



Allele-Specific PCR Shows Low-Level K65R in Treatment-Experienced Patients with L74V in the Absence of TAMs

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Introduction

- Prior abacavir or ddI therapy can result in development of either L74V/I or K65R mutations in RT
- L74V shows full *in vitro* susceptibility to tenofovir; however, patients with L74V have shown significantly reduced response to tenofovir DF therapy¹
- Prior abacavir therapy also predicts poor treatment response to tenofovir DF²

¹Miller et al., JID, 2004; Masquelier et al., Antiviral Therapy, 2004

²Staszewski et al., 2nd European Resistance Workshop, 2004

Introduction (cont'd)

- Previous analyses by single-genome sequencing identified two patients in study 907 with L74V/I by population sequencing and low-level K65R at baseline who had poor treatment response and full development of K65R¹

¹Bae et al., XIII Int'l HIV Drug Resistance Workshop, 2004

Objective

- Assess the frequency of low-level K65R in the plasma HIV-1 of treatment-experienced and treatment-naïve patients and correlate with response to TDF treatment

Methods

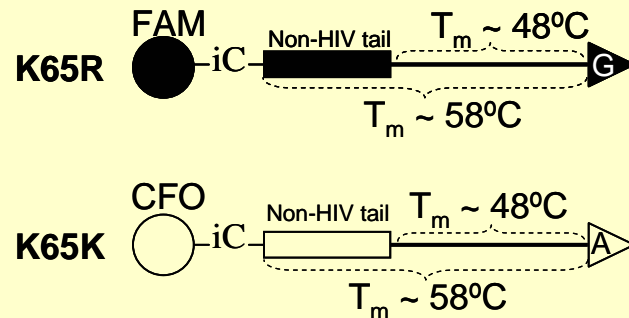
- Study 907 was a randomized, placebo-controlled study of tenofovir DF
 - > 8 weeks prior stable background ART to which tenofovir DF or placebo was added
 - HIV RNA > 400 and < 10,000 copies/mL
 - Mean 5.4 years prior ART

Methods (cont'd)

- An allele-specific PCR (AS-PCR) assay was developed to detect the K65R mutant using the MultiCode RTx real-time PCR platform (EraGen Biosciences, Madison, WI)
 - For viral loads $< 100,000$ copies/mL, plasma HIV-1 RNA was pre-amplified by standard procedures
 - Viral standard curve stocks were obtained by electroporation of NL4-3 plasmids into MT2 cells

MultiCode RTx Assay Description

Allele-specific primers



K65R FAM-GACATGAGTATTTGCCATAAAGAG

K65K CFO-ACAGGTAGTATTTGCCATAAAGAA

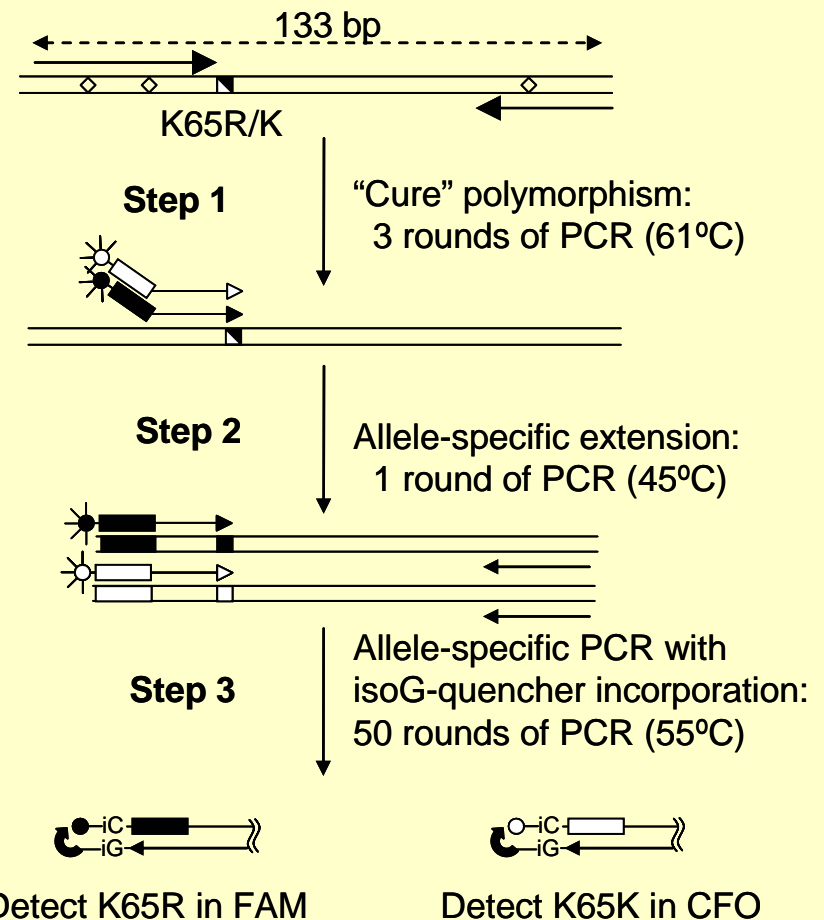
Curative primer:

