

HIV-1 RT Mutations K70E and K65R are Not Present on the Same Viral Genome when Both Mutations are Detected in Plasma

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Introduction

The high incidence of virologic non-response observed in the ABC/3TC/TDF arm of ESS30009 has been associated with selection for the K65R and M184V RT mutations.¹ The RT mutation K70E was selected prior to therapy switch in naive subjects failing on ABC/3TC/TDF. This mutation may represent an alternative resistance pathway associated with the observed non-response to this regimen.²

K70E has primarily been noted in association with adefovir resistance *in vitro*³ and *in vivo*.^{4,5} Recently, selection of K70E has been reported in subjects treated with TDF. In one instance, a subject initially was on a virologically stable regimen of LPV/r, ddI, ABC, and 3TC. After the protease inhibitor was replaced with TDF, the subject experienced viral rebound with selection of K65R + K70E + M184V by population genotype.⁶ K70E has also been reported to have been selected in a treatment-naïve subject failing on a TDF + 3TC + EFV regimen.⁷

In ESS30009, K70E mixtures were sometimes detected with K65R mixtures by population genotype. Clones were made from samples in which K70E and K65R mixtures had been previously detected, and the clonal genotypes were analyzed to determine whether K65R and K70E (+/- M184V) would occur on the same viral genome.

Methods

Plasma HIV drug susceptibility and population genotyping (PhenoSense GT™) were performed by ViroLogic, Inc. on the baseline and on-therapy samples from subjects enrolled in the ESS30009 study on the ABC/3TC/TDF arm. The emergence of resistance mutations as defined by the IAS-USA Panel in subjects with post-baseline genotype was also quantitated. Binomial distribution was used to test if the proportion of K70E mutation occurrence is 0.

The ViroLogic population sequence data were used to identify samples in which K70E was detected. Plasma was available from six of these samples and was sent to Research Think Tank, Inc. (RTT) for clonal analysis.

A minimum of 50 HIV clones were obtained by RTT from each of these six samples and analyzed using the Gene Tanker™ methodologies, with HIV-1 clonal sequencing results obtained from an additional two subjects after abstract submission. Clonal analysis was also performed on one additional sample identified with K65R and K70E by population sequencing, which was submitted to RTT for routine genotypic analysis.

HIV site-directed mutants containing K70E were created at ViroLogic, Inc. and analyzed in triplicate for phenotypic impact on study drugs and replicative capacity. The mean fold changes are reported.

Results

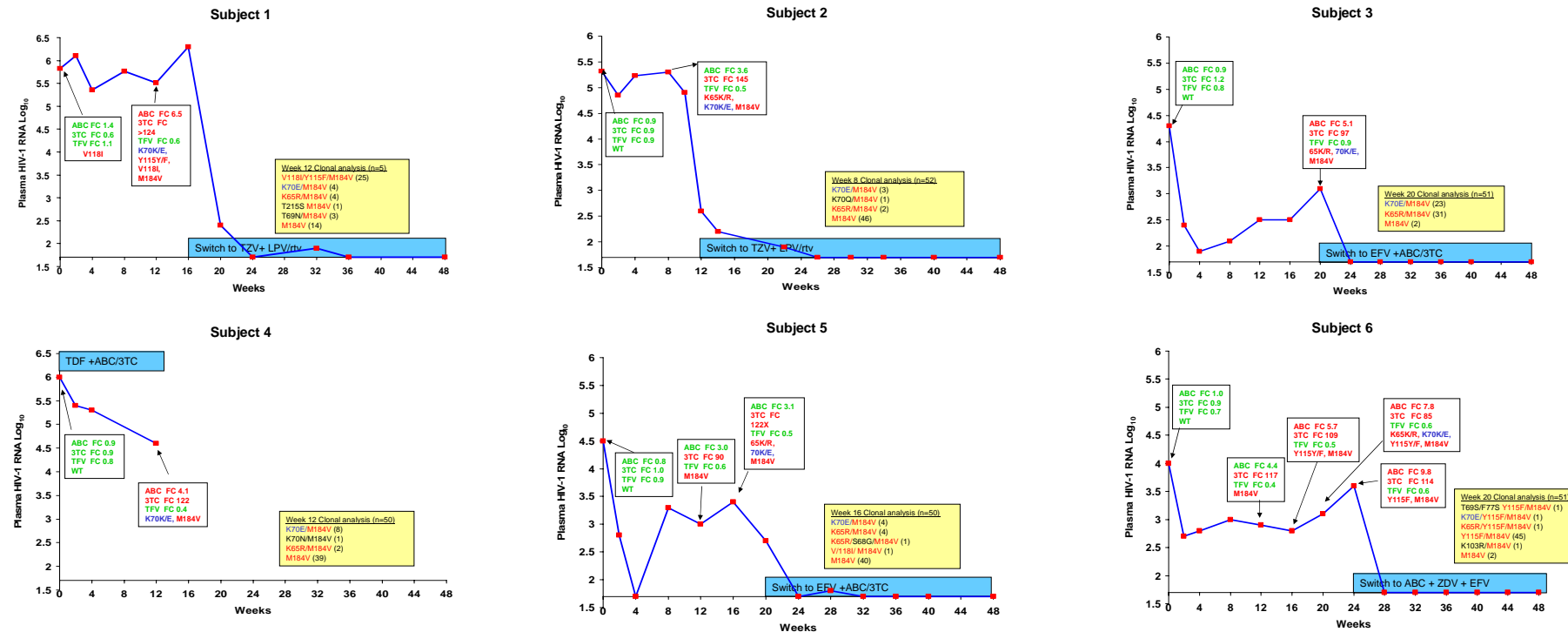
IAS-Defined Treatment Emergent Resistance to TDF/ABC/3TC:

■ There were 81 subjects with both baseline and post-baseline (pre-therapy switch) genotypes in the ESS30009 on the TDF/ABC/3TC arm. Available on-therapy time points with genotype ranged from Weeks 4 through 30.

