Diagnostic and biotechnologies for HIV management in resource-poor settings

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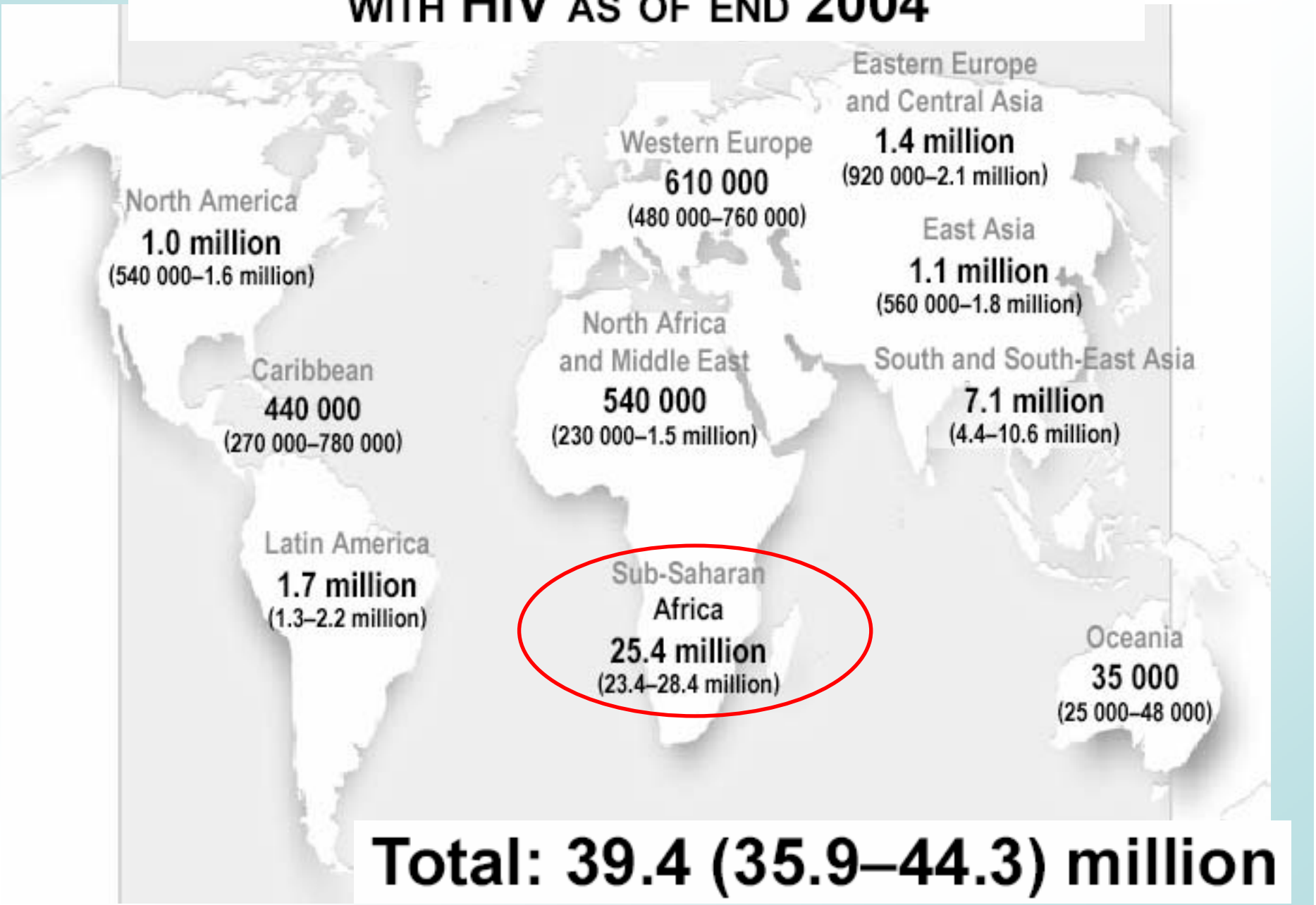
The target of treating 3 million people in developing countries with antiretroviral therapy by the end of 2005 is a necessary, achievable target on the way to the ultimate goal of universal access to antiretroviral treatment for everyone who requires it.

ADULTS AND CHILDREN ESTIMATED TO BE LIVING WITH HIV AS OF END 2004



Total: 39.4 (35.9–44.3) million

-All these people will not take advantage from a prophylactic vaccine against HIV infection.

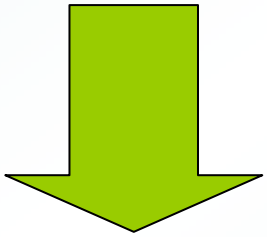
- Vaccines will not be available before 8-10 years

- The large majority will die within few years

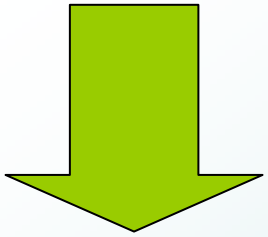
Can a low-profile containment be successful?

- If a low-profile containment of AIDS would have been done in western countries, today we would have millions of death in Europe and United States

- In developed countries:
  - Heavy investment on research



  - Discovery of diagnostic tools and antiviral therapies



  - Death rate dropped from about 100% to <5%

North America, Western and Central Europe

HIV and AIDS statistics and features, end of 2002 and 2004

	Adults and children living with HIV	Number of women living with HIV	Adults and children newly infected with HIV	Adult prevalence (%)	Adult and child deaths due to AIDS
2004	1.6 million [1.1–2.2 million]	420 000 [290 000–570 000]	64 000 [34 000–140,000]	0.4 [0.3–0.6]	23 000 [15 000–32 000]
2002	1.6 million [1.1–2.2 million]	390 000 [270 000–550 000]	62 000 [33 000–140 000]	0.4 [0.3–0.6]	22 000 [15 000–31 000]

- International guidelines of antiviral therapy for developed countries are remarkably different from those of developing countries:
  - **Developed countries**:
    - Triple therapy is the only rational and acceptable approach against the progression of HIV disease
    - Triple therapy must be accessible for all (free of charge in the majority of countries)
    - Result: great success, limited death rate

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  - **Developing countries**:
    - **Single or dual therapy is considered better than nothing**
    - **Give antiviral therapy whenever possible, with what is available**
    - **…….exactly what was done in Europe in early '90s (mono- or dual-therapy, followed by triple therapy when made available)**
      - **Results: No improvement of survival**
        - » **Massive development of resistance to antiviral drugs**
        - » **Rate of mortality far greater than in patients that started treatment directly with triple therapy**
  - **Should not we use the experience of Europe and US to avoid the same mistakes in developing countries?**

- International guidelines of antiviral therapy for developed countries are completely different from those of developing countries:

- **Developed countries**:
  - Assessment of viral load in plasma is considered mandatory during the course of disease
  - CD4 lymphocytes must be evaluated every 2-3 months, always
  - Viral load and CD4 lymphocytes are necessary for a correct monitoring of antiviral therapy

  - **Effect:** A rational approach to antiviral therapy, with three drugs, adjusted on the basis of the results of laboratory analysis (CD4 lymphocytes and viral load) is the accepted routine
  - **Results**: Low mortality, low progression of the disease, long-term success of antiviral therapy

- **Developing countries:**
  - **Viral load is not mandatory for starting and monitoring antiviral therapy.**
  - **CD4 lymphocytes should be counted (if possible).**
  - **Therapy is mainly given on the basis of clinical situation.**

- International guidelines of antiviral therapy for developed countries are remarkably different from those of developing countries:

- **Developed countries**: Prevention of mother-to-child transmission is granted by triple antiviral therapy + cesarean section + artificial formula feeding
  - The phylosophical approach is “save the child by saving the mother”
  - **Results**: Rare (or even no) cases of vertical transmission of HIV are reported in developed countries (in Italy <10 cases in the last 5 years)
  - Vertical transmission is almost “eradicated”

Public Health Service Task Force Recommendations for Use of Antiretroviral Drugs in Pregnant HIV-1-Infected Women for Maternal Health *and* Interventions to Reduce Perinatal HIV-1 Transmission in the United States

- **All pregnant women should be offered highly active antiretroviral therapy to maximally suppress viral replication, reduce the risk of perinatal transmission, and minimize the risk of development of resistant virus.**

- **Antiretroviral treatment is recommended for all pregnant women with viral load >1,000 copies/ml**

- **Beginning of treatment: week 10-12 of gestation**

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- **Developing countries:**
  - Mother-to-child transmission causes millions of infection each year, that accounts for millions of death in infant because of HIV infection.
  - The main approach is “try to save the child, no chance for the mother”
  - Prevention of mother-to-child transmission is made by using approaches considered heavily inappropriate in western countries
  - **Results**: Vertical transmission is only marginally affected
  - Millions of orphans have been generated by not treating mothers.
  - Children surviving HIV will die of starvation.

- Reasons for the differences between western and developing countries:
  - Therapy is expensive
    - A country without young productive people will be dismantled by poor economy, lack of food production, industry not served, services not granted, instruction abandoned
    - Therapy for AIDS-associated opportunistic infections and cancer may cost even more than antiviral therapy (that prevents the progression of AIDS and then the appearance of opportunistic infections)
  - Diagnostic tools cannot be afforded
    - But without them we trash the money invested to buy drugs inappropriately used
    - Without diagnosis, we'll generate new strains of HIV resistant to current therapies
      - All efforts currently devoted to let african have access to triple therapy are at risk to become useless in the future

Diagnostic tools

- Their availability in developing countries is a formidable but necessary task.
- Without them, antiviral therapy as it is conceived in developed countries, cannot be afforded and is not feasible.

