Clinical Impact of HIV-1 Resistance against Non-nucleoside Analogue Reverse Transcriptase Inhibitors.

Impacte clínic de la resistència del VIH-1 als inhibidors de la transcriptassa inversa no anàlegs de nucleòsids

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PhD Thesis / Tesi Doctoral

Universitat Autònoma de Barcelona

2015

Several circles. Wassily Kandinsky, 1926. Munich, Germany.

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Programa de doctorat en Medicina

Tesi presentada per a obtenir el grau de Doctor.

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2015

Supreme excellence consists in breaking the enemy's resistance without fighting.

The Art of War. Sun Tzu. Chinese strategist and tactician. 544–496 BC.

Als pares, per la seva lluita per aconseguir portar a la Universitat a tots els seus fills.

A la Meme, la Majka i l'Aina, per compartir i donar sentit a aquesta aventura que és la vida i pel seu suport incondicional.

A en Pol i la Dido, per la seva joia constant, petits projectes que la vida portarà qui sap on.

ABBREVIATIONS

AIDS	Acquired Immune Deficiency Syndrome
ART	Antiretroviral treatment
$CD4^+$ count	CD4 receptor positive T lymphocyte cell fraction
DF	Disoproxil fumarate (tenofovir)
ENV	Envelope
HIV-1	Human Immunodeficiency virus type 1
NNRTI	Non-nucleoside reverse transcriptase inhibitor
NRTI	Nucleos/tide reverse-transcriptase inhibitors
PBMCs	Peripheral blood mononuclear cells
PI	Protease inhibitors
PI/r	Ritonavir-boosted protease inhibitors
RAM	Resistance-associated mutation
RT	Reverse transcriptase
TAM	Thymidine analogue associated resistance mutations

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ACKNOWLEDGEMENTS

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The thesis is based on research performed in the HIV Unit of the Internal Medicine Department in the Hospital Universitari Germans Trias i Pujol, in Badalona, Barcelona, Spain.

From my onset in the HIV Unit I have been devoted to the analysis of the clinical impact of HIV-1 resistance to antiretrovirals, its consequences on treatment success and failure, and on the efficacy of antiretroviral agents and regimens.

I have had the privilege and opportunity to collaborate with national and international experts within the field. This possibility that I've cherished allowed me to grow in this area and has entailed long term commitments and collaborations in this challenging discipline of the HIV medicine.

I am indebted to Bonaventura Clotet, director of the HIV Unit and architect and chair of the HIV/AIDS medical treatment and investigation in the Hospital and the country. Endless entrepreneur, his impressive insight, spectacular energy remain a unique inspiration, as does his constant focus and perseverance. He transmitted me the go-ahead strategy in life in order to struggle to achieve your targets. He has been able to gather a unique scientific team around, both in the clinic and in the lab, creating a motivating research environment.

I am also grateful for the constructive criticism of Roger Paredes. He has provided unyielding support and excellent scientific collaboration along these years. Unique scientifically-minded physician and lab researcher, he helped me in structuring the findings of many parts of the research.

Roger and Bonaventura were devoted mentors on my PhD, and continue to be key collaborators.

Over these years, I've received tutoring from Jordi Tor, director of the Internal Medicine Department. Both Jordi and Bonaventura gave me the most important ingredient to cook a PhD thesis: time.

José Ramón Santos has been an awesome collaborator though these years. I am indebted to him for his pleasant and tireless help in research. José Moltó helped me as well in critical issues with formatting of the PhD Thesis.

Acknowledgements

Jonathan M Schapiro inspired me many good discussions, both on research areas and in clinical decisions based on resistance tests in HIV patients. His constant insights on the clinical implications of tiny issues found in HIV-1 genotypes have been really rewarding.

There are many bright colleagues who contributed to these research projects. I am grateful to all of them, particularly all my co-authors. It has been a privilege to collaborate with this exquisite group of people and overcome together the obstacles we encountered during these years. I am particularly thankful to Isabel Bravo, Jordi Puig, tireless study monitors, data managers, and lovely nurses, as well as to Núria Pérez-Alvarez and Arelly Ornelas, statistician masters in the "Lluita contra la SIDA" Foundation in Badalona, Barcelona.

I am also indebted to investigators in the city of Barcelona participating in the analysis of the efficacy of etravirine, particularly to Pere Domingo (Hospital Sant Pau), Esteve Ribera (Hospital Universitari Vall d'Hebrón), and Arkaitz Imaz, Elena Ferrer and Daniel Podzamczer (Hospital Universitari de Bellvitge).

I also have a debt of gratitude to Raquel Martin Iguacel (Infectious Diseases Dept, Odense University Hospital, Odense, Denmark) for his work in database management with the analysis of the effectiveness of nevirapine plus tenofovir and emtricitabine in switch/simplification.

Finally, I am also thankful to researchers in the Drug Resistance Platform of the Spanish AIDS Research Network, particularly to Lourdes Anta, and Carmen de Mendoza. They have an impressive overview of the organization and coordination of a National project and tasks, and created a structured and motivating research environment.

Many other colleagues also collaborated in different parts of the research. I'm expressly obliged to all of them for providing me with awesome ideas, projects and challenges.

Josep M Llibre

Barcelona, August 2015

PREFACE

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Sometime around the early 1900s, the virus that sparked the AIDS epidemic likely spread from a chimpanzee to a human in south-eastern Cameroon. Researchers have documented at least 13 different cases in which a simian immunodeficiency virus has jumped from monkeys, chimpanzees, or gorillas into humans. But only the virus known as HIV-1 group M (for “major”) travelled far and wide and was able to create a worldwide epidemic. In roughly 1920, someone infected with it travelled down the Sangha River and its tributaries from Cameroon to Léopoldville, today known as Kinshasa, the capital of Democratic Republic of the Congo. Later on HIV left Kinshasa and spread through Central Africa in the first stage of an epidemic that less than a century later has infected nearly 75 million people worldwide.

HIV-1 is characterised by its great genetic diversity. Its continuous evolution, since the beginning of the pandemic, has given rise to many subtypes and circulating recombinant forms. The widespread use of and selective pressure imposed by ART has also contributed to this diversity with drug-resistant mutations spreading.

Today, an estimated 13.6 million people worldwide are receiving ART, 700,000 having initiated therapy over the past year alone. New goals for universal global access to ART are the “90-90-90” targets: by 2020, 90% of all people living with HIV should know their HIV status, 90% of those who test positive for HIV should be provided therapy, and of those, 90% should achieve virologic suppression (levels of virus below those detectable by standard tests). Achieving these targets would be a major step towards a world without AIDS.

Commensurate with the increase in new antiretroviral drugs and antiretroviral combinations, there has been an increase in knowledge about drug-resistance

mutations. Many new treatment-associated NRTI, NNRTI, PI, ENV and integrase RAMs have recently been described and there has been a growing appreciation of the effects that different amino acid substitutions at the same position often have on drug susceptibility.

The establishment of a clear therapeutic endpoint – complete virological suppression – to prevent treatment failure and HIV transmission necessitates a new framework in which the vast knowledge of these RAMs should be cast and incorporated into routine clinical practice.

NNRTIs are popular components of combination antiretroviral therapy due to their efficacy and simplicity. For NNRTIs, more than for any other antiretroviral class, resistance is caused only by specific mutations at drug-resistance positions.

Despite its proven efficacy, the clinical use of first-generation NNRTIs, as nevirapine and efavirenz, has been limited by side effects, low barrier to resistance and broad cross-resistance. To try to overcome these limitations, a second-generation of NNRTIs has been developed that includes etravirine and rilpivirine, both of which were recently approved as therapy for HIV-1 infection.

Despite being a second generation NNRTI, rilpivirine also depicts a low genetic barrier to drug resistance development. Like nevirapine and efavirenz, complete drug resistance can arise with only one or two resistance-associated mutations (RAMs). In addition, there is a considerable degree of class cross-resistance among all NNRTIs, nearly complete between nevirapine and efavirenz, and more limited from first to second generation NNRTIs. Therefore, the knowledge of RAMs selected by first generation NNRTIs that have a potential to impact the activity both rilpivirine or etravirine in subsequent treatments is of paramount importance.

Genotypic scores are now fully developed for all these drugs, therefore allowing the resistance analyses in clinical samples.

As a result of all this, NNRTIs offer a unique opportunity to investigate the clinical impact of HIV-1 resistance on treatment response both in initial, salvage or simplification ART, as well as the degree of cross resistance among first and second generation NNRTIs.

In the following pages, we will discuss the relevance of RAMs on treatment response, we will pinpoint the RAMs and patterns of RAMs selected at virologic failure with specific regimens including NNRTIs, and the consequent risk of virologic failure of treatment regimens based on NNRTIs.

The first chapter of this PhD thesis will evaluate the effectiveness of etravirine in salvage regimens in treatment-experienced subjects recruited at HIV Units of four acute-care University hospitals in Barcelona, in routine clinical practice. Factors associated with treatment failure to etravirine will be assessed using a multivariate (Cox regression) analysis.

The second chapter will assess the RAMs selected in subjects failing an NNRTI-based treatment (with nevirapine, efavirenz or etravirine) at 22 clinics in Spain. The aim will be the identification of the mutations and their potential impact on the activity of rilpivirine, the last NNRTI introduced in the clinic. We will ascertain if failures to nevirapine, efavirenz or etravirine select different patterns of mutations and have therefore a differential impact on rilpivirine's activity.

The third chapter will estimate the effectiveness of a nevirapine-based switch regimen in subjects with suppressed viremia, combined with tenofovir and emtricitabine (or lamivudine). The analysis will be done in our clinic in Barcelona and will identify factors associated with virological failure in a multivariate analysis as well as the resistance

selection at failure. RAMs and patterns of RAMs isolated in the reverse transcriptase against both NRTIs and NNRTIs will be of principal interest to increase the knowledge on the potential impact on the activity of etravirine in subsequent treatments. Further on this, a greater understanding of the most common emergent mutations in subjects treated with nevirapine and tenofovir will be of great interest in developing countries, where the use of nevirapine is extensive and resistance testing is often unavailable.

We will finish this thesis by presenting the main conclusions derived from our work and outlining future research stemming from the knowledge gathered during this research

INTRODUCTION

INTRODUCTION

HIV-1 has a high mutation rate, accumulating nearly one nucleotide mutation per replication cycle [1]. Because HIV's reverse transcriptase lacks proofreading ability, 10^3 to 10^4 mutations (one or two per genome) are spontaneously generated per replication cycle [2]. Given the high replication rate, any single mutant and some dual mutants could potentially be generated per day [3]. Most mutations are deleterious and drive mutant viruses to extinction. Others have neutral or beneficial effects on the replicative capacity and remain incorporated in the quasispecies. However, HIV poses fabulous challenges with drug resistance. Multidrug-resistant variants of HIV-1 can exist in cells as defective quasispecies and be rescued by superinfection with other defective HIV-1 variants [4]. Indeed, drug-resistance phenotyping revealed that the multidrug-resistant viruses dominated if even single RT inhibitors were present, reflecting linkage of the various RAMs on a single viral nucleic acid backbone. These results are most likely attributable to recombination during second rounds of infection and suggest that defective HIV-1 variants may nonetheless constitute part of the HIV-1 reservoir.

HIV is characterized by a high genetic diversity. Approximately one-half of RT codons from non-B subtypes and circulating recombinant forms (CRFs) are polymorphic in antiretroviral-naive patients [5]. This rate is even higher for the protease gene. Naturally occurring changes may appear at positions associated with drug resistance.

ART tackles the adaptive mechanisms of HIV stemming from two main aspects: the pre-existence of viral variants resistant to three drugs in untreated subjects is highly unlikely, and the rate of evolution is highly dependent on the viral replication rate [2, 6]. Therefore, mathematical modelling shown that resistance selection is very unlikely in subjects with suppressed plasma HIV-1 RNA.

Given the molecular structure similarities within compounds of the same antiretroviral class and their interaction with similar target sites, the emergence of resistance to one drug often leads to variable degrees of cross-resistance to other drugs in the same family [3, 7]. This reduces the therapeutic arsenal available for salvage therapy leading to the prescription of more complex, expensive and often worse tolerated regimens [8].

The efficacy of an ART regimen depends on the activity of the regimen's individual drugs and the number of HIV-1 mutations required for the development of resistance to each antiretroviral – the so-called barrier to resistance development [8-12]. An estimated genetic barrier derived from fitness landscapes may contribute to an improvement of predicted treatment outcome. Antiretroviral resistance impairs the response to therapy in patients with transmitted resistance, unsuccessful initial ART and multiple virological failures [13, 14]. Genotypic resistance testing is used to identify transmitted drug resistance, provide insight into the reasons for virological failure in treated patients, and help guide second-line and salvage therapies [15-17].

Individuals with HIV-1 drug resistance face antiretroviral therapy with a lower genetic barrier to resistance development, a higher risk of virological failure, have more limited options of active drugs, and eventually have a higher risk of clinical progression and death [18-25]. Actually, use of genotypic and phenotypic susceptibility testing has been independently associated with improved survival among ART-experienced patients, although use of resistance tests was not randomized in the analysis and residual confounding might exist [26]. Obviously, non-adherence to ART was soon identified as a major cause of HIV drug resistance and is the major bias existing in all these cohort studies [27].

Therefore, an understanding of the basic principles of HIV resistance and resistance testing is crucial for providing optimal care, particularly for antiretroviral-experienced patients [15-17, 28, 29].

NNRTIs are an antiretroviral class where this has been clearly demonstrated. The risk of virologic failure for subjects with baseline NNRTI resistance was higher than that for subjects without such resistance (hazard ratio 2.27 [95% confidence interval], 1.15–4.49; P=018), in a study done in a population with a prevalence of baseline NNRTI resistance of only 5% [30]. Even among HIV-infected patients with confirmed virologic failure on first-line ART, remaining on first-line therapy led to an increase in mortality relative to switching [23, 31].

Although the rates of multiple regimen failure have decreased dramatically over the past decade, mortality rates for those who have experienced failure of at least 2 regimens have remained high. Indeed, viremia copy-years – identified as a better predictor of long-term clinical outcomes – predicted all-cause mortality independent of traditional, cross-sectional viral load measures and time-updated CD4+ T-lymphocyte count in treated patients, suggesting cumulative HIV replication causes harm independent of its effect on the degree of immunodeficiency [32].

In the EuroSIDA cohort, by 96 months from baseline, the proportion of patients with a new AIDS diagnosis or death was 20.3% (95% CI:17.7–22.9) in patients with no evidence of virological failure and 53% (39.3–66.7) in those with virological failure and mutations to three drug classes (P=0.0001). An almost two-fold difference in risk was confirmed in the multivariable analysis (adjusted relative hazard=1.8, 95% CI:1.2–2.7, P=0.005) [20].

Consequently, HIV drug resistance has an outstanding impact on ART efficacy. Antiretroviral drug resistance is both of individual and public health concern: clinicians must address both levels simultaneously [3, 13, 33].

At the individual level, clinicians must seek to maximize the potency and durability of the antiviral activity of ART by providing patients with antiretroviral regimens to which the virus retains maximum susceptibility.

At a population level, complete viral suppression must be pursued in all individuals, including those with triple-class HIV-1 resistance, not only for the patient's safety, but also to prevent HIV transmission at a global population level [34-36]. Mathematical transmission models coupled with a statistical approach that enabled inclusion of a high degree of uncertainty in the potential treatment effects of ART (in terms of infectivity and survival), increase in risky behaviour, and rate of emergence of drug resistance elegantly demonstrated that usage of ART in a given population (i.e. San Francisco) would decrease the AIDS death rate and could substantially reduce the incidence rate [37-39].

The proportion of treated individuals with suppressed plasma HIV-1 RNA has increased worldwide in developed countries. In a study done in British Columbia, Canada, the percentage of subjects with suppressed viremia increased from 24% to 80% from 1997 to 2010, therefore limiting the spread of HIV in one of the major achievements in HIV medicine [40]. Further on this, the prevalence of antiretroviral resistance among persons with both suppressed and unsuppressed HIV plasma viremia decreased markedly over the study period, even as resistance testing rates increased significantly. This finding has been the initial major driver for the recent shift toward earlier initiation [41-43]. Two other major findings have established the universal need for ART in all subjects with HIV infection. The first one was the evidence that early initiation of ART reduced rates of sexual transmission of HIV-1 and clinical events, indicating both personal and public health benefits from such therapy [34]. The second one were findings from the START study, showing that the initiation of ART in HIV-positive adults with a CD4+ count of more than 500 cells per cubic millimeter provided net benefits over starting such therapy in patients after the CD4+ count had declined to 350 cells per cubic millimetre [44]. Therefore, administering ART to all HIV-infected individuals makes makes now solid scientific sense.

At the public health level, clinicians must seek to reduce the incidence and prevalence of antiretroviral resistance in the society (circulating viruses), so that more individuals retain fully susceptible viruses, less transmit resistant viruses, and more can be effectively treated when needed [13, 33, 37, 38, 40, 42]. Not unexpectedly, subjects with unrecognised transmitted resistance have poorer responses if their ART regimens do not include fully susceptible agents [45].

An HIV-1 genotype must be performed in all treatment-naive subjects before initial ART commencement, ideally as soon as they enter clinical care in order to maximize detection of transmitted drug-resistant HIV (strong recommendation, high quality evidence) [14-17, 46-48]. If therapy is deferred, repeat testing at the time of ART initiation should be considered because of the potential for superinfection (weak recommendation, low quality evidence).

Drug-resistant variants are frequently present in both recently and chronically infected treatment-naive patients, and are most commonly seen in patients infected with subtype B virus, probably because of longer exposure of these viruses to drugs [13, 49-59]. With variable degrees, drug-resistant HIV-1 is present in approximately 10%–20% of new infections in Western countries and in 60% of patients failing ART [53]. Both types of resistance are public health concerns and have the potential to reverse the impressive efficacy of ART [8, 49]. However, the global prevalence of RAMs to tenofovir, lamivudine/emtricitabine and efavirenz in Europe – the most commonly used drugs during the study period – decreased over time between 2005 and 2010 [11]. Despite a stable rate of efavirenz and protease inhibitor use, this phenomenon could potentially be explained by an increased use of single-tablet regimens, which simplify drug intake and maximize adherence, and prevent occult monotherapy due to patient differential non-adherence [60].

Obviously, subjects with virological failure habour significantly higher rates of RAMs [21, 61, 62]. Even in a short period of 6 months, patients kept on the same virologically failing ART regimen had a considerable accumulation of RAMs, particularly in patients with initial low-level of resistance to the failing regimen [62]. In a recent EUROSIDA cohort analysis, the prevalence of NRTI, NNRTI and PI resistance was estimated as 43% (95% confidence interval: 39%-46%), 15% (13%-18%) and 25% (22%-28%), respectively [19].

Recent data suggest that antiretroviral regimens with a high barrier to resistance development combined with improved patient adherence may mitigate transmitted drug resistance increases by reducing the generation of new antiretroviral-resistant strains [52, 63-65]. In a recent study, the overall prevalence of transmitted drug resistance was 10.1%, more commonly to NNRTIs (4.5%) and NRTIs (4%) compared with PIs (2.8%). The most frequent transmitted RAMs observed were M41L, D67N/G/E, T215F/Y/I/S/C/D/E/V/N, 219Q/E/N/R, K103N/S, and G190A/S/E in RT, and M46I/L and L90M in the protease [49]. Reassuringly, universal downtrends or stabilization in transmitted HIV resistance have been reported in Europe, even in subjects likely to have acquired their HIV-1 infection abroad [48, 53, 57, 66]. However, reported resistance remains confined mainly to one antiretroviral class: NNRTIs.

Resistant viruses are usually transmitted less efficiently than wild type although multidrug-resistant variants are sometimes transmitted [67, 68]. Conversely, resistant mutants generated through replication errors often co-exist and compete with the wild-type in the quasispecies [3]. As a result, mutants often become extinct or, sometimes, persist in the viral quasispecies at very low frequency, as predicted from the Poisson distribution [53, 68]. Whereas transmitted resistant variants can contain several resistance mutations in various genes, mutants generated spontaneously in the absence of antiretroviral pressure rarely accumulate more than 2 resistance-associated substitutions in the same genome [2].

The mean time to switching from a pure to a mixture population of wild-type and drug-resistant viruses in the absence of ART is highly variable, but has been calculated roughly in about 96 weeks after the estimated date of primary infection [51, 69]. The median time to loss of detectable drug resistance using population-based assays ranged from 4.1 years (conservative estimate) to longer than the lifetime of the individual. The impact of each RAM on the virus fitness will eventually determine the time of persistence of the mutation without exposure to treatment [1, 3, 12, 70-77]. In an analysis done in UK, RAMs persisted over time in most patients studied. In particular, M41L, T69N, K103N, and T215 variants within RT and multidrug resistance demonstrated little reversion to wild-type virus [77]. By contrast, Y181C and K219Q in RT, occurring alone, disappeared within 25 and 9 months, respectively.

The rapid replacement of M184V/I mutations is fully consistent with known fitness costs. The long-term persistence of NNRTI and PI mutations suggests a risk for person-to-person propagation [69, 75]. Host and/or viral factors not accounted for by viral load or mutation class are likely influencing mutation replacement, and are still incompletely understood.

A complete and sustained suppression of HIV-1 replication is currently the final target of ART, including treatments for patients with advanced multi-resistant HIV-1 infection [16, 17]. This unambiguous therapeutic endpoint necessitates a new framework in which the vast knowledge of drug resistance mutations should be cast [8, 16, 36, 78-80].

Therefore, monitoring and overcoming HIV-1 drug resistance is crucial for guiding every ART, either in an initial, a simplification, or a salvage strategy.

Emergence of any resistance has eventually been associated with mortality (hazard ratio: 1.75 [95% confidence interval: 1.27, 2.43]) [18]. When each class of resistance was considered separately, persons who exhibited resistance to NNRTIs had the

highest risk: mortality rates were 3.02 times higher (95% confidence interval: 1.99, 4.57) for these patients than for those who did not exhibit this type of resistance, even after adjustment for plasma HIV-1 RNA levels, adherence, and $CD4^+$ cell counts [18]. Other studies have also reported on the association between drug resistance and risk of death [81, 82]. Obviously, these cohort studies could have uncontrolled baseline bias influencing the analysis. NNRTI resistance might be an independent marker for poor adherence in the population [83], but a detailed knowledge of the clinical impact of NNRTI resistance is mandatory.

In some cases, RAMs are not detected in genotypic tests despite the presence of confirmed virological failure. The absence of detectable viral resistance after treatment failure may result from any combination of [80, 84]:

- the presence of drug-resistant minority viral populations (usually below the 20% of the viral population), that remain undetected in population (Sanger) genotypes
- a prolonged interval between the time of antiretroviral drug discontinuation and genotypic testing in mutations entailing a fitness cost, that allow a waning of those mutations
- non-adherence to medications
- use of drugs with high genetic barrier to resistance in individuals without any baseline resistance
- laboratory error
- lack of current knowledge of the association of certain mutations with drug resistance
- the occurrence of relevant mutations outside the regions targeted by routine resistance assay[85, 86]
- compartmental issues indicating that drugs might not reach optimal levels in specific cellular or tissue reservoirs where RAMs could be circumscribed

PIs are a typical case where the identification of RAMs in subjects with virological failure could be suboptimal. A recent report has proposed that approximately half of the inhibitory potential of PIs is manifest at the entry step, likely reflecting interactions between the uncleaved Gag and the cytoplasmic tail (CT) of the Env protein, where mutations could occur [85]. Therefore, RAMs in subjects with virological failure to PI-based regimens could be occurring at sites not currently included in genotypes.

NNRTIs offer a unique possibility to study the clinical impact of HIV-1 resistance, with efavirenz, nevirapine and rilpivirine being the most appealing drugs to look at, due to their low genetic drug barrier to resistance despite their high efficacy [8]. Etravirine displays a higher barrier to resistance development, but it can also be jeopardised by transmitted drug-resistance mutations or mutations selected in previous failures with regimens including nevirapine or efavirenz [87, 88].

NNRTIs are the class with the higher impact on clinical response of transmitted drug resistance. Actually, a small number of NNRTI-resistance mutations were responsible for most cases of high-level transmitted drug resistance in a recent GenBank analysis including 50.870 individuals from 111 countries [52, 89]. Four NNRTI RAMs - K101E, K103N, Y181C, and G190A - accounted for >80% of NNRTI-associated transmitted drug resistance in all regions and subtypes [5, 52].

General principles of HIV resistance

Determination of genotypes is preferable to analysis of phenotypes because of lower cost, faster turnaround time, availability of commercial assay kits or in-house protocols, and greater sensitivity for detecting mixtures of wild-type and resistant virus [16, 16, 90, 91]. Therefore, genotypes are the standard resistance tests used in clinical practice in most settings, and are the preferred resistance tests in Europe [10]. Mutation regression coefficients showed that, within a drug class, cross-resistance patterns differ for different RAMs subsets and that cross-resistance has been initially underestimated in these studies [80].

Phenotypes can provide additional information about complex mutational patterns, particularly regarding resistance mutations to new drugs with limited experience, and with PIs.

Both genotypes and phenotypes are unable to detect minority variants, ie, those present in <20% of the viral population [64, 92-97]. Technologies continue to evolve with the ability to sequence and detect extremely small minority populations ("ultradeep sequencing"). Their ultimate clinical role remains to be determined, but they are an important research tool and have demonstrated clinical relevance for pre-therapy mutation screening and predicting treatment response in initial ART with first-generation NNRTIs [92-94, 96, 97]. At least one study with ultrasensitive HIV-1 genotyping has demonstrated an improved genotypic sensitivity score prediction deriving in improved virological outcomes of antiretroviral salvage treatment [98]. However, minority protease RAMs do not impact the efficacy of initial PI-based ART [55, 99, 100].

All things considered, this technology has not been implemented yet into routine clinical practice and remains a high-tech tool in investigation labs.

Interpretation of the resistance test results is complex. Thus, algorithms and software programs are continually being designed and fine-tuned [46, 84, 101]. Despite this, the final interpretation of resistance tests and the design of an optimal suppressive salvage regimen can be challenging.

Distance consultations with use of e-mail and conference calls are a feasible strategy when local availability of experts is lacking, providing expert advice along with continued education in challenging cases with limited options left [15, 17, 102, 103].

Some guidelines still recommend a plasma viral load ≥1000 copies/mL in samples for genotypic testing. However, rates of amplification >70% can usually be obtained with viral loads >100 copies/mL, when HIV-1 RNA is extracted from 3 mL of plasma after centrifugation, particularly with HIV-1 RNA levels >400 copies/mL [16, 104, 105]. In our current clinical practice, HIV genotypes are ordered in all confirmed virological failures (a confirmed HIV-1 plasma RNA>50 copies/mL).

Tropism testing must be routinely assessed in all virological failures and naive subjects with transmitted drug resistance [106-108]. An R5-only tropism result will allow use of maraviroc either in the initially planned regimen or as an alternative if toxicity to another drug is encountered and suppressed plasma viremia has been maintained since the tropism test [109]. Tropism testing is not standardized yet once viral load becomes undetectable [110]. The absence of tropism shifts during viremia suppression suggests that, when available, testing of stored baseline plasma samples is generally safe and informative, provided that HIV-1 suppression is maintained thereafter. Tropism testing in PBMCs may not necessarily produce equivalent biological results to plasma, because the structure of viral populations and the diagnostic performance of tropism assays may sometimes vary between compartments.

The inclusion of new drugs in the treatment regimen without or with limited cross resistance (i.e. enfuvirtide, etravirine, maraviroc, darunavir, an integrase inhibitor) has been associated with significant increases in response rates in all salvage trials. Although the use of enfuvirtide is currently vestigial because of treatment inconvenience and widespread substitution with alternative oral drugs (mainly raltegravir or dolutegravir), its contribution to regimen activity should not be forgotten when options are limited [111-117].

In all ART regimens every active drug protects the rest of the regimen. While usually this protector role is attributed to the “anchor” drug (usually a boosted PI), randomized clinical trials have also demonstrated that active “accompanying” drugs also protect the boosted PI and reduced the resistance selected in the protease [118]. In an analysis done in the DUET studies, of those subjects experiencing virological rebound, fewer etravirine-treated than placebo-treated patients developed RAMs associated with resistance to PIs in general and to darunavir in particular, and more patients in the etravirine than the placebo-group maintained baseline darunavir susceptibility at study endpoint.

The success of salvage therapy lies in closely adhering to a series of basic principles (Table 1). It is crucial to design an optimal regimen which allows for effective and durable viral suppression while minimizing toxicity, inconvenience, and cost.

Table 1. Important steps to be checked for successfully designing salvage antiretroviral therapy regimens.

Step no	Description
1	Determine the cause of the current regimen failure. Take measures to resolve it and avoid its recurrence in the subsequent new regimen.
2	Review all previous resistance test results available as well as the current one. Compile all results and interpret. Prior documented mutations remain in small undetectable subpopulations but will emerge when suboptimal drug pressure is exerted again, even if undetected by the present tests.
3	Thoroughly review the full treatment history, and specifically indentify all drugs included in failing regimens, and those associated with intolerance. Suspect the presence of mutations against drugs with a low genetic barrier to resistance (lamivudine, emtricitabine, nevirapine, efavirenz, rilpivirine, enfuvirtide, raltegravir, elvitegravir) included in regimens which have previously failed, despite the fact that mutations were not detected in genotypes.
4	Review all potential drug-drug interactions and consider therapeutic drug monitoring if available and indicated.
5	Focus on maximizing patient's adherence to treatment. Poorly adherent patients are overrepresented among those with virological failure.
6	Strive to include three fully active drugs or their equivalent in the new treatment regimen in all patients with prior triple-class failure and resistance.
7	Consider obtaining a tropism test in all patients with virological failure or transmitted resistance, even if a CCR5 antagonist use is not initially planned.
8	Patients with high viral loads and lower CD4 cell counts have consistently lower response rates. Take special care with this subset of patients. To optimize salvage regimens including three fully active agents.
9	In complex cases with multidrug resistance, obtain the opinion and the support of an expert in HIV resistance.

The evaluation of the expected activity of second generation drugs in clinical samples, according to mutational patterns associated with decreased virological response has been of paramount importance. PIs and etravirine have been a clear example of this, and the residual activity of the new drug is determined by the RAMs and patterns of RAMs selected in subjects with virological failure while receiving first-generation drugs [87]. These studies have elegantly shown that every NNRTI selects somewhat specific

patterns of RAMs, with differential impact on the predicted activity of etravirine, a clinically relevant information [119, 120].

Genotypic resistance scores.

Whereas early in the HIV epidemic the output of HIV resistance tests had been based on therapeutically arbitrary criteria, there has been a quick move towards correlating test interpretation with virological outcomes on treatment. This approach is undeniably superior, in principle, for tests intended to guide drug choices. There has been a global collaboration to become involved in constructing such tools, with particular emphasis on establishing validated mutation score lists and rules, continuously updating the key issues and confounding factors that influence predictive accuracy outside the originating dataset [46].

