HIGH VIRAL LOAD AND TREATMENT RESPONSE

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Presented at the 10th Eu Meeting on HIV & Hepatitis, 28-30 March 2012, Barcelona
CONSIDERATIONS

• High VL (>100,000 c/mL) and/or low CD4 (<100/mm3) count are associated with higher risk of treatment failure
• Will a quick control of VL help to reduce the risk of emergence of resistance?
• Rapid selection of drug-resistant HIV-1 during the first weeks of suppressive ART in naïve patients. Role for intensification during initial treatment?
• Is there something at a PK level that causes insufficient drug delivery into cells when HIV VL is very high?

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• Is there something at a PK level that causes insufficient drug delivery into cells when HIV VL is very high?
Predictors of Virological Success and Ensuing Failure in HIV-Positive Patients Starting Highly Active Antiretroviral Therapy in Europe

Results From the EuroSIDA Study

Roger Paredes, MD; Amanda Mastroi, PhD; Ole Kirk, MD; Adriano Lazzerini, MD; Simon E. Barton, MD; Jan van Lunzen, MD; Terese L. Katzenstein, PhD; Francisco Antunes, PhD; Jens D. Lundgren, MD, DMSc; Bonaventura Clotet, PhD; for the EuroSIDA Study Group

Background: Predictors of virological response to highly active antiretroviral therapy (HAART) have never been systematically evaluated in a large continental multicenter cohort of unselected human immunodeficiency virus (HIV)-infected people.

Objectives: To determine the factors related to achieving and maintaining undetectable plasma HIV-1 RNA levels among HIV-1-infected patients first starting protease inhibitor– or nonnucleoside retrotranscriptase inhibitor–containing HAART in Europe.

Design: Prospective multicenter cohort study.

Setting: Fifty-two clinical centers in 17 European countries included in the EuroSIDA Study Group, from August 1996 to April 1999.

Patients: A total of 1469 HIV-positive patients first starting HAART recruited from an unselected cohort of more than 7300 HIV-positive patients.

Main Outcome Measure: Detection of factors related to virological success after first starting HAART (baseline) and ensuing failure by standard survival techniques, including Kaplan-Meier techniques and Cox proportional hazards models. All analyses were intention to treat.

Results: Most patients (80%) achieved plasma HIV-1 RNA levels of less than 500 copies/mL during follow-up (60.4% at 6 months from the onset of HAART). Patients with higher baseline HIV-1 RNA levels (relative hazard [RH], 0.78 per log higher; 95% confidence interval [CI], 0.69-0.84; P<.001) and those taking saquinavir mesylate hard gel as a single protease inhibitor (RH, 0.82; 95% CI, 0.47-0.82; P<.001) were less likely to reach undetectable HIV-1 RNA levels. Conversely, higher CD4+ lymphocyte counts (RH per 50% higher, 1.09; 95% CI, 1.02-1.16; P = .008) and the initiation of 3 or more new antiretroviral drugs (RH, 1.29; 95% CI, 1.03-1.61; P = .02) were independent predictors of higher success. Once success was achieved, HIV-1 RNA levels rebounded in more than one third of all patients during follow-up (24% at 6 months). Antiretroviral-naive patients (RH, 0.50; 95% CI, 0.29-0.87; P = .01), older patients (RH, 0.86 per year older; 95% CI, 0.73-0.98; P = .04), and those starting a protease inhibitor other than saquinavir hard gel (RH, 0.66; 95% CI, 0.44-0.98; P = .04) were at decreased hazard for virological failure. Higher baseline HIV-1 RNA level (RH, 1.18 per log higher; 95% CI, 0.99-1.40; P = .08) and a longer time to achieve virological success (RH per 12 months, 1.53; 95% CI, 1.02-2.38; P = .06) were marginally significant predictors of a decreased hazard of ensuing virological failure.

Conclusions: HAART is associated with a favorable virological response if started when the baseline HIV-1 RNA level is low, if at least 2 new nucleoside retrotranscriptase inhibitors are added, and if standard doses of saquinavir hard gel capsule are avoided as a single protease inhibitor. Older patients are more likely to achieve virological success. However, the duration of virological response is predicted by an antiretroviral-naive status and by the use of specific regimens. Lower baseline HIV-1 RNA levels and rapid maximal viral suppression seem to be other important factors in the durability of virological response.