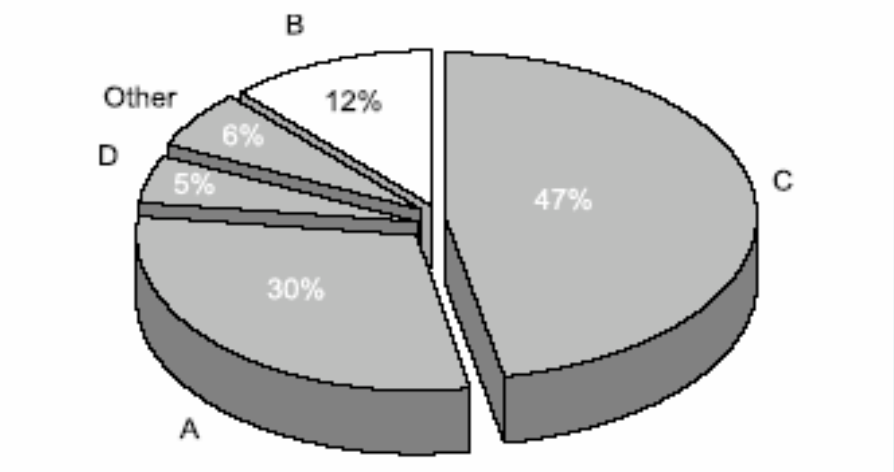
Viral Load and Resistance

- Viral load is essential for monitoring antiviral therapy

  ….but

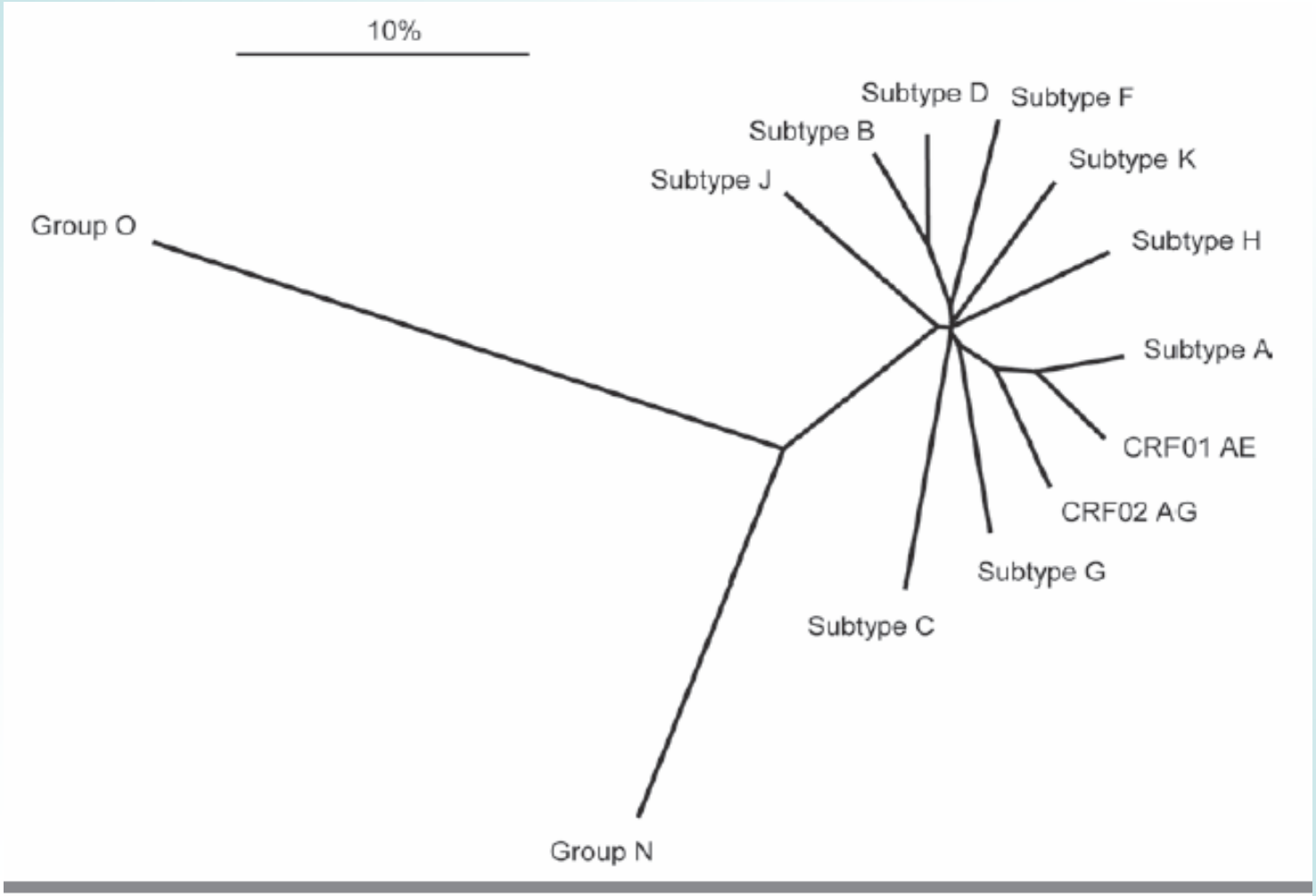
  – Some subtypes and strains may be less recognized by classical HIV testing
- Monitoring and analysis of resistance
  – Mandatory in countries where the classical B strain is virtually absent

Non-B subtypes account for 88% of HIV infections in the world



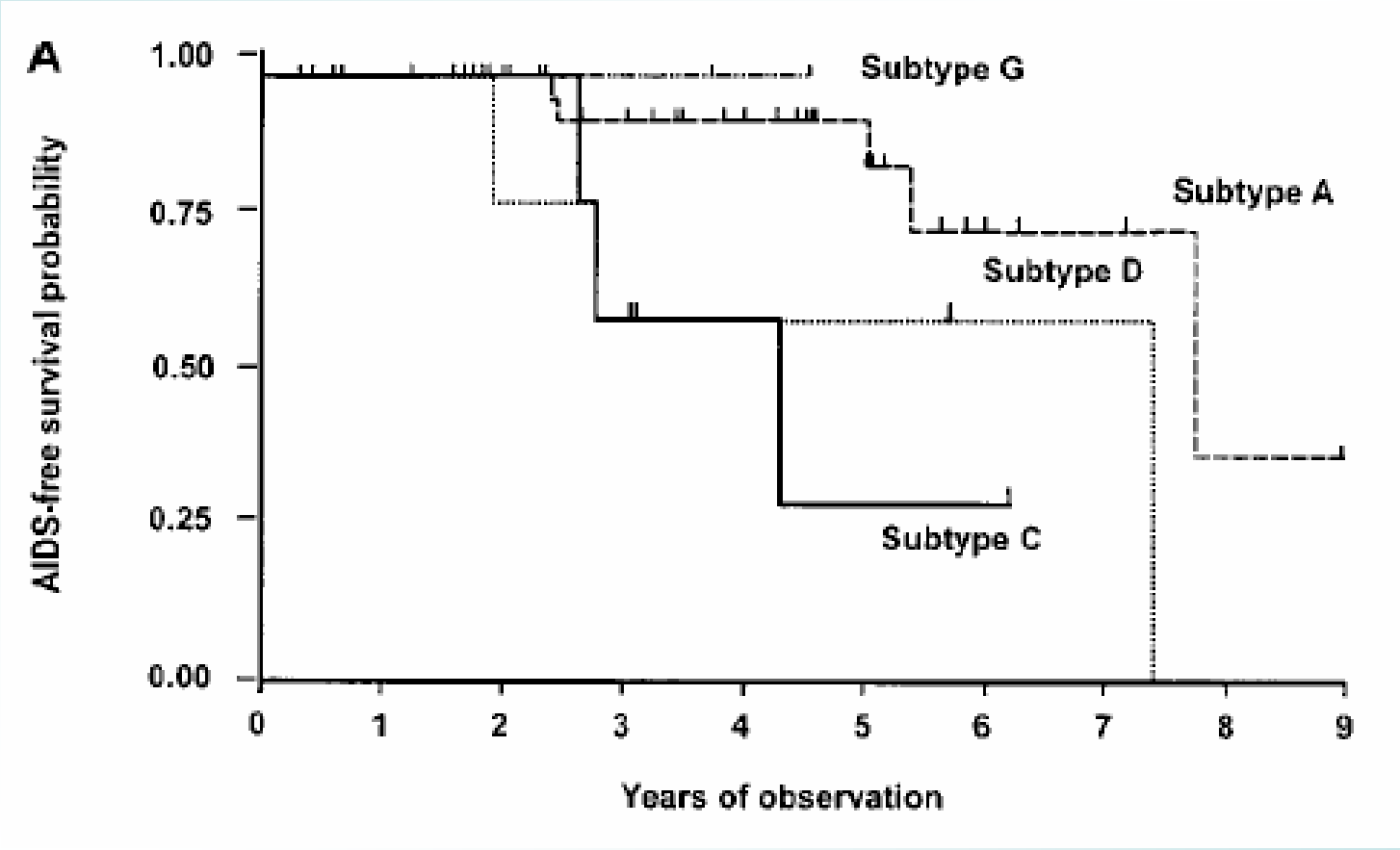
Osmanov et al., 2002

Phylogenetic relationship between HIV subtypes



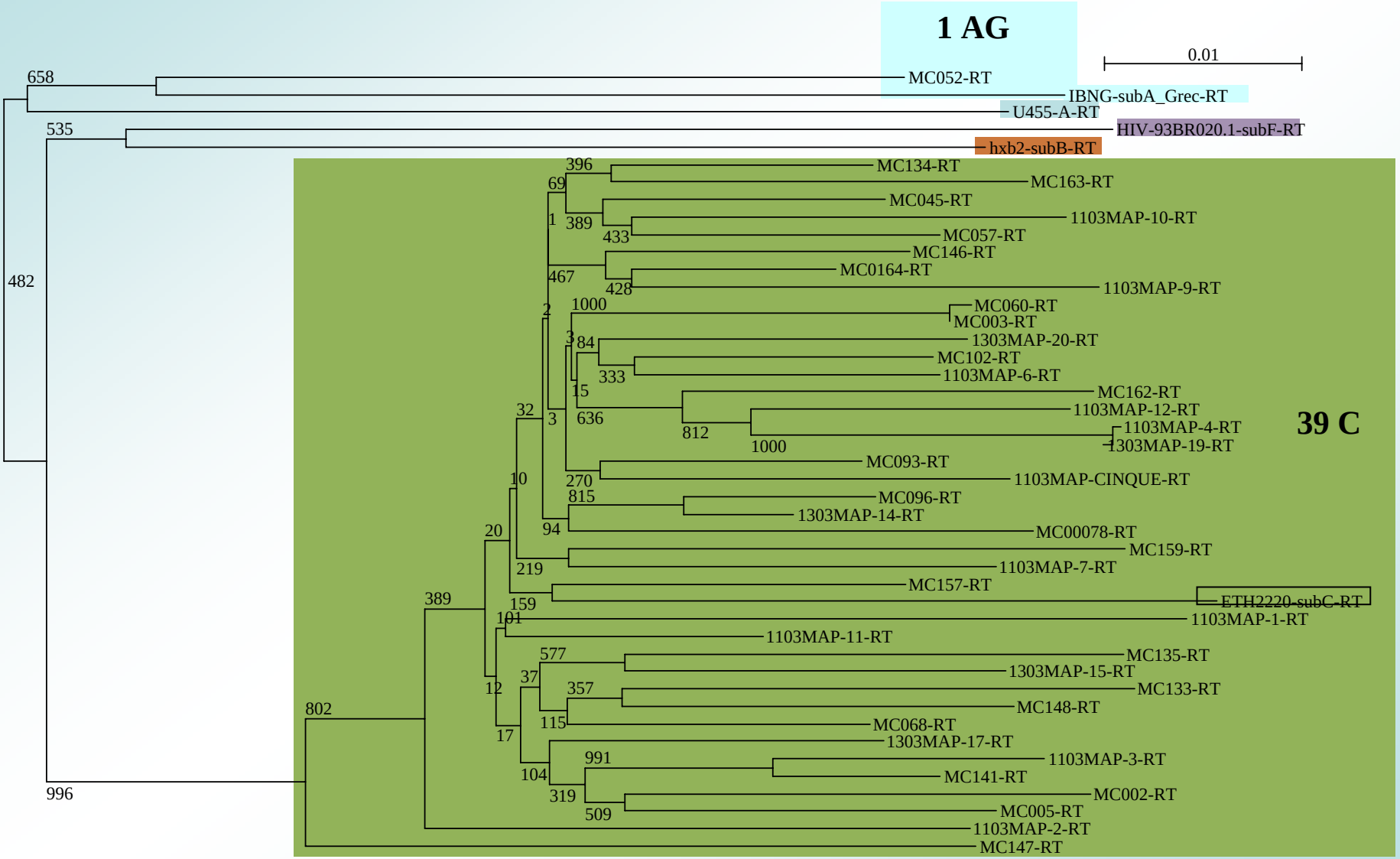
Neighbor-joining tree constructed from pol sequences of 13 reference isolates (subtype A isolate U455, CRF01_AE isolate U54771, CRF02_AG isolate L39106, subtype B isolate HXB2, subtype C isolate C2220, subtype D isolate NDK, subtype F isolate 93BR020, subtype G isolate SE6165, subtype H isolate 90CR056, subtype J isolate SE9173c, subtype K isolate 97EQTB11C, group N isolate YBF30, group O isolate AZT70C). The scale bar represents a 10% nucleotide difference.

