

Prevalence of Low-level Variants with RT K65R Among Different HIV Subtypes and the Effects of ARV-exposure on Variant Levels

Michael J Kozal¹, Jen Chiarella¹, Elizabeth P St. John², Lisbeth A Blake²,
Birgitte B Simen², Michael Egholm², and Max Lataillade^{1,3}

¹Yale University School of Medicine & VA CT Healthcare System, New Haven, CT, United States. ²454 Life Sciences – A Roche Company, Branford, CT, United States. ³Bristol-Myers Squibb, Research and Development, Wallingford, CT, United States.

Background

- Studies have shown an increased frequency of K65R in subtype C ARV failures (noted with 2NRTIs & WHO first line regimens)¹⁻⁴⁺
- CROI 2010: several researchers reported increased rates of K65R in subtype C and investigated possible mechanisms⁵⁻⁸ contributing to K65R detection in subtype C
- Codon 64-65-66 region appeared to be a “hot spot”⁵ for mutations with.....
 - template dependent mutagenesis⁷
 - decreased transcription RT fidelity⁸
 - frame shifts⁵
 - polymerization assay & pyrosequencing errors⁶all possibly contributing to the ↑ detection of K65R in subtype C.

Background

- A common theme of clinical questions/issues were raised by these authors⁵⁻⁸ :
 - The clinical significance of low-level K65R variants in subtype C requires better clinical data with longitudinal sampling to assess the persistence and impact of these variants on ARV therapy outcomes.

Background

Given our large repository of UDS sequences we set out to determine the prevalence of K65R in different subtypes, evaluating level of detection by UDS, and the effects of ARV selection pressure on these variants.

- Is K65R detection dependent upon subtype, assay, or both?
- Are low-level K65R variants clinically significant?

Objective

To determine the prevalence of low-level K65R variants within different HIV subtypes infecting ARV-naïve subjects and the effects of ARV-exposure on K65R variant levels.

Results: Prevalence of RT K65R variants among different HIV subtypes

- 411 ARV-naïve subjects were evaluated by UDS for K65R to 1% levels. 4 subjects (0.97%) had K65R variants at $\geq 1\%$ or had a very high mutation load (ML):

4 subjects	subtype C	K65R at 3.11% (ML 23,325) & 0.69% (5,175) ^{#*}
	subtype C	K65R at 1.22% (ML 684) [#] & 0.49% (ML 275)
	subtype B	K65R at 0.9% (ML 15,481) *
	subtype BF	K65R at 7.85% (ML 693) [#] & 0.31% (ML 27)

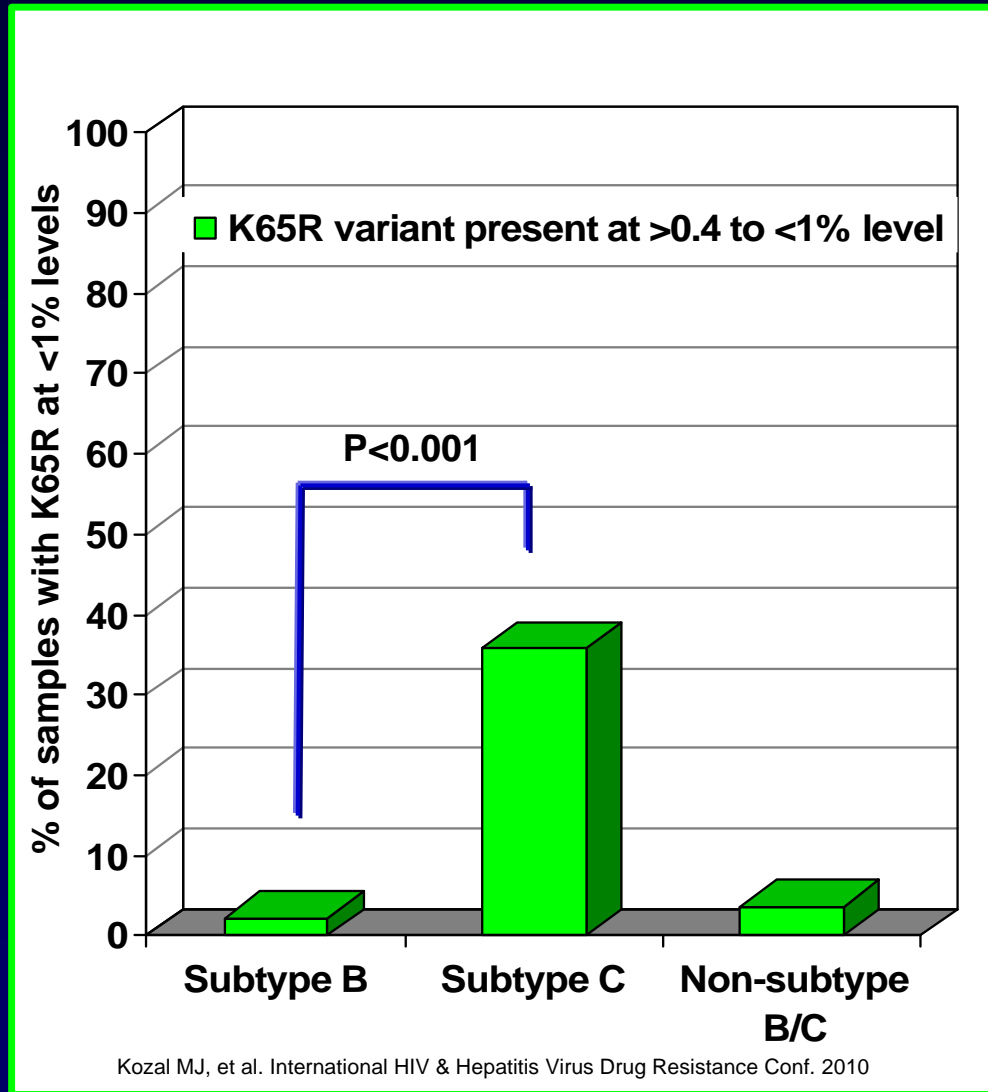
- All 4 subjects had variants with linked Transmitted Drug Resistance (TDR) mutations (e.g. multiple linked NRTI, NNRTI, or PI mutations suggesting TDR variants)

