Shifting paradigms: the resistance profile of etravirine

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The raised genetic barrier of etravirine relative to first-generation compounds indicates that it may now be possible to sequence drugs within the non-nucleoside reverse transcriptase inhibitor (NNRTI) class. Available evidence from clinical trials provides guidance for the use of etravirine in NNRTI-experienced persons, with sustained virological suppression demonstrated in combination with other active drugs in the background regimen, most commonly including a ritonavir-boosted protease inhibitor with or without enfuvirtide. Cross-resistance occurs however, and the drug is vulnerable to loss of activity in the absence of a supportive background regimen. In order to optimize the use of etravirine in clinical practice, it is important to understand how current predictors of virological activity in NNRTI-experienced persons were developed, how they can be applied, and the adjustments and improvements they require.

Keywords: non-nucleoside reverse transcriptase inhibitors, genotype, mutations

Introduction

The introduction of etravirine in routine clinical care requires a shift in our current understanding of antiretroviral treatment strategies. The raised genetic barrier of etravirine relative to first-generation compounds indicates that it may now be possible to sequence drugs within the non-nucleoside reverse transcriptase inhibitor (NNRTI) class. The drug was selected through the optimization of a series of di-amino-pyrimidines against NNRTI-resistant clinical isolates of HIV-1, including mutants with K103N, Y181C or both K103N and Y181C, the most common resistance mutations in NNRTI-experienced patients. In vitro, multiple mutations are required to confer high-level resistance to the drug, reflecting compact design and flexible conformational interactions with the NNRTI-binding hydrophobic pocket in the reverse transcriptase enzyme. However, progressive loss of susceptibility is seen as resistance mutations accumulate and certain double mutations such as Y181C + V179F reduce etravirine susceptibility by >100-fold. It is important therefore to define the optimal use of etravirine in NNRTI-experienced patients as guiding evidence has started to emerge from clinical trials.

Evidence from clinical trials of NNRTI-experienced persons

Study TMC125-C207 was an open-label Phase 2 trial that investigated 16 patients on failing antiretroviral therapy with ≥2 nucleoside reverse transcriptase inhibitors (NRTIs) and either nevirapine (81%) or efavirenz (19%), and with ≥10-fold documented resistance to efavirenz (Table 1). At baseline, the patients had an average of two NNRTI resistance-associated mutations (RAMs), including three patients with 1, eight patients with 2, three patients with 3 and one patient with 4 NNRTI RAMs. The patients showed a median fold-change (FC) >100 for both nevirapine and efavirenz, whereas the median FC for etravirine was 2.2 (range 0.5–8.5), indicating full to partial susceptibility. The patients received etravirine (900 mg twice daily) for 7 days, and during this time showed a median viral load change from baseline of −0.89 (range −1.71 to −0.18) log_{10} copies/mL. The response compares with the median viral load change of −1.92 log_{10} copies/mL observed in 12 drug-naïve persons over 7 days of therapy with etravirine at the same dose. These proof-of-principle data, combined with a good tolerability profile, provided the rationale for the further development of etravirine.

The exploratory TMC125-C227 Phase 2 trial investigated 116 patients who had experienced failure of first-line NNRTI-based HAART and had documented NNRTI resistance either historically or at screening. Of these, 59 received etravirine (800 mg twice daily), with two NRTIs selected on the basis of the resistance profile and treatment history. The mean viral load declined by 1.3 log_{10} copies/mL, but the response was not sustained past week eight. At baseline, the median number of NNRTI RAMs was 2 (range 0–4), with a median FC of 130 for efavirenz, 88 for nevirapine and 2 for etravirine. The median number of NRTI RAMs was 1 (range 0–7), and 9% of the...
### Table 1. Clinical trials of etravirine in NNRTI-experienced persons

