Commonly Transmitted HIV-1 Drug Resistance Mutations in Reverse Transcriptase and Protease in Antiretroviral Treatment-Naïve Patients do not Affect Response to Tenofovir Disoproxil Fumarate- or Tenofovir Alafenamide-Containing Regimens

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ABSTRACT

Background. The presence of transmitted drug-resistance mutations (TDRM) in antiretroviral (ARV) treatment-naïve patients can adversely affect the outcome of ARV therapy.

Methods. Resistance testing was conducted in 6704 ARV-naïve subjects predominantly from the U.S. and Europe in 9 Gilead clinical studies from 2000 to 2013.

Results. The presence of TDRM increased during this period (5.2% to 11.4%), primarily driven by non-nucleoside RT inhibitor resistance mutations (NNRTI; 0.3% to 7.1%), particularly K103N/S (0.3% to 5.3%). NRTI mutations were found in 3.1% of patients. Only 1 patient had K65R (0.01%) and 7 patients had M184V/I (0.1%) despite high use of tenofovir disoproxil fumarate (TDF), emtricitabine, and lamivudine and potential transmission of resistance to these drugs. One or more thymidine analog mutations (TAMs) were present in 2.7% of patients with 0.07% harboring T215Y/F and 2.7% harboring T215 revertant mutations (T215rev). Patients with the combination of M41L+L210W+T215rev showed full HIV RNA suppression while receiving a TDF- or tenofovir alafenamide (TAF)-containing regimen.

Conclusions. There was an overall increase of TDRMs among patients enrolling in clinical trials from 2000 through 2013 driven primarily by an increase in NNRTI-R. However, the presence of common TDRMs, including TAMs/T215rev, showed no impact on response to TDF- or TAF-containing regimens.

INTRODUCTION

The research and discovery of chemical entities targeting HIV replication have provided the medical community with an arsenal of drugs to combat the advance of the AIDS epidemic [1]. However, concurrent to the use of antiretroviral (ARV) therapies with suboptimal viral suppression and owing to the error-prone replication of HIV reverse transcriptase, drug-resistant strains of HIV have emerged [2-4]. A direct consequence of the emergence of drug resistance is that transmission of drug-resistant viruses has the potential to fuel the epidemic with viruses harboring pre-existing drug resistance. Indeed, the risk of transmission of drug-resistant viruses in ARV treatment-naïve patients has been documented as far back as 1992 [5], and more recent reports have estimated that close to 10% of ARV treatment-naïve patients harbor HIV with transmitted drug resistance mutations (TDRM) [6-9].

The extent to which TDRM persist as a major species in the absence of selective pressure from drug treatment after transmission depends mostly on the fitness costs associated with the mutations and the time that has elapsed since the transmission event. For example, the M184V/I RT substitution conferring resistance to emtricitabine (FTC) and lamivudine (3TC), which is known to impart a replication defect to the virus [10, 11], was found to revert to wild-type more readily than mutations with little or no fitness defects such as the M41L and K103N RT substitutions, or the protease substitution L90M [10, 12-14]. Similarly, the T215Y/F RT thymidine analog mutation (TAM) was found to evolve away from the mutant residues at a comparable rate as M184V/I [14], but as the resistance mutations that confer T215Y or T215F are the product of 2-nucleotide changes from wild-type, resistant mutants do not typically revert to wild-type, but to non-resistant mutants with a 1-nucleotide change from the resistant mutants such as T215C/D/S/E, termed T215 revertants (T215rev) [15].

It was recognized in the late 1990s that testing patients for the presence of ARV resistance mutations prior to the initiation of ARV therapy would be key to selecting the most appropriate treatment regimen and thus ensure the best treatment outcomes for HIV-infected patients [16]. As a consequence, resistance testing by population-based viral sequencing has become routine practice in many regions [17-19], using either local methods or commercial assays [20, 21]. The interpretation of the sequencing data is fairly straightforward in the presence of signature ARV resistance mutations–e.g. presence of K103N indicates resistance to the non-nucleoside RT inhibitor (NNRTI) efavirenz (EFV); or presence of K65R indicates resistance to the nucleotide RT inhibitor (NRTI) tenofovir disoproxil fumarate (TDF). However, complex mixtures of mutations may be harder to interpret. Thus, genotypic algorithms for the interpretation of the mutation data have been developed, including proprietary methods (linked to commercial assays) and open source methods (Stanford HIV database, Rega Institute, ANRS). For TDF, one key aspect of these resistance algorithms involves the presence of 3 or more TAMs, notably M41L + L210W + T215Y/F. However, T215 revertants are often included along with T215Y/F even though these substitutions have not been shown to confer resistance to TDF in vitro.

In the present study, we have performed a retrospective analysis of viral genotypes obtained after resistance testing of baseline plasma samples from a large cohort of ARV treatment-naïve patients prior to enrollment in clinical studies that were conducted at Gilead Sciences from 2000-2013. We have looked at the overall prevalence of TDRM at baseline with a special focus on the prevalence of TAMs and T215 revertants, and have studied the response to TDF- or tenofovir alafenamide (TAF)-containing regimens in the presence of TDRM.

MATERIALS AND METHODS

Study subjects: Antiretroviral (ARV) treatment-naïve patients with pretreatment genotypic data from 9 TDF- and/or TAF-based phase 3 studies initiated at Gilead Sciences between 2000 and 2013 were included in the analysis (Table 1) [22-29]. All 9 studies contained an active control treatment group, which was provided either as an open-label treatment or double-blind, double dummy treatment. Studies were conducted at multiple sites across multiple countries (except studies 236-0102 and 216-0130, which were in the U.S. only). Patients' informed consent for the use of biological samples had been obtained at the time of initiation of the studies. In total, the dataset comprised pretreatment genotypic data from 6704 patients, of which 5990 were enrolled in the studies.

Genotypic data: Patients' HIV-1 RNA was extracted from plasma samples and amplified by RT-PCR as previously described [20, 21, 30]. Population sequencing of the RT and protease (PR) segments of HIV-1 *pol* was conducted either at Virco laboratories (Mechelen, Belgium), Monogram Biosciences (South San Francisco, CA), or Gilead Sciences (Foster City, CA). Sequences were compared to the HIV-1_{NL4-3} reference sequence and amino acid changes from reference at resistance residues were tabulated. Amino acid mixtures of mutant and wild-type were counted as mutant.

