Published in final edited form as:
HIV Ther. 2009 November 1; 3(6): 583–594. doi:10.2217/hiv.09.40.

The K65R mutation in HIV-1 reverse transcriptase: genetic barriers, resistance profile and clinical implications

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Abstract

Resistance to antiviral therapy is the limiting factor in the successful management of HIV. In general, the K65R mutation is rarely selected (1.7–4%) with tenofovir disoproxil fumarate (TDF), abacavir (ABC), didanosine (ddI), and stavudine (d4T), as compared with the high incidence (>40%) of thymidine analog mutations associated with zidovudine and d4T. The high barrier to the development of K65R may reflect a combination of factors, including the high potency of K65R-selecting drugs, including recommended TDF/emtricitabine and ABC/lamivudine (ABC/3TC) combinations; the partial (low–intermediate level) profile of cross-resistance conferred by K65R to TDF, ABC and 3TC; the favorable viral fitness constraint imposed by K65R and the 3TC/emtricitabine-associated M184V mutations; the bidirectional antagonism between the K65R and thymidine analog mutation pathways; and unique RNA structural considerations in the region surrounding codon 65. Nevertheless, surprisingly high levels of treatment failures and K65R resistance may be associated with triple nucleoside analog regimens. The use of TDF + ABC, TDF + ddI and ABC + d4T in combination with 3TC or emtricitabine should be avoided. This selection of K65R may be reduced by the inclusion of zidovudine in two–four nucleoside reverse-transcriptase regimens. Clinical studies have demonstrated an increased frequency of K65R in association with suboptimal d4T and ddI regimens, as well as nevirapine and its resistance mutations Y181C and G190A. The potential for the development of the K65R mutation in subtype C is particularly problematic wherein a signature KKK nucleotide motif, at codons 64, 65 and 66 in reverse transcriptase, appear to lead to template pausing, facilitating the selection of K65R. Optimizing regimens may attenuate the emergence of K65R, leading to better long-term treatment management in different geographic settings. TDF-based regimens are the leading candidates for first- and second-line therapy, microbicides and chemoprophylaxis strategies.

Keywords

HIV-1 drug resistance; K65R; nucleoside analogs; subtype C; tenofovir

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Financial & competing interests disclosure: Bluma Brenner is Assistant Professor in the Department of Surgery/Medicine and the McGill AIDS Centre, McGill University, Canada. She is presently directrice de L'axe de résistance, Réseau SIDA et Malades Infectieuses, Fonds de la recherche en santé du Québec (FRSQ), and co-coordinator of Quebec Genotyping Program under the Institut nation de sansté publique du Québec (INSQ) and has received collaborative research funding with Mark Wainberg from the Canadian Institutes for Health Research (CIHR), the Fonds de la Recherche en Santé du Québec-Réseau SIDA (FRSQ-SIDA) and the NIH. Dimitrios Coutsinos has received an MD/PhD CIHR fellowship award and is affiliated with the Departments of Medicine, Microbiology and Immunology, McGill University. The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.
No writing assistance was utilized in the production of this manuscript.

Combination antiretroviral therapy (ART) has led to marked decreases in mortality and morbidity in both western and developing world settings. The failure to suppress viral replication during therapy leads to the selection and expansion of drug-resistant viruses. Control of the emergence of drug resistance has become an integral part of the successful management of HIV infection.

Nucleoside reverse-transcriptase inhibitors (NRTIs) remain the most commonly utilized components of HIV antiretroviral combinations. Most dual–dual NRTI combinations consist of a primary NRTI with lamivudine (3TC) or emtricitabine (FTC) [1,101]. The M184V mutation, conferring high-level resistance to 3TC and FTC, develops rapidly in approximately 50% of treated persons but remains a clinical benefit [2–5]. This mutation impairs the enzymatic function of HIV reverse transcriptase (RT), reduces viral replicative capacity, delays the appearance of thymidine analog mutations (TAMs), and improves drug susceptibility to zidovudine (AZT), stavudine (d4T) and tenofovir [2,3]. The M184V mutation can confer 0.7–1.0 log-fold diminution in viral load in the presence of other NRTIs [2,3].

The earliest antiviral drugs used in clinical practice were the thymidine analogs, AZT and d4T used in combination with 3TC [4]. Two distinct TAM (TAM-1 and -2) pathways lead to the stepwise accumulation of major (M41L, K70R and T215Y/F), minor/secondary (D67N and L210W) and compensatory (E44D, V118I and H208Y) mutations that confer a 5–500-fold reduced susceptibility to AZT and broad cross-resistance between NRTIs (Figure 1) [4–6]. In addition, AZT and d4T may also select for the more rarely observed Q151M nucleoside analog mutational (NAM) pathway (Q151M, A62V, V75I, F77L and F116Y), which confers broad-spectra NRTI resistance [6].

