XLVI Congresso Nazionale AMCLI
Rimini, 11-14 Novembre 2017

Update sui test di resistenza ai farmaci antivirali

Cosa ci ha insegnato l'esperienza su HIV

M. Zazzi – Siena

ui test di resistenza ai farmaci antivirali

ha insegnato l'esperienza su HIV

iena
unisi.it

XLVI Congresso Nazionale AMCLI

11 - 14 Novembre 2017

Palacongressi di Rimini

Outline

- The need for HIV drug resistance testing
- The options for HIV drug resistance testing
  - Genotyping replacing phenotyping
- The science of HIV genotype interpretation
  - The multidisciplinary approach and the expert systems
- Drug resistance testing as a key component of the success of antiretroviral therapy
  - The driver for cART
  - The driver for novel drug classes
  - The focus on genetic barrier, adherence and STR
- Evolution of HIV drug resistance testing
  - NGS to replace Sanger?
- Opportunities from the largest ever bug sequence collection
  - Research platforms
  - Molecular epidemiology and surveillance

HIV drug resistance testing

- The need
- The options
- A key component of ART success
- Evolution of HIV drug resistance testing
- The largest ever bug sequence collection

The need

HIV drug resistance testing

- The need
- The options
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First drug approved to treat HIV infection

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HIV/AIDS News

Home > HIV/AIDS News > Approval of AZT

Approval of AZT

Date: March 20, 1987
Source: Department of Health and Human Services (DHHS)
Author: Public Health Service (PHS)

Robert E. Windom, M.D., assistant secretary for health, today announced that the Food and Drug Administration has approved the drug zidovudine, commonly known as azidothymidine, or AZT, to help certain patients with Acquired Immunodeficiency Syndrome (AIDS) and advanced AIDS-Related Complex (ARC).

> AZT could offer about a year of prolonged life in those early days.

First evidence of HIV drug resistance

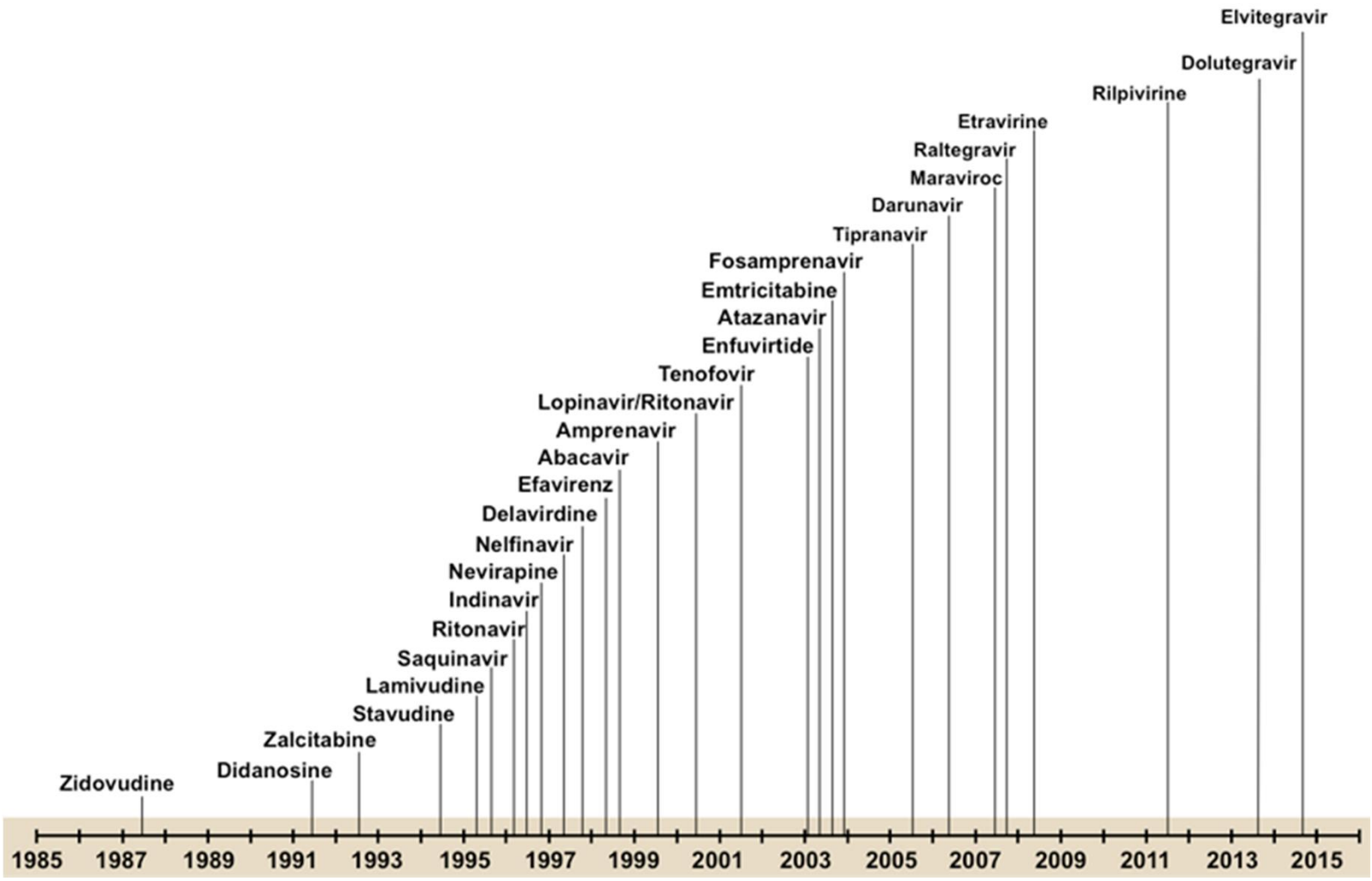
HIV with Reduced Sensitivity to Zidovudine (AZT) Isolated During Prolonged Therapy

BRENDAN A. LARDER, GRAHAM DARBY, DOUGLAS D. RICHMAN

The drug sensitivities of human immunodeficiency virus (HIV) isolates from a group of patients with acquired immunodeficiency syndrome (AIDS) or AIDS-related complex (ARC) who were receiving zidovudine (3′-azido-3′-deoythymidine, AZT) therapy were tested by means of a newly developed plaque assay in $CD4^+$ HeLa cells. Fifty percent inhibitory dose (ID_{50}) values of 18 isolates from untreated individuals ranged between 0.01 μM and 0.05 μM. In contrast, most isolates from patients who had received zidovudine for 6 months or more exhibited decreased sensitivity characterized by changes in ID_{50} or ID_{95} values (or both), with isolates from several patients (5/15) showing 100-fold increases in ID_{50}. The latter isolates were also insensitive to 3′-azido-2′,3′-dideoxyuridine; however, the isolates were still sensitive to 2′,3′-dideoxycytidine, 2′,3′-dideoxy-2′,3′-didehydrothymidine, or phosphonoformate. It cannot be determined from this small sample of patients whether development of a less sensitive virus phenotype results in clinical resistance. Appearance of such variants was not associated with a consistent increase in viral p24 concentrations in patient plasma and did not herald any sudden deterioration in clinical status. More extensive studies are required to determine the clinical significance. Thus, it would be premature to alter any treatment protocols for HIV-infected individuals at present.

SCIENCE, VOL. 243 31 MARCH 1989

HIV drug development timeline

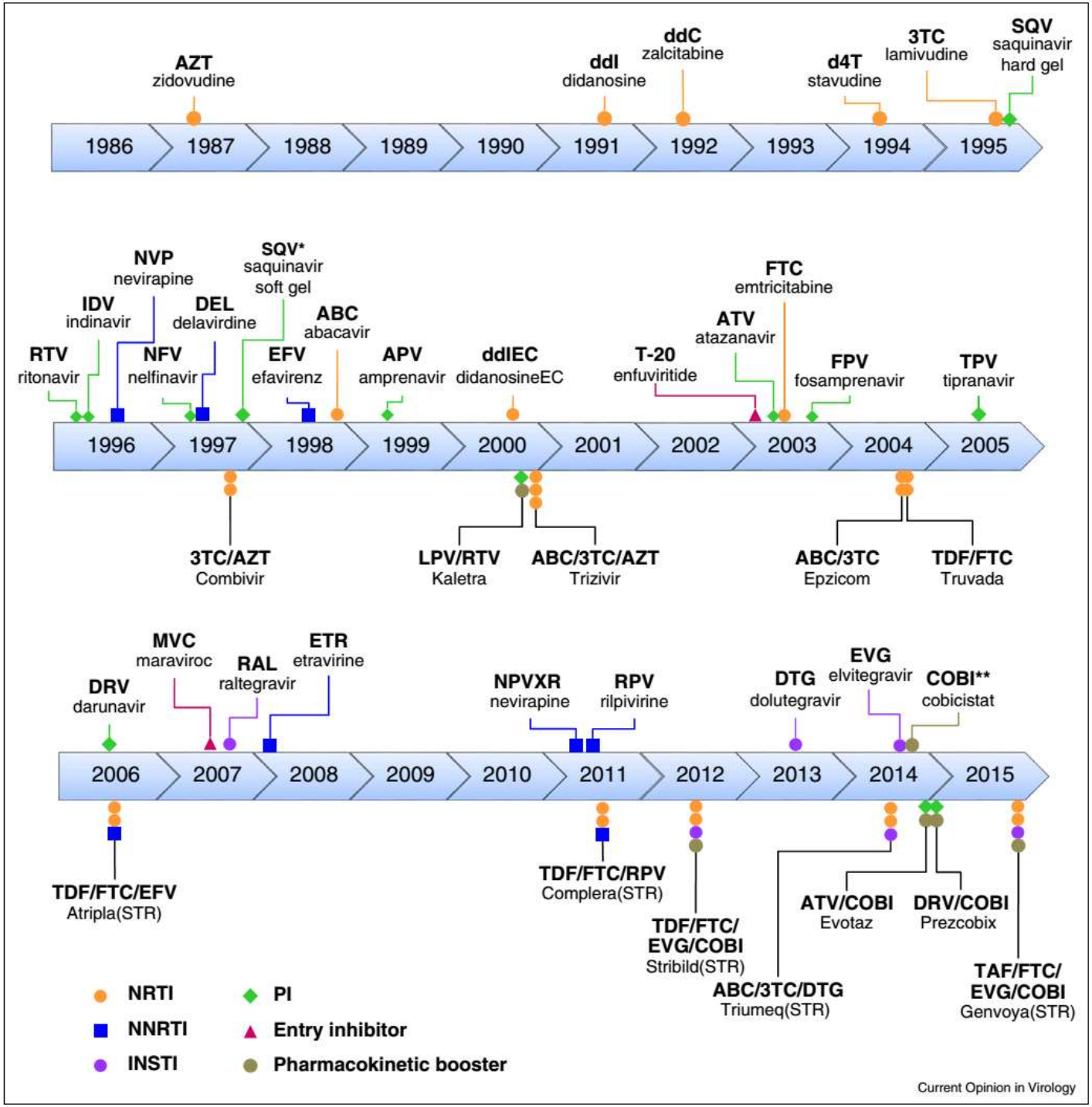


Anti-HIV compounds & HIV resistance

"An antiretroviral drug is something to which HIV becomes resistant"

(Douglas Richman)

ART progress



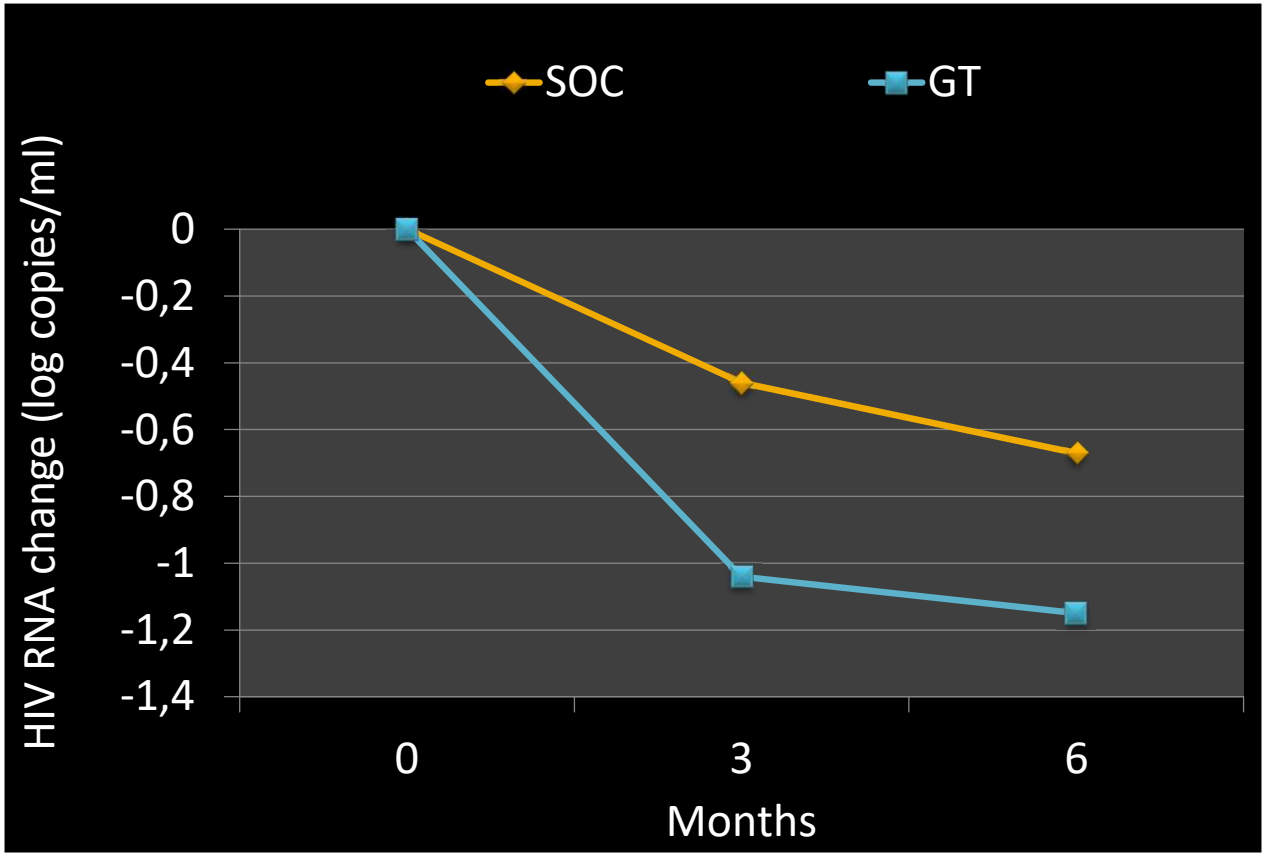
- Only **NRTIs** up to 1995
- 1995 → **PIs**
- 1996 → **NNRTIs**

- Drug resistance regularly documented for each drug

- Also, much cross-resistance within each class

Drug-resistance genotyping in HIV-1 therapy: the VIRADAPT randomised controlled trial

- Prospective, open, randomised, controlled study
- Patients on treatment failure randomly assigned to standard care (SOC, n=43) or treatment according to the resistance mutations in protease and reverse-transcriptase genes (genotypic group, n=65)
- Endpoint: change in HIV-1 RNA viral load by intention to treat



Adapted from Durant, Lancet 1999

Pivotal studies supporting the value of drug resistance testing at treatment failure

Study	Patients	Outcome
VIRADAPT (Durant 1999)	108	GENO >SOC
CPCRA 046 (Baxter 2000)	153	GENO > SOC
HAVANA (Tural 2002)	326	GENO+EXPERT OPINION > SOC
CCTG 575 (Haubrich 2005)	256	PHENO = SOC
ARGENTA (Cingolani 2002)	174	GENO > SOC
VIRA3001 (Cohen 2002)	221	PHENO > SOC

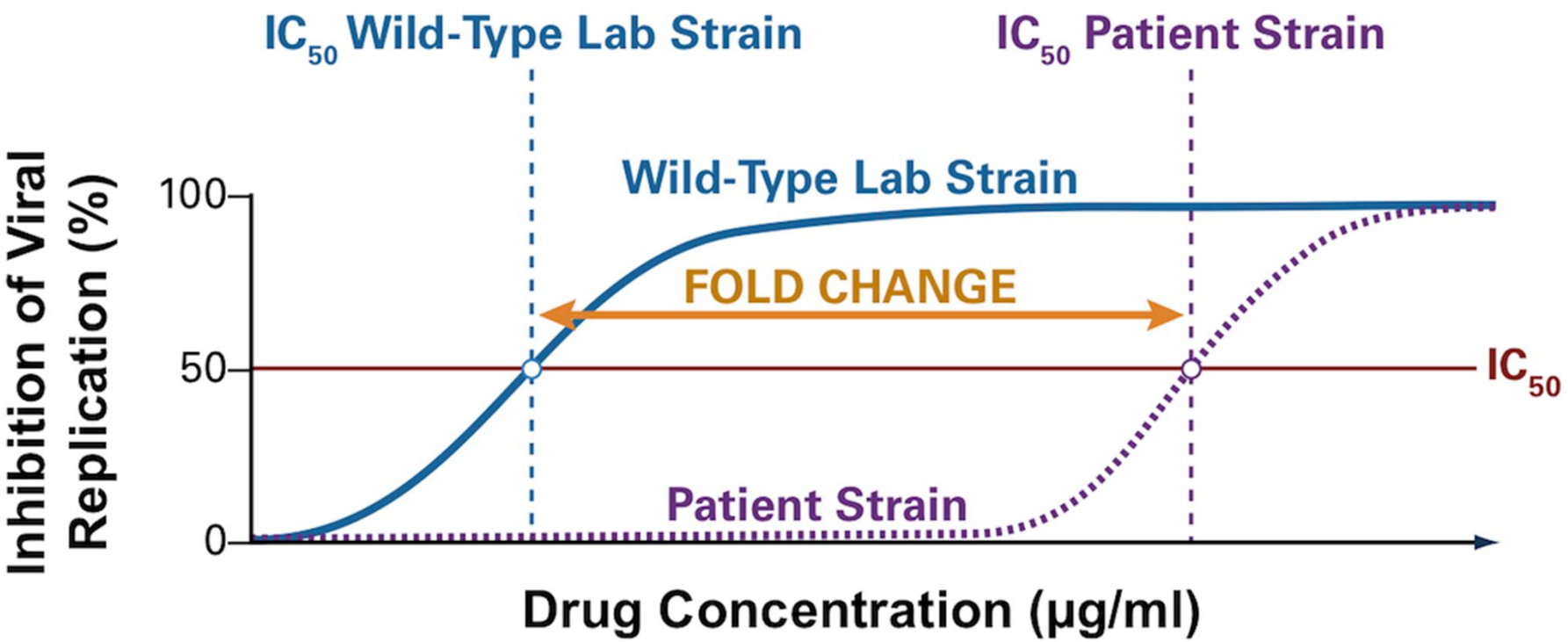
GENO = Genotype; PHENO = Phenotype; SOC = ‘standard of care’

The options

HIV drug resistance testing

- The need
- The options
- A key component of ART success
- Evolution of HIV drug resistance testing
- The largest ever bug sequence collection

Measurement of HIV drug resistance in vitro

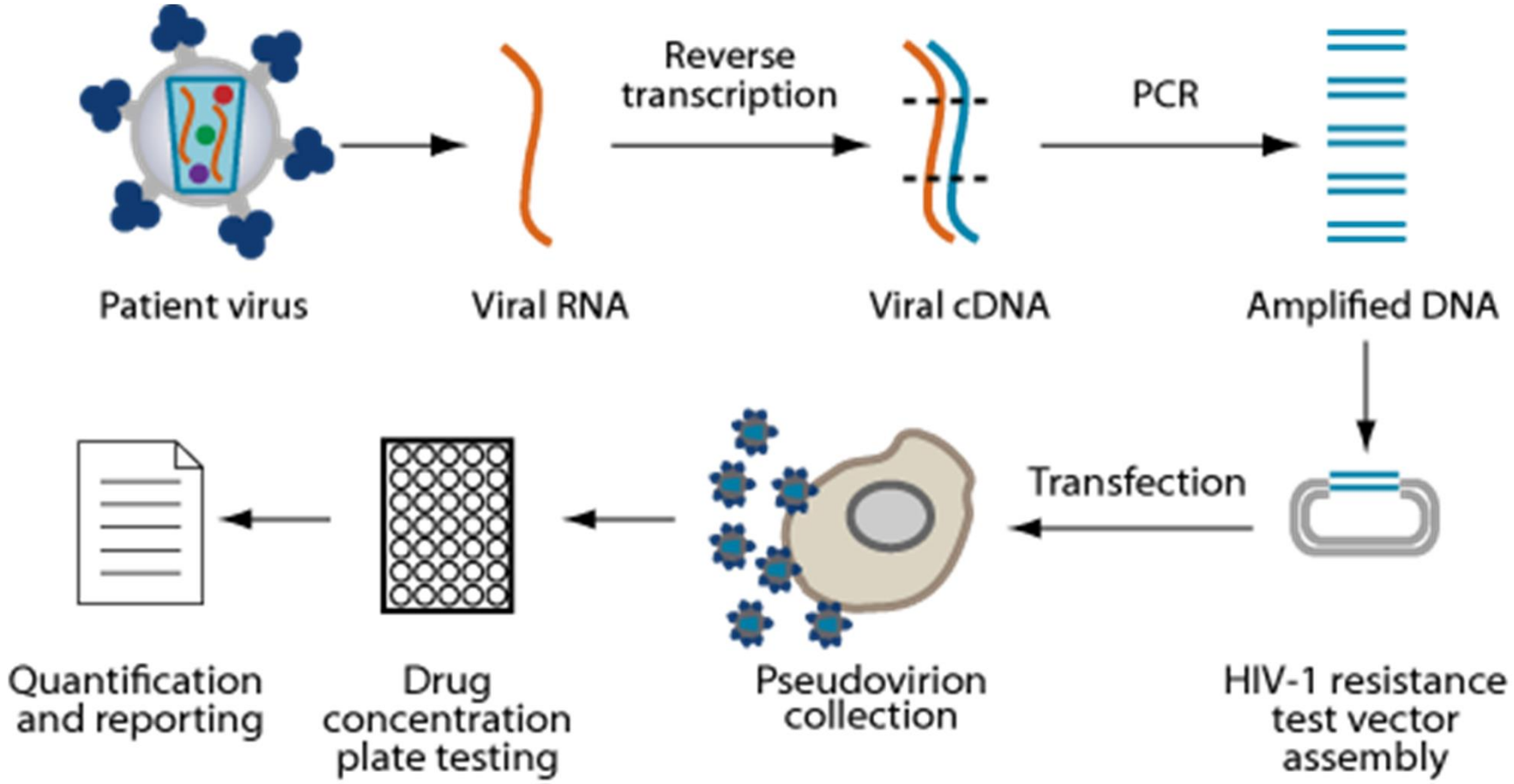


www.hiv.uw.edu

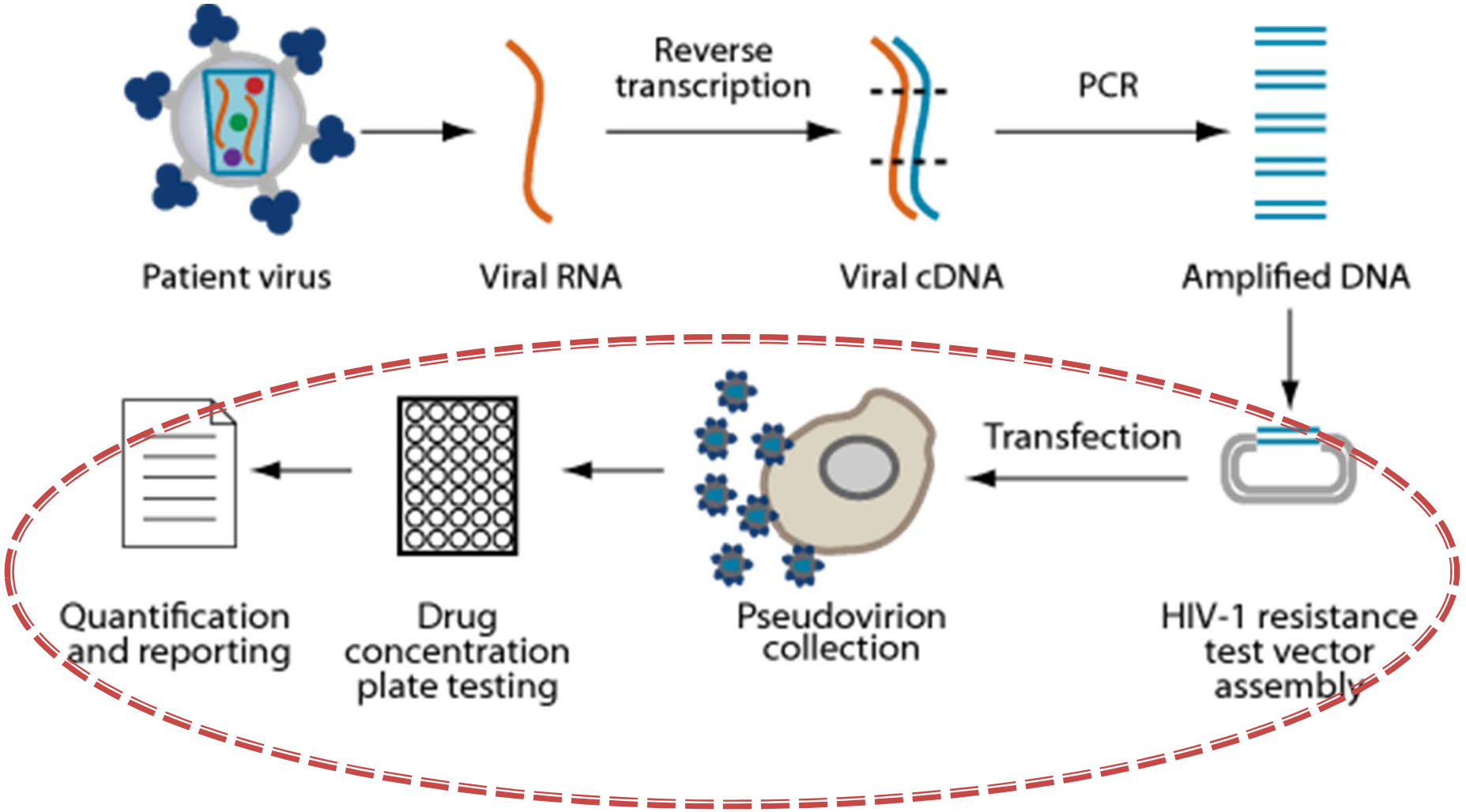
Measurement of HIV drug resistance in vitro
A complicated story

- Most primary isolates do not grow well in cell lines
- Co-culture with (CD8 cell depleted) patient PBMCs and HIV-negative donor PBMCs is required
- An HIV isolate may grow with a different kinetics in different donor PBMCs

