Prevalence of resistance mutations related to integrase inhibitor S/GSK1349572 in HIV-1 subtype B raltegravir-naïve and -treated patients

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Complete List of Authors:	Malet, I; Pitie-Salpetriere hospital, virology Wirden, Marc; Pitie-Salpêtriere hospital, virology Fourati, Slim; Université Pierre et Marie Curie-Paris 6/INSERM/APHP, UMR S-943/U943/virology laboratory Armenia, Daniele; University of Rome Tor Vergata, Experimental Medicine Masquelier, Bernard; CHU de Bordeaux, Laboratoire de Virologie Fabeni, Lavinia; University of Rome Tor Vergata, experimental Medicine Sayon, Sophie; Pitie-Salpetriere hospital, virology Katlama, christine; Groupe Hospitalier Pitié-Salpêtrière, service des Maladies Infectieuses et Tropicales Perno, Carlo-Federico; University of Rome, Department of experimental medicine calvez, vincent; pitie-Salpetriere hospital, virology Marcelin, Anne-Genevieve; Pitie-Salpetriere Hospital, Virology Ceccherini-Silberstein, Francesca; University of Rome, Department of Biochemical Sciences and Experimental Medicine
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Prevalence of resistance mutations related to integrase inhibitor S/GSK1349572 in HIV-1 subtype B raltegravir-naïve and -treated patients

Isabelle Malet[1*], Marc Wirden[1], Slim Fourati[1], Daniele Armenia[2], Bernard Masquelier[3], Lavinia Fabeni[2,4], Sophie Sayon[1], Christine Katlama[1], Carlo Federico Perno[2,4], Vincent Calvez[1], Anne-Geneviève Marcelin[1] and Francesca Ceccherini-Silberstein[2]

[1]Laboratoire de Virologie, AP-HP, Groupe Hospitalier Pitié-Salpêtrière, UPMC Univ Paris 06, INSERM U943, Paris, France; [2]Department of Experimental Medicine, University of Rome Tor Vergata, Rome, Italy; [3]Laboratoire de Virologie, CHU de Bordeaux, EA 2968, Université Victor Segalen, Bordeaux, France; [4]National Institute for Infectious Diseases (INMI) L. Spallanzani, Rome, Italy

*Corresponding author. Mailing address: hôpital Pitié-Salpêtrière, Service de Virologie, 83, bld de l'hôpital, 75013 Paris, France. Phone: 0033 142177401. Fax: 0033 142177411. E-mail: isabelle.malet@psl.aphp.fr

Short title: S/GSK1349572 mutations in HIV-1 patients

Keywords: resistance, failure, polymorphism, prevalence

Objectives: To compare the frequency of previously *in vitro* selected integrase mutations (T124A, T124A/S153F, S153Y, T124A/S153Y and L101I/T124A/S153Y) to S/GSK1349572 between HIV-1 subtype B integrase inhibitor (INI)-naïve and raltegravir (RAL)-treated patients.

Methods: Integrase (IN) sequences from 650 INI-naïve patients and 84 raltegravir-treated patients were analyzed.

Results: T124A mutation alone and the combination T124A/L101I were more frequent in raltegravir-failing patients than in INI-naïve patients (39.3% *versus* 24.5%, respectively, with p=0.005 for T124A and 20.2% *versus* 10%, respectively, with p=0.008 for T124A/L101I) as the S153Y/F mutations have never been detected in any integrase sequence.

Conclusions: T124A and T124A/L101I, more frequent in raltegravir-treated patients, could have some effect on raltegravir response and their presence could play a role in the selection of other mutations conferring S/GSK1349572 resistance. The impact of such changes mediated by raltegravir should be further studied on the virological response to S/GSK1349572.

Introduction

Integrase, the HIV-1 enzyme responsible for the integration of the viral genome into the chromosomes of infected cells, is the target of the recently approved antiretroviral raltegravir (RAL) and currently investigated elvitegravir (EVG). Despite activity against viruses resistant to other antiretrovirals, failures against integrase inhibitors (INIs) therapy were observed, in association with the emergence of resistance due to mutations in the integrase gene.[1] S/GSK1349572 is a next generation HIV-1 strand transfer INI with high potency (IC_{50} measured in presence of human serum = 38 nM).[2] *In vitro,* serial passage experiments identified five single or combined amino acid substitutions that could confer S/GSK1349572 resistance: T124A, T124A/S153F, S153Y, T124A/S153Y and L101I/T124A/S153Y.[2] S/GSK1349572, showing low fold changes in activity against site directed molecular clones, including Y143C/H/R, Q148K/R/H and N155H, seems to have limited cross-resistance to raltegravir- and elvitegravir-resistant mutants[3] and may have a higher genetic barrier to resistance than raltegravir.[4] *In vivo*, preliminary results in 10 HIV-1 infected patients INI naïve and treated by S/GSK1349572 in monotherapy (50 mg once daily) during 10 days reported a HIV-1 plasma viral load decrease of -2.46 $\log_{10}$ copies/mL.[5] Another recent study evaluated the short-term antiviral activity of S/GSK1349572 (at day 11) in 27 raltegravir-experienced patients with raltegravir-resistant viruses. Results showed a HIV-1 plasma viral load decrease of -1.45 $\log_{10}$ copies/mL in 100% of patients harboring mutations linked to the N155 and Y143 pathways. In contrast, a viral load decrease of -0.72 $\log_{10}$ copies/mL was observed only in 33% of patients harboring the Q148 pathway associated with L74, E138 or G140 mutations.[6]