While AZT and d4T were of considerable importance in early stages of the epidemic, their use has declined in western-world settings owing to drug toxicities, clinical complications and the introduction of newer TAM-sparing NRTIs [1,7,101]. AZT is associated with anemia and lipoatrophy, while d4T regimens show significant toxicity, including mitochondrial-associated complications, peripheral neuropathy, lipoatrophy, triglyceride elevations and pancreatitis [8, 101]. In resource-poor settings, lower doses may reduce toxicity without adversely affecting antiviral activity or increasing the emergence of resistant viruses. The optimum doses of AZT and d4T are being assessed in South Africa and other parts of the world, in well-controlled clinical trials [4,8].

Tenofovir disoproxil fumarate (TDF) was first introduced into clinical practice in 2001, with proven antiviral activity against HIV and HBV [7]. This second-generation NRTI showed improved antiviral activity against infections harboring TAMs (D67N, K70R and T215Y) and NAMs (Q151M) [7,9,10]. Unlike other NRTIs, TDF requires one, rather than two, phosphorylation steps, resulting in high intracellular concentrations, allowing for antiviral activity in resting and activated $CD4^+$ cells [7,9]. TDF regimens combined with 3TC or FTC are the dual NRTI combination of choice for the management of HIV-1 infection [101]. This is based on its once-daily formulation, its prolonged intracellular half-life and more favorable lipid profiles than AZT/d4T [7,9].

Resistance to TDF is rarely selected through the point mutation K65R (AAA→AGA) in reverse transcriptase (RT) [7,9–12]. While the K65R mutation is often considered the TDF resistance mutation, it also arises with other nucleoside analogs, including abacavir (ABC), didanosine (ddI), d4T and amdoxovir [7,11–13]. The K65R resistance pathway has a favorable resistance profile conferring low- to intermediate-level phenotypic resistance to most NRTIs while hypersensitizing viruses to AZT [3–7]. The K65R pathway may be associated with the NAM pathway, which is associated with the Q151M, A62V, V75I, F77L and F116Y mutations [7].

Clinical studies and large genotypic databases show a low incidence of K65R resistance in drug-naive and treatment-experienced patients [7,9,14,102]. The K65R mutation is rarely selected with an overall incidence in 2–5% in genotyped patients, despite the increasing use of TDF and ABC since 2001 [14,102]. This compares to the frequency of TAMs in 40–60% in genotyped patients failing earlier AZT- and d4T-based regimens.

The profiles for resistance to different NRTIs and interactions amongst resistance pathways are summarized in Figure 1. TDF/FTC (Truvada™; Gilead, CA, USA) and TDF/FTC/efavirenz (EFV) (Atripla™; Bristol-Myers Squibb, NY, USA and Gilead) are the NRTIs of choice for first-line therapy according to the latest Department of Health and Human Services (DHHS) guidelines [101]. This is based on viral regimen potency, intracellular half-life, single-drug dosing, the low risk of K65R development and a more favorable phenotypic resistance profile. Cumulative findings from a number of clinical trials demonstrate a benefit of TDF-based regimens in treatment-naive patients, in switch studies replacing AZT and d4T regimens and in treatment failures harboring NRTI-resistant infections [7,9]. Intensified virological response can be observed with K65R in the presence of the M184V mutation [7]. Drug-related toxicities include decreased bone mineral density (osteopenia/osteoporosis) and potential renal toxicity [101]. Data on TDF safety during pregnancy and for children are yet to be established [101].

Abacavir/3TC (Epzicom™; GlaxoSmithKline, NC, USA) with lopinavir has been used extensively as an alternate NRTI regimen with a favorable resistance profile that includes K65R. ABC requires *HLA-B**5701 testing to avoid a rash and is not recommended for individuals with pre-existing cardiac risk factors [101]. AZT/3TC remains the preferred treatment for pregnant women and newborns [101]. While ddI + 3TC/FTC remains the recommended treatment option, ddI is associated with an increased risk of pancreatitis and peripheral neuropathy [101].

Barrier to K65R selection is dependent on NRTI & NNRTI regimens

The low incidence of the K65R mutation in drug-naive and treatment-experienced patients cannot be directly attributed to its' high genetic barrier. Indeed, the barrier to K65R selection is very low, requiring a single transition of AAG→AGG in subtype C and, AAA→AGA in subtype B and other non-C subtypes [15]. Moreover, such transitions (replacements of a purine by another purine: A–G) are, for steric reasons, 2.5-times more frequent than transversions (the replacement of a purine by a pyrimidine) [15].

The low incidence and high genetic barrier to K65R selection in drug-naive and treatment-experienced patients may be attributed to multiple factors including: impaired K65R viral replicative capacity, viral fitness constraints imposed by K65R in the presence of M184V – conferring resistance to 3TC and FTC, counter-selection of K65R- and TAM-resistance pathways, regimen potency and viral subtype (Figures 1 & 2).

