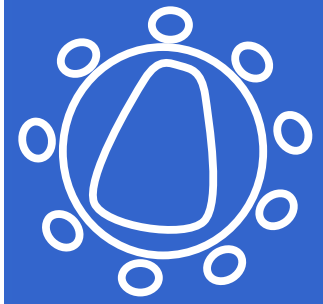

IrsiCaixa!
Grup d'Epidemiologia Molecular

CHAIN
Collaborative HIV and Anti-HIV Drug Resistance Network

Clinical application of ultradeep sequencing in the management of HIV infection

Roger Paredes, MD, PhD

HIV Unit & IrsiCaixa AIDS Research Institute
Hospital Universitari Germans Trias i Pujol
Badalona, Catalonia, Spain

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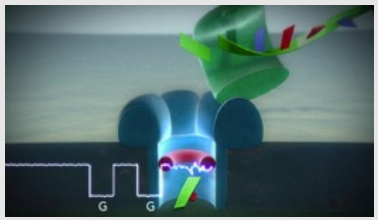
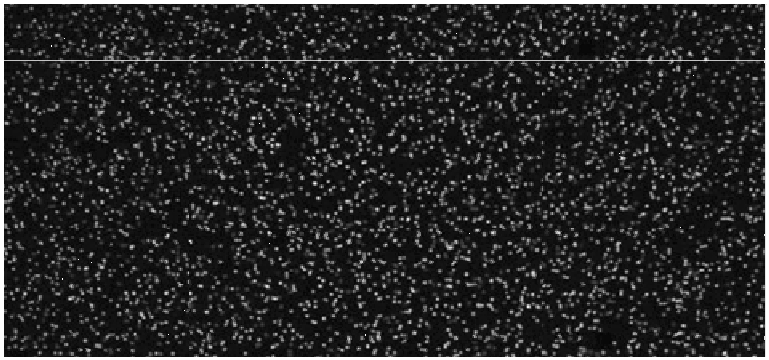


NEWSFOCUS



GENE SEQUENCING

The Race for the $1000 Genome

Fast, cheap genetic analyses will soon become a reality, and the consequences—good and bad—will affect everybody

MARCO ISLAND, FLORIDA—Computers aren't the only things getting better and cheaper every time you turn around. Genome-sequencing prices are in free fall, too. The initial draft of the first human genome sequence, finished just 5 years ago, cost an estimated $300 million. (The final draft and all the technology that made it possible came in near $3 billion.) Last month, genome scientists completed a draft of the genome sequence of the second nonhuman primate—the rhesus macaque—for $22 million. And by the end of the year, at least one company expects to turn out a full mammalian genome sequence for about $100,000, a 3000-fold cost reduction in just 6 years.

It's not likely to stop there. Researchers are closing in on a new generation of technology that they hope will slash the cost of a genome sequence to $1000. "Advances in this field are happening fast," says Kevin McKernan, co–chief scientist at Agencourt Bioscience in Beverly, Massachusetts. "And they are coming more quickly than I think" says Harvard University sequencing pioneer George Church.

Even today, the declining cost of genome sequencing is triggering a flowering of basic research, looking at broad-ranging topics such as how the activation of genes is regulated and understanding genetic links to cancer. And as prices continue to drop, sequencing will revolutionize both the way biologists hunt for disease genes and the way medical professionals diagnose and treat diseases. In fact, some researchers say cheap sequencing technology could finally usher in personalized medicine in a major way. "The promise of cheap sequencing is in the understanding of disease and biology, such as cancer, where the genome changes over time," says Dennis Gilbert, chief scientist of Applied Biosystems, the leading gene-sequencing-technology company based in Foster City, California. "It will enable different kinds of science to be done." Of course, as with other forms

illumina

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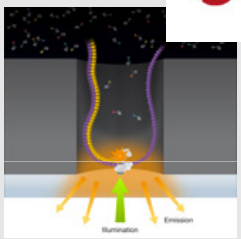
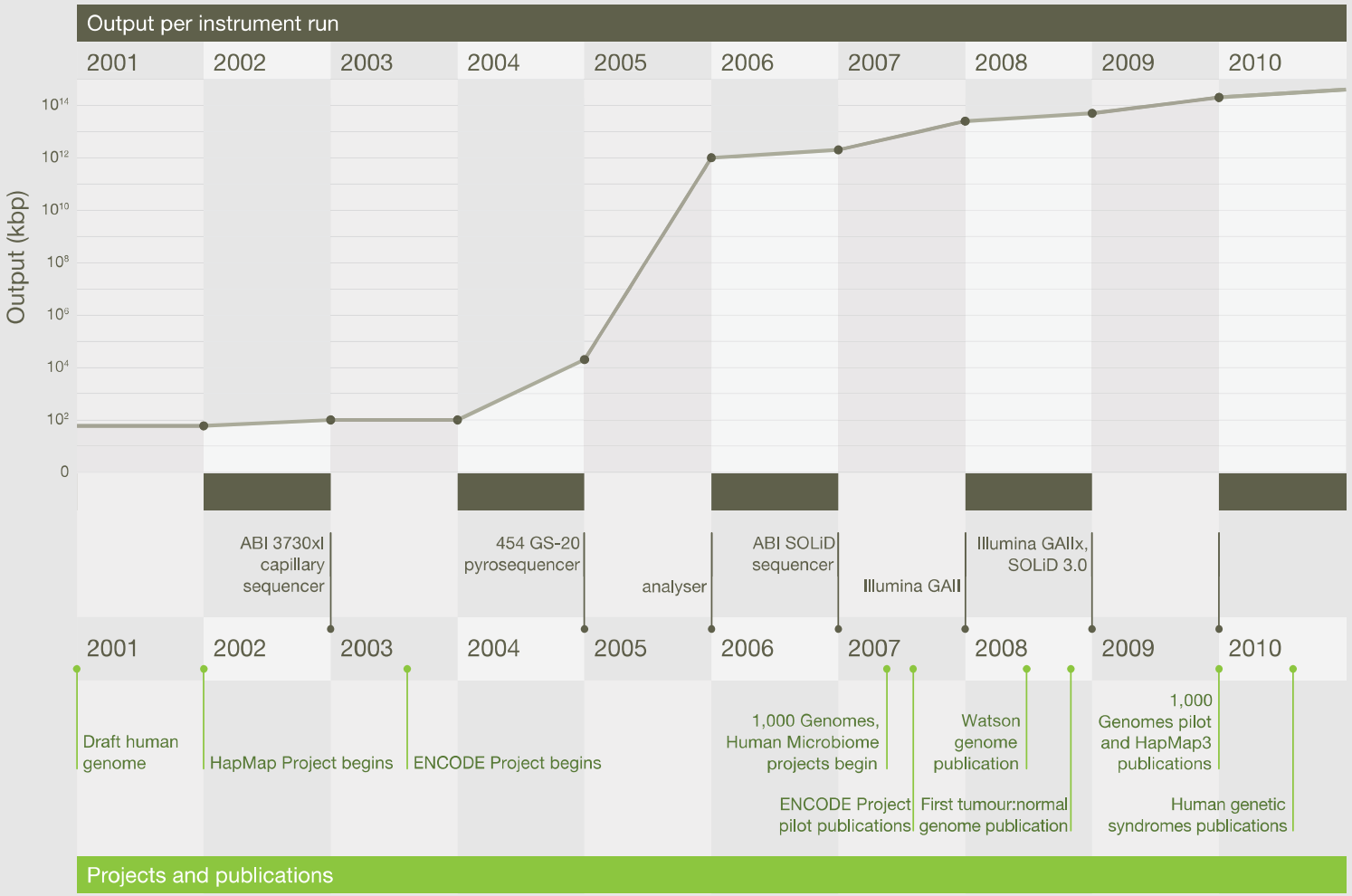


SEQUENCING OUTPUT HAS DRAMATICALLY INCREASED

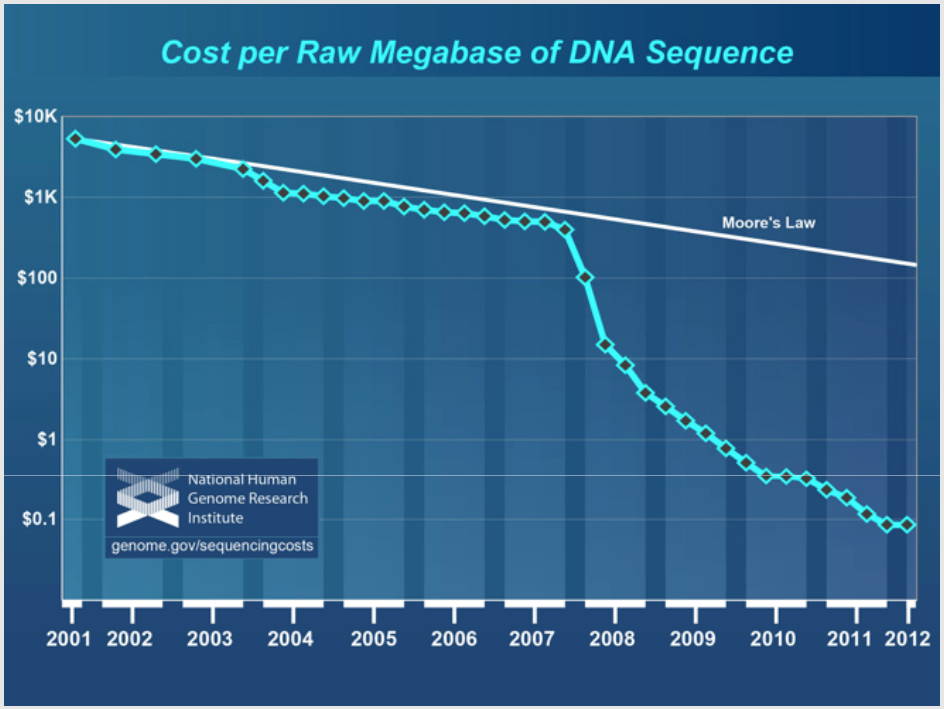


Mardis E. Nature 2011

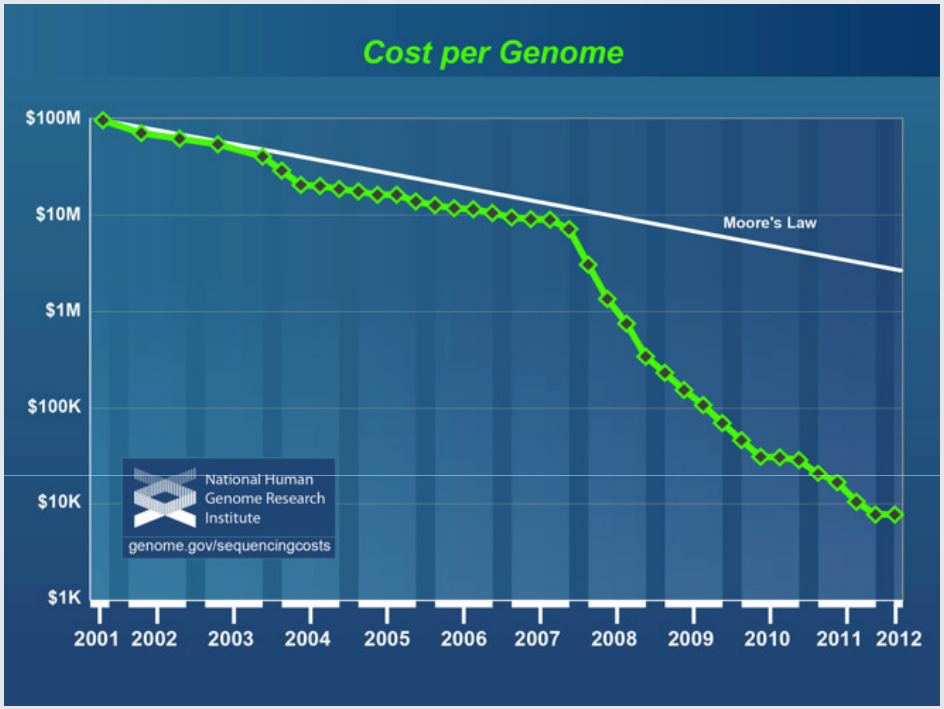
SEQUENCING COSTS HAVE DECREASED DRAMATICALLY

January 2012

Cost per Mb: $0.09



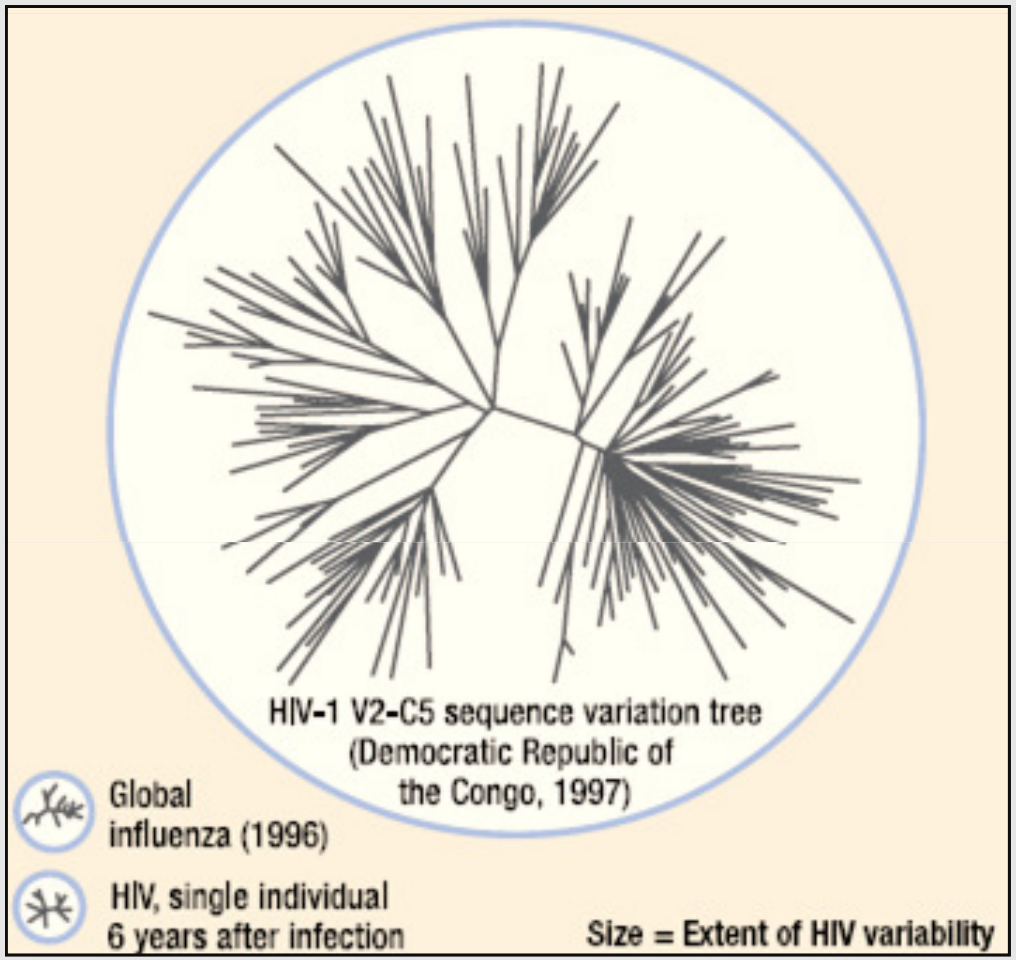
Cost per Human Genome:$7,666



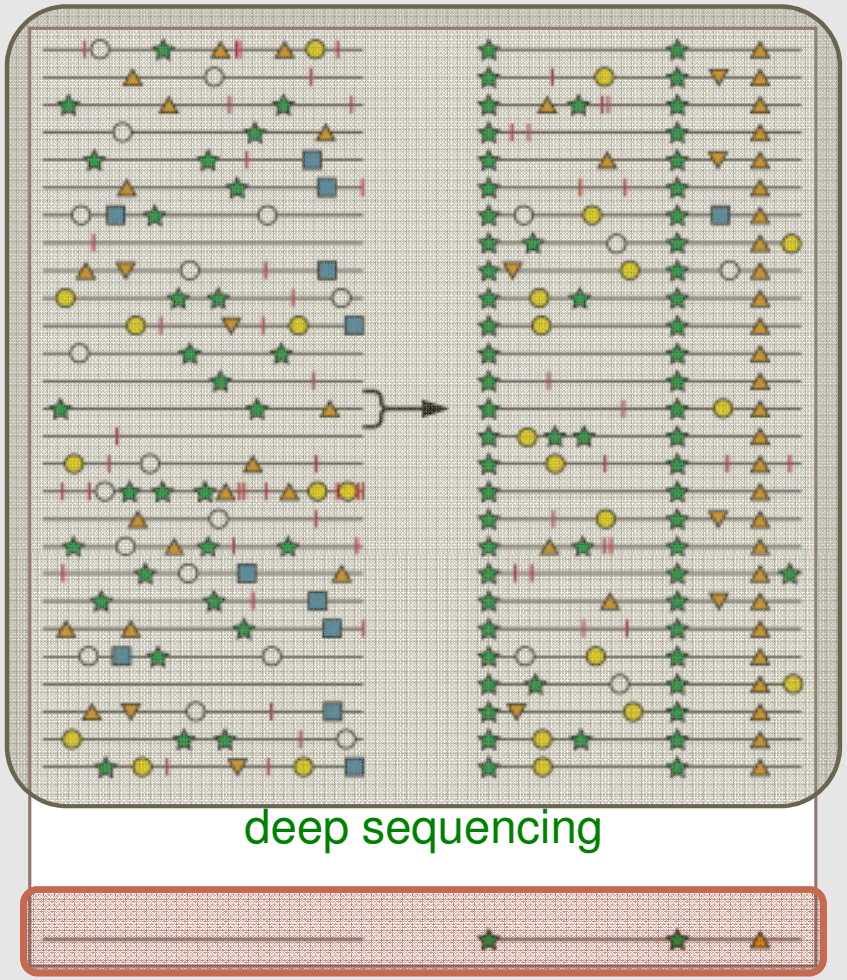
National Human Genome Research Institute: genome.gov/sequencingcosts

