ANTİRETROVİRAL DİRENÇ

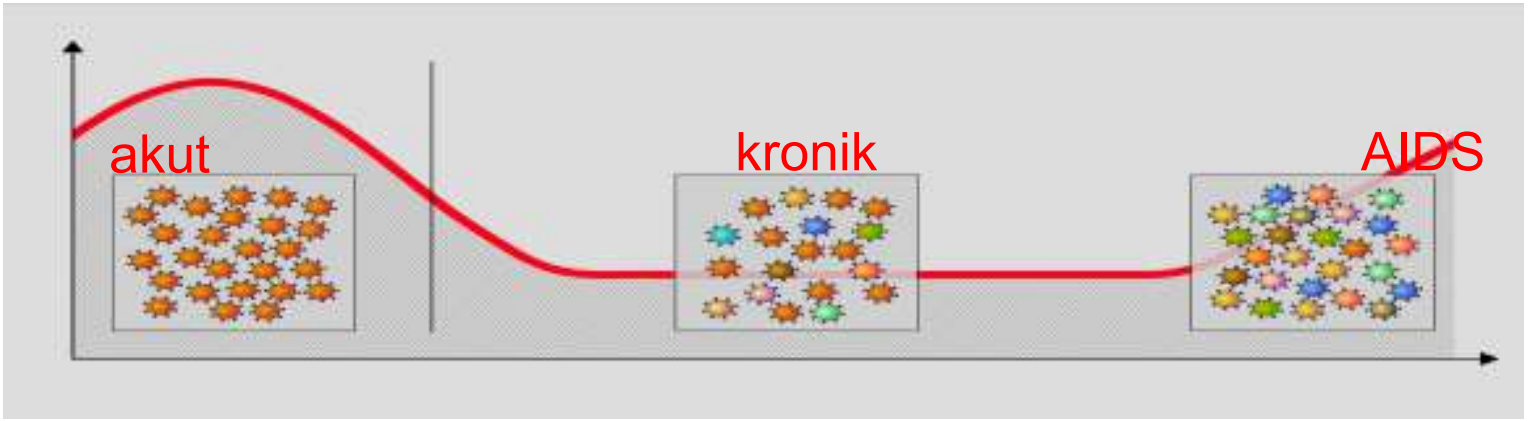
Dr. Ahmet Çağkan İnkaya

AIDS PANDEMIC

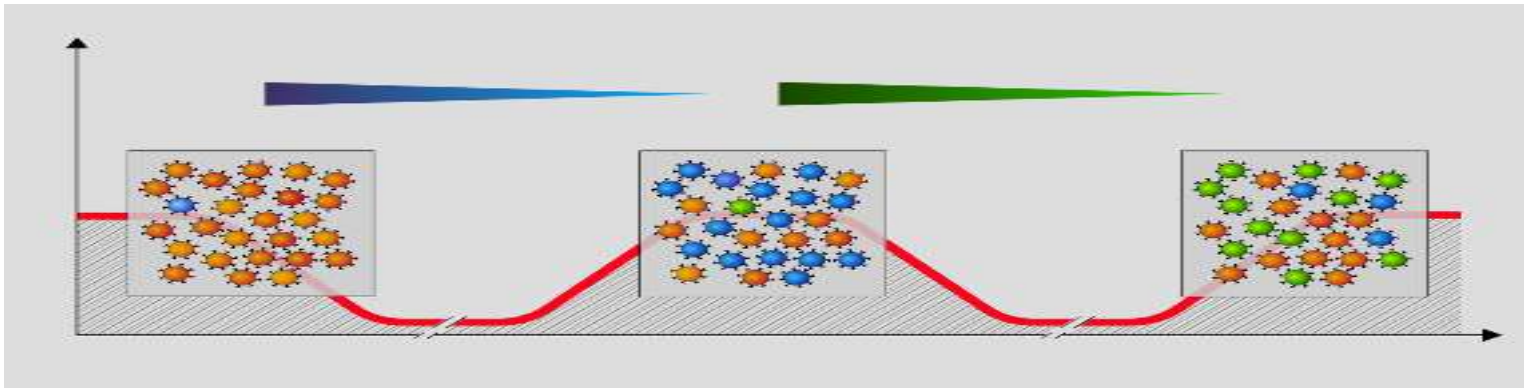
Viral mutasyonların gelişimi

- HIV1 RT hata yapar ($1/10^4$)
- HIV-1 genom 10 000 (10^4)
- 10^9 to 10^{10} virion/gün → quasispecies
- ART öncesi pek çok mutasyon gelişebilir.

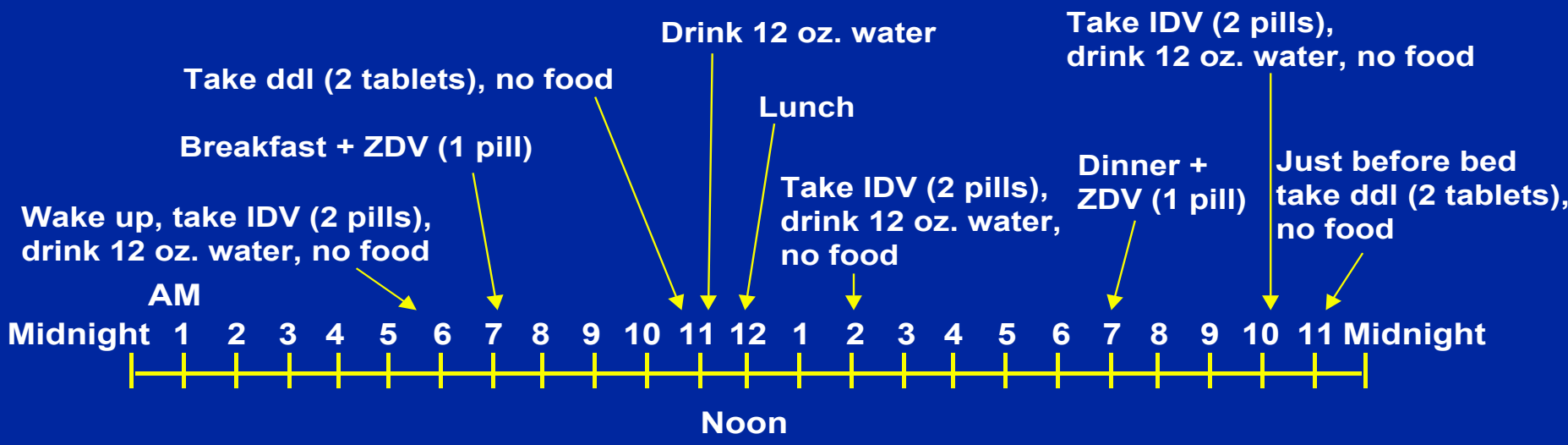
HIV-1 Quasi Species in Untreated and Treated HIV Infection: Heterogeneity vs. Selection of Resistant Strains



