UMC Utrecht

HIV drug resistance

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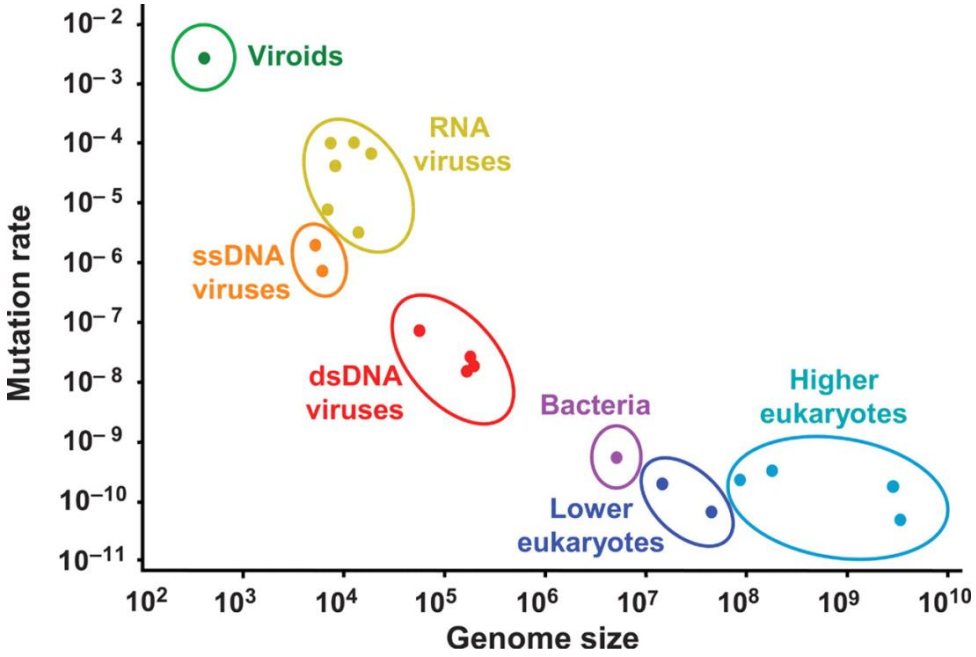
Disclosure

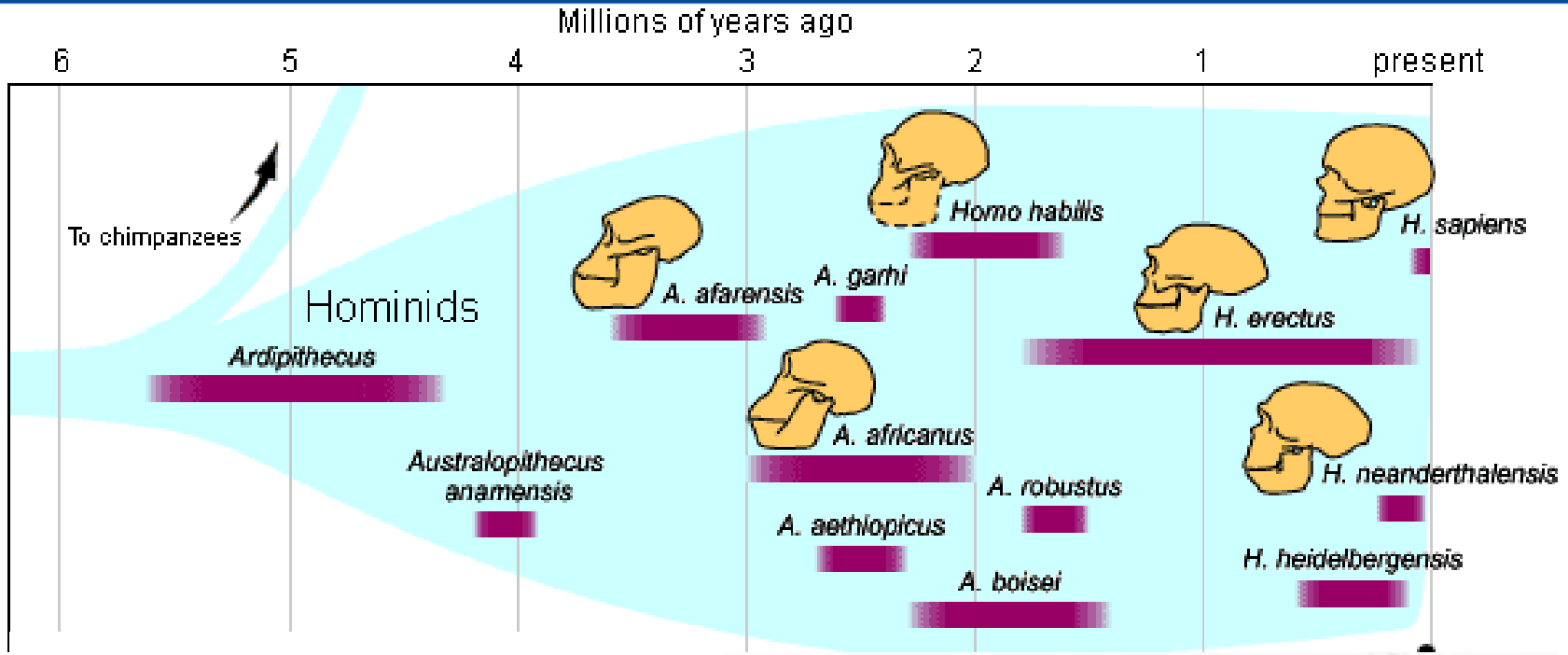
Relations that could be relevant for the meeting	Company names
Sponsorship or refund funds INVESTIGATOR INITIATED RESEARCH GRANTS* UNRESTRICTED EDUCATIONAL GRANTS*	• Janssen, Viiv Healthcare, Merck, Gilead
Payment or other financial remuneration CONSULTANCY FEES*	• Gilead, Viiv Healthcare, Janssen, Merck
Shareholder rights	• None
Others: CONFERENCE TRAVELING/MASTERCLASS FEE*	• Virology Education
* All paid to my institution	

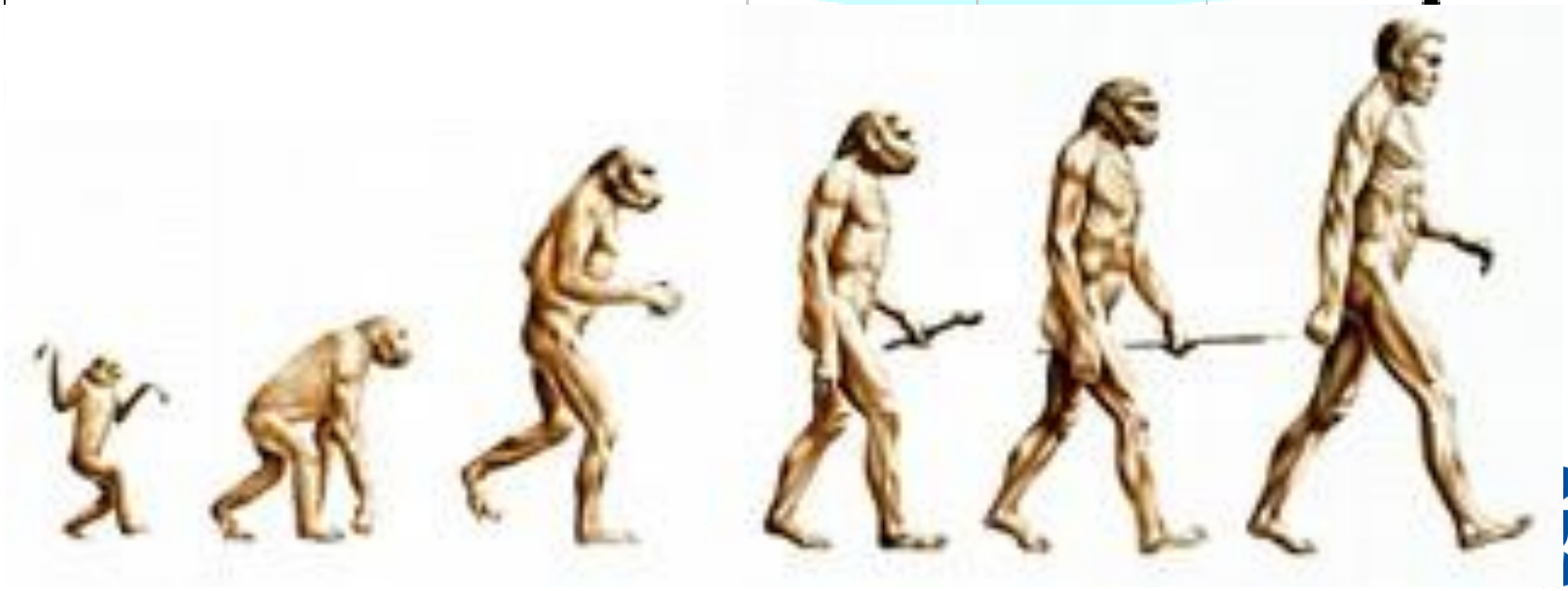
Mutation rate RNA viruses > DNA viruses > bacteria > humans



Even among RNA viruses HIV is highly variable:
the HIV population present in a single individual six years after infection is comparable with the global variation of an influenza outbreak

D. Burke Emerging Inf Dis 1997


Millions of years ago
6
5
4
3
2
1
present
To chimpanzees
Hominids
Ardipithecus
Australopithecus anamensis
A. afarensis
A. garhi
A. africanus
A. aethiopicus
A. robustus
A. boisei
Homo habilis
H. erectus
H. sapiens
H. neanderthalensis
H. heidelbergensis



