Klinik HIV/AIDS Sempozyumu 2010

Antiretroviral Direnç Tespiti

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5 sınıftan 24 ilaç

- NRTI 7
- NNRTI 4
- PI 10
- FI 1
- CRA 1
- INI 1

Antiretroviral tedavi ile

- HIV'e bağlı morbiditede düşüş
- Sağkalım süresinin uzaması
- Yaşam kalitesinde iyileşme
- İmmun restorasyon
- HIV bulaşmasının engellenmesi

- **Antiretroviraller direnç gelişimine yol açmazlar**
- **Varolan dirençli suşları seçtirirler**


Konak bağışıklık sistemi
Yüksek mutasyon hızı
heterojen bir virus topluluğu
Dirençli mutantların seçilmesi
Yüksek viral turnover
İlaç tedavisinin baskısı

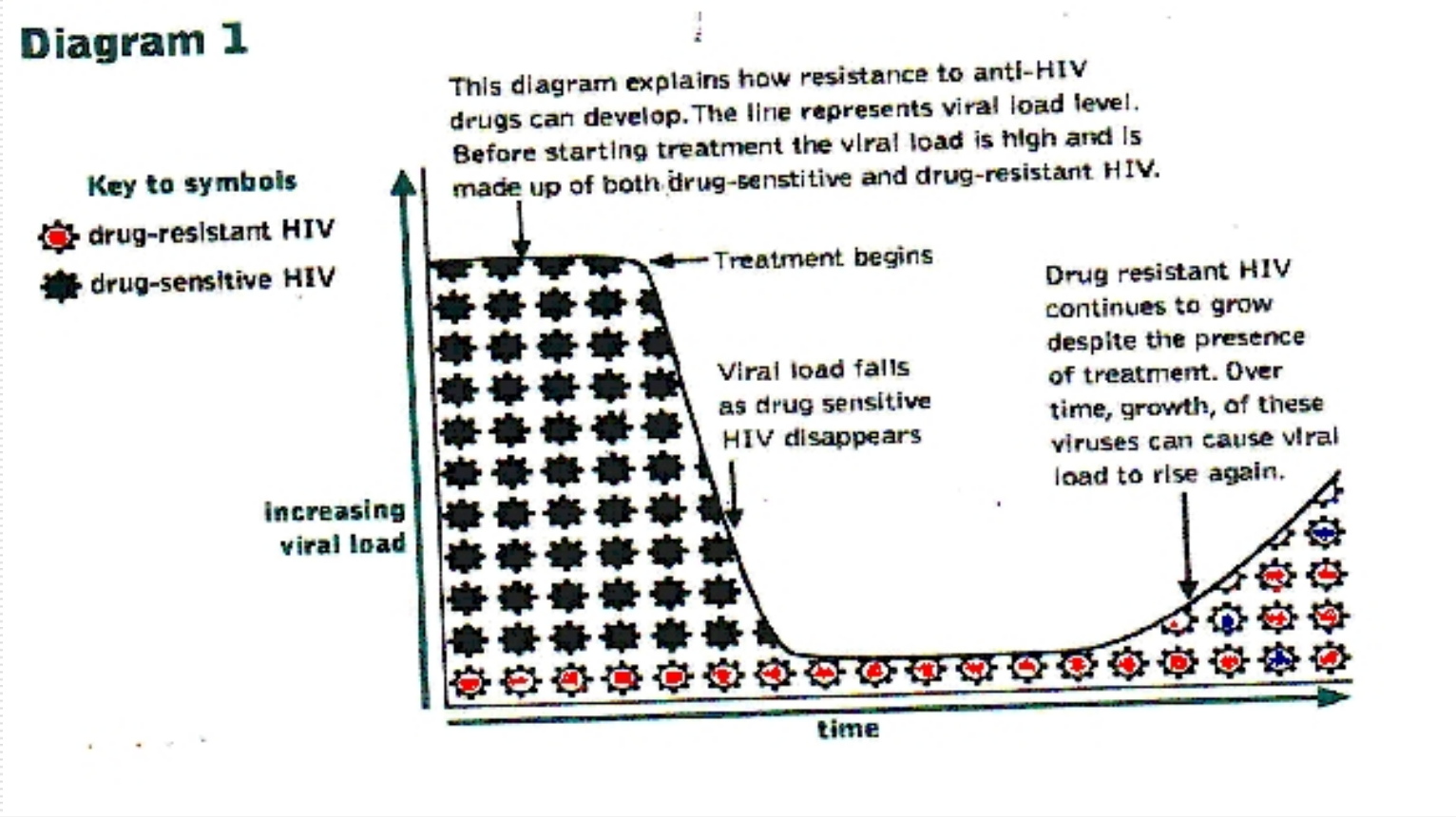
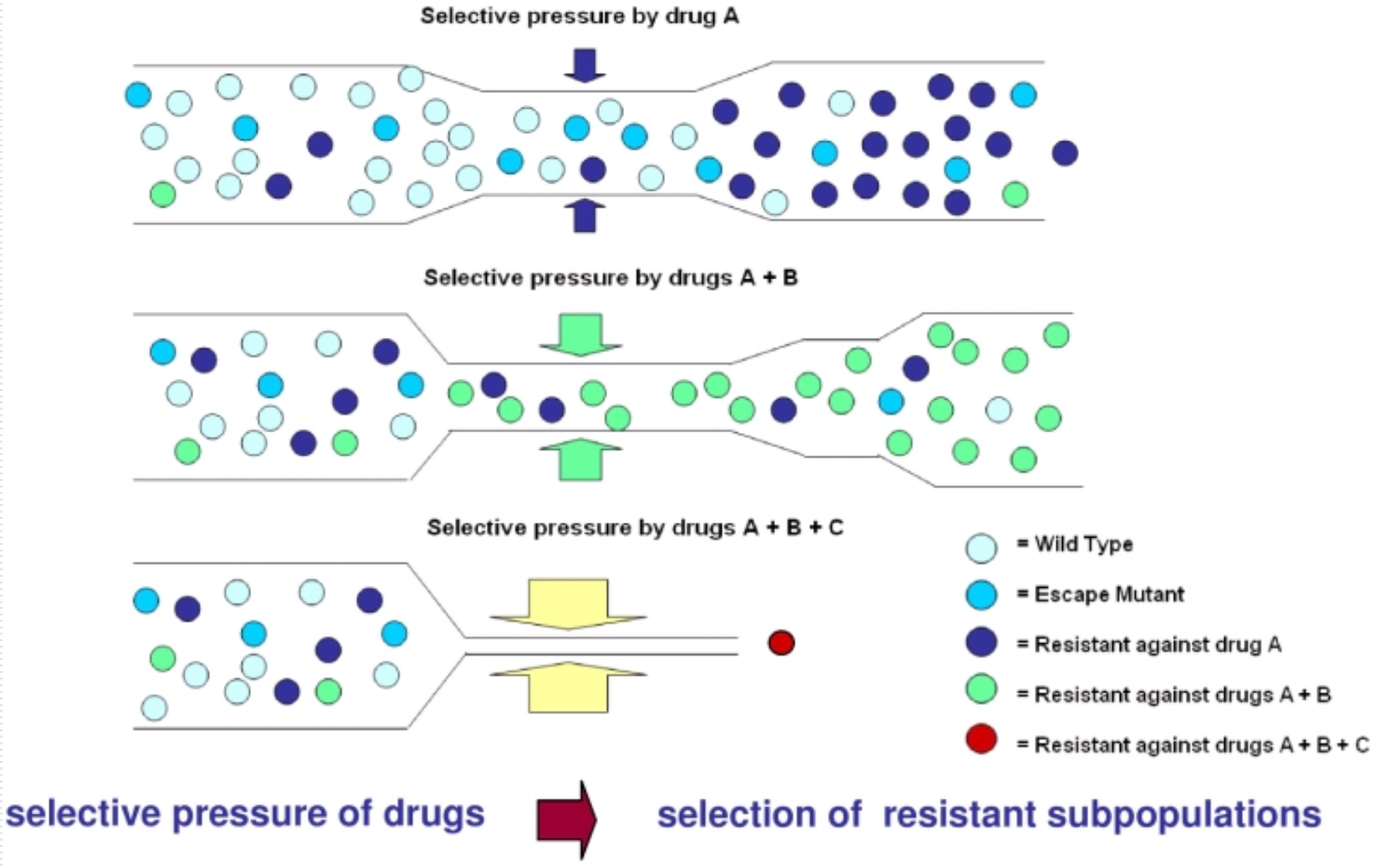
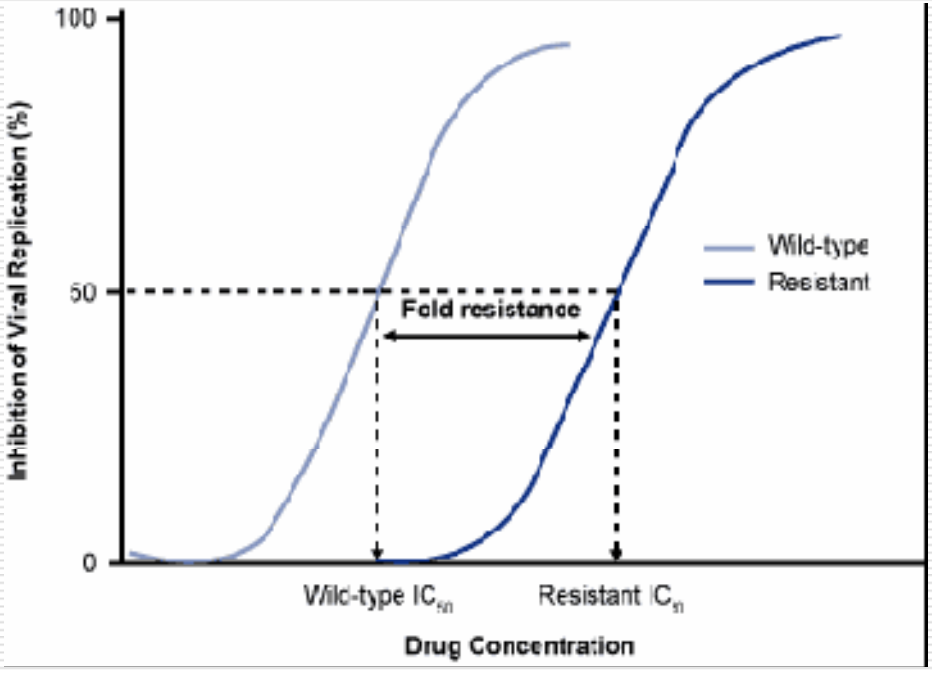


Diagram 1
This diagram explains how resistance to anti-HIV drugs can develop. The line represents viral load level. Before starting treatment the viral load is high and is made up of both drug-senstitive and drug-resistant HIV.
Key to symbols
drug-resistant HIV
drug-sensitive HIV
Treatment begins
Viral load falls as drug sensitive HIV disappears
Drug resistant HIV continues to grow despite the presence of treatment. Over time, growth, of these viruses can cause viral load to rise again.
increasing viral load
time


Selective pressure by drug A
Selective pressure by drugs A + B
Selective pressure by drugs A + B + C
= Wild Type
= Escape Mutant
= Resistant against drug A
= Resistant against drugs A + B
= Resistant against drugs A + B + C
selective pressure of drugs
selection of resistant subpopulations

- **Antiretrovirallere direnç: Virusun baskılanması için gereken ilaç konsantrasyonunda artış**

İlaç duyarlılığı: Yabanıl kökenin %50'sini baskılamak için gerekli ilaç konsantrasyonu (IC50)



İlaç direncinin kaynakları

- Dirençli suşun bulaşmış olması sonucu
- Daha önceki tedaviler sırasında seçilmiş
- Süperinfeksiyonla yeni dirençli bir suşun alınması (Özellikle ani VY yükselmelerinde)

Antiretroviral direnç gelişimi

- Kullanılan tedavinin göreceli potensi
- Tedaviye rağmen devam eden virus replikasyonunun derecesine bağlıdır.