GAAAATCCATACAATACTCCAGTATTTGCCATAAAGA

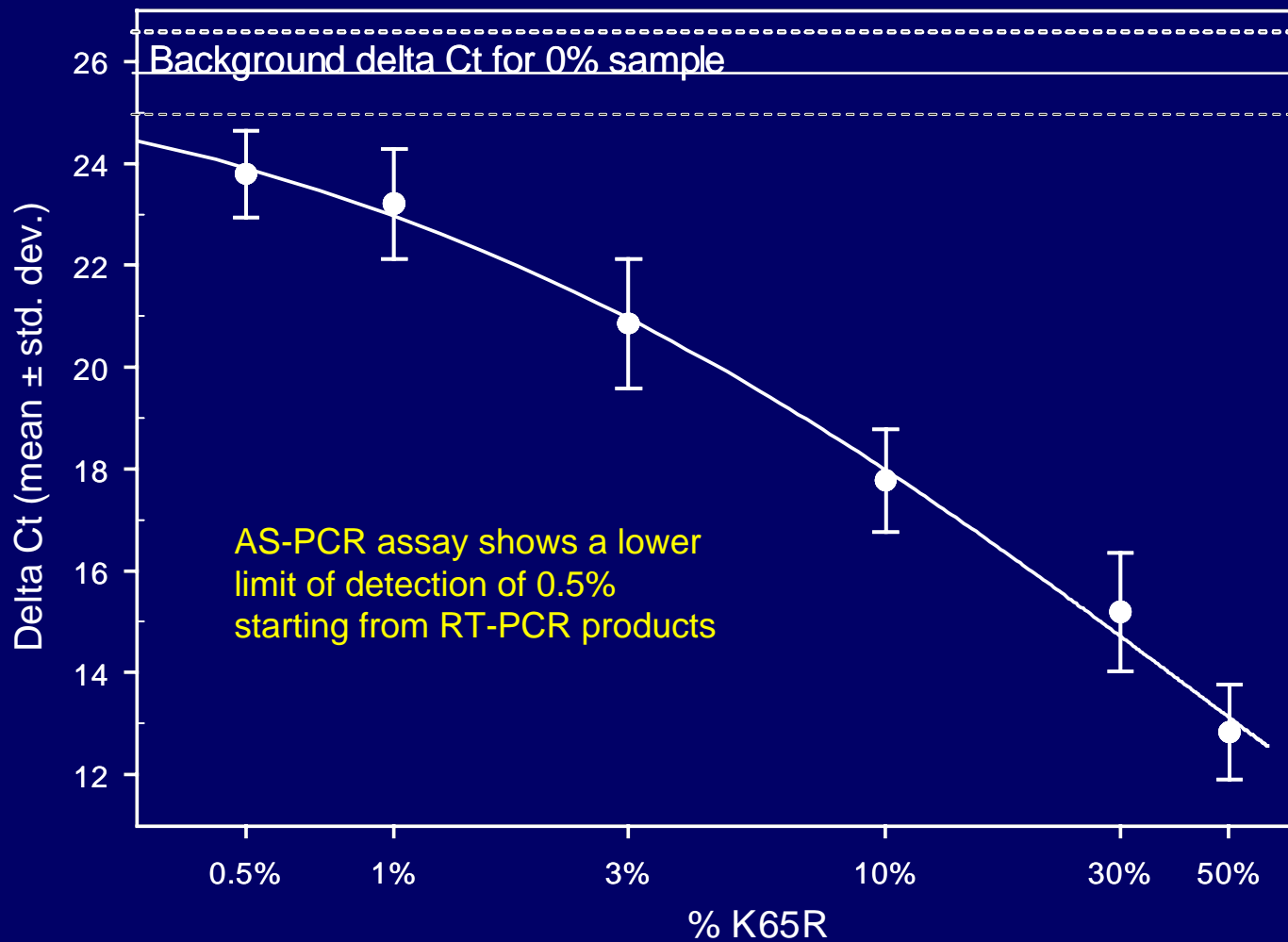
Sample preparation

- RNA extraction (QiaGen)
- RT-PCR of 1.8 kb of PR-RT
- MultiCode RTx assay

MultiCode RTx assay



MultiCode RTx Assay Standard Curve



Study 907 Baseline Samples Analyzed

