What's New in HIV Drug Resistance?

Urvi M. Parikh, PhD & John W. Mellors, MD
MTN Virology Core
University of Pittsburgh

Outline

- What's old in HIV drug resistance?
  - Quick refresher
- What's new?
  - Does prior resistance matter?
  - Do minor resistant variants matter?

Origins of HIV Drug Resistance

- Large, diverse population of HIV variants within a chronically infected individual
  - High viral replication: ~10^{11} virions produced per day
  - sloppy RT: ~3 errors per 100,000 bases copied
  - RT doesn't correct its errors
  - No two genomes are the same!
  - Differ on average by one base out of ~10,000

Billions of mutants produced daily!

- For many ARV, a single nucleotide change results in resistance:
  - TNV (K65R): AAA to AGA
  - FTC (M184V): ATG to GTG
  - EFV (K103N): AAA to AAC
- With 10^{11} genomes produced daily:
  - All possible single mutants produced daily
  - Double mutants probably also exist
  - Triple mutants probably do not
    - » $P = 10^{-12}$ (10^{-4} x 10^{-4} x 10^{-4}) < 10^{11} genomes/day

Lessons Learned from ART

- Resistant variants are rapidly selected by monotherapy with drugs for which 1 mutation confers resistance
- Incomplete suppression of viral replication results in accumulation of multiple mutations, more resistance and broader cross-resistance

Principles of Successful ART

- Cover all pre-existing mutants
  - Single and double drug-resistant mutants
- Suppress new cycles of HIV replication
  - Plasma HIV RNA < 50 copies/ml
- Generally requires 3 potent drugs
  - With non-overlapping resistance mutations

ART MANTRA

No Replication = No Resistance

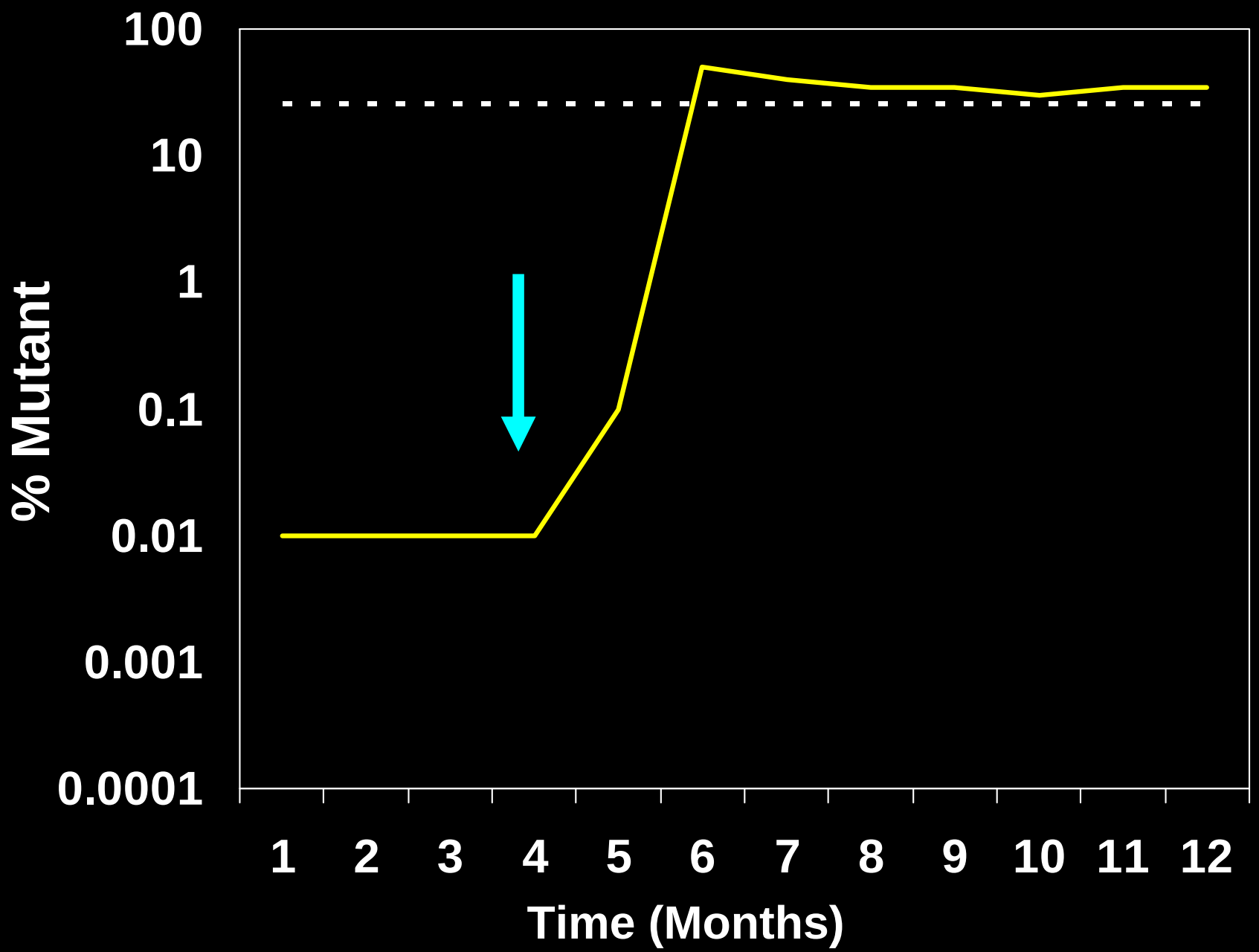
Relevant Issues for PrEP

- Individuals who are put on PrEP with undiagnosed HIV infection will develop resistance
  - Unless PrEP is equivalent to ART (impractical)
- Route of PrEP administration may affect resistance
  - Systemic vs. local
- Individuals who become infected on PrEP will likely develop resistance unless it is stopped promptly
  - Impact of resistance on future response to ART???
    - » Next part of talk!!!!

