The coding regions of the transpeptidase domain of the \textit{pbp1a}, \textit{pbp2b} and \textit{pbp2x} genes of the two GBS isolates were amplified and sequenced according to previously outlined methods. Amino acid sequences were deduced and analysed using the ClustalW alignment tool included in the Lasergene software (DNAnstar, Madison, WI, USA). Nucleotide and deduced amino acid sequences were compared with those of the reference penicillin-susceptible strains 2603V/R (GenBank accession number: NC_004116) and NEM316 (GenBank accession number: NC_004368). DNA analysis revealed that the \textit{pbp} genes of the clinical GBS isolates possessed many amino acid substitutions compared with the corresponding genes of the reference strains 2603V/R and NEM316. Five previously described amino acid substitutions were observed in both the penicillin-susceptible GBS and penicillin G-non-susceptible GBS isolates (S453N and N682D in PBP1a, V625I in PBP2b, and I377V and G627V in PBP2x). However, there were three novel substitutions (T526A in PBP1a, P278L in PBP2b and N575D in PBP2x) found exclusively in the penicillin G-non-susceptible GBS 2007 isolate.

In previous studies, the V405A and Q557E substitutions adjacent to the conserved SSN and KSG motifs in PBP2x, considered to form the active site of the enzyme, were found in invasive GBS with elevated, but still susceptible, MICs of one or multiple \(\beta\)-lactam antibiotics, in 21 penicillin G-non-susceptible GBS isolated from the respiratory tract and in a penicillin G-non-susceptible GBS recurrently isolated from a sacral ulcer. Several amino acid substitutions in PBP1a, PBP2a and PBP2b were also found in penicillin G-non-susceptible GBS isolated from the respiratory tract. However, the PBP2a amino acid substitutions were documented in only two GBS with penicillin MICs of 1 mg/L. In the present study, the penicillin G-non-susceptible GBS 2007 isolate did not harbour the PBP2x V405A and Q557E substitutions previously associated with reduced susceptibility to penicillin. Instead, the penicillin G-non-susceptible GBS 2007 isolate possessed three novel substitutions (T526A in PBP1a, P278L in PBP2b and N575D in PBP2x). At this time it is not known whether these substitutions can actually increase resistance to penicillin since they were not found within or in the proximity of the putative conserved motifs, and their significance needs to be assessed in future studies. Moreover, it is possible that the penicillin G-non-susceptible GBS 2007 isolate harbour mutations in other \textit{pbp} or non-\textit{pbp} genes that are responsible for the observed phenotype. To our knowledge, that is the first report of development of invasive GBS not susceptible to penicillin G and ceftriaxone after prolonged low-dose oral penicillin V.

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**Transparency declarations**

None to declare.

**References**


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**Raltegravir: is a 400 mg once-daily dose enough?**

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Sir,

In the STARTMRK trial, 400 mg of raltegravir twice daily + tenofovir/emtricitabine showed non-inferior efficacy versus efavirenz. The lack of teratogenicity and favourable drug–drug interaction profile of raltegravir supports first-line use, but the current cost prevents widespread use of raltegravir for first-line treatment.

In dose-ranging studies, raltegravir has shown strong efficacy at doses of 100–400 mg twice daily, with no clear correlation...
**Table 1. Summary efficacy data from dose-ranging trials of raltegravir**

<table>
<thead>
<tr>
<th>Raltegravir dose (twice daily), mg</th>
<th>100</th>
<th>200</th>
<th>400</th>
<th>600</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phase 1b trial: raltegravir monotherapy—10 day efficacy results(^2)</td>
<td>n</td>
<td>7</td>
<td>6</td>
<td>8</td>
</tr>
<tr>
<td>% HIV RNA &lt; 400 copies/mL</td>
<td>57</td>
<td>57</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>decrease in log(_{10}) HIV RNA</td>
<td>-1.93</td>
<td>-1.98</td>
<td>-1.66</td>
<td>-2.16</td>
</tr>
<tr>
<td>Naive patient trial: TDF/3TC/raltegravir—48 week efficacy results(^3)</td>
<td>n</td>
<td>39</td>
<td>40</td>
<td>40</td>
</tr>
<tr>
<td>% HIV RNA &lt; 400 copies/mL</td>
<td>97</td>
<td>85</td>
<td>98</td>
<td>90</td>
</tr>
<tr>
<td>% HIV RNA &lt; 50 copies/mL</td>
<td>85</td>
<td>83</td>
<td>88</td>
<td>88</td>
</tr>
<tr>
<td>CD4 rise (mean) (cells/mm(^3))</td>
<td>+221</td>
<td>+146</td>
<td>+144</td>
<td>+187</td>
</tr>
<tr>
<td>Merck 005 trial: experienced patients—24 week efficacy results(^4)</td>
<td>n</td>
<td>43</td>
<td>40</td>
<td>40</td>
</tr>
<tr>
<td>% HIV RNA &lt; 400 copies/mL</td>
<td>70</td>
<td>71</td>
<td>71</td>
<td></td>
</tr>
<tr>
<td>% HIV RNA &lt; 50 copies/mL</td>
<td>65</td>
<td>56</td>
<td>67</td>
<td></td>
</tr>
<tr>
<td>CD4 rise (mean) (cells/mm(^3))</td>
<td>+63</td>
<td>+113</td>
<td>+94</td>
<td></td>
</tr>
</tbody>
</table>

between the raltegravir dose used and either reductions in HIV RNA or rises in CD4 counts. Summary results from the three main dose-ranging trials are shown in Table 1.