HIV-1 cycle

No proofreading: 2-20 billion mutations a day

2 RNA copies are present in the virion

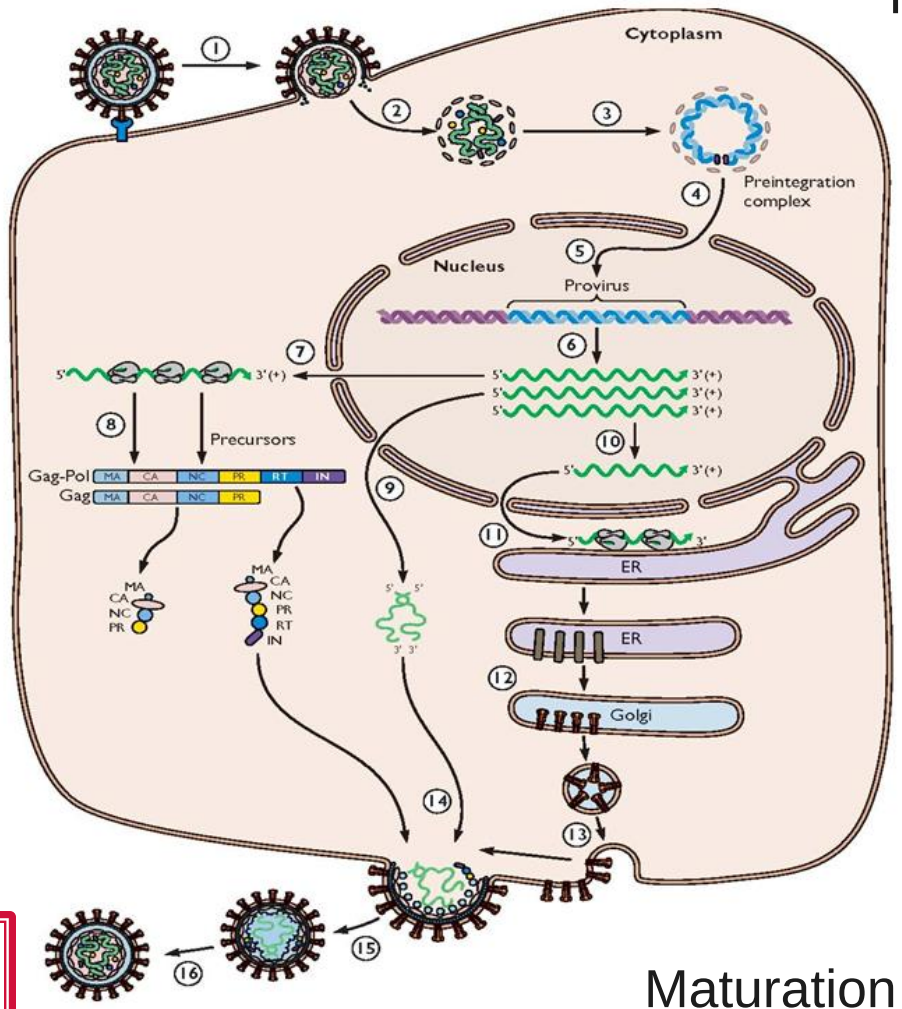
Frequent Recombination

Integration in HIV DNA: Persistent infection

Enormous production of 1-10 bilion HIV particles

Entry

Reverse Transcription



Integration

Maturation

Viral heterogenity

2-20 billion mutations a day!

Viral population is characterised by extreme genetic diversity resulting in rapid evolution and quick adaptation to a new situation:

Viral Quasispecies

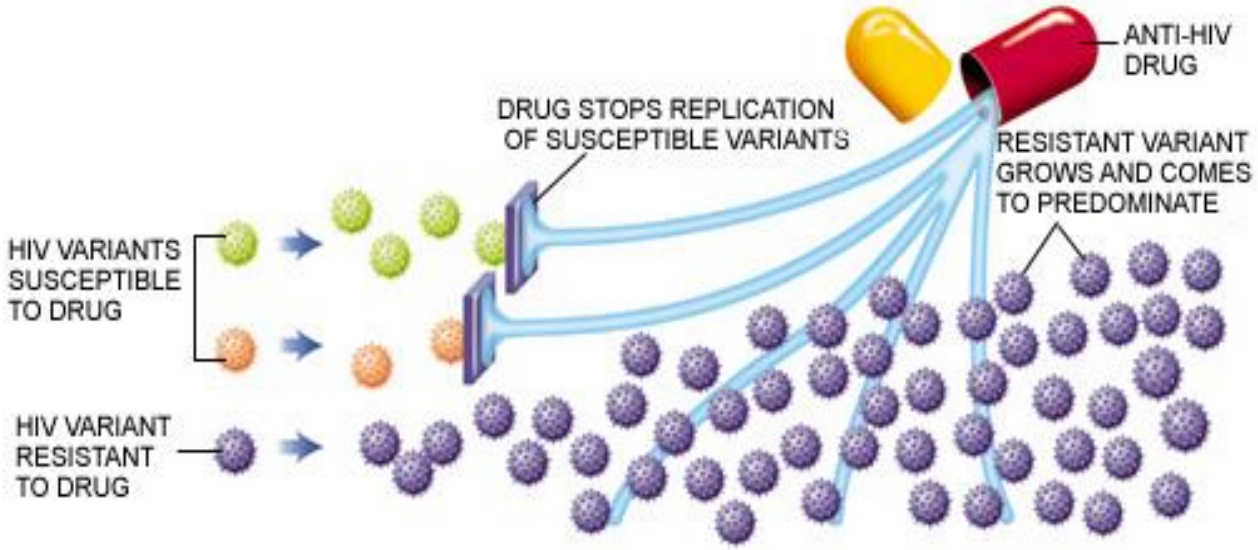
Every possible mutation is present

Selection of drug resistance

- Drug are generally designed to target conservative sites
- Major mutations resulting in drug resistance are selected at a fitness cost
- One and possibly two primary drug resistance mutations may be present per HIV-RNA copy before therapy at very low level <0.2%
- These variants are not detected with standard resistance testing

Selection of drug resistance during cART

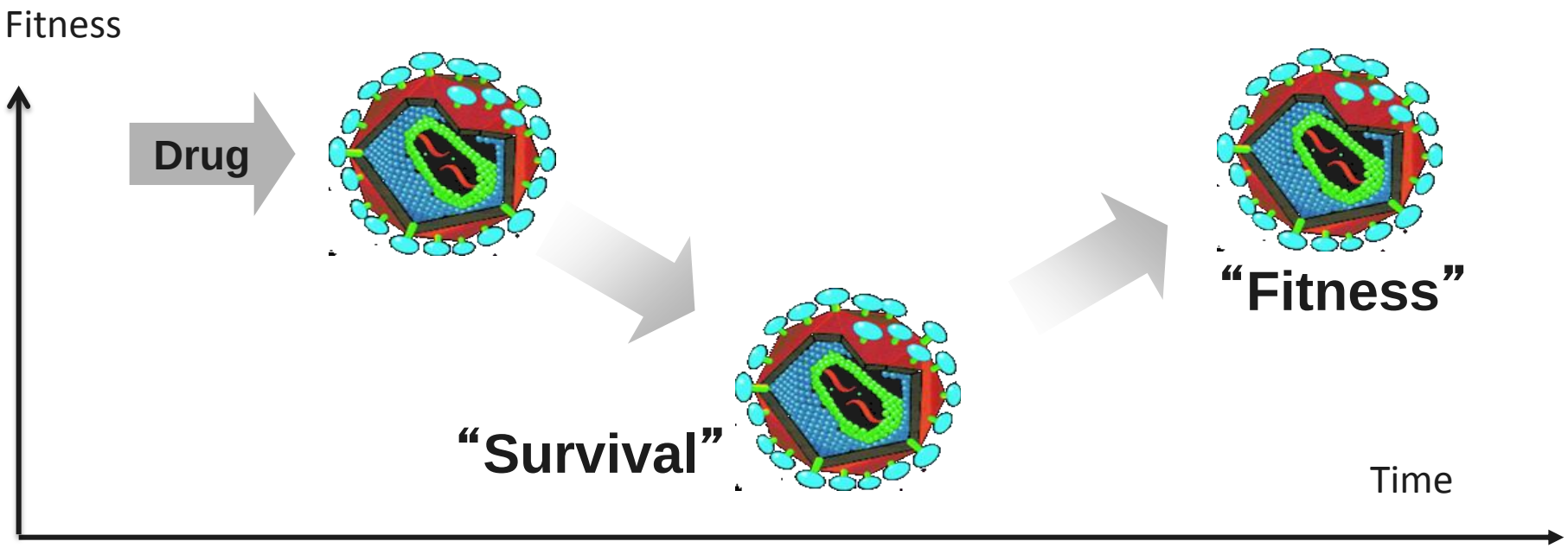
Insufficient suppression of HIV replication: resistant variants that are pre-existing in the viral quasispecies become the dominant viral variant.



Selection of drug resistance

- For some drugs single mutations can confer high-level resistance
- For other drugs or combination of drugs, high-level resistance requires several mutations within a single genome
- For successful therapy the barrier to resistance of cART should exceed the level of resistance present in the quasispecies

Viral escape to drug pressure



Drug places pressure on virus to escape inhibition

Drug pressure results in selection of primary escape mutations

Secondary mutations selected to compensate loss of fitness

Genetic Barrier to resistance

- the number of mutations required for resistance to develop

AND

- the likelihood with which such mutations are likely to occur
  - depending on the level of resistance
  - replication capacity (fitness) of the variants
  - drug level
  - Level of replication

Genetic barrier in patients with HIV replication

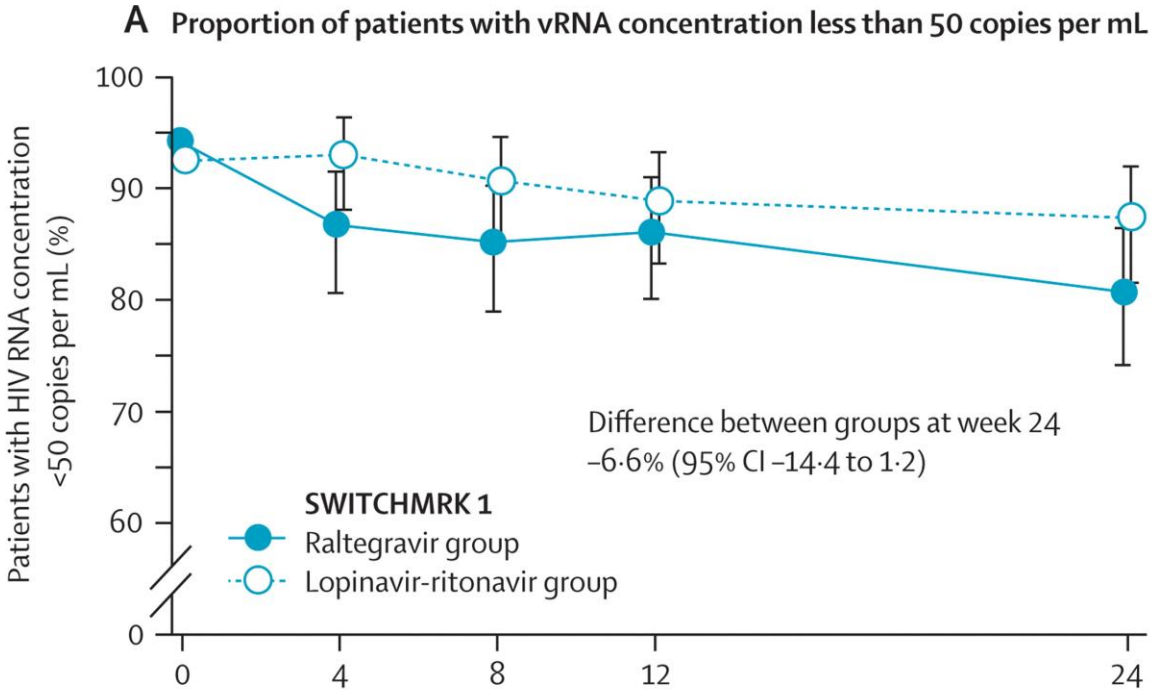
RC is more important than level of resistance

- 70R in RT gives less resistance than 215Y, but has a smaller effect on resistance and is selected first

TDF + FTC genetic barrier seems low since 65R in RT results in resistance to both drugs