HIV RESISTANCE STEMS FROM THE HUGE ABILITY OF THE VIRUS TO GENERATE DIVERSITY

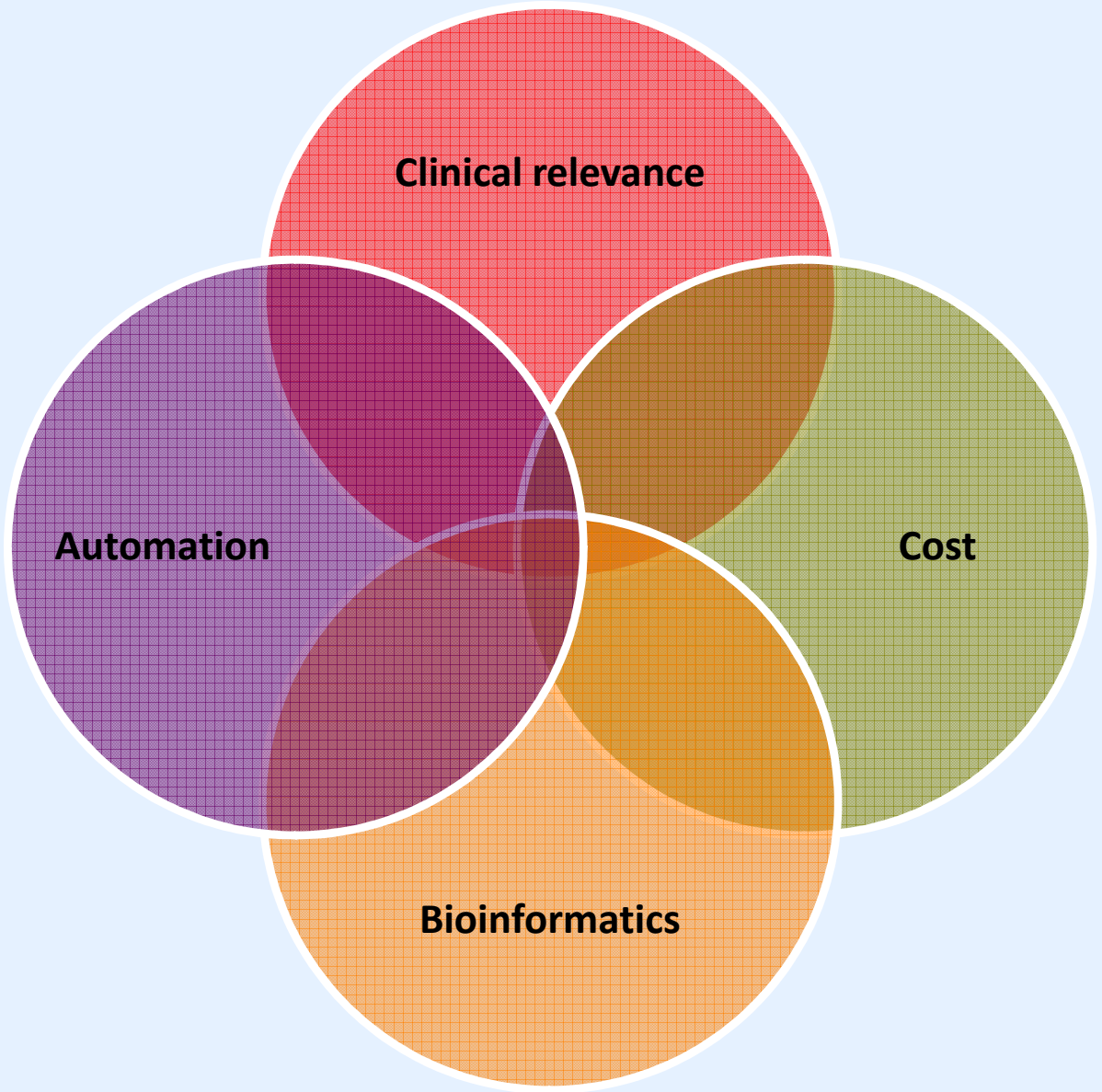
Population level



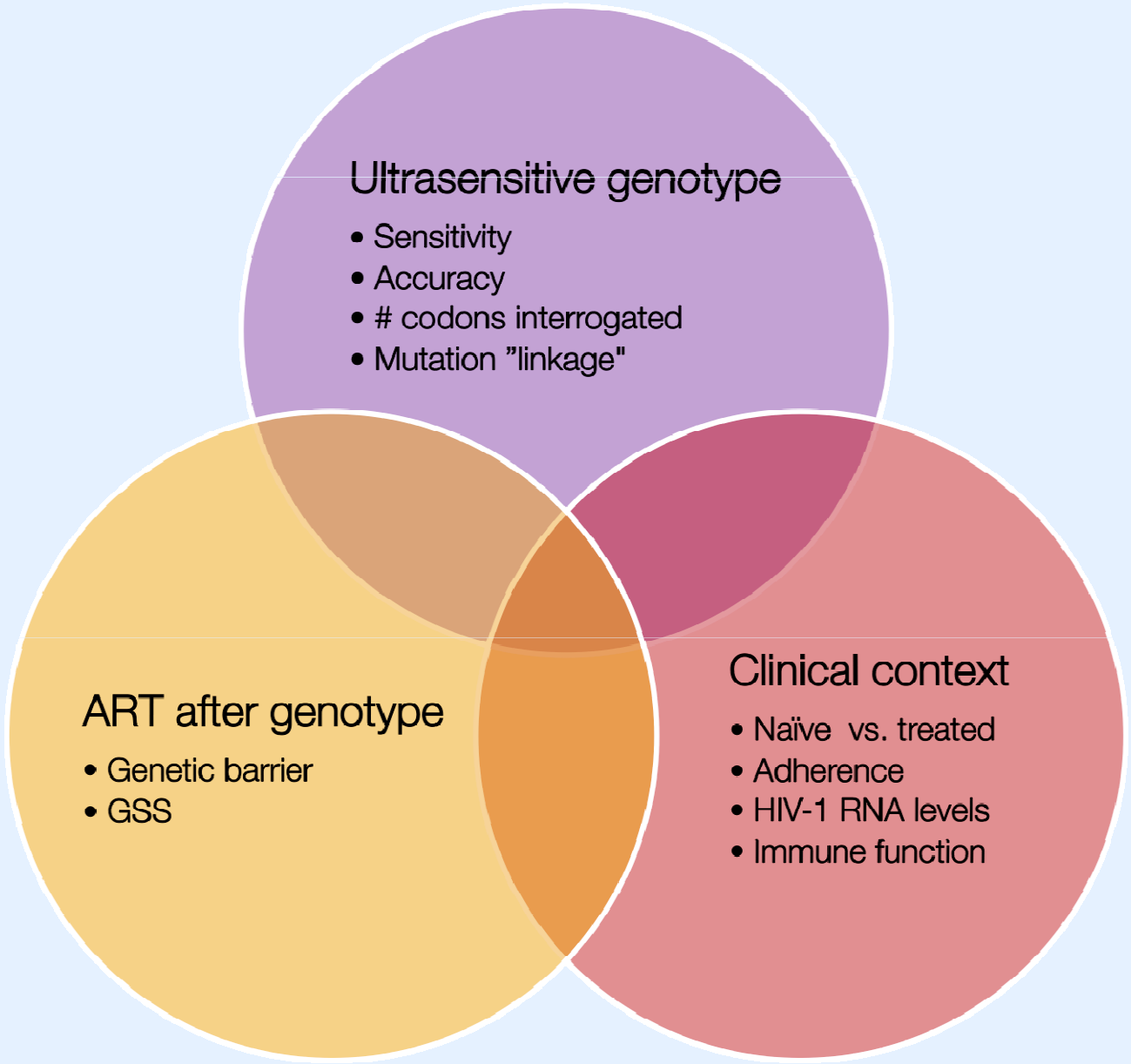
Individual level

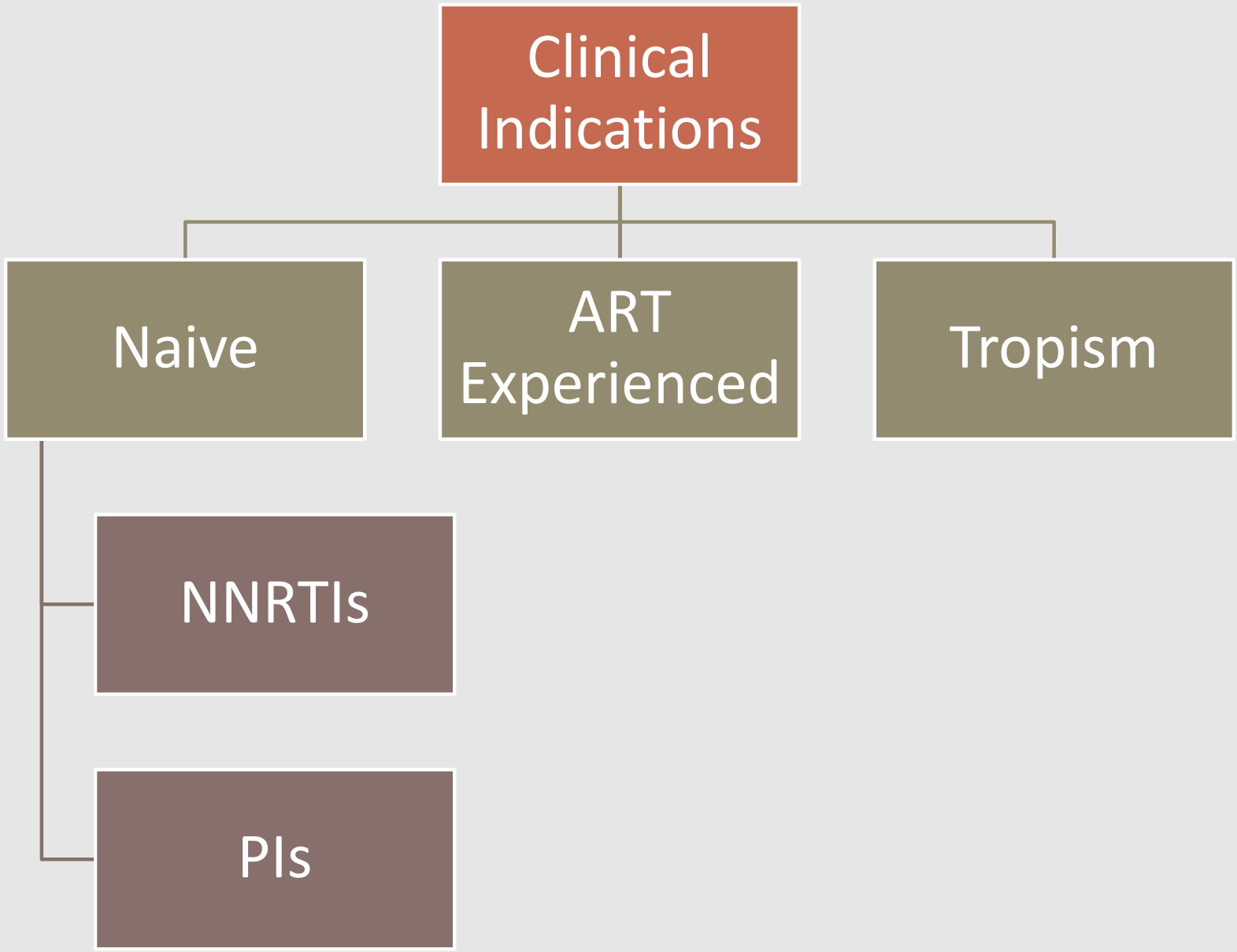


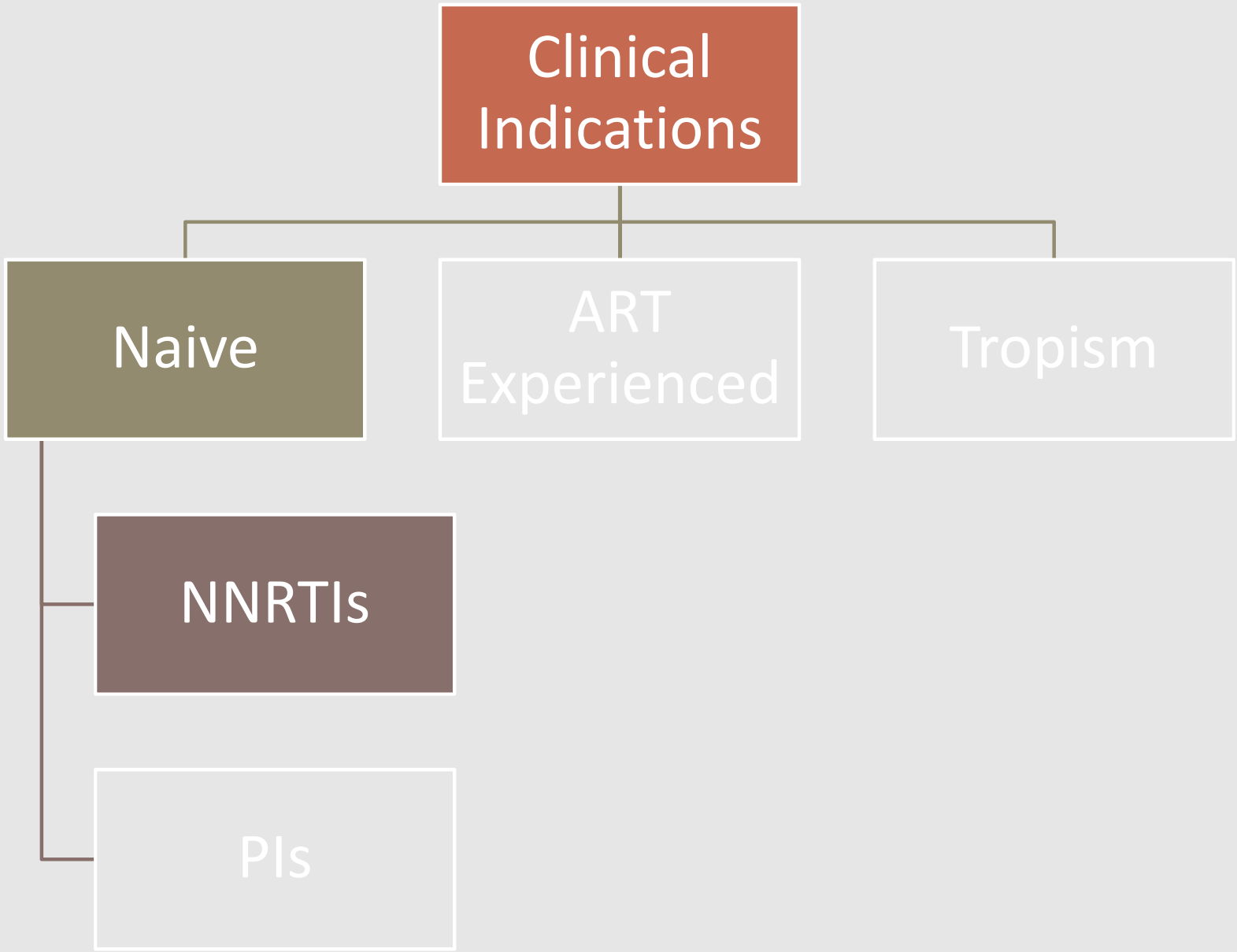
The main challeges with UDS



The “cut-off” issue




Clinical Indications
Naive
ART Experienced
Tropism
NNRTIs
PIs


Clinical Indications
Naive
ART Experienced
Tropism
NNRTIs
PIs

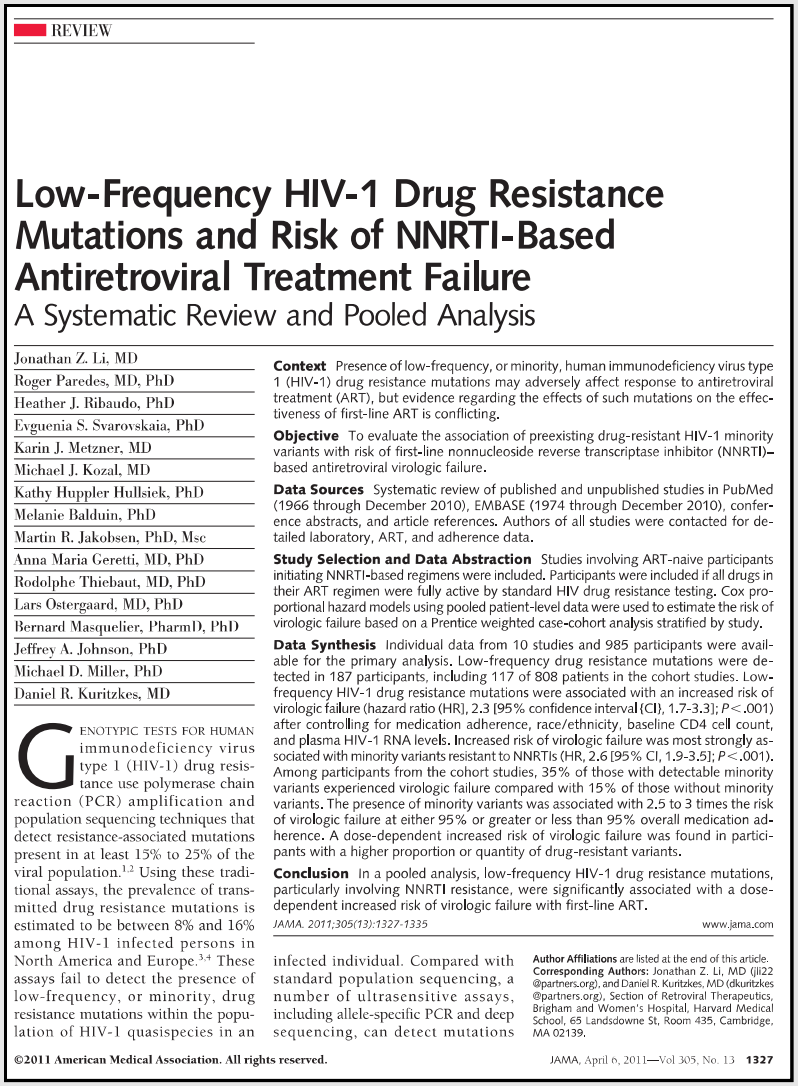
Systematic review – efv / Nvp

INCLUSION

- Cohort or case-control studies
- that evaluated the effects of low-frequency HIV-1 NRTI and NNRTI DRMs on the rate of virologic failure
- in treatment-naive adults
- receiving an initial NNRTI-based ART.

EXCLUSION

- No comparison group
- No treatment outcome data
- Focused solely on primary infection
- Cross-sectional design



REVIEW

Low-Frequency HIV-1 Drug Resistance Mutations and Risk of NNRTI-Based Antiretroviral Treatment Failure

A Systematic Review and Pooled Analysis

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Michael D. Miller, PhD
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Context Presence of low-frequency, or minority, human immunodeficiency virus type 1 (HIV-1) drug resistance mutations may adversely affect response to antiretroviral treatment (ART), but evidence regarding the effects of such mutations on the effectiveness of first-line ART is conflicting.

Objective To evaluate the association of preexisting drug-resistant HIV-1 minority variants with risk of first-line nonnucleoside reverse transcriptase inhibitor (NNRTI)–based antiretroviral virologic failure.

Data Sources Systematic review of published and unpublished studies in PubMed (1966 through December 2010), EMBASE (1974 through December 2010), conference abstracts, and article references. Authors of all studies were contacted for detailed laboratory, ART, and adherence data.

Study Selection and Data Abstraction Studies involving ART-naive participants initiating NNRTI-based regimens were included. Participants were included if all drugs in their ART regimen were fully active by standard HIV drug resistance testing. Cox proportional hazard models using pooled patient-level data were used to estimate the risk of virologic failure based on a Prentice weighted case-cohort analysis stratified by study.

Data Synthesis Individual data from 10 studies and 985 participants were available for the primary analysis. Low-frequency drug resistance mutations were detected in 187 participants, including 117 of 808 patients in the cohort studies. Low-frequency HIV-1 drug resistance mutations were associated with an increased risk of virologic failure (hazard ratio (HR), 2.3 [95% confidence interval (CI), 1.7-3.3]; $P<.001$) after controlling for medication adherence, race/ethnicity, baseline CD4 cell count, and plasma HIV-1 RNA levels. Increased risk of virologic failure was most strongly associated with minority variants resistant to NNRTIs (HR, 2.6 [95% CI, 1.9-3.5]; $P<.001$). Among participants from the cohort studies, 35% of those with detectable minority variants experienced virologic failure compared with 15% of those without minority variants. The presence of minority variants was associated with 2.5 to 3 times the risk of virologic failure at either 95% or greater or less than 95% overall medication adherence. A dose-dependent increased risk of virologic failure was found in participants with a higher proportion or quantity of drug-resistant variants.

Conclusion In a pooled analysis, low-frequency HIV-1 drug resistance mutations, particularly involving NNRTI resistance, were significantly associated with a dose-dependent increased risk of virologic failure with first-line ART.

JAMA. 2011;305(13):1327-1335 www.jama.com

GENOTYPIC TESTS FOR HUMAN immunodeficiency virus type 1 (HIV-1) drug resistance use polymerase chain reaction (PCR) amplification and population sequencing techniques that detect resistance-associated mutations present in at least 15% to 25% of the viral population.[1,2] Using these traditional assays, the prevalence of transmitted drug resistance mutations is estimated to be between 8% and 16% among HIV-1 infected persons in North America and Europe.[3,4] These assays fail to detect the presence of low-frequency, or minority, drug resistance mutations within the population of HIV-1 quasispecies in an infected individual. Compared with standard population sequencing, a number of ultrasensitive assays, including allele-specific PCR and deep sequencing, can detect mutations

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Increased risk of virological failure

Risk of virological failure NOT increased

Risk increased in some subjects or non-significant trend towards increased risk

Table 1. Baseline Characteristics of Studies Included in the Pooled Analysis

Characteristic	Peuchant et al,[16] 2008	Simen et al,[15] 2009	Balduin et al,[17] 2009	Jakobsen et al,[18] 2010	Metzner et al,[19] 2011	Goodman et al,[20] 2011	Paredes et al,[21] 2010	Johnson et al,[22] 2008	Geretti et al,[23] 2009	Metzner et al,[24] 2009	Total
Study design	Cohort	Cohort	Cohort	Cohort	Cohort	Cohort	Case cohort	Case-control	Case-control	Case-control	
Virologic failure, No.	2	45	7	1	1	44	150	52	14	3	315
Total participants, No.	13	70	54	20	56	423	280	240	89	18	1263
Age, mean (SD), y	38 (16.8)	37 (8.8)	41 (11.7)	43 (12.3)	42 (11.1)	38 (9.4)	37 (9.6)	37 (9.5)	38 (8.5)	43 (9.5)	38 (9.8)
Men, No. (%)	12 (92)	56 (80)	41 (76)	19 (95)	45 (80)	365 (86)	227 (81)	196 (82)	78 (88)	13 (72)	1052 (83)
Race/ethnicity, No. (%)											
Participants, No.	13	70	52	NR	NR	422	279	240	89	17	1182
White	12 (92)	16 (23)	39 (75)			253 (6)	110 (39)	132 (55)	78 (88)	14 (82)	654 (55)
Black	1 (8)	38 (54)	11 (21)			94 (22)	110 (39)	61 (25)	10 (11)	3 (18)	328 (28)
Hispanic	0	14 (20)	0			61 (14)	54 (19)	42 (18)	0	0	171 (14)
Other	0	2 (3)	2 (4)			14 (3)	5 (2)	5 (2)	1 (1)	0	29 (2)
CD4 cell count, median (IQR), cells/mm^3	426 (303-522)	247 (38-344)	251 (196-326)	200 (48-278)	279 (191-368)	227 (127-319)	202 (69-331)	243 (145-327)	222 (126-299)	222 (59-249)	229 (125-324)
$\log_{10}$ HIV RNA, median (IQR), copies/mL	4.4 (4.2-5.3)	5.3 (4.9-5.8)	4.7 (4.0-4.9)	5.1 (4.6-5.8)	4.9 (4.5-5.3)	5.0 (4.6-5.4)	4.8 (4.4-5.4)	5.1 (4.5-5.5)	5.2 (4.9-5.5)	5.4 (4.9-5.9)	5.0 (4.6-5.4)

Abbreviations: HIV, human immunodeficiency virus; IQR, interquartile range; NR, not reported.

Increased risk of virological failure

Risk of virological failure NOT increased

Risk increased in some subjects or non-significant trend towards increased risk

Table 2. Characteristics of Minority Variants by Study[a]

Characteristic	Peuchant et al,[16] 2008	Simen et al,[15] 2009	Balduin et al,[17] 2009	Jakobsen et al,[18] 2010	Metzner et al,[19] 2011	Goodman et al,[20] 2011	Paredes et al,[21] 2010	Johnson et al,[22] 2008	Geretti et al,[23] 2009	Metzner et al,[24] 2009	Total for Cohort Studies[b]
Study design	Cohort	Cohort	Cohort	Cohort	Cohort	Cohort	Case cohort	Case-control	Case-control	Case-control	
Method of detection[c]	AS-PCR	454	AS-PCR	SNaPshot	AS-PCR	AS-PCR	AS-PCR	AS-PCR	AS-PCR	AS-PCR	
Limit of detection (% of viral population)											
K103N	0.4	1.0	0.2	2.0	0.01	0.5	0.003	0.9	0.9	0.01	
Y181C		1.0		2.0			0.03	1.0	1.0	0.2	
M184V	0.3	1.0		2.0	0.2			0.5	0.5	0.2	
K65R		1.0		2.0	0.4				0.3	0.4	
Other NNRTI[d]		1.0		2.0					0.9		
No. with MVs and VF/total No. with MVs[e]											
K103N	1/3	1/1	3/13	1/2	0/2	5/14	27/39	1/1	3/3	1/1	17/53
Y181C		0/0		0/0			83/123	1/1	0/0	1/1	25/65
M184V	0/3	0/0		1/1	0/3			1/1	0/0	2/2	1/7
K65R		0/0		0/0	0/2				0/0	0/0	0/2
Other NNRTI[d]		3/3		0/0					0/0		3/3

PREVALENCE & OUTCOMES

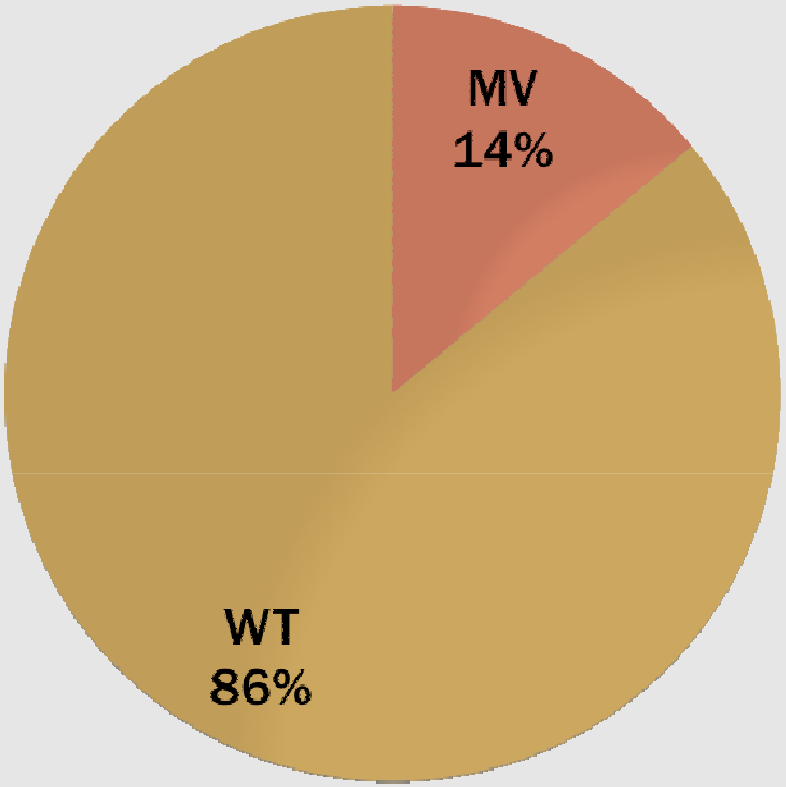
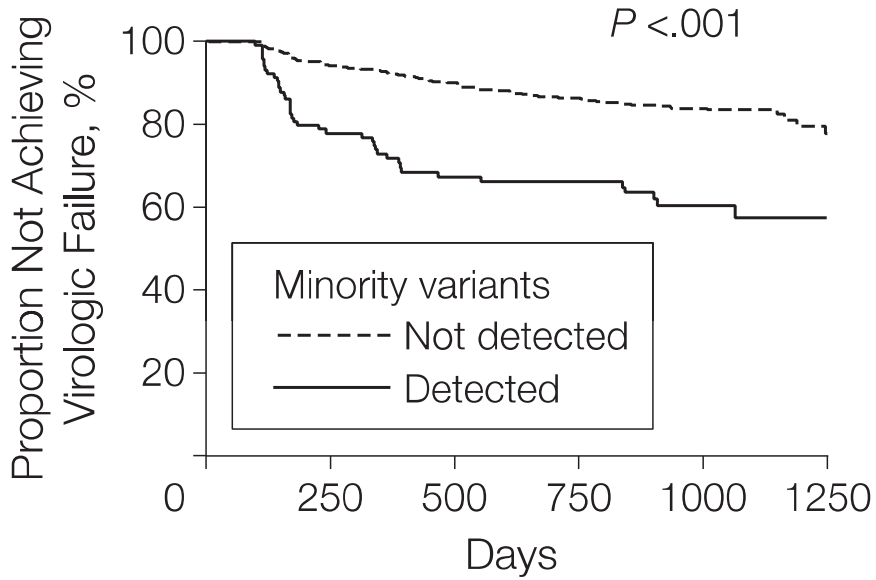


Figure 2. Kaplan-Meier Curves for Proportion of Patients Without Virologic Failure by Presence of Drug-Resistant HIV-1 Minority Variants

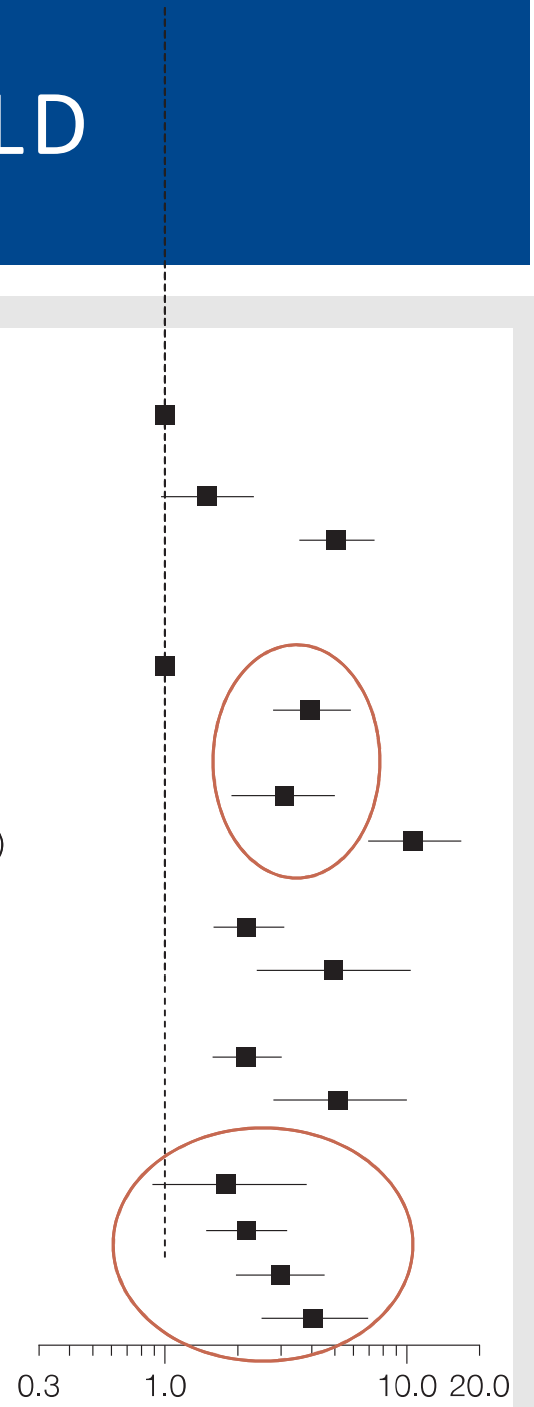


No. at risk

Minority variants						
Not detected	691	620	455	398	344	46
Detected	117	86	60	53	37	7

ADHERENCE & THRESHOLD

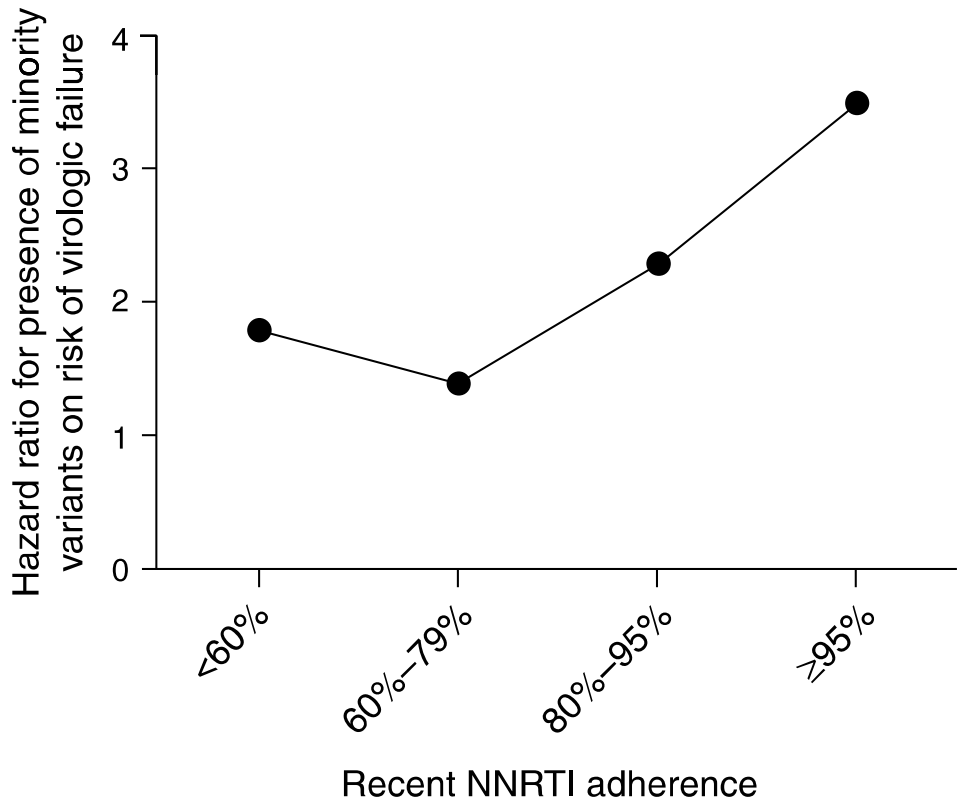
Minority variant and adherence					
No minority variant and any adherence					1 [Reference]
Any minority variant					
Adherence ≥95%	35	138	73	617	1.5 (0.98-2.3)
Adherence <95%	63	138	79	617	5.1 (3.6-7.2)
No minority variant					
Adherence ≥95%					1 [Reference]
Adherence <95%		43	231	386	4.0 (2.8-5.8)
Any minority variant					
Adherence ≥95%	35	43	73	386	3.1 (1.9-5.0)
Adherence <95%	63	43	79	386	10.6 (6.9-16.4)
Minority variant, %					
<1	91	209	154	781	2.2 (1.6-3.1)
≥1	18	209	30	781	5.0 (2.4-10.3)
<0.5	86	107	143	654	2.2 (1.6-3.0)
≥0.5	14	107	32	654	5.2 (2.8-9.8)
Minority variant copies, No.					
1-9	8	148	15	720	1.8 (0.9-3.8)
10-99	41	148	71	720	2.2 (1.5-3.2)
100-999	35	148	55	720	3.0 (2.0-4.5)
≥1000	20	148	38	720	4.1 (2.5-6.8)



