Selection for S68G/N/D Mutations of HIV-1 Reverse Transcriptase (RT) in Antiretroviral-Naïve Subjects Treated with Tenofovir (TDF)/Abacavir (ABC)/Lamivudine (3TC) Therapy

Poster Number
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Introduction

Selection for mutations at the HIV reverse transcriptase codon S68 have been observed after virologic failure on tenofovir, abacavir, didanosine or stavudine containing regimens and in some cases linked with K65R selection.[1,2] K65R was detected in the majority of the subjects with virologic non-response receiving TDF/ABC/3TC in ESS30009.[3]

For this analysis, HIV genotypes from subjects while on TDF/ABC/3TC therapy were compared with baseline results and examined for selection of S68 mutations. Where available, sequential timepoints while on therapy were examined. The association of this mutation with selection of K65R, as well as other relevant reverse transcriptase mutations, was examined to determine if it was linked in this study with resistance mutation selection and if, as suggested, this mutation might play a compensatory role in restoration of viral fitness in the presence of some resistance associated mutations.[1,3]

Methods

- Plasma HIV phenotype and population genotype were analyzed by ViroLogic. Per protocol, samples were collected for virologic analysis at baseline and at time points after 12 weeks on therapy.
- HIV clonal analysis was performed at GlaxoSmithKline, as described by Rouse et al[4] and was similar to the methods reported by members of the Demeter and Siliciano labs.[5,6]
- Additional resistance profile data for K65R with and without S68G was examined using the ViroLogic, Inc. database of patient samples analyzed for HIV protease and RT mutations and for phenotypic resistance. Mixtures of K65R/K were excluded. Drug susceptibility was expressed as the median of the IC_{50} fold-change (clinical isolate IC_{50} divided by IC_{50} of simultaneously tested drug-sensitive reference virus [NL4-3]) values (Median Fold Change; MFC).

Results

- 81 subjects had both baseline and post-baseline population genotype, ranging from Week 4-30.
- The most commonly observed, treatment emergent, IAS USA-defined NRTI-associated resistance mutations for these 81 subjects were M184I/V (78/81, 96%), K65R (43/81, 53%) and Y115F (9/81, 11%)
- By population genotype, HIV-1 coding changes at codon 68 have been detected in 6/43 (14%) of the subjects, all of whom also harbored the K65R and M184V mutations. The enrichment for mutations at 68 appears to be delayed and follows the initial selection for K65R and M184V, at times ranging from between Week 10-21. The sequential mutational profiles and associated virologic response in each of these six subjects are shown in Figure 1.
- The sequential selection for coding changes at 68 appears to be associated with viral rebound since at the time of detection, the change in viral load from baseline was less than a 0.3 $\log_{10}$ reduction compared to baseline in 5 of 6 subjects. Subject 5 had a 1.0 $\log_{10}$ drop from baseline, but rebounded three weeks later to within 0.4 $\log_{10}$ of baseline.

References

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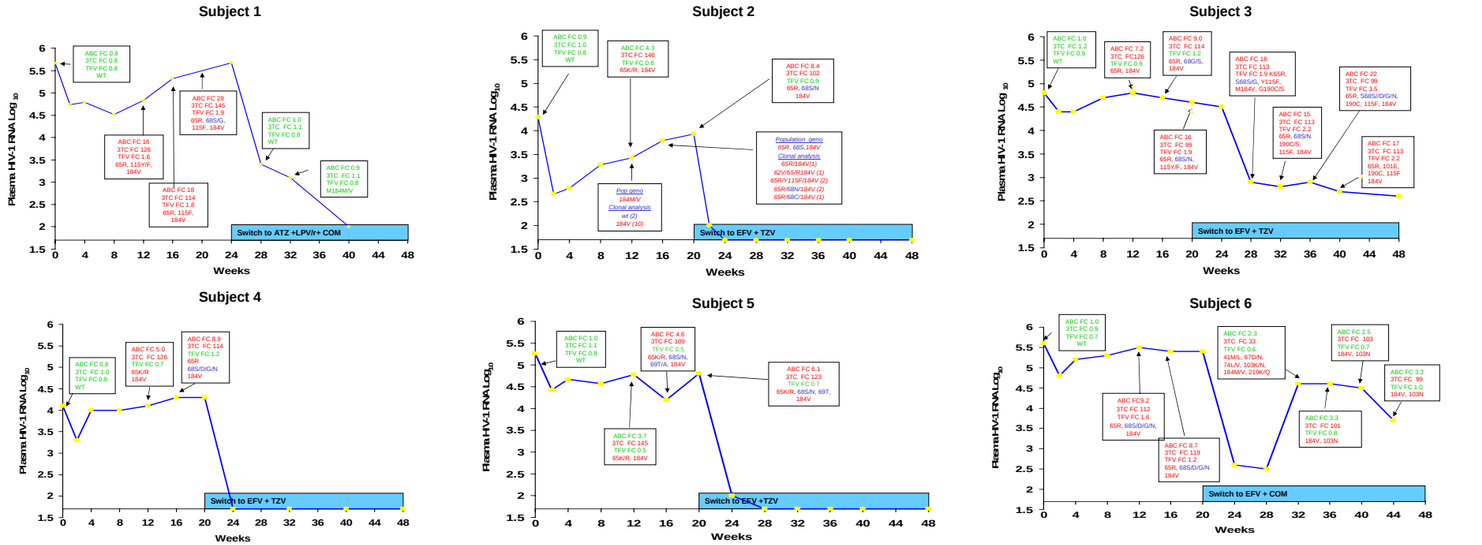
Figure 1. Longitudinal Profiles of HIV RT S68 Mutation Selection in Six Patients Being Treated with Tenofovir/Abacavir/Lamivudine (TDF/ABC/3TC) as Detected by Population Genotyping

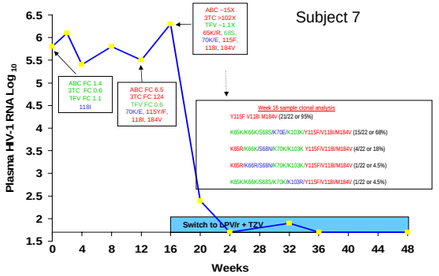
Plasma HIV-RNA, population genotype and phenotype fold resistance to study drugs are shown. Red font indicates decreased phenotypic susceptibility or presence of a known drug resistance-associated mutation. The fold change (FC) in susceptibility compared with to control is also shown. Green font indicates phenotypic drug susceptibility. Clonal analysis results and accompanying population genotype presented in italics for Subject 2.



