 3TC</td>
<td>100% 3TC</td>
</tr>
</tbody>
</table>

High level of NNRTI mutations: 58%: 2 NNRTI 26%: 3NNRTI in absence of NRTI related TAMS or 65R

Conclusions

Drug resistance in treated patients is manageable, but information on the genetic barrier of new drugs is still limited

Transmitted resistance is stable, but clinical relevant depending on the genetic barrier of the initial therapy

Less strict criteria in RLS facilitates accumulation of resistance mutations

Acknowledgements

- Monique Nijhuis
- Axel Fun
- Roos Barth
- Andy Hoepelman
- Hugo Tempelman
- Jens Verheyen
- Jurgen Vercauteren
- Charles Boucher
- EC supported
  - EuropeHIVResistance Network
  - LSHP-CT-2006-518211

Cohort data: Ndlovu care group in South Africa, Barth AIDS 2008

- 3 sites in Limpopo: data Elandsdoorn
- Ca 2000 patients on therapy: NNRTI + 2 NRTIs
- Criteria: CD4 <200/WHO stage 4, therapy-buddy, attending of 3 appointments, distance
- Frequent monitoring CD4 count and plasma HIV-RNA:
- bsl, 6 wks, 3, 6, 9, 12 m and thereafter every 6 months
- Virological failure: after initial suppression <50, rebound >1000 copies/ml
- Evaluation of therapy response in the first year: 313 patients