Two Examples of Impact

- Impact of NNRTI resistance from prior sdNVP on response to initial ART (Lockman et al. CROI 2008)
- Impact of low frequency NNRTI resistant variants on response to multidrug regimens in treatment-experienced patients (Halvas et al. JID in press)

Transient Monotherapy Selects Pre-existing Mutant



Lopinavir/ritonavir (LPV/r) + Tenofovir/Emtricitabine (TDF/FTC) is Superior to Nevirapine (NVP)+TDF/FTC For Women With Prior Exposure to Single-Dose Nevirapine:

AIDS Clinical Trials Group A5208

Optimal
Combination
Therapy
After
Nevirapine
Exposure

ACTG
AIDS CLINICAL TRIALS GROUP

Acknowledgements

Study participants!

Study site staff

Study vice chairs:
Judy Currier
James McIntyre

Study team
John Mellors
Sue Eshleman
Fran Aweeka
Farida Amod
Aida Asmelash
Tom Campbell
Tsungai Chipato
Francesca Conradie
Monica Carten
Elizabeth Dangaiso
Robin DiFrancesco
Betty Dong
James Hakim
Lou Halvas
Scott Hammer
Jane Hitti
Bill Holmes
Mina Hosseinipour
Lynn Kidd-Freeman
Cissy Kityo

Study team, continued
Cecilia Kanyama
Christine Kaseba
Dan Kuritzkes
Chiedza Maponga
Cheryl Marcus
Mary Marovich
Rosie Mngqbisa
Lerato Mohapi
Peter Mugyenyi
Bev Putnam
Michael Saag
Bob Salata
Ian Sanne
Fred Sawe
Chip Schooley
Doug Shaffer
Abraham Siika
Elizabeth Stringer
Heather Watts
Carolyn Wester
Kara Wools-Kaloustian
Peter Ziba
Beth Zwickl

SDAC
Michael Hughes
Evelyn Zheng

ACTG Ops
Evelyn Hogg
Christina Blanchard-Horan
Nikki Gettinger
Yvette Delph
Linda Berman

FSTRF
Ann Walawander
Apsara Nair
Laura Smith
Jimi Tutko

NIAID, DAIDS
Beverly Alston-Smith
Eva Purcelle
Elaine Ferguson
Lynette Purdue
Ana Martinez
Bola Adedeji
Sandy Lehrman
Jeffrey Nadler

Pharmaceutical supporters
Abbott: Sibtain Rahim
Boehringer Ingelheim: Carolyn Connor, Marita McDonough
Gilead: Jim Rooney, Audrey Shaw
Glaxo SmithKline: Navdeep Thoofer, Wendy Snowden
Bristol-Myers Squibb: Awny Farajallah, Kristy Grimm

NATIONAL INSTITUTES OF HEALTH

ACTG AIDS CLINICAL TRIALS GROUP

Background

- Single dose nevirapine (SD NVP) is frequently used to prevent mother to child transmission (MTCT) of HIV-1, where resources are limited
- NVP is also a component of first-line antiretroviral treatment (ART) globally
- NVP-resistant virus is detected in up to 75% of women after SD NVP, but “fades” from plasma over time
- A5208 was designed to study whether prior SD NVP exposure compromises subsequent virologic response to NVP-containing ART

Study Design

Trial 1: 240 women with prior SD NVP (superiority)

- LPV/r + TDF/FTC n=120
- NVP + TDF/FTC n=120

Trial 2: 500 women with NO prior SD NVP (equivalence)