The K65R mutation is associated with reduced viral replication capacity and fitness, similar to the 3TC/FTC-associated M184V mutation – hallmarks that can be demonstrated at the enzymatic level [16–19]. Viruses harboring the K65R mutation show:

- Decreased incorporation rate (k_{pol}) of dNTPs
- Decreased excision of NRTIs
- Increased fidelity
- Decreased viral replication capacity

The severe compromise in replicative fitness imposed by K65R contributes to the bidirectional phenotypic antagonism between K65R and TAM pathways [16,17]. K65R reduces enzymatic

excision of chain-terminating thymidine analogs. TAMs counterbalance K65R by decreasing enzymatic discrimination of D-nucleotide analogs and by increasing rates of nucleoside excision. Single-genome sequence analysis demonstrates a negative association of K65R with TAMs (T215Y/F + ≥2 TAMs) on the same genome, except when facilitated with Q151M complex mutations [17]. In addition, there is a strong negative association of K65R with the M184V, L74V and K70E mutations, conferring resistance to 3TC/FTC, ddI and TDF, respectively. These four mutations severely compromise viral fitness, wherein double mutants impose a marked diminution in replicative capacity and may be mutually deleterious [2,3,18–21]. While M184V and K65R may prevail in patients, these mutations markedly compromise viral replicative capacity [2,3,18].

The accumulation of secondary/compensatory mutations following acquisition of K65R is rare and quite distinct from the observed step-wise accumulation of TAMs. The mutation S68G appears to partially compensate for the replication defect associated with K65R [22]. The most common coselected mutation is Q151M and the Q151M–NAM resistance pathway [23–26], which confers a reduced susceptibility to all NRTIs, with the exception of 3TC and TDF. The presence of K65R and Q151M counter-selects M184V and confers more resistance than either of the single mutations alone [23–26].

The replicative compromise conferred by K65R is reflected by the strong selective pressure driving its rate of disappearance/reversion upon treatment interruption – K65R (1 month) > M184I/V (3 months) > TAMs (4–6 months) and Q151-NAMs (5.6 months) [27]. The low incidence and rapid disappearance of K65R is important with respect to second-line and salvage treatment options.

Surprisingly, the development of K65R is strongly NRTI regimen-dependent. The use of triple nucleoside analog combinations facilitates the selection of K65R. Several clinical studies were designed to test the clinical benefit of Trizivir® (GlaxoSmithKline; AZT/3TC/ABC), TDF/ABC/3TC, TDF/ddI/ABC and TDF/ddI/3TC as TAM-sparing regimens in treatment-naive subjects [28–31]. These studies were terminated after unexpectedly early failure rates (30–60%) occurred in association with K65R resistance.

The mechanism underlying the increased emergence of K65R and the unanticipated interaction between TDF + ABC and TDF + ddI, remain unclear [28–33]. Poor virological suppression could not be directly associated with either circulating drug levels or intracellular NRTI triphosphate levels [28]. Virological failure was initially associated with the rapid emergence of M184V and K65R on separate viral genomes [28–33].

Early virological failure occurred despite apparent susceptibilities to two or three drugs in regimens, according to genotypic algorithms. The rapid selection of K65K/R and M184M/I/V as minority species emerging on separate clones underestimated resistance to TDF, ABC and ddI in phenotypic assays [28–33]. This caveat of phenotypic assays should be recognized for K65R-selecting drugs, including TDF, ABC, d4T and ddI [33]. Testing for minority K65R and M184V species using ultrasensitive allele-specific PCR may be important in ascribing the role of resistance in virological failure to K65R-selecting drugs [34–36]. Changing to AZT-containing four-drug regimens has been demonstrated to be successful in regaining virological suppression [28,37,38].

Indeed, combining AZT with K65R-selecting drugs may be useful in preventing the evolution of K65R resistance [31,37,38]. A composite retrospective analysis of data from patients receiving a TDF + ABC regimen revealed a success rate of 86% when the regimen contained AZT, compared with 62% when it did not, and no K65R mutations were observed in subjects on regimens containing AZT [31]. Similarly, a reduced selection of the K65R mutation was observed when AZT was added to an ABC-containing regimen [37]. There was a lower

incidence of the K65R and L74V in ABC regimens containing AZT than in those without [39]. In several clinical studies involving a triple-NRTI regimen without AZT, 24–92% of patients identified as treatment failures had a virus with the K65R mutation [32,40–44]. Only one out of 90 patients who failed therapy in clinical studies using three or four NRTIs, which included AZT, had the K65R mutation and this person had received only once-daily AZT [45,46]. Moreover, recent findings in the COL40263 study looking at a ABC/3TC/ZDV + TDF-combined regimen, found that therapeutic failure was more likely to occur via the TAM pathway rather than K65R [38].