■ Treatment emergent HIV-1 RT resistance mutations, as defined by the IAS Drug Resistance Guidelines, were observed in 79 of 81 (98%) subjects. They were as follows, in order of decreasing incidence:

M184I/V (79/81; 98%), K65R (43/81; 53%), Y115F (9/81; 11%), M41L (1/81), T69A/D/N/T (1/81), V106A (1/81), V118I (1/81) and Y181F/I/N (1/81).

Figure 1. Longitudinal Profiles of HIV RT K70E Mutation Selection in Six Patients Being Treated with Tenofovir/Abacavir /Lamivudine (TDF/ABC/3TC) as Detected by Population Genotyping (ViroLogic, Inc.) and Clonal Analysis (Research Think Tank, Inc.). Treatment regimen (blue bars, top), plasma HIV-RNA, population genotype and phenotypic fold resistance to study drugs are shown. Red font indicates decreased phenotypic susceptibility or presence of a known drug resistance mutation. The fold change (FC) in susceptibility compared with to control is also shown. Clonal analysis results for specific time points are shown in the inset yellow box in each of the graphs.



Identification and Characterization of Treatment Emergent K70E:

By population sequencing, isolates with a glutamic acid (E) substitution for lysine at codon 70 were detected in 8 of 81 subjects (10%) at post-baseline time points only.

In all cases, K70E was observed as a mixture of wild-type and mutant subpopulations (i.e. K70K/E). K65K/R mixtures were also observed in the majority of these isolates, and M184V was detected as an apparently homogeneous mutant population.

In an attempt to determine genotypic linkage between K65R and K70E, *pol* gene clones were prepared and analyzed. Of 305 clones analyzed from six patient isolates, K65R and K70E were never observed on the same genome.

A sample from another subject that was submitted to RTT for routine genotypic analysis was also examined by clonal analysis after population genotyping detected K65R + K70E (+M184V, L74V, K100I, and K103N). Clonal analysis indicated that 5/10 clones (50%) had K70E, while 5/10 had K65R (50%) with the same backbone mutations, again with the K65R and K70E mutations always detected on separate viruses.

Table 1. Mean Fold Change (FC) in Drug Susceptibility and Replicative Capacity (RC) in HIV with Site Directed Mutations (SDM)

SDM	ABC FC	3TC FC	TDF FC	RC
K70E	1.4	3.4	1.2	97.3%
K65R+K70E	3.1	8.8	2.3	2.4%
K65R+K70E+M184V	RC too low to determine IC ₅₀			0.01%

■ To further examine whether the K70E and K65R mutations could co-exist on the same virus, HIV mutants with the following site directed mutations (SDMs) were created: K70E, K70E + K65R, and K70E +K65R+ M184V. Each SDM was analyzed in triplicate for replicative capacity (RC) and for phenotypic susceptibility to study drugs. As shown in Table 1, in the presence of both the K65R and K70E mutations, the RC dropped to <2.4%, and to <0.01% when all three mutations were present, making it unlikely that these mutations would be detected occurring together in clinical samples as a majority viral species.

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Discussion

■ While K70E has previously been reported to be selected by and associated with resistance to adefovir^{3,4,5}, after adefovir was withdrawn from the market, K70E was no longer reported as a resistance associated mutation on HIV genotype reports. K70E is not present on the most widely used resistance mutations list, such as the IAS-USA Panel's list of drug resistance-associated mutations. While a few reports have emerged recently noting the presence of the K70E mutation in patients being treated with tenofovir^{6,7}, it is possible that K70E selection under drug pressure has been under reported. The prevalence of both the K65R and the K70E mutation have been rising yearly over the past three years since tenofovir was introduced, and this rise has been correlated with an increase in utilization of tenofovir.⁸

■ In the absence of prior drug treatment, K70E appears to be rarely detected. In an analysis of 721 ART naïve subjects (pre-therapy ESS30009, APV30001 and APV30002 samples) K70E was detected in only one subject.²

■ Since tenofovir was one component of this regimen (with abacavir/lamivudine), selection for K70E after failure on abacavir/lamivudine containing therapies has also been examined. This mutation was not detected in ART-naïve subjects treated with a backbone of abacavir/lamivudine who experienced virologic failure through 48 weeks in APV 30001 or APV 30002, nor was it observed in subjects with virologic failure on the abacavir/lamivudine + efavirenz arm of ESS30009.² While it appears unlikely that abacavir/lamivudine will select for the K70E mutation, it may still impact susceptibility to these drugs once selected.

Conclusions

■ By population genotyping, K65R, K70E and M184V mutations were selected in subjects failing on tenofovir + abacavir/lamivudine therapy.

■ While K70E is not currently listed as a drug resistance associated mutation by the IAS USA, it was previously associated with resistance to adefovir and K70E selection on tenofovir-containing regimens has been reported.

■ Using comprehensive and sensitive clonal genotypic analyses employed here, no evidence was found for linkage between K70E and K65R. Though both the HIV RT mutations K70E and K65R were detected by clonal genotyping in these subjects, they were never detected on the same genome.

■ The site directed mutagenesis data suggests that there is some reduction in susceptibility to tenofovir, abacavir and lamivudine when K70E is present.

■ The reduction in replicative capacity for the site directed mutants containing both K65R + K70E or K70E+K65R+M184V make it unlikely that these mutations would occur together as a majority species in a clinical sample.

■ Together with data previously reported, these clonal genotyping data provide further evidence for a possible alternative resistance pathway to K65R for TDF with exclusivity between K65R and K70E at the genomic level.

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