- LPV/r + TDF/FTC n=250
- NVP + TDF/FTC n=250

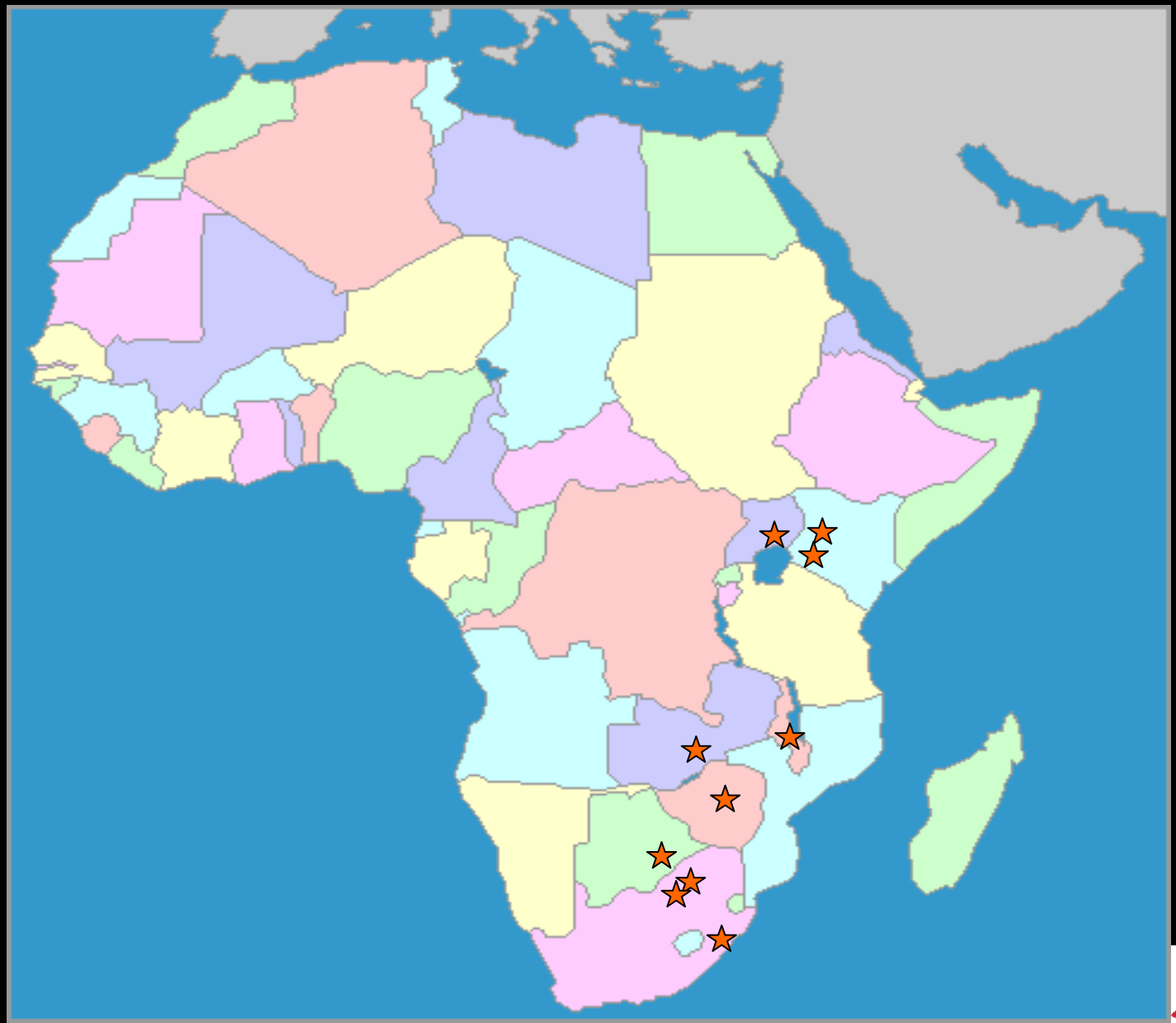
Only Trial 1 results presented today

ACTG AIDS CLINICAL TRIALS GROUP

Selected Eligibility Criteria

- HIV-1-infected women
- CD4 < 200 cells/mm^3 in past 90 days
- No prior ART
- Trial 1: prior SD NVP at least 6 months previously
- Estimated creatinine clearance $\geq$ 60 mL/min

10 Study Sites, 7 Countries in Africa



ACTG
AIDS CLINICAL TRIALS GROUP

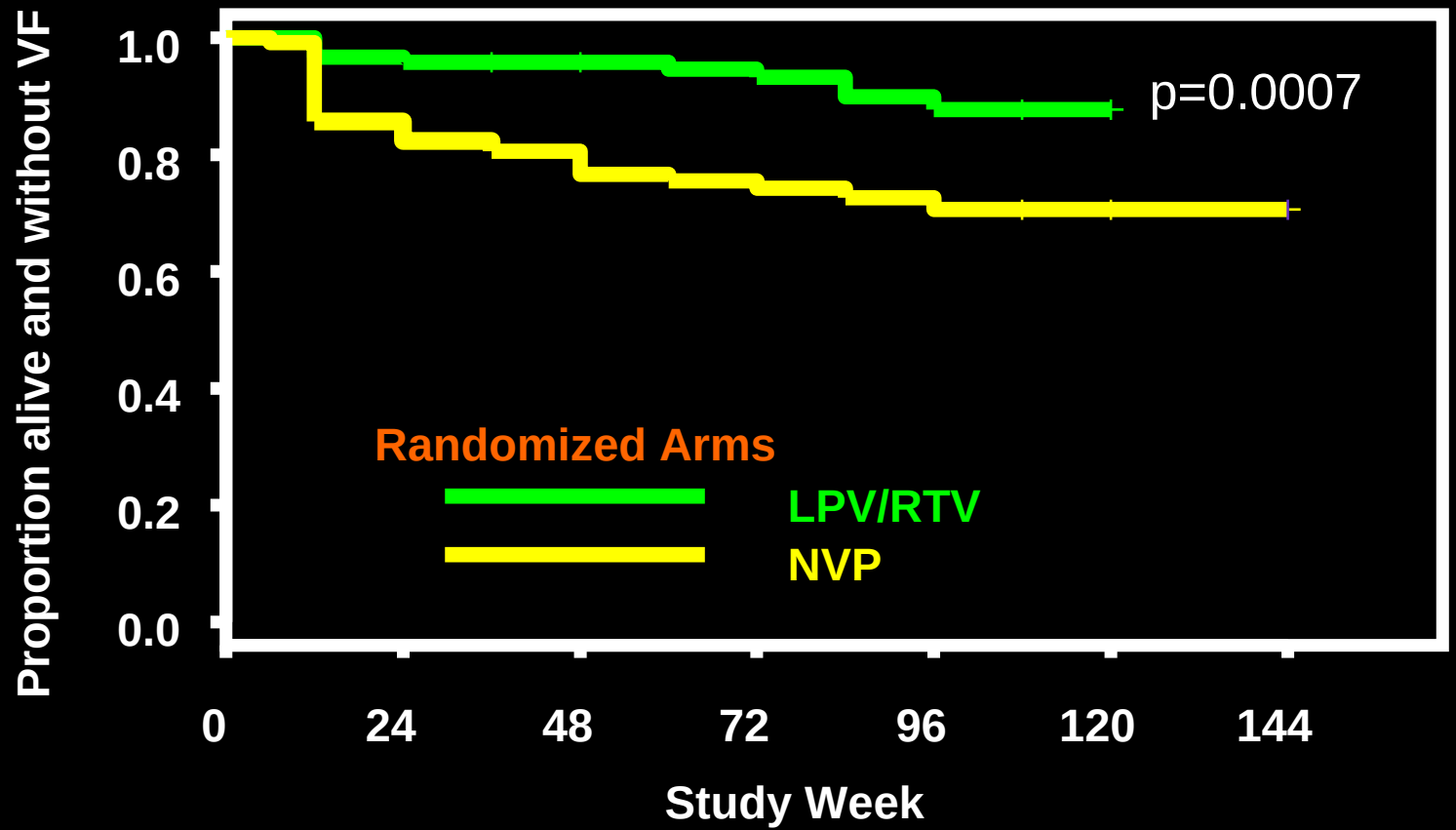
Baseline Characteristics

Characteristic	NVP arm n=121	LPV/r arm n=120	Total n=241
Age (median years)	30	31	31
CD4 (median cells/mm^3)	141	138	139
HIV-1 RNA (median log_{10})	5.20	5.14	5.15
Time from most recent SD NVP (months)	16	17	17
Previous zidovudine exposure	11%	10%	10%
HIV-1 subtype C	73%	72%	72%
Written documentation of SD NVP receipt	71%	75%	73%

