Drugs inside: intracellular penetration

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10TH RESIDENTIAL COURSE ON CLINICAL PHARMACOLOGY OF ANTIRETROVIRALS

2005
2006
2007
2009
2010
2011
2012
2013
2014

21-22-23 January 2015

TDM
Therapeutic Drug Monitoring

TDM: search for better PK-PD markers the « quest of the GRAAL »

• Since the years '70, permanent search for optimal marker of efficacy/toxicity e.g.:

– Plasma, whole blood, free vs total fraction

– Sampling time: Cmin, C2, Cmax, full AUC …

• Progress in pharmacokinetics

– Mathematics: Pop PK, Bayesian estimates, short AUC,…

• Pharmacogenetics (SNPs):

- Biotransformation enzymes

- Drug transporters

• Progress in analytical methods (automation, LC-MSMS, MALDI, SIMS …)

Utility of intracellular concentrations

May be only a speculative issue?

Intracellular Drug Concentrations

CT Dollery[1]

Many drug targets are intracellular. To access them, a drug molecule must pass through the cell membrane, a process often facilitated or impeded by transporters. Once in the cytoplasm, basic molecules may become concentrated in organelles. To predict the pharmacologic effect accurately, there must be data concerning the concentration at the target, which is difficult to measure. Techniques that combine mass spectrometry and imaging techniques (matrix-assisted laser desorption/ionization, secondary ion mass spectrometry (SIMS), and nanoSIMS) have promise in addressing this problem.

Modified from *Pr Pierre Wallemacq,*

Utility of intracellular concentrations

the topic is interesting and recently it is being developed

Intracellular Pharmacokinetics of Antiretroviral Drugs in HIV-Infected Patients, and their Correlation with Drug Action

Caroline Bazzoli,[1,2] Vincent Jullien,[3,4,5] Clotilde Le Tiec,[6] Elisabeth Rey,[3,4,5] France Mentré[1,2] and Anne-Marie Taburet[6]

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Journal of Pharmaceutical and Biomedical Analysis

journal homepage: www.elsevier.com/locate/jpba

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Review

Therapeutic drug monitoring of intracellular anti-infective agents☆

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SITE OF ACTION OF ACTUAL ANTIRETROVIRAL DRUGS

Class	Target	Site of Action	N° drugs	
N(t)RTI	Reverse Transcriptase	Intracellular	9	
NNRTI	Reverse Transcriptase	Intracellular	5	26
PI	Protease	Intracellular	9	
II	Integrase	Intracellular	3	
FI	Gp41	Extracellular	1	
CCR5 Antagonist	CCR5	Extracellular	1	2

The majority of antiviral drugs act at the intracellular level
…then intracellular PK is important!!!

Clinical relevance - Compartmental viral evolution

We can assume PBMC (or tissue) as a compartment, and each drug has different capacities to penetrate inside cells

Biological / anatomical / physiological barriers

TRAFFIC

Cell associated DNA

Target Cells

Cell Free RNA

Drug 1

Drug 1

R

Drug 2 Drug 3

PBMC
Compartment
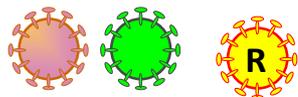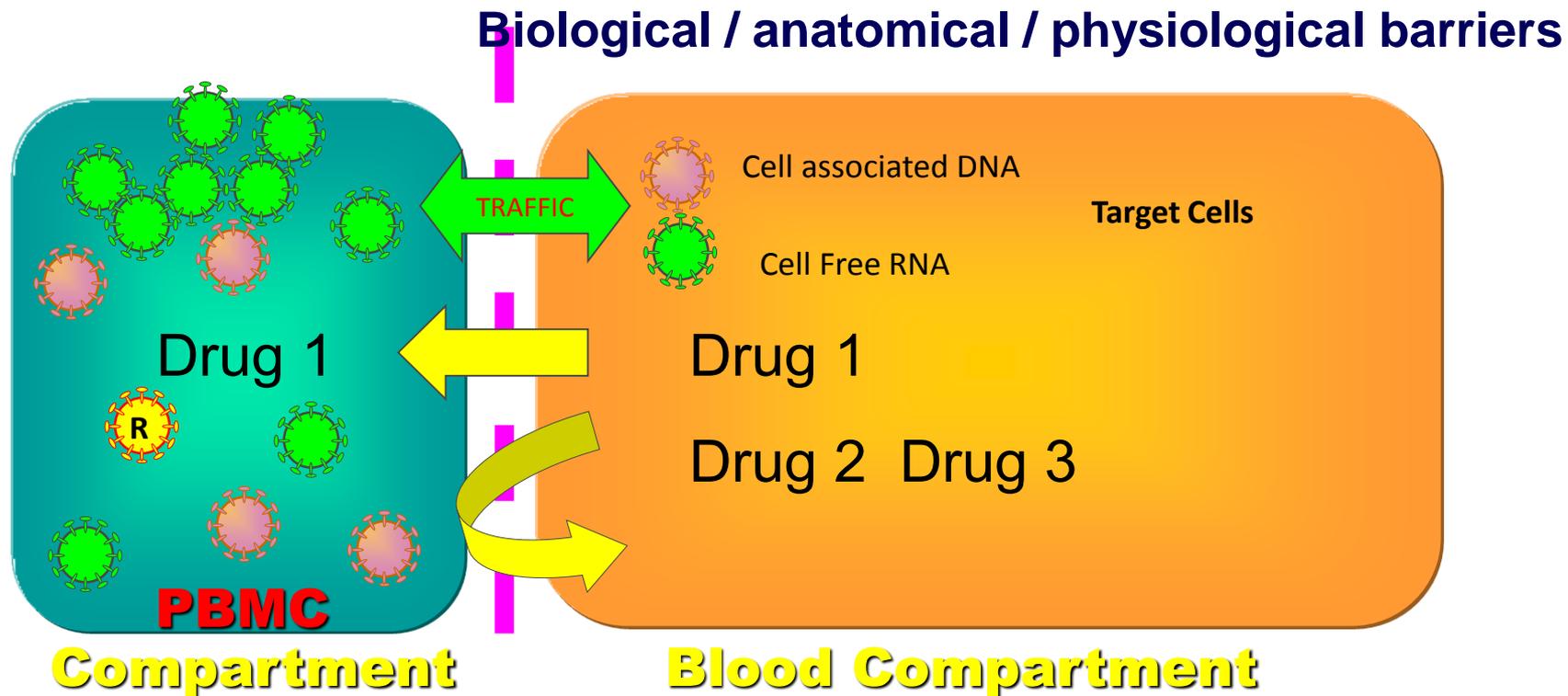
Blood Compartment

Insufficient intracellular drug concentrations may select resistant strains.

Remember compartment…

Each drug has own chemical and physical characteristics influencing passive and active diffusion (inside-outside compartments and cells).

Hydrophilic antibiotics

blood — interstitial fluid — cell
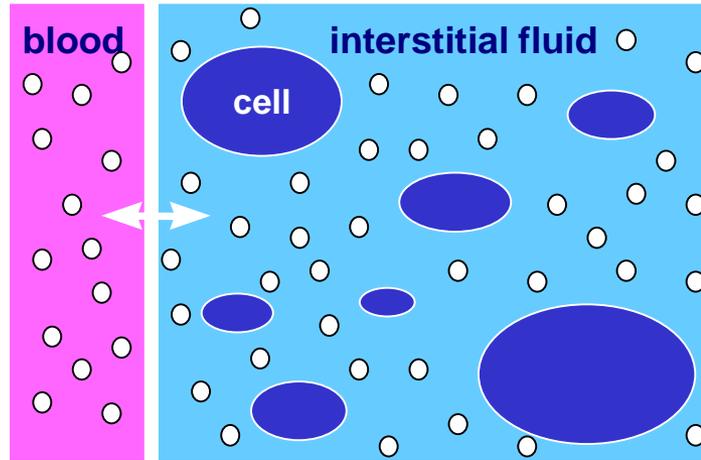
**Betalactams
Aminoglycosides
Glycopeptides**

Lipophilic antibiotics

blood — interstitial fluid

**Macrolides
Quinolones**

D.E. Nix et al., Antimicrob. Ag. Chemother., 1991, modified

Membrane transporters

Uptake transporters
SLC

Efflux transporters
ABC

SLC22A, OCT1,2
OCT substrates: NRTIs

ABCB1, P-gp
Substrates: PIs

ABCC1–5, MRP1–5
Substrates:
MRP1, MRP2 PIs
MRP4, MRP5 NRTIs

SLC28, hCNT1–3
Na+ dependent
substrates: NRTIs

ABCG2, BCRP
Substrates: NRTIs

SLC29, hENT1 or 2
Facilitated diffusion
substrates: NRTIs
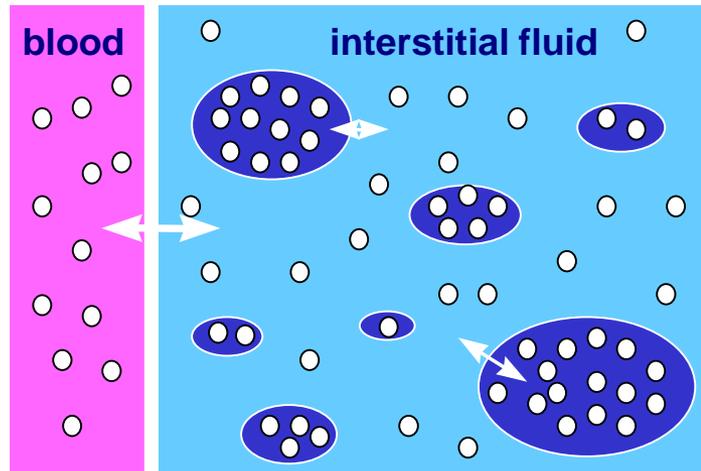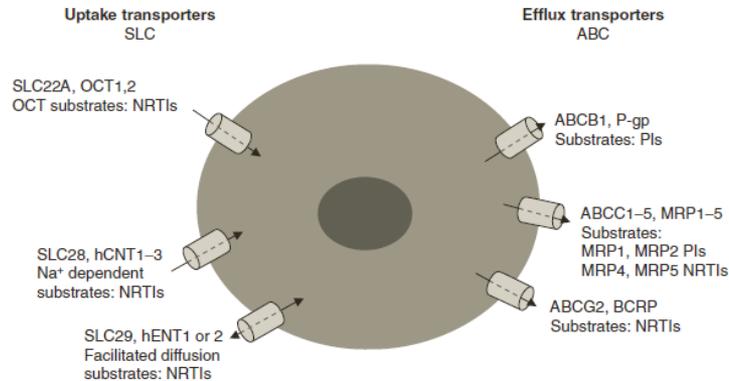
Bazzoli C. et al 2010

Fig. 2. Schematic representation of uptake and efflux transporters that may influence intracellular concentrations of antiretroviral drugs in peripheral blood cells. Transporters are named by gene and proteins (adapted from Ford et al.,[53] with permission, and updated[55-57]). **ABC** = adenosine triphosphate binding cassette; **BCRP** = breast cancer resistance protein; **hCNT** = human concentrative nucleoside transporter; **hENT** = human equilibrative nucleoside transporter; **MRP** = multidrug resistance protein; **NRTI** = nucleoside reverse transcriptase inhibitor; **OCT** = organic cation transporters; **P-gp** = P-glycoprotein; **PI** = protease inhibitor; **SLC** = solute carrier.

all drugs are affected by passive and active transport using several transport proteins

| Key	Process	Example Transporter
A	Intestinal Uptake	OATPs
B	Intestinal Efflux	MDR1*, BCRP
C	Hepatic Uptake	OATPs
D	Hepatic Efflux	MRP3
E	Biliary Secretion	MDR1, MRP2
F	Renal Uptake	OAT3
G	Renal Secretion	MDR1, MRP2
H	Renal Reabsorption	SVCT1
I	Brain Uptake	LAT1
J	Brain Efflux	MDR1, BCRP

*Commonly called P-glycoprotein

ABCB1 (P-gp efflux pump, MDR1), ABCC2 (MRP2 efflux pump)
BCRP (breast cancer resistance protein)
Organic anion transporter (OAT)P1B1, OATP1B3
Organic cation transporter 2