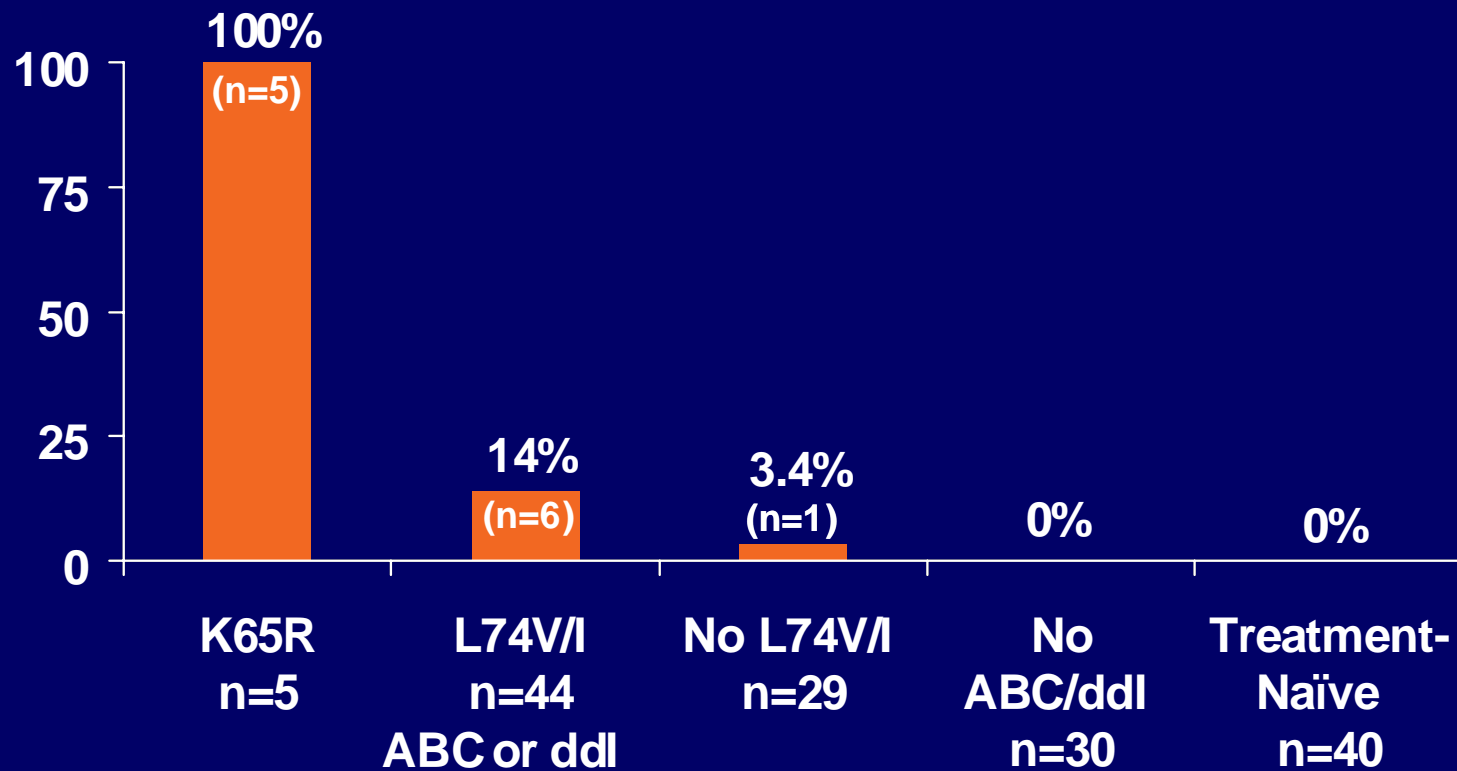
	Total (n)	With TAMs (% Total)
Taking ABC or ddl		
All L74V (8% of study)	44	33 (75%)
L74 wt / K65 wt ^a	29	23 (79%)
K65R (by pop. sequencing)	5	1 (20%)
No ABC or ddl		
L74 wt / K65 wt ^a	30	19 (63%)
Total	108	76 (70%)