ACTG AIDS CLINICAL TRIALS GROUP

KM Plot of Time to Primary Endpoint (Virologic Failure or Death)

- 41 women reached an endpoint:
  - -31 (26%) in NVP and 10 (8%) in LPV/r arms
- Hazard ratio 3.55 (95% CI 1.71, 7.34)



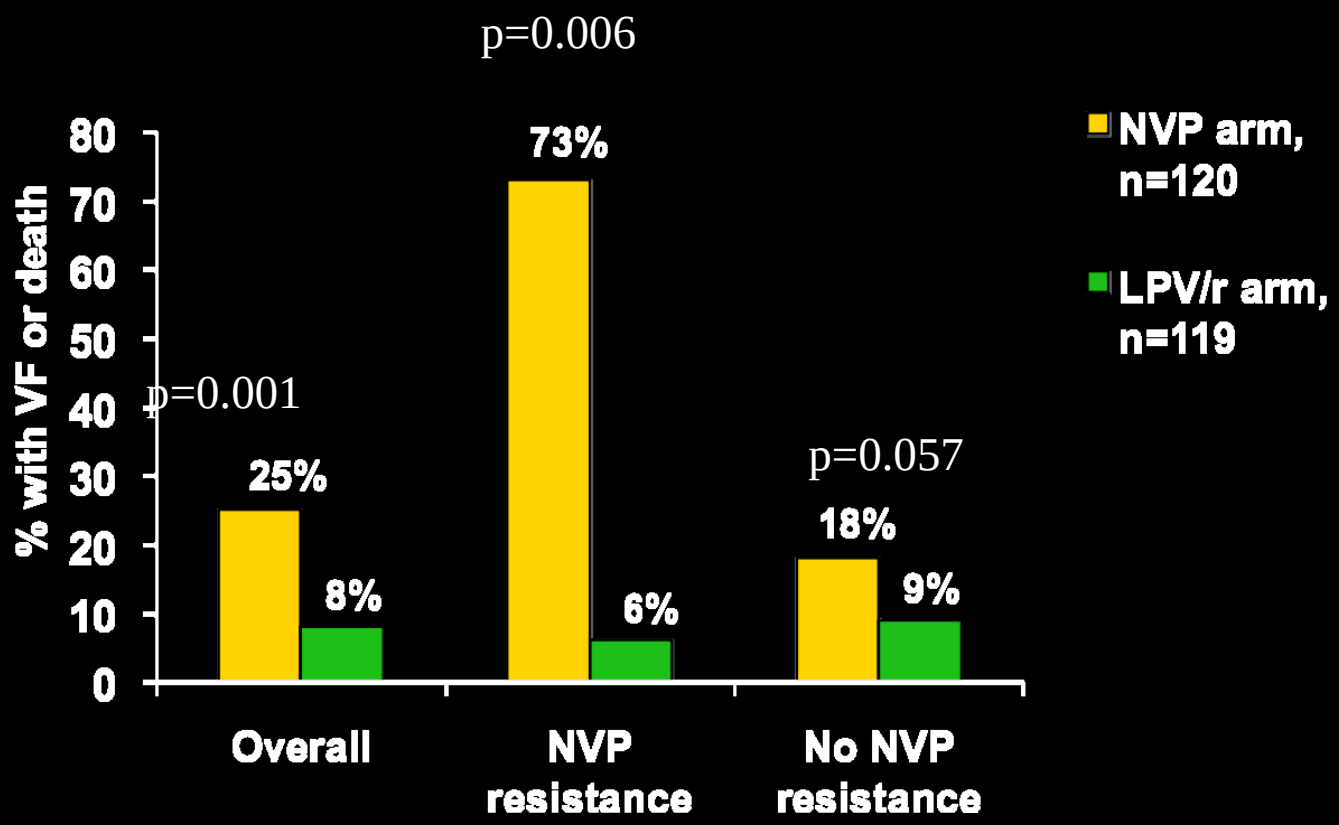
ACTG AIDS CLINICAL TRIALS GROUP

Baseline NVP Resistance

- Pre-planned HIV drug resistance testing (ViroSeq) of baseline samples (run retrospectively)
- Interpreted using modified IAS-USA tables
- Results available for 239 of the 241 participants
  - 33 (14%) had NVP resistance mutations at baseline (K103N in 28, Y181C in 5)
- Median time since last SD NVP exposure:
  - 11 months in 33 women with NVP resistance
  - 17 months in 206 without resistance (p=0.024)

