Review

Challenges for the clinical development of new nucleoside reverse transcriptase inhibitors for HIV infection

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There is a need for new antiretroviral drugs with activity against HIV isolates resistant to currently available agents and improved short and long-term tolerability profiles. Clinical trial designs for nucleotide and nucleoside reverse transcriptase inhibitors (NRTIs) are restricted by the characteristics of these agents (for example, their cross-resistance, resistance threshold and interaction profiles), the ethical need to ensure that patients are not maintained on suboptimal regimens, and regulatory requirements (for example, with regards to trial designs and patient populations). For example, consideration of cross-resistance profiles must influence the way in which an NRTI in development is sequenced to minimize any impact on future treatment options. The resistance threshold is determined by the number of mutations required to diminish sensitivity to a given drug. Pharmacokinetic or pharmacodynamic interactions restrict how NRTIs may be combined during clinical development. Doses may be selected on the basis of results from short-term monotherapy studies in treatment-naive patients, but such studies cannot establish the long-term efficacy or tolerability of new agents used in combination regimens. Confirmatory studies in treatment-naive populations do not meet the medical and regulatory needs for clinical data in treatment-experienced populations, while studies in treatment-experienced populations are subject to numerous clinical and logistical difficulties. Intensification, switch and hybrid study designs all offer suitable approaches to the evaluation of NRTIs with novel resistance profiles. Switch studies are particularly useful for agents with resistance profiles that suggest a specific sequencing approach in treatment and for those with the potential, based on pharmacokinetic data, for interactions with other agents. The successful development of new NRTIs will depend upon a thorough appreciation of these many and complex issues, not only among those involved in the design of clinical studies, but also those contributing to their review and conduct.

Introduction

Nucleoside and nucleotide reverse transcriptase inhibitors (NRTIs and NtRTIs) are the backbone of highly active antiretroviral therapy (HAART) for HIV disease. They are currently recommended for use within HAART regimens for the treatment of anti-retroviral-naive and -experienced HIV-infected patients [1,2], based on substantial evidence that their use in combination improves virological, immunological and clinical endpoints [3–5].

The prolonged use of HAART has resulted in substantial reductions in morbidity and mortality from HIV disease [6,7]. Accordingly, the problems associated with maintaining the effectiveness and safety of life-long therapy are now viewed with increased priority. Many patients who achieve undetectable plasma HIV-1 RNA levels during treatment ultimately experience virological failure, often as a result of antiretroviral resistance. A 6-year genotypic survey in
France found that almost 80% of clinical HIV samples collected up to 2002 had mutations conferring some degree of resistance to NRTIs [8]. A quarter of the isolates had multiple mutations conferring resistance to all three major antiretroviral classes, that is, NRTIs, non-nucleoside reverse transcriptase inhibitors (NNRTIs) and protease inhibitors (PIs). Patients with virological failure for all three classes are at high risk of death [9]. Increasing rates of primary infection with antiretroviral-resistant HIV-1 strains have also been reported in many countries, including those in North America and Western Europe [10–14]. Thus, the extent of NRTI resistance and cross-resistance must be confirmed to allow the choice of therapy in treatment-experienced patients and the design of clinical trials of new antiretrovirals. Moreover, there is a need for new antiretrovirals that possess not only activity against HIV isolates resistant to currently available agents, but also with improved long-term tolerability profiles [15].

Unfortunately, the clinical development of new NRTIs is subject to various challenges, many of which relate to the difficulty in demonstrating the attributable efficacy of a new agent within combination HAART regimens, and to problems associated with cross-resistance and interactions between NRTIs. As predicted [16], these issues have become more complex in recent years as HIV treatment strategies have advanced. This paper explores the current challenges in designing clinical studies of new NRTIs, focusing in particular on the implications of the resistance profiles of new compounds.

New and marketed NRTIs and NtRTIs

Currently, eight NRTIs and one NtRTI are approved in the United States (Table 1). Two (tenofovir and emtricitabine) have been introduced only recently, while at least six additional agents are in clinical development. All NRTIs are structural analogues of endogenous nucleic acids, that is, deoxyadenosine, deoxycytidine, deoxyguanosine or thymidine. Following a series of intracellular phosphorylation reactions, the triphosphate (TP) form of the NRTI competes with endogenous deoxynucleoside-TPs as a substrate for HIV reverse transcriptase, the virus-encoded DNA polymerase enzyme responsible for viral DNA synthesis. Incorporation of the NRTI into the nascent DNA chain results in premature chain termination and hence impaired transcription. Tenofovir differs from the other NRTIs in that it is an acyclic, phosphonate analogue of deoxyadenosine, that is, an NtRTI. The presence of the phosphonate group eliminates the need for phosphorylation by a nucleoside or deoxynucleoside kinase and the active intracellular metabolite of tenofovir is its diphosphate [17].

Despite their common mechanism of action, NRTIs vary in terms of their efficacy, tolerability, long-term safety, pharmacokinetic properties (and hence dosing convenience), drug interactions, food restriction and resistance profiles. The resistance profile of a new NRTI has important implications for the future clinical role of the agent and, by implication, for its clinical development. The clinical development of agents with resistance profiles very similar to those of existing agents, and agents with resistance profiles that differ substantially from those of others, are therefore subject to different influences.

