Pr Vincent Calvez

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IAS Résistance aux antirétroviraux

ANRS AC11 Résistance aux antirétroviraux

ANRS AC5 Essais Thérapeutiques

Variabilité du VIH

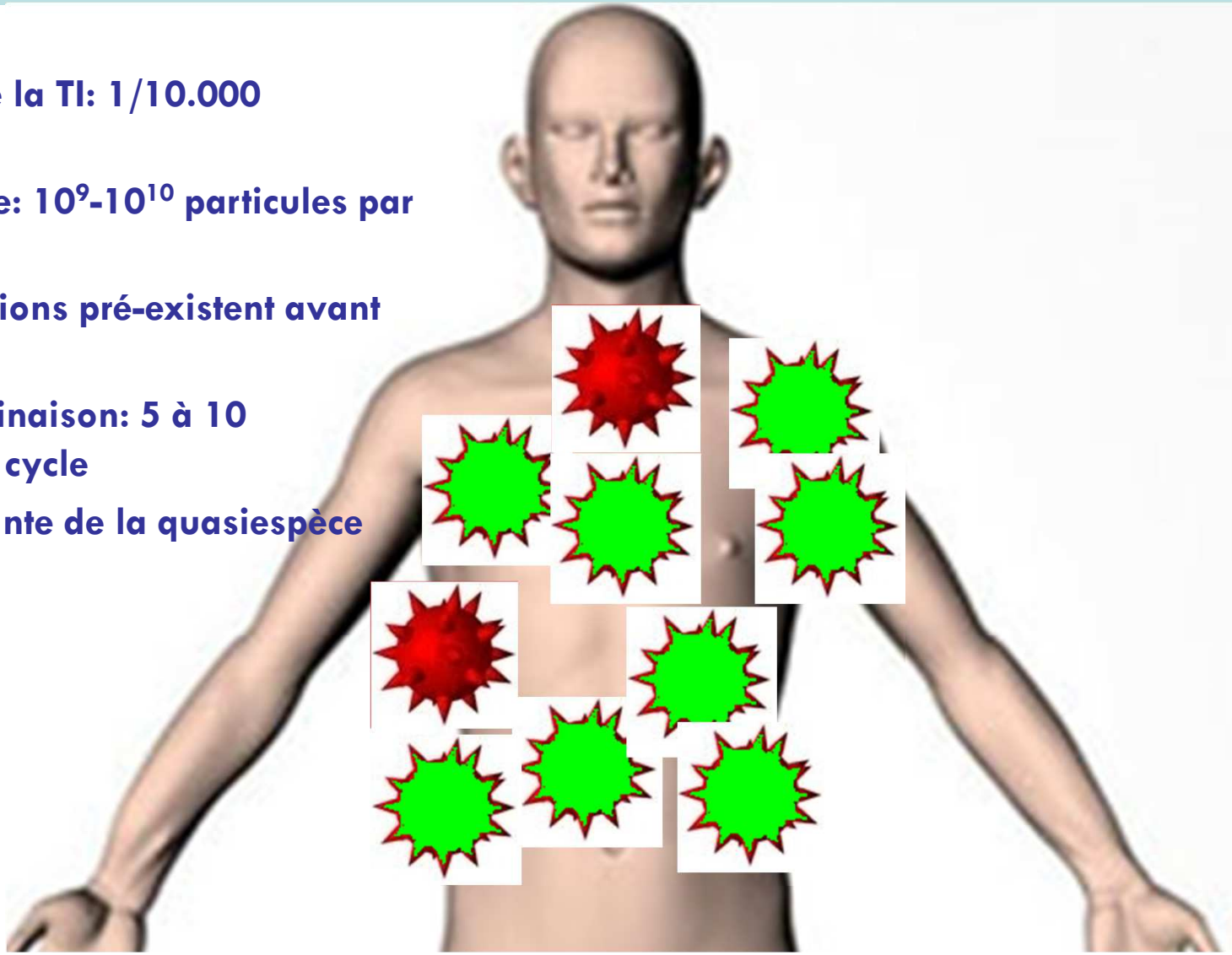
- Absence d'activité correctrice de la transcriptase inverse
- Erreurs d'appariement : $1/10^5$ nucléotides
- Production journalière : 10 milliards de particules virales
- Recombinaisons génétiques : 10 événements par cycle

POPULATION VIRALE HETEROGENE : QUASI-ESPECE

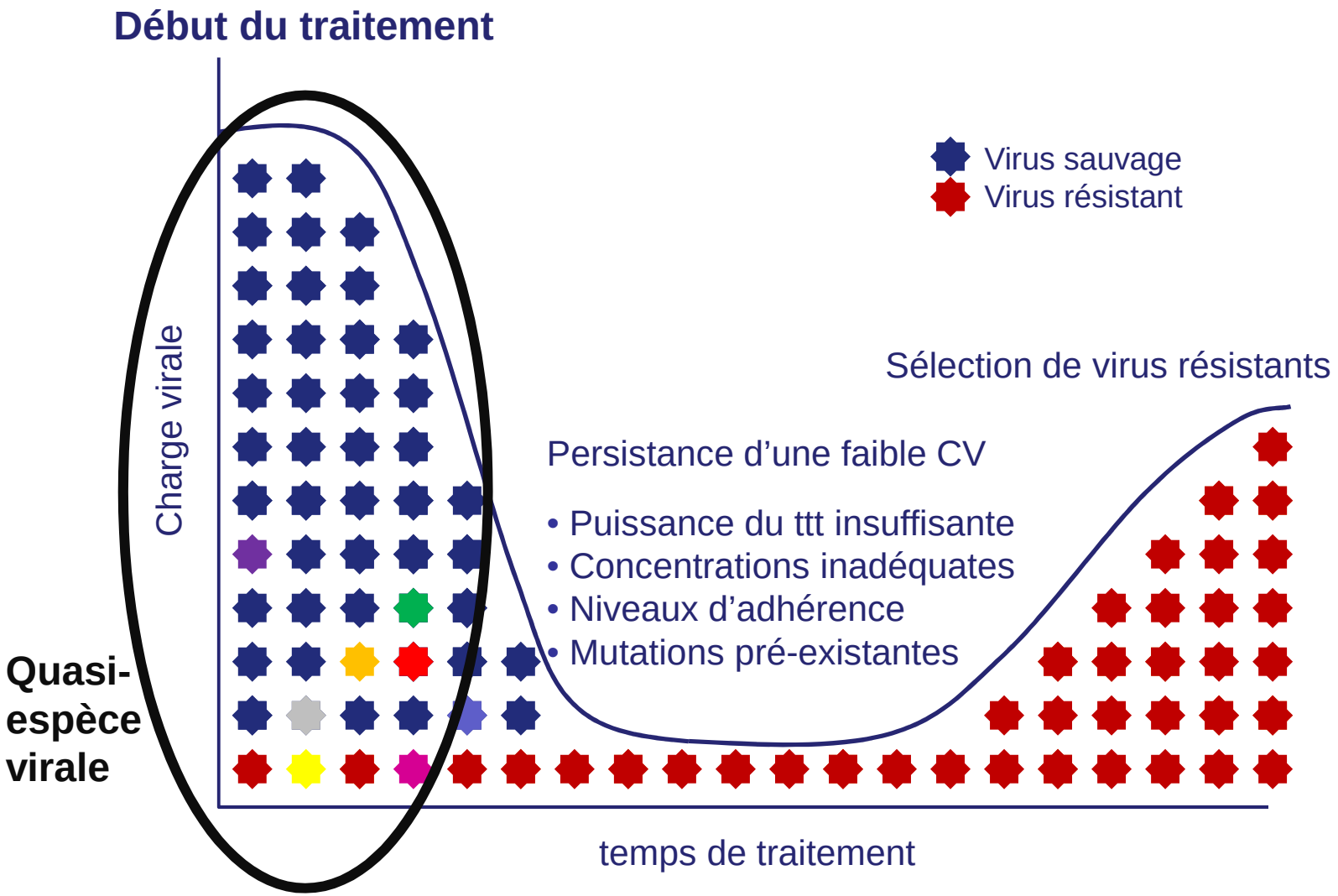
- Echappement au système immunitaire
- Obstacle à la mise au point des vaccins et des tests diagnostiques
- Résistance aux antirétroviraux ++++