Modified from *Pr Pierre Wallemacq,*
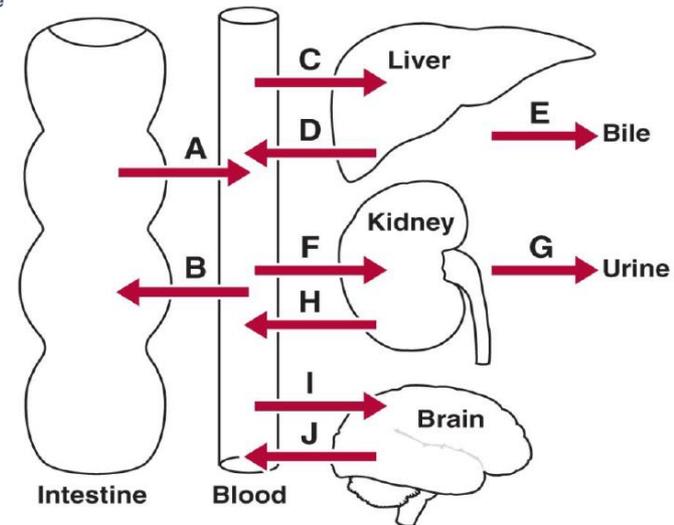
Drug cell entry
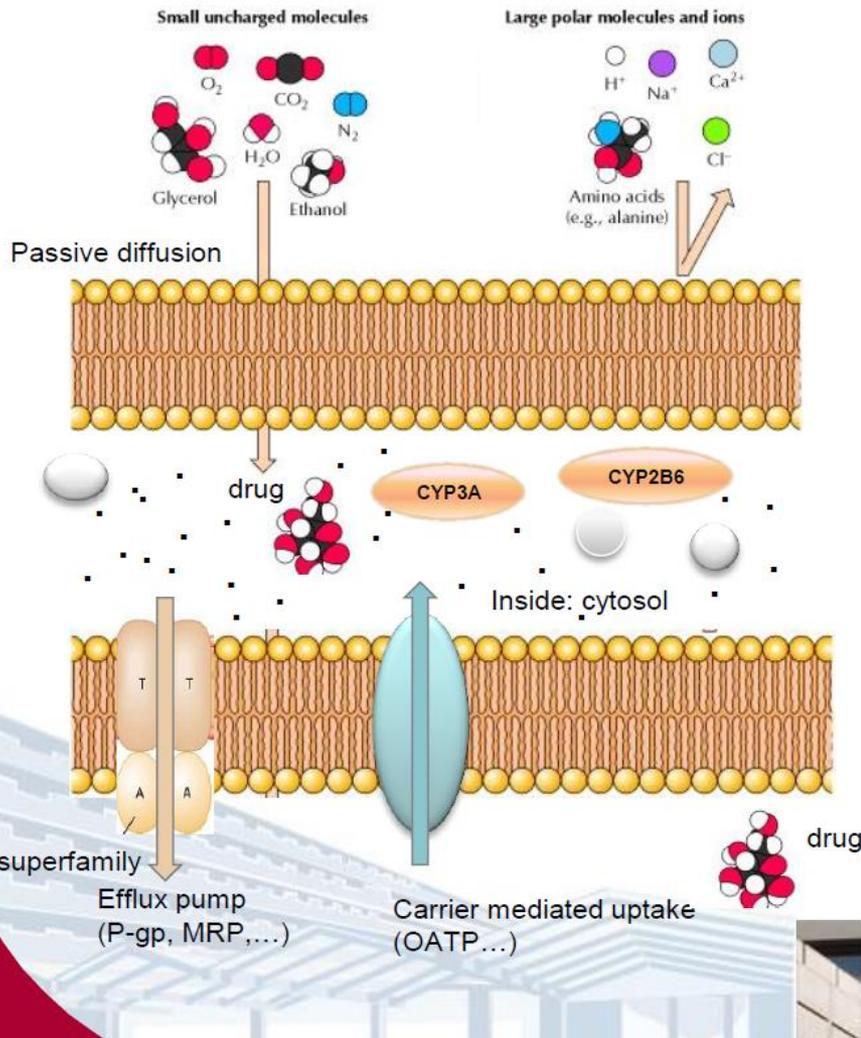
- Intracellular drug conc are regulated by passive or active processes
  - Blood or plasma free conc
  - Physicochemical factors across bilayer membranes (mw, pKa, pH, logP)
  - Carrier-mediated transport (efflux or influx pumps,…)
  - Local biotransformation (CYP)
- Cellular PK and PG
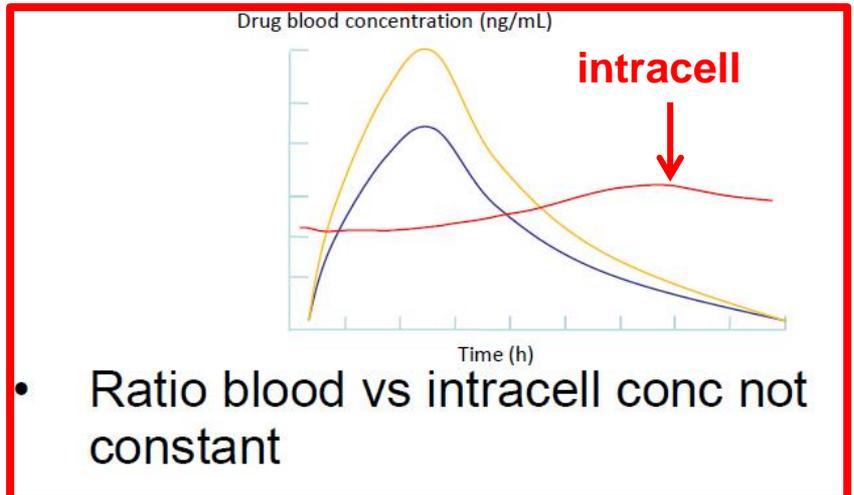
- Ratio blood vs intracell conc not constant

Blood/Plasma concentration does not necessarily predict target site drug concentration

Immunosuppressant Oral Dose 100%

Enterocytes

P-gp

P-gp

CYP3A

CYP3A

GI Tract

Intestinal first pass effect & efflux pump -30-50%

Liver

Systemic circulation Drug bioavailability 5-40%

CYP3A4/5 Hepatic first pass effect -10-30%

Portal vein
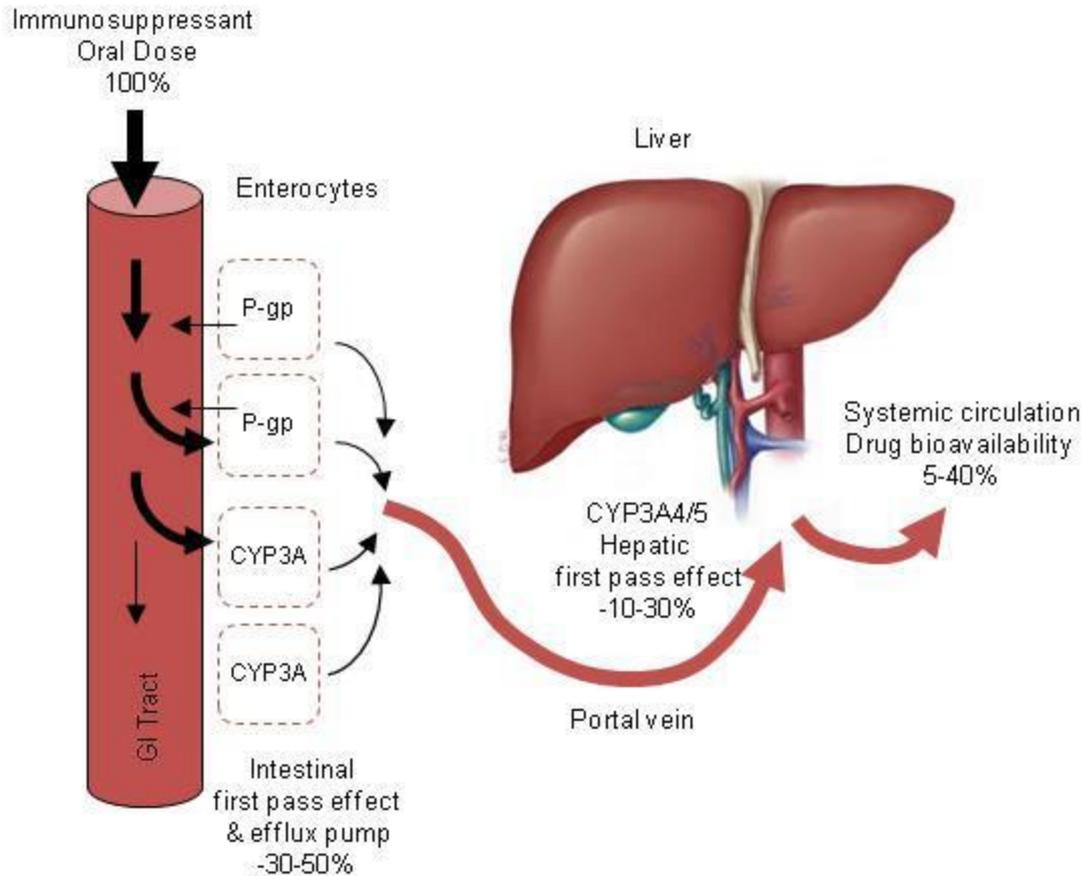
Blood concentrations are regulated by PK and PG

– Bioavailability (first pass effects)
– Distribution (fu,..)
– Drug transporters (P-gp,…)
– Biotransform enzymes (CYP3A…) and clearance
– Drug interactions

• Typical time-conc profile

Drug blood concentration (ng/mL)

Time (h)

Modified from *Pr Pierre Wallemacq,*
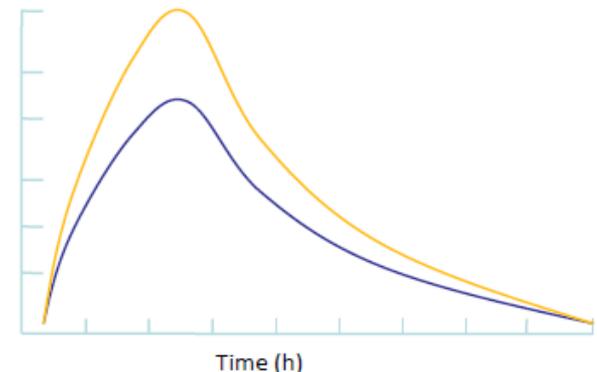
Evaluation of intracellular drug concentrations could be very important!

Intracellular drug concentrations

First reports for the interest of intracellular drug conc.

– Lithium in RBC in 1977 (Hisayusu GH et al, Clin Chem, 1977, 23, 41-5)

– CsA in hepatocytes in 1992 (Sandborn et al, Hepatology 1992, 15,1086-91)

– Fluoxetine in RBC in 1993 (Amitai Y et al, Vet Hum Toxicol, 1993, 35, 134-6)

– CsA in lymphocytes in 1998 (Masri et al, Transplant Proc, 1998,30,3561-2)

– Lamivudine in lymphocytes in 1999 (Moore et al, AIDS, 1999, 13,2239-50)

– Protease inhibitors in lymphocytes in 2002 (Chaillou S, HIV Clin trials, 2002,3,493-501)

– MPA in lymphocytes in 2007 (Benech H, J Chromatogr B Analyt Technol Biomed Life Sci, 2007,853,168-74)

…rate of accumulation of ARVs within the cells is still debated due to **scarce and controversial data** and **methodological limitations.**

Modified from *Pr Pierre Wallemacq,*

Intracellular Tacrolimus

Would intracellular tacrolimus better predict efficacy or toxicity than whole blood?

Tacrolimus concentration in liver biopsies
Relationship with histologic rejection score

Validation of a Liquid Chromatography-Mass
Spectrometric Assay for Tacrolimus in Liver Biopsies After
Hepatic Transplantation: Correlation With
Histopathologic Staging of Rejection

Arnaud Capron, MSc,* Jan Lerut, MD, PhD,† Catherine Verbaandert, MD,‡ Jules Mathys, MD,†
Olga Ciccarelli, MD,† Roger Vanbinst, MSc,* Francine Roggen,† Chantal De Reyck,†
Julien Lemaire, MD,† and Pierre E. Wallemacq, PhD*

Choice of alternative biological matrix
– **Better correlation between Tac tissue levels [<30 pg/mg] (hepatic biopsies) and score for rejection than with whole blood**

– **Surrogate marker for lymphocytes?**
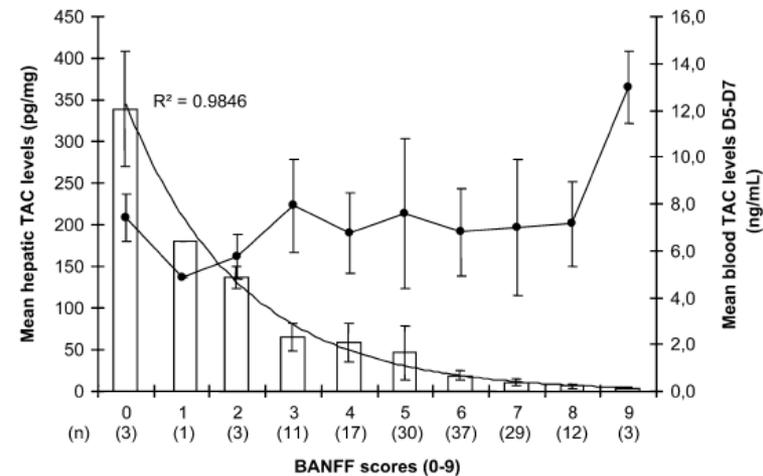
FIGURE 3. Correlations between either mean hepatic tacrolimus concentrations at day 7 (open boxes) or mean blood tacrolimus obtained between day 5 and 7 (black plots) and BANFF scores determined at day 7 as histologic marker of rejection. Values are expressed as mean ± SD. Standard deviations reported correspond to interpatient variability both in hepatic tissue and whole blood. Number of patients (n) is represented for each BANFF subgroup. First-order exponential correlation between hepatic concentrations and BANFF scores displays a R^2 of 0.9846 ($P = 0.002$).