The development of new NRTIs with resistance profiles similar to those of existing agents may be warranted if they offer other advantages [15]. However, the development of these agents is likely to be subject to the following limitations: i) they are unlikely to confer significantly enhanced efficacy over the existing agent; ii) co-administration with the existing agent may not be possible owing to the combined pressure for the selection of resistant viral mutants; iii) they cannot usually be administered sequentially with the existing agent owing to cross-resistance between these agents. [This is true for lamivudine and emtricitabine (see below), but not necessarily for others]; iv) demonstration of tolerability and safety advantages over related or existing NRTIs

<table>
<thead>
<tr>
<th>Adenosine</th>
<th>Cytidine</th>
<th>Guanosine</th>
<th>Thymidine</th>
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<tr>
<td>Didanosine (dDI)</td>
<td>Lamivudine (3TC)</td>
<td>Abacavir (ABC)</td>
<td>Zidovudine (AZT, ZDV)</td>
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<td>Tenofovir (PMPA)</td>
<td>Zalcitabine (ddI)</td>
<td>Amdoxovir (DAPD)</td>
<td>Stavudine (d4T)</td>
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<td></td>
<td>Emtricitabine (FTC)</td>
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<td>Alovudine (MIV-310, FLT)</td>
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<td></td>
<td>SPD754</td>
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<td></td>
<td>D-d4FC (OPC-817, Reverset&lt;sup&gt;™&lt;/sup&gt;, RVT)</td>
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<td></td>
<td>Elvucitabine (ACH-126, 443, β-L-d4FC)</td>
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<td></td>
<td>Racivir&lt;sup&gt;™&lt;/sup&gt; (racemic FTC)</td>
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will be difficult and require long-term studies in large numbers of patients; and 6) their clinical utility can best be demonstrated in a treatment-naive population, hence at least some clinical trials must be conducted within this group.

For example, emtricitabine is structurally related to lamivudine and has a similar resistance profile to this agent. The key resistance mutation selected by both emtricitabine and lamivudine is M184V in RT [18,19]. Thus, emtricitabine is very unlikely to be effective in patients with lamivudine-resistant HIV-1. As lamivudine is widely used within current HAART regimens, and the M184V mutation is often the first mutation to occur during lamivudine-containing HAART [20,21], emtricitabine is therefore likely to be of limited usefulness for treatment-experienced patients whose viral load is not controlled. Accordingly, the clinical development of emtricitabine primarily focused on its use in place of lamivudine in treatment-naive patients and in well-controlled, experienced patients [24,29,30].

In vitro passaging studies give useful early information about the key mutations that may be selected by new NRTIs during clinical studies and subsequent clinical use. Some developmental NRTIs have resistance profiles suggesting full or partial cross-resistance to some of the existing NRTIs. These agents include Racivir™ (racemic emtricitabine) [31] and elvucitabine (ACH-126,443 or β-L-d4FC) [32–35]. Conversely, several developmental NRTIs have in vitro resistance profiles that suggest a lower potential for cross-resistance with marketed agents (Table 2). These include SPD754 [36–39], alovudine (MIV-310) [40,41], D-d4FC (formerly known as DPC-817) [42–45] and amdoxovir (DAPD) [46–49]. These resistance profiles may confer clinical advantages in terms of efficacy against HIV resistant to some or all other NRTIs. Since these agents may have favourable cross-resistance profiles with respect to other NRTIs, they may have a role similar to that of the first NRTIs introduced, that is, in the management of both treatment-naive and -experienced patients.

In addition to in vitro data, results from long-term clinical studies are required to fully characterize the resistance profile of a new drug in terms of the rate and types of mutations selected. For example, although tenofovir selects the K63R mutation in RT in vitro [50], this mutation is infrequently selected in patients with resistance to NRTIs caused by thymidine analogue mutations (TAMs) [51]. However, it is associated with the emergence of resistance to tenofovir used in combination with lamivudine and efavirenz [52]. The prevalence of the K63R mutation remains low (approximately 4% of patient-derived isolates), perhaps owing to the comitant use of zidovudine in

### Table 2. Resistance profiles of marketed and developmental nucleoside and nucleotide reverse transcriptase inhibitors according to the effect of reverse transcriptase mutations on viral susceptibility

<table>
<thead>
<tr>
<th>Mutational pattern</th>
<th>M184V alone</th>
<th>M184V + three TAMs</th>
<th>Three TAMs alone</th>
<th>K65R</th>
<th>T69 insertions</th>
<th>Q151M</th>
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</tr>
<tr>
<td>Abacavir</td>
<td>+</td>
<td>++</td>
<td>++</td>
<td>+</td>
<td>++</td>
<td>+++</td>
</tr>
<tr>
<td>Didanosine</td>
<td>++</td>
<td>+</td>
<td>+++</td>
<td>++</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>Emtricitabine</td>
<td>+++</td>
<td>+++</td>
<td>+</td>
<td>+++</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Lamivudine</td>
<td>+++</td>
<td>+++</td>
<td>+</td>
<td>+</td>
<td>++</td>
<td>+</td>
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<tr>
<td>Stavudine</td>
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<td>++</td>
<td>++</td>
<td>+</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>Tenofovir</td>
<td>−</td>
<td>++</td>
<td>++</td>
<td>+</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>Zalcitabine</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>+++</td>
<td>+</td>
</tr>
<tr>
<td>Zidovudine</td>
<td>−</td>
<td>++</td>
<td>+++</td>
<td>−</td>
<td>+</td>
<td>+</td>
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<td><strong>Developmental</strong></td>
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<tr>
<td>Alovudine (MIV-310)</td>
<td>++</td>
<td>0</td>
<td>+++</td>
<td>ND</td>
<td>+</td>
<td>0</td>
</tr>
<tr>
<td>Amdoxovir</td>
<td>+</td>
<td>+</td>
<td>++</td>
<td>++</td>
<td>++</td>
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<tr>
<td>D-d4FC (DPC-817)</td>
<td>+</td>
<td>+</td>
<td>++</td>
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<tr>
<td>Elvucitabine (ACH-126,443, β-L-d4FC)</td>
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<td>+++</td>
<td>+</td>
<td>++</td>
<td>0</td>
<td>0</td>
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<tr>
<td>Racivir</td>
<td>+</td>
<td>+</td>
<td>++</td>
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<td>+</td>
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<td>++</td>
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</table>