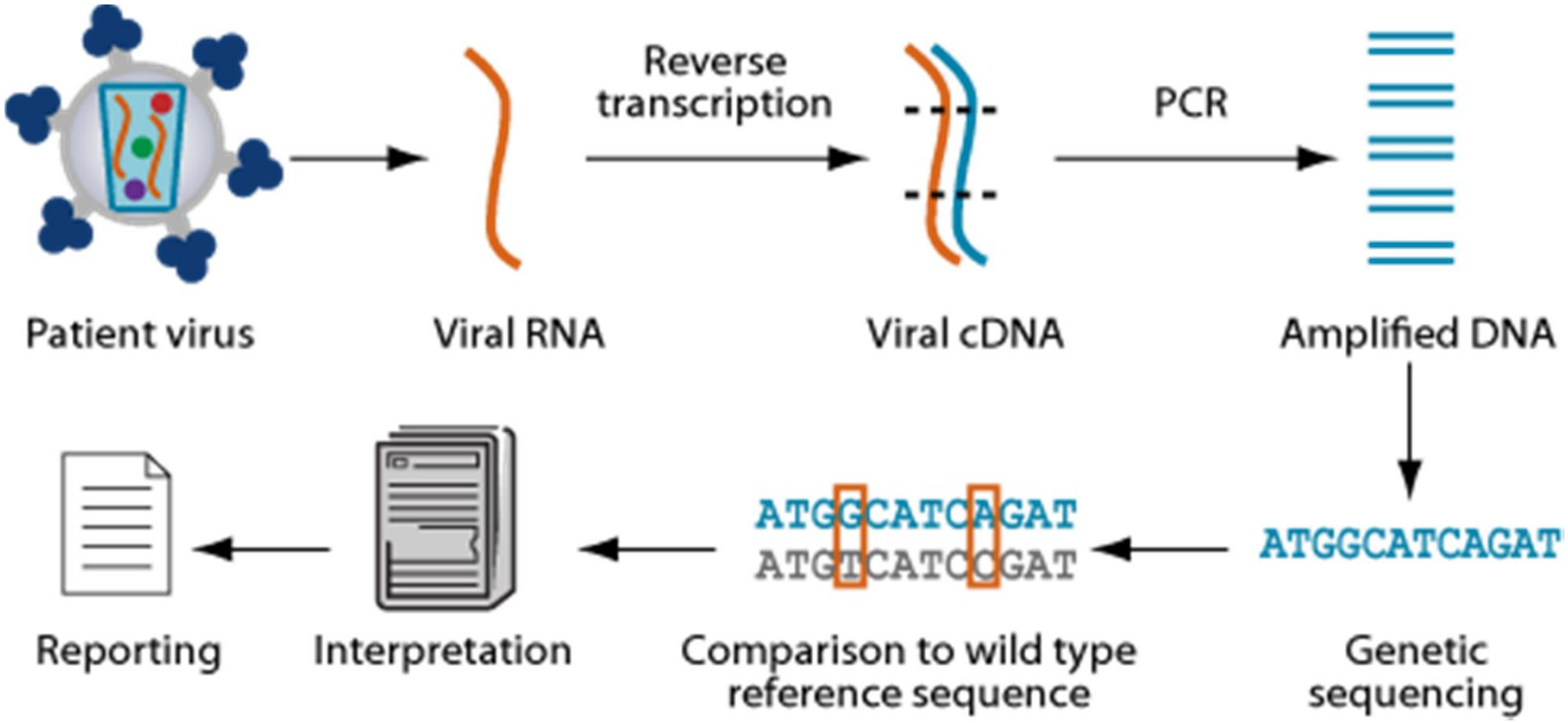
Phenotypic HIV drug resistance testing



Phenotypic HIV drug resistance testing



Genotypic HIV drug resistance testing

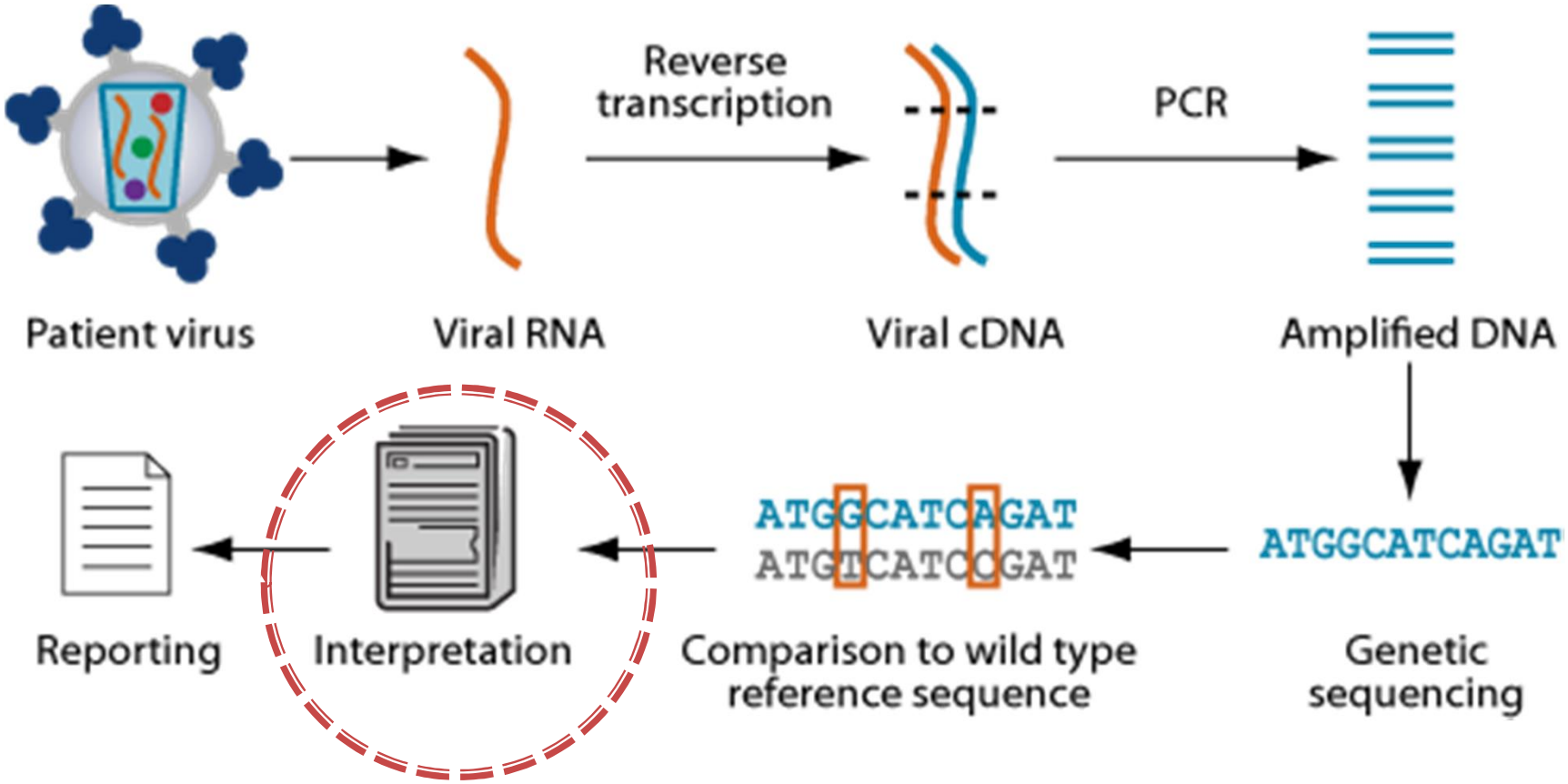


IVD CE marked and FDA approved systems for HIV drug resistance genotyping

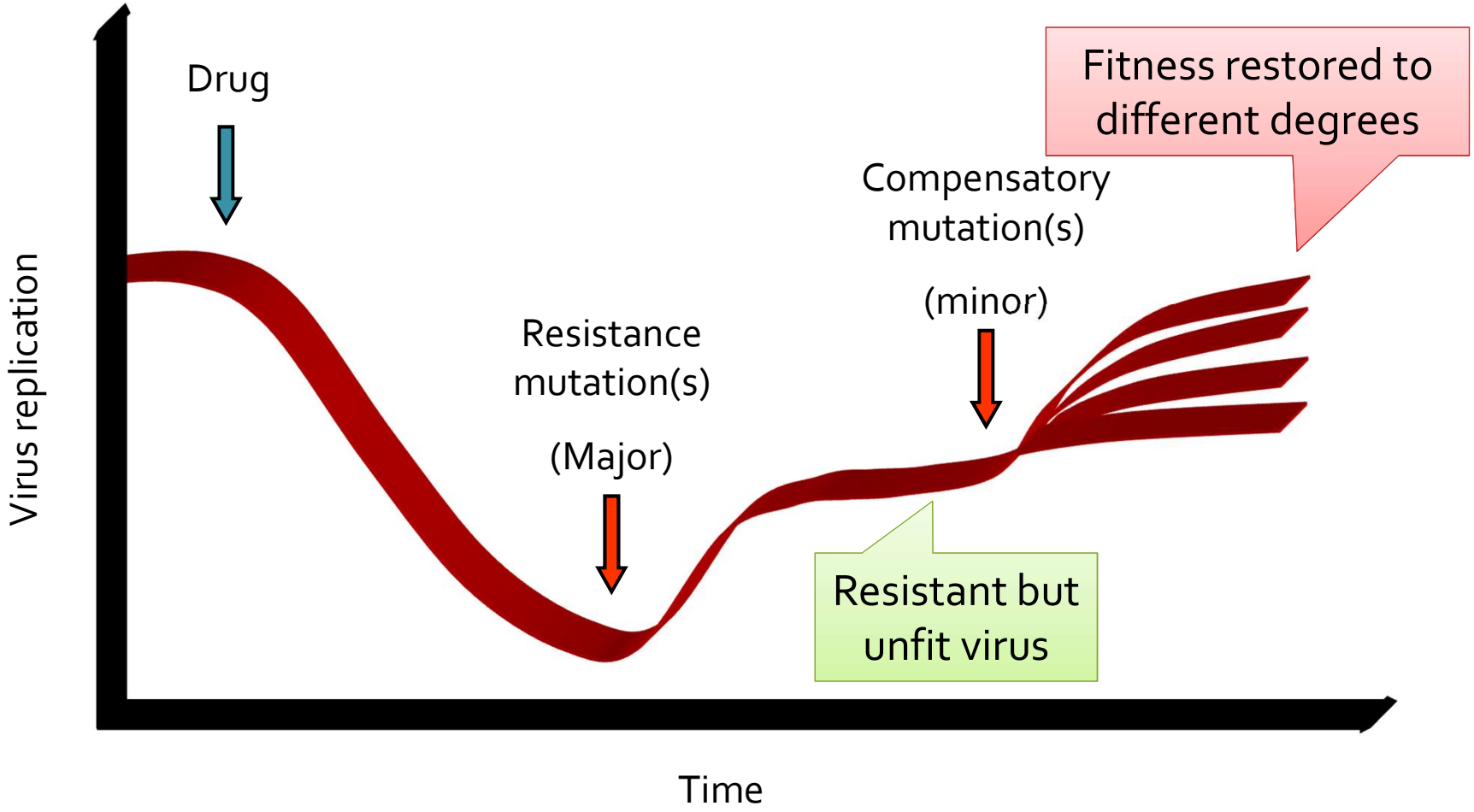
- First DNA sequencing IVD products in infectious diseases
  - Goal: to have HIV genotyping available at every lab with minimal molecular diagnostics background
  - Result: most cases successful but also some failures
- Yet, "expert" labs continues to use homebrew technology

GISs

Genotypic HIV drug resistance testing

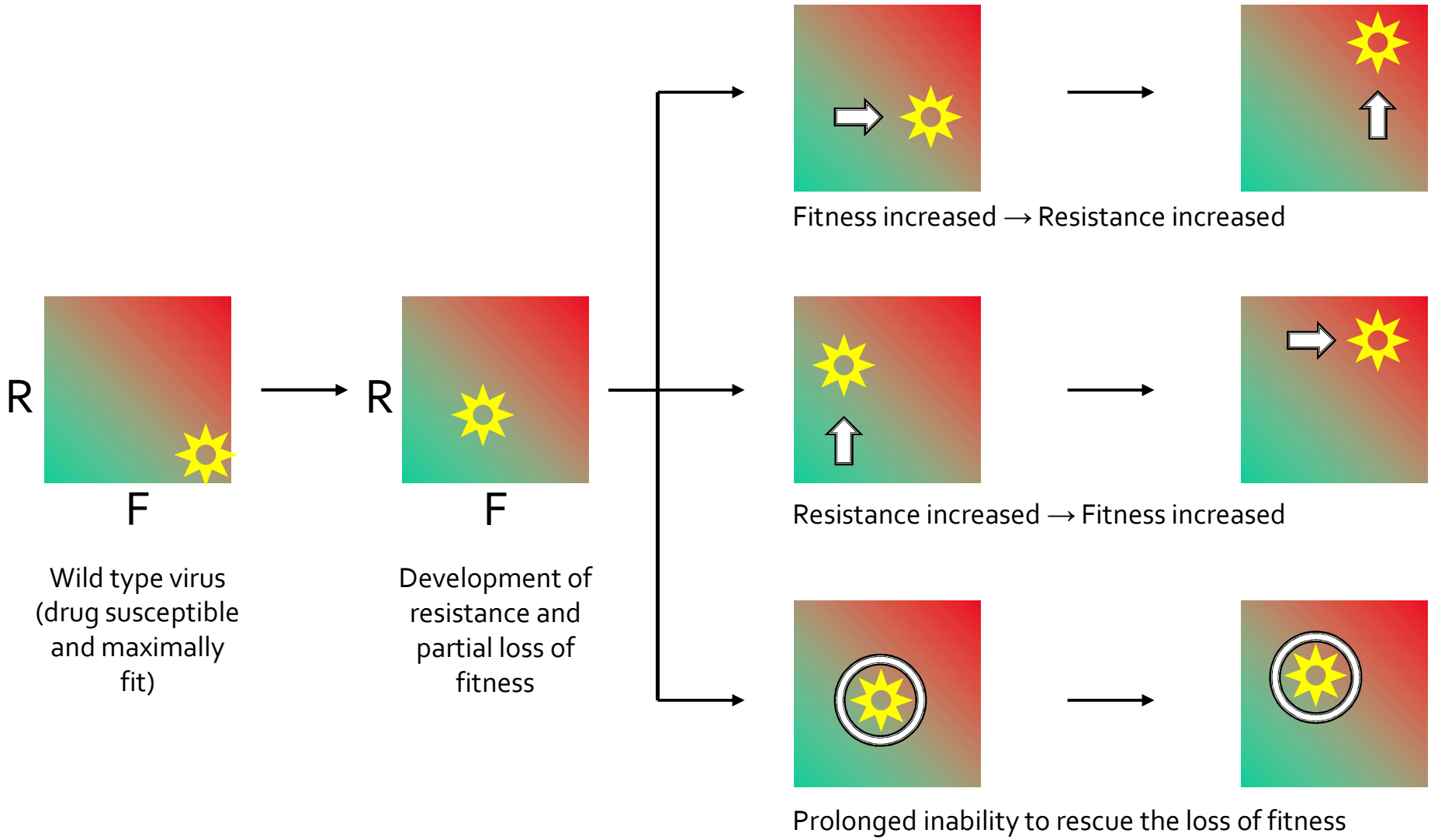


Resistance and compensatory mutations

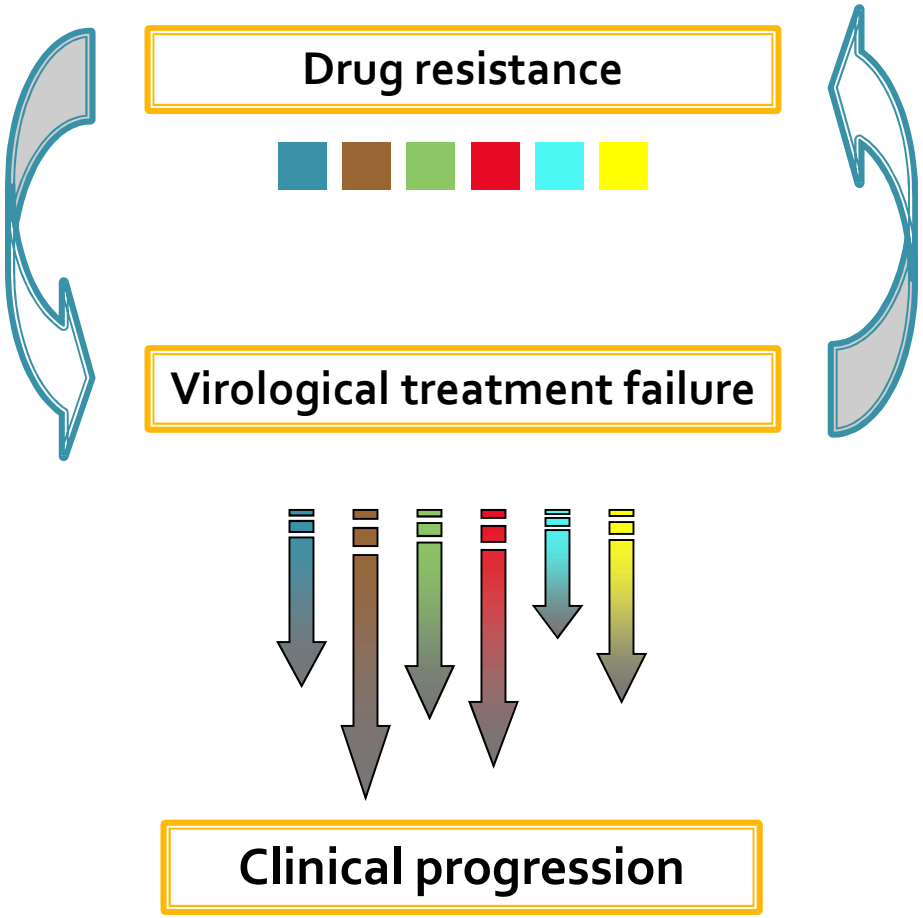


- **Major** mutation → Confers resistance on its own, may often decrease fitness
- **Minor** mutation → Does not confer resistance on its own but may modulate resistance and/or (partially) restore fitness which was decreased by a major mutation

Drug resistance (R) vs. fitness (F)



Drug resistance and clinical progression

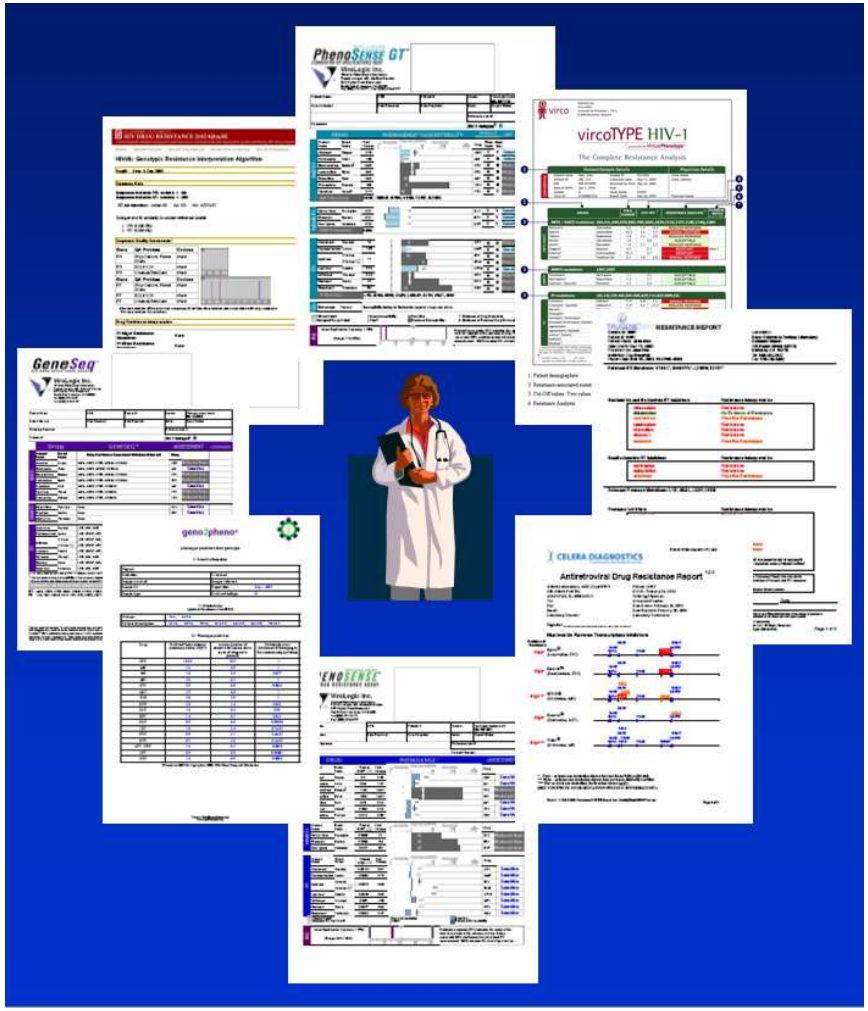


Virological failure is not always / immediately associated with clinical progression

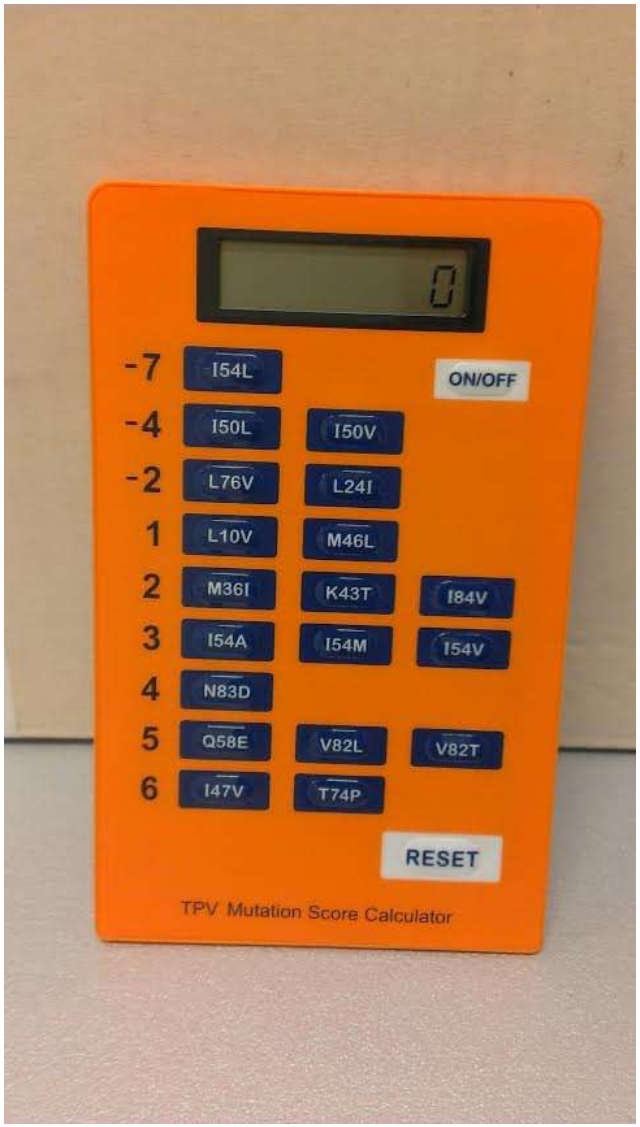
Most drug-resistant variants have reduced pathogenecity

How to infer drug resistance from HIV sequence analysis

- Multiple options
  - Mutation lists
  - Computer programs
  - Commercial and academic systems
- Some discordances
  - High genetic barrier drugs
- Nevertheless excellent support
  - A must for proper management of treatment choices



How to infer drug resistance from HIV sequence analysis



Data sources for HIV genotype interpretation

Stanford University

HIV DRUG RESISTANCE DATABASE

A curated public database to represent, store and analyze HIV drug resistance data.

Query Pages

Genotype-treatment

Retrieve sequences (and/or mutations) from persons receiving selected HIV drugs

Retrieve sequences and treatments from viruses with specific mutations

Genotype-phenotype

Retrieve drug susceptibility data for isolates with selected mutations

Download genotype-phenotype research datasets

Genotype-clinical

Summaries of genotype-clinical outcome studies

Genotype-clinical outcome datasets (download)

References

Published drug resistance studies in HIVDB

Published studies by Stanford database group

Most widely used HIV genotype interpretation systems

HIValg Program

Comparison of Genotypic Resistance Algorithms

HIVdb version 8.4 (last updated 2017-06-16)

HIValg compares HIVdb results to those of 2 other algorithms: i. Rega Institute (rules), and ii. Agence Nationale de Recherches sur le SIDA (ANRS rules). However, it does not provide the complete HIVdb report. HIValg also allows users to interpret sequences using any algorithm created using the Algorithm Specification Interface (ASI). A detailed description of the program as well as all updates can be found in the Release Notes.

Protease, RT, and integrase mutations can be entered using either the text box or auto-suggestion boxes. To use the text box, type each mutation separated by one or more spaces. The consensus wildtype and separating commas are optional. If there is a mixture of more than one amino acid at a position, write both amino amino acids (an intervening slash is optional). Insertions should be indicated by "Insertion" and deletions by "Deletion".

Select algorithms

Select two or more previously published algorithms and/or upload one or more ASI-encoded interpretation algorithms from your computer using the file selection box below.

☑ HIVdb ☑ ANRS ☑ rega Choose Local ASI2 File(s)

Input mutations | Input sequences

Reverse Transcriptase	Protease	Integrase
Input mutation(s)	Input mutation(s)	Input mutation(s)

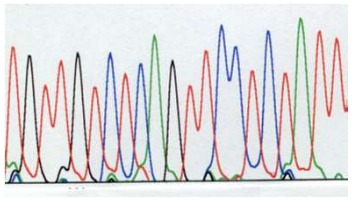
https://hivdb.stanford.edu/hivalg

HIV genotype is a necessary guide to treatment choices

- Best example of successful introduction of **DNA sequence based diagnostics** in the routine management of an infectious disease
- Recommended in all HIV **treatment guidelines** since 2000
  - At treatment failure to detect **acquired resistance**
  - At treatment initiation to detect **transmitted resistance**
  - At pregnancy to adjust treatment and minimize **mother-to-child HIV transmission**

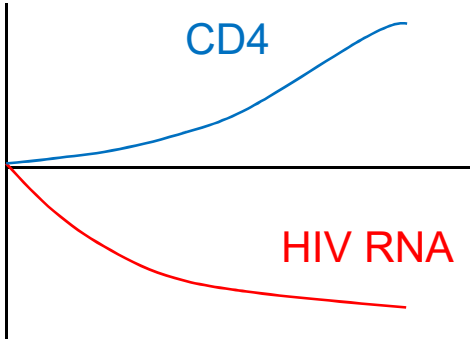
TOSs

From HIV genotype to response to treatment



Baseline HIV genotype, viral load, CD4, previous genotypes and drug exposure...

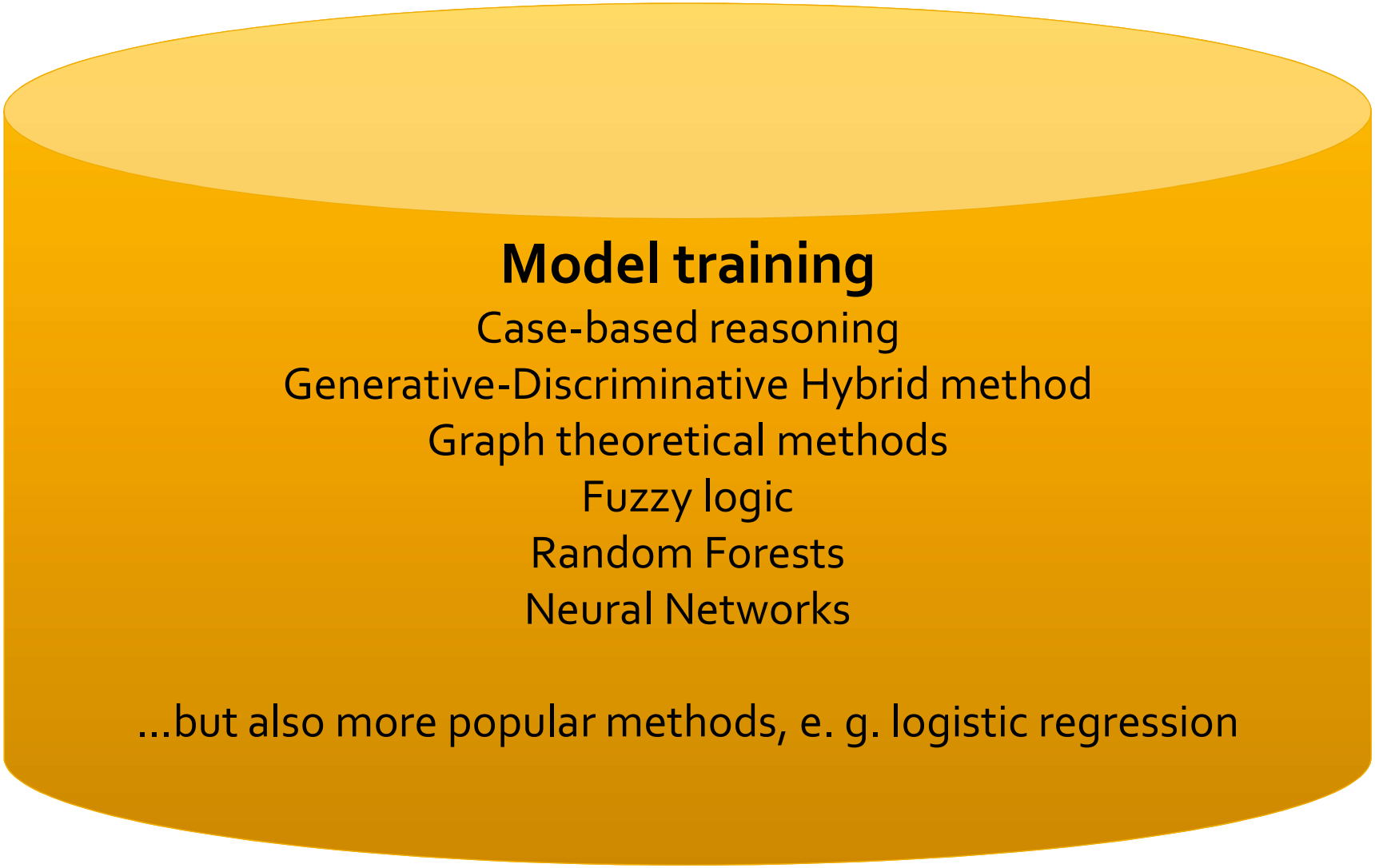
Treatment switch



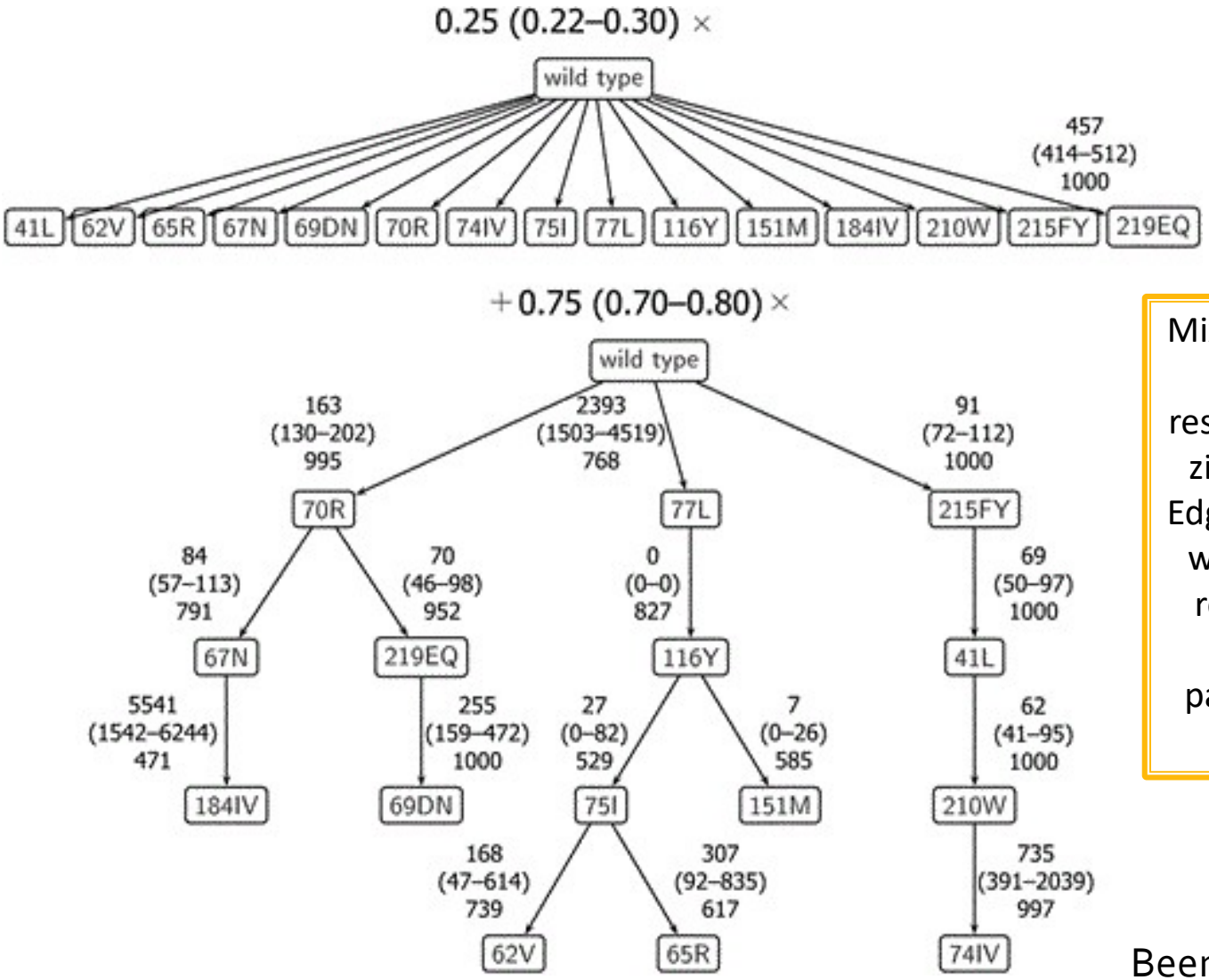
Follow-up viral load, CD4, ...

Model training

From HIV genotype to response to treatment



Estimating HIV Evolutionary Pathways and the Genetic Barrier to Drug Resistance



Mixture model of mutagenetic trees for the evolution of resistance during therapy with zidovudine plus didanosine. Edge weights denote expected waiting times in weeks (first row), their 95% confidence intervals (second row, in parentheses), and bootstrap support (third row).