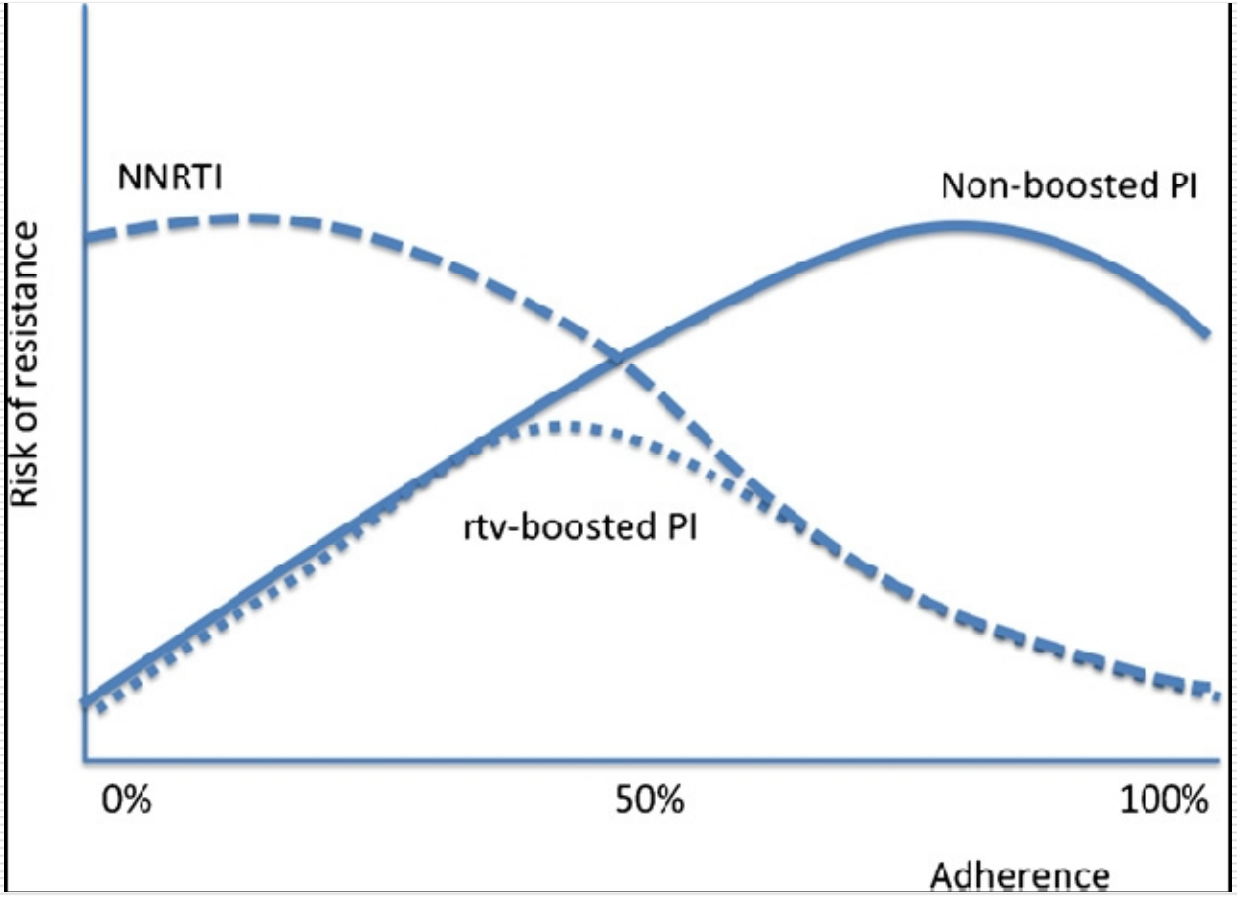
- **Düşük potensli bir tedavide: Seçtirici baskı düşüktür ve replikasyona rağmen R gelişimi yavaş**
- **Viral replikasyonu baskılayamayan fakat daha potent bir tedavide: Seçtirici baskı artacak ve R gelişimi hızlanacak**
- **Virusu baskılayabilecek yüksek potense sahip bir tedavide ise yüksek seçtirici baskıya rağmen R gelişimi yavaş olacaktır**

ARV ilaçların genetik bariyeri: Direnç için gerekli mutasyon sayısı

- RTI'lerin bazıları (LAM,FTC, nvp, dlv, efv) ve FI (t-20): Yüksek düzeyde fenotipik R için tek bir mutasyon yeterli -> Düşük genetik bariyer
- Ritonavir ile güçlendirilmiş PI ve NRTI'lerden AZT ve d4T -> Yüksek genetik bariyer
- Diğerleri orta derecede genetik bariyer

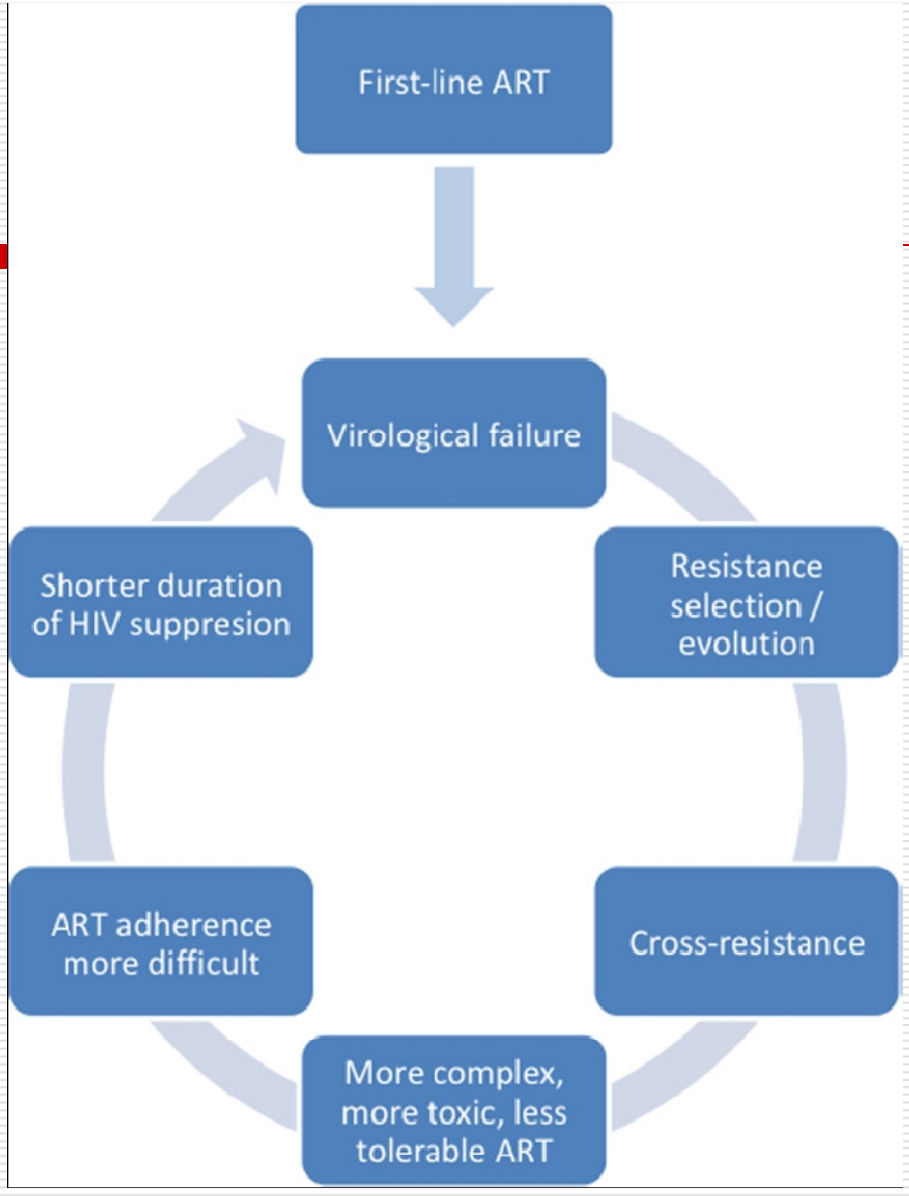
Her ilaç sınıfı kendine özgü bir tedavi uyumu-direnç gelişimi ilişkisine sahiptir.

- Yüksek tedavi uyumunda NNRTI direnç gelişimi nadir
- Tek başına PI alanlarda yüksek tedavi uyumunda direnç gelişimi sık


NNRTI
Non-boosted PI
rtv-boosted PI
Risk of resistance
0%
50%
100%
Adherence

Viral başarısızlığın olası nedenleri

- Direnç
- Yeterli ilaç düzeylerinin sağlanamaması
- Uygulanan tedavinin yetersiz olması


First-line ART
Virological failure
Resistance selection / evolution
Cross-resistance
More complex, more toxic, less tolerable ART
ART adherence more difficult
Shorter duration of HIV suppresion

Direnç gelişiminin klinik sonuçları:

- Tedavi etkililiğinin yitirilmesi
- Çapraz direnç gelişimi
- Mortalitede artış
- Dirençli suşun bulaşması
- Sonraki ART'nin etkililik süresinde kısalma
- Bir sonraki ART altında direnç gelişme riskinde artış
- Aşırı duyarlılık mutasyonları

Direnç Tespiti

- **Tüm hasta gruplarında direnç testinin klinik yararlılığı ve ekonomik olduğu gösterilmiştir à Tüm kılavuzlarda tıbbi bakım altına giren tüm hastalarda tedaviye başlanacak olsun ya da olmasın öneriliyor.**
- **ART alan hastalarda tedaviyi kesmeden önce yapılması gerekli.**

- **Virolojik başarısızlık olanlarda:**

- **VY > 1000 kopya/ml ise evet**
- **VY 500-1000 kopya ise yapılabilir**

- **Direnç testleri hangi ilacın etkili olacağından çok hangisinin ETKİSİZ olacağının belirlenmesine olanak verir**

- **Değerlendirmeler önceki ilaç tedavisi öyküleri ve daha önceki direnç testlerinin sonuçları gözönünde bulundurularak yapılmalıdır**

- İlaç direnci mutasyonu saptanmasa bile bazen revertan mutasyonların saptanması, daha öncesinde direnç mutasyonlarının dolayısıyla direnç gelişmiş olduğunun bir göstergesi olabilir

Direnç testleri

- Fenotipik testler
- Genotipik testler

Table 4
Principal mechanisms of antiretroviral drug resistance[a].

Drug family	Mechanism of action	Mechanism of resistance
NRTIs and NtRTIs	NRTIs and NtRTIs are chain terminators. They are incorporated into the nascent chain of viral DNA. Because they lack a 3′ hydroxyl group, no additional nucleotides can be appended.	Impaired nucleotide incorporation; M184V, K65R and the Q151M complex selectively impair RT's ability to incorporate an analogue into DNA. Nucleotide excision; TAMs allow ATP to bind RT near the 3′ end of viral DNA terminated by the incorporation of a nucleoside analogue. ATP then excises the analogue from viral DNA, allowing reverse transcription to proceed normally.
NNRTIs	Small molecules with strong affinity for a hydrophobic pocket located near the catalytic domain of RT. Inhibitor binding affects the flexibility of the enzyme, thereby blocking its ability to synthesize DNA.	Most NNRTI resistance mutations affect residues that are directly involved in inhibitor binding. Few have been found to act indirectly, by changing the position or the orientation of the aminoacids involved with direct contact with the inhibitor. Etravirine (ETV) is a diarylpyrimidine with some conformational isomerism that can bind RT in multiple conformations, allowing for a more robust interaction between ETV and the enzyme, even in the presence of some mutations (K103N).
PIs	PIs mimic the structure of the natural viral substrates of the HIV PR, competing with them for binding in the enzyme's active site.	Mutations in direct contact with the inhibitor or of distant aminoacids that modify the overall shape of the PR cavity disrupt fitting of the PI within the cavity and induce resistance.
InSTI	Raltegravir and Elvitegravir are DNA strand-transfer inhibitors that block the joining of the processed viral DNA ends into the host chromosome.	Binding requires divalent metal and resistance is metal dependent with active site mutants displaying resistance only when the enzymes are evaluated in the context of Mg(2+). There is extensive cross-resistance between the two drugs.
Fusion inhibitors (enfuvirtide)	Enfuvirtide is a 36-mer synthetic oligopeptide that binds to the trimeric HR-1 complex, preventing the association of HR-1 with HR-2 and inhibiting fusion.	Changes in a conserved amino acid triad (GIV) at positions 36–38 and in amino acids 39–45 in the HR1 region of gp41 prevent ENF binding.
CCR5 antagonists	CCR5 antagonist binding alters the conformational state of the CCR5 receptor, inhibiting the binding of gp120 to CCR5 by an allosteric mechanism.	Resistant viruses acquire the ability to recognize receptor conformations stabilized by CCR5 antagonists.

