Tenofovir (TDF)- or Abacavir (ABC)-selected Minority Subpopulations in Viremic Subjects Detected by Ultra-deep Sequencing

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Disclosures

- Study funded by GlaxoSmithKline
- R. D'Aquila relationships
  - Boehringer Ingelheim, Bristol Myers Squibb. Gilead, GlaxoSmithKline, Merck, Monogram, Tibotec, Virco
- E. Rouse, J. Horton, K. Oie, K. Pappa, L.L. Ross are each employed by GlaxoSmithKline

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Background

- RT point mutations can concurrently reduce NRTI susceptibility and restrict HIV replication *in vitro*.
  - HIV quasi-species can harbor minority variants
- TDF and ABC are associated with selection of K65R and L74V.
- Using ultra-deep sequencing, we tested the hypothesis that minor sub-populations with these mutations are often currently missed by population GT during viremia.

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Methods

- Plasma-derived HIV was obtained from 16 viremic subjects who had received ABC, TFV or ZDV/3TC (control) based ART.
- Clones encompassing RT amino acids 56-120 were analyzed by high-throughput sequencing at ≥1 timepoint (454 Life Sciences).
- The percentages above calculated error rates* are reported (to exclude mutations produced by in vitro errors; probability <0.001)

***as per Wang C, Mitsuya Y, Gharizadeh B, Ronaghi M, Shafer RW, 2007, Genome Res.**

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Study Population

- Inclusion criteria:
  - Specimens archived (Vanderbilt/CCC and GSK) during failure of regimens with TDF or ABC;
  - Response to therapy with undetectable viremia;
  - Subsequent loss of virologic control (HIV-1 RNA levels above the limit of detection);
  - Subjects could have received a protease inhibitor or non-nucleoside inhibitor as part of their antiretroviral regimen along with TFV or ABC.
- Exclusion criteria:
  - >2 regimen failures

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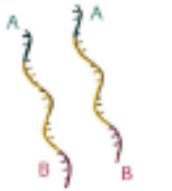
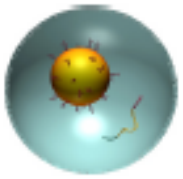
Key Concept

454 Sequencing from individual DNA molecules



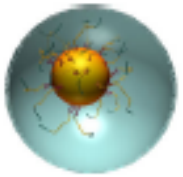
Library of single stranded DNA molecules

↓



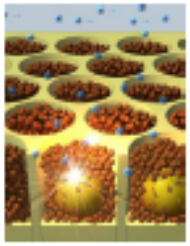
One DNA molecule per bead

↓



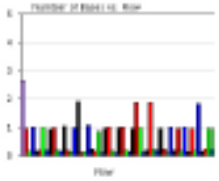
Clonal amplification of that single molecule to ~10 million copies

↓



Independent sequencing of each bead

↓



One Bead = One Read = One DNA molecule

RESULTS

- A median of 39,508 clones were analyzed per timepoint.
  - 9/16 subjects were on first line ART at failure.