a. Randomly selected control population

Results

- Samples containing an AAA AAG sequence (vs. AAG AAA) at codons 64 and 65 resulted in higher background amplification and redesign of K65R specific-primers
 - 4/108 samples analyzed had this natural polymorphism in subtype B
 - AAA AAG is consensus in subtype C

Detection of Low-Level K65R by AS-PCR



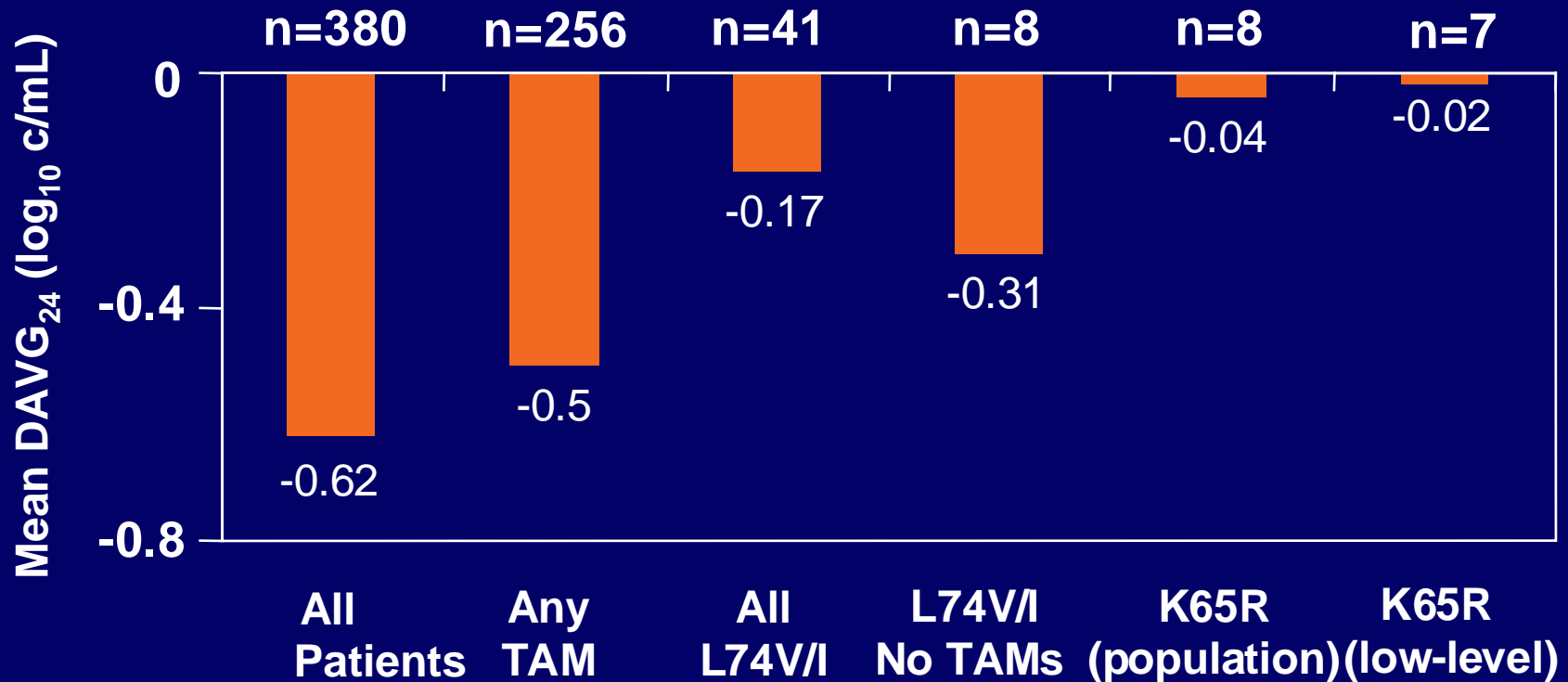
- Range of low-level detection: 1.2 to 25% K65R (n=7)