The scores or weighted lists of mutations that confer resistance include mutations selected *in vitro* by passage experiments, those that result in drug resistance *in vitro* and decreased response *in vivo*, and those that appear in patients who experience virological failure [8, 46, 121].

The University of Stanford (CA,US) has strived to maintain his genotypic database as a publicly available resource. In his claim, an HIV drug-resistance database that provides unfettered access to all known types of data on HIV resistance must be publicly available to the broadest number of users to promote discovery in the most efficient manner. Proprietary databases that deny access to the majority of researchers are not only inefficient but also counterproductive: the company or small group of researchers with a stake in such a database will typically act to thwart the non-proprietary dissemination of data, to maintain the perceived commercial or research value of their monopoly [122].

The identification of specific drug resistance mutations in the HIV-1 genome (amino acid differences from wild-type reference sequences, most commonly viruses HXB2 and NL43, and a consensus subtype B reference virus sequence) is a complicated way of facing a very simple issue: the selection and evaluation of HIV-1 drug resistance against antiretroviral drugs. However, the clinical interpretation of genotypic drug resistance testing remains challenging. Some common terminology used in the analysis of RAMs in HIV resistance is listed in Table 2.

Table 2. Definitions of relevant terms in understanding genotypic HIV drug resistance. Adapted from RW Shafer et al [156].

Primary or transmitted drug resistance: Drug resistance in previously untreated persons. Because drug resistance seldom occurs without drug exposure, primary drug resistance implies that a virus with RAMs was transmitted either directly, or through one or more intermediates, from a person with acquired drug resistance. Previously untreated persons include drug-naïve persons with laboratory evidence for recent infection (e.g. within the preceding 6 to 18 months depending on the particular study); newly diagnosed with infection of uncertain duration; and previously diagnosed with infection of uncertain duration.
Acquired or secondary drug resistance: Drug resistance developing in a person who has received ART. Acquired drug resistance results from the generation of genetic variation in the population of viruses within a person followed by the selection of drug-resistant variants during therapy.
Mutation: Because HIV-1 is highly variable, there is no standard wild-type strain. Therefore for drug-resistance studies, mutations are defined as amino acid differences from one of several wild-type reference sequences. The most commonly used reference sequences are of the laboratory viruses HXB2 and NL43 and a consensus reference sequence comprising the most common amino acid at each position in wild-type subtype B viruses (subtype B consensus). These sequences are nearly identical, differing at only a few amino acids not involved in drug resistance. The use of subtype B sequences as reference sequences is based on historical precedence.
Polymorphism: Polymorphisms are mutations occurring frequently in viruses not exposed to selective drug pressure. A non-polymorphic mutation is one that does not occur in the absence of therapy. No frequency cut-off has been proposed to distinguish polymorphic from nonpolymorphic positions.
Electrophoretic mixture: The presence of more than one fluorescent peak at the same position in a dideoxynucleoside sequence indicates that two populations of viruses with different nucleic acids at the same position are each present in large enough proportions (>10–20%) to be detected by sequencing.

Computerized rules-based algorithms are needed to characterize virus as "susceptible", "possibly resistant" or "resistant" to each antiretroviral drug. The algorithms need to take into account not only the impact of every single mutation, but also the existence of synergism or antagonism between particular patterns of mutations, thus complicating the interpretation beyond a simple list of mutations [121].

The development of these rule-based algorithms is a difficult and lengthy process, and requires frequent updating [8, 78, 84, 89, 101]. The vast majority of genotypic algorithms are based on data that were obtained using subtype B viruses. However, the level of resistance to antiretroviral drugs may differ among HIV variants. Indeed, we have limited knowledge of resistance mutations in non-B subtypes of HIV-1 and their clinical relevance, despite the fact that more than 90% of patients with HIV-1 infection worldwide have non–subtype B variants of HIV-1 [3, 5, 89]. The potential for genetic differences among subtypes to yield different patterns of resistance-conferring mutations is supported by natural variation among HIV subtypes in genetic content. For example, 40% variation in the viral envelope (env) gene and 8 to 10% variation in the polymerase (pol) and group-specific-antigen (gag) genes [5]. This issue acquires special relevance in view of the fact that the HIV pol gene encodes each of the reverse-transcriptase, protease, and integrase enzymes that are the major targets of ART. Hence, some polymorphisms can act as the equivalent of secondary resistance mutations in some HIV subtypes, particularly for protease inhibitors [5, 49, 53].

Most reports on drug resistance deal with subtype B infections in developed countries.

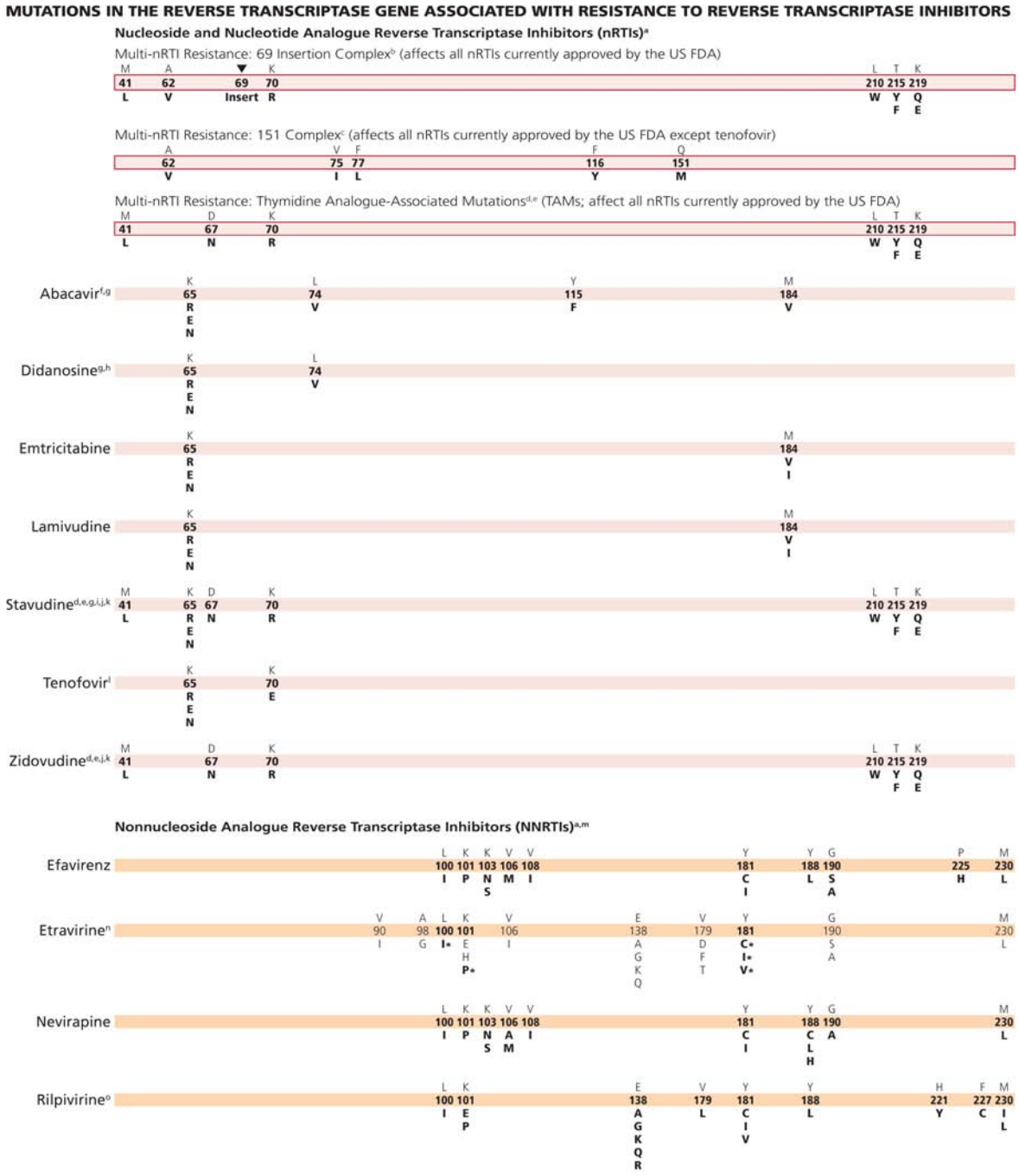
The most commonly used resources are lists of mutations related to resistance to every particular drug [84]. The mutations included in the list have been typically identified by one or more of the following criteria [50, 78, 84, 101]:

- *in vitro* passage experiments or validation of contribution to resistance by using site-directed mutagenesis

- *in vitro* susceptibility testing of stored laboratory samples or clinical isolates, usually with one or a maximum of two mutations
- nucleotide sequencing of viruses from patients in whom the drug is failing to
- ascertain the mutations typically selected at virological failure
- correlation studies between genotype at baseline and virologic response in patients exposed to the drug to fine-tune a “weighted” score

Clinicians routinely use these lists as a very helpful resource in their clinical practice when designing initial, switch or salvage antiretroviral regimens (Figure 1).

Figure 1. Mutations in HIV-1 associated with an impact on susceptibility to antiretroviral agents, by HIV gene target [84].



The letter above each position is the wild-type amino acid and the letter(s) below each position indicate the substitution(s) associated with drug resistance. **Amino acid abbreviations**: A, alanine; C, cysteine; D, aspartate; E, glutamate; F, phenylalanine; G, glycine; H, histidine; I, isoleucine; K, lysine; L, leucine; M, methionine; N, asparagines; P, proline; Q, glutamine; R, arginine; S, serine; T, threonine; V, valine; W; tryptophan; Y, tyrosine.

MUTATIONS IN THE PROTEASE GENE ASSOCIATED WITH RESISTANCE TO PROTEASE INHIBITORS[p,q,r]

	L 10	G 16	K 20	L 24	V 32	L 33	E 34	M 36	M 46	G 48	I 50	F 53	I 54	D 60	I 62	I 64	A 71	G 73	V 82	I 84	I 85	N 88	L 90	I 93
Atazanavir +/- ritonavir[s]	I F V C	E	R M I T V	I	I	I F V	Q	I L V	I L	V	L	Y	L V M T A	E	V	L M V	V I T L	C S T A	A T F I	V	V	S	M	L M
Darunavir/ ritonavir[t]	V 11 I				V 32 I	L 33 F			I 47 V		I 50 V		I 54 M L					T 74 P	L 76 V	I 84 V			L 89 V	
Fosamprenavir/ ritonavir	L 10 F I R V				V 32 I				M 46 I L	I 47 V	I 50 V		I 54 L V M					G 73 S	L 76 V	V 82 A F S T	I 84 V			L 90 M
Indinavir/ ritonavir[u]	L 10 I R V		K 20 M R	L 24 I	V 32 I			M 36 I	M 46 I L				I 54 V				A 71 V T	G 73 S A	L 76 V	V 77 I	V 82 A F T	I 84 V			L 90 M
Lopinavir/ ritonavir[v]	L 10 F I R V		K 20 M R	L 24 I	V 32 I	L 33 F			M 46 I L	I 47 V A	I 50 V	F 53 L	I 54 V L A M T S		L 63 P		A 71 V T	G 73 S	L 76 V	V 82 A F T S	I 84 V			L 90 M
Nelfinavir[u,w]	L 10 F I			D 30 N				M 36 I	M 46 I L								A 71 V T		V 77 I	V 82 A F T S	I 84 V	N 88 D S		L 90 M
Saquinavir/ ritonavir[u]	L 10 I R V			L 24 I						G 48 V			I 54 V L		I 62 V		A 71 V T	G 73 S	V 77 I	V 82 A F T S	I 84 V			L 90 M
Tipranavir/ ritonavir[x]	L 10 V					L 33 F		M 36 I L V	K 43 T	M 46 L	I 47 V		I 54 A M V	Q 58 E		H 69 K R		T 74 P		V 82 L T	N 83 D	I 84 V		L 89 I M V	

MUTATIONS IN THE ENVELOPE GENE ASSOCIATED WITH RESISTANCE TO ENTRY INHIBITORS

	G 36	I 37	V 38	Q 39	Q 40	N 42	N 43
Enfuvirtide[y]	D S	V	A M E	R	H	T	D
Maraviroc[z]	See User Note						

MUTATIONS IN THE INTEGRASE GENE ASSOCIATED WITH RESISTANCE TO INTEGRASE STRAND TRANSFER INHIBITORS[aa]

	T 66	E 92	T 97	F 121	E 138	G 140	Y 143	S 147	Q 148	N 155
Dolutegravir[bb]				Y	A K	S A			H	
Elvitegravir[cc]	I A K	Q G	A	Y				G	R H K	H
Raltegravir[dd]				Y						

Weighted genotypic lists or scores constitute a step towards scientific excellence, because the impact of every single mutation on drug susceptibility varies significantly [101].

These lists represent valuable and straightforward point-of-care resources used to maximize the virological response in complicated cases, thus narrowing the gap existing between complicated and sophisticated studies of basic HIV-1 resistance knowledge and its translation into routine clinical care, and are therefore very helpful for clinicians and for investigations in HIV resistance.

Although some outstanding publicly available resources offer systems of scoring for all available antiretroviral drugs [101], specific weighted genotypic scores have been specifically developed for a limited number of drugs, usually when enough experience is compiled.

In some cases, the scores can include mutations associated with increased susceptibility, therefore granting negative points in the final score. In addition, many resistance-conferring mutations decrease the replication capacity of HIV in comparison with the wild-type virus [78]. The clinical correlates of this mutation-derived "hypersusceptibility" and replication capacity measurements, however, remain largely unknown. Weighted sensibility scores do not currently take into account the impact on fitness or replicative capacity of the mutations. Nevertheless, De Luca *et al*. nicely idenfied that after normalizing for viral susceptibility to the employed regimen or in patient subsets with suboptimal virologic response, higher viral replication capacity may predict worse subsequent treatment outcomes [123].

Etravirine is a typical case of the construction of these scores, with data coming from all the inputs previously defined [120, 124-129].

In Table 3, we show an updated correlation between three different scores to estimate the activity of etravirine, and the rules for their interpretation [8, 101].

These weighted scores are highly predictive of treatment response and are continuously updated paralleling the accumulation of the clinical experience with the drug and research results on resistance mutation knowledge.

Evaluation of resistance to the newer antiretroviral drugs in subjects with virological failure.

The nearly simultaneous launch of a plethora of new antiretroviral agents with expanded activity in existing (tipranavir, darunavir, and etravirine) and novel classes (raltegravir, dolutegravir, and maraviroc) has resulted in unprecedented success for HIV-1-infected patients who have received and failed multiple treatments [8, 130]. Currently achieved virological suppression rates in patients with triple-class failure were until recently only seen in drug-naive patients with wild-type HIV [33, 131]. It is critical that clinicians use the available agents carefully and become familiar with the complexity of dealing with their resistance patterns.

These new drugs have demonstrated superiority in key efficacy parameters in their salvage trials until recently, when it has become no longer ethical to compare new drugs against placebo [111, 113, 127, 128, 132-138]. Unfortunately, we lack head-to-had comparative trials between many of these new antiretrovirals in salvage. Trials generally have evaluated only a single new drug, the exception being darunavir and etravirine in the DUET trials [127-129].

Reports of combined use of these new drugs in routine clinical practice show very promising results [130, 139], and constitute one of the few scenarios where routine clinical practice can show better results than randomized clinical trials.

The optimal choice of drugs relies on the evaluation of resistance that compromises their activity and varies depending on previous drug exposure and virological failure [87]. Importantly, viral load suppression to <50 copies/mL must be aggressively pursued in salvage regimens to preclude the emergence of resistance to these life-saving agents.

The inclusion of at least 1 drug of a new class is strongly recommended, as is the presence of 3 active drugs in the regimen. Failure of these drugs can quickly lead to loss of activity and even class cross-resistance, leaving patients with few if any options for the near future.

To maximize the success of the new fully suppressive regimen, clinicians should preferably prescribe drugs from new families without cross resistance to previous drug exposure (integrase inhibitors like dolutegravir, or the CCR5 antagonist Maraviroc), drugs with high genetic barrier to resistance (e.g. boosted protease inhibitors like darunavir/ritonavir, second-generation NNRTIs like etravirine and strand-transfer integrase inhibitors like dolutegravir), and seek for the highest antiviral activity of the regimen [140]. In settings with full availability of new ARVs, it should often be possible to design a regimen containing three drugs to which the virus remains fully susceptible even in subjects with multidrug resistance [10, 63].

The principles of integrase inhibitor resistance parallel those of NRTI, NNRTI, and PI resistance. It is caused by primary mutations that reduce integrase inhibitor susceptibility in combination with secondary mutations that further decrease virus susceptibility and/or compensate for the decreased fitness associated with the primary mutations [141]. There is a genetic barrier to integrase resistance, defined by the number of mutations required for the loss of clinical activity of integrase inhibitors and there is extensive but incomplete cross-resistance among the integrase inhibitors [63]. Paralelling the situation with NNRTIs, raltegravir and elvitegravir (the so-called first-

generation integrase inhibitors) share high degrees of cross-resistance, while the later is limited from those drugs to dolutegravir, which remains active against some clones resistance to first-generation integrase inhibitors [142]. Actually, dolutegravir data are the first clinical demonstration of the activity of any integrase inhibitor in subjects with HIV-1 resistant to raltegravir [132].

NON-NUCLEOSIDE REVERSE TRANSCRIPTASE INHIBITORS (NNRTIs).

Clinical efficacy and clinical implication of resistance data.

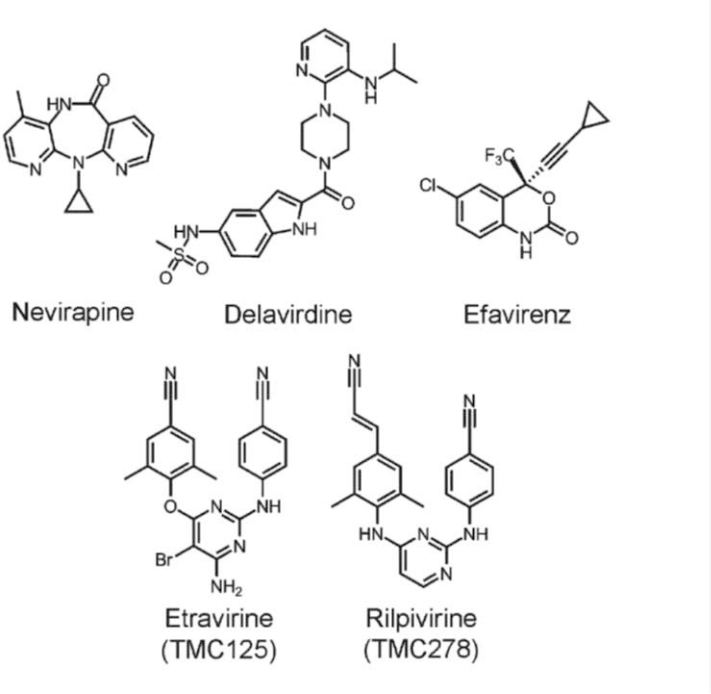
Reverse transcription begins when the RNA viral particle enters the cytoplasm of a human target cell as part of a nucleoprotein reverse transcription complex. This complex includes matrix and capsid structural proteins and the accessory Vpr protein, together with the reverse transcriptase and the integrase. The capside protein dissociates soon during uncoating from this complex. The process of reverse transcription generates a linear DNA duplex with terminal duplications known as long terminal repeats (LTRs) [143].

The NNRTI-binding pocket resides in the palm domain of RT, an especially flexible portion of the protein, where NNRTIs bind approximately 10A from the polymerase active site, disrupting RT polymerase function allosterically [144].

NNRTIs are small molecules with strong affinity for a hydrophobic pocket located close to, but not contiguous with, the catalytic domain of RT, thereby inhibiting the RT. Inhibitor binding affects the flexibility of the enzyme, thereby blocking its ability to synthesize DNA. Nearly all of the NNRTI resistance mutations are within or adjacent to this NNRTI-binding pocket [145, 146].

The chemical structure of NNRTIs is shown in Figure 2.

Figure 2. Chemical structures of the NNRTI compounds.



NNRTIs have a low genetic barrier to resistance. High-level resistance to nevirapine generally requires one mutation, high-level resistance to efavirenz and rilpivirine generally requires one to two mutations, and high-level resistance to etravirine requires two or more mutations [3, 10, 78, 101]. Although etravirine and rilpivirine have similar chemical structures, rilpivirine has a lower genetic barrier to resistance than etravirine and is approved solely for first-line therapy in subjects with a baseline plasma HIV-1 RNA <100.000 copies/mL. Indeed, a newly recognized RAM (E138K) has been shown to emerge in about one-half of patients developing virological failure while receiving rilpivirine and with successful genotypic amplification results [147-154].

In an unusual example of 'cross-talk' between the NRTI and NNRTI-resistance mutations, subjects with tenofovir DF/emtricitabine/rilpivirine failure are more likely to develop M184I (rather than the more common RAM M184V) in combination with E138K [147-149, 152, 153]. Actually, in an analysis done in routine clinical samples

with RAMs to nevirapine and efavirenz we found that most samples (69%) harboured more than one RAM to first-generation NNRTIs: 42% harboured two RAMs, 21% three RAMs and 6% four or more RAMs [87]. Therefore, RAMs accumulation is a speedy and active process in subjects failing an NNRTI-based regimen if drug pressure is maintained.

The low genetic barrier to resistance of some NNRTIs makes it possible for multiple independent NNRTI-resistant lineages to emerge *in vivo*, even if not all of these will be detected by standard genotypic resistance testing, as previously discussed [93-97, 155].

In treatment-naive patients, ultra-deep sequencing did not detect additional major NNRTI-resistant mutations, suggesting that etravirine might be effective in patients with K103N as the only transmitted DRM. However, in NNRTI-experienced patients, ultra-deep sequencing often detected additional major NNRTI-resistant mutations, including some mutations impacting etravirine, suggesting that etravirine might not be fully active in patients with acquired K103N in population genotypes [155]. Y188L is another RAM selected by rilpivirine. It causes high-level resistance to rilpivirine, nevirapine and efavirenz but only potential low-level resistance to etravirine [9, 84].

The NNRTI RAMs can be classified into the following categories [78, 156]:

- primary NNRTI RAMs that cause high-level resistance to one or more NNRTI and that are among the first to develop during NNRTI therapy
- secondary NNRTI RAMs that usually occur in combination with primary NNRTI RAMs, but that also have clinically significant implications for choosing an NNRTI, particularly etravirine
- minor non-polymorphic mutations that may occur alone or in combination with other NNRTI RAMs and that cause consistent but low-level reductions in NNRTI susceptibility

- polymorphic accessory mutations that modulate the effects of other NNRTI RAMs

The most common NNRTI mutations seen in clinical practice are L100I, K101E/P, K103N/S, V106A/M, Y181C/I/V, Y188L, G190A/S/E and M230L.

All these mutations cause high-level resistance to nevirapine with the exception of L100I, and all of them cause intermediate- or high-level resistance to efavirenz excluding V106A and Y181C/I/V [9, 78].

Each of the primary NNRTI resistance mutations – K103N/S, V106A/M, Y181C/I/V, Y188L/C/H, and G190A/S/E – cause therefore high-level resistance to nevirapine and variable but significant resistance to efavirenz, ranging from about twofold for V106A and Y181C, sixfold for G190A, 20-fold for K103N, and more than 50-fold for Y188L and G190S [78]. On clinical grounds nevertheless, anyone of these RAMs contraindicates the use of both nevirapine or efavirenz. In contrast, patients with any single primary NNRTI RAM could benefit from etravirine salvage therapy, although RAMs at position 181 and to a lesser extent 190 compromise etravirine response and may provide the foundation for the development of high-level etravirine resistance [87].

L100I, K101P, P225H, F227L, M230L, and K238T are secondary mutations that usually occur in combination with one of the primary NNRTI RAMs.

There are also minor NNRTI RAMs. A98G, K101E, V108I, and V179D/E are common NNRTI RAMs that reduce susceptibility to nevirapine and efavirenz about twofold to fivefold. For instance, while K103R alone, occurring in about 1% of untreated persons, has no effect on NNRTI susceptibility, the combination of K103R plus V179D reduces nevirapine and efavirenz susceptibility by 15-fold, in a clear example of synergy among RAMs [157]. Actually, among samples with no known NNRTI mutations, the most resistant samples contained K101P or a combination of K103R and V179D.

V179D, and rarely A98G and V108I, are observed in patients who have never been treated with NNRTI [156]. This is a challenging situation, because the optimal management of patients with viruses containing these RAMs is not known.

Finally, several highly polymorphic RT mutations, such as K101Q, I135T/M, V179I, and L283I, reduce susceptibility to nevirapine and efavirenz by about twofold and may act synergistically with primary NNRTI RAMs.

In opposition to protease and some NRTI RAMs, NNRTI RAMs have a minimal impact on HIV's replication activity and fitness, and so can persist stable and for long term in the absence of treatment [158, 159].

There is also an interaction between NRTI RAMs and NNRTI susceptibility. There is a large body of evidence showing that type I TAM increase NNRTI susceptibility [160, 161]. Mutations at positions 215, 208 and 118 were independently associated with NNRTI hypersusceptibility in a cohort of 444 NRTI-experienced, NNRTI-naive patients with paired baseline genotypes and phenotypes [162]. Furthermore, it is readily seen in multicycle susceptibility assays and in enzyme inhibition assays [163]. It occurs in more than 20% of NRTI-experienced patients and was associated with greater reduction of plasma HIV RNA and increase in CD4+ cells in a clinical trial [161].

NEVIRAPINE

Nevirapine was one of the first NNRTIs to be studied in humans and the first one to receive approval in 1997 in Europe. Together with efavirenz became a standard of care in ART, both in initial treatment and in switch/simplification strategies [164-170].

It was the first anchor drug to demonstrate that triple drug therapy with nevirapine and a backbone of two NRTIs (zidovudine and didanosine) led to a substantially greater and sustained decrease in plasma viral load than nevirapine plus only 1 NRTI, paving

the way to the successful era of triple drug therapy [170]. It also demonstrated that achieving an undetectable HIV-1 RNA could at least forestall the development of resistance [169, 171].

Nevirapine-based antiretroviral regimens have demonstrated comparable efficacy to indinavir, nelfinavir, efavirenz, lopinavir/ritonavir and atazanavir/ritonavir in initial treatment [167-173]. Despite having similar efficacy to efavirenz, it did not met formal noninferiority within the predefined 10% limit in the 2NN study, stemming a long-standing debate about the comparative efficacy of nevirapine vs efavirenz [169, 174]. More recently, some studies have confirmed a high efficacy of nevirapine plus tenofovir DF and emtricitabine in initial ART [167, 168, 175].

The rates of selection of resistance at virological failure with nevirapine were greater than with the boosted PIs lopinavir/ritonavir or atazanavir/ritonavir [167, 172]. These studies established indeed the different behaviour of a first generation NNRTI and a boosted PI if virological failure occurs. While subjects with nevirapine failures commonly selected NNRTI mutations (mainly Y181C/I/V/C, and other mutations such as K103N/S/T, V106A/M, V108I/M/V and K101E/R), no protease mutations were selected in subjects failing in the boosted PI arms in initial ART [167]. A similar finding was seen in the NRTI component of the regimen. Subjects failing with nevirapine usually selected M184V/I and more rarely K65R, while subjects on the boosted PI arm did not select NRTI-associated RAMs if treated with atazanavir/ritonavir, or these RAMs were rarely seen when treated with lopinavir/ritonavir [172]. The same difference has been observed between the other first generation NNRTI efavirenz and a boosted PI (lopinavir/ritonavir and atazanavir/ritonavir) in the ACTG studies 5142 and 5202 [176, 177]. The resistance lower of nelfinavir, when compared with nevirapine, was significantly lower than a boosted PI, with greated selection of M184V and the selection of some primary protease RAMs (D30N) [178].

Therefore, it was soon recognised that initial therapy with boosted PI-based regimens resulted in less resistance within and across drug classes, a finding of particular significance for the developing world, where rates of resistance to NRTIs and NNRTIs at 48 weeks are much higher than has been seen in both cohorts and clinical trials in well-resourced countries due to limited, if any, HIV-RNA monitoring. [179-181].

In a systematic overview evaluating the resistance consequences after virologic failure on initial ART, first generation NNRTIs (both nevirapine and efavirenz) and boosted PI regimens provided the highest rates of virological suppression in treatment-naive HIV-infected persons. Treatment option scores were higher in subjects who failed boosted PI-containing regimens versus NNRTI-containing regimens, however [19, 182].

In a systematic review and meta-analysis done in ART-naives, efavirenz-based first line ART was significantly less likely to lead to virologic failure compared to nevirapine-based ART, though differences were marginally significant in both randomised controlled trials (RR 1.04 [1.00–1.08] and observational studies (RR 1.06 [1.00–1.12] [183, 184]. In a cohort study, Virological outcomes of nevirapine-based ART were comparable to efavirenz-based regimens in initial ART or when tuberculosis developed while taking established nevirapine- or efavirenz-based therapies [185].

The virological efficacy of the drug has been outstanding in all trials. Nevirapine showed similar rates of initial plasma HIV-1 RNA decline during the first 2 weeks of treatment than efavirenz [186], and is one of the antiretroviral drugs that achieve higher reductions of residual plasma viremia to below 1 copy/mL and are associated with higher rates of undetectable HIV-DNA [187, 188].

However, nevirapine treated subjects had a fourfold discontinuation rate caused by adverse events than those treated with atazanavir/ritonavir [167].

An extended-release (XR) formulation has been developed for once daily administration, providing reduced nevirapine exposure with lower maximum plasma concentration while maintaining adequate steady-state through levels. Based on the pharmacokinetic findings of the 2NN study, the target pharmacokinetic profile of the nevirapine XR formulation was a median C_{min} of 3 µg/mL, which is >15 fold higher than the 95% inhibitory concentration for wild type HIV-1 [169, 189]. In combination with emtricitabine and tenofovir DF it demonstrated noninferior efficacy to the twice daily immediate release formulation in initial ART, with a similar safety and adverse event profile, and with the better convenience of once-daily dosing [168, 190, 191].

In international treatment guidelines nevirapine has been initially rated as a preferred regimen, and in more recent years rated as an alternative component in initial ART [15, 16].

Currently available as a generic drug, generic NVP plus branded tenofovir/emtricitabine (TDF/FTC) constitutes one of the most cost-effective treatments in Europe [192].

NVP has been a drug commonly used in switching strategies [166, 193-196]. In ART simplification nevirapine displayed similar rates of efficacy at 12 and 36 months against efavirenz, though nevirapine achieved the lowest rates of virological failure and higher lipid benefits in the extended three-year follow-up [197-201].