Transplant International

Correlation of tacrolimus levels in peripheral blood mononuclear cells with histological staging of rejection after liver transplantation: preliminary results of a prospective study

Arnaud Capron,[1,2] Jan Lerut,[3] Dominique Latinne,[4] Jacques Rahier,[5] Vincent Haufroid[1,2] and Pierre Wallemacq[1,2]

1 Clinical Chemistry Department, Cliniques universitaires St Luc, Université catholique de Louvain – UCL, Brussels, Belgium
2 Louvain centre for Toxicology and Applied Pharmacology, Cliniques universitaires St Luc, Université catholique de Louvain – UCL, Brussels, Belgium
3 Starzl Abdominal Transplantation Unit, Cliniques universitaires St Luc, Université catholique de Louvain – UCL, Brussels, Belgium
4 Immuno-haematology Department, Cliniques universitaires St Luc, Université catholique de Louvain – UCL, Brussels, Belgium
5 Department of Pathology, Cliniques universitaires St Luc, Université catholique de Louvain – UCL, Brussels, Belgium

Monitoring of tacrolimus concentrations in peripheral blood mononuclear cells: Application to cardiac transplant recipients

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a Rennes University Hospital, Department of Clinical and Biological Pharmacology and Pharmacovigilance, Pharmacoepidemiology and Drug Information Center, Rennes, France
b Rennes 1 University, Faculty of Medicine, Laboratory of Experimental and Clinical Pharmacology, Rennes, France
c Inserm, CIC-P 0203, Clinical Investigation Center, Rennes, France
d EA4123, Barrières physiologiques et réponses thérapeutiques, Faculty of Pharmacy, Paris 11 University, Châtenay-Malabry, France
e Pharmacy Department, Saint-Antoine Hospital, Assistance Publique Hôpitaux de Paris APHP, Paris, France

ABSTRACT

Objectives: Despite the intensive therapeutic drug monitoring of tacrolimus (TAC) using trough whole blood concentrations, graft rejections occur in transplant recipient patients. Thus, other ways to monitor closely immunosuppressive treatments are necessary. A promising way of monitoring TAC treatments could be the measure of its concentrations inside the lymphocyte cell. Whereas the pharmacokinetics of TAC in peripheral blood mononuclear cells (PBMCs) was evaluated in renal and liver transplant recipients, data regarding PBMC concentrations of TAC in cardiac transplant recipients are lacking.
This study aimed, in cardiac transplant recipients: to validate a method for determination of TAC in PBMC, to investigate PBMC trough concentrations of TAC, and to evaluate their relationship with trough whole blood concentrations.

Design and method: We developed and validated a High-performance-liquid-chromatography tandem mass-spectrometry method of TAC quantitation in PBMC. The method was then evaluated by determining TAC concentrations in PBMC of 24 cardiac transplant recipients.

Results: Twenty-four patients were prospectively included in the study. Tacrolimus PBMC concentrations displayed a large inter-individual pharmacokinetic variability (CV = 71.4%) in the cohort. A lack of correlation between TAC whole blood trough concentrations and TAC trough concentrations in PBMCs was found (r = 0.259; p = 0.183).

Conclusion: Further studies should be implemented to evaluate the correlation between TAC concentrations in PBMC and clinical outcomes in cardiac transplant recipients to allow concluding whether monitoring TAC concentrations in PBMC is a good tool to prevent graft rejection in cardiac recipients.

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There are some strong correlations between intracellular concentrations and toxicity and/or the risk of organ rejection.

the key factors (correctly used) of these works are:
1) **the intracellular values were expressed per million cells**
2) **the cell count was performed with an automated system**

Other drugs?

Other clinical (infective) areas?

Multiple Classes of Direct Acting Antiviral Agents

| 5'UTR | | Core | E1 | E2 | p7 | NS2 | NS3 | | NS4B | NS5A | NS5B | | 3'UTR |

| | | Protease | | NS5A Replication Complex Inhibitors | Polymerase | |
| Ribavirin | NS3 protease inhibitors | | | | NS5B NUC Inhibitors | NS5B Non-NUC Inhibitors |

NS3 protease inhibitors	NS5A Replication Complex Inhibitors	NS5B NUC Inhibitors	NS5B Non-NUC Inhibitors
Telaprevir	Daclatasvir	Sofosbuvir	ABT-333
Boceprevir	Ledipasvir	VX-135	Deleobuvir
Simeprevir	ABT-267	IDX20963	BMS-791325
Asunaprevir	MK-8742	ACH-3422	PPI-383
ABT-450	GS-5885		GS-9669
MK-5172	GS-5816		TMC647055
Faldaprevir	ACH-3102		
Sovaprevir	PPI-668		
ACH-2684	GSK2336805		
	Samatasvir		

HCV

Table 1 A summary of product and dosing information of the medications currently approved by the US FDA and/or EMA

Drug	Brand name	Pharmaceutical form	Route of administration	Dose	Remarks	References
Peginterferon-α_{2a}	Pegasys®	180 µg/mL, 180 µg/0.5 mL or 135 µg/0.5 mL solution for injection	Subcutaneous	180 µg once weekly	Duration of treatment depends on viral genotype, viral response, co-morbidities and whether it is combined with other anti-HCV medication	[34, 35]
Peginterferon-α_{2b}	PegIntron®	50 µg/0.5 mL, 80 µg/0.5 mL, 120 µg/0.5 mL, 150 µg/0.5 mL powder and solvent for solution for injection	Subcutaneous	1.5 µg/kg body weight once weekly	Duration of treatment depends on viral genotype, viral response, co-morbidities and whether it is combined with other anti-HCV medication	[33, 36]
Ribavirin	Copegus®	200 mg tablet	Oral	800–1,200 mg (in divided doses)	Dose is based on body weight and genotype Should be taken with food Should be given in combination therapy Treatment duration depends on viral genotype, viral response, co-morbidities and treatment combination	[47]
Ribavirin	Rebetol®	200 mg capsule 40 mg/mL oral solution	Oral	800–1,400 mg (in divided doses)	Dose is based on body weight and genotype Should be taken with food Should be given in combination therapy Treatment duration depends on viral genotype, viral response, co-morbidities and treatment combination	[48, 49]
Boceprevir	Victrelis®	200 mg capsule	Oral	800 mg three times daily (every 7–9 h)	Must be taken with food (a meal or light snack) Should be administered with peginterferon-α and ribavirin Peginterferon and ribavirin are given for 4 weeks before the addition of boceprevir, then boceprevir is added and duration of triple therapy is based on viral response, prior response status and presence of cirrhosis[a] Only for HCV genotype 1	[59, 60]
Telaprevir	Incivek®, Incivo®	375 mg tablet	Oral	1,125 mg twice daily (10–14 h apart)	Must be taken with food (not low fat) Should be administered with peginterferon-α and ribavirin Triple therapy for 12 weeks, followed by 12 or 36 weeks of peginterferon-α and ribavirin, depending on viral response and prior response status[a] Only for HCV genotype 1	[63, 64]
Simeprevir	Olysio™	150 mg capsule	Oral	150 mg once daily	Must be taken with food Should be administered with peginterferon and ribavirin Triple therapy for 12 weeks, followed by 12 or 36 weeks of peginterferon-α and ribavirin, depending on prior response status Only for HCV genotype 1	[9]
Sofosbuvir	Sovaldi™	400 mg tablet	Oral	400 mg once daily	Can be taken with or without food Combination with peginterferon-α and/or ribavirin and treatment duration depend on viral genotype	[10, 11]

HCV hepatitis C virus

[a] For schedules of boceprevir- and telaprevir-based regimens see Fig. 2 in reference [145]

REVIEW ARTICLE

Viral Hepatitis C Therapy: Pharmacokinetic and Pharmacodynamic Considerations

Clara T. M. M. de Kanter · Joost P. H. Drenth ·
Joop E. Arends · Henk W. Reesink · Marc van der Valk ·
Robert J. de Knegt · David M. Burger

HCV - Telaprevir & Boceprevir in Human Plasma

Validation of an electrospray ionisation LC-MS/MS method for quantitative analysis of telaprevir and its R-diastereomer.

Penchala SD[1], Tjia J, El Sherif O, Back DJ, Khoo SH, Else LJ.

Highly sensitive determination of HCV protease inhibitors boceprevir (SCH 503034) and telaprevir (VX 950) in human plasma by LC-MS/MS.

Farnik H[1], El-Duweik J, Welsch C, Sarrazin C, Lötsch J, Zeuzem S, Geisslinger G, Schmidt H.

Determination of telaprevir in plasma of HCV-infected patients by HPLC-UV.

Tempestilli M[1], Milano E, D'Offizi G, Montalbano M, D'Avolio A, Gasperi T, Narciso P, Ascenzi P, Pucillo LP.

A UPLC-MS/MS method for the simultaneous plasma quantification of all isomeric forms of the new anti-HCV protease inhibitors boceprevir and telaprevir.

D'Avolio A[1], De Nicolò A, Agnesod D, Simiele M, Mohamed Abdi A, Dilly Penchala S, Boglione L, Cariti G, Di Perri G.

Telaprevir-S isomer enhances ribavirin exposure and the ribavirin-related haemolytic anaemia in a concentration-dependent manner.

De Nicolò A[1], Boglione L[2], Ciancio A[3], Cusato J[2], Strona S[3], Cardellino CS[2], Abdi AM[2], Cariti G[2], Troshina G[3], Caviglia GP[3], Smedile A[3], Rizzetto M[3], Di Perri G[2], D'Avolio A[2].

ONLY PLASMA PK DATA ARE AVAILABLE…

PATIENTS (FROM AMEDEO DI SAVOIA HOSPITAL)

Characteristics	Median Values Or Frequencies (number of cases)	
	BOC	TEL
Number of patients	25	10
Weight (kg)	78.0 IQR (70.0-83.5)	80.0 IQR (74.3 – 87.5)
BMI	25.5 IQR (23.9-28.6)	27.6 IQR (24.9 – 29.8)
Sex (M/F)	18/7	9/1
Age (years)	46.0 IQR (42.0-52.5)	43.0 IQR (37.0 – 57.3)
Viral Genotype (1a/1b)	12/13	4/6
RBV Dose (mg)	1200 IQR (1000-1200)	1000 IQR (1000 – 1200)
PEG-INF Dose	120 IQR (100-150)	180
Type of PEG-INFα (2a/2b)	2/23	10/0
Metavir (F_0/F_1/ F_2/ F_3/ F_4)	1/5/4/5/10	2/3/0/2/3