ACTG AIDS CLINICAL TRIALS GROUP

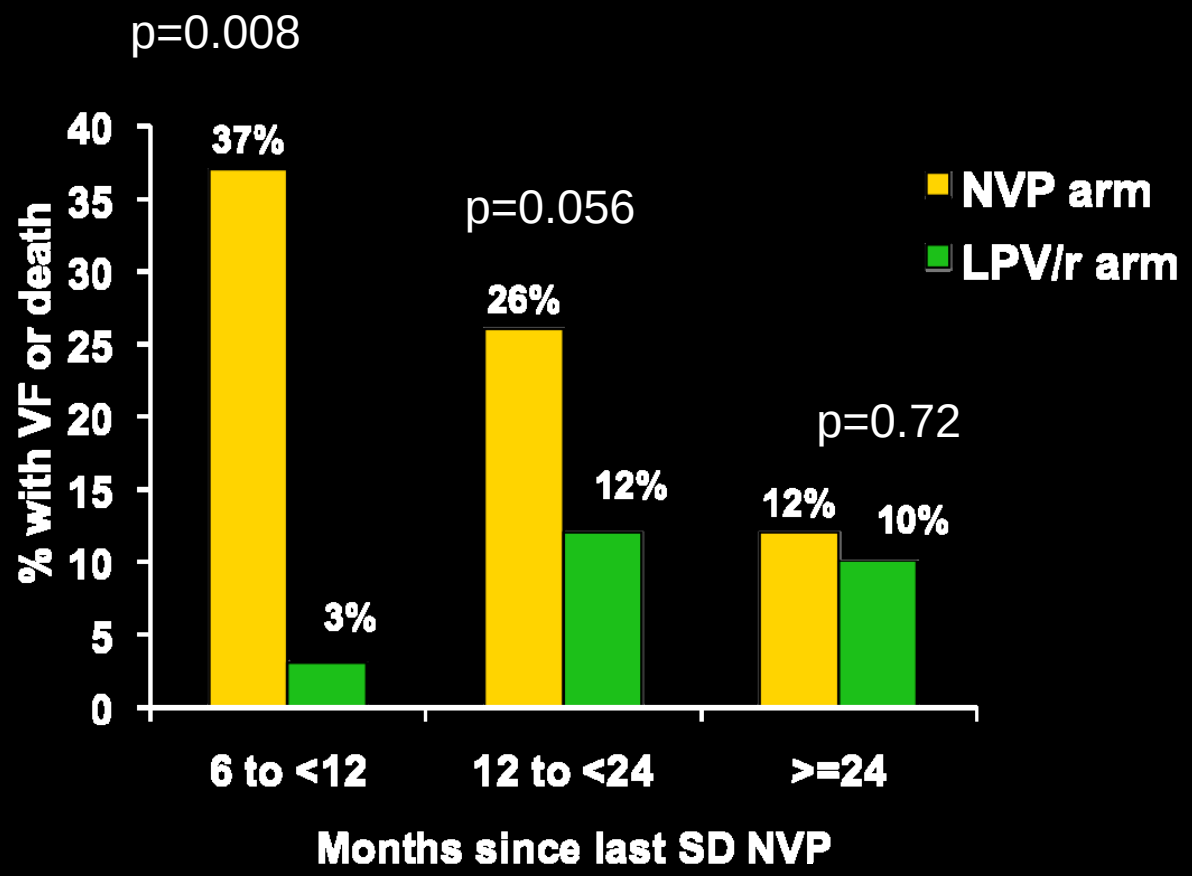
Proportions With Virologic Failure or Death, By Presence of NVP Resistance at Baseline



P value for interaction (of difference between treatment arms and presence/absence of resistance) = 0.040

ACTG AIDS CLINICAL TRIALS GROUP

Proportions With Virologic Failure or Death, By Time Since Last SD NVP Exposure



n = 78 98 65

P value for interaction (of difference between treatment arms and continuous time since last SD NVP) = 0.20

ACTG AIDS CLINICAL TRIALS GROUP

A5208/OCTANE Conclusions

- Treatment with LPV/r+TDF/FTC is superior to treatment with NVP+TDF/FTC among women with prior SD NVP exposure and CD4 < 200 cells/mm^3
- The difference between regimens is greater for women with pre-treatment NVP resistance than for women without resistance
- The difference between treatment regimens appears persists at least 2 years after prior sdNVP
- Detection of low frequency drug-resistant variants is in progress for women with negative standard genotypes