- In practice: 184V which gives resistance to FTC only is selected first
- In the background of 184V, 65R is not easily selected in subtype B

Genetic barrier in patients with HIV suppression: Switch of bPI to INI

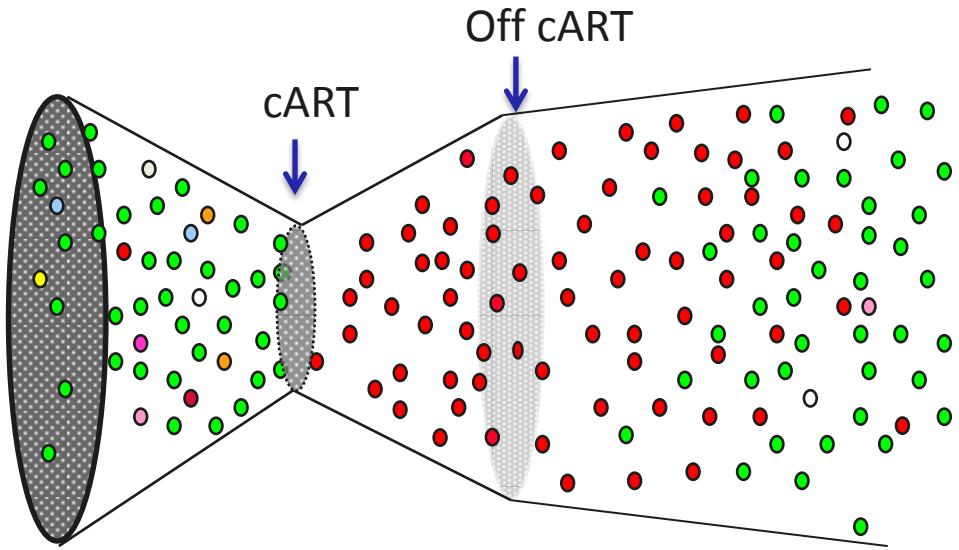


Number of contributing patients					
Raltegravir group	174	166	169	173	172
Lopinavir-ritonavir group	174	171	171	171	174

Eron JJ et al. *Lancet. 2010 Jan 12.*

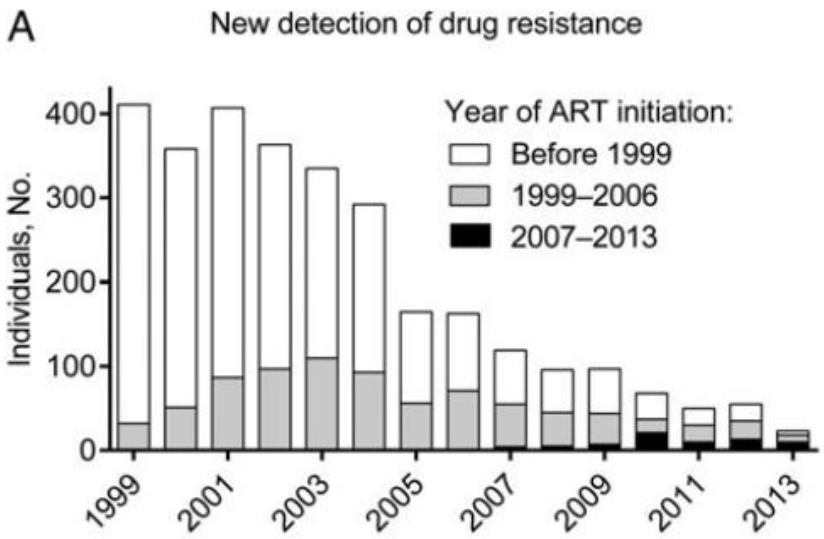
Evolution of resistance off drug pressure

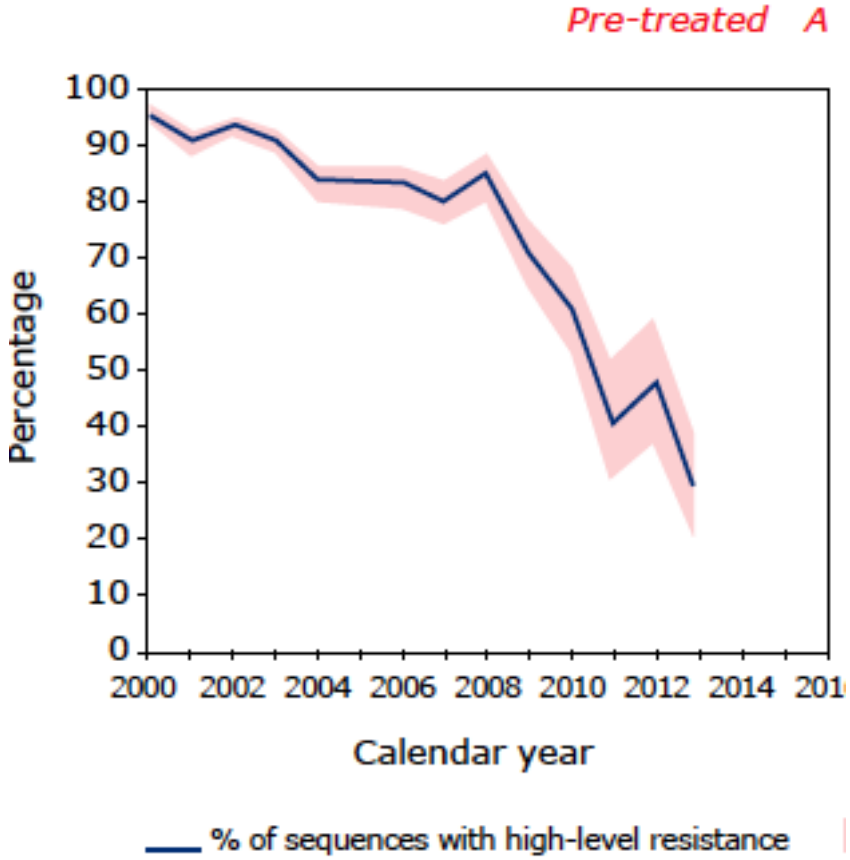
- After interruption of therapy in therapy-experienced patients: wildtype regains dominance
- Resistant variants remain archived as proviral DNA and may also circulate as minority variants
- Reintroduction of therapy: rapid selection and dominance of resistant quasispecies (Kijak, J Vir 2002)



Susceptible virus (wild type)
Resistant virus (mutant)

Emergence of Acquired HIV-1 Drug Resistance decreased dramatically





Scherrer et al. Clin Infect Dis. 2016;SHM (Athena) Report 2017

Resistance can be selected to high genetic barrier drugs

Clin Infect Dis. 2018 Jul 28. doi: 10.1093/cid/ciy589. [Epub ahead of print]

Evolution of protease inhibitor resistance in HIV-1-infected patients failing protease inhibitor monotherapy as second-line therapy in low-income countries: an observational analysis within the EARNEST randomised trial.

Thompson JA[1,2], Kityo C[3], Dunn D[1], Hoppe A[1,4], Ndashimye E[3], Hakim J[5], Kambugu A[6], van Oosterhout JJ[7,8], Arribas J[9], Mugyenyi P[3], Walker AS[1], Paton NI[1,10]; Europe Africa Research Network for Evaluation of Second-line Therapy (EARNEST) Trial Team.

Author information

Abstract

Open/close author information list

Lancet HIV. 2017 Dec;4(12):e547-e554. doi: 10.1016/S2352-3018(17)30152-2. Epub 2017 Oct 26.

Dolutegravir as maintenance monotherapy for HIV (DOMONO): a phase 2, randomised non-inferiority trial.

Wijting I[1], Rokx C[1], Boucher C[2], van Kampen J[2], Pas S[2], de Vries-Sluijs T[1], Schurink C[1], Bax H[1], Derksen M[1], Andrinopoulou ER[3], van der Ende M[1], van Gorp E[4], Nouwen J[1], Verbon A[1], Bierman W[5], Rijnders B[6].

Author information

HIV drug resistance: Africa

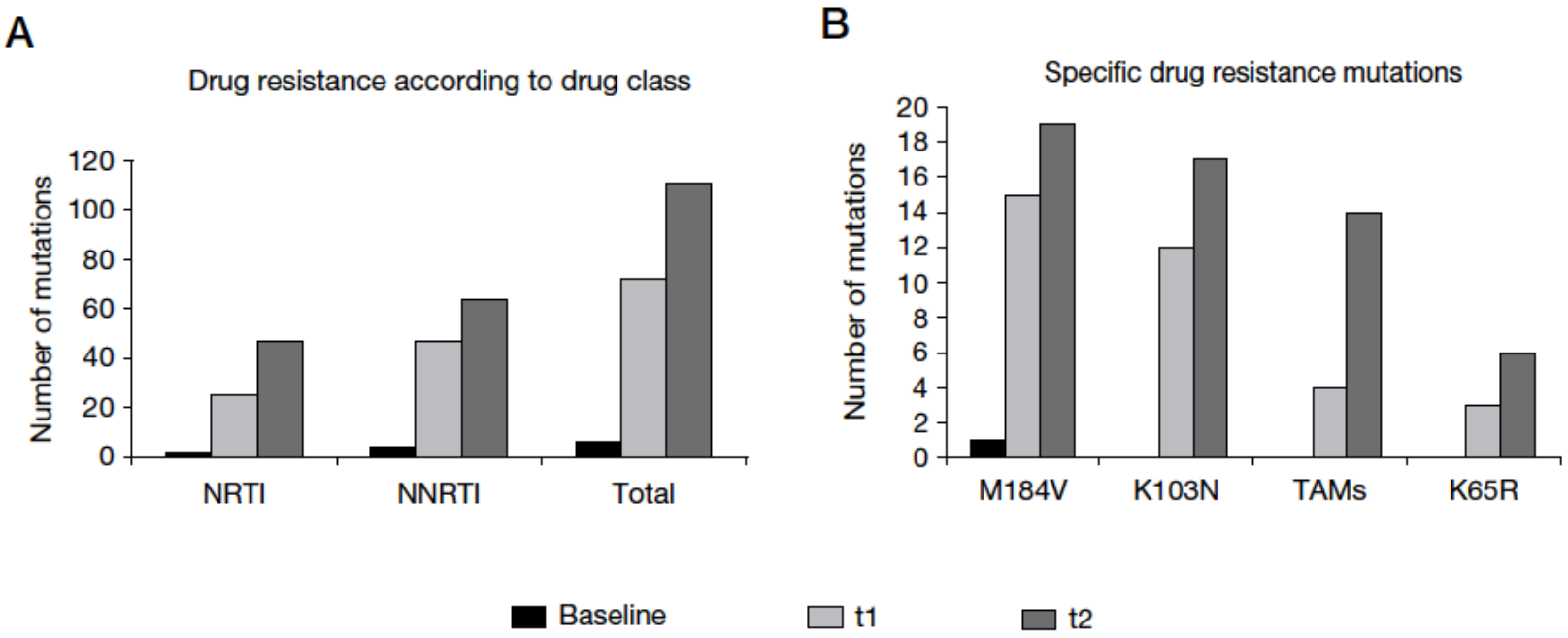
Cameroon: VF on 1st line after 12 months: 16% with viral load >1,000 HIV-RNA copies/mL, **63% NRTI or NNRTI mutations**

Mali: Virological failure on 1st line:
78% NRTI mutations
82% NNRTI mutations

South Africa:Virological failure with **HIV-1 subtype C** during 1st line TDF+3TC+NNRTI:
70% K65R + 93% NNRTI mutations

Fofana DB et al. J Antimicrob Chemother 2014; 69:2531-35
Zoufaly A et al. J Antimicrob Chemother. 2015 Mar;70(3):922-5.
Casadella M et al. AIDS 2016; 30:1137-1140

Delayed Switch: Accumulation of resistance



Barth et al. Antiviral Ther 2012

Absence of resistance: adherence?

