UMC Utrecht

HIV drug resistance

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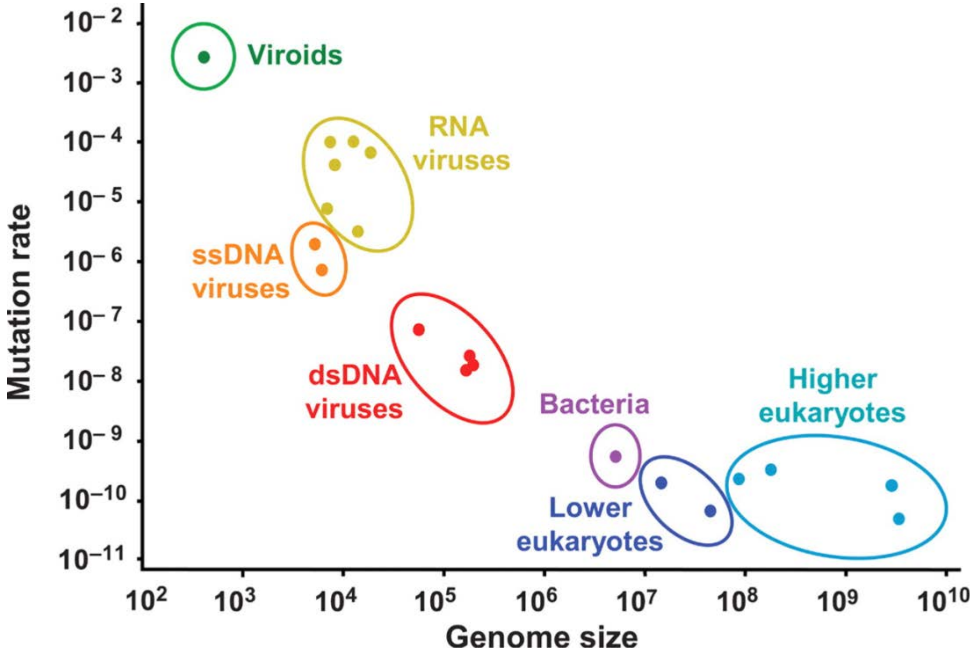
Should you know something about resistance?

95% suppression on ART in many countries

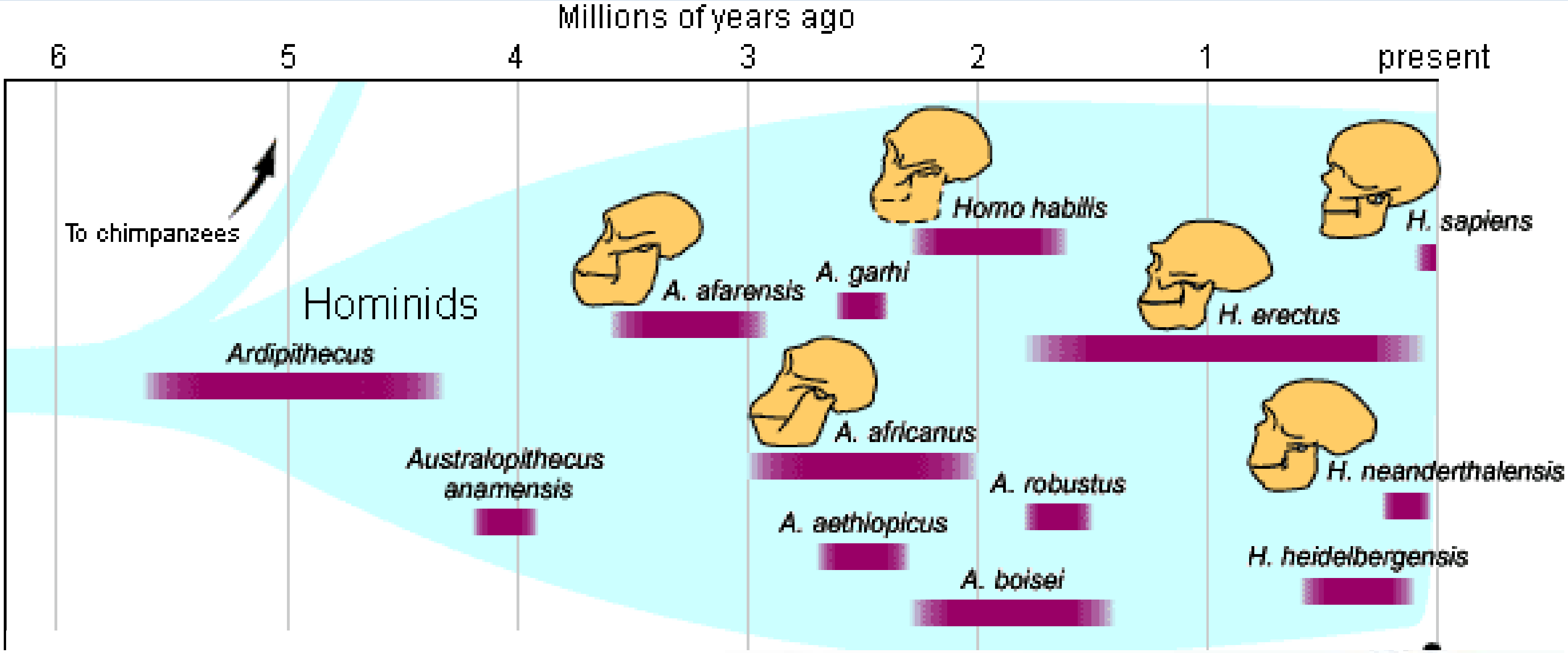
- Failure although rare still occurs
- Simplification of therapy
- Use of new combinations outside the scope of clinical trials
- Suppression rates are not like this worldwide, viruses travel with humans
- Natural variation/transmitted resistance may affect response
- Use of PREP
- New techniques

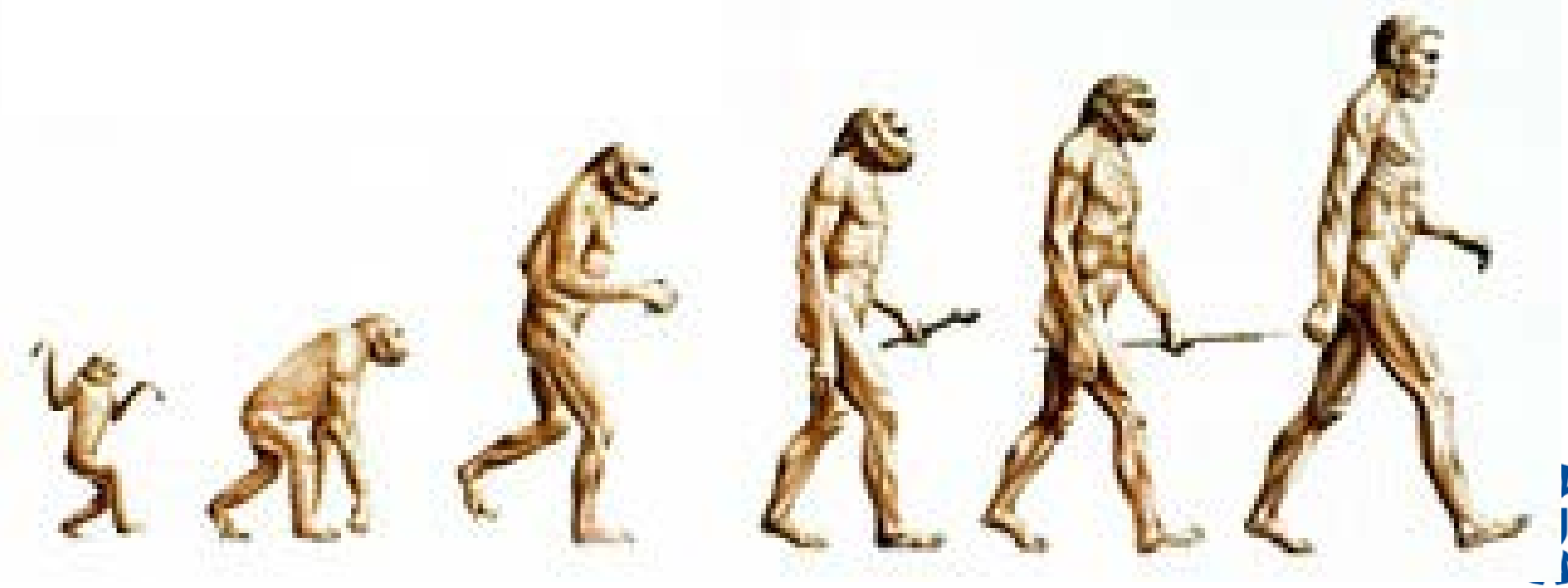
Mutation rate RNA viruses > DNA viruses > bacteria > humans



Even among RNA viruses HIV is highly variable:
the HIV population present in a single individual <u>six years</u> 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
Homo habilis
A. africanus
A. aethiopicus
A. robustus
A. boisei
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