VIH et populations minoritaires

- Taux d'erreur de la TI: 1/10.000 nucléotides
- Production virale: 10^9-10^{10} particules par jour
- Toutes les mutations pré-existent avant traitement
- Taux de recombinaison: 5 à 10 évènements par cycle
- Evolution constante de la quasiespèce

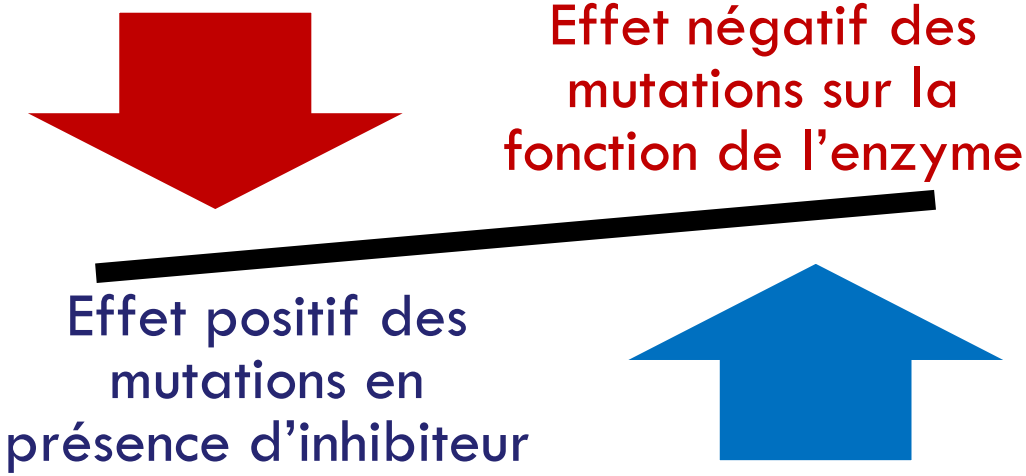


Résistance


Début du traitement
Charge virale
Virus sauvage
Virus résistant
Sélection de virus résistants
Persistance d'une faible CV
• Puissance du ttt insuffisante
• Concentrations inadéquates
• Niveaux d'adhérence
• Mutations pré-existantes
Quasi-espèce virale
temps de traitement

Résistance et niveaux de rebond de la charge virale (virémie)

- Sélection de mutations dans les gènes viraux codant pour les protéines cibles des antirétroviraux
- Mutations affectent la liaison du substrat naturel à l'enzyme et la spécificité enzyme/substrat

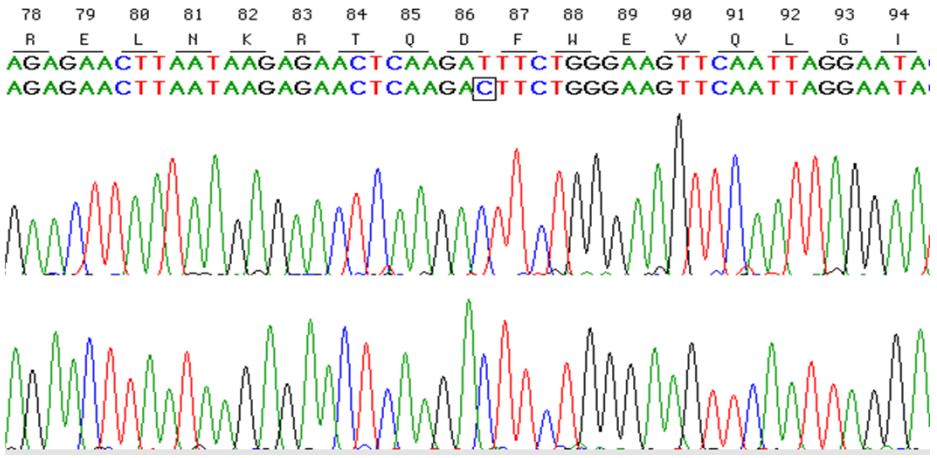


Résistance

- Résistance acquise
- Résistance transmise ou primaire

Résistance

- Evaluée par des tests phénotypiques ou génotypiques
- Tests génotypiques : consistent à rechercher par séquençage dans les gènes cibles des mutations connues comme associées à la résistance
- Limite : manque de sensibilité pour les variants < 15 à 20% de la quasi-espèce



ANRS - AC 11 : RESISTANCE GROUP
GENOTYPE INTERPRETATION: NUCLEOSIDE AND NUCLEOTIDE REVERSE TRANSCRIPTASE INHIBITORS

	Mutations associated to resistance	Mutations associated to « possible resistance »
ZDV	T215Y/F At least 3 mutations among : M41L, D67N, K70R, L210W, T215A/C/D/E/G/H/I/L/N/S/V, K219Q/E [1, 2, 3, 4] Q151M Insertion at codon 69	T215A/C/D/E/G/H/I/L/N/S/V [1, 2, 3, 4]
3TC/FTC	M184V/I Insertion at codon 69	K65R [11, 12, 16] Q151M
ddI	At least a score of + 2 among: M41L + T69D + L74V + T215Y/F + K219Q/E – K70R – M184 V/I [5, 14, 15, 17, 18] L74V without any mutations among M41L, T69D, K70R, M184 V/I, T215Y/F, K219Q/E [19] Q151M Insertion at codon 69	K65R [11, 12]
d4T	V75A/M/S/T T215Y/F [6] At least 3 mutations among : M41L, D67N, K70R, L210W, T215A/C/D/E/G/H/I/L/N/S/V, K219Q/E [4, 7, 14, 15] Q151M Insertion at codon 69	T215A/C/D/E/G/H/I/L/N/S/V [4, 7]
ABC	At least 5 mutations among : M41L, D67N, L74V, M184V/I, L210W, T215Y/F [8, 19] K65R and L74V and Y115F and M184V/I Q151M Insertion at codon 69	4 mutations among : M41L, D67N, L74V, M184V/I, L210W, T215Y/F [8, 19] K65R [9, 11, 12]
TDF	At least 6 mutations among: M41L, E44D, D67N, T69D/N/S, L74V, L210W, T215Y/F [13] K65R [9, 10, 11, 12] Insertion at codon 69	3, 4 or 5 mutations among: M41L, E44D, D67N, T69D/N/S, L74V, L210W, T215Y/F [13]

Havana Trial : Results

Patients with HIV RNA <400 c/ml at Week 24 (ITT)

	Genotype	No Genotype	
Expert	69%	49%	69%
No Expert	46%	36%	41%
	58%	42¨%	

p = 0.02

p = 0.002

Tural, AIDS 2002

Echappements de première ligne

- La résistance n'est pas la cause initiale du 1er échappementObservance +++
  - Pb "pharmacologique"
- Peu ou pas de mutations
- Efficacité des molécules en général non entamée
  - à condition de ne pas attendre trop longtemps !

First line virologic failures: adherence is the main factor

2 Dianes JAMA issue in 2000

Mechanisms of Virologic Failure in Previously Untreated HIV-Infected Patients From a Trial of Induction-Maintenance Therapy

Diane Descamps, MD
Philippe Flandre, PhD
Vincent Calvez, MD, PhD
Gilles Peytavin, MD
Vincent Meiffredy, MD
Gilles Collin, MS
Constance Delaugerre, MD
Sandrine Robert-Delmas, MD
Brigitte Bazin, MD
Jean Pierre Aboulker, MD
Gilles Pialoux, MD
François Raffi, MD
Françoise Brun-Vézinet, MD, PhD
for the Trilège (Agence Nationale de Recherches sur le SIDA 072) Study Team

Context In the Trilège trial, following induction with a zidovudine, lamivudine, and indinavir regimen, human immunodeficiency virus (HIV) replication was less suppressed by 2-drug maintenance therapy than by triple-drug therapy.