HIV-1 subtypes differ in disease progression



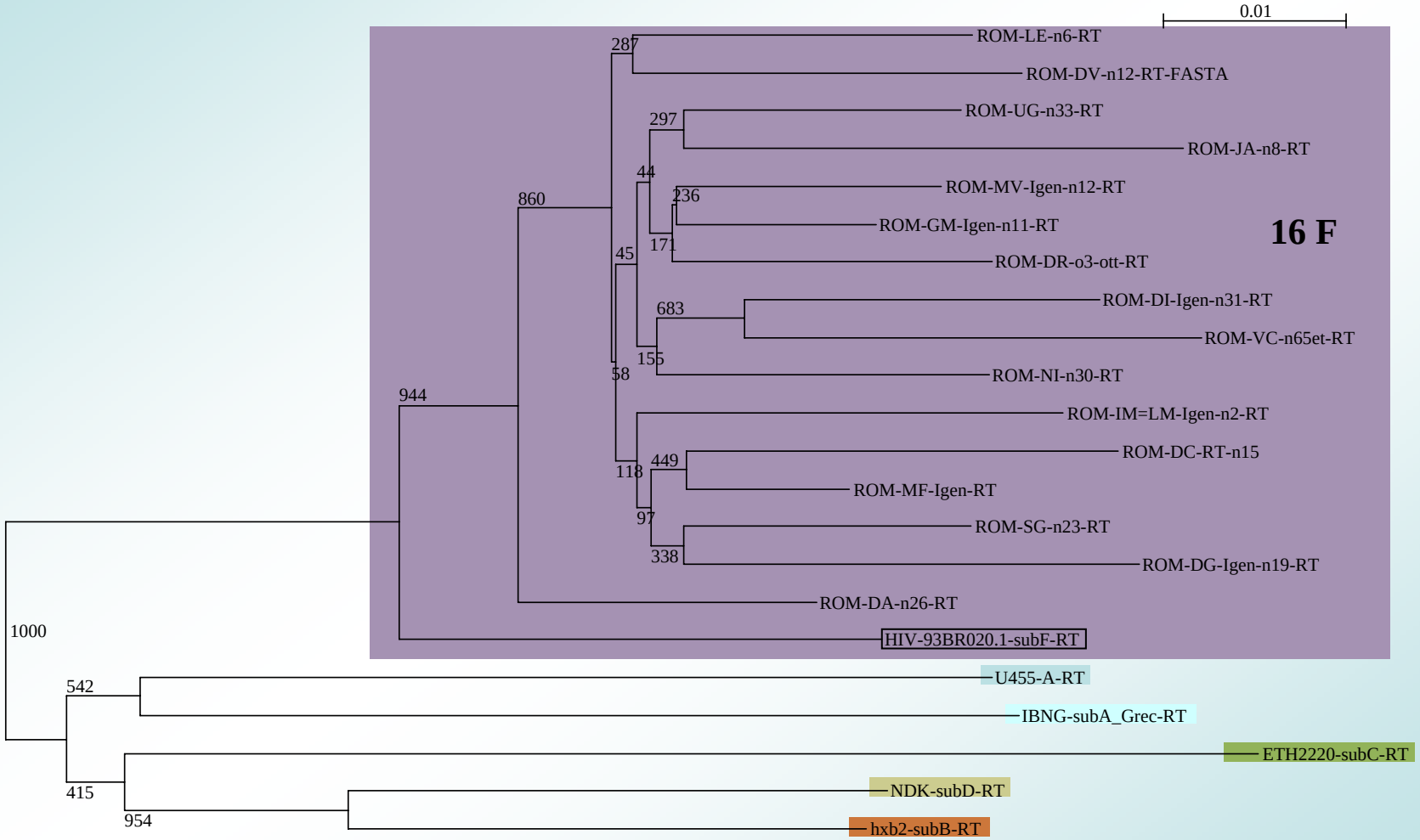
Kanki et al., 1999. J Infect Dis

Mainly C subtype in Mozambique



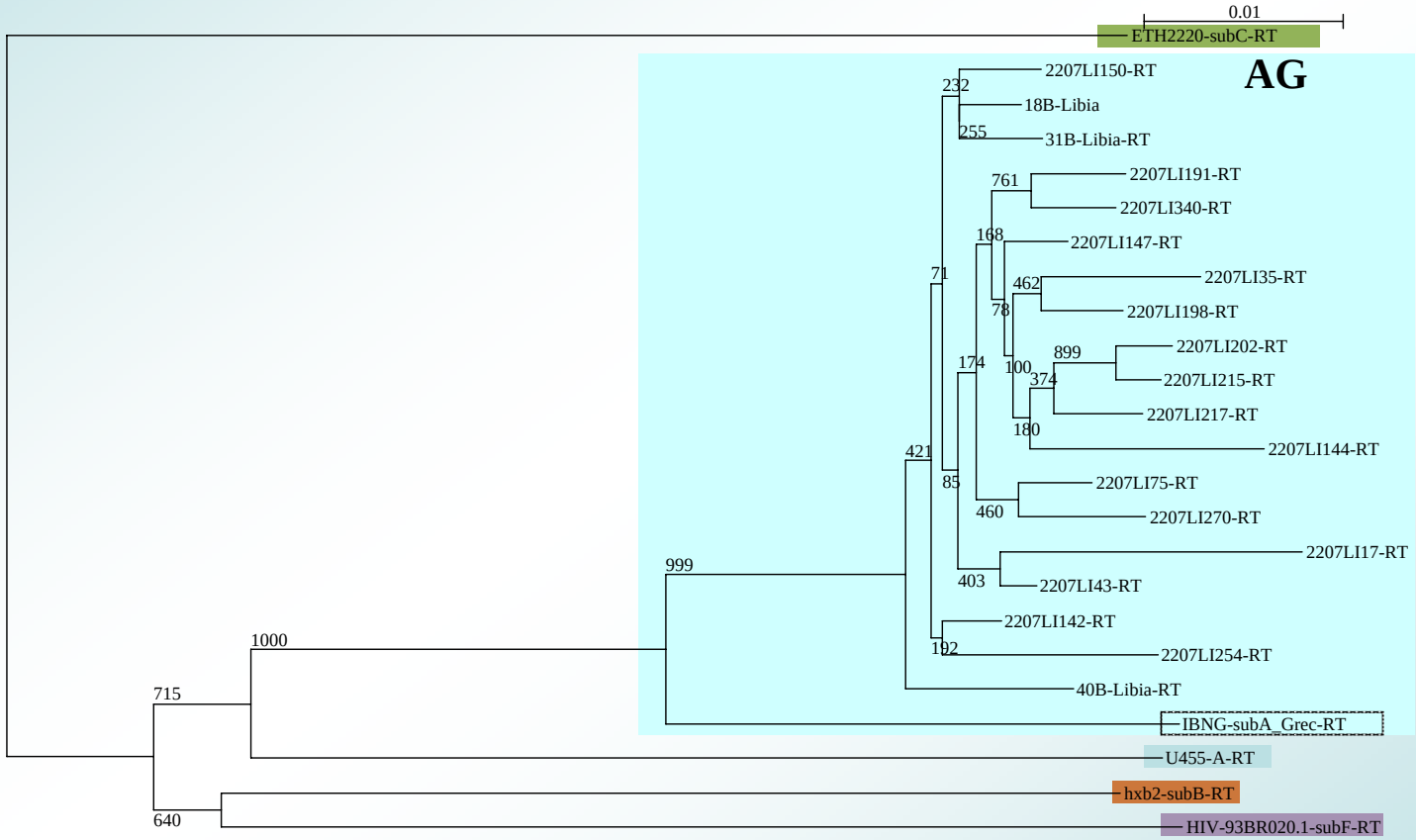
RT phylogenetic characterization of 40 HIV-1 isolates from naive patients of Mozambico using neighbor joining analysis

F subtype in Romania



RT phylogenetic characterization of 16 HIV-1 isolates from treated patients of Romania using neighbor joining analysis

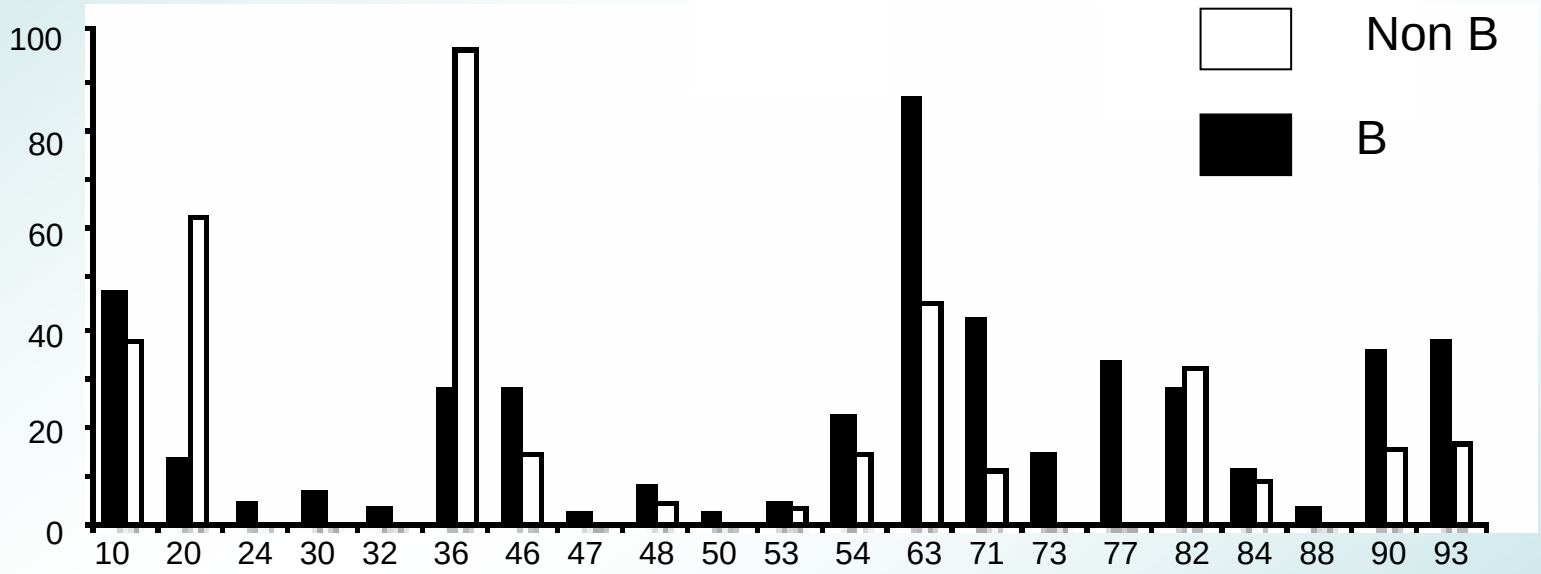
AG subtype in Ivory Coast, Burkina Faso and Lybia



RT phylogenetic characterization of 19 HIV-1 isolates from treated patients of Libia using neighbor joining analysis. The numbers near the nodes are the percentage of bootstrap replicates supporting the clade to the right???.