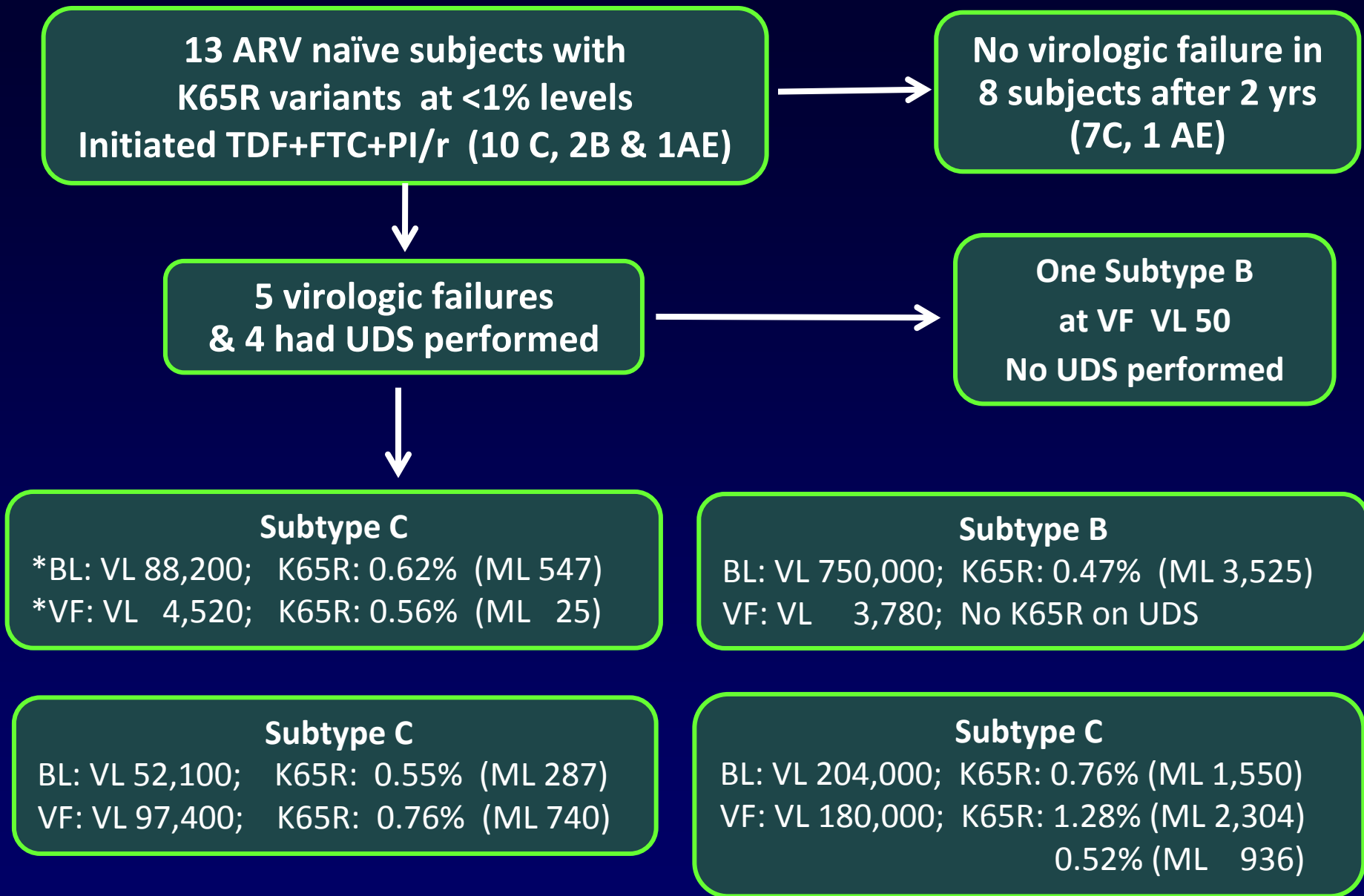
Results: Samples sequenced to 0.4% to <1%