+++ , high-level reduction in susceptibility in vitro (clinically resistant); ++, moderate reduction in susceptibility in vitro (potentially clinically resistant); +, low-level reduction in susceptibility in vitro (clinically susceptible in most cases); 0, no detectable effect; −, increase in susceptibility in vitro. Note that this table gives estimated effects for illustrative comparison only. Variations exist between drugs in the quantity and quality of data available. The clinical significance of these susceptibility reductions has not yet been confirmed for agents in development. ND, no data available; TAMs, thymidine analogue mutations.
background regimens [53,54]. Nevertheless, it has recently increased, probably because of increased use of NRTIs that select it, that is, abacavir, didanosine and tenofovir [55]. It is worth noting that the K65R mutation was commonly recorded in patients failing triple thymidine-sparing NRTI treatment with tenofovir/ lamivudine/abacavir [56,57], tenofovir/lamivudine/ didanosine [58] and tenofovir/abacavir/didanosine [59] – all regimens composed entirely of NRTIs to which resistance is conferred by K65R. This mutation has also been selected during treatment with stavudine/ didanosine/abacavir [60]. The impact of K65R on the activity of NRTIs is still being debated [54]. Stavudine and zidovudine are not affected, while most other available NRTIs are. Developmental agents are affected to differing degrees.

Therefore, depending on the NRTI combination used, three major resistance profiles may occur: M184V, K65R or TAMs. Of these, the last two occur rarely, if ever, during first-line antiretroviral therapy. Changes in the epidemiology of these NRTI resistance profiles in experienced patients will depend on the extent of use of various NRTI combinations and may be predicted with the advent of novel fixed combinations, such as tenofovir plus emtricitabine and abacavir plus lamivudine. The incidence of other mutations that may affect NRTI cross-resistance, such as L74V, may also increase with the use of novel combinations. These epidemiological considerations should be taken into account when determining the potential usefulness of developmental NRTIs.

Dose selection

The relationship between the dose and efficacy of new agents is usually established in Phase I or II studies. Generally, such studies evaluate a range of doses with the objective of showing a dose–response slope. The use of more than one dose in longer-term confirmatory Phase III studies may also be worthwhile [61]. The tolerability and safety of the new agent are of central concern. The need to minimize the risk of unexpected toxicity from nucleoside analogues was highlighted by reports in 1995 of severe mitochondrial damage produced by fialuridine [62]. In some circumstances, a sequential-dose cohort study design might be appropriate if there are concerns about the level of risk of unexpected toxic effects. However, this approach is not essential. For agents that have demonstrated acceptable safety margins during tests of cyto-, mitochondrial and systemic toxicity in appropriate in vitro and in vivo pre-clinical studies, parallel-group studies may be conducted. The choice between sequential-dose cohort and parallel-group studies should be discussed with regulatory authorities and ethics review panels.

Long-term data are essential to provide an understanding of the efficacy and tolerability of a drug in clinical practice. Long-term studies can only evaluate a new NRTI within a HAART regimen, owing to the increased risk of resistance development during prolonged monotherapy. However, the potential for pharmacodynamic and/or pharmacokinetic interactions between antiretroviral agents confounds the interpretation of combination dose-ranging studies and thereby necessitates the use of short-term monotherapy trials [16,61]. Functional monotherapy trials of any duration must only be undertaken after careful assessment of the risk and consequences of resistance selection. They must be designed to minimize patient exposure to single-agent therapy and the risk of suboptimal dosing in confirmatory trials [61].

The risk of resistance development during functional monotherapy is a semi-qualitative evaluation in the early stages of clinical development, as this is related to the potency and in vitro resistance profile of the drug, the duration of monotherapy and the genotypic profile of the patient population. The results of in vitro passaging studies with regard to how quickly any mutations are selected and the level of resistance they confer to the new NRTI and other members of the class are of most use in this assessment. For example, the early appearance, in passaging studies, of any mutation is likely to represent a concern. As additional clinical information is generated, the risks of functional monotherapy may be more easily anticipated. Recent Phase I/II dose-ranging studies for emtricitabine [27] and D-d4FC [44,45] have treated patients for 10 days. In some circumstances, the choice of the active dose for Phase II/III studies may be made on the basis of short-term clinical data alone, including measurement of intracellular NRTI-TP concentrations. For example, the selection of the marketed dose of emtricitabine (200 mg once daily) was based in part on evidence that emtricitabine-TP concentrations reach a plateau above this dose with little increase in antiviral effect [23].

In principle, dose selection for NRTIs with resistance profiles distinct from existing agents may be performed either in treatment-naïve or -experienced patients. Although prolonged monotherapy with these agents in naïve patients risks the selection of resistance, monotherapy studies using such compounds are still ethically acceptable if treatment is limited to 2 weeks or less [39,46,47] and the risk of resistance selection is not thought to be high. Studies in treatment-naïve patients have fewer confounding factors (for example, baseline resistance mutations), and hence are likely to provide a clearer signal of a drug’s efficacy. For example, in a randomized, double-blind study, Cahn et al. demonstrated a dose–response profile for SPD754 (400, 800, 1200 or 1600 mg/day) administered as monotherapy.
in naive patients (n=63) [39]. After 10 days’ therapy, all doses studied were statistically superior to placebo, while the top two doses (1200 mg and 1600 mg/day) were superior to the lowest dose (400 mg/day; all P<0.05). Viral load reductions with the highest two doses suggested that a plateau in dose response was achieved. In a recent 10-day study of D-d4FC monotherapy, similar magnitudes of viral load reduction were observed [44], but without a clear dose response.