Prevalence studies of first-line ART virological failure cases without detected drug resistance

Author	Journal	Year	Setting	% without resistance	n=
Kantor	AIDS Res Hum Retrov	2002	Zimbabwe	19%	21
Weidle	Lancet	2002	Uganda	35%	94
Marconi	CID	2008	SA (KZN)	17%	124
Murphy	AIDS	2010	SA (KZN)	13%	115
Van Zyl	J Med Virol	2011	SA (W Cape)	17%	167
Manasa	PLoS ONE	2013	SA (KZN)	14%	222
Aghokeng	CID	2014	various countries	21%	433

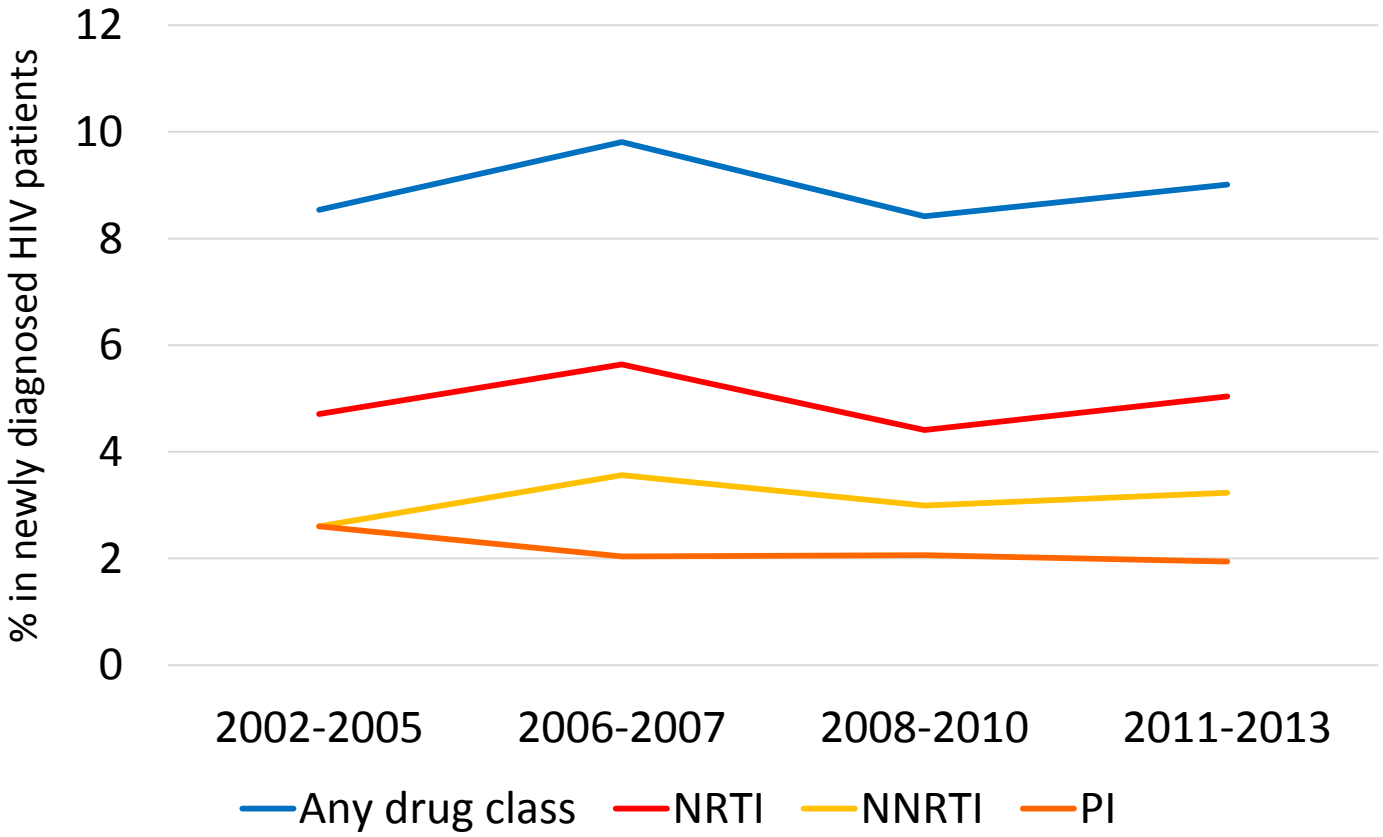
Baseline resistance (= primary resistance)

* Secondary/compensatory mutations may be present as polymorphisms and should not be included to assess transmitted resistance from an “epi” point of view

* Major mutations: profound effect on drug susceptibility in vitro (and RC). Not present as polymorphisms.

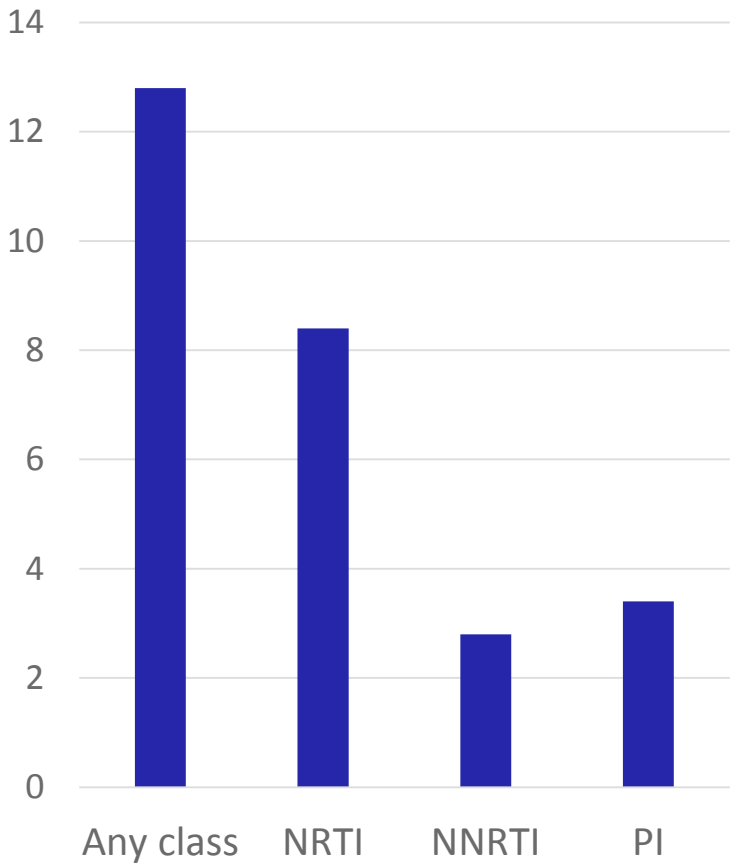
  Major mutations in naive patients are an indicator of exposure to ART ot the virus in a previous host = transmitted resistance