Statistical Analysis of Baseline K65R

	Number of Patients with K65R vs. Total		P-Value ^a
	Yes	No	
Presence of L74V/I	6 / 44	1 / 59	0.041
Presence of TAMs	2 / 76	5 / 27	0.013
+ Full K65R patients	3 / 77	9 / 31	< 0.001
ABC or ddl Use	7 / 73	0 / 30	0.11
+ Full K65R patients	12 / 78	0 / 30	0.035

a. Fisher's Exact Test

Treatment Response to TDF at Week 24 (combined 902 and 907 studies)



Resistance Development in Patients with Low-Level K65R

Patient #	Baseline NRTI Genotype	Baseline % K65R	Week 24 TDF Response (log ₁₀ c/mL)	Absolute Week 48 Response (log ₁₀ c/mL)	Treatment Regimen	Resistance Development (pop. seq.)
1278	L74V	11.1%	-0.13	+0.44	ddl, EFV, SQV/RTV	Full K65R
2076	L74V, V118I, M184V	1.2%	-0.22	-0.01	3TC, ABC, APV	Full K65R, Y115F
2264	M41L, D67N, L210W, T215Y	2%	-0.02	-0.41	ABC, d4T, EFV	K219K/R
2656	L74V, M184V	25%	+0.80	-0.94	ABC, d4T, ddl, EFV+ Kaletra	< 50 c/mL
3030	L74V, M184V	2.4%	-0.48	-0.01	ddl	M41L
3034	L74V	6.8%	-0.27	-0.21	ddl, EFV	Assay Failure
5288	D67N, K70R, L74I, T215F	2.8%	+0.15	-1.75	ABC, d4T, EFV+ Kaletra	V118I/V

Baseline Clonal Analysis Results

- Patient 2264 with low-level K65R (2%) and TAMs showed K65R exclusively on same genome with M41L, D67N, L210W, and T215Y (n=4/84)
- Patient 2656 with the highest % K65R and full L74V by population sequencing showed independent genomes of K65R and L74V (n=89)

HIV RNA Response to TDF

Final Multivariate Linear Regression Model

Parameter	Parameter Estimate (log ₁₀)	P-Value
TDF treatment	-0.66	< 0.0001
BL HIV RNA	-0.20 / log increase	< 0.0001
BL CD4	-0.03 / 100 cell increase	0.0006
4 TAMs (M41L, D67N, L210W, T215Y)	+0.14 / mutation	< 0.0001
K65R	+0.61	0.0003
L74V	+0.29	< 0.0001
M184V	-0.13	0.0026

- Multivariate statistical analysis confirms K65R, L74V, and multiple TAMs as independent predictors of reduced TDF response

Conclusions

- **No K65R was detected among >40 treatment-naïve patients by an AS-PCR technique with a lower cut-off of 0.5%**
 - **Strong “A” rich region limits sensitivity of assay at K65**
- **Natural polymorphism at codon 65 (AAG) can interfere with AS-PCR for K65R**
 - **AAG is predominant sequence for subtype C**

Conclusions (cont'd)

- **Low-level K65R (1.2% - 25%) detected in 7 of 73 patients (9.6%) pre-treated with ddl or ABC**
 - **Positively associated with L74V and negatively associated with TAMs**
 - **No detection among NRTI-experienced patients not taking ddl or ABC**
 - **Reduced treatment responses to TDF with 2 patients developing full K65R**
- **Along with baseline K65R and multiple TAMs, L74V is an independent predictor of reduced response to TDF therapy in heavily treatment-experienced patients**

Acknowledgements

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