Arch Intern Med. 2000;160:1123-1132

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Pooled ECHO & THRIVE: Response by Baseline CD4 and HIV RNA

Randomized, double-blind, double-dummy, multicenter, 96-week study

Objective: virologic outcomes; univariate

Cohen C, et al. 19th CROI; Seattle, WA; March 5-8, 2012. Abst. 626.
Pooled ECHO & THRIVE: Response by Baseline CD4 and HIV RNA

Randomized, double-blind, double-dummy, multicenter, 96-week study

Objective: virologic outcomes; univariate

Cohen C, et al. 19th CROI; Seattle, WA; March 5-8, 2012. Abst. 626.
Results from a Single Arm Study of Darunavir/Ritonavir plus Raltegravir in treatment-naïve HIV-1 infected patients (ACTG A5262)

Efficacy of a nucleoside-sparing regimen of darunavir/ritonavir plus raltegravir in treatment-naïve HIV-1-infected patients (ACTG A5262)


Objective: To explore darunavir/ritonavir (DRV/r) plus raltegravir (RAL) combination therapy in antiretroviral-naïve patients.

Design: Phase III, single-arm, open-label, multicenter study.

Methods: One hundred and twelve antiretroviral-naïve, HIV-1-infected patients received DRV/r 800/100mg once daily and RAL 400mg twice daily. Primary endpoint was virologic failure by week 24. Virologic failure was defined as confirmed viral load of 1000 copies/ml or more at week 12, or an increase of more than 0.5 log10 copies/ml in viral load from week 4 to 12, or a confirmed viral load of more than 50 copies/ml or after week 24. Protease and integrase genes were sequenced in patients experiencing virologic failure.

Results: Virologic failure rate was 16% (95% confidence interval (CI) 10–24%) by week 24 and 26% (95% CI 19–36%) by week 48 in an intent-to-treat analysis. Viral load at virologic failure was 51–200 copies/ml in 17/28 failures. Adjusting for age and sex, virologic failure was associated with baseline viral load of more than 100 000 copies/ml (hazard ratio 3.76, 95% CI (1.52–9.31), P = 0.004) and lower CD4 cell count (0.77 per 100 cells/μl increase [95% CI 0.61–0.98], P = 0.037). When trough RAL concentrations were included as a time-varying covariate in the analysis, virologic failure remained associated with baseline viral load more than 100 000 copies/ml [hazard ratio = 4.67 (95% CI 1.93–11.25), P < 0.001], whereas RAL level below detection limit in plasma at one or more previous visits was associated with increased hazard [hazard ratio = 3.42 (95% CI 1.41–8.26), P = 0.006]. All five participants with integrase mutations during virologic failure had baseline viral load more than 100 000 copies/ml.

Conclusion: DRV/r plus RAL was effective and well tolerated in most patients, but virologic failure and integrase resistance, in particular in patients with baseline viral load more than 100 000 copies/ml.

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AIDS 2011, 25:2113–2122

Presented at the 10th Eu Meeting on HIV & Hepatitis, 28-30 March 2012, Barcelona
QDMRK, A Phase III Study of the Safety & Efficacy of Once Daily (QD) Versus Twice Daily (BID) Raltegravir (RAL) in Combination Therapy for Treatment-Naïve HIV-Infected Patients (Pts)

J. Eron¹, J. Rockstroh², J. Reynes³, J. Andrade⁴, J. Madruga⁵, J. Zhao⁶, P. Sklar⁶, B-Y. Nguyen⁶ for the QDMRK Study Team

¹Univ. of North Carolina, Chapel Hill, NC, USA; ²Univ. of Bonn, Bonn-Venusberg, Germany; ³Montpellier Univ. Hospital, Montpellier, France; ⁴Universidad de Guadalajara, Mexico; ⁵Centro de Referência DST/AIDS, São Paulo, Brazil; and ⁶Merck Research Laboratories, North Wales, PA, USA

Presented at the 10th Eu Meeting on HIV & Hepatitis, 28-30 March 2012, Barcelona
All patients received TDF/FTC FDC

† Non-completer equals failure (NC=F) approach treats all discontinuations as failures