The efficacy of raltegravir was first evaluated in a 10 day monotherapy study, in 35 treatment-naive, HIV-infected individuals.\(^2\) The doses evaluated were 100, 200, 400 and 600 mg twice daily. After 10 days of dosing, the log\(_{10}\) reductions in HIV RNA and the percentage of patients with HIV RNA <400 copies/mL were similar at the four doses evaluated (Table 1). Subsequently, a 48 week trial in treatment-naive patients\(^1\) showed no differences in efficacy between raltegravir doses of 100, 200, 400 and 600 mg twice daily, given with tenofovir and lamivudine (Table 1). The increases in CD4 count were greatest for the 100 mg twice-daily dose (+221 cells/mm\(^3\)) and lowest for the 400 mg twice-daily dose (+144 cells/mm\(^3\)). In addition, a 24 week trial was conducted in treatment-experienced patients, evaluating raltegravir doses of 200, 400 and 600 mg twice daily.\(^4\) This trial showed no difference in HIV RNA suppression rates between the doses (Table 1). In this trial, the increases in CD4 count were greater at the 400 mg twice-daily dose (+113 cells/mm\(^3\)) relative to the 200 mg twice-daily dose (+63 cells/mm\(^3\)). Across the three dose-ranging trials, there is no consistent trend for improved HIV RNA reductions or greater increases in CD4 counts with increasing doses of raltegravir. The combined sample size for these dose-ranging trials—312 patients—provides strong evidence for this lack of correlation between raltegravir dosing and efficacy.

Raltegravir cannot be dosed at 100 mg or 200 mg twice daily, because the only tablets commercially available have a strength of 400 mg. However, the pharmacokinetics of raltegravir, with its long terminal elimination half-life, support once-daily dosing.\(^5\) A large clinical trial of first-line raltegravir at doses of 800 mg once daily versus 400 mg twice daily is ongoing. A clinical pharmacology trial has evaluated raltegravir at the 400 mg once-daily dose in combination with atazanavir/ritonavir.\(^6\)

A 41-year-old man (CDC A3), HIV infected from 2000, without co-infections (hepatitis B virus and hepatitis C virus infections) and co-morbidities, started first-line antiretroviral therapy in October 2008 for a decline of his CD4 count to <250/mm\(^3\) and HIV RNA of 284,346 copies/mL. A genotypic resistance test showed no resistance mutations for nucleoside reverse transcriptase inhibitors (NRTIs) and protease inhibitors but resistance mutations for non-nucleoside reverse transcriptase inhibitors (K103N). The patient refused all conventional and approved antiretroviral regimens due to a fear of lipodystrophy, so we began an NRTI-sparing regimen of raltegravir (400 mg twice daily) with atazanavir/ritonavir 300/100 mg daily.

At week 1, the patient came back complaining of jaundice. Clinical chemistry showed a value of total bilirubin of 3.9 mg/dL (grade 3). We decided to reduce the atazanavir dosage to 200 mg daily boosted with 100 mg daily of ritonavir. One month later his CD4 cell count rose to 319 cells/mm\(^3\) and HIV RNA was undetectable (<50 copies/mL) with a total bilirubin value of 2.06 mg/dL. After 6 months the CD4 count was 331 cells/mm\(^3\) with HIV RNA <50 copies/mL. The patient revealed that he had never taken raltegravir at the correct dosage but he took only one raltegravir tablet daily. The patient continued on raltegravir at the 400 mg once-daily dose thereafter.

After 9 months we performed a pharmacokinetic analysis to assess C\(_{\text{trough}}\) of raltegravir and atazanavir through a validated HPLC method with UV detection. The results showed an atazanavir C\(_{\text{trough}}\) of 232 ng/mL, superior to 150 ng/mL (the minimum effective concentration of atazanavir), and a raltegravir C\(_{\text{trough}}\) of 26 ng/mL which exceeded the concentration required to inhibit 95% of viral replication (IC95) (14.6 ng/mL).

Two months later, HIV RNA was still undetectable and CD4 cells were 383 cells/mm\(^3\). After another 2 months (October 2009), HIV RNA was <50 copies/mL, with a CD4 cell count of 459 cells/mm\(^3\).

During a year of follow-up, plasmatic total cholesterol and low-density lipoprotein (LDL)-cholesterol rose above the upper limit of the normal range. Total cholesterol rose from 178 mg/dL at baseline to 236 mg/dL (upper limit <200 mg/dL); LDL-cholesterol rose from 91 mg/dL at baseline to 140 mg/dL (upper limit <100 mg/dL).

The results from the dose-ranging trials and this case report provide preliminary evidence that a 400 mg once-daily dosage of raltegravir, combined with atazanavir/ritonavir, could be a first-line treatment option. This strategy needs to be confirmed in large, well-powered efficacy trials, compared with a recognized standard of care. At this lower dosage, the NRTI-sparing combination of raltegravir with atazanavir/ritonavir would lower pill counts and cost, and could therefore be suitable for wide-scale use.

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**Transparency declarations**

None to declare.
References