ADHERENCE & MINORITY VARIANTS

- The presence of minority NNRTI DRMs and NNRTI adherence were found to be independent predictors of virologic failure, but also modify each other's effects on virologic failure

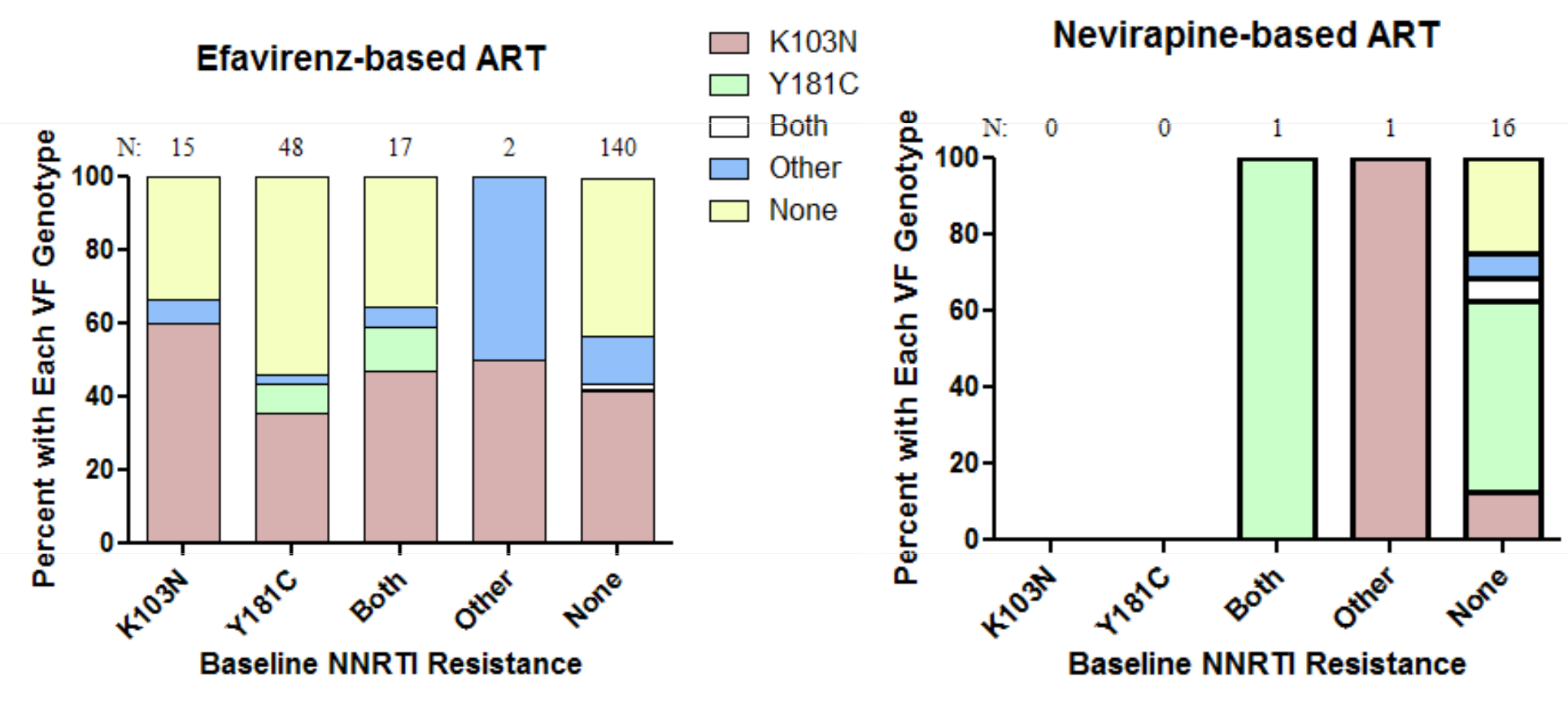
- In addition to the focus on medication adherence counseling, ultrasensitive HIV-1 drug resistance assays could play a role in optimizing the success rates of first-line ART



Hazard ratios	1.8	1.4	2.3	3.5
95% CI	1.1–2.9	0.5–3.7	0.9–5.8	2.3–5.4

Li J et al. AIDS 2012

BASELINE MVS AND ART: EFFECT ON RESISTANCE GENOTYPE AT VF

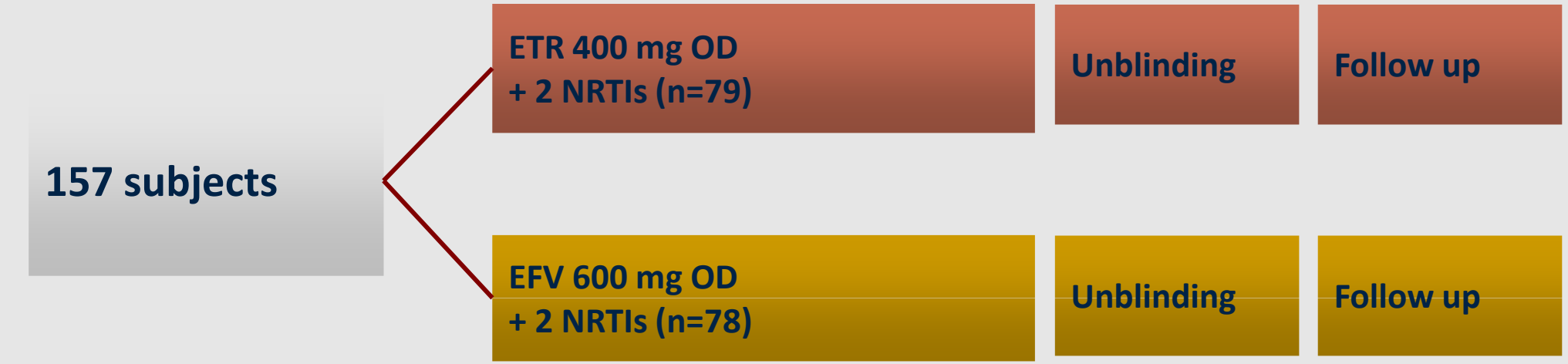


- If EFV-based regimen, baseline Y181C associated with a higher rate of Y181C detection at VF (18% vs. 3%, Fisher's P = 0.01).
- No BL mutation → Y181C at VF: 75% (9/12) NVP vs. 4% (3/79) EFV (Fishers P <0.001)

Li J et al. IHHDRW 2012

SENSE STUDY (ETRAVIRINE)

Inclusion: Treatment naïve, HIV RNA >5,000 copies/mL
No resistance mutations to NRTIs, NNRTIs or PIs (WHO list)
and predicted phenotypic sensitivity to NNRTIs and selected NRTIs
(vircoTYPE HIV-1)

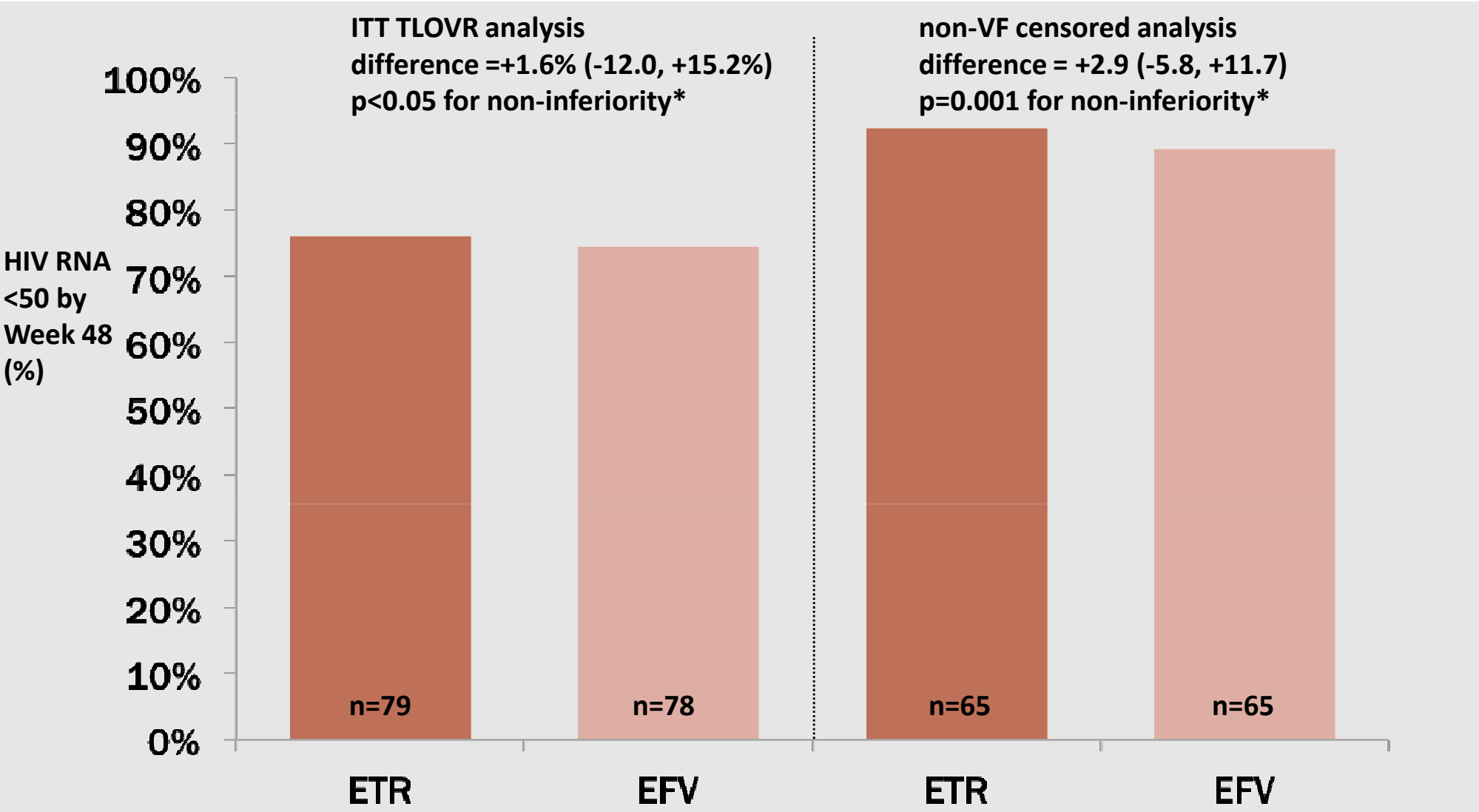


← 6 weeks → ← 48 wks → 2-8 wks 4 wks

Double-blinded, active controlled to Week 48
Two investigator-selected NRTIs (TDF+FTC; ABC+3TC; ZDV+3TC)
Primary endpoint: Neuropsychiatric adverse events up to Week 12

Geretti AM et al ICAAC 2011 [abstract H1-373]

SENSE: HIV RNA <50 copies/mL at Week 48, TLOVR, ITT Population – All Patients Randomised and Treated



* p values from logistic regression, adjusted for baseline HIV RNA. Adjusted response for TLOVR: 76.1% vs 74.5%

SENSE: TREATMENT-EMERGENT RESISTANCE MUTATIONS AMONG VIROLOGICAL FAILURES BY TLOVR

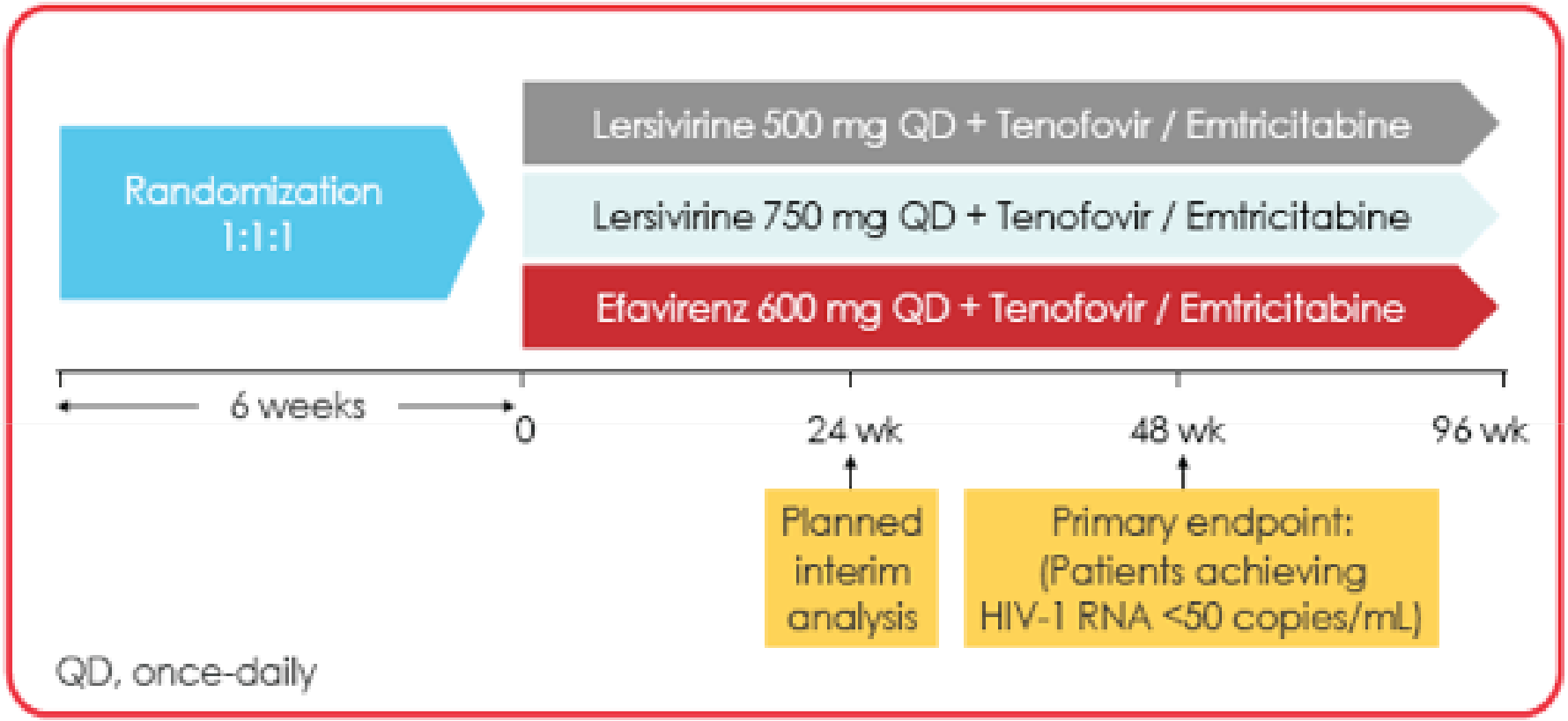
Geretti AM et al ICAAC 2011 [abstract H1-373]

- EFV ARM (N=7)
  - 4 No mutations
  - 1 V106I + M184I
  - 1 K103N
  - 1 L74V + M184V + K103N + P225H
- ETR ARM (N=4)
  - 4 No mutations
- NONE had pre-existing mutations at baseline by either assay

- CONCLUSION: NO EFFECT OF PRE-EXISTING RESISTANCE MUTATIONS BY EITHER POPULATION OR ULTRASENSITIVE SEQUENCING

A5271015 LERSIVIRINE STUDY DESIGN

Figure 1. Study design



Craig et al. IAS Rome 2011

VIROLOGICAL OUTCOMES

Table 6. A total of seven patients with NNRTI or lersivirine RAMs at Screening experienced TLOVR50 failure, including three with virologic failure

Treatment group	PID/Outcome	Screening genotype (Population or UDS)	On-treatment genotype (Population)
Lersivirine 500 mg	1/Discontinued (AE)	V90I	NA[a]
	2/Virologic Failure	L210W/V90I	NA[a]
	3/Discontinued (pregnancy)	V106I	NA[a]
	4/Virologic Failure		M184V/V90I/F227C
Lersivirine 750 mg	5/Virologic Failure	K101E/F227L	M184V/V106M/F227L
	6/Discontinued (AE)		NA[a]
Efavirenz 600 mg	7/Discontinued (AE)	A62V/L100I/K101E	NA[a]

[a]Insufficient plasma HIV-1 RNA on failure

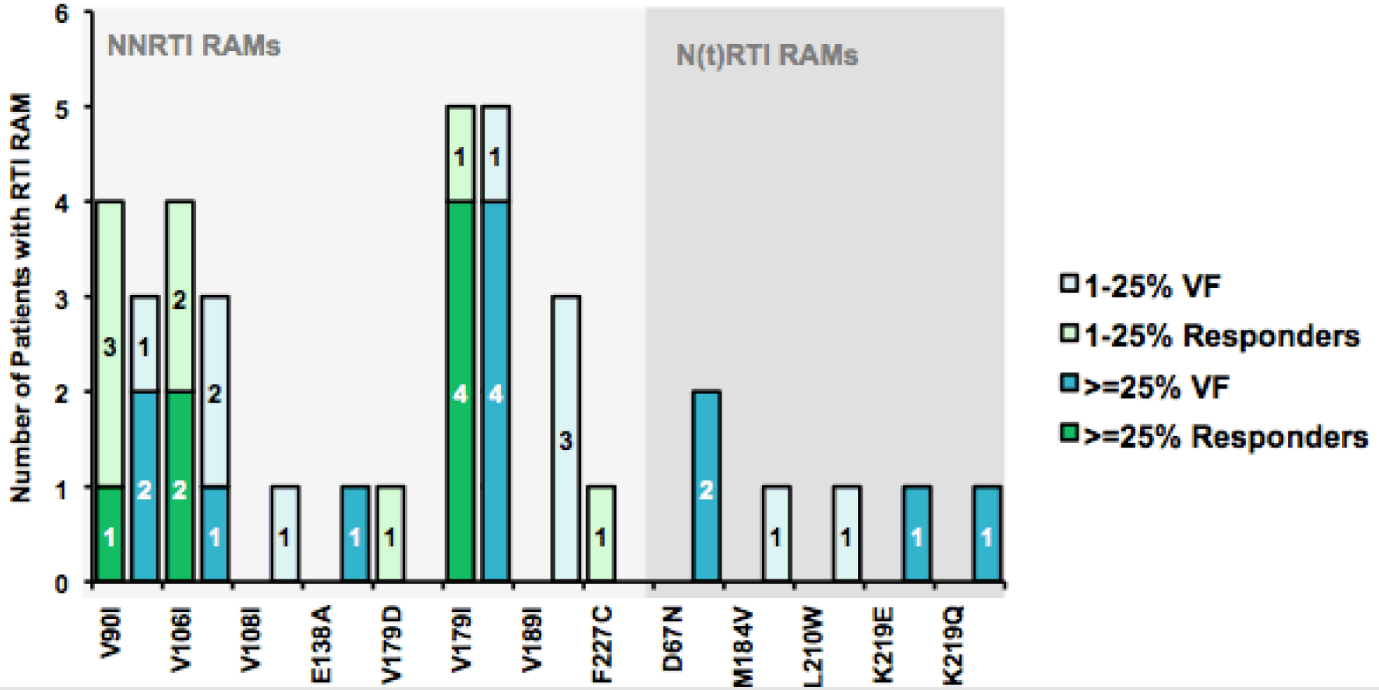
Mutations detected using both population genotyping and UDS are shown in square brackets '[ ]'

AE, adverse event; NA, not available; PID, patient identification; TLOVR, time to loss of virologic response; UDS, ultra deep sequencing

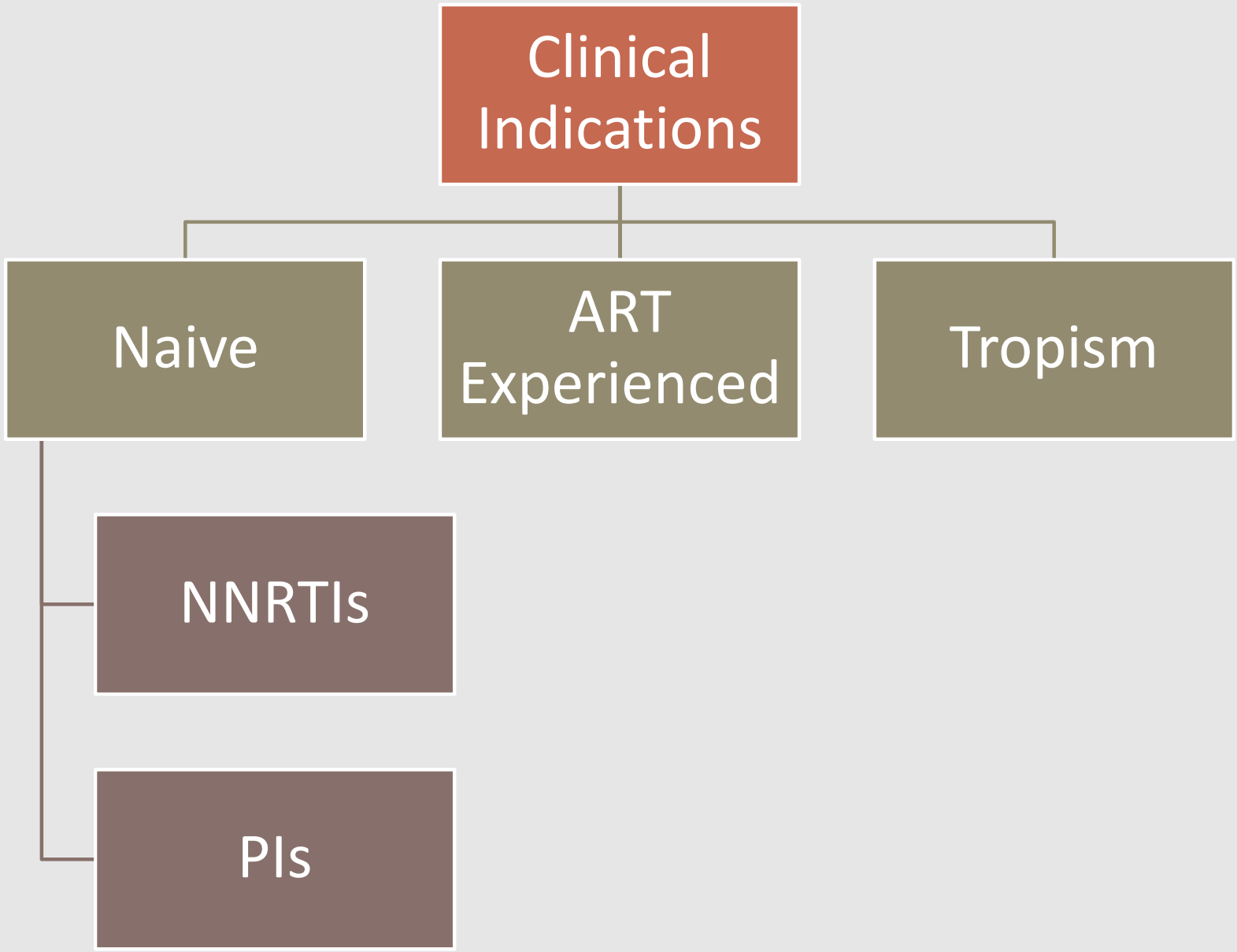
Craig et al. IAS Rome 2011

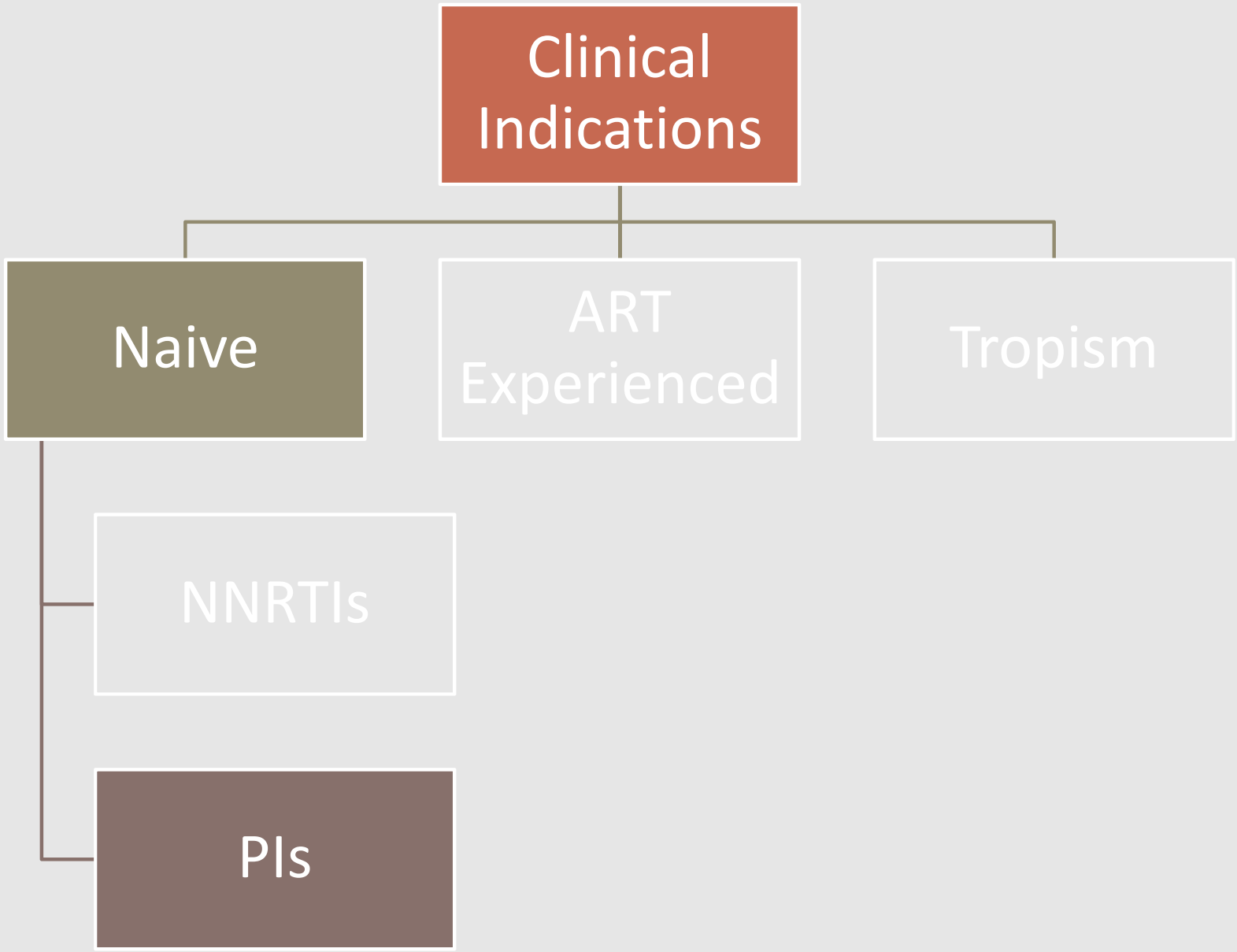
SIMILAR RILPIVIRINE MUTS IN RESPONDERS AND VFS BY 454

Patients, n, (%)	DS Frequency ≥25% / By PS		DS Frequency 1–25%	
	Responders N=49	VFs N=47	Responders N=49	VFs N=47
NNRTI RAMs[1]	7 (14.3)	7 (14.9)	6 (12.2) ↔	8 (17.0)
N(t)RTI RAMs[2]	0	2 (4.3)	0 ↔	2 (4.3)