[a] RT: Reverse transcriptase; PR: protease; IN: integrase; HR1: first heptad repeat; HR2: second heptad repeat; NRTI: nucleoside reverse transcriptase inhibitor; NtRTI: nucleotide reverse transcriptase inhibitor; NNRTI: non-nucleoside reverse transcriptase inhibitor; PI: protease inhibitor; InSTI: integrase strand-transfer inhibitor; NVP: nevirapine; EFV: efavirenz; ETV: etravirine; ENF: enfuvirtide.

Fenotipik testler

- **Antivirogram (Tibotec-Virco)**
- **Pheno-Sense (Monogram-Biosciences) : env**
- **Phenoscript (Viralliance): gag, PR, Rt, env**

- **İlgili gen bölgeleri klonlama ya da in vitro rekombinasyonla bir HIV klonuna sokulur ve ilaçların belli konsantrasyonlarının varlığında işaretlibir "reporter" gen aracılığı ile üreme kontrol edilir**

Fenotipik testlerde İlaç varlığında IC50 değeri belirlenir

- **Teknik sınır değer: Referans kökenle yinelenen ölçüm sonuçlarına dayanır; yinelenebilirlik ve değişkenliğin göstergesidir; %95 GA belirlenir.**
- **Biyolojik sınır değer: Farklı yabanıl tipte suşların IC50'lerinin değişkenliğini gösterir**
- **Klinik sınır değer: Bir ilacın sınır değerinde klinik korelasyona dayalı olarak yapılan düzeltmeler ile elde edilen IC 50 değeri; İki farklı klinik sınır değer var: İlacın aktivitesinin azalmaya başladığı değer ve ilacın artık aktivitesini bütünüyle yitirdiği değer.**

Fenotipik testler

- **NRTI direncini özellikle TAM mutasyonları ile bağlantılı olanları belirlemede daha başarısız**
- **Ayrıca test hücrelerinde dNTP konsantrasyonu yüksek olduğundan NRTI'ler *in vitro* daha etkin göründükleri halde bu etkinlik *in vivo* koşullarda daha düşük olabilir**
- **Revertan mutasyonların ya da aşamalı olarak gelişmekte olan mutasyonları erken aşamalarda saptanması olanaklı değil**

Genotipik testler

- **Dizi analizine dayalı:**
  - **Ticari testler:** **HIV-1 Trugene (Bayer)**
    ViroSeq (Abbott)
    Virco Type HIV-1 (Virco)
    GenoSure (Plus) (LabCorp)
    Gene-Seq (Monogram)
  - **Home-made**
- **Hibridizasyona dayalı:**
  Inno-LIPA-HIV-1 (Murex-Innogenetics)

Fenotipik testler

- Daha pahalı ve özel lab. Koşulları gerektirir
- Daha az duyarlı
- Daha uzun sürede sonuç alınır

FAKAT

- Doğrudan ölçüm yöntemi
- Çapraz direncin değerlendirilmesi için daha uygun
- Yeni ARV ya da B dışı genotiplerin değerlendirilmesi için uygun

Genotipik testler

- **Daha kısa sürede sonuç alınır**
- **Daha ucuz**
- **Teknik olarak daha kolay**
- **Mutant + yabanıl tip karışımlarında direncin saptanmasına olanak verir**

FAKAT

- **Yorumlanmaları veri kalitesine, kullanılan algoritma ve uzman görüşlerine bağımlı**
- **Yeni ARV ve B dışı genotipler, yeni mutasyonlar açısından yetersiz kalabilir**
- **Kısmi ilaç etkinliği ile mutasyonların birbirleri üzerindeki etkilerini saptama konusunda daha az duyarlı**

Genotipik Testler

- Aşamalı olarak ortaya çıkan mutasyonlar (TAM'lar için K70R ve multinükleozid direnç yolizinde Q151M)

ya da

- Revertan mutasyonlar (T215 A/C/D/S)

  saptanabilir.

Kurala dayalı yorumlar

- Genotip-fenotip korelasyonları
- Tedavi öyküleri ile klinik yanıt arasındaki korelasyonlar

gibi çeşitli bilgi/verilere dayanan uzman görüşleri şekindedir.

X mutasyonu varsa x ilacına karşı yüksek düzeyde R, fakat y ve benzeri mutasyonlardan x kadarı varsa ...., ancak şunların varlığı/yokluğunda gibi yorumlardan oluşur

Veri tabanlarında bilgisayar üzerinden yürütülen yorumlar (virtüel fenotip tayinleri)

- İncelenen dizi veritabınındaki dizilerle karşılaştırılır ve uyumlu bulunan dizilerin direnç durumundan incelenen dizinin direnç durumu hakkında bir tahminde bulunulur
- Veritabanının çok sayıda dizi ve olası tüm mutasyon kombinasyonlarını içermesi gerekir.

Çeşitli yaklaşımlar var

- Decision tree model: Her bir poizisyonun direnç açısından anlamı üzerinden işleyen bir sistem. Veri setleri yinelenerek altsetlere bölünür
- SVM (support vector machines): Belli pozisyonlardaki amino asitler ile fenotip arasındaki korelasyona dayanan bir sistem
- Neuronal networks:

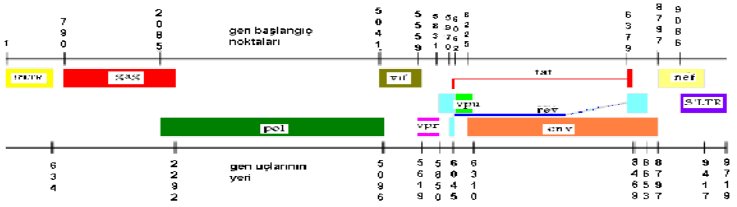
Bu sistemlerde her pozisyon yayımlanmış verilerden bağımsız olarak direnç açısından eşit olarak değerlendirilir; dirençle ilişkili yeni pozisyonların tanımlanmasına olanak verir

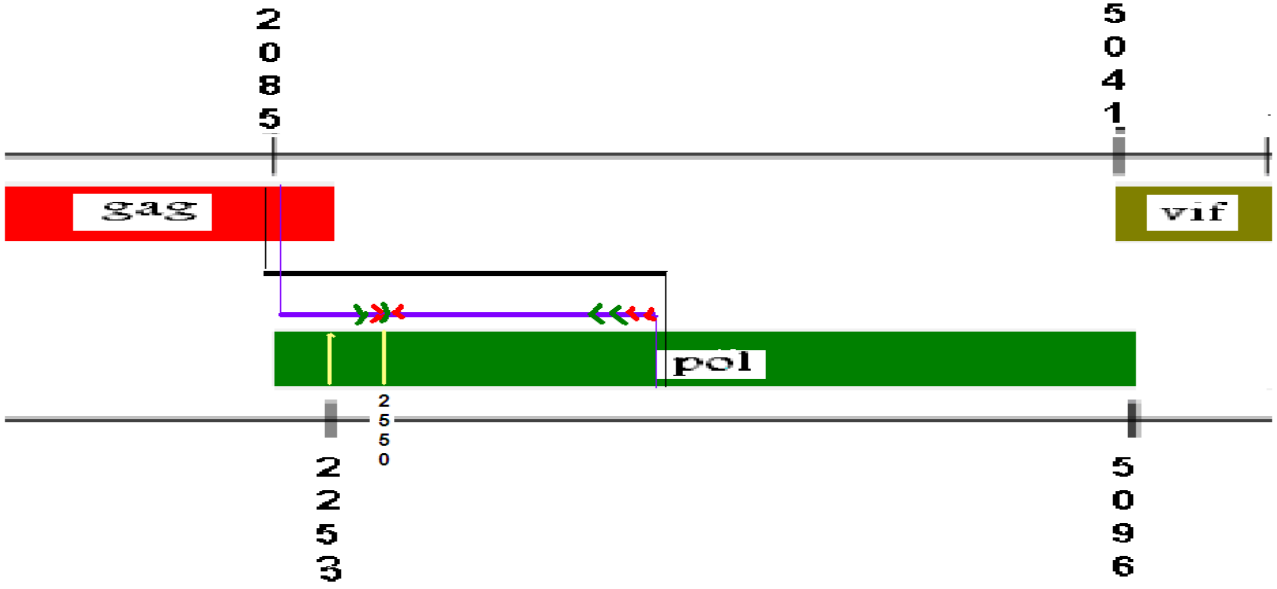
Duyarlılık sınır değerleri

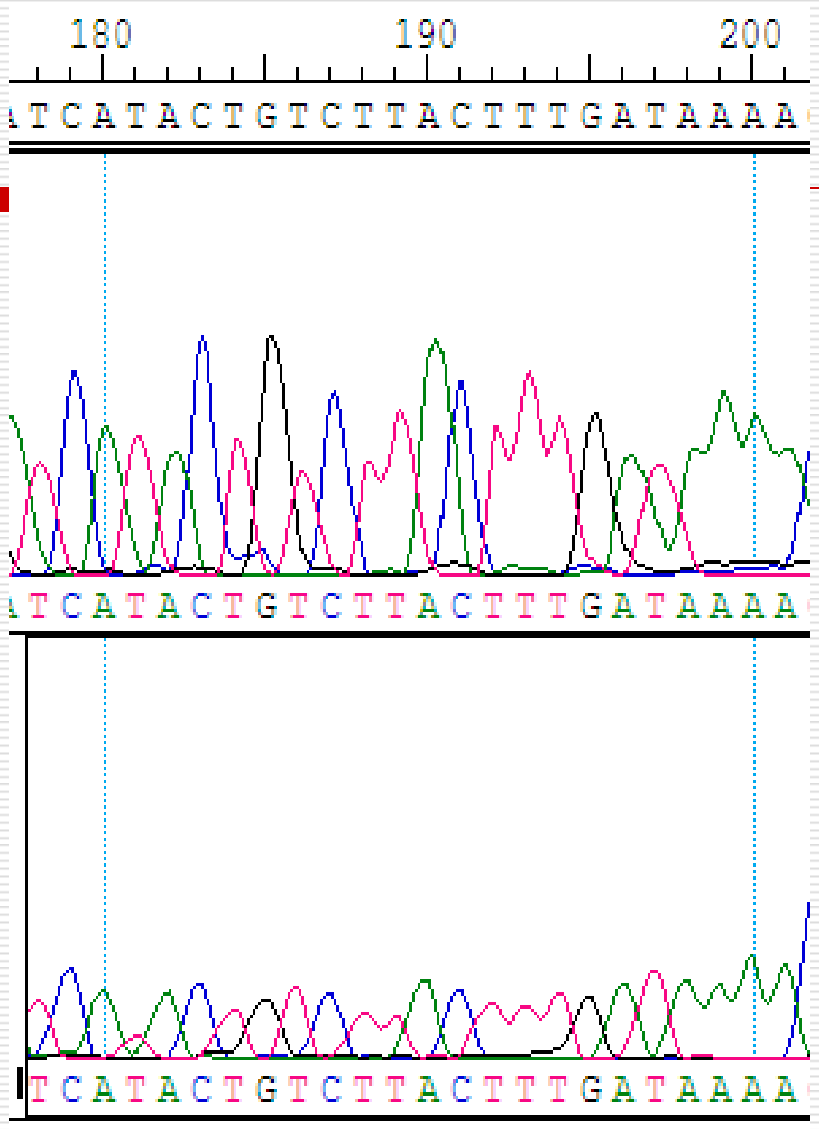
- En basit şekil “duyarlı” vs “dirençli” şeklinde olabilirdi.
- Oysa tüm yorum sistemlerinde en az 3 kategori var: Duyarlı – orta derecede duyarlı- dirençli ya da duyarlı-duyarlılığı azalmış- orta derecede dirençli-yüksek düzeyde dirençli.