Presented at the 10th Eu Meeting on HIV & Hepatitis, 28-30 March 2012, Barcelona
### QDMRK – HIV RNA < 50 copies/mL at Week 48 by Subgroup (NC=F)

<table>
<thead>
<tr>
<th>Baseline HIV RNA (copies/mL)</th>
<th>Response</th>
<th>Difference</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>RAL QD</td>
<td>RAL BID</td>
</tr>
<tr>
<td></td>
<td>n/N</td>
<td>n/N</td>
</tr>
<tr>
<td>&gt; 100,000 copies/mL</td>
<td>113/152</td>
<td>128/152</td>
</tr>
<tr>
<td>≤ 100,000 copies/mL</td>
<td>205/230</td>
<td>215/234</td>
</tr>
</tbody>
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<tr>
<th>Baseline CD4 (cells/mm³)</th>
<th>Response</th>
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</tr>
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<tbody>
<tr>
<td></td>
<td>RAL QD</td>
<td>RAL BID</td>
</tr>
<tr>
<td></td>
<td>n/N</td>
<td>n/N</td>
</tr>
<tr>
<td>≤ 200 cells/mm³</td>
<td>63/89</td>
<td>80/99</td>
</tr>
<tr>
<td>&gt; 200 cells/mm³</td>
<td>254/292</td>
<td>262/286</td>
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<tr>
<td></td>
<td>n/N</td>
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</tr>
<tr>
<td>RAL QD</td>
<td>113/152</td>
<td>74.3</td>
<td>128/152</td>
</tr>
<tr>
<td>RAL BID</td>
<td>205/230</td>
<td>89.1</td>
<td>215/234</td>
</tr>
</tbody>
</table>

#### Baseline HIV RNA (copies/mL)

- **> 100,000 copies/mL**
  - 113/152 (74.3%)
  - 128/152 (84.2%)
  - Difference: -9.9% (95% CI: -19.0, -0.8)

- **≤ 100,000 copies/mL**
  - 205/230 (89.1%)
  - 215/234 (91.9%)
  - Difference: -2.7% (95% CI: -8.3, 2.7)

#### Baseline CD4 (cells/mm³)

- **≤ 200 cells/mm³**
  - 63/89 (70.8%)
  - 80/99 (80.8%)
  - Difference: -10.0% (95% CI: -22.3, 2.2)

- **> 200 cells/mm³**
  - 254/292 (87.0%)
  - 262/286 (91.6%)
  - Difference: -4.6% (95% CI: -9.8, 0.4)
Patients with high viral load (>100K) do worse than those with low VL (<100K) and QD regimens are even worse that BID in this setting for achieving virologic success
High pre-therapy viral load is associated with delayed and decreased control of HIV replication also at the time of modern HAART

Carlo F Perno

Presented at the 10th Eu Meeting on HIV & Hepatitis, 28-30 March 2012, Barcelona
The time to achieve virological undetectability and the rate of success at 48 weeks are pre-HAART viremia dependent.

Kaplan Meyer survival curves (Event viremia < 50 copies/mL)

Pre-HAART Viremia 30K ÷ 100K cps/mL

15 weeks

98% of success

On treatment analysis

Perno et al., EACS 2011

Presented at the 10th Eu Meeting on HIV & Hepatitis, 28-30 March 2012, Barcelona
The time to achieve virological undetectability and the rate of success at 48 weeks are pre-HAART viremia dependent.

Kaplan Meyer survival curves (Event viremia < 50 copies/mL)

Pre-HAART Viremia 100K ÷ 300K cps/mL

On treatment analysis

18 weeks

93% of success
The time to achieve virological undetectability and the rate of success at 48 weeks are pre-HAART viremia dependent.

Kaplan Meyer survival curves (Event viremia<50 copies/mL)

On treatment analysis

Pre-HAART Viremia 300K ÷ 500K cps/mL

22 weeks

93% of success

Perno et al., EACS 2011

Presented at the 10th Eu Meeting on HIV & Hepatitis, 28-30 March 2012, Barcelona
The time to achieve virological undetectability and the rate of success at 48 weeks are pre-HAART viremia dependent.