However, one may reasonably question whether a dose–response profile established in treatment-naive patients is relevant to the treatment of experienced patients. Treatment-experienced patients are likely to have lower CD4+ cell counts and more NRTI resistance mutations than naive patients (Figure 1). The presence of increasing numbers of multiple nucleoside-associated mutations (NAMs) generally reduces viral susceptibility to individual NRTIs. This would necessitate the use of higher doses to achieve the same effect and thereby alter the dose–response curve. Thus, it may be appropriate to study higher doses of an investigational agent in heavily pretreated populations, depending on the tolerability and safety of the compound [15]. On the other hand, the risk of compromising future options through the use of a subtherapeutic dose may be more limited in heavily pre-treated patients with no or few remaining treatment options. Thus, the investigation of doses at the lower end of the expected therapeutic dose range of the new agent can also be justified in this population.

Dose selection in experienced patients has additional advantages and disadvantages. The present standard of care requires the use of combination HAART in this population and thereby introduces the confounding effect of other new antiretrovirals. In principle, the incremental efficacy of each dose of an investigational agent could be identified, provided the background therapy is consistent. In practice, the activity of the new background therapy may mask that of the investigational agent and prevent differentiation between doses. This is of particular importance during short-term studies. Nevertheless, the fact that other agents may mask the effects of a study agent does not mean these effects are not there or are not valuable. An alternative approach involves maintaining the failing background regimen and switching the drug belonging to the same class as the tested agent, the latter being administered at various doses for an initial period of 2–3 weeks before the background regimen is optimized. This initial period of functional monotherapy with the new agent allows a good estimate of potency and tolerability in a highly experienced population. This approach has been used to evaluate lopinavir/ritonavir [63] and tipranavir/ritonavir [64].

For NRTIs, an approach used in experienced patients involves the addition of the experimental NRTI at various doses to a failing regimen [41,65].

The question of whether short-term data are sufficient to identify a dose for all eventualities and patient populations is of increased relevance for developmental agents whose potential utility encompasses both treatment-naive and -experienced populations. A potential advantage of dose selection where the test agent is part of a new combination regimen in experienced patients is that such studies may be of more prolonged duration, and hence may be more clinically relevant, owing to the relatively low risk of selection for resistance. However, differences between the treatment arms in patterns of cross-resistance may also introduce bias, in which case stratifying the analysis according to the presence of resistance mutations at entry might give useful information. Thompson et al. reported preliminary results of a 12-week, uncontrolled study in treatment-experienced patients of amdoxovir (300 or 500 mg twice daily) added to existing HAART, which could be optimized at the discretion of the investigator [49]. Although the published data did not differentiate between the doses used in terms of outcome, a long-term treatment study comparing a single dose of amdoxovir with placebo in addition to a new regimen was initiated.

Pharmacodynamic modelling techniques may help in the design of Phase I/II studies and the selection of active doses. For example, the in vitro ‘hollow-fibre’ system uses computerized pumps to modulate antiretroviral exposure within a range of hollow-fibre bioreactor cell cultures, thereby simulating variable clinical drug concentrations under different conditions [66]. However, further clinical validation of this method is required.

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**Figure 1.** Hypothetical natural history of CD4+ cell count and NRTI resistance genotype in treated, HIV-infected patients

![Figure 1](image-url)
Endpoints

Before discussing the complex issues surrounding the designs of clinical trials of new NRTIs, it is worthwhile considering the factors affecting the central decision regarding the endpoints to be measured and the timeframe of assessment.

Selection of endpoint

In light of the success of combination antiretroviral therapy in reducing the incidence of HIV-related illnesses, it is now deemed unfeasible for new antiretrovirals to be assessed according to clinical disease progression [15]. Instead, the efficacy of new agents is assessed using a variety of ‘surrogate’ endpoints. Primary endpoints are based on the measurement of viral load, in light of evidence that viral load reductions are highly predictive of meaningful clinical benefit [15]. The specific endpoints used include absolute reduction in plasma viral load, time-weighted reduction in viral load, proportion of patients with undetectable viral load (defined either as <400 or <50 HIV RNA copies/ml) and time to virological treatment failure.

The absolute reduction in viral load is a versatile endpoint that can be used in all studies. It is particularly useful in studies in which the goal of treatment is not the achievement of undetectable viral loads and/or when the distribution of baseline viral loads in the study population is difficult to predict. The time-weighted reduction in viral load is useful for assessing the duration of response, as it avoids the limitations of assessing outcome at a single time point only.

Measuring the proportion of patients with undetectable viral load levels is essential for patients who are treatment-naive or at first treatment failure, and is consistent with the goals of therapy in clinical practice [2]. It may also be useful in ‘intensification’ studies (see below) and other trials in which the treatment goal is to regain full viral control. However, in this setting the utility of this endpoint can be diminished by the problems associated with unpredictable baseline viral loads. Time to virological failure is a particularly important endpoint in ‘switch’ studies (see below) conducted in patients whose viral load is well controlled. However, in other situations this may not provide as early an indication of differences between treatment groups as other endpoints described.

Although viral load assessments constitute the primary endpoints of antiretroviral studies, it is recommended that changes in CD4 cell counts and clinical endpoint data be reported as well [15,61].

Endpoint determination

Ideally, a clear demonstration of the effect of the study drug during long-term treatment (24–48 weeks) should be supported by analysis of data that are blinded during this time. It may be appropriate to measure primary endpoints at earlier times in some situations, for example, when the study drug is added to or switched for existing therapy for a short period in order to demonstrate its contribution while minimizing the confounding effects of combination therapy. However, the measurement of 24-week outcome in these studies remains important to assess the durability of response.

NRTIs with resistance profiles similar to existing agents

The only suitable designs for large-scale, controlled trials of NRTIs with resistance profiles similar to existing agents are direct comparative (‘head-to-head’) studies in treatment-naive patients or ‘switch’ studies in treated patients who have not yet experienced virological failure. Ideally, all trials should be randomized and blinded [15,61].