Objective To identify mechanisms of virologic failure in the 3 arms of the Trilège trial.

Design Case-control study conducted from February to October 1998.

Setting Three urban hospitals in Paris, France.

Patients Fifty-eight case patients with virologic failure (HIV RNA rebound to >500 copies/mL in 2 consecutive samples) randomized to 3 therapy groups: triple drug (zidovudine, lamivudine, and indinavir), 8; zidovudine-lamivudine, 29; and zidovudine-indinavir, 21; the case patients were randomly matched with 58 control patients with sustained viral suppression.

Main Outcome Measures At virologic failure (S1 sample) and 6 weeks later (S2 sample), assessment of protease and reverse transcriptase gene mutations, plasma indinavir level, and degree of viral load rebound; pill count during induction and maintenance periods.

Results Only 1 primary resistance mutation, M184V, was detected in S1 plasma samples from 4 of 6 patients in the triple-drug and in all 22 in the zidovudine-lamivudine therapy groups and in S2 plasma samples from 3 of 6 in the triple-drug and 20 of 21 in the

Drug Susceptibility in HIV Infection After Viral Rebound in Patients Receiving Indinavir-Containing Regimens

Diane V. Havlir, MD
Nick S. Hellmann, MD
Christos J. Petropoulos, PhD
Jeannette M. Whitcomb, PhD
Ann C. Collier, MD
Martin S. Hirsch, MD
Pablo Tebas, MD
Jean-Pierre Sommadossi, PhD
Douglas D. Richman, MD

Context Loss of viral suppression in patients infected with human immunodeficiency virus (HIV), who are receiving potent antiretroviral therapy, has been attributed to outgrowth of drug-resistant virus; however, resistance patterns are not well characterized in patients whose protease inhibitor combination therapy fails after achieving viral suppression.

Objective To characterize drug susceptibility of virus from HIV-infected patients who are failing to sustain suppression while taking an indinavir-containing antiretroviral regimen.

Design and Setting Substudy of the AIDS Clinical Trials Group 343, a multicenter clinical research trial conducted between February 1997 and October 1998.

Patients Twenty-six subjects who experienced rebound (HIV RNA level ≥200 copies/mL) during indinavir monotherapy (n = 9) or triple-drug therapy (indinavir, lamivudine, and zidovudine; n = 17) after initially achieving suppression while receiving all 3 drugs,

Echappements de de 2, 3ème ….. lignes

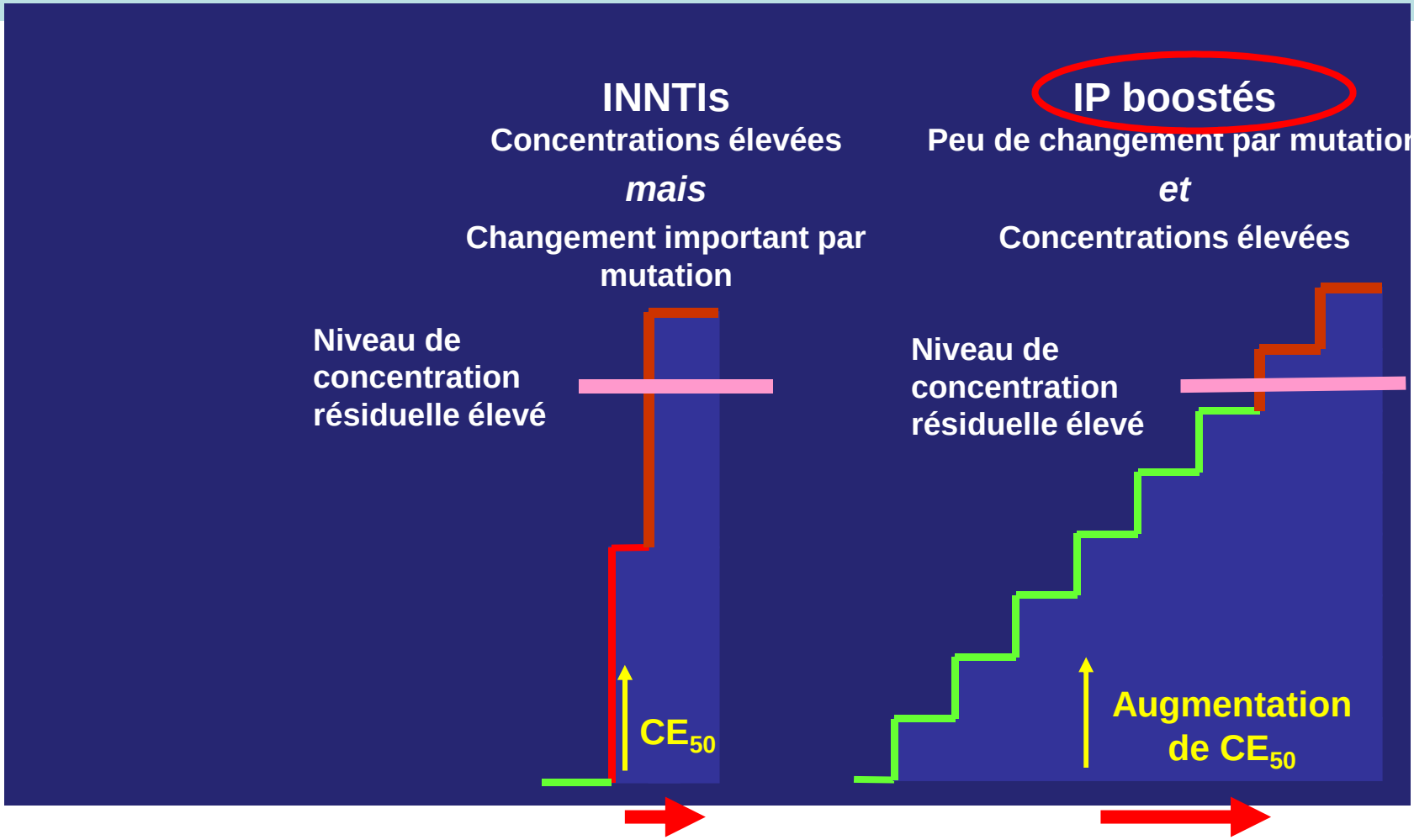
- Si le virus a pu se répliquer suffisamment longtemps
  - acquisition de mutations de résistance
- Le pb ici va être progressivement dominé par les résistances croisées ++
- Tolérance encore plus faible aux défauts d'observance ++