Definition of resistance: The list of resistance-associated mutations (RAMs) used in these analyses was identical to the 2009 update of the list published by the World Health Organization [31], except for position RT T215 where T215Y/F and T215rev (any substitution other than Y or F at position RT T215) were tabulated separately. Nucleoside/nucleotide RT inhibitor (NRTI)

resistance associated mutations were: M41L, K65R, D67N/G/E, T69 insertions, T69D, K70R/E, L74V/I, V75M/T/A/S, F77L, Y115F, F116Y, Q151M, M184V/I, L210W, T215Y/F, and K219Q/E/N/R in RT. Non-NRTI (NNRTI) resistance associated mutations were: L100I, K101E/P, K103N/S, V106A/M, V179F, Y181C/I/V, Y188L/C/H, G190S/A /E, P225H, and M230L in RT. Protease inhibitor (PI) primary resistance associated mutations were: L23I, L24I, D30N, V32I, M46I/L, I47V/A, G48V/M, I50L/V, I54M/L/V/A/T/S, G73S/T/C/A, L76V, V82A/F/T/S/L/C/M, N83D, I84V/A/C, I85V, N88D/S, and L90M in protease.

Treatment response and statistical analyses: Response to TDF- or TAF-based treatment at Week 48 was calculated using FDA algorithms as originally defined in the clinical study protocols [32]. Earlier studies (Studies 99-903 and 01-934) used the FDA Time to Loss of Virologic Response (TLOVR) algorithm and later studies (all others) use the FDA snapshot analysis algorithm. The resistance trend analysis over time was conducted using the Cochran-Armitage trend test. The statistical analysis of treatment response at week 48 per baseline virologic category and all other comparisons were conducted using the Fisher's exact test.

RESULTS

Dataset characteristics: We investigated the prevalence of transmitted RT and PR resistance mutations in HIV-infected ARV treatment-naïve patients who were enrolled or screened in 9 Gilead clinical studies between 2000 and 2013 (Table 1). The majority of patients who were screened in the course of the various studies (screened dataset, n = 6704) fit the study criteria and were eventually enrolled in the studies (enrolled dataset, n = 5990). Within the enrolled dataset, 4586 patients received a TDF-containing regimen and 865 received a TAF-containing regimen. HIV-1 subtype B was the most prevalent subtype (89.9 % of screened patients, 6026/6704), followed by HIV-1 subtype AE (3.8%, 255/6704), subtype C (1.4%, 91/6704), and subtype AG (1.3%, 87/6704). The predominance of HIV-1 subtype B was consistent with the geographical origin of the patients who mostly came from North America or Western Europe. The median age of enrolled patients ranged from 34 to 38 years across all 9 studies.

Prevalence of pretreatment genotypic resistance: The overall prevalence of any genotypic resistance across all studies was 11.2% in the screened dataset (Table 2). Single drug-class resistance was found in 9.9% of patients, dual drug-class resistance in 1.1%, and triple drug-class resistance in 0.3% of patients. Resistance to the NNRTI class was the most prevalent (6.0%), with 4.5% of screened patients harboring K103N/S substitutions in RT. The prevalence of NNRTI resistance (NNRTI-R) was significantly higher among U.S. patients than among European patients (8.0% vs. 2.1%; $p < 0.0001$), with K103N/S found in 6.1% of U.S. patients compared to 1.4% of European patients ($p < 0.0001$). NRTI resistance (NRTI-R) was detected in 3.1% of screened patients, with 182 patients (2.7% of screened patients) harboring one or more thymidine analog mutations (TAMs), and 183 patients (2.7% of total) harboring T215rev

mutations. T215rev mutations were enriched in patients with HIV-1 subtype B, with 97.8% of subjects (179/183) with T215rev having HIV-1 subtype B compared to 89.9% of subjects overall (6026/6704) with HIV-1 subtype B ($p = 0.0006$). The most frequent T215rev mutations were T215S (n = 67), T215D (n = 48), T215E (n = 28), T215L (n = 21), and T215C (n = 20); others included T215I/A/V/H/N/P/G/Q or R. Five patients had 3 or more TAMs, including 3 patients with M41L + L210W + T215Y. Only one patient carried the K65R substitution (1/6704, 0.01%), and 7 patients (0.1%) carried the M184V/I substitutions. Primary PI resistance (PI-R) was detected in 3% of patients, with M46I/L (0.9%) and L90M (0.8%) being the most prevalent. The proportion of patients harboring NRTI-R or PI-R was similar between U.S. and European patients.

There was a statistically significant increase in the presence of any resistance mutations (5.2% to 11.4%), NNRTI resistance (0.3% to 7.1%), and/or K103N/S (0.3% to 5.3%) over time from 2000 to 2013 (Figure 1A; $p <0.0001$). The presence of PI-R also showed an increase (0.7% to 2.4%) from 2000 to 2013 ($p = 0.02$). The presence of NRTI resistance, TAMs, and T215rev remained essentially unchanged during the period (4.2% to 3.6%, 2.8% to 2.3%, and 2.5% to 1.8% from 2000 to 2013, respectively). The trend analysis for U.S. patients (Figure 1B, n = 4414) showed an increase over time in the Any Resistance category (8.2% to 14.6%, $p = 0.0003$), the NNRTI-R category (0.7% to 9.8%, $p <0.0001$), and the K103N/S category (0.7% to 7.5%, $p <0.0001$). For European patients (Figure 1C, n = 1191), the trend analysis showed no statistically significant changes over time across any of the resistance categories.

Among the 6704 ARV treatment-naïve patients who were screened to potentially receive a regimen containing FTC + TDF or FTC + TAF, only 8 patients (0.1%) were excluded from enrollment per protocol due to the presence of TDRM in their screening genotype and potential

TDF, TAF, or FTC resistance (2 with M184V/I, 2 with M184V/I + ≥ 3 TAMs, 3 with ≥ 3 TAMs, and 1 with K65R). In contrast, of the 2803 of 6704 patients who were screened to potentially receive an NNRTI (Studies 903, 934, 236-0102, and 264-0110), 78 patients (2.8%) were excluded from enrollment due to the presence of NNRTI-R in the screening genotype. Non-enrollment for the remainder of patients was due to other protocol-based reasons.

Distribution of TAMs at baseline: As the presence of ≥ 3 TAMs has been documented to affect treatment response to TDF in ARV-experienced patients with incomplete viral suppression [34], we studied the distribution of TAMs in the ARV treatment-naïve patients who were enrolled in the studies described here (Figure 2). Using the strict definition of TAMs (M41L, D67N, K70R, L210W, T215Y/F, and K219Q/E/N/R in RT), 112 patients had 1 TAM and 33 patients had 2 TAMs at enrollment (presence of ≥3 TAMs was an exclusion criteria) (Figure 2). Among patients with 2 TAMs, most carried the D67N + K219Q combination (20/33, 61%) (Figure S1A), followed by the M41L + L210W combination (10/33, 30%). Among patients with 1 TAM, M41L was the most frequent (65/112, 58%), followed by K219Q/E/N/R (24/112, 21%). Upon inclusion of T215 revertants in the count of TAMs (TAMs/rev), the number of patients with 2 TAMs/rev increased to 70, and 17 patients were found to carry 3 TAMs/rev. Most of the patients with 2 TAMs/rev carried an M41L + T215rev combination (43/70, 61%) (Figure S1B), followed by patients carrying a D67N + K219Q combination (16/70, 23%). The majority of patients with 3 TAMs/rev carried a combination of M41L + L210W + T215rev (T215D or S or C or D/E) (10/17, 59%), followed by patients with a combination of D67N + K219Q + T215 rev (T215S or L or A/V) (4/17, 24%).