In the former approach, the developmental NRTI and a marketed NRTI are compared within a standard-of-care HAART regimen as initial therapy in naive patients. For example, the efficacy of emtricitabine (comparison with lamivudine (both plus stavudine and either nevirapine or efavirenz) [24] and stavudine (both plus didanosine and efavirenz) [25,26] has been evaluated. A smaller study compared emtricitabine with abacavir (both plus stavudine and efavirenz) [28].

Using the second approach, patients whose initial NRTI-containing regimen is successful (for example, whose viral load is undetectable) are allocated either to continue their current therapy or to have the NRTI in development substituted for an NRTI within the current regimen. NRTIs are unlikely to show marked differences in efficacy in this setting. Hence, in order to demonstrate a statistically significant benefit of a new agent, such studies may require large numbers of patients or long study durations. Two prospective, open-label Phase III studies of emtricitabine have used this type of design. In a 48-week study, patients on a lamivudine-containing triple-therapy HAART regimen with viral load levels <400 copies/ml were randomized to continue their current regimen or to switch from lamivudine to emtricitabine [24,30]. In the second study, patients with viral loads <400 copies/ml were randomized to continue their current PI-based HAART regimen or to switch to the combination of emtricitabine plus didanosine and efavirenz [29].

Retrospective analyses have also been used to provide supportive data during NRTI development. For example, Sanne et al. compared the results of therapy with emtricitabine plus stavudine and abacavir with data from previous studies of other triple-NRTI HAART regimens [67]. However, there

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are limitations on the interpretation of external or historical controls, not least due to the heterogeneity within these populations [15].

**NRTIs with distinct resistance profiles**

Initial long-term studies of NRTIs with distinct resistance profiles are likely to take place in experienced patients, since regulatory agencies in the United States [15] and Europe [61] stress the need for new agents that are effective in the treatment of patients with few remaining approved treatment options. The term ‘treatment-experienced’ is used to cover a wide spectrum of patients with various levels of medical need. For the purposes of drug development, distinct populations may be defined according to the complex interplay of such variables as HAART exposure, viral load, immune status, and the viral genotype and phenotype.

Regulatory agencies have recently offered useful advice for the design of clinical development programmes for new antiretrovirals in treatment-experienced patients [15,61]. Although the advice is generally consistent with regard to the principles involved, there are differences in the phraseology used. The United States Food and Drugs Administration (FDA) recommends studies in a broad range of populations, including ‘advanced and early disease’ and ‘heavily pretreated and naive patients’, but does not specify how subgroups within the spectrum of experienced patients should be defined [15]. The European Medicines Evaluation Agency categorizes experienced patients according to whether they are failing on their current regimen or not [61]. Those failing are subdivided into three groups: i) those who are naive to at least one class and who have various remaining treatment options; ii) those who are heavily pre-treated who have failed on regimens including at least one compound in all licensed classes as of March 2003, but still have remaining options; and iii) those who are heavily pre-treated and who have no or very limited remaining therapeutic options. When designing clinical studies, drug developers may need to use patient entry criteria that bridge these international variations in definitions.

There are five options for the design of controlled, comparative studies of new antiretrovirals in treatment-experienced patients: i) add-on designs: a new drug is provided in addition to a new HAART regimen; ii) intensification designs: a new drug is provided to intensify a current failing regimen; iii) switch designs: one drug in the existing regimen is switched for the new drug; iv) hybrid designs: combinations of either option (ii) or (iii) followed by the start of a new regimen; and v) head-to-head evaluations within a new or optimized (but individualized) HAART regimen: a new drug compared with another agent as part of a triple HAART regimen.

**Addition to a new HAART regimen**

In this design, the addition of the investigational agent to a new background HAART regimen is compared with the addition of a comparator or placebo (Figure 2A). This type of study can show a clear treatment effect of the investigated agent in a relatively simple, ‘real world’ setting. It is most readily applicable to the evaluation of new classes of drug with novel mechanisms of action or classes with which the patients have not yet been treated. Such agents are likely to show a clear treatment effect because no resistance is expected in the study population and the investigational agent is likely to be the most effective agent in the regimen. For example, this design was used to advantage in the T20 Optimised Regimen Only

**Figure 2. Schematic summary of (A) ‘add-on’ and (B) ‘head-to-head’ trial designs for studying new NRTIs for patients failing on their current regimen**

![Diagram](image-url)

Note: *Ideal outcome measure: reduction in viral load. Other endpoints (for example, proportion with undetectable viral load) may be appropriate, depending on the population studied. Primary endpoint could be at week 24 or 48. (A) Randomization between trial NRTI and placebo (double-blind). To select new background therapy. (B) Randomization between trial NRTI and comparator (double-blind). To select new background therapy and new comparator NRTI.
(TORO) trials of the HIV fusion inhibitor, enfuvirtide [68,69]. To a large extent, this situation also applies to the development of new NNRTIs and PIs. These classes are recommended alternatives for administration in combination with a ‘backbone’ of NRTIs [1,2]. Hence, it is still possible to avoid cross-resistance and to find treatment-experienced patients not previously exposed to each class of antiretrovirals. In studies of this type, it is advisable to limit the number of active drugs in the background regimen and to preserve the numerical balance of active drugs in the two compared regimens. Stratification at randomization based on genotypic and/or phenotypic score is useful in achieving this requirement.

In contrast, NRTIs are almost ubiquitous in current recommended treatment regimens and NRTI-naive, treatment-experienced patients are rare. The efficacy of an NRTI under investigation is likely to be affected by baseline mutations in RT, and this may vary between treatment groups depending on the distribution of baseline mutations. Hence, pilot studies are advisable prior to larger clinical trials of this type and stratification according to key mutations (for example, M184V) or number of TAMs may also be beneficial. In this situation, a new background regimen should not include NRTIs other than the tested NRTI.