<table>
<thead>
<tr>
<th>Trial</th>
<th>Study population</th>
<th>Arms</th>
<th>Outcome</th>
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<tr>
<td>TMC125-C207</td>
<td>• failing efavirenz or nevirapine-based therapy &lt;br&gt; • ≥10-fold resistance to efavirenz</td>
<td>• etravirine for 7 days (n = 16)</td>
<td>• viral load decay rate: 0.13 log_{10} copies/mL per day &lt;br&gt; • median viral load decline over 7 days: 0.89 log_{10} copies/mL</td>
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<td>TMC125-C227</td>
<td>• ≥1 NNRTI RAM (historical or at screening) &lt;br&gt; • PI naive</td>
<td>• etravirine (n = 57) or PI (n = 59) plus two optimized NRTIs</td>
<td>• etravirine arm prematurely discontinued due to suboptimal virological responses compared with control arm at week 24 (ITT): &lt;br&gt; • mean viral load decline (log_{10} copies/mL): −1.04 (etravirine 400 mg), −1.18 (etravirine 800 mg), −0.19 (control); (P = 0.005) and (P &lt; 0.001), respectively &lt;br&gt; • viral load &lt;400 copies/mL: 30% (etravirine 400 mg), 38% (etravirine 800 mg), 7.5% (control); (P = 0.018) and (P = 0.002), respectively &lt;br&gt; • viral load &lt;50 copies/mL: 21.3% (etravirine 400 mg), 17.7% (etravirine 800 mg), 7.5% (control); (P = 0.133) and (P = 0.218), respectively &lt;br&gt; • responses not statistically different between etravirine doses proportion with viral load &lt;50 copies/mL at week 24 (ITT): &lt;br&gt; • DUET-1: etravirine arm 56% versus placebo arm 39% (P = 0.005) &lt;br&gt; • DUET-2: etravirine arm 62% versus placebo arm 44% (P = 0.0003)</td>
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<td>TMC125-C223</td>
<td>• ≥1 NNRTI RAM (historical or at screening) &lt;br&gt; • ≥3 primary PI mutations at screening</td>
<td>• etravirine 400 mg twice daily (n = 80) or 800 mg twice daily (n = 79) plus optimized regimen with ≥2 active agents including NRTIs and/or LPV/r (n = 104), and/or T20 (n = 122) &lt;br&gt; • active control: ≥3 optimized antiretroviral drugs including NRTIs, and/or PIs (tipranavir not available) and/or T20 (n = 40)</td>
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<td>• active control: ≥3 optimized antiretroviral drugs including NRTIs, and/or PIs (tipranavir not available) and/or T20 (n = 40)</td>
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<tr>
<td>DUET-1 and</td>
<td>• ≥1 NNRTI RAM (historical or at screening) &lt;br&gt; • ≥3 primary PI mutations at screening</td>
<td>• etravirine (n = 304 + 295) or placebo (n = 295 + 296) plus DRV/r with optimized NRTIs and optional T20</td>
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<tr>
<td>DUET-2</td>
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NNRTI, non-nucleoside reverse transcriptase inhibitor; NRTIs, nucleoside reverse transcriptase inhibitors; RAM, resistance-associated mutation; PI, protease inhibitor; optimized, selected on the basis of the resistance profile and treatment history; LPV/r, ritonavir-boosted lopinavir; T20, enfuvirtide; ITT, intention to treat analysis; DRV/r, ritonavir-boosted darunavir.
patients did not receive two active NRTIs; 37% and 9% recycled one or both NRTIs, respectively. Relating the relative contribution of NRTI versus NNRTI resistance to the poor outcome of the study is complicated by the fact that patients with multiple NNRTI resistance mutations also had extensive NRTI resistance. Increased numbers of NRTI and NNRTI RAMs, use of inactive NRTIs and higher etravirine FC were associated with virological failure.

The Phase 2 study TMC125-C223 investigated 199 NNRTI- and protease inhibitor (PI)-experienced patients with documented NNRTI resistance, who received etravirine (400 or 800 mg twice daily) together with an optimized background regimen consisting of ≥2 approved antiretroviral drugs: NRTI and/or lopinavir/ritonavir or enfuvirtide, in any combination selected according to the resistance profile and treatment history.1 At baseline, patients showed a median of 2 (range 0–5) NNRTI, 6 NRTI and 9 PI RAMs, and a median FC of 1.7 (range 0.1–399) for etravirine and 83.6 for lopinavir. After 24 weeks, patients in the etravirine arm (n = 159) showed a mean drop in viral load of 1.2 log_{10} copies/mL and responses were overall maintained at week 48, when 22% (800 mg arm) and 23% (400 mg arm) showed an undetectable viral load (<50 copies/mL).8 Viral load responses were improved by the presence of active drugs in the background regimen, but reduced by an increasing number of NNRTI RAMs at study entry.10,11 The viral load decline was not affected by the pre-existence of K103N but was reduced in persons with Y181C, albeit less so after adjustment for the use of enfuvirtide and viral load, CD4 count and number of NNRTI RAMs at study entry.11