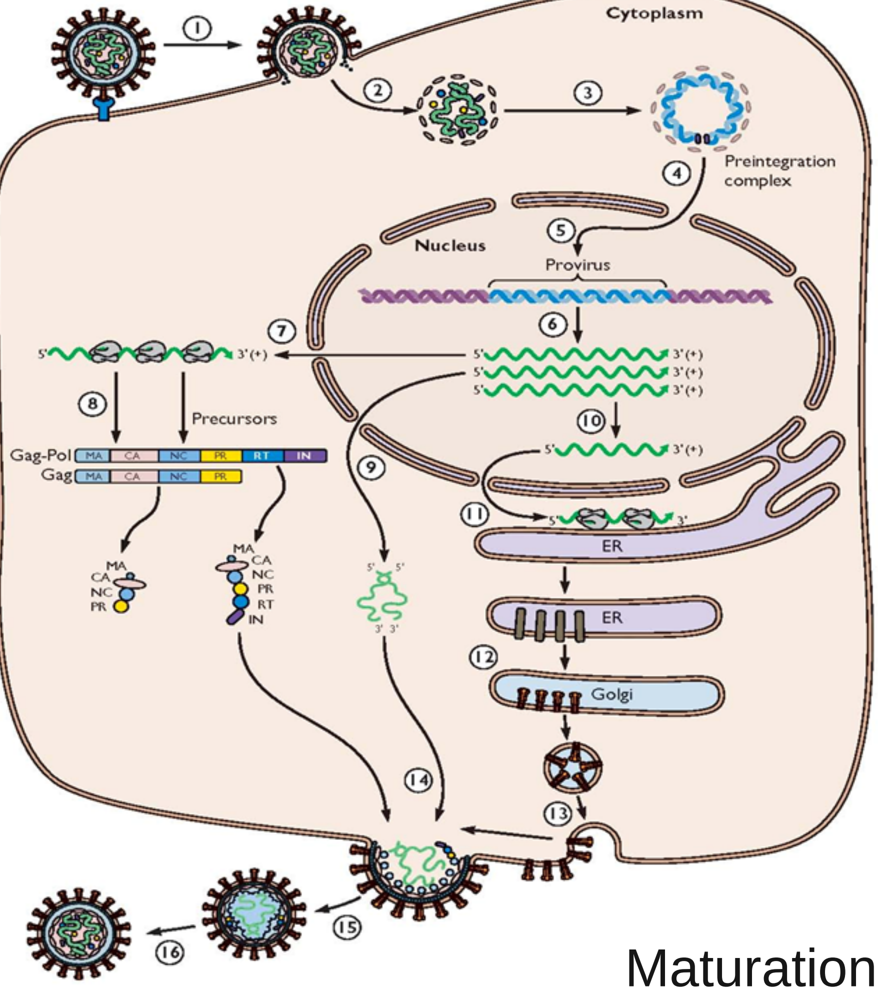
Frequent Recombination

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

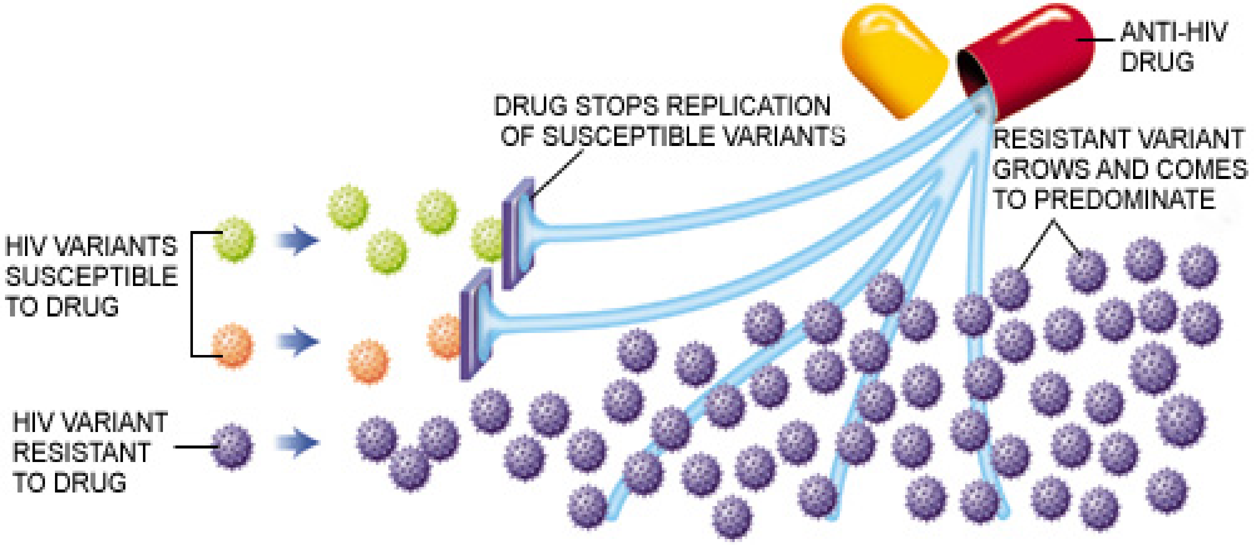
Variability of HIV:

- Intrinsic variability differs per nucleotide position
    - Polymorphic sites: high plasticity
    - Conservative sites: low plasticity
- Many variants are not replication competent
- The effective population size (viruses that are replication competent) is relatively small: 1000 to 10000 variants
- Among those, one is most fit and therefore most abundant: **wild type** virus