Viremi

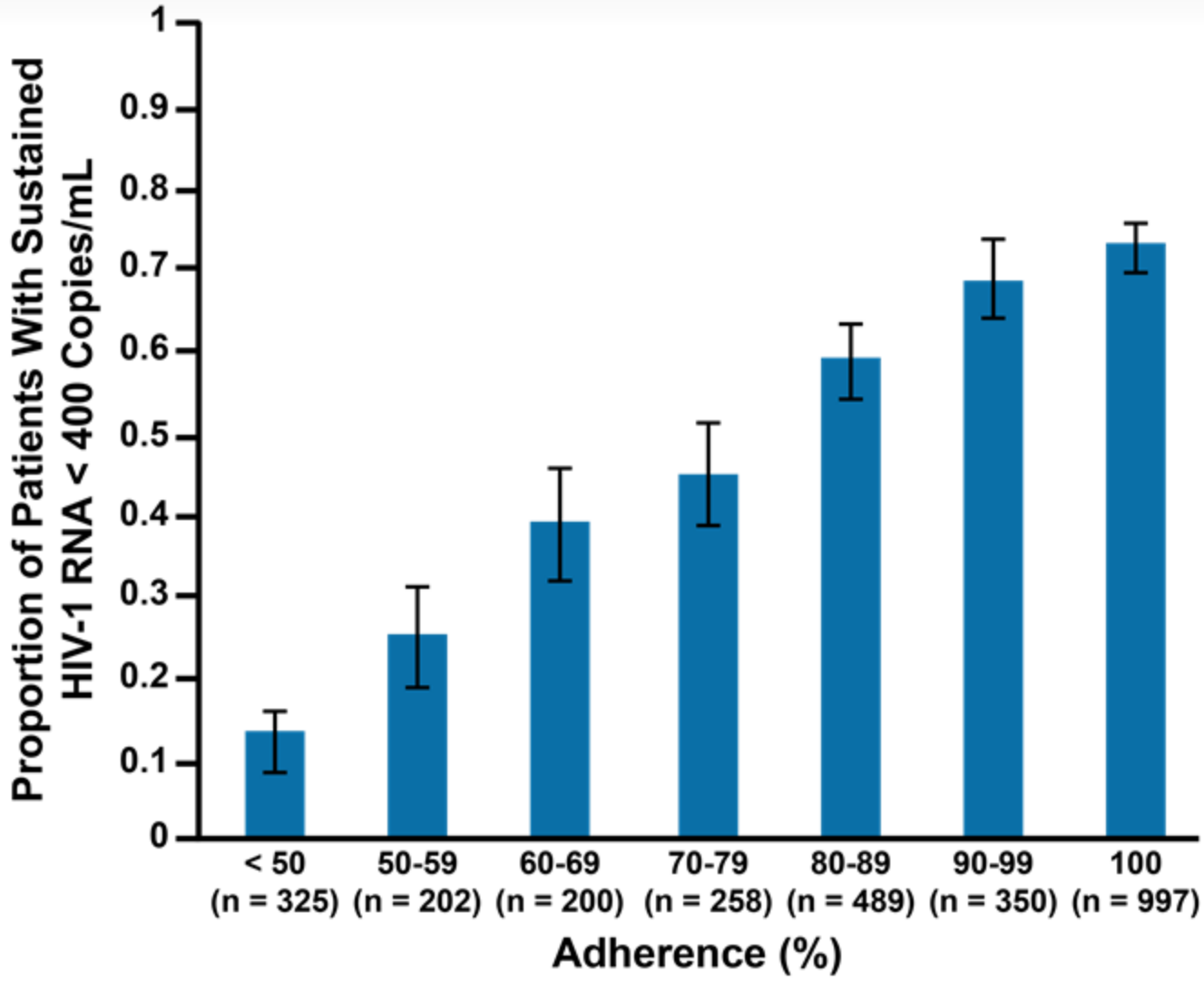


Adherence Issues: ZDV + ddI + IDV



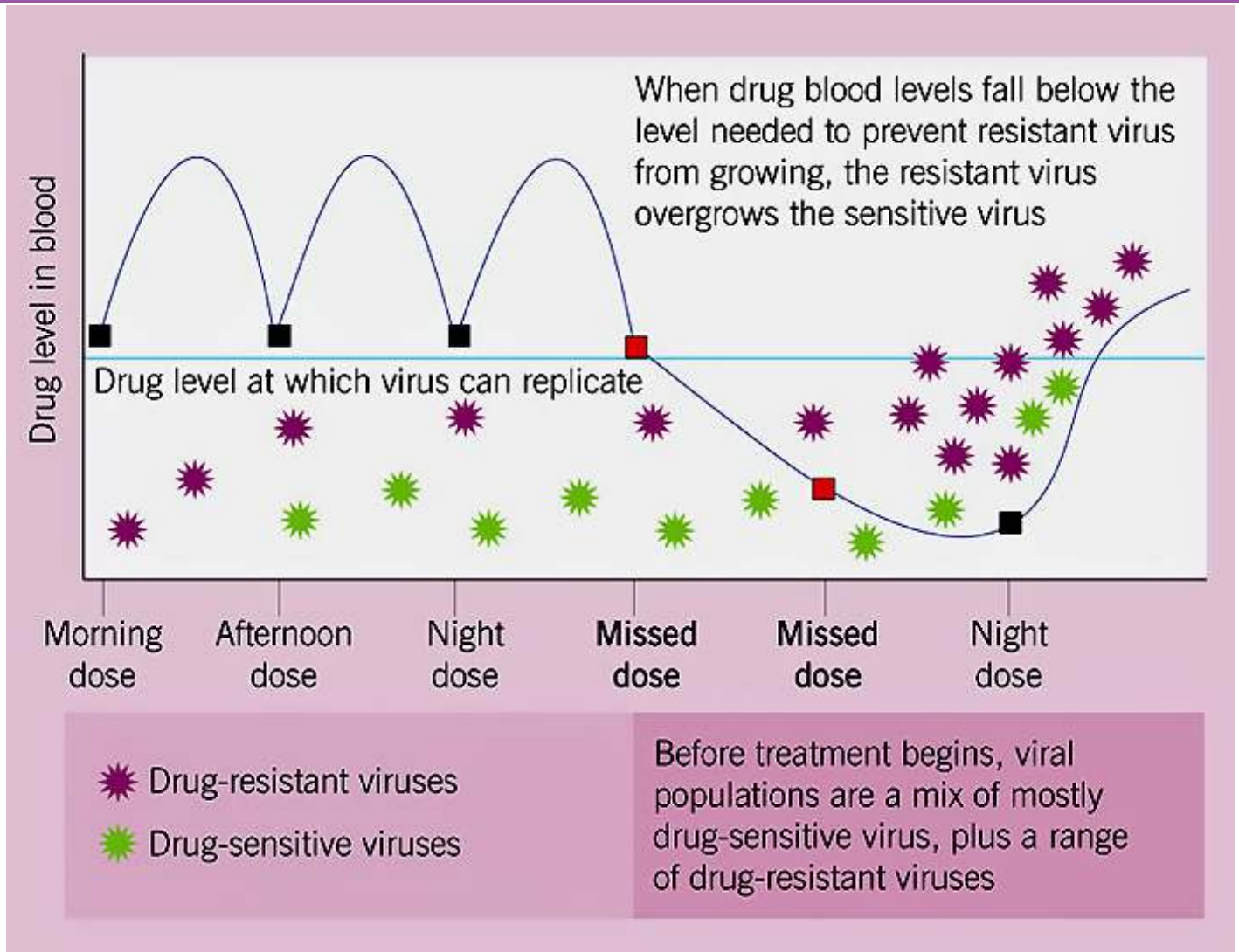
Source: *Physicians' Desk Reference*®. Medical Economics Co; 1997.

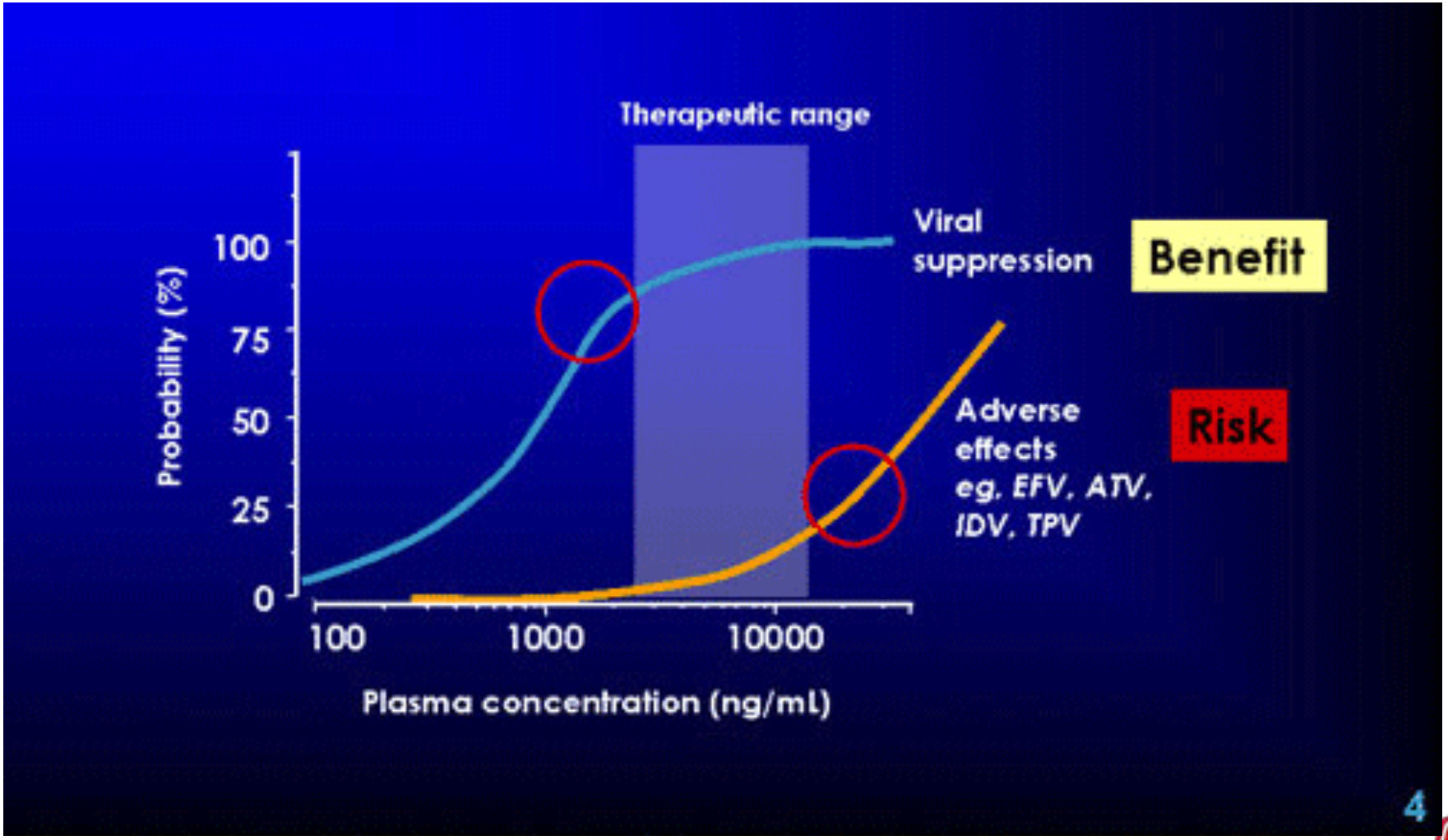
ART Adherence Predicts Virologic Response



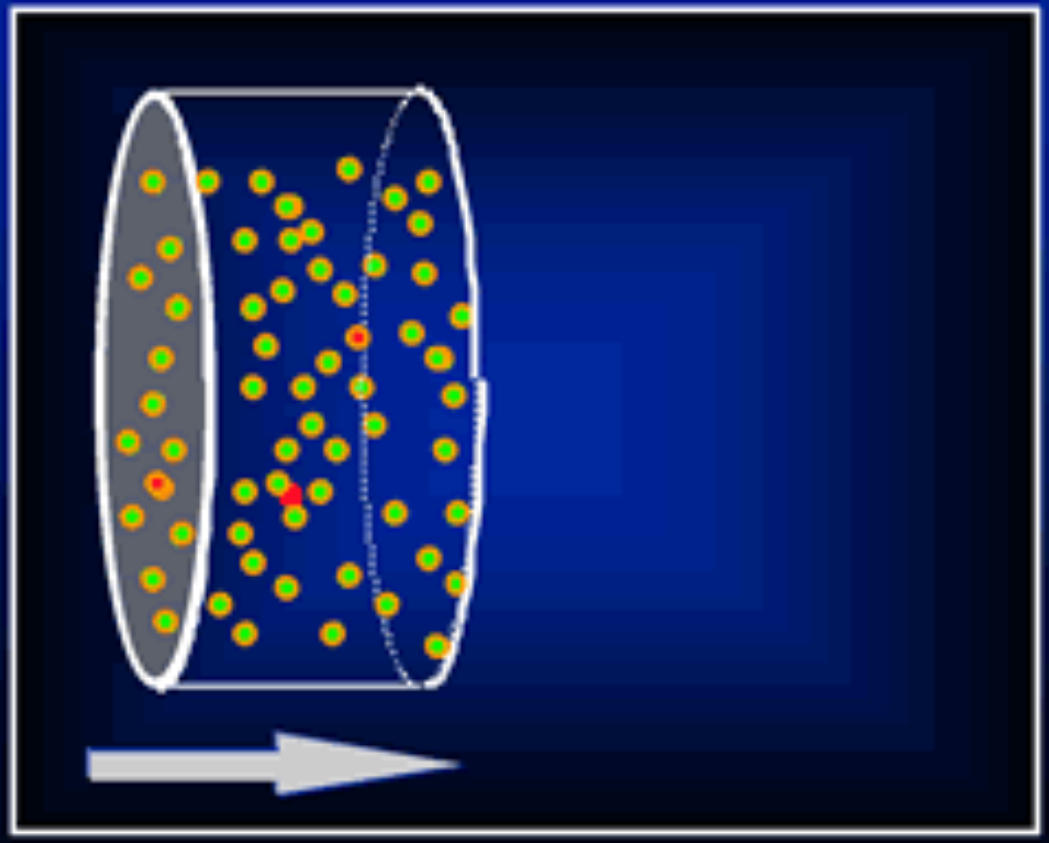
Nachega JB, et al. *Ann Intern Med*. 2007;146:564-573.