Barrière Génétique : Définition

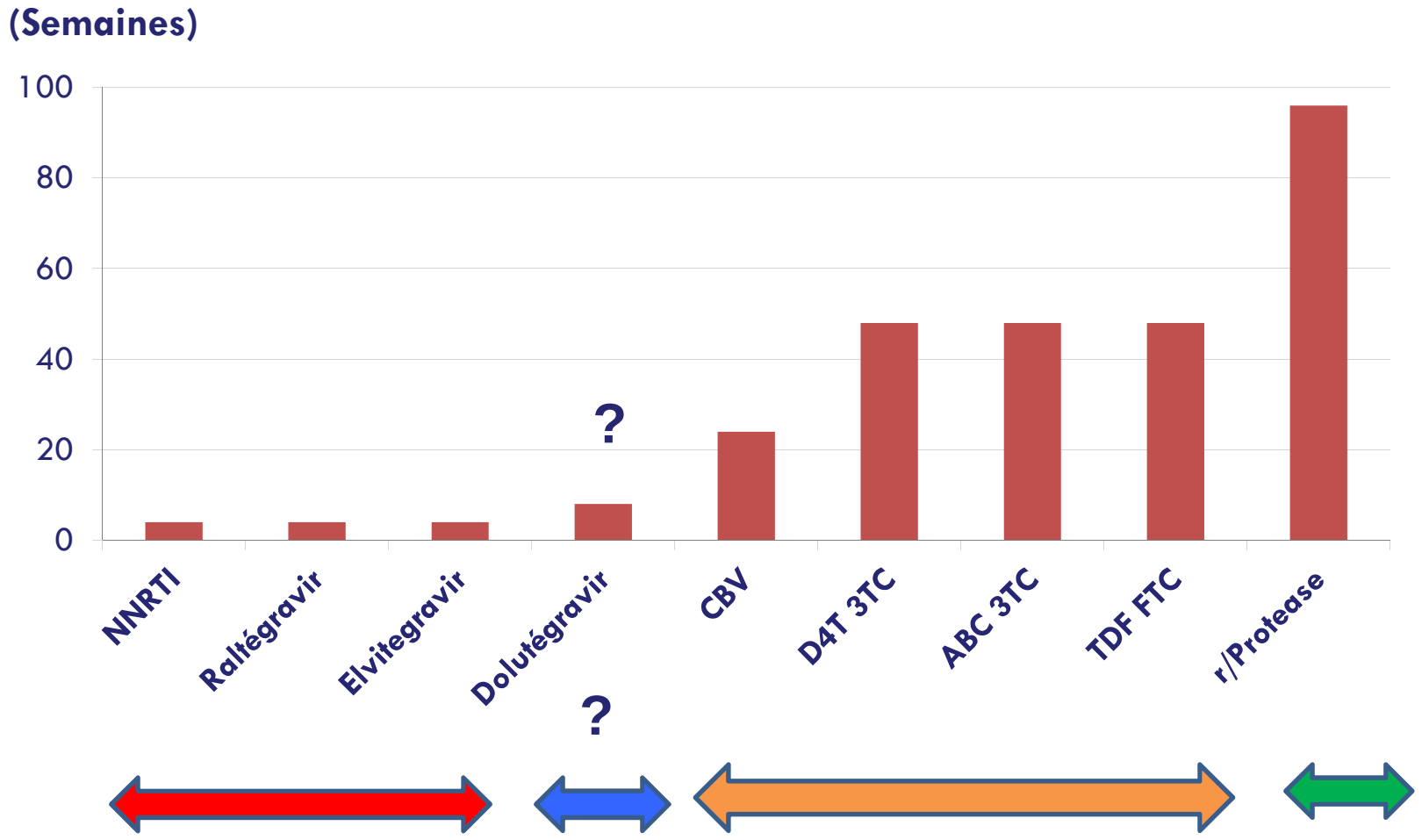
= capacité d'un médicament antirétroviral a être robuste vis-à-vis de la résistance

- Distance génétique en codon : nombre de sauts
- Type de mutation : transition/transversion
- Nombre de mutations requises pour rendre le virus résistant : 1, 2, 3 etc …
- Cinétique de sélection
- Pharmacologie des médicaments
  - Effet positif d'une longue demi vie si le produit est associé à d'autres produits à longue demi vie
  - Effet négatif d'une longue demi vie si le produit est associé à d'autres produits à courte demi vie
- Puissance intrinsèque antivirale du produit

Des molécules différentes ont des barrières génétiques à la résistance différentes



Cinétiques d'acquisition de la résistance lors d'échecs virologiques= 3 comportements différents



Expected NRTIs sensitivity spectrum after 1 year of treatment if virological failure HIV-1

	Starting with				
	AZT (Zidovudine)	d4T (Stavudine)	TDF (Tenofovir)	ABC (Abacavir)	ddI (Didanosine)
Cross Resistance ⇩					
AZT					
d4T					
TDF					
ABC					
ddI					

- **Opposite behavior between Thymidine analogs (ATZ and d4T) and the others (TDF, ABC and ddI) : these drugs are selecting antagonist mutations**

Pr V Calvez

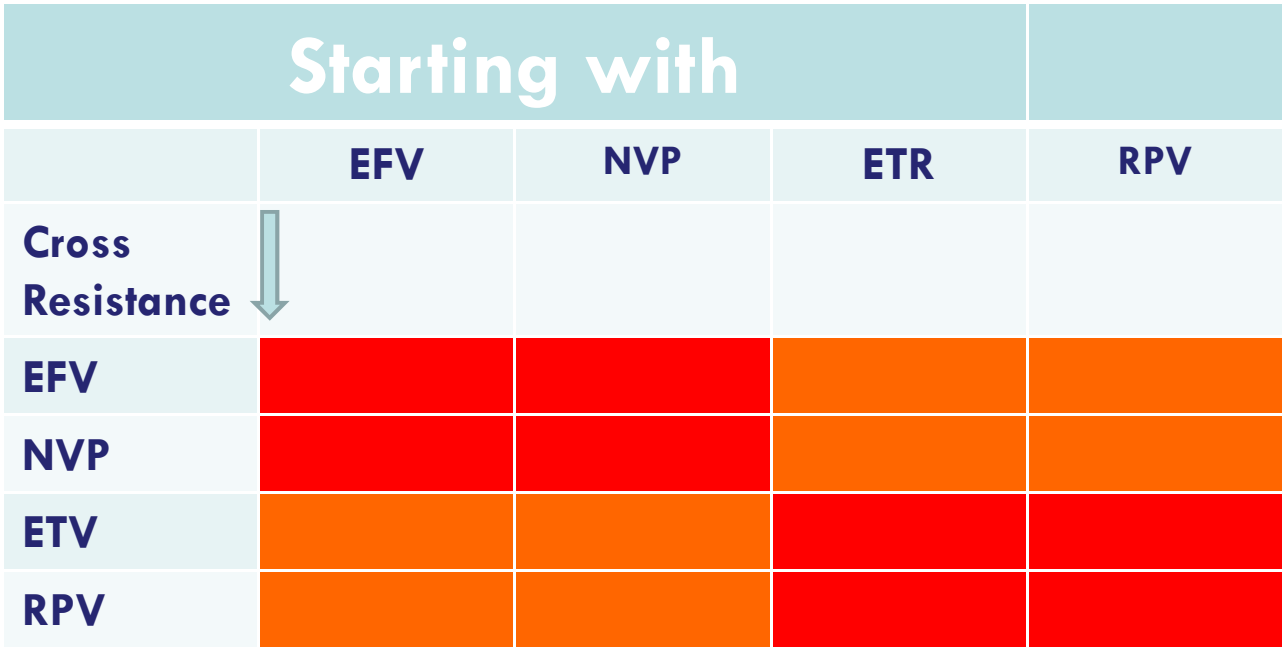
Expected NRTIs sensitivity spectrum after 2 years of treatment if virological failure HIV-1

	Starting with				
	AZT (Zidovudine)	d4T (Stavudine)	TDF (Tenofovir)	ABC (Abacavir)	ddI (Didanosine)
Cross Resistance ⇓					
AZT					
d4T					
TDF					
ABC					
ddI					

- TDF,ABC or ddI induce less cross resistance even after a long period of replication
- However after years of replication under AZT or d4T => TDF, ABC and ddI are resistant

Pr V Calvez

Expected NNRTIs sensitivity spectrum after 1 year of treatment if virological failure



- **Same low genetic barrier and weak robustness of EFV and NVP**
- **ETR (Etravirin) is not affected at least at the beginning if the failure**

Pr V Calvez

Expected NNRTIs sensitivity spectrum after 2 years of treatment if virological failure

Starting with				
	EFV	NVP	ETR	RPV
Cross Resistance ⇓				
EFV				
NVP				
ETR				
RPV				

- ETV become resistant after long duration failure to EFV or NVP

Pr V Calvez

Expected PIs sensitivity spectrum after 1 year of treatment if virological failure HIV-1

Cross Resistance ⇓	Starting with LPV (Lopinavir)	ATV (Atazanavir)	DRV (Darunavir)	SQV (Saquinavir)
LPV				
ATV				
DRV				
SQV				

- After one year of failure, the sensitivity of LPV is partially affected
- DRV is the most robust PI for the selection of resistance
- ATV does not induce any cross resistance to other PIs

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Expected PIs sensitivity spectrum after 2 years of treatment if virological failure HIV-1

	Starting with			
	LPV (Lopinavir)	ATV (Atazanavir)	DRV (Darunavir)	SQV (Saquinavir)
Cross Resistance ⇓				
LPV				
ATV				
DRV				
SQV				

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Niveaux d'intervention

A quel niveau de virémie devons nous craindre la sélection de résistance ?