Treatment response at week 48: Most of the 5990 patients who met the enrollment criteria in any of the 9 studies received a treatment regimen that contained either TDF (n = 4586) or TAF (n = 865). We investigated whether the presence of TDRM affected treatment response at Week 48 (Table 3). The overall proportion of patients with treatment success at week 48 was 85.7% and 92.5% for patients receiving a TDF- or TAF-containing regimen, respectively. Only 1.7% and 0.8% of patients receiving a TDF- or TAF -containing regimen, respectively, had virologic failure with emerging resistance to their treatment regimen. Non-success in the remaining 12.6% and 6.7% of TDF or TAF patients, respectively, was due to other events such as noncompliance, adverse events, or withdrawal of consent [25, 29, 35-40]. Similarly as for the overall population, the presence of pre-existing NNRTI resistance, K103N/S, NRTI resistance, TAMs (excluding or including T215 revertants), or PI-R had no significant impact on the observed response to treatment. Of note, patients with pre-existing NNRTI resistance did not receive a treatment regimen that contained an NNRTI, and patients with pre-existing PI-R may have received a treatment regimen that contained a PI (either atazanavir or darunavir) to which they were considered sensitive based on genotype. Patients with treatment failure in the TAMs + T215rev group (17 of 177 receiving a TDF-based regimen and 1 of 28 receiving a TAF-based regimen) mostly failed for reasons other than virologic failure and did not develop additional resistance, including no additional TAMs or T215Y/F (data not shown). In addition, similar treatment responses to either TDF- or TAF-containing regimens were observed in patients with HIV-1 subtype B or non-B. Notably, all 14 of the 17 patients with 3 TAMs/rev (Table 4) who received a TDF- or TAF-containing regimen were virologically suppressed with < 50 copies/mL of HIV RNA though the duration of the studies, including 8 patients with M41L + L210W + T215rev who remained suppressed through maximum follow-up of ≥144 weeks.

DISCUSSION

We have conducted a retrospective analysis of the genotypic resistance observed in 6704 patients prior to enrollment in clinical studies conducted at Gilead Sciences between 2000 and 2013. We observed an increase in the overall presence of transmitted drug resistance mutations throughout the period, from 5.2% of screened patients in 2000 to 11.4% of screened patients in 2013. The increase in pretreatment resistance during the period was primarily driven by an increase in the number of patients with NNRTI resistance (6.0% overall), notably with the K103N/S substitutions in RT (4.5% overall).

The prevalence of NNRTI-R and K103N/S was significantly higher in U.S. patients (6.1% of patients with K103N/S) compared to European patients (1.4% of patients with K103N/S). In the U.S., the prevalence of K103N/S increased from <1% in 2000 to 7.4% in 2010 and remained mostly stable through 2013. In contrast, the prevalence of K103N/S in European patients remained stable near 2% or below from 2003 through 2013. Longitudinal studies from the SPREAD study conducted in Europe from 1996 to 2010 have shown a similar trend for the presence of K103N/S over time, which has remained stable around 1.5% in the European ARV treatment-naïve population [6, 8, 41]. Similar to our observations, studies conducted in the U.S. found a consistently higher prevalence of K103N/S than those conducted in Europe with a prevalence of 5.2% [9], or 8.6% to 10.1% [42] at similar time periods to our analysis. These differences are likely multifactorial, including differential management of HIV treatment and differential ARV drug usage between the U.S. and Europe.

In contrast to NNRTI-R, prevalence of NRTI-R was very similar between U.S. and European patients. Signature NRTI-R mutations such as K65R or M184V/I were very rarely observed in our dataset (0.01% and 0.1%, respectively), despite the prevalent use of drugs

potentially selecting for these mutations during the period studied, such as TDF (K65R or K70E) and emtricitabine, lamivudine, or abacavir (M184V/I). Other studies have reported consistently low frequency or absence of K65R (<0.5%), while the frequency of M184V/I has shown more variability across studies (from 0.3% to 4.3%) [6, 8, 9, 41, 42]. The higher frequency of M184V/I over K65R correlates with reported rates of emergence of these mutations in clinical studies [26, 27, 35]. In addition, the overall rare observation of these mutations in ARV treatment-naïve patients is likely a consequence of the reduced fitness of viruses harboring these mutations [10, 11, 43], resulting in reduced transmission rates and/or a higher rates of reversion to wild-type in the absence of drug pressure. Indeed, M184V/I was found at higher frequencies in acutely/recently infected ARV treatment-naïve patients compared to chronically infected ARV treatment-naïve patients (8.2% and 2.5%, respectively) [44], and M184V/I was reported to have a particularly high reversion rate in the absence of drug pressure [14] compared to more stable mutations such as the PI-R L90M or the NNRTI-R K103N [13]. Overall, it appears that the impairment conferred by the M184V/I or K65R substitutions on viral fitness reduces their prevalence in ARV treatment-naïve patients.

Thymidine analog mutations (TAMs: M41L, D67N, K70R, L210W, T215Y/F, and K219Q/E/N/R in RT) represent another set of NRTI-R signature mutations. In addition to conferring resistance to AZT, with the T215Y/F substitutions playing a major role [2], TAMs have been associated with cross-resistance to most NRTIs [45, 46]. In particular, the combination of 3 or more TAMs including M41L, L210W, and T215Y was associated with significantly reduced response to TDF treatment in heavily ARV treatment-experienced patients [34]. TAMs were altogether found in 2-3% of ARV treatment-naïve patients throughout the period studied here with only minor variation between the U.S. and Europe, indicating relative ease of

transmissibility and persistence in the ARV treatment-naïve population compared to M184V/I or K65R. The M41L substitution was the most persistently observed TAM in our dataset (1.5%) in agreement with published data (1.5%, [41]). On the other hand, T215Y/F was very rarely observed (<0.05%), likely as a result of the impaired fitness associated with these substitutions in the absence of drug selective pressure [10], and consequently, T215 revertants (T215A/C/D/E/G/H/I/L/N/P/Q/R/S or V) were readily detected (2.7% of patients), similar to prior data (2.5%, [41]). Some current resistance algorithms include all changes from wild-type at position T215 (Y/F as well as revertants) as potential resistant mutants. However, the observed extended virologic response to TDF- or TAF-containing regimens through ≥144 weeks in patients with 2 TAMs + T215 revertants in our dataset suggest that the T215 revertants did not act as resistant mutants against TDF or TAF. In addition, the overall response to TDF- or TAF-containing regimens at week 48 was slightly higher when T215 revertants were included in the TAMs total (Table 3), and back-reversion of T215rev to T215Y/F was never observed in patients with virologic failure. Taken together, these data suggest that T215rev mutants are not associated with resistance to TDF or TAF and therefore resistance algorithms may need to be revised to reflect this lack of correlation. These findings are supported by a prior study [47] that also showed the absence of a resistance phenotype linked to the presence of T215rev mutants.