The rising incidence of K65R from 0.4 to 3.6% between 1998 and 2003 was linked to ddI therapy [47,48]. There was also a strong association for K65R selection with nevirapine (NVP) and the Y181C/G190A/S mutations associated with non-NRTI (NNRTI) resistance [49–51]. A synergistic fitness interaction has been observed for K65R and Y181C mutations [49–51]. The selection of K65R is negatively associated with K103N [48].

Altogether, ddI, NVP and triple NRTI therapy may be associated with higher frequencies of selection of K65R. Careful avoidance of coadministration of TDF with ABC or ddI in first-line treatment regimens and genotypic resistance testing for NNRTIs has led to decreasing trends in K65R appearance (<2% of the treated population) in the years following 2005 [47–52]. A total of four NRTI-containing regimens, including TDF coformulated Trizivir have been recently proposed as a more stable regimen that may offset the development of K65R, while representing TAM-sparing regimens [53–55].

The aforementioned considerations provide clinicians with reasonable clinical approaches in preventing and managing HIV drug resistance. Highly potent TDF/FTC and ABC/3TC coformulated regimens with EFV or protease inhibitors prevent the advent of K65R resistance and are the drug combinations of choice in resource-rich settings.

Accelerated risk for the development of K65R in the developing world

The growing genetic diversification of HIV-1 represents one of the most serious challenges of the ongoing pandemic. ARTs and emergent drug-resistance profiles have been established on paradigms of subtype B infections present in western-world settings, with little comparative information available for non-B subtypes in developing-world settings. The epicenters of the HIV-1 pandemic are concentrated in the resource-constrained nations, which have limited access to healthcare services and treatment. As access to antiviral therapy in developing-world settings increases, it remains imperative to establish appropriate treatment strategies for long-term clinical benefit and limit the emergence of drug resistance. Subtype differences, suboptimal therapies and deficiencies in healthcare delivery systems can create conditions for the accelerated development of resistance [56–59].

In resource-poor settings, patients at multiple disease stages with significant comorbidities and harboring numerous subtypes are treated with diverse antiretroviral regimens. While the conclusions of early studies suggest significant virological, immunological and clinical benefits of antiviral therapy, aggregating data from numerous clinical studies may mask intersubtype and drug regimen differences [56–60].

Drug regimens provided to resource-poor nations are often suboptimal and may be associated with significant toxicity that affects drug adherence. Whereas TDF and ABC are the backbone NRTIs used in resource-rich settings, d4T, ddI, AZT and NVP represent the major drugs used in resource-limited settings [8,60,101]. These regimens may be suboptimal and fail to adequately achieve viral suppression, leading to a more rapid emergence of K65R resistance in non-B subtypes [52,56].

Genetic diversity amongst subtypes may facilitate the development of resistance [52,56,58–60]. The fastest growing epidemics are subtype C and subtype A variants (A1, A2, CRF01_AE and CRF02_AG) representing approximately 50 and 30% of global infections, respectively [56–58]. These epidemics represent 30–50% of new infections in Europe and 10–20% of new infections in the Americas [56,58,59]. The 10–15% sequence diversity and polymorphisms amongst non-B subtypes may contribute to differences in resistance profiles to NRTIs, as well as interactions between NRTI and NNRTI resistance. Differential replicative fitness and pathogenesis may also affect the duration to develop resistance.

Facilitated development of K65R in non-B subtype infections

The impact of HIV-1 subtypes on drug resistance is most noteworthy in viral resistance to NNRTIs. NVP has become widely used in developing countries as an effective and inexpensive drug for prophylaxis to prevent mother–child transmission [56,59]. In this regard, the acquisition of resistance to NNRTIs is somewhat unique, in that single point mutations, including K103N, Y181C and V106M, arise within days or weeks and confer more than 100–1000-fold resistance. Since NNRTIs are noncompetitive inhibitors, they do not significantly impose fitness constraints on the viral RT enzyme. The slow rates of NVP clearance lead to a rapid appearance and retention of NNRTI mutations, which can facilitate the emergence of K65R resistance in developing countries where NVP is extensively used [49–51]. In this regard, retrospective analysis has revealed significant intersubtype differences in the acquisition of NVP resistance in mothers and infected children, involving K103N or Y181C in 69, 36, 19 and 21% of women with subtype C, D, A and CRF02_AG infections, respectively [56]. Ultrasensitive PCR detection procedures identified minority NNRTI species (K103N or Y181C) in 70–87% of persons with subtype C, as compared with 42% of persons with subtype A/AE infections [56].

In addition, high rates of virological failure have been observed for once-daily TDF/3TC/NVP regimens with K65R in subtype B and non-B subtype infections [50,51]. Virological failure was linked to the synergistic selection of K65R, Y181C and/or G190A resistance.