Direncin gelişimi




Therapeutic range
Probability (%)
100
75
50
25
0
100
1000
10000
Plasma concentration (ng/ml)
Viral suppression
Benefit
Adverse effects eg. EFV, ATV, IDV, TPV
Risk
4

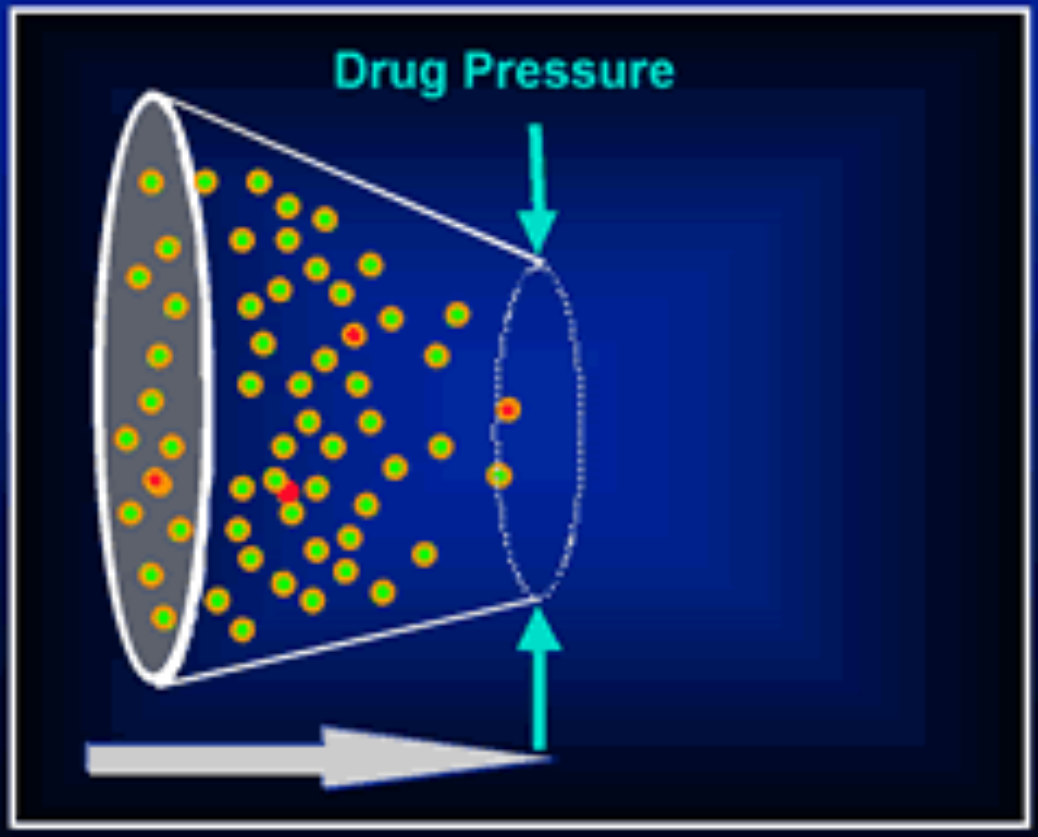
Development of Drug Resistance



- "wild" type virus (drug sensitive)
- "mutant" virus (drug resistant)

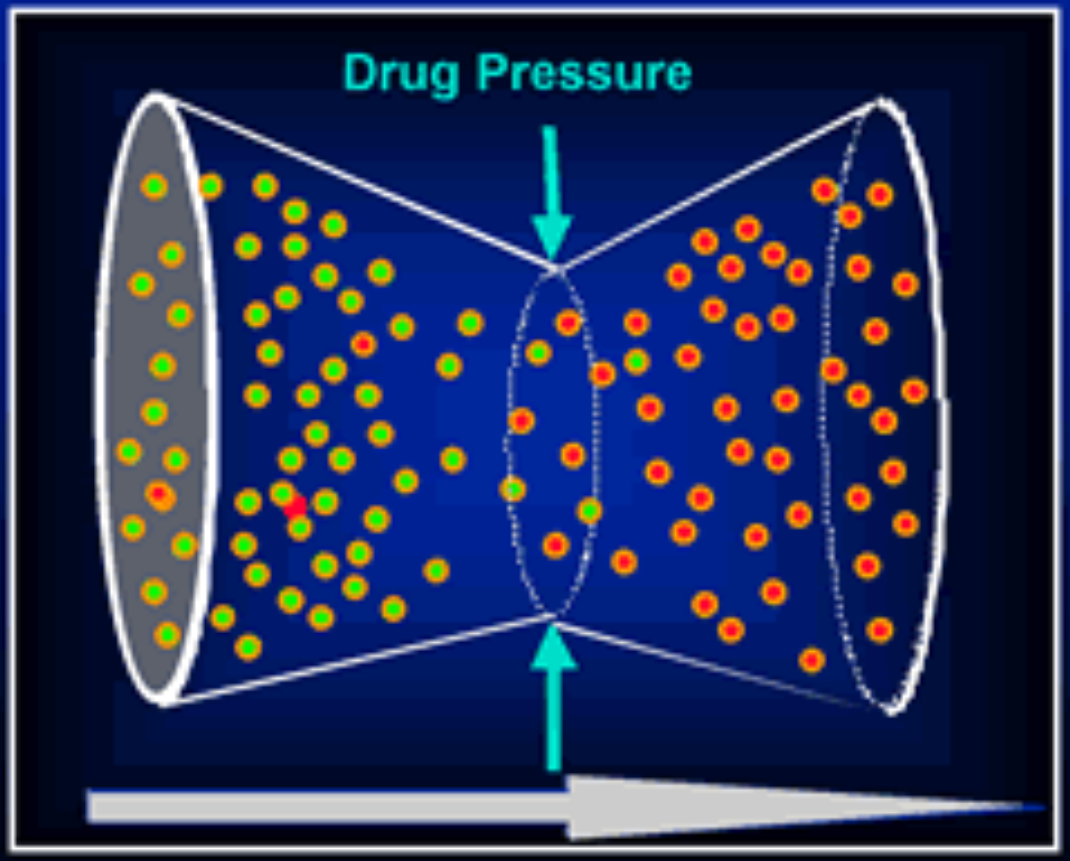
Time

Development of Drug Resistance



- "wild" type virus (drug sensitive)
- "mutant" virus (drug resistant)

Development of Drug Resistance

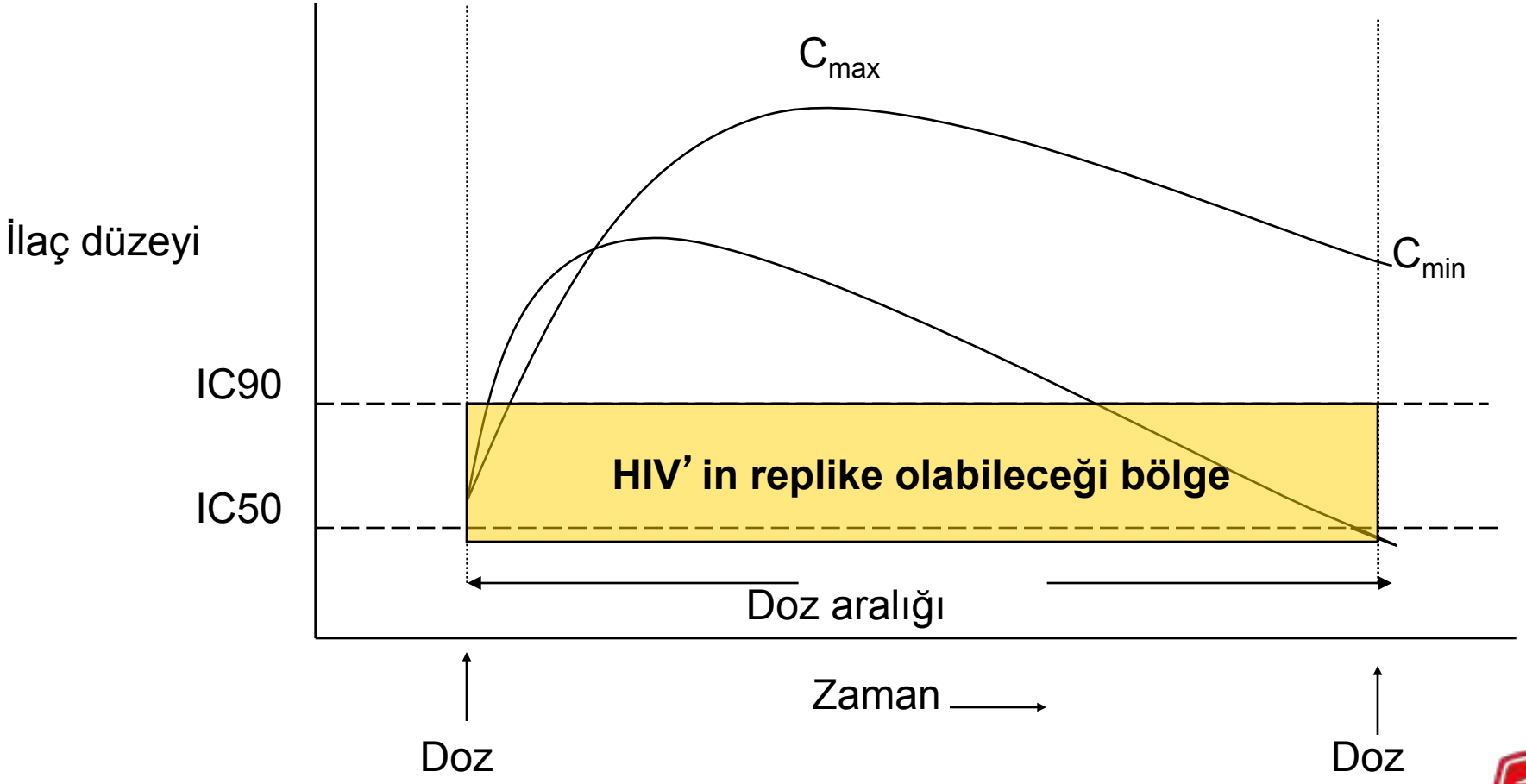


- "wild" type virus (drug sensitive)
- "mutant" virus (drug resistant)

Direncin ortaya çıkması

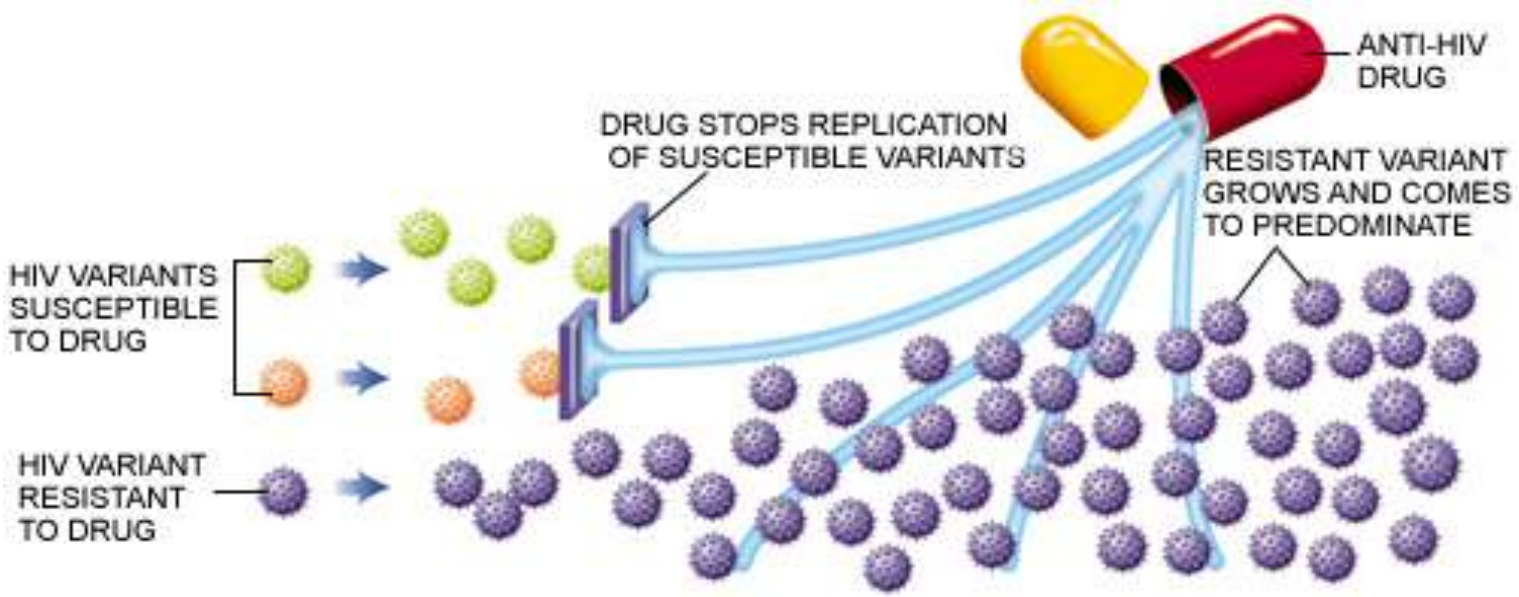
- Dirençli virüsün ortaya çıkışı
  - Genetik bariyer (seçicici baskı).
  - Rezidüel replikasyon (potens).