Transmitted resistance is stable in Europe



Hofstra et al. SPREAD program

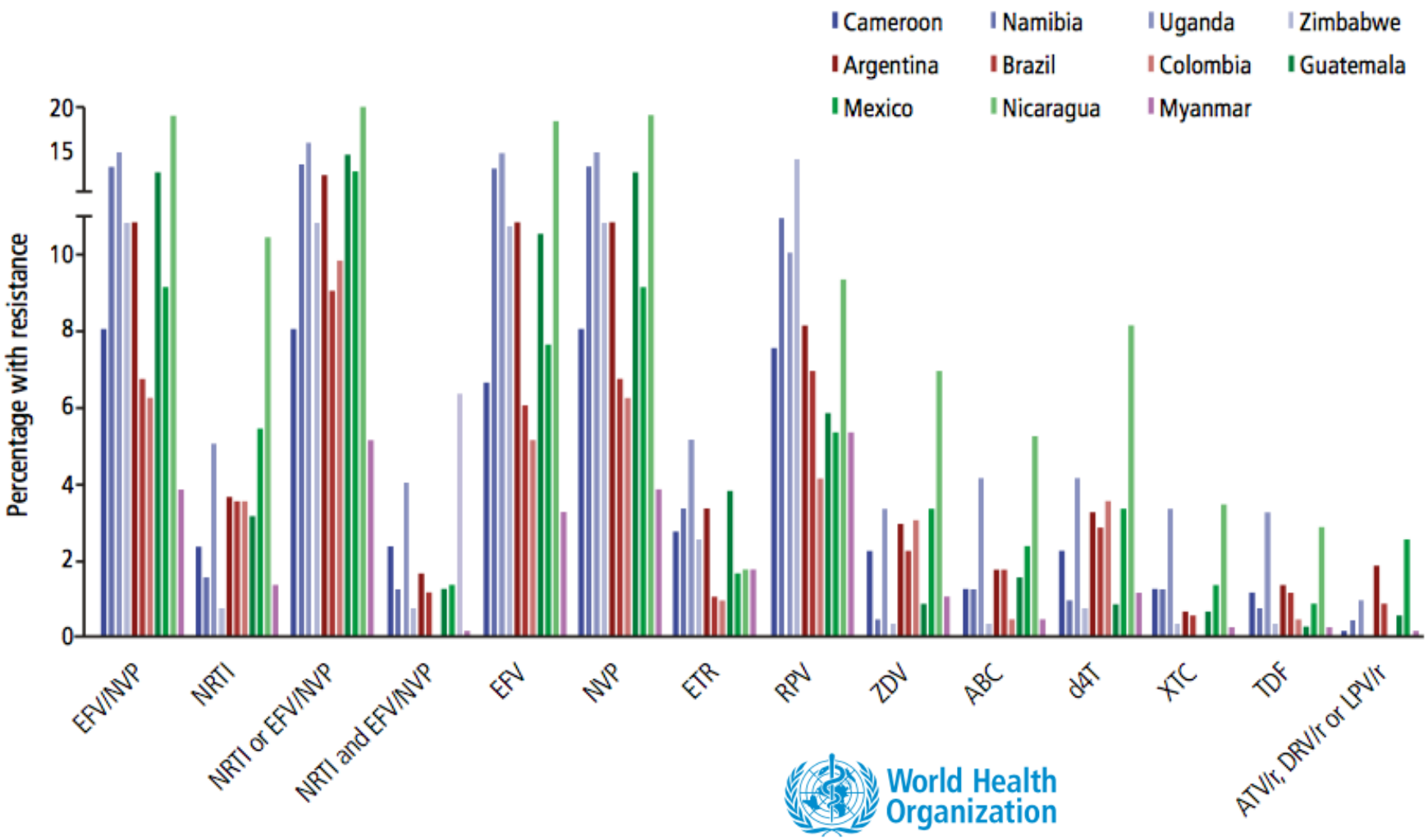
Transmitted drug resistance



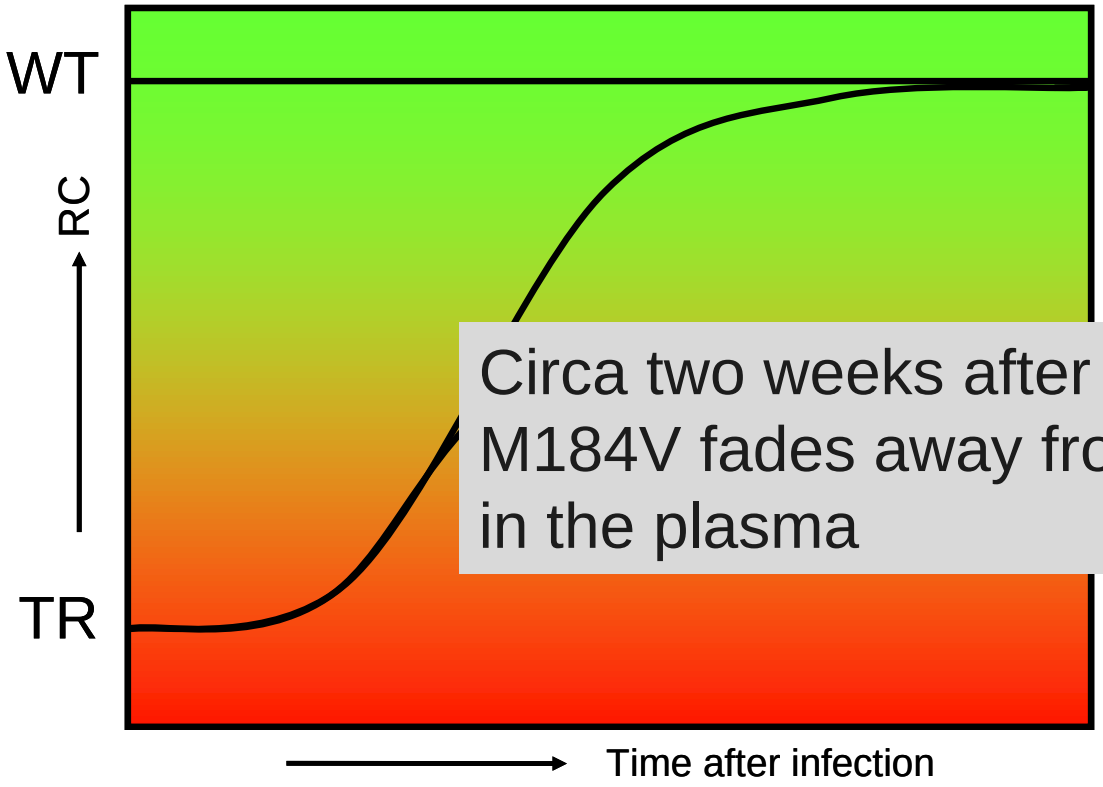
- 3 large subtype B clusters with TDR:
  - M41L: n=28, 42% recent
  - T215S: n= 15, 31% recent
  - M46L: n=10, 24% recent
- These 3 clusters include 42.4% of all patients with TDR

HIV drug resistance report WHO 2017

Fig. 4: Prevalence of pretreatment HIV drug resistance by country



Reversion –
evolution towards wildtype

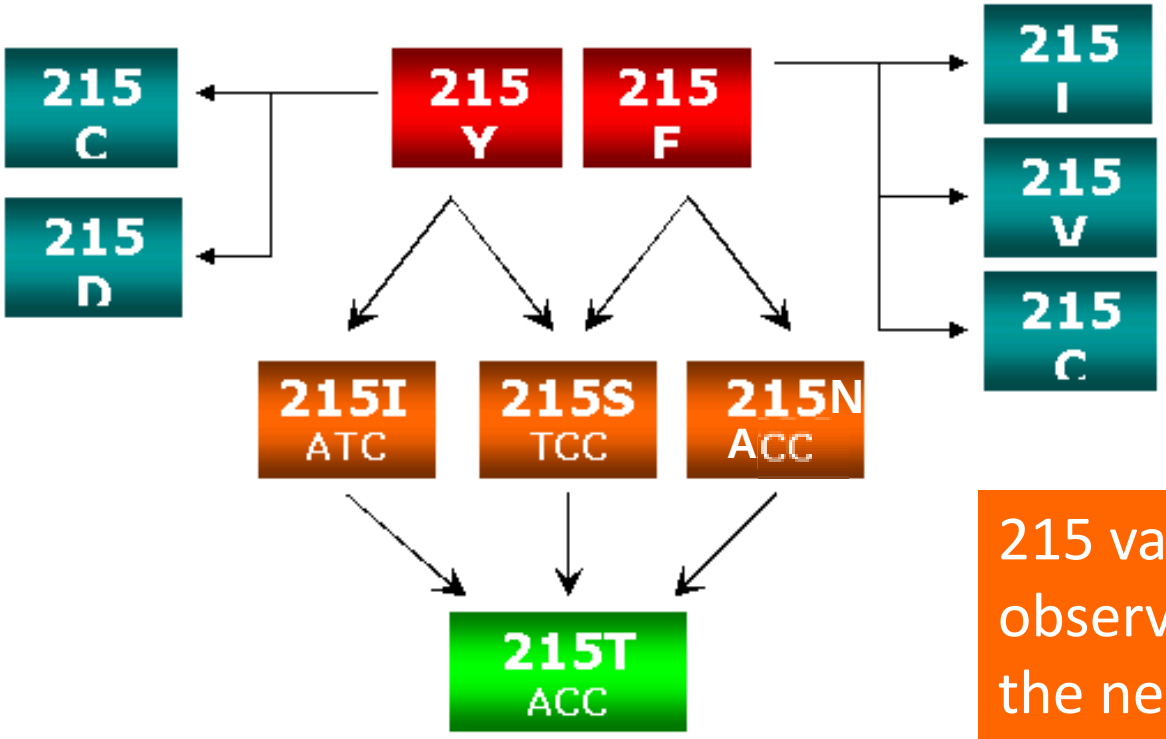


Circa two weeks after infection
M184V fades away from detection
in the plasma

M. Pingen et al., **JAC 2011**

Evolution of DR in naïve patients revertants/atypical variants

Figure 2. Evolution of transmitted variants at position 215 of reverse transcriptase



215 variants are observed inn 3% of the newly diagnosed individuals in Europe

Wensing et al AIDS 2008

Clinical Infectious Diseases

MAJOR ARTICLE

High Rates of Transmission of Drug-resistant HIV in Aruba Resulting in Reduced Susceptibility to the WHO Recommended First-line Regimen in Nearly Half of Newly Diagnosed HIV-infected Patients

L. Marije Hofstra,[1,2] Elena Sánchez Rivas,[3] Monique Nijhuis,[1] Leonie E. A. Bank,[1,4] Eduan Wilkinson,[5,6] Karina Kelly,[3] Tania Mudrikova,[4] Rob Schuurman,[1] Tulio de Oliveira,[5,6] Jaclyn de Kort,[3] and Annemarie M. J. Wensing[1]

[1]Virology, Department of Medical Microbiology, University Medical Center Utrecht, The Netherlands; [2]Department of Infection and Immunity, Luxembourg Institute of Health, Esch-sur-Alzette, Luxembourg; [3]Department of Internal Medicine, Dr Horacio E. Oduber Hospital, Oranjestad, Aruba; [4]Department of Internal Medicine and Infectious Diseases, University Medical Center Utrecht, The Netherlands; [5]Africa Centre for Population Health, and [6]School of Laboratory Medicine and Medical Sciences, College of Health Sciences, University of KwaZulu-Natal, Durban, Republic of South Africa

Background. In Western countries emergence of human immunodeficiency virus (HIV) drug resistance has tremendously decreased, and transmission of drug resistance has merely stabilized in recent years. However, in many endemic settings with limited resources rates of emerging and transmitted drug resistance are not regularly assessed.

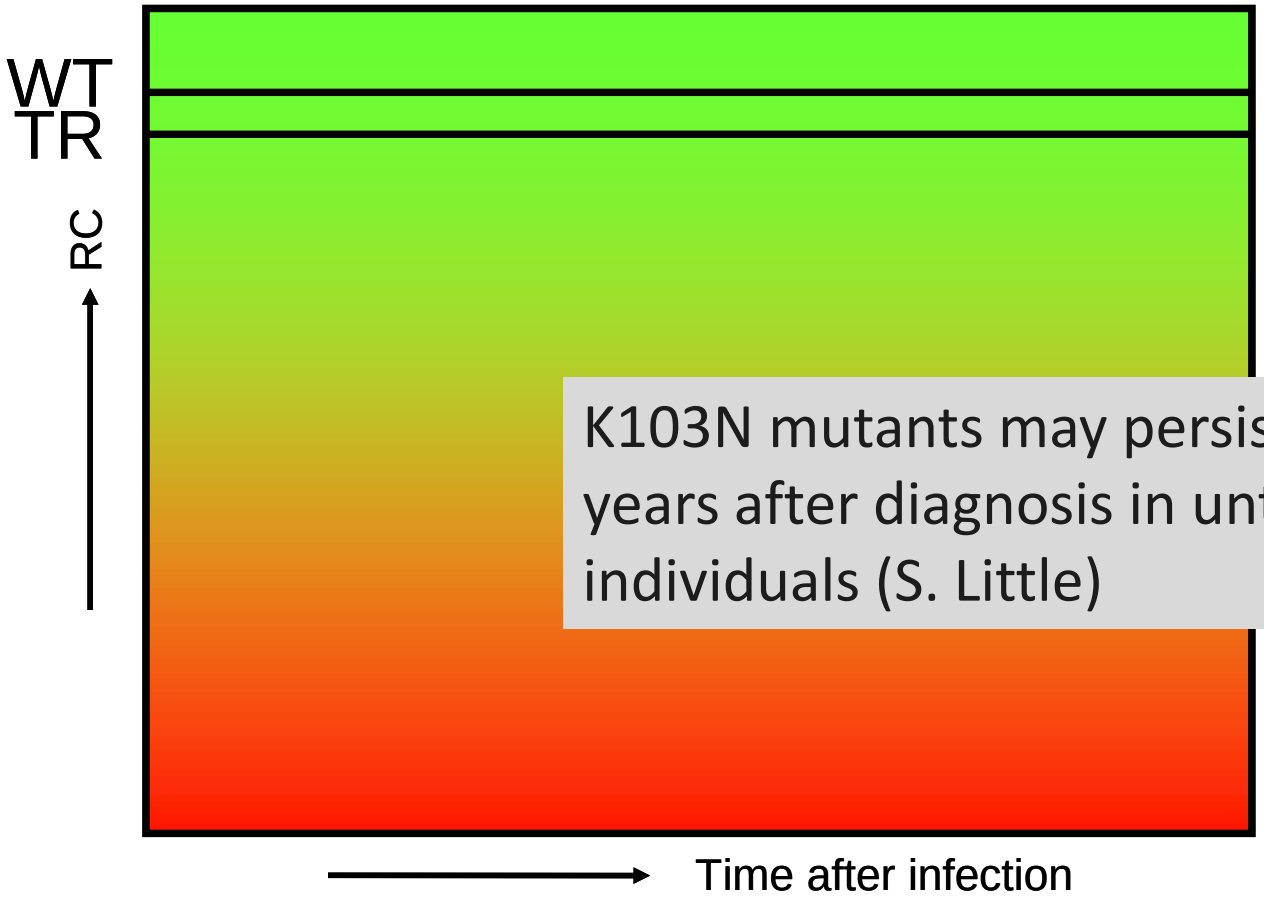
Methods. We performed a survey including all HIV-infected individuals who received resistance testing in 2010–2015 in Aruba, a highly endemic HIV area in the Caribbean. Transmitted HIV drug resistance was determined using World Health Organization (WHO) criteria. Transmission dynamics were investigated using phylogenetic analyses. In a subset, baseline samples were re-analyzed using next generation sequencing (NGS).