- OMS: 1000 copies/ml
- USA: 200 copies/ml
- Europe: 50 copies/ml

Niveaux d'intervention

A quel niveau de virémie devons nous craindre la sélection de résistance ?

- OMS: 1000 copies/ml
- USA: 200 copies/ml
- Europe: 50 copies/ml

Qui a raison ??

Analyses of Emergent Drug Resistance from the NEAT 001/ANRS 143 trial: Raltegravir + Darunavir/ritonavir vs. Tenofovir/Emtricitabine + Darunavir/ritonavir

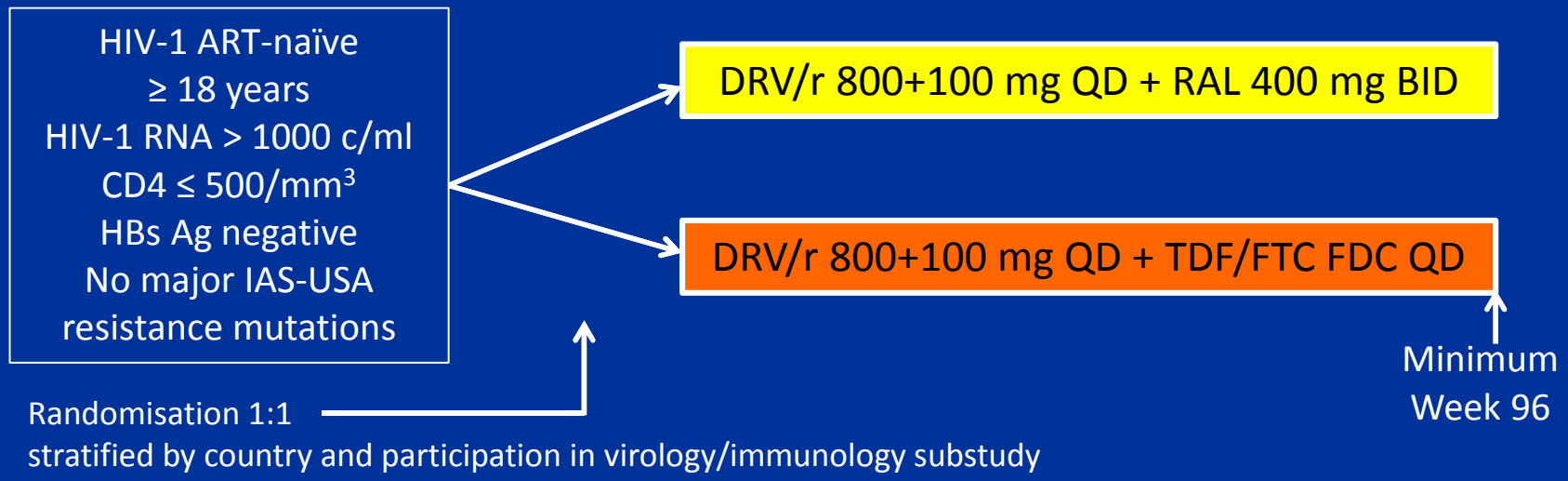
S. Lambert-Niclot [1], AG Marcelin[1], EC George[2], E White[2], C Schwimmer[3], H Jessen[4], D Dunn[2], CF Perno[5], B Clotet[6], M Johnson[7], L Richert[3], A Pozniak[8], V Calvez[1], F Raffi[9],

and the NEAT 001/ANRS 143 Study Group

1 Inserm UMR_S 1136, Hôpital Pitié-Salpêtrière, Service de Virologie, Paris,, France; 2 MRC Clinical Trials Unit at UCL, London, United Kingdom; 3 Inserm U897 Epidemiologie-Biostatistique, University of Bordeaux, Bordeaux, France; 4 Gemeinschaftspraxis Jessen-Stein, Berlin, Germany; 5 Department of Experimental Medicine, University of Rome Tor Vergata, Rome, Italy ; 6 HIV Unit and Retrovirology Laboratory 'Irsicaixa' Foundation, Hospital Universitari Germans Trias i Pujol, UAB, Badalona, Catalonia, Spain ; 7 Royal Free Hospital, London, United Kingdom ; 8 Chelsea and Westminster Hospital, London, United Kingdom ; 9 Infectious diseases department, University Hospital of Nantes, Nantes, France

NEAT 001/ANRS 143 study design

- Phase III, randomised, open-label, multicenter, parallel-group, non-inferiority, strategic trial
- 78 sites, 15 countries (Austria, Belgium, Denmark, France, Germany, Great Britain, Greece, Hungary, Ireland, Italy, Netherlands, Poland, Portugal, Spain, Sweden)



- Composite virological and clinical primary endpoint (6 components)

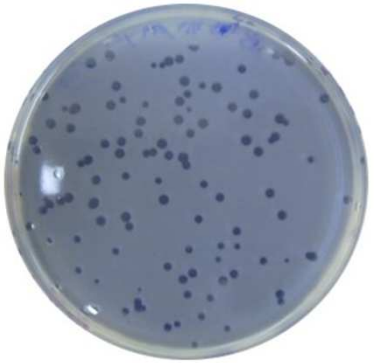
Raffi F et al. Lancet 2014; 384: 1942-51

Resistance mutations in the RAL+ DRV/r arm n = 17 pts (continued)

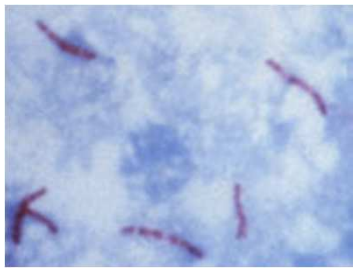
	Genotypic testing Time	VL (c/ml)	IN	Subsequent regimen	VL at W96 (c/ml)
5	W58	60	Y143C	TDF/FTC + DRV/r	90
6	W32	85	N155H	TDF/FTC + DRV/r	<50
7	W34	148	N155H	No treatment after W67	227185
8	W64	192	N155H	TDF/FTC + DRV/r	68
9	W62	406	N155H	TDF/FTC + DRV/r	<50
10	W29	442	N155H + Q148R	Missing data	Missing data
11	W49	498	N155H	RAL + DRV/r	<50
12	W80	731	N155H	ABC/3TC + DRV/r	<50
13	W32	1311	N155H	TDF/FTC + DRV/r	<50
14	W34	1900	N155H	TDF/FTC + ETR	<50
15	W21	14864	N155H	TDF/FTC + EFV	<50
16	W19	52857	N155H	AZT/3TC + DRV/r + NVP	<50
17	W74	129000	N155H	RAL + DRV/r	50

Minority variants with drug resistance mutation common problem in infectious diseases

- Rare generation of resistant variants of bacteria in the absence of drug exposure was first described in the 40s
  - Luria Se,et al. Genetics **1943**



- In the 1950s, the clinical relevance of rare drug-resistant populations of *Mycobacterium tuberculosis* formed the rationale for combination therapy
  - Coates EO, N Engl J Med **1953**
  - Cohn ML, J Clin Invest **1959**



MRVs frequency seems to be very high (NNRTIs)

Poster # 77

Low-frequency NNRTI-resistant HIV-1 Variants and Mutational Load Relationship in ART-naïve Subjects

Michael J Kozal[1*], Tassos Kyriakides[1], Jennifer Chiarella[1], Elizabeth P St. John[2], Birgitte B Simen[2], Suzin Webb[2], Elizabeth A Moreno[2] and Max Lataillade[1,3]

1. Yale University School of Medicine and Veterans Affairs Healthcare System, New Haven, CT, U.S. 2. 454 Life Sciences – A Roche Company, Branford, CT, U.S. 3. Bristol-Myers Squibb, Research and Development, Wallingford, CT, U.S.

