Two-drug combination passage among dolutegravir, rilpivirine, elvitegravir and Lamivudine

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Background

- A two-drug regimen may provide an option for less adverse events and reduced administrative burden; however, durable virologic suppression and the risk for emergence of drug resistant virus is of concern.

- Recently, results of LATTE study as two-drug oral maintenance therapy of INSTI, cabotegravir (CAB) + NNRTI, rilpivirine (RPV) has demonstrated non-inferiority to the preferred 3-drug regimen through 96 weeks. (CROI 2015, poster 554LB).

- Regimen switch study to INSTI, dolutegravir (DTG) + RPV from current antiretroviral regimen (SWORD-1, 2) are ongoing.

- In this study, *in vitro* passage studies of two-drug combinations were conducted to investigate viral growth and drug resistance emergence pattern by passaging under 2-drug combination compared with that of under a single drug.
**Materials and Methods in a concentration constant method**

**Virus and cell:** NL432 and MT-2  
**Drugs:** single: RPV, 3TC, EVG, DTG  
combination: RPV + DTG, 3TC + DTG, RPV + 3TC, RPV + EVG, 3TC + EVG  
**Drug concentration:**  
single: 0.5, 1, 2, 4, 8, 16, 32, 64x EC50* for RPV, EVG, DTG  
0.5, 1, 2, 64, 320, 640x EC50* for 3TC  
combination: 1, 2, 4, 8, 16x cEC50# for each drug in the combination  

#Combination EC50_{Dn} (cEC50_{Dn}) = EC50_{Dn} x Fractional Inhibitory Concentration Index (FICI),  
where FICI of drug 1 is equal to FICI of drug 2 for each drug combination. (n= 1 or 2)

**Passage condition:** Passage under constant drug concentrations through 85 days

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<table>
<thead>
<tr>
<th>drug</th>
<th>EC50 nM</th>
<th>EC90 nM</th>
</tr>
</thead>
<tbody>
<tr>
<td>3TC (Lamivudine)</td>
<td>3310</td>
<td>9055</td>
</tr>
<tr>
<td>RPV (Rilpivirine)</td>
<td>1.2</td>
<td>2.6</td>
</tr>
<tr>
<td>DTG (Dolutegravir)</td>
<td>2.1</td>
<td>5.3</td>
</tr>
<tr>
<td>EVG (Elvitegravir)</td>
<td>1.4</td>
<td>4.4</td>
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</table>

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<table>
<thead>
<tr>
<th>D1 + D2</th>
<th>FICI</th>
<th>cEC50 D1 nM</th>
<th>cEC50 D2 nM</th>
</tr>
</thead>
<tbody>
<tr>
<td>EVG + 3TC</td>
<td>0.431</td>
<td>0.60</td>
<td>1426</td>
</tr>
<tr>
<td>DTG + 3TC</td>
<td>0.428</td>
<td>0.89</td>
<td>1416</td>
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<tr>
<td>EVG + RPV</td>
<td>0.509</td>
<td>0.71</td>
<td>0.63</td>
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<tr>
<td>DTG + RPV</td>
<td>0.489</td>
<td>1.0</td>
<td>0.60</td>
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<tr>
<td>RPV + RPV</td>
<td>0.499</td>
<td>0.62</td>
<td>0.62</td>
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<tr>
<td>3TC + RPV</td>
<td>0.452</td>
<td>1496</td>
<td>0.56</td>
</tr>
</tbody>
</table>

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cEC50 is roughly equal to \(\frac{1}{2} \times EC50\).
Passage results of single compounds

**3TC**
- More than 64x EC50
- RT: M184V at day 14
- RT: M184I at day 57
- RT: M184I at day 85

**RPV**
- More than 4x EC50
- RT: K101E at day 14
- RT: Y181C at day 57
- RT: Y181C at day 85

**EVG**
- More than 32x EC50
- IN: Q148R at day 28
- IN: T66I at day 28
- IN: T66I at day 85

**DTG**
- 4x EC50 is enough to stop HIV proliferation
- No mutation
Passage results of combination with 3TC

A) 3TC+EVG

- More than 4x cEC50
- RT: M184I at day 28
- IN: S147G
- RT: M184I at day 57

B) 3TC+DTG

- More than 4x cEC50
- RT: M184I at day 28
- IN: No mutation
- RT: M184I at day 14
- RT: M184I at day 57

- Both combination could stop HIV proliferation at more than 4x cEC50.
- No INSTI resistant virus emerged in the combination of 3TC and DTG.
Passage results of combination with RPV

A) RPV+EVG

* FC of R263K against EVG is 1.3.

More than 4x cEC50

RT: T240I at day 57
IN: R263K*

RT: None
IN: T66I

B) RPV+DTG

More than 2x cEC50

RT: No mutation
IN: No mutation

Neither NNRTI nor INSTI resistant virus emerged in the combination of RPV and DTG.
Summary Results of Concentration Constant Method

Single drug

<table>
<thead>
<tr>
<th>Fold concentration of EC50</th>
<th>3TC</th>
<th>RPV</th>
<th>EVG¹)</th>
<th>DTG</th>
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<tbody>
<tr>
<td>320x</td>
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<td>ND</td>
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<td>ND</td>
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<tr>
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<td>ND</td>
<td>14</td>
<td>14*</td>
<td>ND</td>
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<table>
<thead>
<tr>
<th>Fold concentration of cEC50</th>
<th>3TC+RPV</th>
<th>3TC+DTG</th>
<th>3TC+EVG</th>
<th>RPV+DTG</th>
<th>RPV+EVG</th>
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</thead>
<tbody>
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<td>57</td>
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No replication
Resistant virus emerged (the first day when a primary resistant virus was isolated)
Replicated without resistant mutation
ND: No data

1) Q148R was isolated in a well, however T66I emerged at day 14 in another well (*data not shown).

2-drug combination

DTG: 8.4nM > 4.0nM
RPV: 9.6nM > 2.4nM

Combination of RPV and DTG is the best among those investigated in this study.
Summary Results of Concentration Stepwise Increasing Method

* IN/R263K emerged at day 85 after RT/M184I emerged at day 14. FC of R263K against DTG is 1.5.

- Combination of RPV and DTG is the best in concentration stepwise increasing method also.
Conclusions & Discussion

• Two-drug combination could stop HIV proliferation at lower concentration than single drug.

• Combination of RPV and DTG is the best among those investigated in this study because neither NNRTI nor INSTI resistant virus emerged and because HIV could not replicate at the lowest concentration.

  Single $\Rightarrow$ two-drug
  
  RPV: 8xEC50(9.6nM)  4xEC50(2.4nM)
  DTG: 4xEC50(8.4nM)  4xEC50(4.0nM)

• Concentration stepwise increasing method showed the similar results as the constant concentration method with
  • Resistant virus emerged timing is the same as that with the highest drug concentration condition in the constant concentration method.
  • Combination of RPV+DTG could stop HIV proliferation at the lowest concentration among combinations investigated in this method although RT/E138K or Y181C emerged.