Nijhuis et al, PNAS, 1998

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

- 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

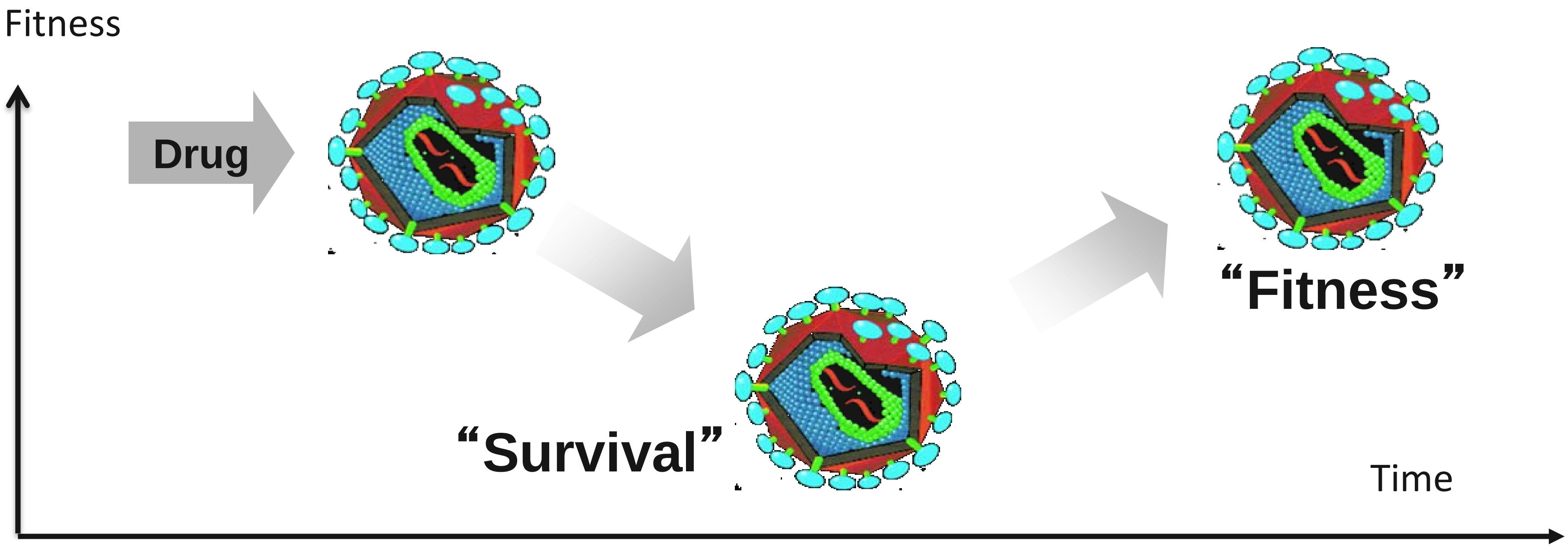
Selection of drug resistance

- 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

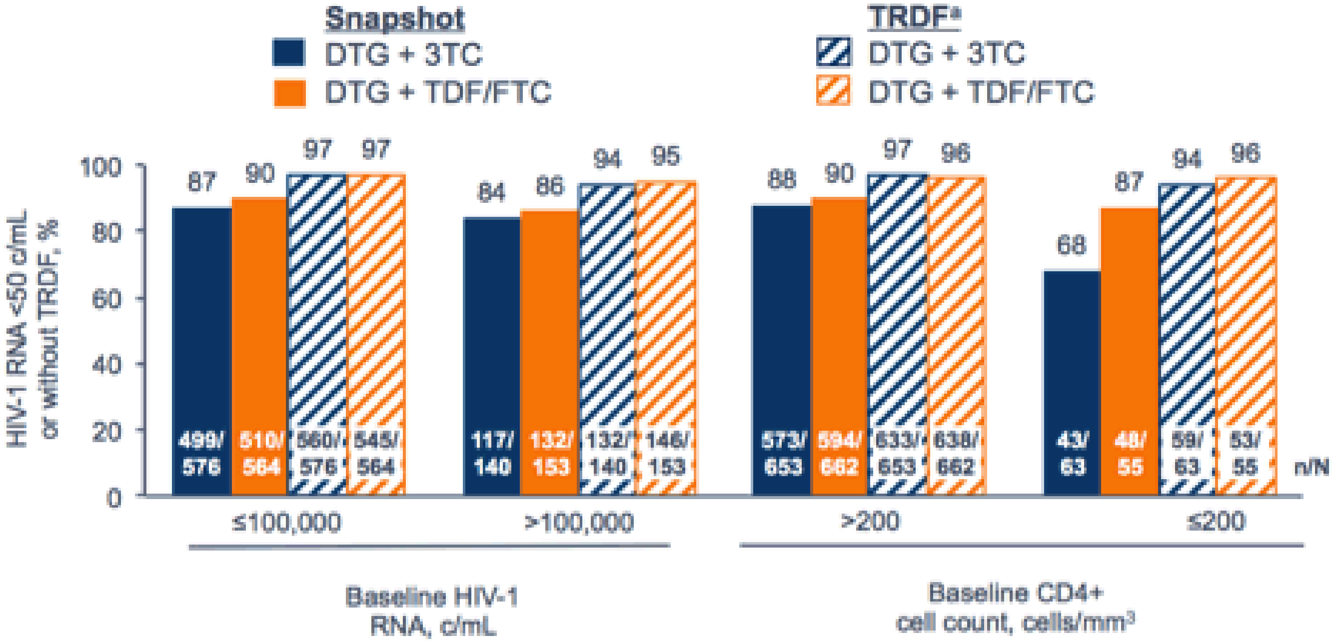
- In practice: 184V which gives resistance to FTC only is selected first
- In the background of 184V, 65R is not easily selected in subtype B

Genetic barrier in patients with HIV suppression: Switch of bPI to 1st gen INI

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

Genetic barrier concept does not support dogma of 3 drugs: Gemini data

PROPORTION OF PARTICIPANTS WITH HIV-1 RNA <50 C/ML BY BASELINE VIRAL LOAD AND CD4+ CELL COUNT AT WEEK 96: SNAPSHOT AND TRDF ANALYSIS



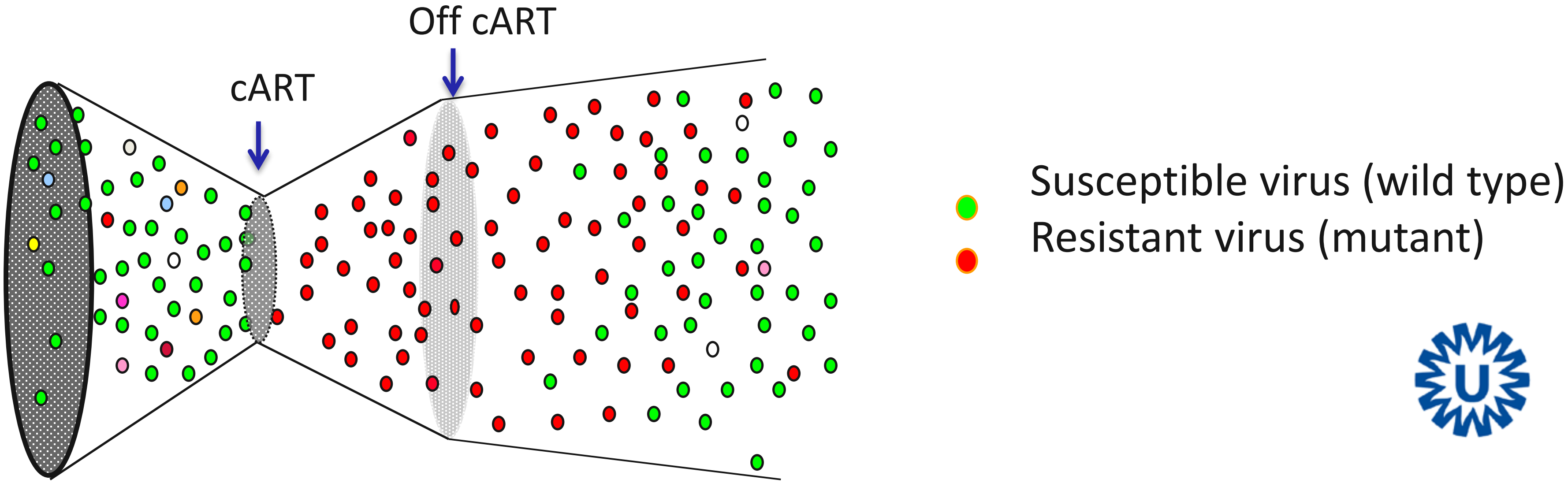
- **At Week 96, there were 3 confirmed virologic withdrawals in the DTG + 3TC group and 2 in the DTG + TDF/FTC group in the CD4 ≤ 200 stratum**

TRDF, treatment-related discontinuation equals failure. *TRDF was a pre-planned analysis at Week 96.

Cahn IAS 2019

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)

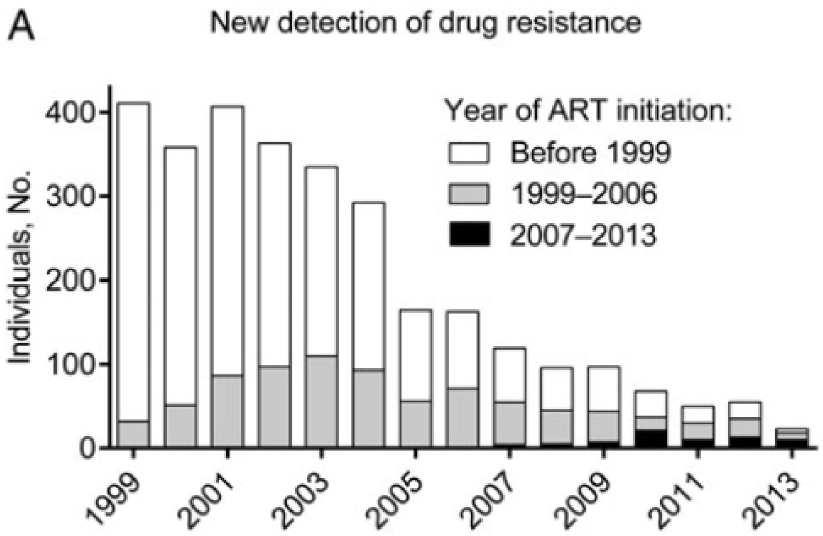


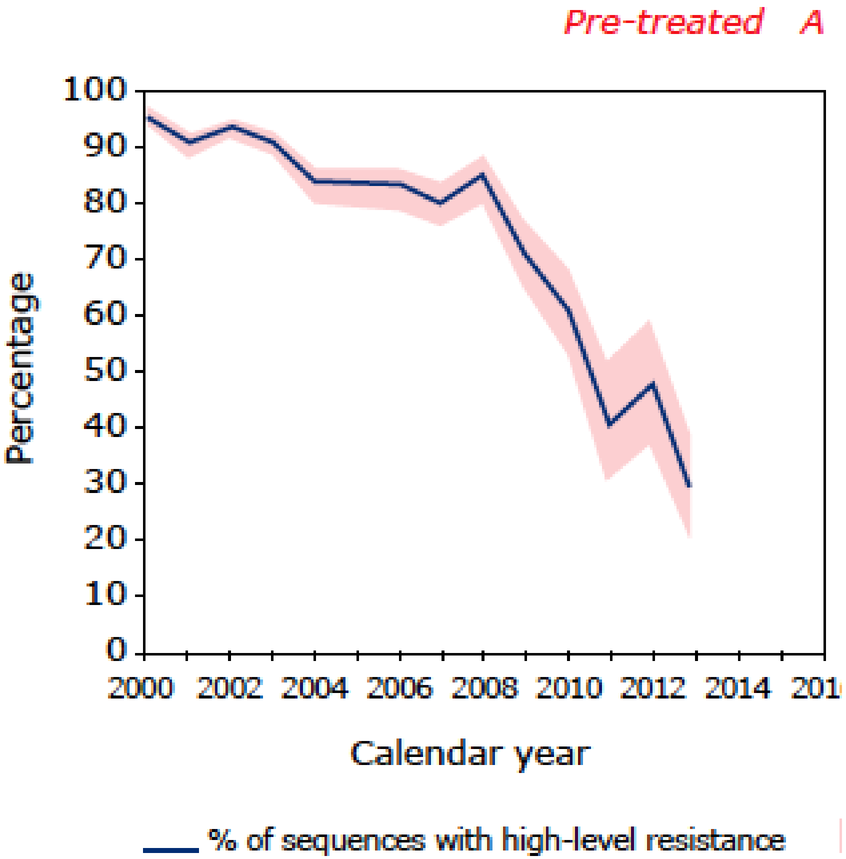
Absence of resistance: adherence?