Van Eygen V et al. IHHDRW 2012 Abstract 27


Clinical Indications
Naive
ART Experienced
Tropism
NNRTIs
PIs


Clinical Indications
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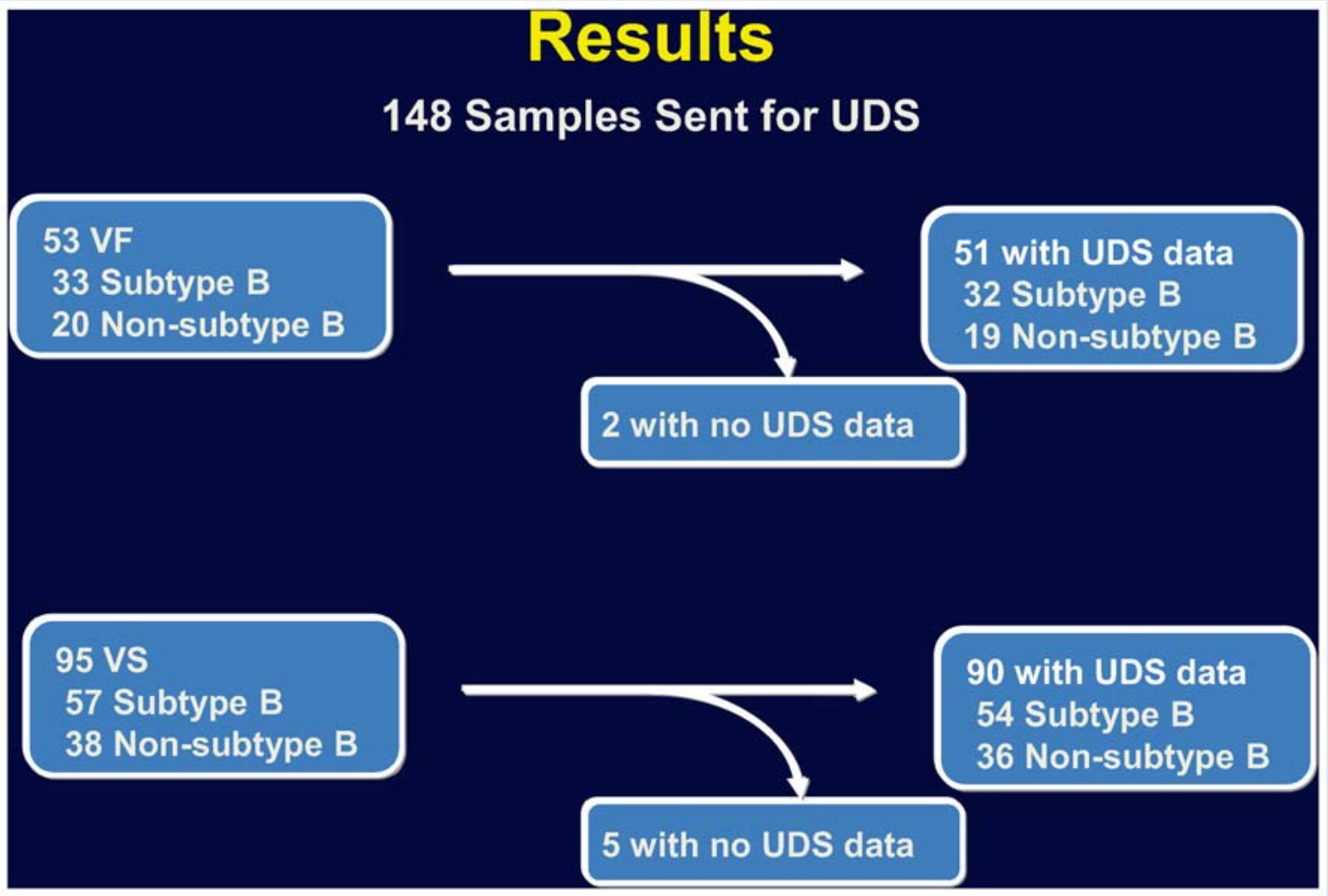
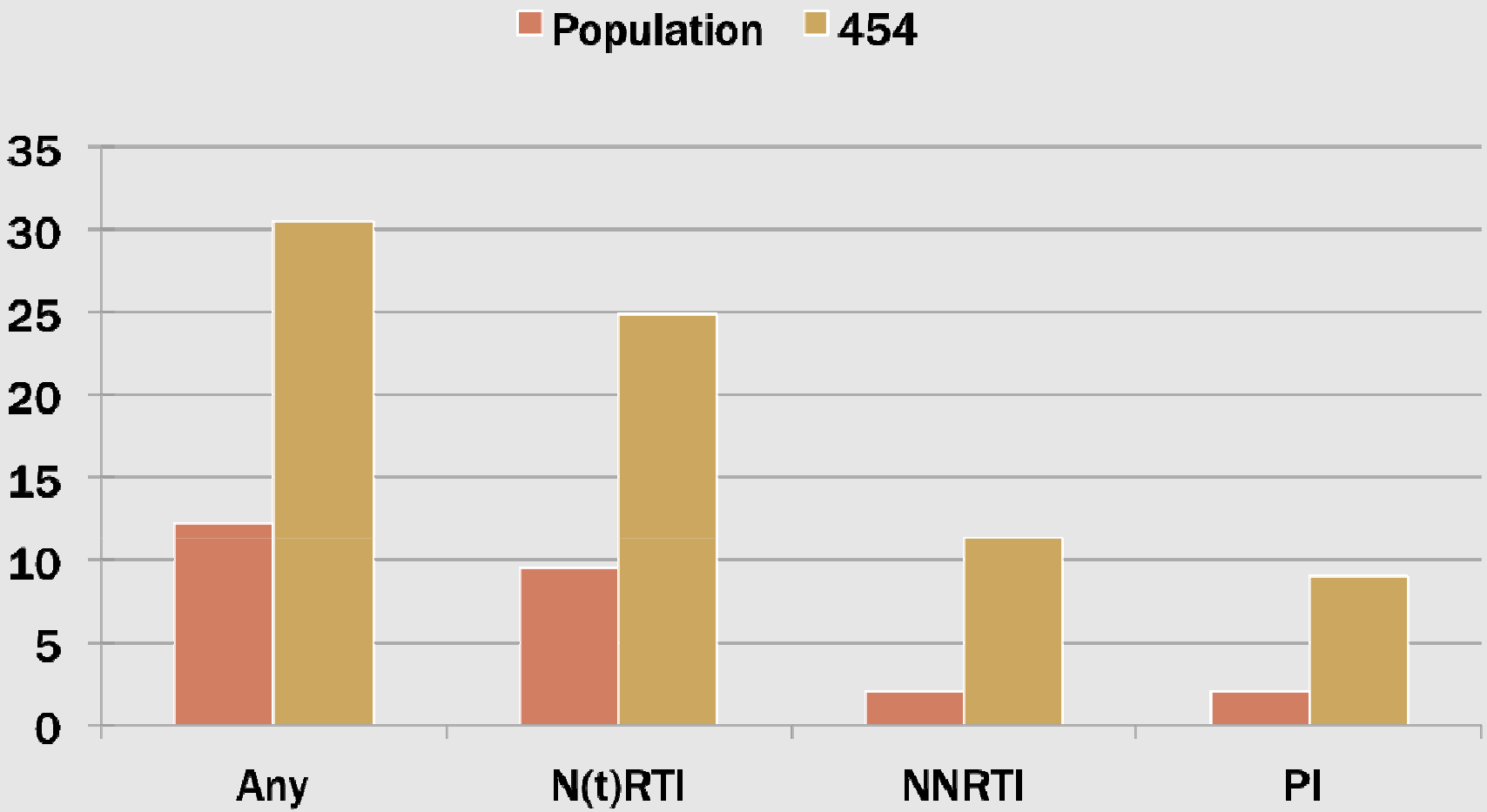
PIs

CASE-CONTROL SUBANALYSIS OF THE CASTLE STUDY



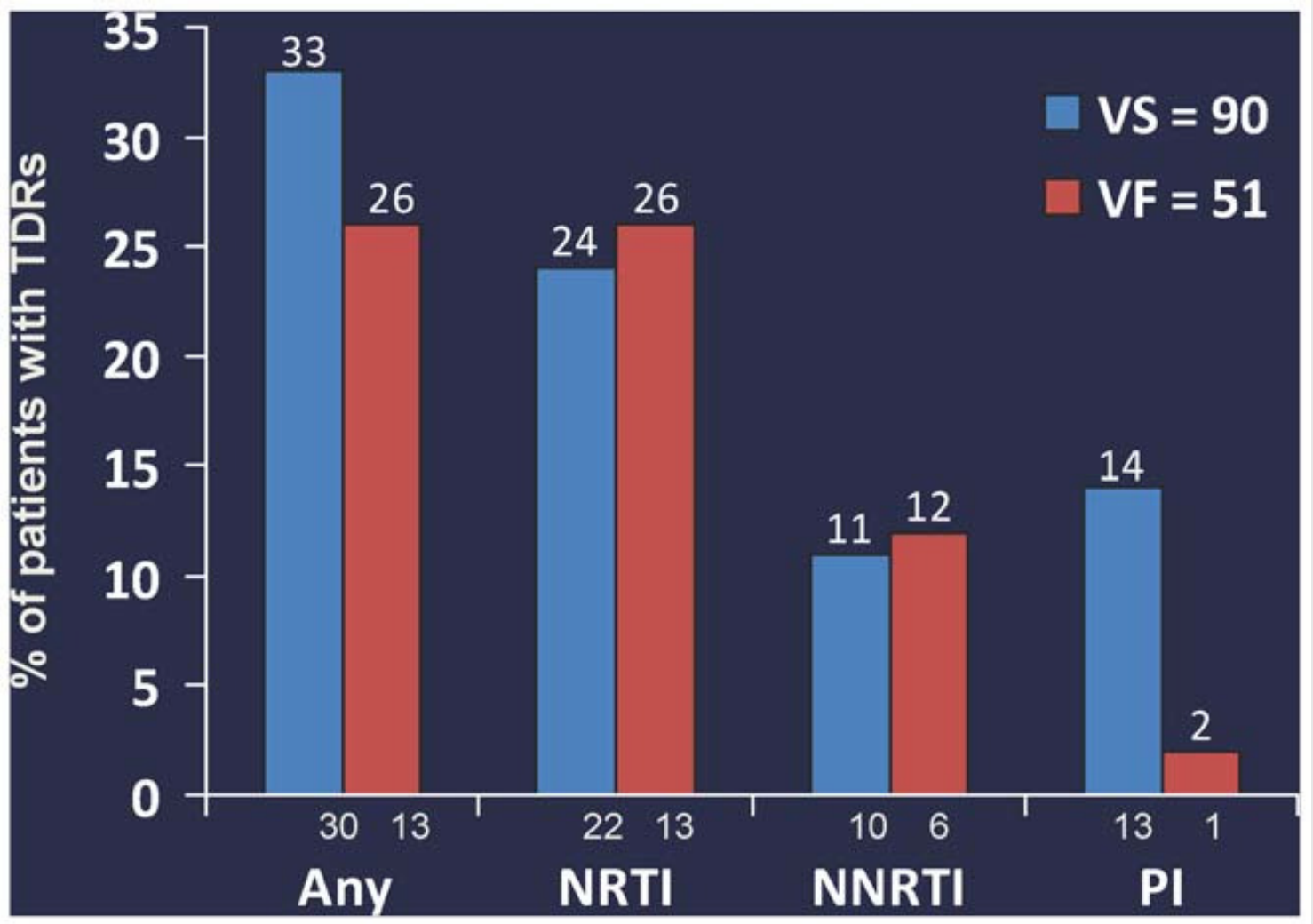
Lataillade M, et al, PLoS ONE 2010

PREVALENCE OF RESISTANCE – castle study

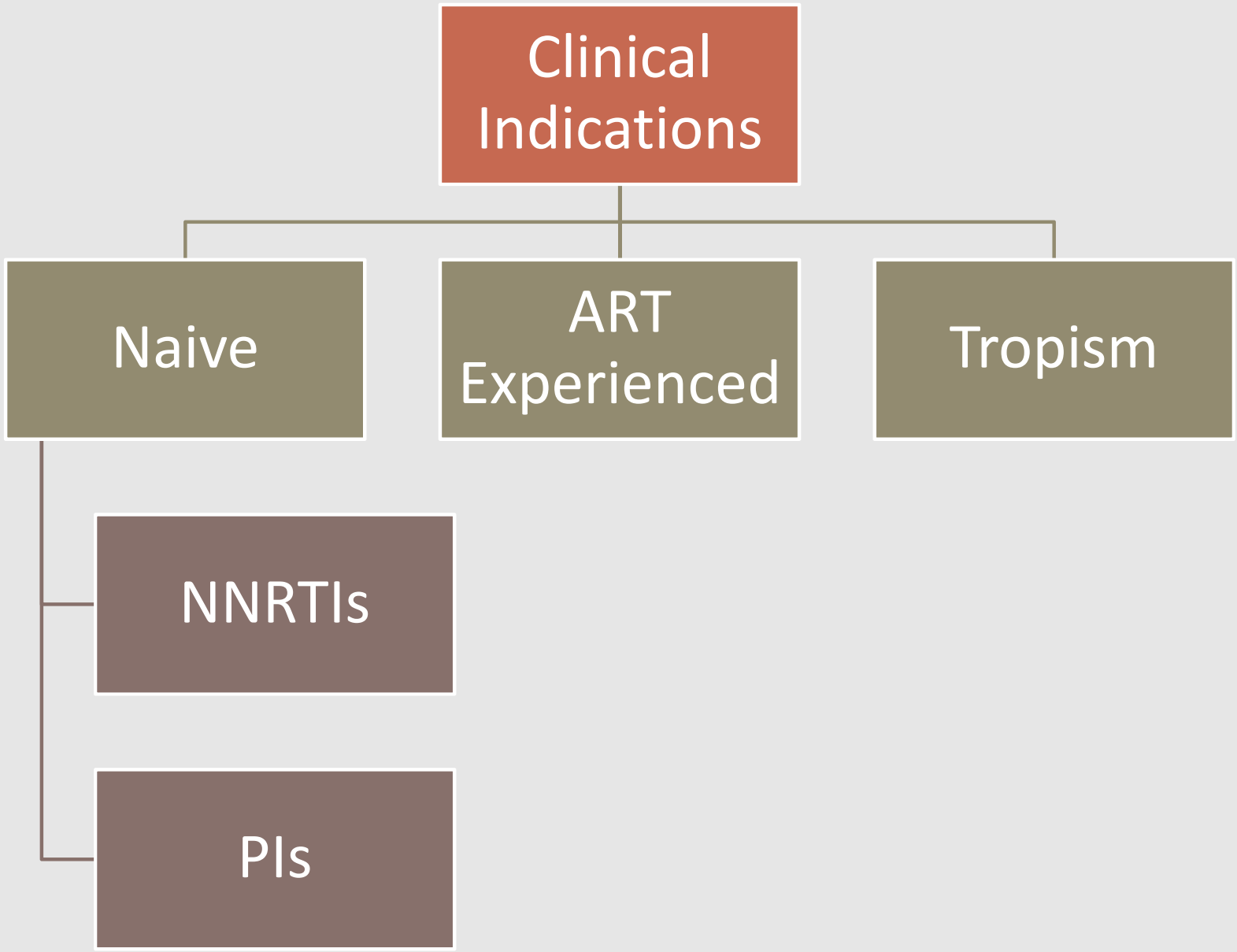


Lataillade M, et al, PLoS ONE 2010

DRUG RESISTANCE BY ARM



Lataillade M, et al, PLoS ONE 2010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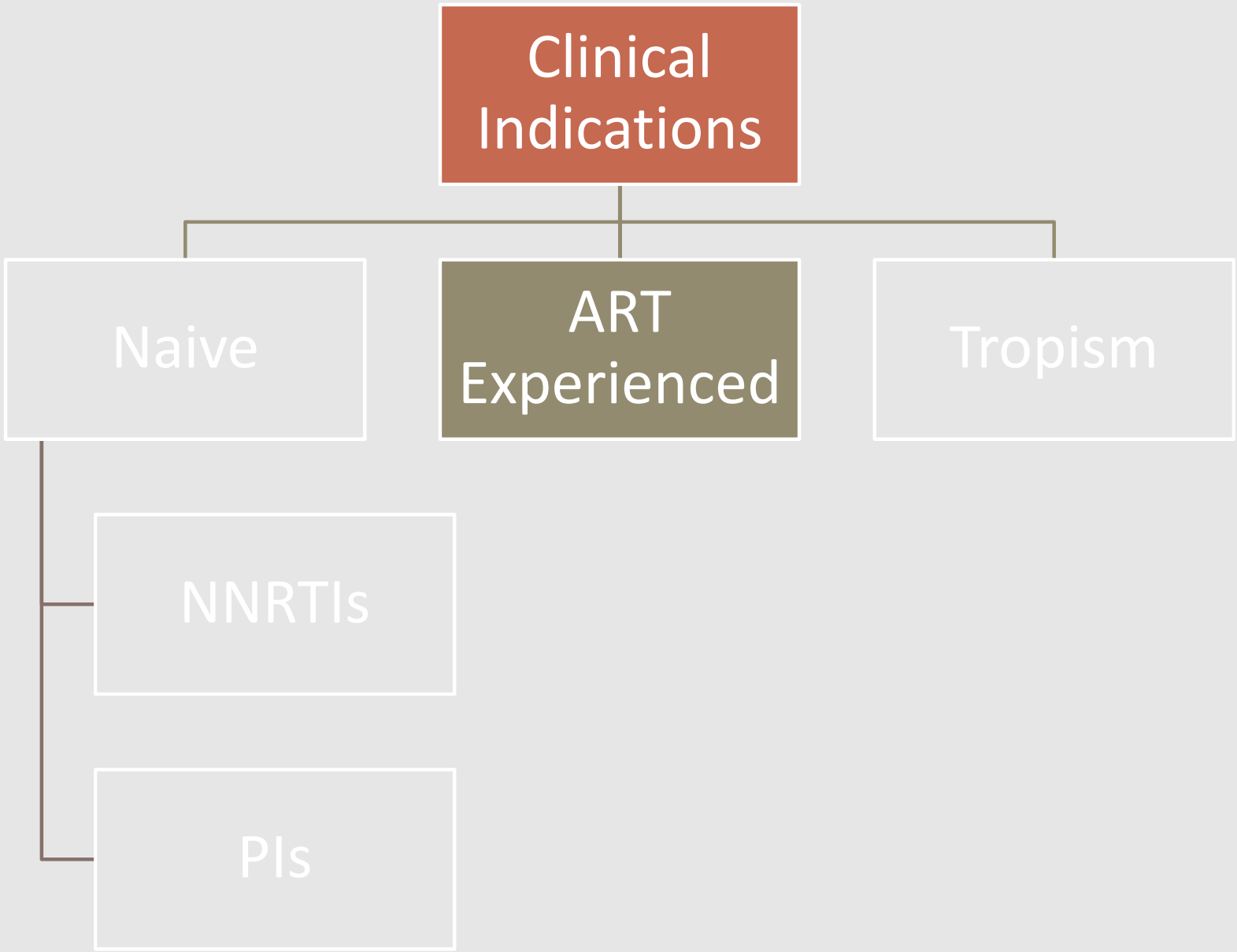
Clinical Indications
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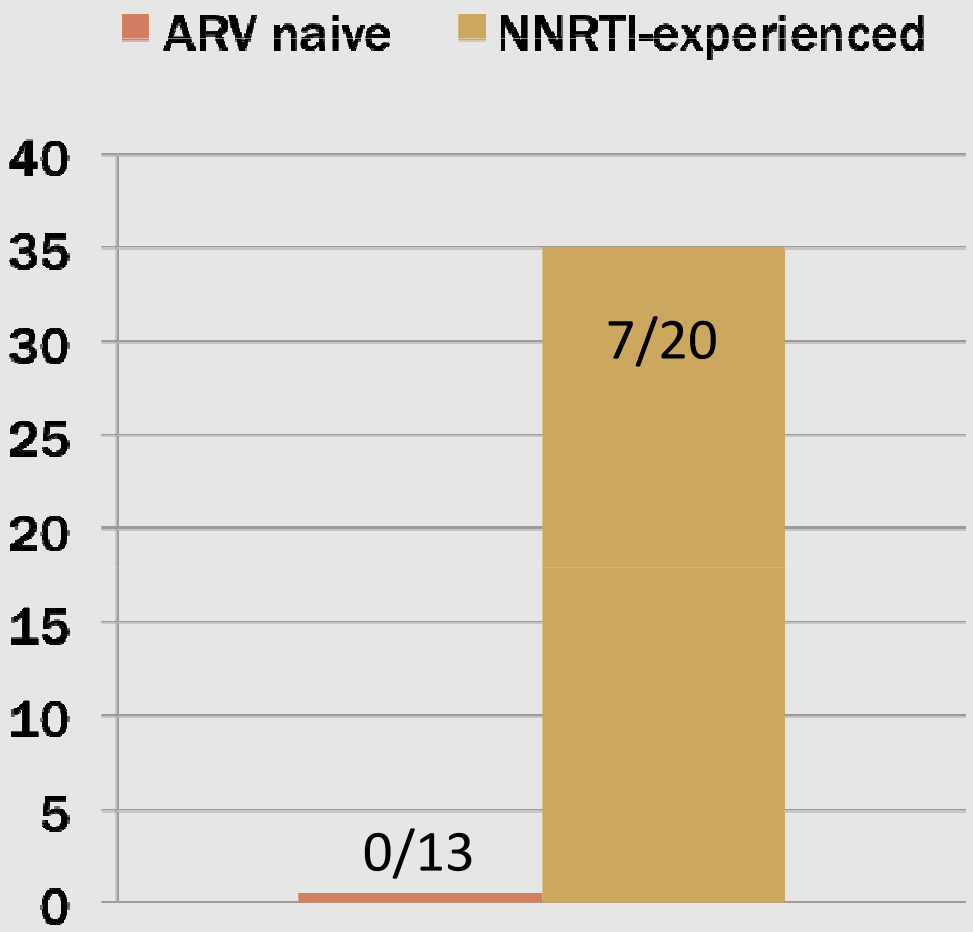
Tropism
NNRTIs
PIs

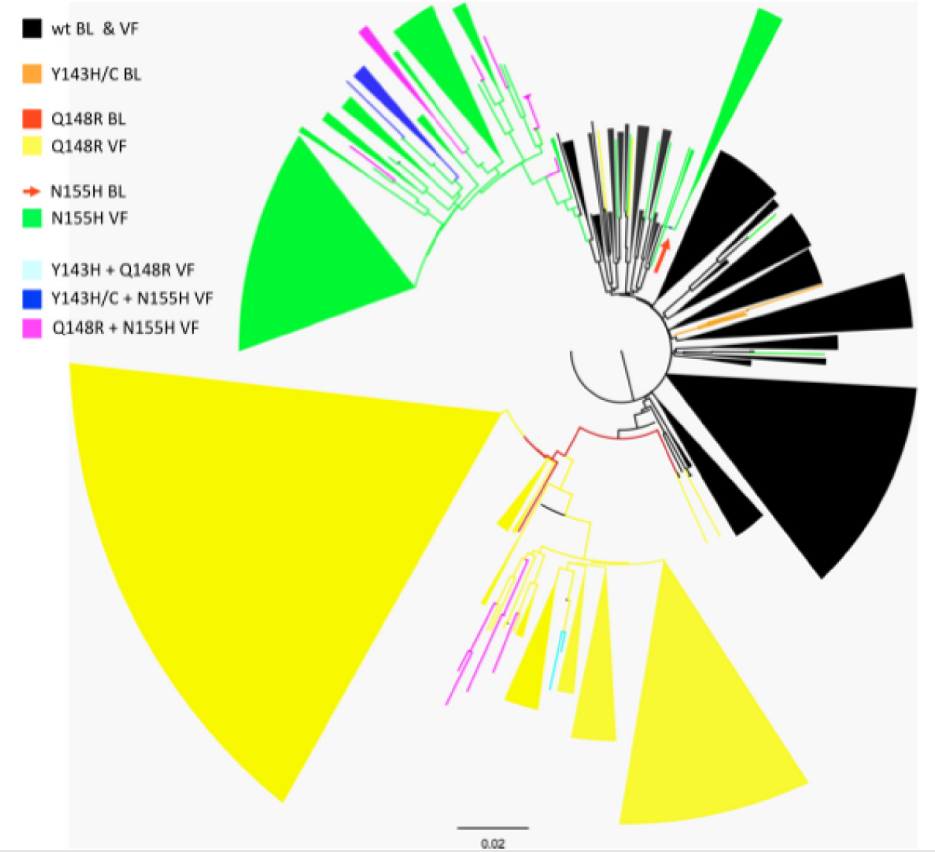
ETR MUTATIONS IN ARV-NAIVE VS. NNRTI-EXPERIENCED

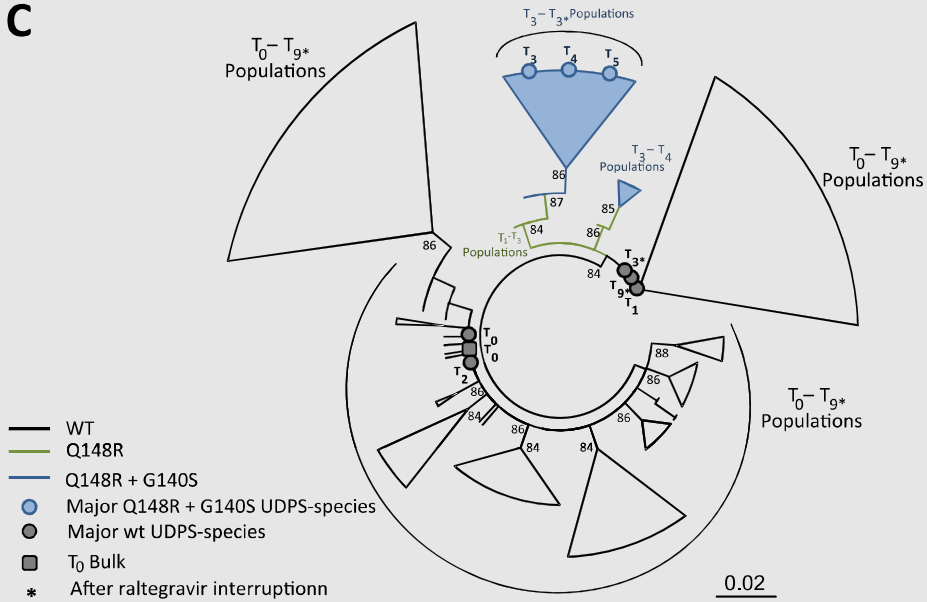


MUTATIONS (LEVEL)
Y181C (7%)
Y181C (3.6%) + G190A (3.2%)
L100I (14%)
L100I (32%) + G190A (5.4%)
K101E (3.8%) + G190A (4.9%)
K101E (4.0%) + G190A (4.8%)
G190S (3.1%)