Temel farmakolojik prensipler



1. İlaçlar genetik bariyerlerine göre farklılaşırlar
    1. Tek bir nükleotid değişimiyle direnç gelişenler
    2. 2' den fazla nükleotid değişimiyle direnç gelişenler
    3. 1 den fazla mutasyon gerekenler

2. Escape mutantlar, replikasyona devam edebilmek için yeni mutasyonlar geliştirir.



How drug resistance arises. Richman, DD. Scientific American , July 1998

Direnç mutasyonlarının kaybolması

- Tedavi kesilirse
- Tedavi değiştirilirse
- Wild-type baskın hale geçerse
- Minority variant halde kalırlar

Ne zaman test yapalım?

- İlk saptandığında
- Tedavi başlanmadan önce
- Bazalde INSTI direncine bakmaya gerek yok
- Virolojik başarısızlık anında
- INSTI altında virolojik başarısızlık varsa INSTI sekans
- Suboptimal supresyon
- Viral yüksınırı 500kopya/ml

Tedavi almamış birine neden test yapalım?

ABD ve Fransa'da aktarılan direnç

CDC: Prevalence of TDR in MSM (*N*= 10,894) in US (2008-2011)

≥ 1 RAM	#	%
Any	1,894	17.4%
NNRTI	984	9.0%
NRTI	722	6.6%
PI	498	4.6%

ANRS Survey: TDR from Patients (*N*=799) with Primary HIV Infection in France (2010-2013)

≥ 1 RAM, %	10.6
PI	2.0
NRTI	5.1
NNRTI	4.0
INSTI	1.5

- Risk factors for transmitted resistance include: MSM and B subtype
- Prevalence of TDR mutations increased slightly in the US as observed by the CDC, whereas this has remained stable in France since 1996
- NNRTI transmitted resistance continues to be most prevalent in the US, so other treatment options may be considered for first-line therapy

1. Banez-Ocfemia C, et al. CROI; 2014 Boston. #579
2. Chaix M, et al. CROI 2014; Boston. #582

Türkiye' de aktarılan direnç 2014

- 10/190 örnekte direnç mevcut

No	City/Region	Subtype	NRTI mutation	NNRTI mutation	PI mutation
1	Adana/Med.	C	M184V	K103N, Y181C	-
2	Adana/Med.	B	L210W, T215S	-	-
3	Adana/Med.	CRF_BF	-	K103S	-
4	Amasya/Blacksea	A1	-	-	M46L
5	Ankara/Middle	B	M41L, T215C	-	-
6	Ankara/Middle	CRF_B	K70E	-	-
7	Ankara/Middle	A1	-	Y181C	-
8	Ankara/Middle	B	-	-	L90M
9	Ankara/Middle	CRF_BF	-	K103N	-
10	Ankara/Middle	A1	-	-	D30DN
11	Ankara/Middle	B	-	K103N	-
12	Erzurum/East	B	T215E	-	-
13	Erzurum/East	CRF_BF	-	K103KN	-
14	İstanbul/Marmara	B	M41L, T215D	-	-
15	İstanbul/Marmara	B	T215E	-	-
16	Mersin/Med.	B	M41L, T215C	-	-
17	Mersin/Med.	B	M41L, T215CS	-	-
18	Samsun/Blacksea	A1	-	-	M46L
19	Tokat/Blacksea	A1	K70E	-	-

Direnç nasıl çalışılır?

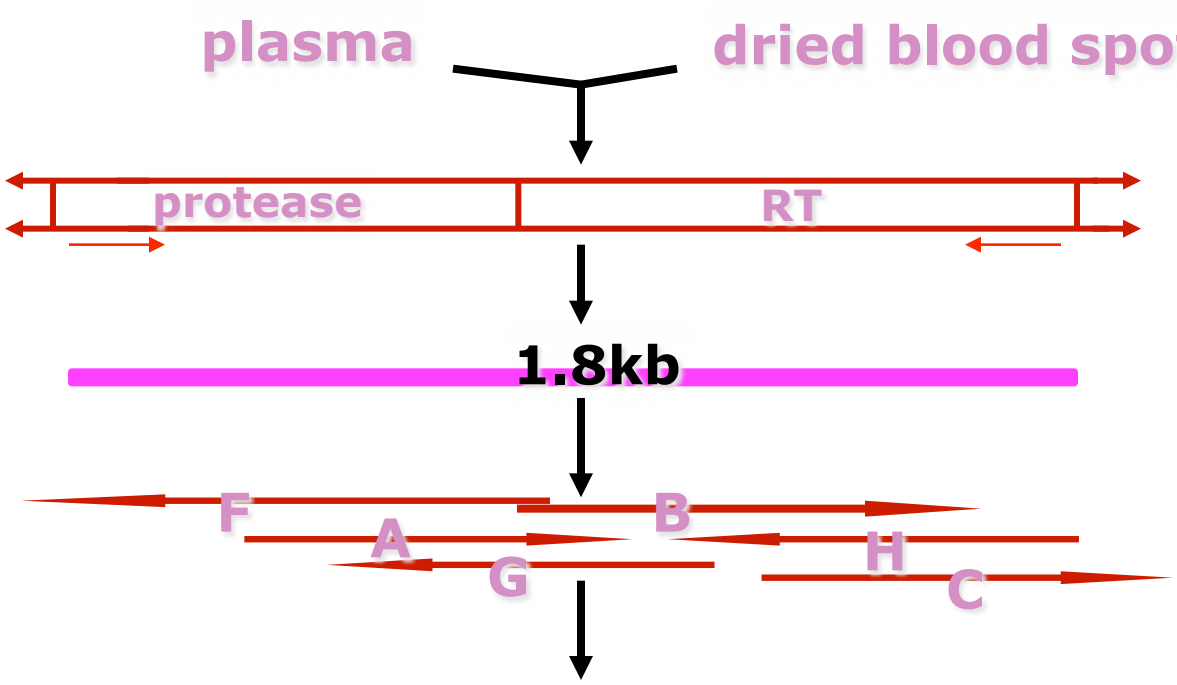
Genotipik direnç testleri

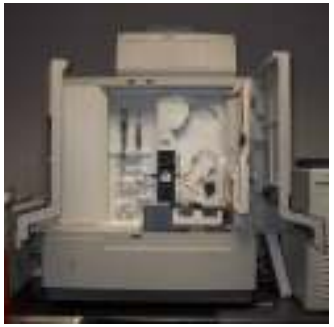
1. Tüm nükleotid değişimlerini gösterebilir
2. %20' lik popülasyonu gösterir
3. Erken uyarı
4. Raporları anlamak güç, lablar arası tartışma
5. Yüksek viral yük gerekir

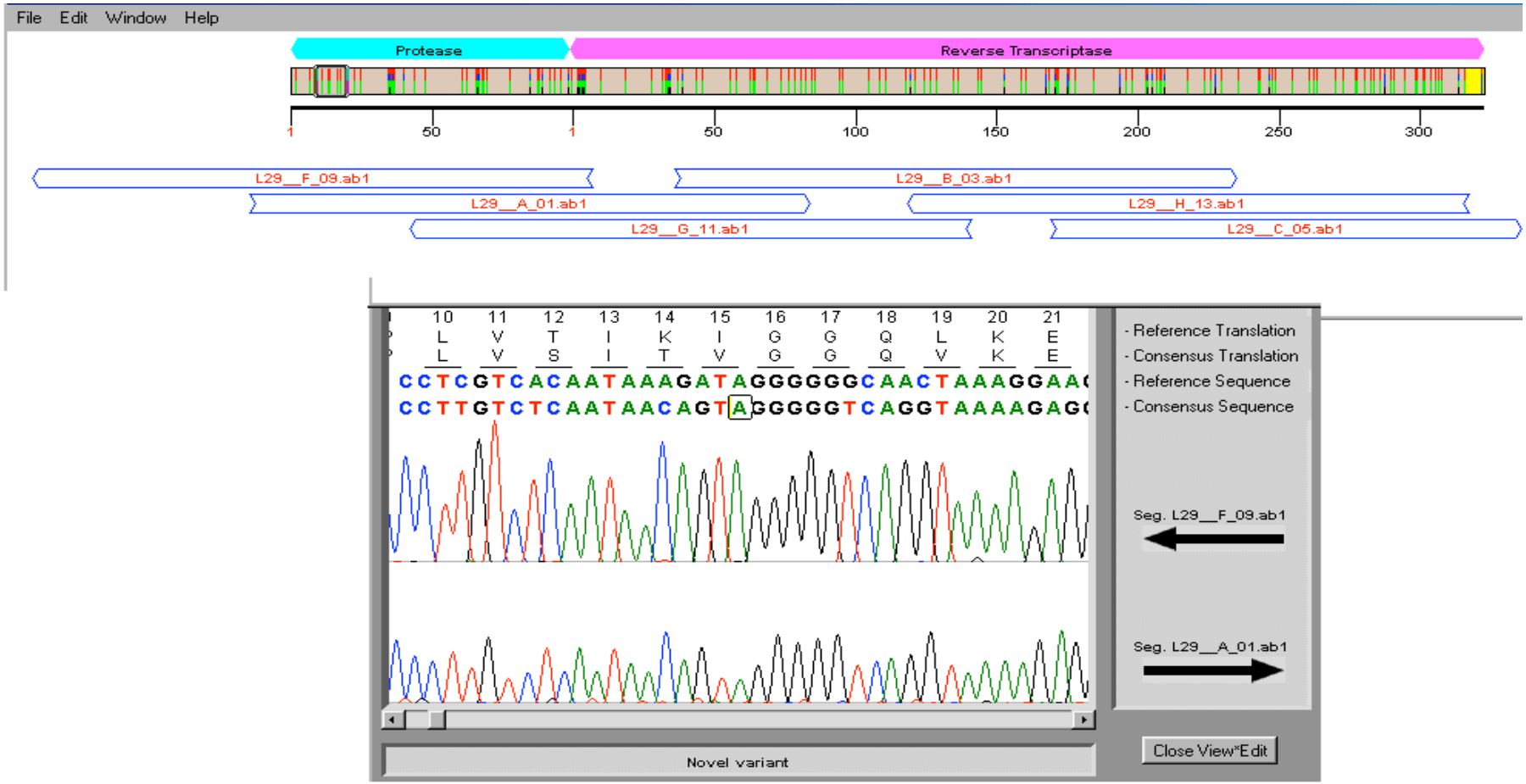
Fenotipik direnç testleri

1. Tüm virüsler hakkında bilgi verir
2. Yapılması güç ve zahmetli
3. Çapraz direnç araştırmaları için ideal
4. Birden fazla direncin olduğu durumlarda kullanışlı