Beerenwinkel, J Infect Dis 2005

More and more drug resistance mutations
More and more interactions among mutations

RESEARCH Open Access

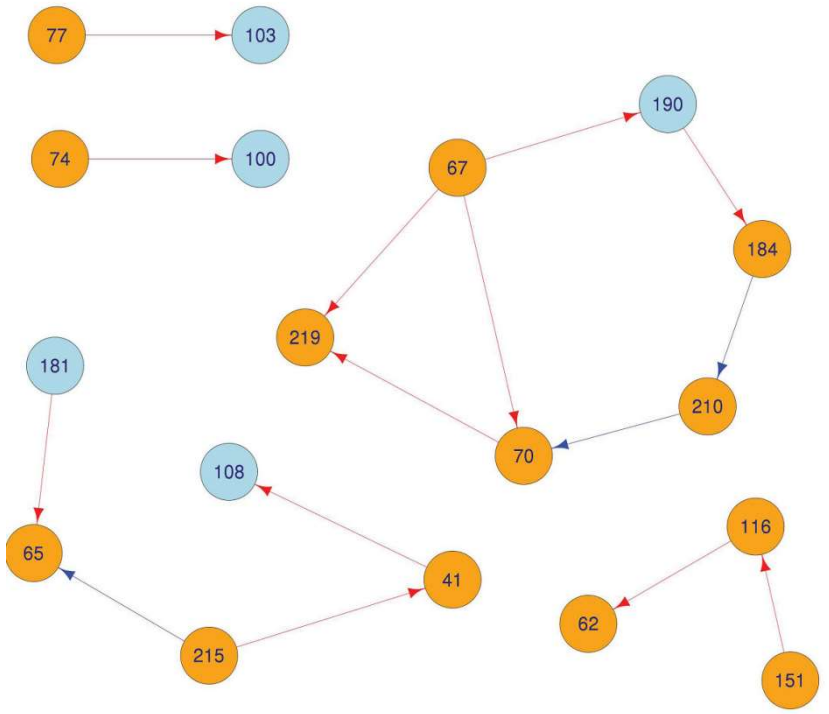
HIV-1 mutational pathways under multidrug therapy

Glenn Lawyer[1*], André Altmann[2], Alexander Thielen[1], Maurizio Zazzi[3], Anders Sönnerborg[4] and Thomas Lengauer[1]

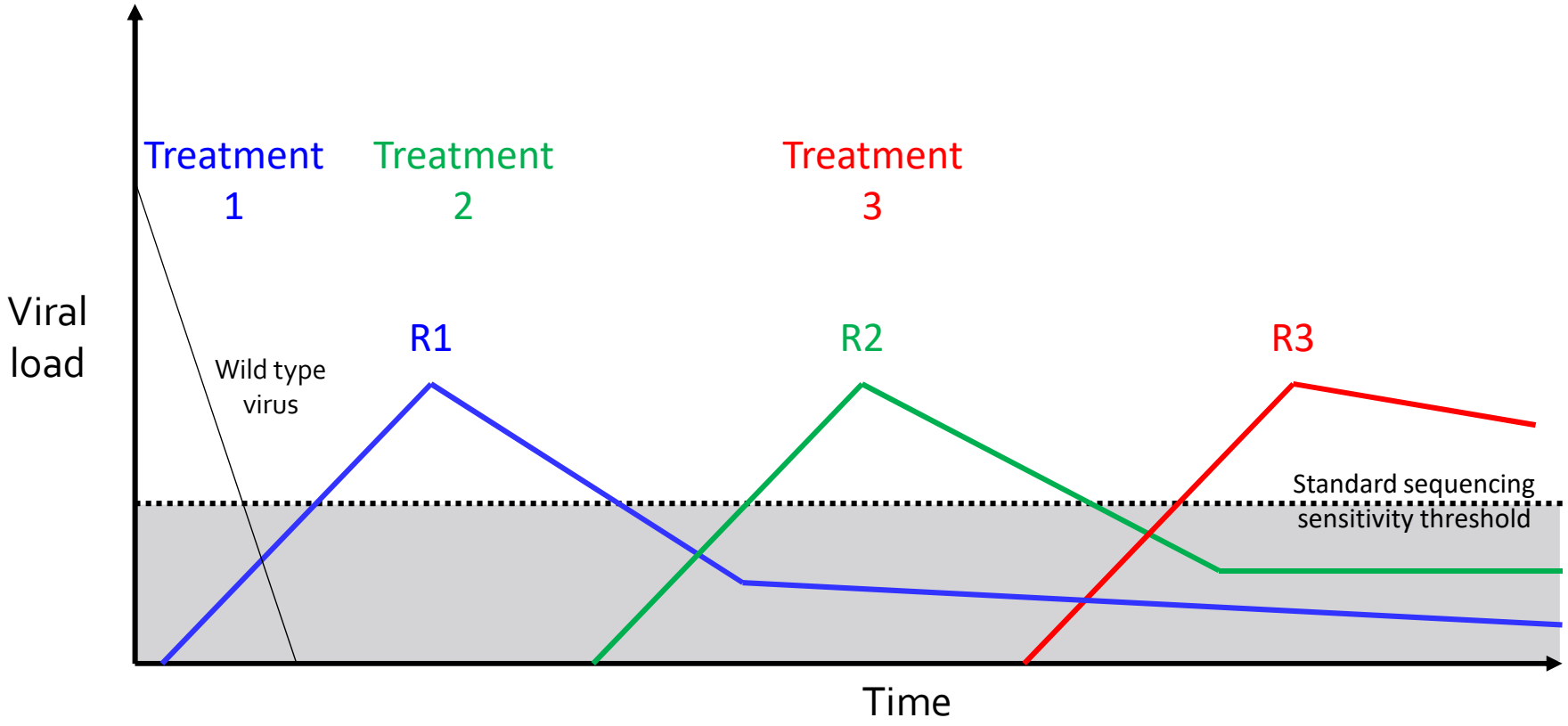
AIDS Research and Therapy 2011, **8**:26

Resistance mutations adjacency graph. Red pathways indicate an increase in hazard, blue a reduction.

Orange nodes are locations hosting NRTI resistance mutations, blue nodes are NNRTI locations.



Kinetics of drug resistance species following multiple treatment failures



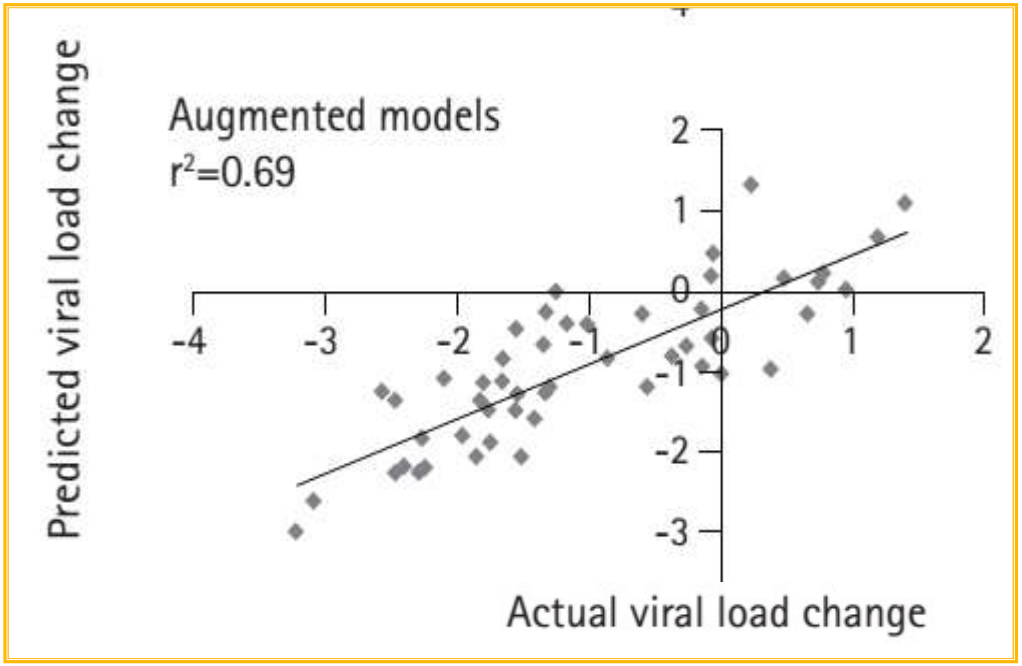
How to infer response to treatment from HIV sequence analysis (from GIS to TOS)

- From **Genotype Interpretation** Systems to **Treatment Optimization** Systems
- Many "complex" learning methods
  - Neural networks
  - Support Vector Machines
  - Random Forests
  - …
- Partly successful

Antiviral Therapy **12:15–24**

The development of artificial neural networks to predict virological response to combination HIV therapy

Brendan Larder[1], Dechao Wang[1], Andrew Revell[1], Julio Montaner[2], Richard Harrigan[2], Frank De Wolf[3], Joep Lange[4], Scott Wegner[5], Lidia Ruiz[6], María Jésus Pérez-Elías[7], Sean Emery[8], Jose Gatell[9], Antonella D'Arminio Monforte[10], Carlo Torti[11], Maurizio Zazzi[12] and Clifford Lane[13]*



www.hivrdi.org

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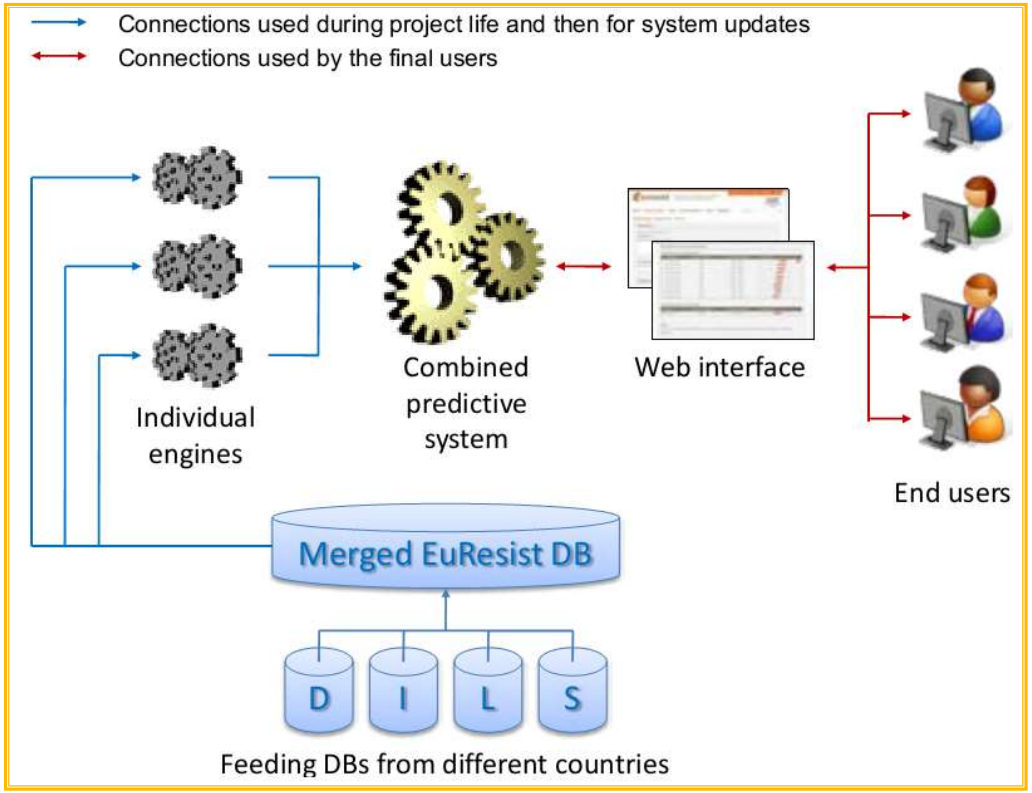
BIOINFORMATICS

Vol. 24 ISMB 2008, pages i399–i406
doi:10.1093/bioinformatics/btn141

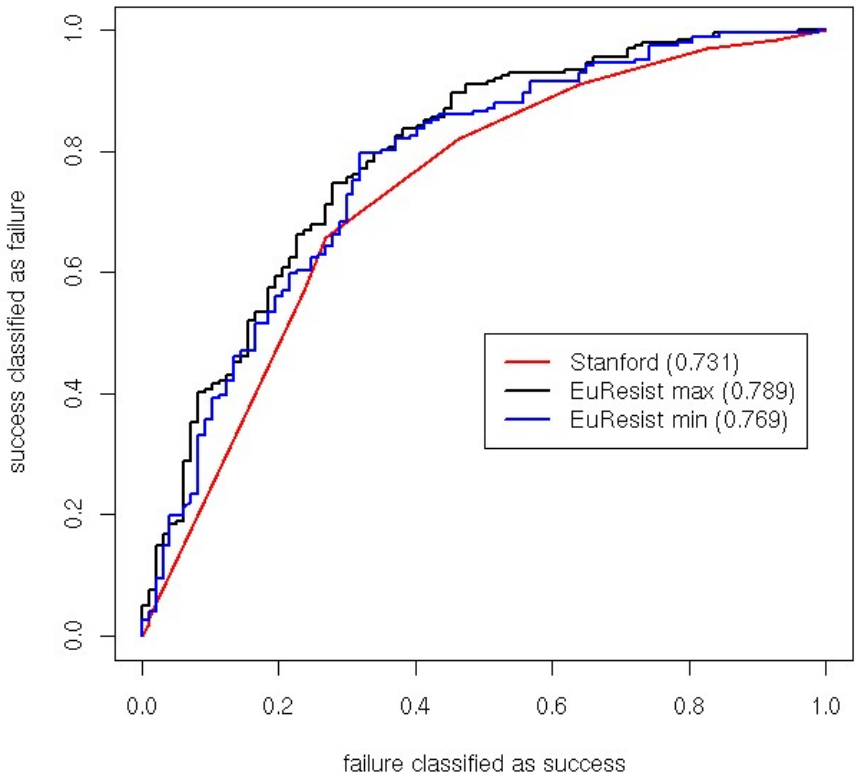
Selecting anti-HIV therapies based on a variety of genomic and clinical factors

Michal Rosen-Zvi[1,*], Andre Altmann[2], Mattia Prosperi[3], Ehud Aharoni[1], Hani Neuvirth[1], Anders Sönnerborg[4], Eugen Schülter[5], Daniel Struck[6], Yardena Peres[7], Francesca Incardona[8], Rolf Kaiser[5], Maurizio Zazzi[9] and Thomas Lengauer[2]



www.euresist.org

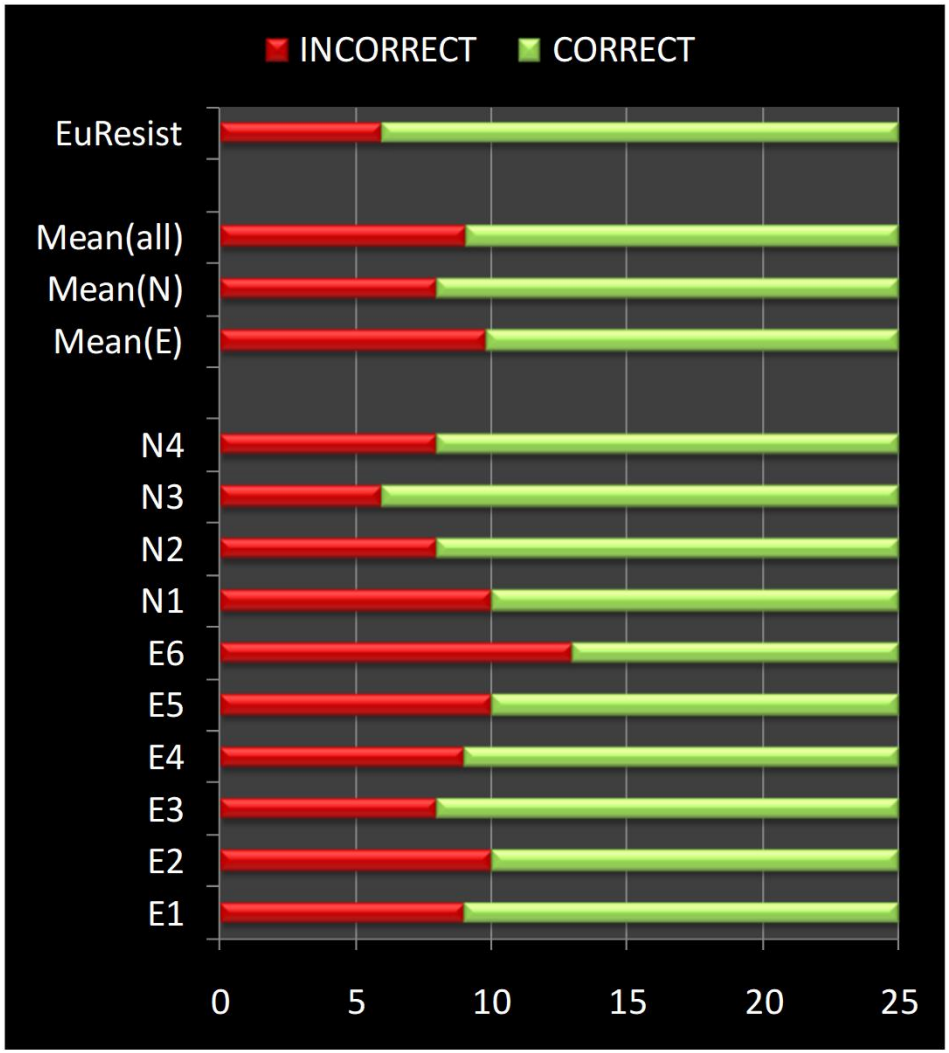
How to infer response to treatment from HIV sequence analysis



3143 therapies, Short-term outcome (8 weeks)

Rosen-Zvi, Bioinformatics 2008

EuResist vs. Expert interpretation (EvE study)



- 25 ART cases randomly selected form the EuResist db
  - Obsolete therapies and wild type genotype excluded
  - All clinical and virological information available
- EuResist engine used to predict success/failure
- 10 leading experts examining patient charts to predict success/failure
  - On-line anonymous rating
  - Only European (E) vs. non-European (N) setting traceable
  - Use of any interpretation system allowed
- Top accuracy (76%) reached by EuResist and one expert

Why "Treatment Optimization Systems" have not gained wide attention

- **Genotype interpretation systems** are well consolidated and **work satisfactorily** (i.e. HIVdb, REGA, ANRS)
- TOSs are based on complex and non-transparent functions (**black box**), the user is not comfortable with
- The increase in accuracy is not dramatic and is more relevant with **old therapies** (larger training datasets)
- The potential benefits may come at the **expense of providing additional patient data**
- **The incidence and impact of drug resistance have decreased over time**

GRT as a key for ART success

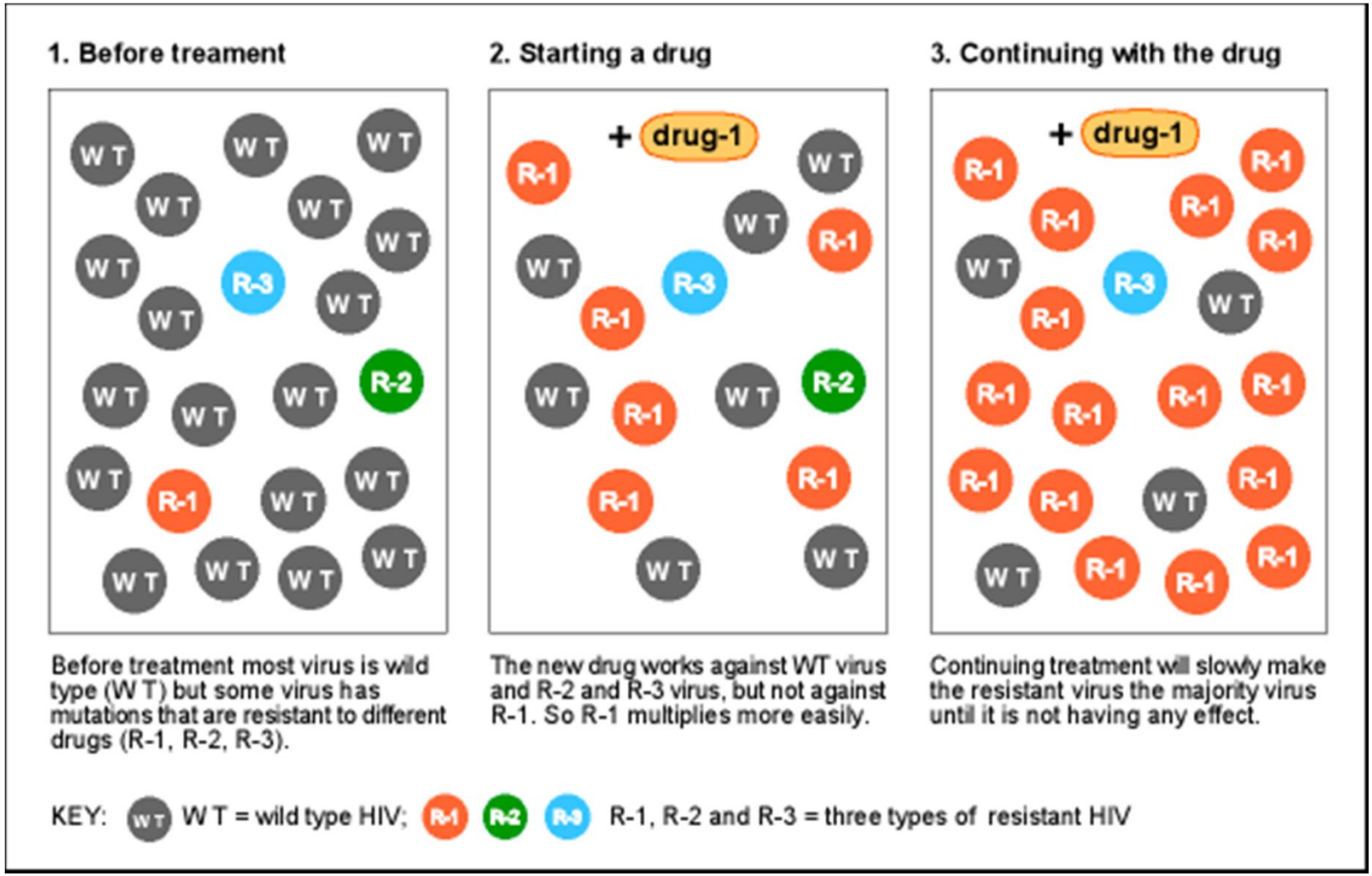
HIV drug resistance testing

- The need
- The options
- **A key component of ART success**
- Evolution of HIV drug resistance testing
- The largest ever bug sequence collection

Drug resistance testing as a key component of the success of antiretroviral therapy

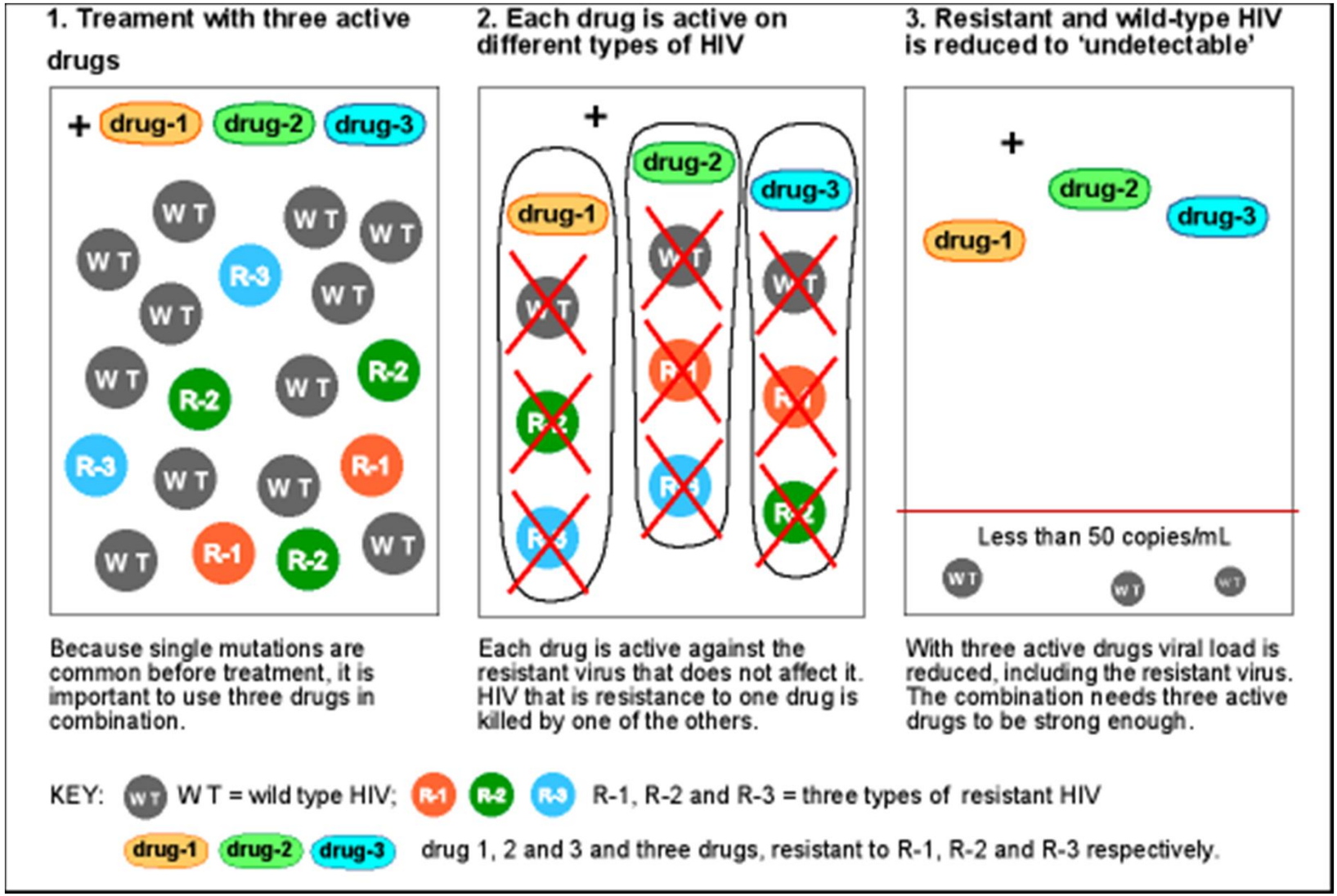
Drug resistance testing provided a basis for combination ART

Drug resistance emerging as a variability and selection process



http://i-base.info/hiv-and-drug-resistance/

Drug resistance is the driver for combination therapy



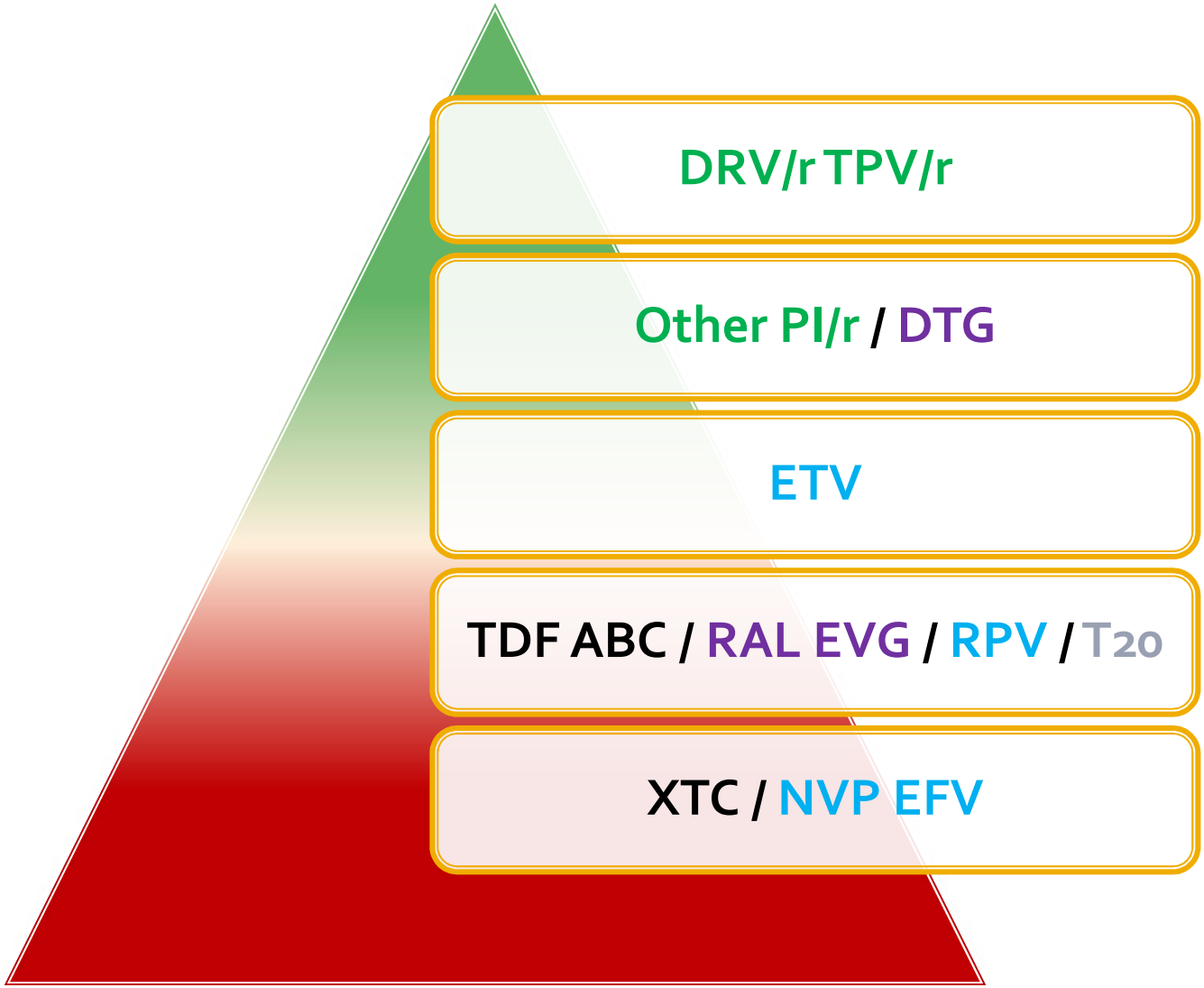
Drug resistance testing as a key component of the success of antiretroviral therapy

Drug resistance testing highlighted the key concept of genetic barrier

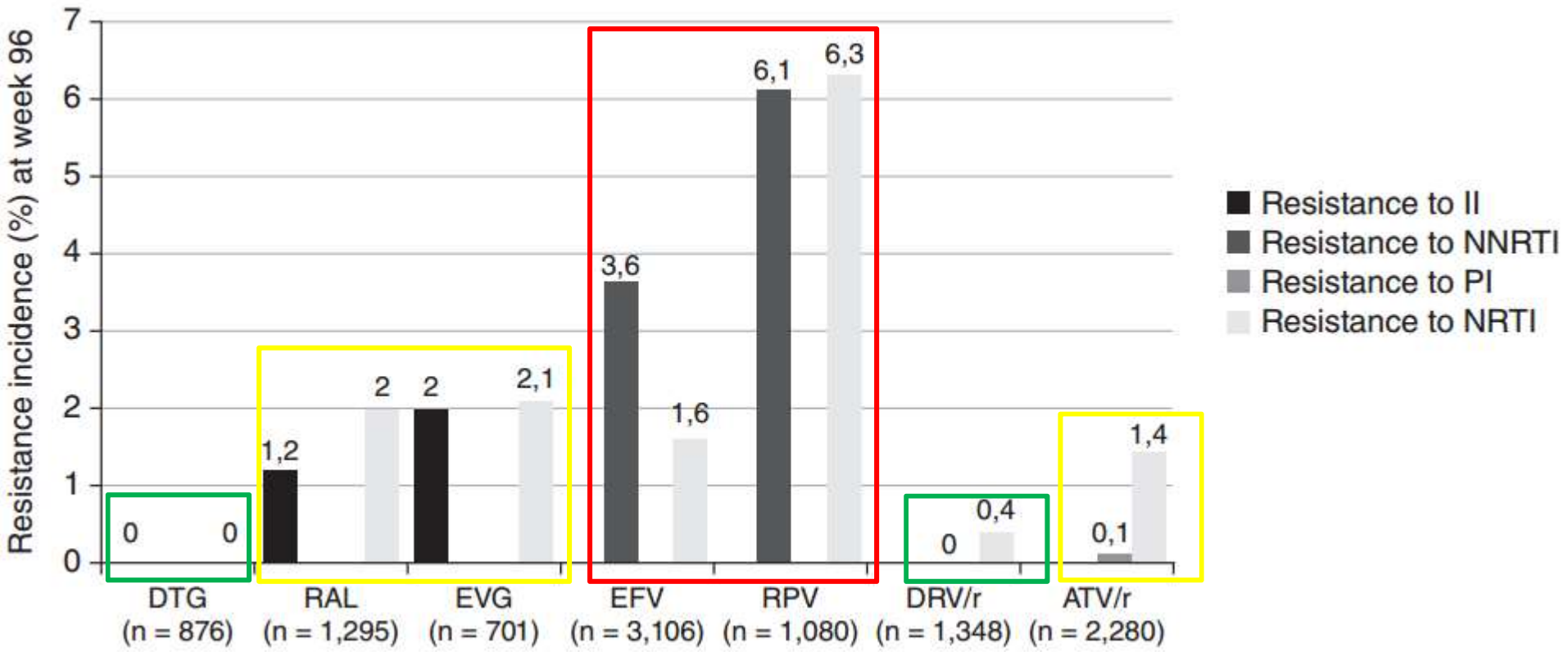
How can we define the genetic barrier to resistance?