Results. Baseline resistance testing was performed in 104 newly diagnosed untreated individuals (54% of all newly diagnosed individuals in 2010–2015): 86% were men, 39% were foreign-born, and 22% had AIDS at diagnosis. And 33% (95% CI: 24–42%) was infected with a drug-resistant HIV variant. The prevalence of resistance to non-nucleoside reverse transcriptase inhibitors (NNRTIs) reached 45% (95% CI: 27–64%) in 2015, all based on the prevalence of mutation K103N. NGS did not demonstrate additional minority K103N-variants compared to routine resistance testing. K103N-harboring strains were introduced into the therapy-unexposed population via at least 6 independent transmissions epidemiologically linked to the surrounding countries. Virological failure of the WHO-recommended first-line NNRTI-based regimen was higher in the presence of K103N.

Conclusions. The prevalence of resistant HIV in Aruba has increased to alarming levels, compromising the WHO-recommended first-line regimen. As adequate surveillance as advocated by the WHO is limited, the Caribbean region could face an unidentified rise of NNRTI-resistant HIV.

Persistence

due to limited effect on fitness

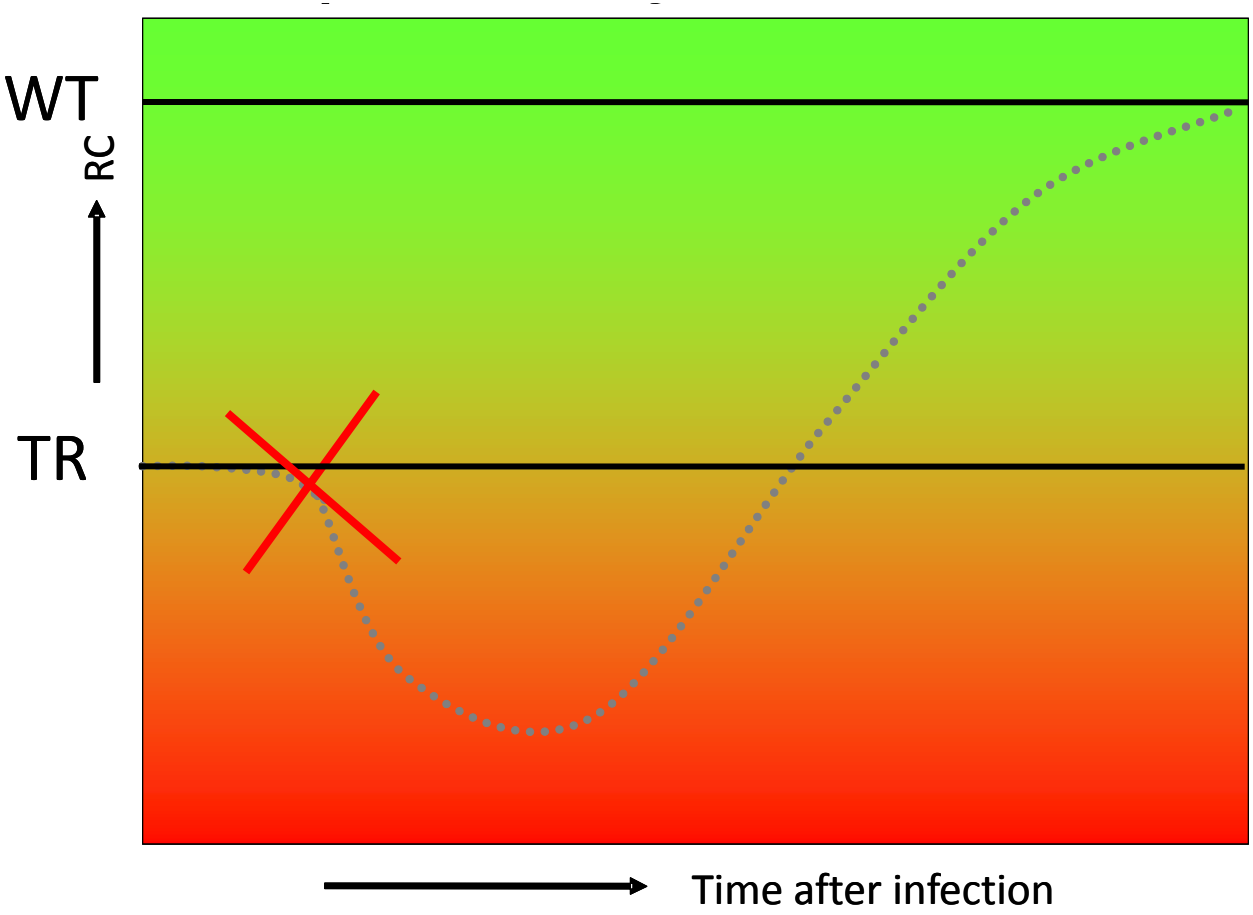


K103N mutants may persist up to 7 years after diagnosis in untreated individuals (S. Little)