1 month and 3 months

Median PBMCs/Plasma Ratio: 2.6
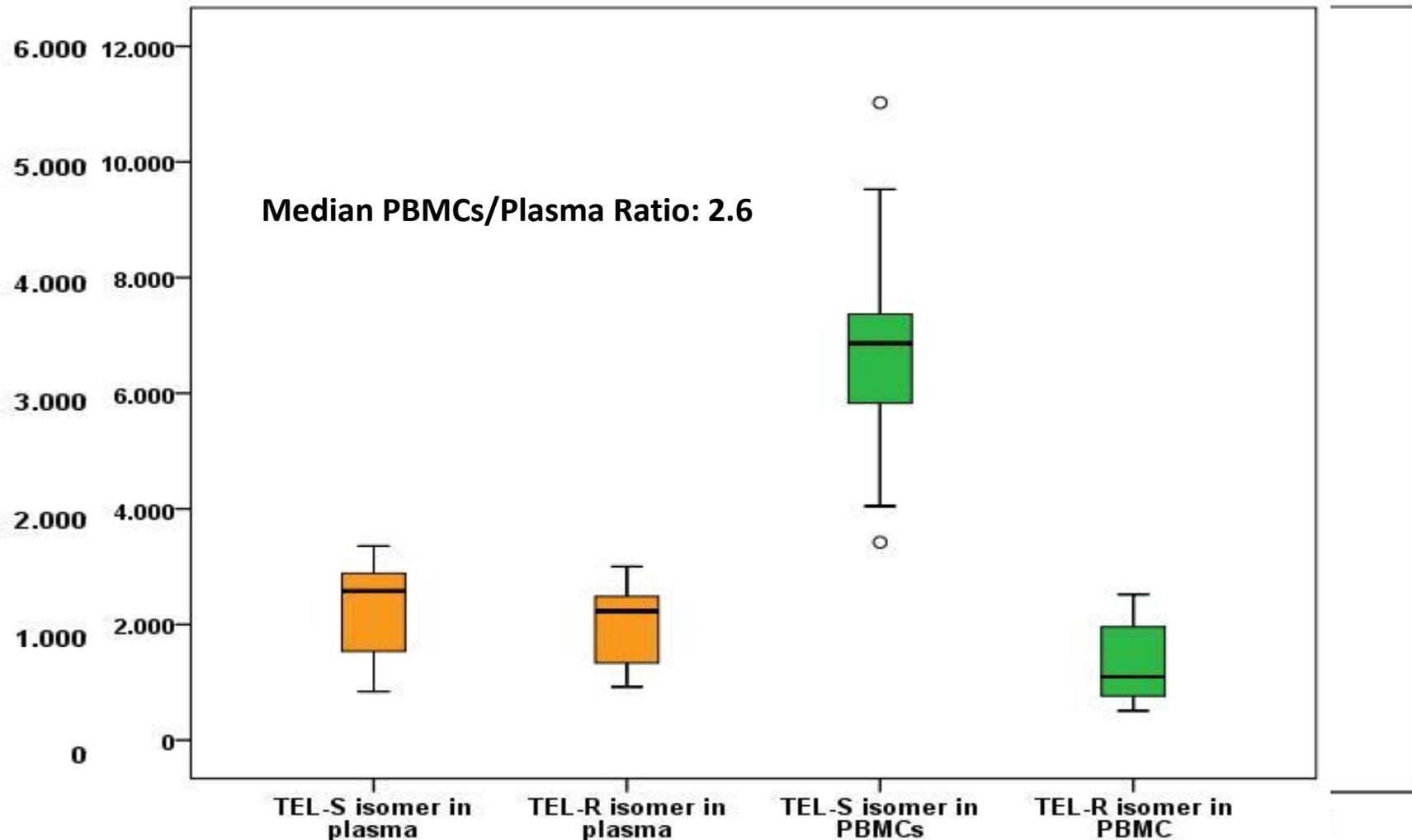
- ➤ **ACCUMULATION OF BOTH DRUGS ACTIVE ISOMER (-S) IN PBMCs WHEN COMPARED TO PLASMA CONCENTRATIONS**
- ➤ **GREATER PRESENCE OF THE –S ISOMERS, COMPARED WITH THE –R ONES**

HCV - Discussion

BOC and TEL accumulate in PBMCs

Outstanding greater presence of -S isomers, justifying antiviral S isomers activity both for TEL and BOC

The low number of patients can not allow a significant correlations with toxicity or therapeutic response

maybe the same happens in hepatocytes (?) explaining why it is S-isomer works

….useful data for other isomeric drugs (sofosbuvir -S, GS-7977)?

HBV - Entecavir

Entecavir significantly accumulates inside PBMC
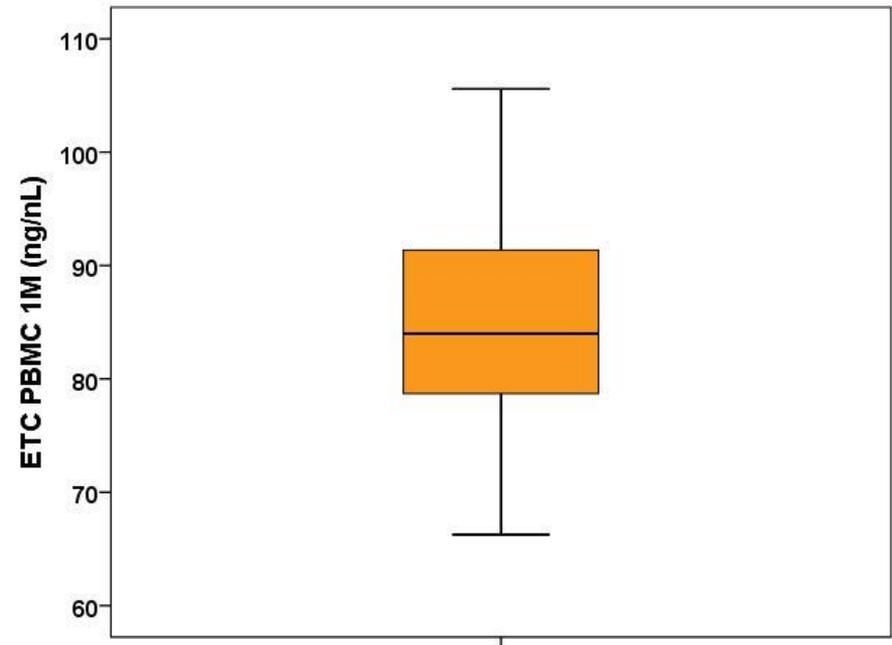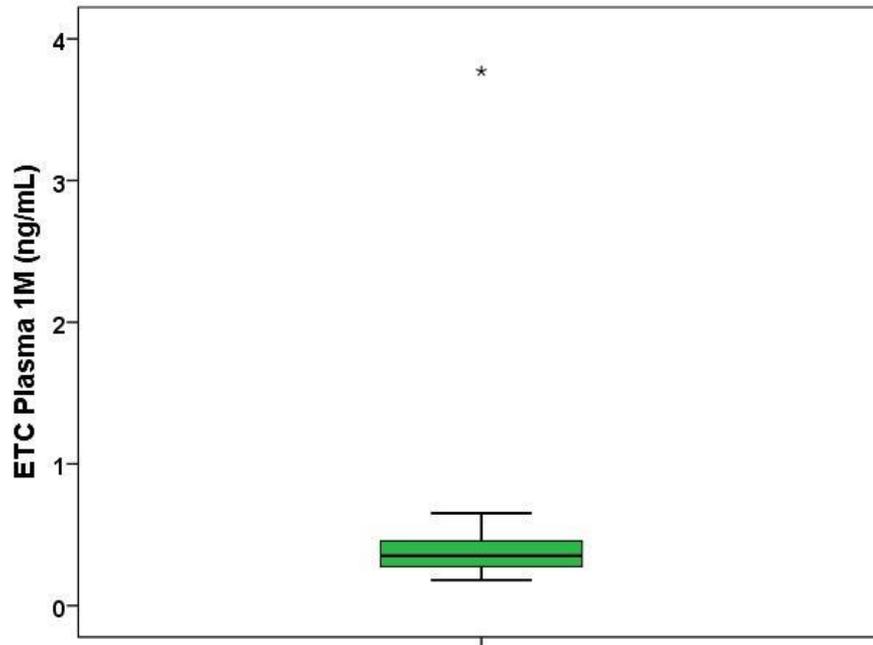
HBV

HBV - Entecavir

PBMC/Plasma Ratio is around 250

Strong correlation between RATIO (1 month) and logarithmic HBV-DNA decline (3 months)
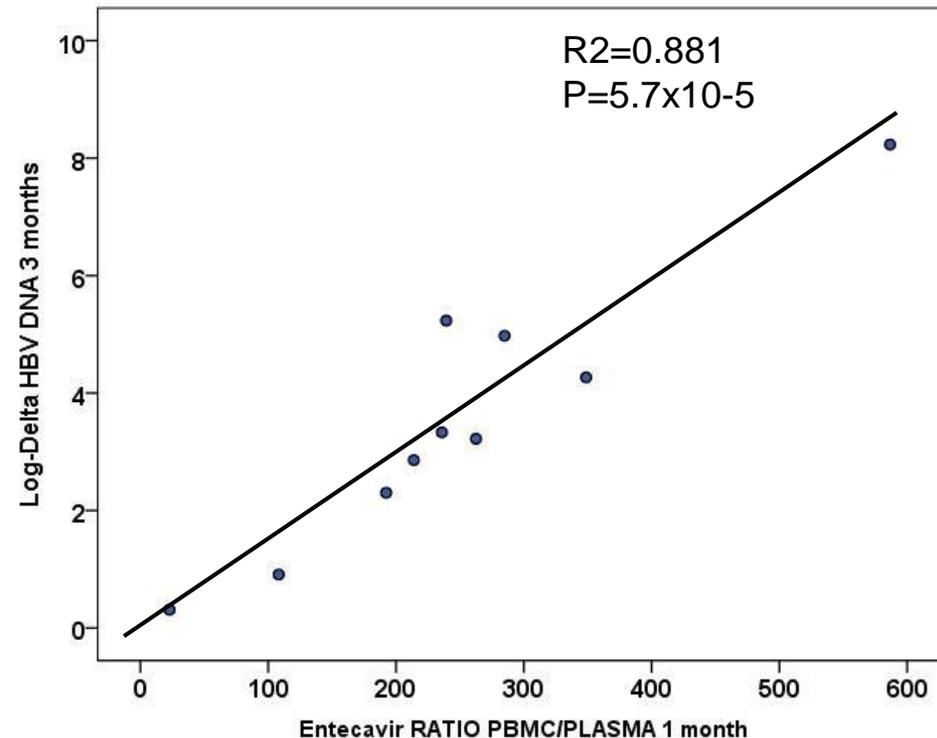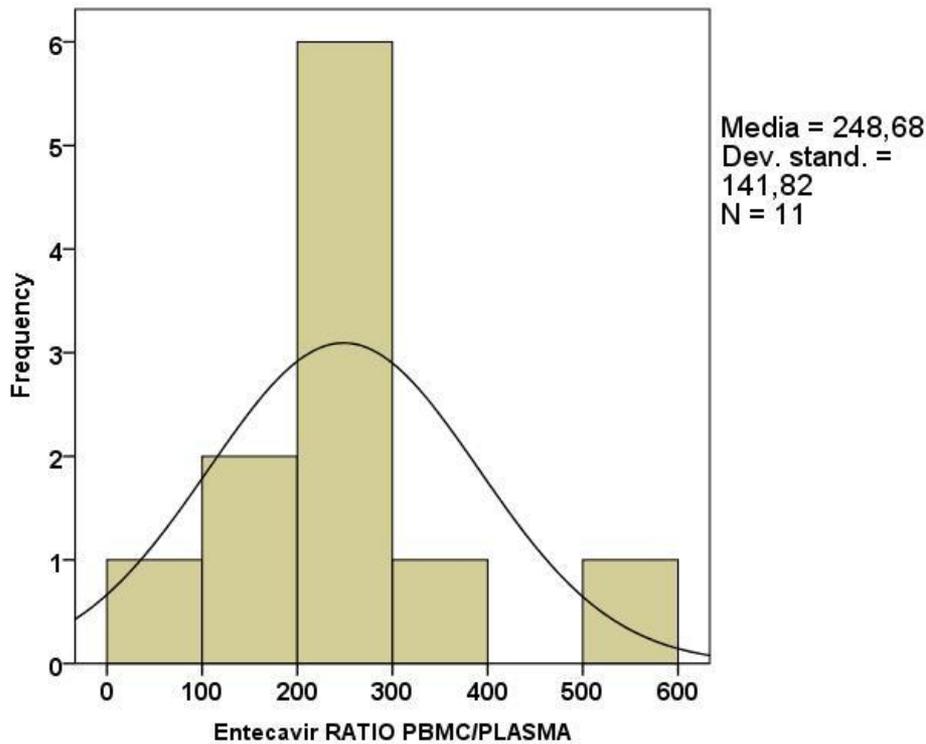
Antitubercular drugs

INTRACELLULAR/PLASMA Ratio (n=10 patients)