- HIV-RNA was ≥3.8 $\log_{10}$ copies/mL for all timepoints analyzed

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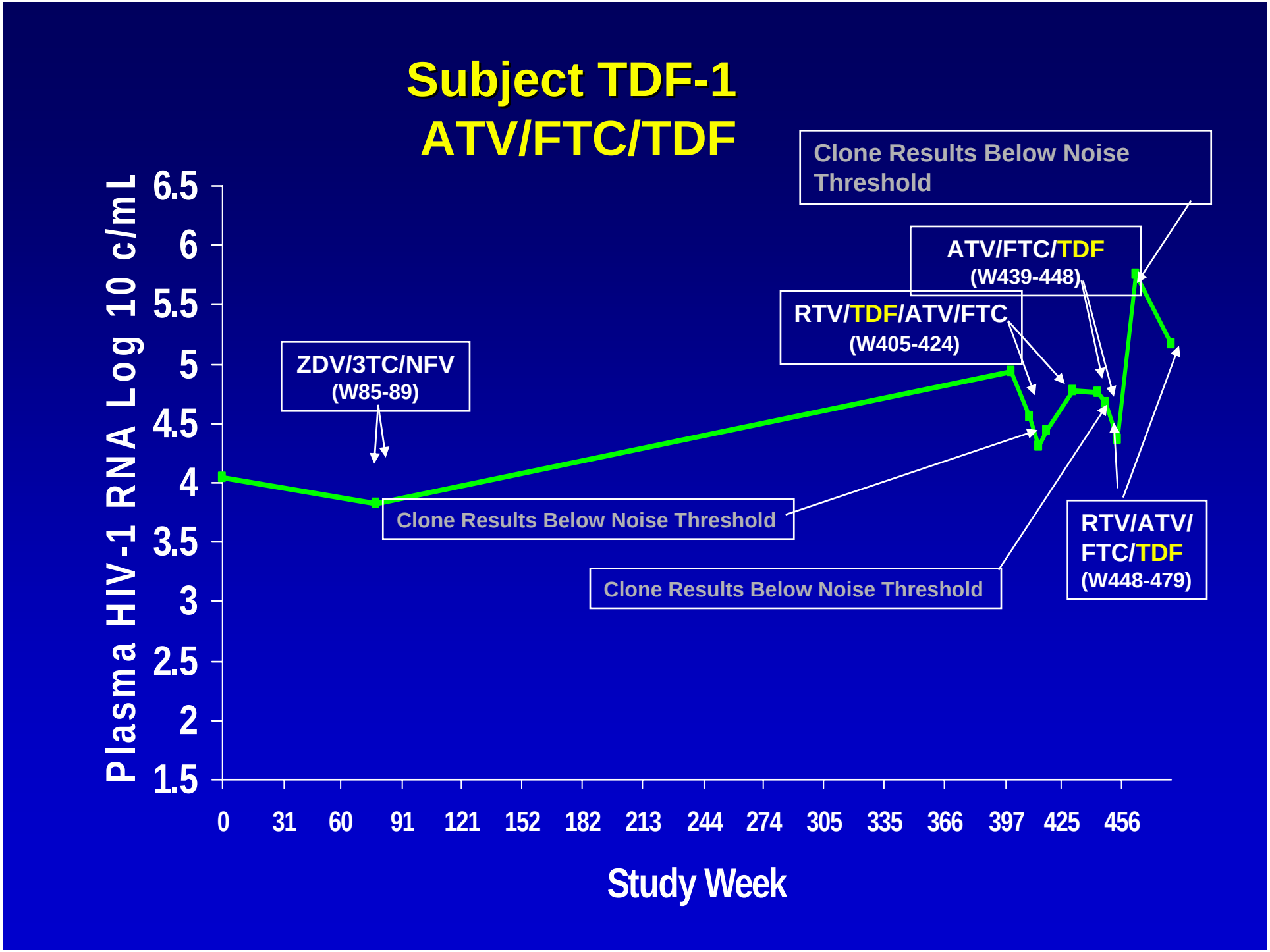
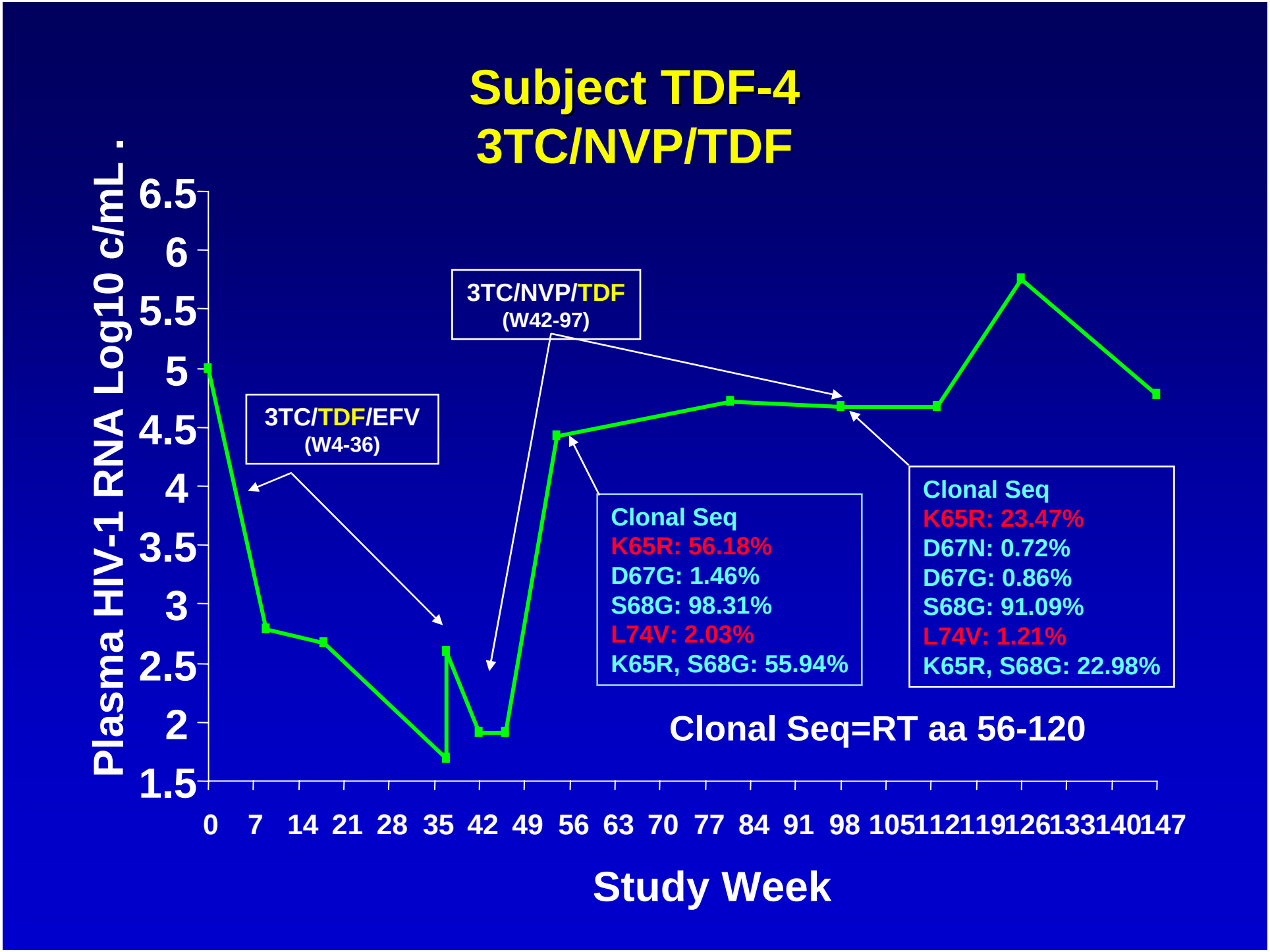
Clonal Analysis for Subjects Receiving a TDF-Based Regimen