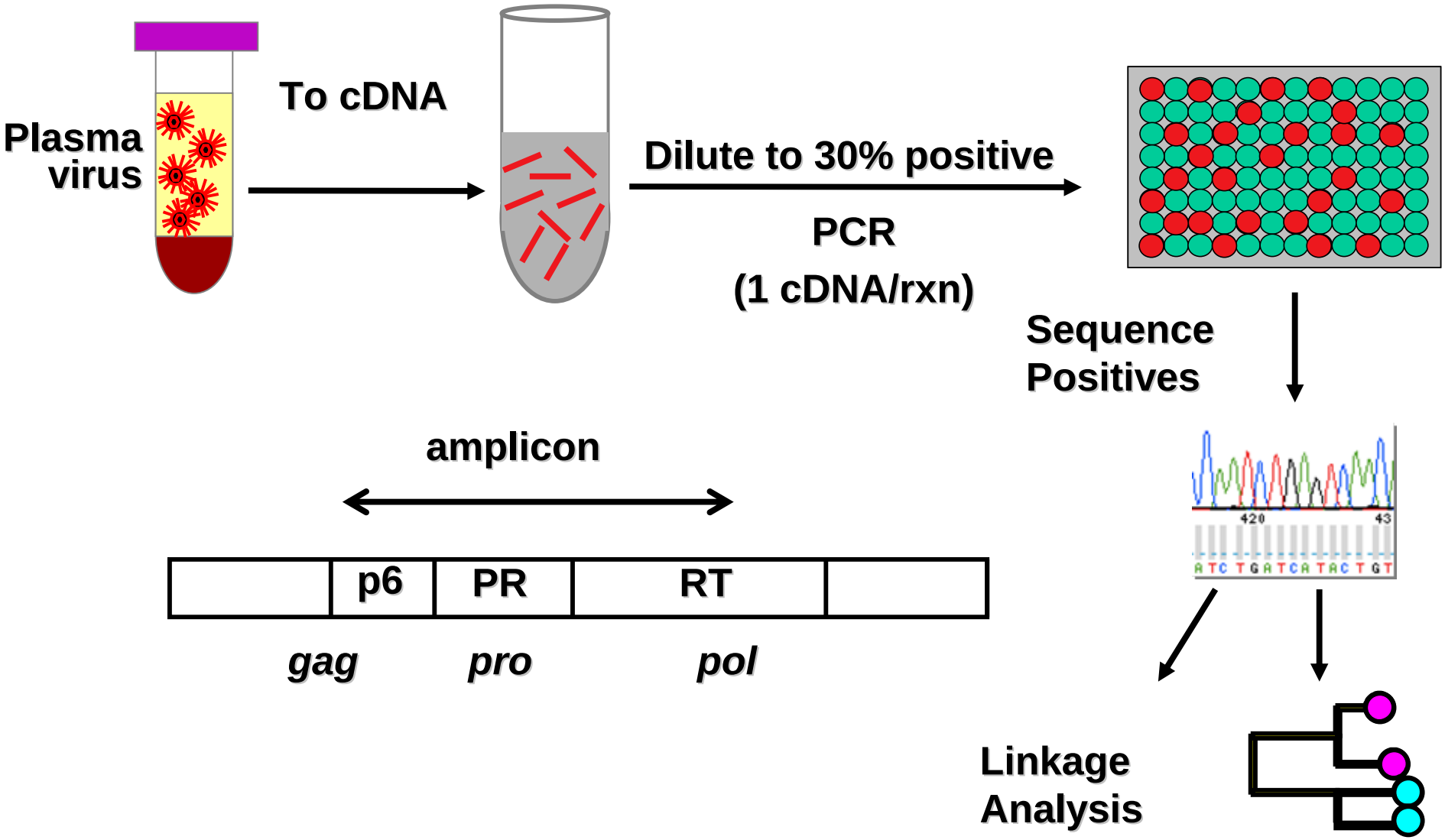
ACTG
AIDS CLINICAL TRIALS GROUP

What are low frequency drug-resistant variants?

- Cannot be detected by standard genotype
- For standard genotype:
  - HIV RNA is extracted, reverse transcribed, PCR amplified, and sequenced as a population and not as individual molecules
    - » Termed "Bulk, population, or composite" genotype analysis
  - Alleles that are present in <25% of the RNA are not reliably detected above background

How can low frequency drug-resistant variants be detected?

Single Genome Sequencing



SGS vs. Standard Genotype in Patients with Suspected MDR (N = 26)

	Standard Genotype	
% Mutant by SGS	Detected	Not Detected*
1-10%	1%	99%
>10% - 35%	25%	75%

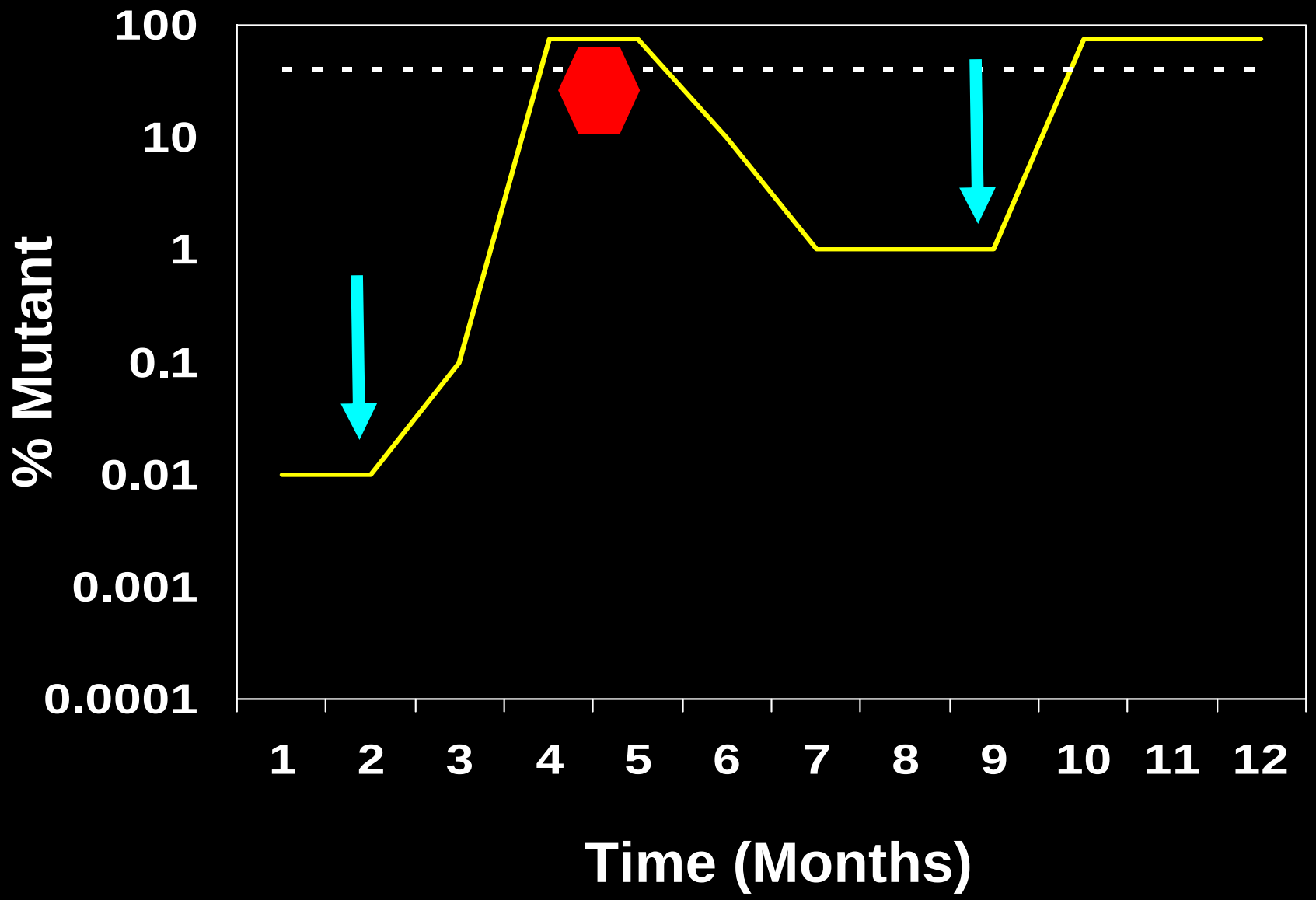
*Including multiple, linked resistance mutations