Viroseq ile genotiplendirme



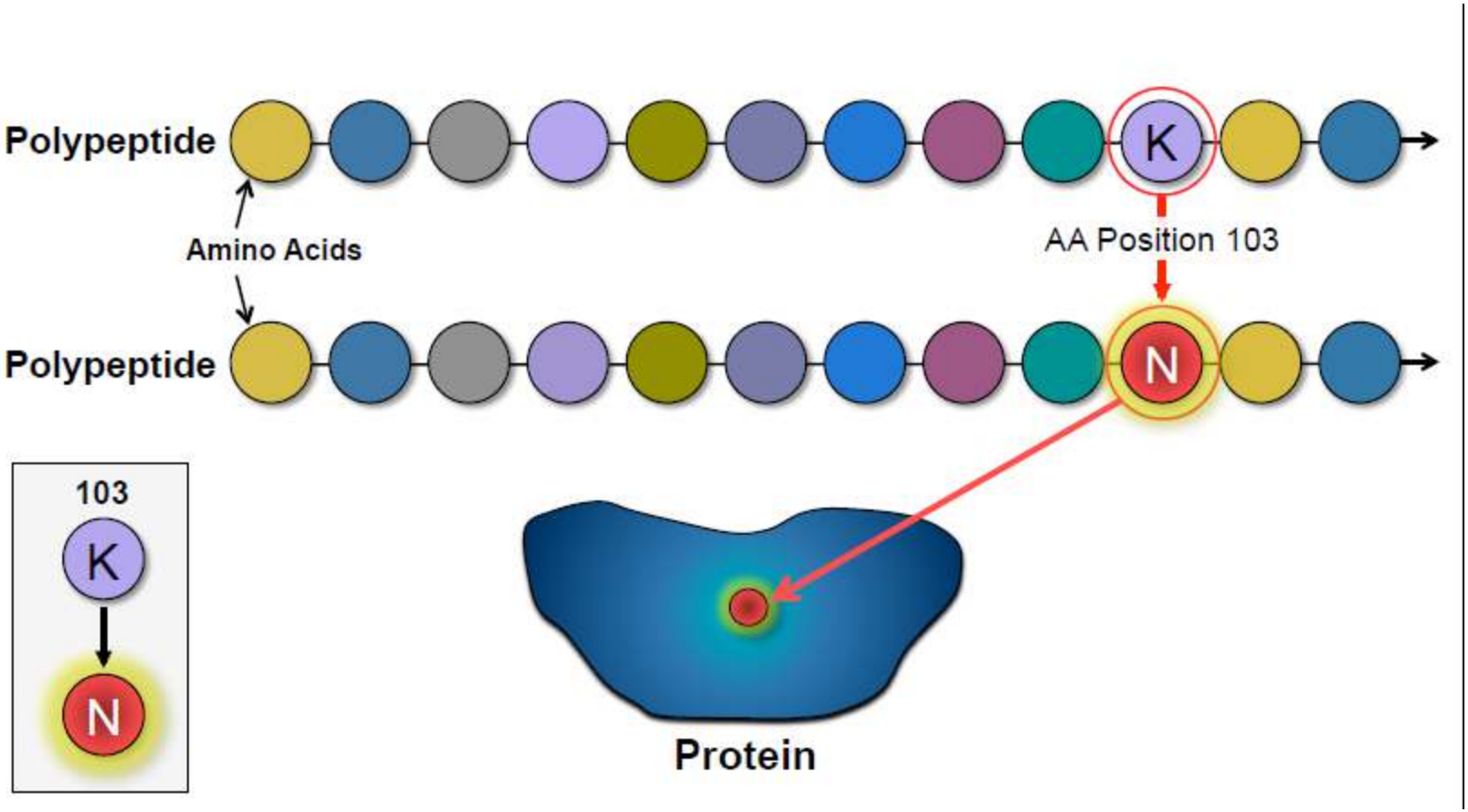


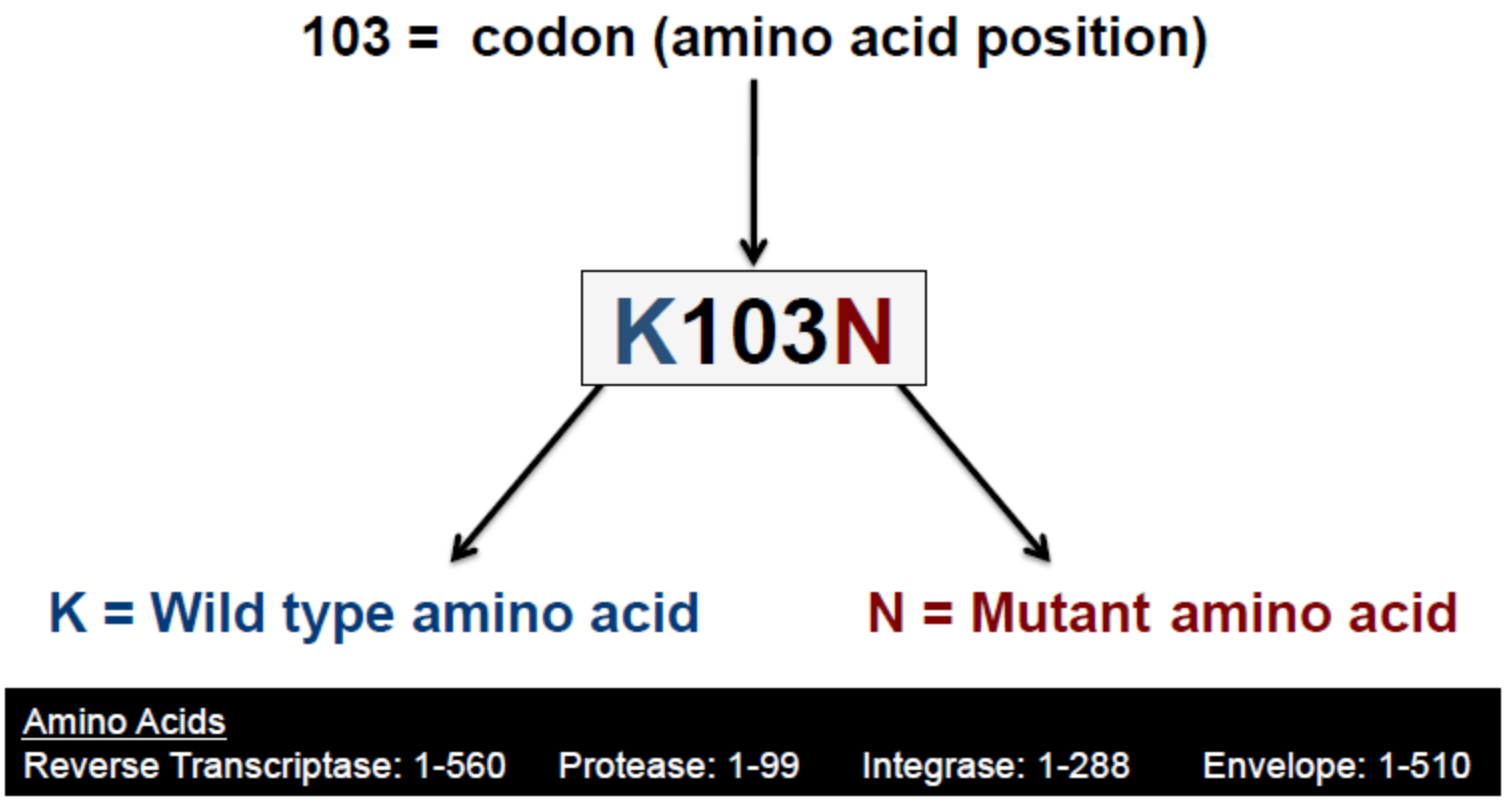

File Edit Window Help
Protease
Reverse Transcriptase
1 50 1 50 100 150 200 250 300
L29__F_09.ab1
L29__B_03.ab1
L29__A_01.ab1
L29__H_13.ab1
L29__G_11.ab1
L29__C_05.ab1
10 11 12 13 14 15 16 17 18 19 20 21
L V T I K I G G Q L K E
L V S I T V G G Q V K E
CCTCGTCACAATAAAGATAGGGGGGCAACTAAAGGAAG
CCTTGTCTCAATAACAGTAGGGGGTCAGGTAAAAGAGG
- Reference Translation
- Consensus Translation
- Reference Sequence
- Consensus Sequence
Seg. L29__F_09.ab1
Seg. L29__A_01.ab1
Novel variant
Close View*Edit

Genotipik direnç testinin değerlendirilmesi

- **Kural zeminli okuma**
  - TruGene (Bayer)
  - Viroseq (Celera)
  - HIVdb (Stanford)
  - ANRS (French)
  - Rega (Belgium)

Direnç nasıl okunur?




103 = codon (amino acid position)
K103N
K = Wild type amino acid
N = Mutant amino acid
Amino Acids
Reverse Transcriptase: 1-560
Protease: 1-99
Integrase: 1-288
Envelope: 1-510

http://hivdb.stanford.edu/

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.

HOME | GENOTYPE-RX | GENOTYPE-PHENO | GENOTYPE-CLINICAL | HIVdb PROGRAM

HIVdb Program Integrase Update
Mutation classification, Scores, Comments and References.

HIVdb User Guide (link to PDF)
Database query and reference pages, Interactive program, Educational resources

Crystallographic Structures
RT, protease, and integrase

More news »

GENOTYPE-TREATMENT CORRELATIONS

- Retrieve sequences (and/or mutations) from persons receiving selected HIV drugs
- Retrieve sequences and treatments from viruses with specific mutations

GENOTYPE-CLINICAL CORRELATIONS

- Summaries of genotype-clinical outcome studies
- Genotype-clinical outcome datasets (download)

GENOTYPE-PHENOTYPE CORRELATIONS

- Retrieve drug susceptibility data for isolates with selected mutations
- Download genotype-phenotype research datasets

REFERENCES

- Published drug resistance studies in HIVRT&PrDB
- Published studies by Stanford database group

NEW SUBMISSIONS

- Fujisaki, et al. 11-year surveillance of HIV subtypes in Japan

SURVEILLANCE MUTATIONS

- World Health Organization 2009 Mutation List

HIVdb PROGRAM Genotype Resistance Interpretation

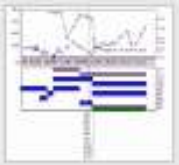
This program interprets user-entered mutations to infer the level of resistance to NRTIs, NNRTIs, PIs. Web Service is available.