Methods

ART-naïve subjects from 3 studies enrolled between 2005 – 2010 (Castle, Spartan, and HRDB) were evaluated by ultra-deep sequencing (UDS; 454 Life Sciences-Roche) for low-frequency variants possessing any NNRTI-resistance mutation.

Major NNRTI-resistance mutations were defined as having a Stanford-HIVdb algorithm[13] value ≥30 which confer at minimum intermediate resistance to EFV or NVP (e.g. mutations at positions: 100, 101, 103, 106, 179, 181, 188, 190, 225 and 238).

An estimated mutational load was calculated by multiplying variant frequency by HIV RNA copies/mL.

Results

A total of 206 ART-naïve subjects were evaluated by UDS for NNRTI-resistant variants; 49(23.8%) subjects had a major NNRTI-resistant variant detected with 11 (11/206=5.3%) possessing multiple NNRTI-mutations.

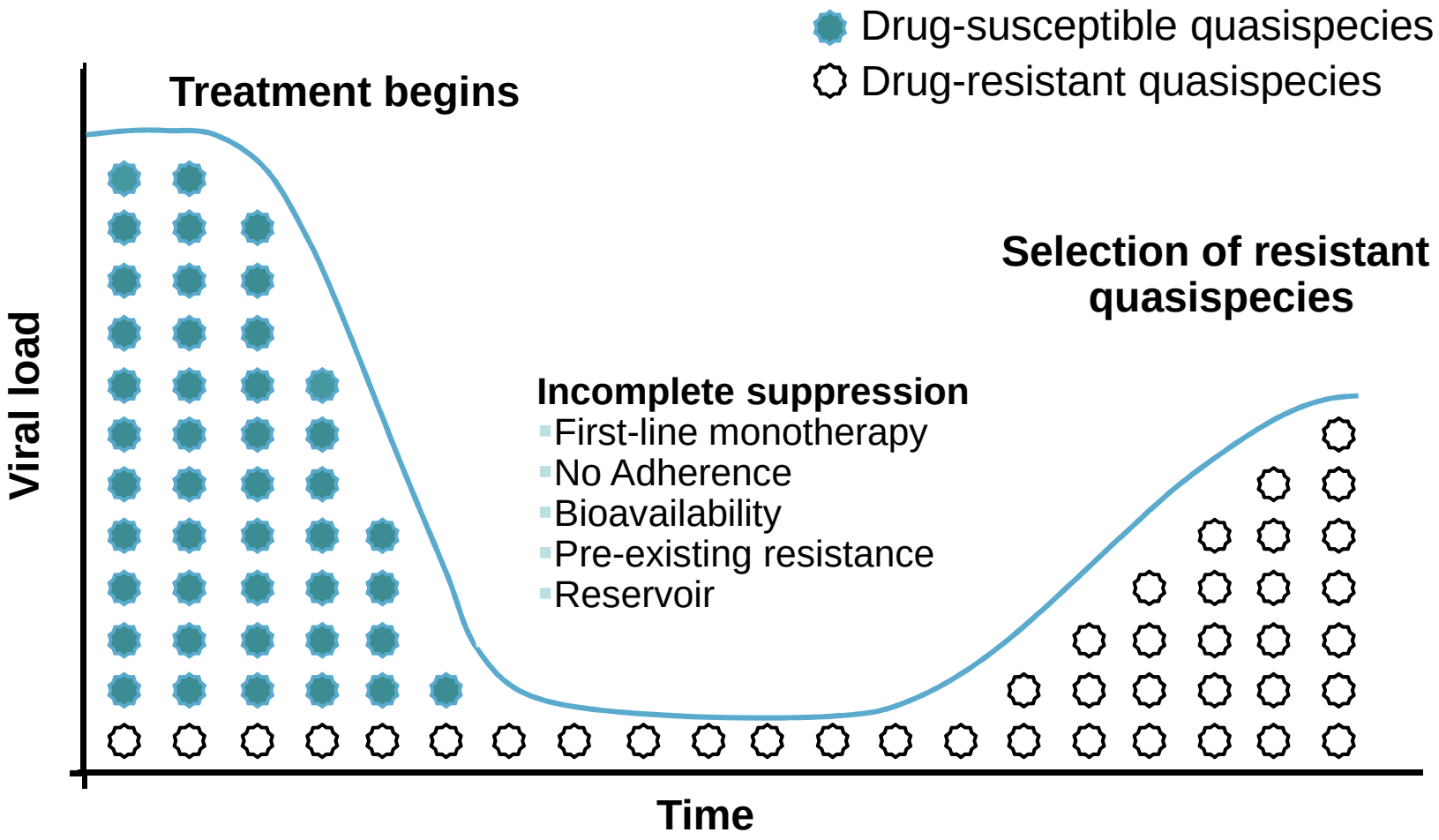
Fourteen different types of mutations were identified at 9 major NNRTI-resistance sites (totaling 69 mutations): K103N/S/T (n=24), G190A/E (n=13), Y181C/I (n=12), K101E (n=6), P225H (n=6), Y188H/C (n=4), L100I (n=2), V106A (n=1) and K238N (n=1) (Table 1).

Table 1. NNRTI mutations identified in ARV-naïve subjects

NNRTI Mutation	Frequency	Percent
L100I	2	2.90
K101E	6	8.70
K103N	20	28.99
K103S	2	2.90
K103T	2	2.90
V106A	1	1.45
Y181C	10	14.49
Y181I	2	2.90
Y188C	1	1.45
Y188H	3	4.35
G190A	5	7.25
G190E	8	11.59
P225H	6	8.70
K238N	1	1.45
TOTAL	69	100