The extent of variation required for a susceptible virus to become resistant to a specific drug or drug combination

Genetic barrier of individual antiretrovirals



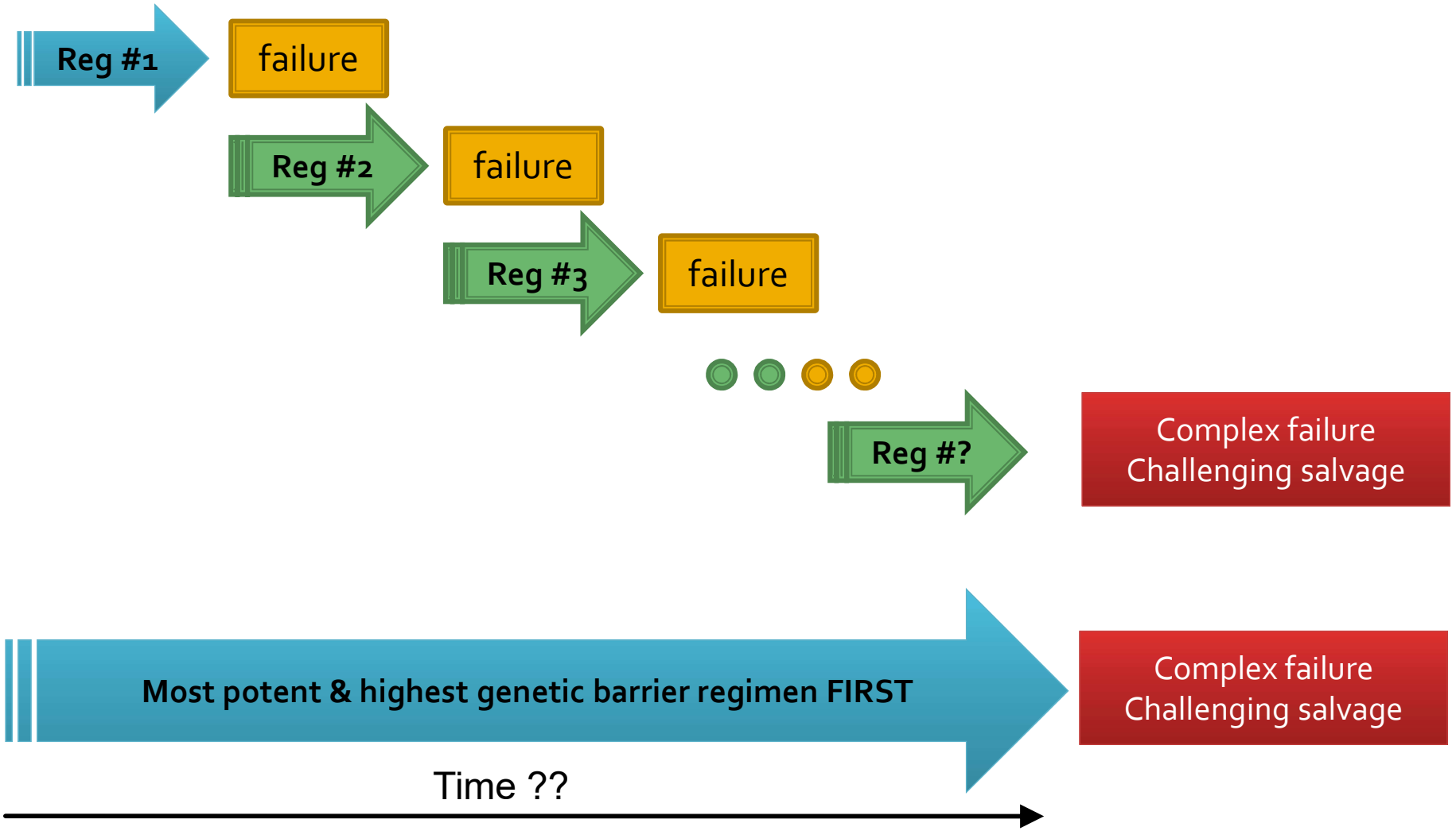
Incidence of resistance at week 96 in pivotal first-line treatment trials



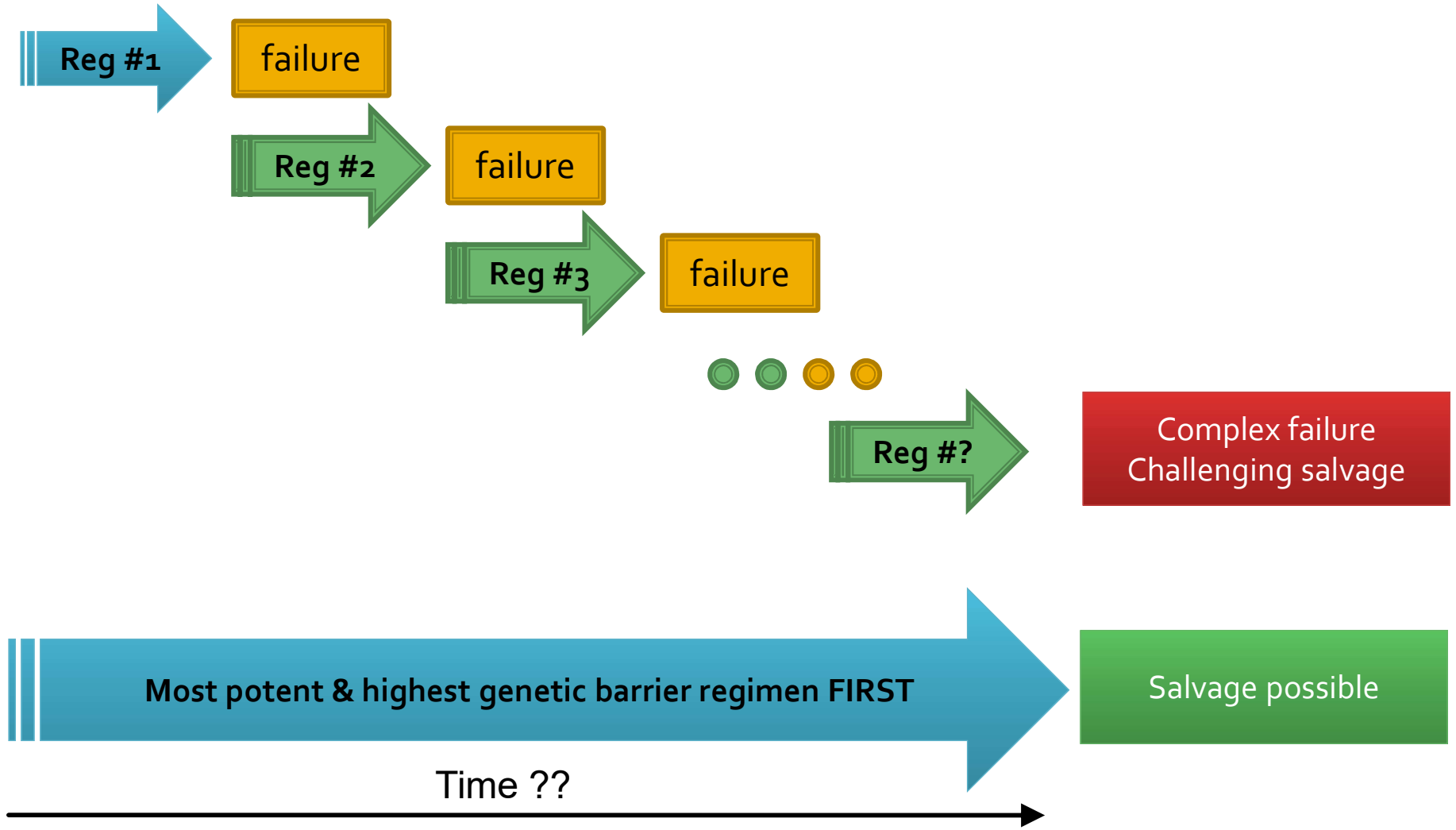
CAUTION: different definitions of virological failure, different proportions of advanced patients, different procedures to collect samples at failure.

Llibre, AIDS Rev 2015

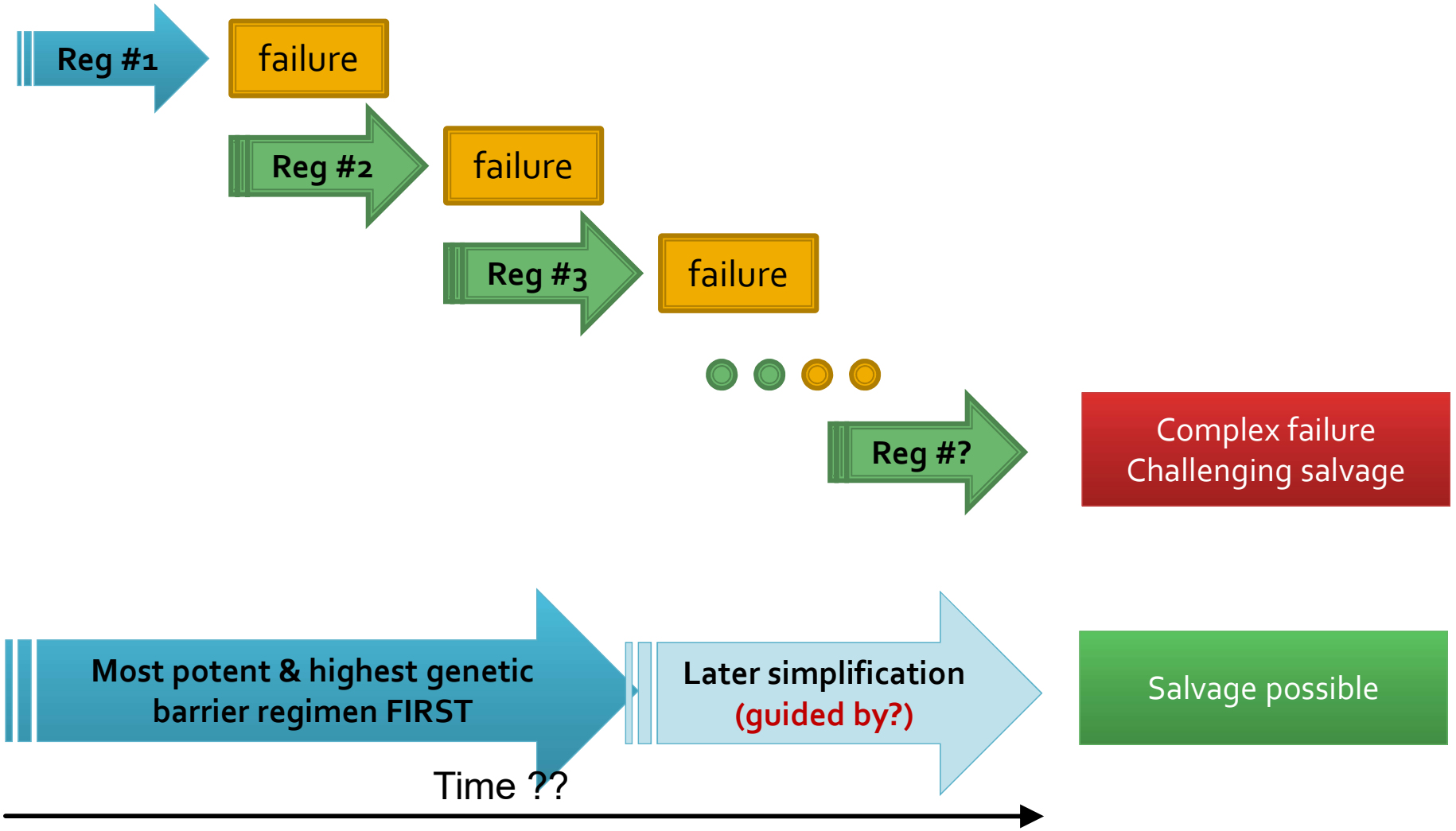
Durability and sequencing of antiretroviral regimens



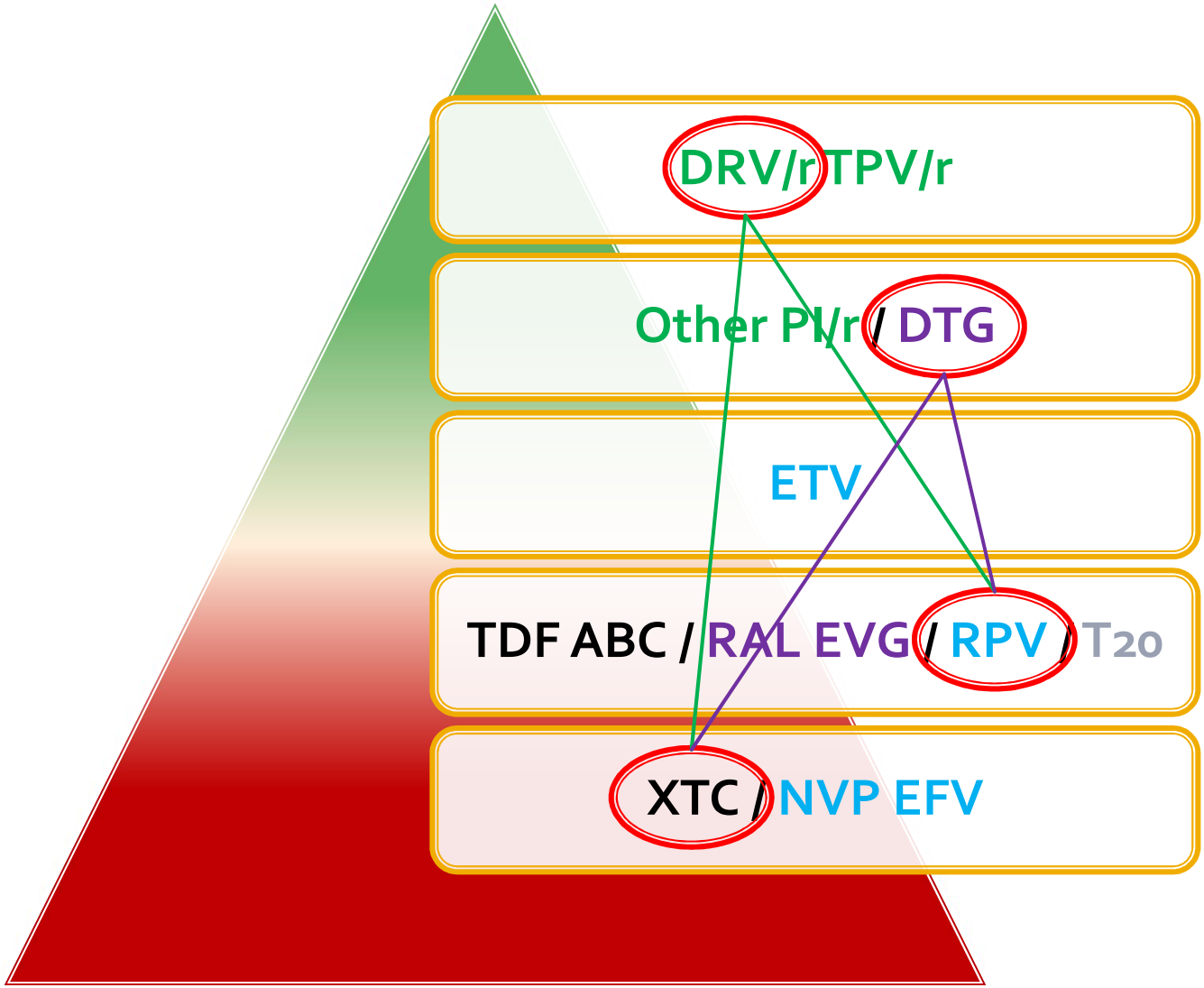
Durability and sequencing of antiretroviral regimens



Durability and sequencing of antiretroviral regimens



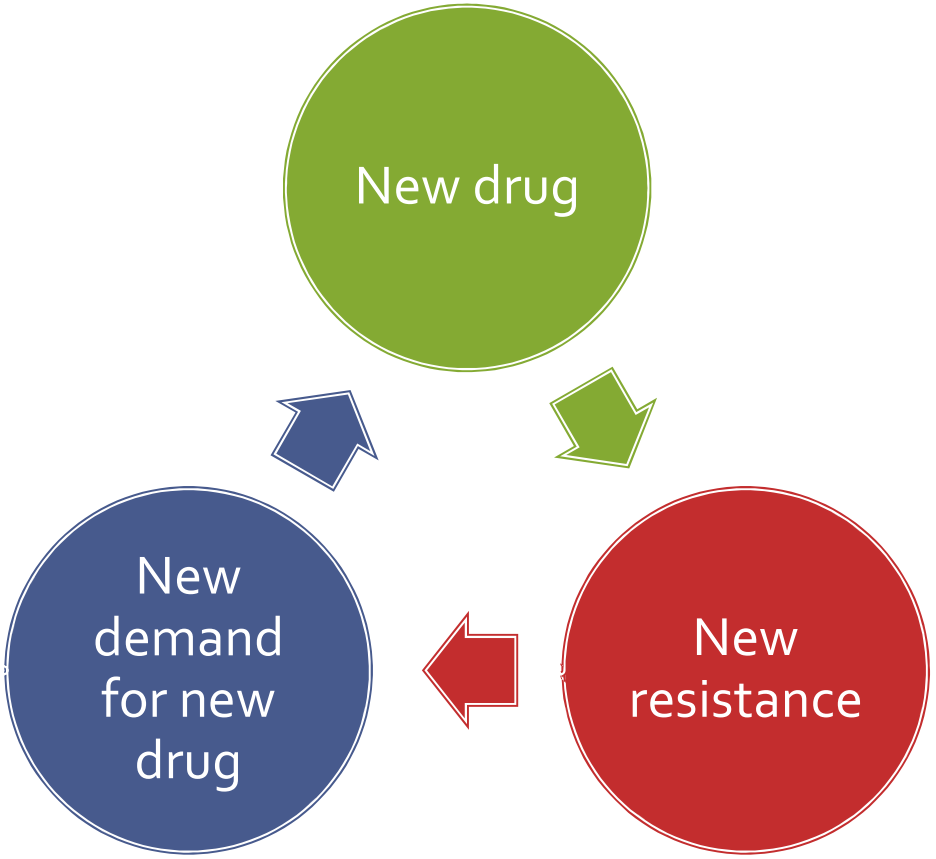
Genetic barrier of individual antiretrovirals & how to build a robust 2-drug regimen



Drug resistance testing as a key component of the success of antiretroviral therapy

Drug resistance testing urged development of novel drug classes

Anti-HIV compounds & HIV resistance



Cross-resistance within antiretroviral classes

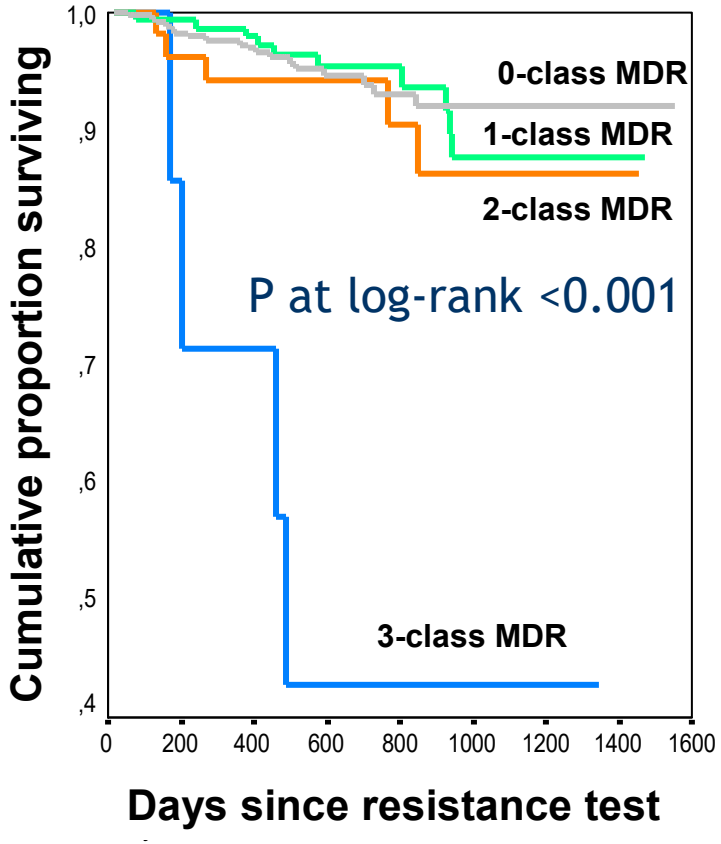
Major Nucleoside RT Inhibitor (NRTI) Resistance Mutations													
	Non-TAMs					TAMs						MDR	
	184	65	70	74	115	41	67	70	210	215	219	69	151
Cons	M	K	K	L	Y	M	D	K	L	T	K	T	Q
3TC	VI	R										Ins	M
FTC	VI	R										Ins	M
ABC	VI	R	E	VI	F	L			W	FY		INS	M
TDF	***	R	E		F	L		R	W	FY		INS	M
ZDV	***	***	*	*		L	N	R	W	FY	QE	INS	M

Major Non-Nucleoside RT Inhibitor (NNRTI) Resistance Mutations								
	100	101	103	106	181	188	190	230
Cons	L	K	K	V	Y	Y	G	M
NVP	I	EP	NS	AM	CIV	LCH	ASEQ	L
EFV	I	EP	NS	AM	CIV	LCH	ASEQ	L
ETR	I	EP			CIV	L	ASEQ	L
RPV	I	EP			CIV	L	ASEQ	L

Major Protease Inhibitor (PI) Resistance Mutations												
	24	32	46	47	48	50	54	76	82	84	88	90
Cons	D	V	M	I	G	I	I	L	V	I	N	L
ATV/r		I	IL	V	VM	L	VTALM		ATFS	V	S	M
DRV/r		I		VA		V	LM	V	F	V		
LPV/r	I	I	IL	VA	VM	V	VTALM	V	AFTS	V		M

Major Integrase Inhibitor (INI) Resistance Mutations									
	66	92	138	140	143	147	148	155	263
Cons	T	E	E	G	Y	S	Q	N	R
RAL	AIK	Q	KAT	SAC	RCH		HRK	H	K
EVG	AIK	Q	KAT	SAC		G	HRK	H	K
DTG	K	Q	KAT	SAC			HRK	H	K

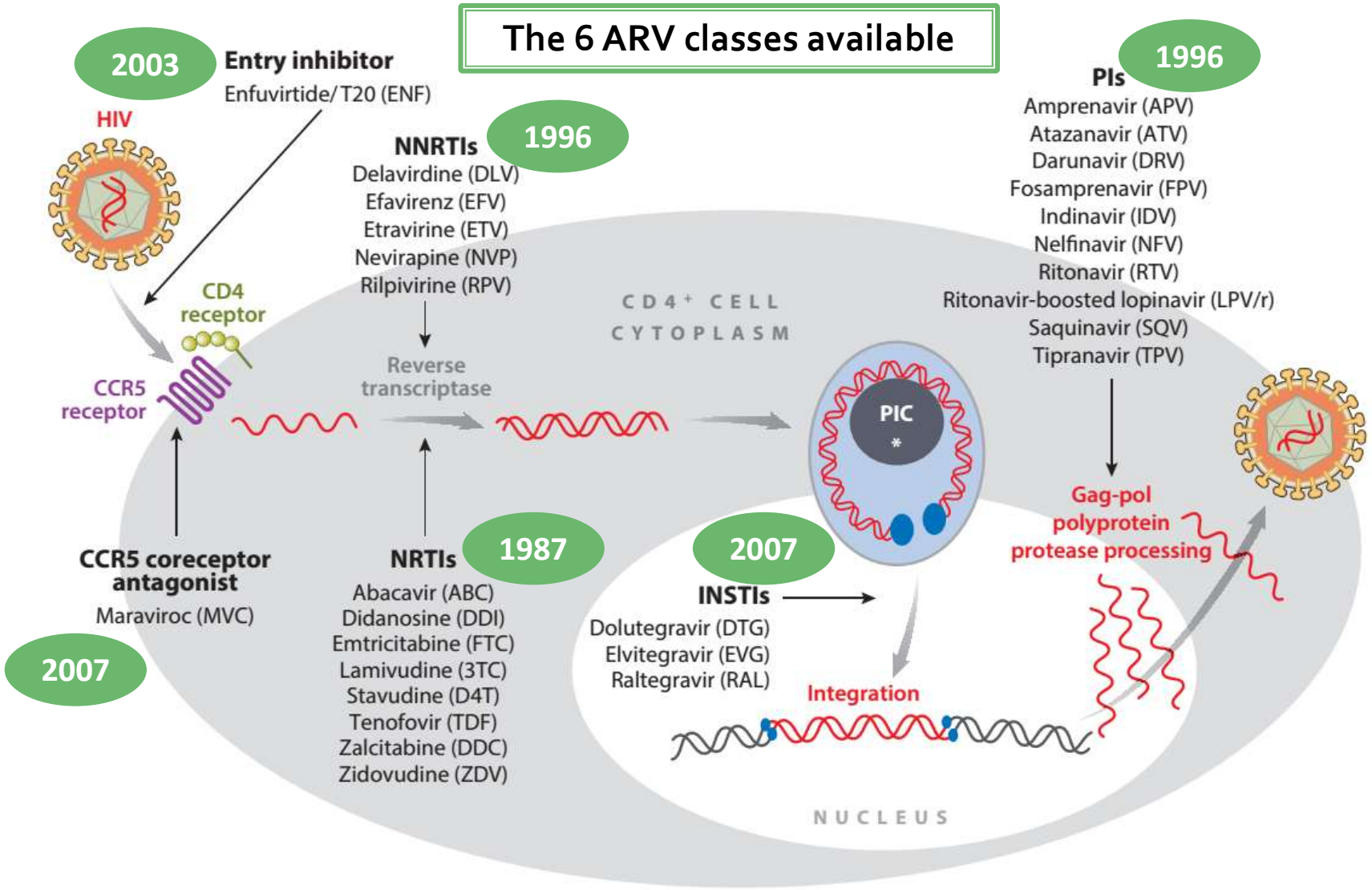
Drug resistance impacts on HIV related mortality



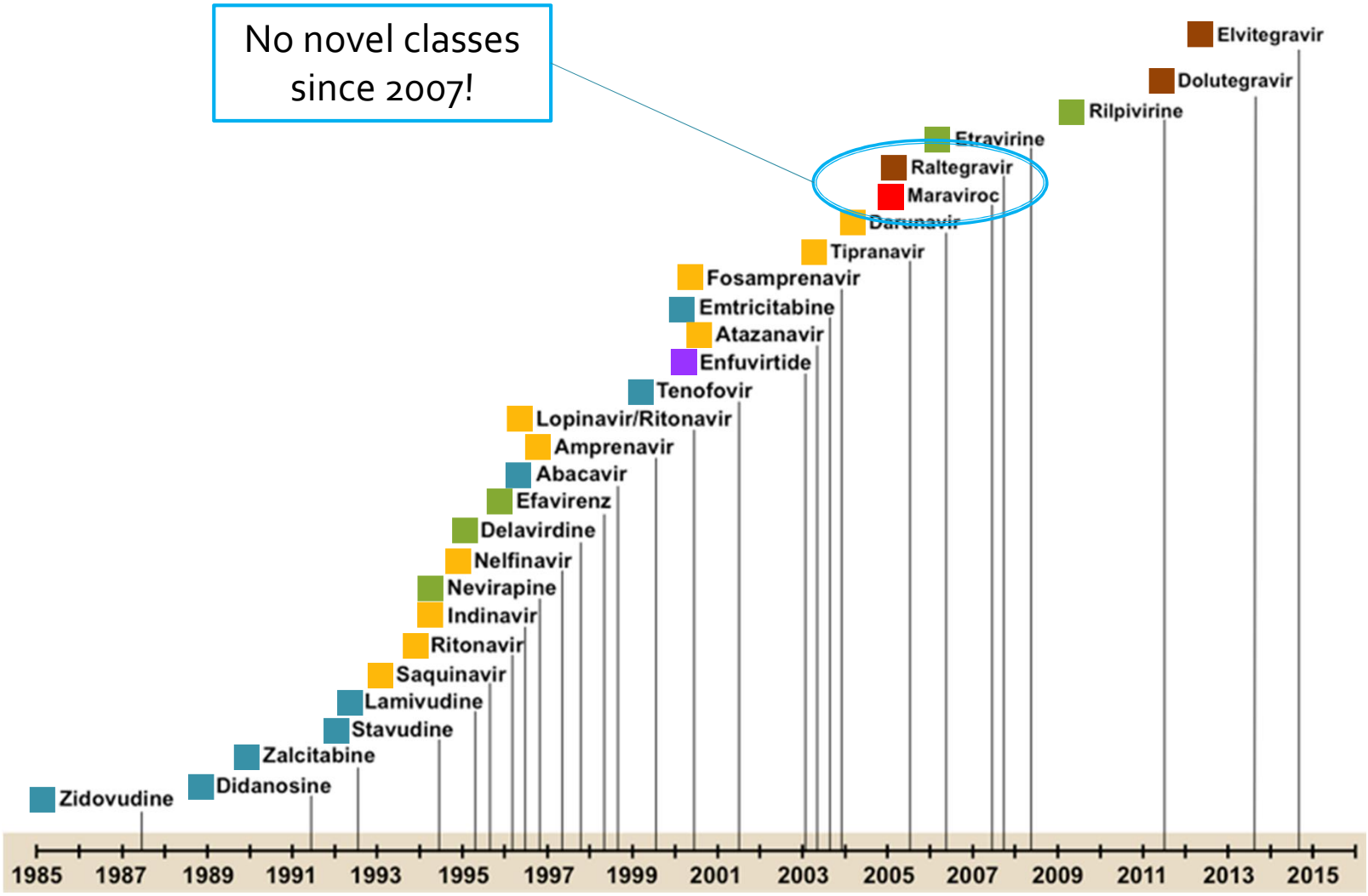
Multidrug resistance predicts mortality

(Zaccarelli, AIDS 2007)