Short-term toxicity-related issues (such as the development of cutaneous reactions and liver toxicity are known safety concens of nevirapine [202]. In a meta-analysis in initial ART nevirapine was associated with a higher frequency of severe adverse events, in particular treatment discontinuations [203]. In April 2005, the European Medicines Agency (EMA) issued an amendment in the package insert information of tablets and oral solution concerning the CD4+ cell count limits that should prevent the initiation of any treatment with the drug, mainly based on information from a meta-analysis of several studies done by the manufacturer [204-206]. Due to greater risk of symptomatic

hepatic events, including serious and life-threatening events, nevirapine should only be initiated in antiretroviral-naive women with CD4 counts <250 cells/mm^3 or in antiretroviral-naïve men with CD4 counts >400 cells/mm^3 [207].

HLA-C*04:01 carriage was identified as a risk factor for nevirapine-induced hypersensitivity reactions in a Malawian HIV cohort, but was never validated in a larger cohort of patients and the screening of this allele was not introduced in clinical practice [208]. HIV treating physicians highly experienced with nevirapine use quickly realised after the amendment of the nevirapine package information that this increased risk had not been observed in pre-treated patients starting nevirapine as a simplification switch strategy. This is, having achieved undetectable plasma HIV-1 RNA levels with another regimen, and subsequently changing to a simpler, more convenient, and with a more favourable lipid profile nevirapine-based regimen. Data emerged from some cohorts, randomized clinical trials, and a meta-analysis of randomized clinical trials indicating that pre-treated individuals with high CD4+ cell counts have no increased risk for hypersensitivity reactions or treatment-limiting toxicity provided there is no detectable viremia at inititation of nevirapine [194, 209-214]. Considering that there was enough scientific evidence to support that pre-treated subjects starting a nevirapine-based regimen with “high” (above the gender-specific thresholds) CD4+ cell counts and an undetectable plasma HIV-1-RNA did not have higher risks of treatment-limiting toxicity due to hypersensitivity reactions or hepatotoxicity than naive patients with “low” (below threshold) CD4 cell counts (for whom the risk is around 1%) an investigator sponsored initiative contacted the EMA who eventually agreed on changing the package insert information accordingly (application number II/0094) [215].

Nevirapine has a well-known initial potential toxicity profile, but has not been associated to any specific long-term toxicity, including central nervous system, bone, kidney, liver, lipodystrophy or cardiovascular, and has an optimal lipid profile, safety during pregnancy, and a favourable penetration in the seminal fluid and the central

nervous system [216-220]. Cohort studies have reported a low rate of discontinuation caused by toxicity in the long-term follow-up with nevirapine-containing ART together with the maintenance of virological suppression in patients switched with undetectable viral loads [216, 221].

Indeed, first-line nevirapine treatment is associated with a favourable lipoprotein profile, i.e., an increase in HDL-cholesterol and apo A1 plasma levels, mainly due to an increase in apoA-I production while ApoA-I catabolism remains unchanged [218, 219, 222-224]. Some studies have also observed a modest increase of lecithin:cholesterol acyltransferase and cholesteryl ester transfer protein activity [224]. The lipid profile observed in patients who are switched from a PI-based regimen to a nevirapine-based regimen improves in a very similar fashion, reducing the number and lipid content of atherogenic LDL particles, and increasing the protective HDL fraction. Although total triglyceride levels remained unchanged, a reduction in the VLDL-1 fraction contributes to the reduction of LDL particles. [164, 197, 218, 220, 222, 225]. A a small 52-week randomized study also reported that switching from efavirenz to nevirapine was associated with significantly decreased low-density lipoprotein cholesterol levels, compared with continuation of efavirenz therapy [223].

First-generation NNRTIs nevirapine and efavirenz bind to the NNRTI-binding protein with relying heavily on π-stacking interactions to Y181 and Y188, which can easily mutate, conferring resistance [144]. RAMs selected after nevirapine (or efavirenz) failure are all located in the drug-binding pocket. While most affect residues directly involved in inhibitor binding, a few have been found to act indirectly, by changing the position or orientation of the aminoacids involved with direct contact with the drug [226, 227]. Due to the interaction between every NNRTI and the hydrophobic pocket, emerging RAMs tend to be somewhat drug-dependent [145, 146, 228, 229].

The list of RAMs impacting nevirapine susceptibility is currently fully understood and includes L100I, K101P, K03NS, V106AM, V108I, Y181C/I, Y188C/L/H, G190A, and M230L, all them occurring in the pocket where the drug is attached [84]. All the above-mentioned RAMs generate cross-resistance except Y181C, which causes only a two-fold decrease in phenotypic susceptibility of efavirenz *in vitro* .

The A376S substitution in the connection subdomain of HIV-1 RT has been shown to cause selective nevirapine resistance and confer an increased risk of virological failure to nevirapine-based ART [230, 231].

Connection domain RAMs are a typical example of mutations than despite potentially having a clinical impact can remain unnoticed in routine genotypes [231-235]. Resistance testing of the HIV-1 RT gene has often included approximately the first 250 amino acids of the RT enzyme and has excluded the C-terminal RT domain, comprising amino acids 289–560. The connection domain is located between codons 316 and 437 of HIV-1 RT, connecting the DNA polymerase (codons 1–315) and the RNase H (codons 438–560) domains. Dau *et al*. have recently reported that connection domain mutations are frequent in treatment-experienced populations, and are associated with reduced susceptibility to some NRTIs and with a diminished response to ARV therapy [86].

Data on the safety, efficacy and resistance patterns of the combination of nevirapine plus emtricitabine and tenofovir DF have been scarce despite being a combination extensively used as a long-term simplification regimen, a scenario where the risk of viral rebound decreases with longer duration of viral suppression [236]. In a recent simplification trial comparing switching the couple of NRTIs to once-daily fixed dose abacavir/lamivudine or tenofovir DF/emtricitabine (TDF/FTC), 118 out of 333 patients were actually receiving nevirapine [237]. In addition, the trial only evaluated the switch in NRTIs, while nevirapine remained unchanged. Furthermore, 34% of patients in the

tenofovir DF/emtricitabine group were actually maintaining tenofovir from their pre-randomisation regimen, thus further limiting the power to analyse the performance of nevirapine plus tenofovir regimens. Reassuringly, the rates of virological failure and resistance selection were low.

Some recent small reports have found an unexpectedly high rate of virologic failure in treatment-naïve participants who received nevirapine plus tenofovir DF and either lamivudine or emtricitabine. In the first report (only presented in a meeting) seven out of 23 (30%) virological faiures occurred in naive patients, five of whom developed the Y181C mutation [238]. In the second one, 8 out of 36 patients (22%) presented an early non-response or viral rebound [239]. All of them showed at failure one or more NNRTI RAMs absent at baseline by standard bulk genotyping (Y181C, G190A and K103N), and most of them also selected K65R and M184V. Factors associated with failure were once daily administration of nevirapine, higher baseline viral load, lower initial CD4+ cell counts, and non-B (CRF or C) HIV-1 subtypes. In the last report, 3 out of 7 patients experienced virological rebound at week 12, and also selected for the same spectrum of new treatment limiting resistance [240].

These high rates of virologic failure with regimens including nevirapine plus tenofovir plus emtricitanine or lamivudine have not been seen in routine clinical practice in countries where these regimens have been commonly used.

Although the reasons for these unprecedented rates of failure remain unclear and were not seen in other naives trials [167, 168], they forced the issue of a warning in the DHHS guidelines that advised caution and close monitoring of virologic responses with these regimens because of those reports of early virologic failure, while awaiting further information [16].

So, there is a great need of accurate data on the long term efficacy, rates of virological failure and patterns of RAMs detected in subjects receiving regimens including

nevirapine and tenofovir DF plus emtricitabine or lamivudine, and these data must be searched in cohorts as no randomized clinical trials have been done with this combination used in switch/simplification.

EFAVIRENZ

Efavirenz has been a preferred regimen in initial ART in all international treatment guidelines since the approval of the drug in 1998. The demonstration in 1999 of its greater antiviral activity and better tolerability than indinavir established efavirenz-based triple ART as the gold-standard comparator in all antiretroviral regimens in initial ART [241]. The long-term follow-up confimed the greater antiviral efficacy and tolerability of the regimen [242].

Later on, the combination the combination of tenofovir DF, lamivudine, and efavirenz demonstrated higher efficacy in antiretroviral-naive patients, with better lipid profiles and less lipodystrophy than its comparators with zidovudine or stavudine [243-245]. Those practice-changing trials established this regimen as the gold standand of initial ART until 2015 [15-17, 243].

It has been better or as good virologically than all its comparators for years and years, including indinavir, nevirapine, lopinavir/ritonavir, maraviroc, atazanavir/ritonavir, rilpivirine, elvitegravir/cobicistat [148, 152, 153, 169, 176, 177, 246-248]. Efavirenz-based regimens have strong virologic efficacy, including in patients with high plasma HIV-1 RNA and severe CD4 depletion [249].

In summary, large randomized, controlled trials in ART-naive patients have demonstrated potent and durable viral suppression in patients treated with efavirenz plus two NRTIs with superiority or non-inferiority to:

• In ACTG 5142, efavirenz was superior to lopinavir/ritonavir, although drug resistance was more common after efavirenz failure [176]

• In the 2NN study, compared to efavirenz, nevirapine did not meet non-inferiority criteria [169]

• In ACTG 5202, efavirenz was comparable to atazanavir/ritonavir when each was given with either tenofovir DF/emricitabine or abacavir/lamivudine [177]

• In the ECHO and THRIVE studies, efavirenz was non-inferior to rilpivirine, with less virologic failure but more discontinuations due to adverse events. The virologic advantage of efavirenz was most notable in participants with pre-ART viral loads >100,000 copies/mL, and NRTI and NNRTI resistance was more frequent with rilpivirine failure [147, 151-153].

• In the GS 102 study, the co-formulated single tablet regimen efavirenz/tenofovir DF/emtricitabine was non-inferior to elvitegravir/cobicistat/tenofovir DF/emtricitabine in an open-label study, at both 48, 96 and 144 weeks [148, 250]

In 2015, efavirenz has been recommendation has been downgraded for the first time ever to an alternative regimen due to its inferior efficacy to the strand transfer integrase inhibitors dolutegravir in the main study endpoint in the phase III Single study, as well as its inferior efficacy versus raltegravir in the long-term follow-up in the Startmark study [15, 16, 251-255]. Concerns about the tolerability of efavirenz in clinical trials and practice, especially the high rate of central nervous system related toxicities, and a possible association with suicidality observed in one analysis of four clinical trials have also had a role in this decision [256].

Efavirenz is available co-formulated with emtricitabine and tenofovir DF in a once-daily single tablet regimen.

Efavirenz is effective against many point mutations; however, efficacy is strongly compromised by the K103N, the most prevalent NNRTI RAM. It has a low genetic barrier to resistance, especially in patients with suboptimal adherence.

The list of RAMs impacting efavirenz susceptibility is well-known and includes L100I, K101P, K103N/S, V106M, V108I, Y181C/I, Y188L, G190S/A, P225H and M230L [3, 84, 101].

A K103N substitution is the HIV-1 RT RAM most frequently observed among plasma samples from patients for whom combination therapy including efavirenz failed, occurring in most cases. V108I and P225H mutations are observed frequently, predominantly in viral genomes that also contained other NNRTI RAMs. L100I, K101E, K101Q, Y188H, Y188L, G190S, G190A, and G190E mutations are also observed. V106A, Y181C, and Y188C RAMs, which have been associated with high levels of resistance to other NNRTIs, are more infrequently seen [3, 227].

ACTG studies 5142 and 5202 have been pivotal in understanding the different behaviour of drugs with low or high barrier to resistance development when virological failure occurs. Despite having similar or even superior overall efficacy, among patients with virologic failure, emergent resistance mutations were less frequent in those assigned to receive a boosted PI (atazanavir plus ritonavir or lopinavir/ritonavir) than those assigned to receive efavirenz [176, 177, 257]. A similar finding has arised from the randomized comparison against dolutegravir, resulting in inferior efficacy of efavirenz with higher rates of resistance selection in subjects with virological failure [63, 253].

Variant strains of HIV-1 constructed by site-directed mutagenesis confirmed the role of K103N, G190S, and Y188L substitutions in reduced susceptibility to efavirenz [258]. Further, certain secondary RAMs (V106I, V108I, Y181C, Y188H, P225H, and F227L) conferred little resistance to efavirenz as single mutations but enhanced the level of

resistance of viruses carrying these mutations in combination with K103N or Y188L [157]. Viruses with K103N or Y188L mutations, regardless of the initial selecting NNRTI, exhibited cross-resistance to first-generation NNRTIs.

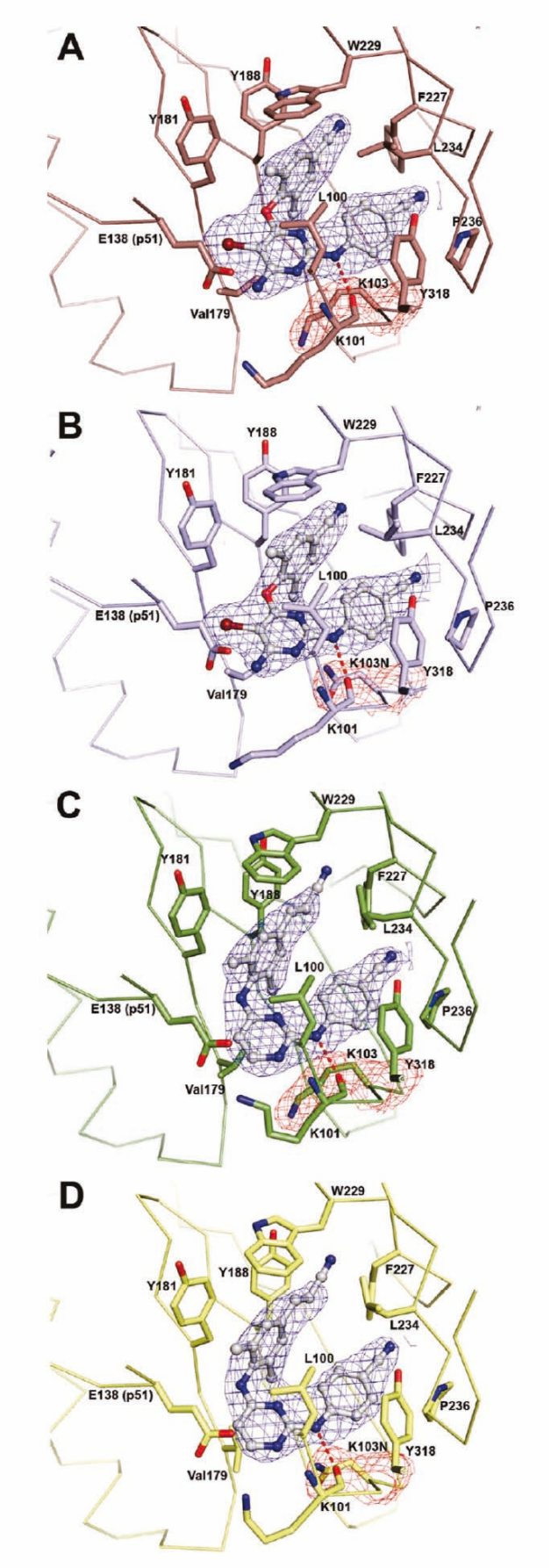
ETRAVIRINE

Etravirine is a diarylpyrimidine NNRTI with activity against some HIV-1 strains resistant to first-generation NNRTIs, mainly due to its inherent flexibility between aromatic rings, allowing the compound to adopt multiple conformations, helping to maintain activity against a wide range of RAMs [124, 144]. Its design endeavoured to reduce the interactions with Y181 and Y188 positions, which can easily mutate and confer resistance, relying more on W229, which is less prone to mutation. The crystal structure of both etravirine and rilpivirine are shown in Figure 3.

Approved for patients with previous treatment failures, it retains full activity against K103N mutants, the most common mutation seen with resistance to first-generation NNRTIs, and retains expanded activity against some HIV-1 resistant to nevirapine and efavirenz [87, 124, 126, 259, 260].

However, some active drugs with no or limited cross-resistance to prior antiretroviral drugs were not used in the pivotal studies for etravirine registration and the knowledge of the impact of every RAM on the activity of etravirine has improved. Therefore, there was scarce information about the real efficacy of etravirine-based salvage regimens in current clinical practice.

Figure 3. Cocrystal structures of (A) HIV-1 RT with wild-type-etravirine, (B) K103N-etravirine, (C) wild-type-rilpivirine, and (D) K103N-rilpivirine. Shown in bluemesh and contoured at 1.0σ is the composite omit map drawn around the inhibitor. The omit map for the K103(N) side chain is shown in red mesh. The hydrogen bond between the secondary amine and the main chain carbonyl of Lys101 is illustrated with a dashed line. As described by EB Lansdon et al (structure figures generated by Pymol; www.pymol.org) [144].


A
W229
Y188
F227
Y181
L234
L100
P236
E138 (p51)
K103
Y318
Val179
K101
B
Y188
W229
Y181
F227
L234
L100
P236
E138 (p51)
K103N
Y318
Val179
K101
C
W229
Y181
F227
Y188
L234
L100
E138 (p51)
P236
K103
Y318
Val179
K101
D
W229
Y188
Y181
F227
L234
L100
E138 (p51)
P236
K103N
Y318
Val179
K101

Etravirine activity varies according to the number and type of NNRTI mutations selected. It has a higher genetic barrier than older NNRTIs, requiring multiple mutations for loss of activity [8, 64, 127-129].

Its clinical efficacy and safety have been demonstrated in the TMC125-C223 and the pivotal double-blind, placebo-controlled trials with treatment-experienced patients DUET studies and in the STAR study as well [127, 128, 148, 151, 153, 259, 261], which included patients pre-treated with NRTIs, NNRTIs, and protease inhibitors. In the DUET studies, all patients also received darunavir/ritonavir, resulting in much higher rates of efficacy. High-degree resistance was uncommon in an analysis of a big dataset of 1586 routine clinical samples with RAMs to nevirapine and efavirenz, even in patients with proven resistance to first-generation NNRTIs, whereas low-to-intermediate etravirine resistance was more common [87].

Conversely, etravirine was inferior to a PI in PI–naive patients with previous NNRTI failures in a study (TMC125-C227 trial) prematurely stopped after a median of 14.3 weeks in an unplanned interim analysis, indicating that a certain degree of cross-resistance exists within the class [126]. A *post hoc* analysis identified the presence of Y181C, a fold change ≥10, and a higher number of etravirine mutations, with a diminished response to etravirine salvage.

The development of resistance to etravirine is complex and requires the coexistence of several specific RAMs.

Using pooled data from the DUET studies, only 13 of those RAMs at eight positions were associated with decreased virological response at week 24 [125]. The mutational pattern of etravirine is well characterized now, although uncommon mutations may not be represented in some scores [87, 119, 125, 262]. The list includes V90I, A98G, L100I, K101E/H/P, V106I, E138A, V179D/F/T, Y181C/I/V, G190A/S, M230L,

V179X is the most common position where mutations are selected after etravirine failures [262].

V179F, F227C, L234I, and L318F are rare mutations that are of increased importance now that etravirine is licensed. V179F occurs solely in combination with Y181C/I/V and acts synergistically to increase etravirine resistance from fivefold to 10-fold with Y181C/I/V alone to more than 100-fold [120]. F227C, a so far exceedingly rare RAM, reduces etravirine susceptibility 10-fold to 20-fold [78, 263]. L234I, which has been selected *in vitro* by etravirine, acts synergistically with Y181C to reduce etravirine susceptibility. Finally, L318F, which was intitially reported to reduce delavirdine and nevirapine susceptibility by 15-fold and threefold, respectively, has also been selected *in vitro* by etravirine and found to reduce etravirine susceptibility synergistically with Y181C [264].

The interaction between K103N and L100I is the typical situation where synergy must be captured by genotypic scores, as the resultant resistance is by far more severe than just the sum of the effect of both RAMs separately. Actually, subjects with baseline L100I at baseline in the DUET studies had a response rate above the threshold defined for the inclusion of the RAM in the response analysis [265]. However, although viruses with K103N are fully susceptible to etravirine, viruses with L100I plus K103N display about 10-fold decreased susceptibility [87, 125, 266, 267].

V90I and V106I are highly polymorphic mutations that were associated with decreased virologic response to etravirine in the DUET clinical trial, but could owe this association to their correlation with other NNRTI resistance mutations [129, 263, 267].

Essentially, 3 independent genotypic scores have been developed (Table 3). The first one has been correlated with treatment response [125]. Overall, the presence of 3 etravirine mutations was associated with a reduced response, though the weight for each mutation was different. Seventeen mutations have now been identified. The most

common are Y181C and G190A. More recently, E138G/Q substitutions have also been associated with etravirine resistance [268]. Intermediate etravirine activity is not uncommon among patients who have accumulated mutations after first-generation NNRTI failures [87]. This must be considered when designing the new regimen in subjects with treatment failure, to obtain an optimal response. Nevertheless, complete etravirine resistance is also uncommon [87]. In the DUET studies, etravirine protected the activity of darunavir, reducing the proportion of patients developing darunavir mutations [118].

A second score based on the correlation with 4248 phenotypes identified 30 mutations [269]. Although the mutations included and the scores given differ slightly, the final interpretation of both scores and the Stanford Database one is very similar (Table 4) [101, 125, 268]. The last score does not grant points to polymorphic mutations (V90I and V106I). Initially, it granted 10 points to K103N (a marker of previous NNRTI exposure and risk of coexistence of further mutations). However, the scoring for this RAM has recently been removed.

In an analysis including 1586 routine clinical samples with RAMs to nevirapine and efavirenz (K103N 60%, Y181C 37%, G190A 27%, V108I 13%), the prevalence of 13 specific etravirine RAMs was V179F 0.12%, G190S 3.9%, Y181V 0.1%, V106I 2.6%, V179D 1.6%, K101P 2.0%, K101E 10.1%, Y181C 36.9%, A98G 5.9%, V90I 6.9%, Y181I 3.6%, G190A 27% and L100I 9.1% (Figure 4) [87]. The five RAMs with the most impact on virologic response (V179F/D, G190S, Y181V and V106I) occurred less often.

Table 3. Weighted scores of the mutations conferring resistance to etravirine.

Mutation	Tibotec	Monogram	Stanford
V90I	1	1	0
A98G	1	-	10
L100I	2.5	4	30
K101E	1	2	15
K101H	1	1	10
K101N/Q	-	-	0
K101P	2.5	4	45
K103N/S/T	-	-	0
V106A	-	2	0
V106I	1.5	-	0
V106M	-	1	0
E138A	1.5	3	10
E138G	-	3	10
E138K	-	2	10
E138Q	-	1	10
V179D	1	1	10
V179E	-	3	10
V179F	1.5	1	15
V179L	-	2	10
V179M	-	1	0
V179T	1	-	10
Y181C	2.5	4	30
Y181I	3	4	60
Y181F	-	1	30
Y181S	-	-	15
Y181V	3	4	60
Y188C/H	-	-	0
Y188L	-	2	15
V189I	-	1	0
G190A	1	-	15
G190C	-	-	10
G190E	-	1	45
G190Q	3	-	45
G190S	1.5	-	15
G190T/V	-	-	10
H221Y	-	1	10
P225H	-	1	0
F227C	-	-	30
F227L	-	-	0
M230L	2.5	3	30
L234I	-	-	0
K238N	-	3	0
K238T	-	1	5
Y318F	-	-	0

NOTE. Tibotec, Monogram and Stanford scores are described in [125], [268], [101], respectively.

Some NRTI mutations (M41L, D67N, T69D/N, K70R, L74I/V, M184V, L210W, T215F/Y, K219N/Q/R and H208Y) confer NNRTI (including etravirine) hypersusceptibility, an issue not yet assessed in any score [84, 272]. The presence of these RAMs may increase subsequent virologic response to NNRTI-based regimens including nevirapine and efavirenz, as was seen in a prospective clinical trial cohort [161]. Indeed, in the DUET studies, 34% of all samples displayed a fold change <0.4 [270]. The clinical significance of this finding is not defined yet.

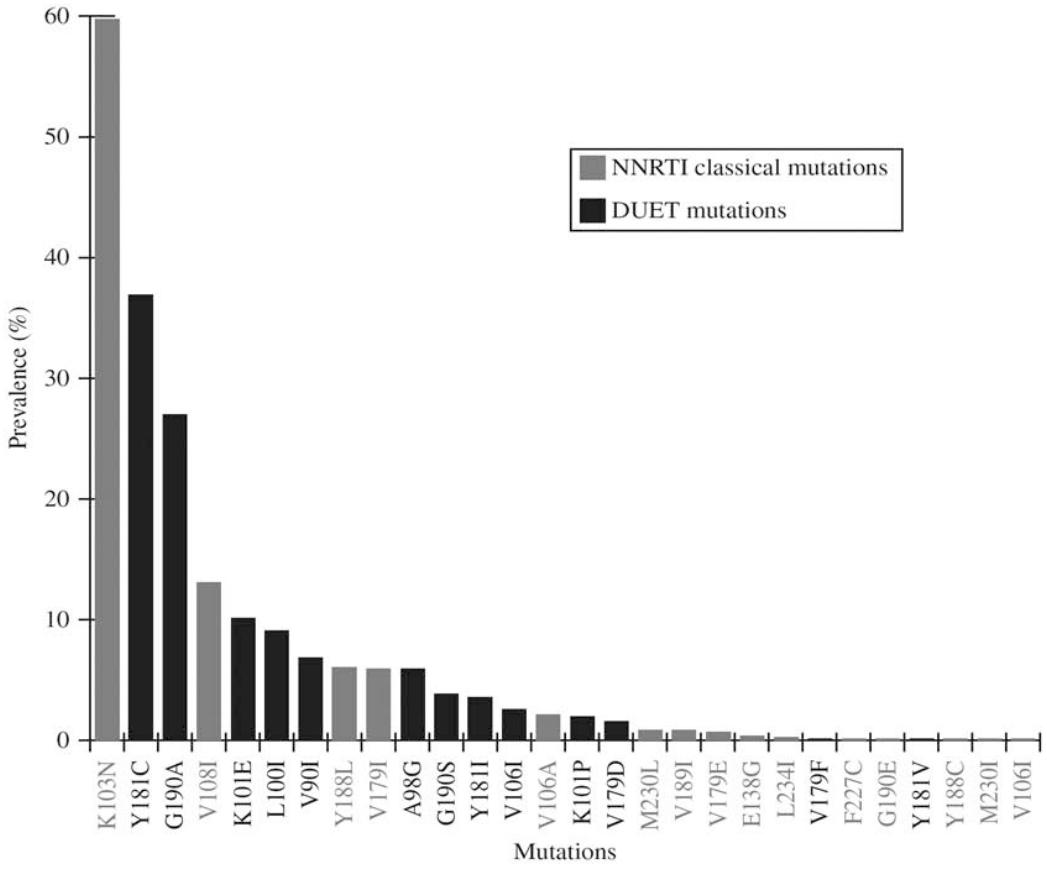
Table 4. Interpretation of the score points obtained in the calculus of the degree of resistance against etravirine.

Score	*Points*	*Interpretation*
Tibotec		
Correlation with the rate of virological response observed with etravirine in the DUET studies.	0-2	Highest response (74%)
	2.5 – 3.5	Intermediate response (52%
	≥4	Reduced response (38%), similar to the rate seen in the comparator arm without etravirine.
Monogram *		
Correlates with the phenotypic fold change in a huge independent database, independent of the DUET studies.	<4	Susceptible, correlates to a fold change <2.9.
	≥4	Intermediate resistance, correlates with a fold change > 2.9 *.
Stanford		
Etravirine resistance estimates drawn up by continuous updates done by the database team with different clinical or phenotypic correlations.	0-9	Susceptible
	10-14	Potential low-level resistance. The virus is likely to be fully susceptible yet it contains mutations that may be indicative of previous exposure to drugs in the same class.
	15-29	Low-level resistance.
	30-59	Intermediate resistance.
	≥ 60	High-level resistance.

* This score could not identify the cut-off for complete or high level resistance.

These data have increased our knowledge on the RAMs and patters of RAMs found in routine clinical practice in subjects with virological failure while receiving first-generation NNRTIs, and are useful in many countries where these drugs are still included in all preferred initial ART regimens [273]. From the perspective of drug resistance and on the basis of limited virologic monitoring data, optimal sequencing in these countries seems to involve use of a a tenofovir-containing NNRTI-based first-line regimen, followed by a zidovudine-containing, PI-based second-line regimen [273].

Figure 4. Prevalence of first-generation NNRTI and specific etravirine-related mutations with clinical impact on etravirine response in routine clinical samples with resistance to nevirapine or efavirenz (1998–2006) [87].



The study found a high prevalence of Y181C associated with two additional NNRTI mutations, and patients with four or more NNRTI mutations or etravirine-specific RAMs usually harboured Y181C, therefore highlighting the critical role of Y181C in the genesis of etravirine resistance, particularly in subjects failing nevirapine-based regimens. L100I (prevalence 9%) was another mutation with impact on etravirine resistance, usually selected with efavirenz failures.

Overall, 8.2% of the samples had three or more etravirine RAMs and only 1.1% had four or more. In addition, patterns of RAMs previously associated with intermediate etravirine resistance were present in 26.2% of the samples, whereas 4.85% displayed patterns of high-degree resistance. Etravirine resistance rates were lower than previously reported [87]. High-degree resistance was uncommon, even in patients with resistance to first-generation NNRTIs, whereas low-to-intermediate etravirine resistance was more common.

RILPIVIRINE.

Rilpivirine is a novel diarylpyrimidine derivative with a molecular structure very similar to etravirine. It inhibits HIV-1 replication by non-competitive inhibition of HIV-1 RT. It shows subnanomolar 50% effective concentrations (EC_{50} values) against wild-type HIV-1 group M isolates (0.07 to 1.01 nM) and nanomolar EC_{50} values against group O isolates (2.88 to 8.45 nM) [124, 274].

The discovery and development of the drug has elegantly shown how subtle variations between analogue compounds in structure activity relationships and X-ray crystallography determine their ability to inhibit wild type RT and several clinically relevant RT mutants [275].

The approval of rilpivirine was based on the results from the 'Rilpivirine versus efavirenz with tenofovir DF and emtricitabine in treatment-naive adults infected with HIV-1 (ECHO), and the 'Rilpivirine versus efavirenz with two background NRTIs in treatment-naive adults infected with HIV-1 (THRIVE), two twin phase 3, randomized, double-blind, double-dummy, active controlled noninferiority trial, which assessed the efficacy and safety of the drug in nearly 1400 antiretroviral-naive patients [151-153]. The subsequent STAR study confirmed the efficacy and safety of rilpivirine administered as a single tablet regimen once daily in an open study, the only one so far comparing two single tablet regimens head to head in an open study without the need to take a placebo pill [148].

Each tablet contains 27.5 mg of rilpivirine hydrochloride, which is equivalent to 25 mg of rilpivirine, the recommended daily dose.