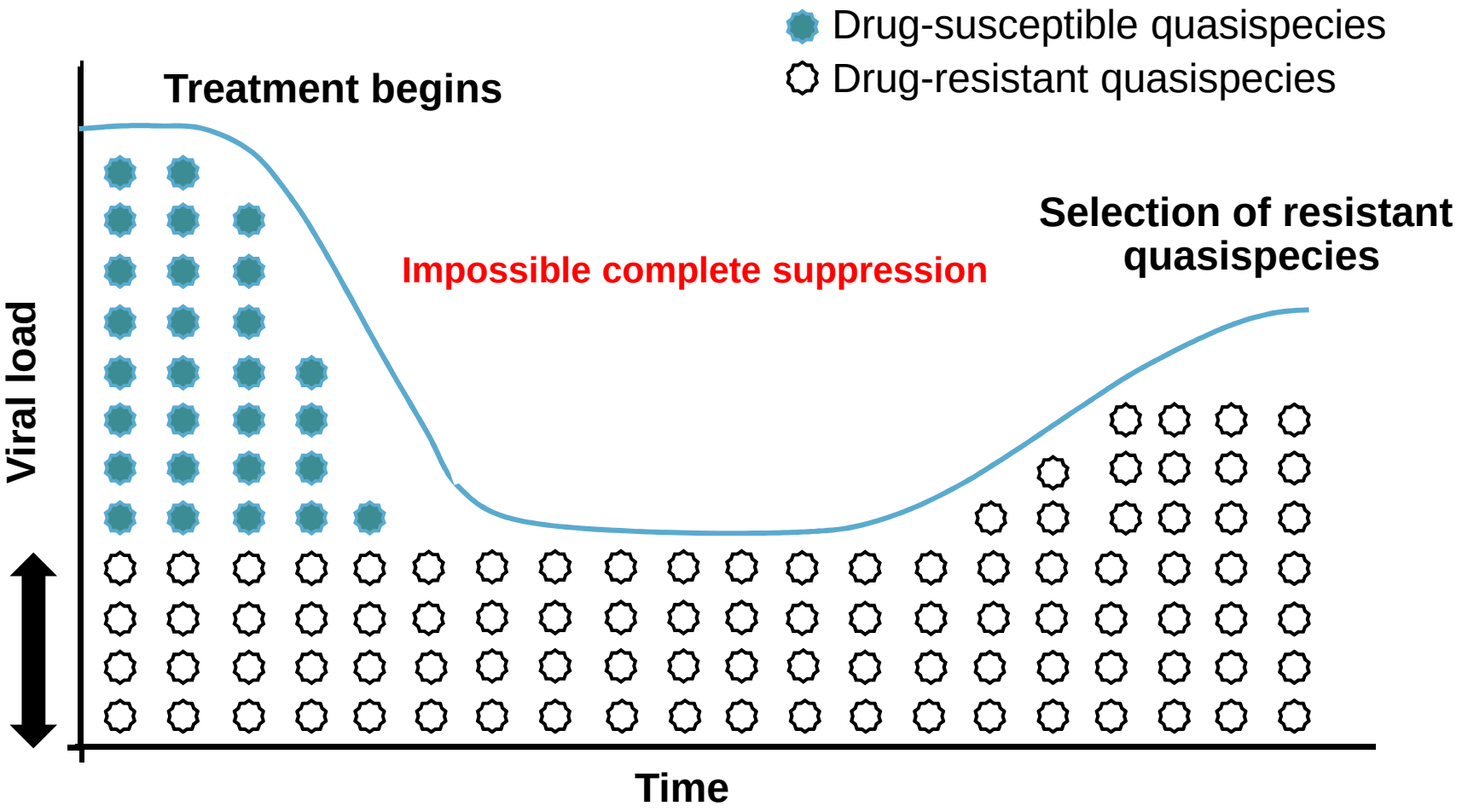
Low levels of MRVs consequences

Low levels of MRVs (no transmitted drug resistance)
In cases of incomplete viral replication suppression resistant quasispecies will lead to expansion of resistant viruses



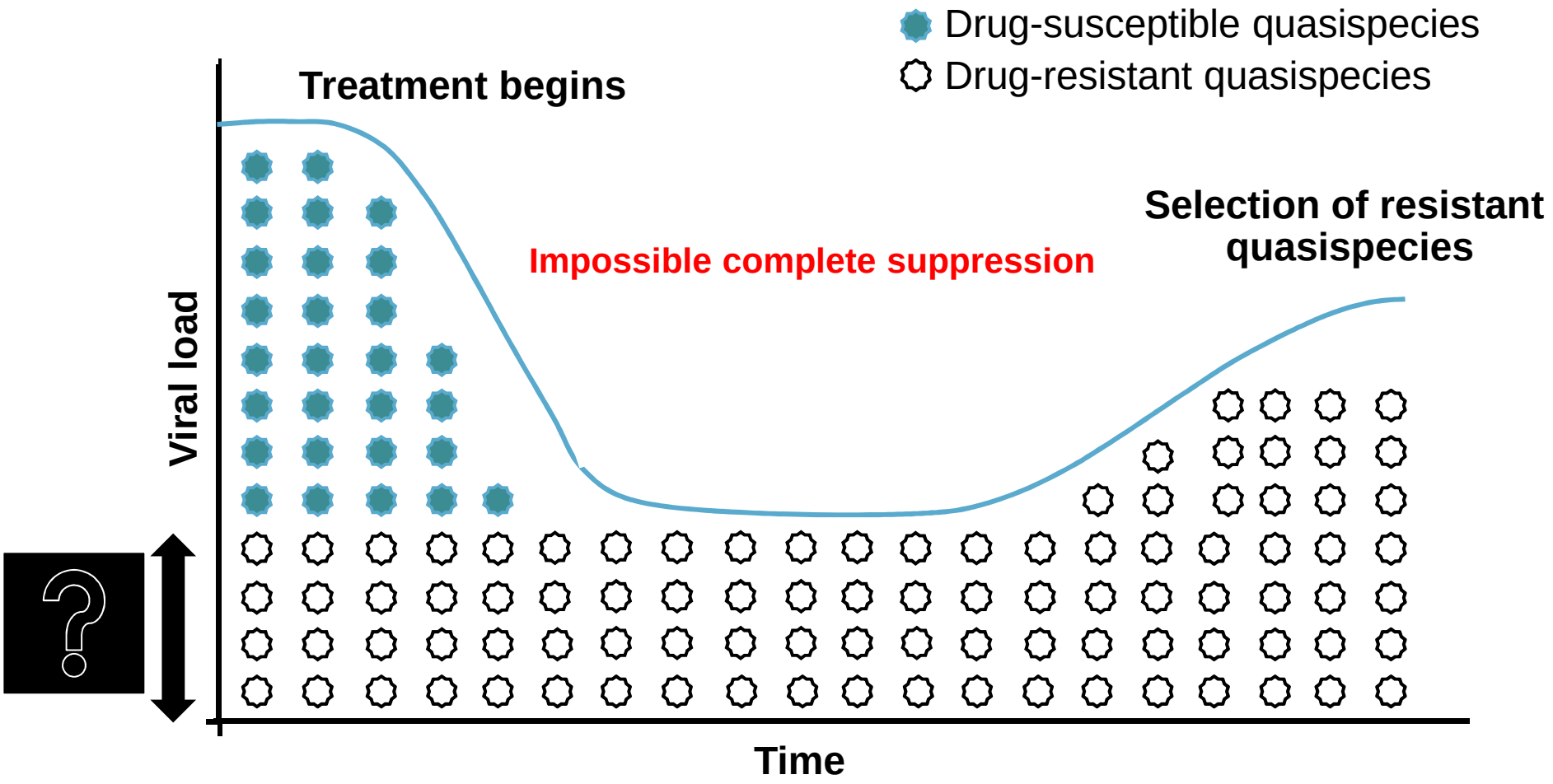
Higher levels of MRVs consequences

In cases of higher levels of MRVs but not detectable using classical tests (transmitted resistance or treatment experience), complete viral suppression will not be achieved leading more rapidly to a virologic failure and an expansion of resistant viruses



Higher levels of MRVs consequences

In cases of higher levels of MRVs but not detectable using classical tests (transmitted resistance or treatment experience), complete viral suppression will not be achieved leading more rapidly to a virologic failure and an expansion of resistant viruses



What is the clinical cut off of MRVs leading to a virologic failure ??

OPEN ACCESS Freely available online PLOS MEDICINE

Minority HIV-1 Drug Resistance Mutations Are Present in Antiretroviral Treatment–Naïve Populations and Associate with Reduced Treatment Efficacy

Jeffrey A. Johnson[1*], Jin-Fen Li[1], Xierong Wei[1], Jonathan Lipscomb[1], David Irlbeck[2], Charles Craig[3], Amanda Smith[1], Diane E. Bennett[1], Michael Monsour[1], Paul Sandstrom[4], E. Randall Lanier[2], Walid Heneine[1]

1 Division of HIV/AIDS Prevention, National Center for HIV, STD, and TB Prevention, Centers for Disease Control and Prevention, Atlanta, Georgia, United States of America, **2** GlaxoSmithKline, Research Triangle Park, North Carolina, United States of America, **3** GlaxoSmithKline, Stevenage, United Kingdom, **4** National HIV and Retrovirology Laboratories, Public Health Agency of Canada, Ottawa, Ontario, Canada

Mutation Status	Treatment Success ($n = 221$)	Treatment Failure ($n = 95$)
No detectable drug resistance mutation	219 (99.1%)	88 (92.6%)
Minority drug resistance mutation	2 (0.9%)	7 (7.4%)

Johnson et al Plos Med 2008

Low-Frequency HIV-1 Drug Resistance Mutations and Risk of NNRTI-BasedAntiretroviral Treatment Failure
A Systematic Review and Pooled Analysis