		Ratio C_{trough} intracellular/C_{trough} plasma (Median, IQR)	Ratio C_{max} intracellular/C_{max} plasma (Median, IQR)
Week 2	INH	-	0.03 (0.16-0.055)
	RFP	0.73 (0.27-1.54)	1.3 (0.9-1.72)
	PZA	0.05 (0.04-0.8)	0.04 (0.03-0.07)
	ETB	34.5 (27.4-61.4)	15.1 (3.7-40.3)
Week 4	INH	-	0.02 (0.015-0.2)
	RFP	0.73 (0.29)	1.12 (0.8-2.65)
	PZA	0.07 (0.05-0.9)	0.07 (0.045-0.185)
	ETB	35.3 (19.2-81.59)	32.5 (18-36.83)

intracell

Drug blood concentration (ng/mL)
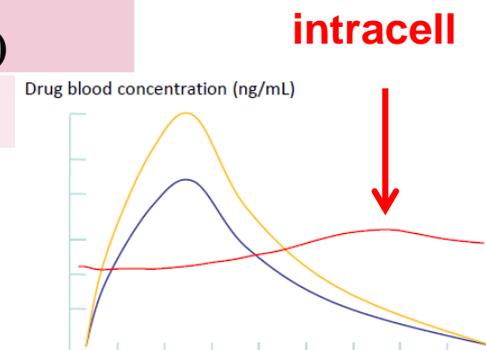
➢ **High inter-individual variability of the ratio**
➢ **Isoniazid seems to not accumulate within cells**
➢ **Rifampicin seem to a little accumulate within cells (Cmax only)**
➢ **Pirazinamide seems to not accumulate within PBMCs**
➢ **Ethambutol accumulation of 30-15 times in PBMCs**

ARVs Examples

PIs

Intracellular Concentration of Protease Inhibitors in HIV-1–Infected Patients: Correlation with -1 Gene Expression and ow Dose of Ritonavir
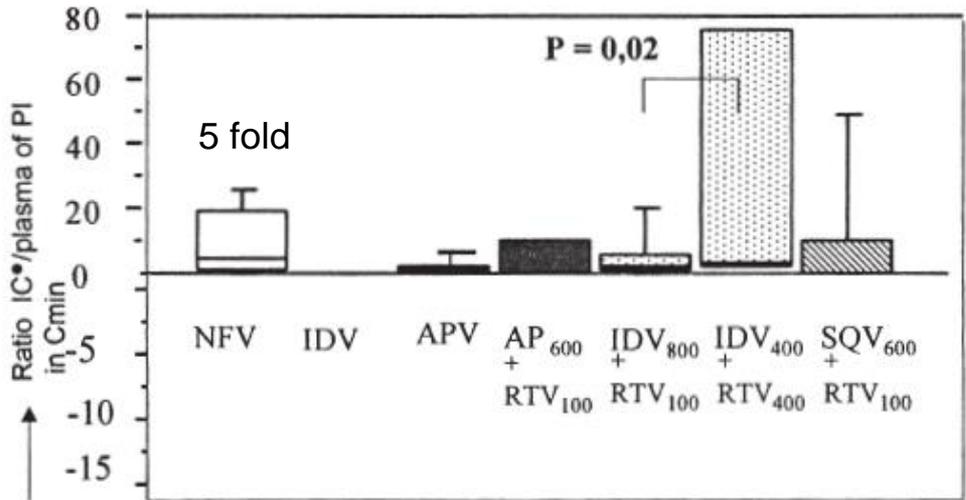
R. Garraffo,[2] E. Georgenthum,[1] C. Roptin,[2] P. Clevenbergh,[1] V. Mondain,[1] P.M. Roger,[1] and P. Dellamonica[1]

s, Hôpital Archet 1, Nice, France; [2]Service de Pharmacologie, Hôpital Pasteur, , France; [3]CISIH- Hôpital Archet 1, Nice, France

Figure 1. Ratio of different protease inhibitors within the cells and in the plasma in HIV-positive patients at C_{min} (A) and at C_{max} (B). IC concentration was determined for each PI used alone or with ritonavir (RTV). Intracellular RTV concentration is not represented here. Results are presented as median with 25th and 75th percentiles. Attached horizontal bars represent 10th and 90th percentiles. •IC: intracellular concentration of PI was interpreted for 10^6 cells.
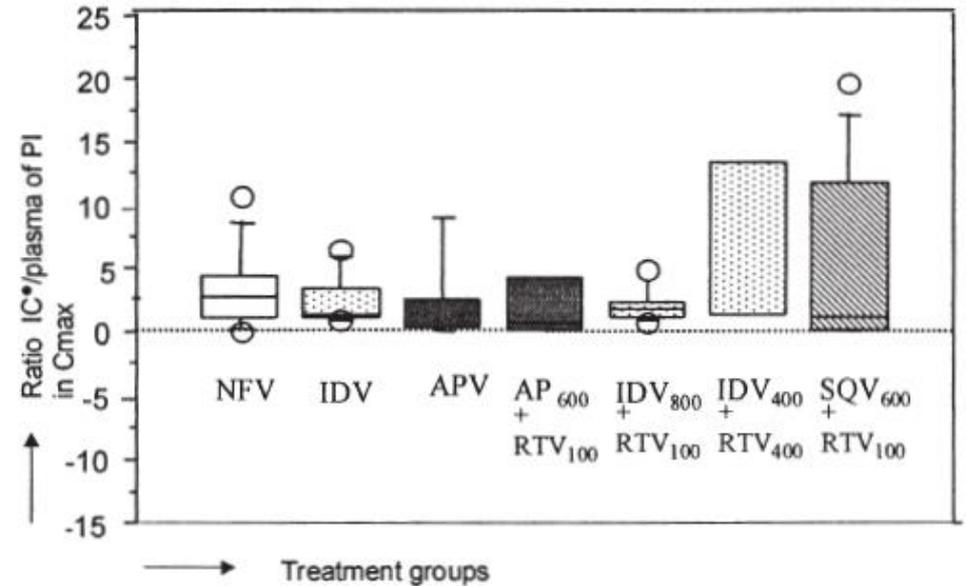
Different accumulation ratios between protease inhibitors in Cmin and Cmax with a high variability.

PI - Ritonavir

Intracellular accumulation of ritonavir combined with different protease inhibitors and correlations between concentrations in plasma and peripheral blood mononuclear cells

The different penetration capacity of molecules are influenced by concomitant medications of each patient.

9.6 (7.5–12.1)
7.6 (6.3–8.9)
9.2 (6.0–12.7)
6.1 (5.1–9.5)
5.0 (3.2–6.3)

0.22 (0.05–0.3)
0.12 (0.06–0.3)
2.44 (1.7–5.1)
0.35 (0.16–0.93)
0.14 (0.13–0.18)

etween darunavir-600 and
en atazanavir and lopinavir

on than previously reported,
V. The ratio varied according
avir intracellular penetration.
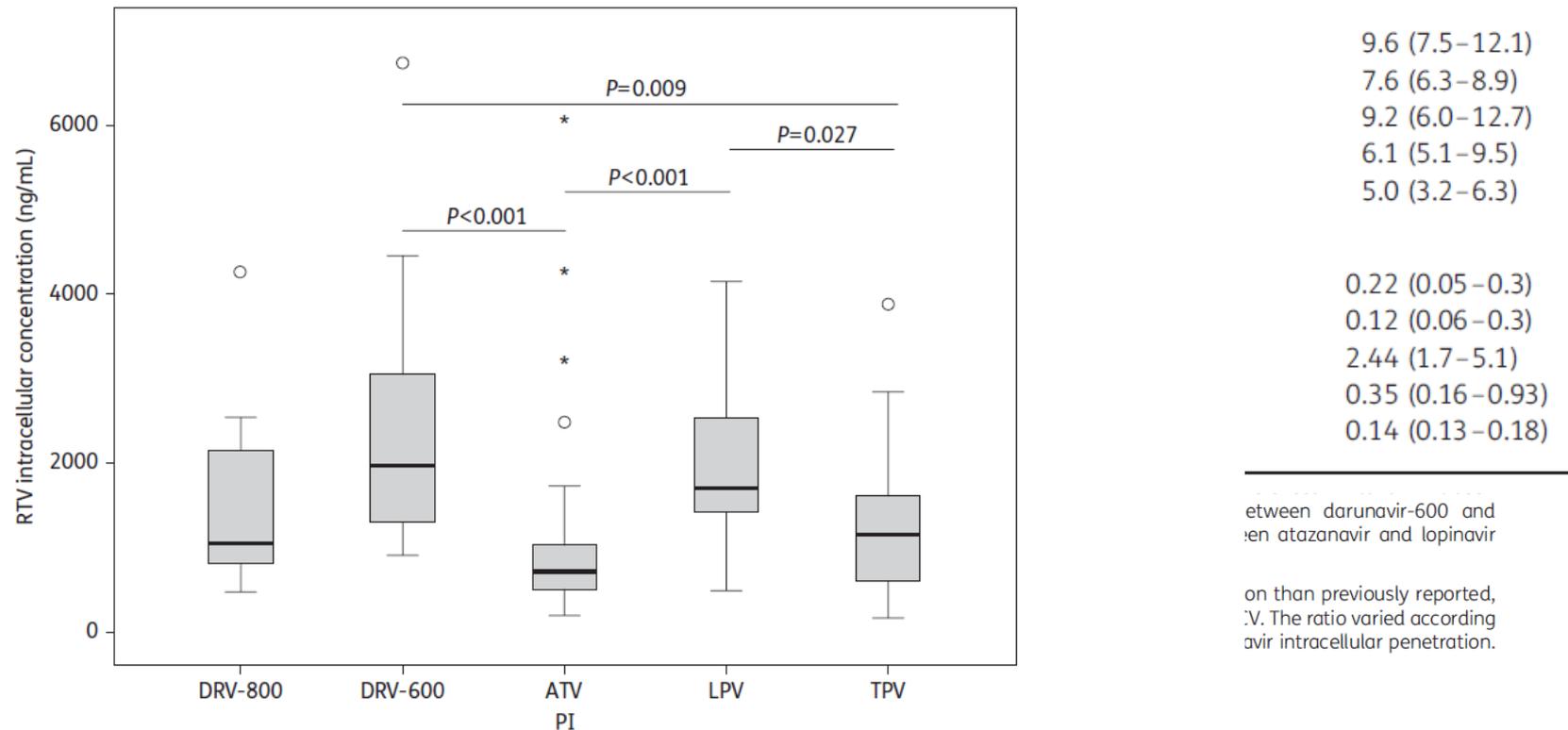
Figure 1. Ritonavir intracellular concentrations (ng/mL) according to concomitant PIs. Mild outliers (>1.5 × IQR from other values) are shown by open circles. Extreme outliers (>3 × IQR from other values) are shown by asterisks. Ritonavir, RTV; darunavir, DRV; atazanavir, ATV; lopinavir, LPV; tipranavir, TPV.

Intracellular Antiviral Activity of Low-Dose Ritonavir in Boosted Protease Inhibitor Regimens

Amedeo De Nicolò, Marco Simiele, Andrea Calcagno, Adnan Mohamed Abdi, Stefano Bonora, Giovanni Di Perri, Antonio D'Avolio

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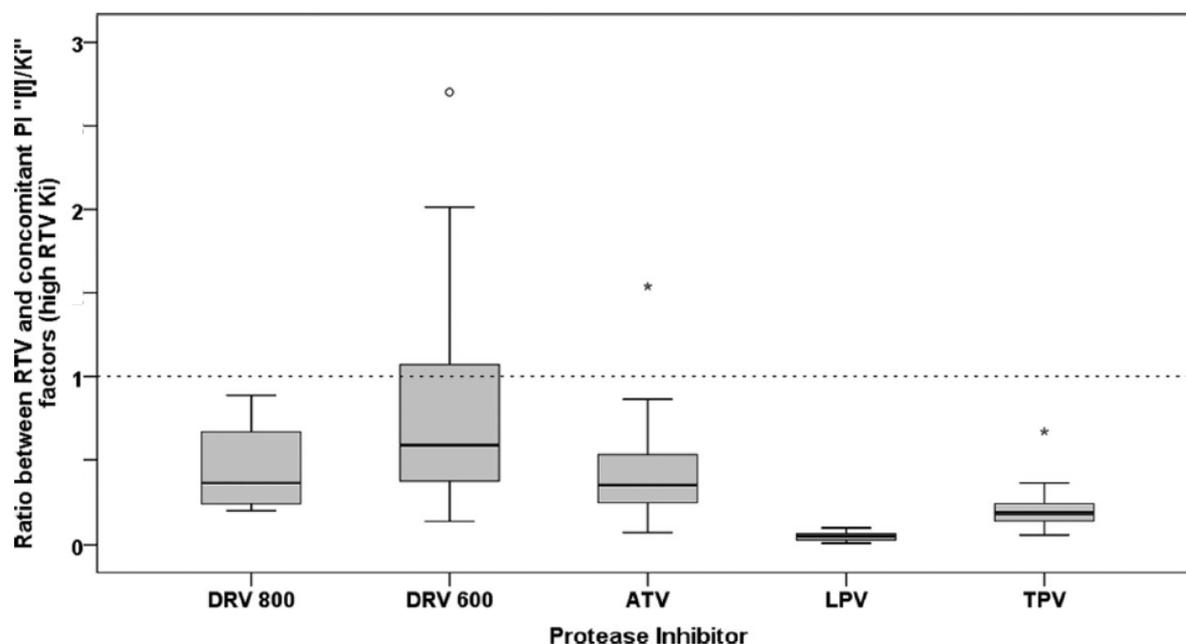
FIG 2 Distribution of the ratio between the $[I]/K_i$ factor of RTV (considering the highest RTV K_i) and that of the other PIs among the patients. The dashed line marks the cutoff of 1, indicating a higher activity of RTV than that of the concomitant PI. DRV800, DRV-RTV 800 and 100 mg once daily; DRV600, DRV-RTV 600 and 100 mg twice daily. Circles and asterisks indicate mild and extreme outliers, respectively.