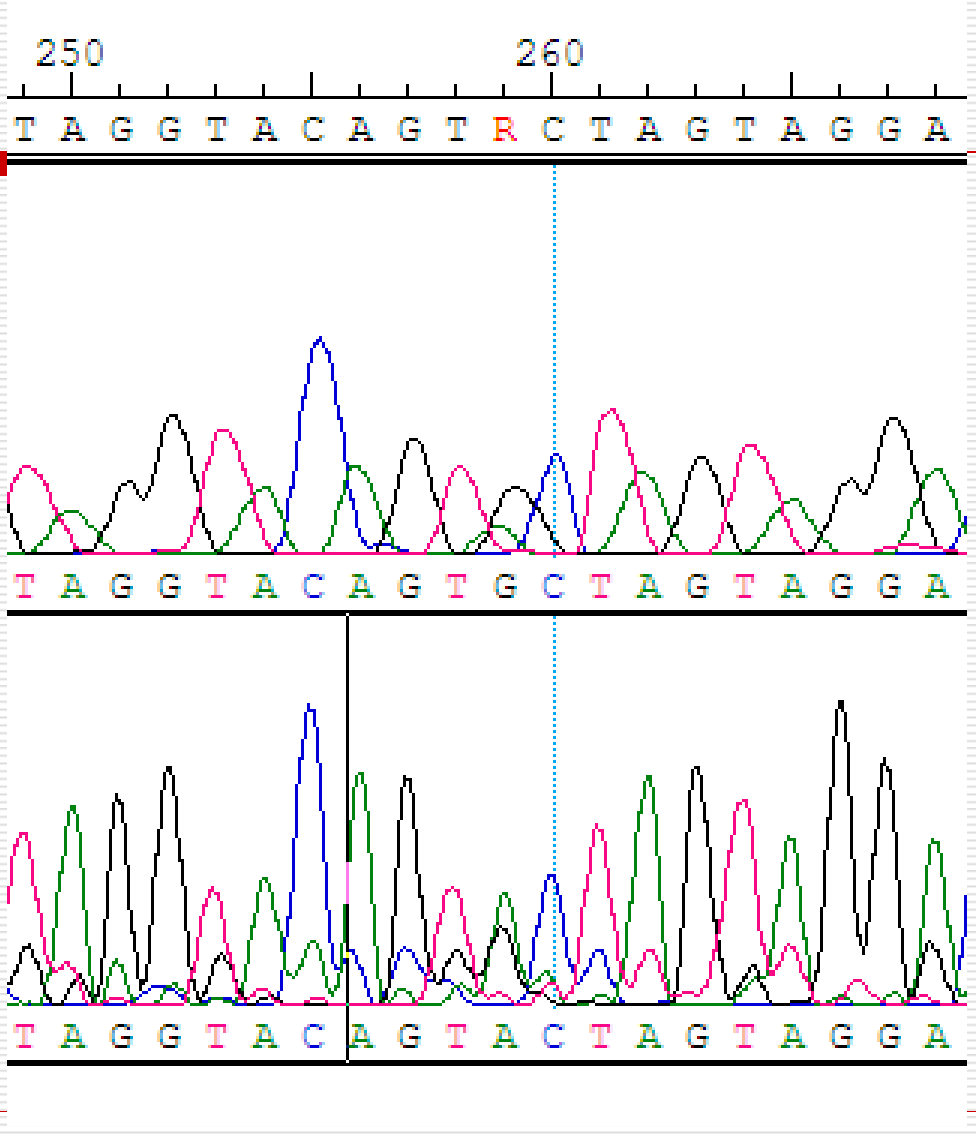
Home made genotipik direnç tayini

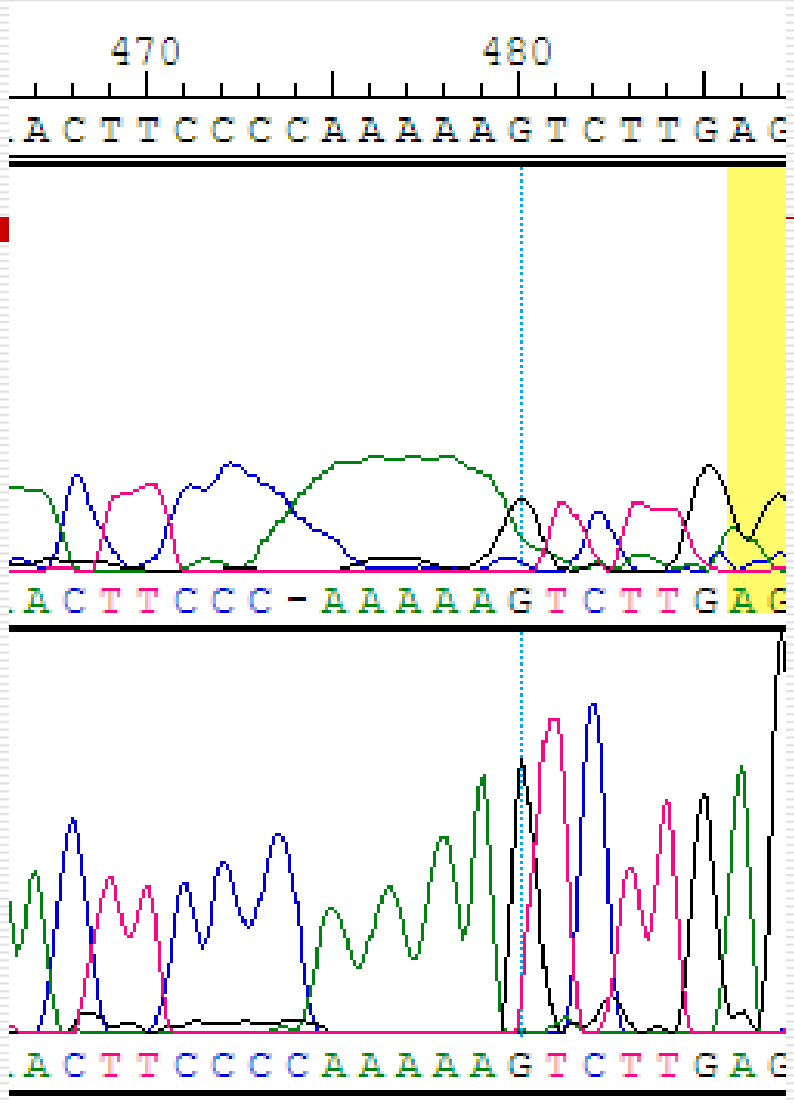
- Bu yöntem, proteaz bölgesinin tamamı ile RT bölgesinin ilk 220-40 amino asitlik bölgesinin RT-PCR ile çoğaltıldıktan sonra dizi analizine dayanmaktadır.

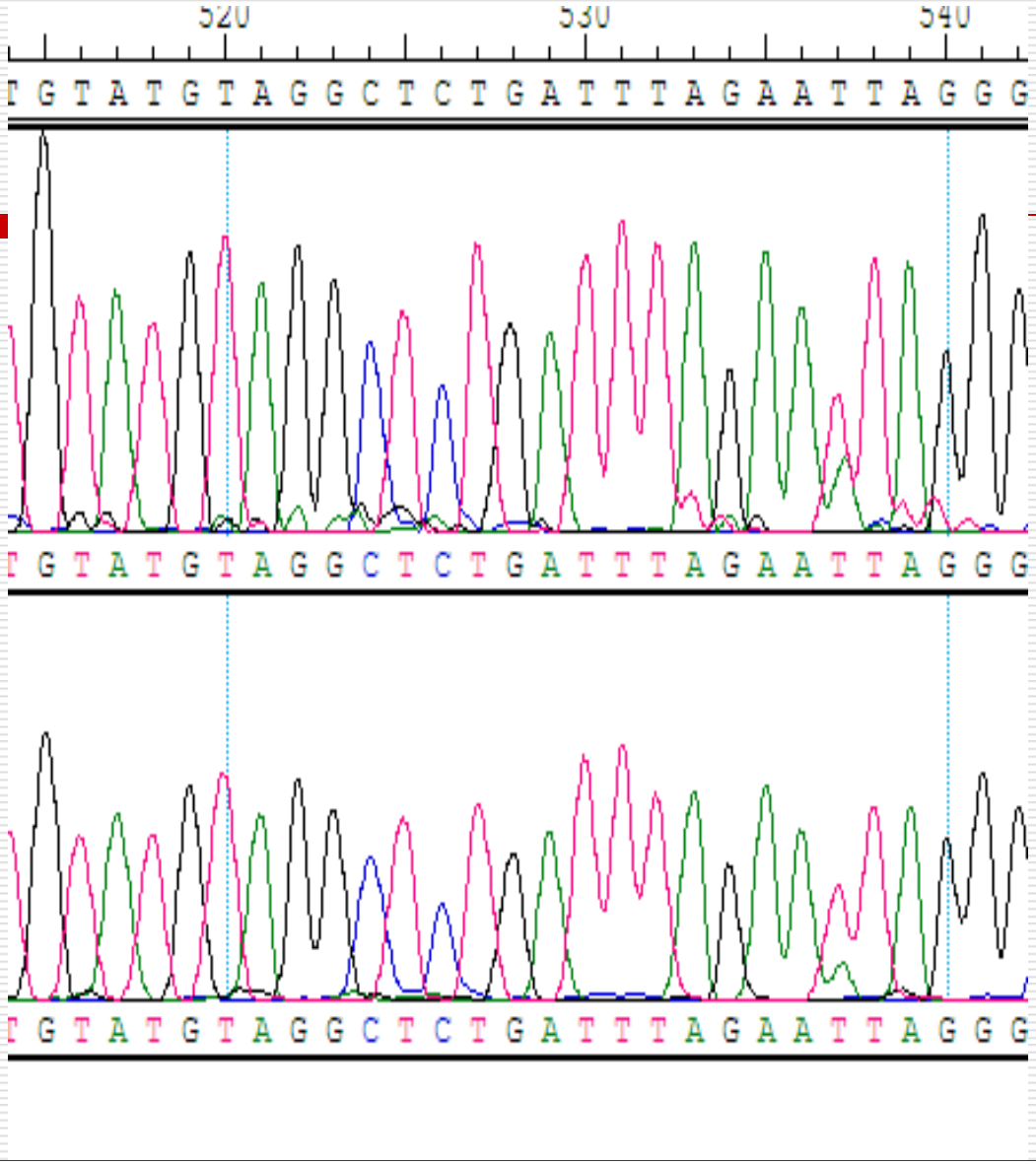



2085
5041
gag
vif
pol
2550
2253
5096


180
190
200
T C A T A C T G T C T T A C T T T G A T A A A A
T C A T A C T G T C T T A C T T T G A T A A A A
T C A T A C T G T C T T A C T T T G A T A A A A


250
260
T A G G T A C A G T R C T A G T A G G A
T A G G T A C A G T G C T A G T A G G A
T A G G T A C A G T A C T A G T A G G A


470
480
ACTTCCCCAAAAAGTCTTGAG
ACTTCCC-AAAAAGTCTTGAG
ACTTCCCCAAAAAGTCTTGAG


520
530
540
T G T A T G T A G G C T C T G A T T T A G A A T T A G G G
T G T A T G T A G G C T C T G A T T T A G A A T T A G G G
T G T A T G T A G G C T C T G A T T T A G A A T T A G G G

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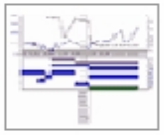
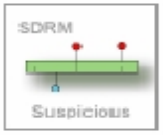
HIV DRUG RESISTANCE DATABASE

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

Three new programs launched: ART-AiDE, eCARE, and CPR



Antiretroviral Therapy - Acquisition and Display Engine (ART-AiDE) makes it possible to generate a permanent electronic and graphical record of a patient's antiretroviral treatment (ARV) history, plasma HIV-1 RNA levels,... More »



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

- Church, et al. NVP Mutations in Treatment-naive Patients with Subtypes A, C, and D
- Ibe, et al. Genotypes from Nagoya, Japan, 1999-2006
- Malet, et al. Genotypes from Patients who Failed Raltegravir Treatment

SURVEILLANCE

- World Health Organization 2008 Mutation List
- Calibrated Population Resistance (CPR) Version 3.0 beta
- Mutation Prevalence
- Surveillance Drug Resistance Mutation (SDRM) Worksheet

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.