Finally, we have shown that the overall presence of commonly transmitted drug resistance mutations, most notably NRTI resistance mutations, had no impact on treatment response to TDF- or TAF-containing regimens at week 48. Of note, patients with specific drug resistance mutations to any of the components of the study regimens, such as K65R or ≥3 TAMs, were excluded from the trials and the impact of these mutations on treatment response to TDF- or TAF-containing regimen could not be assessed here. However, this resulted in only minimal

exclusions (8 patients) based on TDF, TAF, or FTC resistance given the very low prevalence of K65R, M184V, and ≥ 3 TAMs. In contrast, many more patients with NNRTI-R were excluded from enrollment in NNRTI-containing clinical studies due to transmitted NNRTI resistance (78 patients). Importantly, patients with transmitted NNRTI-R were treated in our studies with integrase strand transfer inhibitor-based or PI-based regimens and obtained high virologic success rates, indicating the absence of cross-class resistance. In summary, we have showed that the presence of common TDRM, particularly the T215 revertant mutations, in ARV treatment-naïve patients had no measurable impact on treatment-response to TDF- or TAF-based regimens. This further suggests that the presence of T215rev mutations should not be a factor in the estimation of genotypic resistance to TDF or TAF.

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REFERENCES

1. Cihlar T, Fordyce M. Current status and prospects of HIV treatment. Current opinion in virology **2016**; 18:50-6.

2. Larder BA, Kemp SD. Multiple mutations in HIV-1 reverse transcriptase confer high-level resistance to zidovudine (AZT). Science **1989**; 246:1155-8.

3. Wensing AM, Calvez V, Gunthard HF, et al. 2015 Update of the Drug Resistance Mutations in HIV-1. Topics in antiviral medicine **2015**; 23:132-41.

4. Iyidogan P, Anderson KS. Current perspectives on HIV-1 antiretroviral drug resistance. Viruses **2014**; 6:4095-139.

5. Erice A, Mayers DL, Strike DG, et al. Brief report: primary infection with zidovudine-resistant human immunodeficiency virus type 1. N Engl J Med **1993**; 328:1163-5.

6. Vercauteren J, Wensing AM, van de Vijver DA, et al. Transmission of drug-resistant HIV-1 is stabilizing in Europe. J Infect Dis **2009**; 200:1503-8.

7. Descamps D, Assoumou L, Chaix ML, et al. National sentinel surveillance of transmitted drug resistance in antiretroviral-naive chronically HIV-infected patients in France over a decade: 2001-2011. J Antimicrob Chemother **2013**; 68:2626-31.

8. Wensing AM, van de Vijver DA, Angarano G, et al. Prevalence of drug-resistant HIV-1 variants in untreated individuals in Europe: implications for clinical management. J Infect Dis **2005**; 192:958-66.

9. Poon AF, Aldous JL, Mathews WC, et al. Transmitted drug resistance in the CFAR network of integrated clinical systems cohort: prevalence and effects on pre-therapy CD4 and viral load. PLoS ONE **2011**; 6:e21189.

10. Cong ME, Heneine W, Garcia-Lerma JG. The fitness cost of mutations associated with human immunodeficiency virus type 1 drug resistance is modulated by mutational interactions. J Virol **2007**; 81:3037-41.

11. Deval J, White KL, Miller MD, et al. Mechanistic basis for reduced viral and enzymatic fitness of HIV-1 reverse transcriptase containing both K65R and M184V mutations. J Biol Chem **2004**; 279:509-16.

12. Jain V, Sucupira MC, Bacchetti P, et al. Differential persistence of transmitted HIV-1 drug resistance mutation classes. J Infect Dis **2011**; 203:1174-81.

13. Pingen M, Wensing AM, Fransen K, et al. Persistence of frequently transmitted drug-resistant HIV-1 variants can be explained by high viral replication capacity. Retrovirology **2014**; 11:105.

14. Yang WL, Kouyos RD, Boni J, et al. Persistence of transmitted HIV-1 drug resistance mutations associated with fitness costs and viral genetic backgrounds. PLoS pathogens **2015**; 11:e1004722.

15. Garcia-Lerma JG, Nidtha S, Blumoff K, Weinstock H, Heneine W. Increased ability for selection of zidovudine resistance in a distinct class of wild-type HIV-1 from drug-naive persons. Proc Natl Acad Sci U S A **2001**; 98:13907-12.

16. Hirsch MS, Conway B, D'Aquila RT, et al. Antiretroviral drug resistance testing in adults with HIV infection: implications for clinical management. International AIDS Society--USA Panel. JAMA **1998**; 279:1984-91.

17. Hirsch MS, Brun-Vezinet, Clotet B, et al. Antiretroviral drug resistance testing in adults infected with human immunodeficiency virus type 1: 2003 recommendations of an International AIDS Society-USA Panel. Clin Infect Dis **2003**; 37:113-28.

18. Vandamme AM, Sonnerborg A, Ait-Khaled M, et al. Updated European recommendations for the clinical use of HIV drug resistance testing. Antivir Ther **2004**; 9:829-48.

19. Department for Health and Human Services (DHHS). Guidelines for the Use of Antiretroviral Agents in HIV-1-Infected Adults and Adolescents. Developed by the DHHS Panel on Antiretroviral Guidelines for Adults and Adolescents – A Working Group of the Office of AIDS Research Advisory Council (OARAC). Downloaded from http://aidsinfo.nih.gov/guidelines on 5/11/2015. Last Updated **2015**.

20. Hertogs K, de Bethune MP, Miller V, et al. A rapid method for simultaneous detection of phenotypic resistance to inhibitors of protease and reverse transcriptase in recombinant human immunodeficiency virus type 1 isolates from patients treated with antiretroviral drugs. Antimicrob Agents Chemother **1998**; 42:269-76.

21. Petropoulos CJ, Parkin NT, Limoli KL, et al. A novel phenotypic drug susceptibility assay for human immunodeficiency virus type 1. Antimicrob Agents Chemother **2000**; 44:920-8.

22. Cohen C, Wohl D, Arribas JR, et al. Week 48 results from a randomized clinical trial of rilpivirine/emtricitabine/tenofovir disoproxil fumarate vs. efavirenz/emtricitabine/tenofovir disoproxil fumarate in treatment-naive HIV-1-infected adults. AIDS **2014**; 28:989-97.

23. Gallant JE, Staszewski S, Pozniak AL, et al. Efficacy and safety of tenofovir DF vs stavudine in combination therapy in antiretroviral-naive patients: a 3-year randomized trial. JAMA **2004**; 292:191-201.

24. Gallant JE, DeJesus E, Arribas JR, et al. Tenofovir DF, emtricitabine, and efavirenz vs. zidovudine, lamivudine, and efavirenz for HIV. N Engl J Med **2006**; 354:251-60.