MARVEL

MARVEL (Mutation ARV Evidence Listing) » Go To Program

ART-AiDE

Antiretroviral Therapy - Acquisition & Display Engine
» Go To Program



HIVseq Program

Provides mutation frequencies by subtype. » Go To Program

HIValg Program

Compare HIVdb, ANRS, Rega, or create

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.

HIVdb Program

Genotypic Resistance Interpretation Algorithm

Version 6.0.3 (last updated 09/01/09)

HIVdb accepts user-submitted protease and RT sequences and returns inferred levels of resistance to 19 commonly used protease and RT inhibitors. Its purpose is educational and as such it provides extensive comments and a highly transparent scoring system that is hyperlinked to data in the HIV Drug Resistance Database. In clinical settings, genotypic data must be used in conjunction with a patient's clinical history (including past treatments) and a solid understanding of the principles of antiretroviral treatment (http://www.aidsinfo.nih.gov/guidelines/).

The drug resistance interpretation system used here is similar to the one used by the Stanford University Hospital (SUH) Diagnostic

Choose A Method

ANALYSIS MUTATION LIST — Enter Protease & RT Mutations

OR

ANALYSIS SEQUENCES — Enter Complete Sequences

Seq·ID:·234-14-VETA· VETA¶

¶

Summary·Data¶

Sequence includes PR: codons: 1 -- 99¤

Sequence includes RT: codons: 1 -- 335¤

¶

There are no insertions or deletions¤

¶

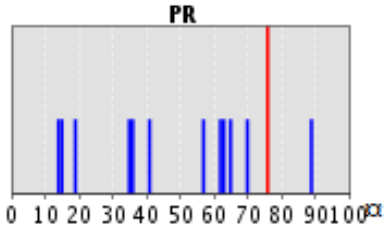
Subtype and % similarity to closest reference isolate¶

1.→*PR: F (91.2%)*¶
2.→*RT: B (95.0%)*¤

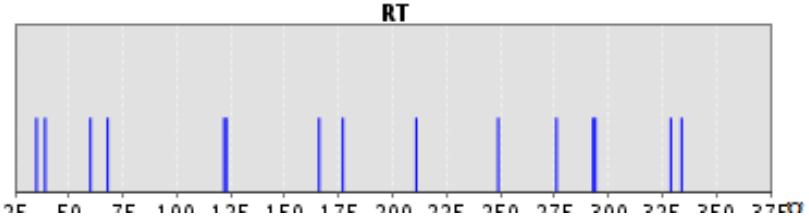
¶

Sequence·Quality·Assessment¶

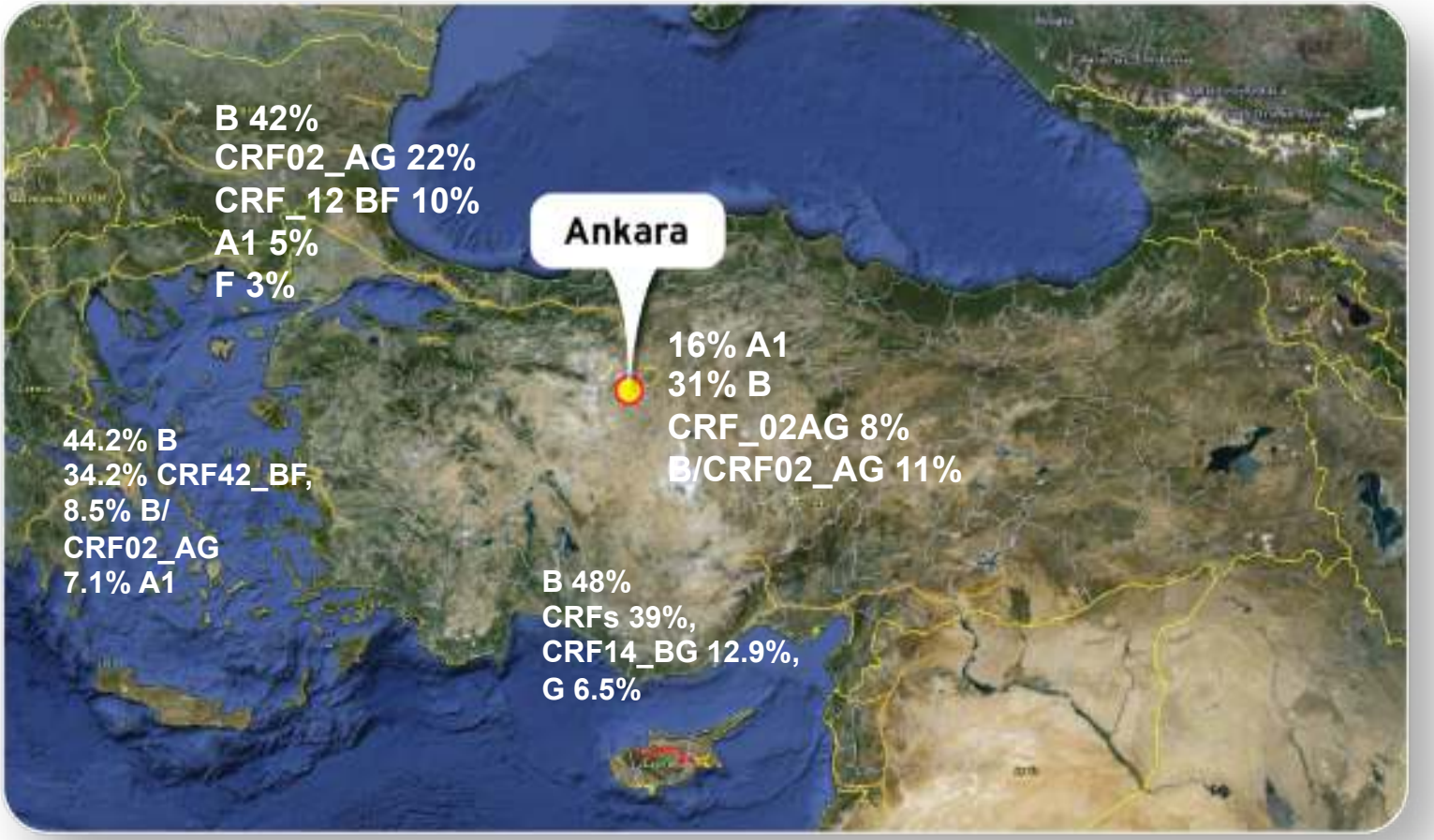
Gene	QA Problem	Codons
PR	Stop Codons, Frame Shifts:	76
PR	Ambiguous Positions:	*None*
PR	Unusual Residues:	*None*



Gene	QA Problem	Codons
RT	Stop Codons, Frame Shifts:	*None*
RT	Ambiguous Positions:	*None*
RT	Unusual Residues:	*None*



Türkiye' de HIV genotipleri

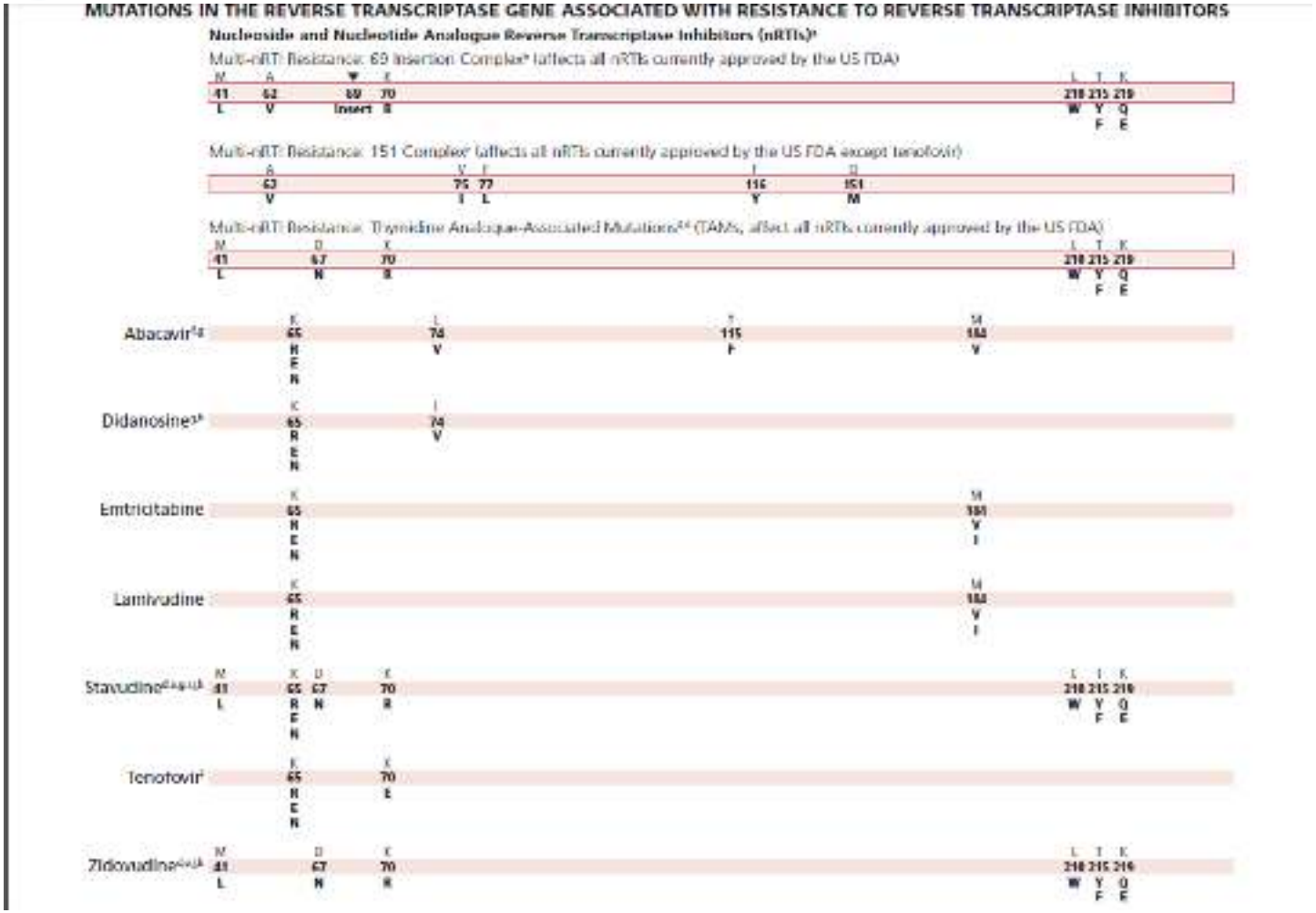


M Sayan 2013, KT Yalçınkaya 2014, D İnan 2014, M Sayan 2014, S Uluer Biçeroğlu 2014

Special Contribution

2014 Update of the Drug Resistance Mutations in HIV-1

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



Drug Resistance Interpretation: PR

PI Major Resistance Mutations:	**M46I, I54V, V82A**
PI Minor Resistance Mutations:	**L10F, T74S**
Other Mutations:	T12S, I15V, L19I, K20R, E35D, M36I, R41K, L63S, H69K, L89M, I93L

Protease Inhibitors	
atazanavir/r (ATV/r)	Intermediate resistance
darunavir/r (DRV/r)	Potential low-level resistance
fosamprenavir/r (FPV/r)	Intermediate resistance
indinavir/r (IDV/r)	High-level resistance
lopinavir/r (LPV/r)	Intermediate resistance
nelfinavir (NFV)	High-level resistance
saquinavir/r (SQV/r)	Intermediate resistance
tipranavir/r (TPV/r)	Low-level resistance

PR Comments

PIMajor

- M46I/L decreases susceptibility to IDV/r, NFV, FPV/r, LPV/r, and ATV/r when present with other mutations.
- I54V contributes resistance to each of the PIs except TPV/r and DRV/r.
- V82A reduces susceptibility to IDV/r and LPV/r. With other mutations it is associated with reduced susceptibility to NFV, ATV/r, SQV/r, and FPV/r.