Treatment intensification

In intensification studies, the addition of an investigational agent to a stable – that is, not optimized – existing regimen is compared with the addition of an additional agent to a stable – that is, not optimized – background regimen and to preserve the numerical balance of active drugs in the two compared regimens. Stratification at randomization based on genotypic and/or phenotypic score is useful in achieving this requirement.

In the context of a clinical trial, safeguards can be used to protect patients from the risks of sub-optimal

Figure 3. Schematic summary of (A) ‘intensification’ design (for patients failing on their current regimen) and (B) ‘switch’ trial design (for well-controlled patients or patients failing on their current regimen) for studying new NRTIs

(A) *Ideal outcome measure: reduction in viral load. Other endpoints (for example, proportion with undetectable viral load) may be appropriate, depending on the population studied. Primary endpoint should be at week 24 or 48. †Randomization between trial NRTI and placebo (double-blind). ‡To select optimized background therapy. (B) *Ideal outcome measure: reduction in viral load (patients failing current regimen) or time to treatment failure (for well-controlled patients). Latter may be a composite endpoint, including outcomes such as loss of viral control, change in treatment and withdrawal due to adverse effects. Primary endpoint could be at week 24 or 48. § Randomization between trial NRTI and continued comparator (double-blind). ¶ For analysis according to phenotypic activity and to allow optimization if necessary.

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therapy. Firstly, a relatively low upper limit for HIV viral load (for example, 5000–10 000 HIV RNA copies/ml) may be applied, although this may delay patient recruitment and give rise to perceptions that the data cannot be generalized to populations with higher viral loads. Secondly, assessments can be scheduled a priori to identify poorly responding patients and to rapidly improve their case. For example, a ‘bail-out’ criterion might stipulate that patients whose viral load has not fallen sufficiently after 2–4 weeks’ treatment are provided with a revised background regimen. These patients may continue within the study to allow the collection of long-term safety data required by regulatory authorities. Allowing patients to further optimize their background regimen at a subsequent predetermined point is another option in this setting.

Switch designs
In switch (or ‘substitution’) studies, the investigational agent is substituted for a component of a standard regimen in one treatment arm (Figure 3B). This regimen is then compared against continued treatment with the standard regimen. This approach may be applied in several ways.

An NRTI in a failing HAART regimen (the comparator) may be switched in one treatment arm for the agent under investigation, while the failing regimen is continued unchanged in the other arm. Implicit in this design is the assumption that resistance to the comparator agent is responsible for the loss of viral control. Clearly, this raises the possibility of only two phenotypically active agents being used in patients treated with the continued regimen. This is ethically unacceptable unless the study duration is limited, and is probably unethical even for a short duration if more than one agent in the regimen has a low genetic barrier for resistance (for example, an NNRTI). However, this option could be acceptable in patients with high CD4 cell counts and multiple resistance mutations associated with resistance to the ongoing background regimen such that continuation of the failing treatment, for a short period, would not expose them to the risk of accumulation of further mutations.

This approach has been used to evaluate NRTIs active against lamivudine-resistant HIV [33] carrying the M184V mutation. Thus, patients with M184V-positive HIV failing on a lamivudine-containing regimen would be randomized to continue therapy with lamivudine or to switch to the investigational agent. The ethical acceptability of this approach is supported by arguments that HIV containing M184V is less fit [69] and increasing evidence that lamivudine retains some residual antiretroviral activity, despite the presence of the M184V mutation [70,71].

The interpretation of a trial evaluating a switch versus continued lamivudine is complicated by the possible indirect antiviral effect of this agent. M184V mutants show reduced replicative fitness in vitro [72]. This mutation also enhances the susceptibility of TAM-containing HIV-1 strains to other agents, including zidovudine and tenofovir [73]. Thus, if an investigational NRTI is compared with continued lamivudine, there may be a confounding effect if the former does not maintain the M184V mutation and reversion to wild-type occurs. Ultimately, however, it is the difference in viral load outcome between groups that is most important, irrespective of any changes in circulating genotype.

A second switch approach involves randomizing failing patients to switch one NRTI for either the investigational agent or another marketed NRTI comparator. This approach is notionally attractive as both treatment arms are ‘active’. However, such an approach would require considerable efforts to identify eligible patients in whom both the comparator and investigational agent could be expected to be effective and would carry a risk that patients would not be provided with optimal care unless the comparator NRTI could be selected according to the resistance profile of each individual patient’s HIV. Also, the clinical imperative to ensure baseline sensitivity to the comparator agent would present logistical challenges. Ultimately, this design is unlikely to be practical as it will require high numbers of patients to show differences in outcome.

A third option is to switch an NRTI in patients who are well controlled on their existing HAART, as previously described for investigational NRTIs with resistance profiles similar to marketed agents. In such cases, the incentive for patients to change may be improved tolerability or dosing convenience rather than efficacy. These attributes may be less apparent for some agents with novel resistance profiles and hence patient recruitment might be difficult.

Hybrid study designs
The FDA has acknowledged that ‘controlled, comparative studies in patients who have exhausted many treatment regimens may call for innovations in study design’ [15]. For example, the addition of an investigational drug to an existing regimen may be compared with the continuation of the existing regimen for an initial 14-day ‘switch’ or ‘intensification’ phase, after which there would be a further ‘add on’ phase in which all patients receive the investigational agent together with an optimized regimen (Figure 4) [15]. A dose-ranging study with this design investigating the efficacy and safety of D-d4FC is currently ongoing (www.pharmasset.com). The initial phase identifies the new agent’s efficacy contribution, while the second assesses the durability of the effect. The results may be analysed according to the
genotypic profile and/or the phenotypic viral susceptibility to the investigational agent at baseline.