Palmer et al, J Clin Microbiol 2005; 43:406-413

Two Examples of Impact

- Impact of NNRTI resistance from prior sdNVP on response to initial ART (Lockman et al. CROI 2008)
- Impact of low frequency NNRTI resistant variants on response to multidrug regimens in treatment-experienced patients (Halvas et al. JID in press)

Re-selection of “Low Frequency” Mutant



Low Frequency NNRTI-Resistant Variants Contribute to Failure of Efavirenz-Containing Regimens in NNRTI-Experienced Patients

Elias K. Halvas, Ann Wiegand, Valerie F. Boltz, Mary Kearney, Dwight Nissley, Michael Wantman, Scott M. Hammer, Sarah Palmer, Florin Vaida, John M. Coffin and John W. Mellors

aactg Adult AIDS Clinical Trials Group

ACTG 398 Study Population

- N = 481 enrolled
- HIV RNA ≥1,000 c/ml on PI-containing regimen
- No prior abacavir, amprenavir, efavirenz
- Enrollment stratified by NNRTI experience
  - 56% naïve
  - 44% experienced (>7days)
- Standard baseline genotype (ABI ViroSeq v2.0)
  - N = 452 (94%)

ACTG 398: Study Arms

Hammer et al., JAMA 2002

Efavirenz 600 mg QD + Abacavir 300 mg BID +

Adefovir 60 mg QD + Amprenavir 1200 mg BID

Randomized to:

Saquinavir 1600 mg BID

or

Indinavir 1200 mg BID

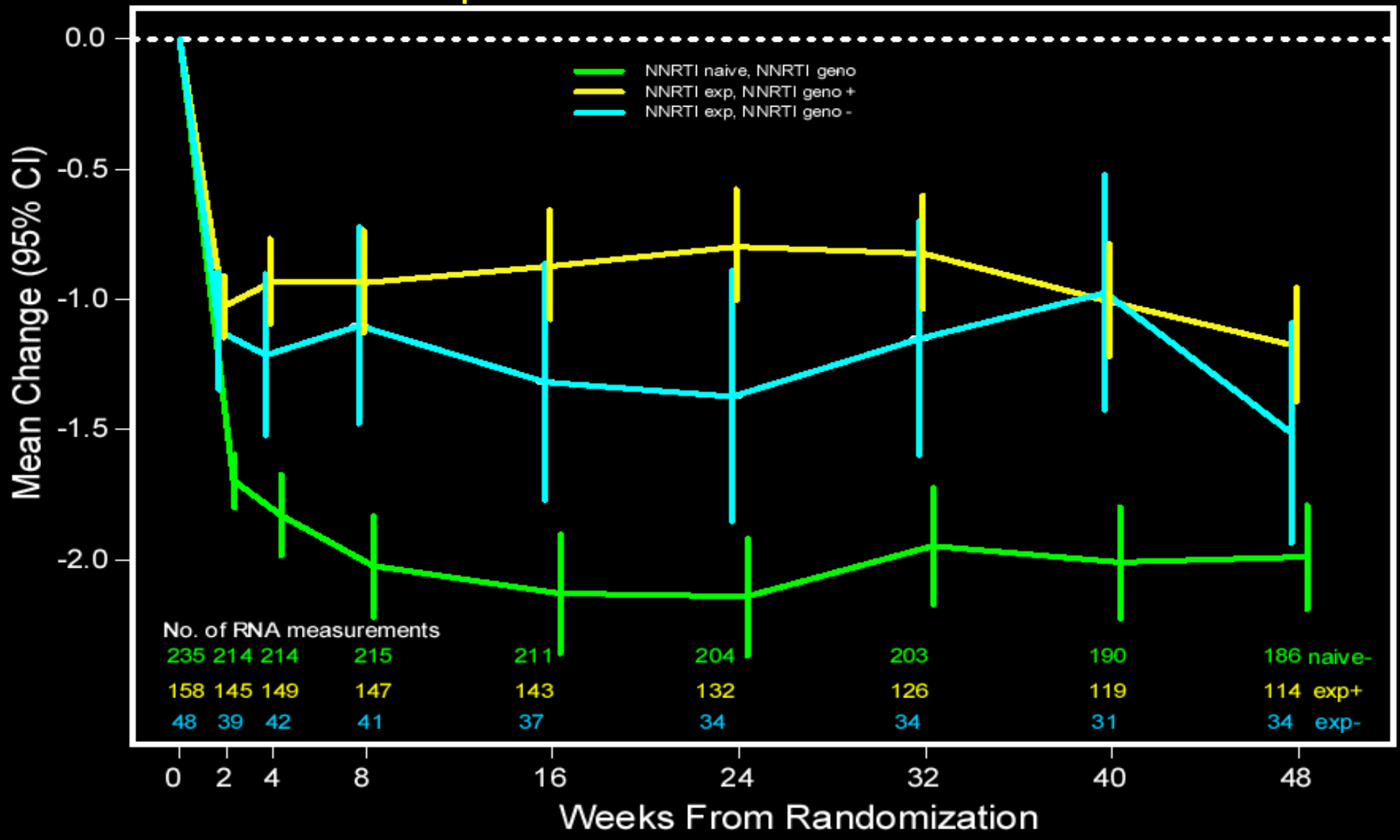
or

Nelfinavir 1250 mg BID

or

Matched PI Placebo

ACTG 398: HIV-1 RNA response (log_{10} copies/ml) by NNRTI Experience and NNRTI mutations



Methods (Sample Selection)

- Random sample of baseline specimens
  - Negative for NNRTI mutations (ABI v2.0)
  - Experienced virologic failure
  - Duration off NNRTI:
    - » Median 336 days (range 0 – 555 days)