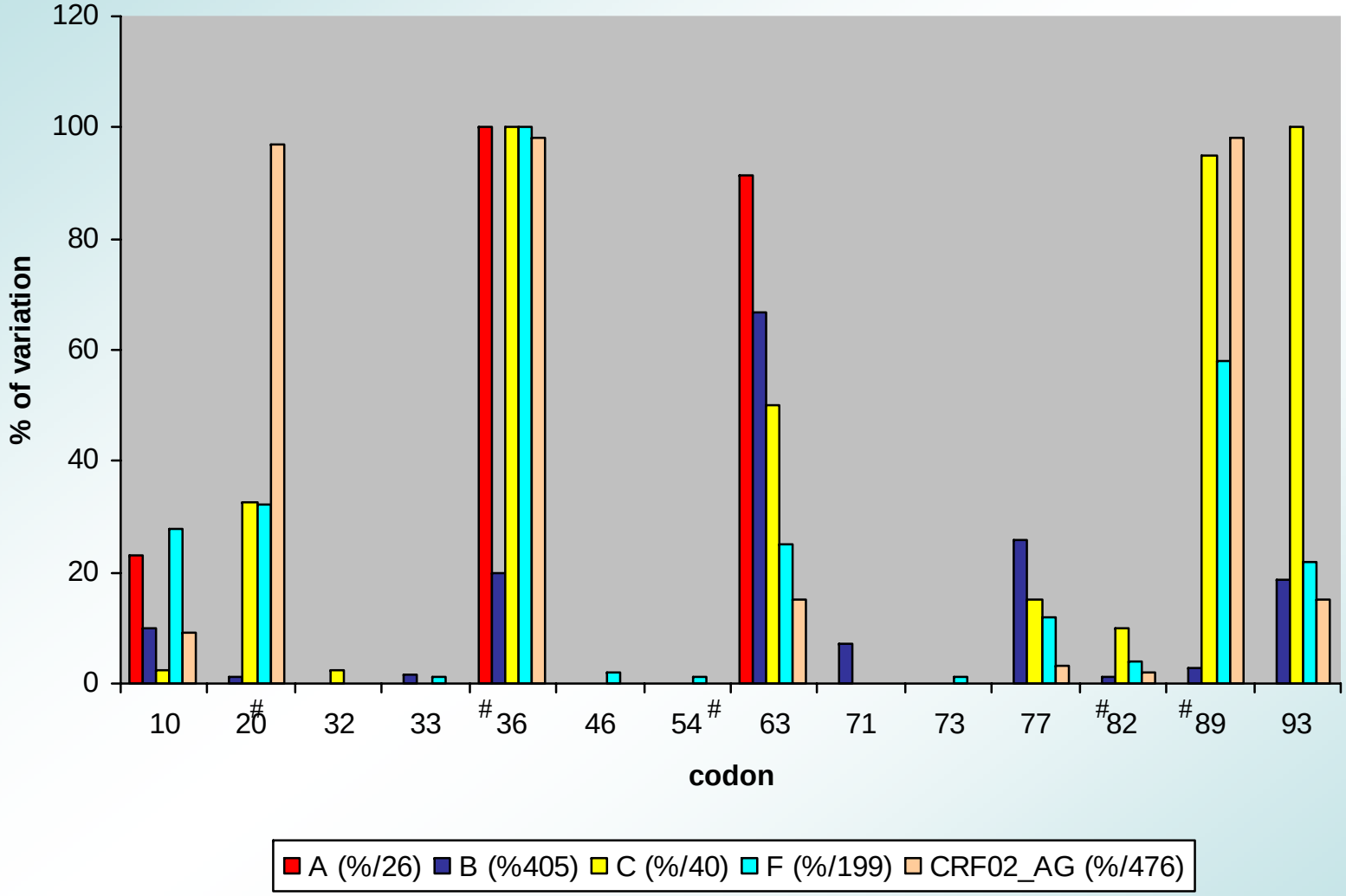
Several amino acid substitutions occur at high rate in certain non-B subtype viruses at positions associated with subtype B drug resistance

Frequency of PI-resistance mutations in both B and non-B isolates from treated patients



Kantor and Katzenstein, 2004.J Clin Vir

Prevalence of drug resistance-associated mutations in HIV-1 PR in different subtypes from naive patients



Results from F subtype and CRF02_AG are from Stanford database

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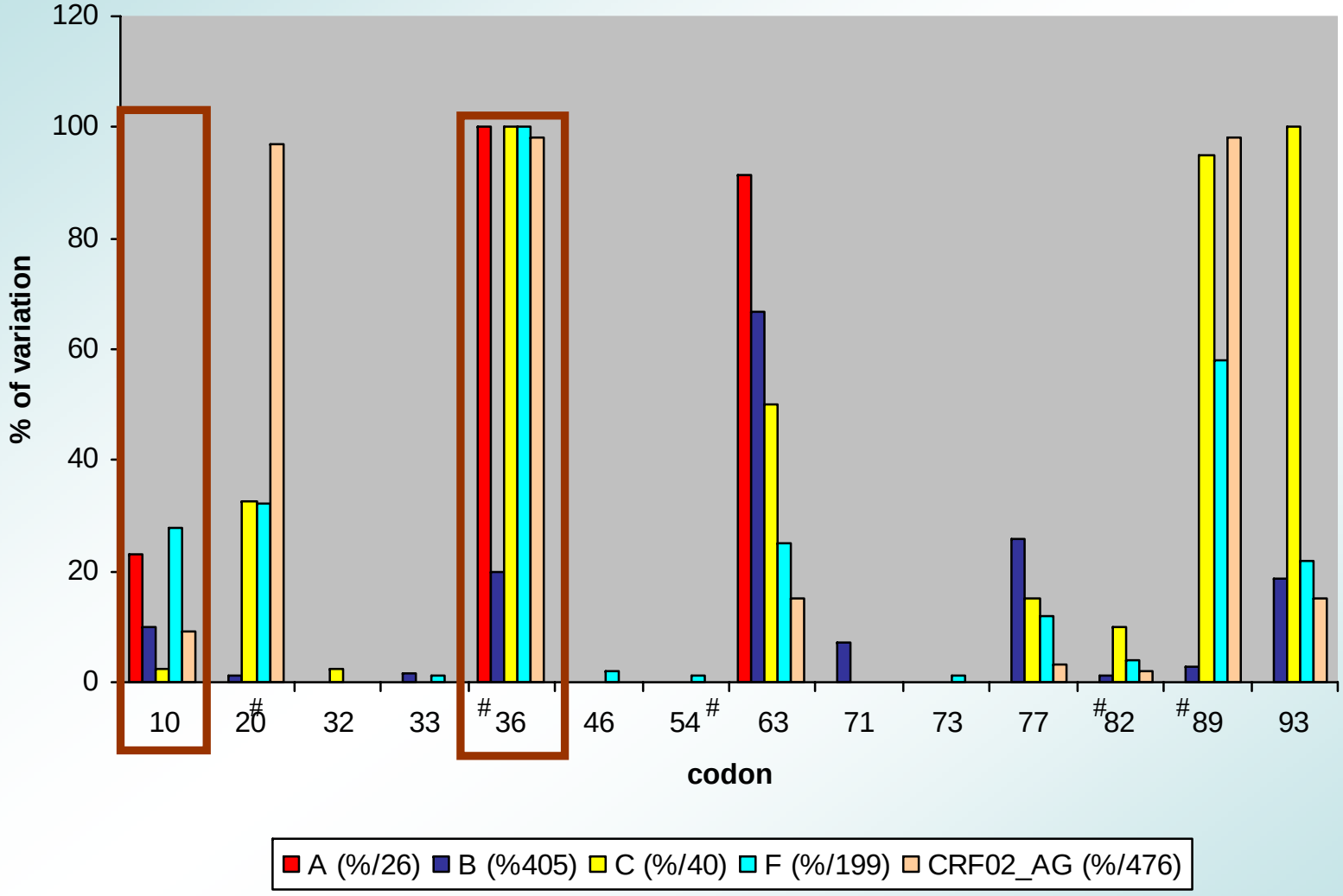


Results from F subtype and CRF02_AG are from Stanford database

- **Mutations at positions 10/36 of the protease are associated with increased risk of virological failure in patients treated with protease-inhibitors (PI) as part of their first regimens**

Perno et al JID 2001.

