

Monitoring of intracellular triphosphorylated anabolites of didanosine EC (ddA-TP) and stavudine (d4T-TP): result of a pilot clinical study in HIV infected patients.

F. BECHER¹, R. LANDMAN², A. CANESTRI², S. MBOUP³, C. NDEYE TOURE KANE,³ F. LIEGEOIS⁴, M. VRAY⁵, C. DALBAN⁵, MH PREVOT⁶, G. LELEU⁶ and H.BENECH¹

1: CEA, Pharmacology and Immunology unit, DSV/DRM, 91191 Gif sur Yvette Cedex, France, 2: IMEA, Bichat-Claude Bernard Hospital, France, 3: PLNS, Dakar, Senegal, 4: IRD, France, 5: INSERM SC4, France, 6: Bristol-Myers-Squibb, La Défense Paris, France.

Background: A direct Liquid Chromatography tandem Mass Spectrometry (LC/MS/MS) assay was recently developed and validated to measure the active intracellular triphosphorylated (TP) anabolites of NRTIs in PBMC^{1,2} such as ddA-TP for ddI and d4T-TP for d4T.

Aim: To verify the relevance of this assay in samples coming from d4T and ddI treated patients and to approach the inter patient variability (IPV) of the measure.

Patients and method: Naïve adult patients were enrolled in an efficacy and tolerance open pilot study in Dakar, Senegal of d4T (40/30 mg bid), ddI (400/250 mg qd) and efavirenz (600 mg qd) regimen (IMEA 012-ANRS1206). A pharmacokinetic sub study was conducted to assess d4T-TP and ddA-TP intracellular concentration (ICC) at M6. Delay between dosing to sampling was recorded. PBMCs were obtained by FicolI on EDTA. Limits of LC/MS/MS quantification (LOQ) are 61 and 53 fmol/sample for d4T-TP and ddA-TP, respectively. d4T and ddI plasma concentrations (PC) were simultaneously measured using a validated LC/MS/MS method (LOQ 0.5 ng/mL). Viral load (VL) and CD4 cells count were available at baseline and M6.

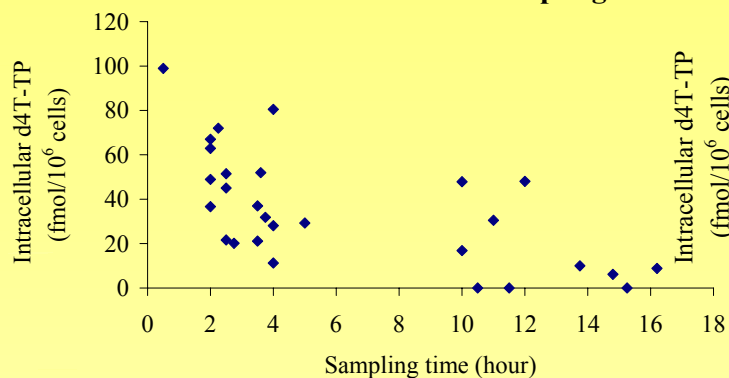
Results: All samples were available for the 28/40 first patients (12 male). At baseline, CDC stage C= 46%, and mean values \pm sd : weight = 54 \pm 8 kg, VL = 5.5 \pm 0.4 log cp/mL and CD4 = 134 \pm 86/mm³. At M6, CD4 mean \pm sd = 226 \pm 153/mm³, VL mean \pm sd = 2.3 \pm 1.0 log cp/mL. VL remained >200 cp/mL in 6 pts. d4T-TP and ddA-TP ICC were above the LOQ in 25/28 and 26/28 pts respectively (median [range]d4T-TP and ddA-TP: 31[0-99] and 8[0-23] fmol/10⁶ cells). Two of the 3 d4T-TP undetectable pts had discontinued their treatment according to medical assessment, confirmed by high VL and undetectable d4T and ddI PC.

In the 17 samples collected 2 to 5 h after last dosing, d4T-TP IC median[range] was 37[11-81] and d4T PC 281[47-670] fmol/10⁶ cells. In the 22 samples collected 10 to 16 h after last dosing, ddA-TP IC median[range] was 9.8[0-23] and ddI PC 29[2-165] fmol/10⁶ cells. ICC IPV (CV%) were 59 and 48% for ddA-TP and d4T-TP respectively. Significant correlation was found between ICC d4T-TP and PC d4T (r=0.6, p=.005), but not for ICC ddA-TP dl and PC ddI.