Kaplan-Meyer survival curves (Event viremia < 50 copies/mL)

Pre-HAART Viremia > 500 K cps/L

On treatment analysis

23 weeks

84% of success

Perno et al., EACS 2011

Presented at the 10th Eu Meeting on HIV & Hepatitis, 28-30 March 2012, Barcelona
Success achieved in patients with BL VL >100K (2008-2011)

HIV Unit. “irsicaixa” Foundation
Hospital Universitari “Germans Trias i Pujol”. Badalona. Barcelona.
Methods

Inclusion criteria:

- All naïve patients (>18y/o) from our HIV-Unit starting HAART from 2008-2011 with BL VL > 100K
- VL measurements available every 12 wks (or at least 2 VL values during all follow-up)
- Patients changing therapy were also included (OT)
- Survival analysis:
  - Time-to undetectability (first VL <50) was assessed by Kaplan-Meier. Groups comparison by Breslow test.
NAIVE PATIENTS INITIATING TREATMENT IN 2008-2011

CV <100K
79% (n:711)

CV >100
21% (n:189)
Patients >100k characteristics (2008-2011)

<table>
<thead>
<tr>
<th>VARIABLE</th>
<th>N=189</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male, n(%)</td>
<td>169, (89%)</td>
</tr>
<tr>
<td>Age, years</td>
<td>41</td>
</tr>
<tr>
<td>Pre HAART plasma HIV-RNA median</td>
<td>5,65</td>
</tr>
<tr>
<td>Pre HAART CD4 (cels/mm) Median</td>
<td>73.5</td>
</tr>
</tbody>
</table>

**Drug Administered**

**NRTIs**
- FTC TDF 88%
- TDF 3TC 6%
- ABV 3TC 6%
- AZT 3TC 1%
- OTHERS 3%

**THIRD DRUG**
- IP/r 56%
- MRV 5%
- RAL 8%
- NNRTI 31%
Patients >100k characteristics (2008-2011)

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Drug Administered

**NNRTI**
- EFV 79%
- NVP 21%

**IP**
- DRV 55%
- LPV 25%
- ATV 16%
- FOSAPV 4%
Pre-Haart Viremia

- >500: 28% (n:52)
- 100-300: 53% (n:100)
- 300-500: 19% (n:37)

Presented at the 10th Eu Meeting on HIV & Hepatitis, 28-30 March 2012, Barcelona
Time to achieve virological undetectability and the rate of success at 48 weeks (in patients with VL > 100K)

On treatment analysis

Kaplan Meier survival curves (Event viremia<50 copies/mL)

Success 83.6%

Success <100K: 98%
MTU: 14 wks
(IQR: 11-20)

Median (IQR) time to undetectability (MTU): 25wks (18;38)

Presented at the 10th Eu Meeting on HIV & Hepatitis, 28-30 March 2012, Barcelona
Efficacy according to the initial HAART approach (48 wks)

Presented at the 10th Eu Meeting on HIV & Hepatitis, 28-30 March 2012, Barcelona
Time to achieve virological undetectability and the rate of success at 48 weeks are pre-HAART viremia dependent

<table>
<thead>
<tr>
<th>Viremia Range</th>
<th>Median</th>
<th>25% Percentile</th>
<th>75% Percentile</th>
<th>Rate of success</th>
</tr>
</thead>
<tbody>
<tr>
<td>&gt;500</td>
<td>30</td>
<td>22</td>
<td>&gt;48</td>
<td>73.1</td>
</tr>
<tr>
<td>300-500</td>
<td>24</td>
<td>17</td>
<td>35</td>
<td>89.2</td>
</tr>
<tr>
<td>100-300</td>
<td>23</td>
<td>16</td>
<td>31</td>
<td>83.7</td>
</tr>
<tr>
<td>Global</td>
<td>25</td>
<td>18</td>
<td>38</td>
<td>83.6</td>
</tr>
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</table>

Kaplan Meier survival curves by pre-HAART viremia (Event viremia < 50 copies/mL)

Breslow p-value = 0.012
Pre-HAART viremia 100K-300K

Success 83.7%

Median (IQR) time to undetectability: 23 wks (16;31)
Pre-HAART viremia 300K-500K

Median (IQR) time to undetectability: **24 wks (17;35)**

Success 89.2%
Time to achieve virological undetectability and the rate of success at 48 weeks are pre-HAART viremia dependent.