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

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

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

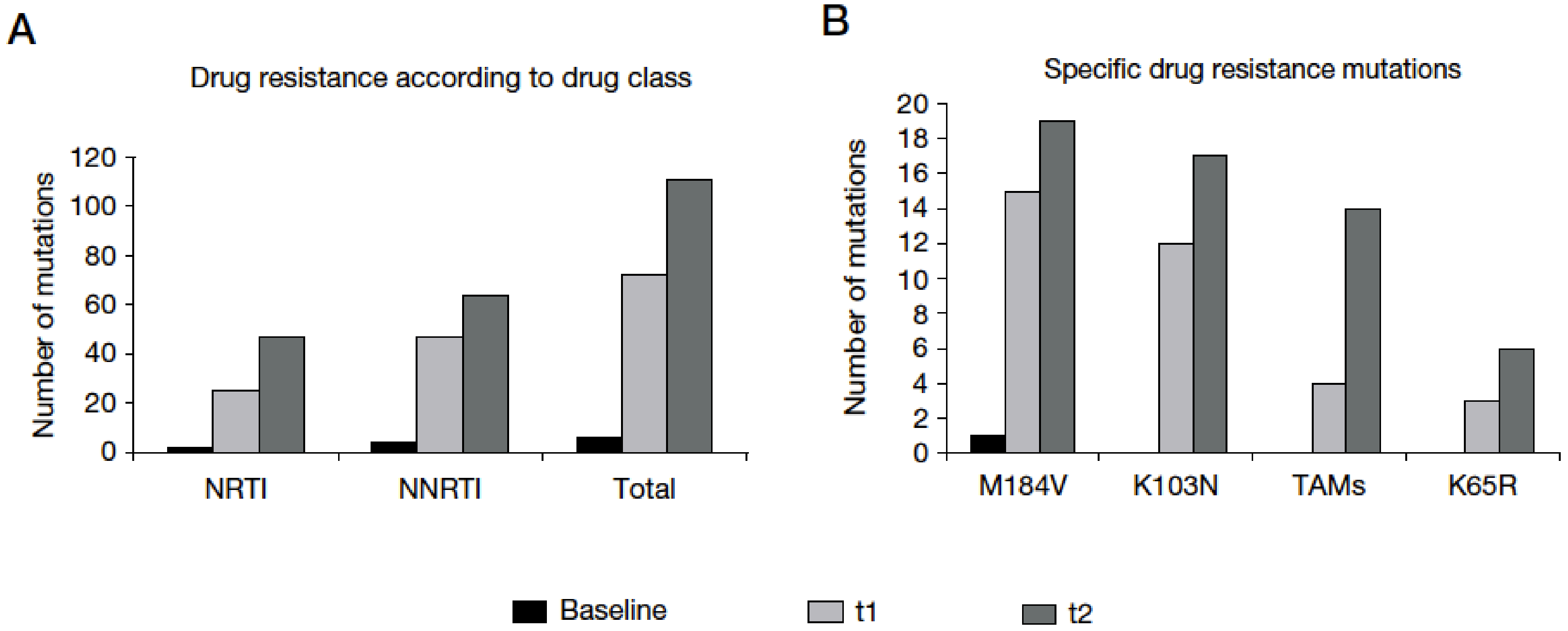
Cameroon: VF on 1st line after 12 months: 16% with viral load >1,000 HIV-RNA copies/mL, **63% NRTI or NNRTI mutations**

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

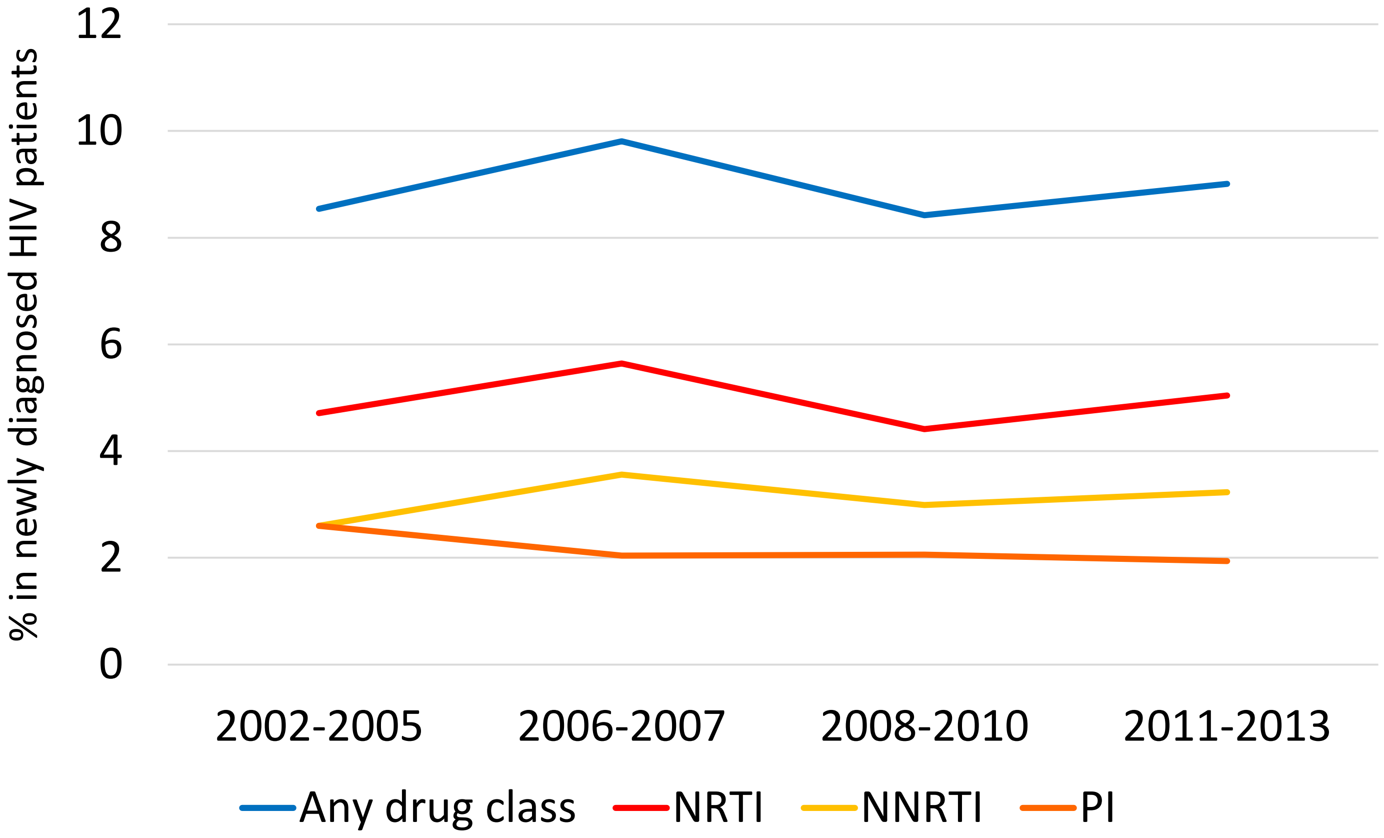
Baseline resistance (= primary resistance)