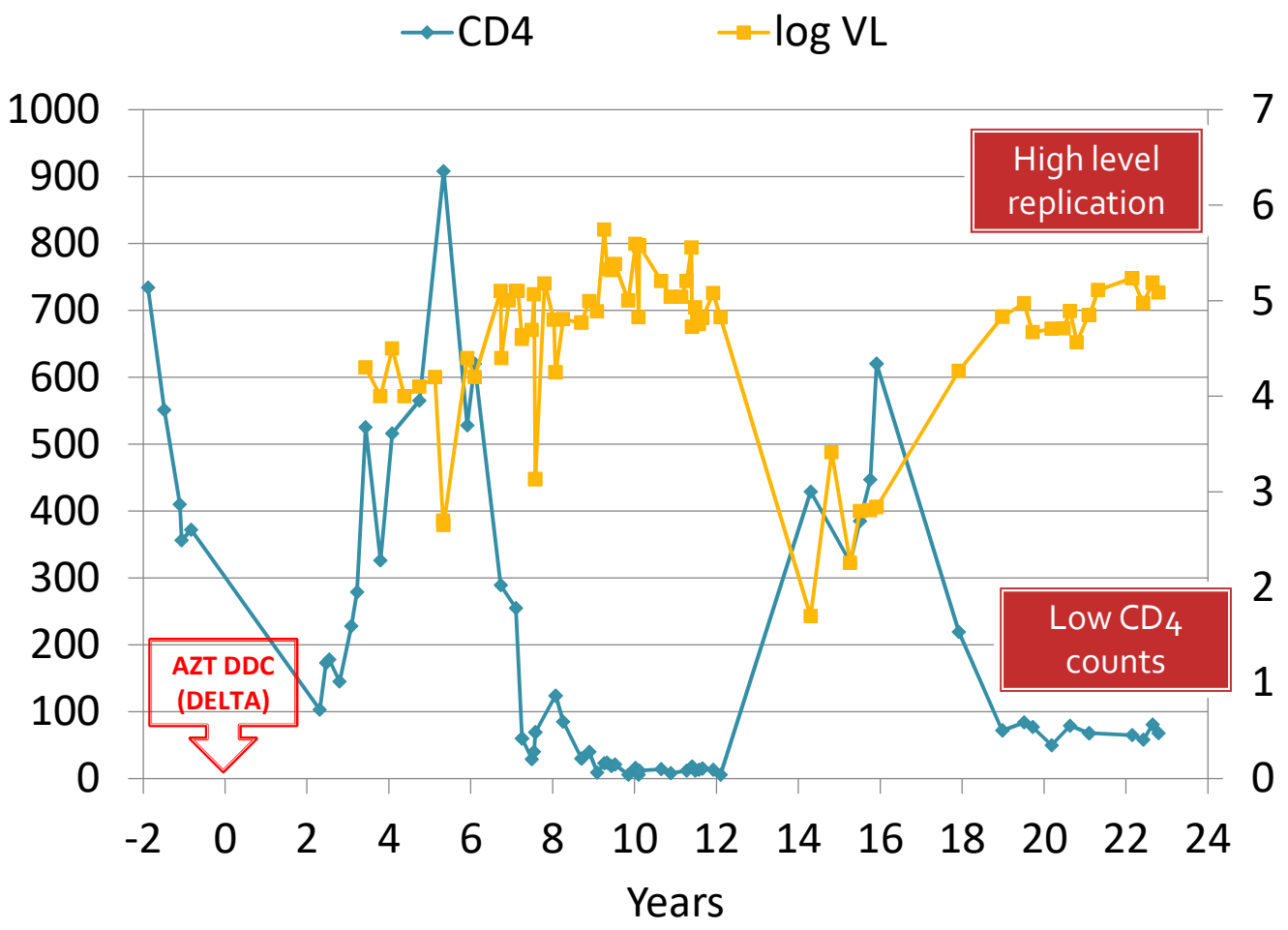
Antiretroviral drug classes



HIV drugs timeline



Difficult-to-treat 6-class resistant viruses



- Born in 1961, HIV-1 diagnosis in 1990, IDU
- Therapy started in 1993, >20 treatment changes (including DELTA trial)
- Multiple treatment failures due to poor adherence
- 6-class experienced
- Extrapulmonary TB in 2008, anal cancer in 2013
- From 2012, VL~10^5 cp/ml and CD4+ <100 cells/µl

Difficult-to-treat 6-class resistant viruses

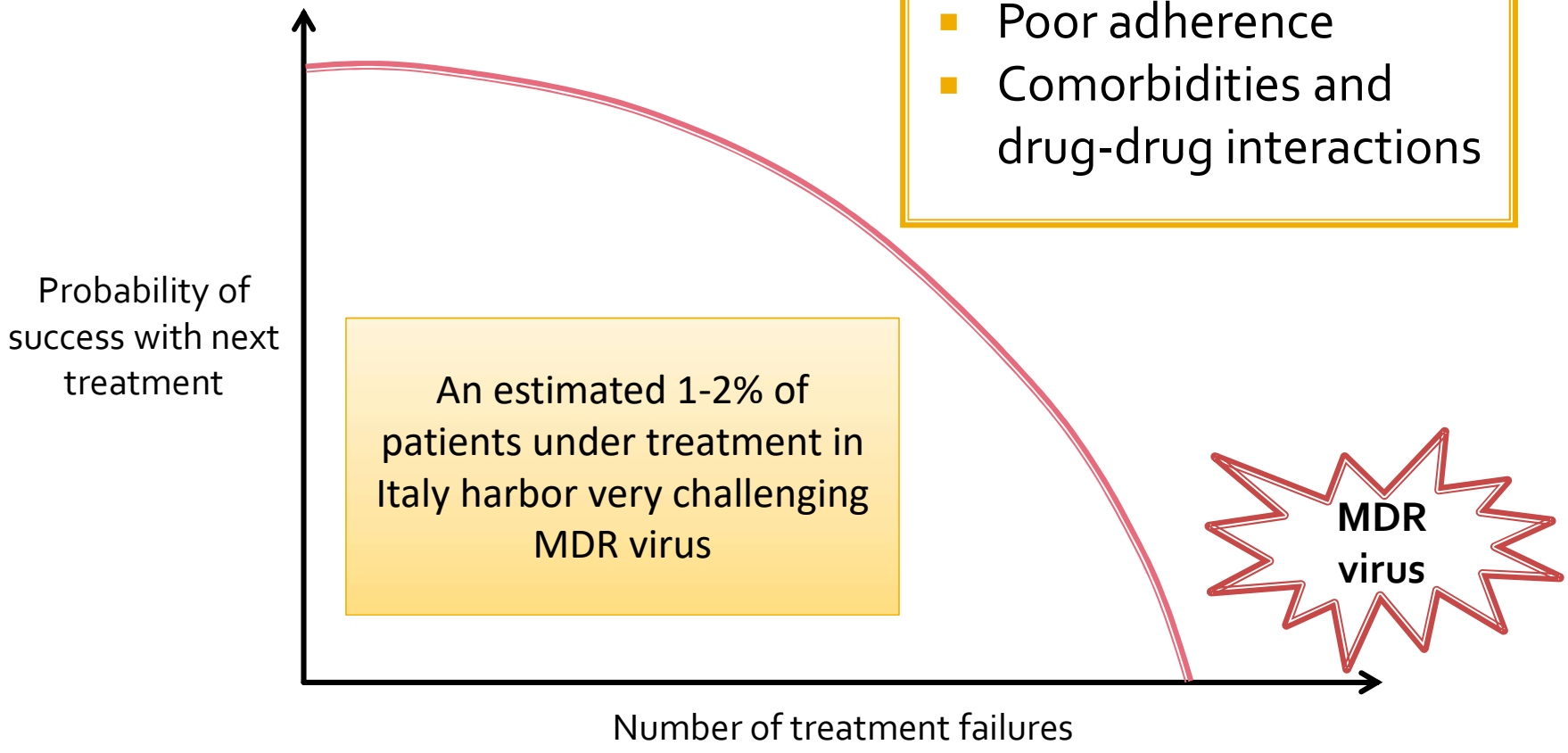
NRTI							NNRTI				PI									INI			FI	CA
3TC	ABC	AZT	D4T	DDI	FTC	TDF	EFV	ETR	NVP	RPV	ATV	ATV /rtv	DRV /rtv	FPV /rtv	IDV /rtv	LPV /rtv	NFV	SQV /rtv	TPV /rtv	DTG	EVG	RAL	T20	MVC
0	0	0,25	0	0	0	0,5	0	0,6	0	0,3	0	0	0	0	0	0	0	0	0	0,5	0,25	0	0,25	0

Mutazioni PR:	10I 10V 11I 13V 20I 20T 32I 33F 36I 43T 46I 54L 54V 60E 63P 71I 77I 82A 82I 82T 84V 89V 90M 93L
Altre mutazioni PR:	12P 12S 15V 16R 19P 37D 37E 41K 55N 62V 72R 73D 79A 79S 91S 92K 94D
Mutazioni RT:	66d 67E 69G 70R 74I 103N 108I 118I 179I 181C 184V 215F 219E 228H
Altre mutazioni RT:	37F 39K 40A 40T 43Q 47F 67K 69R 98S 100F 111L 122E 135T 165A 177N 201I 223Q 245E
Mutazioni IN:	140S 148H
Altre mutazioni IN:	49S 49T 61L 101I 116G
Mutazioni GP41:	43D
Altre mutazioni GP41:	7L 23A 24L 32L

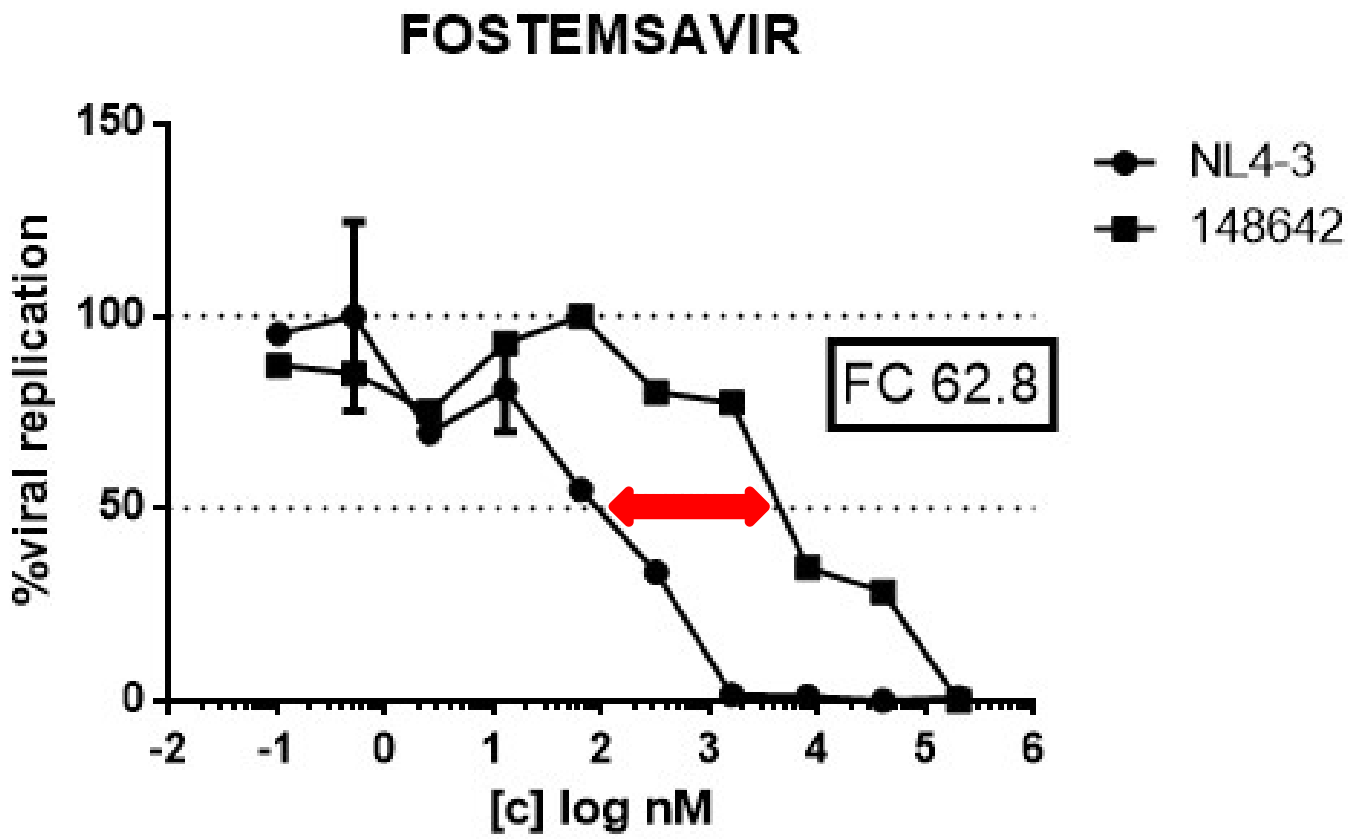
- Cumulative (1999-2017) genotype based on
  - 18 PR/RT sequences (note that 33 of 99 PR aminoacids have changed!)
  - 6 IN sequences
  - 2 gp41 sequences
  - 4 V3 sequences

How MDR builds up

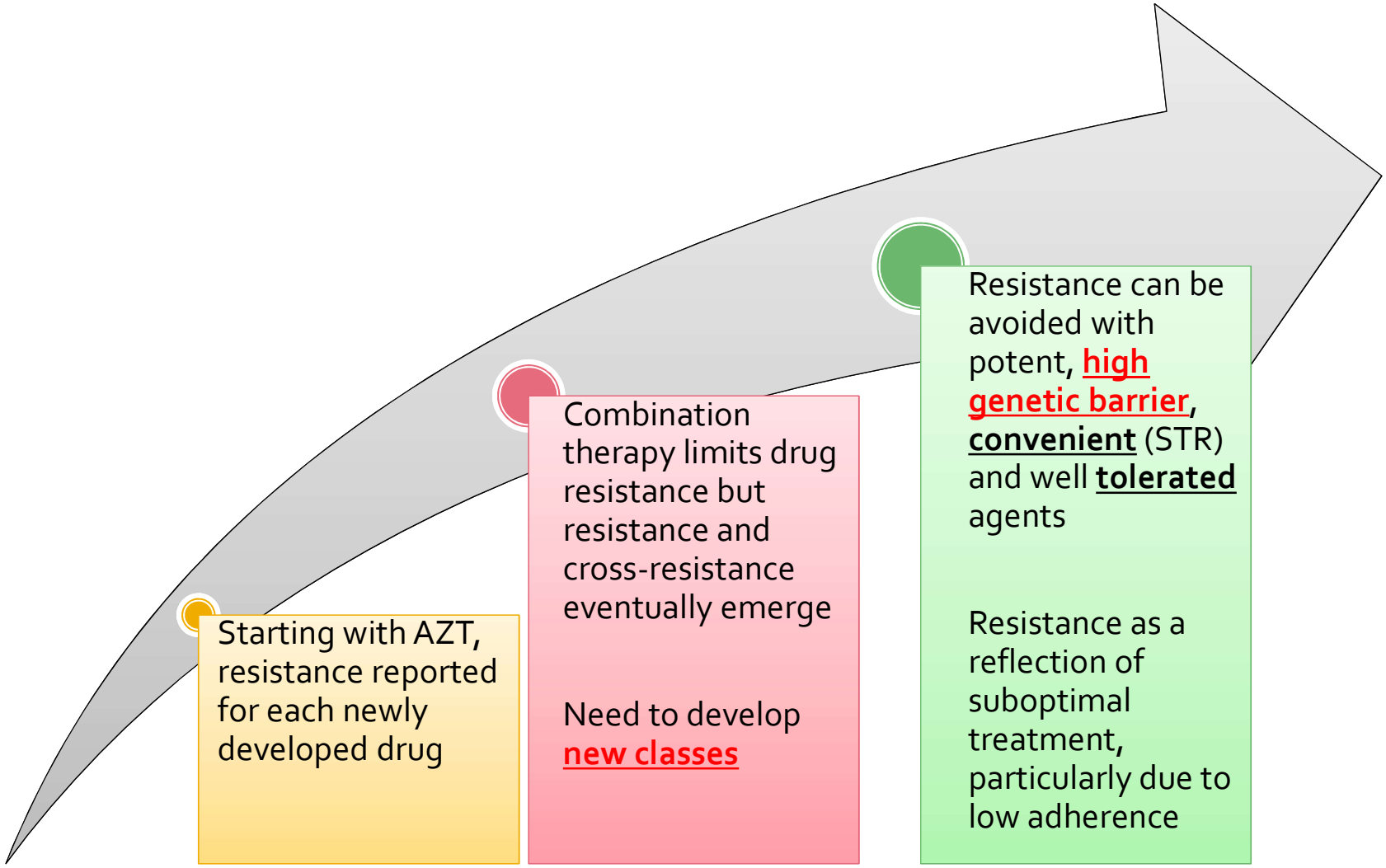
- Suboptimal therapy
- Add-on therapy
- Poor adherence
- Comorbidities and drug-drug interactions



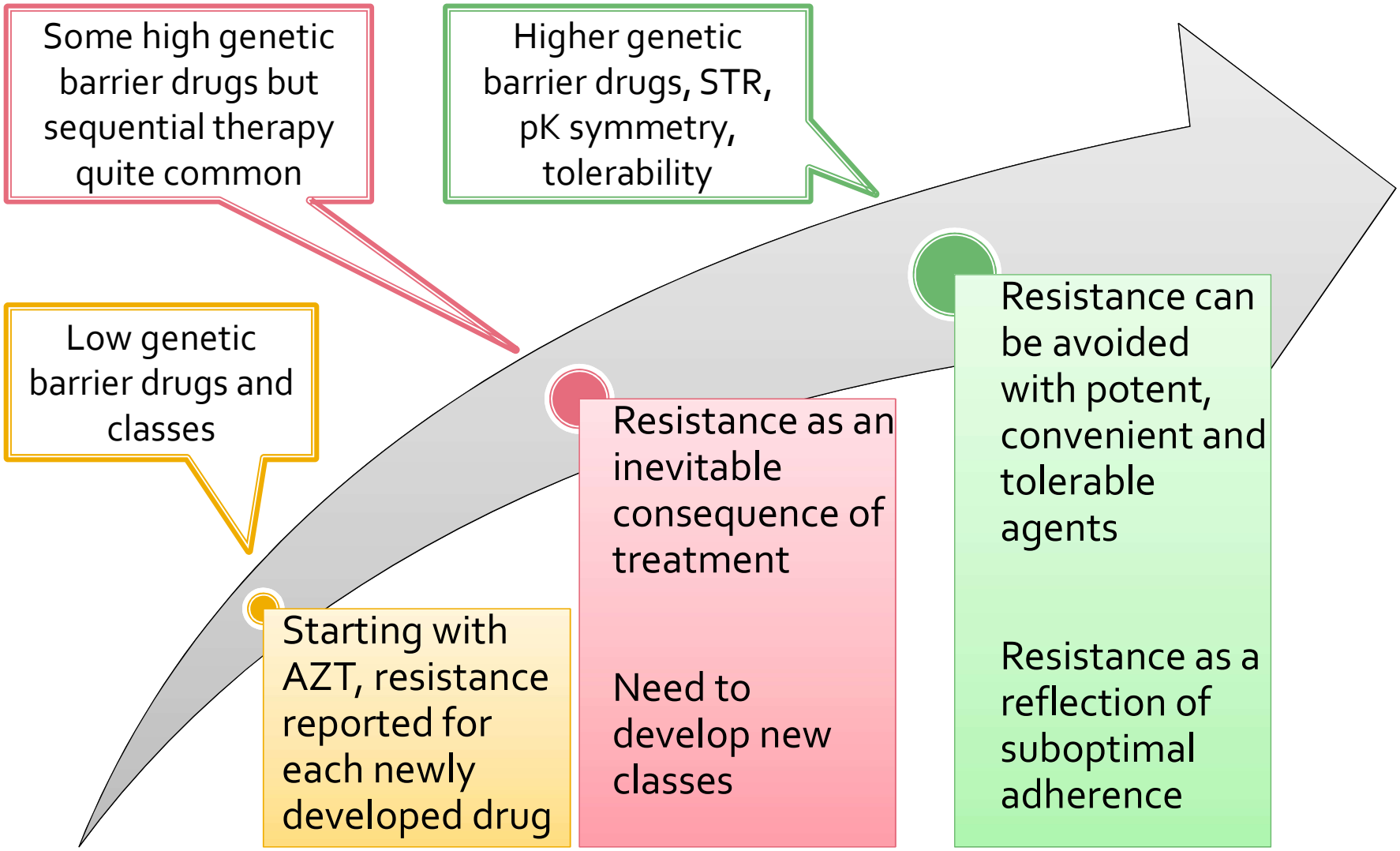
Difficult-to-treat 7-class resistant virus



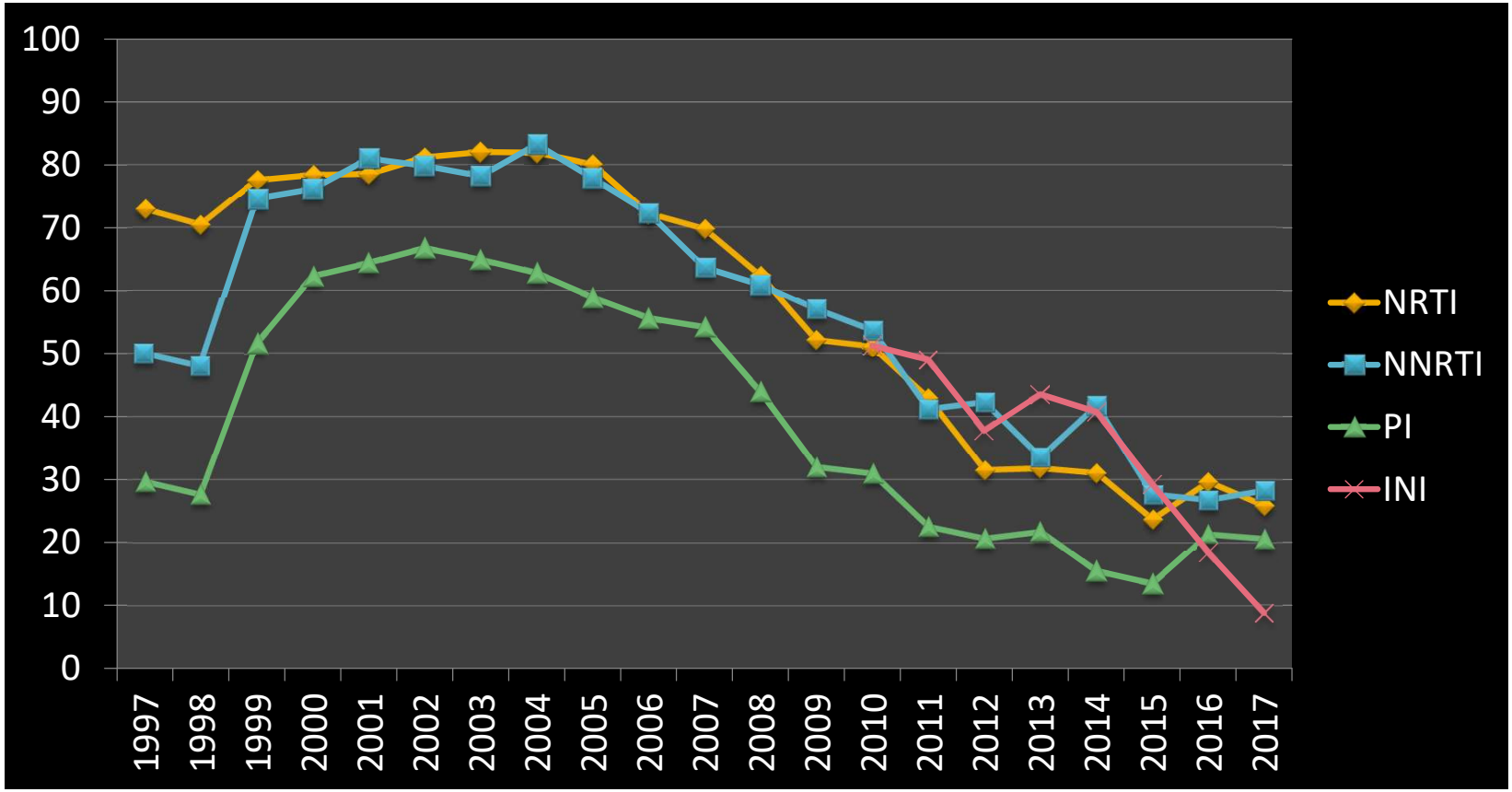
Evolution of HIV treatment and drug resistance over antiretroviral therapy eras



Evolution of HIV treatment and drug resistance over time

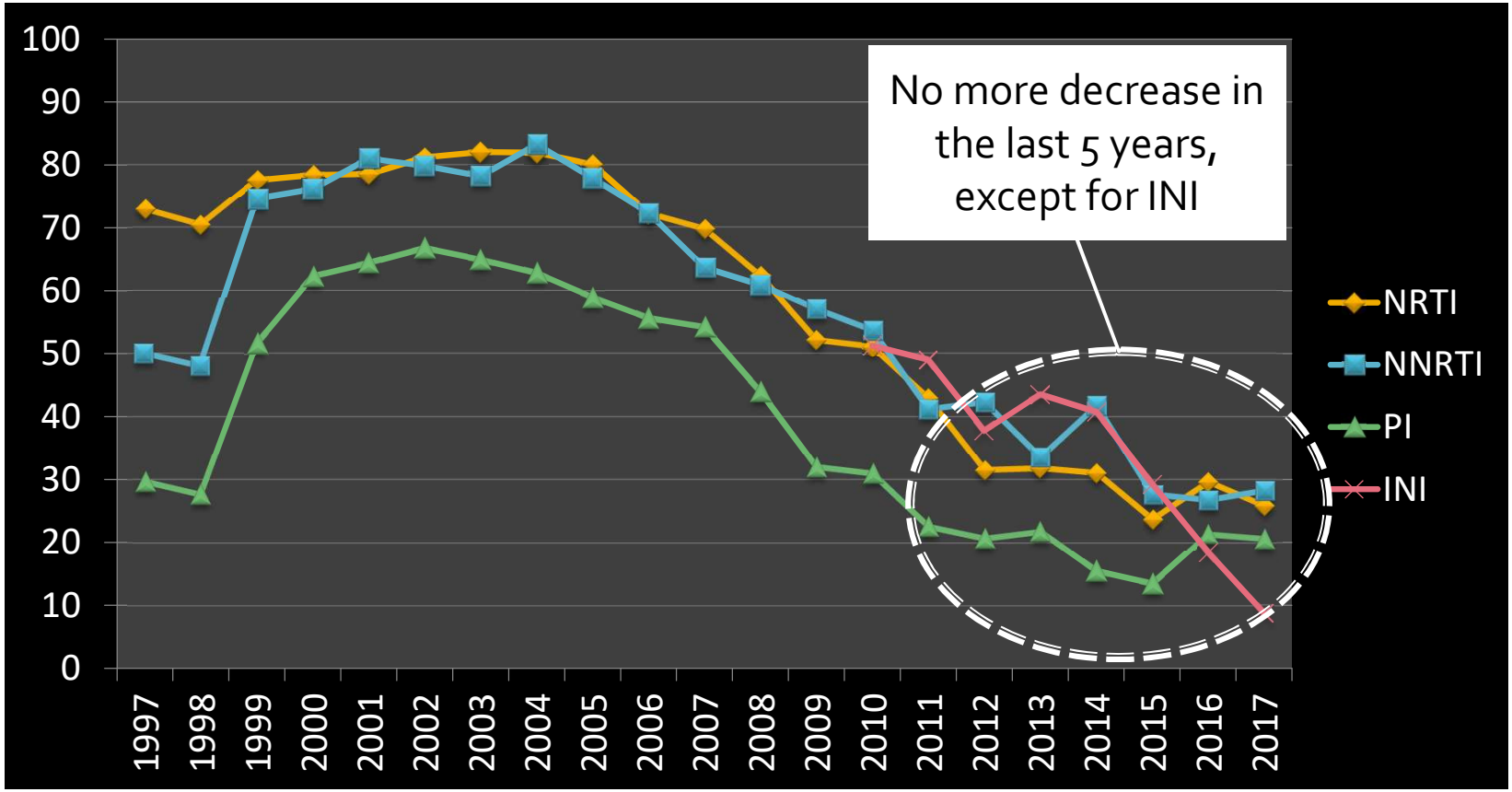


Time trends of emergent resistance at failure with the corresponding drug class in Italy



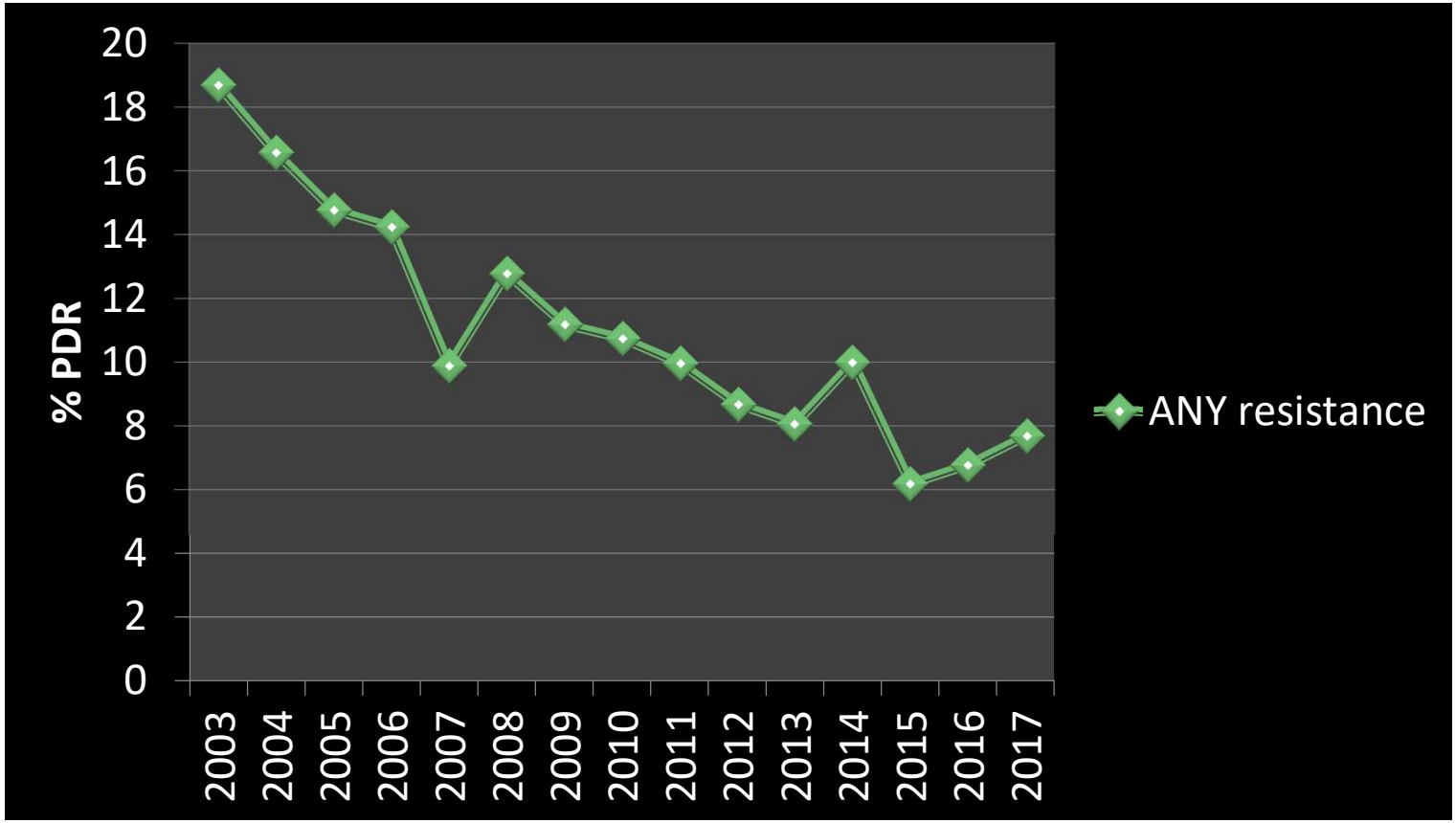
ARCA, accessed October 10, 2017; based on 14,947 sequences from patients on NRTI, 4,357 on NNRTI, 9,619 on PI, 565 on INI

