Frequency of amino acid changes associated with resistance to attachment inhibitor BMS-626529 in R5- and X4-tropic HIV-1 subtype B

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Objectives: Resistance to attachment inhibitor BMS-626529, which inhibits the binding of HIV to CD4, involves mutations in the HIV-1 gp120 gene. There is a lack of information on the primary resistance of HIV-1 subtype B to attachment inhibitors, so we decided to investigate.

Methods: Sequences from 109 attachment-inhibitor-naive patients infected with HIV-1 subtype B were analysed for the presence of previously described in vivo resistance mutations associated with attachment inhibitor BMS-626529 and tropism determination.

Results: The M426L substitution associated with a reduced efficacy of the attachment inhibitor BMS-626529 was present at 7.3%. There was no difference in mutation distribution according to virus tropism (R5 or X4).

Conclusions: The attachment inhibitor BMS-626529 is suitable for most patients infected with HIV-1 subtype B.

Keywords: primary resistance, gp120, tropism

Introduction

BMS-626529 is a novel attachment inhibitor; BMS-663068 is the phosphonooxymethyl prodrug. These compounds represent a potential new class of drug under investigation for the treatment of HIV infection that are able to inhibit its binding to CD4+ T cells.1 The CD4 binding site as the coreceptor binding site (including the V3 loop used for tropism determination) is located in HIV gp120.

The in vitro antiviral activity of BMS-626529 exhibited low cytotoxicity in cell culture and demonstrated activity against the majority of laboratory strains of HIV-1 and clinical isolates of HIV in peripheral blood mononuclear cells. The in vitro passage experiments demonstrated substitutions in the CD4 binding site in HIV gp120 that confer resistance to BMS-626529 (L116P, A204D, M426L, M434I-V506M and M475I).2 Furthermore, the in vitro antiviral activity of BMS-626529 was generally not associated with either tropism or subtype (with the exception of subtype CRF01_AE and possibly HIV-1 group O).2

The BMS-626529 molecule, dosed as BMS-663068, has demonstrated potent antiviral activity when administered once or twice daily, with and without ritonavir, in an 8 day monotherapy study in treatment-naïve and treatment-experienced subjects infected with HIV-1 subtype B viruses.3 In the in vivo study of BMS-626529 (8 days of monotherapy), administered as its produg form to patients infected with HIV-1 subtype B, env substitutions M426L and S375M were found to be strongly associated with low susceptibility to BMS-626529. M434I, S375T and M475I also contributed to loss of phenotypic susceptibility in some non-responders.4

A recent study described the subtype-related polymorphisms associated with in vitro resistance to BMS-626529 in HIV-1 ‘non-B’-infected patients and demonstrated the presence of M426L substitutions in gp120 in 46% and 7% of subtype D and CRF02_AG samples, respectively.2 Currently there is a lack of information on primary resistance of HIV-1 subtype B to attachment inhibitors, suggesting the need for such analyses. In the present study we investigated the presence of env substitutions described as associated with the in vivo resistance of BMS-626529 in patients infected with HIV-1 subtype B according to the tropism of the viruses.

Methods

This study was approved by the Ethics Committee of the AC11-Agence Nationale de Recherche sur le Sida et les hépatites virales (ANRS). One
hundred and nine patients followed at the Internal Medicine and Infectious Diseases Departments of the Pitié-Salpêtrière Hospital signed individual consent forms. The 109 patients were either treatment naive or treated experienced, were infected with HIV-1 subtype B and they had never been treated with attachment inhibitors or CCR5 antagonists.

Sequences of env gp120 were analysed both for tropism and the presence of previously described combinations of mutations linked to in vivo resistance to BMS-626529 (S375M/T, M426L, M434I and M475I). Briefly, the fragment was amplified with RT–PCR and nested PCR using the following outer primers: E56 (5’-CCATAGAATGTGATCCACACCTAAAG-3’) and ED12 (5’-AGTGGTCTGTGCTCTCCGACAGA-3’); and the following inner primers: E5 (5’-GTGTTGTTGTCATGAATCAGATCA-3’) and E4 (5’-CAGTTCTCAATTGTCCCTCA-3’). The reverse transcription step (Titan One Tube RT–PCR Kit, Roche, Mannheim, Germany) was carried out at 55°C for 60 min, followed by 94°C for 5 min. PCR cycling parameters were 50 repeat cycles (94°C for 45 s, 65°C for 60 s and 68°C for 3 min) and 68°C for 10 min. Nested PCRs were performed with Expand High Fidelity (Roche) and 1.5 μL of the inner primers described above (10 μM). The PCR conditions consisted of an initial denaturation at 94°C for 5 min followed by 50 repeated cycles (94°C for 45 s, 51°C for 1 min and 72°C for 3 min) and 72°C for 10 min. The sequencing reactions were performed using the E5 and E4 primers. Sequencing reactions were run using the ABI Prism Dye Terminator Kit. The genotypic determination of tropism was performed using the Geno2pheno algorithm with a false positive rate of 10%.