* 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

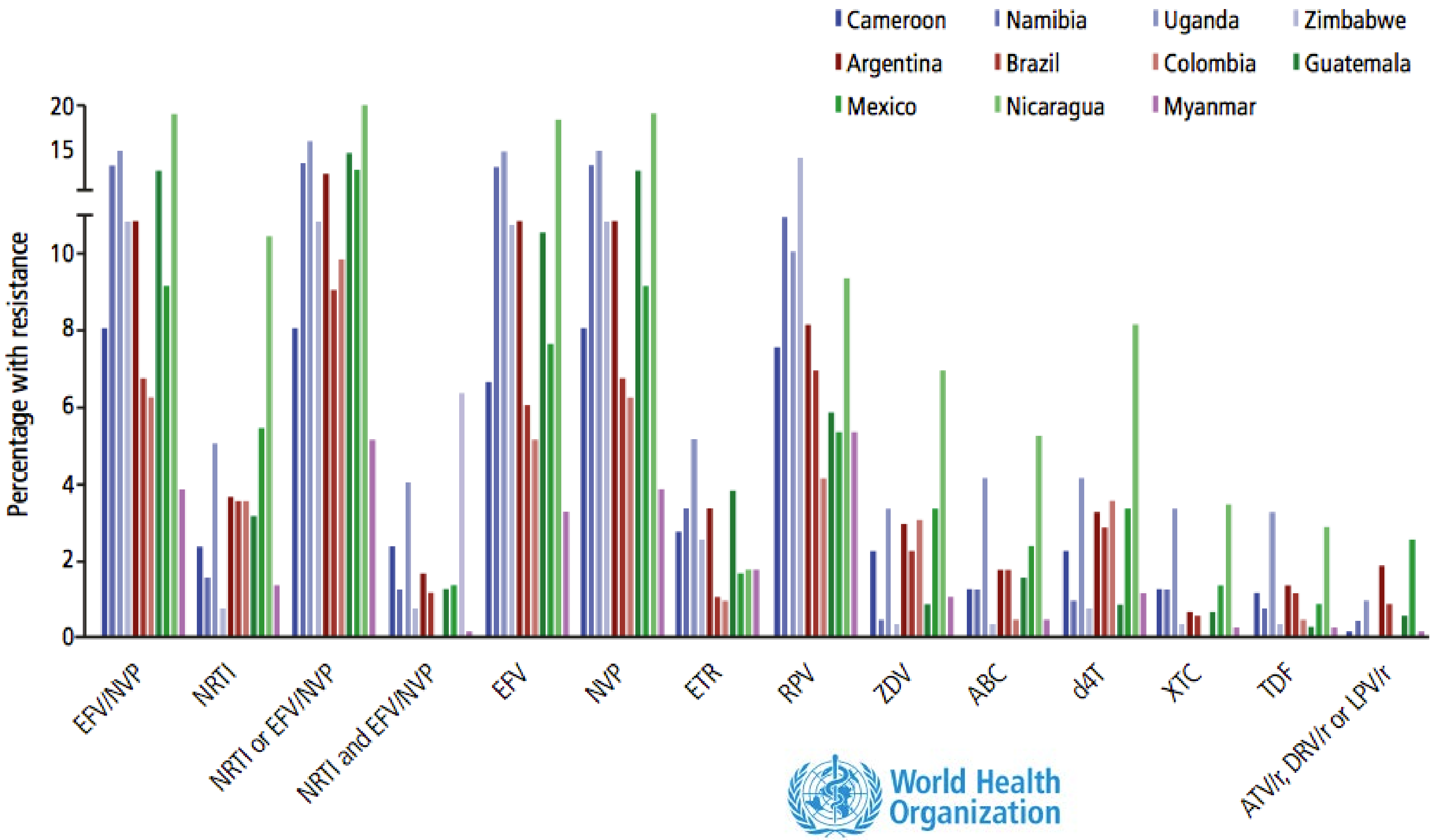
Transmitted resistance is stable in Europe