Varguese V, et al, JAIDS 2009

EMERGING RAL-RESISTANT MUTANTS ORIGINATE FROM PRE-EXISTING VIRUSES





Codoñer et al, Antiv Res 2010; Armenia D et al., JID 2012

Detection of Minority HIV-1 Drug-Resistant Variants Moderately Improves the Prediction of Salvage Antiretroviral Therapy Outcomes: The PRIUS Study

C Pou[1], M Noguera-Julian[1], S Pérez-Álvarez[1], F García[2]; R Delgado[3]; D Dalmau[4], M Álvarez-Tejado[5], C Rodríguez[1], JR Santos[2], B Clotet[1;2], R Paredes[1;2]

[1]IrsiCaixa AIDS Research Institute, Hospital Universitari Germans Trias i Pujol, Badalona, Spain,[2]Hospital Universitario San Cecilio, Granada, Spain, [3]Hospital 12 de Octubre, Madrid, Spain, [4]Hospital Universitari Mútua de Terrassa, Spain, [5]Roche Diagnostics, SL, Spain, [6]HIV Unit, Hospital Universitari Germans Trias i Pujol , Badalona, Spain

International HIV Drug Resistance Meeting, Sitges 2012

PRIUS STUDY

DESIGN

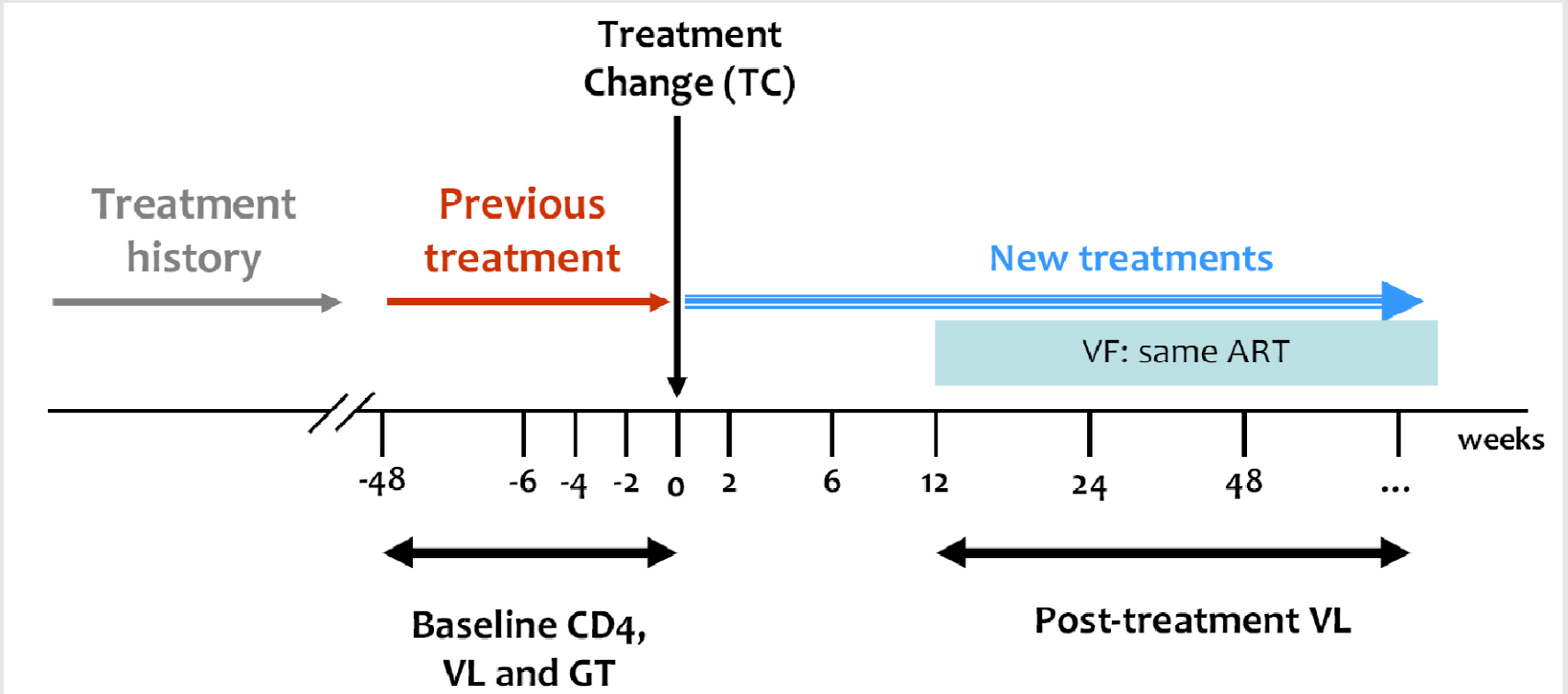
- Retrospective, multicenter cohort study in Badalona, Madrid, Terrassa and Granada, Spain (Clinicaltrials.gov ID: NCT01346878)

SUBJECTS

- ART-experienced adults
- Initiating salvage ART including PI/r, raltegravir (RAL) or etravirine (ETR)
- HIV-1 RNA (VL) ≥5000 copies/mL and 1 mL of plasma available for testing within 12 months before treatment change (TC)
- Clinical follow-up available through at least 48 weeks after TC
- Good adherence to therapy in clinical records

Pou C et al. IHHDRW 2012

PRIUS STUDY



Virological Failure defined as:

- 2 consecutive measurements of VL >200 copies/ml
- ≥week 12 (3rd month) after a treatment change (TC)
- with no changes on ART

Pou C et al. IHHDRW 2012

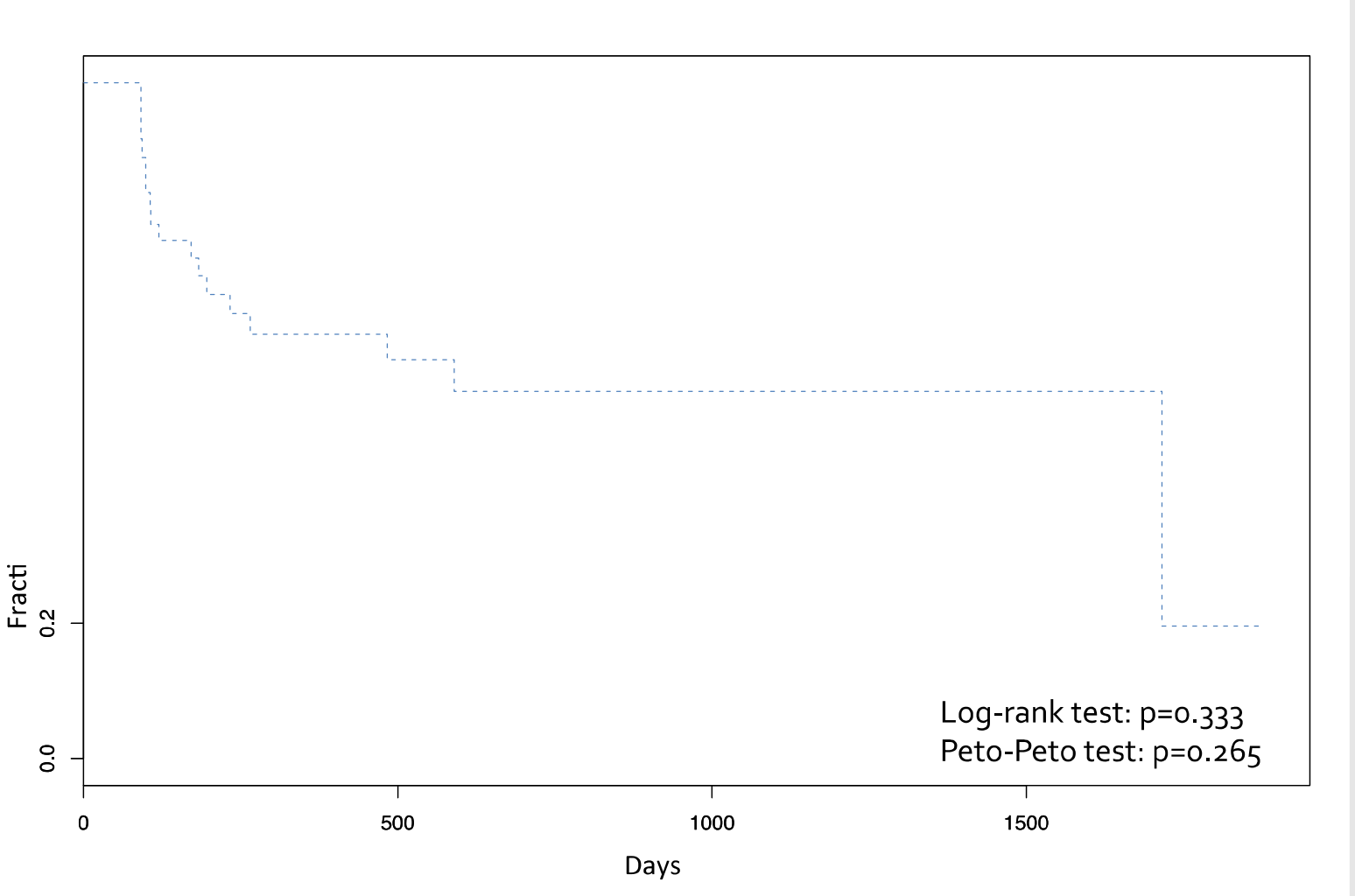
PRIUS STUDY

- 146 subjects.
- Pre-TC genotypes were obtained a median of 48 (0;135) days before TC.
- Virological outcomes evaluable in 138 individuals.
- 41% developed VF

Gender, %			Age at diagnosis, years, median (IQR)	29 (24;33)
Female	26		Age at TCE, years, median (IRQ)	43 (38;47)
Male	74		Time since diagnosis, years, median (IRQ)	15 (11;14)
Mode of infection, %			Follow-up, years, median (IQR)	13 (9;15)
HTS	14		Number of previous drugs, median (IQR)	13 (9;17)
MSM	25		CD4, cells/mm^3, median (IQR)	
IVDU	36		Nadir	39 (26;177)
Transfusion	1		Baseline	232 (104;388)
Unknown	24		Baseline VL, c/mL, median (IQR)	39905 (17,000; 100,665)
Prior AIDS, %	64			