The development of K65R and Q151M may be facilitated for HIV-2 variants originating from West Africa (Senegal and Portugal), harboring NNRTI mutations (K101A, V106I, V179I, Y181I, Y188L and G190A) and TAMs/NAMs (T69N, V75I, V118I, L210N, T215S and K219E) as natural polymorphisms [61–63]. K65R has been reported to occur in 20% of patients receiving TDF at some point during their treatment. In addition, three single point mutations, K65R, Q151M and M184V, can confer high-level cross-class resistance to all commercially available NRTIs for HIV-2 infections [62].

K65R development in subtype C HIV-1

There is clinical and tissue culture evidence to indicate an accelerated risk in developing the K65R mutation in subtype C infections [64–69]. The first reported study demonstrated that 30% of Botswanan patients failing ddI- or d4T-based regimens developed K65R within 8 months of treatment [64]. While one report disputed a facilitated selection of K65R in subtype C in early TDF trials, recent clinical studies demonstrates a higher incidence of K65R in subtype A and C infections receiving d4T- and ddI-based regimens [64,66–69]. The incidence of K65R in genotyped persons failing treatment was 7, 9, 14, 23 and 30% in clinical studies in Thailand, Senegal, South Africa, Malawi and Botswana, respectively [63,64,66–68]. Although no direct head-to-head comparisons of subtype C versus B have been conducted clinically, the available data suggest that an HIV-1 subtype plays an associative role in the accelerated development of K65R resistance.

Tissue culture selections of virus isolates from Botswana and Ethiopia confirmed the facilitated development of K65R in subtype C relative to subtype B [65,70–72]. Development of K65R in subtype C occurred regardless of regimen, including TDF, d4T, ddI, ABC, TDF + 3TC and d4T + ddI [70–72].

Cell culture studies, site-directed mutagenesis and enzymatic studies have begun to unravel the novel molecular mechanisms that may account for the more rapid appearance of K65R in subtype C [70–73]. Subtype C has a unique KKK nucleotide motif at codons 64, 65 and 66 (AAA–AAG–AAG) when compared with subtype B and other non-B subtypes (AAG–AAA–AAA). The rapid selection of K65R in subtype C strains (AAG→AGG), as compared with the slow evolution of K65R in subtype B (AAA→AGA), cannot be explained by codon usage [70–72]. Enzymatic analyses of subtype B- and subtype C-derived RT enzymes reveal that, mechanistically, the biochemical profiles of both enzymes are very similar [70,74].

The development of K65R between subtypes may be explained by the nucleotide sequence dissimilarities that exist in subtype-specific templates (Figure 2). Introduction of the 64/65 nucleotide polymorphisms of subtype C into subtype B HIV-1 accelerates the selection of the K65R mutation in subtype B to levels observed for subtype C [71]. Preferential selection of K65R in subtype C HIV-1 may be attenuated by silent mutations at codons 70, 210 and 219, implicated in the TAM-resistance pathway [72].

The higher propensity for K65R selection in subtype C, relative to other subtypes, may be related to differences in the poly-adenine stretches within the RT template spanning codons 63–68 (Figure 2) [70]. The RT enzyme is known to exhibit characteristic pausing at the end of such sequences – these pausing events, in turn, contribute to mutagenesis [70,73–80]. In the case of the subtype C sequence, a homopolymeric stretch of adenine bases is present and ends at the exact nucleotide that is responsible for the AAG to AGG transition that gives rise to K65R. By contrast, the subtype B sequence homopolymeric region begins later and stretches beyond codon 65, to include codon 66, and end at codon 67 (Figure 2).

When subtype C RT was used to synthesize DNA on the subtype C template containing the K65 homopolymeric region, strong pausing was seen at the exact nucleotide position responsible for K65R development. When subtype B RT was used to synthesize DNA on the subtype B template containing the K65 homopolymeric region, a ladder of pausing events was observed that started at codon 65 and ended at codon 67, which may be important for the selection of D67N associated with the TAM-1 pathway (Figure 2). No matter what subtype RT enzyme was used to synthesize DNA, the pausing patterns were determined exclusively by the subtype of the template [70,79].

This facilitated selection of K65R in subtype C is a grave concern, given that this subtype accounts for 50% of the worldwide pandemic. It is clear that this subtype may be ‘less forgiving’ for suboptimal regimens. The 23% incidence of K65R in the Malawi study was associated with a d4T/3TC/NVP first-line regimen [68]. This drug combination led to rapid treatment failure by either K65R or TAM pathways. This consequently led to limited treatment options. The long duration of this study (36 months), as compared with the Thai, Kenyan and South African studies, clearly demonstrate the dangers of d4T-based regimens in accelerating the development of resistance, including the K65R pathway [8,64–69,81]. This provides a strong argument for replacing d4T in first-line regimens for TDF-based substitutes [8]. Similarly, AZT/3TC/NVP remains a cost-effective, viable treatment alternative to d4T/3TC/NVP in resource-poor settings until access to TDF is addressed. At least this way, K65R is not likely to be selected, even in the presence of Y181C and/or G190A, and AZT will remain active against M184V viruses.