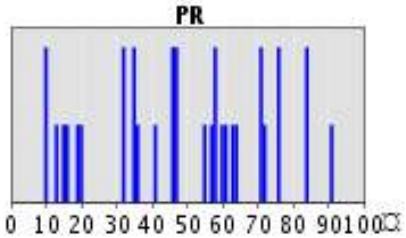
PIMinor

- L10I/V/F/R/Y are associated with resistance to most PIs when present with other mutations. L10I/V occur in 5-10% of untreated persons. L10F/R/Y are nonpolymorphic.
- T74S is associated with reduced NFV susceptibility. It occurs in untreated persons with subtype C viruses.

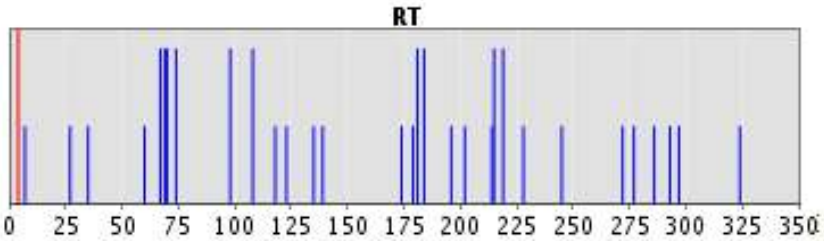
Örnek olgu

Sequence Quality Assessment

Gene	QA Problem	Codons
PR	Stop Codons, Frame Shifts:	*None*
PR	Ambiguous Positions:	*None*
PR	Unusual Residues:	*None*



Gene	QA Problem	Codons
RT	Stop Codons, Frame Shifts:	*None*
RT	Ambiguous Positions:	4
RT	Unusual Residues:	*None*



Blue lines indicate differences from consensus B; tall blue lines indicate sites associated with drug resistance. Red lines indicate QA problems.

Drug Resistance Interpretation: PR

PI Major Resistance Mutations:	**V32IV, M46I, I47V, L76V, I84V**
PI Minor Resistance Mutations:	**L10F, E35DG, Q58EQ, A71AITV**
Other Mutations:	I13V, I15V, G16E, L19I, K20I, M36I, R41K, K55R, R57K, D60E, Q61H, L63N, I64V, I72R, T91A

atazanavir/r (ATV/r)	High-level resistance
darunavir/r (DRV/r)	High-level resistance
fosamprenavir/r (FPV/r)	High-level resistance
indinavir/r (IDV/r)	High-level resistance
lopinavir/r (LPV/r)	High-level resistance
nelfinavir (NFV)	High-level resistance
saquinavir/r (SQV/r)	High-level resistance
tipranavir/r (TPV/r)	High-level resistance

PR Comments

PIMajor

- V32I is a substrate cleft mutation which is associated with reduced susceptibility to all PIs except SQV/r.
- M46I/L decreases susceptibility to IDV/r, NFV, FPV/r, LPV/r, and ATV/r when present with other mutations.
- I47V decrease susceptibility to FPV/r, ATV/r, IDV/r, LPV/r, TPV/r, and DRV/r.
- L76V reduces susceptibility to FPV/r, IDV/r, LPV/r, and DRV/r and increases susceptibility to SQV/r, ATV/r, and TPV/r.
- I84V causes intermediate/high-level resistance to ATV/r, FPV/r, IDV/r, NFV, and SQV/r; and low-level resistance to LPV/r, TPV/r, and DRV/r.

Mutation Scoring

PR	ATV/r	DRV/r	FPV/r	IDV/r	LPV/r	NFV	SQV/r	TPV/r
V32IV	15	20	30	15	15	15	0	5
M46I	10	0	10	10	10	15	0	0
I47V	5	10	30	10	15	15	0	20
L76V	-5	20	60	30	30	0	-5	-5
I84V	40	10	60	30	15	60	60	30
L10F	0	0	10	5	5	10	0	0
E35DG	0	0	0	0	0	5	0	5
Q58EQ	5	0	0	0	0	5	0	10
A71AITV	5	0	5	0	0	5	5	0
K20I	-	-	-	-	-	10	-	-
L76V+M46I	-	-	-	10	10	-	-	-
I47V+V32IV	-	-	-	10	10	-	-	-
Total:	75	60	205	120	110	140	60	65

RT	3TC	ABC	AZT	D4T	DDI	FTC	TDF	EFV	ETR	NVP	RPV
D67N	0	5	15	15	5	0	5	-	-	-	-
T69D	0	0	0	10	30	0	0	-	-	-	-
K70R	0	5	30	15	5	0	10	-	-	-	-
L74I	0	30	0	0	60	0	0	-	-	-	-
M184V	60	15	-10	-10	10	60	-10	-	-	-	-
T215F	5	15	45	45	15	5	15	-	-	-	-
K219Q	0	5	10	10	5	0	5	-	-	-	-
A98G	-	-	-	-	-	-	-	5	5	15	5
V108I	-	-	-	-	-	-	-	5	0	10	0
Y181C	-	-	-	-	-	-	-	30	30	60	30
V118I	5	5	5	5	5	5	5	-	-	-	-
D67N+K70R+K219Q	-	10	10	10	10	-	10	-	-	-	-
L74I+M184V	-	15	-	-	-	-	-	-	-	-	-
Total:	70	105	105	100	145	70	40	40	35	85	35

Bu tablo kime ait?

A. Michelangelo
B. Leonardo da Vinci
C. Marcel Duchamp
D. Frida Kahlo

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1919, L.H.O.O.Q

Minority variant

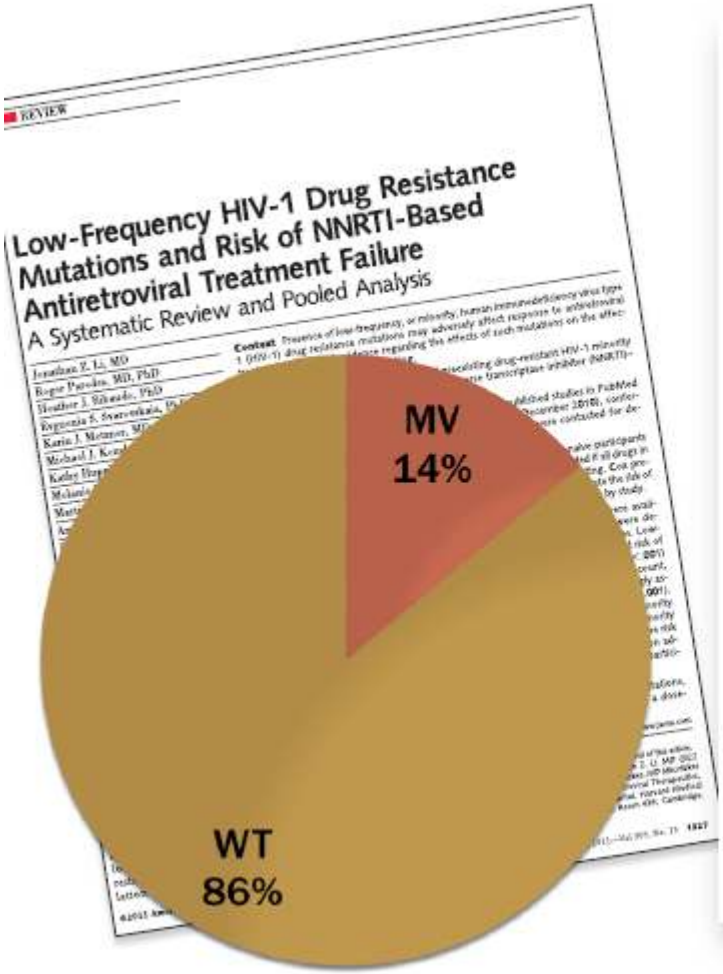
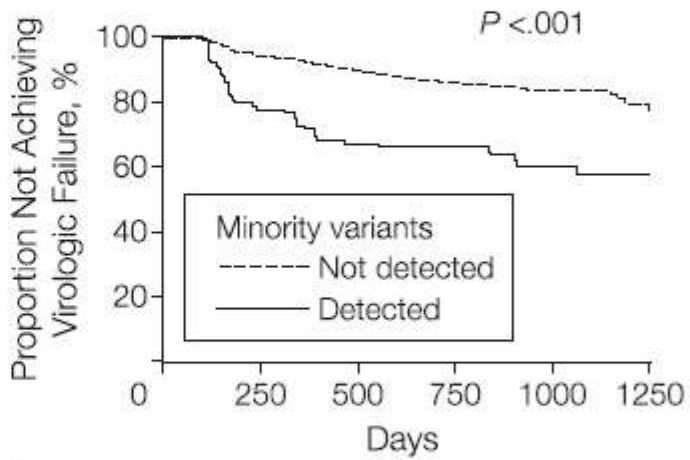


Figure 2. Kaplan-Meier Curves for Proportion of Patients Without Virologic Failure by Presence of Drug-Resistant HIV-1 Minority Variants