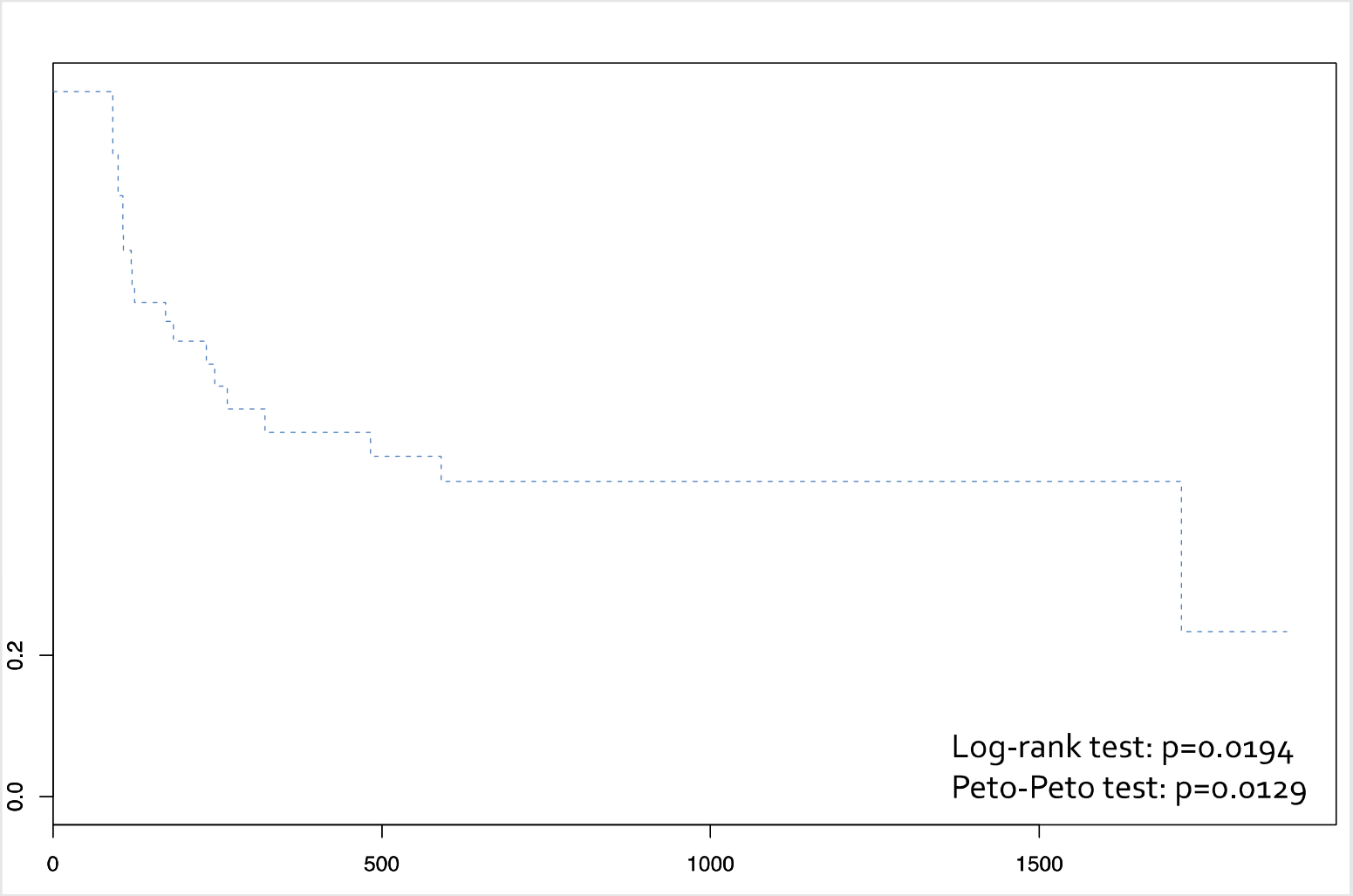
Pou C et al. IHHDRW 2012

PRIUS STUDY



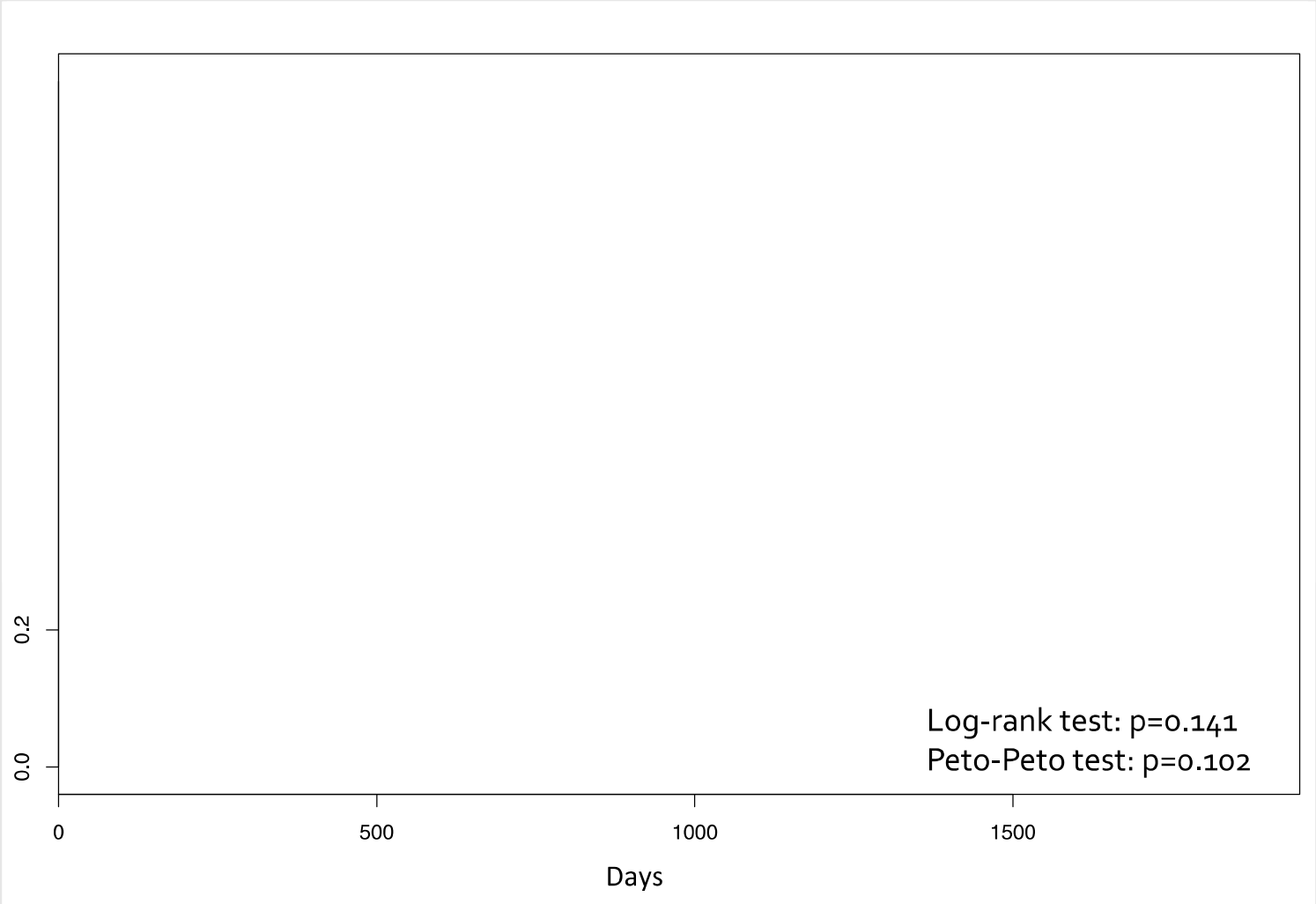
Pou C et al. IHHDRW 2012

PRIUS STUDY

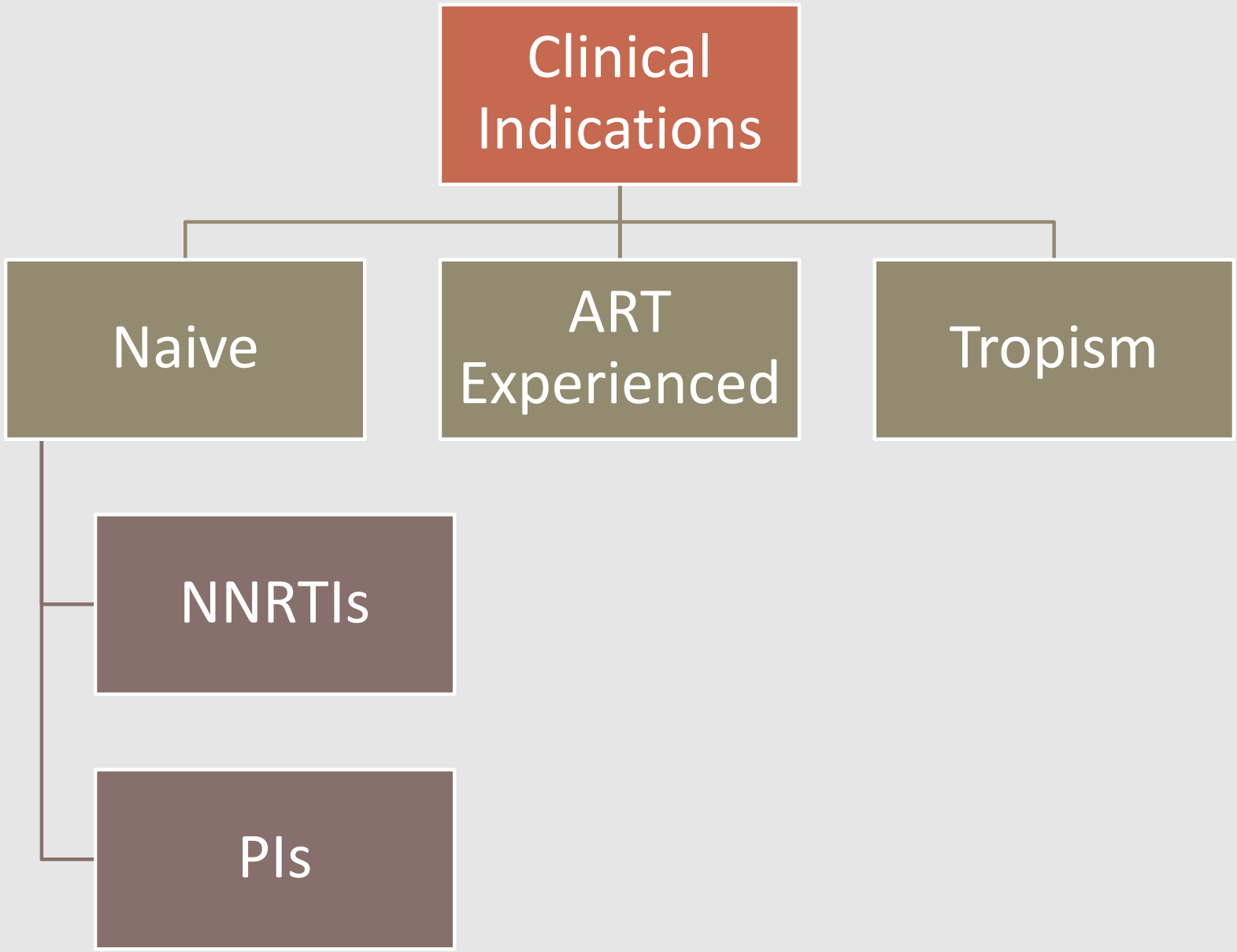


Pou C et al. IHHDRW 2012

PRIUS STUDY



Pou C et al. IHHDRW 2012


Clinical Indications
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ART Experienced
Tropism
NNRTIs
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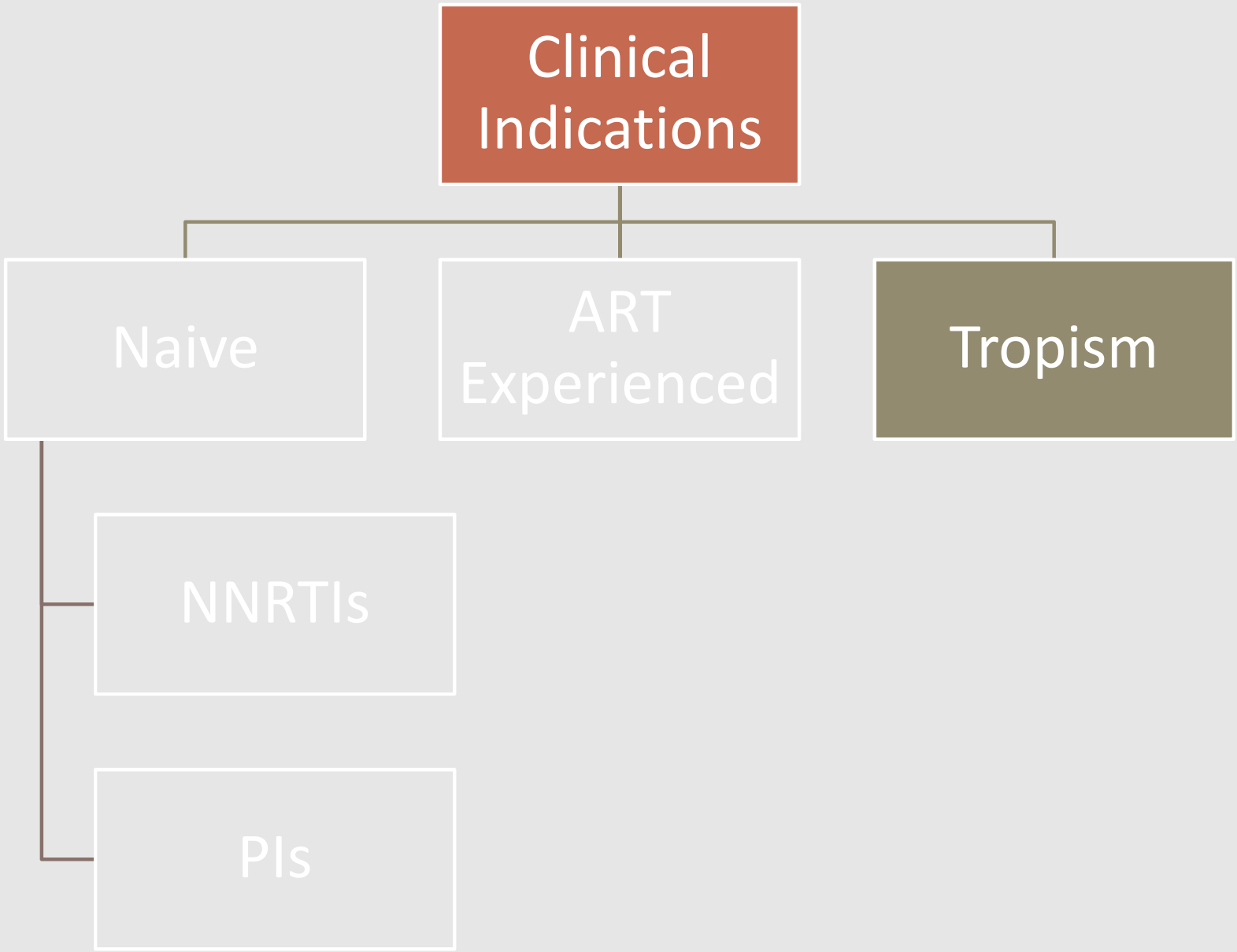
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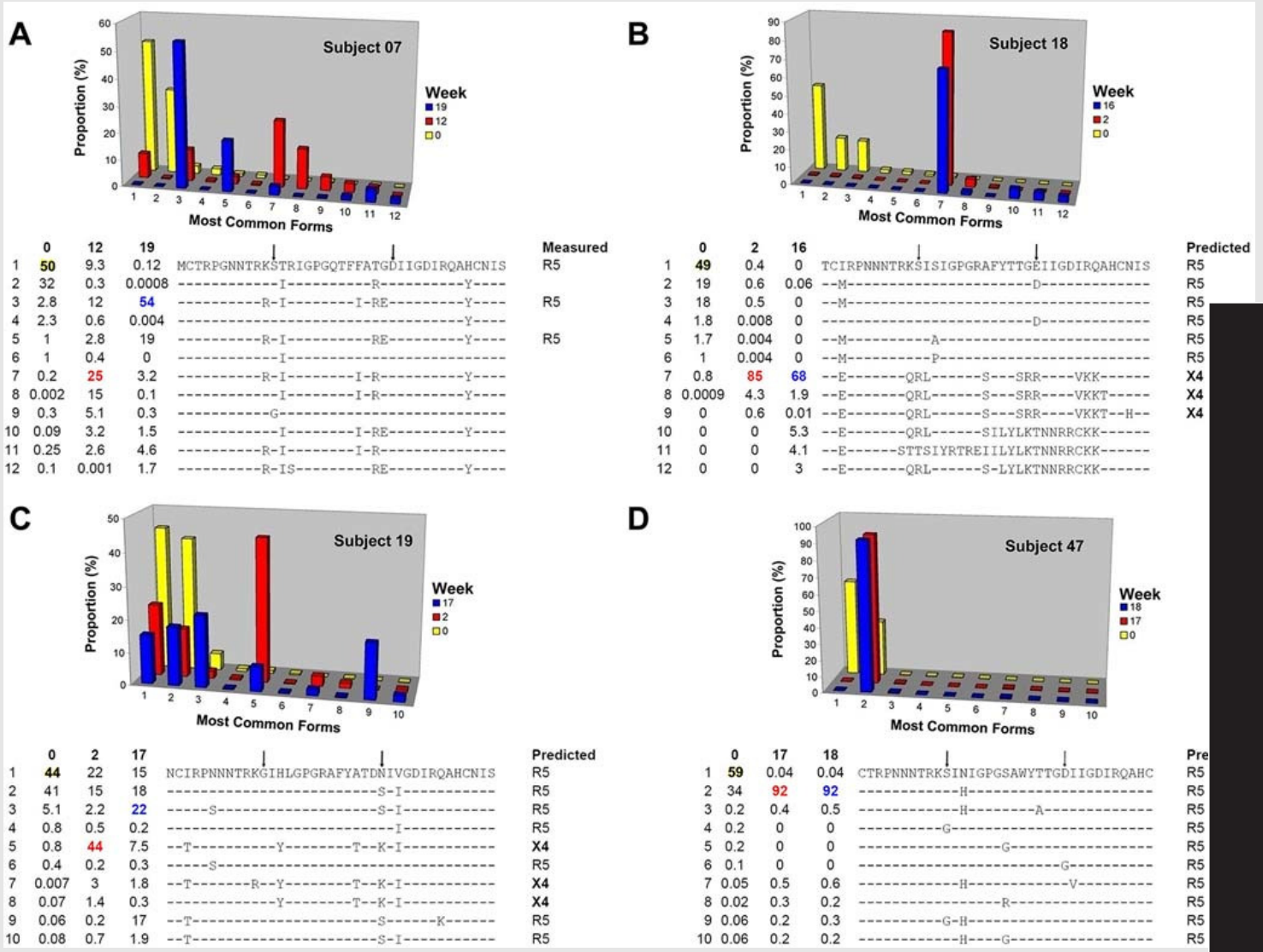
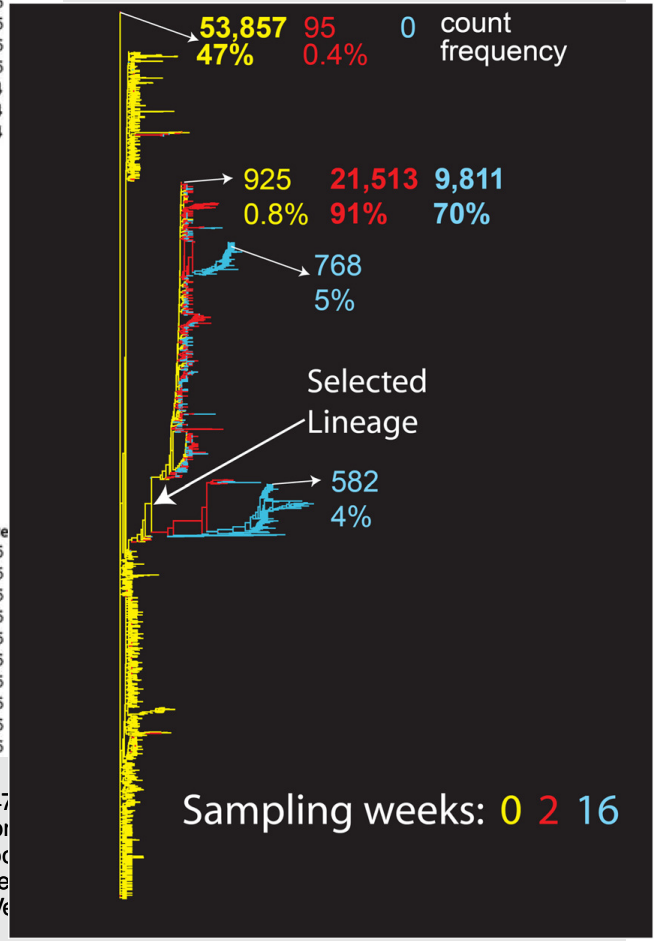


Figure 2. Longitudinal changes in V3 loop forms and proportions. (A) Subject 07, (B) Subject 18, (C) Subject 19, and (D) Subject 47 most common V3 loop sequences across all three time points are numbered and displayed along the x-axis of the 3D-bar graph; correspon amino acid sequences are shown below the graphs. The relative contribution of each sequence is plotted on the y-axis and displayed as a propo of the total population. Time in weeks are shown on the z-axis. A coreceptor usage prediction using PSSM is shown for each sequence Coreceptor usage was measured phenotypically in sub07 by generating recombinant viruses that incorporated each V3 loop sequence. Ve arrows denote positions 11 and 25 in the V3 loop, respectively.

doi:10.1371/journal.pone.0005683.g002



Tsibris AMN, et al, PLoS One 2009

Pou C et al. High Resolution Tropism Kinetics by Quantitative Deep Sequencing in HIV-1 Infected Subjects Initiating Suppressive First-Line Antiretroviral Therapy. CROI 2010

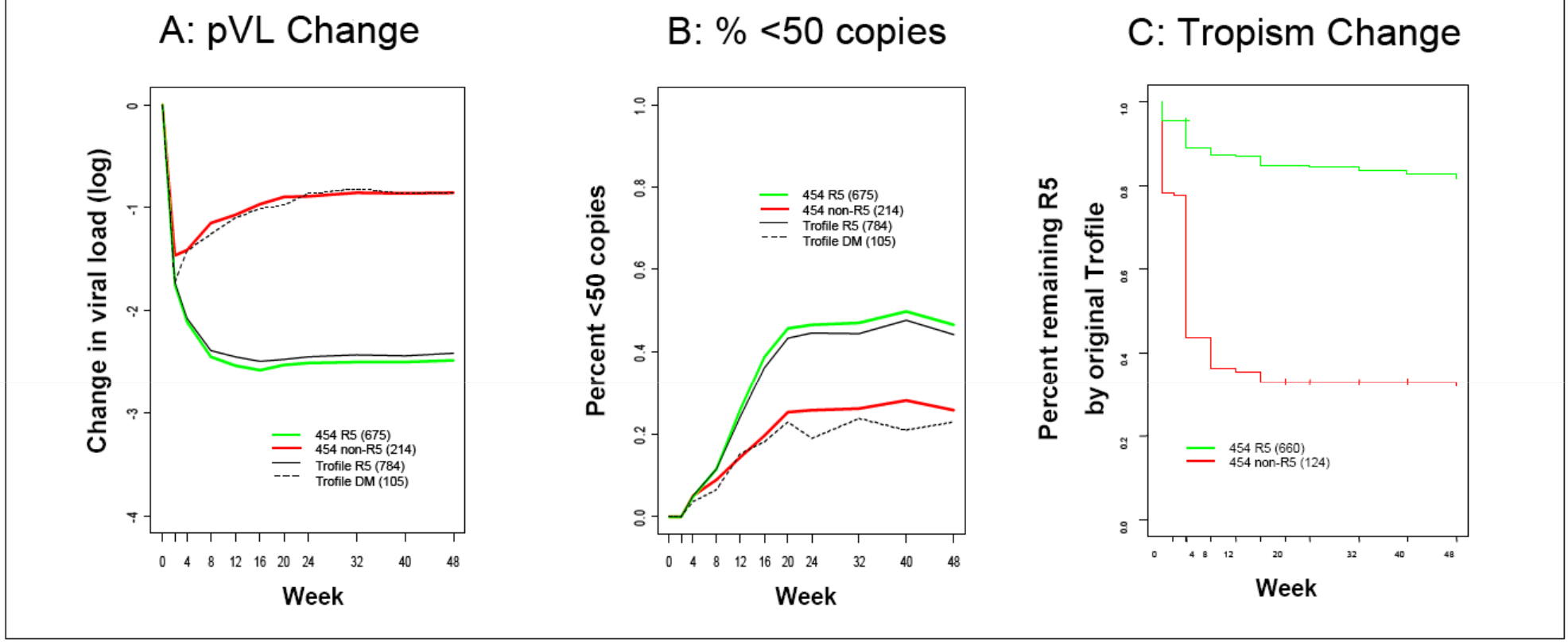
	PLASMA			PBMC		
	ESTA	PS*	QDS**	PS*	QDS**	MT2
Sensitivity	-	36.4	90	36.3	90.9	45.5
Specificity	-	94.4	82.4	100	55.6	100
PPV	-	80	75	100	55.6	100
NPV	-	70.8	93.3	73.1	90.9	76

* FPR 20%

** FPR 10%, cut-off X4: 1%

V3-454 IN MERIT & MOTIVATE & 1029

Figure 3: Virological Response of Treatment-Experienced Patients in MOTIVATE-1, -2, and A4001029



Swenson et al. JID 2011

SUMMARY

Setting	Clinical Value of Minority Variants	Degree of Evidence
ARV-naive initiating EFV or NVP	Added value	IIa
ARV-naive initiating ETV, RPV or LSV	No added value – underpowered	IIb
ARV-naïve initiating PI/rtv	No added value – underpowered	III
ARV-experienced	Potential added value - To be proven	III
HIV-1 Tropism	Added value	IIa

SHORTER HANDS-ON TIME IS REQUIRED

Steps	Ultra-deep sequencing protocol	Time/sample (h)		TRUGENE HIV-1 Genotyping	Time/sample (h)
Sample preparation	High Pure Viral Nucleic Acid Large Volume Kit	2		MagNA Pure Compact Nucleic Acid Isolation Kit I	0.75
Amplification	cDNA generation and PCRs	4		RT-PCR	4
Purification	Agencourt AMPure kit	12	1	–	–
Quantitation	PicoGreen measurement	2	1	–	–
Dilution	Dilution and pooling	4		–	–
Sequencing	Emulsion-PCR and pyrosequencing	11		Sequencing and electrophoresis	4
Data analysis	Amplicon Variant Analyzer software and Stanford University HIV database	2		OpenGene DNA system software Guidelines 15.0	0.5

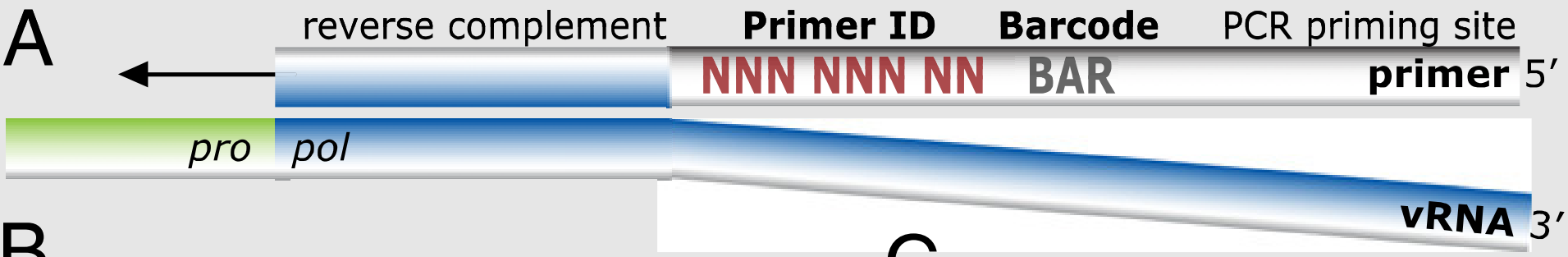
37 h 25 h 9.25 h

irsiCaixa (96 samples)

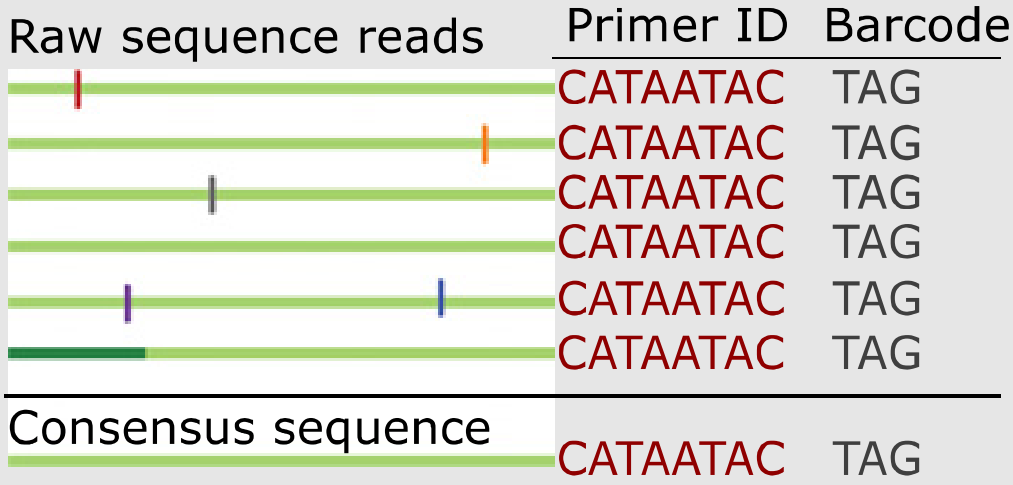
E. Stelzl et al. / Journal of Virological Methods 178 (2011) 94–97

PRIMER-ID HELPS QUANTIFYING INITIAL COPY NUMBERS FOR SINGLE AMPLICONS

A



B



C

Sample	T1	T2	T3
Ritonavir	-	-	+
Total reads	20,429	24,658	27,075
Consensus sequences	857	1,609	2,213

Jabara CB, et al PNAS 2011; 108:20166–71.

OPEN ACCESS Freely available online

PLoS one

HIV Drug Resistance Surveillance Using Pooled Pyrosequencing

Hezhao Ji[1], Nathalie Massé[1], Shaun Tyler[2], Ben Liang[3], Yang Li[1], Harriet Merks[1], Morag Graham[2,3], Paul Sandstrom[1], James Brooks[1]*

1 National HIV and Retrovirology Laboratories, National Microbiology Laboratory, Public Health Agency of Canada, Ottawa, Canada, **2** Genomics Core Facility, National Microbiology Laboratory, Public Health Agency of Canada, Winnipeg, Canada, **3** Department of Medical Microbiology, University of Manitoba, Winnipeg, Canada

96 specimens from HIV+ ART-naive patients tested for PR resistance with Sanger and Pooled 454 genotyping

Ji H, et al, PLoS One 2010

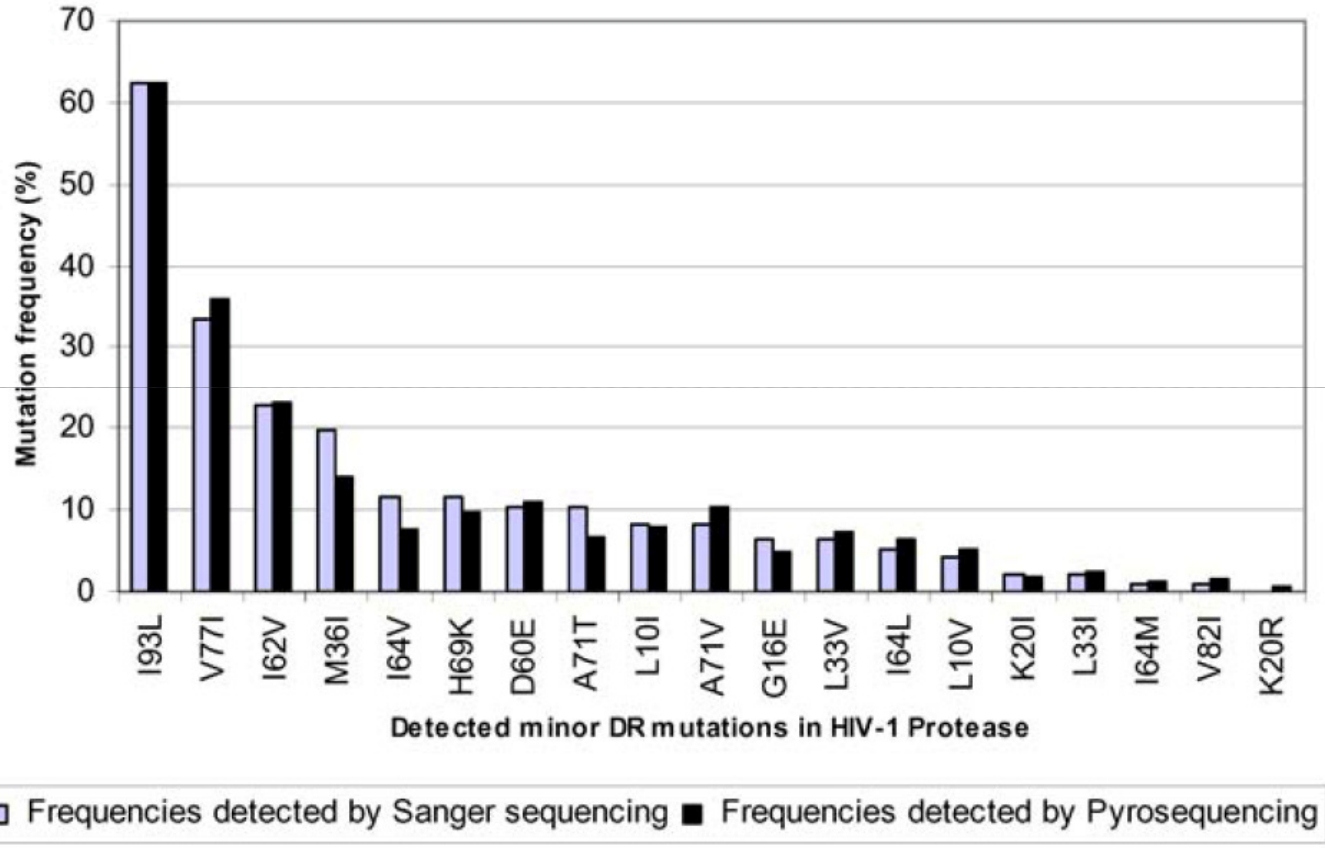


Figure 5. Consistent and comparable frequency readouts for minor protease DRMs by the two approaches. Eighteen minor DR mutations (IAS-USA 2008) were detected by either pyrosequencing or Sanger sequencing among the 96 specimens. Individual mutations are plotted against thee frequency detected by each Chart shows the frequency at which the individual mutations were detected by each method.

doi:10.1371/journal.pone.0009263.g005

Ji H, et al, PLoS One 2010

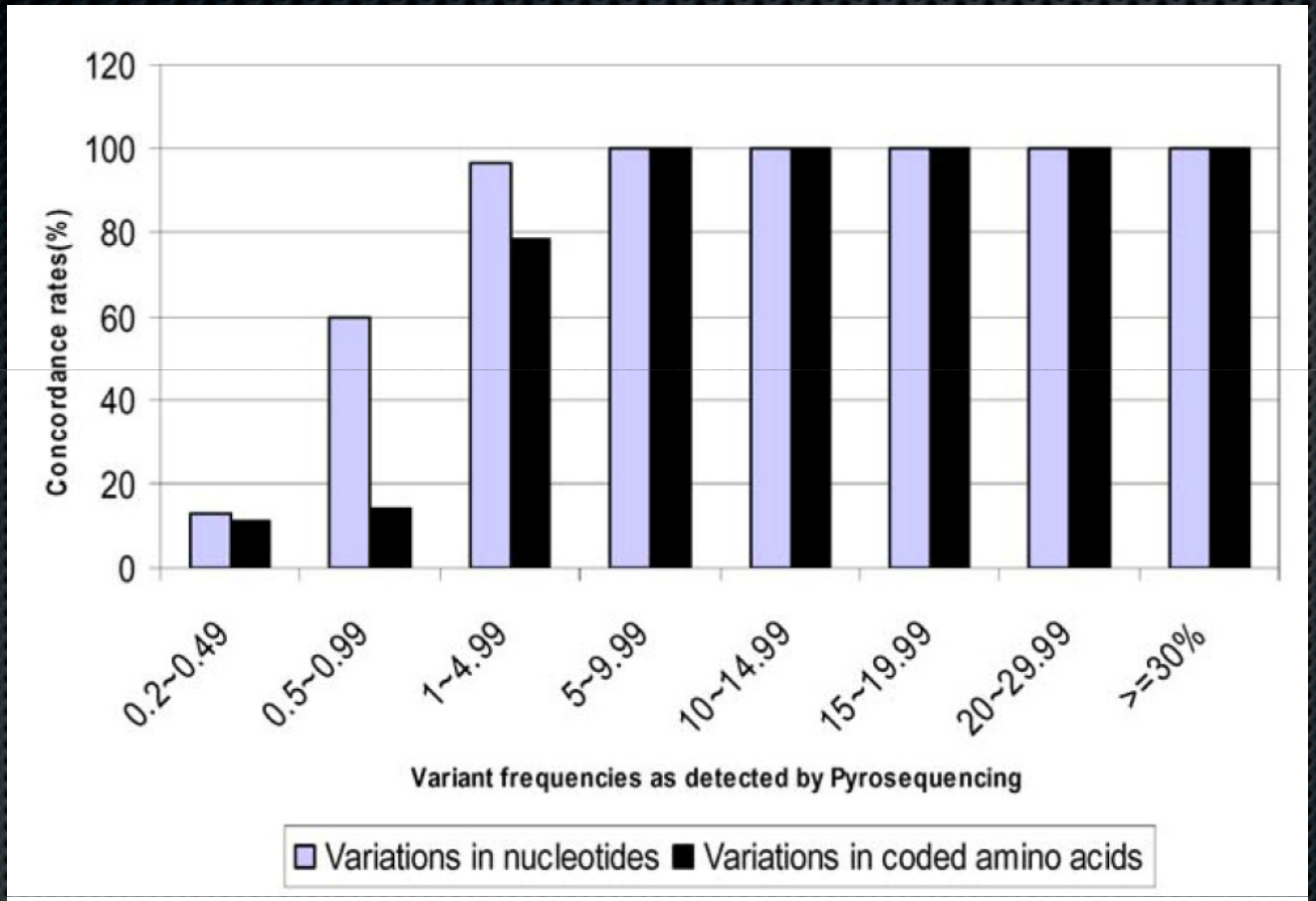


Figure 1. Concordance of variations detection by pyrosequencing and Sanger sequencing. The concordance rates were calculated as percentage of pyrosequencing detected sequence variations that were also observed in Sanger sequencing. Frequency ranges are categorized based on those detected by pyrosequencing.
doi:10.1371/journal.pone.0009263.g001

Ji H, et al, PLoS One 2010

Table S3 Comparison of total cost for DR testing of 96 specimens.