- 147 ARV-naïve subjects were sequenced to 0.4% to <1%
 - Average VL: 230,465 c/mL (IQR 42,300 – 378,500)
- 13 of 147 (8.8%) had K65R low-level variants identified
 - Mean VL: 212,215 c/mL (IQR 52,100 to 341,000)
 - Average # of reads through K65R: 1,563 (IQR 1,032– 1,911)
 - Average % of reads with K65R: 0.58% (range 0.41 – 0.76%)
 - Average mutational load: 1,299 (range 147 – 4,510)

Prevalence of low-level HIV variants with K65R mutation among different HIV subtypes

- 147 ARV-naïve subjects samples were sequenced to 0.4% to 1%
- 8.8% (13/147) had RT K65R low-level variants identified
- 2.2% (2/92) in subtype B, 35.7% (10/28) in subtype C ($p < 0.001$ for B vs C), and 3.7% (1/27) in non-B/C subtypes (BF, A, AE & F1)





* Baseline (BL) and Virologic Failure (VF) HIV viral load c/mL (VL); Mutational load (ML)

Conclusions

- Low-level RT K65R variants were more frequently identified in subtype C.
- K65R variants at >1% levels likely represent transmitted resistant variants.
- The implication of low-level K65R variants below 1% in ARV-naïve subjects who receive TDF/FTC+PI/r remains to be determined as the majority are very low-level and did not increase after ARV exposure.
- The lack of K65R enhancement suggests that other active components of the regimen may be able to prevent selection and/or alternatively their identification may be a result of viral or assay polymerization errors especially in subtype C.

Specific Issues and Future Study

- Clinically relevant K65R levels for specific ARV regimens require individual study. The impact of low level resistant variants on therapy is a multi-factorial process with ML, linkage, and the genetic barrier of the regimen all contributing.
- Levels below 1% did not seem to enhance after exposure to TDF+FTC+PI/r, however, this may not be the same for other ARV regimens with lower genetic barrier or poor adherence.
- PI/r-based therapy and specific regimens have been reported to protect against the development of K65R and VF (lower rates than with older NRTIs and NNRTIs regimens)¹³⁻¹⁶.
- K65R at <0.4% identified in additional samples from all subtypes (data not shown). No K65R enhancement seen at VF by UDS with these variants. Levels <0.4% are likely heavily affected by assay error.

Issues and Limitations

- UDPS important limitations¹²: RNA extraction & cDNA synthesis limits, assay polymerization & pyrosequencing errors - likely contributing.
- Issues with typical pyrosequencing error is over or under call of one of the two A-stretches (homopolymeric regions);
- typical UDPS error occur in one direction (forward or reverse) or look different in the two directions; if found in both directions, errors more likely due to PCR error or alignment. For K65R in subtype C, most confidence in AGG codon change, and reads seen in both directions if above PCR error rate (Simen BB, personal communication).

Acknowledgements

- **FIRST Team**
- **CASTLE Team**
- **454 Life Sciences – a Roche Co.**
- **Funding: NIH, VA, & Bristol Myers Squibb**

Methods

- 433 total subjects from multiple studies⁹⁻¹¹; 411 ARV naïve subjects with longitudinal data. Virologic failure (VF) specimens from 60 subjects.
- UDS was performed as described in previous studies⁹⁻¹¹. Samples were further evaluated for low-level variants to 0.4% depending upon HIV viral load (VL).
- UDS results are analyzed by the depth of sequencing and by VL. Estimated mutational load (ML) was calculated by percent (%) of variant detected x sample VL.
- VF specimens were evaluated by UDS; given stochastic effects of RNA sampling, for VF samples with VL<10,000 c/mL, K65R mutation levels reported represent the proportion of PCR amplicons containing the mutation.
- Please note the data is slightly different than the submitted abstract as the wrong abstract was uploaded. The new data does not change any findings in the study.