Bulk and Clonal Resistance Analyses in Highly Experienced Patients Failing Raltegravir, Etravirine and Darunavir/ritonavir Containing Regimen (ANRS 139 TRIO Trial)

B Roquebert, C Colin, AM Taburet, C Fagard, C Katlama, JM Molina, C Jacomet, G Chêne, Y Yazdanpanah, D Descamps and the ANRS 139 TRIO Trial
Background

✓ **Aim of ANRS 139 TRIO trial**: to assess the safety and efficacy of a regimen containing raltegravir, etravirine and darunavir/ritonavir in multidrug-resistant treatment-experienced patients

✓ **Primary outcome**: proportion of patients reaching plasma HIV-1 RNA < 50 copies/mL at week 24

✓ **Results**: Potent virologic efficacy at week 24 as well as at week 48: 93% and 86% of patients had undetectable plasma viral load (VL), respectively

Yazdanpanah Y, 2009
Objectives

✓ Analyze the emergence of resistant mutant viruses at the time of virological failure

✓ Investigate the impact of baseline integrase (IN) polymorphism on virological failure

Virological Failure (VF) was defined as a plasma VL > 50 copies/mL at week 24 or between week 24 and week 48
Patients and Methods

✓ Patients:
  - 14 patients presented a VF (10 at W24 and 4 between W24 and W48)
  - Median (IQR) plasma VL was 90 [IQR: 60-783] copies/mL at time of VF

✓ Methods:
  - Bulk sequencing: www.hivfrenchresistance.org
  - ANRS algorithm and IAS-USA list
  - IN clonal analyses: IN gene PCR products were cloned into the pCR4-TOPO® vector (Invitrogen) and the purified clonal fragments were sequenced and analyzed
### Results: bulk sequencing

<table>
<thead>
<tr>
<th>Patient</th>
<th>VL (copies/mL)</th>
<th>Protease mutations</th>
<th>Reverse transcriptase mutations</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>NRTI</td>
</tr>
<tr>
<td>1</td>
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<td>L10V; I15V; K20T; <strong>L33F</strong>; M36I; I54V; A71I; V77I; N88S; L90M</td>
<td>M41L; L74V; M184V; T215Y</td>
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<td></td>
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<td>L10V; I15V; K20T; <strong>L33F</strong>; M36I; I54V; <strong>I62V</strong>; A71I; V77I; N88S; <strong>L89I</strong>; L90M</td>
<td>NS</td>
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<tr>
<td>2</td>
<td>D0 29228</td>
<td>L10I; <strong>L33F</strong>; M46L; I54A; D60E; I62V; L63P; A71T; G73S; V77I; <strong>I84V</strong>; L90M</td>
<td>M41L; M184V; T215Y</td>
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<td>VF 50975</td>
<td>I62V; A71T; V77I</td>
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</tr>
<tr>
<td>6</td>
<td>D0 610000</td>
<td>G16E; <strong>L33F</strong>; M46I; I54V; H69K; V77I; V82A</td>
<td>M41L; M184V; T215Y</td>
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<tr>
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<td>7</td>
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<td>D67N; K70R; T215F; K219Q</td>
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<td>D67N; K70R</td>
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<td>M41L; D67N; M184V; L210W; T215Y; <strong>K219E</strong></td>
</tr>
</tbody>
</table>

Presented at the 8th European HIV Drug Resistance Workshop, March 17-19 2010, Sorrento, Italy
Results: bulk sequencing

For 3 of them, accumulations of resistance associated mutations in connection with investigational drugs were observed:

- **Darunavir** resistance mutations: emergence of I50V mutation at W16 replaced with L76V at W24 in one patient
- **Etravirine** resistance mutations: emergence of V179D; V90I and Y181C