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 your own algorithm.
» Go To Program

Drug Resistance Summaries

PIs
NRTIs
NNRTIs
FIs
IIs

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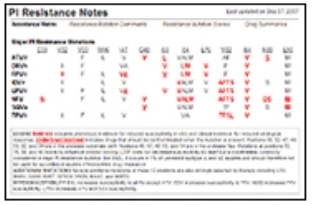
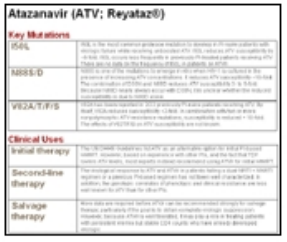
HIV Drug Resistance Summaries

Drug resistance mutation tables



Nucleoside RT Inhibitors (NRTIs)
Non-nucleoside RT inhibitors (NNRTIs)
Protease inhibitors (PIs)
Fusion inhibitors (FIs)
Integrase inhibitors (IIs)
CCR5 inhibitors

Antiretroviral drug summaries



Summary of the key mutations associated with each antiretroviral drug. Indications for drug use in first-line, second-line, and salvage therapy regimens.

NRTIs

- Abacavir (ABC)
- Didanosine (ddI)
- Emtricitabine (FTC)
- Lamivudine (3TC)
- Stavudine (d4T)
- Tenofovir (TDF)
- Zidovudine (ZDV)

NNRTIs

- Delavirdine (DLV)
- Efavirenz (EFV)
- Etravirine (ETR)
- Nevirapine (NVP)

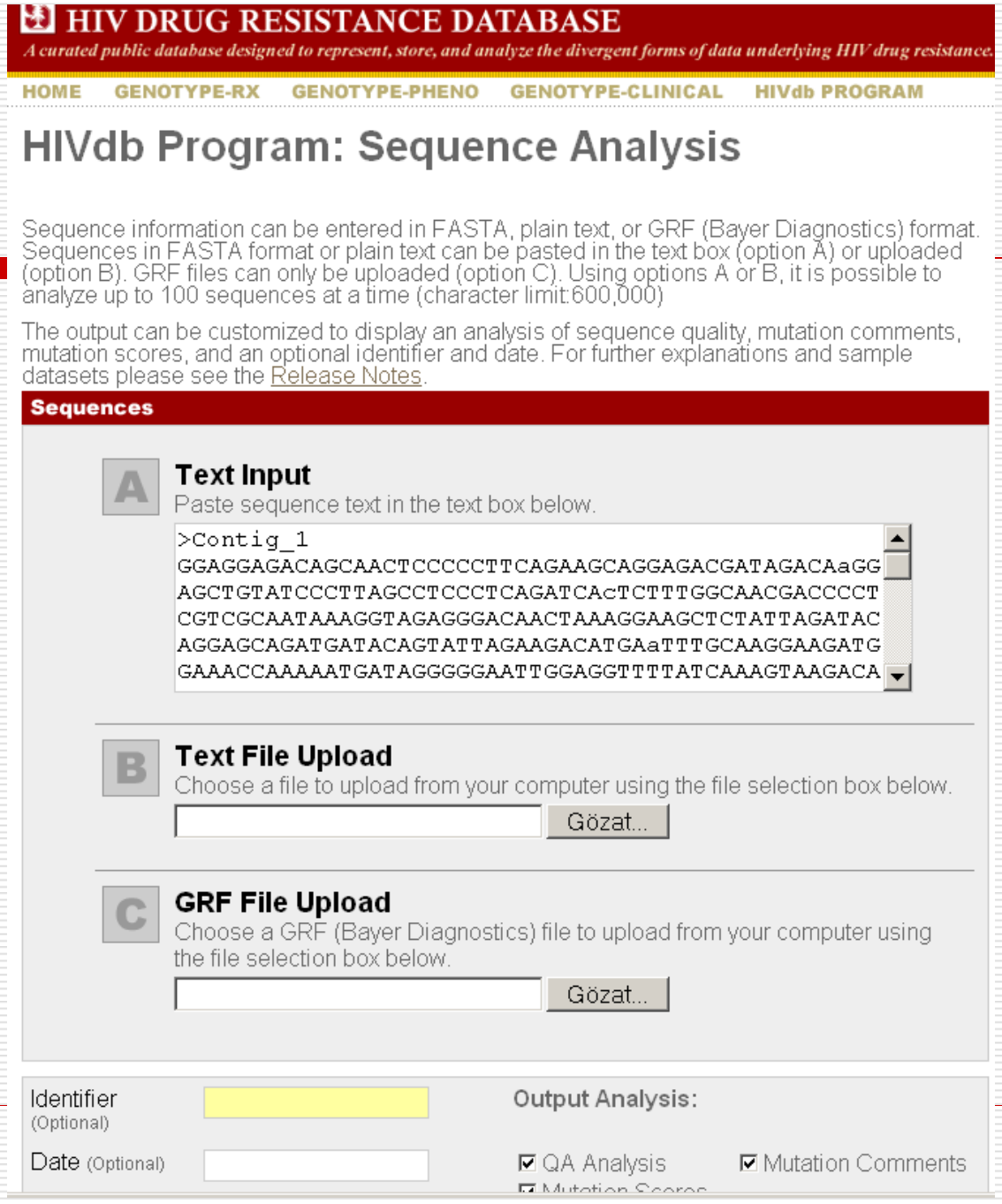
PIs

- Atazanavir (ATV)
- Darunavir (DRV)
- Fosamprenavir (fAPV)
- Indinavir (IDV)
- Lopinavir (LPV)
- Nelfinavir (NFV)
- Saquinavir (SQV)
- Tipranavir (TPV)

FIs

- Enfuvirtide (ENF)

Clinical studies of genotypic predictors of virological outcome


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: Sequence Analysis
Sequence information can be entered in FASTA, plain text, or GRF (Bayer Diagnostics) format. Sequences in FASTA format or plain text can be pasted in the text box (option A) or uploaded (option B). GRF files can only be uploaded (option C). Using options A or B, it is possible to analyze up to 100 sequences at a time (character limit:600,000)
The output can be customized to display an analysis of sequence quality, mutation comments, mutation scores, and an optional identifier and date. For further explanations and sample datasets please see the Release Notes.
Sequences
A
Text Input
Paste sequence text in the text box below.
>Contig_1
GGAGGAGACAGCAACTCCCCCTTCAGAAGCAGGAGACGATAGACAaGG
AGCTGTATCCCTTAGCCTCCCTCAGATCAcTCTTTGGCAACGACCCCT
CGTCGCAATAAAGGTAGAGGGACAACTAAAGGAAGCTCTATTAGATAC
AGGAGCAGATGATACAGTATTAGAAGACATGAaTTTGCAAGGAAGATG
GAAACCAAAAATGATAGGGGGAATTGGAGGTTTTATCAAAGTAAGACA
B
Text File Upload
Choose a file to upload from your computer using the file selection box below.
Gözat...
C
GRF File Upload
Choose a GRF (Bayer Diagnostics) file to upload from your computer using the file selection box below.
Gözat...
Identifier (Optional)
Date (Optional)
Output Analysis:
QA Analysis
Mutation Comments

- **HIVdb (Stanford)**

Her bir mutasyon için ilaçlara karşı kazandırdığı dirence göre bir puan veriliyor ve bu değerlendirme sonucunda belirtiliyor.

Ayrıca mutasyonlar "majör" ve "minör" mutasyonlar şeklinde kategorize ediliyor.

0-9 arası : Duyarlı (duyarlılıkta azalma yok)

10-15 arası: Potansiyel düşük düzeyde direnç

15-29 arası: Düşük düzeyde direnç

30-60 arası: Orta derecede direnç

> 60: Yüksek düzeyde direnç

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: Genotypic Resistance Interpretation Algorithm

SeqID: Contig_1 Date: 7-Sep-2008

Summary Data

Sequence includes PR: codons: 1 - 99
Sequence includes RT: codons: 1 - 252

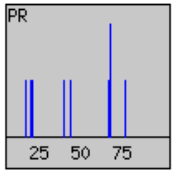
There are no insertions or deletions

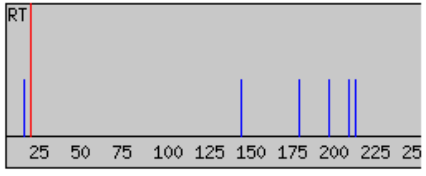
Subtype and % similarity to closest reference isolate:

1. *PR: B (96.0%)*
2. *RT: B (95.9%)*

Sequence Quality Assessment

Gene	QA Problem	Codons
PR	Stop Codons, Frame Shifts:	*None*
PR	B,D,H,V,N:	*None*
PR	Unusual Residues:	*None*
Gene	**QA Problem**	**Codons**
RT	Stop Codons, Frame Shifts:	*None*
RT	B,D,H,V,N:	15
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

PI Major Resistance Mutations:	None
PI Minor Resistance Mutations:	None
Other Mutations:	T12A, I15V, G16E, E35D, P39Q, I62V, L63H, I72V

	Protease Inhibitors
atazanavir (ATV)	Susceptible
darunavir (DRV)	Susceptible
fosamprenavir (FPV)	Susceptible
indinavir (IDV)	Susceptible
lopinavir (LPV)	Susceptible
nelfinavir (NFV)	Susceptible
saquinavir (SQV)	Susceptible
tipranavir (TPV)	Susceptible

PR Comments

- G16E is a highly polymorphic mutation occurring in 2% to 20% of viruses depending on subtype. It may be weakly associated with ATV therapy and response.
- I62V is a highly polymorphic mutation that is more common in PI-treated compared with untreated persons. It was weakly associated with a decreased virologic response to ATV in one of three retrospective analyses.

Drug Resistance Interpretation

NRTI Resistance Mutations:	None
NNRTI Resistance Mutations:	None
Other Mutations:	K11Q, G15X, I142IV, D177G, I195T, Q207E, R211K

Nucleoside RTI		Non-Nucleoside RTI	
lamivudine (3TC)	Susceptible	**delavirdine (DLV)**	Susceptible
abacavir (ABC)	Susceptible	**efavirenz (EFV)**	Susceptible
zidovudine (AZT)	Susceptible	**etravirine (ETR)**	Susceptible
stavudine (D4T)	Susceptible	**nevirapine (NVP)**	Susceptible
didanosine (DDI)	Susceptible		
emtricitabine (FTC)	Susceptible		
tenofovir (TDF)	Susceptible		

RT Comments

None

Mutation Scoring

	ATV	DRV	FPV	IDV	LPV	NFV	SQV	TPV
Total:	0	0	0	0	0	0	0	0

	3TC	ABC	AZT	D4T	DDI	FTC	TDF	DLV	EFV	ETR	NVP
Total:	0	0	0	0	0	0	0	0	0	0	0

The Team

- Who We Are & How to Contact Us
- Publications
- Collaborators
- Acknowledgements

The Data

- User Guide & Database Documents
- Database Statistics
- HIV Related Links
- Additional Resources

Drug Resistance Interpretation

PI Major Resistance Mutations: **M46I, I54V, V82A**
PI Minor Resistance Mutations: **L10I, L24I, G73C**
Other Mutations: T12S, I15V, G16E, K20M, R41K, K55R, Q61E, I62V, L63N, I72V

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

PR Comments

- This sequence has 1 major TPV-resistance mutations (I54V), 0 minor TPV-resistance mutations, and 1 mutations associated with increased TPV responsiveness (L24I). RESIST study (Baxter J et al J Virology 2006 and Scherer J et al EACS 2007).
- L10I/V/F/R are associated with resistance to each of the PIs when present with other mutations. L10I/V occur commonly in 5-10% of untreated persons. L10FRY are nonpolymorphic.
- G16E is a highly polymorphic mutation occurring in 2% to 20% of viruses depending on subtype. It may be weakly associated with ATV therapy and response.
- K20R/M/I/T are weakly associated with resistance to each of the PIs when present with other mutations. Many variants at this position occur commonly in non-subtype B viruses.
- L24I is associated with reduced susceptibility to IDV and possibly RTV, LPV, SQV, and ATV when present with other mutations.
- M46I/L decreases susceptibility to IDV, NFV, FPV, LPV, ATV, TPV, and possibly SQV and DRV when present with other mutations.
- I54V contributes resistance to each of the PIs except possibly DRV when present with other mutations.
- I62V is a highly polymorphic mutation that is more common in PI-treated compared with untreated persons. It was weakly associated with a decreased virologic response to ATV in one of three retrospective analyses.
- G73S/T/C/A are selected by and appear to be associated with decreased susceptibility to most, if not all, PIs.
- V82A reduces susceptibility to IDV and LPV. With other mutations it is associated with reduced susceptibility to NFV, ATV, SQV, FPV, and TPV.