Referring to the intracellular concentrations of ritonavir and PIs, ritonavir could have a possible additive and / or synergistic antiviral activity.

Intracellular Antiviral Activity of Low-Dose Ritonavir in Boosted Protease Inhibitor Regimens

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TABLE 2 Observed RTV dispositions in combination with each concomitant PI and comparison between RTV and concomitant PI effect on viral protease

Parameter	Median (interquartile range) for concomitant PI				
	ATV	DRV600[a]	DRV800[b]	LPV	TPV
No. of samples	40	22	4	21	16
RTV intra-PBMC concn (μM)	0.90 (0.61–1.40)	2.96 (1.82–4.95)	1.45 (1.16–4.47)	2.60 (1.86–3.76)	1.65 (0.93–2.30)
RTV plasma concn (μM)	0.09 (0.06–0.19)	0.48 (0.26–0.55)	0.17 (0.14–0.61)	0.39 (0.22–0.65)	0.38 (0.20–0.70)
RTV intra-PBMC/plasma ratio	9.07 (5.97–12.83)	7.59 (6.20–9.97)	7.561 (6.60–11.11)	7.21 (5.71–10.45)	5.27 (3.12–6.39)
Intracellular [RTV]/[PI]	0.42 (0.29–0.64)	4.04 (2.48–8.03)	2.49 (1.49–5.35)	0.57 (0.29–0.76)	0.19 (0.13–0.25)
$[I]/K_i$ (RTV)/$[I]/K_i$ (PI) (high RTV K_i)	0.35 (0.24–0.54)	0.59 (0.36–1.17)	0.36 (0.22–0.78)	0.05 (0.03–0.07)	0.19 (0.13–0.25)
$[I]/K_i$ (RTV)/$[I]/K_i$ (PI) (mean RTV K_i)	0.49 (0.36–0.80)	0.91 (0.61–2.24)	0.53 (0.32–1.14)	0.07 (0.06–0.10)	0.29 (0.25–0.37)
Reaction speed ratio with/without RTV	0.74 (0.65–0.80)	0.63 (0.46–0.74)	0.74 (0.57–0.82)	0.95 (0.94–0.96)	0.84 (0.80–0.88)
Fraction of antiviral effect accounted for by RTV (%)	26 (20–35)	37 (26–54)	26 (18–43)	5 (4–6)	16 (12–20)

[a] DRV600, DRV-RTV 600 and 100 mg BID.
[b] DRV800, DRV-RTV 800 and 100 mg QD.

Ritonavir PBMC/Plasma Ratio changes according to the concomitant PI

INTRACELLULAR PHARMACOKINETICS OF RILPIVIRINE IN HIV-POSITIVE PATIENTS TREATED WITH SINGLE-TABLET REGIMEN FIXED-DOSE COMBINATION

L. Marinaro, A. Calcagno, M. Simiele,
L. Trentini, M.C. Tettoni, C. Alcantarini,
S. Raviolo, M. Ferrara, J. Cusato,
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PHARMACOKINETICS		
RPV pC_{12} ng/ml	med, IQR	190 (43.3-52.9)
RPV iC_{12} ng/ml	med, IQR	1366 (943-2776)
RPV i/p ratio	med, IQR	6.3 (4.7-9.1)
TDF pC_{12} ng/ml	med, IQR	73 (58-103)
FTC pC_{12} ng/ml	med, IQR	350 (242-453)
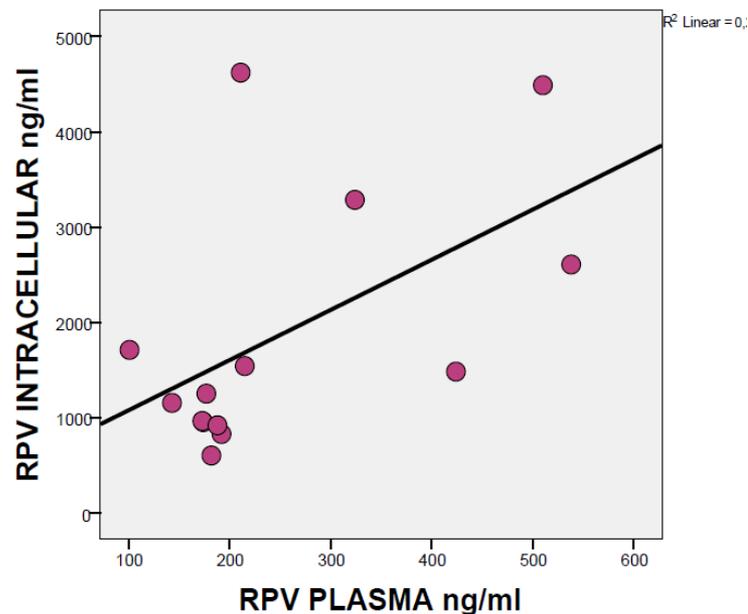
- ❑ These are the first data on intracellular rilpivirine penetration.
- ❑ Plasmatic and intracellular RPV concentrations showed a trend towards correlation (p=0.078, r: 0.486).
- ❑ RPV showed an high accumulation rate in PBMCs, with a ratio (6.3) well above those reported for other NNRTIs (nevirapine, efavirenz and etravirine), ranging from 1.3 to 0.05[*].
- ❑ Further clinical studies are warranted in order to elucidate clinical implications of RPV intracellular exposure.

[*]Bazzoli C, et al. Clin Pharmacokinet 2010; 49 (1): 17-45

Table III. Relationships between intracellular concentrations and efficacy of antiretroviral drugs in patients

Study	Primary objective (yes/no); type of trial	Intracellular moieties	Dosage regimen	Patients	Studies parameters from intracellular concentrations	Efficacy criteria	Results[a]
NRTIs							
Moore et al.[85]	Yes; clinical trial substudy				Wk 28: average of 1 h and 4 h concentrations postdose	Change between wk 0 and wk 24 in: (i) log plasma HIV RNA (ii) CD4 cell count	
		3TC-TP	3TC 150 mg + ZDV 300 mg bid or 3TC 150 mg + d4T 40 mg bid	39 treatment-naïve			(i) $p < 0.02$ (ii) $p = NS$
		ZDV-TP	ZDV 300 mg + 3TC 150 mg bid	10 treatment-naïve			(i) $p < 0.02$ (ii) $p = NS$
		d4T-TP	d4T 40 mg + 3TC 150 mg bid	15 treatment-naïve			(i) $p = NS$ (ii) $p = NS$
Aweeka et al.[125]	No; cross-sectional analysis	ZDV-TP	Any regimen containing ZDV	13 HCV- or HBV-co-infected, with stable regimen >4wk	AUC (NCA) from 5 samples: predose and 1, 4, 6 and 8 h postdose	CD4 cell count at time of PK sampling	$p = NS$
Anderson et al.[8]	Yes; clinical trial substudy	ZDV-TP	ZDV 300 mg bid + 3TC 150 mg bid + IDV 800 mg tid; or concentration-controlled ZDV-3TC-IDV regimen	33 treatment-naïve	Median concentration above threshold (yes/no) from samples 2 h postdose at wk 2, wk 28 and wk 56 and at 2–8 h postdose at 9 visits from wk 8 to wk 80 Thresholds: ZDV-TP: 30 fmol/10^6 3TC-TP: 7017 fmol/10^6	(i) Time to reach <50 copies/mL of HIV RNA (ii) Undetectable HIV RNA (<50 copies/mL) at wk 24 and wk 52 (iii) Time to loss of virological response in patients achieving undetectable HIV RNA (iv) CD4 cell counts at wk 24 and wk 48	(i) $p = 0.01$ (ii) wk 24: $p = 0.009$; wk 52: $p = NS$ (iii) $p = 0.02$ (iv) $p = NS$
		3TC-TP					(i) $p = 0.02$ (ii) wk 24: $p = 0.002$; wk 52: $p = 0.0008$ (iii) $p = 0.002$ (iv) $p = NS$
Fletcher et al.[89]	Yes; clinical trial substudy	ZDV-TP	ZDV 300 mg bid + 3TC 150 mg bid + IDV 800 mg tid; or concentration-controlled ZDV-3TC-IDV regimen	8 treatment-naïve	Median concentration from samples 2 h postdose at wk 2, wk 28 and wk 56 and at 2–8 h postdose at 9 visits from wk 8 to wk 80	Change between wk 0 and wk 24 in: (i) log HIV RNA (ii) CD4 cell count	(i) $p = 0.03$ (ii) $p = 0.001$

Bazzoli C. et al 2010

Table III. Contd

Study	Primary objective (yes/no); type of trial	Intracellular moieties	Dosage regimen	Patients	Studied parameters from intracellular concentrations	Efficacy criteria	Results[a]
		3TC-TP					(i) p = 0.003 (ii) p = NS
Stretcher et al.[135]	Yes; PK trial	Total ZDV phosphates	ZDV only: 800 mg/day, 100 mg every 4 h while awake	21 ZDV-naïve	AUC (NCA) from 6 samples: predose and 1, 2, 4, 6 and 8 h postdose at wk 4 and >wk 24	Change between wk 0 and wk 4 or wk 0 and wk 24 in: (i) % CD4 cells (ii) CD4/CD8 ratio	(i) wk 4: p = 0.029; wk 24: p = NS (ii) wk 4: p = 0.028; wk 24: p = NS
PIs							
Lamotte et al.[103]	No; secondary objective of clinical trial	SQV	SQV 1600 mg + RTV 100 mg od + 2 or 3 NRTIs/NTRTIs/NNRTIs	13 treatment-naïve	C_{trough} (24 h postdose) at wk 2, wk 4 or wk 12	Change in plasma HIV RNA between wk 0 and wk 12	p = NS for all periods of C_{trough} measurements
Breilh et al.[105]	Yes; observational study	LPV	LPV 400 mg + RTV 100 mg bid + 2 or 3 NRTIs/NTRTIs/NNRTIs	38 LPV-naïve	C_{trough} (12 h postdose) at wk 4 and wk 24	Virological success: (i) HIV RNA <50 copies/mL before wk 4 (ii) HIV RNA <50 copies/mL before wk 4 and during all follow-up until wk 24	(i) p < 0.0002 (ii) p < 0.002
Chaillou et al.[81]	No; secondary objective of cross-sectional trial	NFV, IDV, APV, SQV, RTV	NFV 750 mg tid or IDV 800 mg tid or APV 1200 mg bid or IDV 800 mg + RTV 100 mg bid or SQV 600 mg + RTV 100 mg bid or APV 600 mg + RTV 100 mg bid or IDV 400 mg + RTV 400 mg bid + 2 or 3 NRTIs/NNRTIs	49 treatment-experienced	Ratio of intracellular to plasma C_{trough} and C_{max} (1.5–3 h postdose) on day of study: (i) main PI (ii) RTV	Undetectable HIV RNA (<40 copies/mL) on day of study	(i) p = NS for PI ratio (ii) p = 0.04 for presence of intracellular RTV, p = 0.029 with RTV ratio in 28 patients receiving RTV

a Significant results always associated better efficacy with higher intracellular concentrations.

3TC = lamivudine; **APV** = amprenavir; **AUC** = area under the concentration-time curve; **bid** = twice daily; **C_{max}** = maximum concentration; **C_{trough}** = trough concentration; **d4T** = stavudine; **HBV** = hepatitis B virus; **HCV** = hepatitis C virus; **IDV** = indinavir; **LPV** = lopinavir; **NCA** = noncompartmental analysis; **NFV** = nelfinavir; **NNRTI** = non-NRTI; **NRTI** = nucleoside reverse transcriptase inhibitor; **NS** = nonsignificant; **NTRTI** = nucleotide reverse transcriptase inhibitor; **od** = once daily; **PI** = protease inhibitor; **PK** = pharmacokinetic; **RTV** = ritonavir; **SQV** = saquinavir; **tid** = three times daily; **TP** = triphosphate; **ZDV** = zidovudine.