SGS in NNRTI-Naïve

PID	NNRTI Mutations at Baseline by Std Sequencing	NNRTI Mutations at Baseline by SGS	Total # of Mutants	NNRTI Mutations at Failure by Std Sequencing
1N	None	None	0 of 52	K103N, V108I
2N	None	None	0 of 52	K103N
3N	None	None	0 of 49	K103N, V108I
4N	None	None	0 of 55	K103N
5N	None	P225H	1 of 53	K103N
6N	None	None	0 of 51	K103N
7N	None	K103N	1 of 40	K103N, M230L
8N	None	None	0 of 50	K103N, G190A
9N	None	None	0 of 63	K103N
10N	None	None	0 of 46	K103N
11N	None	None	0 of 51	L100I, K103N
12N	None	L100I	2 of 48	G109S
13N	None	None	0 of 48	K103N, Y181C
14N	None	None	0 of 45	K103N, V108I
15N	None	None	0 of 70	K103N, G190A
Total	0 of 15	3 of 15	3 of 773	1 of 3 Match

SGS in NNRTI-Experienced

PID	NNRTI Mutations at Baseline by Std Sequencing	NNRTI Mutations at Baseline by SGS	# Mutant of Total	NNRTI Mutations at Failure by Std Sequencing
1E	None	V108I	2 of 32	K103N, V108I
2E	None	None	0 of 48	L100I, K103N
3E	None	K101E	8 of 41	L100I, K101E, Y188H/L
4E	None	None	0 of 45	K103N, P225H
5E	None	K101E, Y181C, G190A	10 of 30	K101E, V108I, Y181C, G190A/S
6E	None	Y181C	3 of 19	K103N, Y181C
7E	None	K103N	1 of 33	K103N, V108I
8E	None	K103N	1 of 34	K103N, V108I
9E	None	None	0 of 46	K103N
10E	None	None	0 of 48	L100I, K103N
11E	None	G190E	1 of 45	K103N
12E	None	Y181C, G190A	5 of 47	K101E, Y181C, G190A
Total	0 of 12	8 of 12	31 of 468	7 of 8 Match

Association of Low Frequency Mutants with NNRTI Experience

NNRTI-Naïve	NNRTI-Experienced	P-value
3/15	8/12	P=0.022
3/773	31/468	P<0.0001

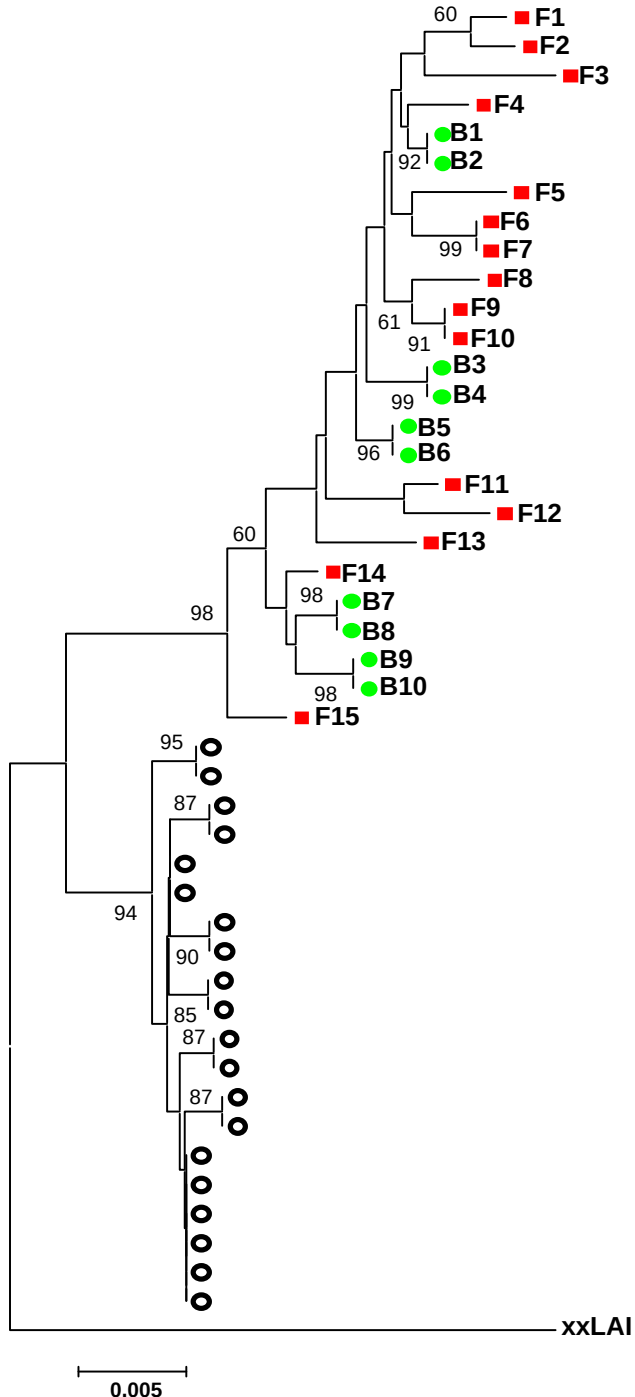
D.

Patient 5E: NNRTI Experienced

- Baseline Wild Types
- Baseline Mutant
- Failure Mutants

Genotypes

B1-B10: K101E, Y181C, G190A
F1, F2, F4, F8-F10, F12-F14 : K101E, V108I, Y181C, G190A
F3, F5-F7: K101E, Y181C, G190S
F11, F15: K101E, Y181C, G190A



Low Frequency NNRTI-Resistant Variants

- Are missed by standard genotyping but can be detected by SGS
- Are associated with reduced virologic response to efavirenz-containing therapy
- Can be linked to the dominant virus population at virologic failure

Clinical Implications

Prior NNRTI exposure matters

"What you can't see can hurt"

Implications for PrEP Trials and PrEP

- Transient resistance may impact future response to ART
  - Resistance to TNV or FTC may differ from NNRTI
    - »Less fit virus, decline faster to lower frequency
  - Resistance to topical product may also differ
    - »May not disseminate
- Nevertheless, must be diligent in detection of resistance from PrEP (MTN-003) and perform long-term follow-up of seroconverters (MTN-015)

Thank You