Odds ratio of having L90M at the time of virological failure from fitting a logistic regression model

Covariate	Crude analysis		Adjusted analysis	
	OR (95% CI)	p-value	OR (95% CI)	p-value
CD4 count, Per 100 cells/μl higher	0.93 (0.7-1.2)	0.60	0.82 (0.6-1.2)	0.32
Viral load reduction <1 or increase 1-1.4 ≥1.5 log10 copies/mL	4.13 (0.91-18.7) 1.42 (0.29-6.86)	0.07 0.66	1.00 15.5 (1.64-146.8) 1.54 (0.23-10.5)	0.02 0.65
10I/V No Yes	1.00 3.70 (0.80-17.2)	0.09	1.00 9.11 (1.09-76.1)	0.04
36I No Yes	1.00 3.18 (0.94-10.7)	0.06	1.00 13.5 (1.89-95.6)	0.009
77I no Yes	1.00 0.51 (0.11-2.50)	0.41	1.00 0.60 (0.07-5.3)	0.64
63P No Yes	1.00 1.23 (0.38-3.97)	0.73	1.00 1.21 (0.29-5.07)	0.80
Other PR mutations One additional	0.48 (0.06-3.63)	0.48	0.99 (0.13-7.42)	0.99

Perno et al

JID 2004

L89I/V: a novel mutation selected by a protease inhibitor therapy in subtype G, but not in subtype B-infected patients

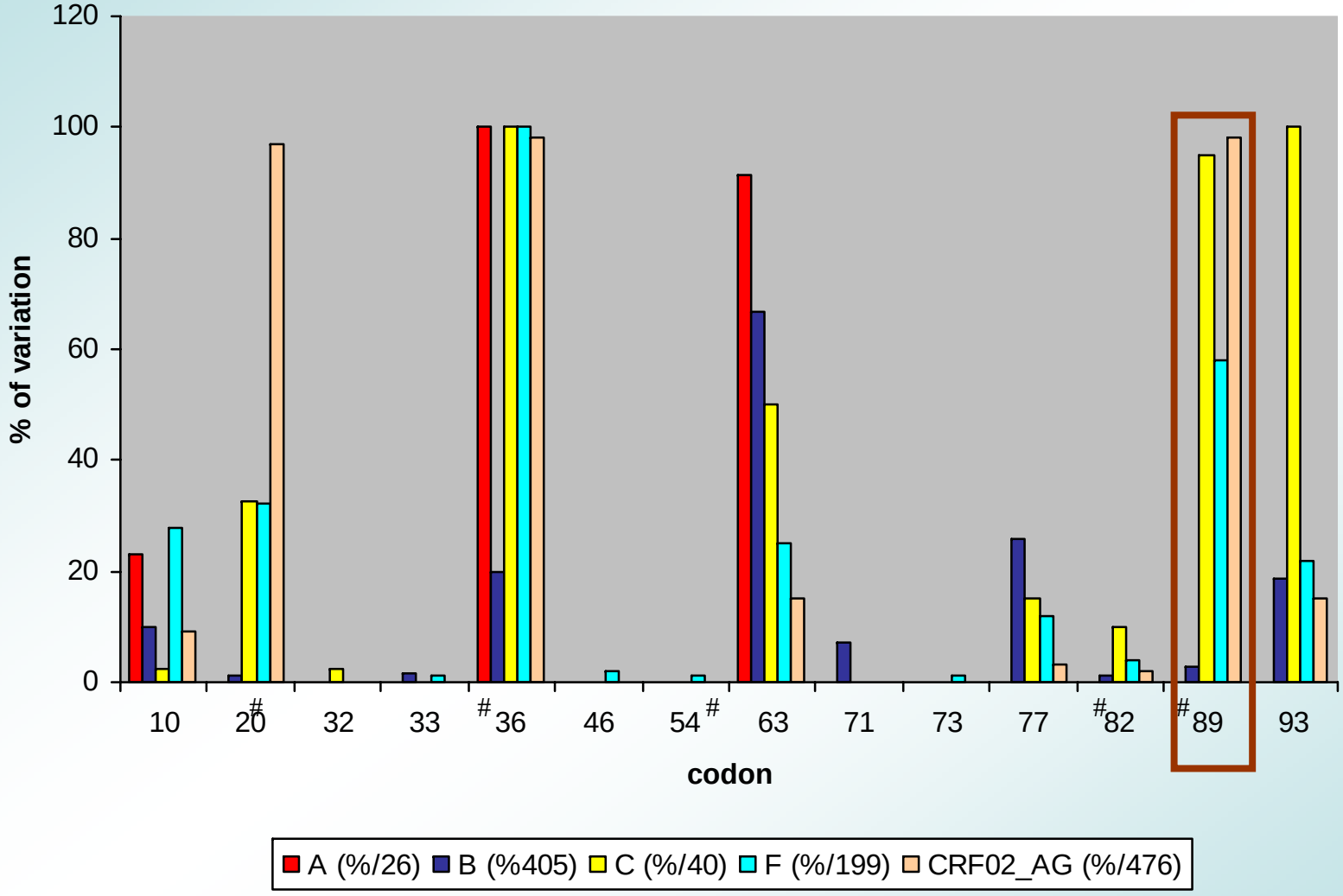
		drug-naive pts	**PI-treated pts**
Residue 89:	B-subtype	L (96.6%)	L (94.2%)
	G-subtype	M (96.2%)	I/V (72.4%)

In subtype B no mutation is selected at PR codon 89 under PI pressure

In subtype G the prevalence of mutations I/V is high in patients failing a PI-regimen, but only in presence of major PI-mutations

(Abecasis A et al., 2003. Antivir Ther, Los Cabos. Ab 126)

Prevalence of drug resistance-associated mutations in HIV-1 PR in different subtypes from naive patients



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Subtype-dependent Resistance to Protease Inhibitors by the HIV-1 Protease L89M Polymorphism.

A Calazans et al., 2004. XI Conference on Retroviruses and Oppurtunistic Infections. San Francisco. Ab692

Background: In this work, we describe the effect of HIV-1 protease L89M substitution (recognized as a molecular signature of many non-B HIV-1 subtypes) over the phenotypic EC50 values for each of the 6 FDA-approved PI. This Leu to Met polymorphism is the same of its neighbor amino acid 90, conferring resistance to Saquinavir (SQV) and Nelfinavir (NFV).

Results: The L89M mutation conferred 4.5-, 2-, 6-, 4.7-, 4.5-, and 3.4-fold increases in EC_{50} for IDV, SQV, NFV, RTV, APV, and LPV, respectively, on *Fwt* 89M90L virus; comparable to the fold resistance values obtained with *Bwt* 89L90M virus. Surprisingly, *Bwt* 89M90L clone behaved phenotypically similar to the susceptible *Bwt* 89L90L virus for the latter 4 PI. The *Fwt* 89M90L also showed a replicative fitness comparable to the PI-susceptible *Bwt* 89L90L and were more fit than its counterpart *Bwt* 89M90L. Computational molecular dynamics analysis of F and B subtype proteases have pointed significant differences, mainly the hydrogen bond between the catalytic residue Asp25 and the hydroxyl group of Nelfinavir.

Conclusions: The L89M mutation impacts phenotypic resistance to SQV, NFV, RTV, APV, and LPV differently for F and B clade viruses.

RT consensus positions which differ from consensus B in drug-naïve patients

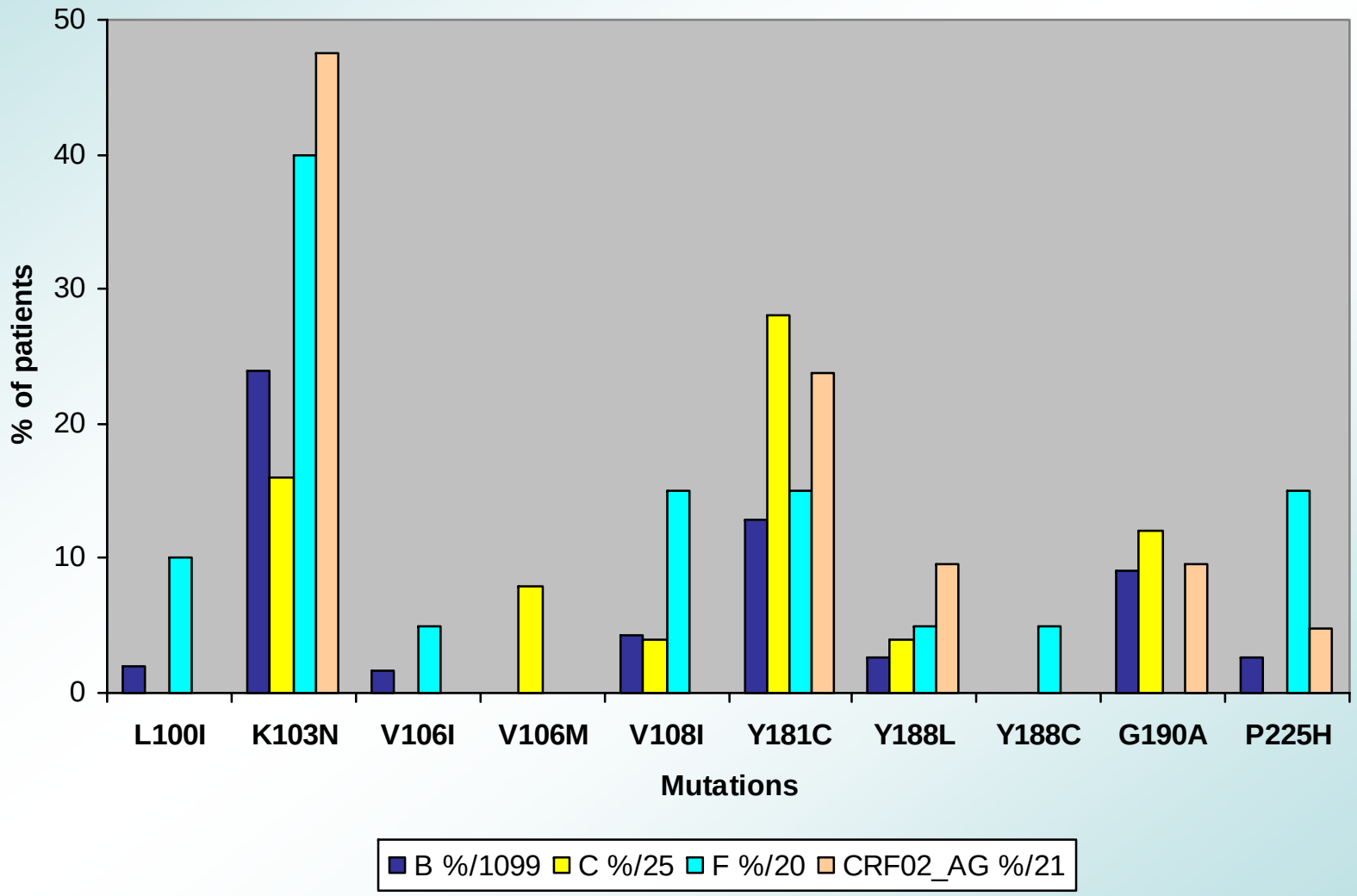
	35	36	39	48	60	122	123	135	162	173	174	177	179	200	207	211	245	248	250	272	277	286	291
A	**T**	E	T	S	**I**	**E**	**S**	**T**	S	**S**	**K**	**E**	**I**	T	**A**	**S**	**E**	E	**E**	A	K	**A**	**D**
B	**V**	E	**T**	S	**V**	**K**	**D**	**I**	**S**	**K**	**Q**	**D**	**V**	**T**	**Q**	**R**	**V**	**E**	D	**A**	**K**	**T**	**E**
C	**T**	**A**	**E**	**T**	V	**E**	D	I	S	**A**	Q	**E**	V	**A**	**E**	**K**	**Q**	E	D	**P**	**R**	**A**	**D**
F1	**T**	E	T	S	V	K	D	**V**	**C**	**A**	**K**	D	V	T	**E**	**K**	**Q**	**D**	D	**P**	K	**A**	**D**
AG	**T**	**D**	T	S	V	K	D	**V**	**A**	**T**	**K**	**E**	V	**A**	**E**	**K**	**Q**	E	D	A	K	**A**	**D**
				*	*	*	*				*	*	*	*				*	*	*	*	*	*