Rilpivirine has also been marketed as a single tablet regimen in co-formulation with emtricitabine and tenofovir disoproxil fumarate. These once-daily drug co-formulations reduce the risk of treatment error, are associated with a lower risk of hospitalization, and can lessen the possibility of covert monotherapy in situations of selective noncompliance [60].

The drug is also considered in other clinical scenarios, such as in simplification strategies, having demonstrated non-inferiority in switching to the single table regimen rilpivirine/emtricitabine/tenofovir DF (RPV/FTC/TDF) from a ritonavir-boosted PI regimen in virologically suppressed, HIV-1-infected individuals [276]. The primary objective of non-inferiority at week 24 was met: plasma HIV-1RNA < 50 copies/mL by Snapshot analysis, 93.7%of RPV/FTC/TDF versus 89.9% of PI/r plus two NRTIs (difference 3.8%, 95% confidence interval -1.6 to 9.1%). The new regimen maintained virologic suppression with a low risk of virologic failure, while improving total cholesterol, LDL, and triglycerides. In this scenario, the overall development of RAMs

after switching to RPV/FTC/TDF was low. Through week 48, seven (1.5%, 7/469) had their viral isolates analyzed for resistance and of those, only four (0.9%, 4/469) had evidence of NNRTI and/or NRTI RAMs [276]. M184V/I + E138K in RT was the most common pattern of resistance [277]. All four participants with emergent resistance in their HIV had M184V/I substitutions and three also had emergent NNRTI RAMs (E138E/K; L100I + K103N with preexisting V90V/I; V108V/I + E138K with preexisting K103N and V179V/I). Among the 24 participants with a preexisting K103N RAM in their historical genotype (while still antiretroviral-naive), 18 were in the immediate switch arm and all were virologically suppressed at week 24. At week 48, 17 of 18 participants in this group maintained virologic suppression. One participant (1/18) who had preexisting K103N and V179I/V demonstrated virologic non-suppression at week 48 and developed additional RAMs (M184V, E138K, and V108V/I). All five participants with preexisting K103N in the delayed switch arm with data available were virologically suppressed 24 weeks after switch. These data strongly suggest that a historical K103N RAM does not impact the efficacy of rilpivirine in a treatment switch. Cross-resistance to other NNRTIs was observed in subjects with phenotypic resistance to rilpivirine at failure [277].

In naives, the frequent emergence of E138K, especially in combination with M184I, in rilpivirine virologic failures is a unique finding of these trials [260, 278]. As discussed before, it constitutes one of the best examples of ‘cross-talk’ between the NRTI and NNRTI-resistance mutations, as subjects with tenofovir DF/emtricitabine/rilpivirine failure were more likely to develop M184I -rather than the more common RAM, M184V- in combination with E138K. Actually, the E138K substitution alone in RT does not have an impact on emtricitabine, and therefore the virus evolution leads to the selection of M184I [279]. Using *in-vitro* experiments and analyzing patients PBMC Fourati et al. have been able to demonstrate that M184I and E138K RAMs may pre-exist in proviral reservoir at a high frequency prior to drug exposure, as a result of APOBEC3 editing

[280]. Thus, incomplete neutralization of one or more APOBEC3 proteins may favour viral escape to rilpivirine-emtricitabine. As compared to wild-type, the E138K/M184I mutant had a greater replicative advantage than the E138K/M184V mutant at higher etravirine concentrations tested (25 to 100 nM) with the order E138K/M184I > E138K/M184V > E138K >> M184V≥ M184I [70].

These data have forced the evaluation of the frequency of E138 RAMs in baseline genotypic resistance profiles of antiretroviral-naives, which has been set at 1.9% [281].

Among 686 patients receiving rilpivirine, 72 (10%) experienced virologic failure versus 39/682 (6%) receiving efavirenz. In patients with low baseline viral load (defined as ≤100,000 copies/mL, the proportions of rilpivirine virologic failures (19/368) and efavirenz virologic faiures (16/330) were similar (5%) [147, 260]. However, in patients with high baseline viral load (>100,000 copies/mL), the proportion of virologic failures was higher with rilpivirine (53/318; 17%) than efavirenz (23/352; 7%). This has led to the approval of the drug in only treatment-naïve subjects with low baseline viral load (defined as ≤100,000 copies/mL). In ART guidelines it is recommended as well in subjects with a CD4+ cell count >200 cells/μL due to limited experience and potential lower response in those with low baseline CD4+ counts [15, 16, 282].

The rate of rilpivirine virologic failure was comparable between patients infected with HIV-1 subtype B (11%) and non-B subtype (8%). The absolute number of virologic failures with treatment-emergent NNRTI RAMs was higher for rilpivirine (most commonly E138K or K101E) than efavirenz (most commonly K103N), but relative proportions were similar [63% (39 of 62) vs. 54% (15 of 28), respectively]. More rilpivirine virologic failures had treatment-emergent NRTI RAMs than efavirenz virologic failures [68% (42 of 62) versus 32% (9 of 28), respectively], most commonly M184I and M184V. The proportion of rilpivirine virologic failures with RAMs in patients with low baseline viral load was lower than in those with high baseline viral load: 38% (6 of 16)

versus 72% (33 of 46) for NNRTI RAMs, and 44% (7 of 16) versus 76% (35 of 46) for NRTI RAMs, respectively.

A total of 24 changes at 14 positions in the HIV-1 RT gene have been associated with a decreased susceptibility to rilpivirine [84, 84, 283, 284]. NNRTI RAMs emerging in HIV-1 under selective pressure from rilpivirine included combinations of V90I, L100I, K101E/P, V106A/I, V108I, E138A/G/K/Q/R, V179F/I/L, Y181C/I/V, Y188L, V189I, G190E, H221Y, F227C, and M230I/L. Y188L has been the last RAM incorporated on board, being the fourth in elevated fold change (6.2 to 9) after K101P, Y181I and Y181V, and the third in frequency [283].

By far, the RAM E138K was the most frequently selected (45%) in antiretroviral-naive patients that failed on rilpivirine therapy in the pivotal ECHO and THRIVE and the STAR studies [147, 148, 152, 153].

Despite all this, there is an incomplete knowledge of the prevalence of rilpivirine RAMs in routine clinical samples in subjects with virological failure to regimens with other NNRTIs, as well as the potential impact on the predicted activity of rilpivirine in those subjects.

Even though rilpivirine is not approved for salvage ART, RAMs become archived and persist for lengthy periods of time in the long-term latent reservoir, probably indefinitely [285, 286]. These RAMs massively fuel the cellular reservoir, and their prolonged persistence is supported by the early expansion of a dominant homogenous and resistant viral population in subjects with transmitted RAMs acquired at the time of primary infection [287]. Thus, once resistance to a particular drug arises, the patient will always carry that RAM. Interruption in treatment results in the re-emergence of the original wild-type virus, which often replicates better than drug-resistant virus, but drug-resistance HIV will have a replication advantage once the drug is resumed [288]. In fact, the reservoir serves as a permanent archive for all wild-type and drug-resistant

viruses that have circulated at significant levels during the course of the infection [71, 289]. Actually, it has been shown that a single dose of nevirapine can establish antiretroviral resistance within the latent reservoir, resulting in a potentially lifelong risk of re-emergence of nevirapine-resistant virus [290].

Therefore, some of these subjects with archived NNRTI-associated RAMs would potentially be switched subsequently to rilpivirine-containing regimens in the future, having increased risks of suboptimal ART efficacy and virological failure.

ANNEX. PUBLICATIONS TO THE INTRODUCTION.

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Josep M Llibre, JR Santos, T Puig, J Molto, L Ruiz, R Paredes and B Clotet. Prevalence of etravirine-associated mutations in clinical samples with resistance to nevirapine and efavirenz. J Antimicrob Chemother 2008;62 (5):909-913.

Josep M Llibre, Jonathan M Schapiro, and Bonaventura Clotet. Clinical Implications of Genotypic Resistance to the Newer Antiretroviral Drugs in HIV-1–Infected Patients with Virological Failure. Clin Infect Dis 2010; 50:872–88.

Journal of Antimicrobial Chemotherapy Advance Access published July 23, 20

JAC antiviral

Journal of Antimicrobial Chemotherapy
doi:10.1093/jac/dkn297

Prevalence of etravirine-associated mutations in clinical samples with resistance to nevirapine and efavirenz

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Received 4 March 2008; returned 7 May 2008; revised 24 June 2008; accepted 30 June 2008

***Objectives*: To evaluate the expected activity of etravirine in clinical samples, according to mutational patterns associated with decreased virological response (VR).**

***Methods*: We identified 1586 routine clinical samples with resistance-associated mutations (RAMs) to nevirapine and efavirenz (K103N 60%, Y181C 37%, G190A 27%, V108I 13%). Concerning *in vitro* identified etravirine mutations, samples with F227C, Y181I, M230L or L100I plus K103N plus Y181C were considered highly resistant. Samples with two RAMs plus Y181C or V179D or K101E or Y188L were considered intermediate. The prevalence of 13 RAMs recently associated with decreased VR to etravirine in the DUET clinical trials was also investigated.**

***Results*: Most samples (69%) harboured more than one IAS-USA RAM to first-generation non-nucleoside reverse transcriptase inhibitors (NNRTIs): 42% harboured two RAMs, 21% three RAMs and 6% four or more RAMs. The prevalence of 13 specific etravirine RAMs was V179F 0.12%, G190S 3.9%, Y181V 0.1%, V106I 2.6%, V179D 1.6%, K101P 2.0%, K101E 10.1%, Y181C 36.9%, A98G 5.9%, V90I 6.9%, Y181I 3.6%, G190A 27% and L100I 9.1%. The five RAMs with the most impact on VR (V179F/D, G190S, Y181V and V106I) occurred less often. Overall, 8.2% of the samples had three or more etravirine RAMs and only 1.1% had four or more. In addition, patterns of RAMs previously associated with intermediate etravirine resistance were present in 26.2% of the samples, whereas 4.85% displayed patterns of high-degree resistance.**

***Conclusions*: For RAMs associated with decreased VR, etravirine resistance in routine clinical samples was lower than previously reported. High-degree resistance was uncommon, even in patients with resistance to first-generation NNRTIs, whereas low-to-intermediate etravirine resistance was more common.**

Keywords: non-nucleoside reverse transcriptase inhibitors, TMC125, resistance-associated mutations

Introduction

The efficacy of first-generation non-nucleoside reverse transcriptase inhibitors (NNRTIs) is limited by their low genetic barrier to resistance, resulting from the relatively easy selection of single mutations that confer nearly complete cross-resistance. Resistance to first-generation NNRTIs among patients with treatment failure is widespread, given that they have been extensively used in clinical practice.[1,2] Etravirine is a new NNRTI with expanded activity against HIV-1 resistant to current NNRTIs and has demonstrated its efficacy and favourable safety profile in double-blind, placebo-controlled trials with treatment-experienced patients (DUET studies).[3,4]

The development of resistance to etravirine is complex and requires the coexistence of several specific resistance-associated mutations (RAMs). *In vitro* studies identified mutational patterns associated with increased resistance. Using pooled data from DUET studies, only 13 of those RAMs at eight positions were associated with decreased virological response (VR) at week 24.[5]

We assessed the expected activity of etravirine in samples with resistance to first-generation NNRTIs by searching for mutational patterns described both *in vitro* during etravirine development and those validated *in vivo* in DUET trials.

Methods

In a systematic database search of 4981 samples from patients, which had been submitted to our laboratory for routine clinical resistance testing between 1998 and 2006, we identified 1586

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different patients with documented RAMs conferring resistance to nevirapine or efavirenz. The search included any RAM confering resistance to the first-generation NNRTIs nevirapine or efavirenz present in the IAS-USA Drug Resistance Mutation List, namely L100I, K103N, V106A/M, V108I, Y181C/I, Y188C/H/L, G190S/A or P225H.[6]

The IrsiCaixa Foundation based in Barcelona, Spain, is a reference retrovirology laboratory receiving samples for routine genotyping. HIV-1 DNA coding for amino acids between positions 37 and 247 of reverse transcriptase (RT) is routinely sequenced and genotyped using the FDA-approved TRUGENE™ HIV-1 genotyping kit (Siemens).

Sets of mutations evaluated

Mutational patterns conferring resistance to etravirine were identified by comprehensively searching peer-reviewed journals and presentations at medical conferences. The survey gathered mutations identified *in vitro* and *in vivo*. *In vitro* studies, including standardized sequential passage experiments at low and high multiplicity of infection as well as site-directed mutant analysis, undertaken during drug development identified mutations specifically selected by etravirine. High-level resistance was associated with the presence of either F227C, Y181I or M230L mutations alone or L100I plus K103N plus Y181C mutations, or the presence of two or more first-generation NNRTI RAMs associated with K101P or V179D/E/F/I or Y181I/V or G190S.[7,8] Two first-generation NNRTI RAMs plus Y181I/V or V179D/E/I/F or K101E/P or Y188L were considered as conferring intermediate resistance and were associated with fold change (FC) increases in EC_{50} values of 4–10.[7,9] L100I plus K103N has also been identified as conferring low intermediate resistance to etravirine.[7]

Vingerhoets *et al.*[5] found a correlation between 13 RAMs and clinical response (decreased VR) to etravirine in the phase III DUET trials. They identified the following mutations: V90I, A98G, L100I, K101E/P, V106I, V179D/F, Y181C/I/V and G190A/S. The investigators analysed only 26 of 44 potential RT RAMs present at study entry in five or more participants. VR was defined as HIV-1 RNA <50 copies/mL at 24 weeks, and an arbitrary line was drawn at a 25% response reduction. Patterns of RAMs were, therefore, only judged to be associated with a significant loss of activity if $<75\%$ of the patients achieved <50 copies/mL. No single RAM had a significant impact on VR by itself. However, all etravirine RAMs occurred mainly with other NNRTI RAMs. In the multivariate analysis, patients with one or two etravirine RAMs displayed a 19% decrease in VR, whereas the VR dropped to below 75% in patients with three or more RAMs. Therefore, the presence of one to two of these etravirine RAMs was considered as partial or low-level resistance and the presence of three or more RAMs as high-level resistance.

We analysed descriptively the etravirine RAMs, calculating means and percentages.

Results

Of the 4981 samples submitted for routine clinical resistance testing, 1586 (31.8%) had mutations conferring resistance to nevirapine and efavirenz. Of these, 97.2% were subtype B. Among these non-B samples, subtypes were 25% CRF02_AG, 16% F1, 13.6% C, 11.3% CRF12_BF, 2.3% A2, 2.3% D; in 29.5% the subtype could not be assigned. The most frequent mutations were K103N (59.7%) and Y181C (37%). The frequencies of all mutations are depicted in Figure 1. In total, 31%

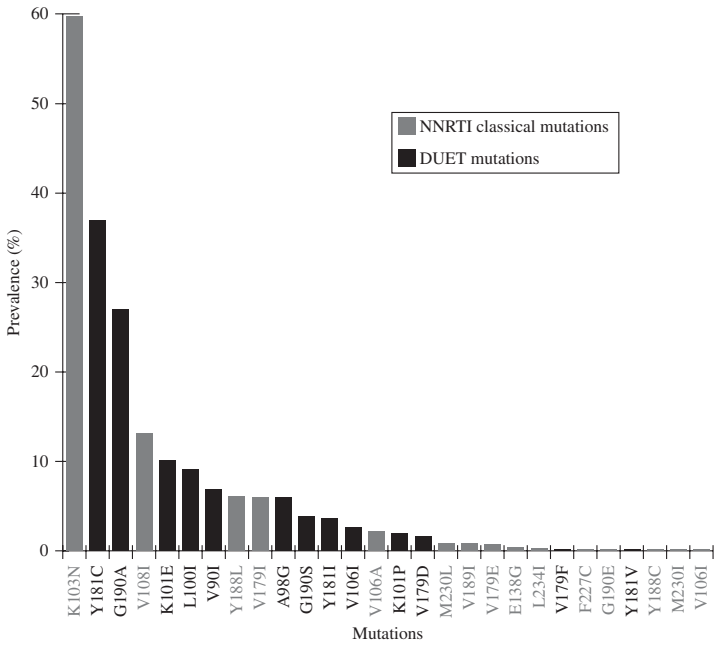

Figure 1. Prevalence of first-generation NNRTI and specific etravirine-related mutations with clinical impact on etravirine response in routine clinical samples with resistance to nevirapine or efavirenz (1998–2006).

JAC antiviral

Table 1. Frequency of patterns of combinations of mutations conferring resistance to etravirine

RAMs	*In vitro* combinations		Clinically validated (DUET studies) resistance and loss of VR [*n* (%)]
	high-degree resistance [*n* (%)]	intermediate-degree resistance [*n* (%)]	
K101P + ≥2 mut	32 (2.01)	—	10 (0.63)
V179E + ≥2 mut	5 (0.31)	—	—
V179F + ≥2 mut	5 (0.31)	—	4 (0.25)
Y181V + ≥2 mut	2 (0.12)	—	2 (0.12)
Y181I + ≥2 mut	41 (2.58)	—	15 (0.94)
G190S + ≥2 mut	19 (1.2)	—	14 (0.88)
M230L + ≥2 mut	7 (0.4)	—	—
F227C alone	2 (0.13)	—	—
Y181I alone	57 (3.6)	—	—
Y181C + ≥2 mut	—	251 (15.82)	104 (6.55)
K101E + ≥2 mut	—	105 (6.62)	79 (4.98)
L100I + ≥2 mut	—	104 (6.55)	—
Y188L + ≥2 mut	—	28 (1.76)	—
V179D + ≥2 mut	—	18 (1.13)	8 (0.50)
G190A + ≥2 mut	—	—	15 (0.94)
V106I + ≥2 mut	—	—	11 (0.69)
A98G + ≥2 mut	—	—	30 (1.89)
V90I + ≥2 mut	—	—	26 (1.63)
L100I + ≥2 mut	—	—	8 (0.5)
≥3 Etravirine mut	—	—	130 (8.1)

mut, mutation(s); *n*, number of patients; RAMs, resistance-associated mutation(s); VR, virological response.

($n = 491$) of the samples had only one NNRTI RAM, 42% ($n = 670$) had two, 21% ($n = 333$) had three and 6% ($n = 90$) of the samples had four or more mutations.

The most frequent RAMs related to any decreased etravirine activity were Y181C (36.9%), G190A (27%), K101E (10.1%), L100I (9.1%), V90I (6.9%), Y188L (6.1%), V179I (6%), A98G (5.9%), G190S (3.9%), Y181I (3.6%), V106I (2.6%) and K101P (2%). The prevalence of the remaining RAMs was <1% (Figure 1).

Analysis of RAMs identified during etravirine drug development (in vitro)

The general prevalence of any mutation or combination of mutations reported to confer high or intermediate degrees of resistance was 31%.

Mutational patterns reported to confer high-degree resistance were found in 4.85% of the samples (77/1586). The prevalence of single RAMs associated with high-degree etravirine resistance was 0.1% for F227C, 3.6% for Y181I and 0.8% for M230L. The most frequent combinations were Y181I plus two or more mutations (2.58%), K101P plus two or more mutations (2.01%) and K103N plus L100I plus Y181C (0.36%). The remaining mutational patterns appeared in <1% (Table 1). The combination of V179E/D/F or Y181I or G190S or M230L plus four mutations, reported to confer even higher resistance to etravirine, was extremely rare (0.75% overall).

RAM patterns associated with intermediate resistance were identified in 26.3% of the samples (417/1586), the most frequent being the Y181C plus two or more mutations (15.82%), K101E plus two or more mutations (6.62%), L100I plus K103N (6.55%), Y188L plus two mutations (1.76%) and V179D plus two mutations (1.13%) (Table 1).

Analysis of clinically validated etravirine RAMs in the DUET studies

The most frequent mutations validated to confer resistance in the DUET studies were Y181C (36.9%), G190A (27%), K101E (10.1%) and L100I (9.1%), but the five RAMs with the highest impact on VR (V179F/D, G190S, Y181V and V106I)[5] were found less frequently in our clinical samples (Figure 1). With regard to combinations, 8.1% of the samples had three or more specific etravirine RAMs, Y181C plus two or more mutations (6.5%) and K101E plus two or more mutations (4.98%) being the most frequently identified. Only 1.13% of the samples had four or more etravirine-associated RAMs. The remaining combinations are depicted in Table 1.

Combinations with four or more NNRTI RAMs were found in 90 (6%) samples, and 54 (60%) of them shared Y181C. Likewise, 18 (1.14%) samples had four or more etravirine-specific RAMs and 67% ($n = 12$) contained the Y181C mutation.

Discussion

According to our analysis, high-level etravirine resistance was uncommon in HIV-1 infected patients with resistance to first-generation NNRTIs in routine clinical practice, regardless of

whether mutation patterns reported during drug development or those clinically validated in the DUET trials are considered. Samples with low-to-intermediate resistance are much more prevalent, although when only RAMs associated with decreased VR in the DUET trials are considered, the prevalence of etravirine resistance is lower than previously reported during drug development.

After K103N, Y181C was the most frequent NNRTI-related mutation (37%), in line with previous reports,[9–11] perhaps related to the wider use of nevirapine in Spain. Neither individual mutation has been associated with etravirine resistance by itself, although most highly resistant clones *in vitro* contain Y181C.[8] In addition, Y181C in combination with two or more additional mutations has been associated *in vitro* and *in vivo* with increased etravirine resistance.[5,7,8,10] We found a high prevalence of Y181C associated with two additional NNRTI mutations, and patients with four or more NNRTI mutations or etravirine-specific RAMs usually harboured Y181C.[11] When such mutations are present, etravirine would not be a preferred drug to include in salvage regimens.

In our analysis, the frequency of L100I was 9%, which is slightly higher than other reports but similar to another one in which the prevalence was calculated from a database containing 7144 clinical samples.[6,9] *In vitro*, intermediate etravirine resistance was reported for L100I plus K103N, for which a fold change (FC) of 11 was reported, although the FC for each individual mutation was 2.1 and 0.5, respectively.[8] In our analysis, L100I plus K103N constituted the most frequent combination associated with etravirine resistance. However, in DUET trials, L100I was associated with the smallest decrease in VR among the identified set of mutations. It rarely appears alone, and it is associated with a median of two NNRTI RAMs. A more detailed analysis of its impact with every specific RAM is required because it is very common in patients with prior failure to first generation NNRTIs.

V179I, another mutation reported during etravirine development and a common polymorphism in HIV subtype A, was present in 5.9% of the patients. However, this mutation has not been validated in DUET studies, even though insertions F and D at position 179 were included, and V179E is under evaluation. Although a recent study has also associated V179I with etravirine resistance, its role remains unclear and should be studied further.[6]

Other mutations, both clinically validated (K101P, Y181I/V, G190S and V179F) and reported *in vitro* (V179E, G190E, F227L and M230L), had frequencies below 2% in our study (except Y181I), in agreement with previous reports.[2,5,6,9,10] The prevalence of combinations of V179E/D/F or Y181I or G190S or M230L plus four mutations, reported to confer even higher resistance to etravirine, was extremely rare (0.75% overall).

DUET trials showed that an increasing number of baseline etravirine RAM was associated with a steady decrease in VR, with the greatest impact in patients with three etravirine RAMs.[5] However, the specific relevance of each mutation is still to be determined in the unweighted score. In our analysis, 27% of the samples had three or more of these etravirine mutations. This is slightly higher than reported in DUET trials, perhaps because of more widespread prior use of nevirapine in Spain driving Y181C selection. In contrast, rates of V106I, G190S, V179F and Y181V, which had the most pronounced effect on etravirine VR, were lower in our study.[5]

Our analysis is limited in that etravirine is a novel drug with modest clinical experience with unweighted mutation scores, pending further fine-tuning in the future. Both *in vitro* and clinically validated scores have advantages and drawbacks, and *in vitro* experiments do not always correspond with *in vivo* results, particularly in salvage trials with complex antiretroviral regimens. Previous studies had based the analysis of etravirine resistance on a phenotypic FC >10, but this is an arbitrary unvalidated threshold.[10,11] The prevalence of additional mutations such as T386A and Y318A[8] could not be assessed in our study, because mutations beyond the 247 position were not routinely amplified.

This analysis shows that the prevalence of high-level resistance to etravirine is low in routine clinical practice, and the drug should retain activity in most patients with resistance to first-generation NNRTIs. Nevertheless, the prevalence of mutations or their combinations associated with low-to-intermediate etravirine resistance is quite common. Our findings, therefore, support the recommendation of early withdrawal of first-generation NNRTIs from non-suppressive antiretroviral regimens to avoid the accumulation of further mutations that would jeopardize future etravirine activity.

Acknowledgements

These data were presented in part at the Sixteenth International HIV Drug Resistance Workshop, Barbados, West Indies, 2007 (Abstract 65) and the Fifteenth Conference on Retroviruses and Opportunistic Infections, Boston, MA, 2008 (Poster 868).

We wish to acknowledge Greg Morley for assistance with English language and Luis Cortina (Siemens, Spain) for assistance with the conduct of this study.

Funding

This work was funded in part by grants from the Spanish AIDS Network 'Red Temática Cooperativa de Investigación en SIDA' (RIS, RD06/0006) and NEAT (European AIDS Treatment Network).

Transparency declarations

None to declare.

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HIV/AIDS INVITED ARTICLE
Kenneth H. Mayer, Section Editor

Clinical Implications of Genotypic Resistance to the Newer Antiretroviral Drugs in HIV-1–Infected Patients with Virological Failure

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Virological suppression rates achieved with the new antiretroviral drugs in patients with virological failure and resistance to multiple drug classes are nearly matching the rates seen in treatment-naive patients. Knowledge of cross-resistance patterns to drugs of the same class is key for successful use of etravirine, tipranavir, and darunavir in treatment-experienced patients. Determination of human immunodeficiency virus type 1 (HIV-1) tropism is cardinal for maraviroc. The impressive potency of raltegravir must not preclude its use with other active drugs because of its limited genetic barrier. These new agents have demonstrated superiority in virtually all efficacy parameters in their pivotal salvage trials, but comparative data between them are still very scarce. This review discusses the clinical implication of resistance to these new drugs. Specific genotypic resistance scores have been developed for tipranavir and etravirine, and mutations conferring resistance to darunavir are well understood. Determining the most active drugs and successfully combining them is the key challenge in salvage regimens.

The nearly simultaneous launch of a plethora of new antiretroviral agents with expanded activity in existing (tipranavir, darunavir, and etravirine) and novel classes (raltegravir and maraviroc) has resulted in unprecedented success for human immunodeficiency virus type 1 (HIV-1)–infected patients who have received multiple treatments. Currently achieved virological suppression rates in patients with triple-class failure were until recently only seen in drug-naive patients [1, 2]. It is critical that clinicians use the available agents carefully and become familiar with the complexity of dealing with their resistance patterns.

These new drugs have demonstrated superiority in key efficacy parameters in their salvage trials [3–10]. Unfortunately, we lack comparative trials between them. Trials generally have evaluated only a single new drug, the exception being darunavir and etravirine in the DUET trials. Preliminary reports of combined use of these new drugs in routine clinical practice show very promising results [11].

The optimal choice of drugs relies on the evaluation of resistance that compromises their activity and varies depending on previous drug exposure and virological failure. Importantly, viral load suppression to <50 copies/mL must be aggressively pursued in salvage regimens to preclude the emergence of resistance to these life-saving agents.

GENERAL PRINCIPLES

Determination of genotypes is preferable to analysis of phenotypes because of lower cost, faster turnaround time, and greater sensitivity for detecting mixtures of wild-type and resistant virus [12–14]. Phenotypes can provide additional information about complex mutational patterns, particularly regarding resistance mutations to protease inhibitors. Both are unable to detect minority variants, ie, those present in <20% of the viral population. Technologies continue to evolve with the ability to sequence and detect extremely small minority populations ("ultradeep sequencing"). Their ultimate clinical role remains to be determined, but they are an important research tool and have demonstrated clinical relevance for pretherapy mutation screening [15, 16].

Interpretation of the resistance test results is complex. Thus,

Received 11 September 2009; accepted 9 December 2009; electronically published 8 February 2010.

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Clinical Infectious Diseases 2010;50:872–881

1058-4838/2010/5006-0013$15.00
DOI: 10.1086/650732

Table 1. Important Steps to Be Checked for Successfully Designing Salvage Therapy Regimens

Step no	Description
1	Determine the cause of the current regimen failure. Take measures to resolve it and avoid its recurrence in the subsequent new regimen.
2	Review all previous resistance test results available as well as the current one. Compile all results and interpret. Prior documented mutations remain in small undetectable subpopulations but will emerge when suboptimal drug pressure is exerted again, even if undetected by the present tests.
3	Thoroughly review the full treatment history, and specifically identify all drugs included in failing regimens, and those associated with intolerance. Suspect the presence of mutations against drugs (lamivudine, emtricitabine, nevirapine, efavirenz, enfuvirtide, raltegravir) included in regimens which have previously failed, despite the fact that mutations were not detected in genotypes.
4	Review potential drug-drug interactions and consider therapeutic drug monitoring if available and indicated.
5	Focus on maximizing patient's adherence to treatment. Poorly adherent patients are overrepresented among those with virological failure.
6	Strive to include 3 fully active drugs or their equivalent in the new treatment regimen in all patients with prior triple-class failure and resistance.
7	Consider obtaining a tropism test in all patients, even if a CCR5 antagonist use is not planned initially.
8	Patients with high viral loads and lower CD4 cell counts have consistently lower response rates. Take special care with this subset of patients to optimize salvage regimens including three fully active agents.
9	In complex cases with multidrug resistance, obtain the opinion and the support of an expert in human immunodeficiency virus resistance.

algorithms, software programs, and virtual phenotype are continually being designed and fine-tuned [13, 14, 17, 18]. Distance consultations with use of e-mail and conference calls are a feasible strategy when local availability of experts is lacking, providing expert advice along with continued education [19].

Some guidelines still recommend a viral load >500–1000 copies/mL for genotypic testing. However, rates of amplification >70% can usually be obtained with viral loads >100 copies/mL [14, 20].

Tropism testing must be routinely assessed. An R5-only tropism result will allow use of maraviroc either in the initially planned regimen or as an alternative if toxicity to another drug is encountered. Tropism testing is not standardized once viral load becomes undetectable [21, 22].

The inclusion of enfuvirtide in treatment has been associated with significant increases in response rates in all salvage trials. Although its use is currently vestigial because of treatment inconvenience and widespread substitution with alternative oral drugs (mainly raltegravir), its contribution to regimen activity should not be forgotten when options are limited [3–10, 23–27].

The success of salvage therapy lies in closely adhering to a series of basic principles (Table 1). It is crucial to design an optimal regimen which allows for effective and durable viral suppression while minimizing toxicity, inconvenience, and cost.

The scores, or lists, of mutations that confer resistance include mutations selected in vitro by passage experiments, those that result in drug resistance and decreased response in vivo, and those that appear in patients who experience virological failure.