Bazzoli C. et al 2010

Table IV. Relationships between intracellular concentrations and toxicity of antiretroviral drugs in patients

Study	Primary objective (yes/no); type of trial	Intracellular moieties	Dosage regimen	Patients	Studied parameters from intracellular concentrations	Toxicity criterion	Results[a]
NRTIs							
Anderson et al.[8]	Yes; clinical trial substudy	ZDV-TP, 3TC-TP	ZDV 300 mg bid + 3TC 150 mg bid + IDV 800 mg tid; or concentration-controlled ZDV-3TC-IDV regimen	33 treatment-naïve	Median concentration above threshold (yes/no) from samples 2 h postdose at wk 2, wk 28 and wk 56 and at 2–8 h postdose at 9 visits from wk 8 to wk 80 Thresholds: ZDV-TP: 30 fmol/10^6 3TC-TP: 7017 fmol/10^6	Occurrence of a grade I laboratory event (haemoglobin, absolute neutrophil count, AST, ALT)	NS
Stretcher et al.[135]	Yes; PK trial	Total ZDV phosphates	ZDV only: 800 mg/day; 100 mg every 4 h while awake	21 treatment-naïve	AUC (NCA) from 6 samples: predose and 1, 2, 4, 6 and 8 h postdose at wk 4 and wk 24	Change between wk 0 and wk 4 or wk 0 and wk 24 in: (i) neutrophils (ii) red blood cells (iii) haemoglobin	(i) p = NS (ii) p = NS (iii) p = NS
Durand-Gasselin et al.[83]	Yes; PK trial	ZDV-TP, 3TC-TP	ZDV (8 mg/kg/day in 4 daily doses) ±3TC (4 mg/kg/day in 2 daily doses)	49 neonates	Single-point concentration (time of sampling NR)	Proportions of the haematological toxicity grade between neonates with intracellular concentrations above or below the observed median	NS
NNRTI							
Rotger et al.[101]	Yes; PK trial	EFV	EFV + ZDV + 3TC EFV + abacavir + 3TC EFV + d4T + ddI ± PI (doses NR)	55	Intracellular AUC obtained by Bayesian estimation (no. of samples per patient and sampling times NR)	Presence of grade I to IV of: (i) sleep disorder (ii) mood disorder (iii) fatigue	(i) p = NS (ii) p = 0.02 (iii) p = NS

a   Significant results always associated increased risk of toxicity with higher intracellular concentrations.

3TC = lamivudine; **AUC** = area under the concentration-time curve; **bid** = twice daily; **d4T** = stavudine; **ddI** = didanosine; **EFV** = efavirenz; **IDV** = indinavir; **NCA** = noncompartmental analysis; **NR** = not reported; **NS** = nonsignificant; **PI** = protease inhibitors; **PK** = pharmacokinetic; **tid** = three times daily; **TP** = triphosphate; **ZDV** = zidovudine.

Bazzoli C. et al 2010

TDM…

The correlation between plasma and intracellular drug concentrations are essential to <u>not consider useful</u> the TDM in PBMCs.

Utility and futility of plasma concentrations

Interspecies comparisons in toxicology: the utility and futility of plasma concentrations of the test substance.

Monro AM.

Pfizer Central Research, Groton, Connecticut 06340.

Abstract
A classical dilemma in toxicology is how the dose administered relates to the dose delivered to the target site. Plasma concentrations of the test substance may be misleading since the concentration of any given substance in the plasma may not be representative of its concentration in tissues. Furthermore, a given tissue concentration of a xenobiotic can evoke responses which are highly species-dependent. While evaluating toxicity data

Often lack of correlation between plasma concentration and tissue concentration (site of action)

Modified from *Pr Pierre Wallemacq,*

Plasma and intracellular drug concentrations were "often" statistically correlated (but with <u>controversial data!</u>; No NRTIs and many times PIs [Bazzoli C. et al 2010; D'Avolio A. et al 2014]).

Ritonavir considering all boosted PIs ➡️

rho = 0.842 p < 0.001

RTV Intracellular Ctrough (ng/mL)

RTV Plasmatic Ctrough (ng/mL)
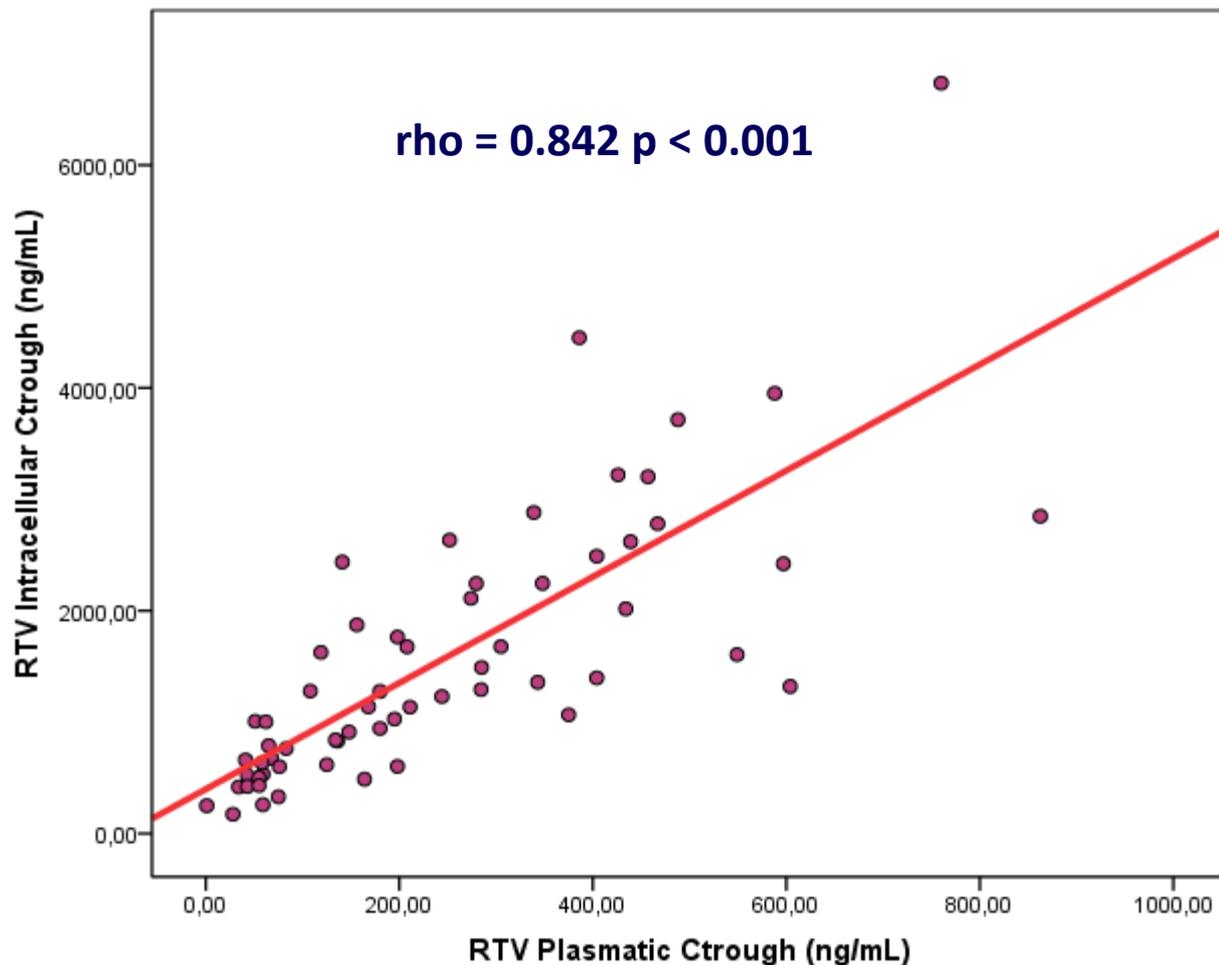
D'Avolio A. et al - JAC 2012

D'Avolio A. et al - ICAR 2011

Technical and Methodological Issues

The raltegravir "paradigm"

Plasma and Intracellular (Peripheral Blood Mononuclear Cells) Pharmacokinetics of Once-Daily Raltegravir (800 Milligrams) in HIV-Infected Patients[∇]

study. No differences in the times to the maximum concentration of raltegravir in plasma or the raltegravir half-lives were observed between plasma and PBMCs. The geometric mean raltegravir maximum concentration, the concentration at the end of the dosing interval, and the area under the concentration-time curve during the dose interval in plasma versus PBMCs were 2,640 ng/ml (range, 887 to 10,605 ng/ml) versus 199 ng/ml (range, 82 to 857 ng/ml) (geometric mean ratio [GMR], 13.30; 95% confidence interval [CI], 3.11 to 56.89; $P = 0.003$); 89 ng/ml (range, 51 to 200 ng/ml) versus 7 ng/ml (range, 2 to 15 ng/ml) (GMR, 13.21; 95% CI, 3.94 to 44.26; $P = 0.001$); and 12,200 ng · h/ml (range, 5,152 to 30,130 ng · h/ml) versus 909 ng · h/ml (range, 499 to 2,189 ng · h/ml) (GMR, 13.43; 95% CI, 5.13 to 35.16; $P < 0.001$), respectively. Raltegravir does not accumulate in PBMCs, with intracellular concentrations being about 1/10 of the concentrations in plasma.
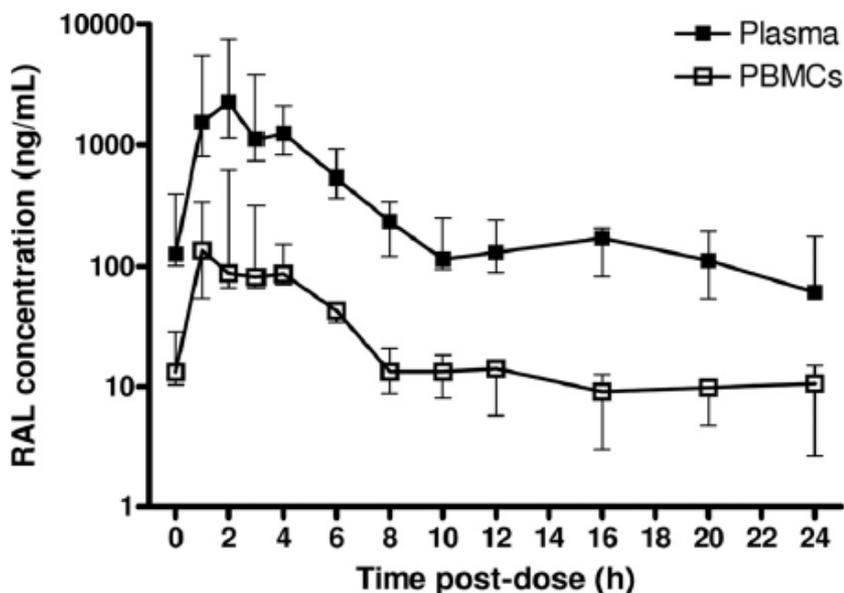
s added to the antiretroviral regimen at a dose of 800 mg once daily from days 0 to macokinetic profile was obtained for each participant. Raltegravir concentrations in od mononuclear cells (PBMCs) were determined by high-performance liquid chro-cence detector and by liquid chromatography-tandem mass spectrometry (LC-MS/ lues of the raltegravir pharmacokinetic parameters in plasma and PBMCs were mental analysis. Raltegravir was well tolerated, and all participants completed the e times to the maximum concentration of raltegravir in plasma or the raltegravir etween plasma and PBMCs. The geometric mean raltegravir maximum concentra-the end of the dosing interval, and the area under the concentration-time curve n plasma versus PBMCs were 2,640 ng/ml (range, 887 to 10,605 ng/ml) versus 199 g/ml) (geometric mean ratio [GMR], 13.30; 95% confidence interval [CI], 3.11 to l (range, 51 to 200 ng/ml) versus 7 ng/ml (range, 2 to 15 ng/ml) (GMR, 13.21; 95% 01); and 12,200 ng · h/ml (range, 5,152 to 30,130 ng · h/ml) versus 909 ng · h/ml /ml) (GMR, 13.43; 95% CI, 5.13 to 35.16; $P < 0.001$), respectively. Raltegravir does , with intracellular concentrations being about 1/10 of the concentrations in plasma. mean raltegravir concentrations at the end of the dosing interval in plasma and orted protein-binding-adjusted 95% inhibitory concentration (IC_{95}) and IC_{50} for pectively.