30/291 residues (10%) of RT are known to present different aa in different HIV-1 subtypes
Among these subtypes:

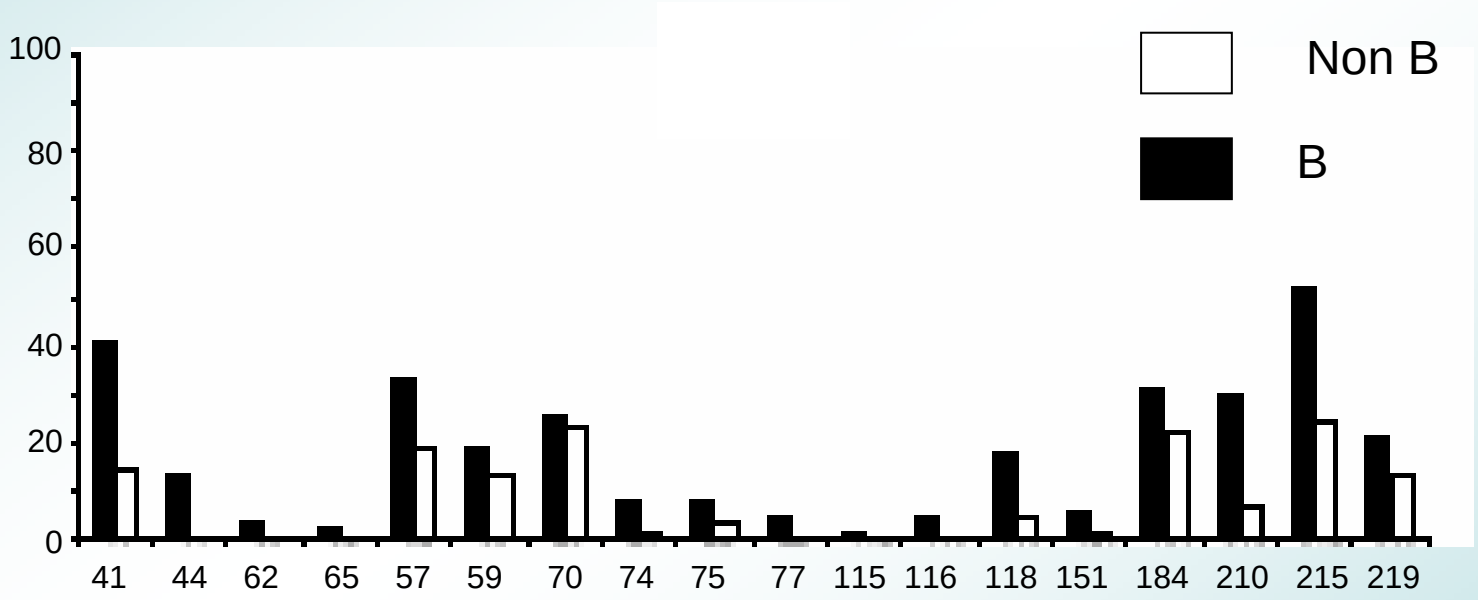
14/23 residues (61%) present conservative substitutions *

0/23 residues are “IAS” drug-resistance associated

Prevalence of drug resistance-associated mutations in HIV-1 RT in different subtypes from NNRTI-treated patients



Frequency of NRTI-resistance mutations in both B and non-B isolates from treated patients



Kantor and Katzenstein, 2004.J Clin Vir

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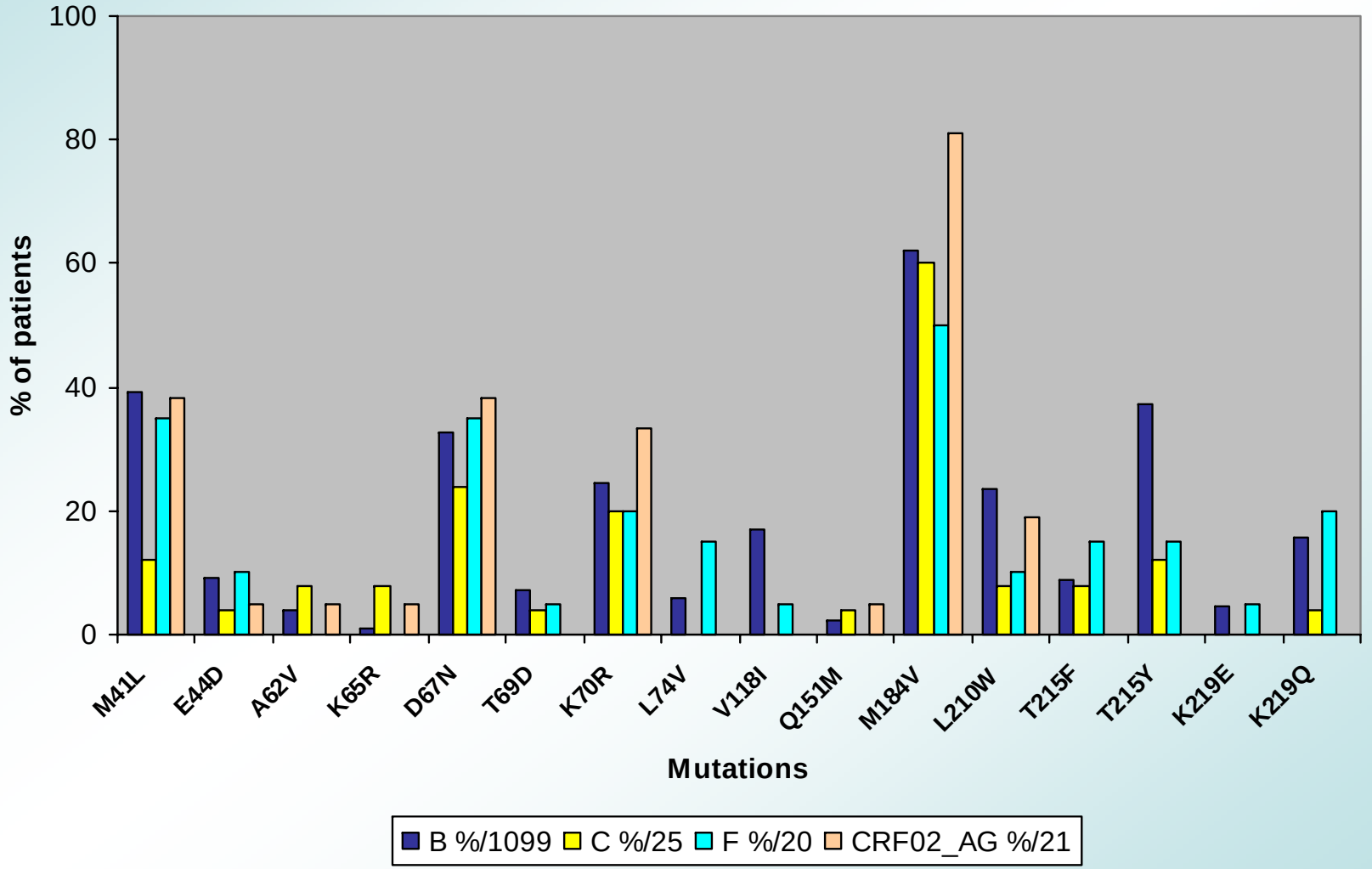

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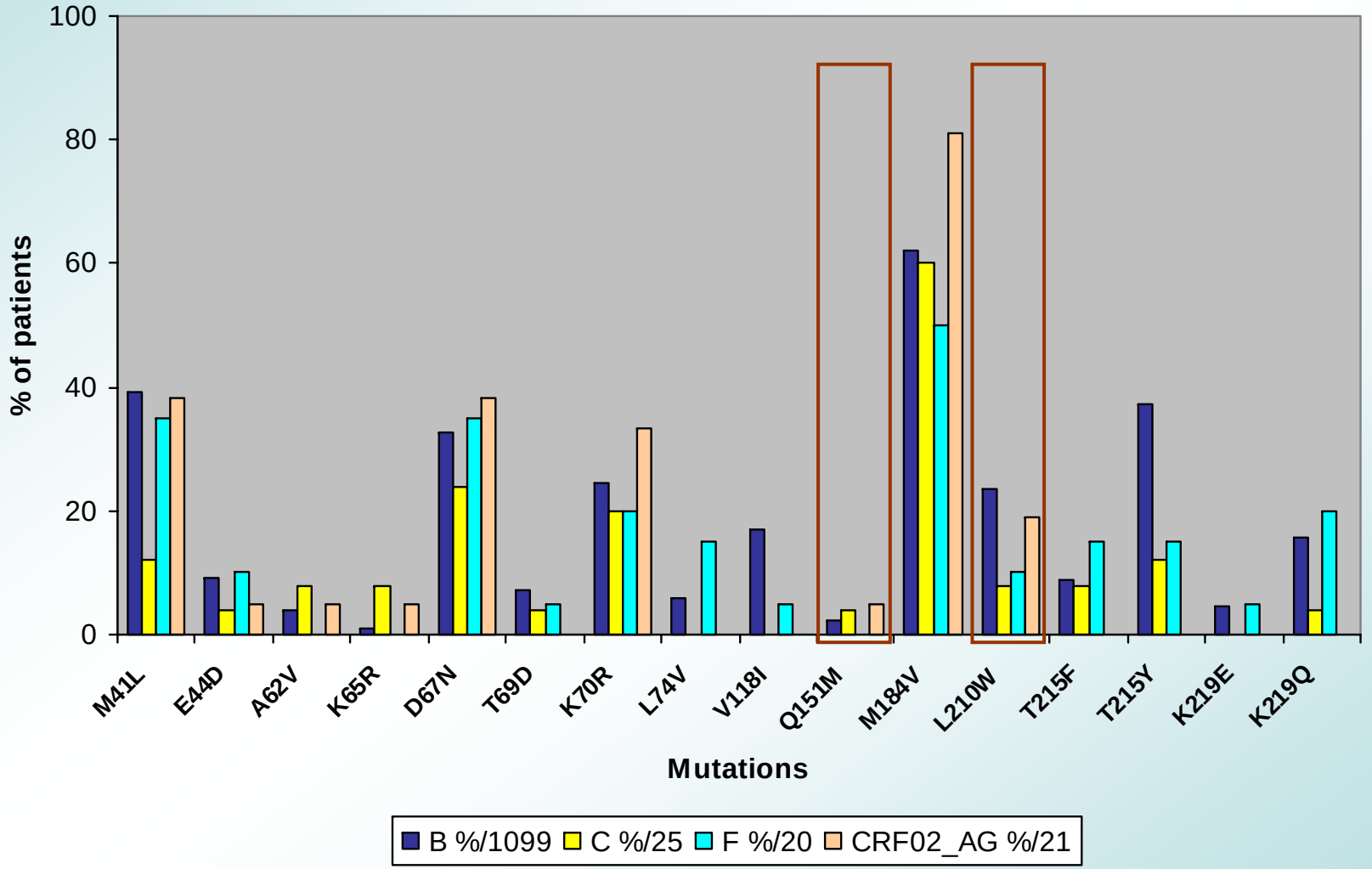


Synonomous genetic changes in subtype F HIV-1 may influence mutational routes to drug resistance

L210W :

In B subtype TTG → TGG; required 1 transition

In F subtype CTG → TGG; required 1 transversion and 1 transition

Q151M:

In B subtype CAG → ATG; required 2 transversions

In F subtype CAA → ATG; required 2 transversions and 1 transition

Differential genetic barrier between RT of subtype B and F may lead to altered rate of emergence of resistance-associated mutations at positions 210 and 151

Tanuri A et al., 2003. Antivir Therapy, Los Cabos. Ab 128

Are existing drugs equally effective against Pr and RT from different HIV-1 subtypes?