Pre-HAART viremia >500K

Median (IQR) time to undetectability: **30 wks** (22;>48)

Success 73.1%
CONSIDERATIONS

- High VL (>100,000 c/mL) and/or low CD4 (<100/mm3) count are associated with higher risk of treatment failure
- Will a quick control of VL help to reduce the risk of emergence of resistance?
- Rapid selection of drug-resistant HIV-1 during the first weeks of suppressive ART in naïve patients. Role for intensification during initial treatment?
- Is there something at a PK level that causes insufficient drug delivery into cells when HIV VL is very high?
Rapid selection of drug-resistant HIV-1 during the first months of suppressive ART in treatment-naive patients

Karin J. Metzner\textsuperscript{a}, Kristina Allers\textsuperscript{a}, Pia Rauch\textsuperscript{a} and Thomas Harrer\textsuperscript{b}

**Objective:** Efficient antiretroviral therapy (ART) of HIV-1 infection reduces the viral load to undetectable levels and restores the immune system. However, therapy failure appears in a substantial fraction of patients and is mostly associated with the appearance of drug-resistant viruses. It is still not clear when the drug pressure leads to the earliest selection and appearance of drug-resistant HIV-1 populations. In this study, we wanted to determine whether drug-resistant viruses are already selected during viral decline within the first months of ART.

**Design and methods:** Fifteen mostly chronically HIV-1 infected patients were included. None had received ART prior to this study. The selection of three key resistance mutations, L90M (protease), K103N and M184V (reverse transcriptase), were measured by allele-specific real-time PCR allowing us to track minority quasispecies with a discriminative power of 0.01–0.2%.

**Results:** Drug-resistant HIV-1 variants were found in 7/15 patients (46.7%) prior to ART. Rapid selection of drug resistance was detected in six patients (40%) independent of the presence of drug-resistant HIV-1 prior to ART. The risk for the selection of drug resistant viruses was correlated with the time until viral load became undetectable ($P = 0.02$). Besides the proportional increment of drug-resistant viruses, we observed in two patients a quantitative increase of this virus population while the total viral load decreased.

**Conclusions:** Drug-resistant viruses can be selected and replicate even in the first weeks of suppressive ART, thus, intensification of ART during the initial treatment period should be considered and further evaluated in clinical studies.

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*AIDS* 2007, 21:703–711

**Keywords:** HIV-1, allele-specific real-time PCR, selection, drug resistance, minority quasispecies

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• Is there something at a PK level that causes insufficient drug delivery into cells when HIV VL is very high?
Increased antiretroviral potency by the addition of enfuvirtide to a four-drug regimen in antiretroviral-naive, HIV-infected patients

José Molto, Lidia Ruiz, Marta Valle, Javier Martinez-Picado, Ana Bonjoch, Isabel Bravo, Eugenia Negredo, Gabrielle M Heikle-Neider and Bonaventura Clotet

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2IRSCaixa Foundation, Germans Trias i Pujol Hospital, Badalona, Spain
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*Corresponding author: Tel: +34 93 497 8887; Fax: +34 93 465 7602; E-mail: jmolto@nshugtip.sc.es

Objective: To assess if enfuvirtide (ENF) increases antiviral activity of a highly active four-drug antiretroviral (ARV) regimen containing lopinavir/ritonavir, efavirenz, lamivudine and tenofovir in ARV-naive, HIV-infected patients.

Methods: Pilot study in ARV-naive, HIV-infected patients with viral load (VL) >10,000 copies/ml and no documented resistance to any of the study drugs. Patients were randomized to receive ENF (ENF Group) or not (Control Group) in combination with lopinavir/ritonavir, efavirenz, lamivudine and tenofovir as a backbone. The primary endpoint was to assess differences in the HIV-1 RNA decay rate during the first phase of viral decay. VL and treatment adherence were measured at baseline, every 6 h during the first 3 days, and once daily from day 3 to 6. Individual HIV-1 RNA decay rates were obtained using a non-linear least squares regression model.