The issues of costs and access to TDF in resource-poor settings needs to be addressed [8]. The DART studies in Uganda and Zimbabwe using TDF/3TC/AZT regimens show low K65R development [66]. Such regimens are well tolerated with low renal toxicity [82].

Future perspective

Over the past decade, advances in HAART have led to significant improvements of survival rates in resource-poor settings, as well as in developed-world settings. In general, emergent resistance to K65R is rare. The K65R mutation results in diminutions in viral replicative capacity similar to that observed for the M184V mutation [2,3,7,9]. These mutations confer severe fitness constraints and are rapidly lost upon treatment interruption. The high barrier to K65R development and the benefit of K65R and M184V in viral suppression, have led to the hope that TDF and TDF/FTC regimens may be used in microbicide- and chemoprophylaxis-prevention strategies [83–85].

The bidirectional antagonism between K65R and TAM pathways hold the promise of developing NRTI resistance-sparing regimens that combine AZT/TDF/ABC with FTC or 3TC. While clinical trials with triple-drug combinations had to be discontinued because of early treatment failure, quadruple-drug combinations appear to lead to more favorable outcomes in treatment-naive patients and treatment-experienced patients harboring multi-drug-resistant infections [53–55]. In a 96-week prospective trial, ZDV/3TC/ABC + TDF in treatment-naive patients provided a well-tolerated NRTI/NNRTI/protease inhibitor-sparing regimen, even for patients with a high viral load [54]. TDF–AZT combined regimens may block the emergence of NRTI resistance, as well as offer combination regimens towards salvage therapies in individuals harboring multidrug resistance.

The facilitated development of K65R, M184V and/or L74V as a minority species leads to early treatment failure with suboptimal treatment regimens, in particular d4T, ddI, NVP and triple-NRTI drug combinations [21,28–36]. This may be accelerated in non-B subtype infections, notably HIV-2 and subtype C [61–69]. The recent report of transmitted K65R resistance in four out of four breast-feeding infants from mothers harboring K65R illustrates the potential public health concern of transmitted drug resistance [86].

Most importantly, the use of ddI/d4T and d4T/3TC/NVP regimens in resource-poor settings needs to be questioned [8,64–69,81]. The combined dangers of TAM and K65R resistance, facilitated by synergistic NVP resistance (Y181C/G190A), may lead to situations where the choice of second-line regimens is limited [68]. The reasons for replacing d4T–NVP and ddI–NVP regimens with TDF-based regimens in resource-limited settings are compelling and therefore must be addressed [8]. AZT/3TC/NVP is a viable treatment alternative to d4T/3TC/NVP in resource-limited settings and is cost effective until access to TDF occurs.

Currently, AZT is the only licensed antiretroviral for use in pregnancy [87]. In macaque models, perinatal exposure to a very high dose of TDF results in bone toxicity in some offspring [87]. Single-dose (SD) NVP is the only antiretroviral option in many resource-poor settings worldwide. Recent clinical trials in Africa demonstrate that SD TDF used with SD NVP may be of benefit in further reducing mother–child transmission of HIV while preventing emergent NNRTI resistance [87].

In summary, the rarity of K65R development with TDF-based regimens has enormous clinical potential towards the development of cost-effective HIV therapeutic and prevention strategies.

Executive summary

K65R resistance pathway

- K65R is selected by tenofovir disoproxil fumarate (TDF), abacavir, didanosine (ddI), stavudine (d4T) and amdoxovir.
- K65R confers partial resistance to most nucleoside analogs while remaining susceptible to zidovudine (AZT).
- TDF exhibits improved antiviral activity against isolates harboring some thymidine analog mutations (TAMs).

Barrier to K65R is regimen associated

- The overall incidence of K65R is in 1–4% of the genotyped population.
- The infrequent development of K65R in drug-naive and treatment-experienced patients is due to impaired K65R replicative capacity and fitness constraints imposed by K65R and M184V – associated with resistance to lamivudine (3TC) and emtricitabine (FTC) and regimen potency (e.g., TDF–FTC).
- Bidirectional antagonism of TAMs and K65R pathways lead to a counter selection of K65R in AZT/d4T-experienced patients.
- Clinical trials with triple nucleoside reverse-transcriptase inhibitors (NRTIs) regimens were discontinued due to virological failure with high rates of K65R. TDF + abacavir, TDF + ddI and d4T + ddI in combination with 3TC or FTC should be avoided.
- Inclusion of AZT may offset virological failure and K65R resistance in two–four NRTI regimens containing K65-selecting drugs.
- Higher frequencies of K65R are linked to suboptimal ddI and d4T regimens.
- Increased selection of K65R with nevirapine (NVP) is associated with the Y181C and/or G190A mutations.