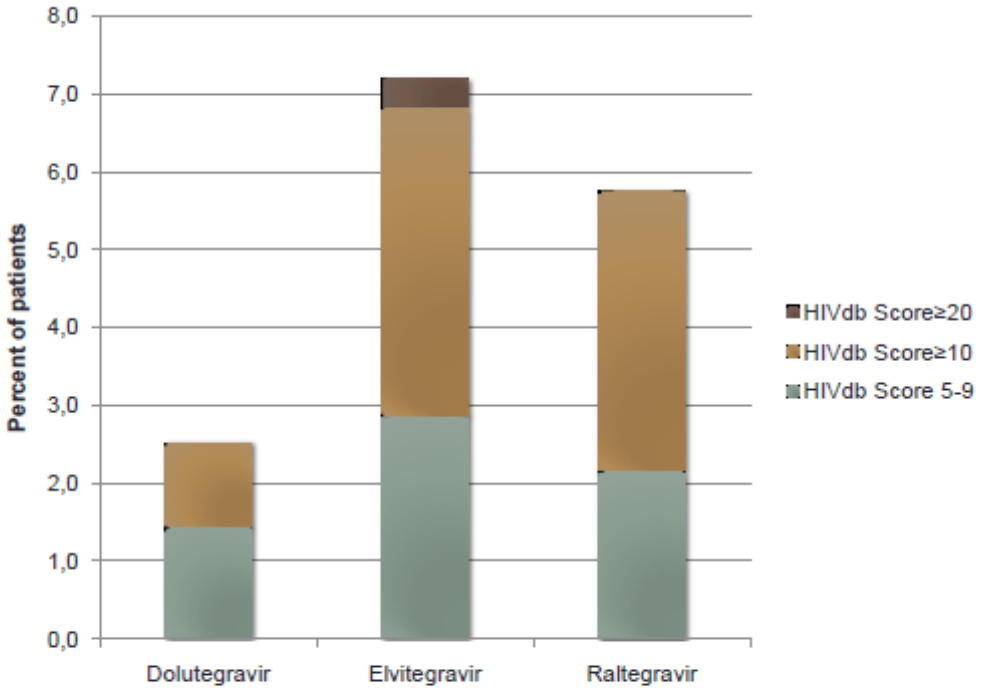
No. at risk

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

Li J et al. JAMA 2011

INSTI: Sanger, n=300 Europeans

	n		
IAS USA Integrase mutations	5	2%	74M (2), 97A (2), 138A
Any INI related mutation	42	15%	

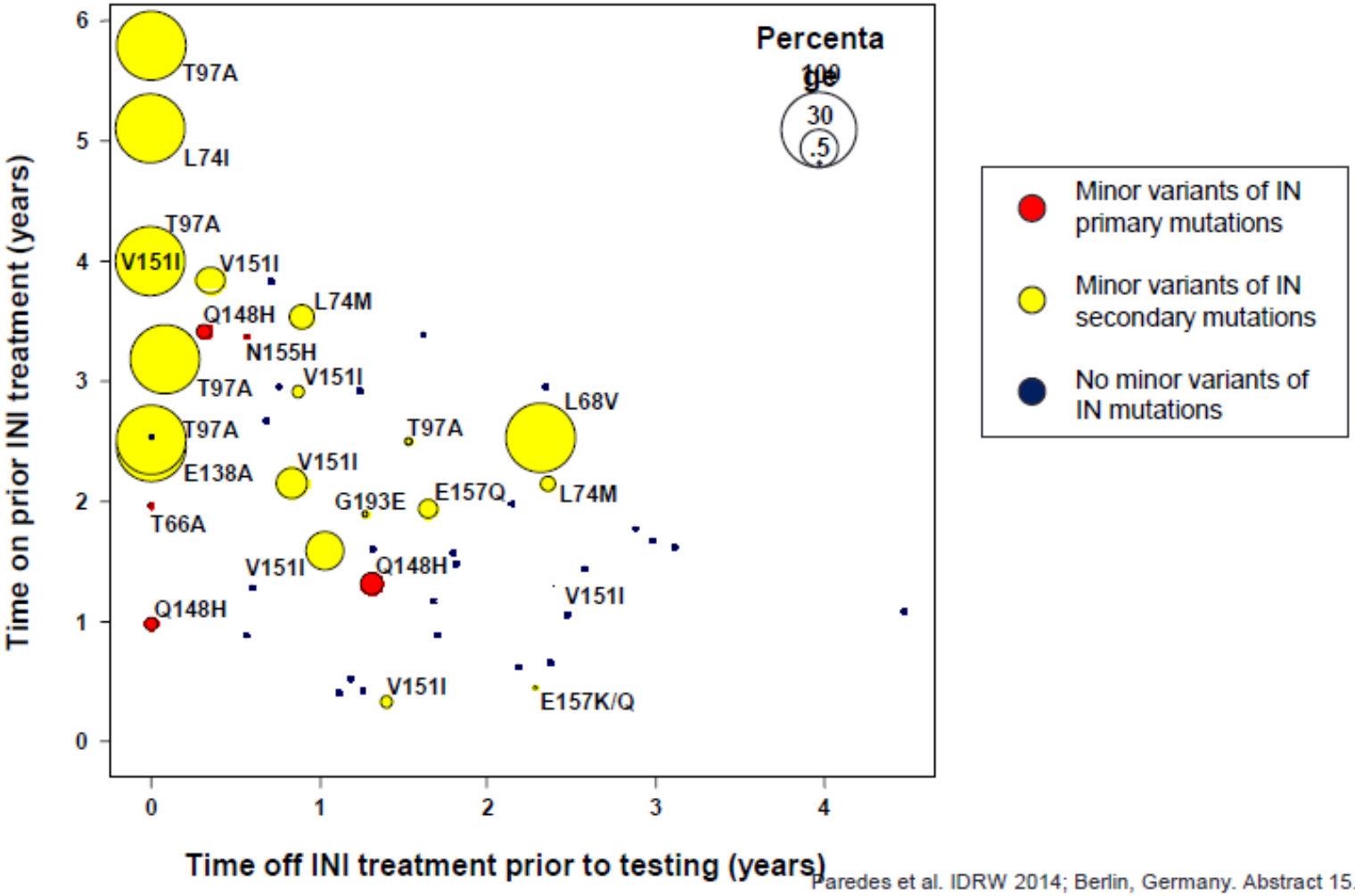


HIV db score ≥10:
L74M (n=2; 1%), T97A (n=2; 1%), E138A, A153F, E157Q (n=2; 1%), G163KT, R263K, V151I + G163EKR (n=1; 0.3%)

HIVdb score <10:
L68I, L68V, L74I (n=13; 4.6%), L74V, T97S, A128T, E138D (n=2; 1%), E138G, G140W, V151I (n=6; 2%), 68V + L74V, 143DN, Q146E (n=1)

Casadellà M, JAC 2015 (submitted)

Minor Variant Detection and Frequency by Duration of Prior INI Treatment



Paredes et al. IDRW 2014; Berlin, Germany. Abstract 15.

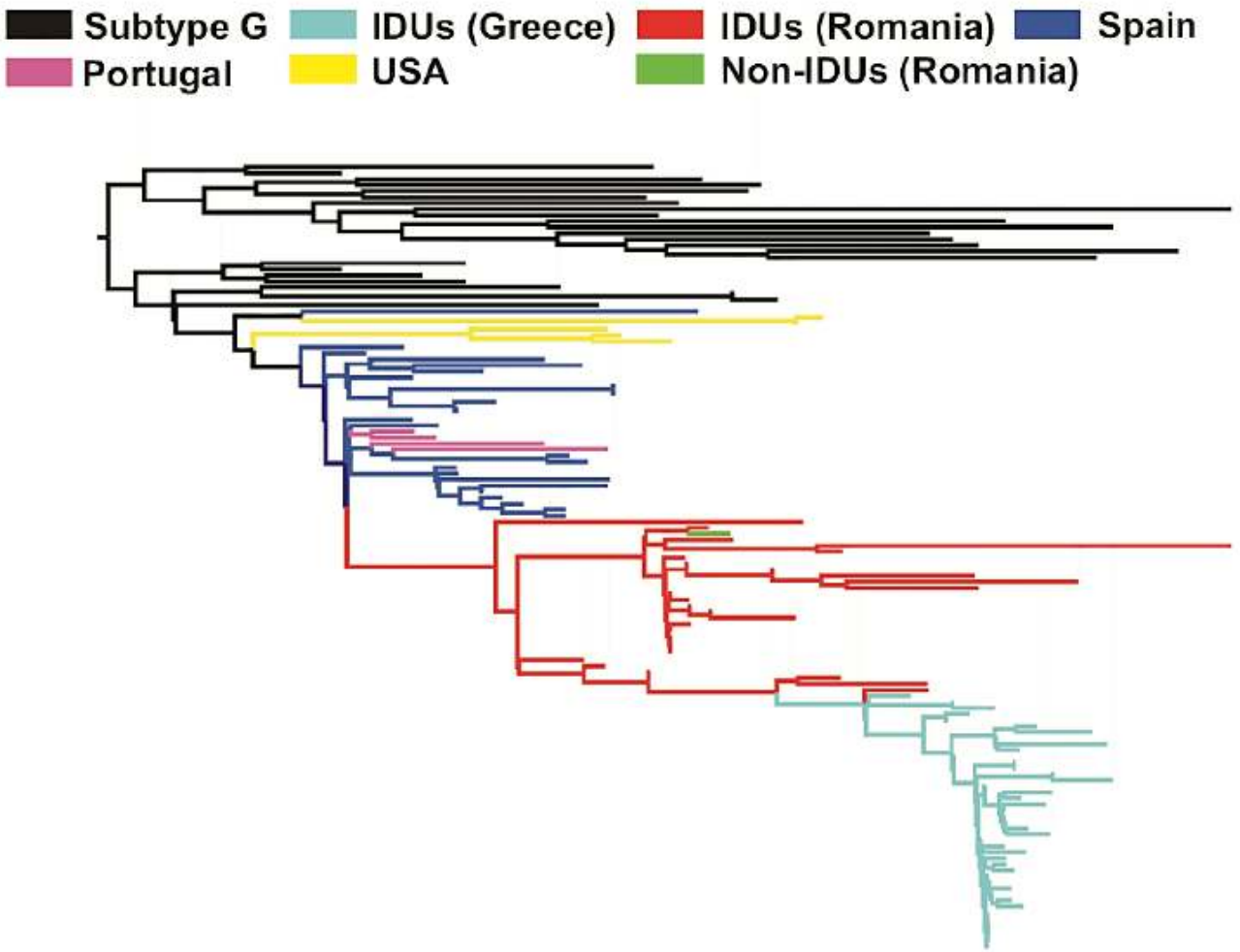
Direnç testinin faydaları

Patterns of HIV-1 transmission

POPULATIONS	HIV SPREAD (n, %)		
	Clustered infections	Non-clustered infections	Total
IDUs 1998-2009	4 (5.3%)	72 (94.7%)	76
IDUs 2010	5 (41.3%)	7 (58.3%)	12
IDUs 2011	112 (91.1%)	11 (8.9%)	123
IDUs 2012	251 (94.7%)	14 (5.3%)	265
IDUs (11th 2013)	159 (94.1%)	10 (5.9%)	169

Paraskevis *et al*, Plos One 2013

CRF14_BG



Paraskevis *et al*, submitted

IDU Clusters	IDUs within the cluster (%)	Geographic origin	*Re max. (range)*	Sampling proportion	tMRCA (95% HPD)
CRF14_BG	415 (50.1%)	Portugal Romania	3.36 (0.64-3.36)	0.637	10/2009 (12/2008-4/2010)
CRF35_AD	128 (15.5%)	Afghanistan /Iran	3.60 (0.72-3.60)	0.631	4/2010 (9/2009-9/2010)
Subtype B	109 (13.2%)	Greece	2.92 (0.49-2.92)	0.602	5/2008 (11/2006-9/2009)
Subtype A	50 (6.0%)	Greece	3.37 (0.74-3.37)	0.633	6/2010 (9/2009-12/2010)

Bilgilendirme

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