Time trends of emergent resistance at failure with the corresponding drug class in Italy



ARCA, accessed October 10, 2017; based on 14,947 sequences from patients on NRTI, 4,357 on NNRTI, 9,619 on PI, 565 on INI

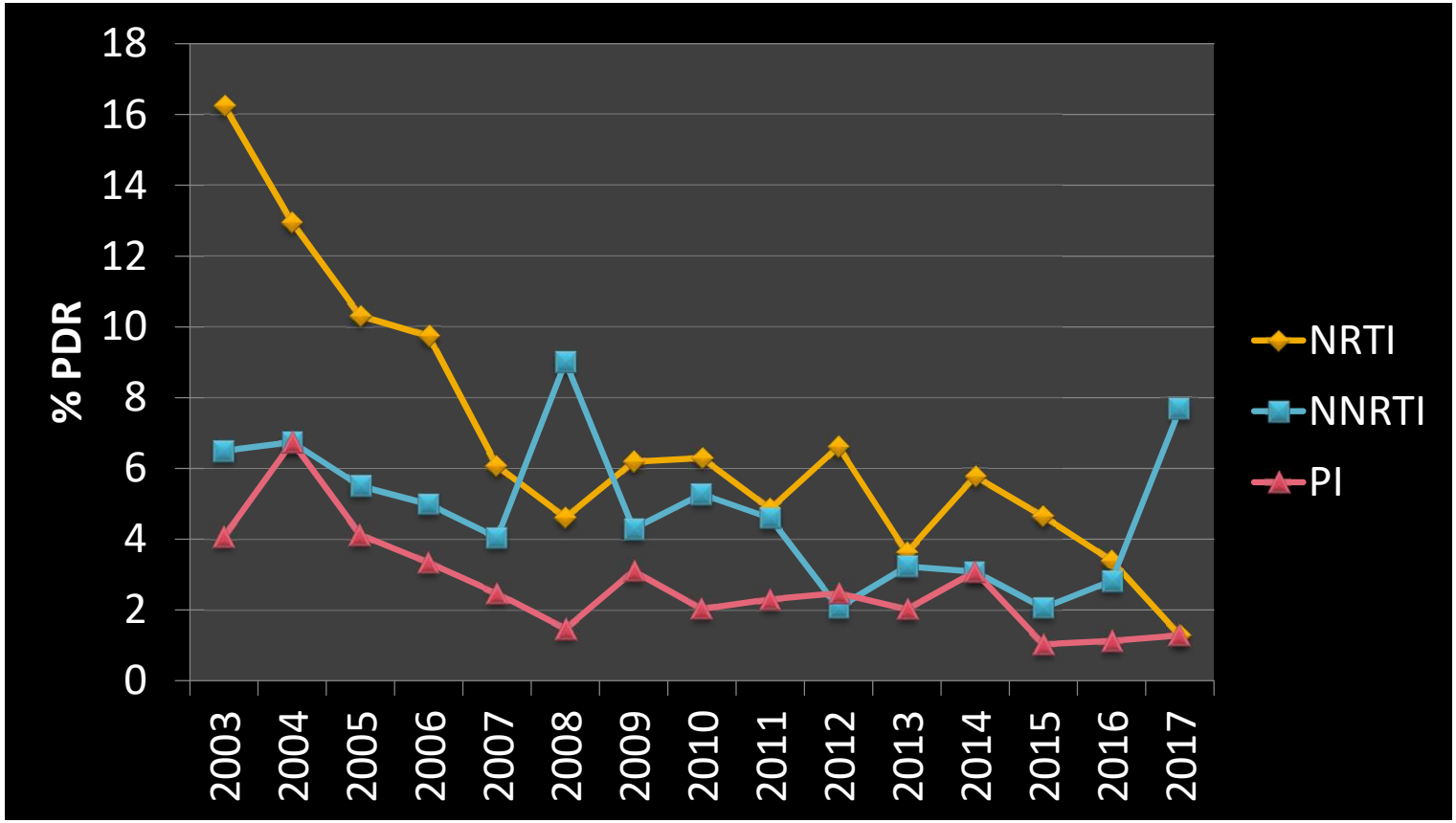
HIV pre-treatment drug resistance in Italy



Based on 4453 PR/RT sequences from drug naïve individuals

ARCA, accessed 06/10/2017

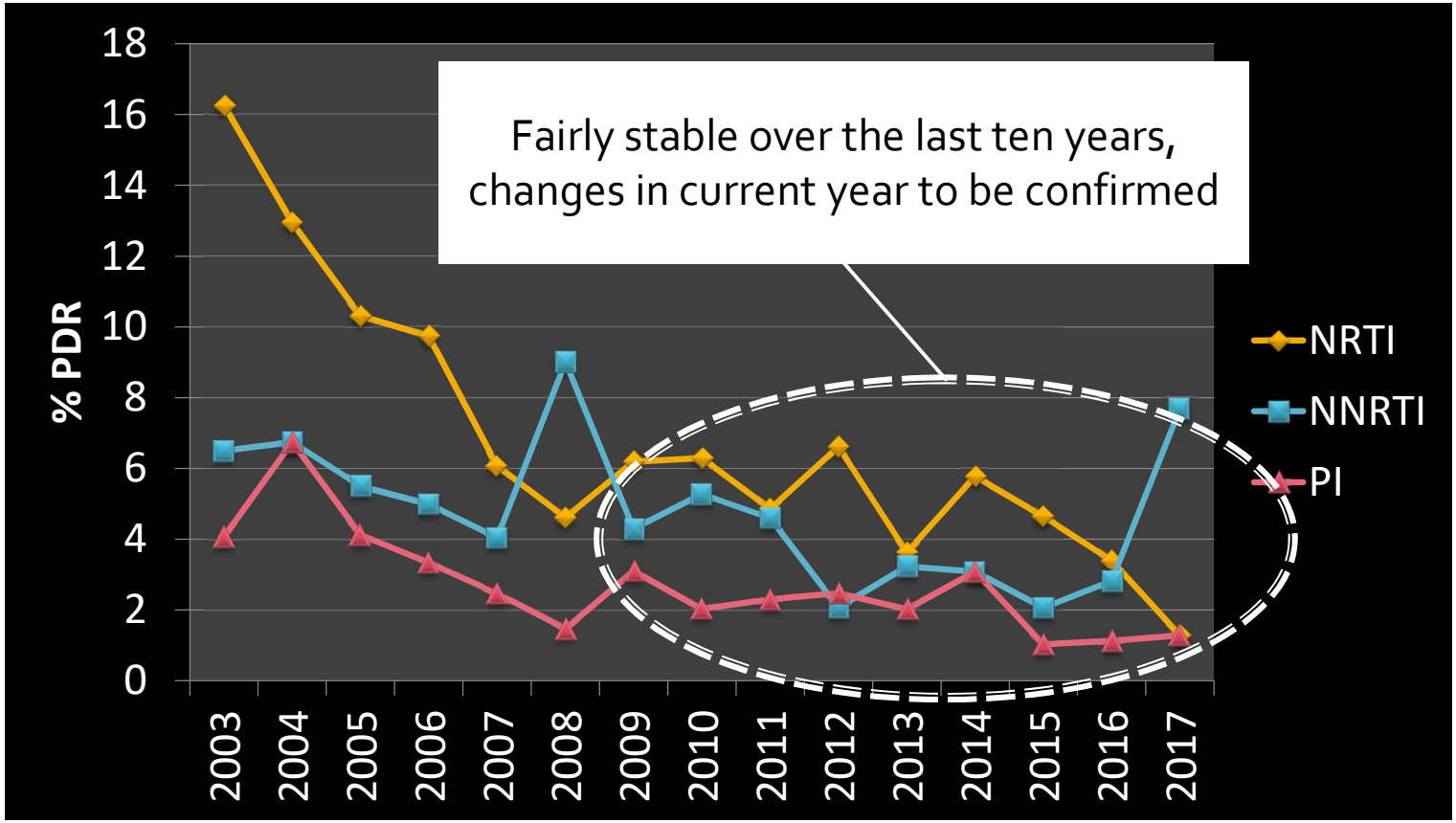
HIV pre-treatment drug resistance in Italy



Based on 4453 PR/RT sequences from drug naïve individuals

ARCA, accessed 06/10/2017

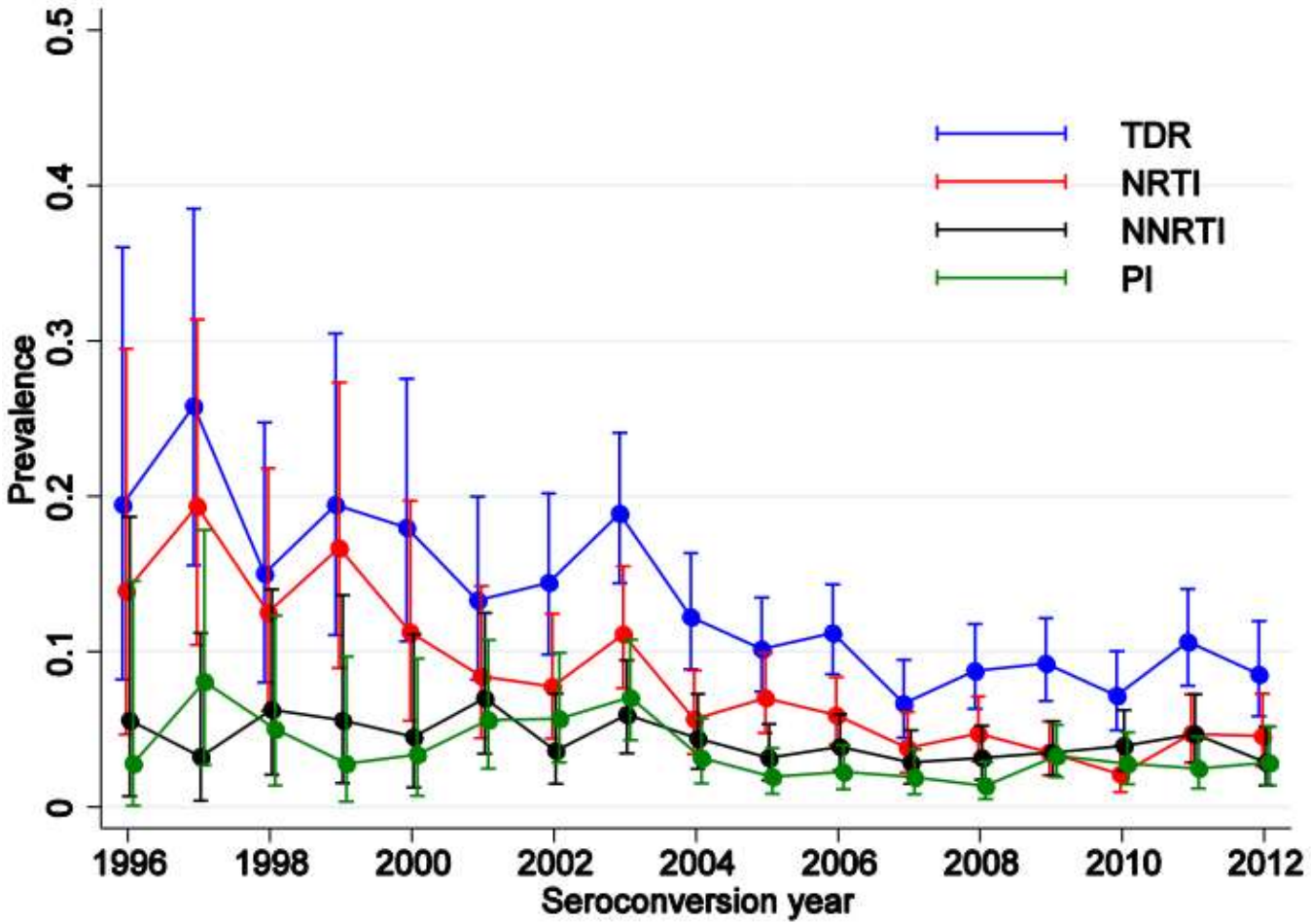
HIV pre-treatment drug resistance in Italy



Based on 4453 PR/RT sequences from drug naïve individuals

ARCA, accessed 06/10/2017

HIV pre-treatment drug resistance in European seroconverters (CASCADE cohort)

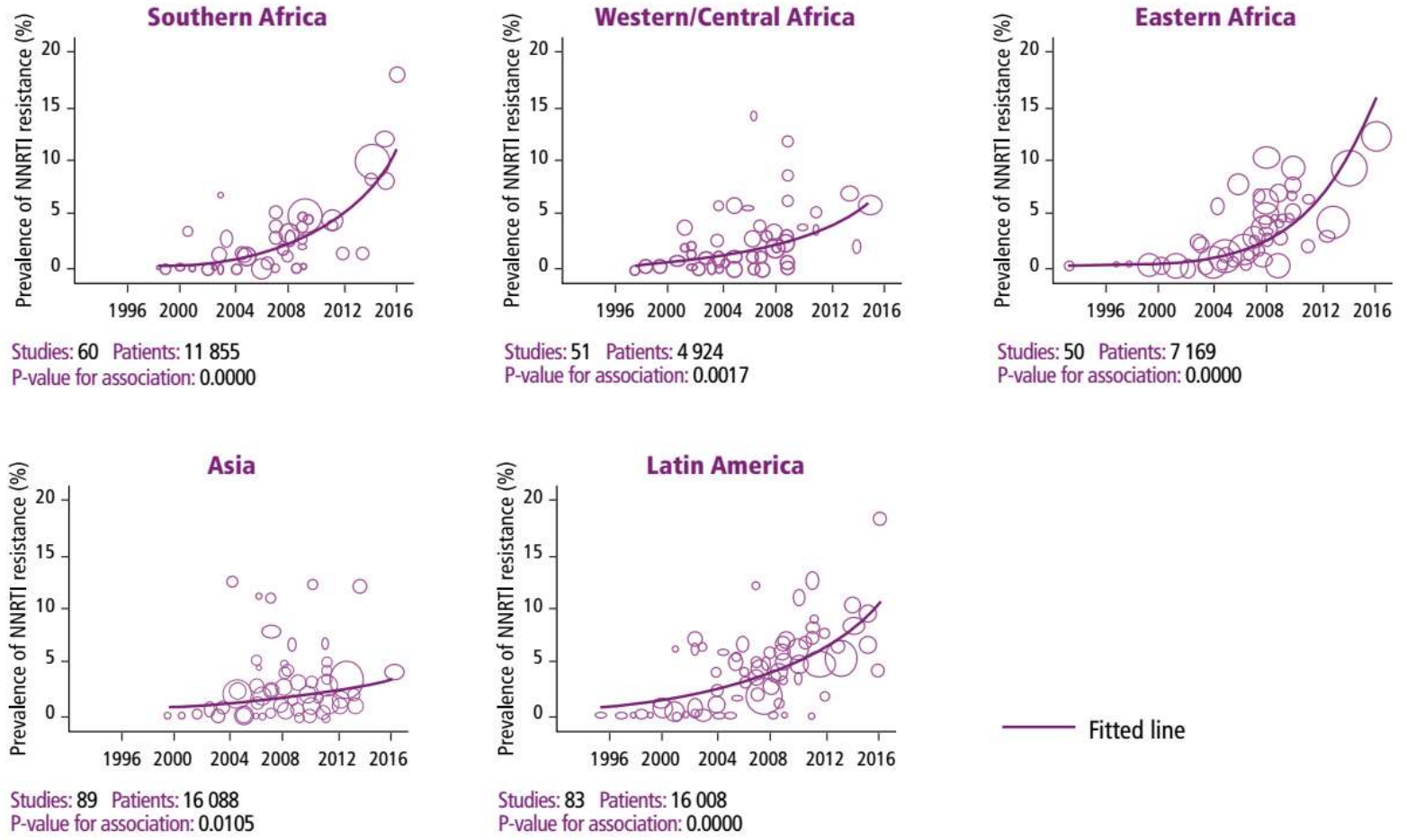


- 4717 individuals seroconverting between 1996-2012
- MSM 80%
- Subtype B 73%
- TDR prevalence significantly decreasing from 19.4% in 1996 to 8.5% in 2012

Olson, AIDS 2017

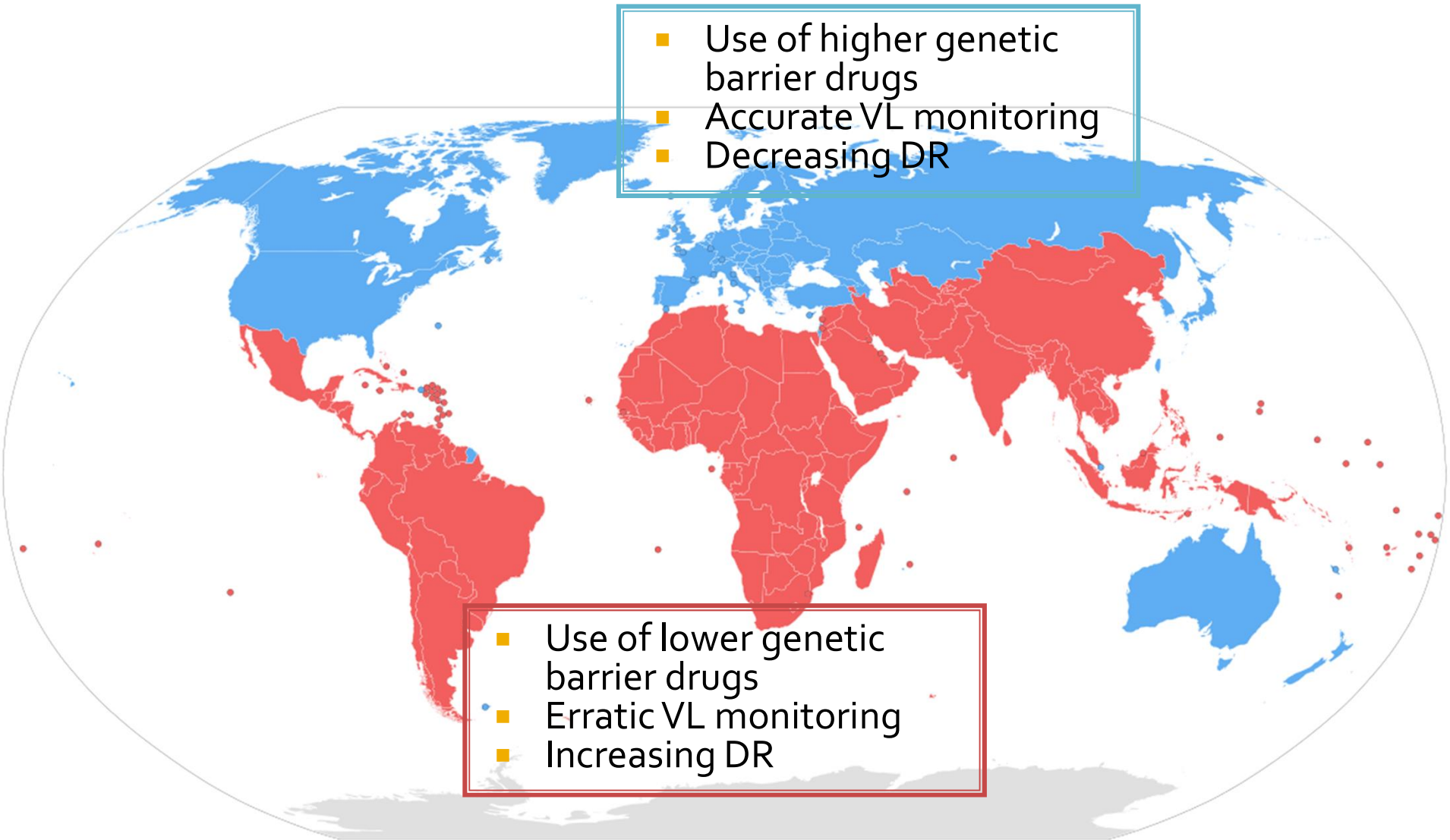
HIV PDR in low/middle income countries

Fig. 7: Prevalence of NNRTI pretreatment resistance by calendar year across studies included in the systematic review

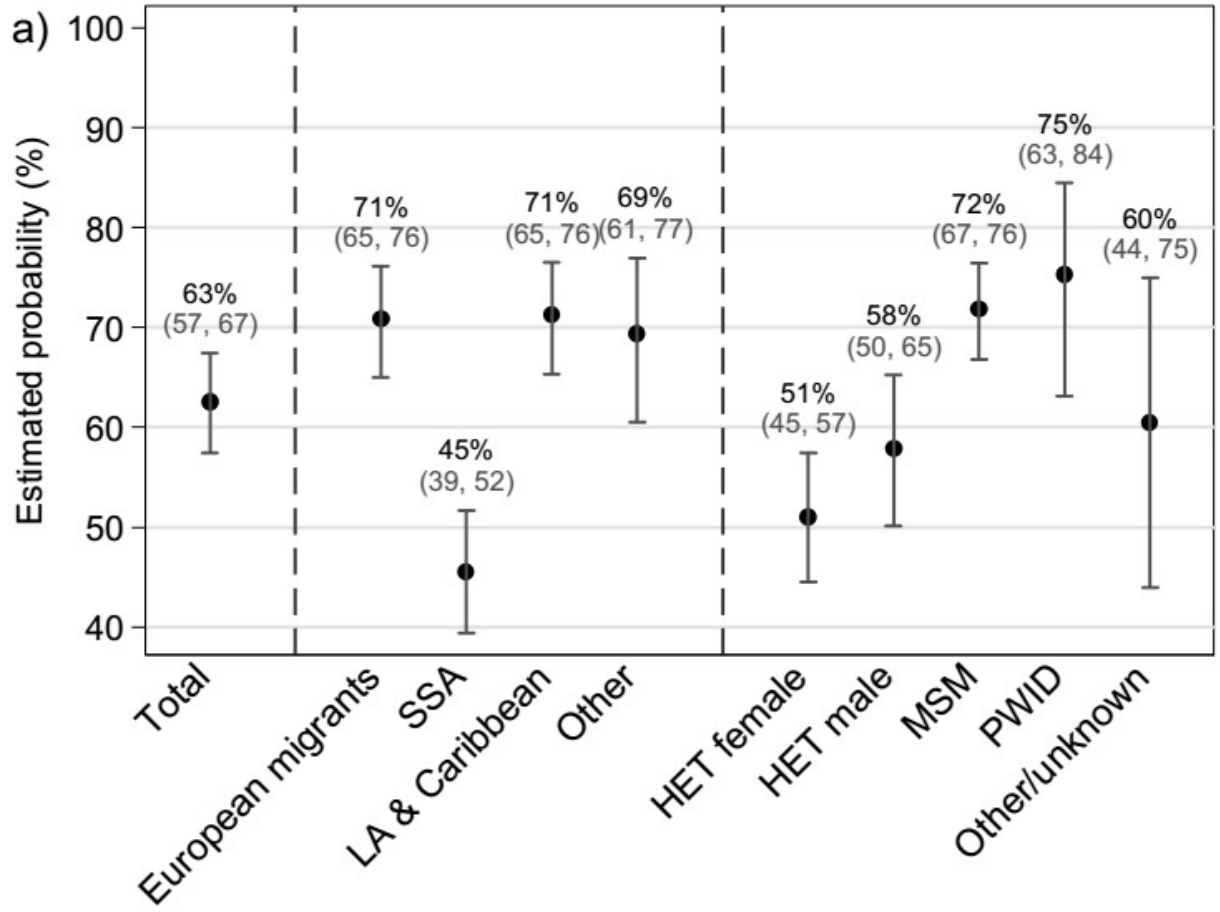


WHO HIV drug resistance report - 2017

HIV import from migrants and implications on PDR



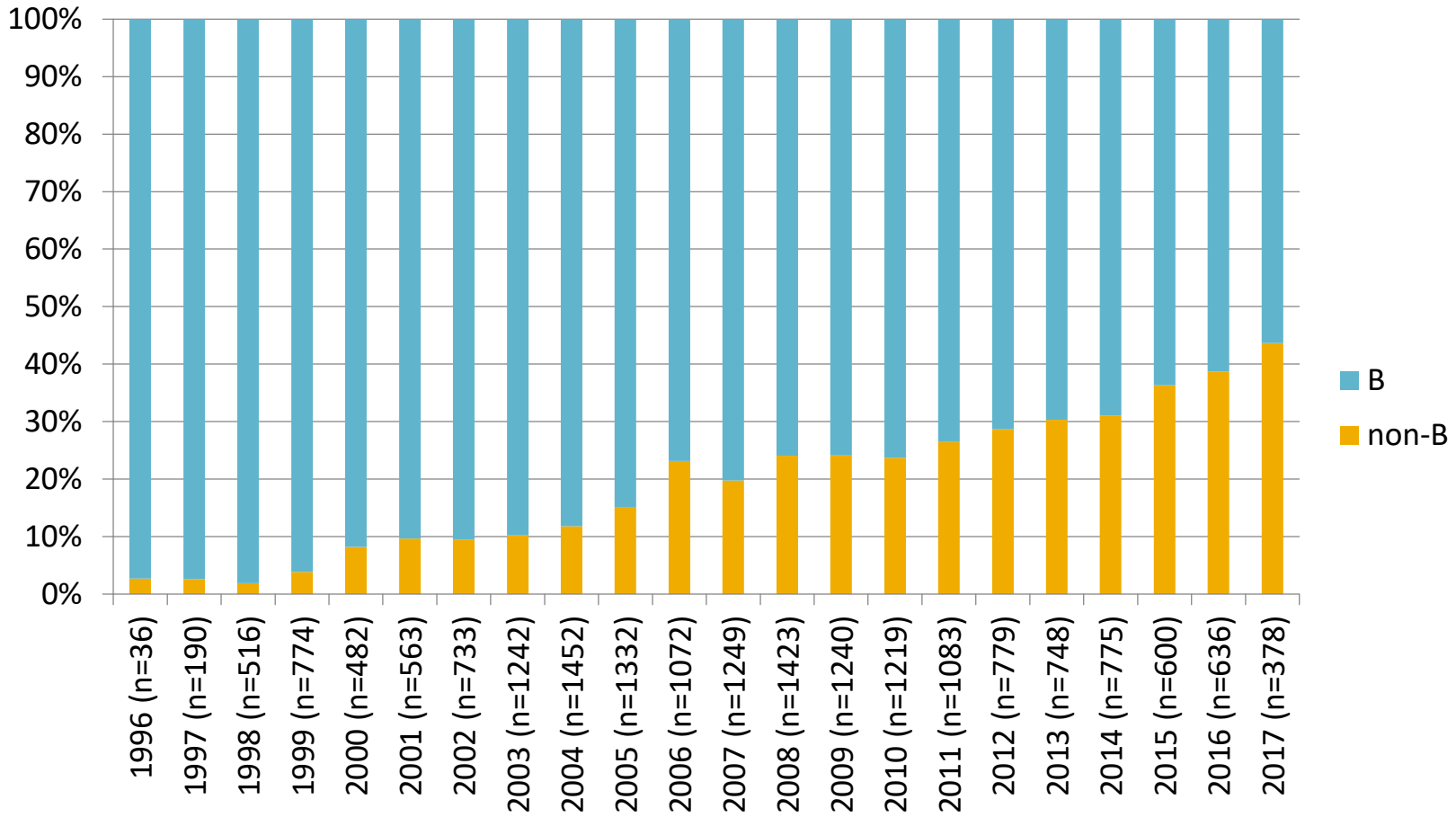
HIV acquisition in migrants



Estimated post-migration HIV acquisition probability (95% CI) by a) mode of transmission and geographical origin

Alvarez-del Arco, AIDS 2017

Time trends of B and non-B HIV subtypes in Italy



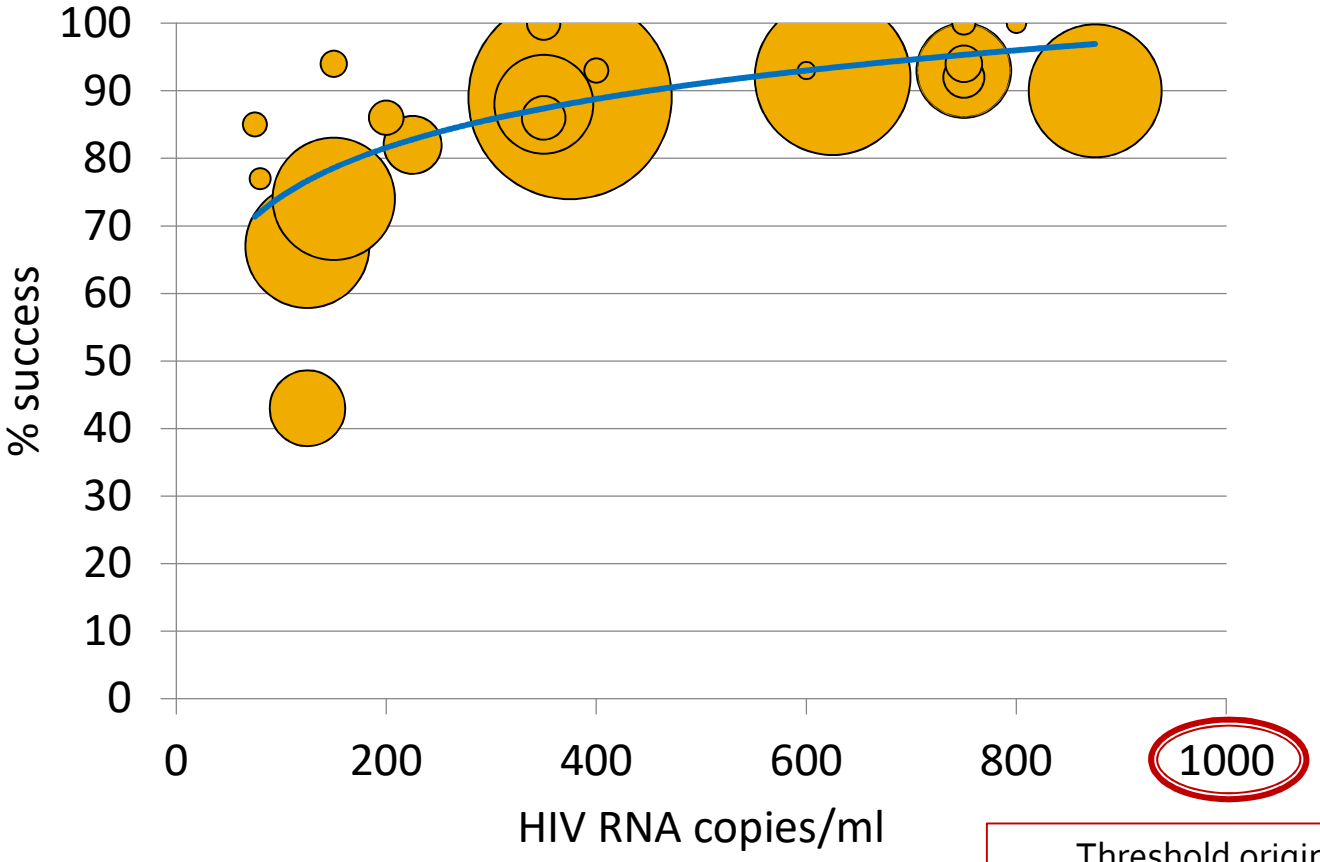
ARCA, accessed October 10, 2017; based on first sequence available per individual patient (n=23,588)