Results: Eight subjects were included in each study group. Mean (SD) baseline VL was 4.98 (0.38) log_{10} copies/ml in the ENF Group and 5.10 (0.49) log_{10} copies/ml in the Control Group (P=0.607). Baseline CD4+ cell count was 463 (306) and 362 (225) cells/mm³ in the ENF and the Control Group, respectively (P=0.484). First phase HIV-1 RNA decay rate was 0.802 (0.127) d⁻¹ in the ENF Group and 0.624 (0.182) d⁻¹ in the Control Group (P=0.045). By day 6, VL was 3.55 (0.40) and 3.92 (0.36) log_{10} copies/ml in the ENF and the Control Group, respectively (P=0.079).

Conclusion: The addition of ENF increased the antiviral potency of a highly active four-drug ARV regimen by 28.5% in ARV-naive, HIV-infected patients. The clinical impact of this finding should be assessed.
Presented at the 10th Eu Meeting on HIV & Hepatitis, 28-30 March 2012, Barcelona

Control Group

Enfuvirtide Group

Mean day 1-6 slope
HIV-1 RNA decay
p:0.025
Protocol 004 - Treatment Naïve Study
Proportion (95% CI) with HIV RNA < 50 copies/mL
[Non-Completer = Failure]

Efficacy was observed at all doses studied

Markowitz M, et al. 4th IAS, Sydney 2007, #TUAB104
CONSIDERATIONS

- High VL (>100,000 c/mL) and/or low CD4 (< 100/mm³) count are associated with higher risk of treatment failure
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- Is there something at a PK level that causes insufficient drug delivery into cells when HIV VL is very high?
PK issues

• The major target of most antiretrovirals (ARVs) is within cells infected with HIV and the clinical outcome of ARV therapy is related to intracellular (IC) drug concentrations.

• IC concentrations are likely to be influenced by different pharmacological aspects:
  – drug oral bioavailability,
  – plasma protein binding,
  – drug physiochemical characteristics (i.e. lipophilicity or ionization),
  – involvement of multidrug transport proteins responsible for drug cellular influx/efflux.

• Methods to assay IC concentrations are still a major challenge and have not been standardized.
The intracellular disposition of raltegravir is dependent on P-glycoprotein (P-gp; ABCB1) activity and is significantly reduced in primary CD4+ P-gp<sup>high</sup> cells

Gerard Minuesa<sup>1</sup>, Itziar Erkizia<sup>1</sup>, Cristina Arimany-Nardi<sup>2</sup>, Marçal Pastor-Anglada<sup>3</sup>, Bonaventura Clotet<sup>1</sup>, Javier Martinez-Picado<sup>1,3</sup>

<sup>1</sup>ADIS Research Institute, Ir 地球, Hospital Universitari Germans Trias i Pujol, Badalona, Spain
<sup>2</sup>University of Barcelona and IIBB, Barcelona, Spain
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**INTRODUCTION**

Raltegravir (RAL), the first integrase inhibitor used to combat HIV-1 infection, works by inhibiting the insertion of viral genomic into human DNA with a very potent in vitro activity against HIV-1 (50% Inhibitory Concentration - IC<sub>50</sub> in human T-cell cultures of 33 nM).<sup>(1)</sup>

P-glycoprotein (P-gp; ABCB1) and Multidrug Resistance Protein 1 (MRP1; ABCC1) are the best known efflux transporters from the ATP-binding cassette (ABC) family and both have been involved in HIV protease inhibitors (PIs) and non-nucleoside RT inhibitors (NNRTIs) efflux. (2)

Drug intracellular drug efficacy represents an interplay between entry and efflux processes that regulate drug disposition and response. The mechanisms by which RAL effluxes from cellular compartments and tissues are still poorly understood.(1)

**OBJECTIVES**

To study the role of the efflux pumps P-glycoprotein (P-gp) and Multidrug Resistance Protein 1 (MRP1) in RAL cellular efflux and disposition in CD4+ T cells.

**MATERIALS & METHODS**

Cell lines and primary cultures: We used the CEM-SS (p3HR1 iodinated) cell line (DAPI, CM-H2DCFDA, CM-H2-DCFDA, CM-VIE, DHE, DHE DiOC<sub>18</sub>(3), and DHE DiOC<sub>2</sub>(5)) and DHE DiOC<sub>18</sub>(3), and DHE DiOC<sub>2</sub>(5) cells in Cellular Accumulation Ratio assays.