Protease inhibitors are highly constrained molecules preshaped to bind specific sites in B subtypes protease.

Small geometric distortions in other subtypes binding sites may reduce the efficacy of such drugs

Current PIs have higher inhibition costants for A and C subtype than B subtype protease

Inhibitor	K_i (nM), subtype B	K_i (nM), subtype A	K_i (nM), subtype C
Indinavir	0.8 ± 0.06	5.1 ± 0.2	2.5 ± 0.2
Ritonavir	0.05 ± 0.005	0.36 ± 0.03	0.23 ± 0.02
Saquinavir	0.5 ± 0.03	1.2 ± 0.08	1.1 ± 0.05
Nelfinavir	1.0 ± 0.07	2.6 ± 0.2	2.1 ± 0.1

Inhibition constants, K_i, for the inhibitors indinavir, ritonavir, saquinavir, and nelfinavir were obtained at 25°C by measuring the rate of substrate hydrolysis using 15–30 nM Protease in 10 mM sodium acetate, 1 M NaCl, pH 5.0, and 42.5 μM substrate plus increasing amounts of inhibitor.

Velazquez-Campoy et al., 2001. PNAS

- Despite a similar rate of success in a short term follow-up, it is conceivable that the long-term success of antiviral therapy may be different in B- vs non-B strains

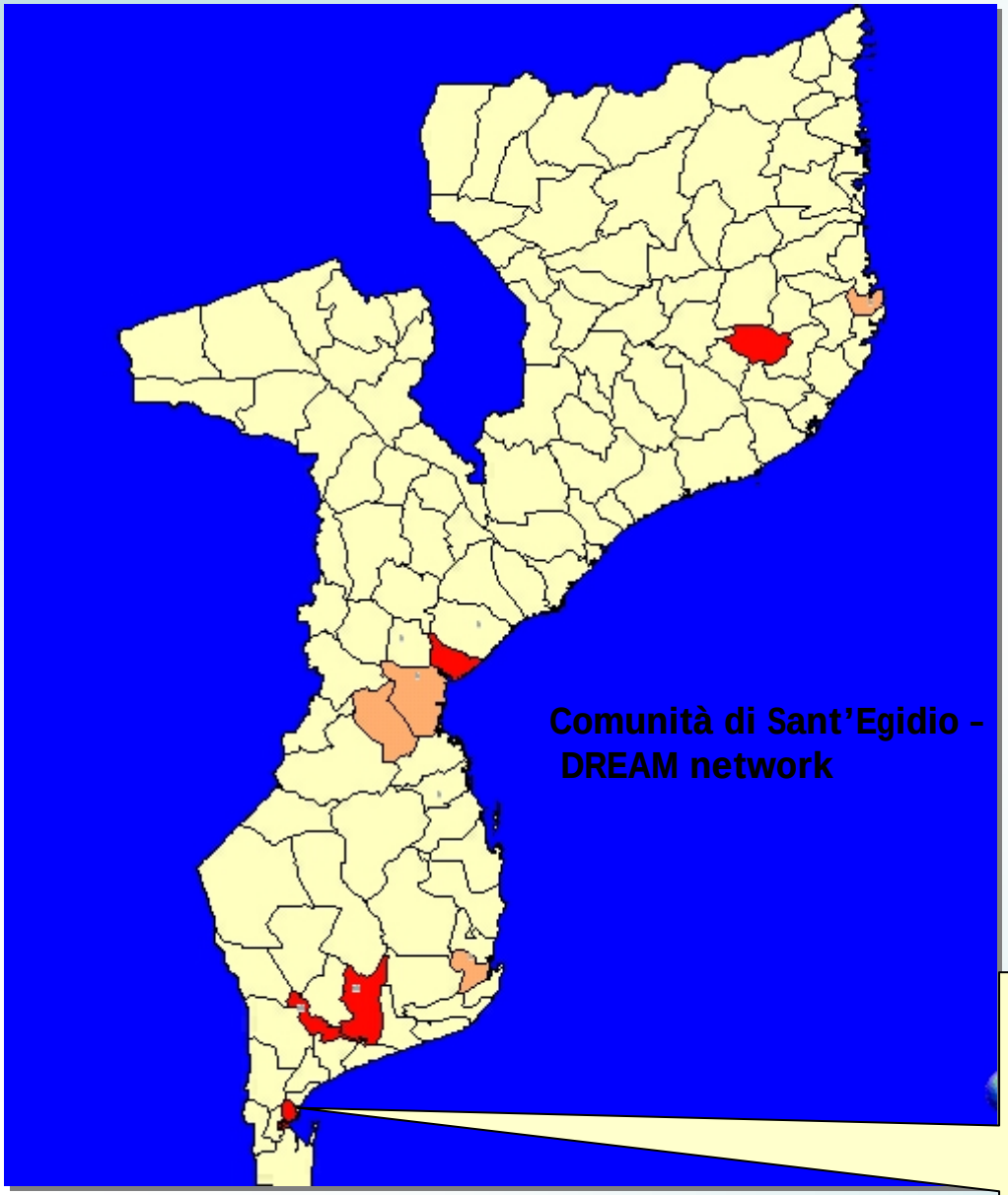
  – Reasons for that:
    - Therapeutic strategies not appropriately started and implemented
    - Adequate diagnostic tools not made available
    - Knowledge of the characteristics of non-B strains missing

- Antiviral therapy should be provided since the beginning according with appropriate standards of quality, and taking into account the experience of developed countries

Survival, Virological and Immunological Results of DREAM Program in Adults and Children in Mozambique

Palombi L., et al.
Community of Sant' Egidio

DREAM Centers



Activity data 31/12/2004	No°
VCT	15,547
HAART	2,782
Pregnancies	1,432
Deliveries	923

Maputo:
Matola 2
Machava
Polana Canisso
Hulene
Mahotas

Newly active

Active centres

First Line Treatment

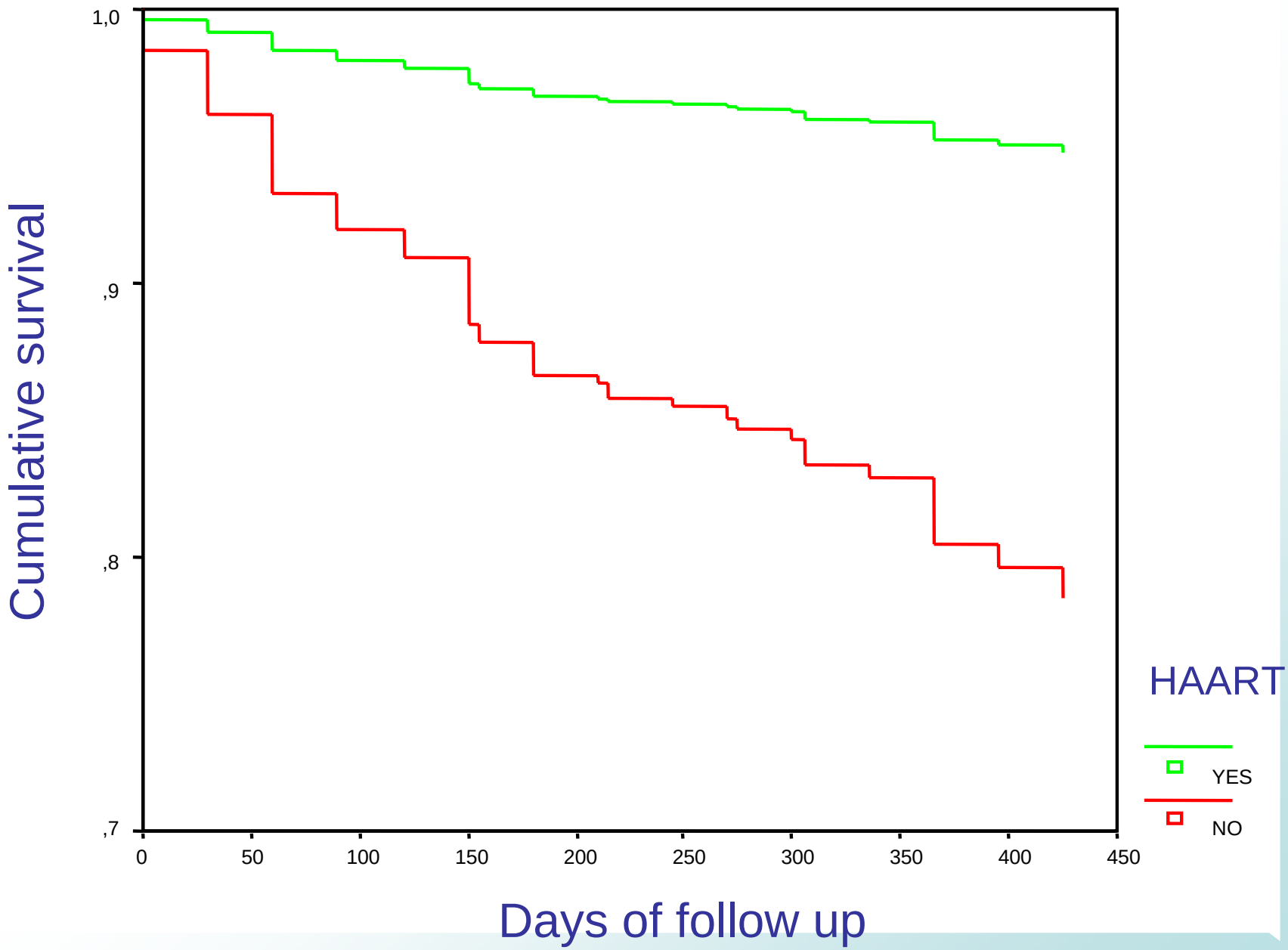
- AZT, or d4T (30 and 40 mg)
  - +
  - 3TC
  - +
  - NVP