Facilitated development of K65R in non-B subtype infections

- Elevated K65R selection in resource-limited settings may be related to suboptimal ddI, d4T and NVP regimens.
- K65R is selected in 9–30% of subtype C patients failing therapy in Africa.
- A signature mutational motif in subtype C accelerates K65R selection.
- AZT/3TC/NVP is a viable treatment alternative in resource-poor settings and is cost effective until access to TDF is addressed.

Conclusion

- Optimizing NRTI and NRTI/non-NRTI regimens deter the emergence of K65R.
- The favorable barrier to K65R development makes TDF-based regimens leading candidates for microbicides and pre- and postexposure prophylaxis.
- Recent clinical trials are evaluating the efficacy of quadruple-NRTI analog combinations as TAM/K65R-sparing regimens.

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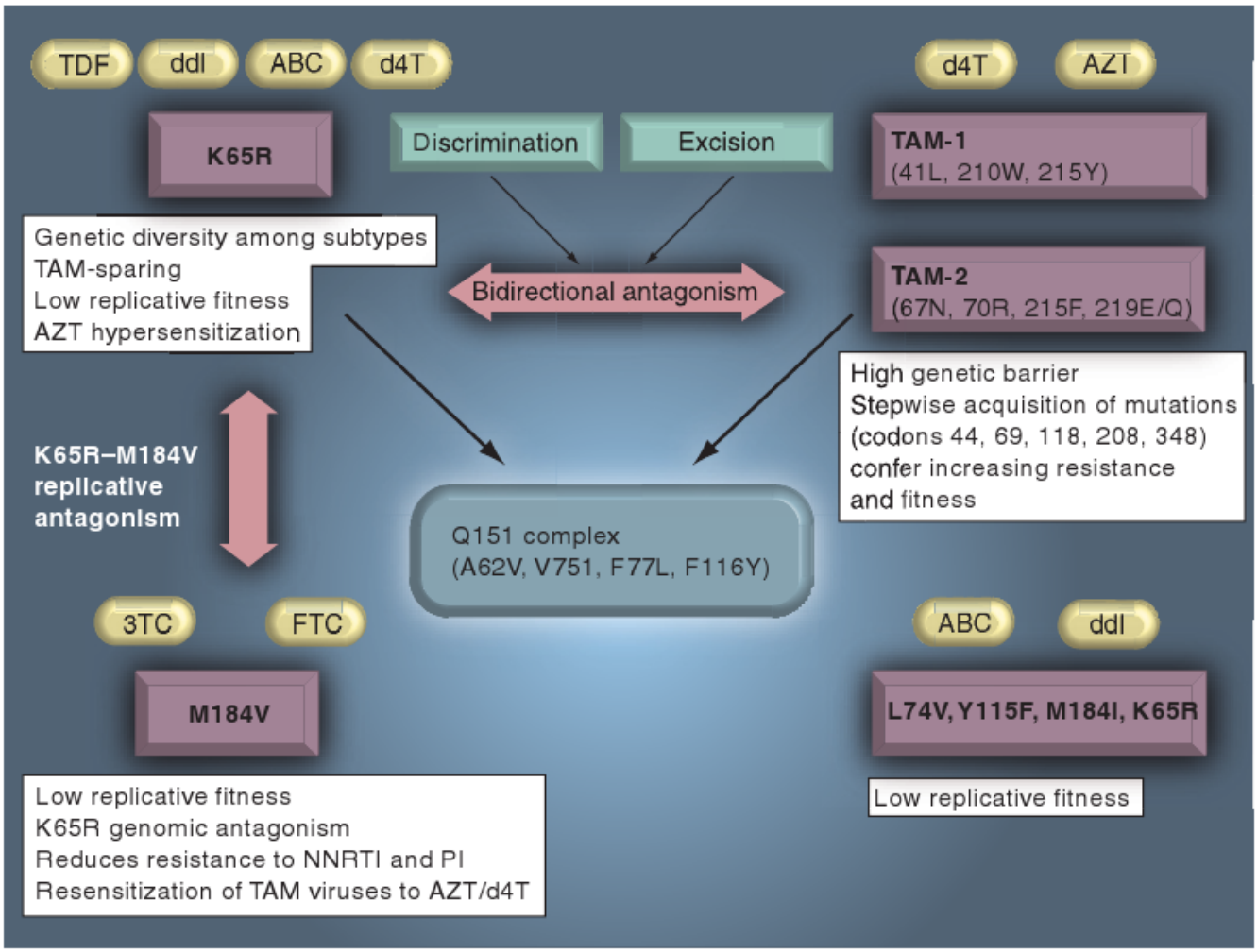

Figure 1. Interactive pathways of resistance to the nucleoside reverse-transcriptase inhibitors, including TDF, ddI, ABC, d4T, AZT, 3TC and FTC

3TC: Lamivudine; ABC: Abacavir; AZT: Zidovudine; d4T: Stavudine; ddI: Didanosine; FTC: Emtricitabine; NNRTI: Non-nucleoside reverse-transcriptase inhibitor; PI: Protease inhibitor; TAM: Thymidine analog mutation; TDF: Tenofovir.