TIPRANAVIR

Tipranavir is a nonpeptide protease inhibitor with activity against strains with multiple protease mutations, approved for use in treatment-experienced patients. A full resistance score was initially derived, with the following 21 mutations: 10V, 13V, 20M/R/V, 33F, 35G, 36I, 43T, 46L, 47V, 54A/M/V, 58E, 69K, 74P, 82L/T, 83D, and 84V [3, 28]. A weighted score then assigned 5 mutations (24I, 50V/L, 54L, and 76V) a negative score (eg, increased response to treatment) [29, 30]. The score has recently been updated (Table 2), achieving a better prediction of response [31, 33]. The most commonly selected mutations

Table 2. Calculation of the Score for Each Tipranavir Resistance Mutation in the New Weighted Score

Mutation	Initial score[a]	Current score[b]
10 V	1	1
24I	−2	−2
33F	**0**	**1**
36I	2	2
43T	2	2
46L	1	1
47V	**6**	**4**
50L/V	−4	−4
54A/M/V	**3**	**2**
54L	**−7**	**−6**
58E	**5**	**3**
74P	**6**	**4**
76V	−2	−2
82L/T	**5**	**4**
83D	4	4
84V	**2**	**3**

NOTE. Mutations with updated scores are indicated by bold-faced type. A clinical interpretation of the final result is as follows: ≤3 points, sensitive; 4–10 points, intermediate or partially sensitive; and >10 points, resistant.

[a] From [31].

[b] From [32].

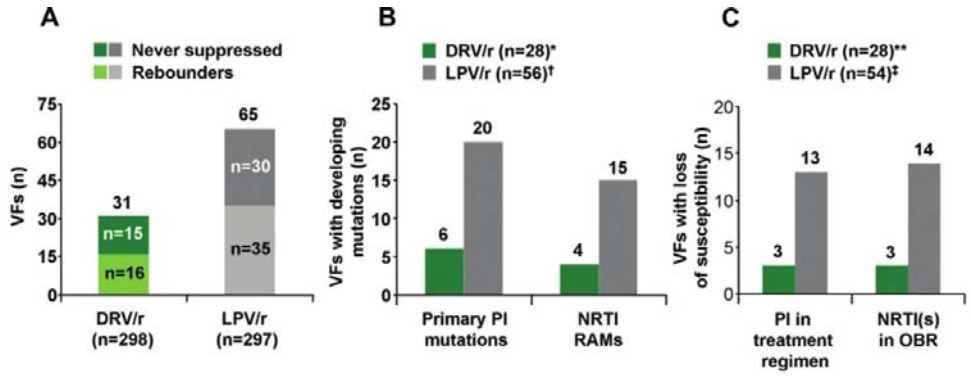

Figure 1. Numbers of virological failures (VFs) (*A*) and development of resistance (*B, C*) in the patients with VF observed in the TITAN study [41]. DRV/r, darunavir/ritonavir; LPV/r, lopinavir/ritonavir; PI, protease inhibitor; NRTI, nucleoside reverse-transcriptase inhibitor; OBR, optimized background regimen; RAM, resistance-associated mutation. *Baseline and end point genotype available for 28 of 31 VFs; †Baseline and end point genotype available for 56 of 65 VFs; **Baseline and end point phenotypes available for 28 of 31 VFs (2 of 28 had decreased susceptibility to DRV at baseline); ‡Baseline and end point phenotype available for 54 of 65 VFs (12 of 54 had decreased susceptibility to LPV at baseline).

in patients with virological failure are A82T, I84V, and I24L/M [32].

Phenotypic clinical cut-offs associated with a 20% (clinical cut-off 1) and 80% (clinical cut-off 2) loss of response have been established at 1.2–2 and 5.4–8 [34]. A phenotypic fold change <0.5 has been associated with more durable response [35].

The use of tipranavir is limited to those cases who meet 2 criteria: (1) its predicted activity significantly surpasses that of darunavir, and (2) etravirine use is not planned. There is a clinically relevant pharmacokinetic interaction that reduces etravirine exposure, precluding their coadministration [36]. Of importance, I50V, I54L, and L76V confer resistance to darunavir but hypersusceptibility to tipranavir, which can have a discriminatory role in their selection, particularly following treatment failures with amprenavir, lopinavir, or darunavir.

DARUNAVIR

Darunavir has been approved in treatment-naive patients and in initial and advanced salvage regimens, where it is the standard-of-care protease inhibitor [4, 10, 37]. Activity is preserved among non-B HIV-1 subtypes [38, 39].

In naive patients, it is administered once-daily at doses of darunavir/ritonavir 800/100 mg. In all other situations, the approved dose is 600/100 mg twice daily. However, there are prospects regarding the use of the once-daily dose in selected pretreated patients without significant darunavir resistance, with ongoing trial results eagerly awaited [40]. This is based on the high inhibitory quotient achieved, its long plasma half-life (~15 h), and preliminary findings of phase II studies. Similar to other boosted protease inhibitors, neither primary mutations nor phenotypic resistance to darunavir are selected naive patients who experience virological failure [38].

In lopinavir-naive, treatment-experienced patients (TITAN study), darunavir was superior to lopinavir [10]. It was also superior provided there was at least 1 baseline primary protease mutation (IAS-USA list), 3 lopinavir resistance mutations, or the lopinavir fold change was >10.

New mutations in cases of treatment failure and loss of susceptibility to the protease inhibitor were lower with darunavir (Figure 1) [41]. Thus, darunavir protects the background regimen activity better than lopinavir in patients who experience early failure. In patients with advanced HIV-1 infection, darunavir also demonstrated superiority to the comparator protease inhibitor [4].

The current score of mutations conferring resistance include V11I, V32I, L33F, I47V, I50V, I54L/M, T74P, L76V, I84V, and L89V [4, 10, 42]. They have been associated with a reduction in the in vitro sensitivity and clinical response and appeared in at least 10% of patients with virological failure (V32I, L33F, I47V, I54L, and L89V appeared most frequently).

An optimal response to darunavir was associated with a phenotypic fold change ⩽10 (upper clinical cut-off defined at 90) [41]. A linear loss of response begins to occur with the first mutation, and beyond 3 mutations, the response is greatly reduced (Figure 2). Nonetheless, complete resistance to darunavir is rare, and its exclusion in a salvage regimen must be carefully assessed [44].

N88S is associated with a reduction in the phenotypic fold change. Statistically, the presence of V82A has been associated with a lower fold change, a higher rate of response, and a greater viral load decrease [45]. These data are relevant, because V82A

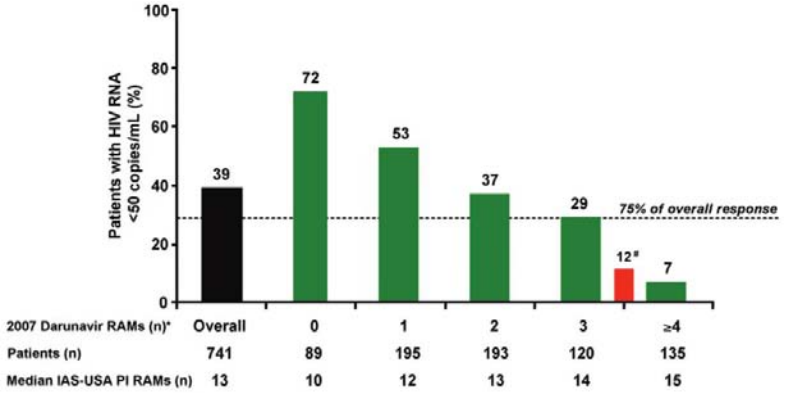

Figure 2. Virological response at week 24 by number of baseline mutations for darunavir in patients who did not receive enfuvirtide (or reused it). Analysis was done by TLOVR among those with human immunodeficency virus (HIV) RNA levels <50 copies/mL at 24 weeks (non–virological failures were censored) and was performed in the POWER 1, 2 (darunavir arms), and 3 and DUET 1 and 2 studies. *In those cases in which there was a mixture of mutations in a certain position, only one mutation was counted per position; #The reference response rate of the comparator arm without darunavir in studies POWER 1 and 2 was 12%. Note that patients had a median of 13 IAS-USA protease mutations (DRM). The 2007 set of mutations for darunavir included V11I, V32I, L33F, I47V, I50V, I54L/M, G73S, L76V, I84V, and L89V. G73S currently substituted by T74P [43]. PI, protease inhibitor; RAM, resistance-associated mutation. Derived from De Meyer et al [42].

is one of the most common protease mutations seen in clinical practice [46].

There is a significant overlap between the molecular structures and mutational resistance patterns of darunavir and amprenavir. Although darunavir's inhibitory quotient is far higher, in patients with fosamprenavir failure, the efficacy of darunavir could be reduced, particularly when I50V or V32I plus I47V are present [47].

Fortunately, darunavir and tipranavir mutation patterns are somewhat different, each of them being a potential candidate in the event of failure of the other [48]. I50V, I54L, and L76V confer significant resistance to darunavir but hypersusceptibility to tipranavir and, therefore, may be of substantial importance in this scenario. L33F, I54M, I47V, T74P, and I84V confer resistance to both drugs. I84V favored darunavir in a multivariate analysis, whereas I47V favored tipranavir [49].

Virtually all pivotal salvage trials have included a protease inhibitor [3, 6, 7, 9, 23]. However, in unusual situations (patient intolerance and established resistance) physicians may be forced to use protease inhibitor–sparing regimens. Because of the availability of etravirine, maraviroc, raltegravir, and enfuvirtide, these regimens may actually include 3 fully active drugs even in heavily pretreated individuals. Preliminary reports have described very high rates of virological suppression when the sensitivity score of the regimen was similar to that of patients receiving a protease inhibitor [50]. The number of active antiretrovirals, but not the inclusion of a protease inhibitor, was a predictor of response, suggesting that the crucial point for success lies in the inclusion of multiple fully active drugs.

ETRAVIRINE

Etravirine has activity against HIV-1 strains resistant to first-generation nonnucleoside reverse-transcriptase inhibitors (NNRTIs) [51]. Approved for patients with previous treatment failures, it retains full activity against K103N mutants, the most common mutation seen with resistance to first-generation NNRTIs [52].

Etravirine activity varies according to the number and type of NNRTI mutations selected. It has a higher genetic barrier than older NNRTIs, requiring multiple mutations for loss of activity [16, 53].

Its clinical efficacy and safety have been demonstrated in the TMC125-C223 and DUET studies [5, 6, 54, 55], which included patients pretreated with nucleoside reverse-transcriptase inhibitors (NRTIs), NNRTIs, and protease inhibitors. In the DUET studies, all patients also received darunavir/ritonavir, resulting in much higher rates of efficacy.

Conversely, etravirine was inferior to a protease inhibitor in protease inhibitor–naive patients with previous NNRTI failures (TMC125-C227 trial), indicating that a certain degree of cross-resistance exists within the class [56]. A post hoc analysis identified the presence of Y181C, a fold change ⩾10, and a higher number of etravirine mutations, with a diminished response. The mutation pattern of etravirine is well characterized, although uncommon mutations may not be represented in some scores [52, 57].

Essentially, 3 independent genotypic scores have been developed. The first has been correlated with treatment response [57]. Overall, the presence of 3 etravirine mutations was as-

Table 3. Weighted Scores of the Mutations Conferring Resistance to Etravirine

Mutation	Tibotec	Monogram	Stanford
V90I	1	1	…
A98G	1	…	5
L100I	2.5	4	20
K101E	1	2	15
K101H	1	1	5
K101N	…	…	5
K101P	2.5	4	20
K101Q	…	…	5
K103N	…	…	10
K103S	…	…	10
K103T	…	…	10
V106A	…	2	5
V106I	1.5	…	…
V106M	…	1	10
E138A	1.5	3	5
E138G	…	3	5
E138K	…	2	10
E138Q	…	1	10
V179D	1	1	10
V179E	…	3	10
V179F	1.5	1	25
V179L	…	2	…
V179M	…	1	…
V179T	1	…	…
Y181C	2.5	4	35
Y181I	3	4	35
Y181F	…	1	…
Y181S	…	…	20
Y181V	3	4	35
Y188C	…	…	10
Y188H	…	…	15
Y188L	…	2	20
V189I	…	1	…
G190A	1	…	15
G190C	…	…	10
G190E	…	1	25
G190Q	3	…	15
G190S	1.5	…	15
G190T	…	…	10
G190V	…	…	10
H221Y	…	1	…
P225H	…	1	10
F227C	…	…	15
F227L	…	…	5
M230L	2.5	3	20
L234I	…	…	10
K238N	…	3	5
K238T	…	1	5
Y318F	…	…	10

NOTE. Tibotec, Monogram, and Stanford scores are described in [58], [59], and [60], respectively.

sociated with a reduced response, though the weight for each mutation was different. Seventeen mutations have now been identified (Tables 3 and 4) [57]. The most common are Y181C and G190A. More recently, E138G/Q substitutions have also been associated with etravirine resistance [58]. Intermediate etravirine activity is commonly observed in patients who have accumulated mutations after first-generation NNRTI failures [52]. This must be considered when designing the regimen to obtain an optimal response. Nevertheless, complete etravirine resistance is uncommon [52]. In the DUET studies, etravirine protected the activity of darunavir, reducing the proportion of patients developing darunavir mutations (Figure 3) [61].

A second score based on the correlation with 4248 phenotypes identified 30 mutations (Tables 3 and 4) [62]. Although the mutations included and the scores given differ slightly, the final interpretation of both scores and the Stanford Database one is very similar (Tables 3 and 4) [59, 60]. The last score does not grant points to polymorphic mutations (V90I and V106I) but grants 10 points to K103N (a marker of previous NNRTI exposure and risk of coexistence of further mutations). Phenotypic lower and higher clinical cut-offs have been set at 1.6–3 and 13–27.6, respectively, depending on the test manufacturer [34, 63, 64].

Some NRTI mutations (M41L, D67N, T69D/N, K70R, L74I/V, M184V, L210W, T215F/Y, and K219N/Q/R) confer etravirine hypersusceptibility, an issue not yet assessed in any score. In the DUET studies, 34% of all samples displayed a fold change <0.4 [65]. The clinical significance of this not defined yet.

Efavirenz failures select for K103N, L100I, Y188L, G190A, and K101E in subtype B virus, whereas nevirapine selects Y181C, K103N, G190A, K101E, and A98G [66]. Whether one or the other are associated with higher rates of etravirine failure is still uncertain [67, 68]. Although nevirapine selects for mutations with higher impact on etravirine (particularly Y181C), the rate of selection of mutations was higher for efavirenz [69]. In the analysis with the higher degree of evidence (599 etravirine-treated patients), the rate of response was virtually the same with both, and a prior nevirapine failure was not a predictor of response [70].

RALTEGRAVIR

Raltegravir is the first approved HIV-1 integrase strand-transfer inhibitor for both naive and treatment-experienced patients, with elvitegravir in late clinical development. The HIV-1 integrase catalyses the insertion of viral complementary DNA into host DNA and was not routinely sequenced in genotypes [7, 71, 72].

The drug is active against wild-type HIV-1; viruses with resistance against NRTIs, NNRTIs, and protease inhibitors (no cross-resistance with other classes); and viruses with CCR5 or CXCR4 tropism. It suppresses plasma HIV-1 RNA levels sig-

Table 4. Interpretation of the Weighted Scores of the Mutations Conferring Resistance to Etravirine

Score, points	Interpretation
Tibotec[a]	
0–2	Highest response (74%)
2.5–3.5	Intermediate response (52%
⩾4	Reduced response (38%), similar to the rate seen in the comparator arm without etravirine
Monogram[b]	
<4	Susceptible, correlates to a fold change <2.9
⩾4	Intermediate resistance, correlates with a fold change >2.9[c]
Stanford[d]	
0–9	Susceptible
10–14	Potential low-level resistance; the virus is likely to be fully susceptible, yet it contains mutations that may be indicative of previous exposure to drugs in the same class
15–29	Low-level resistance
30–59	Intermediate resistance
⩾60	High-level resistance

[a] Correlation with the rate of virological response observed with etravirine in the DUET studies.
[b] Correlates with the phenotypic fold change in a huge independent database, independent of the DUET studies.
[c] This score could not identify the cut-off for complete or high level resistance.
[d] Etravirine resistance estimates drawn up by the database team with different clinical or phenotypic correlations.

nificantly faster than other current drugs, though the clinical relevance of this is unknown [73, 74].

Raltegravir does not demonstrate the high genetic barrier to resistance seen with a boosted protease inhibitor. Thus, it is critical to secure its protection with other active agents. On treatment failure, resistance mutations are seen to accumulate, and considerable reductions in susceptibility are seen with single key mutations. Phenotypic clinical cut-offs have not yet been determined.

Genotypic resistance commonly emerges in patients with virological failure, with substantial cross-resistance to elvitegravir (Figure 4) [76]. A higher baseline viral load and a background regimen without active agents were associated with the development of mutations, the most common being N155H (incidence, ~40%) and Q148H/R/K (incidence, 28%–30%), representing 2 mostly exclusive pathways. Other less common resistance pathways are Y143R/C (7%), E157Q, and E92Q.

Q148 substitutions are associated with increases in resistance of up to 25-fold, compared with an average of 10-fold with N155H. Isolated N155H mutations might potentially be overcome by other integrase inhibitors—opening the door to possible sequential use of newer agents of the class. In patients experiencing treatment failure, accessory mutations accumulate, which either increase the degree of resistance or restore viral fitness (eg, G140S rescues the integration defect induced by Q148H) [77].

In patients experiencing early failure, N155H predominates because selective advantage, but under continued treatment, there is often a gradual replacement by Q148H/R/K [78]. The degree of resistance increases if virological failure persists. The rate at which resistance increases varies substantially between patients, and high-level resistance could sometimes require months of persistent replication [77]. Early withdrawal of raltegravir would help in preventing high levels of integrase resistance. Integrase resistance testing or storing of samples from patients with treatment failure of integrase inhibitor–based regimens should always be considered.

MARAVIROC

Maraviroc is currently the only approved CCR5 coreceptor antagonist, with vicriviroc in advanced clinical development, representing the first antiretroviral class that does not target the virus. Rather, it acts as a noncompetitive inhibitor on a human cell coreceptor required for viral entry. CCR5 and CXCR4 are cell surface receptors for various natural ligands [21]. It binds only to the CCR5 receptor and, therefore, has no activity against X4-tropic viruses [79]. It has been approved in both treatment-naive and treatment-experienced patients [8, 9, 80, 81]. Approximately 85% of treatment-naive and 50% of treatment-experienced individuals harbor R5-only tropic viruses [82].

Intrinsic resistance to maraviroc with CCR5 tropism appears

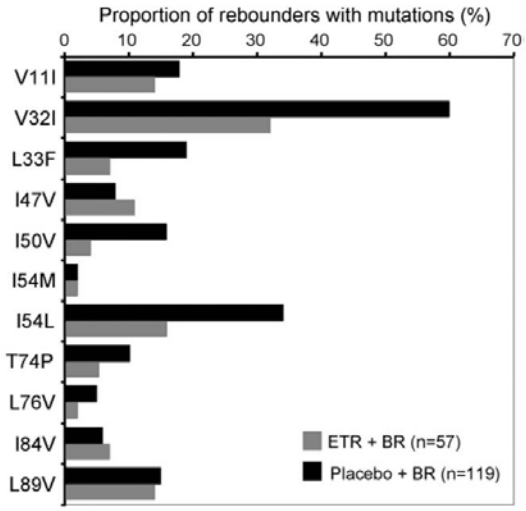

Figure 3. Emerging darunavir mutations in patients with virological failure in the DUET study. The most frequently emerging darunavir mutations in both arms were V32I and I54L [10, 61]. BR, background regimen; ETR, etravirine.

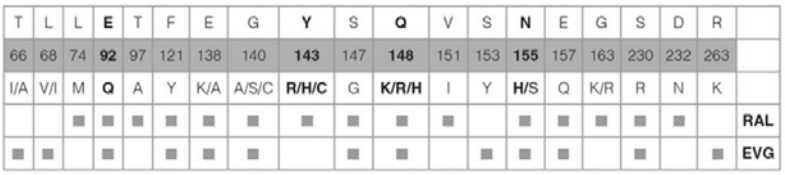

Figure 4. Substitutions in the integrase associated with resistance to the integrase inhibitors. RAL, raltegravir; EVG; elvitegravir. Previously published in [75]. Used with permission of the publisher.

to be rare. There is no cross-resistance with enfuvirtide, which selects for resistance in the gp41 envelope region [21].

Prior to its use, the presence of CCR5 tropism needs to be confirmed. Currently, the only validated test is the Trofile phenotypic assay (Monogram). The original assay (no longer available) has been replaced by a more sensitive version (ES Trofile), which detects strains with R5/X4 dual/mixed tropism or X4 tropism present in 0.1%–0.3% of the viral population. However, this technology is propriety and is performed only in California, requires worldwide sample shipment, and is expensive, with a long turnaround time.

A variety of genotyping techniques to predict tropism have been tested [83]. Envelope V3 loop sequence is determined and interpreted using a public domain software (http://www.geno2pheno.org/) [84]. The gp41 transmembrane subunit and other sequences of the envelope external to V3 may also be determining factors in tropism switches [85, 86]. The overall correlation has shown a sensitivity of 60%, with 90% specificity, of population-based V3 genotyping algorithms, compared with the original Trofile assay [87]. However, their clinical utility was much the same, with all of them similarly discriminating short-term responders. Genotypic assays are less expensive, have faster turnaround times, and can be performed in local labs. Furthermore, massive parallel pyrosequencing with 454 technologies will allow more sensitive genotypic detection of CXCR4-tropic variants [88].

Clinical resistance to CCR5 antagonists emerges through 2 different mechanisms. The first one consists of a change to the use of CXCR4 coreceptor. This occurred in 57% of patients experiencing failure with maraviroc; it is apparently not selected de novo during treatment failure but is attributable to preexisting minority X4 populations [8, 88]. The development of the more sensitive ES Trofile assay may reduce the number of treatment failures that occur through X4-tropic virus emergence [81].

The second mechanism involves true viral resistance through mutations in gp120. This results in a plateau effect in the dose-response curves [89]; ie, increasing concentrations do not increase the percentage of viral inhibition because HIV-1–resistant clones are able to bind to the receptor occupied by maraviroc (allosteric inhibition).

The base of the V3 loop remains generally intact, with mutations concentrating in the stem of the loop. The residues 316 and 323 seem to play a key role, and mutations commonly described in V3 are I20F, A25D, and I26V. However, much remains to be discovered, and the pattern of mutations seems to be very heterogeneous, with changes outside V3 having a contribution as well. High rates of viral suppression are seen when maraviroc is combined with the equivalent of 2 active drugs, with genotypic and phenotypic weighted susceptibility scores equally predicting response [90, 91].

X4 usage increases spontaneously with duration of HIV-1 infection. Therefore the potential benefit of CCR5 antagonists is greatest early in infection, suggesting that efforts to encourage usage in this scenario should be further pursued.

CONCLUSIONS

The entrance of potent new antiretroviral agents into the clinical realm has revolutionized the care of treatment-experienced patients. Proper use of these new drugs and drug classes requires a basic understanding of their resistance characteristics. Pivotal studies indicate that salvage regimens in patients with prior triple-class failure and resistance optimally should include 3 active agents or their equivalent. However, active drugs in these studies were often enfuvirtide, the study drug, and recycled NRTIs. Combinations of multiple new antiretroviral drugs have not yet been evaluated in randomized studies, but preliminary data suggest outstanding results when used in combination [11, 70]. The inclusion of at least 1 drug of a new class is strongly recommended. Failure of these drugs can quickly lead to loss of activity and even class cross-resistance, leaving patients with few if any options for the near future. The need for continued use of inactive NRTIs and non–protease inhibitor containing regimens remain to be determined.

Acknowledgments

We thank Xavier Soler (University of California, San Diego) for his support in preparing the manuscript.

Financial support. Partially supported by the "Red Temática Cooperativa de Grupos de Investigación en Sida del Fondo de Investigación Sanitaria (RIS)," and grant ISCIII-RETIC RD06/006 from the Instituto de Salud Carlos III, Madrid (Spain).

Potential conflicts of interest. J.M.L. has received research funding,

consultancy fees, or lecture sponsorships from, or has served on advisory boards for Abbott, Boehringer-Ingelheim, Gilead Sciences, GlaxoSmith-Kline, Jansen-Cilag, Merck Sharp & Dohme, Pfizer, and Roche; J.M.S. has received research support, honorarium, or consulting fees from Abbott, Merck, Roche, Gilead, GSK, Pfizer, Monogram Biosciences, Tibotec, Siemens, Ambrillia, and BMS; B.C. has received research funding, consultancy fees, or lecture sponsorships from, or has served on advisory boards for Abbott, Boehringer-Ingelheim, Gilead Sciences, GlaxoSmithKline, Janssen-Cilag, Merck Sharp & Dohme, Panacos, Pfizer, Roche, and Tibotec.

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HYPOTHESIS AND STUDY OBJECTIVES.

HYPOTHESIS AND STUDY OBJECTIVES.

Study 1. Effectiveness of Etravirine in Routine Clinical Practice in Treatment-Experienced HIV Type 1-Infected Patients.

Hypothesis.

The safety and efficacy of etravirine in antiretroviral salvage regimens had not been evaluated outside the strictly controlled conditions of a clinical trial and the availability of new antiretroviral drugs could improve the efficacy of these treatments. The efficacy of regimens containing etravirine in routine clinical practice when combined with new active drugs could be higher that what was observed in registrational etravirine trials.

Study objectives.

- The primary endpoint was the proportion of patients receiving a salvage regimen containing etravirine with an HIV-1 viral load <50 copies/ml at 48 weeks in a multicenter retrospective study done in at the HIV units of four acute-care university hospitals in Barcelona, Spain.
- Secondary endpoints included the relationship between treatment response and the number of active antiretrovirals at baseline, CDC stage, CD4+ T-cell count, and viral load ≥100,000 copies/ml at baseline, number of previous lines of treatment, number of previous antiretrovirals, number of NNRTIs/NRTIs/PIs, previous failure or interruptionwith efavirenz or nevirapine, adverse events leading to discontinuation of therapy, and changes in CD4+ T-cell counts.

Study 2. Rilpivirine resistance mutations selected in HIV patients failing non-nucleoside reverse transcriptase inhibitor-based therapies.

Hypothesis.

Rilpivirine is the latest approved NNRTI. It displays *in-vitro* activity extending over other NNRTI-resistant HIV strains. Patients failing other NNRTI-based regimens could select for variable degrees of rates of RPV resistance-associated mutations.

Study objectives.

- To examine the existence of RPV resistance-associated mutations and the proportion of estimated RPV resistance in plasma samples collected from HIV patients that had recently failed NNRTI-based regimens in a large network of 22 clinics in Spain.
- To identify the mutations most commonly selected in subjects failing every NNRTI in nevirapine-, efavirenz-, or etravirine-based regimens.
- To pinpoint the mutations causing cross-resistance between rilpivirine and etravirine among these failures.

Study 3. Effectiveness of a treatment switch to nevirapine plus tenofovir DF and emtricitabine (or lamivudine) in adults with HIV-1 suppressed viremia.

Hypothesis.

Switching subjects with persistently undetectable HIV-1 viremia under ART to once-daily tenofovir DF/emtricitabine (or lamivudine) + nevirapine could be a safe, effective, cost-effective and well-tolerated strategy.

Study objectives.

- To evaluate the rates of treatment failure, virological failure, and variables associated with virological failure, in all subjects initiating this switch combination in our clinic since 2001. The main endpoint was plasma HIV-RNA < 50 copies/mL.
- To identify the most frequently isolated drug-resistance mutations selected in the reverse transcriptase in subjects with virological failure, both against NNRTIs and NRTIs.

PhD THESIS PUBLICATIONS.

PhD THESIS PUBLICATIONS.

José R. Santos, Josep M. Llibre, Pere Domingo, Arkaitz Imaz, Elena Ferrer, Daniel Podzamczer, Isabel Bravo, Esteban Ribera, Sebastià Videla, and Bonaventura Clotet. High Effectiveness of Etravirine in Routine Clinical Practice in Treatment-Experienced HIV Type 1-Infected Patients. AIDS Research and Human Retroviruses 2011;27(7):713-717.

Lourdes Anta, Josep M. Llibre, Eva Poveda, José L. Blanco, Marta Alvarez, María J. Pérez-Elíase, Antonio Aguilera, Estrella Caballero, Vicente Soriano , Carmen de Mendoza, on behalf of the Resistance Platform of the Spanish AIDS Research Network. Rilpivirine resistance mutations in HIV patients failing non-nucleoside reverse transcriptase inhibitor-based therapies.AIDS 2013, 27:81–85.

Josep M. Llibre, Isabel Bravo, Arelly Ornelas, José R. Santos, Jordi Puig, Raquel Martin-Iguacel, Roger Paredes, Bonaventura Clotet. Effectiveness of a Treatment Switch to Nevirapine plus Tenofovir and Emtricitabine (or Lamivudine) in Adults with HIV-1 Suppressed Viremia. PLoS ONE 2015; 10(6): e0128131.

AIDS RESEARCH AND HUMAN RETROVIRUSES
Volume 27, Number 7, 2011

DOI: 10.1089/aid.2010.0283

Short Communication: High Effectiveness of Etravirine in Routine Clinical Practice in Treatment-Experienced HIV Type 1-Infected Patients

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Abstract

The effectiveness of etravirine has not been thoroughly investigated in routine clinical practice, where adherence rates and the heterogeneous nature of patients differ from the clinical trial setting. We evaluated the effectiveness of rescue regimens containing etravirine and the factors associated with treatment response. Multicenter retrospective cohort of all consecutive patients was recruited in a routine clinical practice setting. Patients were taking rescue regimens containing etravirine plus an optimized background regimen. The primary endpoint was the percentage of patients with HIV-1 RNA <50 copies/ml at week 48. The secondary endpoints were those factors associated with treatment response to etravirine. Endpoints were evaluated using univariate and multivariate analysis. A total of 122 patients were included with a median viral load of 11,938 (1055–55,500) copies/ml at baseline. The most frequent drugs in the backbone were darunavir/ritonavir in 98 (80.3%) patients and raltegravir in 76 (62.3%). In the full dataset analysis, 73% (89/122; 95% CI, 64–81%) of patients responded to treatment at week 48; in the on-treatment analysis, 82% (89/109; 95% CI, 71–87%) responded. The factors associated with treatment failure to etravirine [HR (95% CI)] were baseline $CD4^+$ T cell count <200 cells/mm^3 [2.45 (1.17–5.16)] and use of raltegravir [0.47 (0.22–0.99)] and darunavir [0.45 (0.21–0.98)] as backbone drugs. Skin rash was the only adverse event directly related to etravirine and led to withdrawal in three patients (2.5%). In routine clinical practice, rescue ETR-containing regimens are well tolerated and achieve rates of virological suppression higher than those observed in its pivotal clinical trials, especially when combined with darunavir and raltegravir.

