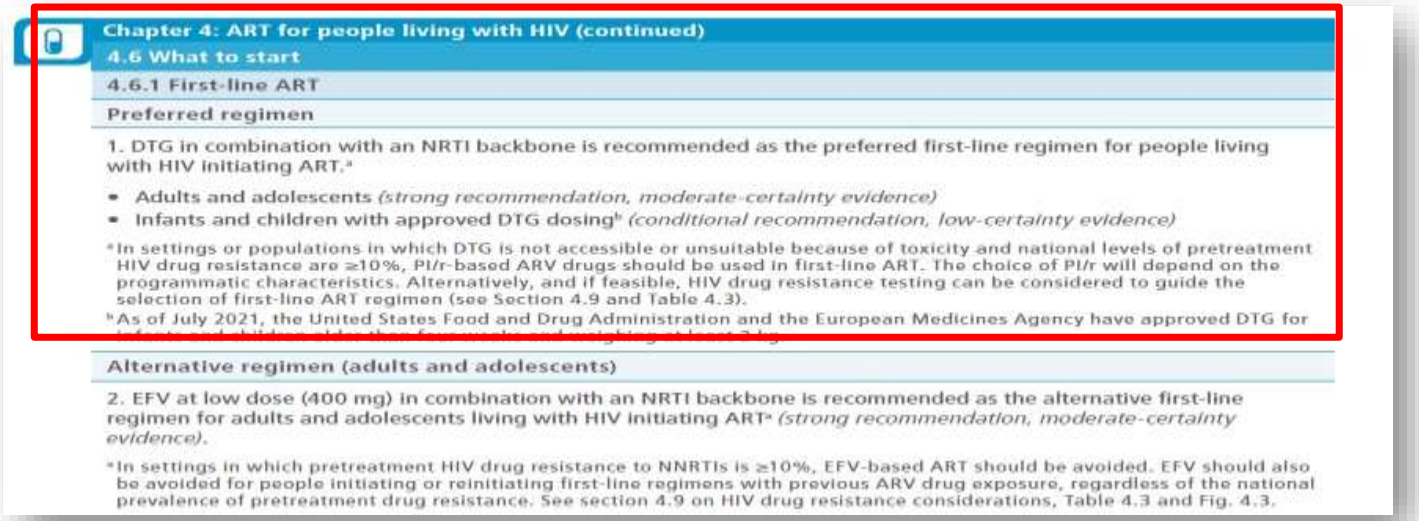
Türkiye'deki ARV Direnç Durumu ve Direnç Testleri

Dr. Tülin DEMİR

ART Temel hedef: Sürdürülebilir, tam bir viral baskılama sağlanması



Chapter 4: ART for people living with HIV (continued)

4.6 What to start

4.6.1 First-line ART

Preferred regimen

1. DTG in combination with an NRTI backbone is recommended as the preferred first-line regimen for people living with HIV initiating ART.[a]

- Adults and adolescents *(strong recommendation, moderate-certainty evidence)*
- Infants and children with approved DTG dosing[b] *(conditional recommendation, low-certainty evidence)*

[a] In settings or populations in which DTG is not accessible or unsuitable because of toxicity and national levels of pretreatment HIV drug resistance are ≥10%, PI/r-based ARV drugs should be used in first-line ART. The choice of PI/r will depend on the programmatic characteristics. Alternatively, and if feasible, HIV drug resistance testing can be considered to guide the selection of first-line ART regimen (see Section 4.9 and Table 4.3).

[b] As of July 2021, the United States Food and Drug Administration and the European Medicines Agency have approved DTG for

Alternative regimen (adults and adolescents)

2. EFV at low dose (400 mg) in combination with an NRTI backbone is recommended as the alternative first-line regimen for adults and adolescents living with HIV initiating ART[a] *(strong recommendation, moderate-certainty evidence)*.

[a] In settings in which pretreatment HIV drug resistance to NNRTIs is ≥10%, EFV-based ART should be avoided. EFV should also be avoided for people initiating or reinitiating first-line regimens with previous ARV drug exposure, regardless of the national prevalence of pretreatment drug resistance. See section 4.9 on HIV drug resistance considerations, Table 4.3 and Fig. 4.3.