The evolution of GRT

HIV drug resistance testing

- The need
- The options
- A key component of ART success
- Evolution of HIV drug resistance testing
- The largest ever bug sequence collection

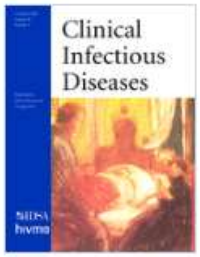
Drug resistance genotyping at low-level viremia



- Based on modified Viroseq or homebrew methods
- Overall success rate 84% with <1000 HIV RNA copies/ml
  - Mackie 2004
  - Balestrieri 2010
  - Santoro 2014
  - Gonzalez-Serna 2014
  - Bruzzone 2014
  - Armenia 2015

Threshold originally recommended to ask for drug resistance test

Drug resistance can be detected at low-level viremia



Volume 39, Issue 7
1 October 2004

Genotypic Resistance in HIV-1–Infected Patients with Persistently Detectable Low-Level Viremia while Receiving Highly Active Antiretroviral Therapy

Richard E. Nettles,[1] Tara L. Kieffer,[1] Rachel P. Simmons,[1] Joseph Cofrancesco, Jr.,[1] Richard D. Moore,[1] Joel E. Gallant,[1] Deborah Persaud,[2] and Robert F. Siliciano[1,3]

Departments of [1]Medicine and [2]Pediatrics, Johns Hopkins University School of Medicine, and [3]Howard Hughes Medical Institute, Baltimore, Maryland

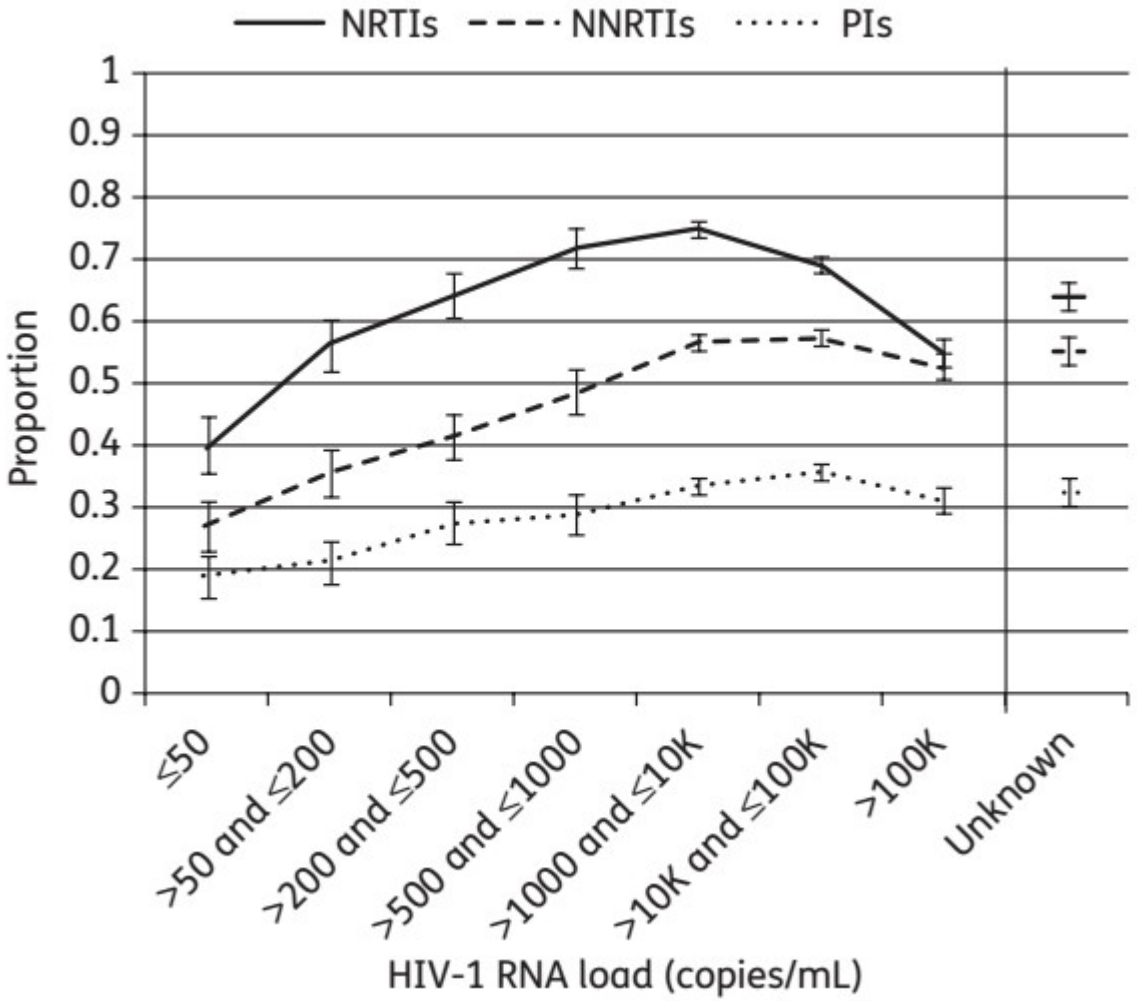
Background. Technical limitations in the sensitivity of commercial genotyping methods may prevent clinicians from determining whether drug-resistant human immunodeficiency virus type 1 (HIV-1) is present in patients with low-level viremia. We performed ultrasensitive HIV-1 genotyping for patients with persistent plasma virus loads of 50–400 copies/mL to better define the prevalence of drug resistance and the most common resistance mutations during persistently detectable low-level viremia.

Methods. Genotyping of HIV-1 was performed with an ultrasensitive clonal genotyping method.

Results. We studied 21 patients who had persistent, detectable, low-level viremia for a median of 11 months. Nine (43%) of 21 patients had HIV-1 isolates with significant resistance mutations. The most common mutations were M184V, K65R, and M41L/T215Y.

Conclusions. The finding that clinically significant resistance mutations were present in some but not all patients with persistent viremia (range, 50–400 copies/mL) highlights the need to improve the sensitivity of current clinical assays for detection of drug resistance.

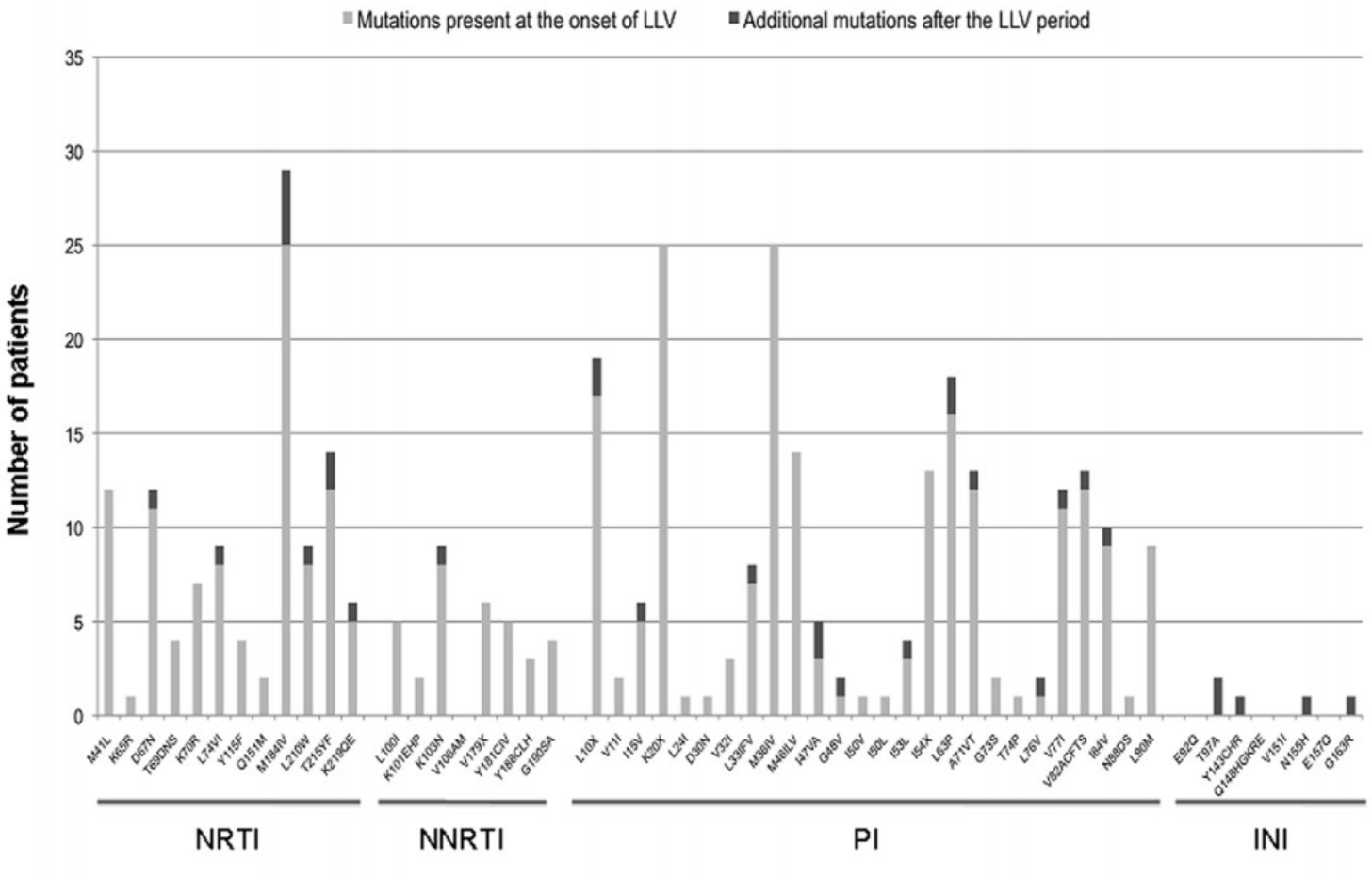
Drug resistance can be detected at low-level viremia



- SEHERE consortium (I, UK, P, D, B, E, S)
- 16,511 PR/RT sequences from 11,492 treatment-experienced patients
- 2,500/16,511 (15.14%) test results were obtained at a viral load <1,000 copies/mL

Prosperi, JAC 2011

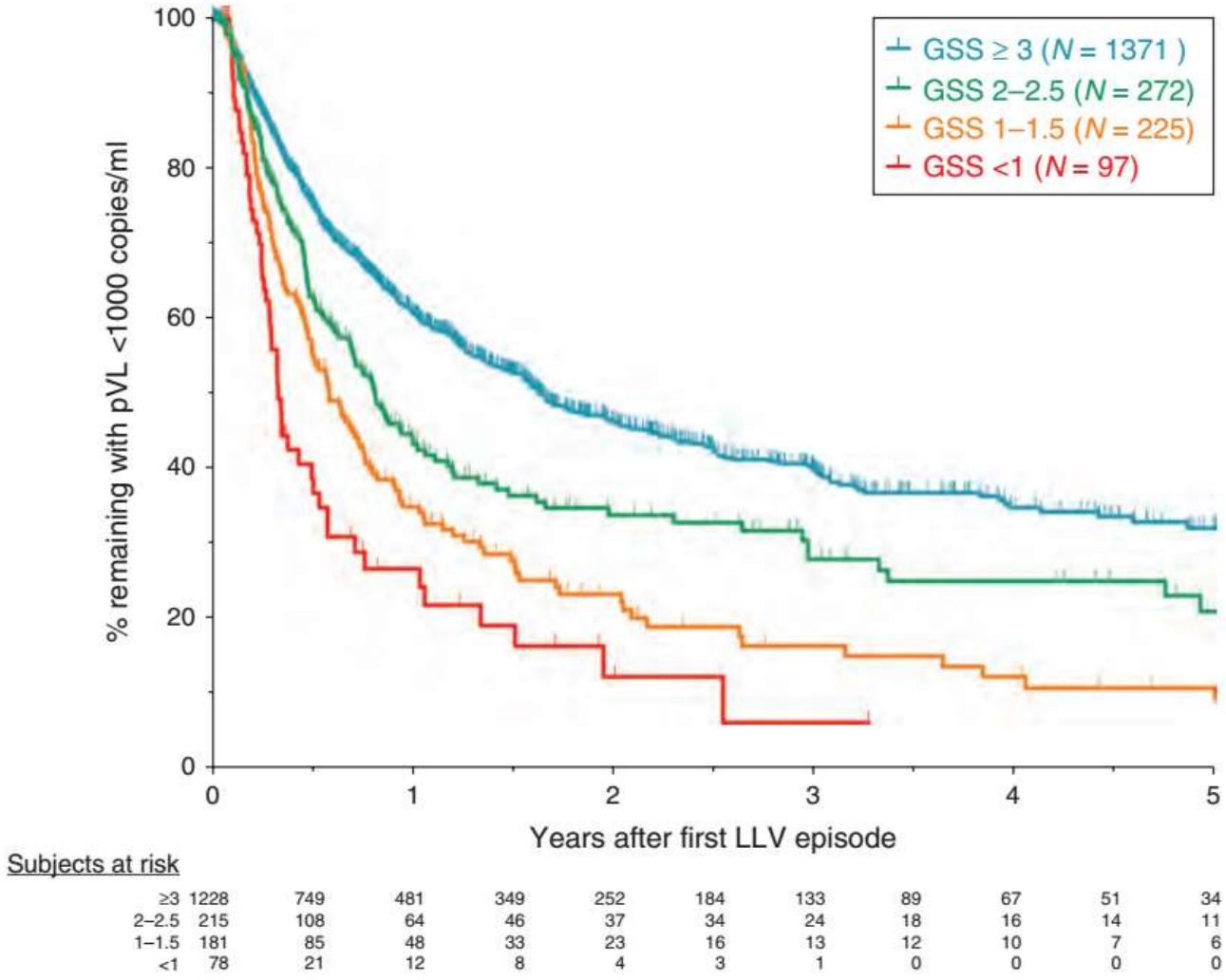
Drug resistance can emerge during persistent low-level viremia



- 48 patients (4 naive and 44 pretreated) with LLV episode with a median duration of 11 months
- Successful resistance testing at both onset and end of the LLV episode obtained for 37 patients (77%)
- 11 (30%) acquired at least 1 DRAM during the LLV period: for NRTI in 6, for NNRTI in 1, for PI in 4, and for raltegravir in 2

Delaugerre, PLoS ONE 2012

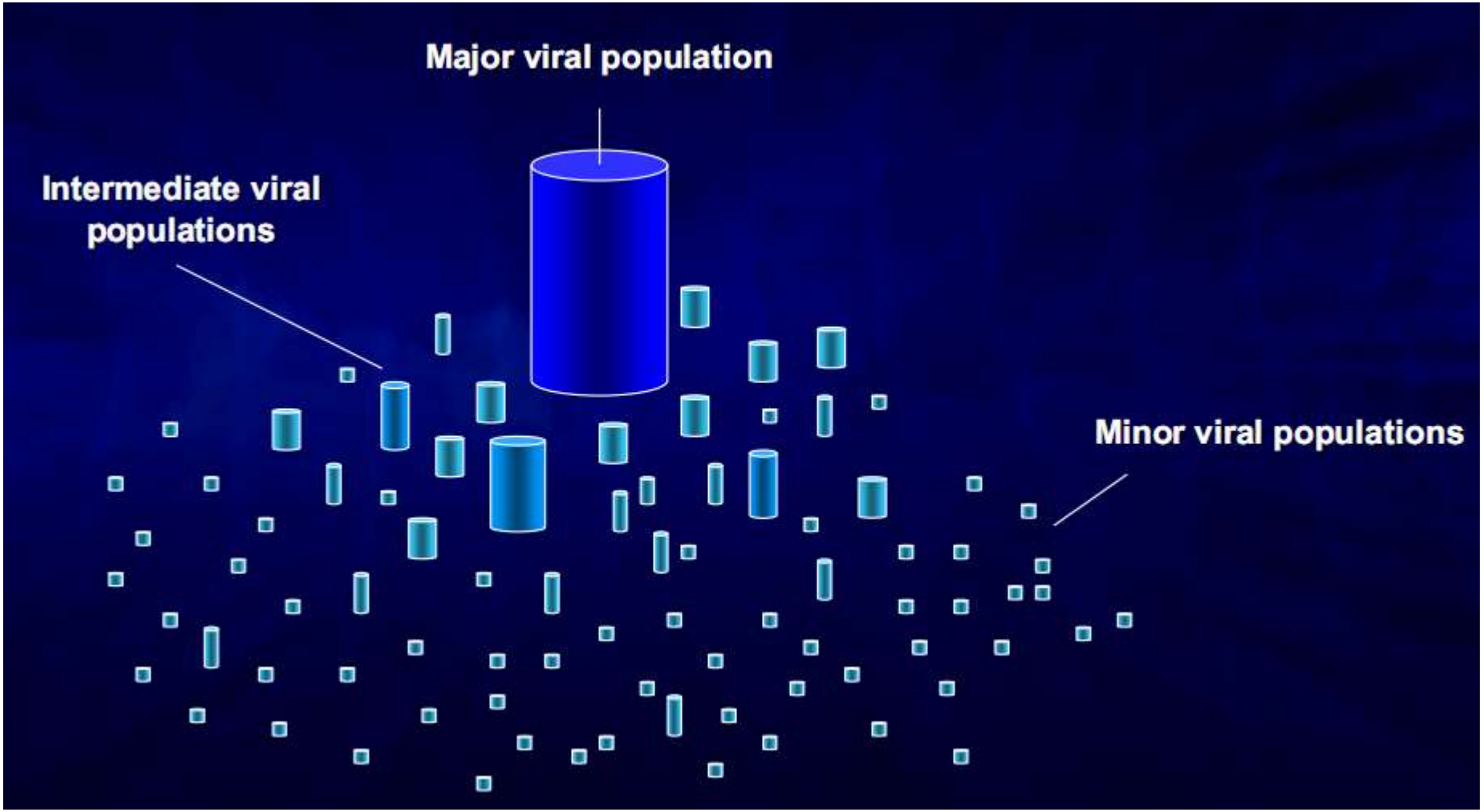
Drug resistance at low-level viremia predicts virological failure



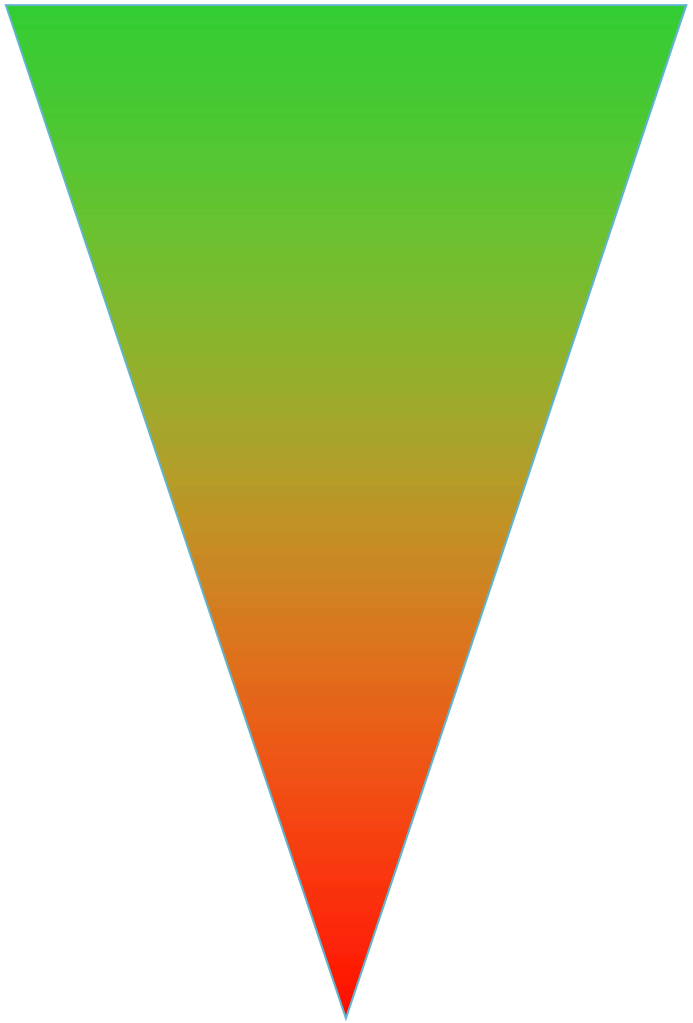
- LLV defined as <1000 copies/mL
- 1702 patients with follow-up on constant therapy eligible for analysis
- 'dose-dependent' increase in the hazard ratio for virologic failure with susceptibility categories at LLV (<1000 copies/ml)

Swenson, AIDS 2014

HIV replicates as quasispecies



Detectability of drug-resistant variants
Available methods



>25% of virus population

Standard population sequencing

>5% of virus population

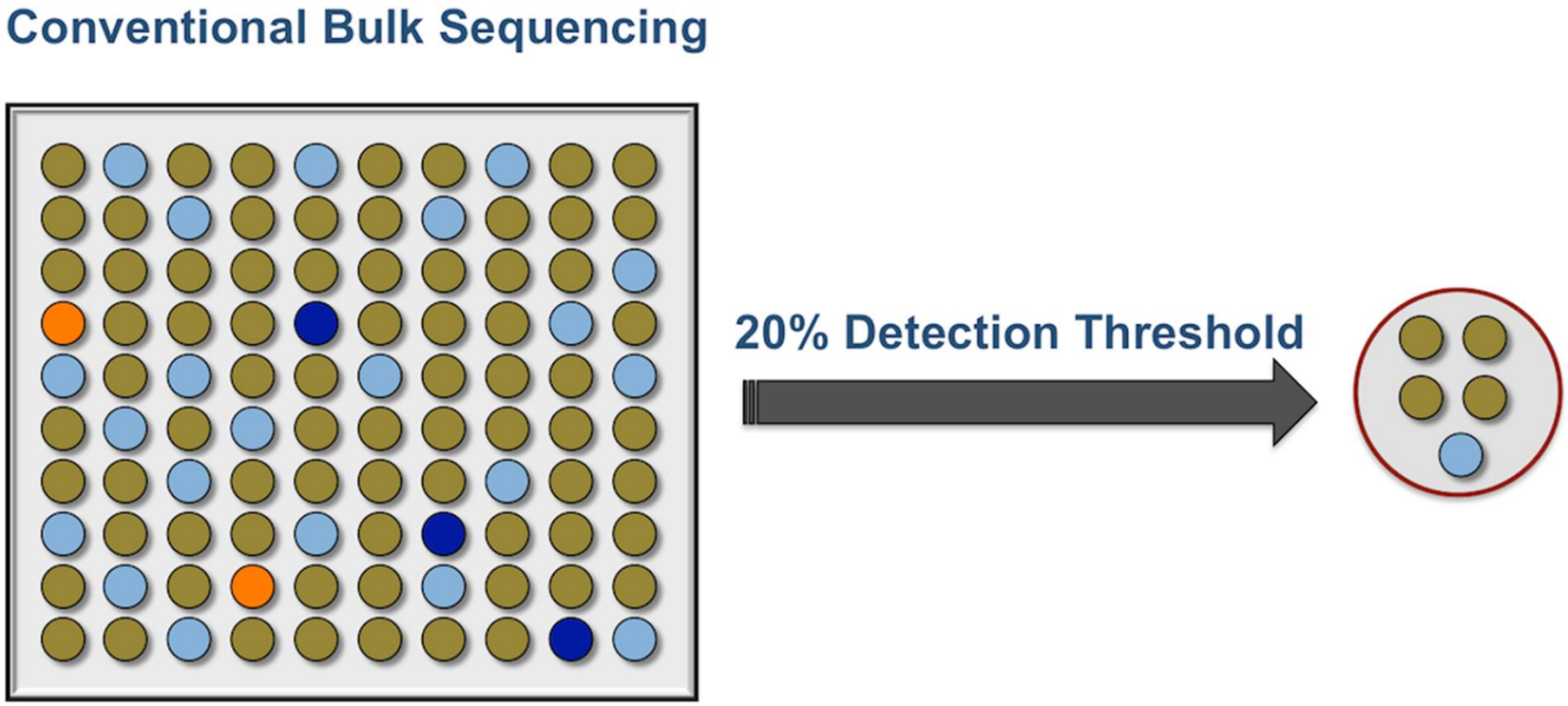
Multiple clones or “single genome sequencing”

>0.1% of virus population

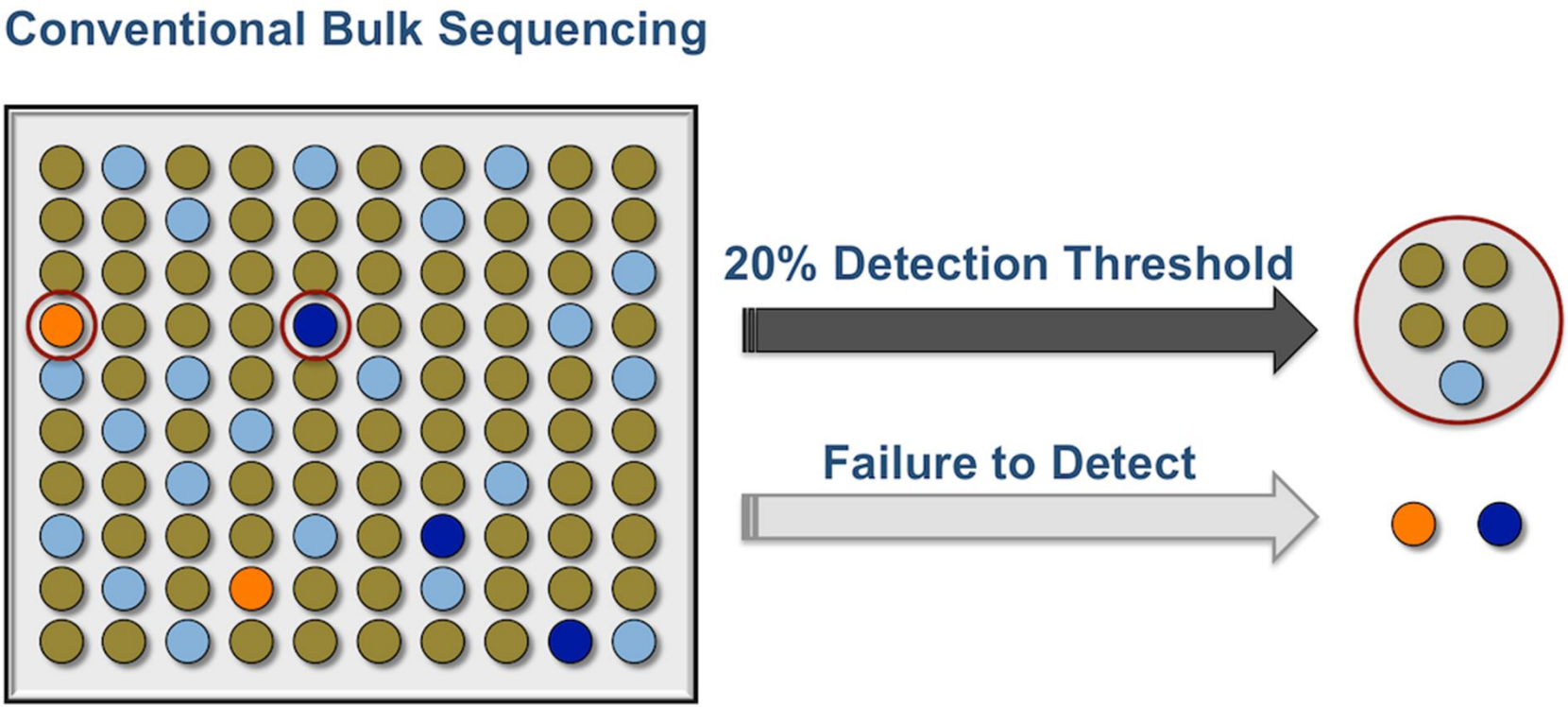
Point mutation assays (apply to defined mutations)

Next generation sequencing

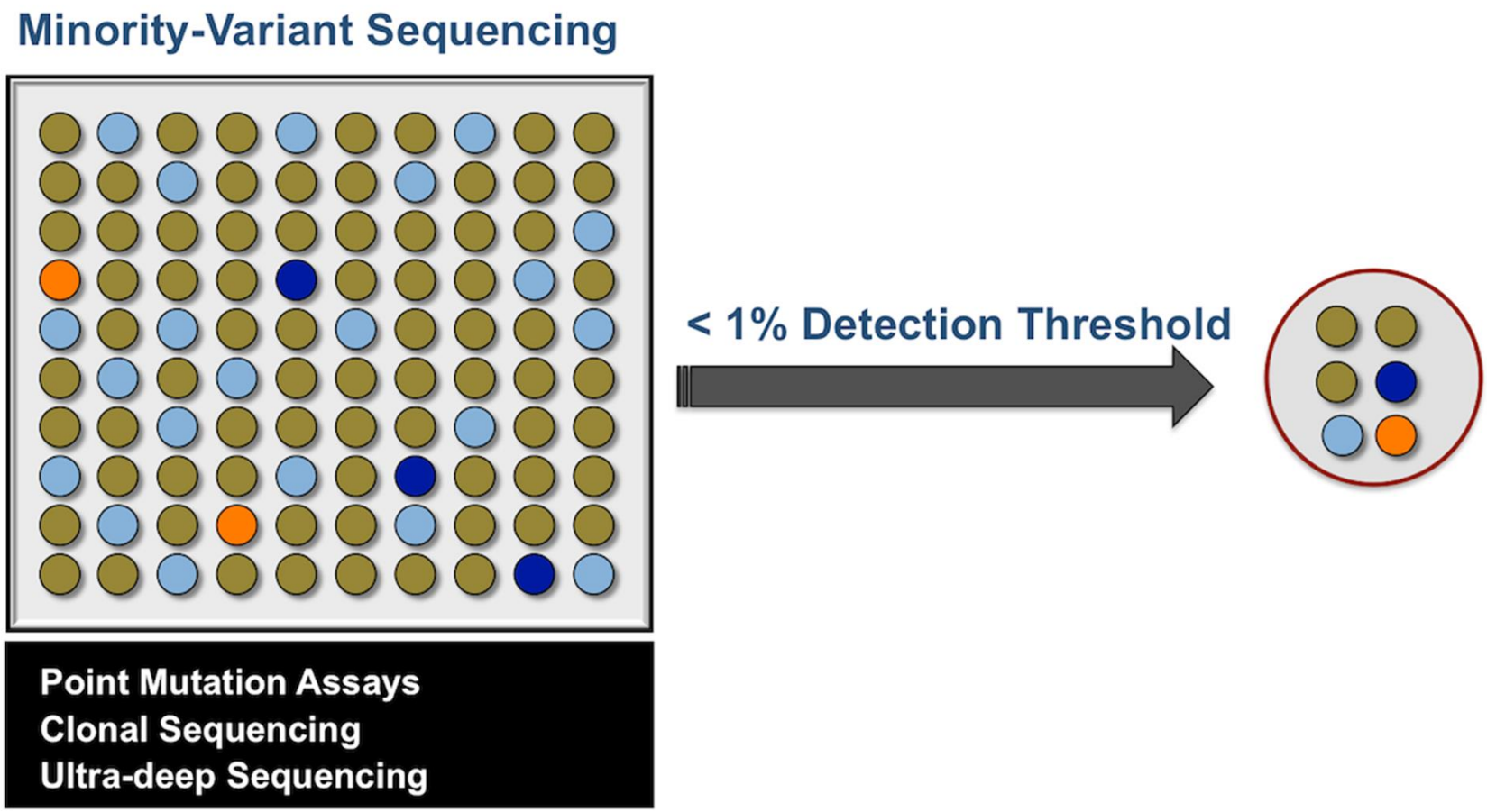
Sensitivity of genotypic HIV drug resistance testing