Introduction

The availability of new drugs, in both existing or novel antiretroviral classes, with expanded activity against triple-class resistant HIV-1 makes it possible to achieve sustained virologic suppression in multitreated patients in routine clinical practice. New agents have demonstrated superiority in all efficacy parameters in their pivotal salvage trials.[1–6] The combination of these drugs allows us to construct regimens with at least two—and preferably three—fully active drugs, even in very treatment-experienced individuals.[7,8] However, with the exception of the DUET-1 and DUET-2 studies,[2,3] no trials have compared the efficacy of the different combinations of these drugs to date. In the DUET studies, darunavir was combined with etravirine (ETR) in all patients, and neither raltegravir nor maraviroc was available. The efficacy of etravirine at 24 weeks rose to 66% in patients from DUET-1 and 80% from DUET-2. In both trials, patients achieved sustained virological suppression with regimens containing three or more active agents.[2,3] The safety and efficacy of ETR in combination with the remaining new antiretrovirals have not been evaluated outside the strictly controlled conditions of a clinical trial, although preliminary reports on the combination of these new drugs have shown promising results.[9–12]

We evaluated the effectiveness of rescue regimens containing ETR combined with all the available active agents in routine clinical practice. We also analyzed the relationship between the

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number of additional active drugs in the optimized regimen and other factors associated with the response to ETR.

Materials and Methods

We conducted a multicenter retrospective study of HIV-1-infected patients aged at least 18 years who started an antiretroviral rescue regimen containing ETR between June 2003 and November 2009. Patients were recruited at the HIV units of four acute-care university hospitals in Barcelona, Spain. Patients with virological failure (at least two successive HIV-1 plasma RNA measurements >50 copies/ml) who had started a rescue therapy were selected through a systematic search of the electronic files at each center. All patients were treatment experienced and had resistance to three antiviral classes: nucleoside reverse transcriptase inhibitors (NRTIs), nonnucleoside reverse transcriptase inhibitors (NNRTIs), and protease inhibitors (PIs). Clinicians selected the backbone regimen according to genotyping results and treatment history, and ETR was started at doses of 200 mg orally twice daily. Demographic characteristics, treatment history, historical and current HIV-1 genotypic resistance test results, and tropism (Trofile; Monogram Biosciences, Inc., CA) were recorded. HIV-1 RNA (Roche HIV-1 RNA Ultrasensitive PCR assay; Hoffmann-La Roche, Basel, Switzerland) measurements and $CD4^+$ T cell counts were recorded at baseline and every 12 weeks thereafter.

The primary endpoint was the proportion of patients with an HIV-1 viral load <50 copies/ml at 48 weeks. Secondary endpoints included the relationship between treatment response and the number of active antiretrovirals at baseline, CDC stage, $CD4^+$ T cell count and viral load ≥100,000 copies/ml at baseline, number of previous lines of treatment, number of previous antiretrovirals, number of NNRTIs/NRTIs/PIs, previous failure or interruption with efavirenz or nevirapine, adverse events leading to discontinuation of therapy, and changes in $CD4^+$ T cell counts. Treatment failure was defined as a confirmed viral load >50 copies/ml before week 48 or early discontinuation of ETR for any reason. The number of active drugs was calculated using the HIV Drug Resistance Database, Stanford (version 6.0.8). We assigned 1, 0.5, or 0 points to drugs with scores of <15, 15–59, and ≥60 points, respectively. Enfuvirtide and raltegravir were considered active in those patients using the drugs for the first time. Maraviroc was considered active in those patients who had CCR5 tropism.

The primary endpoint was measured in all patients who started treatment (full dataset). The last observation carried forward was used if no information was available at week 48. Quantitative variables are expressed as mean (±SD) or median and interquartile range, and qualitative variables as percentages. Normally distributed variables were compared using the *t* test; nonnormally distributed variables were compared using the Wilcoxon rank sum test. The relationship between treatment response, clinical characteristics, and number of active drugs at baseline was assessed using univariate and multivariate (Cox regression) analysis. The hazard ratio and its 95% confidence interval (95% CI) were also calculated. All statistical analyses were performed using SPSS version 15.0 for Windows (SPSS, Chicago, IL). Statistical significance was set at $p < 0.05$.

Results

A total of 122 patients with virological failure started a rescue regimen containing ETR. Patients had received a median of 8 (4–10) antiretroviral regimens over a mean of 11.9 (4.2) years and had a median HIV-1 RNA of 11,938 (1055–55,500) copies/ml. When rescue therapy was started, 82 (67.2%) and 67 (54.9%) patients had experienced failure or interruption of previous regimens with nevirapine or efavirenz, respectively. Darunavir and raltegravir were the most frequent drugs in the backbone regimens, and were taken by 98 (80.3%) and 76 (62.3%) patients, respectively. Only 11 (9%) patients took maraviroc, 8 (6.6%) lopinavir/ritonavir, and 5 (4.1%) atazanavir/ritonavir. Baseline characteristics are shown in Table 1.

Of the 122 patients included in the study, 89 (73%, 95% CI: 64–81%) achieved virological suppression in the full dataset analysis and 33 (27%, 95% CI: 19–35%) experienced treatment failure at 48 weeks. Of these, 17 (51.5%) had confirmed virological failure, 11 (33.3%) were lost to follow-up, 3 (9.1%) experienced a toxicity-limiting adverse event with ETR, and 2 (6%) stopped treatment. These last two patients had achieved complete viral suppression when they voluntarily decided to discontinue. As a result, the proportion of patients who achieved treatment response at week 48 in the on-treatment analysis was 89/109 (82 %).

Factors found to predict treatment failure at 48 weeks in the univariate analysis were baseline $CD4^+$ T cell count <200 cells/mm^3 [(HR = 2.148; 95% CI, 1.029–4.483); $p = 0.042$], use of raltegravir [(HR = 0.452; 95% CI, 0.225–0.908); $p = 0.026$] and darunavir [(HR = 0.380; 95% CI, 0.184–0.783); $p = 0.009$] as backbone drugs, and time on antiretroviral treatment (risk per year) [(HR = 0.921; 95% CI, 0.851–0.998); $p = 0.043$]. In the multivariate analysis, only the baseline $CD4^+$ T cell count <200 cells/mm^3 [(HR = 2.458; 95% CI, 1.170–5.166); $p = 0.018$] and use of raltegravir [(HR = 0.459; 95% CI, 0.214–0.985); $p = 0.046$] and darunavir [(HR = 0.474; 95% CI:0.226–0.994), $p = 0.048$] were identified as predictors of treatment response. The factors not identified as predictors of treatment response in the univariate analysis were a viral load >100,000 copies/ml (HR = 1.056, 95% CI, 0.406–2.751), overall time since HIV-1 diagnosis, prior interruption or failure on regimens containing nevirapine and efavirenz, and number of previous antiretroviral regimens, number of fully active drugs (≥3 at baseline), and number of previous PIs/NRTIs/NNRTIs (Table 2).

According to the Stanford HIVDB score (version 6.0.8), ETR was fully active in 56/122 (45.9%) patients, intermediate in 49/122 (40.2%), and resistant in 8/122 (6.6%). The baseline genotyping result was not available in 9/122 (7.4%) patients.

As for the number of active antiretrovirals in the backbone regimen, 10 of the 17 patients who experienced virological failure (58.82%) had ≤2.5 active drugs and 7/17 (41.17%) had ≥3.0 active drugs. Of these, two patients who were on maraviroc, four on lopinavir/ritonavir, and two on atazanavir/ritonavir experienced virological failure despite taking ≥2.5 active drugs at baseline. In addition, according to their medical records, these six (35.29%) patients had poor adherence, which could explain their treatment failure, even though they were taking active drugs at baseline. Unfortunately, the design of the study and the heterogeneity of the medical records meant that it was not possible to correctly evaluate adherence in the remaining patients.

In addition to virological efficacy, rescue regimens containing ETR resulted in a significant overall increase in $CD4^+$

Table 1. Baseline Characteristics of HIV-1-Infected Patients Taking Rescue Regimens Containing Etravirine Plus an Optimized Antiretroviral Regimen[a]

Baseline characteristic	N = 122
Male[b]	97 (79.5)
Age (years)[c]	44.5 (9.1)
HCV[b]	79 (64.8)
HBV[b]	7 (5.7)
CDC stage[b]	
A	24 (19.7)
B	17 (13.9)
C	54 (44.3)
Time since HIV diagnosis (years)[d]	15.5 (12.2–18.5)
Time on treatment (years)[c]	11.9 (4.2)
No. of previous antiretroviral regimens[d]	7 (4–10)
No. of previous antiretroviral drugs[c]	10.6 (3.7)
No. of previous NRTIs[d]	6 (5–7)
No. of previous NNRTIs[d]	1 (1–2)
No. of previous PIs[c]	3.5 (2.0)
Interruption/failure of previous NNRTIs[b]	
NVP	82 (67.2)
EFV	67 (54.9)
ARV at baseline[b]	
Darunavir/ritonavir	98 (80.3)
Lopinavir/ritonavir	8 (6.6)
Atazanavir/ritonavir	5 (4.1)
Saquinavir/ritonavir	1 (0.8)
Enfuvirtide	9 (7.4)
Raltegravir	76 (62.3)
Maraviroc	11 (9.0)
Tenofovir	62 (50.8)
Lamivudine	53 (43.4)
Zidovudine	10 (8.2)
Abacavir	9 (7.4)
Didanosine	8 (6.6)
Stavudine	5 (4.1)
Baseline active drugs[d]	2.5 (2–3)
≤1.5 active drugs[b]	18 (14.9)
2 active drugs[b]	21 (17.2)
2.5 active drugs[b]	39 (32)
3 active drugs[b]	19 (15.6)
≥3.5 active drugs[b]	24 (19.7)
CCR5 tropism[e] ($n = 36$)[b]	
CCR5	15 (41.6)
CXCR4 and D/M	10 (27.7)
Non-reportable	11 (30.5)
$CD4^+$ T cell count (cells/mm^3)[c]	274.4 (213.3)
Viral load (copies/ml)[d]	11,938 (1055–55,500)

[a]ARV, antiretroviral drugs; HCV, hepatitis C virus; HBV, hepatitis B virus; NRTIs, nucleoside reverse transcriptase inhibitors; NNRTIs, nonnucleoside reverse transcriptase inhibitors; PI, protease inhibitor; D/M, dual/mixed.
[b]*n* (%).
[c]Mean (standard deviation).
[d]Median (interquartile range).
[e]Measured by Trofile.

T cell count during follow-up from 274 (213) cells/mm^3 at baseline to 417 (231) cells/mm^3 at week 48 ($p < 0.0001$).

There were no unexpected adverse events. Sixteen (13.11%) patients presented side effects associated with their antiretroviral treatment. Only three (2.5%) presented adverse events leading to discontinuation of therapy and the remaining 13 (10.65%) maintained their ETR-based regimens. Rash was the most frequent adverse event and was observed in eight (6.5%) patients, of whom only three were women ($p = 0.359$) and six were also using DRV in the backbone. Three of these eight patients presented a moderate diffuse rash[13] and discontinued ETR (two of them had started darunavir and ETR simultaneously at baseline), four had mild and transient ETR-related rash, and one experienced a confirmed darunavir-related rash after week 24 leading to discontinuation of darunavir, while ETR was maintained. NRTI-associated adverse events were reported in six patients (worsening of neuropathy in three, dizziness caused by emtricitabine in one, and anemia caused by zidovudine in two). Diarrhea due to boosted PI was reported in two patients.

Discussion

Rescue regimens containing ETR plus optimized antiretroviral drugs in heavily treatment-experienced individuals show higher effectiveness rates than those observed in pivotal ETR trials.[2,3,14] In our analysis, 73% of patients achieved a viral load of <50 copies/ml at 48 weeks in a restrictive full dataset analysis. This result is higher than the rates observed in the pooled DUET-1 and −2 trials, where 61% of patients receiving ETR achieved complete viral suppression.[15] This is contrary to what normally happens in treatment-naive patients, in whom the excellent response rates seen in clinical trials are difficult to match in routine clinical practice. This higher effectiveness of ETR, when prescribed as rescue treatment in clinical practice, is probably due to the availability of more active agents than were available during the initial clinical trials.

With the exception of the DUET trials, in which ETR proved to be more effective than placebo,[2,3] few studies have evaluated the efficacy and safety of ETR. ETR with raltegravir and darunavir (or other boosted PIs) has shown outstanding efficacy rates and a good safety profile in preliminary clinical trials and different expanded-access programs, achieving undetectable viral loads at 48 weeks in as many as 70% and 81% of patients after 48 weeks of treatment.[9,10,12,16] Our results are consistent with these findings, and darunavir and raltegravir were the most frequently used antiretroviral agents in the optimized baseline treatments, with high rates of treatment response and virological suppression. In addition, viral suppression has also been observed in 92% of patients in a setting with more limited therapeutic options, namely, a PI and NRTI-sparing regimen containing ETR plus maraviroc and raltegravir.[11] In our series, the number of patients taking maraviroc or other boosted PIs (not darunavir) plus ETR was too low for conclusions to be drawn about efficacy.

The pooled 48-week results from the DUET studies showed that baseline HIV-1 RNA and $CD4^+$ T cell count, adherence, number of active agents in the background regimen, and use of enfuvirtide were predictors of virological response with ETR in rescue regimens.[15] We also found a relationship between baseline $CD4^+$ T cell count <200 cells/mm^3 and treatment response at week 48, which is consistent with the fact that advanced stages of HIV infection are associated with poorer treatment response rates. The DUET trials revealed a significantly greater response in the ETR group than in the placebo group, irrespective of the number of active

Table 2. Factors Predicting Treatment Failure in HIV-1-Infected Patients Taking Rescue Regimens Containing Etravirine Plus an Optimized Antiretroviral Regimen[a]

	Treatment failure (n = 122)			
	Yes N = 33	*No* N = 89	*Univariate analysis HR (95% CI)*	*Multivariate analysis HR (95% CI)*
Baseline active drugs[b]				
≤1.5 active drugs	7	11	1.86 (0.8–4.33)	1.07 (0.24–4.70)
≤2 active drugs	11	28	1.17 (0.56–2.45)	1.20 (0.29–4.96)
≤2.5 active drugs	20	58	0.83 (0.41–1.68)	0.47 (0.12–1.81)
≤3 active drugs	26	71	1.04 (0.45–2.44)	2.07 (0.45–9.48)
Viral load ≥ 100,000 copies/ml	5	14	1.05 (0.40–2.75)	1.28 (0.37–4.35)
$CD4^+$ T cell count ≤200 (cells/mm^3)	20	36	**2.14 (1.02–4.48)**	**2.45 (1.17–5.16)**
CDC stage				
A	9	15		
B	3	14		
C	19	35	1.16 (0.52–2.59)	1.10 (0.40–3.01)
Time since HIV diagnosis (years)[c]			1.0 (0.96–1.04)	0.94 (0.8–1.1)
Time on treatment (years)[c]			0.92 (0.85–0.99)	1.04 (0.84–1.29)
No. of previous antiretroviral regimens			1.0 (0.92–1.08)	0.99 (0.9–1.09)
No. of previous antiretroviral drugs			0.97 (0.87–1.07)	1.03 (0.53–1.97)
No. of previous NRTIs			0.95 (0.73–1.22)	0.83 (0.39–1.76)
No. of previous NNRTIs			1.4 (0.72–2.7)	<0.001 (<0.001–>1000)
No. of previous PIs			0.93 (0.78–1.12)	0.89 (0.43–1.86)
Interruption/failure of previous NNRTIs				
NVP	22	60	1.0 (0.46–2.18)	1.99 (0.24–1.51)
EFV	21	46	0.85 (0.39–1.85)	0.88 (0.4–1.92)
ARV at baseline				
Darunavir/ritonavir	20	78	**0.38 (0.18–0.78)**	**0.459 (0.214–0.985)**
Raltegravir	76	61	**0.45 (0.22–0.90)**	**0.47 (0.22–0.99)**

[a]ARV, antiretroviral drugs; CDC, Centers for Disease Control and Prevention; EFV, efavirenz; NRTIs, nucleoside reverse transcriptase inhibitors; NNRTIs, nonnucleoside reverse transcriptase inhibitors; NVP, nevirapine; PI, protease inhibitor; HR, hazard ratio; CI, confidence interval.
[b]Baseline genotyping result was not available in nine patients ($n = 113$).
[c]Risk per year.

background agents. However, consistent with current HIV-1 treatment guidelines, the use of an increasing number of other active antiretrovirals with ETR was associated with an increased likelihood of treatment response.[7,15,17,18]

Similarly, we also found a higher proportion of patients whose regimen had failed with ≤2.5 active drugs than with ≥3 active drugs at baseline, although we were unable to demonstrate a statistically significant difference ($p = 0.802$). The low number of patients taking ≤2.5 active drugs could have masked any existing differences, as has been observed in other salvage studies. In addition, we were unable to find a relationship between response to treatment and baseline HIV-1 RNA >100,000 copies/ml, a predictor that is universally associated with higher rates of treatment failure. In our series, the number of individuals with baseline viral load >100,000 copies/ml was very low (5/122 patients); therefore, the difference was not statistically significant. This major drawback has also been encountered in many current clinical rescue trials reporting lower median viral loads in patients whose current antiretroviral treatment has failed in recent years.[19,20] These data suggest that the number of active drugs is probably a stronger predictor of response than a higher viral load in treatment-experienced patients.

We found no relationship between prior interruption or failure with NVP or EFV and response to ETR. This is concordant with what was observed in the DUET trials.[21]

As expected, and consistent with the results of other studies,[2,3,14,15] there was a significant increase in $CD4^+$ T cell count during follow-up. The only adverse event related to the administration of ETR was rash, which occurred in 50% of patients who experienced possibly or probably drug-related side effects, although this led to discontinuation in only three patients (2.5%), while the remaining five patients presented mild and transient rash that did not require discontinuation. An association between female gender and ETR-related rash has been reported,[14] although we were not able to observe this relationship in our study, probably due to the low number of women included and the low prevalence of rash. No other unexpected side effects leading to discontinuation of ETR were observed. However, the rescue regimen was modified during follow-up, due to the side effects induced by other families of antiretrovirals: zidovudine-related anemia; peripheral neuropathy associated with zidovudine, didanosine, and abacavir; dizziness caused by emtricitabine; and gastrointestinal disorders induced by PIs.

In conclusion, in conditions of routine clinical practice, ETR-containing rescue regimens are generally well tolerated and achieve rates of virological suppression that exceed those observed in clinical trials. This is probably due to a higher number of new active drugs in the regimen. Darunavir and raltegravir are safe and very effective antiretrovirals when administered in combination with ETR. Studies that evaluate

the efficacy and safety of ETR in combination with other PIs and maraviroc are still needed.

Acknowledgments

We are grateful to Arelly Ornelas for statistical advice and Thomas O'Boyle for editorial assistance. This study was funded in part by grants from the Spanish AIDS Network "Red Temática Cooperativa de Investigación en SIDA" (RIS, RD06/0006), NEAT (European AIDS Treatment Network), and Lluita contra la SIDA Foundation.

Author Disclosure Statement

No competing financial interests exist.

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CONCISE COMMUNICATION

Rilpivirine resistance mutations in HIV patients failing non-nucleoside reverse transcriptase inhibitor-based therapies

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Objective: Rilpivirine (RPV) is the latest approved nonnucleoside reverse transcriptase inhibitor (NNRTI). It displays in-vitro activity extending over other NNRTI-resistant HIV strains. There is scarce information about the rate of RPV resistance-associated mutations (RAMs) in patients failing other NNRTIs.

Methods: RPV RAMs were examined in plasma samples collected from HIV patients that had recently failed NNRTI-based regimens at 22 clinics in Spain.

Results: Resistance tests from a total of 1064 patients failing efavirenz (EFV) (54.5%), nevirapine (NVP) (40%) or etravirine (ETR) (5.5%) were examined. The prevalence of RPV RAMs was K101E (9.1%), K101P (1.4%), E138A (3.9%), E138G (0.3%), E138K (0.3%), E138Q (0.8%), V179L (0.2%), Y181C (21.8%), Y181I (0.5%), Y181V (0.2%), H221Y (8.3%), F227C (0.1%) and M230L (1.5%). K101E/M184I was seen in 1%. E138K/M184I were absent. Mutations L100I and V108I were significantly more frequent in patients failing EFV than NVP (7.9 vs. 0.2 and 12.2 vs. 7.3%, respectively). Conversely, Y181C, Y181I, V106A, H221Y and F227L were more prevalent following NVP than EFV failures. Using the Spanish resistance interpretation algorithm, 206 genotypes (19.3%) from patients failing NNRTI (NVP 52%, EFV 40.8% and ETR 7.8%) were considered as RPV resistant. In patients with ETR failure, cross-resistance to RPV was seen in 27.6%, mainly as result of Y181C (81.3%), V179I (43.8%), V90I (31.3%) and V108I (18.8%).

Conclusion: RPV resistance is overall recognized in nearly 20% of patients failing other NNRTIs. It is more common following ETR (27.6%) or NVP (25%) failures than EFV (14.5%). E138 mutants are rarely seen in this context.

AIDS 2013, **27**:81–85

Keywords: drug resistance, efavirenz, etravirine, nevirapine, rilpivirine

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Received: 4 May 2012; revised: 16 July 2012; accepted: 18 July 2012.

DOI:10.1097/QAD.0b013e3283584500

ISSN 0269-9370 © 2012 Wolters Kluwer Health | Lippincott Williams & Wilkins

Introduction

Nonnucleoside reverse transcriptase inhibitors (NNRTI) are popular components of combination antiretroviral therapy. Despite its proven efficacy, the clinical use of first-generation NNRTI, as nevirapine (NVP) and efavirenz (EFV), has been limited by side effects, low barrier to resistance and broad cross-resistance. To try to overcome these limitations, a second-generation of NNRTI has been developed that includes etravirine (ETR) and rilpivirine (RPV), both of which were recently approved as therapy for HIV-1 infection.

The approval of RPV was based on the results from the 'Rilpivirine versus efavirenz with tenofovir and emtricitabine in treatment-naive adults infected with HIV-1 (ECHO): a phase 3 randomised double-blind active-controlled trial' and the 'Rilpivirine versus efavirenz with two background nucleoside or nucleotide reverse transcriptase inhibitors in treatment-naive adults infected with HIV-1 (THRIVE): a phase 3, randomized, non-inferiority trial', which assessed the efficacy and safety of the drug in nearly 1400 antiretroviral-naive patients [1–3]. Although the resistance profile for RPV has not been well defined yet, there is information suggesting that the susceptibility to the drug is not compromised or only marginally affected by the presence of single NNRTI resistance-associated mutations (RAMs). A total of 15 changes at the HIV-1 reverse transcriptase gene have been associated with a decreased susceptibility to RPV [4]. By far, mutation E138K was the most frequently selected (45%) in antiretroviral-naive patients that failed on RPV therapy in ECHO and THRIVE studies. Interestingly, this change was generally seen along with M184I (34%), which confers lamivudine and emtricitabine resistance [5]. The combination E138K/M184I confers a 6.7-fold reduced phenotypic susceptibility to RPV compared with a 2.8-fold reduction for E138K alone. Mutation K103N, which is associated with clinical resistance to EFV and NVP, does not reduce susceptibility to RPV.

There is scarce information about the rate of RPV RAMs in HIV-1-infected patients with prior history of NNRTI failure. Likewise, very few studies have examined the clinical outcome of patients harbouring NNRTI-resistant viruses that subsequently received RPV [3]. Drug resistance interpretation systems for antiretroviral agents (i.e., Stanford, ANRS, and so on) have recently incorporated predictions of virological response to RPV based on the available information derived from the ECHO and THRIVE trials, from in-vitro studies and from expert opinion. The Drug Resistance Platform of the Spanish AIDS Research Network (http://www.retic-ris.net) has weighted NNRTI RAMs [6], and for considering resistance to RPV at least two RT mutations must be present. Changes with the greatest impact on RPV susceptibility are at four codons (K101E/P/T, E138A/G/K/R, Y181C/I/V and M230L), whereas changes at other nine positions display a lower impact (V90I, L100I, V106A/I, V108I, V179F/I/L, Y188I, G190E, H221Y and F227C/L). However, in the presence of M184I, only one of two changes (either E138K or K101E) is enough to produce high-level RPV resistance. This information is important for clinicians, particularly when simplification strategies using coformulations with RPV or rescue interventions in patients failing on NVP, EFV or ETR are being considered.

The aim of this study was to examine the rate of RPV RAMs and the proportion of estimated RPV resistance in HIV-1-infected patients who had failed other NNRTI-based regimens in a large network of HIV-1 clinics in Spain.

Patients and methods

Study population

The RT genotypes and clinical information from all HIV-1-infected patients on regular follow-up at 22 different HIV clinics in Spain who had failed NNRTI-based regimens were identified at the Spanish national resistance database (ResRIS) [6,7]. This is a large clinical database that records information from HIV-1 patients treated outside clinical trials. Data recorded includes drug resistance mutations, antiretroviral therapy, HIV clade, viral load and CD4 cell counts. Ultimately it produces back a virtual interpretation of the resistance mutation profile for all antiretroviral agents for a given sample, which is then send back to clinicians.

Drug resistance mutations and interpretation

The prevalence of RPV RAMs as well as the proportion of estimated RPV-resistant samples, as reported using the ResRis national algorithm (http://www.retic-ris.net), were assessed in the whole population of HIV-1 patients that had failed on NNRTI-based regimens. Drug resistance mutations were examined taking into account the updated mutation list from the IAS-USA panel (December 2011), also other recent changes that have been highlighted from the ECHO and THRIVE trials as well as in-vitro studies [8]. Briefly, these changes are the following: V90I, L100I, K101E/P/T, V106A/I, V108I, E138A/G/K/Q/R, V179F/I/L, Y181C/I/V, Y188I, and M230L. All these changes are considered in the current Spanish resistance interpretation algorithm, which additionally provides a weighting impact for each mutation (Table 1).

Statistical analyses

All results are expressed as absolute numbers and percentages. The prevalence of RPV RAMs in patients who had failed on EFV, NVP or ETR was compared using χ^2 tests. Significant differences were only considered for P values below 0.05. All statistical analyses were performed using SPSS v15.0 (SPSS Inc., North Chicago, Illinois, USA).

Table 1. Drug resistance interpretation for nonnucleoside reverse transcriptase inhibitors. Spanish drug resistance algorithm.

Drug	Nevirapine	Efavirenz	Etravirine	Rilpivirine
Group 3 (3 points)	L100I K101P K103N/S/T V106A/M Y181C/I/S/V Y188C/H/L G190A/C/E/Q/S/T/V M230L	L100I K101P K103N/S/T V106A/M Y181C/I/S/V Y188C/H/L G190A/C/E/Q/S/T/V M230L		
Group 2 (2 points)	K101E V179F F227C K238N/T Y318F	K103I/P P225H	L100I K101P E138K Y181C/I/V M230L	K101E[a]/P/T E138A/G/K[a]/R Y181C/I/V M230L
Group 1 (1 point)	A98G L100V K101H/N K103R V108I E138K/Q V179D/E/M P225H F227L/Y	A98G L100V K101E/H/N K103R V108I E138K/Q V179D/E/F/M K238N/T F227C Y318F	A98G L100V K101E/H V106A/I/M E138A/G/Q V179D/E/F/I/L/M/T Y181S Y188C/H/L G190A/C/E/Q/S/T/V P225H F227C K238N/T	V90I L100I V106A/I V108I V179F/I/L Y188I G190E H221Y F227C/L

Interpretation: $\geq$3 points = resistance (R); $\leq$2 points = susceptible (S).
[a]Mutation M184I causes Rilpivirine resistance when present along with E138K or K101E.

Results

From a total of 8200 RT genotypes derived from 5873 different HIV-1 individuals recorded at ResRIS, 1064 belonged to HIV-1-infected patients that had failed NNRTI-based regimens. Overall, 27.1% ($n = 288$) of specimens did not harbour any NNRTI RAMs. Among the 1064 genotypes examined, 580 (54.5%) had failed on EFV, 426 (40%) on NVP and 58 (5.5%) on ETR. Up to 45.9% ($n = 488$) were on their first NNRTI treatment, 39.8% ($n = 424$) had previously been exposed to another NNRTI and 14.3% ($n = 152$) had received two NNRTI.

Figure 1 records the prevalence of distinct RPV RAMs in the study population. The most prevalent mutations were Y181C (21.8%), V108I (10.2%), K101E (9.1%), V90I (7.9%) and V179I (6.1%). All other RPV RAMs examined were present at rates below 5%, being mutations E138R and Y188I absent in our study population. Only three patients (0.3%) harboured mutation E138K, and two of them had failed on ETR. The NRTI resistance mutation M184I was present in 3.4% of the whole genotypes, whereas M184V was seen in 36.2%. The combination K101E/M184I was seen in 1% of specimens, being absent E138K/M184I.

Mutations L100I and V108I were significantly more frequent in patients failing on EFV than NVP (7.9 vs. 0.2 and 12.2 vs. 7.3%, respectively). Conversely, Y181C, Y181I, V106A, H221Y and F227L were significantly more prevalent in patients failing on NVP than EFV. Interestingly, the lamivudine/emtricitabine RAM M184V was more frequent in patients failing on NVP than EFV (43.7 vs. 32.1%; $P < 0.001$). Finally, changes at positions V90I, E138K, V179I and Y181C were more common in patients failing on ETR than EFV ($P < 0.05$).

Based on the virtual reports produced by the Spanish drug resistance interpretation system, a total of 206 genotypes (19.3%) from patients failing NNRTIs should be considered as RPV-resistant. They corresponded to failures on NVP (51.5%), EFV (40.8%) or ETR (7.8%). When the proportion of RPV resistance was considered for distinct NNRTI failures, figures were as follows: 14.5% for EFV, 25% for NVP and 27.6% for ETR. Of note, cross-resistance between RPV and ETR (27.6% in 58 ETR failures) was reported mainly as result of changes Y181C (81.3%), V179I (43.8%), V90I (31.3%) and V108I (18.8%).

Discussion

RPV is the latest approved NNRTI for the treatment of HIV-1 infection [3]. Virologic responses as well as selection of drug resistance up to week 96 have recently been reported for ECHO and THRIVE trials [9]. The combination E138K + M184I was the most frequently selected failing on RPV (44.2%) in this population [9].