The DUET-1 and DUET-2 trials investigated 1203 NNRTI- and PI-experienced patients with NNRTI resistance documented either at screening or in historical genotypes and ≥3 primary PI RAMs. Patients received placebo or etravirine 200 mg twice daily, in a new formulation with improved bioavailability, giving an exposure equivalent to the previous 800 mg dose. Both arms received darunavir/ritonavir and NRTIs were chosen on the basis of the resistance profile, with or without enfuvirtide.12,13 In a combined analysis,14 the mean viral load decline after 24 weeks was significantly greater in the etravirine arm (2.4 log_{10} copies/mL) than in the placebo arm (1.7 log_{10} copies/mL). Despite this being a highly treatment-experienced population, between 74% and 45% of the patients in the etravirine arm achieved an undetectable viral load (<50 copies/mL), with the best responses seen in patients who received active drugs in the background regimen. Importantly, responses were sustained through week 48 when the proportion with viral load <50 copies/mL was 61% in the etravirine arm (n = 599) and 40% in the placebo arm (n = 604) (P < 0.0001).15–17

Genotypic resistance

To determine the impact of NNRTI resistance on virological responses, analyses were performed in the subset of patients in the DUET studies who were not using enfuvirtide as a new drug and excluding patients who discontinued for reasons other than virological failure. From an initial list of 44 NNRTI RAMs, 26 mutations were selected for being present in five or more patients, including common mutations such as K103N and Y181C, and 13 were found to be associated with a response to etravirine that was at least 25% lower than the reference response at week 24 (Figure 2).18 The greatest added benefit of etravirine relative to placebo was seen in patients with ≤2 etravirine RAMs at study entry. In a combined analysis, the proportion of patients showing virological responses <50 copies/mL was consistently >50% in the presence of <3 etravirine RAMs and <3 darunavir RAMs.17 Importantly, in the presence of Y181C as the only etravirine RAM, the virological response was comparable with that observed in the overall group (Figure 3).

Mutations at codon 181, most commonly Y181C but also Y181S/I/F, are selected in vitro by etravirine.2,19 The initial selection of Y181C may be followed by the emergence of Y181I.19 Other NNRTI RAMs selected by etravirine include: (i) L100I and V179F, which are part of the current genotypic score; (ii) V179I and F227L, which are not part of the score as they did not predict responses in the DUET studies when present at baseline; and (iii) E138K, G190E and M230I/L, which were not evaluated in the DUET studies. Etravirine can select additional mutations in vitro, most commonly T386A (not evaluated in the DUET studies), and less commonly V90I (part of the current score), and several other changes with uncertain resistance effects.2,19,20 Some of the mutations may have a role in resistance or may compensate for reduced fitness of resistant strains.

Four NNRTI RAMs emerged in at least 10% of the people in the DUET studies who experienced virological failure on etravirine: V108I in 11%, V179I in 17%, V179F in 17%, and

<table>
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<tr>
<th>V90I</th>
<th>A98G</th>
<th>L100I</th>
<th>K101E</th>
<th>K101P</th>
<th>K101Q</th>
<th>K103N</th>
<th>K103S</th>
<th>V106I</th>
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<tr>
<td>V108I</td>
<td>E138Q</td>
<td>V179D</td>
<td>V179F</td>
<td>V179I</td>
<td>Y181C</td>
<td>Y181I</td>
<td>Y181V</td>
<td>Y188L</td>
</tr>
<tr>
<td>V189I</td>
<td>G190A</td>
<td>G190S</td>
<td>H221Y</td>
<td>P225H</td>
<td>F227L</td>
<td>K238T</td>
<td>Y318F</td>
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Figure 2. Resistance analysis of the DUET studies. Mutations that impacted on responses to etravirine among the 26 analysed are indicated in bold in the shaded boxes. The 13 mutations form the current etravirine genotypic score. Adapted from reference 18 with permission.
Y181C in 12%. Of these, only V179F and Y181C are part of the etravirine score, as neither V108I nor V179I predicted responses when present at baseline. Notably, V179I also emerged with either Y318F (FC 11), V179I (FC 11) or L100I (FC 43).2