Hybrid designs such as these provide a clear, if short-term, signal of a new agent’s activity within a regimen comprising three or more antiretroviral agents while limiting the period for which patients receive treatment that does not fulfil the ‘standard of care’. The contribution of the investigational agent beyond the initial period, that is, after optimization, may be hard to identify unless a control group is added, with the advantages and disadvantages outlined above under ‘Addition to a new HAART regimen’. The outcome at 24 weeks and beyond is probably not influenced by changes in viral load that occur during the first phase of the study (Figure 5). Thus, if a control group is added, a hybrid design might be a useful model for studies involving optimization of background therapy at baseline.

Head-to-head studies within a new triple HAART regimen

Finally, a new NRTI may be compared with a comparator as part of a new background regimen in which all drugs are changed (Figure 2B). The background combination may be fixed for all patients (for example, comprising two defined antiretrovirals) or individualized according to genotype testing. The former approach may be applicable for studies involving only patients with well-defined phenotypes, whereas the latter is likely to be the most appropriate for studies involving a broader range of treatment-experienced patients. Changing all the components of a regimen removes much of the risk associated with single additions or substitutions in failing patients and is likely to be acceptable as the standard of care. It also limits withdrawal rates as most patients are likely to respond to trial therapy.

A disadvantage, as previously mentioned, is that it may be difficult to isolate the contribution of an investigational NRTI within a new, optimized HAART regimen. Superiority is unlikely to be attainable – especially if genotyping is used to ensure baseline susceptibility to optimized therapy – necessitating the use of an equivalence design and large numbers of patients to provide sufficient statistical power. The use of a fixed background regimen is notionally attractive as this reduces the scope for heterogeneity among the population and aids interpretation. However, recruitment would be hindered by the inevitable restrictions this places on the availability of eligible patients.

Pharmacokinetics and formulation

The pharmacokinetic/pharmacodynamic (PK/PD) relationships for NRTIs are extremely complex and depend not only upon the plasma PK profile of the parent compound but also that of the active intracellular triphosphate (TP) metabolite. When these factors are coupled with natural variations in the susceptibility of the ‘wild-type’ viruses of different individuals, it is understandable that it has hitherto been impossible to establish PK/PD relationships for NRTIs used to treat HIV disease, and that in vitro modelling approaches require clinical validation [66]. Similarly, because in vitro 50% inhibitory concentration (IC50) values are not necessarily representative of in vivo requirements for NRTIs, it is not as simple to establish

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**Figure 4.** Schematic summary of a ‘hybrid’ trial design for studying new NRTIs for patients failing on their current regimen

**Figure 5.** Comparison of hypothetical response rate profiles with an NRTI and placebo in a hybrid study (comprising an initial phase) and a controlled trial with optimization at baseline
target ranges for these agents as it is for PIs. However, it is clear that a degree of correlation may exist between parent NRTI concentrations in plasma and those of the intracellular TP metabolite.

Longer elimination half-lives in either the plasma or intracellular compartments may support a reduced frequency of dosing, as the goal is to sustain high intracellular TP metabolite concentrations. The intracellular TP half-life is the more important variable because ‘forgiveness’ of therapy, that is, the extent to which effective drug concentrations are maintained following a missed dose, will be driven by the rate of elimination of the intracellular TP metabolite rather than the parent NRTI in plasma. Conversely, prolonged exposure to suboptimal therapeutic concentrations once a drug has been stopped might lead to concerns regarding the selection of mutations, as has been observed following single-dose administration of nevirapine for prophylaxis of mother-to-child transmission [74]. None of the NRTIs in development at present raise this concern.

Once-daily formulations, including combined formulations of multiple antiretroviral agents, are becoming the ‘norm’ for use in treatment-naive patients, in whom dosing convenience is considered most important. However, combinations of NRTIs within the same subclass may be unsuitable owing to pharmacological interactions (see below). Treatment choices in treatment-experienced patients are influenced by previous therapy and associated resistance profiles. Hence, for this population, fixed combinations in which each drug has meaningful activity are commonly harder to identify. Moreover, in light of the need to tailor therapy to individuals’ needs, twice-daily administration may be perfectly acceptable in experienced patients.

Interactions

Pharmacokinetic and pharmacodynamic interactions between antiretrovirals, and between antiretrovirals and other medications, are an important consideration for the choice of therapy in both clinical practice [2] and drug development. PIs have a well-established potential for metabolic drug interactions involving the hepatic cytochrome P-450 enzyme system. However, the adverse clinical impact of interactions between PIs is limited, because the only common reason to combine these agents is to exploit the effect of ritonavir in boosting plasma concentrations of other PIs within double [75] or even triple PI combinations [76,77].

As NRTIs are always co-administered with members of the same class, interactions between members have important implications for treatment selection. There is the potential for intracellular interactions between NRTIs that are phosphorylated by the same enzyme. An interaction between zidovudine and stavudine [78,79] was first identified from adverse outcomes in clinical trials and later explained by a competing phosphorylation reaction [80]. More recently, a Phase III trial comparing tenofovir plus abacavir and lamivudine versus efavirenz plus abacavir and lamivudine in treatment-naive patients was halted after an interim analysis revealed a significantly higher rate of virological non-response in the triple NRTI regimen [56]. Since tenofovir and lamivudine have been shown to be as effective as stavudine and lamivudine, and since abacavir and lamivudine have formed the NRTI backbone of effective HAART regimens, a possible interaction between tenofovir and abacavir is being investigated. Preliminary data show no pharmacokinetic effect of tenofovir on single-dose abacavir pharmacokinetics in healthy volunteers [81]. Although a negative intracellular and/or transporter interaction has not been fully ruled out, the current hypothesis proposes that co-administration of these non-thymidine analogues may result in a significant synergism that promotes the rapid selection of M184V and K65R mutations [82]. The protective role of zidovudine against the selection of the K65R mutation in triple NRTI regimens may also explain the discordance between outcomes from different regimens of this type.