Subj. ID	A62V	K65R	D67G	D67N	S68G	K70R	L74V	K103N	Y115F
TDF 1-3	**No mutations detected**								
TDF 4		**56.18%**	1.46%		98.31%		**2.03%**		
TDF 5	0.76%	**95.54%**	18.54%		0.42%				6.16%
TDF 6		**98.05%**		3.22%					

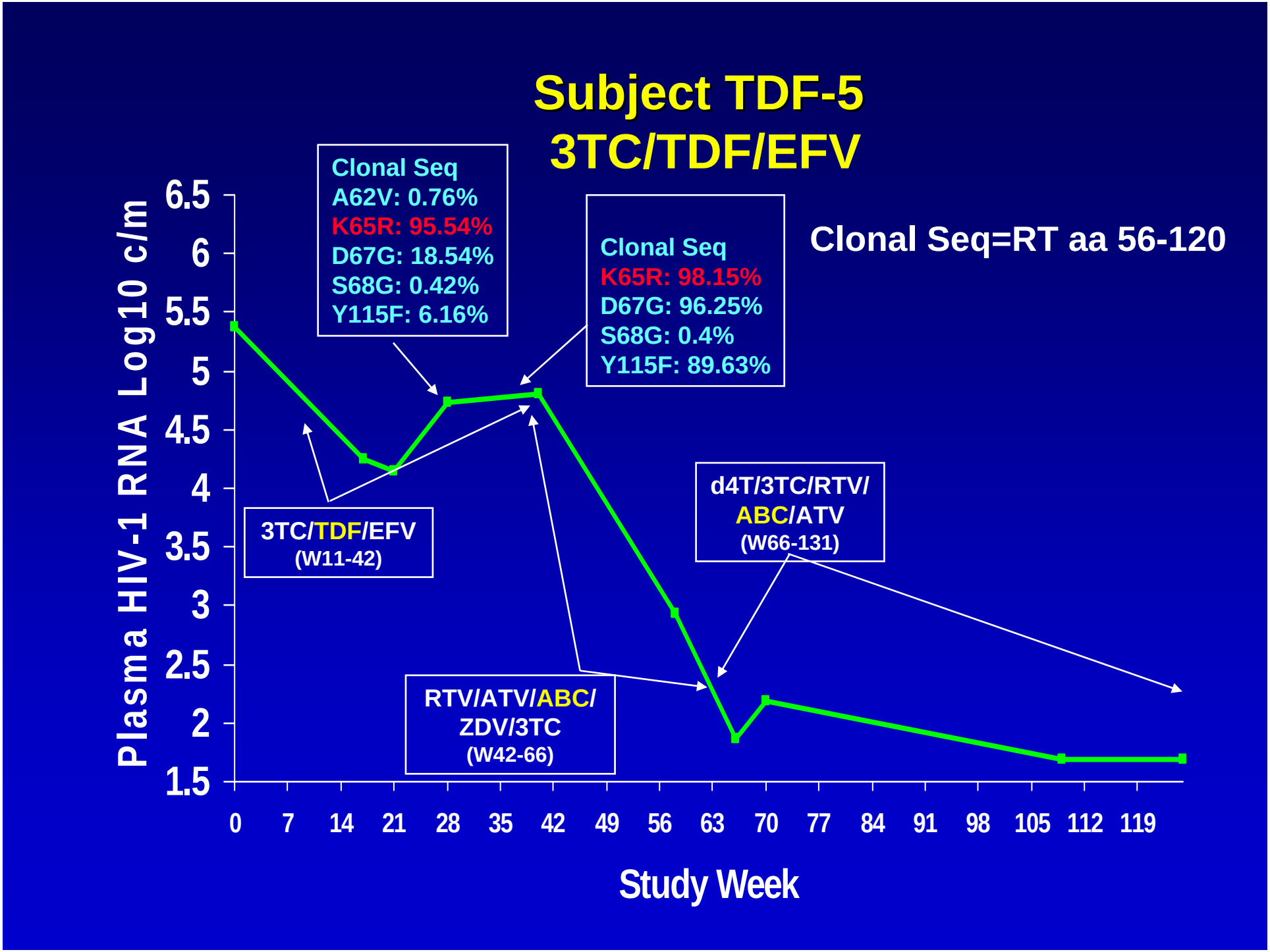
Representative Response Profiles for Subjects on TDF-Containing Regimens

Subject TDF-1
ATV/FTC/TDF




Subject TDF-4
3TC/NVP/TDF
Plasma HIV-1 RNA Log10 c/mL .
6.5
6
5.5
5
4.5
4
3.5
3
2.5
2
1.5
3TC/TDF/EFV
(W4-36)
3TC/NVP/TDF
(W42-97)
Clonal Seq
K65R: 56.18%
D67G: 1.46%
S68G: 98.31%
L74V: 2.03%
K65R, S68G: 55.94%
Clonal Seq
K65R: 23.47%
D67N: 0.72%
D67G: 0.86%
S68G: 91.09%
L74V: 1.21%
K65R, S68G: 22.98%
Clonal Seq=RT aa 56-120
0 7 14 21 28 35 42 49 56 63 70 77 84 91 98 105 112 119 126 133 140 147
Study Week