HAART and non – HAART groups

	HAART (SD)	**NON HAART (SD)**	p	TOTAL
AGE	**34,3 (±9.4)**	**32,8 (±9,3)**	0.003	
Body Mass Index (BMI)	**20,5 (±3.4)**	**21,9 (±3.5)**	<0.001	
Hb	**10.1 (±4.6)**	**10.7 (±2.7)**	0.004	
CD4	**192 (± 189)**	**434 (± 326)**	<0.001	
VL copies/ml	**135,200 (± 126.400)**	**82,200 (± 75,600)**	0.832	
TOTAL	**643**	**705**		1.348

Non-HAART patients at baseline were at lower risk of progression and death

Cox Proportional Hazard Model - Adults



CD4 and VL patterns in 413 HAART patients

HAART Mean Duration (days):	0	113	267

Secondary mutations/polymorphisms in HIV-protease and RT of 42 drug-naive subtype-C patients

PROTEASE			REVERSE TRANSCRIPTASE		
Mutation	**N°**	**% of mutations**	**Mutation**	**N°**	**% of mutations**
10I	1	(2,4%)	44K	1	(2,4%)
20R	12	(28,6%)	98S	1	(2,4%)
20I/M	2	(4.8%)	101R	1	(2,4%)
32G	1	(2,4%)	118I	2	(4.8%)
36I*	40	(95.2%)	179E	1	(2,4%)
36L	2	(4,8%)	179D	1	(2,4%)
36T*	1	(2,4%)	179I	1	(2,4%)
60E	17	(40,5%)	210F	1	(2,4%)
63P	13	(31,0%)	333E	1	(2,4%)
63V	3	(7,1%)			
63S/T/E/A	6	(14.3%)			
82I	5	(12,0%)			
93L	40	(95.2%)			

Resistance-associated mutations in patients failing antiretroviral treatment in Mozambique

PI Resistance Mutations	n°samples	%	NRTI Resistance Mutations	n° samples	%	NNRTI Resistance Mutations	n° samples	%
K20R	2	10	A62V	1	5	A98G	2	10
K20I	1	5	K65R	1	5	K101E	3	15
K20M	1	5	D67G	1	5	K103N	2	10
M36I	15	75	D67N	3	15	K103N/T	1	5
M36L	1	5	T69N	1	5	K103N/R/S	1	5
D60E	3	15.	K70R	3	15	K103R	1	5
L63P	4	20	M184I	2	10	V106M	2	10
L63S	1	*5*	M184V	15	75	V179D	2	10
L63I	1	5	T215F/Y	1	5	Y181C	4	20
L63T	1	5	K219T	1	5	Y181D/F/V	1	5
L63V	3	15	K219E	1	5	Y188L	1	5
V77I	2	10				G190A	6	30
V82I	1	5				G190S	1	5
I93L	17	85						
20 sequences			20 sequences			20 sequences		

- HAART in developing countries based upon a thymidine analogue + 3TC + nevirapine…
  - Is feasible
  - Is tolerated
  - Is potent

And…

- Provides a rate of success (both clinical and immunovirological) similar to that achieved in Europe and US in a medium time period

HAART in Pregnancy: Safety, Effectiveness, and Protection from Viral Resistance: Results from the DREAM Cohort

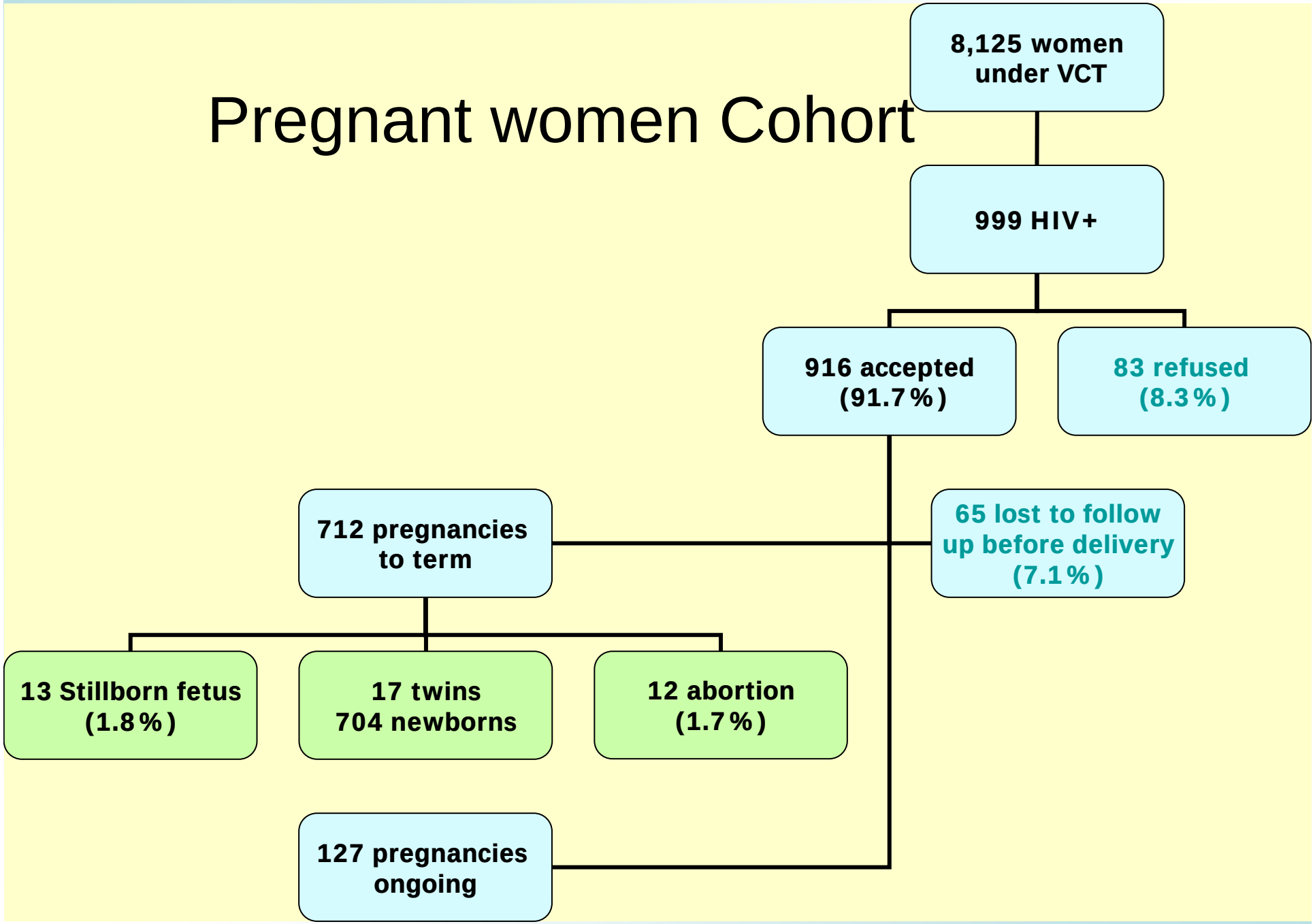
Palombi L et al.

Community of Sant' Egidio

The DREAM Approach:
Protecting the mother-and-child duo

- Triple therapy starting from the 25th week of pregnancy and for 6 months after delivery
- Fixed Dose Combinations/bd:
  - Zidovudine 300 mg (AZT), Lamivudine 150 mg (3TC) and Nevirapine 200 mg (NVP)
  - Stavudine 30/40 mg (d4T), Lamivudine 150 mg (3TC) and Nevirapine 200 mg (NVP)
- nutritional evaluation & supplementation
- CD4 cell count, Viral Load, Hematology, Liver Function closely monitored
- Health Education, water filter, bednets

Pregnant women Cohort



Base-line parameters (712 pregnancies)

Parameter	Median	IQ 25-75
Age	25	22-29
HAART start (week of pregnancy)	26.8	25.1-31.0
Pre-HAART CD4 count	498	322-695
Pre-HAART Viral Load $_{Log}$	4.15	3.6-4.6
Pre-HAART Hb	9.7	8.6-10.6
Length of pregnancy (weeks)	38.5	36.1-40.2
Pre-delivery days of HAART	74	42-98

Children Cohort: outcome of intervention

	No.(%) of HIV positive children		
	1st Month	6th Month	12th month
Children tested	519	283	67
(ITT) Incidence rate	21(4.1%)	4 (1.4%)	0
Cumulative incidence rate	4.1%	5.5%	5.5%
On treatment Incidence rate	10 (1.9%)	0	0

MTCT risk factors (one-month VL assessment)

		HIV neg		HIV pos		OR
		N	%	N	%	
Mother pre-HAART VL	<10,000 c/ml	198	98.5	3	1.5	4.7 (1.3-16.3)
	>10,000 c/ml	226	93.4	16	6.6	
HAART	AZT-containing	421	96.3	16	3.7	3.2 (1.1-9.5)
	d4T-containing	40	88.9	5	11.1	
Pre-HAART Haemoglobin	> 8 gm/100 cc	418	96.5	15	3.5	2.7 (1.1-6.9)
	< 8 gm/100 cc	71	91.0	7	9.0	
STD	No	451	96.6	16	3.4	3.5 (1.3-9.4)
	Yes	48	88,9	6	11.1	
Pre-delivery length of HAART	<70 days	212	93.8	14	6.2	0.3 (0.1-0.9)
	>70 days	240	98.0	5	2.0	

MTCT risk factors (one-month VL assessment) - 2

NVP to the baby	Yes	307	95.6	14	4.4	1.03 (0.4-2.5)
	No	180	95.7	8	4.3	
Place of delivery	Reference centre	186	95.9	8	4.1	0.9 (0.4-2.2)
	Others	270	95.4	13	4.6	

Logistic Regression Analysis – forward stepwise

Logistic Regression Analysis						
Variable	**B**	**E.S.**	**Sig.**	**Exp(B)**	**95% CI per Exp (B)**	
Viral Load (c/ml)	**1.416**	**0.649**	**0.029**	**4.120**	**1.155**	**14.696**
HAART - AZT	**- 1.450**	**0.571**	**0.011**	**0.235**	**0.068**	**0.866**

Resistance

42 unselected women that completed the protocol were assessed for genotypic resistance, in a time period between 2-6 months after therapy interruption:

All carried a subtype C strain

37 (88.1%) showed no mutations associated with resistance

5 (11.9%) carried mutations associated with resistance to nevirapine

- 3 : K103N
- 2 : G190S

Resistance to 3TC and AZT-D4T was not detectable

•……

• If, by 2025, millions of African people are still becoming infected with HIV each year, these scenarios suggest that it will not be because there was no choice.
• It will not be because there is no understanding of the consequences of the decisions and actions being taken now, in the early years of the century.

•It is not inevitable.

- Introducing antiviral therapy in Africa is not a money problem…
  - **…but a real committment to protect human life**