Results
The sequence analysis showed a total conservation for one of the four analysed amino acid residues (position M475). Fifty of 109 (45.9%) patients showed no modifications of amino acid residues at the four analysed positions in comparison with an HXB2 reference sequence. Fifty-nine (54.1%) sequences showed some variations at these positions, associated with resistance to the attachment inhibitor (Table 1). The variations were detected in isolation, in pairs or in triplets. M426L, considered as the key resistance substitution, was observed in eight viruses without variations at the other studied positions (1.8%). M426L is considered a key resistance substitution. S375M is responsible for reduced susceptibility to the attachment inhibitor, was also present in this population of HIV-1 subtype B, but the impact on the efficacy of attachment inhibitor BMS-626529 as described below.

The tropism of the 109 patients was analysed and 69 (63.3%) sequences with no variation and 32 with variation at one or more positions were determined to be R5- and X4-tropic, respectively. The frequency of primary resistance mutations was not different between R5- and X4-tropic viruses: 37 R5/22 X4 and 32 R5/18 X4 with or without variations at the four positions, respectively (P=0.889). The distribution of the mutations according to tropism is described in Table 1.

Discussion
Actually, three antiretrovirals are known to target HIV-1 entry: attachment inhibitor (BMS-626529) (inhibits HIV gp120 attachment to CD4), CCR5 inhibitor (the use of which depends on virus tropism) and fusion inhibitor.1,6–8 The attachment inhibitor and the CCR5 inhibitor are linked by HIV gp120.

Some primary resistances to BMS-626529 were observed for naive patients. The M426L key resistance substitution was present at 7.3% in this group of subtype-B-infected patients, which is the same as the primary genotypic resistance of HIV-1 to CCR5 antagonists in CCR5 treatment-naïve patients.9 The prevalence of the primary resistance to this attachment inhibitor was lower than the prevalence of X4-tropic viruses observed in this population (36.7%). The presence of attachment inhibitor mutations before introducing the treatment may lead the virus to become resistant, as for all antiretrovirals, and genotype determination is recommended before treatment initiation.

M426L is considered a key resistance substitution. S375M is responsible for reduced susceptibility to the inhibitor. S375T and M434I contributed to loss of phenotypic susceptibility to the attachment inhibitor. M475I could contribute to loss of phenotypic susceptibility in some non-responders. For the 59 sequences presenting variations, 43 of them have isolated variations and 16 of them have pairs or triplets of variations.

Table 1. Distribution of the mutations associated with resistance to the attachment inhibitor BMS-626529

<table>
<thead>
<tr>
<th></th>
<th>All patients, n=109 (50 sequences with no variation and 59 sequences with variation at one or more positions)</th>
<th>Patients with R5-tropic viruses, n=69 (32 sequences with no variation and 37 sequences with variation at one or more positions)</th>
<th>Patients with X4-tropic viruses, n=40 (18 sequences with no variation and 22 sequences with variation at one or more positions)</th>
</tr>
</thead>
<tbody>
<tr>
<td>S375H, n (%)</td>
<td>3 (2.8)</td>
<td>1 (1.4)</td>
<td>2 (5.0)</td>
</tr>
<tr>
<td>S375I, n (%)</td>
<td>1 (0.9)</td>
<td>1 (1.4)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>S375M, n (%)</td>
<td>2 (1.8)</td>
<td>1 (1.4)</td>
<td>1 (2.5)</td>
</tr>
<tr>
<td>S375N, n (%)</td>
<td>9 (8.3)</td>
<td>7 (10.1)</td>
<td>2 (5.0)</td>
</tr>
<tr>
<td>S375T, n (%)</td>
<td>31 (28.4)</td>
<td>19 (27.5)</td>
<td>12 (30.0)</td>
</tr>
<tr>
<td>M426A, n (%)</td>
<td>0 (0)</td>
<td>1 (1.4)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>M426L, n (%)</td>
<td>3 (3.6)</td>
<td>1 (1.4)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>M426K, n (%)</td>
<td>0 (0)</td>
<td>1 (1.4)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>M426L, n (%)</td>
<td>8 (7.3)</td>
<td>4 (5.8)</td>
<td>4 (10.0)</td>
</tr>
<tr>
<td>M426R, n (%)</td>
<td>10 (9.2)</td>
<td>7 (10.1)</td>
<td>3 (7.5)</td>
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<tr>
<td>M434I, n (%)</td>
<td>7 (6.4)</td>
<td>4 (5.8)</td>
<td>3 (7.5)</td>
</tr>
<tr>
<td>M434K, n (%)</td>
<td>1 (0.9)</td>
<td>1 (1.4)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>M434T, n (%)</td>
<td>1 (0.9)</td>
<td>1 (1.4)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>M475I, n (%)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
</tbody>
</table>

In bold: amino acid changes described to possibly impact the effect of the attachment inhibitor as described below.
presence or absence of the mutations described to be associated with resistance to the attachment inhibitor \textit{in vivo}, allowing a possible concomitant use of the attachment inhibitor and CCR5 antagonists.

In conclusion, primary resistance to BMS-626529 was observed for this population of patients infected with HIV-1 subtype B, allowing the prescription of the inhibitor in most cases. Furthermore, there was no difference in mutation distribution according to virus tropism.

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\section*{Transparency declarations}
None to declare.

\section*{References}