25. Gallant JE, Koenig E, Andrade-Villanueva J, et al. Cobicistat versus ritonavir as a pharmacoenhancer of atazanavir plus emtricitabine/tenofovir disoproxil fumarate in treatment-naive HIV type 1-infected patients: week 48 results. J Infect Dis **2013**; 208:32-9.

26. DeJesus E, Rockstroh J, Henry K, et al. Co-formulated elvitegravir, cobicistat, emtricitabine, and tenofovir disoproxil fumarate versus ritonavir-boosted atazanavir plus co-formulated emtricitabine and tenofovir disoproxil fumarate for initial treatment of HIV-1 infection: a randomised, double-blind, phase 3, non-inferiority trial. Lancet **2012**; 379:2429-38.

27. Sax PE, DeJesus E, Mills A, et al. Co-formulated elvitegravir, cobicistat, emtricitabine, and tenofovir versus co-formulated efavirenz, emtricitabine, and tenofovir for initial treatment of HIV-1 infection: a randomised, double-blind, phase 3 trial, analysis of results after 48 weeks. Lancet **2012**; 379:2439-48.

28. Sax PE, Wohl D, Yin MT, et al. Tenofovir alafenamide versus tenofovir disoproxil fumarate, coformulated with elvitegravir, cobicistat, and emtricitabine, for initial treatment of HIV-1 infection: two randomised, double-blind, phase 3, non-inferiority trials. Lancet **2015**; 385:2606-15.

29. Tashima K, Crofoot G, Tomaka FL, et al. Cobicistat-boosted darunavir in HIV-1-infected adults: week 48 results of a Phase IIIb, open-label single-arm trial. AIDS research and therapy **2014**; 11:1-12.

30. Goodman DD, Zhou Y, Margot NA, et al. Low level of the K103N HIV-1 above a threshold is associated with virological failure in treatment-naive individuals undergoing efavirenz-containing therapy. AIDS **2011**; 25:325-33.

31. Bennett DE, Camacho RJ, Otelea D, et al. Drug resistance mutations for surveillance of transmitted HIV-1 drug-resistance: 2009 update. PLoS ONE **2009**; 4:e4724.

32. Smith F, Hammerstrom T, Soon G, et al. A meta-analysis to assess the FDA DAVP's TLOVR algorithm in HIV submissions. Drug Information Journal **2011**; 45:291-300.

33. Hertogs K, Bloor S, De Vroey V, et al. A novel human immunodeficiency virus type 1 reverse transcriptase mutational pattern confers phenotypic lamivudine resistance in the absence of mutation 184V. Antimicrob Agents and Chemother **2000**; 44:568-73.

34. Miller MD, Margot N, Lu B, et al. Genotypic and phenotypic predictors of the magnitude of response to tenofovir disoproxil fumarate treatment in antiretroviral-experienced patients. J Infect Dis **2004**; 189:837-46.

35. Margot NA, Kitrinos KM, Fordyce M, McCallister S, Miller MD, Callebaut C. Rare emergence of drug resistance in HIV-1 treatment -naive patients after 48 weeks of treatment with elvitegravir/cobicistat/emtricitabine/tenofovir alafenamide. HIV Clin Trials **2016**; 17:78-87.

36. Margot NA, Enejosa J, Cheng AK, Miller MD, McColl DJ. Development of HIV-1 Drug Resistance Through 144 Weeks in Antiretroviral-Naive Subjects on Emtricitabine, Tenofovir Disoproxil Fumarate, and Efavirenz Compared With Lamivudine/Zidovudine and Efavirenz in Study GS-01-934. J Acquir Immune Defic Syndr **2009**; 52:209-21.

37. Margot NA, Lu B, Cheng A, Miller MD. Resistance development over 144 weeks in treatment-naive patients receiving tenofovir disoproxil fumarate or stavudine with lamivudine and efavirenz in Study 903. HIV Med **2006**; 7:442-50.

38. Porter DP, Kulkarni R, Fralich T, Miller MD, White KL. Characterization Of HIV-1 Drug Resistance Development Through Week 48 In Antiretroviral Naive Subjects On Rilpivirine/Emtricitabine/Tenofovir DF Or Efavirenz/Emtricitabine/Tenofovir DF In The STaR Study (Gs-Us-264-0110). Journal of Acquired Immune Deficiency Syndromes **2014**; 65:318-26.

39. White KL, Kulkarni R, McColl DJ, et al. Week 144 resistance analysis of elvitegravir/cobicistat/emtricitabine/tenofovir DF versus efavirenz/emtricitabine/tenofovir DF in antiretroviral-naive patients. Antivir Ther **2015**; 20:317-27.

40. Kulkarni R, Abram ME, McColl DJ, et al. Week 144 Resistance Analysis of Elvitegravir/Cobicistat/Emtricitabine/Tenofovir DF Versus Atazanavir+Ritonavir+Emtricitabine/Tenofovir DF in Antiretroviral-Naive Patients. HIV Clin Trials **2014**; 15:218-30.

41. Hofstra LM, Sauvageot N, Albert J, et al. Transmission of HIV Drug Resistance and the Predicted Effect on Current First-line Regimens in Europe. Clin Infect Dis **2016**; 62:655-63.

42. Kassaye SG, Grossman Z, Balamane M, et al. Transmitted HIV Drug Resistance Is High and Longstanding in Metropolitan Washington, DC. Clin Infect Dis **2016**.

43. Svarovskaia ES, Feng JY, Margot NA, et al. The A62V and S68G Mutations in HIV-1 Reverse Transcriptase Partially Restore the Replication Defect Associated With the K65R Mutation. J Acquir Immune Defic Syndr **2008**; 48:428-36.

44. Metzner KJ, Scherrer AU, Preiswerk B, et al. Origin of minority drug-resistant HIV-1 variants in primary HIV-1 infection. J Infect Dis **2013**; 208:1102-12.

45. Lanier ER, Ptak RG, Lampert BM, et al. Development of hexadecyloxypropyl tenofovir (CMX157) for treatment of infection caused by wild-type and nucleoside/nucleotide-resistant HIV. Antimicrob Agents Chemother **2010**; 54:2901-9.

46. Li Z, Terry B, Olds W, et al. In vitro cross-resistance profile of nucleoside reverse transcriptase inhibitor (NRTI) BMS-986001 against known NRTI resistance mutations. Antimicrob Agents Chemother **2013**; 57:5500-8.

47. Mitsuya Y, Varghese V, Wang C, et al. Minority human immunodeficiency virus type 1 variants in antiretroviral-naive persons with reverse transcriptase codon 215 revertant mutations. J Virol **2008**; 82:10747-55.

48. Ruane PJ, Dejesus E, Berger D, et al. Antiviral Activity, Safety, and Pharmacokinetics/Pharmacodynamics of Tenofovir Alafenamide as 10-Day Monotherapy in HIV-1-Positive Adults. J Acquir Immune Defic Syndr **2013**; 63:449-55.