M. Pingen et al., **JAC 2011**

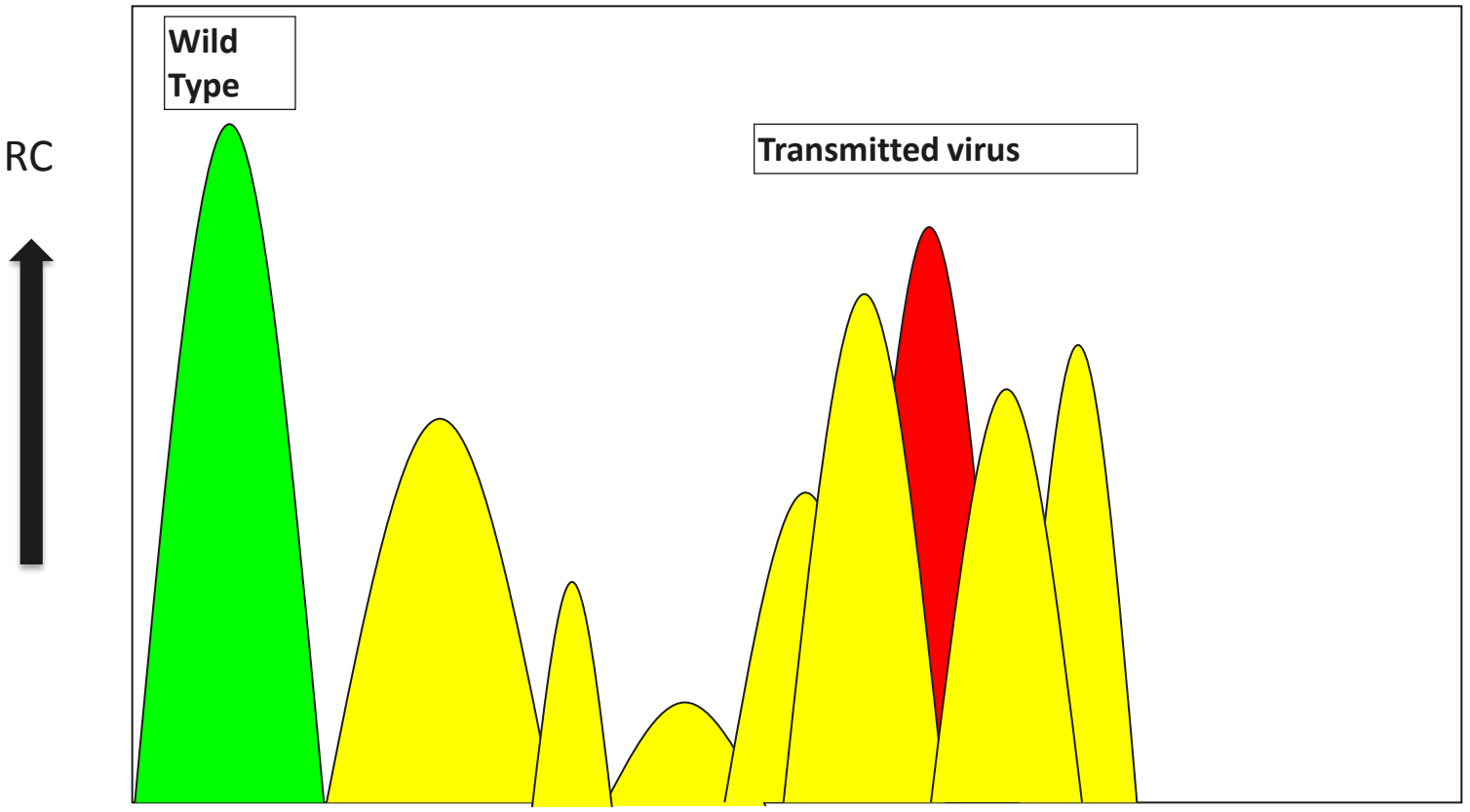
Persistence

due to compensatory fixation

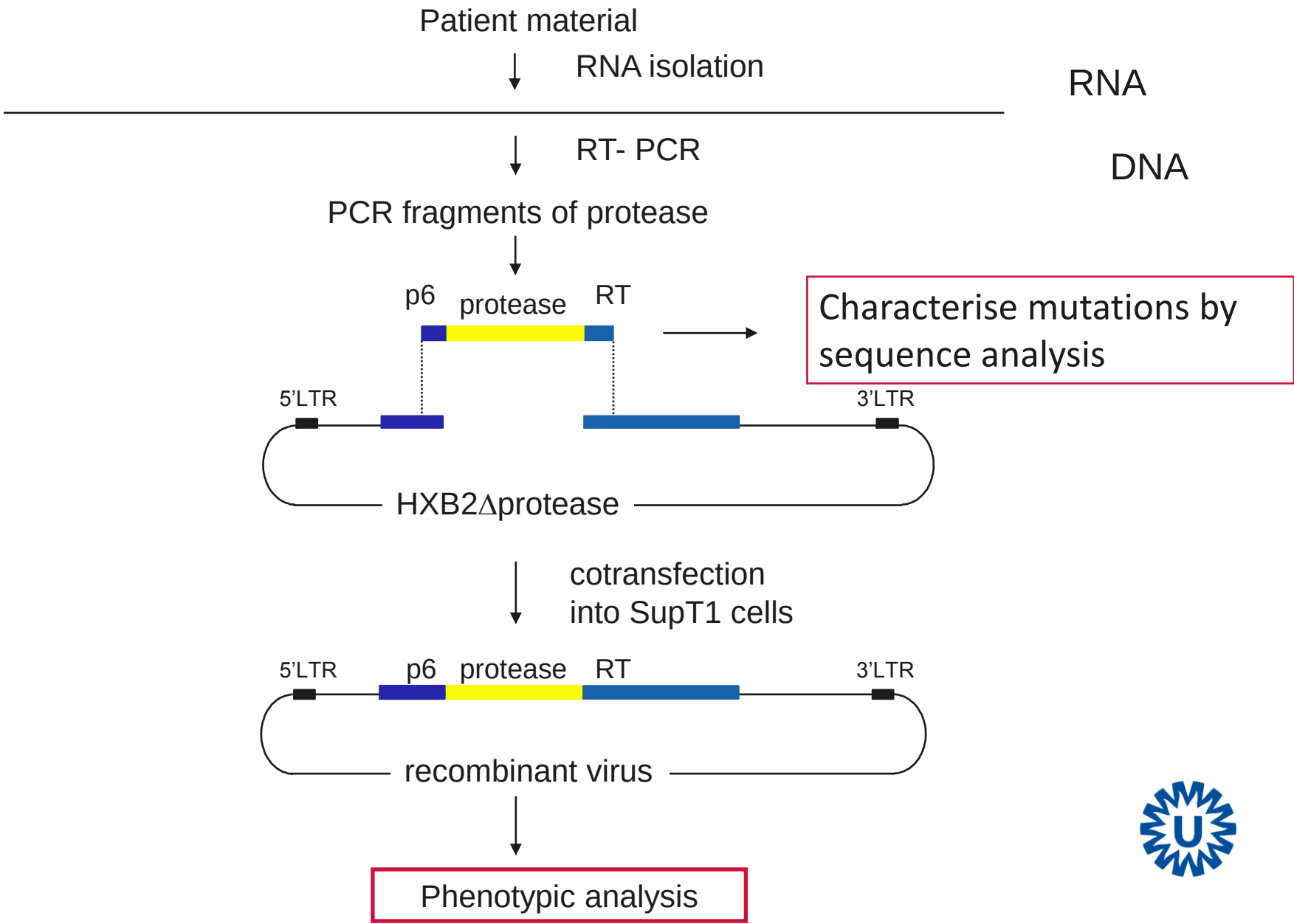


M. Pingen et al., **JAC 2011**

Mechanismof Compensatory fixation

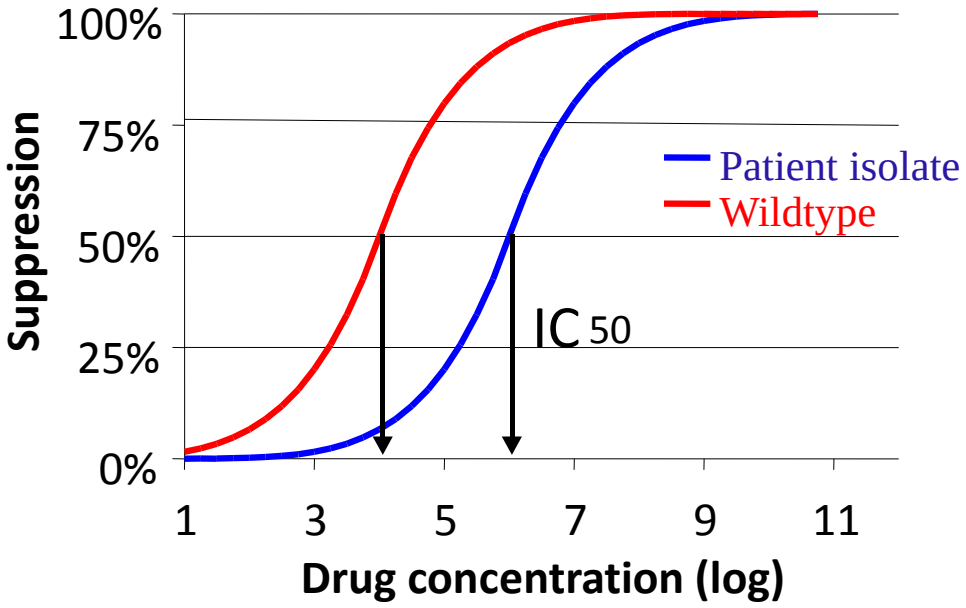


Resistance testing



Resistance test: Fenotype

- Fenotypering determines the viral susceptibility for drugs in cell culture: Direct measurement of the concentration drug needed to inhibit viral replication (IC50/IC90)
- Time consuming, recombinant assays only, limited correlation with clinical outcome
- Indicated when effect of mutations is unknown



Resistance test: Genotype

Easy to perform, rapid

Indirect measurement: Prediction

Knowledge on mutation patterns and therapy outcome needed

Knowledge on interaction between mutations needed

Excellent correlation with outcome in clinical trials

Population sequencing: detects mutant if they make up 10-15% of the viral population

Low-frequency drug-resistant HIV-1 and risk of virological failure to first-line NNRTI-based ART: a multi-cohort European case-control study using centralized ultrasensitive 454 sequencing

A Cozzi-Lepri[1], M Noguera-Julian[2], F Di Giallonardo[3], R Schuurman[4], M Däumer[5], S Aitken[4], HF Günthard[3], F Brun-Vezinet[6], KJ Metzner[3], R Paredes[2], and the CHAIN Minority HIV-1 Variants Working Group

1 University College London, London, UK, 2 Institut de Recerca de la SIDA IrsiCaixa, Badalona, Catalonia, Spain, 3 Division of Infectious Diseases and Hospital Epidemiology, University Hospital Zurich, Zurich, Switzerland, 4 Utrecht Medical Centre, Utrecht, Netherlands, 5 Institut für Immunologie und Genetik, Kaiserslautern, Germany, 6 Association de Recherche en Virologie et Hematologie, France.

Toronto 7 June, 2013; 11:30

Interpretation

IAS–USA *Topics in Antiviral Medicine*

Special Contribution

2017 Update of the Drug Resistance Mutations in HIV-1

Annemarie M. Wensing, MD, PhD; Vincent Calvez, MD, PhD; Huldrych F. Günthard, MD; Victoria A. Johnson, MD; Roger Paredes, MD, PhD; Deenan Pillay, MD, PhD; Robert W. Shafer, MD; Douglas D. Richman, MD

HIV french resistance — *HIV-1 genotypic drug resistance interpretation's algorithms*

anrs France REcherche Nord & sud Sida-hiv Hépatites — Agence autonome de l'Inserm

HIV-GRADE

Sequence Analysis | Mutation List Analysis

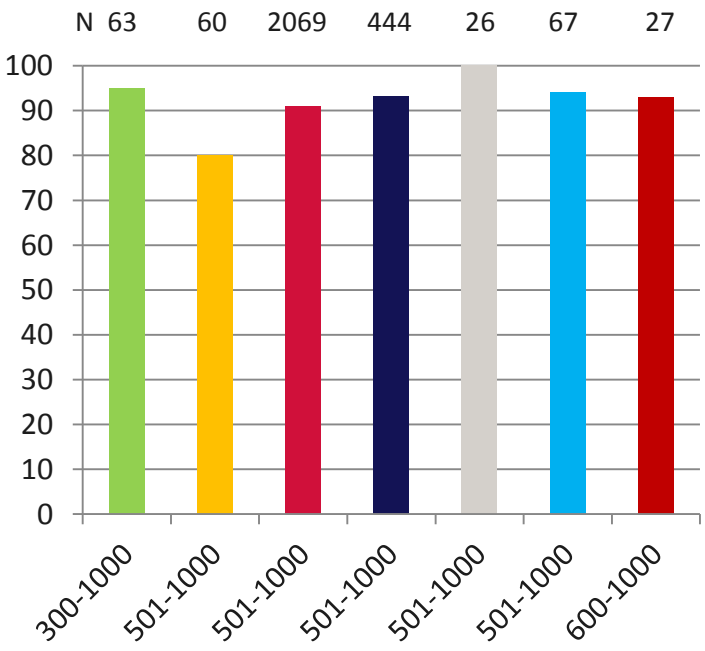
STANFORD UNIVERSITY

HIV DRUG RESISTANCE DATABASE

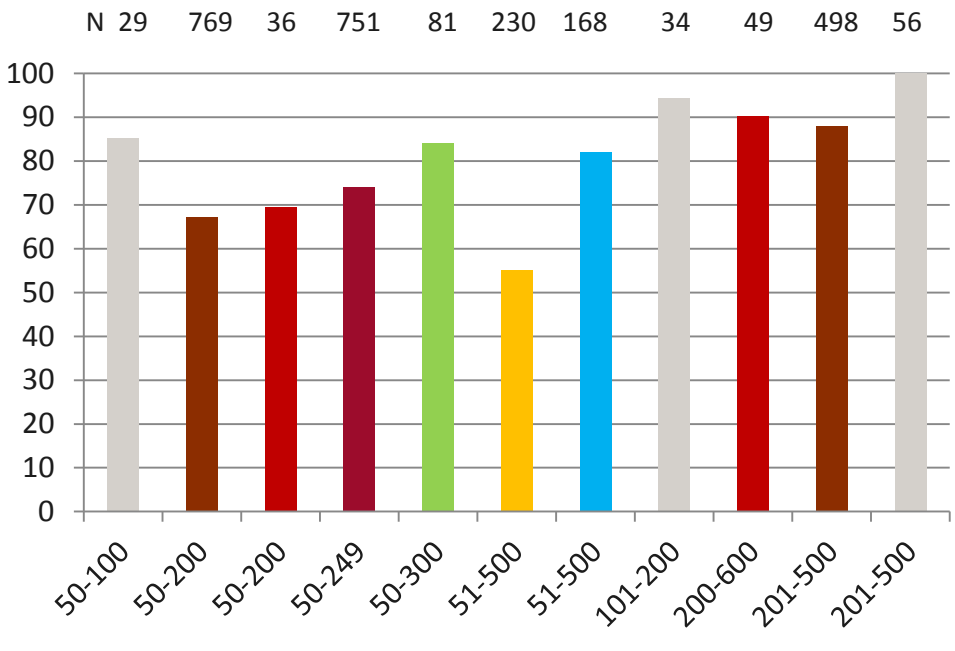
A curated public database designed to represent, store, and analyze the divergent forms of data underlying HIV drug resistance.