Hofstra et al. SPREAD program

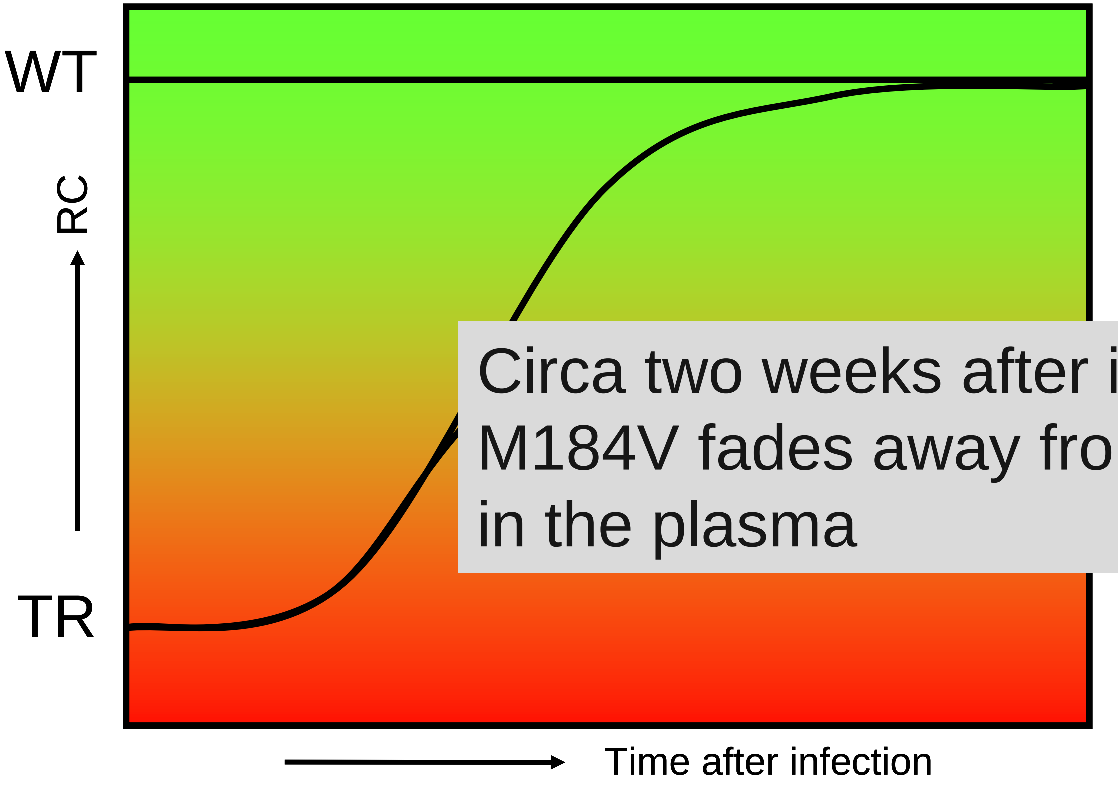
HIV drug resistance report WHO 2017

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



World Health
Organization

Reversion –
evolution towards wildtype

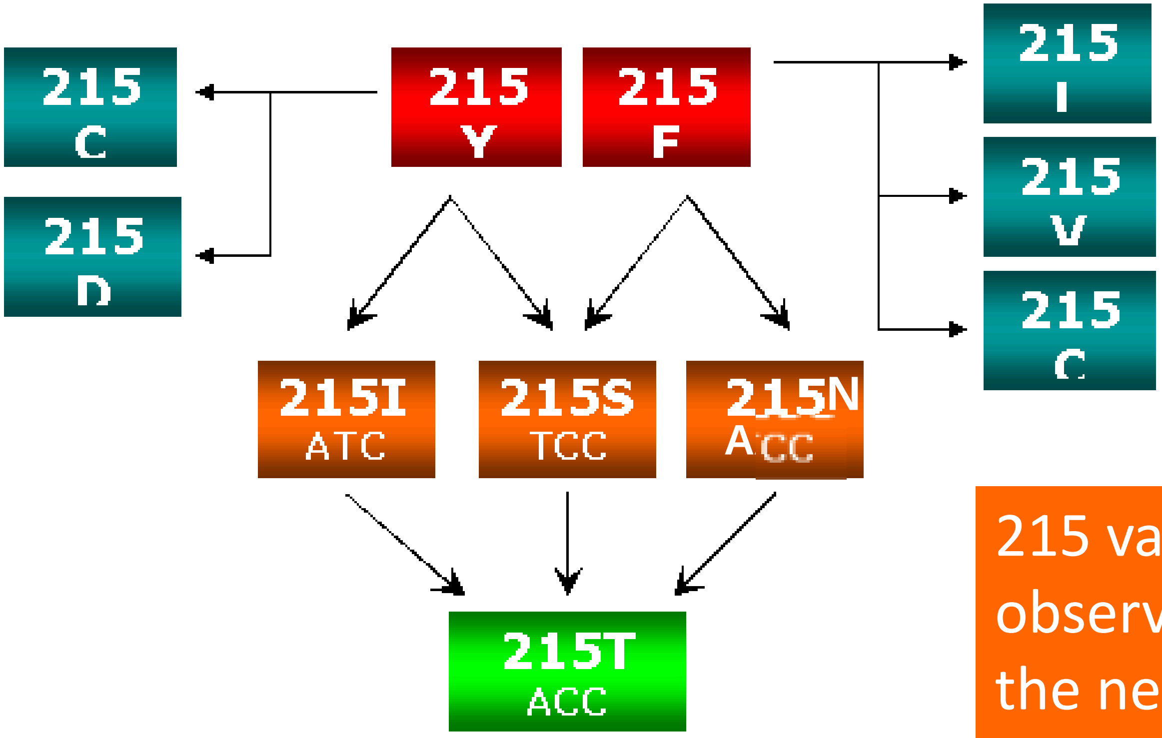


Circa two weeks after infection M184V fades away from detection in the plasma

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

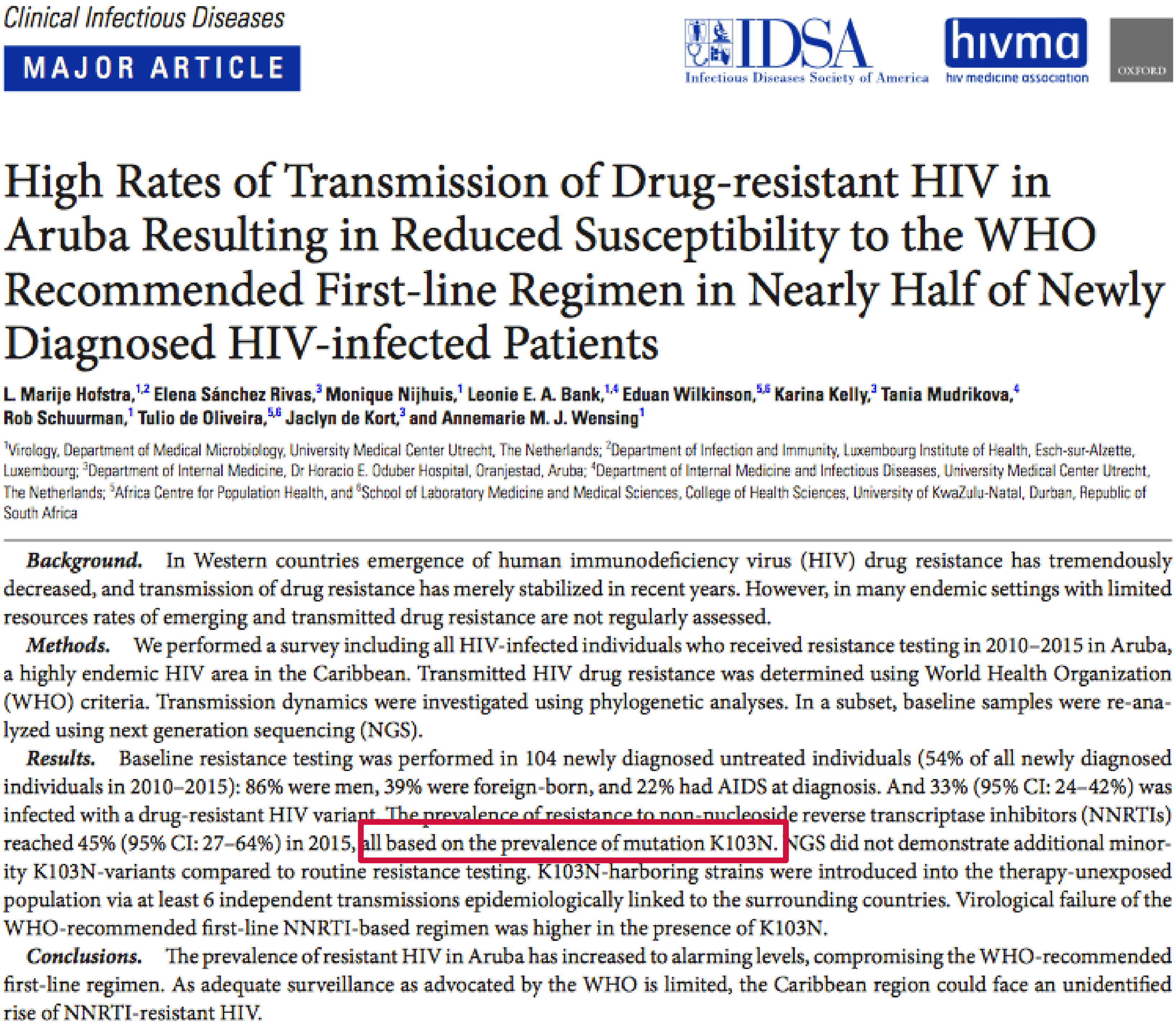
Evolution of DR in naïve patients revertants/atypical variants

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

IDSA Infectious Diseases Society of America

hivma hiv medicine association

OXFORD

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

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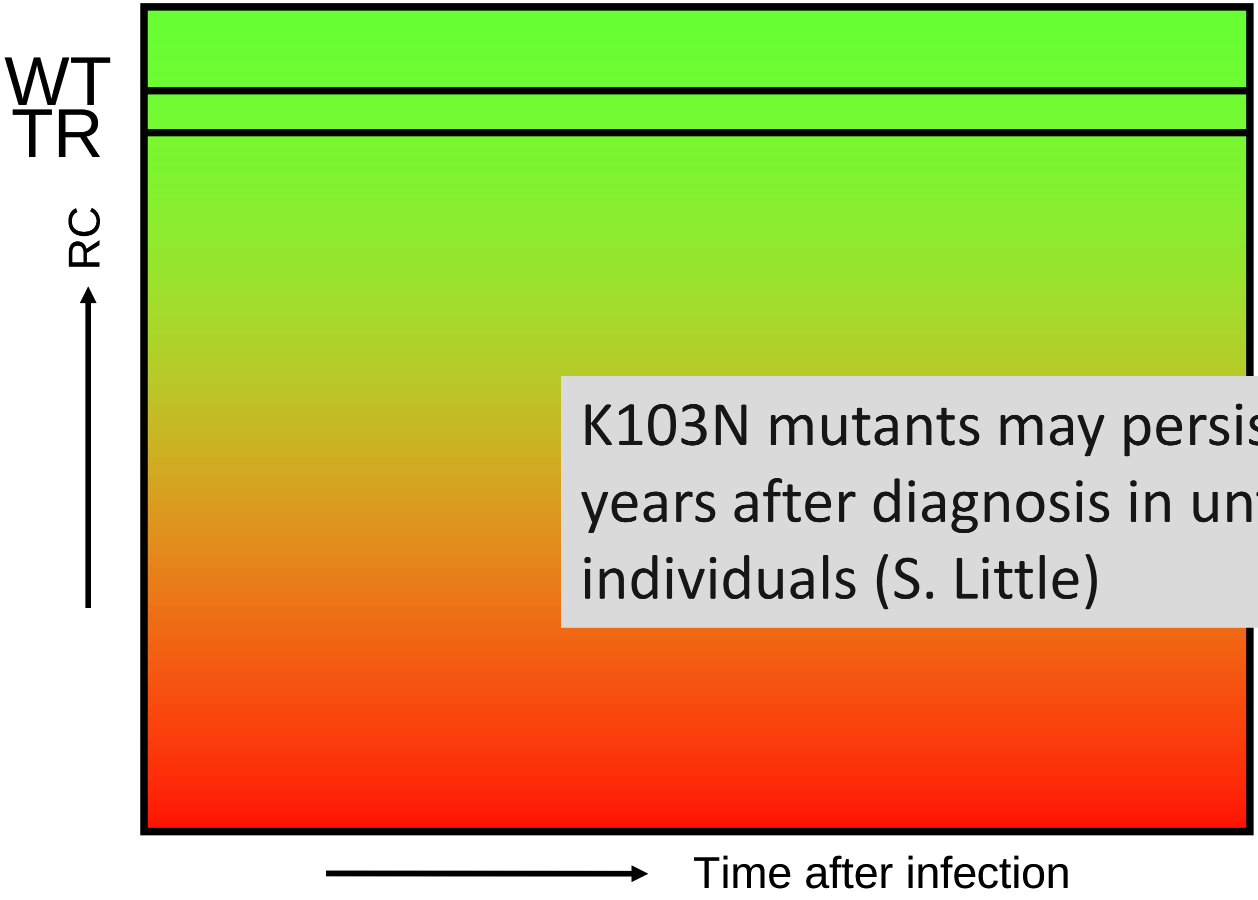
Background. In Western countries emergence of human immunodeficiency virus (HIV) drug resistance has tremendously decreased, and transmission of drug resistance has merely stabilized in recent years. However, in many endemic settings with limited resources rates of emerging and transmitted drug resistance 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.