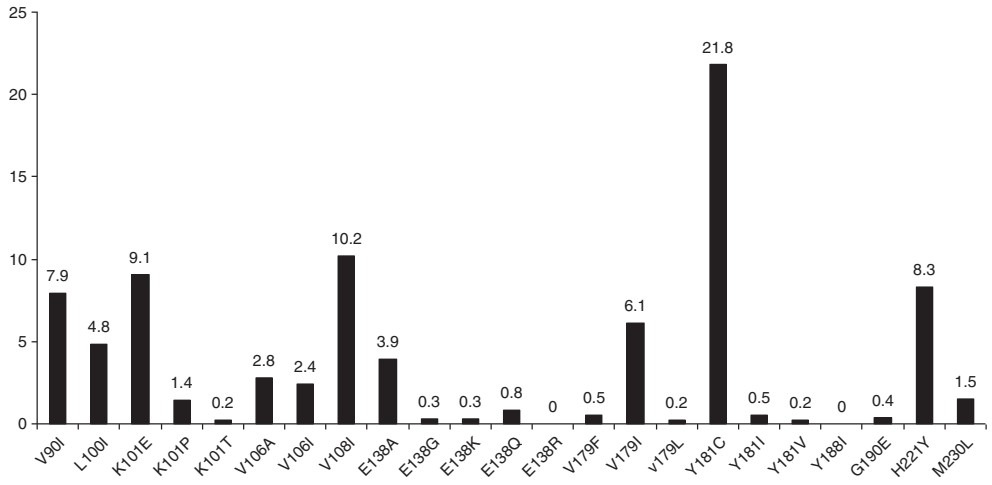

Fig. 1. Prevalence of rilpivirine resistance-associated mutations in 1064 genotypes derived from HIV-1-infected patients failing efavirenz, nevirapine or etravirine in Spain.

In ResRis, the prevalence of codon 138 mutants was very rare (1%) in patients that had failed NNRTI-based regimens other than RPV. Moreover, the combination E138K + M184I was absent in this population. These results are in agreement with those recently reported by German authors that tested a group of antiretroviral-experienced patients and found a very low rate (0.5%) of E138K [10]. Altogether, these results support that this mutation is not selected in patients failing on NVP and EFV and should be considered as specifically selected by ETR [11]. Taking into account that 39.8% of specimens tested come from individuals on their second NNRTI and 14.3% on their third NNRTI, the three individuals we found with viruses harbouring E138K were retrospectively reassessed, and two of them were found to have recently received ETR.

It must be noted that RPV and ETR largely share their respective resistance profile, and that E138K has recently been added to the ETR genotypic score [12]. In our study, 27.6% of 58 ETR failures harboured mutant viruses interpreted as RPV-resistant. Interestingly, loss of RPV susceptibility was mainly interpreted as a result of selecting changes other than E138K, as Y181C, V179I, V90I and/or V108I.

Although RPV has so far been approved as first-line treatment for HIV-1 infection, the drug is currently being considered in other clinical scenarios, such as in simplification strategies or in rescue interventions [13], given its good tolerability and easy to take coformulation as a single-tablet regimen. In-vitro data *per se* are not enough to predict clinical response, but they could support that RPV would be active following EFV failure, acknowledging minor overlap in selected drug resistance mutations; however, clinical data proving this assumption are scarce [14]. To validate the chances of any clinical benefit of RPV based on drug resistance genotyping following NNRTI failure, it would be worth collecting more clinical data in this specific scenario. However, our study is the first to support this hypothesis in a relatively large number of patients examined outside clinical trials. Only 14.5% of 580 EFV failures in our study were considered as RPV-resistant. In contrast, this figure was 25% for 426 NVP failures. Anyway, these rates are not negligible, and, therefore, drug resistance testing should be recommended before considering RPV therapy in patients that had failed on other NNRTIs. Prospective studies evaluating the efficacy of RPV in patients who have failed on EFV, NPV or ETR should be conducted.

Although phenotypic drug resistance testing could be useful to determine the susceptibility of recently approved antiretrovirals, for which the genetic correlates of clinically relevant drug resistance have not yet been well characterized, the situation is distinct for NNRTI. So far, the phenotypic susceptibility data have poorly predicted the efficacy of most NNRTI, as generally these drugs behave as on-off, being active or not, with no room for clinically relevant partial activity. In this situation, genotypic tests perform the best and facilitate the interpretation of mutations in drug resistance algorithms. However, the situation may be different for RPV. There is still scarce information about the distinct weight of mutations leading to resistance and the initial list of RPV RAMs must be refined.

In summary, the rate of E138 mutants is very rare in individuals failing NNRTI-based regimens other than RPV in ResRIS. Almost 20% of patients failing NNRTIs should be considered as RPV resistant, as a result of selecting changes at other positions. The extent of

cross-resistance seems to be higher for ETR and NVP in comparison with EFV, but prospective clinical trials should confirm the clinical value of this observation. The sequential use of RPV in patients that had failed other NNRTIs should not be done in the absence of drug resistance testing excluding cross resistance. Nevertheless, analyses of virological responses in patients treated with RPV following failures to NNRTI are needed and will provide more robust evidence about the impact of distinct resistance changes on RPV activity.

Acknowledgements

The study design and data analysis was made by L.A., C.d.M., V.S. and J.M.L. The manuscript draft was written by L.A., C.d.M. and V.S. Critical reading was contributed by J.M.L. (H. Germans Trias i Pujol, Badalona), E.P. (H. Carlos III, Madrid), J.L.B. (H. Clinic, Barcelona), M.Á. (H. Clínico San Cecilio, Granada), M.J.P.E. (H. Ramón y Cajal, Madrid), A.A. (H. Conxo, Santiago de Compostela) and E.C. (H. Vall d'Hebrón, Barcelona).

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This work was supported in part by grants from Red de Investigación en SIDA (RIS) RD06/0006, European Commission project CHAIN (FP7/2007–2013; grant 223131), and Fundación Investigación y Educación en SIDA (F-IES).

Conflicts of interest

There are no conflicts of interest.

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RESEARCH ARTICLE

Effectiveness of a Treatment Switch to Nevirapine plus Tenofovir and Emtricitabine (or Lamivudine) in Adults with HIV-1 Suppressed Viremia

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OPEN ACCESS

Citation: Llibre JM, Bravo I, Ornelas A, Santos JR, Puig J, Martin-Iguacel R, et al. (2015) Effectiveness of a Treatment Switch to Nevirapine plus Tenofovir and Emtricitabine (or Lamivudine) in Adults with HIV-1 Suppressed Viremia. PLoS ONE 10(6): e0128131. doi:10.1371/journal.pone.0128131

Academic Editor: Mark A Wainberg, McGill University AIDS Centre, CANADA

Received: February 12, 2015

Accepted: April 22, 2015

Published: June 24, 2015

Data Availability Statement: All relevant data are within the paper. All study data will be available on request once published. Researchers who meet the criteria for access to confidential data should contact Isabel Bravo (ibravo@flsida.org) for database requirements, or with Arelly Ornelas (arellyo@gmail.com) for statistical issues, and will need the approval of the Hospital Universitari Germans Trias i Pujol (Badalona, Barcelona, Spain) Institutional Data Access / Ethics Committee. All data will be anonymized in accordance with National standards.

Abstract

Background

Switching subjects with persistently undetectable HIV-1 viremia under antiretroviral treatment (ART) to once-daily tenofovir/emtricitabine (or lamivudine) + nevirapine is a cost-effective and well-tolerated strategy. However, the effectiveness of this approach has not been established.

Methods

We performed a retrospective study evaluating the rates of treatment failure, virological failure (VF), and variables associated, in all subjects initiating this switch combination in our clinic since 2001. Analyses were performed by a modified intention to treat, where switch due to toxicity equalled failure. The main endpoint was plasma HIV-RNA < 50 copies/mL.

Results

341 patients were treated for a median of 176 (57; 308) weeks. At week 48, 306 (89.7%) subjects had HIV-1 RNA <50 copies/mL, 10 (2.9%) experienced VF, and 25 (7.4%) discontinued the treatment due to toxicity. During the whole follow-up 23 (6.7%) individuals (17 on lamivudine, 6 on emtricitabine; p = 0.034) developed VF and treatment modification due to toxicity occurred in 36 (10.7%). Factors independently associated with VF in a multivariate analysis were: intravenous drug use (HR 1.51; 95%CI 1.12, 2.04), time with undetectable viral load before the switch (HR 0.98; 0.97, 0.99), number of prior NRTIs (HR 1.49; 1.15, 1.93) or NNRTIs (HR 3.22; 1.64, 6.25), and previous NVP (HR 1.54; 1.10, 2.17) or efavirenz (HR 5.76; 1.11, 29.87) unscheduled interruptions. VF was associated with emergence of usual nevirapine mutations (Y181C/I/D, K103N and V106A/I), M184V (n = 16; 12 with lamivudine vs. 4 with emtricitabine, p = 0.04), and K65R (n = 7).

Funding: This work was supported by "CHAIN, Collaborative HIV and Anti-HIV Drug Resistance Network" (Integrated Project no. 223131, funded by the European Commission Framework 7 Program) and by "Gala contra la Sida Barcelona 2011". The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript. The authors had full access to the data, and the corresponding author had the final responsibility for submitting the manuscript for publication.

Competing Interests: JML has received research funding, consultancy fees, and lecture sponsorships from or has served on advisory boards for Boehringer Ingelheim, Bristol-Myers Squibb, Gilead Sciences, Janssen-Cilag, Merck Sharp & Dohme, and ViiV Healthcare. JRS has received research funding, consultancy fees, and lecture sponsorships from and has served on advisory boards for Abbott, Boehringer Ingelheim, Gilead Sciences, Janssen-Cilag, Bristol-Myers Squibb, ViiV Healthcare, and Pfizer. RP has received consulting fees from Pfizer and grant support from Pfizer, Roche Diagnostics, Siemens, Merck and Boehringer-Ingelheim. BC has received research funding, consultancy fees, and lecture sponsorships from and has served on advisory boards during the past 5 years for Abbott, Gilead Sciences, ViiV, Janssen-Cilag, and Merck Sharp & Dohme. The remaining authors declare no potential conflicts of interest. This does not alter the authors' adherence to PLOS ONE policies on sharing data and materials.

Conclusions

The rates of treatment failure at 48 weeks, or long-term toxicity or VF with this switch regimen are low and no unexpected mutations or patterns of mutations were selected in subjects with treatment failure.

Introduction

Many subjects on suppressive antiretroviral therapy (ART) may be considered candidates for long-term regimen simplification towards easier to administer, more tolerable, or more cost-effective regimens [1–3]. Treatment guidelines consider that boosted protease inhibitors (PI) or efavirenz may be switched for toxicity, simplification, prevention or improvement of metabolic abnormalities or adherence facilitation to unboosted atazanavir, non-nucleoside reverse transcriptase inhibitors (NNRTIs; NVP, efavirenz, rilpivirine), or integrase inhibitors (raltegravir or elvitegravir/cobicistat) [1–7].

Nevirapine (NVP) displayed similar rates of efficacy at 12 and 36 months against efavirenz in simplification [8–13], and achieved the lowest rates of virological failure and higher lipid benefits in the extended three-year follow-up in a randomized study [12;14;15]. However, some observational cohorts found higher rates of virological failure with nevirapine versus efavirenz [16–18]. Baseline uncontrolled biasing factors, mainly differences in calendar year of prescription, could have an impact on the results of these cohorts. In addition, it is one of the antiretroviral drugs that achieve higher reductions of residual plasma viremia to below 1 copy/mL and a better lipid profile [15;19–21]. Pre-treated individuals with high CD4 cell counts do not have the increased risk for treatment-limiting toxicity seen in naives, provided there is no detectable viremia at initiation of NVP [22]. NVP has a well-known initial potential toxicity profile, and has not been associated to any specific long-term toxicity.

Among the newest drugs, only elvitegravir/cobicistat has been evaluated in randomized studies as a switch strategy for subjects receiving NVP [7]. It demonstrated non-inferior efficacy in the substitution of efavirenz or nevirapine, albeit the sole benefit (lipid profile) in the study was seen only in the efavirenz subgroup. Therefore, maintenance of generic NVP in long-term therapy might offer a powerful approach to cost-savings in well-resourced countries, and be a common strategy in countries with limited treatment options [23]. However, some patients and physicians may believe that a new brand-name drug is superior or more appealing, and could be reluctant to maintain an effective antiretroviral regimen based on a generic drug.

The combination of once-daily NVP plus TDF/FTC (or 3TC) has been extensively used as a long-term simplification regimen in some European countries, however information about the efficacy and long-term toxicity of this regimen is still scant [24;25]. Furthermore, 3TC has been associated with lower virological responses compared to FTC in some reports in naives, including one with NVP and TDF, but data are not available in simplification [26].

Therefore, accurate data on the long-term efficacy and toxicity of NVP plus TDF/FTC (or 3TC) as a switch regimen—with particular focus on the rates of VF or any particular pattern of unexpected mutations—are needed.

Methods

Study design and study subjects

We performed a retrospective cohort study of HIV-infected patients attending a tertiary University Hospital in Barcelona, Spain since 2001, when all drugs became available. All subjects aged ≥18 years with documented HIV-1 infection were included if they started treatment with

NVP plus TDF plus FTC (or 3TC) as a switch from any previous regimen, with an undetectable plasma viral load (pVL), and had at least one subsequent follow-up visit. The inclusion criteria allowed incorporating subjects with early withdrawal of the regimen due to toxicity. Subjects were followed until they stopped the regimen for any reason. The study was approved by the Hospital Research Ethics Committee and conducted in accordance with the Declaration of Helsinki and National standards.

Historical follow-up data were extracted from medical records through a systematic database search. No restriction criteria were included in the search. All causes of treatment discontinuation were registered. VF was defined as two consecutive pVL >50 copies/ml.

Baseline characteristics were gathered, including age, gender, risk factor for HIV acquisition, time of HIV infection, number of prior antiretroviral drugs and antiretroviral regimens, prior NRTI mono or dual therapy, time with HIV-1 RNA suppression before the switch, hep B or C co-infection, and reason to initiate the study regimen. $CD4^+$ cell counts and pVL were collected every 12–24 weeks thereafter, until the last sample available.

The complete previous treatment history was searched, and all previous NNRTI interruptions were recorded, as well as all prior treatment failures.

Genotypic resistance tests prior to the initiation of the regimen and the available resistance studies in those patients who failed were collected.

Statistical analysis

Patients' characteristics were described using medians (IQR) for continuous, non-normal variables and percentages for categorical variables.

The primary endpoint was the proportion of subjects with pVL <50 copies/mL at 48 weeks. We based our efficacy and safety analyses on a modified intent-to-treat (mITT, S = F) exposed or safety populations, which consisted of all patients initiating the regimen with any treatment discontinuation due to toxicity or voluntary treatment discontinuation considered as treatment failure. Subjects substituting 3TC with FTC during the study were not considered failures. Patients lost to follow-up or withdrawing the regimen due to reasons unrelated to toxicity (i.e. recruited into a clinical trial) or efficacy were censored at that time in the analysis, provided they had a pVL <50 c/mL and no toxicity at that visit, considering that this was a retrospective study and subjects were not tied up to an allocated treatment.

VF and factors associated with it were also pre-planned analyses. A secondary analysis assessed the percentage of patients remaining on the same regimen with a pVL < 50 copies/mL at the end of follow-up.

A relevant list of covariates was included in a multivariate Cox proportional model to determine factors independently associated with VF. The model was adjusted for age, intravenous drug use, hepatitis C co-infection, number of prior NNRTIs received, prior NNRTI treatment interruptions, presence of 3TC (versus FTC) in the regimen, inclusion of NVP in the last regimen, and duration of HIV-1 infection.

All variables with a significant association ($p<0.05$) in the univariate analysis were introduced into the multivariate model. The multivariate analysis was run in the overall cohort and also excluding subjects already on NVP at the time of the switch. The duration of treatment and time to VF were estimated using the Kaplan-Meier method. All statistical analyses were performed using SPSS software for Windows (version 15.0; SPSS Inc, Chicago, IL, USA).

Results

Baseline characteristics

We identified 367 patients having started a combination including NVP plus TDF plus FTC (or 3TC). Of them, 26 were treatment naïve when they initiated this combination, and were excluded. Cohort demographics of the remaining 341 are shown in Table 1. Most study subjects were male (72%), with a mean age of 42 years. The mean time with undetectable pVL at regimen initiation was 48 months, and had been exposed to a median number of 6 drugs. Prior VFs before initiating NVP were documented in 24% of them.

Reasons for initiation of the regimen

The main reasons for initiating the switch regimen were prior drug toxicity (169, 49.6%), treatment simplification (149, 43.7%), and pregnancy desire (5, 1.5%).

Patient disposition at 48 weeks

Overall, 295/341 (86.5%) patients had a pVL <50 copies/mL at 48 weeks (mITT, S = F), and 10 (2.9%) experienced confirmed VF at 48 weeks. Drug toxicity led to treatment discontinuation in 22 (6.6%) subjects, and 14 (4.0%) experienced a voluntary treatment discontinuation.

Toxicity was specifically assessed in all NVP-naïve subjects at the initiation of the study regimen (168 out of 341). Of them, NVP was discontinued in 20 (11.9%), mainly due to early development (most of them at first trimester) of rash or laboratory liver abnormalities. Only 3 (2%) subjects developed grade 4 transaminase increases, none a severe clinical liver event, and none grade 4 rash.

In an on-treatment analysis, 96.2% of subjects receiving NVP at 48 weeks had a pVL <50 copies/mL.

Patient disposition at the last follow-up visit

Patients stayed on the regimen for a median of 176 (57; 308) weeks and 215 (63.5%) patients discontinued the study regimen at any time during the follow-up. The reasons for treatment discontinuation at the last available control were: lost to follow-up (43, 12.6%), voluntary treatment interruption (37, 10.9%), recruitment for a randomized clinical trial (37, 10.9%), toxicity (34, 10.1%), confirmed VF (23, 6.7%; 17 on 3TC and 6 on FTC, p = 0.034), subsequent treatment simplification (18, 5.3%). Among individuals with immune discordance despite a suppressed viremia, 21 (6.1%) received proactive treatment changes (most of the latter empirically switched NVP to a PI/r, a common practice during some years). Therefore, only 57 (16.8%) subjects discontinued the treatment due to toxicity or lack of efficacy at 4 years.

Hence 156 (45.8%) patients discontinued the study regimen or follow-up in real clinical practice due to reasons unrelated to treatment efficacy. Of the overall cohort, 126 (37.3%) were still on the same regimen and with an HIV-RNA <50 copies/mL in their last control available (median 4 years). The median time to VF and treatment discontinuation are depicted in Fig 1.

Factors associated with VF

In a multivariate analysis adjusted for variables described in Table 2, factors independently associated with VF were: intravenous drug use (HR 1.51; 95%CI 1.12, 2.04), longer time with undetectable pVL before regimen initiation (HR 0,98; 0.97, 0.99), number of prior NRTIs received (HR 1.49; 1.15, 1.93), number of prior NNRTIs received (HR 3.2; 1.6, 6.3), prior NVP interruptions (HR 1.54; 1.10, 2.17), prior efavirenz interruptions (HR 5.76; 1.11, 29.87), and

Table 1. Patient characteristics at the initiation of NVP plus TDF plus FTC (or 3TC) as a switch strategy (n = 341).

Age (yrs, mean [SD])	42.2 (8.7)
Gender (male, %)	246 (72)
Risk category for HIV acquisition (n, %)	
MSM	125 (37.3)
Intravenous Drug Users	92 (27.5)
Heterosexual	92 (27.5)
Other	32 (9.4)
Hepatitis B or C Co-infection (n, %)	
Hep C	98 (29.8)
Hep B	25 (7.7)
Pregnancy	7 (7.4)
Nadir CD4 cell count (cells, mean [SD])	239 (148)
Baseline CD4 cell count (cells, median [IQR])	492 (331,7)
Time of HIV-1 infection (months, mean [SD])	128 (69)
Time with undetectable viral load at regimen initiation	48 (33)
(months, mean [SD])	
Prior Antiretroviral exposure (n of drugs, median [IQR])	6 (4,8)
Number of prior NRTI	4 (2,5)
Number of prior NNRTI	1 (1,1)
Number of prior PI	1 (1,2)
Prior NRTI mono or dual therapy (n, %)	146 (43)
Both NRTI mono and dual NRTI prior therapy	60 (18)
Prior virologic failures documented (n, %)	79 (24)
Prior NNRTI documented treatment interruption (n, %)	99 (29.2)
NNRTI interruption only once	1 (23)
More than 1 NNRTI interruption	44 (13)
Drug previously interrupted:	
Nevirapine	60 (18)
Efavirenz	18 (5)
Viral load at baseline < 50 copies/mL (n, %) *	264 (78.3)
Lamivudine present in the last regimen	193 (57)
Emtricitabine present in the last regimen	66 (19)
Tenofovir present in the last regimen	126 (37)
Nevirapine present in the last regimen	173 (51)
Drug substituted by NVP	
Efavirenz	56 (33)
Protease inhibitor (indinavir, nelfinavir, saquinavir)	40 (24)
Boosted protease inhibitor (lopinavir, atazanavir, darunavir)	60 (36)
Other (raltegravir, etravirine)	12 (7)
Received 3TC + NVP + TDF	159 (47)
Received FTC + NVP + TDF	182 (53)

Data are median (IQR) or n (%).

* Some individuals in the early calendar years had an undetectable viral load at baseline, but with tests using at that moment a threshold of 80 or 200 copies/mL.

MSM: Men having sex with men; NRTI: Nucleos(t)ide reverse transcriptase inhibitor; NNRTI: non-nucleoside reverse transcriptase inhibitor; PI: Protease inhibitor.

doi:10.1371/journal.pone.0128131.t001

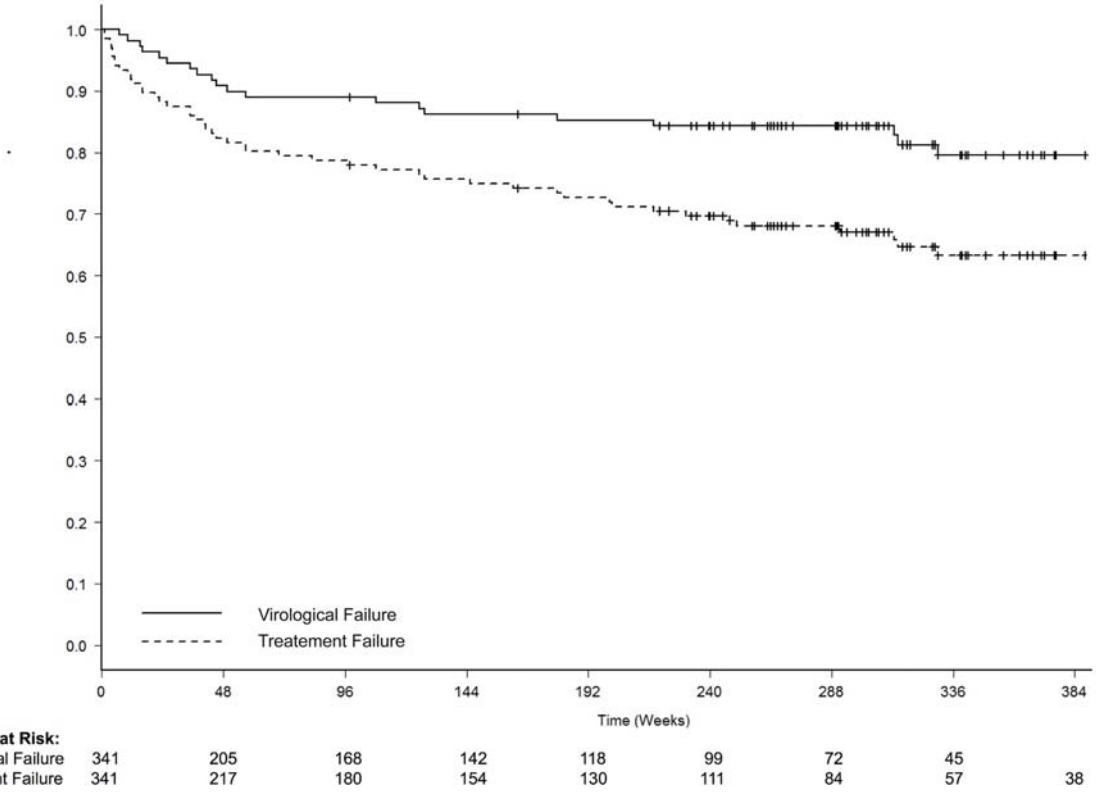

Fig 1. Time to virological failure and treatment failure through the long term follow-up. Virological failure was defined as two consecutive measurements of pVL >50 copies/mL. Treatment failure included subjects with virological failure, treatment discontinuations due to drug toxicity, and death.

doi:10.1371/journal.pone.0128131.g001

NVP being present in the last regimen prior to current simplification (HR 0.57; 0.43, 0.76). Other factors significantly associated with VF in univariate analysis are shown in Table 2.

Resistance selection at failure

Population genotypes at failure were available in all 23 subjects (6.7%) experiencing confirmed VF (Table 3). None of them had previous resistance tests available. Wild-type HIV-1 was seen in 5 (22%) of them. Sixteen out of 23 (70%) had NNRTI mutations (Y181C/I/D: 10; K103N: 6; V106A/I: 3; Y188C/L: 2; K101Q/E: 2; M230L: 1; P225H: 1; A98G: 1; V108I: 1; F227L: 1; K238T: 1), and 10 had >1 NNRTI mutation.

M184V was selected in 16 (70%) subjects: 12 treated with 3TC and 4 with FTC (p = 0.04). Seven patients had K65R (6 associated to M184V, none with thymidine-analogue mutations [TAMs]), 5 of them treated with 3TC and 2 with FTC. Six patients selected A62V, with K65R selected in 4 of them, and none with Q151M or T69 insertions. Five patients harboured TAMs. Three subjects harboured major protease mutations (V32I, M46I, I47V, L90M), selected in prior failures.

Table 2. Factors associated with virologic failure to a switch regimen composed of NVP plus TDF plus FTC (or 3TC) (n = 341).

	Univariate		Multivariate	
Variable	**HR (95% CI)**	**p value**	**HR (95% CI)**	**p value**
Age	0.99 (0.96, 1.03)	0.76		
Gender	1.41 (0.72, 2.78)	0.31		
Intravenous Drug Users	1.98 (1.09, 3.58)	0.02	1.51 (1.12, 2.04)	0.01
Hepatitis B or C Co-infection				
Hep C	1.58 (0.87, 2.87)	0.13		
Hep B	0.90 (0.32, 2.52)	0.85		
Nadir CD4 cell count	0.98 (0.79, 1.22)	0.86		
Baseline CD4 cell count	0.80 (0.69, 0.92)	0.00		
Time of HIV-1 infection	1.00 (1.00, 1.00)	0.92		
Longer time with undetectable VL	0.98 (0.97, 1.00)	0.01	0.98 (0.97, 0.99)	0.01
Prior Antiretroviral exposure	1.01 (0.89, 1.13)	0.93		
number of prior NRTI	1.18 (0.97, 1.43)	0.10	1.49 (1.15, 1.93)	0.00
number of prior NNRTI	2.38 (1.49, 3.85)	0.00	3.22 (1.64, 6.25)	0.00
number of prior PI	1.03 (0.81, 1.31)	0.82		
Prior NRTI mono and dual therapy	1.14 (0.65, 2.01)	0.65		
Prior VF documented	1.76 (0.96, 3.25)	0.07		
Prior NNRTI treatment interruption	2.13 (1.20, 3.78)	0.01		
Nevirapine interruptions	1.72 (0.86, 3.45)	0.13	1.54 (1.10, 2.17)	0.01
Efavirenz interruptions	3.11 (1.23, 7.90)	0.02	5.76 (1.11, 29.87)	0.04
VL at baseline <50 c/mL *	0.22 (0.12, 0.39)	0.00		
3TC present in the last regimen	0.72 (0.40, 1.30)	0.27		
FTC present in the last regimen	0.30 (0.04, 2.18)	0.23		
TDF present in the last regimen	1.01 (0.53, 1.95)	0.97		
NVP present in the last regimen	0.32 (0.18, 0.57)	0.00	0.57 (0.43, 0.76)	0.00
3TC (vs FTC) in the regimen	2.48 (1.38, 4.46)	0.00		

* Some individuals in early calendar years had an undetectable viral load at baseline, but with tests using a threshold of 80 or 200 copies/mL.
VL: viral load; VF: virologic failures; NNRTI: non-nucleoside reverse transcriptase inhibitor; NRTI: nucleoside analogue reverse transcriptase inhibitor; PI: protease inhibitor.

doi:10.1371/journal.pone.0128131.t002

Discussion

The 48-week (2.9%) and long-term (6.7% at 4 years) rates of VF of a switch regimen composed of NVP plus TDF plus FTC (or 3TC) in real clinical practice are low, and similar to those of other common switch strategies seen in similar cohorts and recommended in international guidelines [1;2;27;28]. These strategies include unboosted atazanavir, rilpivirine, raltegravir or elvitegravir/cobicistat, and the corresponding rates of VF in their pivotal clinical trials were 1–8% [7;29;30]. However, data from prospective clinical trials are not comparable to those of cohorts including patients seen in everyday circumstances, and the rates of VF reported in cohorts have been higher and similar to our series [5;7;28–32]. Actually, raltegravir showed higher rates of VF in a randomized switch clinical trial (9.1% at 24 weeks), early terminated by a DSMB because of lower than expected virological efficacy [33;34].

Whilst VF was infrequent, drug resistance mutations against NNRTIs and NRTIs were frequently isolated in patients with VF, in agreement with what has been seen in pivotal trials with NVP in naives, and also with other drugs with a similarly low genetic barrier to resistance (efavirenz, rilpivirine, raltegravir and elvitegravir/cobicistat) in initial therapy [31;33–42]. On

Table 3. Mutations shown at failure in the reverse transcriptase and protease, and NRTI included in the regimen together with NVP and TDF (3TC vs FTC).

3TC/ FTC	Reverse transcriptase	Protease
FTC	A62V,K65R,Y181C,M184V	None
FTC	A62V, K65R, V75I, K103N, Y181C, M184V, M230L,	L63P
3TC	A62V,L74V,K103N,V106A,M184V,T215S, P225H	L63P
3TC	A62V, K65R, K101Q, Y181C	K20M, M36I, M46I, Q58E, L63P, L90M, I93L
3TC	V118I, M184V, Y188C	L63P
FTC	M41L, E44D, D67N, K70R, M184V, T215Y, K219Q	L63P
3TC	Y181I, M184V	G16E
3TC	M41L,A62V,T69N,K70R,K103N,V108I,M184V, T215F,K219E	R41K, L63P, A71V, V77I, L90M, I93L
3TC	D67N, K103N, Y181C, M184V, K219E	I62V, I64V
3TC	None	L63P
3TC	A62V, K65R, A98G, Y181C, M184V	L63P
3TC	K65R, M184V, Y188L	I15V,V77I, I93L
FTC	None (wild-type)	V77I
FTC	L74V/L, Q102K/R, K103K/N, D177E/G, Y181Y/ D, M184V, G190G/A	K20R, M36I, L63P
3TC	K65R, V108I, Y181C, M184V	13V, K20T, V32I, E35D, M36I, K43T, M46I, I47V, F53L, L63P, I66F, A71V, G73S, V77I, L90M,
3TC	M41L, E44D, D67N, T69D, V118I, Y181C, M184V, G190A, L210W, T215Y, V106V/I, F227F/I	L33V
3TC	None	M36I,L63P,V77I
3TC	K103N/S,Y181C,M184V	L33V, R41K, I64V, I13V/I, L63P
FTC	K101E, G190A, K238T	L10V, I13V, L63P
3TC	None	I13V, M36I, L63P
3TC	M41L, M184V, L210W, T215Y	V118I
3TC	None	I13V, L63P, V77I
3TC	K65R, V106A, M184V	None

doi:10.1371/journal.pone.0128131.t003

the other hand, unboosted atazanavir maintains a high genetic barrier to resistance in switch despite the absence of pharmacokinetic boosting, and no major protease mutations isolated in failures in randomised clinical trials even in the long term, albeit they have indeed been reported in some cohorts in real clinical practice [28;29].