In INI-naïve patients, there is a limited degree of natural polymorphisms in the integrase gene from subtype B HIV-1, since 65% of HIV-1 integrase residues are conserved (< 1% variability). Residues involved in protein stability, multimerization, DNA binding, catalytic

activity, and in the binding with the human cellular cofactor LEDGF/p75 are fully conserved.[7] It has also been shown that all primary signature mutations emerging in patients failing raltegravir (Y143C/R, Q148H/K/R, N155H) or elvitegravir (T66I, E92Q, S147G, Q148H/K/R, N155H), as well as secondary mutations (H51Y, T66A/K, E92A/G/Q, F121Y, E138K, G140S/A/C, Y143C/H, K160N, R166S, E170A, S230R, D232N, R263K) were completely absent or highly infrequent (< 0.5%) in INI-naïve patients infected with HIV-1 B subtype.[7] The aims of this study were to explore potential primary genotypic resistance to S/GSK1349572 in INI naïve patients and the ability of this compound to treat patients with raltegravir resistance. Thus, we evaluated the proportion of patients carrying viruses with resistance mutations previously described to S/GSK1349572 in HIV-1 subtype B raltegravir-naïve and -treated patients.

Materials and methods

In this report, sequences of the entire integrase gene from 650 INI-naïve patients and 84 raltegravir-experienced (all raltegravir-failing) patients, all infected with subtype B HIV-1 strains, were analyzed for the presence of previously described *in vitro* mutations to S/GSK1349572. At the time of the genotypic resistance test, INI-naïve patients (143 HAART (Highly Active Antiretroviral Therapy)-naïve and 507 HAART-experienced) and raltegravir-treated patients (all HAART-experienced) received, in their optimized regimen, at least one NRTI (Nucleoside Reverse Transcriptase Inhibitor) with one boosted PI (Protease Inhibitor) or one NNRTI (Non-Nucleoside Reverse Transcriptase Inhibitor) plus, for some of them, enfuvirtide or maraviroc. INI-naïve and raltegravir-treated patients showed a median viral load of 4.2 (3.6 - 4.9) $\log_{10}$ copies/mL and 3.8 (2.5 - 5.1) $\log_{10}$ copies/mL, respectively.

RNA was extracted from 500 µL of plasma, and a 1086 base pair fragment encompassing the entire IN gene was amplified, as described previously.[8] The PCR products were purified and sequenced using a cycle sequencing reaction with the Big Dye terminator kit (Applied Biosystems, Foster City, California, USA). The sequences were aligned using SmartGene software (SmartGene GmbH, Zug, Switzerland) and the amino acid sequence of HIV-1 integrase (288 amino acids) of clade B consensus was considered as a reference.

Results

The prevalence of *in vitro* selected mutations by S/GSK1349572 in naïve and raltegravir-treated patients is presented in Table 1. Mutations L101I and T124A seem to be polymorphic in INI-naïve patients with frequencies of 45.8% and 24.5%, respectively, the two associated mutations L101I/T124A being present with a frequency of 10%. In raltegravir-treated patients, the genotypic resistance test performed at raltegravir failure, showed that mutations L101I, T124A and L101I/T124A occurred with frequencies of 56%, 39.3% and 20.2%, respectively. Consequently, only mutations T124A and L101I/T124A were more frequent in raltegravir-failing patients than in INI-naïve patients ($p = 0.005$ and 0.008, respectively). The mutations S153Y/F, and consequently the profiles T124A/S153F, T124A/S153Y and L101I/T124A/S153Y, have never been detected in any sequence from both INI-naïve and raltegravir-failing patients (except for S153F alone, only detected in one INI-naïve patient).

Discussion

In conclusion, some previously *in vitro* selected mutations by S/GSK1349572 (T124A and L101I/T124A) are polymorphic but significantly more frequent in raltegravir-treated patients

than in raltegravir-naïve patients. This result suggests that these mutations could have some effect on raltegravir response, at least as secondary resistance mutations. The fact that these mutations are increased in raltegravir-failing patients and selected in vitro by S/GSK1349572 also suggests that they can participate to raltegravir and S/GSK1349572 cross-resistance. The mutation T124A, alone or associated with L101I, is among the first mutations that appear in culture, under S/GSK1349572 pressure, at day 56,[2] suggesting a role in the resistance to S/GSK1349572. A recent study has shown that baseline viruses with L101I and/or T124A do not seem to have an impact, at day 10, on S/GSK1349572 response in INI-naïve patients.[9] However, considering the higher prevalence of T124A and L101I/T124A mutations in raltegravir-treated patients, we could not exclude that their presence in raltegravir-failing patients could favour the selection of other mutations conferring S/GSK1349572 resistance. Thereby, it should be interesting to study the response to S/GSK1349572 treatment in patients failing to raltegravir to evaluate the impact of IN polymorphisms and to study if these polymorphisms can affect the selected resistance mutations in case of failure to S/GSK1349572.

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Transparency declarations

None to declare

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183 **Table 1.** Evaluation and comparison of prevalence of L101I, T124A, S153F and S153Y
184 mutations in INI-naïve and RAL-failing patients
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Integrase mutations	Integrase inhibitor-naïve patients (n=650)		Raltegravir failing patients (n=84)		p value
	n	%	n	%	
L101I	298	45.8	47	56.0	0.083
T124A	159	24.5	33	39.3	**0.005**[a]
L101I + T124A	65	10.0	17	20.2	**0.008**[a]
S153Y	0	0	0	0	-
S153F	1	0.2	0	0	-

186 [a] p values shown in bold are valid after multiple comparison tests
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