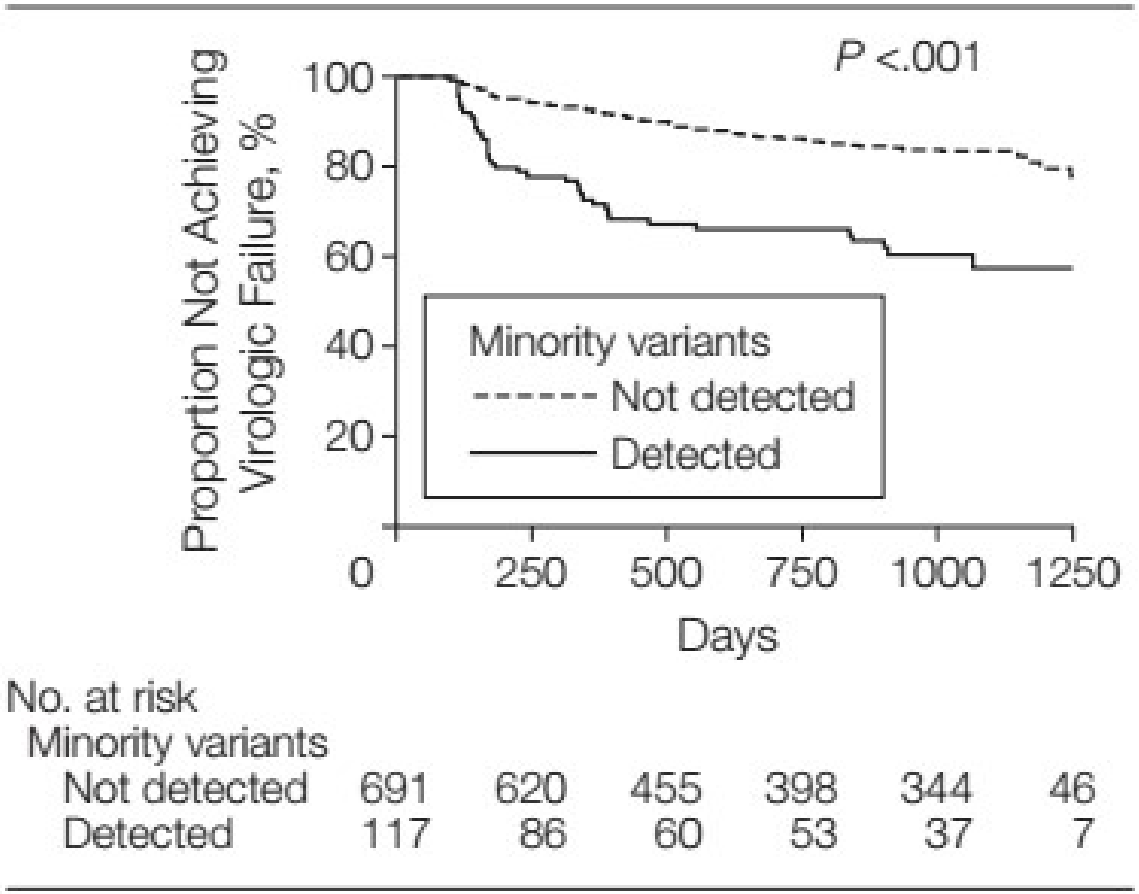
Sensitivity of genotypic HIV drug resistance testing



Sensitivity of genotypic HIV drug resistance testing



Low-Frequency HIV-1 Drug Resistance Mutations and Risk of NNRTI-Based Antiretroviral Treatment Failure – *A systematic review and pooled analysis*

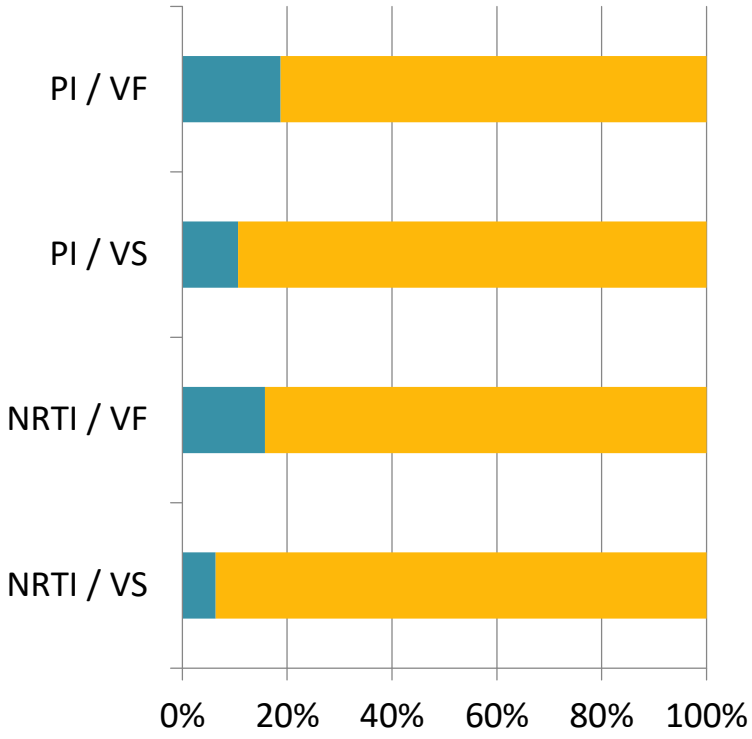


Kaplan-Meier Curves for Proportion of Patients Without Virologic Failure by Presence of Drug-Resistant HIV-1 Minority Variants

- 10 studies, 985 patients, 187 with minority drug resistance mutations (mDRMs)
- mDRMs associated with an increased risk of virologic failure (HR = 2.3; 95% CI 1.7-3.3; P<.001) after controlling for medication adherence, race/ethnicity, baseline CD4 cell count, and plasma HIV-1 RNA levels
- **Risk most strongly associated with NNRTI mDRMs**
- Dose-dependent increased risk of virologic failure found in participants with a higher proportion or quantity of mDRMs

Li, JAMA 2011

No impact of HIV-1 protease minority resistant variants on the virological response to a first-line PI-based regimen containing darunavir or atazanavir

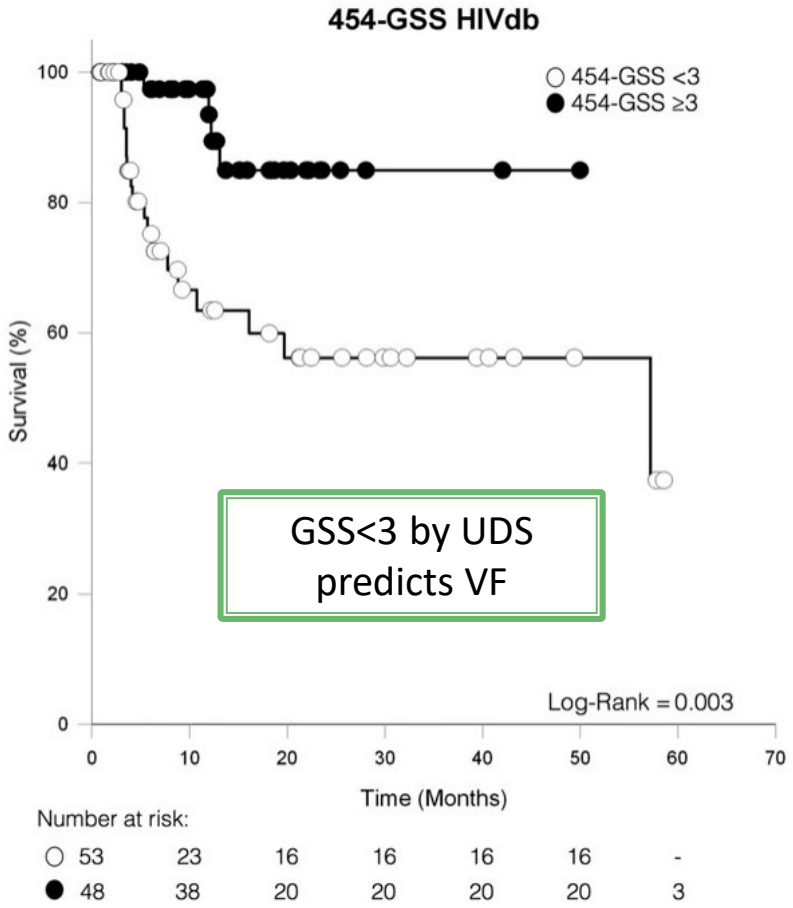


- ART-naive patients initiating a first-line regimen including two NRTI and DRV/r (n = 94) or ATV/r (n = 16), Jan 2012 - Mar 2015
- Ultra-deep sequencing (Illumina)
- Virological failure occurring in 13 patients (13.8%) with DRV and 3 (18.8%) with ATV
- 13 (11.8%) subjects had PI MRV at baseline in the median proportion of 1.3% (IQR = 1.1–1.7)
- The most prevalent PI MRV were G73C (n = 5) and M46I (n = 3)

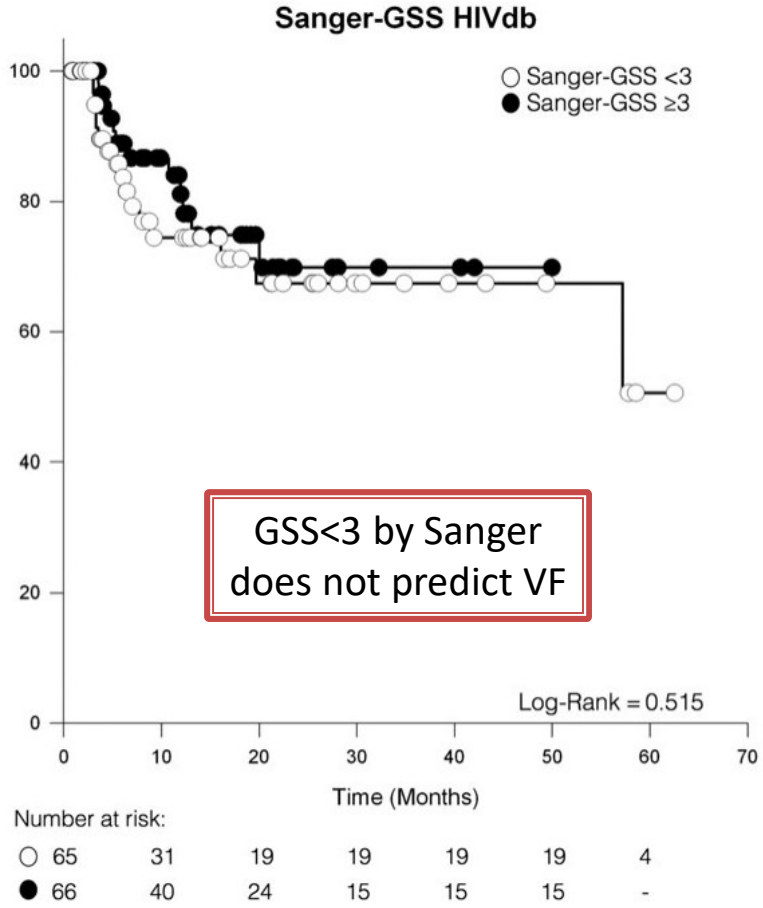
Perrier, JAC 2017

Improved Prediction of Salvage Antiretroviral Therapy Outcomes Using Ultrasensitive HIV-1 Drug Resistance Testing

132 subjects starting salvage therapy with at least one among PI/r, RAL, ETR; 28 (21%) developed VF.



GSS<3 by UDS predicts VF



GSS<3 by Sanger does not predict VF

Pou, CID 2014

Next-Generation Sequencing to Help Monitor Patients Infected with HIV: Ready for Clinical Use?

- **Evidence supporting clinical utility (slowly) increasing**
  - Drug resistance in untreated patients
  - Choice of salvage regimen in highly treatment experienced patients
  - Coreceptor tropism assay for treatment with CCR5 antagonists
- **Barriers to widespread use remaining**
  - Cost of NGS instrumentation plus data analysis and storage solutions
  - Technical expertise required (platforms evolving or superseded rapidly)
  - No clinical trial testing NGS available
  - No IVD approval yet

Gibson, Curr Infect Dis Rep 2014

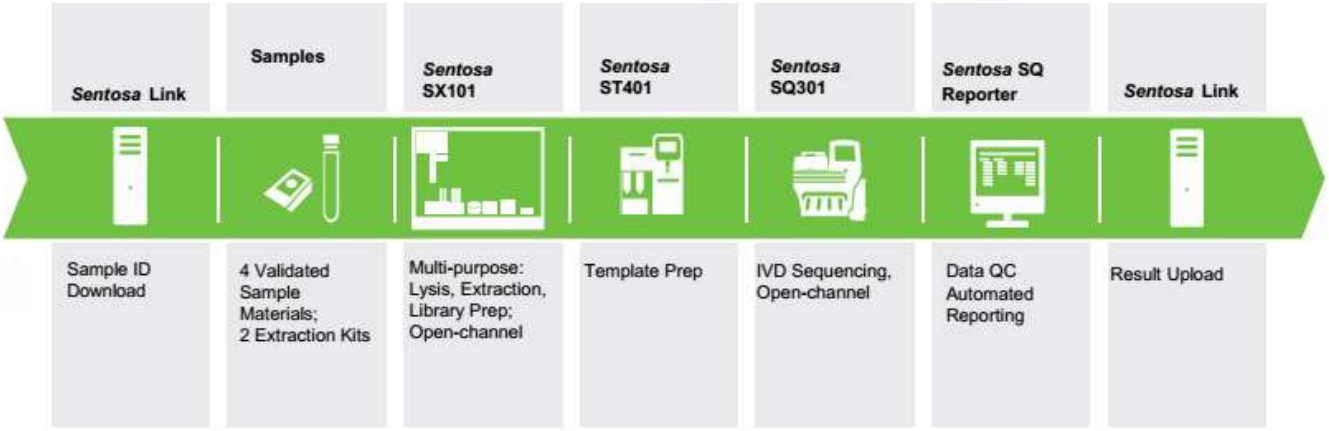
Next-Generation Sequencing to Help Monitor Patients Infected with HIV: Ready for Clinical Use?

Vela Next Generation Sequencing

NGS automation for the IVD routine laboratory

VELA DIAGNOSTICS

CE-IVD marked on Aug 21, 2017

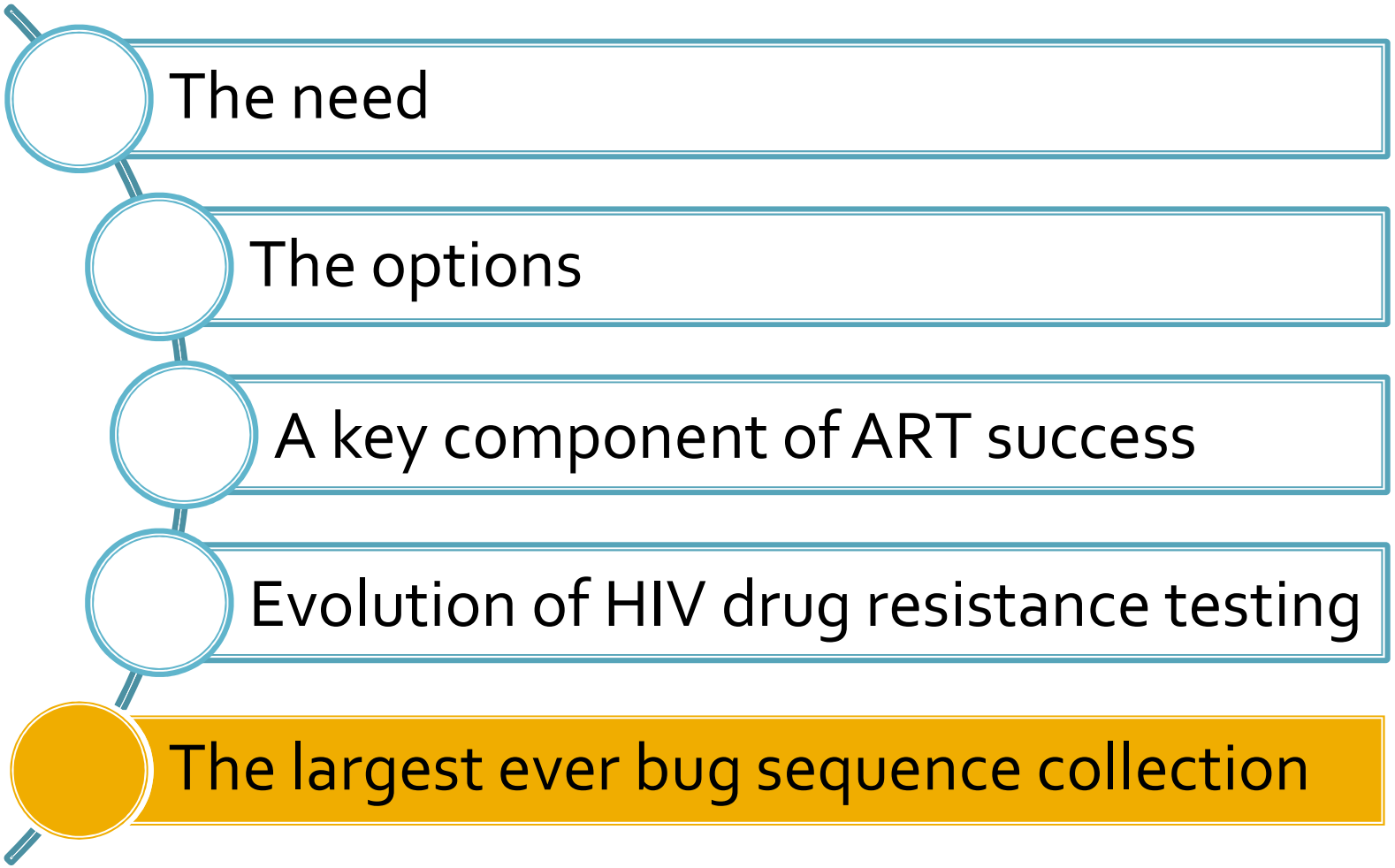


Key Features

- **CE-IVD**
- Ready to use in 2 weeks from installation
- IT Connectivity
- Sample Tracking
- Control System
- Automation
- Ready to use Reagents
- Actionable Design
- Data Analysis
- Data Reporting

Opportunities from the largest ever bug sequence collection

HIV drug resistance testing



Massive use of HIV drug resistance genotyping in the clinic

- Thousands HIV sequences generated in each country
- Huge international databases
  - E.g. Stanford, RDI, EuResist
- Opportunity for novel analysis
  - Phylodynamics, phylogeography, cluster analysis

Challenges from huge HIV (drug resistance) sequence databases

ARTICLE

Received 17 Jan 2011 | Accepted 21 Apr 2011 | Published 24 May 2011

nature COMMUNICATIONS

A novel methodology for large-scale phylogeny partition

Mattia C.F. Prosperi[1,2,3], Massimo Ciccozzi[4], Iuri Fanti[1], Francesco Saladini[5], Monica Pecorari[6], Vanni Borghi[7], Simona Di Giambenedetto[1], Bianca Bruzzone[8], Amedeo Capetti[9], Angela Vivarelli[10], Stefano Rusconi[11], Maria Carla Re[12], Maria Rita Gismondo[13], Laura Sighinolfi[14], Rebecca R. Gray[2,3], Marco Salemi[2,3], Maurizio Zazzi[5] & Andrea De Luca[1,15] on behalf of the ARCA collaborative group

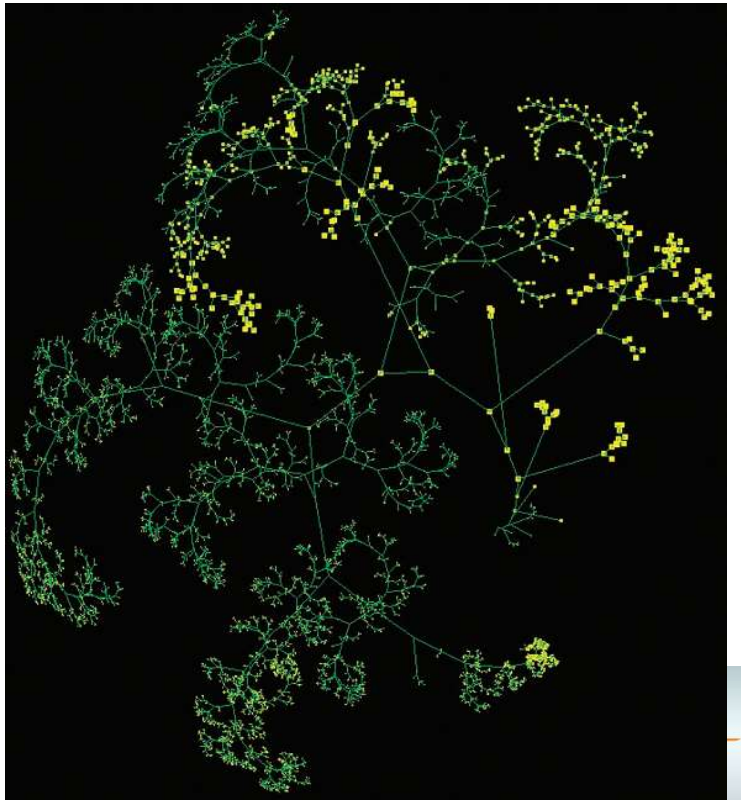


Figure 2 | Phylogeny of Italian HIV-1 subtype B *pol* isolates. Maximum-likelihood phylogenetic tree of 11,541 HIV-1 subtype B *pol* gene sequences from the Italian ARCA cohort. Tree is rooted on subtype J and depicted using three-dimensional hyperbolic geometry. Nodes and leaves are highlighted by yellow points.

Opportunities from huge HIV (drug resistance) sequence databases

Retrovirology

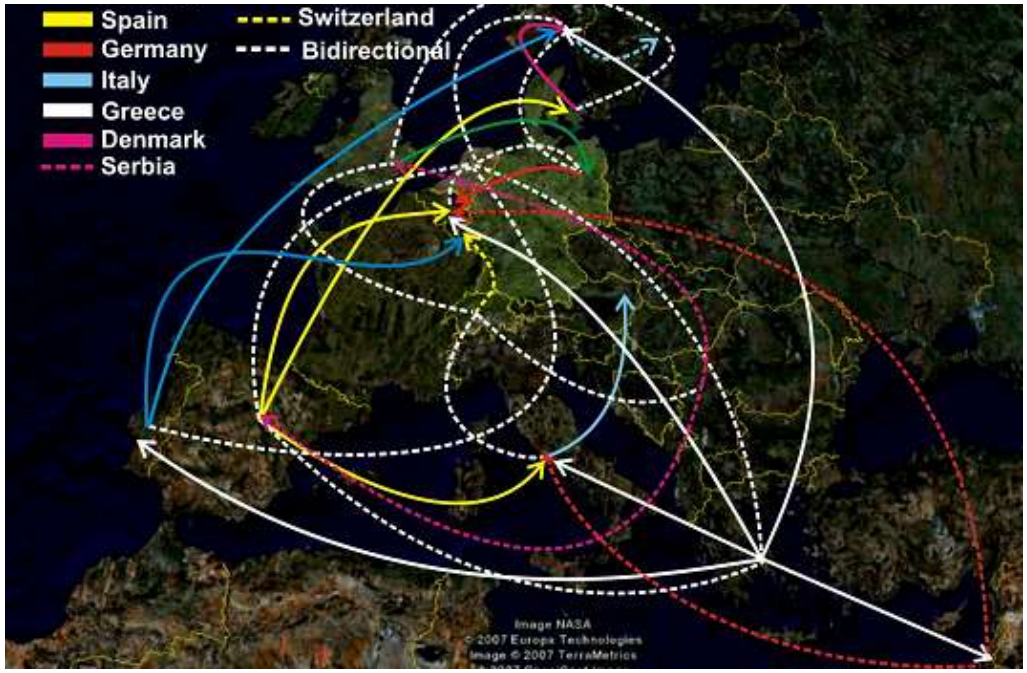
BioMed Central

Research

Open Access

Tracing the HIV-1 subtype B mobility in Europe: a phylogeographic approach

Dimitrios Paraskevis*[1,2], Oliver Pybus[3], Gkikas Magiorkinis[2], Angelos Hatzakis[2], Annemarie MJ Wensing[4], David A van de Vijver[5], Jan Albert[6,7], Guiseppe Angarano[8], Birgitta Åsjö[9], Claudia Balotta[10], Enzo Boeri[11], Ricardo Camacho[12], Marie-Laure Chaix[13], Suzie Coughlan[14], Dominique Costagliola[15], Andrea De Luca[16], Carmen de Mendoza[17], Inge Derdelinckx[18], Zehava Grossman[19], Osama Hamouda[20], IM Hoepelman[21], Andrzej Horban[22], Klaus Korn[23], Claudia Kücherer[20], Thomas Leitner[6,7], Clive Loveday[24], Eilidh MacRae[25], I Maljkovic-Berry[6,7], Laurence Meyer[25], Claus Nielsen[26], Eline LM Op de Coul[27], Vidar Ormaasen[28], Luc Perrin[29], Elisabeth Puchhammer-Stöckl[30], Lidia Ruiz[31], Mika O Salminen[32], Jean-Claude Schmit[33], Rob Schuurman[4], Vincent Soriano[17], J Stanczak[22], Maja Stanojevic[34], Daniel Struck[33], Kristel Van Laethem[1], M Violin[10], Sabine Yerly[29], Maurizio Zazzi[35], Charles A Boucher[4,5], Anne-Mieke Vandamme[1] for the SPREAD Programme



Retrovirology 2009, **6**:49

Mapping HIV TDR in Europe

European Society for TRANSLATIONAL ANTIVIRAL RESEARCH

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Subtype distribution

Prevalence of TDR

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username:

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(support)

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ESAR - SPREAD

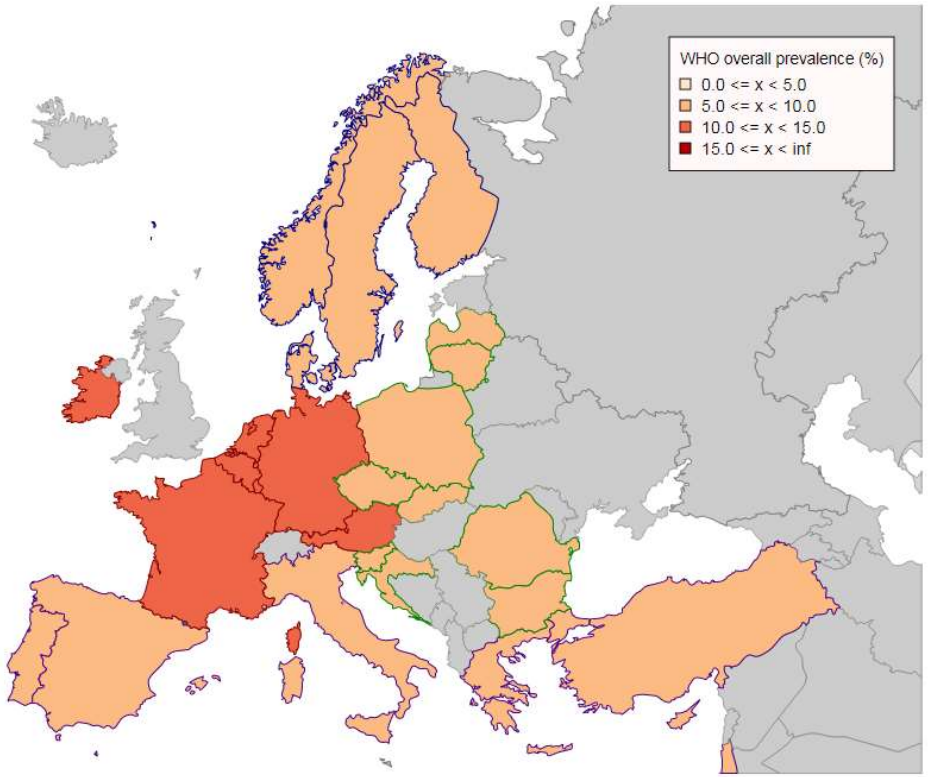
Prevalence of TDR

Route of transmission
- All
- HSX
- MSM
- IDU
- OTHER
- UNK

Gender
- All
- Male
- Female

Year of HIV diagnosis
- All
- 2000-2005
- 2006-2007
- 2008-2010

TDR
- mutations associated with resistance to NRTIs, NNRTIs or PIs
- mutations associated with resistance to PIs
- mutations associated with resistance to NRTIs
- mutations associated with resistance to NNRTIs



Mapping HIV subtype distribution in Europe

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ESAR - SPREAD

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- [ ] Male
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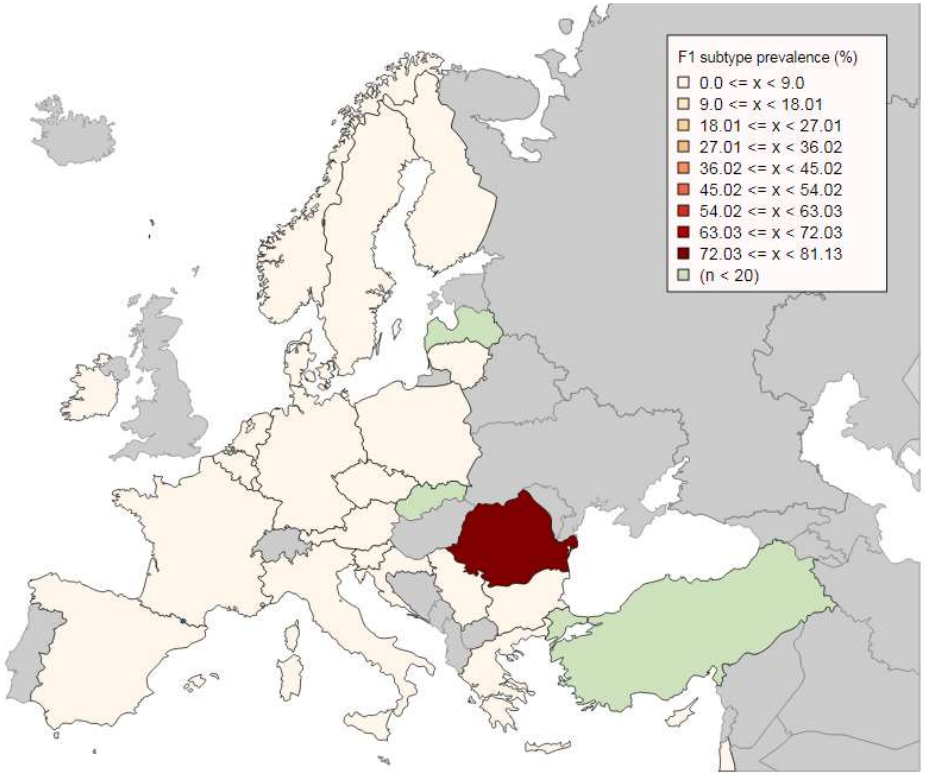
- [ ] All
- [ ] 2000-2005
- [ ] 2006-2007
- [x] 2008-2010

Subtype
B
non-B
A1
C
02_AG
01_AE
F1

submit



Conclusions

Lessons learned: antiretroviral drug resistance testing…

- …has guided **drug development** and shaped the success of antiretroviral therapy
- …has been the first example of integration of a sophisticated molecular assay into **clinical practice** in the infectious diseases domain
- …has generated a huge HIV sequence repositories allowing to track HIV evolution and inform **surveillance and education programs**