FIG. 1. Raltegravir time-concentration curve in plasma and PBMCs. Data are represented as medians and interquartile ranges. RAL, raltegravir.

II - Raltegravir

Research Letter

Measurement of plasma and intracellular concentrations of raltegravir in patients with HIV infection

Uriel Sandkovsky[a,b], Susan Swindells[a,b], Brian Robbins[c,d], Sarah R. Nelson[c], Edward P. Acosta[e] and Courtney V. Fletcher[a,b,c,d]

This paper confirm the previous with no accomulation of raltegravir within the cells
But ratio is different (0.2 – 0.5).

(PBMCs). Secondary objectives were to determine the

Table 1. Plasma and intracellular concentrations in participants receiving raltegravir 400 mg twice daily vs. participants receiving raltegravir 800 mg daily.

| Time (h) No. of samples	Raltegravir 400 mg twice daily				Raltegravir 800 mg once daily			
	Plasma concentrations (ng/ml)				Plasma concentrations (ng/ml)			
	2 $n=3$	4 $n=6$	6 $n=3$	12 $n=12$	2 $n=1$	4 $n=4$	6 $n=2$	24 $n=6$
Mean	1104.2	845.4	1538.3	205.6	7848	617.3	672.2	73.2
SD	1457.4	884.6	1394.3	145.2	–	583.9	633.3	92.5
CV	132%	105%	91%	71%	–	95%	94%	126%
Median	335.8	522.8	1368	165.3	7848	600	672.2	50.8
Min	191.7	80.8	237	34.7	7848	96.1	224.3	7
Max	2785	2316	3010	540.8	7848	1173	1120	230.2
	Intracellular concentrations (ng/ml)				Intracellular concentrations (ng/ml)			
Time (h)	2	4	6	12	2	4	6	24
Mean	227.1	262.7	663.5	67.2	3099.8	184.4	240.9	33.8
SD	195.7	321.1	641.9	70.8	–	135.1	221.6	42
CV	86%	122%	97%	105%	–	73%	92%	124%
Median	184.8	140.3	511	50.8	3099.8	182.7	240.9	16.7
Min	56.1	25.1	111.7	7	3099.8	37	84.2	1.56
Max	440.6	874.9	1367.9	264.6	3099.8	335.3	397.6	109

CV, coefficient of variation; Min, minimum measured concentration; Max, maximum measured concentration SD, standard deviation.

II - Raltegravir*

AIM

To study the steady-state plasma and intracellular pharmacokinetics of raltegravir, etravirine, darunavir and ritonavir in heavily pre-treated patients.

METHODS

Patients on a salvage regimen containing raltegravir, etravirine, darunavir and ritonavir were eligible for inclusion. During a 12 h dosing interval plasma and peripheral blood mononuclear cells were collected. Drug concentrations were measured using a validated LC-MS/MS assay and pharmacokinetic analysis was performed using non-linear mixed

RESULTS

Irregular absorption was observed caused by enterohepatic cycling. P when compared with other ritona pharmacokinetics showed wide in raltegravir concentrations could n The intracellular to plasma ratios 12.9, 1.32 and 7.72, respectively, an estimates were 16.3%, 12.3% and

CONCLUSIONS

The observed distinct intracellular different affinity for the cellular co accumulation of etravirine may ex absence of PK–PD relationships in compared with other non-nucleos intracellular concentrations of rito replication in the cellular compart with HIV harbouring PI resistance.

Correspon
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Table 2

Observed pharmacokinetics in plasma and PBMCs

Mean ± SD	Raltegravir Plasma	Etravirine Plasma	PBMCs	Darunavir Plasma	PBMCs	Ritonavir Plasma	PBMCs
n	11	9		10		10	
$C_{pre-dose}$ (mg l^{-1})	0.0690 ± 0.0507	0.235 ± 0.092	4.53 ± 6.01	1.89 ± 1.27	1.45 ± 1.11	0.124 ± 0.0741	0.976 ± 0.485
C_{max} (mg l^{-1})	0.709 ± 0.760	0.444 ± 0.135	9.40 ± 6.47	4.47 ± 1.82	9.73 ± 5.37	0.350 ± 0.213	2.55 ± 1.22
t_{max} (h)	3.08 ± 2.47	2.89 ± 0.735	2.64 ± 0.907	2.23 ± 0.934	2.26 ± 1.31	3.42 ± 1.66	2.74 ± 2.29

Table 3

A) Estimated pharmacokinetic parameters of raltegravir, etravirine and darunavir			
	Raltegravir (RSE)*	Etravirine (RSE)*	Darunavir (RSE)*
Number of transition compartments	2	4	2
Mean absorption time (h)	1.49 (27.9%)	1.77 (14.8%)	0.965 (22.3%)
CL/F (l h^{-1})	191 (16.0%)	55.3 (9.11%)	21.3 (13.9%)
V/F (l)	820 (25.5%)	781 (13.6%)	220 (14.5%)
Accumulation ratio	–	12.9 (16.3%)	1.32 (12.3%)
IIV MAT (%)	75.0 (59.5%)	36.9 (14.8%)	62.8 (22.3%)
IIV CL/F (%)	49.7 (59.5%)	26.3 (23.2%)	43.0 (31.1%)
Correlation IIV CL/F and IIV V/F	0.273 (47.6%)	0.0697 (45.2%)	0.133 (37.5%)
IIV V/F (%)	72.1 (35.6%)	30.3 (60.3%)	40.5 (38.2%)
IIV accumulation ratio (%)	–	55.1 (52.4%)	35.4 (52.7%)
Exponential residual error plasma observations (%)	68.1	13.2	19.4
Exponential residual error intracellular observations (%)	–	53.3	75.2

II - Raltegravir

Plasma and Intracellular Pharmacokinetics of Darunavir/Ritonavir Once Daily and Raltegravir Once and Twice Daily in HIV-Infected Individuals

Table 1. Plasma and IC Concentrations of Darunavir, Raltegravir, and Ritonavir and PK Parameter Comparison

	Plasma		IC		GM IC to Plasma Ratio	
	Group 1 (n = 13)	Group 2 (n = 12)	Group 1 (n = 13)	Group 2 (n = 12)	Group 1	Group 2
Period 1 (day 21)						
Raltegravir						
GM AUC, $ng \cdot h^{-1} \cdot mL^{-1}$	1944	1635	9010	8780	4.9	5.6
GM C_{max}, ng/mL	533	361	2306	1871	4.6	5.5
GM C_{trough}, ng/mL	23	28	101	115	5.1	4.6
Period 2 (day 35)						
Raltegravir						
GM AUC, $ng \cdot h^{-1} \cdot mL^{-1}$	1759	1979*	9223	11,183	5.8	6.0
GM C_{max}, ng/mL	438	556*	2173	2817	5.6	6.8
GM C_{trough}, ng/mL	20	11*	105	22	4.5	2.4
Darunavir						
GM AUC, $ng \cdot h^{-1} \cdot mL^{-1}$	40,064	50,040	20,2865	26,3148	5.3	5.6
GM C_{max}, ng/mL	4235	5092	20,210	29,293	6.8	6.3
GM C_{trough}, ng						4.3
Ritonavir						
GM AUC, ng·h mL	4299	4559	33,789	28,065	10.0	6.9
GM C ng/mL	482	617	2892	2280	13.8	5.7
GM						1
Period						
Daru						
GM						9
GM						8
GM						4
Ritor						
GM AUC, $ng \cdot h^{-1} \cdot mL^{-1}$	4568	4516	36,769	27,652	9.5	6.5
GM C_{max}, ng/mL	528	527	4041	2607	13.5	5.2
GM C_{trough}, ng/mL	44	36	478	369	11.7	10.6

Which data are correct for raltegravir?

There are key methodological factors:
The wash numbers and time!
Raltegravir goes very easy outside the PBMC.

Technical Questions

Intracellular TDM for ARVs should be in <u>PBMC</u> cells

Open questions…

➢**CPT tube or Ficoll for PBMCs isolation?**

➢**How many times we have to wash PBMCs?**

➢<u>**Cold Ice**</u> **Phosphate buffered saline (PBS), NaCl 0.9%, oil or other solution?**

➢**How to count PBMCs?**

➢**How to converse absolute drug determination in ng/mL?**

Technical Conclusions

…for ARVs (and other drugs) intracellular determinations

To date, there isn't a "gold standard" methodology.

- PBMCs isolation:
  - CPT tube or Ficoll separation?
  - Number of washes?
  - Kind of solution? PBS, NaCl 0.9%, Oil, other?

You have to choose on the basis of your drug!

Contents lists available at ScienceDirect

Journal of Pharmaceutical and Biomedical Analysis

journal homepage: www.elsevier.com/locate/jpba

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Review

Therapeutic drug monitoring of intracellular anti-infective agents☆

Antonio D'Avolio*, Debora Pensi, Lorena Baietto, Giovanni Di Perri

Laboratory of Clinical Pharmacology and Pharmacogenetic, Unit of Infectious Diseases, University of Turin, Department of Medical Sciences, Amedeo di Savoia Hospital, Turin, Italy

CrossMark

Technical Conclusions

Other <u>KEY FACTORS</u> for a correct intracellular drug evaluation are:

- Automated counting with Coulter Counter, with calculation of <u>Mean Cellular Volume</u> [MCV] for a personalized evaluation and data correction in ng/mL).

- No use of 400 fL, it is incorrect! [Simiele et al., AAC 2011] (at least report results per 10^{-6} cells)

- Chromatographic methods… others discussion points

Review

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Pitfalls Associated with the Use of Liquid Chromatography–Tandem Mass Spectrometry in the Clinical Laboratory

Michael Vogeser[1]* and Christoph Seger[2]*

Conclusions

Bazzoli C. et al 2010
D'Avolio A. et al. 2014

•Intracellular concentrations of ARVs could play a major role in their efficacy and toxicity, and are influenced by numerous factors.

•The number of published clinical studies in this area is limited; most studies have been small and not always adequately designed.

• Larger and prospectively designed clinical studies are needed to further investigate the links between intracellular concentrations of ARVs and clinical endpoints.

•**Standardization of assays and PBMC counts and use of MCV should be warranted.**

TAKE HOME MESSAGES

....**routinely** TDM for ARVs
should be performed **only in PLASMA**

despite poor correlation between plasma drug concentrations and intracellular drug concentrations

Actual methods are NOT EASY to perform and they could have high imprecision if not well validated!

Maybe for research only…
excluding immunosuppressive drugs.

Acknowledgement

University of Turin

The Laboratory Team

30/12/13   TDM_home
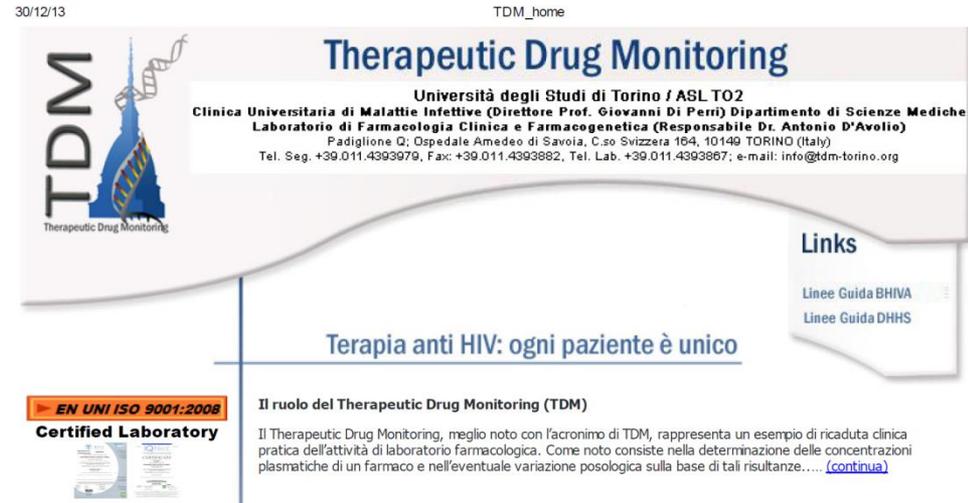
Therapeutic Drug Monitoring

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Links

Linee Guida BHIVA
Linee Guida DHHS

Terapia anti HIV: ogni paziente è unico

EN UNI ISO 9001:2008
Certified Laboratory

Il ruolo del Therapeutic Drug Monitoring (TDM)

Il Therapeutic Drug Monitoring, meglio noto con l'acronimo di TDM, rappresenta un esempio di ricaduta clinica pratica dell'attività di laboratorio farmacologica. Come noto consiste nella determinazione delle concentrazioni plasmatiche di un farmaco e nell'eventuale variazione posologica sulla base di tali risultanze….. (continua)

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