Resistance testing: Success rates (%) at low viral loads

Above 500 copies/mL



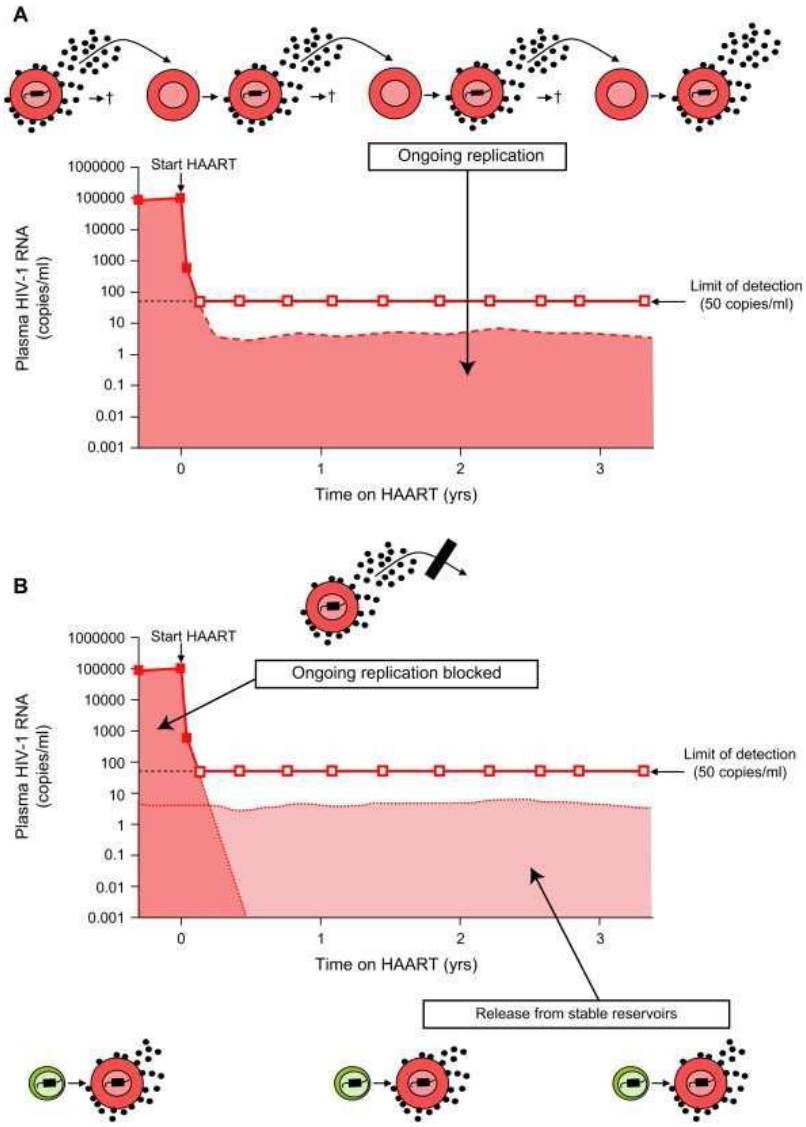
Below 500 copies/mL



Mackie et al, 2004
Waters et al 2006
Assoumou et al, 2010
Gonzalez-Serna et al, 2014
Santoro et al, 2014
Bruzzone et al, 2014
Armenia et al, 2015

Hofstra et al. ESAR guidelines

Nature of low level viremia



- Ongoing replication despite antiretroviral therapy?
- Virus production due to activation of latently infected cells?
- Non-adherence?

Shen, Siliciano, J Allergy Clin Immunol 2008

Effect of HIV-1 low-level viraemia during antiretroviral therapy on treatment outcomes in WHO-guided South African treatment programmes: a multicentre cohort study

Lucas E Hermans, Michelle Moorhouse, Sergio Carmona, Diederick E Grobbee, L Marije Hofstra, Douglas D Richman, Hugo A Tempelman, Willem D F Venter, Annemarie M J Wensing

Lancet Infect Dis 2018; 18: 188–97

Published Online November 17, 2017 http://dx.doi.org/10.1016/S1473-3099(17)30681-3

This online publication has been corrected. The corrected version first appeared at thelancet.com/infection on November 24, 2017

See Comment page 130

Translational Virology, Department of Medical Microbiology (L E Hermans MD, L M Hofstra MD, A M J Wensing MD) and Clinical Epidemiology (Prof D E Grobbee MD), University Medical Center Utrecht, Utrecht, Netherlands; Wits Reproductive Health and HIV Institute (L E Hermans, M Moorhouse MBBCh, W D F Venter MBBCh), and Department of Molecular Medicine and Haematology (S Carmona MBBCh) University of the Witwatersrand, Johannesburg, South Africa; Ndlovu Research Consortium,

Summary

Background Antiretroviral therapy (ART) that enables suppression of HIV replication has been successfully rolled out at large scale to HIV-positive patients in low-income and middle-income countries. WHO guidelines for these regions define failure of ART with a lenient threshold of viraemia (HIV RNA viral load ≥1000 copies per mL). We investigated the occurrence of detectable viraemia during ART below this threshold and its effect on treatment outcomes in a large South African cohort.

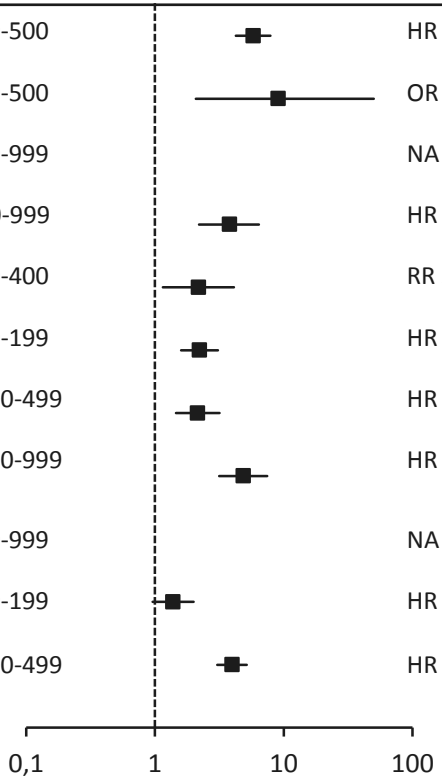
Methods In this observational cohort study, we included HIV-positive adults registered between Jan 1, 2007, and May 1, 2016, at 57 clinical sites in South Africa, who were receiving WHO-recommended ART regimens and viral load monitoring. Low-level viraemia was defined as the occurrence of at least one viral load measurement of 51–999 copies per mL during ART. Outcomes were WHO-defined virological failure (one or more viral load measurement of ≥1000 copies per mL) and switch to second-line ART. Risks were estimated with Cox proportional hazard models.

Findings 70 930 patients were included in the analysis, of whom 67 644 received first-line ART, 1476 received second-line ART, and 1810 received both. Median duration of follow-up was 124 weeks (IQR 56–221) for patients on first-line ART and 101 weeks (IQR 51–178) for patients on second-line ART. Low-level viraemia occurred in 16 013 (23%) of 69 454 patients, with an incidence of 11·5 per 100 person-years of follow-up (95% CI 11·4–11·7), during first-line ART. Virological failure during follow-up occurred in 14 380 (22%) of 69 454 patients on first-line ART. Low-level viraemia was associated with increased hazards of virological failure (hazard ratio [HR] 2·6, 95% CI 2·5–2·8; p<0·0001) and switch to second-line ART (HR 5·2, 4·4–6·1; p<0·0001]) compared with virological suppression of less than 50 copies per mL. Risk of virological failure increased further with higher ranges and persistence of low-level viraemia.

Interpretation In this large cohort, low-level viraemia occurred frequently and increased the risk of virological failure and switch to second-line ART. Strategies for management of low-level viraemia need to be incorporated into WHO guidelines to meet UNAIDS-defined targets aimed at halting the global HIV epidemic.

Virological outcome persistent viremia

First author, year	Cut off LLV	Risk of greater rebound	LLV group	control group	N	FU (mo)
Greub, 2002	50-500	HR 5.80 (4.26-7.90)	NA	NA	2055	17.7
Raboud, 2002	50-500	OR 9.09 (2.08-50.0)	NA	NA	74	12
Karlsson, 2004	50-999	NA	10/18 (55.6%)	0/13 (0%)	31	27
Sungkanuparph, 2006	50-999	HR 3.80 (2.20-6.40)	39.7%	9.2%	362	29.5
Geretti, 2008	50-400	RR 2.18 (1.15-4.10)	12/85 (14.1%)	52/1032 (5.0%)	1386	26.4
Laprise, 2013	50-199	HR 2.22 (1.60-3.09)	22.7%	6.6%	1860	85.2
	200-499	HR 2.15 (1.46-3.17)	24.2%	6.6%		
	500-999	HR 4.85 (3.16-7.45)	58.9%	6.6%		
Hofstra, 2014	50-999	NA	3/16 (19%)	0/79 (0%)	172	34
ART-CC, 2015	50-199	HR 1.38 (0.96-2.00)	49/624 (7.9%)	1745/16796 (10.4%)	17902	28
	200-499	HR 3.97 (3.05-5.17)	109/482 (22.6%)	1745/16796 (10.4%)		
Lo Re, 2004	50-500		37%		79	23.1
Silva, 2014	20-200		0%		61	18
Boillat-Blanco, 2014	21-400		12%		179	11
Charuratananon, 2015	50-999		38.2%		68	68.4



Selection of resistance at low level viremia

First author, year	LLV	N	ART history
Nettles, 2004	50-400	21	19% Naive
Verhofstede, 2007	50-1000	39	22 pts with previous failure
Mackie, 2010	50-300	449	18% Naive
	300-999	552	
Gallien, 2011	50-500	39	Prior history of triple class drug failure
Prosperi, 2011	50-200	2500	Therapy experienced
	200-500		
	500-1000		
Taiwo, 2011	50-1000	54	Naive
Delaugerre, 2012	50-500	37	92% pretreated with at least 1 episode of VF
Li, 2012	50-1000	47	89% treatment experienced
Pellegrino, 2012	200-999	18	Heavily pretreated
Swenson, 2014	50-1000	1965	31% Naive; 20% inititated before cART era
Wirden, 2014	50-1000	171	Treatment experienced
Santoro, 2014	50-200	396	37.9% Naive
	201-500	287	
	501-1000	242	
GonzalezSerna, 2014	50-1000	212	Naive
Vancoillie, 2015	20-250	23	NA
Armenia, 2015	51-500	168	22.6% Naive, 41.4% INSTI naive
	500-1000	67	

Conclusion

- Resistance has become rare in settings with a wide arsenal of drugs and active monitoring
- Baseline resistance is often transmitted by individuals who are therapy naive
- In low and middle income countries both acquired as baseline resistance is accumulating
- Higher genetic barrier drugs will be introduced soon
- Backbones will remain relevant, but the dogma of three drugs may change…

Acknowledgement

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SPREAD programme