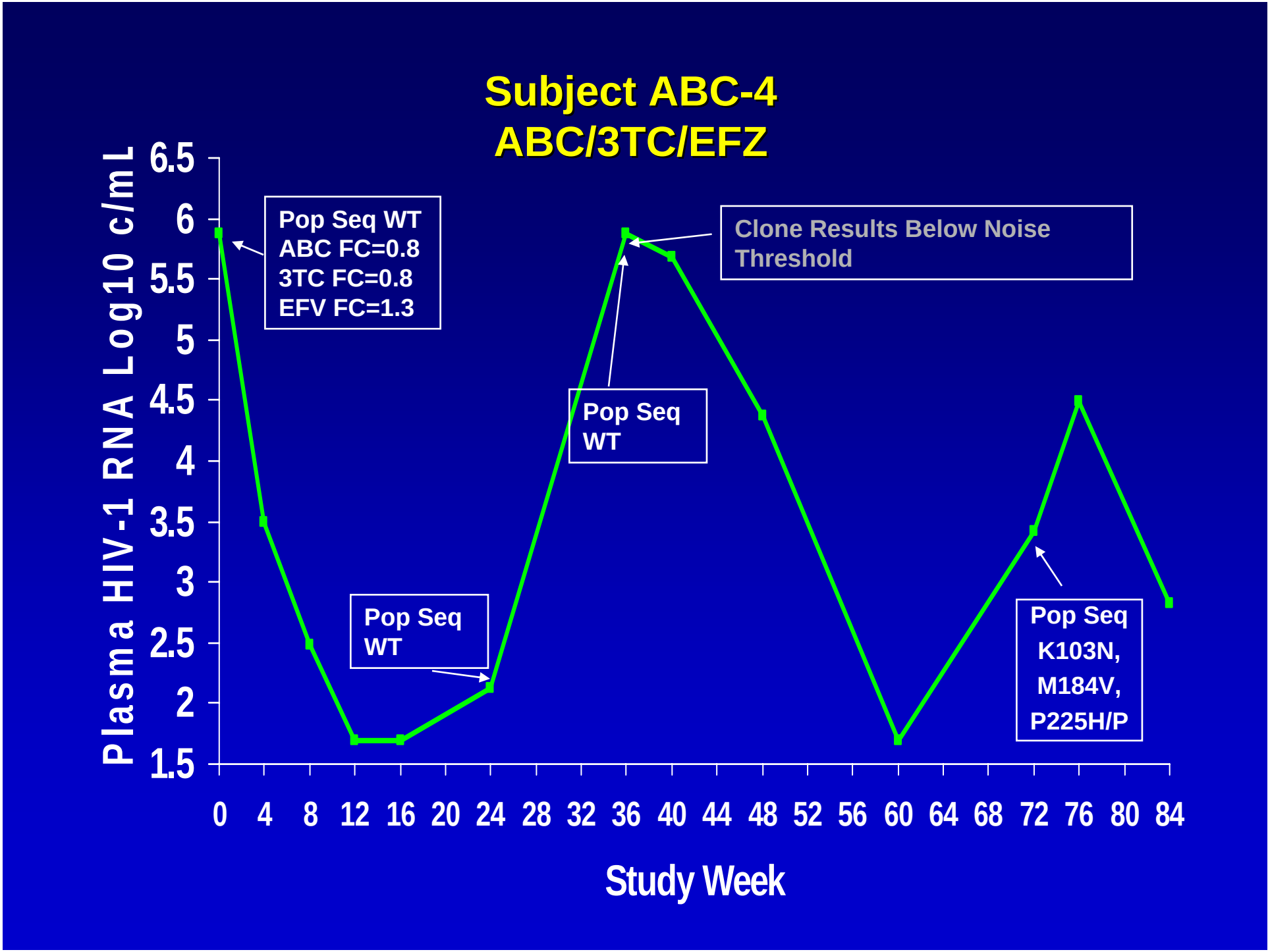
Subject TDF-5
3TC/TDF/EFV



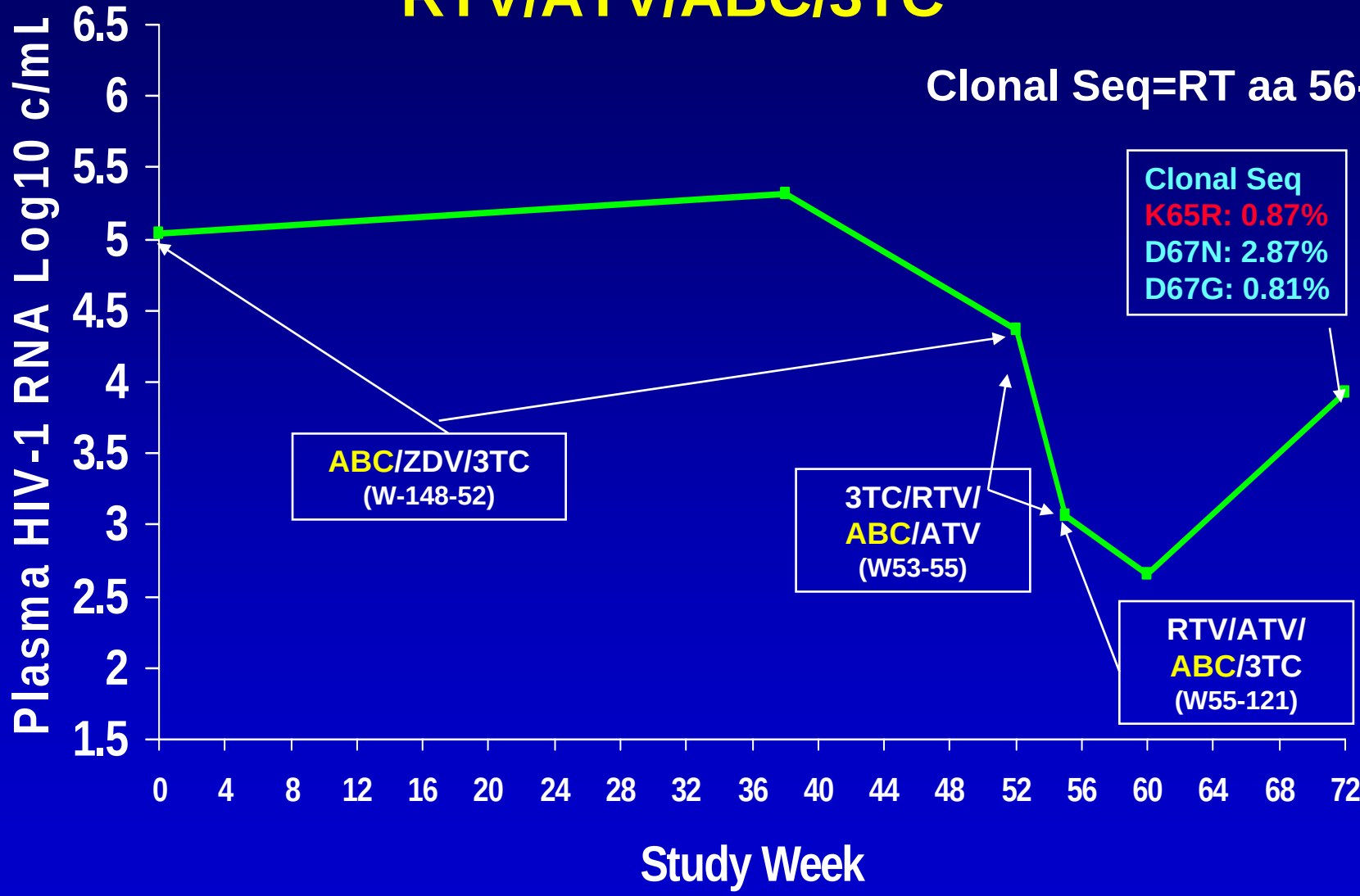
Clonal Analysis for Subjects Receiving an ABC-Based Regimen

Subj. ID	A62V	K65R	D67G	D67N	S68G	K70R	L74V	K103N	Y115F
ABC1-4	**No mutations detected**								
ABC 5			0.45%		0.43%				
ABC 6		**0.87%**	0.81%	2.87%					
ABC 7		**1.73%**	1.59%	6.57%		6.34%			
ABC 8		**1.4%**		97.91%		99.0%			
ABC 9								98.57%	