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<td>NNRTI</td>
</tr>
<tr>
<td>2</td>
<td>D0 29228</td>
<td>L10I; <strong>L33F</strong>; M46L; I54A; D60E; I62V; L63P; A71T; G73S; V77I; <strong>I84V</strong>; L90M</td>
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<td>VF 50975</td>
<td>I62V; A71T; V77I</td>
<td><strong>V179D</strong></td>
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<td>6</td>
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<td>G16E; <strong>L33F</strong>; M46L; I54V; H69K; V77I; V82A</td>
<td>none</td>
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<tr>
<td></td>
<td>VF 1200</td>
<td>G16E; <strong>L33F</strong>; M46L; I54V; H69K; <strong>L76V</strong>; V77I; V82A</td>
<td><strong>V90I</strong></td>
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<tr>
<td>7</td>
<td>D0 69600</td>
<td>L10I; G16E; K20R; <strong>L33F</strong>; M36L; I54T; Q58E; V82A; L90M</td>
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<td>VF 432</td>
<td>L10I; G16E; K20R; <strong>L33F</strong>; M36L; I54T; Q58E; V82A; L90M</td>
<td><strong>Y181C</strong></td>
</tr>
</tbody>
</table>
Results: bulk sequencing

✓ No RAL primary (Y143C/H/R, Q148H/K/R and N155H) resistance associated mutations were detected in any samples neither at baseline, nor at VF

✓ No RAL secondary (L74M, E92Q, T97A, E138A/K and G140A/S) resistance associated mutations were detected in any samples neither at baseline, nor at VF

✓ Follow-up:
  ➢ 3 patients had undetectable plasma VL at W48
  ➢ 1 patient stopped taking the study drugs
  ➢ 1 patient had a detectable VL at W48
Results:
baseline IN polymorphism

- IN polymorphisms >10% were observed at 27 positions.
- Among them, IN polymorphisms >20% were observed at 13 positions: 17, 31, 39, 72, 101, 112, 113, 119, 124, 125, 201, 206 and 234.
- No impact of baseline IN polymorphisms was observed on VF neither at W24 nor at W48.
Results:
Integrase clonal analysis

✓ Successfully performed at baseline and at time of VF for 3 patients

✓ Clonal baseline polymorphisms were similar to bulk baseline polymorphisms with less than 3 clones showing different amino acids for each of the 3 patients

✓ IN clonal analyses showed neither the presence nor the selection of minority variants carrying primary or secondary raltegravir mutations at baseline or at VF, respectively

✓ For 1 patient: G140D substitution was selected in 1/95 clones at VF
Conclusion

- In these highly experienced patients receiving a combination of 3 fully active drugs, virological failure occurred in only 14/103 patients with plasma viral load < 1000 copies/mL.
- Darunavir and etravirine associated mutations were detected at virological failure in only one and 3 patients, respectively.
- No raltegravir resistance mutations were observed using bulk sequencing or clonal analyses.
- No impact of baseline IN polymorphism was observed on virological failure occurrence.
Acknowledgments

The patients for their participation and their commitment during the study and the TRIO Study Group:

**SCIENTIFIC COMMITTEE**
- Y. Yazdanpanah (Chair)
- G. Chêne
- D. Descamps
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**ANRS**
- JF Delfraissy
- MJ. Commoy
- S. Couffin-Cadiergues
- A. Bouxin-Metro
- A. Diallo

**DSMB**
- D. Costagliola
- J. Caron
- F. Berdougo
- D. Rey
- O. Patey

**Merck Sharpe & Dohme-Chibret**
(provided raltegravir)
- A. Aslan
- E. Dohin

**Tibotec, a division of Janssen**
(Cilag provided etravirine)
- A. Cheret
- MB. Hadacek

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- Rennes (F. Souala)
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- Clermont-Ferrand (C. Jacomet)
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- Dijon (L. Piroth)
- Hôtel-Dieu, Paris (A. Compagnucci)
- Perpignan (H. Aumaitre)
- Tours (F. Bastides)
- Pontoise (L. Blum)