Drug Resistance Interpretation

NRTI Resistance Mutations: M184V, T215Y
NNRTI Resistance Mutations: None
Other Mutations: F25AP, I31IL, V35T, T39S, K49R, K122E, I135T, S162C, G196E, T200IT, V245E

Nucleoside RTI	
lamivudine (3TC)	High-level resistance
abacavir (ABC)	Intermediate resistance
zidovudine (AZT)	Low-level resistance
stavudine (D4T)	Low-level resistance
didanosine (DDI)	Low-level resistance
emtricitabine (FTC)	High-level resistance
tenofovir (TDF)	Potential low-level resistance

Non-Nucleoside RTI	
delavirdine (DLV)	Susceptible
efavirenz (EFV)	Susceptible
etravirine (ETR)	Susceptible
nevirapine (NVP)	Susceptible

RT Comments

- M184V/I cause high-level in vitro resistance to 3TC and FTC and low-level in vitro resistance to ddI and ABC. M184V/I increase susceptibility to AZT, TDF, and d4T.
- T215Y causes AZT and D4T resistance and reduces susceptibility to ABC, ddI, and TDF particularly when it occurs in combination with M41L and L210W.
- M184V partially reverses AZT, d4T, and TDF resistance caused by other AZT mutations (TAMs). AZT mutations in this isolate include T215Y.

Mutation Scoring

	ATV	DRV	FPV	IDV	LPV	NFV	SQV	TPV
M46I	15	4	12	12	12	25	5	8
I54V	10	4	10	10	12	15	15	10
V82A	15	4	10	35	20	35	10	10
L10I	2	2	2	2	2	2	2	2
L24I	2	2	2	5	2	2	2	0
G73C	5	2	2	5	2	5	10	2
Total	49	18	38	69	50	84	44	32

	3TC	ABC	AZT	D4T	DDI	FTC	TDF	DLV	EFV	ETR	NVP
M184V	60	12	-8	-5	5	60	-8	_	_	_	_
T215Y	4	20	35	30	20	4	20	_	_	_	_
Total	64	32	27	25	25	64	12	0	0	0	0

geno2pheno

Andre Altmann, Niko Beerenwinkel, Joachim Buech, Martin Däumer, Daniel Hoffmann, Rolf Kaiser, Klaus Korn, Thomas Lengauer, Mark Oette, Barbara Schmidt, Joachim Selbig, Tobias Sing, Hauke Walter

On submitting below an HIV-1 pol-gene DNA sequence you will obtain a sequence alignment to the reference strain HXB2, a list of mutations and different predictions of phenotypic resistance of the respective virus to 17 antiretroviral drugs.

NEW **Features**:

- **PDF Output for phenotype prediction and interpretation, including display of scored mutations**
- **Combination Therapy Optimization**
- **geno2pheno[coreceptor]**: Genotypic prediction of coreceptor usage

1. Identifier (optional): (do not use patient names)

2. Cutoffs: Load entire set: default, low-level, high-level or set individually:

NRTI ZDV 8.5 ddC 2.5 ddI 2.5 d4T 2.5 3TC 8.5 ABC 2.5 TDF 2.5

NNRTI NVP 8.5 DLV 8.5 EFV 8.5

PI SQV 3.5 IDV 3.5 RTV 3.5 NFV 3.5 APV 3.5 LPV 3.5 ATV 3.5

3. Pol-gene (PR and RT) nucleotide sequence: upload from file (plain sequence or FASTA format):

Gözat...

or paste in:

```
>Contig_5
AGAGTTTCAGATCTGgGGAGGAGACAAcaaCTCCCCCTC
```

4. Sequence ambiguities: ☑ use resistance associated mutations at ambiguous sequence positions for phenotype prediction

5. Output: display 60 sequence characters per line. ☐ nice to print (don't use colored backgrounds)

6. Action: align & predict (takes a few seconds) Sifirla Load a sample sequence. Clear

Sequence identifier: Sep

Alignment to reference strain **HXB2**. Position numbering follows Korber *et al.*

Protease

```
ref.   nt: CCTCAGGTCACTCTTTGGCAACGACCCCTCGTCACAATAAAGATAGGGGGGCAACTAAAG 2312
ref.   aa:  P  Q  V  T  L  W  Q  R  P  L  V  T  I  K  I  G  G  Q  L  K    20
query  aa:  P  Q  I  T  L  W  Q  R  P  I  V  S  I  K  V  E  G  Q  L  M
query  nt: CCTCAGATCACTCTTTGGCAGCGACCCATCGTCTCAATAAAGGTAGAGGGACAACTAATG

ref.   nt: GAAGCTCTATTAGATACAGGAGCAGATGATACAGTATTAGAAGAAATGAGTTTGCCAGGA 2372
ref.   aa:  E  A  L  L  D  T  G  A  D  D  T  V  L  E  E  M  S  L  P  G    40
query  aa:  E  A  L  I  D  T  G  A  D  D  T  V  L  E  E  M  N  L  P  G
query  nt: GAAGCTCTAATAGATACAGGAGCAGATGATACAGTATTAGAAGAAATGAATTTGCCAGGA

ref.   nt: AGATGGAAACCAAAAATGATAGGGGGAATTGGAGGTTTTATCAAAGTAAGACAGTATGAT 2432
ref.   aa:  R  W  K  P  K  M  I  G  G  I  G  G  F  I  K  V  R  Q  Y  D    60
query  aa:  K  W  K  P  K  I  I  G  G  I  G  G  F  V  R  V  R  Q  Y  D
query  nt: AAATGGAAACCAAAAATAATAGGGGGAATTGGAGGTTTTGTCAGRGTAAGACAATATGAT

ref.   nt: CAGATACTCATAGAAATCTGTGGACATAAAGCTATAGGTACAGTATTAGTAGGACCTACA 2492
ref.   aa:  Q  I  L  I  E  I  C  G  H  K  A  I  G  T  V  L  V  G  P  T    80
query  aa:  E  V  N  I  E  I  C  G  H  K  A  V  C  T  V  L  V  G  P  T
query  nt: GAGGTAAACATAGAGATCTGTGGACATAAAGCCGTATGTACAGTATTAGTAGGACCTACA

ref.   nt: CCTGTCAACATAATTGGAAGAAATCTGTTGACTCAGATTGGTTGCACTTTAAATTTT    2549
ref.   aa:  P  V  N  I  I  G  R  N  L  L  T  Q  I  G  C  T  L  N  F      99
query  aa:  P  A  N  I  I  G  R  N  L  L  T  Q  I  G  C  T  L  N  F
query  nt: CCTGCCAACATAATTGGAAGAAATCTGTTGACTCAGATTGGGTGCACTTTAAATTTT
```

bad characters removed:	0	matches in reference sequence:	81.82 %
aligned amino acids:	99	matches in alignment:	81.82 %
insertions / amino acids in total:	0 / 0	deletions / amino acids in total:	0 / 0

substitutions: V 3 I, L 10 I, T 12 S, I 15 V, G 16 E, K 20 M, L 24 I, S 37 N, R 41 K, M 46 I, I 54 V, K 55 R, Q 61 E, I 62 V, L 63 N, I 72 V, G 73 C, V 82 A

Reverse transcriptase

```
ref.   nt: CCCATTAGCCCTATTGAGACTGTACCAGTAAAATTAAAGCCAGGAATGGATGGCCCAAAA 2609
ref.   aa:  P  I  S  P  I  E  T  V  P  V  K  L  K  P  G  M  D  G  P  K   20
query  aa:  P  I  S  P  I  E  T  V  P  V  K  L  K  P  G  M  D  G  P  K
query  nt: CCTATTAGTCCTATTGAAACTGTACCAGTAAAATTAAARCCAGGAATGGATGGYCCAAAR

ref.   nt: GTTAAACAATGGCCATTGACAGAAGAAAAAATAAAAGCATTAGTAGAAATTTGTACAGAG 2669
ref.   aa:  V  K  Q  W  P  L  T  E  E  K  I  K  A  L  V  E  I  C  T  E   40
query  aa:  V  K  Q  W  ?  L  T  E  E  K  ?  K  A  L  T  E  I  C  S  E
query  nt: GTTAAACAATGGSCMTTGACAGAAGAAAAAWTAAAAGCATTAACAGAAATTTGCTCAGAA

ref.   nt: ATGGAAAAGGAAGGGAAAATTTCAAAAATTGGGCCTGAAAATCCATACAATACTCCAGTA 2729
ref.   aa:  M  E  K  E  G  K  I  S  K  I  G  P  E  N  P  Y  N  T  P  V   60
query  aa:  M  E  K  E  G  K  I  S  R  I  G  P  E  N  P  Y  N  T  P  V
query  nt: ATGGAAAAGGAAGGAAAAATTTCAAGAATTGGGCCTGAAAATCCATACAATACTCCAGTA

ref.   nt: TTTGCCATAAAGAAAAAAGACAGTACTAAATGGAGAAAATTAGTAGATTTCAGAGAACTT 2789
ref.   aa:  F  A  I  K  K  K  D  S  T  K  W  R  K  L  V  D  F  R  E  L   80
query  aa:  F  A  I  K  K  K  D  S  T  K  W  R  K  L  V  D  F  R  E  L
query  nt: TTTGCCATAAAGAAAAAAGACAGTACTAAATGGAGAAAACTAGTAGATTTTAGAGAACTT

ref.   nt: AATAAGAGAACTCAAGACTTCTGGGAAGTTCAATTAGGAATACCACATCCCGCAGGGTTA 2849
ref.   aa:  N  K  R  T  Q  D  F  W  E  V  Q  L  G  I  P  H  P  A  G  L  100
query  aa:  N  K  R  T  Q  D  F  W  E  V  Q  L  G  I  P  H  P  A  G  L
query  nt: AATAAAAGGACACAAGACTTCTGGGAAGTTCAATTAGGAATACCACACCCCGCAGGGTTA

ref.   nt: AAAAAGAAAAAATCAGTAACAGTACTGGATGTGGGTGATGCATATTTTTCAGTTCCCTTA 2909
ref.   aa:  K  K  K  K  S  V  T  V  L  D  V  G  D  A  Y  F  S  V  P  L  120
query  aa:  K  K  K  K  S  V  T  V  L  D  V  G  D  A  Y  F  S  V  P  L
query  nt: AAAAAGAAAAAATCAGTAACAGTACTAGATGTGGGTGATGCATATTTTTCAGTTCCCCTA

ref.   nt: GATGAAGACTTCAGGAAGTATACTGCATTTACCATACCTAGTATAAACAATGAGACACCA 2969
ref.   aa:  D  E  D  F  R  K  Y  T  A  F  T  I  P  S  I  N  N  E  T  P  140
query  aa:  D  E  D  F  R  K  Y  T  A  F  T  I  P  S  T  N  N  E  T  P
query  nt: GATGAAGACTTCAGAAAGTATACTGCGTTTACCATACCTAGTACAAACAATGAGACACCA

ref.   nt: GGGATTAGATATCAGTACAATGTGCTTCCACAGGGATGGAAAGGATCACCAGCAATATTC 3029
ref.   aa:  G  I  R  Y  Q  Y  N  V  L  P  Q  G  W  K  G  S  P  A  I  F  160
query  aa:  G  I  R  Y  Q  Y  N  V  L  P  Q  G  W  K  G  S  P  A  I  F
query  nt: GGGATTAGATATCAGTACAATGTACTTCCACAGGGATGGAAAGGATCACCAGCAATATTC

ref.   nt: CAAAGTAGCATGACAAAAATCTTAGAGCCTTTTAGAAAACAAAATCCAGACATAGTTATC 3089
ref.   aa:  Q  S  S  M  T  K  I  L  E  P  F  R  K  Q  N  P  D  I  V  I  180
query  aa:  Q  C  S  M  T  K  I  L  E  P  F  R  K  Q  N  P  D  I  V  I
query  nt: CAATGTAGCATGACAAAAATCTTAGAGCCTTTTAGGAAACARAATCCAGACATAGTTATC

ref.   nt: TATCAATACATGGATGATTTGTATGTAGGATCTGACTTAGAAATAGGGCAGCATAGAACA 3149
ref.   aa:  Y  Q  Y  M  D  D  L  Y  V  G  S  D  L  E  I  G  Q  H  R  T  200
query  aa:  Y  Q  Y  V  D  D  L  Y  V  G  S  D  L  E  I  E  Q  H  R  ?
query  nt: TATCAATATGTGGATGATTTGTATGTAGGATCTGACTTAGAAATAGAACAGCATAGAAYA
```

```
ref.   nt: AAAATAGAGGAGCTGAGACAACATCTGTTGAGGTGGGGACTTACCACACCAGACAAAAAA 3209
ref.   aa:  K  I  E  E  L  R  Q  H  L  L  R  W  G  L  T  T  P  D  K  K   220
query  aa:  K  I  E  E  L  R  Q  H  L  L  R  W  G  F  Y  T  P  D  K  K
query  nt: AAAATAGAGGAACTGAGACAGCATCTGTTGAGGTGGGGATTTTACACACCAGACAAAAAG

ref.   nt: CATCAGAAAGAACCTCCATTCCTTTGGATGGGTTATGAACTCCATCCTGATAAATGGACA 3269
ref.   aa:  H  Q  K  E  P  P  F  L  W  M  G  Y  E  L  H  P  D  K  W  T   240
query  aa:  H  Q  K  E  P  P  F  L  W  M  G  Y  E  L  H  P  D  K  W  T
query  nt: CATCAGAAAGAGCCTCCATTCCTTTGGATGGGTTATGAACTCCATCCTGATAAATGGACA

ref.   nt: GTACAGCCTATAGTGCTGCCAGAAAAAGACAGCTGGACTGTCAATGACATACAGAAGTTA 3329
ref.   aa:  V  Q  P  I  V  L  P  E  K  D  S  W  T  V  N  D  I  Q  K  L   260
query  aa:  V  Q  P  I  E  L  P  E  K  D  S  W  T  .  .  .  .  .  .  .
query  nt: GTACAGCCTATAGAACTGCCAGAAAAAGACAGCTGGACT.....................
```