Representative Response Profiles for Subjects on ABC-Containing Regimens

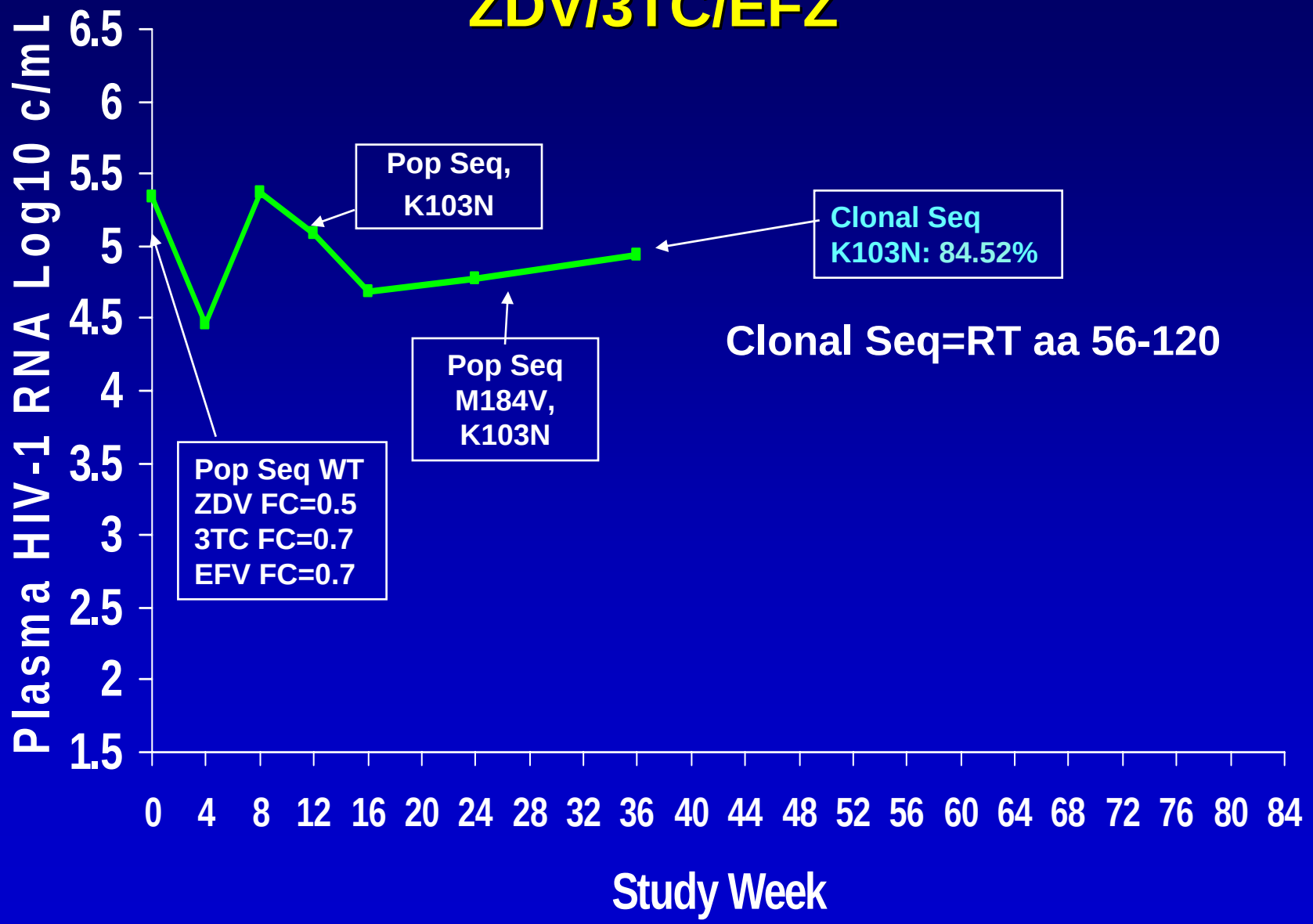

Subject ABC-4
ABC/3TC/EFZ
Plasma HIV-1 RNA Log10 c/mL
6.5
6
5.5
5
4.5
4
3.5
3
2.5
2
1.5
Pop Seq WT
ABC FC=0.8
3TC FC=0.8
EFV FC=1.3
Clone Results Below Noise Threshold
Pop Seq WT
Pop Seq WT
Pop Seq K103N, M184V, P225H/P
0 4 8 12 16 20 24 28 32 36 40 44 48 52 56 60 64 68 72 76 80 84
Study Week

Subject ABC-6
RTV/ATV/ABC/3TC



Representative Response Profile for a Subject not on ABC- or TFV-Containing Regimens

Subject 17
ZDV/3TC/EFZ



Limitation

- No information available about adherence

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Conclusions

- Most viremic subjects (6/9 on ABC, 3/6 on TFV, 1 on ZDV/3TC) had no minority (or majority) variants detected in RT amino acids 56 to 120.
  - Ultra-deep sequencing may not add to management of all
- In some TFV-treated subjects, minority variants with NRTI mutations at 62V, 67G/N, 68G or 74V were detected.
  - One TFV-treated subject with no known prior ABC had a majority 65R with a minority 74V detected at two timepoints.
- In some ABC-treated subjects, minority variants with NRTI mutations at 65R, 67G/N or 70R were detected.
- No K70E detected.

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Additional Background Material

Error Rate Calculation (Subject 17, ZDV/3TC/EFZ)

Estimated error rates for 454 sequencing:	
Reverse transcriptase enzyme	1.00E-03
DNA polymerase	3.40E-05
FLX sequencer	2.00E-03
Estimate of overall error rate	3.03E-03

Assuming this error rate, calculate the probability of observing n or more variants in N reads:

Number of reads (N)	34036
% variants (with K65R)	0.270%
Number of variants (n)	91.8972
Expected number of variants due to errors	9.41E+01
Probability*	0.8961

*** If this probability is small (e.g., less than 0.001) then there are many more variants than we would expect to find due to sequencing and PCR errors. Hence conclude it is an authentic variant. Otherwise, reject.**