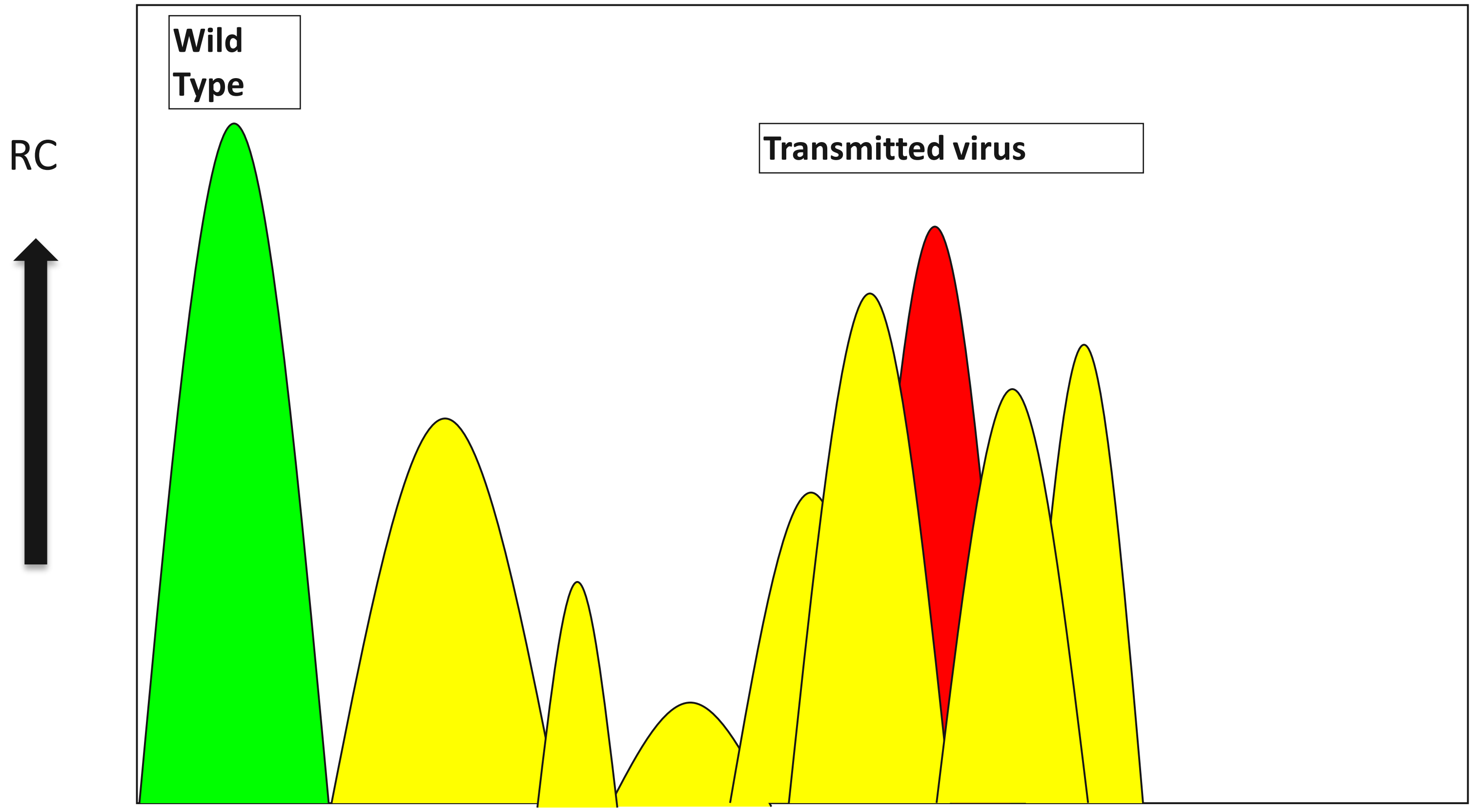
Persistence due to limited effect on fitness



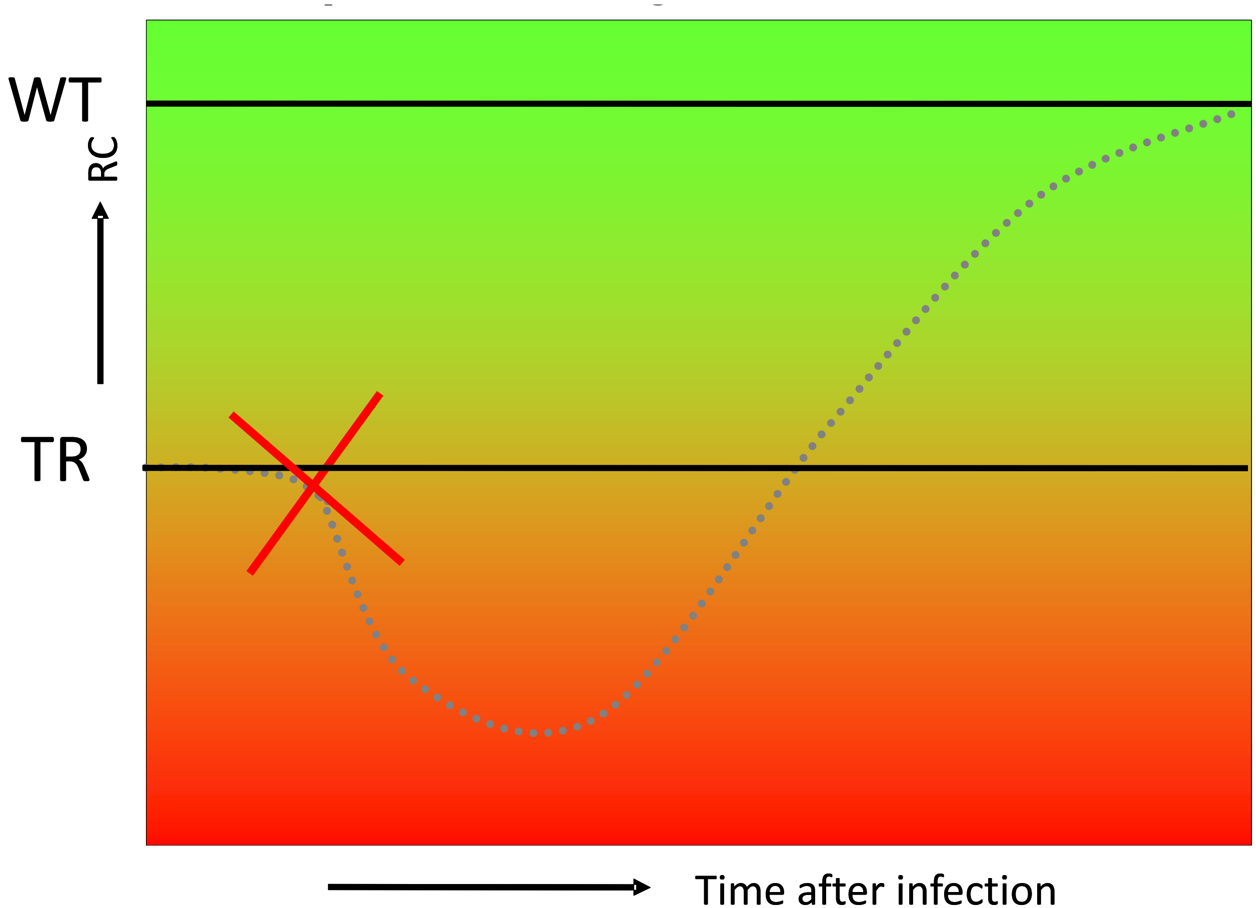
K103N mutants may persist up to 7 years after diagnosis in untreated individuals (S. Little)