bad characters removed:	0	matches in reference sequence:	42.86 %
aligned amino acids:	253	matches in alignment:	94.86 %
insertions / amino acids in total:	0 / 0	deletions / amino acids in total:	0 / 0

substitutions: P 25 A/P, I 31 I/L, V 35 T, T 39 S, K 49 R, I 135 T, S 162 C, M 184 V, G 196 E, T 200 I/T, L 214 F, T 215 Y, V 245 E

Subtype Prediction

Subtype	Probability
B	100.00 %

- All predictions below **aim** at precicting the ***in vitro* resistance phenotype**. Predicting *in vivo* resistance or **clinical utility is not intended**
- The **atazanavir (ATV) model** has been trained on samples derived from atazanavir-naive patients only. Predictions are thus most reliable for this type of samples

Phenotype Prediction

At ambiguous sequence positions (due to a mixed virus population) the resistance associated mutation has been assumed if present.

Drug	Cutoff	Decision tree classification[1] [confidence factor]	SVM classification[2]	Predicted fold-resistance (resistance factor, RF)[2]	z-score (number of standard deviations above mean of drug-naive patients) more	Probability score (likelihood of belonging to the resistant subpopulation) more
ZDV	8.5	susceptible [0.76]	resistant	9.0	3.7	0.98
ddC	2.5	resistant [0.83]	susceptible	1.9	3.9	0.98
ddI	2.5	resistant [0.58]	susceptible	2.2	3.4	0.15
d4T	2.5	susceptible [0.84]	susceptible	1.2	0.2	0.033
3TC	8.5	resistant [0.98]	resistant	61.6	14.4	1
ABC	2.5	resistant [0.79]	susceptible	3.8	8.2	1
TDF	2.5	resistant [0.77]	susceptible	1.4	2.0	0.64
NVP	8.5	susceptible [0.89]	susceptible	2.1	0.3	0.0063
DLV	8.5	susceptible [0.90]	susceptible	1.2	0.5	0.0021
EFV	8.5	susceptible [0.91]	susceptible	0.9	-0.7	0.0011
SQV	3.5	resistant [0.67]	resistant	12.5	8.9	1
IDV	3.5	resistant [0.91]	resistant	70.5	13.2	1
RTV	3.5	resistant [0.92]	resistant	152.4	17.4	1
NFV	3.5	resistant [0.93]	resistant	32.0	7.5	1
APV	3.5	resistant [0.85]	resistant	6.6	4.7	1
LPV	3.5	resistant [0.99]	resistant	72.2	14.5	1
ATV	3.5	resistant [0.99]	resistant	18.0	7.8	1

1: based on C5.0, RuleQuest Research Pty Ltd
2: based on LIBSVM, Copyright (c) 2000, Chih-Chung Chang and Chih-Jen Lin

PDF Output

phenotype prediction from genotype

I. General information

Patient:	Study Id:
Birth date:	Viral load:
Sample received:	Sample collected:
Sample ID:	Predicted subtype: B
Sample type:	Report date: Sep 7, 2008
Physician:	Reported by:

II. Substitutions
(relative to the reference strain HXB2)

Protease:	V3I, L10I, T12S, I15V, G16E, K20M, L24I, S37N, R41K, M46I, I54V, K55R, Q61E, I62V, L63N, I72V, G73C, V82A
Reverse transcriptase:	P254P, I31M, V35T, T39S, K49R, I135T, S162C, M184V, G196E, T200IT, L214F, T215Y, V245E

III. Phenotype prediction

Drug	Resistance Factor RT (*)	z-score	Scored Mutations (**)
ZDV	0.0	3.7	215Y 49R 135T 35T
ddI	2.2	3.4	215Y 184V
d4T	1.2	0.2	215Y 162C 184V 39S 35T
3TC	61.6	14.4	184V
ABC	3.8	8.2	184V 215Y 35T 49R
TDF	1.4	2.0	215Y 35T 184V 135T
NVP	2.1	0.3	135T 31L 35T 162C 200I 196E
EFV	0.9	-0.7	35T 31L 135T 196E
SQV	12.5	8.9	54V 72V 63N 46I 41K 55R 24I 37N
IDV	70.5	13.2	46I 24I 10I 54V 82A 72V 62V
RTV	152.4	17.4	24I 46I 54V 82A 73C 61E
NFV	32.0	7.5	46I 72V 54V 10I 3I 62V 82A
fAPV	6.6	4.7	46I 61E 10I 41K 12S 72V 24I 82A
LPV	72.2	14.5	54V 46I 82A 24I 10I 16E
ATV	16.0	7.8	54V 82A 46I 41K 62V

(*) based on LIBSVM, Copyright (c) 2000, Chih-Chung Chang and Chih-Jen Lin

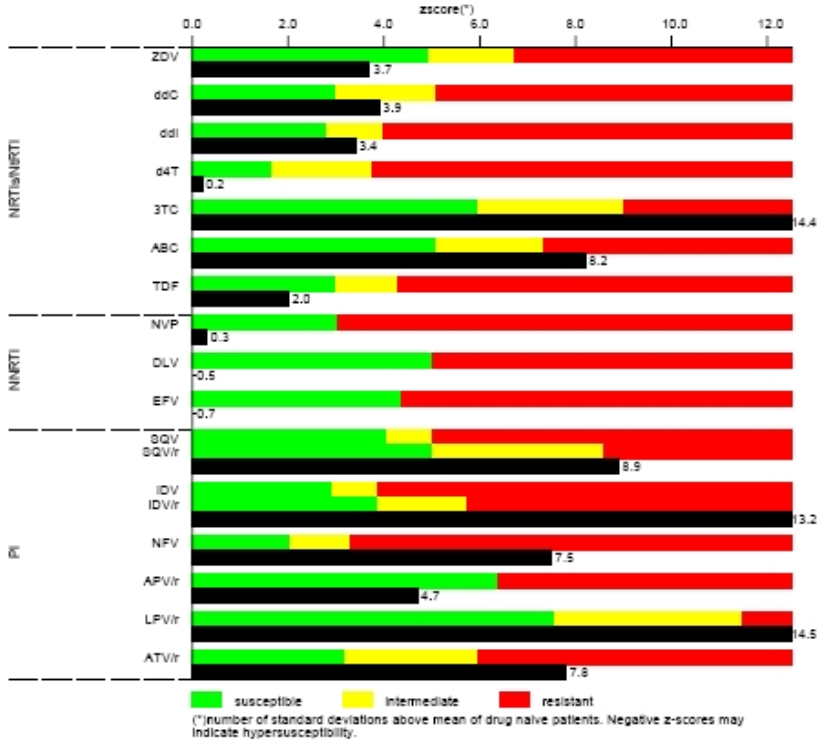
(**) Mutations are ranked according to their impact on the phenotype prediction. Mutations shown in red and green contribute to an increase or decrease in resistance, respectively. Shown mutations sum up to 80% of the total score.

geno2pheno®

phenotype prediction from genotype

IV. Interpretation

Patient:	Birth date:	Sampling date:
Current therapy:		Viral load:



(*)number of standard deviations above mean of drug naive patients. Negative z-scores may indicate hypersusceptibility.

Comments

NRTIs/NtRTI:

NNRTIs:

PIs:

Previous genotypes:

_____ date

_____ signature

(If you see a blank square below, you probably need java runtime environment (JRE) 1.4.2 or later. You can download it here)

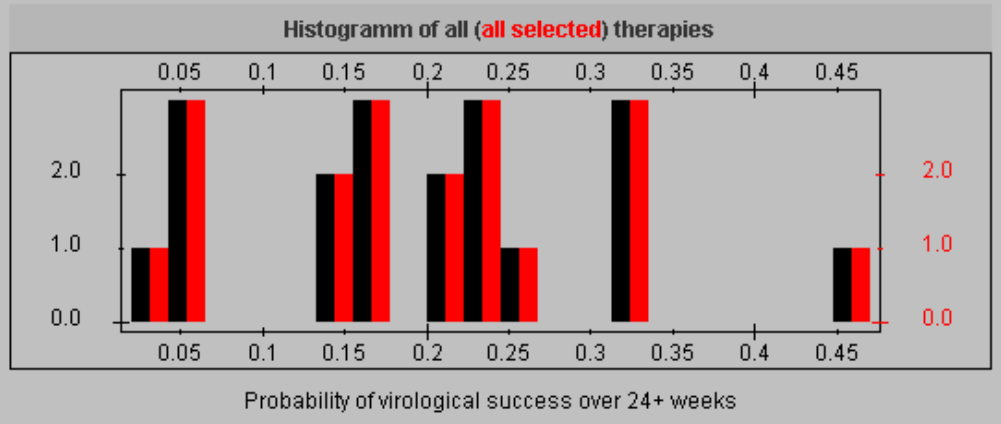
No. of drugs <= - No. of pills per day <= -

NRTIs:		NNRTIs:		PIs:	
>= -	<= -	>= -	<= -	>= -	<= -
ZDV= -		NVP= -		IDV= -	
ddC= -		DLV= -		RTV= -	
ddI= -		EFV= -		SQV= -	
d4T= -				NFV= -	
3TC= -		Reset		APV= -	
ABC= -				LPV= -	
TDF= -		Compute		ATV= -	

Selected drug combinations:

Success*	Regimen	Pills	Comment
0.47	SQV LPV	14	LPV/r(4) SQV(10)
0.33	ddI ABC LPV	7	ddI(1) ABC(2) LPV/r(4)
0.32	ddI 3TC LPV	6	ddI(1) 3TC(1) LPV/r(4)
0.32	ZDV ddI LPV	7	ZDV(2) ddI(1) LPV/r(4)

*) PREDICTED probability of virological success



HIV-GRADE

version 4/2007

Sequence Analysis | Mutation List Analysis

hivalg

1

Name: (optional) Date: (optional)

2

Output Options: Original SpeedBase

3

Additional Algorithms: Select one or more of the previously published algorithms using the checkboxes below to compare their results to HIV-GRADE. Do not select more than 3 additional Algorithms (including geno2pheno) at one time, as this would render the output rather unreadable.