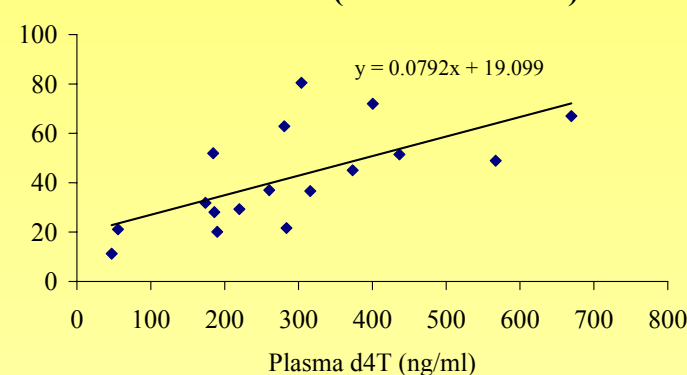
In the 22/28 patients with VL<200 cp/mL at M6, d4T-TP and ddA-TP ICC median[range] were 34[0-99] and 8[0-23] fmol/10⁶ cells.

Conclusion: Excepted the 2 non compliant patients, ICC were quantified in 25/26 pts both for ddA-TP and d4T-TP. The IPV values suggest that this assay could be used to monitor the ddI/d4T active form. PK/PD studies of intracellular anabolites are warranted in order to define the position of this assay in ddI and/or d4T treatment monitoring.

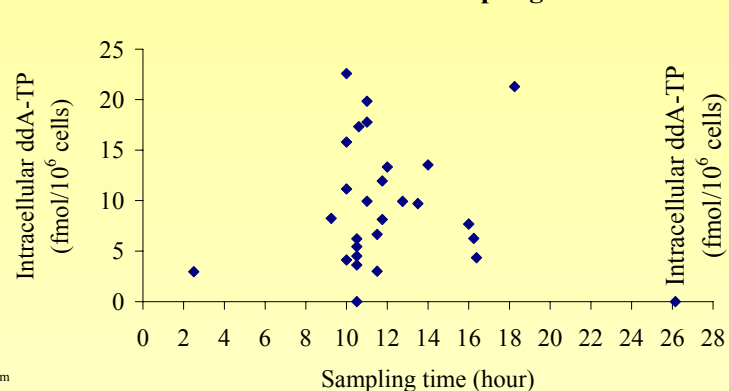
Intracellular d4T-TP vs sampling time



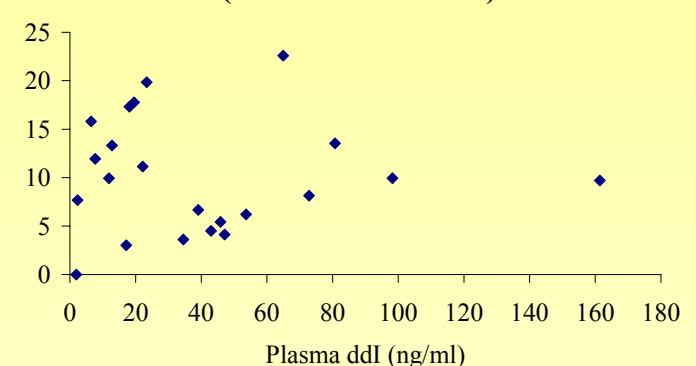
Relationship between plasma d4T vs intracellular d4T-TP (collection 2-5 hr)



Intracellular ddA-TP vs sampling time



Plasma ddI vs intracellular ddA-TP (collection 10-16 hr)



1- Direct determination of phosphorylated intracellular anabolites of stavudine (d4T) by liquid chromatography tandem mass spectrometry

A. Pruvost, F. Becher, P. Bardouille, C. Guerrero, C. Creminon, J.-F. Delfraissy, C. Goujard, J. Grassi and H. Benech. *Rapid Commun. Mass Spectrom.* 15: 1401-1408 (2001)

2- Improved method for the simultaneous determination of d4T, 3TC and ddI intracellular phosphorylated anabolites in human PBMCs using HPLC/MS/MS

F. Becher, A. Pruvost, C. Goujard, C. Guerrero, J.-F. Delfraissy, J. Grassi and H. Benech. *Rapid Commun. Mass Spectrom.* 16: 1-11 (2002)