Cellular Accumulation Ratio (CMR) assays were used to study the role of P-gp and MRP1 in RAL efflux. We analyzed the accumulation of PROA (1 AM) and RAL (0.5-5 AM) in CEM-SS cell lines and effluent cell lines (in the absence of P-gp and MRP1 inhibitors; CMV-842 and AMD3100 respectively) in the CEM-SS and CEM-SS (p3HR1) cell line during 20 min. The CMR was calculated as previously recorded.(5)

CD4+ cells from healthy volunteers were treated with RAL (10 AM) and incubated for 15 min at 37°C in humidified 5% CO<sub>2</sub> incubator. DAPI (1 AM) and DHE (1 AM) were added for 15 min. The cells were then stained with a 1:1600 dilution of anti-HLA class I (BD, San Jose, CA, USA) and anti-HLA class II (BD, San Jose, CA, USA) Ab phycoerythrin conjugated and analyzed by flow cytometry in a BD LSR II flow cytometer.

CD4+ cells were labeled with DHE (1 AM) in the presence and absence of P-gp and MRP1 inhibitors (CMV-842 and AMD3100 respectively) and after 15 min incubation at 37°C. The CMR was calculated as previously recorded.(5)

Calcium retention assays by flow cytometry and microscopic analysis: The retention of calcium fluorescent substance was measured by flow cytometry and use fluorescence microscope for the intracellular calcium accumulation. (3)

**RESULTS**

Time-course and Cellular Accumulation Ratio of [3H]RAL in CEM-SS, CEM-MRP1 and CEM-P-gp cells.

**CD4+ P-gp<sup>high</sup> cells show a higher percentage of early activation markers and TRM and EM T1 cells.

**DISCUSSION**

P-gp and MRP1 can prevent RAL accumulation in CD4+ T cells, which can lead to reduced efficacy and resistance. Our results suggest that P-gp and MRP1 contribute to RAL efflux from CD4+ T cells, and that P-gp blockade using pre-treatment with P-gp inhibitors or genetic modifications may enhance RAL efficacy in vivo.

**CONCLUSIONS**

P-gp and MRP1 can significantly reduce RAL accumulation in CD4+ T cells, which can lead to reduced efficacy and resistance. Our results suggest that P-gp and MRP1 contribute to RAL efflux from CD4+ T cells, and that P-gp blockade using pre-treatment with P-gp inhibitors or genetic modifications may enhance RAL efficacy in vivo.

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**REFERENCES**


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Overview of putative raltegravir (RAL) transporters in T cells

Presented at the 10th Eu Meeting on HIV & Hepatitis, 28-30 March 2012, Barcelona
P-glycoprotein is an efflux transporter for raltegravir

Linfoblastic cell line

CEM-wt (CEM-CRFR)

CEM-MRP1 (CEM-E100)

CEM-P-gp (CEM-VBL100)

MK571 = MRP1 inhibitor
XR9051 = P-gp inhibitor

MK571 + XR9051 = combined inhibitor treatment

CEM-wt
CEM-MRP1
CEM-P-gp

Time-course

30-min transport

Protein expression (Flow Cytometry)

Isotype ctrl (lgG2b):
- - - - CEM-wt
- - - - CEM-MRP1
- - - - CEM-P-gp

Ab-PE stained:
- CEM-wt
- CEM-MRP1
- CEM-P-gp

[^H]Raltegravir
Cellular Accumulation Ratio (%)
High variability among blood donors.

Difference in RAL accumulation:

**CD4+ P-gp<sup>high</sup> cells accumulate between 30-40% less RAL intracellularly.**

As expected, XR9051 (high affinity P-gp inhibitor) blocks RAL efflux.

Presented at the 10th Eu Meeting on HIV & Hepatitis, 28-30 March 2012, Barcelona
Conclusions

- **P-gp efficiently effluxes RAL** both in a T-lymphoblastic cell model and in primary CD4+ T cells with high transporter activity.

- HIV Protease Inhibitors and NNRTIs are **inhibiting P-gp function whereas RAL is not.**

- **CD4+ P-gphigh cells** show a higher percentage of early activation markers and a phenotype of transitional (T_{TM}) and effector memory (T_{EM}) cells.

Intracellular penetration of ARV drugs could be different also at different VL values which could explain the lower efficacy in patients with high viral load values.
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