FIGURE LEGENDS

Figure 1: Proportion of patients with pre-existing resistance mutations over time. Prospective subjects were sequenced at screening (panel A: all patients [n = 6704]; panel B: US patients [n = 4414]; panel C: EU patients [n = 1191]) prior to study entry (Virco, Monogram, or Gilead) from the start of HIV-1 protease (PR-1) through HIV-1 reverse transcriptase up to position 305. The mutations (WHO 2009) included in each category are listed in Materials and Methods, unless specified. Stars (*) indicate a statistically significant increase for specific mutation categories over time ($p \leq 0.01$, Cochran-Armitage Trend test). Panel A p-values: <0.0001 for Any Resistance, NNRTI-R, and K103N/S. Panel B p-values: 0.0003 for Any Resistance; <0.0001 for NNRTI-R, and K103N/S. NRTI: nucleoside/nucleotide RT inhibitor; NNRTI: non-NRTI; TAM: thymidine analog mutation; PI: protease inhibitor; -R: resistance. Total number of sequences is indicated at the bottom of each graph.

Figure 2: Pretreatment distribution of thymidine-analog mutations (TAMs) in enrolled patients (n = 5990). The distributions of TAMs (gray lines) or TAMs including T215 Revertants (such as T215S/D/E/C/L/I/A; solid gray) in the enrollment sequence dataset are shown. TAMs are M41L, D67N, K70R, L210W, T215Y/F, and K219Q/E/N/R in RT.

Table 1. Study Details

Study	Program [a]	Enroll-ment Year	BL HIV-1 RNA [b]	BL CD4 [b]	Geographical Origin [c]	Number of Patients [d]			
						Screened	Enrolled	TDF	TAF
99-903	TDF	2000	4.91	279	US, EU, SA	598	598	299	
01-934	TDF+FTC+ EFV	2003	5.01	237	US, EU	501	479	240	
236-0102	E/C/F/TDF	2010	4.76	380	US	799	700	700	
236-0103	E/C/F/TDF	2010	4.87	357	NA, EU, AU, MX,TH	853	708	708	
216-0114	COBI	2010	4.81	343	NA, EU, AU, BR, MX,DR, TH	772	692	692	
264-0110	RPV/F/TDF	2011	4.80	374	NA, EU, AU	905	786	786	
216-0130	DRV+COBI	2011	4.80	370	US	336	295	294	
292-0104	E/C/F/TAF	2013	4.61	404	NA, EU, AU, JP, TH	972	866	432	434
292-0111	E/C/F/TAF	2013	4.55	406	NA, EU, MX, DR	968	866	435	431
					Total	6704	5990	4586	865

a COBI or C: cobicistat; DRV: darunavir; E: elvitegravir; EFV: efavirenz; FTC or F: emtricitabine; RPV: rilpivirine; TAF: tenofovir alafenamide; TDF: tenofovir disoproxil fumarate.

b Median pretreatment (BL) HIV-1 RNA expressed in $\log_{10}$ copies/mL. Median pretreatment BL CD4 count expressed in cell/mm^3.

c US: United States (may include sites in Puerto Rico); AU: Australia; BR: Brazil; DR: Dominican Republic; EU: Europe; JP: Japan; MX: Mexico; NA: North America (US, Canada); SA: South America; TH: Thailand.

d Patients with available genotypic data.

Table 2. Prevalence of Pre-Treatment Drug Resistance Mutations (2000 to 2013)

Drug Resistance Mutation	Patients, n (%)			
	Screened	Screened (US Only)	Screened (EU Only)	Enrolled
Patients With Data	6704	4414	1191	5990
Any Resistance	752 (11.2%)	607 (13.8%)	76 (6.4%)	604 (10.1%)
NNRTI-Associated [a]	401 (6.0%)	352 (8.0%)	25 (2.1%)	301 (5.0%)
K103N/S	302 (4.5%)	270 (6.1%)	17 (1.4%)	230 (3.8%)
NRTI-Associated [b]	205 (3.1%)	151 (3.4%)	27 (2.3%)	165 (2.8%)
K65R	1 (0.01%)	1 (0.02%)	0	0
K70E	0	0	0	0
M184I/V	7 (0.1%)	4 (0.1%)	2 (0.2%)	3 (0.1%)
TAMs [c]	182 (2.7%)	134 (3.0%)	25 (2.1%)	145 (2.4%)
M41L	99 (1.5%)	78 (1.8%)	9 (0.8%)	77 (1.3%)
D67N	39 (0.6%)	24 (0.5%)	9 (0.8%)	28 (0.5%)
K70R	10 (0.1%)	7 (0.2%)	1 (0.1%)	8 (0.1%)
L210W	32 (0.5%)	25 (0.6%)	4 (0.3%)	21 (0.4%)
T215Y/F	5 (0.07%)	2 (0.05%)	1 (0.08%)	0
K219E/N/Q/R	59 (0.9%)	42 (1.0%)	10 (0.8%)	44 (0.7%)
T215 Revertants [d]	183 (2.7%)	138 (3.1%)	25 (2.1%)	151 (2.5%)
PI-Associated [e]	159 (2.4%)	127 (2.9%)	14 (1.2%)	124 (2.1%)
M46I/L	62 (0.9%)	49 (1.1%)	5 (0.4%)	46 (0.8%)
L90M	56 (0.8%)	48 (1.1%)	4 (0.3%)	43 (0.7%)

a Non-nucleoside RT inhibitor resistance (NNRTI-R) mutations are: L100I, K101E/P, K103N/S, V106M/A, V179F, Y181C/I/V, Y188C/H/L, G190A/E/S, P225H, and M230L in RT (World Health Organization 2009).

b Nucleoside/nucleotide RT inhibitor resistance (NRTI-R) mutations are: M41L, K65R, D67N/G/E, T69 insertion, T69D, K70E/R, L74V/I, V75M/T/A/S, F77L, Y115F, F116Y, Q151M, M184V/I, L210W, T215Y/F, and K219E/Q/N/R in RT (WHO 2009).

c Thymidine-analog mutations are: M41L, D67N, K70R, L210W, T215Y/F, and K219E/Q/N/R in RT.

d RT T215 revertants (T215rev) observed were: T215A/C/D/E/G/H/I/L/N/P/Q/R/S/V.

e Primary protease inhibitor resistance (PI-R) mutations are: L23I, L24I, D30N, V32I, M46I/L, I47V/A, G48V/M, I50V/L, F53L/Y, I54A/V/T/S/M/L, G73S/T/C/A, L76V, V82A/F/L/S/T/C/M, N83D, I84V/A/C, I85V, N88S/D, and L90M in protease (WHO 2009).