A pharmacokinetic interaction between tenofovir and didanosine has been shown. Tenofovir increases exposure to didanosine [83], although the mechanisms and clinical implications of these effects are unclear. A Phase II pilot study has shown poor virological response with the combination of tenofovir, didanosine and lamivudine [84]. An interaction has also been observed between alovudine and stavudine [85].

All theoretical interactions involving investigational NRTIs must be evaluated in vitro prior to any co-administration in vivo. Potential intracellular interactions must be fully characterized in clinical pharmacokinetic and pharmacodynamic studies to determine whether they influence intracellular TP levels and/or affect the antiviral activity of either agent in the short or long term. For example, in vitro studies showed that the intracellular phosphorylation of SPD754 was inhibited by lamivudine, possibly because the phosphorylation of both agents is catalysed by deoxycytidine kinase. This was further investigated within a three-period, cross-over study in which SPD754 and lamivudine were administered singly and in combination in HIV-uninfected volunteers [86]. The plasma pharmacokinetics of both compounds were unaffected by their co-administration. However, intracellular SPD754-TP concentrations were reduced by approximately sixfold in the presence of lamivudine. Lamivudine-TP concentrations were unaffected by co-administration with SPD754.
There are no examples to show how the impact of such interactions upon efficacy may be investigated in clinical trials. It seems prudent to avoid clinical use of the affected combination that reduces the TP levels of one or more NRTIs until guidance on the clinical importance of the interaction can be derived from small exploratory studies. Avoidance of co-administration is clearly possible in clinical practice (as in the case of zidovudine and stavudine) but would be particularly restrictive during the clinical development of new thymidine and cytidine analogues. In these circumstances, the interacting marketed NRTI would have to be the comparator and a switch design would be necessary.

Safety and tolerability

The adverse effects of antiretrovirals present an important obstacle to the long-term suppression of HIV [1,2]. In the case of NRTIs, particular attention has focussed on the problem of mitochondrial toxicity. NRTIs are substrates for human DNA polymerases as well as HIV-1 RT. There is substantial evidence (reviewed elsewhere [87,88]) that many adverse effects associated with NRTIs result from the inhibition of mitochondrial DNA synthesis. Quantification of mitochondrial DNA toxicity can be performed directly by quantitative polymerase chain reaction assay or by determining ratios of mitochondrial DNA to nuclear DNA. A ratio below 20% is thought to be associated with clinical conditions related to mitochondrial DNA depletion [89]. Moreover, mitochondrial toxicity is tissue specific, depending on differences between cell types in NRTI metabolism and mitochondrial function. It is the most likely explanation for the occurrence of lipodystrophy, hepatic steatosis, lactic acidosis, mitochondrial myopathy and peripheral neuropathy during NRTI therapy. Further research is required to investigate its potential role in other adverse effects [87,88].

Differences exist between the mitochondrial toxicity profiles of different antiretroviral classes and between members of each class [87,88,90]. The mitochondrial toxicity of new NRTIs must be evaluated in vitro and in vivo. In vitro data for certain new agents indicate a low propensity for mitochondrial toxicity [34,36]. Unfortunately, current mitochondrial assays remain experimental and inconsistencies exist between different research models, none of which has been validated. Thus, preclinical studies may fail to predict clinical mitochondrial toxicity [88]. Clinical assessment of total body and limb fat and anthropometrics may therefore be helpful for monitoring lipodystrophy. Rarer adverse effects can only be identified through conscientious observation of clinical outcomes during long-term safety studies.

The clinical evaluation of mitochondrial toxicity is best done in treatment-naive patients, in whom there is no pre-existing damage from prior NRTI administration and who will receive only defined combinations of NRTIs. This may not be possible during the initial clinical development of NRTIs with novel resistance profiles, as early studies are often conducted in treatment-experienced patients in whom the use of combination therapy confounds safety assessments of individual agents. Nevertheless, the collection of mitochondrial DNA safety data in the experienced population is important because these patients are generally at greatest risk of long-term adverse effects and have the most to benefit from the development of less toxic agents. Surrogate markers of long-term outcomes, perhaps obtained using cohort databases, may be required to overcome these difficulties.

Conclusions

NRTIs will be integral components of HAART regimens for the foreseeable future. There is an ongoing need for new agents that offer efficacy against NRTI-resistant HIV mutants and improved long-term safety. Unfortunately, the scope for clinical evaluation of NRTIs is restricted by the characteristics of these agents, current management approaches and appropriate regulatory requirements.

Studies in treatment-naive populations alone do not meet the need for clinical data in treatment-experienced populations. Studies in treatment-experienced populations are subject to numerous clinical and logistical challenges. Intensification, switch and hybrid study designs all offer possible approaches to evaluate NRTIs with novel resistance profiles. Switch studies are particularly useful for those drugs with resistance profiles akin to existing agents and when interactions between NRTIs are a concern. Interactions between different NRTIs may become increasingly important as the class continues to expand. In some cases, in vitro and in vivo pharmacokinetic data may be sufficient to make decisions regarding the appropriateness of co-administration. Otherwise, appropriate strategies and clinical trial designs to investigate potential interactions in the clinic will unfold as clinical experience increases.

Successful further development of new NRTIs will depend upon a thorough appreciation of these many complex issues, not only among those involved in the design of clinical studies, but also those contributing to their review and conduct.

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References


Early non-response to tenofovir DF (TDF) + abacavir (ABC) and lamivudine (3TC) in a randomized trial compared to efavirenz (EFV) + ABC and 3TC: ESS30009 unplanned interim analysis. 43rd Interscience Conference on Antimicrobial Agents & Chemotherapy, 14–17 September 2003, Chicago, IL, USA. Abstract H-1722A.