While etravirine resistance appears to move along a continuum, it is possible to identify clinically relevant breakpoints in phenotypic resistance that predict virological outcomes and can assist with treatment decisions. Values of 3 and 13 (Antivirogram phenotypic assay) and 1.6 and 27.6 (VircoType assay) have been proposed as the lower and upper cut-offs, signalling reduced and abolished responses, respectively.22 These cut-offs require further validation and confirmation of applicability in a variety of clinical scenarios.

While multiple mutations are generally required to confer high-level resistance, single NNRTI mutations predicted to cause shifts in etravirine susceptibility >10-fold include K101P, V179F and Y181C/I/V within the current score, and in addition V179Y, Y181F/G/S, G190E, F227C and M230I,22 or M230L.2 Using site-directed mutants (SDM), the combinations Y181C + V179F and Y181I + M230L show strong effects on etravirine susceptibility with FC around 100 or above.2 K103N in isolation can be expected to have no significant impact (FC of SDM 0.5), although in combination with other mutations may reduce susceptibility to etravirine, as seen with the double mutant L100I + K103N (FC 11), and the triple mutants containing L100I + K103N + T386A (FC 16) or K103N + Y181C with either Y318F (FC 11), V179I (FC 11) or L100I (FC 43).2 Other combinations with significant resistance effects include E138K + Y181I (FC 51) and M230L in combination with F227C (FC 19), V106A + F227C (FC 15) or V106A + F227C (FC 46).19 Several of these variants, including Y181I, F227C and L100I + K103N, are present at very low frequency in clinical datasets.1

**Discussion**

What have we learned from these studies? Etravirine can clearly tolerate a certain degree of NNRTI resistance and although cross-resistance does occur, the drug is predicted to retain at least partial activity following most efavirenz, and to a lesser extent, nevirapine failures. Like several other antiretrovirals, etravirine is vulnerable to a rapid loss of response in the absence of other active drugs in the regimen and should not be used as functional monotherapy. Currently, the best evidence of sustained activity in NNRTI-experienced persons comes from its use in combination with darunavir/ritonavir in patients with at least partial susceptibility to both drugs. In this context, a gradual loss of response is seen as the number of etravirine and PI RAMs increase. While it is reassuring that <10% of the samples within clinical databases harbour ≥3 etravirine RAMs,18,23 the prevalence of multiple mutations is likely to be higher in persons who experience prolonged viraemia under NNRTI pressure, including populations with limited access to monitoring tools and treatment options.24

The current etravirine genotypic score can be regarded as predictive of responses in treatment-experienced patients with characteristics similar to those of the DUET cohorts. While accurate weighing is lacking within the score, Y181V, V179F and G190S showed the greatest impact on virological response.18 The clinical impact of less prevalent mutations, including those associated with in vitro resistance to etravirine, has not been determined and these mutations have not necessarily been excluded from the etravirine score. Conversely, some of the mutations included in the score, most notably V90I and V106I, can occur in untreated persons, and in isolation, they are unlikely to have significant effects on drug susceptibility. The role of some mutations such as V179I remains uncertain. The mutation is selected by etravirine in vitro and emerges in patients failing etravirine in vivo, but did not predict responses to etravirine when present at baseline in patients recruited in the DUET studies.

Further data are awaited on the phenotypic and genotypic correlates of drug activity, and the etravirine genotypic score and clinical cut-offs will require validation and updating as new data emerge. The available evidence provides guidance for the use of etravirine in NNRTI-experienced patients with limited NNRTI resistance and in combination with drugs that can provide robust background support. Patients with a history of prolonged viraemia while receiving nevirapine are among those most likely to have reduced susceptibility to etravirine, a further indication of the negative consequences of prolonged treatment failure and the resulting accumulation of resistance.

**Transparency declarations**

The author has received consultancy and speaker honoraria from the following: Abbott Diagnostics, Abbott Pharmaceuticals, Boehringer Ingelheim, Bristol-Myers Squibb, Gilead Sciences, GlaxoSmithKline, Merck, Pfizert, Roche, Tibotec and Virco.

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