Table 3. TDF- and TAF-Based Treatment Response at Week 48 Per Baseline Virologic Category

Category	Proportion of Patients with Treatment Success at Week 48 (n/N) [a]	
	TDF	TAF
All Patients	85.7% (3932/4586)	92.5% (800/865)
B	85.5% (3557/4158)	92.7% (689/743)
Non-B	87.7% (372/424) [b]	91.0% (111/122)
NNRTI-R [c]	89.0% (210/236)	90.6% (58/64)
No NNRTI-R	85.6% (3722/4350)	92.6% (742/801)
K103N/S [c]	87.8% (159/181)	91.7% (44/48)
No K103N/S	85.7% (3773/4405)	92.5% (756/817)
NRTI-R	88.0% (169/192)	96.7% (29/30)
No NRTI-R	85.6% (3763/4394)	92.3% (771/835)
TAMs	87.0% (94/108)	95.2% (20/21)
No TAMs	85.7% (3838/4478)	92.4% (780/844)
TAMs + T215 Revertants	90.4% (160/177)	96.4% (27/28)
No TAMs + T215 Revertants	85.6% (3772/4409)	92.4% (773/837)
PI-R [d]	86.6% (84/97)	84.2% (16/19)
No PI-R	85.7% (3848/4489)	92.7% (784/846)

a Treatment success was determined based on the FDA algorithms (TLOVR 50, Studies 903 and 934; or Snapshot < 50 copies/mL for all other studies).

b Four patients with missing HIV-1 subtype data are not represented.

c Patients with transmitted NNRTI resistance mutations received either an integrase strand transfer inhibitor-based or a PI-based treatment regimen.

d Patients with transmitted PI resistance mutations may have received a PI-based treatment regimen depending on the mutations present pretreatment.

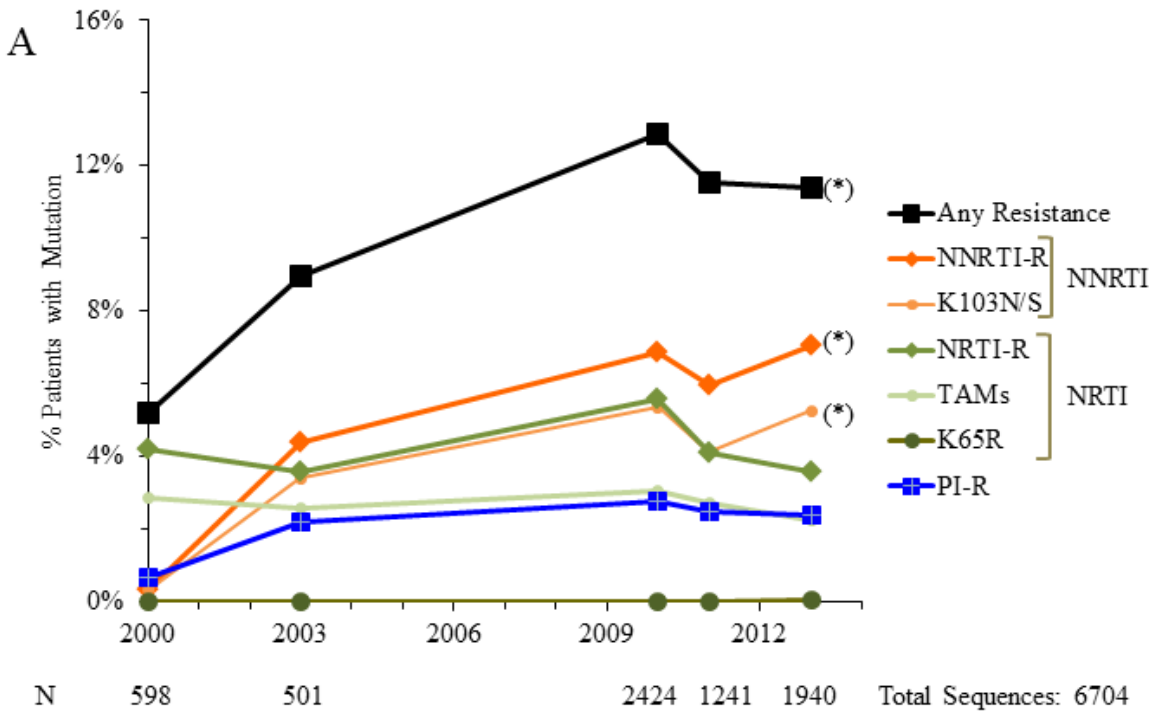
NRTI: nucleoside/nucleotide RT inhibitor; NNRTI: non-NRTI; PI: protease inhibitor; -R: resistance; TAMs: thymidine analog mutations; TAF: tenofovir alafenamide; TDF: tenofovir disoproxil fumarate.

Table 4. TDF- and TAF-Based Treatment Response in Patients with 2 TAMs + T215 Revertants

Patient ID	Mutation at TAM Position						Treatment Regimen	Treatment Response [a]
	M41	D67	K70	L210	T215	K219		
1	M41L			L210W	T215C		EFV/3TC/TDF	<50 through week 144
2	M41L			L210W	T215D		EFV/3TC/TDF	<50 through week 168
3	M41L			L210W	T215C		TDF+FTC+EFV	<50 through week 168
4	M41L			L210W	T215S		ATV/C/F/TDF	<50 through week 156
5	M41L			L210W	T215S		ATV/r/F/TDF	<50 through week 144
6	M41L			L210W	T215S		EFV/F/TDF	<50 through week 144
7	M41L			L210W	T215D		ATV/r/F/TDF	<50 through week 144
8	M41L			L210W	T215D		E/C/F/TDF	<50 through week 168
9	M41L		K70R		T215E		ATV/r/F/TDF	<50 through week 144
10	M41L		K70K/R		T215E		ATV/C/F/TDF	<50 through week 152
11		D67N			T215L	K219Q	E/C/F/TAF	<50 through week 168
12		D67N			T215S	K219Q	EFV/F/TDF	<50 through week 144
13		D67N			T215A/V	K219Q	E/C/F/TDF	<50 through week 24 (DC)
14		D67N			T215S	K219Q	RPV/F/TDF	<50 through week 48

ATV: atazanavir; C: cobicistat; E: elvitegravir; EFV: efavirenz; F: emtricitabine; r: ritonavir; RPV: rilpivirine; TAF: tenofovir alafenamide; TAM: thymidine-analog mutation; TDF: tenofovir disoproxil fumarate; 3TC: lamivudine.