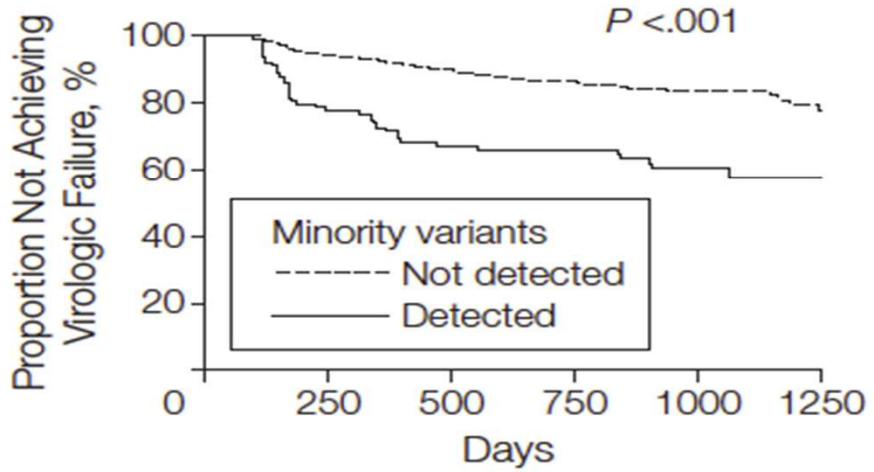
Systematic Review and Baseline Characteristics

10 studies with 985 patients were identified as meeting the inclusion and exclusion criteria.

The median CD4 cell count was 229 cells/mm3 and mean plasma HIV-1 RNA level was 5.0 log10 copies/mL.

All studies evaluated the presence of NNRTI mutations K103N, Y181C (N=435) and NRTI mutations M184V (N=228) and K65R (N=163).

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



No. at risk

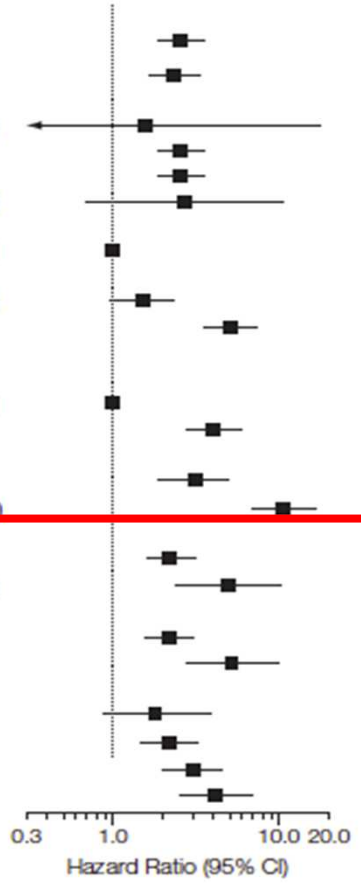
Minority variants						
Not detected	691	620	455	398	344	46
Detected	117	86	60	53	37	7

- **Minority drug-resistant variants were found in 14% (117/808).**

Li et al JAMA 2011

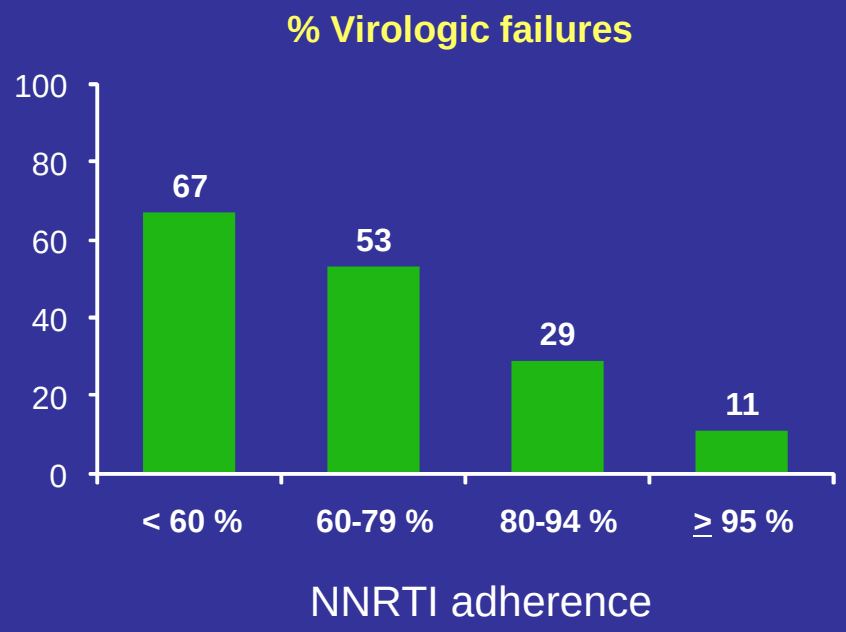
Figure 3. Effect of Minority Variants and Antiretroviral Therapy Adherence on Virologic Failure

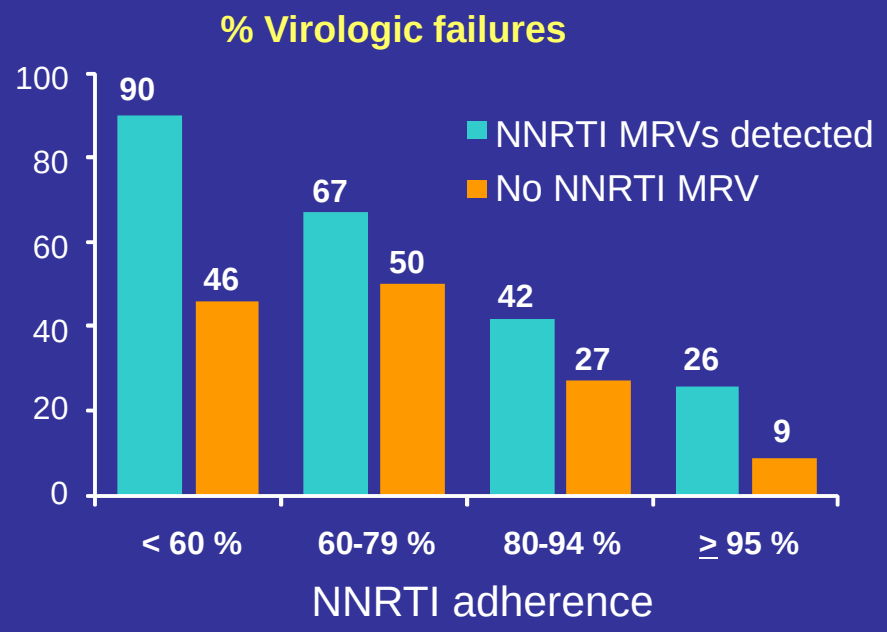
Group vs No Minority Variant[c]	Virologic Failure, No.[a]		Total No.		HR (95% CI)[b]
	Minority Variant	No Minority Variant	Minority Variant	No Minority Variant	
Any minority variant	111	208	187	798	2.6 (1.9-3.5)
Any minority variant (multivariate)[d]	98	138	152	617	2.3 (1.7-3.3)
Minority variant type					
NRTI-resistant	2	104	9	202	1.6 (0.1-17.7)
NNRTI-resistant	109	210	178	807	2.6 (1.9-3.5)
Efavirenz-resistant	105	188	165	723	2.6 (1.9-3.5)
Nevirapine-resistant	4	22	13	84	2.7 (0.7-10.3)
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)



- Presence of MRVs at ≥1% conferred a significantly higher risk of virologic failure as compared to MRVs present at ≤1%.
- A dose-dependent effect on the risk of virologic failure was found when subjects were categorized by the absolute copy numbers of MRVs per ml of plasma : burden of resistance expressed in copies/ml

Influence of MRVs and adherence on virologic response to NNRTI





- NNRTI MRVs increase the risk of virologic failure in all adherence categories even in patients with very high level of adherence

Li J, IHHDRW 2011, Abs. 8

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