Reassuringly, the frequency and type of mutations seen in our series was concordant with what has been previously seen in other NVP studies, with a potential impact of some of them against the activity of etravirine in subsequent treatments [43]. The most common emergent NNRTI mutations were Y181C/I/D, K103N and V106A/I [44].

The rate of K65R selection was low was, but appeared in approximately one of every three failures, usually with M184V, and more frequently in those treated with 3TC (vs FTC) [35;36].

We observed a significantly higher rate of VF in individuals treated with 3TC instead of FTC, with a significantly higher rate of selection of M184V as well. Nevertheless, these data must be interpreted with caution, as calendar years when subjects received 3TC or FTC were

different—as well as the number of pills in the regimen—and unmeasured confounders could exist. However, this is concordant with a double risk of VF (adjusted OR 2.2; 95%CI 1.1, 4.6) for 3TC vs FTC found in treatment-naives [26;45]. Some previous reports have suggested potential differing resistance profiles for FTC and 3TC when administered in combination with TDF [46;47]. Moreover, previous reports have shown differing resistance profiles for FTC and 3TC when administered in combination with TDF [46;47]. Therefore, our findings suggest caution against substituting FTC for 3TC, at least in NVP- and TDF-based regimens, while other series review their data [1]. A shorter intracellular $t_{1/2}$ of activated triphosphate 3TC compared to FTC (15 h versus >39 h), a 4- to 10-fold lower antiviral potency of FTC in a range of cell line *in vitro* passages, and the ability of FTC to inhibit cellular efflux proteins such as the multidrug resistance–associated proteins could account for a lower forgiveness of 3TC, at least when combined with NVP [23;47–49]. This is due to the ability of FTC to inhibit the activity of the cellular efflux proteins, such as the multidrug resistance–associated proteins, that extrude the drugs out of the CD4+ cells [47].

No subjects selected NRTI resistance mutations without NNRTI ones, thus confirming that NNRTI resistance is selected first, in agreement with previous reports [50;51].

In an adjusted multivariate analysis, we found an independent association of VF with intravenous drug use, time with undetectable VL, number of prior NRTIs or NNRTIs received, and prior NVP or efavirenz interruptions. Actually, intravenous drug users and higher antiretroviral drug exposure are variables universally associated to increased rates of VF to any switch regimen [10;34;52]. The long half-life of NNRTIs, as compared with that of some NRTIs, may allow a long terminal tail in plasma pharmacokinetics with suboptimal late NNRTI functional monotherapy in unplanned treatment interruptions. In addition, repeated drug holidays (>48 h of drug cessation) have been previously associated with VF to NVP and efavirenz [52]. These findings have clinical translation indeed. Some studies using standard and ultrasensitive techniques have been able to detect NNRTI-associated mutations in up to 14–16% of individuals who discontinued a NNRTI-based regimen with a pVL <50 copies/mL, particularly with a simultaneous interruption (instead of a staggered interruption) of all drugs in the regimen [53;54]. Moreover, these interruptions led to a 14-fold increased risk of detecting genotypically resistant HIV-1-RNA in female genital tract secretions, therefore potentially increasing the risk of HIV transmission [55].

Therefore, reinitiation of NVP plus TDF plus FTC (or 3TC) should be discouraged in subjects experiencing unplanned treatment interruptions, even with an undetectable pVL at the time of treatment withdrawal.

The main reasons for initiating the regimen in our series were prior toxicity and treatment simplification, which still remain as the main reasons currently.

The rates of treatment discontinuation due to adverse events in our cohort (6.6% at 48 weeks, 10.6% overall at 4 years, and 11.9% in those initiating new NVP) are concordant with rates seen in other NVP or efavirenz studies [10;12;35;36], but are higher than those observed with some other switch strategies and constitute the main limitation of this regimen [29;30;33;34]. This is a drug-related effect of NVP, and suspicion of hypersensitivity reactions or increases in liver transaminases were the most frequent reasons for stopping the regimen, mainly during the first 12 weeks. These early toxicity-associated withdrawals prevented the demonstration of non-inferiority versus efavirenz in initial treatment in the 2NN study as well as in a recent systematic review [56;57]. Grade 4 adverse events were seen in only 2% of the patients and no severe clinical events were reported among the 341 subjects. Not unexpectedly, those receiving NVP in their baseline regimen before the switch were indeed less prone to toxicity. The greater risk of symptomatic hepatic or skin events, including serious and potentially life-threatening events, although the latter not observed in our series, may remain an intrinsic

restriction to new NVP initiation in the future, as compared to the lower intrinsic toxicity of newest drugs.

However, the most frequent reasons for withdrawing the regimen in real clinical practice were not related to toxicity or lack of efficacy, but proactive treatment changes, recruitment for clinical trials, or patients being lost to follow-up, with subjects having suppressed viremia at that time point.

Generic substitution is one mechanism of curtailing prescription drug expenditures. Currently available as a generic and with reference pricing, a cost/efficacy assessment done by the Spanish GESIDA Society has shown that NVP plus TDF/FTC (or 3TC) constitutes a cost-effective treatment in Europe despite the availability of many new regimens [58]. It is administered once-daily as a two-pill regimen, and is commonly used in developing countries as well as developed countries with economic constraints [58].

These findings inform regimen management in clinical practice, and would support the long-term maintenance of this strategy in subjects without initial toxicity. Actually, newer antiretroviral drugs have not demonstrated advantages in switch studies in subjects treated with NVP plus TDF/FTC [7].

Our study is subject to the limitation of its retrospective design, which could lead to bias with unmeasured confounding factors such as treatment adherence or channelling prescription by physicians. We specifically made every effort to capture all subjects receiving the first dose of the regimen, to avoid underestimation of toxicity. The study included subjects who changed the whole regimen and subjects who were already receiving NVP and only changed the NRTI backbone. However, both subgroups have been analysed separately in a sensitivity analysis to pinpoint the toxicity of NVP. Nonetheless, the study reports the largest cohort of patients treated with this switch regimen so far, and the results are consistent and robust through adjusted and sensitivity analyses. Important information gleaned from the study includes higher risk of failure with such a switch in a setting for subjects who had previously been exposed to NNRTI and had a history of treatment interruption.

In conclusion, a simplification regimen with NVP plus TDF and FTC (or 3TC) in pretreated subjects maintained virologic suppression with a low risk of short and long-term treatment or VF in subjects without prior NNRTI treatment interruptions, and a low rate of long-term adverse events. The rates of VF are similar to other switch strategies, and no unexpected mutations or patterns of mutations have been selected at failure. These findings do not suggest increased early or late VF rates with this regimen when used as a simplification strategy. However, the rates of discontinuation due to early toxicity were higher. While potentially severe initial toxicity might limit its new initiation in the future, these data support caution against a systematic proactive switch of those subjects successfully treated with this regimen towards newest drugs until clinical advantages to patients are demonstrated in randomised clinical trials.

Acknowledgments

We are grateful to Nuria Perez-Alvarez for assistance in the statistical analysis, and to Ana Chamorro for patient data management.

Author Contributions

Conceived and designed the experiments: JML. Performed the experiments: JML IB AO JRS JP. Analyzed the data: JML AO JP IB. Wrote the paper: JML JRS RP. Reviewed the manuscript draft: RMI BC.

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DISCUSSION

DISCUSSION

This doctoral thesis shows that the knowledge of RAMs existing at baseline and selected by NNRTIs, as well as the cross-resistance existing among them is a key driver of drug susceptibility and treatment response both in salvage and in simplification ART strategies [291-293]. The differential influence that every RAM or pattern of RAMs will have in either NNRTI has a paramount relevance on the choice of the appropriate NNRTI and backbone treatment regimen in every scenario [293]. We have shown that rilpivirine resistance is not uncommon among subjects failing other NNRTIs. In addition, we have shown a high efficacy of etravirine-based regimens in salvage ART, as well as of nevirapine plus tenofovir DF plus emtricitabine (or lamivudine) in switch ART [291, 292].

Efficacy of salvage regimens with etravirine

We have been able to demonstrate that salvage regimens containing etravirine plus optimized antiretroviral drugs in heavily treatment-experienced individuals show higher effectiveness rates than those observed in the pivotal etravirine trials done in the drug registration process [127, 128, 261]. In our analysis done in 122 subjects treated at the HIV units of four acute-care university hospitals in Barcelona (Spain), 73% of patients had a viral load of <50 copies/ml at 48 weeks in a restrictive full dataset analysis. This result suggests a high efficacy of these drug regimens, and goes beyond the rates observed in the pooled DUET-1 and -2 trials, where 61% of patients receiving etravirine achieved complete viral suppression at 48 weeks [127, 128, 294].

Achieving higher rates of efficacy in real clinical practice than in registrational pivotal randomized trials is the opposite of what normally happens in treatment-naive patients,

in whom the excellent response rates seen in clinical trials are difficult to match in routine clinical practice [16]. This higher effectiveness of etravirine, when prescribed as salvage ART in clinical practice, is probably due to the availability of more active agents than were available during the initial clinical trials, and a better knowledge of the impact of the NNRTI-associated RAMs. Results are even more impressive considering that according to the Stanford HIV database scoring, etravirine was fully active in only 56/122 (45.9%) patients, intermediate in 49/122 (40.2%), and resistant in 8/122 (6.6%). In addition, 58% of the subjects had a CXCR4 or dual-mixed or non-reportable tropism, and had received a median of 11 antiretroviral drugs, hence constituting a heavily pretreated compromised population.

Indeed, preliminary clinical trials and expanded-access programs evaluating the efficacy and safety of etravirine with raltegravir and darunavir (or other boosted PIs) have shown outstanding efficacy rates and a good safety profile, achieving undetectable viral loads at 48 weeks in as many as 70% and 81% of patients after 48 weeks of treatment, in agreement with our data [130, 139, 295]. Darunavir and raltegravir were the most frequently used antiretroviral agents in the optimized baseline treatments in our series, and were associated with significantly higher rates of treatment response and virological suppression in the multivariate analysis. The hazard ratio for treatment failure among those receiving darunavir was 0.46 (95% confidence interval 0.21-0.98), and the hazard ratio for raltegravir was 0.47 (95% confidence interval 0.22-0.99). In our series, the number of patients taking maraviroc or other boosted PIs (not darunavir) plus etravirine was too low for conclusions to be drawn about efficacy.

Baseline plasma HIV-1 RNA and CD4+ T cell count, adherence, number of active agents in the background regimen, and use of enfuvirtide were predictors of virological response with etravirine in salvage regimens in the pooled 48-week results from the

DUET studies [294]. We also found a relationship between low baseline CD4+ T count (<200 cells/mm^3) and treatment response at week 48, which is consistent with the fact that advanced stages of HIV infection are associated with poorer treatment response rates, a common finding in salvage studies [8, 113-115, 132-134, 136].

We also found a higher proportion of patients whose regimen had failed with ≤2.5 active drugs than with ≥3 active drugs at baseline, although we were unable to demonstrate a statistically significant difference (p=0.802), probably due to the low number of subjects receiving ≤2.5 active drugs. In addition, we were unable to find a relationship between treatment response and baseline HIV-1 RNA >100,000 copies/ml, a predictor that is universally associated with higher rates of treatment failure. In our series, the number of individuals with baseline viral load >100,000 copies/ml was very low (5/122 patients), a finding previously reported due to the impact on viral fitness of the RAMs [3].

These data highlight that the number of active drugs is probably a stronger predictor of response than a higher baseline viral load in treatment-experienced patients.

We found no relationship between prior interruption or failure with nevirapine or efavirenz and response to etravirine, in concordance with what was observed in the DUET trials [127, 128, 294].

Not unexpectedly, and consistent with the results of other studies, there was a significant increase in CD4+ T cell count during follow-up. Rash, a known etravirine adverse event [296], occurred in 50% of patients who experienced possibly or probably drug-related side effects, although led to discontinuation in only three patients (2.5%), while the remaining five patients presented mild and transient rash that did not require discontinuation. It has been described more frequently in combination with darunavir and in women [294]. We were not able to confirm this relationship in our study, due to

the low number of women included and the low prevalence of rash. No other unexpected side effects leading to discontinuation of etravirine were observed.

This analysis has the limitations inherent to its retrospective design however the sample size analyzed gives a high certainty to the results.

In summary, in routine clinical practice, etravirine-containing salvage regimens were generally well tolerated and achieved rates of virological suppression that exceed those observed in clinical trials. This is probably due to a higher number of new active drugs in the regimen due to both their current availability and a better knowledge of the scoring of the activity of antiretrovirals in salvage when evaluating compiled genotypic resistance tests. Darunavir and raltegravir were safe and very effective antiretrovirals when administered in combination with etravirine.

Rilpivirine RAMs in subjects failing NNRTI-based therapies

In a total of 1064 samples from patients failing efavirenz (54.5%), nevirapine (40%) or etravirine (5.5%) at 22 clinics in Spain (ResRis research network), rilpivirine resistance has been overall recognized in nearly 20% of patients. It is more common following etravirine (27.6%) or nevirapine (25%) failures than efavirenz (14.5%).

The most prevalent RAMs were Y181C (21.8%), V108I (10.2%), K101E (9.1%), V90I (7.9%) and V179I (6.1%). All other rilpivirine RAMs examined were present at rates below 5%. Codon 138 mutants were very rarely isolated (1%) in patients that had failed NNRTI-based regimens other than rilpivirine, and seen mainly in subjects with etravirine failure. Moreover, the combination E138K+M184I, commonly seen in naives failing a rilpivirine-based ART, was absent in this pre-treated population [150, 153, 154, 260]. Indeed, the NRTI RAM M184I was present in 3.4% of the whole genotypes, whereas M184V was seen in 36.2%.

These results are in agreement with a German cohort reporting also a very low rate (0.5%) of E138K in antiretroviral-experienced patients [297]. Altogether, these results support that this RAM is rarely selected in patients failing on nevirapine and efavirenz and should be considered as specifically selected by etravirine [87, 119, 278].

In this series, 27.6% of 58 etravirine failures harboured mutant viruses interpreted as rilpivirine-resistant, and loss of rilpivirine susceptibility was mainly interpreted as a result of selecting changes other than E138K, as Y181C, V179I, V90I and/or V108I. Efavirenz failures were more common in our database, but only 14.5% of 580 efavirenz failures in our study were considered as rilpivirine-resistant. In contrast, this figure was 25% for 426 navirapine failures. Mutations L100I and V108I were significantly more frequent in patients failing on efavirenz than nevirapine (7.9 vs. 0.2 and 12.2 vs. 7.3%, respectively). Conversely, Y181C, Y181I, V106A, H221Y and F227L were significantly more prevalent in patients failing on nevirapine.

These rates are not negligible, and, therefore, drug resistance testing should be recommended before considering rilpivirine therapy in any patient that had failed on other NNRTIs, particularly due to the ease of performance of genotypic tests, optimal for the interpretation of RAMs in drug resistance NNRTI algorithms [101].

This study has as a limitation the lack of phenotypic drug resistance testing, which could add accuracy to the susceptibility levels identified for rilpivirine. So far, the phenotypic susceptibility data have poorly predicted the efficacy of most NNRTI, as generally these drugs behave as on-off, being active or not, with no room for clinically relevant partial activity. However, this is not exactly the case for rilipivirine, which retains residual activity with certain mutations.

In summary, the rate of E138 mutants is very rare in pretreated individuals failing NNRTI-based regimens other than rilpivirine. Almost 20% of patients failing NNRTIs

should be considered as rilpivirine resistant, as a result of selecting changes at other positions. The extent of cross-resistance seems to be higher for etravirine and nevirapine in comparison with efavirenz. The sequential use of rilpivirine in patients that had failed other NNRTIs should always be guided by resistance testing excluding cross resistance.

Effectiveness of a treatment switch to nevirapine plus tenofovir DF and emtricitabine (or lamivudine) in adults with suppressed HIV-1 viremia.

The rates of virological failure of a switch regimen composed of nevirapine plus tenofovir DF plus emtricitabine (or lamivudine) in real clinical practice are low, 2.9% at 48 weeks and 6.7% at 4 years. These rates are similar to those of other common switch strategies seen in similar cohorts and with drugs recommended in international guidelines [15, 16, 276, 298, 299]. These strategies include unboosted atazanavir, rilpivirine, raltegravir or elvitegravir/cobicistat, and the corresponding rates of virological failure in their pivotal clinical trials were 1–8% [276, 299, 300]. Actually, raltegravir showed even higher rates of virological failure in a randomized switch clinical trial (9.1% at 24 weeks), early terminated by a DSMB because of lower than expected virological efficacy [301]. However, the comparison between clinical trials and cohort has some caveats and must be done with caution. Our results were in complete agreement with other cohorts [298].

Whilst virological failure was infrequent, RAMs against NNRTIs and NRTIs were frequently isolated in patients with virological failure, in agreement with what has been seen in pivotal trials with nevirapine in naives, and also with other drugs with a similarly low genetic barrier to resistance (efavirenz, rilpivirine, raltegravir and elvitegravir/cobicistat) in initial therapy [148, 150, 153, 167-169, 246, 302].

The frequency and type of RAMs seen in our series was concordant as well with what has been previously seen in other nevirapine studies, and no unexpected RAMs or patterns of RAMs have been found, in disagreement with early pivot studies suggesting a high rate of virological failure and RAM selection with this combination [239, 240]. Some of the RAMs found have a potential impact against the activity of etravirine in subsequent treatments [87].

Sixteen out of 23 (70%) subjects had NNRTI mutations at failure (Y181C/I/D: 10; K103N: 6; V106A/I: 3; Y188C/L: 2; K101Q/E: 2; M230L: 1; P225H: 1; A98G: 1; V108I: 1; F227L: 1; K238T: 1), and 10 had >1 NNRTI mutation.

M184V was selected in 16 (70%) subjects: 12 treated with lamivudine and 4 with emtricitabine ($p = 0.04$). Seven patients had K65R (6 associated to M184V, none with TAMs), 5 of them treated with lamivudine and 2 with emtricitabine. An unexpected finding of our study has been this significantly higher rate of virological failure in individuals treated with lamivudine instead of emtricitabine, with a significantly higher rate of selection of M184V as well. These data must be interpreted with caution, as calendar years when subjects received lamivudine or emtricitabine were different—as well as the number of pills in the regimen—and unmeasured confounders could exist. However, these data are concordant with a double risk of virological failure (adjusted OR 2.2; 95%CI 1.1, 4.6) for lamivudine vs emtricitabine found in treatment-naives [303, 304]. The Dutch Athena cohort evaluated 4740 therapy-naive HIV-1-infected patients without baseline resistance initiating lamivudine or emtricitabine with efavirenz/tenofovir or nevirapine/tenofovir. It also identified that the use of lamivudine was associated with more virological failure at week 48 compared to emtricitabine with efavirenz/tenofovir (10.8% vs 3.6%; adjusted odds ratio 1.78; 95% confidence interval, 1.11-2.84) and nevirapine/tenofovir (27% vs 11%; adjusted odds ratio 2.09; 95% CI, 1.25-3.52) [303].

Some previous reports have suggested potential differing resistance profiles for emtricitabine and lamivudine when administered in combination with tenofovir DF [305-307]. Moreover, previous reports have shown differing resistance profiles for emtricitabine and lamivudine when administered in combination with tenofovir DF [46;47]. Therefore, our findings suggest caution against substituting emtricitabine for lamivudine, at least in nevirapine- and tenofovir-based regimens, while other series review their data. A shorter intracellular $t_{1/2}$ of activated triphosphate lamivudine compared to emtricitabine (15 h versus >39 h), a 4- to 10-fold lower antiviral potency of emtricitabine in a range of cell line *in vitro* passages, and the ability of emtricitabine to inhibit cellular efflux proteins such as the multidrug resistance–associated proteins could account for a lower forgiveness of lamivudine, at least when combined with nevirapine [305, 308]. This is mainly due to the ability of emtricitabine to inhibit the activity of the cellular efflux proteins, such as the multidrug resistance–associated proteins, that extrude the drugs out of the CD4+ cells.

No subjects selected NRTI resistance mutations without NNRTI ones, thus confirming that NNRTI resistance is selected first, in agreement with previous reports [309].

In an adjusted multivariate analysis, we found an independent association of virological failure with intravenous drug use, time with undetectable viral load, number of prior NRTIs or NNRTIs received, and prior nevirapine or efavirenz interruptions. Actually, intravenous drug users and higher antiretroviral drug exposure are variables universally associated to increased rates of virological to any switch regimen [164, 301, 310]. The long half-life of NNRTIs, as compared with that of some NRTIs, may allow a long terminal tail in plasma pharmacokinetics with suboptimal late NNRTI functional monotherapy in unplanned treatment interruptions [311]. In addition, repeated drug holidays (>48 h of drug cessation) have been previously associated with virological to nevirapine and efavirenz [310].

These findings might have clinical translation indeed. Some studies using standard and ultrasensitive techniques have been able to detect NNRTI-associated mutations in up to 14–16% of individuals who discontinued a NNRTI-based regimen with a plasma viral load <50 copies/mL, particularly with a simultaneous interruption (instead of a staggered interruption) of all drugs in the regimen [247, 312, 313]. Moreover, these interruptions led to a 14-fold increased risk of detecting genotypically resistant HIV-1-RNA in female genital tract secretions, therefore potentially increasing the risk of HIV transmission [314].

A relevant conclusion of the study is that reinitiation of nevirapine plus tenofovir plus emtricitabine (or lamivudine) should be discouraged in subjects experiencing unplanned treatment interruptions, even with an undetectable plasma viral load at the time of treatment withdrawal.

The rates of treatment discontinuation due to adverse events in our cohort (6.6% at 48 weeks, 10.6% overall at 4 years, and 11.9% in those initiating new nevirapine) are in concordance with rates seen in other nevirapine or efavirenz studies, but higher than those observed with some other switch strategies and constitute a main known limitation of this regimen [167-169, 197, 237, 276, 300, 301, 315]. Grade 4 adverse events were seen in only 2% of the patients and no severe clinical events were reported among the 341 subjects. Not unexpectedly, those receiving nevirapine in their baseline regimen before the switch were indeed less prone to toxicity.

The greater risk of symptomatic hepatic or skin events, including serious and potentially life-threatening events, although the latter not observed in our series, may remain an intrinsic restriction to new nevirapine initiation in the future, as compared to the lower intrinsic toxicity of newest drugs.

However, the most frequent reasons for withdrawing the regimen in real clinical practice in our series were not related to toxicity or lack of efficacy, but proactive treatment changes, recruitment for clinical trials, or patients being lost to follow-up, with subjects having suppressed viremia at that time point.

Generic substitution of branded drugs is one mechanism of curtailing prescription drug expenditures. Currently available as a generic and with reference pricing, a cost/efficacy assessment done by the Spanish GESIDA Society has shown that nevirapine plus tenofovir/emtricitabine (or lamivudine) constitutes a cost-effective treatment in Europe despite the availability of many new regimens [192]. It is administered once-daily as a two-pill regimen, and is commonly used in developing countries as well as developed countries with economic constraints.

These findings inform regimen management in clinical practice, and would support the long-term maintenance of this strategy in subjects without initial toxicity. Actually, newer antiretroviral drugs have not demonstrated advantages in switch studies in subjects treated with this combination [299].

Our study is subject to the limitation of its retrospective design, which could lead to bias with unmeasured confounding factors such as treatment adherence or channelling prescription by physicians. The study included subjects who changed the whole regimen and subjects who were already receiving nevirapine and only changed the NRTI backbone. However, both subgroups have been analysed separately in a sensitivity analysis to pinpoint the toxicity of nevirapine.

Nonetheless, the study reports the largest cohort of patients treated with this switch regimen so far, and the results are consistent and robust through adjusted and sensitivity analyses.

In summary, a simplification regimen with nevirapine plus tenofovir and emtricitabine (or lamivudine) in pretreated subjects maintained virologic suppression with a low risk of short and long-term treatment or virologic failure in subjects without prior NNRTI treatment interruptions, and a low rate of long-term adverse events. The rates of virologic failure are similar to other switch ART strategies, and no unexpected RAMs or patterns of RAMs have been selected at failure. These findings do not suggest increased early or late virological failure rates with this regimen when used as a simplification strategy. However, the rates of discontinuation due to early toxicity were higher. While potentially severe initial toxicity might limit its new initiation in the future, these data support caution against a systematic proactive switch of those subjects successfully treated with this regimen towards newest drugs until clinical advantages to patients are demonstrated in randomised clinical trials.

CONCLUSIONS

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1. In routine clinical practice, etravirine-containing salvage regimens are generally well tolerated and achieve rates of virological suppression that exceed those observed in its pivotal clinical trials. This is probably due to the inclusion of a higher number of active drugs in the regimens.
2. Baseline $CD4^+$ T cell count <200 cells/mm^3 and use of raltegravir and darunavir in the regimen are factors associated with treatment failure to etravirine.
3. In agreement with the DUET studies, we found no relationship between prior interruption or failure with nevirapine or efavirenz and response to etravirine.
4. Rilpivirine resistance is overall recognized in nearly 20% of patients failing other NNRTIs. It is more common following etravirine or nevirapine failures than efavirenz.
5. The most prevalent of rilpivirine RAMs in subjects failing other NNRTIs were Y181C, K101E/P, H221Y and E138A/G/K.
6. E138K/M184I, the most frequently selected combination in initial antiretroviral therapy with rilpivirine, was absent in this treatment-experience population.
7. Mutations L100I and V108I were significantly more frequent in patients failing efavirenz than nevirapine. Conversely, Y181C, Y181I, V106A, H221Y and F227L were more prevalent following nevirapine failures.
8. The rates of treatment failure at 48 weeks, or long-term toxicity or virological failure with a switch to once-daily tenofovir DF/emtricitabine (or lamivudine) + nevirapine are low.
9. No unexpected RAMs or patterns of RAMs were selected in subjects with treatment failure to this regimen. At week 48, nearly 90% of the subjects had HIV-1 RNA <50

copies/mL, virological failure was uncommon, and 25 (7.4%) subjects discontinued the treatment due to toxicity.

10. Factors independently associated with virological failure in a multivariate analysis were intravenous drug use, time with undetectable viral load before the switch, number of prior NRTIs or NNRTIs, and previous nevirapine or efavirenz unscheduled interruptions.

11. There is a significantly higher rate of virological failure in individuals treated with lamivudine instead of emtricitabine with this regimen, with a significantly higher rate of selection of M184V as well. Our findings suggest caution against substituting emtricitabine for lamivudine, at least in nevirapine- and tenofovir-based regimens, while other series review their data.

12. Reinitiation of nevirapine plus tenofovir plus emtricitabine (or lamivudine) should be discouraged in subjects experiencing unplanned treatment interruptions, even with an undetectable plasma viral load at the time of treatment withdrawal.

FUTURE RESEARCH QUESTIONS

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The improved knowledge of the clinical impact of HIV-1 resistance on NNRTI based on the findings of this thesis paves the way for many treatment simplification opportunities and enhanced efficacy in salvage therapies. All this knowledge and study routines have been translated into the ART as a whole and these findings, together with the breakthrough in the knowledge of the barrier to drug resistance of antiretroviral drugs, have been translated into other drug classes as well.

Many research questions are now set out, will potentially translate into new ART regimens or strategies, and will be answered in the near future.

- Will he higher risk of virological failure and resistance selection of lamivudine vs emtricitabine shown in this thesis when combined with nevirapine be identified and confirmed in other cohorts?
- Will this difference be restricted to first-generation NNRTI-based ART, or alternatively will be also seen with second-generation NNRTIs and other drug classes?
- Will rilpivirine-based triple ART regimens used in simplification be effective in subjects with prior NNRTI-based failures but without any RAM on their genotypes?
- Will rilpivirine-based triple ART regimens used in simplification be effective in subjects with prior unplanned NNRTI-based treatment interruptions and with no RAM on their genotypes?
- Would a dual drug NRTI-sparing regimen with rilpivirine and dolutegravir be safe and effective in subjects with prior failures but full activity of both drugs?.

- Could drugs without full activity be withdrawn from suppressive regimens in subjects with prior virological failures who have maintained a safe period with undetectable plasma HIV-1 RNA thereafter?
- What characteristics/requirements would be needed to prevent the risk of virological failure with this strategy?

Many other clinical research questions not necessarily related to NNRTIs remain opened now, usually with the main aim of treatment simplification to reduce drug toxicity, administrative burden and treatment costs. In this scenario, the risk for emergence of drug resistance is a major concern.

- Could drugs with twice-daily dosing approved in salvage (darunavir, dolutegravir) be reduced to once-daily dosing when subjects have maintained a prolonged period with undetectable plasma HIV-1 RNA?
- Would a dual drug regimen with dolutegravir and lamivudine be safe and effective in simplification ART in subjects with undetectable plasma HIV-1 RNA?.
- Would dolutegravir monotherapy be safe and effective in simplification/maintenance ART in subjects with undetectable plasma HIV-1 RNA?.

ADDENDUM I.

OTHER PUBLICATIONS OF THE AUTHOR IN THE FIELD OF HIV RESISTANCE.

Along these years, all the work done in HIV-1 resistance research has generated and/or contributed to these publications, both in medical journals and book chapters.

PUBLICATIONS

1) Genotypic resistance in HIV-infected naïve patients receiving abacavir plus lamivudine and efavirenz. E Ferrer, J Niubo, M Crespo, JM Gatell, J Sanz, S Veloso, **Josep M Llibre**, P Barrufet, P Sanchez, D Podzamczer for the ABCDE Study Team. J Acquir Immune Defic Syndr 2007; 46: 253-255.

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ADDENDUM II. Publications stemming from the knowledge in HIV-1 resistance under journal review as of July 2015.

Josep M Llibre, Hortensia Alvarez, Antonio Antela, Jessica Toro, Antoni Payeras, M Jesús Perez-Elias, Arkaitz Imaz, Mar Masià, Núria Pérez-Alvarez, Joaquin Burgos, Bonaventura Clotet. Withdrawing Inactive Nucleos/tide Reverse Transcriptase Inhibitors in HIV-1 Infected Subjects with Suppressed Viremia. A Randomized Trial.

Submitted, Under review.

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