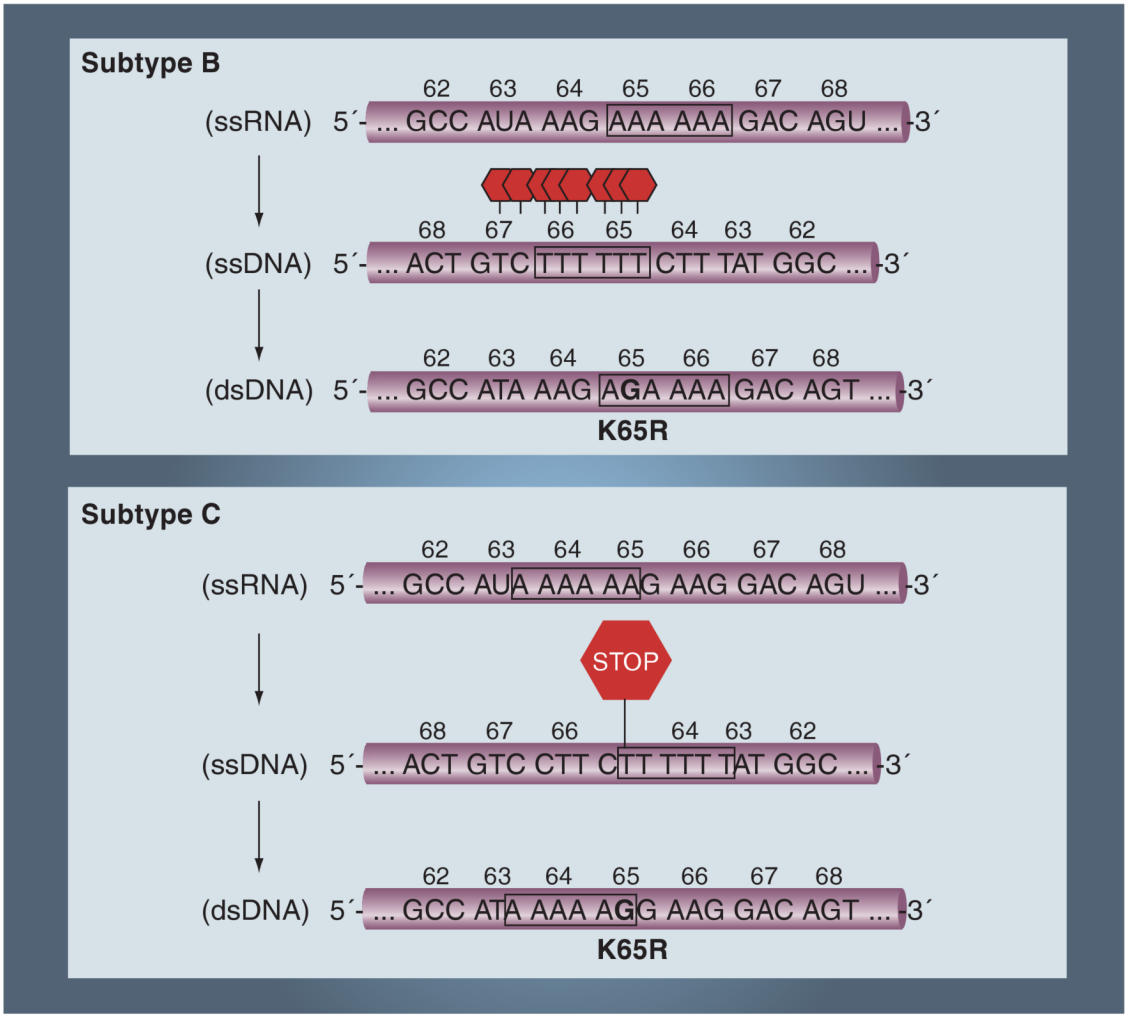

Figure 2. Facilitated selection of K65R in subtype C

The lower genetic barrier in subtype C may be attributed to enzymatic pausing arising at the end of poly-adenine stretches. A strong pausing is observed at codon 65 in subtype C, which may favor the evolution of K65R. By contrast, the homopolymeric region in subtype B (and other subtypes) begins later and stretches beyond codon 65, to include codon 66, and end at codon 67. Selection of K65R in subtype C is facilitated by a poly-A stretch between codons 63 and 65.