a HIV-1 RNA <50 copies/mL through the end of the studies at the time indicated. DC: discontinuation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Any Resistance
NNRTI-R
K103N/S
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NRTI-R
TAMs
K65R
NRTI
PI-R
N
598
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1241
1940
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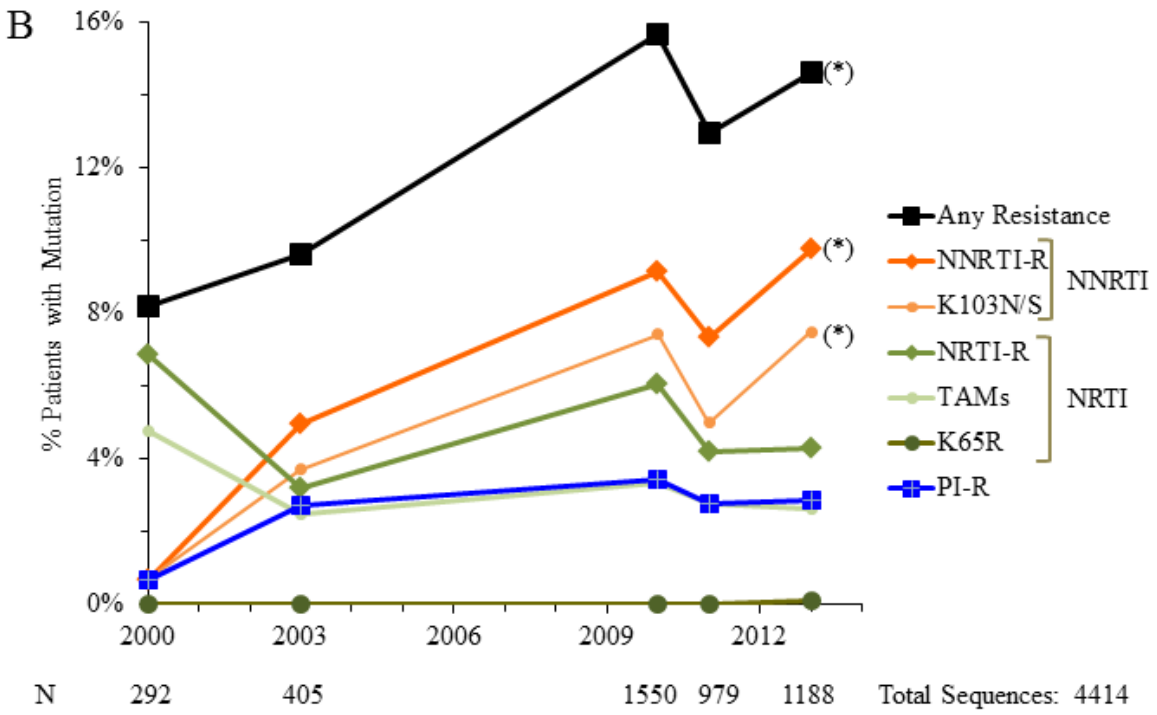
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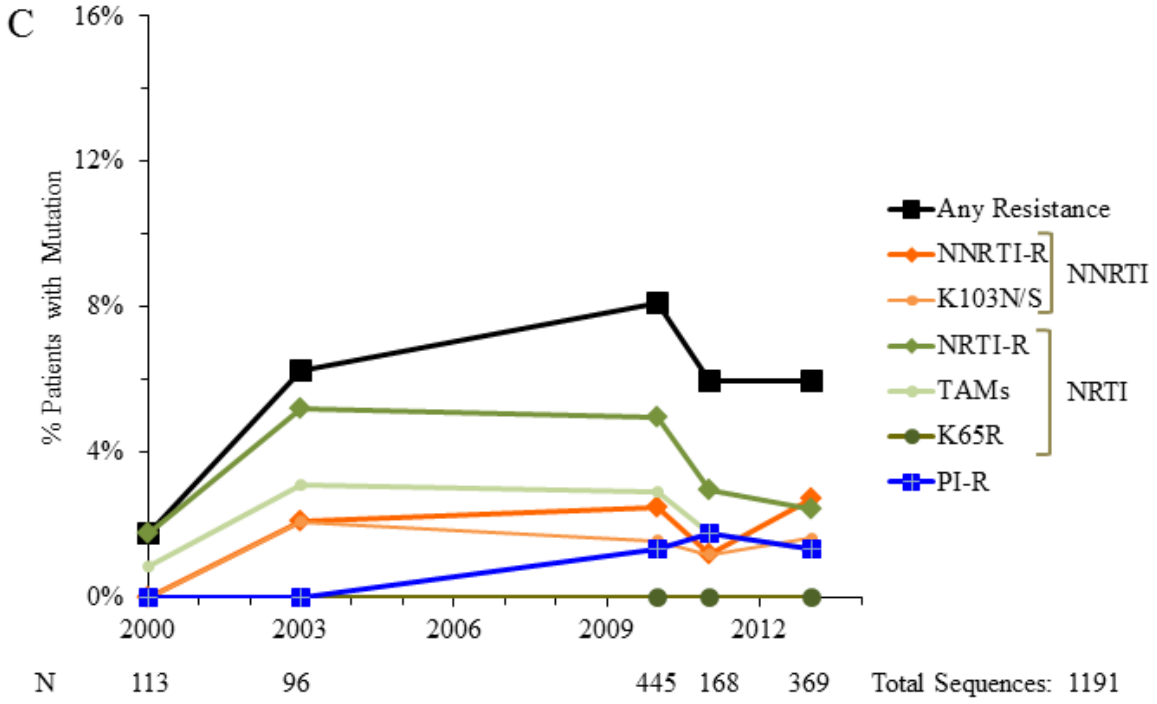
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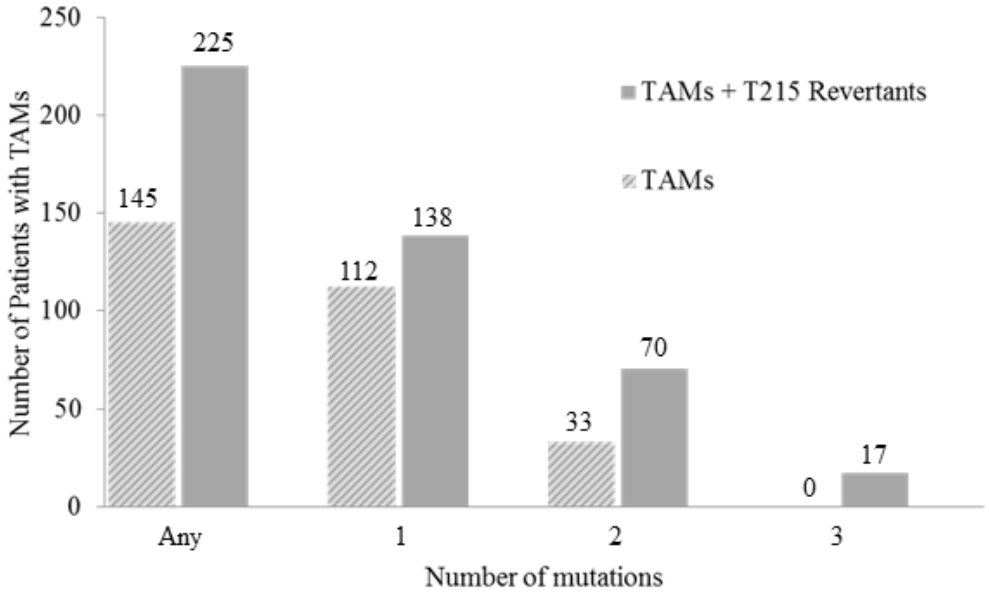
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