GRADE ANRS HIVDB Rega

geno2pheno: To integrate the results of geno2pheno in the Output, activate the checkbox below. geno2pheno data will be fetched over the Internet, so the process will take up to some minutes.

geno2pheno

show Sequence

4

Sequences:

1. Choose a file to upload from your computer using the file selection box below.
2. Paste sequence text in the text box below.

>Contig_3
AGAGTTTCAGATCTGgGGAGGAGACAAcaaCTCCCCCTCAG
AAGCAGGAGCAGAAGGACAGGAACTgATCCTTTaGCTTC
CCTCAGATCACTCTTTGGCAGCGACCCATTCTCTAATAAA
GGTAGAGGGACAACTAATGGAAGCTCTAATAGATACAGG
AGCAGATGATACAGTATTAGAAGAAATGAATTTGCCAGGA
AAATGGAAACCAAAAATATAGGGGGAATTGGAGGTTTT
GTCAGGTAAGACAATATGATGGTAAAGATAGAGATCT
GTGGACATAAAGCCGTATGTAGTGTATTAGTAGGACCTAC
ACCTGCCAACATAATTGGAAGAAATCTGTTGACTCAGATTG

You can also try out some sample sequences. (Warning: You will need javascript for this feature!)
sample 1 sample 2 sample 3 sample 4 sample 5 sample 6 clear

5

ANALYZE RESET FORM

Subtype and % similarity to closest reference isolate (adopted from Stanford hivDB):
PR: B (91.2%)
RT: B (94.1%)

Gene	Differences from Consensus B	Drug Resistance Mutations
PR	L10I, T12S, I15V, G16E, K20M, L24I, R41K, M46I, I54V, K55R, Q61E, I62V, L63N, I72V, G73C, V82A	L10I, K20M, L24I, M46I, I54V, G73C, V82A
RT	P25AP, I31IL, V35T, T39S, K49R, K122E, I135T, S162C, M184V, G196E, T200IT, T215Y, V245E	M184V, T215Y

	GRADE_04/2008			ANRS_10/2007			HIVDB_4.3.6			Rega_V7.1.1			geno2pheno			
NRTI	Mutation List	Algorithm Result	SIR	Mutation List	Algorithm Result	SIR	Mutation List	Algorithm Result	SIR	Mutation List	Algorithm Result	SIR	Predicted Resistance Factor	Z-Score	SIR	Final Rating
3TC	M184V	Resistance	R	M184V	Resistance	R	M184V, T215Y	High-level resistance	R	M184V	Resistant GSS 0	R	61.6 : R (>15.4)	14.4 : R (>9.0)	R	
ABC	M184V, T215Y	limited susceptibility	I		Susceptible		M184V, T215Y	Intermediate resistance	I		Susceptible GSS 1		3.8 : R (>3.4)	8.2 : R (>7.34)	R	
AZT	T215Y	limited susceptibility	I	T215Y	Resistance	R	M184V, T215Y	Low-level resistance	I		Susceptible GSS 1		9.0 : S (<14.5)	3.7 : S (<4.92)		
AZT_SP	T215Y, M184V	Susceptible														
D4T	T215Y	limited susceptibility	I	T215Y	Resistance	R	M184V, T215Y	Low-level resistance	I		Susceptible GSS 1		1.2 : S (<1.5)	0.2 : S (<1.65)		
D4T_SP	T215Y, M184V	Susceptible														
DDC													1.9 : I(1.7-2.2)	3.9 : I (2.99-5.09)	I	
DDI	M184V, T215Y	limited susceptibility	I		Susceptible		M184V, T215Y	Low-level resistance	I		Susceptible GSS 1		2.2 : I(2-2.4)	3.4 : I (2.80-3.99)	I	
FTC	M184V	Resistance	R	M184V	Resistance	R	M184V, T215Y	High-level resistance	R	M184V	Resistant GSS 0	R				
TDF	T215Y	limited susceptibility	I		Susceptible		M184V, T215Y	Potential low-level resistance	S		Susceptible GSS 1		1.4 : S (<1.7)	2.0 : S (<3.0)		
TDF_SP	T215Y	limited	I													

TDF_SP	T215Y	limited susceptibility	I															

Scored mutations for Drugclass NRTI : M184V, T215Y

NRTI Comments

- M184V has resensitising effects on d4T. M184V is selected by 3TC.
- M184V has resensitising effects on AZT. M184V is selected by 3TC.

	GRADE_04/2008			ANRS_10/2007			HIVDB_4.3.6			Rega_V7.1.1			geno2pheno			
NNRTI	Mutation List	Algorithm Result	SIR	Mutation List	Algorithm Result	SIR	Mutation List	Algorithm Result	SIR	Mutation List	Algorithm Result	SIR	Predicted Resistance Factor	Z-Score	SIR	Final Rating
DLV								Susceptible			Susceptible GSS 1		1.2 : S (<9.7)	-0.5 : S (<5)		
EFV		Susceptible			Susceptible			Susceptible			Susceptible GSS 1		0.9 : S (<7)	-0.7 : S (<4.35)		
ETR		Susceptible			Susceptible			Susceptible			Susceptible GSS 1					
NVP		Susceptible			Susceptible			Susceptible			Susceptible GSS 1		2.1 : S (<9)	0.3 : S (<3.02)		

Scored mutations for Drugclass NNRTI :

	GRADE_04/2008	ANRS_10/2007	HIVDB_4.3.6	Rega_V7.1.1	geno2pheno	

NVP		Susceptible		Susceptible		Susceptible		Susceptible GSS 1		2.1 : S (<9)	0.3 : S (<3.02)				

Scored mutations for Drugclass NNRTI :

PI	GRADE_04/2008			ANRS_10/2007			HIVDB_4.3.6			Rega_V7.1.1			geno2pheno			Final Rating
	Mutation List	Algorithm Result	SIR	Mutation List	Algorithm Result	SIR	Mutation List	Algorithm Result	SIR	Mutation List	Algorithm Result	SIR	Predicted Resistance Factor	Z-Score	SIR	
APV/FPV	L24I, M46I, V82A, I54V	Resistance	R				L10I, L24I, M46I, I54V, G73C, V82A	Intermediate resistance	I	L10I, K20M, L24I, M46I, I54V, V82A	Intermediate Resistant GSS 0.5	I				
APV/FPV_RTV	L24I, M46I, I54V, V82A	Intermediate	I	L10I, I54V, I62V, V82A	Resistance	R				L10I, K20M, L24I, M46I, I54V, V82A	Intermediate Resistant GSS 0.75	I	6.6 : S (<12)	4.7 : S (<6.4)		
ATV	L10I, G73C, L24I, M46I, V82A, I54V	Resistance	R				L10I, L24I, M46I, I54V, G73C, V82A	Intermediate resistance	I	L10I, K20M, L24I, M46I, I54V, G73C, V82A	Resistant GSS 0	R				
ATV_RTV	L10I, G73C, L24I, M46I, V82A, I54V	Resistance	R	L10I, G16E, M46I	Resistance	R				L10I, K20M, L24I, M46I, I54V, G73C, V82A	Resistant GSS 0	R	18.0 : R (>10)	7.8 : R (>5.95)	R	
ATV_SP	L10I, G73C, L24I, M46I, V82A,	Resistance	R													

NFV	L10I, L24I, M46I, V82A, I54V, K20M	Resistance	R	L10I, M46I, I54V, V82A	Possible resistance	I	L10I, L24I, M46I, I54V, G73C, V82A	High-level resistance	R	L10I, K20M, L24I, M46I, I54V, I62V, G73C, V82A	Resistant GSS 0	R	32.0 : R (>5.8)	7.5 : R (>3.3)	R
SQV	I54V, G73C	Resistance	R				L10I, L24I, M46I, I54V, G73C, V82A	Intermediate resistance	I				12.5 : R (>4.5)	8.9 : R (>5.02)	R
SQV_RTV	I54V, G73C	Resistance	R	L10I, I15V, K20M, L24I, I62V, V82A	Resistance	R				L10I, K20M, L24I, M46I, I54V, I62V, G73C, V82A	Resistant GSS 0	R	12.5 : R (>11.4)	8.9 : R (>8.58)	R
SQV_SP	I54V, G73C	Resistance	R												
TPV	I54V, K20M	Susceptible			Susceptible		L10I, M46I, I54V, G73C, V82A	Intermediate resistance	I	L10I, G73C, M46I, V82A, I54V, R41K, K20M	Susceptible GSS 1.5				

Scored mutations for Drugclass PI : L10I, I15V, G16E, K20M,

L24I, R41K, M46I, I54V, I62V, G73C, V82A

Combination analysis

Combinations	GRADE				Final Rating
	Mutation List	Algorithm Result	ADS	SIR	
3TC+ABC+AZT+TDF	T215Y, M41L	Potential complete HAART	3		
ABC+3TC	M41L, T215Y	Reduced activity expected	1		
ABC+3TC+AZT	M41L, T215Y	Reduced activity expected	1.5		
AZT+3TC	M41L, T215Y	Reduced activity expected	1		
TDF+3TC	M41L, T215Y	Reduced activity expected	1.5		
TDF+FTC	T215Y, M41L	Additional active drug needed	2		
TDF+FTC+AZT	M41L, T215Y	Reduced activity expected	1.5		

Scored mutations for Drugclass Combinations : M41L, T215Y

Rega- GSS

- GSS = Genotypic Susceptibility Scores

GSS	Dirençli	Orta derece R	Duyarlı
NRTI	0	0.5	1
NNRTI	0	0.25	1
Etravirin	0	0.5	1
PI	0	0.5	1
PI/r	0	0.75	1.5
EI	0	0.25	1

Kombine tedavilerde hedef GSS

Klinik durum	Hedef GSS
o Tedavi görmemiş; fakat dirençli suşla infeksiyona dair ipuçları var	≥ 3.5
o Tedavi görmüş ya da görmemişler	≥ 3
o Tedavi görmüş ve ilaç seçeneği Kısıtlı olan hastalar	≥ 2

Minör popülasyonların saptanması için duyarlı genotipik yöntemler

- Real-time PCR
- Massive parallel sequencing

Diğer direnç testleri

- Füzyon inhibitörü
- Koreseptör antagonistleri
- İntegraz inhibitörleri
- Viral fitnes ve replikasyon kapasitesi
- Aşırı abakavir duyarlılığı

INI ved CRA direnç tesleri

- **CRA için CCR5 ya da CXCR4 reseptör tropsimi**

à V3 loop analizi: A316 ve I 323

Trofile fenotipik test ve genotipik test

- **İntegraz geninde T66, E92, Q148, N 155 ve Y143 poizsyonlarındaki değişimler**

  Genotipik ve fenotipik testler (Monogram)

Füzyon inhibitörü

- Gp41 36-38; 40-45. amino asit mutasyonları