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

Compensatory fixation

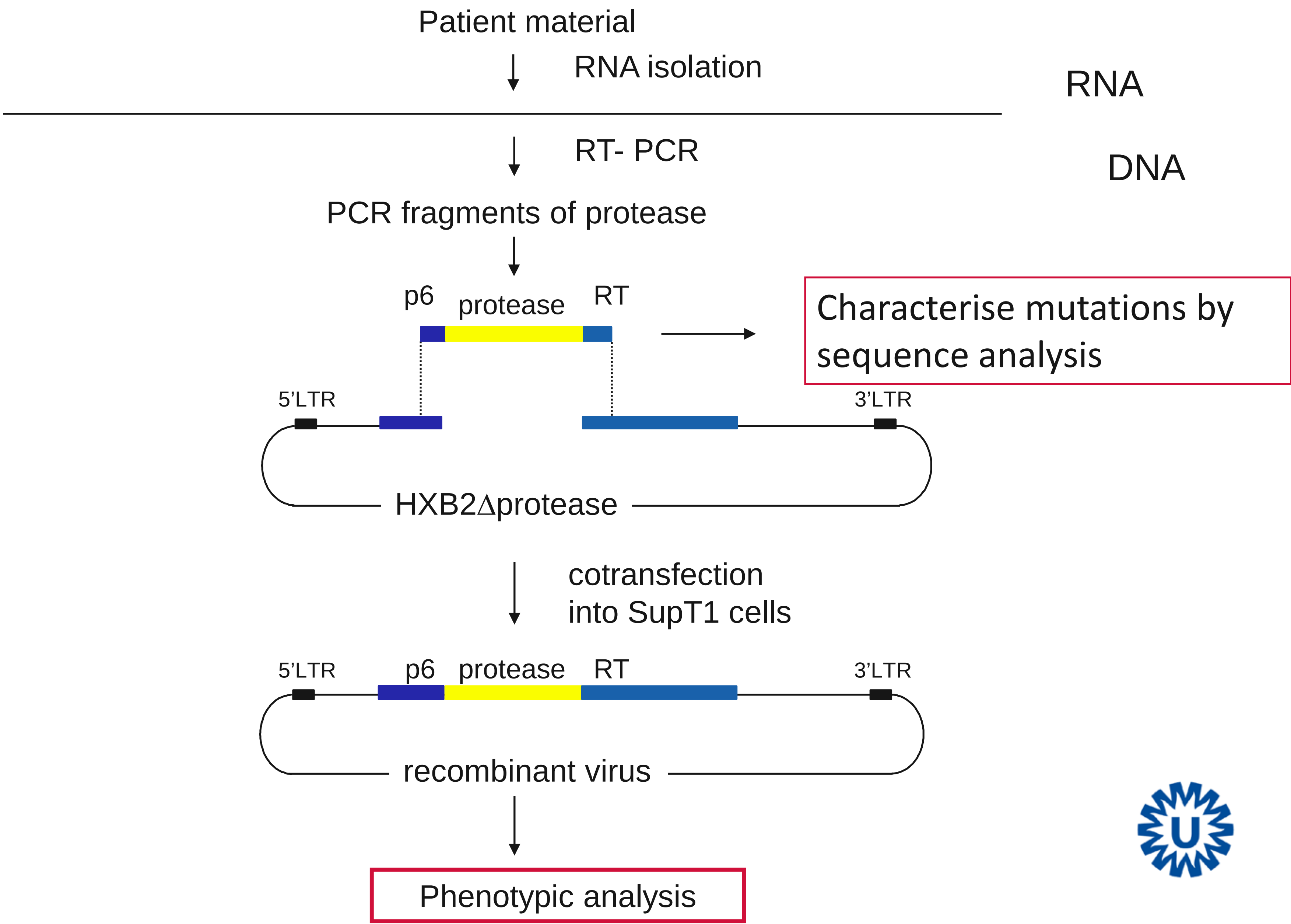


Persistence
due to compensatory fixation



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

Resistance testing



Patient material

RNA isolation

RNA

RT- PCR

DNA

PCR fragments of protease

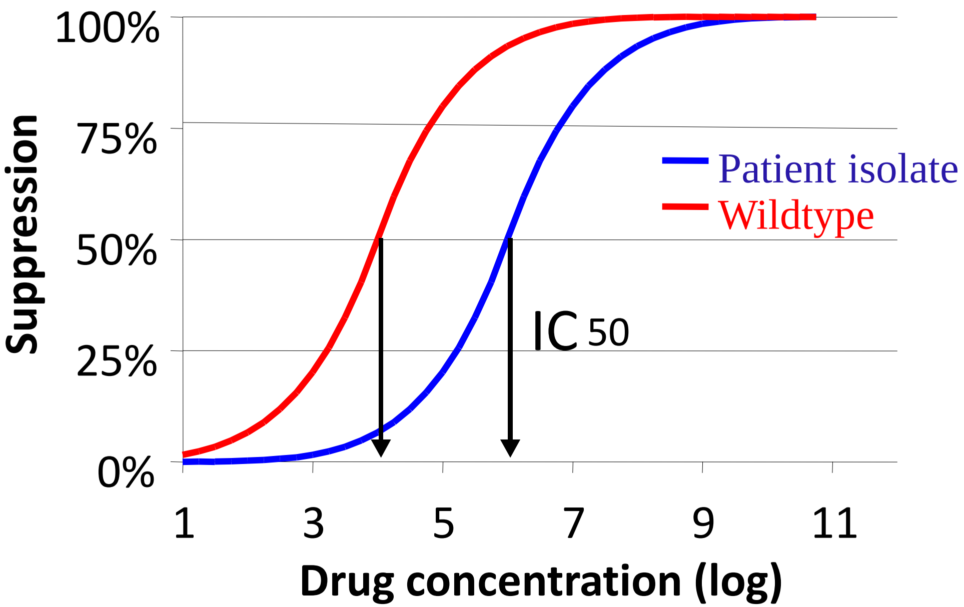
Characterise mutations by sequence analysis

cotransfection
into SupT1 cells

Phenotypic analysis

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

CHAIN

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

SEVENTH FRAMEWORK PROGRAMME

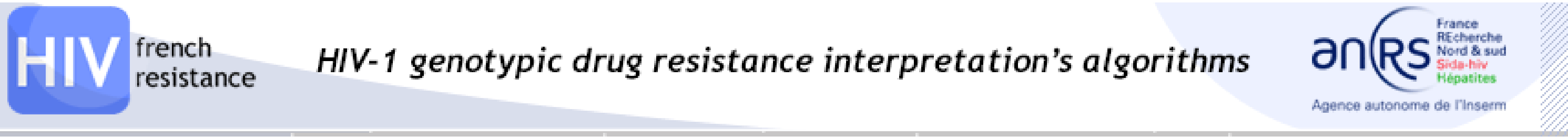
Interpretation

2019 Resistance Mutations Update Volume **27** Issue **3** July/August **2019**

Special Contribution

2019 Update of the Drug Resistance Mutations in HIV-1

Annemarie M. Wensing, MD, PhD; Vincent Calvez, MD, PhD; Francesca Ceccherini-Silberstein, PhD; Charlotte Charpentier, PharmD, PhD; Huldrych F. Günthard, MD; Roger Paredes, 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

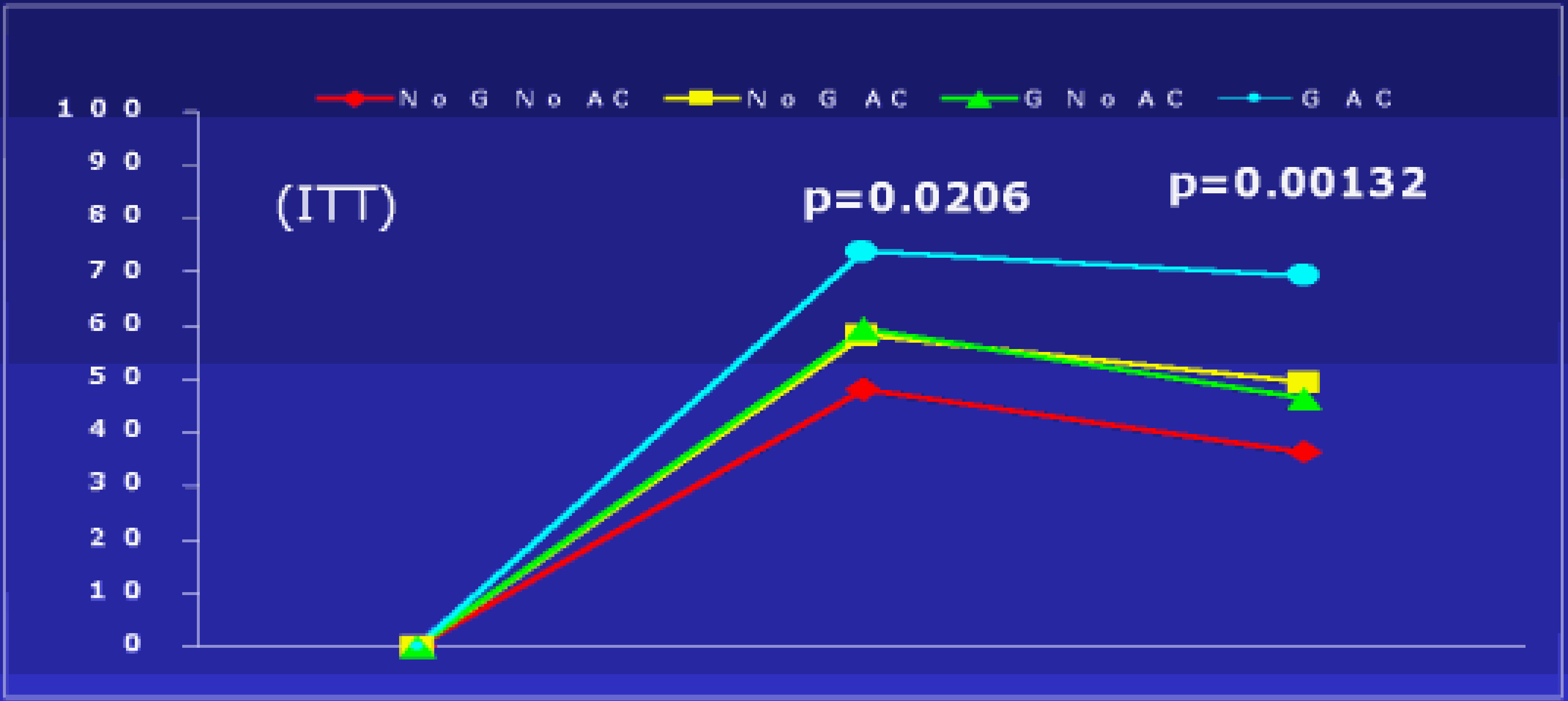
STANFORD UNIVERSITY

HIV DRUG RESISTANCE DATABASE

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

Havana : results (1)

% of patients with therapy success



	BL	Wk12	Wk24
NO G/ NO AC *(N=77)*		*48.1%*	*36.4%*
NO G/ AC *(N=67)*		*58.2%*	*49.3%*
G NO AC *(N=69)*		*59.4%*	*46.4%*
G/AC *(N=65)*		*73.8%*	*69.2%*

Tural AIDS 2002

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 acquired and baseline resistance is a more extensive issue
- Higher genetic barrier are being introduced worldwide, we have to see how they hold up with compromised backbones
- Backbones will remain relevant, but the dogma of three drugs has changed

Acknowledgement

Translational Virology, UMCU