	Cost in CDN$			
	Sanger sequencing (PR only)	Sanger sequencing (PR+RT)	Pyro-sequencing (PR only)	Pyro-sequencing (PR +RT)*
Labour cost / specimen	$4.17	$6.13	$6.39	$9.11
Material Cost/specimen	$47.90	$76.22	$26.07	$43.64
Total cost/specimen	$52.07	$82.35	$32.46	$52.75

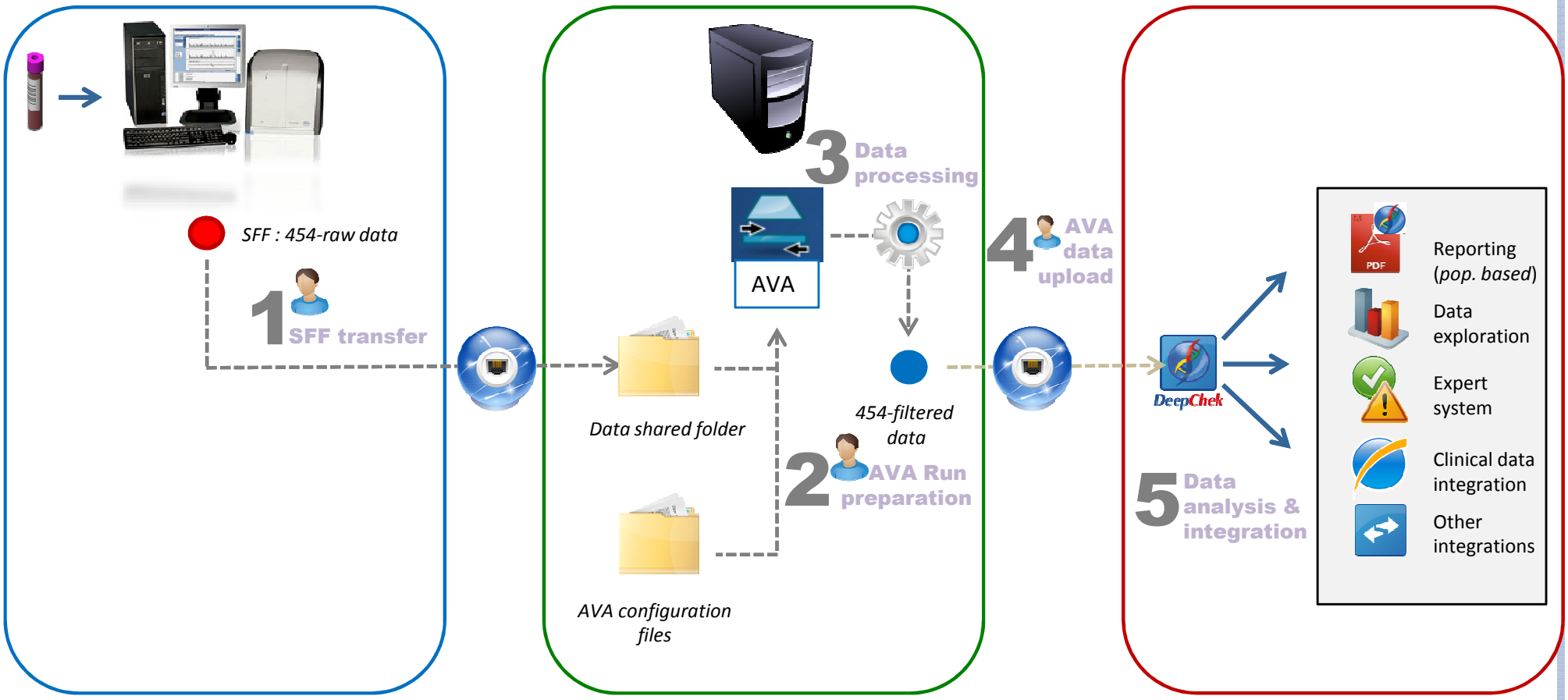
Ji H, et al, PLoS One 2010

THE WAY TO THE CLINIC

NEEDS TO BE MET TO REACH THE CLINIC

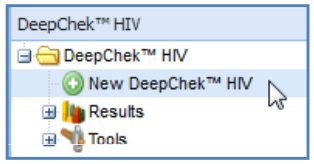
More clinical science	
	Well-powered studies in treatment-naïve and experienced subjects
	Incorporation of minority variant information to drug resistance interpretation algorithms
	PCR adjustments to enable mutational linkage studies / PCR-free methods
	Role of new platforms: Illumina miSeq, PacBio, IonTorrent, Oxford Nanopore
Increased technical robustness	
	HIV-specific quality control panels to certify laboratories
	Clear definition of technical cut-offs: alerts if coverage is not reached
	Quantification of initial copy numbers of genetic material amplified, RT-PCR, ddPCR, primerID
Industrial streamlining	
	Reduce hands-on time and automatize steps, particularly in 454 library/emPCR preparation
	Improve turn-around time to results
	Incorporate validated drug resistance and tropism interpretation systems
Reduce costs: UDS costs must be competitive with population sequencing	

DeepChek®-HIV Methodology

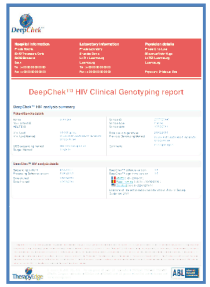


DeepChek®-HIV Data Workflow

Configuration — Load data — Sample information — Setup report — Results



Configuration → Sequences data entry → Data management → Setup report → Analysis



5 to 10 minutes

- Type of use : Diagnostics /Research
- Type of entry : sample alignment/plate raw data (v2)
- Type of alignment (consensus/individual reads)
- Type of genotyping method
- Type of subtyping method
- Options: Sanger comparative analysis…
- Data source: file upload / integration with sequencer (v2)

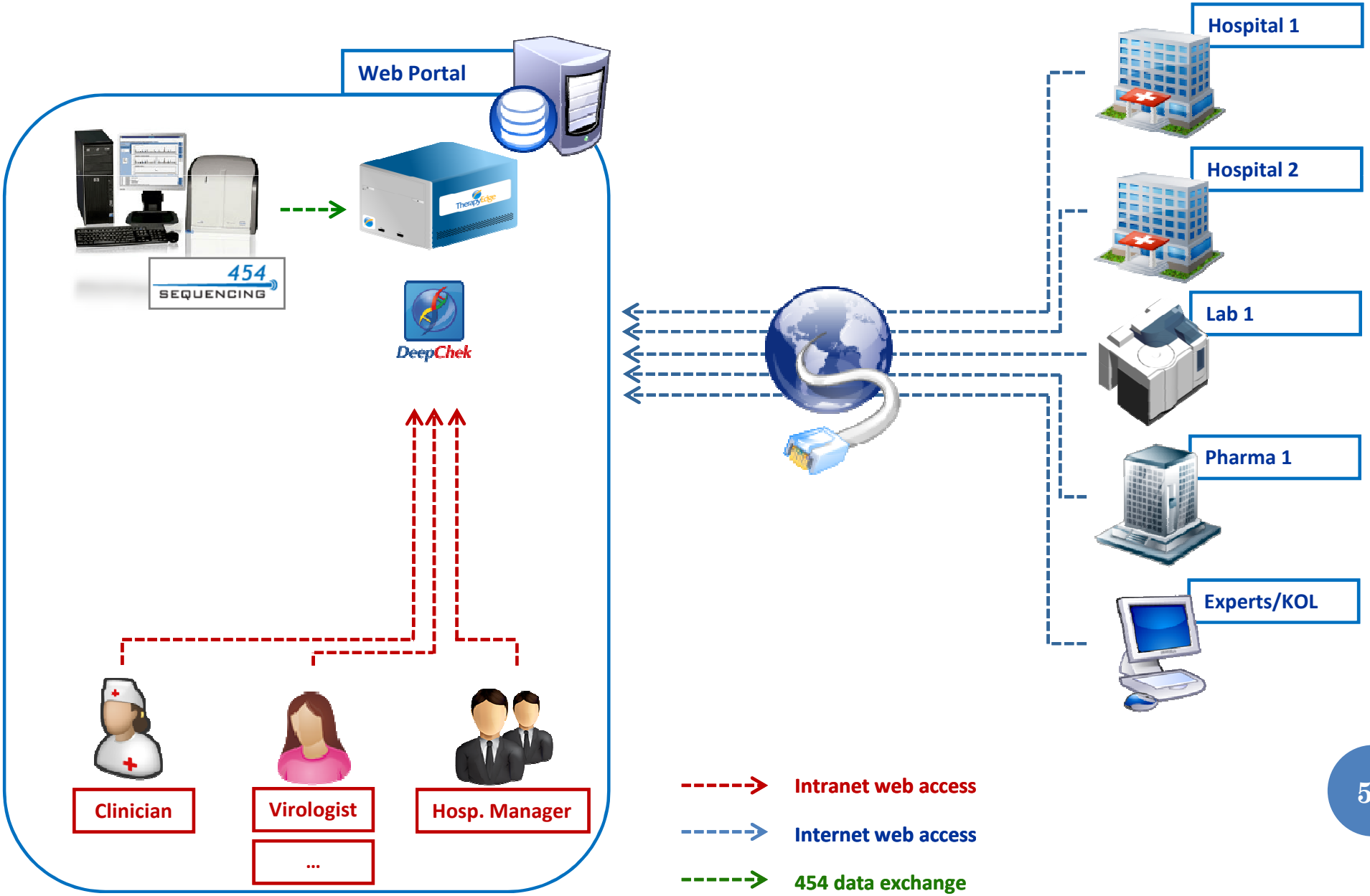
- *NGS* data:
  - PROT
  - RT
  - INT
  - GP41
  - GP120
  - V3
- Sanger data
  - PROT
  - RT
  - INT
  - GP41
  - GP120/V3

- Identifiers
- Sample information
- *NGS* information
- Patient information
- Clinical data
  - Regimen
  - Viral load
  - …
- Physicians details
- Healthcare providers
- *NGS* data management :
  - Thresholds definition

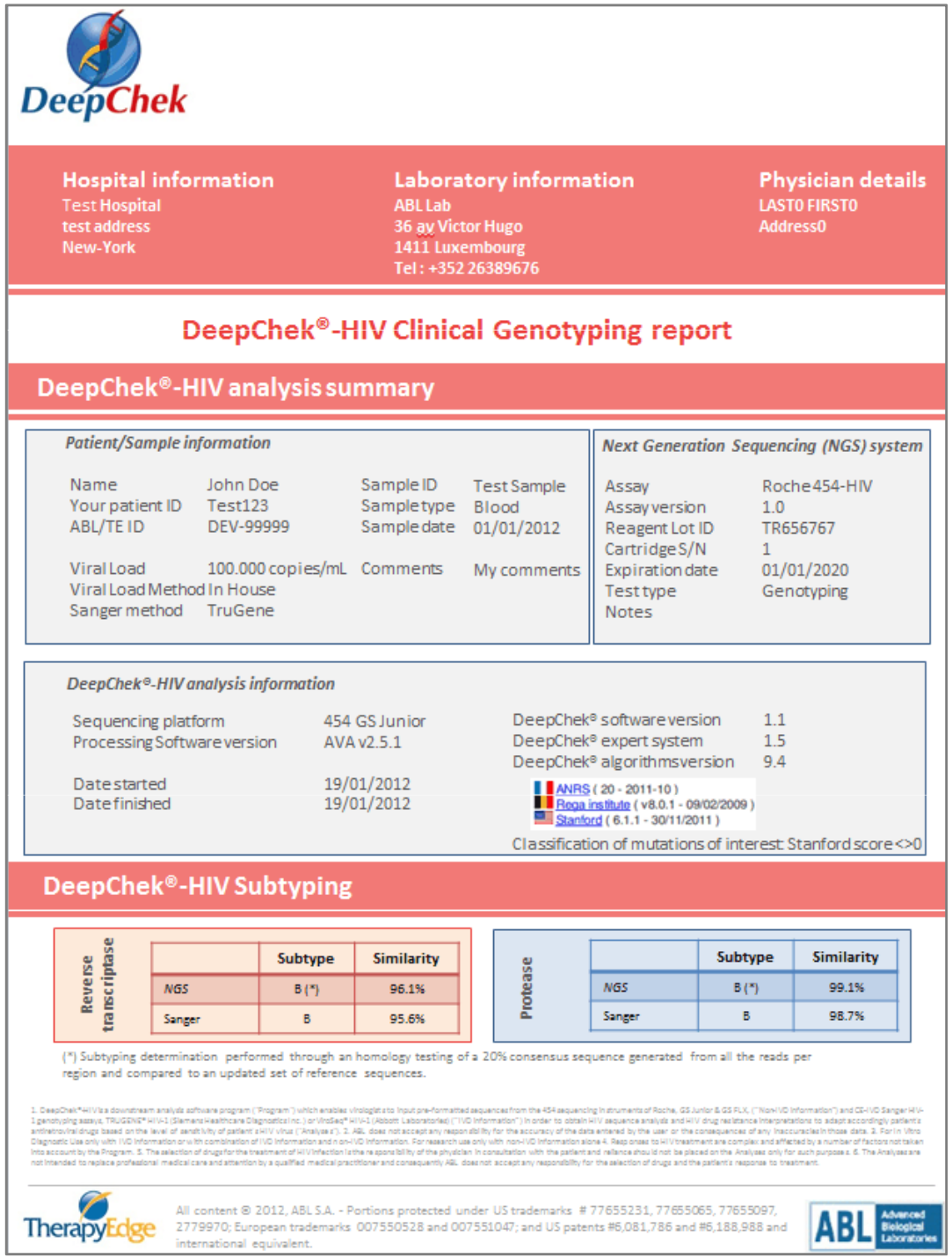
- Algorithms selection
  - ANRS
  - CHL
  - HIV-Grade
  - Rega
  - RenaGeno
  - RIS
  - HIVdb
- Services
  - Geno2Pheno Tropism
  - ViroType
- Report configuration
  - Language
  - GSS cutoffs definition
  - Mutations display
  - Mutational load
  - Disable Expert System
  - Comments

- *NGS* reads alignment analysis
- NGS data QA/QC (DeepChek Expert System)
- Sanger data analysis and QA/QC
- Mutations frequency
- Resistance testing
- Subtyping
- Miscellaneous analysis
  - Coverage
  - FW/RV balance
  - classification mutations of interest
  - Contaminati on check
- DeepChek reporting
- Data storage

CONNECTIVITY



DEEPCHEK-HIV V1.1 REPORT



DeepChek

Hospital information
Test Hospital
test address
New-York

Laboratory information
ABL Lab
36 av Victor Hugo
1411 Luxembourg
Tel : +352 26389676

Physician details
LAST0 FIRST0
Address0

DeepChek®-HIV Clinical Genotyping report

DeepChek®-HIV analysis summary

Patient/Sample information

Name	John Doe	Sample ID	Test Sample
Your patient ID	Test123	Sample type	Blood
ABL/TE ID	DEV-99999	Sample date	01/01/2012
Viral Load	100.000 copies/mL	Comments	My comments
Viral Load Method	In House		
Sanger method	TruGene		

Next Generation Sequencing (NGS) system

Assay	Roche 454-HIV
Assay version	1.0
Reagent Lot ID	TR656767
Cartridge S/N	1
Expiration date	01/01/2020
Test type	Genotyping
Notes	

DeepChek®-HIV analysis information

Sequencing platform	454 GS Junior	DeepChek® software version	1.1
Processing Software version	AVA v2.5.1	DeepChek® expert system	1.5
		DeepChek® algorithms version	9.4
Date started	19/01/2012		
Date finished	19/01/2012		

ANRS (20 - 2011-10)
Rega institute (v8.0.1 - 09/02/2009)
Stanford (6.1.1 - 30/11/2011)

Classification of mutations of interest: Stanford score <>0

DeepChek®-HIV Subtyping

Reverse transcriptase

	Subtype	Similarity
NGS	B (*)	96.1%
Sanger	B	95.6%

Protease

	Subtype	Similarity
NGS	B (*)	99.1%
Sanger	B	98.7%

(*) Subtyping determination performed through an homology testing of a 20% consensus sequence generated from all the reads per region and compared to an updated set of reference sequences.

1. DeepChek®-HIV is a downstream analysis software program ("Program") which enables virologist to input pre-formatted sequences from the 454 sequencing instruments of Roche, GS Junior & GS FLX, ("Non-IVD Information") and CE-IVD Sanger HIV-1 genotyping assays, TRUGENE® HIV-1 (Siemens Healthcare Diagnostics Inc.) or ViroSeq® HIV-1 (Abbott Laboratories) ("IVD Information") in order to obtain HIV sequence analysis and HIV drug resistance interpretations to adapt accordingly patient's antiretroviral drugs based on the level of sensitivity of patient's HIV virus (Analyses). 2. ABL does not accept any responsibility for the accuracy of the data entered by the user or the consequences of any inaccuracies in those data. 3. For In Vitro Diagnostic Use only with IVD Information or with combination of IVD Information and non-IVD Information. For research use only with non-IVD Information alone 4. Responses to HIV treatment are complex and affected by a number of factors not taken into account by the Program. 5. The selection of drugs for the treatment of HIV infection is the responsibility of the physician. In consultation with the patient and reliance should not be placed on the Analyses only for such purposes. 6. The Analyses are not intended to replace professional medical care and attention by a qualified medical practitioner and consequently ABL does not accept any responsibility for the selection of drugs and the patient's response to treatment.

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DEEPCHEK-HIV V1.1 REPORT

DeepChek

Patient Name		*Sample ID*	Test123
Your patient ID		*Sample Date*	19/10/2011
ABL/TE ID			

DeepChek®-HIV *NGS* Mutation Analysis

HIV Reverse transcriptase mutations

Position	Mutation	Comparative Sanger-based sequencing	Threshold 20%	Threshold 10%	Threshold 1%	Prevalence (%)	Mutational load (cp/mL)
K20	M				✓	5	5.000
-	R	✓	✓	✓	✓	21.9	21.900
M41	L				✓	1.7	1.700
M184	V	✓		✓	✓	15.1	15.100
-	I				✓	2.7	2.700
P272 (*)	A			✓	Low Coverage	10.2	10.200
-	C				Low Coverage	2.1	2.100
-	R	✓			Low Coverage	-	-

- Mutations with Stanford score <>0
- (*) Position with low coverage for at least one detection threshold
- Low coverage: Codon where the minimum number of required sequences has not been reached for the selected threshold, and drug resistance assessment derived from such position may not be accurate and therefore is not reported.

HIV Protease mutations

Position	Mutation	Comparative Sanger-based sequencing	Threshold 20%	Threshold 10%	Threshold 1%	Prevalence (%)	Mutational load (cp/mL)
V3	I	✓	✓	✓	✓	25	25.000
L10	I				✓	3.2	3.200
-	V				✓	1.75	1.750
K20	R		Low Coverage	Low Coverage	Low Coverage	1.12	1.120
L90 (*)	M	✓		Low Coverage	Low Coverage	17.1	17.100

- Mutations with Stanford score <>0
- (*) Position with low coverage for at least one detection threshold
- Low coverage: Codon where the minimum number of required sequences has not been reached for the selected threshold, and drug resistance assessment derived from such position may not be accurate and therefore is not reported.

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ABL Advanced Biological Laboratories

DEEPCHEK-HIV V1.1 REPORT

DeepChek

DeepChek

Patient Name
Your patient ID
ABL/TE ID

Sample ID Test123
Sample Date 19/10/2011

DeepChek®-HIV *NGS* Drug Resistance Determination

HIV Nucleoside Reverse Transcriptase inhibitors

	Algorithm	Sanger based sequencing	Threshold 20%	Threshold 1%
Zidovudine	ANRS	S	S	S
	Rega institute	S	S	I
	Stanford	S	S	I
Didanosine	ANRS	S	S	S
	Rega institute	R	R	R
	Stanford	I	I	I
Stavudine	ANRS	S	S	S
	Rega institute	I	I	I
	Stanford	I	I	I
Lamivudine	ANRS	R	R	R
	Rega institute	R	R	R
	Stanford	R	R	R
Emtricitabine	ANRS	R	R	R
	Rega institute	R	R	R
	Stanford	R	R	R
Abacavir	ANRS	S	S	I
	Rega institute	S	S	I
	Stanford	I	I	I
Tenofovir	ANRS	S	S	I
	Rega institute	S	S	S
	Stanford	S	S	S

HIV Non-nucleoside Reverse Transcriptase inhibitors

Nevirapine	ANRS	R	R	R
	Rega institute	R	R	R
	Stanford	R	R	R
Delavirdine	ANRS	N/A	N/A	N/A
	Rega institute	R	R	R
	Stanford	N/A	N/A	N/A
Efavirenz	ANRS	R	R	R
	Rega institute	R	R	R
	Stanford	R	R	R
Etravirine	ANRS	S	S	S
	Rega institute	S	S	S
	Stanford	S	S	S
Rilpivirine	ANRS	S	S	S
	Rega institute	N/A	N/A	N/A
	Stanford	S	S	S

	ANRS	Rega Institute	Stanford
S	Susceptible	Susceptible GSS 1 Susceptible GSS 1.5	Susceptible Potential low-level resistance
I	Possible resistance	Intermediate Resistant GSS 0.75 Intermediate Resistant GSS 0.5 Intermediate Resistant GSS 0.25	
R	Resistance	Resistant GSS 0	High-level resistance
N/C	The resistance profile derived from these positions may be inaccurate , therefore the drug resistance assessment is not conclusive		
N/A	Not available		

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DEEPCHEK-HIV V1.1 REPORT

DeepChek

Patient Name
Your patient ID
ABL/TE ID

Sample ID Test123
Sample Date 19/10/2011

DeepChek®-HIV *NGS* Drug Resistance Determination

HIV Protease inhibitors

	Algorithm	Sanger based sequencing	Threshold 20%	Threshold 1%
Nelfinavir	ANRS	R	R	R
	Rega institute	R	R	R
	Stanford	R	R	R
Fosamprenavir/r	ANRS	S	S	S
	Rega institute	S	S	I
	Stanford	I	I	I
Lopinavir/r	ANRS	I	I	R
	Rega institute	S	S	R
	Stanford	I	I	I
Atazanavir/r	ANRS	S	S	R
	Rega institute	I	I	R
	Stanford	I	I	R
Tipranavir/r	ANRS	S	S	S
	Rega institute	S	S	S
	Stanford	S	S	I
Darunavir	ANRS	S	S	S
	Rega institute	S	S	S
	Stanford	S	S	S

Entry inhibitors

Drug	Algorithm	
Maraviroc	Geno2pheno [coreceptor]	*Not yet available*
	Toulouse Tropism Test	
	PSSM	

GSS*

Nevirapine + Zidovudine + Saquinavir	ANRS	1	1	1
	Rega institute	1	1	0.5
	Stanford	1	1	0.5

- (*) GSS: Genotypic Sensitivity Score on selected ARV regimen

	ANRS	Rega institute	Stanford
S	Susceptible	Susceptible GSS 1 Susceptible GSS 1.5	Susceptible Potential low-level resistance
I	Possible resistance	Intermediate Resistant GSS 0.75 Intermediate Resistant GSS 0.5 Intermediate Resistant GSS 0.25	
R	Resistance	Resistant GSS 0	High-level resistance
N/C	The resistance profile derived from these positions may be inaccurate , therefore the drug resistance assessment is not conclusive		
N/A	Not available		

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ABL Advanced Biological Laboratories

DEEPCHEK-HIV V1.1 REPORT

DeepChek

DeepChek

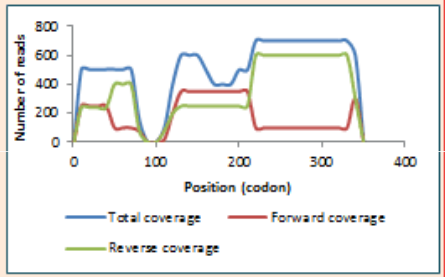
Patient Name
Your patient ID
ABL/TE ID

Sample ID Test123
Sample Date 19/10/2011

DeepChek®-HIV *NGS* Expert system

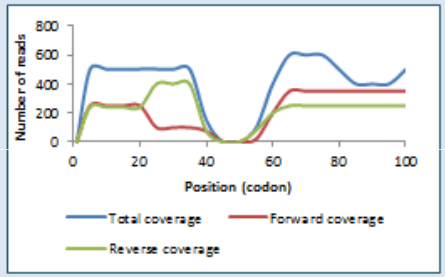
Coverage

Reverse transcriptase



Threshold	Minimum number of required sequences	Low covered positions
20%	25	
10%	50	
1%	500	272

Protease



Threshold	Minimum number of required sequences	Low covered positions
20%	25	20
10%	50	20,90
1%	500	20,90

Discarded mutations

Reverse transcriptase

Reasons excluded	Mutations
Noisy mutations filtering	45 mutations (see details on CSV report)
Forward/Reverse unbalanced frequency	11 mutations (see details on CSV report)

Protease

Reasons excluded	Mutation list
Noisy mutations filtering	12 mutations (see details on CSV report)
Forward/Reverse unbalanced frequency	5 mutations (see details on CSV report)

Reads quality

Reverse transcriptase

	Q1	Q2	Q3
Insertions	0	1	3
Deletions	1	2	5
Stop codons	2	4	7

Protease

	Q1	Q2	Q3
Insertions	0	1	3
Deletions	1	1	4
Stop codons	1	2	4

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DEEPCHEK-HIV V1.1 REPORT

DeepChek

Patient Name
Your patient ID
ABL/TE ID

Sample ID Test123
Sample Date 19/10/2011

DeepChek®-HIV *NGS* Mutation Notes

HIV Reverse transcriptase mutations

Algorithm	Related to	Comments
HIVdb	-	M41L usually occurs with T215Y. Together these mutations confer intermediate-to- high level resistance to AZT and d4T and a lower level of resistance to ddI, ABC, and TDF. M41M is a highly unusual mutation at this position.
HIVdb	-	K103N/ S/ T/ H are NNRTI- resistance mutations. K103R/ E/ Q are variants that usually do not cause NNRTI resistance. K103K is a highly unusual mutation at this position.
Non-described mutations		272F

HIV Protease mutations

Algorithm	Related to	Comments
HIVdb	Tipranavir/r	This sequence has 1 major TPV/r- resistance mutations (I54V). RESIST study (Baxter J et al J Virology 2006 and Scherer J et al EACS 2007).
HIVdb	Tipranavir/r	This sequence has 1 minor TPV/r- resistance mutations (L10V). RESIST study (Baxter J et al J Virology 2006 and Scherer J et al EACS 2007).
HIVdb	-	I54V/ M/ L/ A/ T/ S have diverse effects on multiple PIs. I54I is a highly unusual mutation at this position.
Non-described mutations		36G, 90Y

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DEEPCHEK-HIV V1.1 REPORT

DeepChek

Patient Name		*Sample ID*	Test123
Your patient ID		*Sample Date*	19/10/2011
ABL/TE ID			

DeepChek®-HIV References

References

1. "Low-Frequency HIV-1 Drug Resistance Mutations and Risk of NNRTI-Based Antiretroviral Treatment Failure", Z Li et.al. *JAMA, April 6, 2011—Vol 305, No. 13.*
2. "A Fully Integrated and Simplified HIV Clinical Genotyping Solution Using 454 Ultra-Deep-Sequencing and the DeepChek™-HIV System", D Gonzalez et al. *International Workshop on HIV & HEPATITIS VIRUS – Drug Resistance and Curative Strategies - JUNE 7-11, 2011 | Los Cabos, Mexico.*
3. "Comparison of algorithms that interpret genotypic HIV-1 drug resistance to determine the prevalence of transmitted drug resistance", L Liu et al. *AIDS. 2008 April 23; 22(7): 835–839.*
4. "Genotypic susceptibility scores and HIV type 1 RNA responses in treatment-experienced subjects with HIV type 1 infection." JA Anderson et al. *AIDS Res Hum Retroviruses. 2008 May;24(5):685-94.*
5. "HIV type 1 chemokine coreceptor use among antiretroviral-experienced patients screened for a clinical trial of a CCR5 inhibitor: AIDS Clinical Trial Group A5211." Wilkin TJ, Su Z, Kuritzkes DR, et al. *Clin Infect Dis. 2007;44:591-595.*
6. Deep V3 sequencing for HIV type 1 tropism in treatment-naive patients: a reanalysis of the MERIT trial of maraviroc. Swenson et al. *Clin Infect Dis. 2011 Oct;53(7):732-42.*
7. "Use of the DeepChek®-HIV system in the PRIUS study: validation of a new reliable genotyping solution to streamline the 454 sequencing analysis of HIV drug resistance in routine diagnostics and research applications", R. Paredes et al. *International Workshop on HIV & HEPATITIS VIRUS – Drug Resistance and Curative Strategies - JUNE 5-9, 2012 | Sitges, Spain.*
8. "DeepChek® HIV v1.0., a reliable tool for the bioinformatics analysis and resistance interpretation of Massive Ultra Deep Sequencing of HIV genomes", F. Garcia et al. *10th European meeting on HIV & Hepatitis (Barcelona, March 2012).*
9. "Added value of Ultra Deep Sequencing in patients with HIV-1 Transmitted Drug Resistance mutations in the Reverse Transcriptase", F. Garcia et al. *International Workshop on HIV & HEPATITIS VIRUS – Drug Resistance and Curative Strategies - JUNE 5-9, 2012 | Sitges, Spain..*
10. "Drug resistant minority variants are associated with first-line treatment failure in antiretroviral drug-naive patients", M. Papathanasopoulos et al. *International Workshop on HIV & HEPATITIS VIRUS – Drug Resistance and Curative Strategies - JUNE 5-9, 2012 | Sitges, Spain.*