- In HIV-1 from 5/6 of subjects with both K65R and S68 mutations, K65R was detected prior to S68 mutations. For Subject 6, both mutations were detected at the Week 12 timepoint.
- The Y115F mutation was also selected in 2 of these 6 subjects (Subjects 1 and 3). When present, reduced susceptibility (a fold change greater than the phenotypic clinical cut-offs) was noted for all study drugs. Y115F was detected prior to S68G mutation selection in Subject 1 and after S68S/D/G/N mutation detection in Subject 3.
- Replicative capacity data were available for 5/6 subjects at the last genotype prior to therapy change. The median replicative capacity at that timepoint was 71%, with all samples having K65R and M184V mutations present.
- Post switch, the following regimens were used: Trizivir + efavirenz (Subjects 2, 3, 4, and 5), Combivir + atazanavir + lopinavir/ritonavir (Subject 1), Combivir + efavirenz (Subject 6). Abacavir, lamivudine and zidovudine were included in the NRTI component of 4/6 regimens, while Subjects 1& 6 received only zidovudine and lamivudine.
- As seen in Figure 2, clonal analysis results were available for another subject from this study. Subject 7 did not have evidence of selection for 68 mutations by population genotype, although this subject did have mixtures of K65K/R, K70K/E, Y115F, V118I and M184V present at Week 16 on therapy. Clonal analysis indicated that ~22% of the clones at this timepoint contained both K65R and S68N mutations.
- Clonal analysis results were also available for Subject 2 (shown in Figure 1) at the Week 12 and 16 time points. In this subject, neither K65R nor S68 mutations were present at the Week 12 timepoint; however, 3 of 7 had S68N/C at week 16, consistent with the observed mixture by population sequencing at week 20.
- Analysis of the ViroLogic, Inc. database of samples indicated that there was a modest increase in resistance when K65R was present with the S68G mutation, with the greatest impact on TDF. In samples with K65R without S68G (n=119), 83% were above the TDF cut-off for decreased susceptibility, while the presence of S68G produced a 12% increase (95% of samples). For ABC, no sample was above the cut-off with K65R and without S68G, and only 5% were above the cut-off when present, while for 3TC a slight decrease (3%) was observed when S68G was present (from 98% to 95%).

Figure 2. Clonal Analysis Results for Subject 7

Population genotype profile indicates selection for K65R but without S68 mutations, clonal analysis indicates presence of K65R clones with S68N.



Discussion

- In another multicenter study of 60 antiretroviral naïve subjects randomized to receive didanosine, stavudine and abacavir, Roge and colleagues[1] reported similar findings. In the eight subjects experiencing virologic failure, K65R was selected in HIV from five subjects, with concomitant selection of S68G in four of these subjects. Decreased susceptibility to tenofovir, abacavir, didanosine and lamivudine was reported for subjects with virus harboring both mutations. While our study is also limited in sample size, the findings are in agreement with those of Roge *et al*, and suggest that selection of S68 mutations after K65R in HIV from these subjects offers a selective advantage, perhaps by stabilizing or restoring fitness to the K65R mutation.
- The S68G mutation has previously been observed to be selected in vitro following selection of the Q151L mutation, an intermediate on the pathway to selection of the Q151M mutation associated with multi drug resistance.[7] Replicative fitness of the virus harboring the Q151L intermediate was restored after selection for the S68G mutation.
- Mutations at S68 have also been reported by Schmidt and colleagues[8] in virus from subjects who had selected for Q151M. Sequential analysis of earlier time points detected emergence of the S68N prior to selection of Q151M in one subject, while Larder and colleagues[9] reported an elevated incidence of S68D/N mutations in subjects selecting for multi nucleoside resistance mutations (A62V, V75I, F77L, F116TY, and Q151M).
- In our study, since the TDF/ABC/3TC therapy was discontinued after a relatively short time and before the S68 was present as a fully mutant population, it is unclear whether the Q151M or other multidrug resistance complex mutations would have emerged here.

Conclusions

- Selection of K65R was detected by population genotype in 53% of subjects treated with tenofovir/abacavir/lamivudine post baseline genotype (ranging from 4 to 30 Weeks on therapy).
- Mutations at codon S68 were detected in virus from 14% of subjects in whom K65R had also been selected. Sequential population genotype results indicated S68 mutations were almost always selected after K65R selection. This suggests that enrichment for mutations at 68 follows the initial selection for K65R and M184V, and that there may be a compensatory role for S68 mutations in the presence of K65R. The full phenotypic implications of S68 in conjunction with K65R are not known, as this mutation was detected as an emerging mixture prior to the therapy arm being discontinued. Phenotypic analysis of site directed mutants or of clones will be needed to fully address the implications of treatment emergent variants at S68.
- When the Y115F was also selected in conjunction with K65R and S68G/N, reduced susceptibility to all study drugs (TDF, ABC and 3TC) was observed.

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