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Signature

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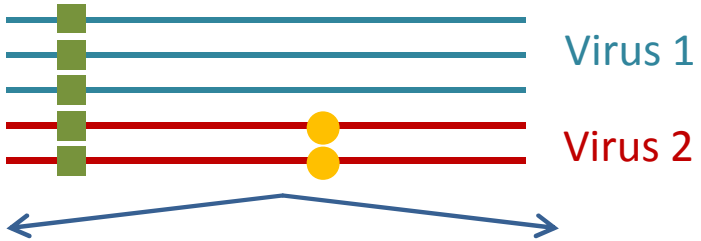
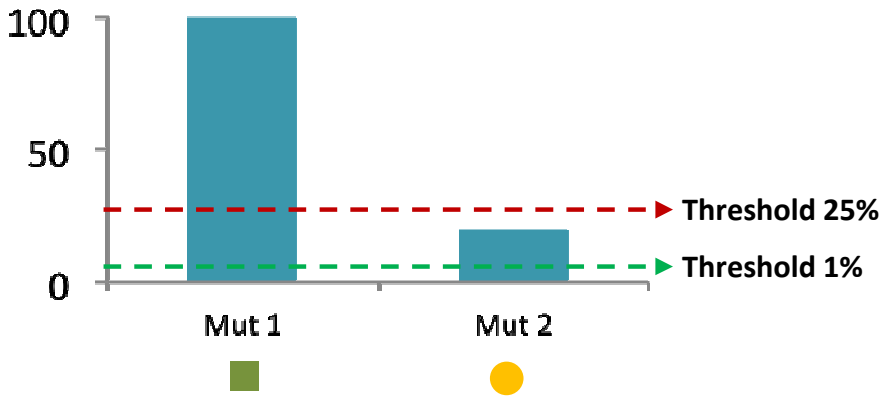
DeepChek-HIV v1.2 – Clonal genotyping

NGS Types of Genotyping Analysis



Variants Population Analysis (from DC v1.1)



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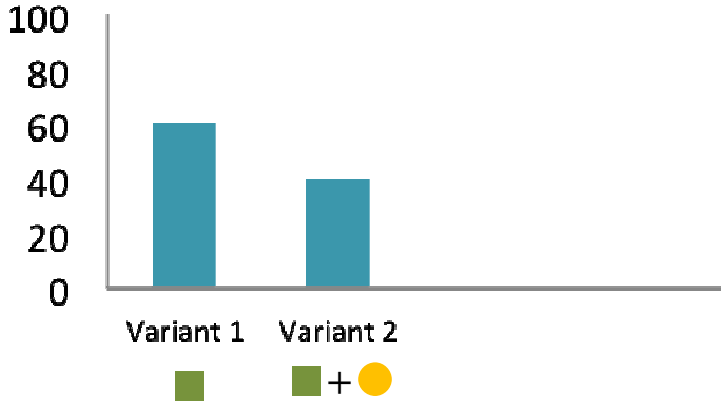


Reporting

Mutations analysis

	25%	1%
■	✓	✓
●		✓

Drug resistance

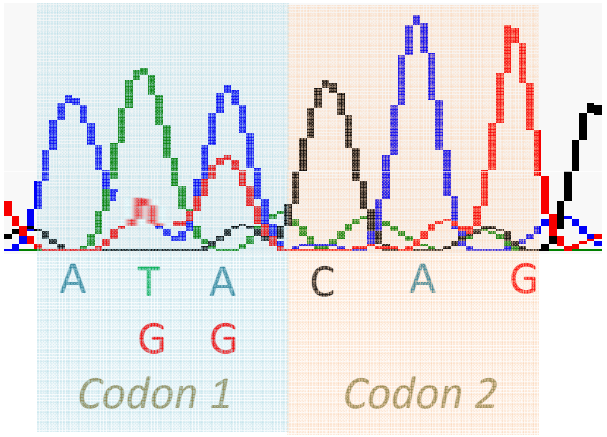
Drug	Algo	25%	1%
3TC	A	S	R
	B	S	I
D4T	A	S	R
	B	S	R

Clonal variants analysis

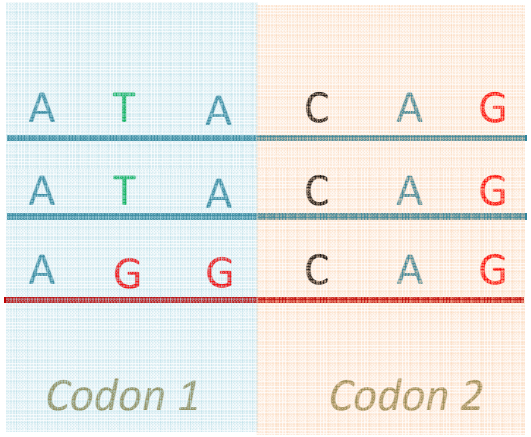
Variant	Freq	Seq	Pattern	FW count	RV count	A 3TC	B 3TC	A D4T	B D4T
1	60%	—	■	2	1	S	S	S	I
2	40%	—	■ + ●	1	1	S	S	R	I

Sanger population based vs. 454/DeepChek variants population based

Sanger population sequencing



454/DeepChek Variants Population sequencing



↓ *Variant calling*

Pos 1	Pos 2
ATA (I)	CAG (Q)
ATG (M)	
AGG (R)	
AGA (R)	

Virtual codons

Mutation list: *1I/M/R, 2Q*

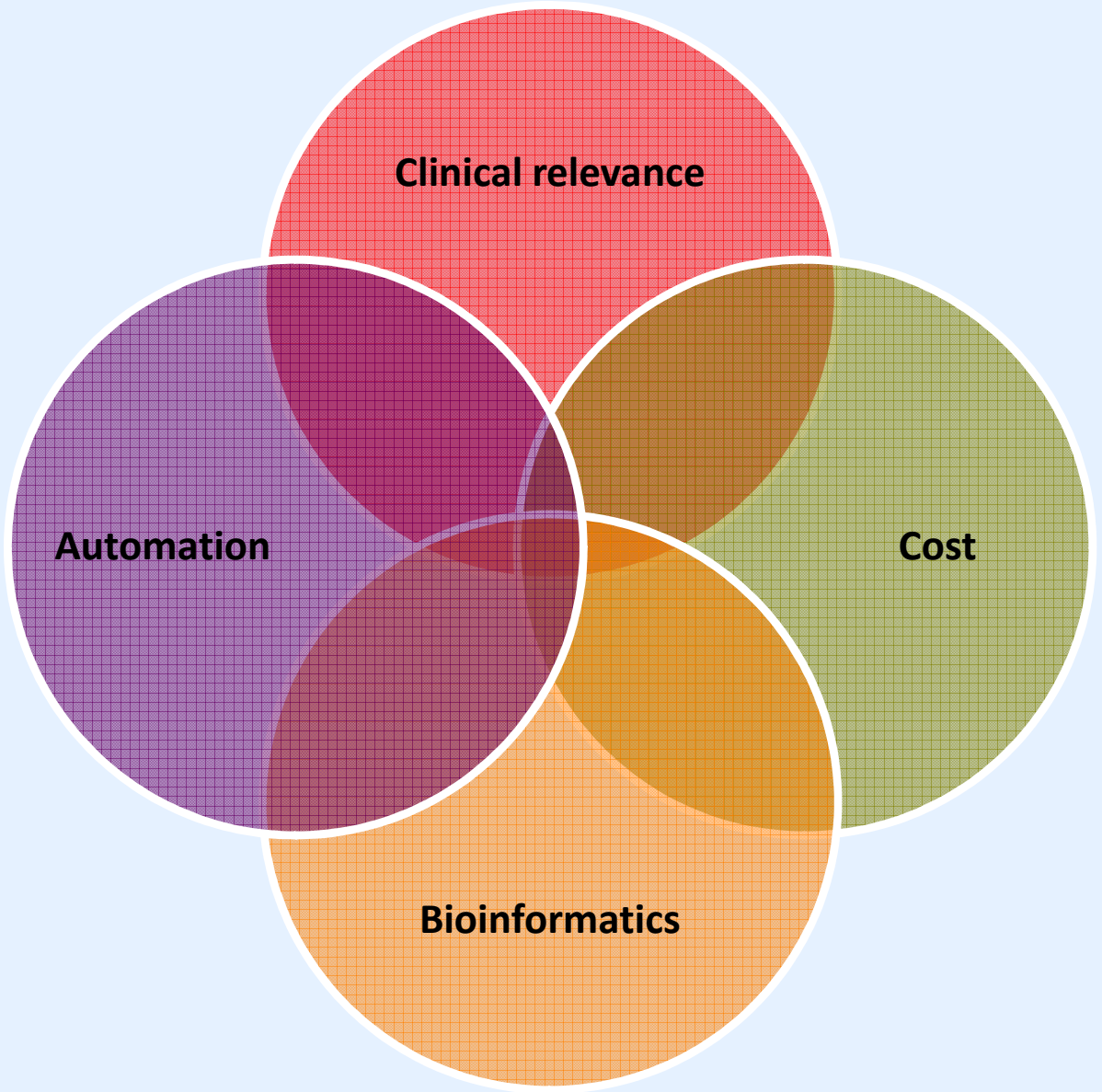
↓

Virtual mutation (false positive)

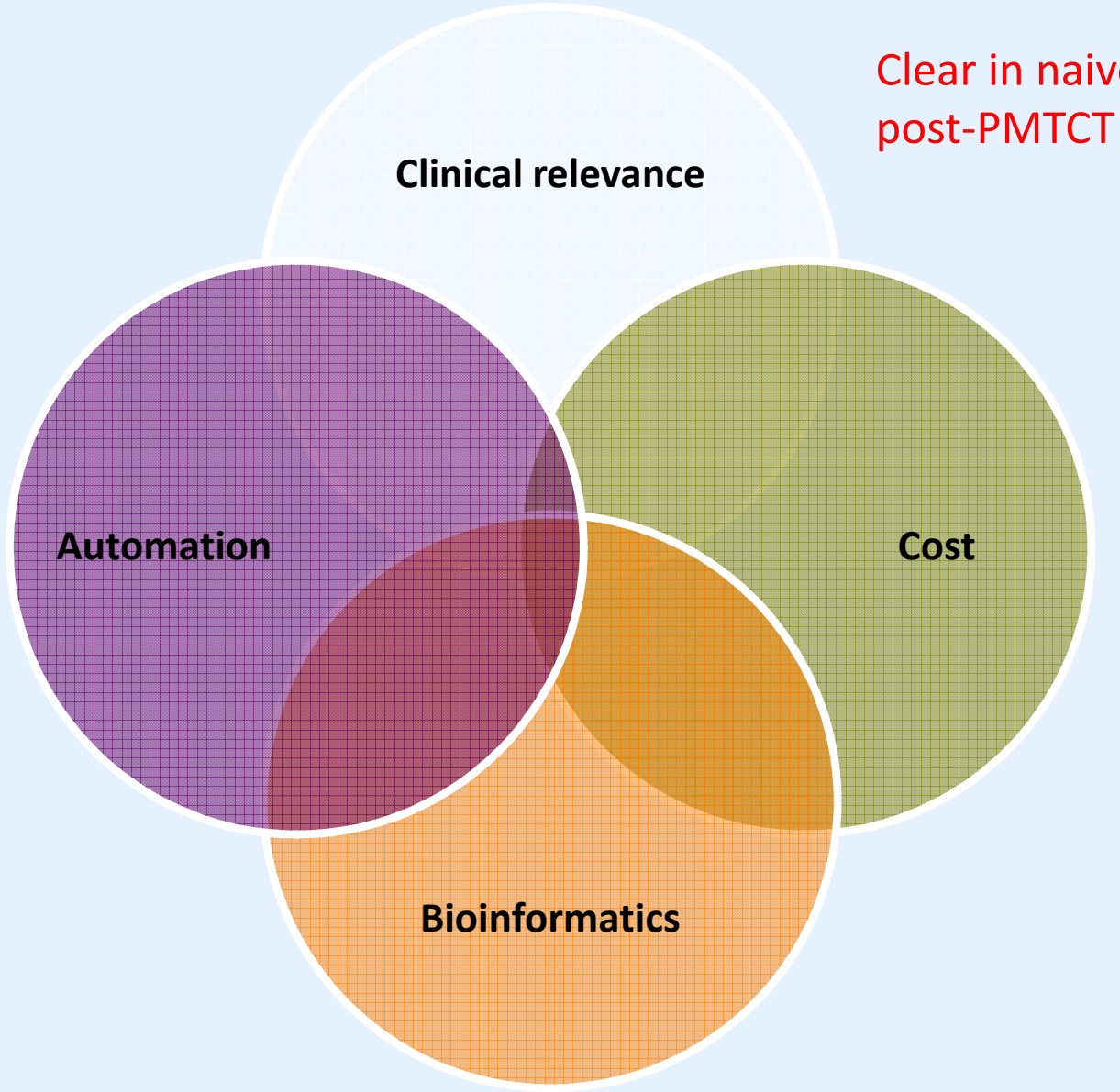
Pos 1	Pos 2
ATA (I)	CAG (Q)
ATA (I)	CAG (Q)
AGG (R)	CAG (Q)

Mutation list: 1I/R, 2Q

The main challeges with UDS

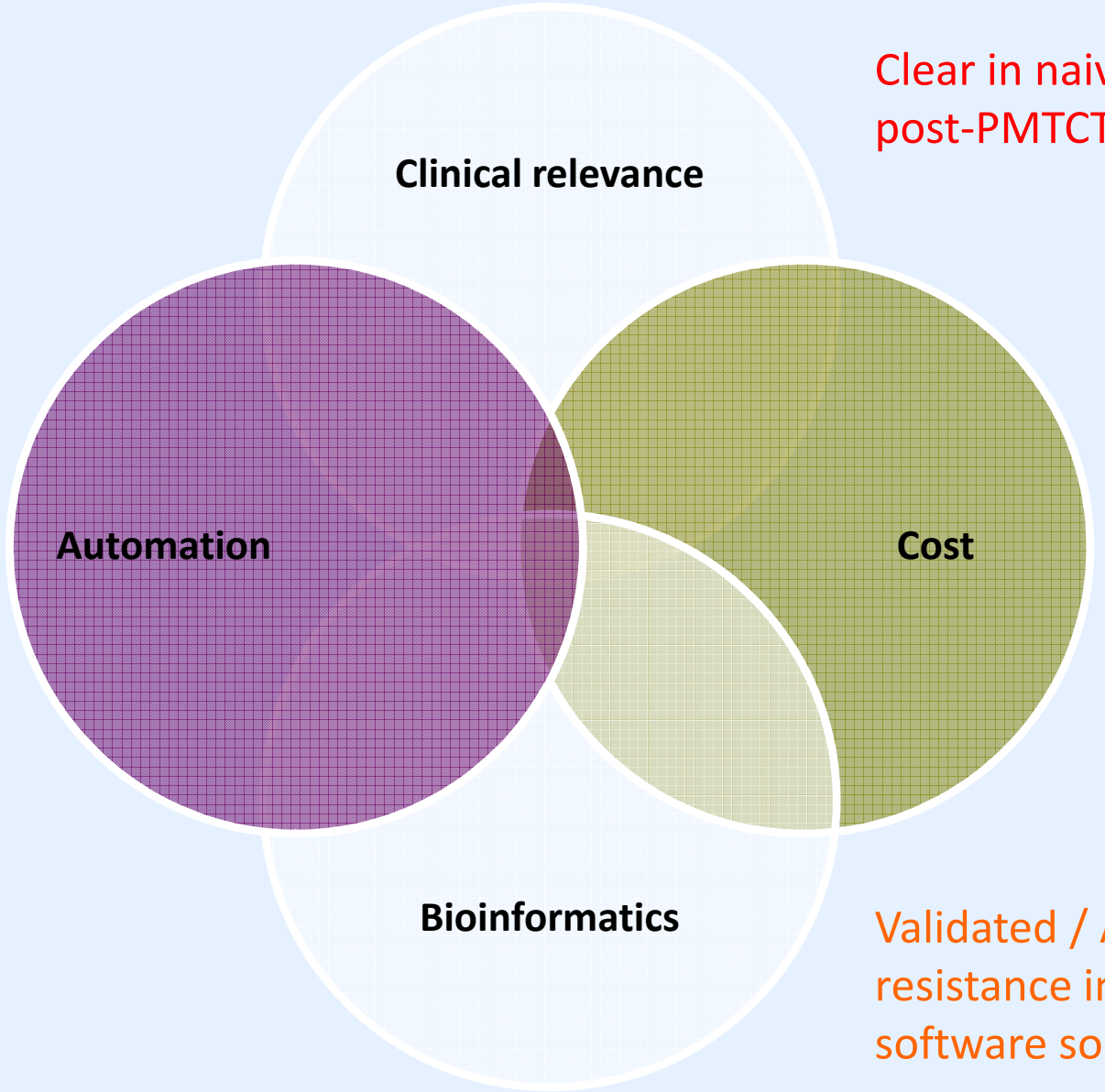


The main challeges with UDS



Clear in naive w NNRTI, post-PMTCT & tropism

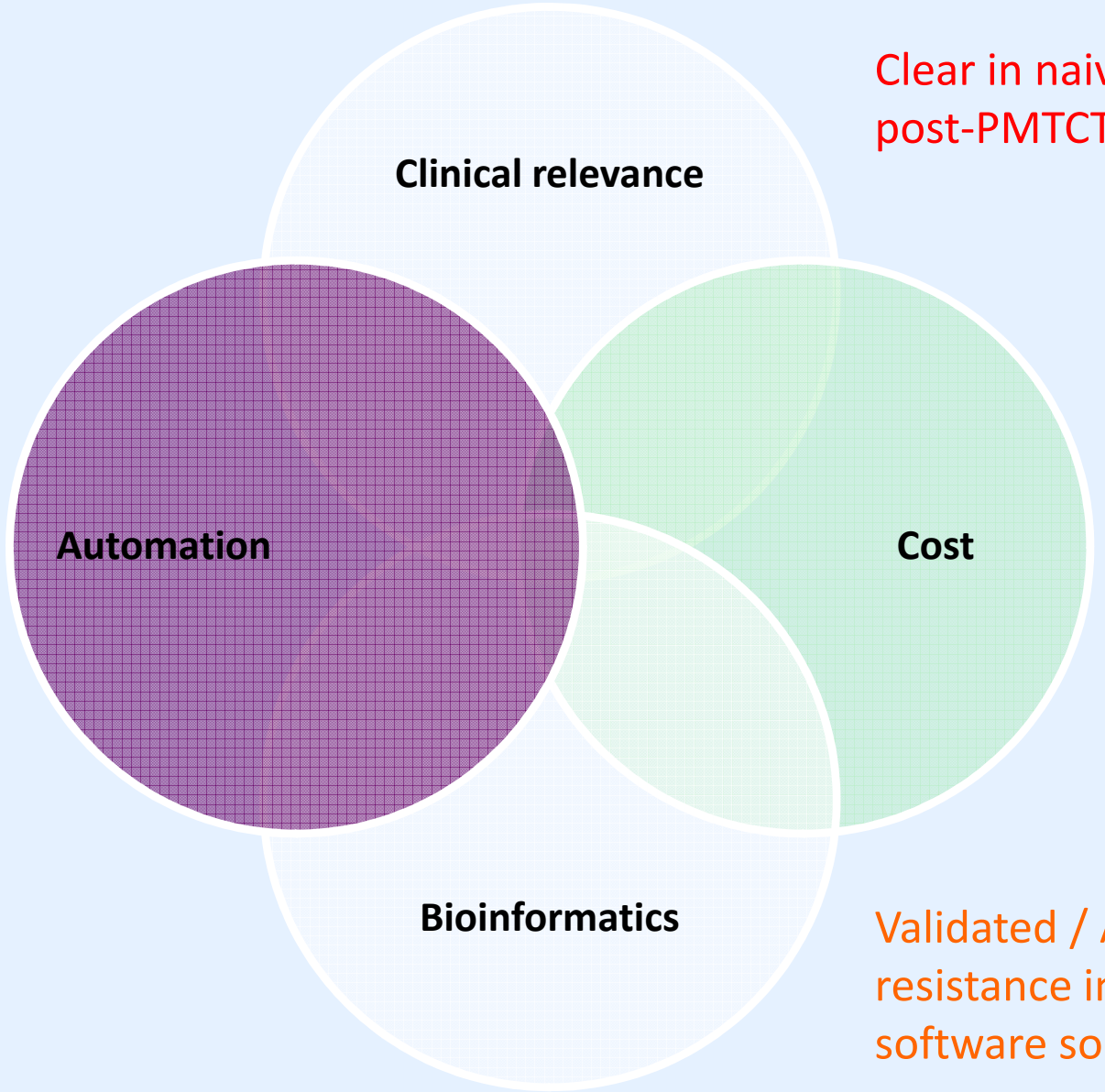
The main challeges with UDS



Clear in naive w NNRTI, post-PMTCT & tropism

Validated / Automated resistance interpretation software solutions

The main challeges with UDS

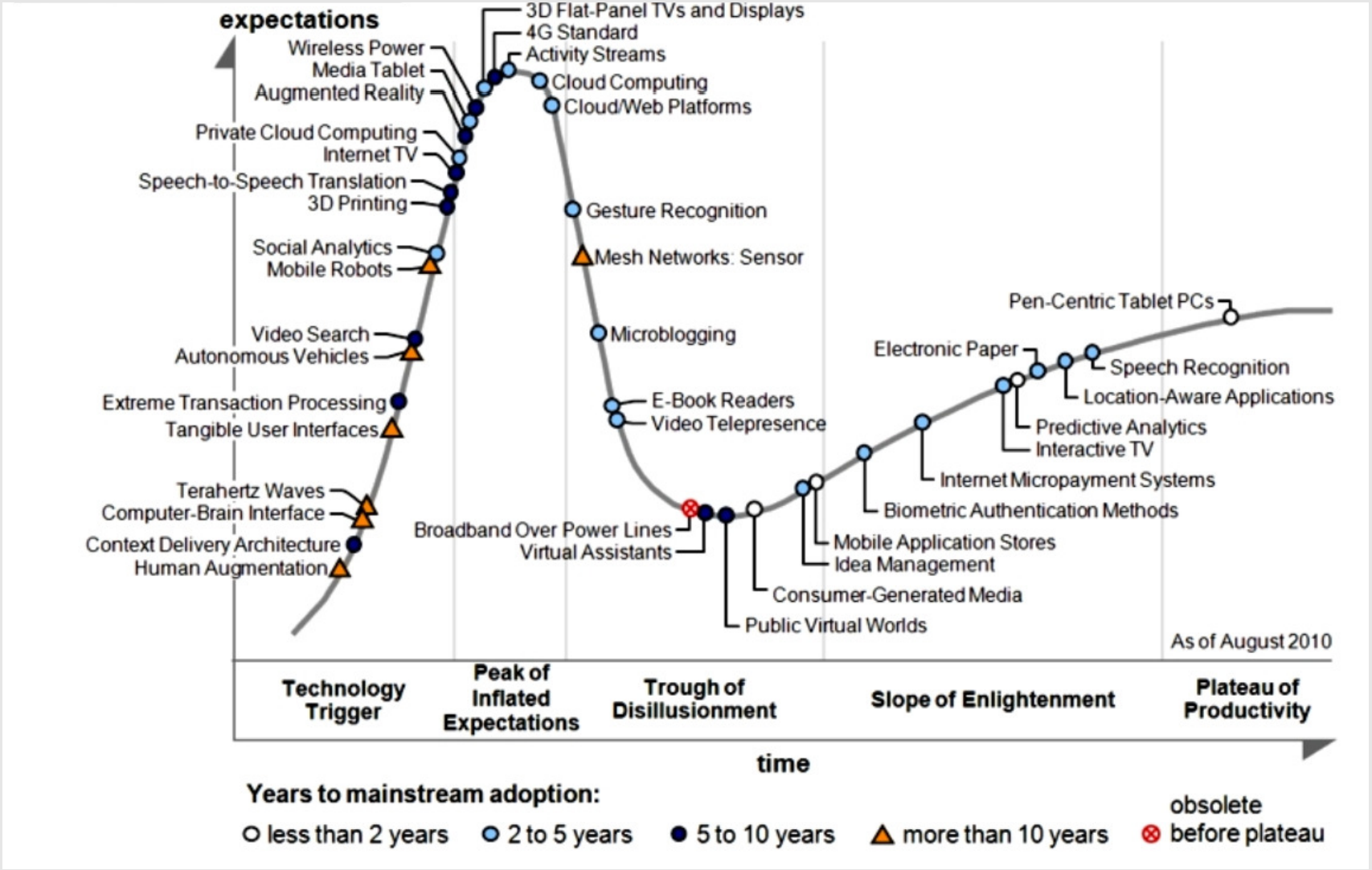


Clear in naive w NNRTI, post-PMTCT & tropism

- Sample pooling
- Centralised testing
- Pop sequencing for most samples / deep sequencing for selected samples (naive with NNRTI, tropism, post-PMTCT)

Validated / Automated resistance interpretation software solutions

THE HYPE AND HOPE CYCLE



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- Roche Diagnostics S.L., Spain
- ABL - Advanced Biological Laboratories, Luxembourg

- Center for Technological and Industrial Development (CDTI) Spanish Ministry of Science and Innovation

- CHAIN
- EuroSIDA
- EuroCOORD

THE MOLECULAR EPIDEMIOLOGY GROUP AT IRSICAIXA

IrsiCaixa Institut de Recerca de la Sida

EuroSIDA

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CHAIN Collaborative HIV and Anti-HIV Drug Resistance Network

CDTI