- **CD4 sayısından bağımsız erken ART (HIV controller grup hariç)**
- **Genotipik ilaç duyarlılık test sonucu beklenmemeli**
- **Genetik bariyeri yüksek ajanlarla tedavi başlanmalı (NRTI&2. gen INSTI)**

Consolidated guidelines on HIV prevention, testing, treatment, service delivery and monitoring: recommendations for a public health approach. Geneva: World Health Organization; 2021. Licence: CC BY-NC-SA 3.0 IGO.

Günthard HF, Calvez V, Paredes R, Pillay D, Shafer RW, Wensing AM, Jacobsen DM, Richman DD. Human Immunodeficiency Virus Drug Resistance:2018 Recommendations of the International Antiviral Society–USA Panel. CID 2019:68: 177-87.

DHHS. Guidelines for the Use of Antiretroviral Agents in Adults and Adolescents Living with HIV, June 2021.

4.9 ARV drug resistance

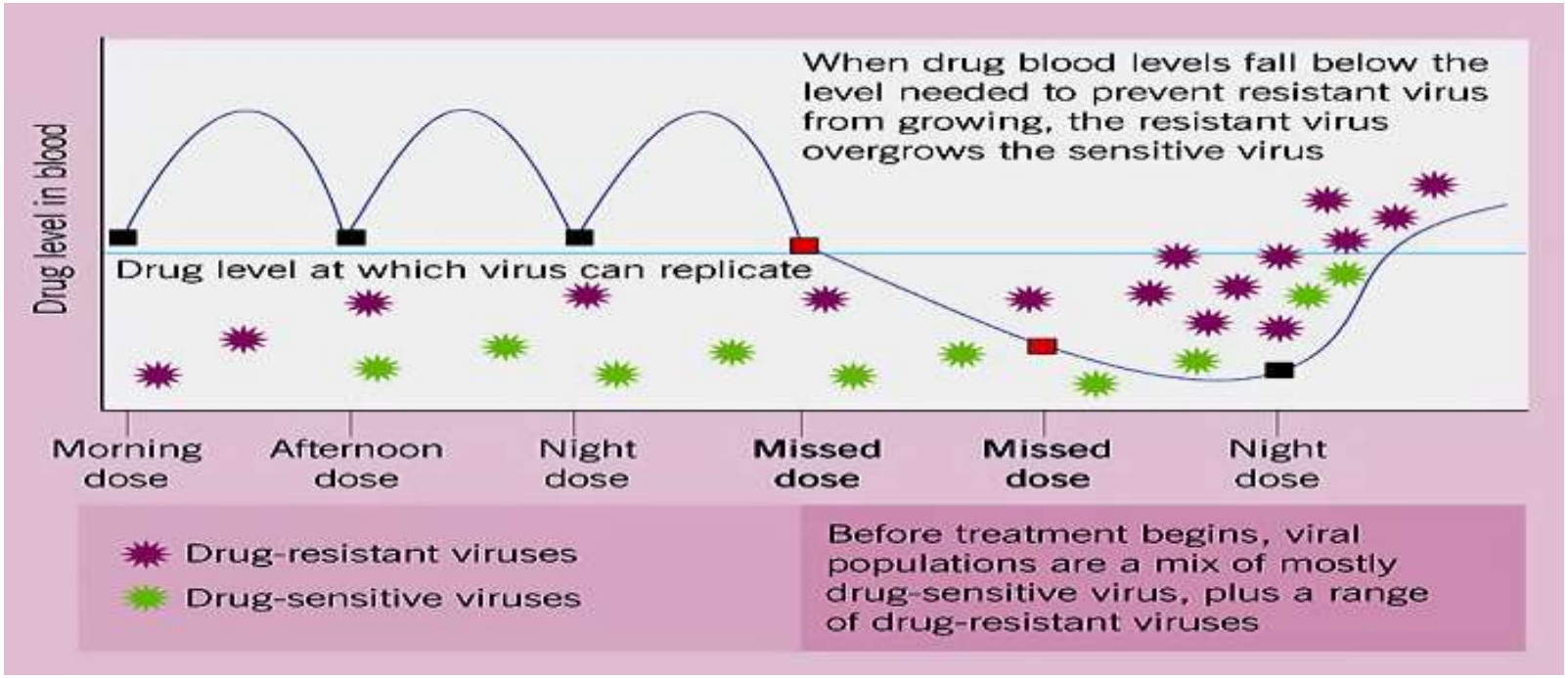
For people initiating first-line ART with pretreatment HIV drug resistance to NNRTIs, a NNRTI-containing regimen should be avoided *(conditional recommendation, low-certainty evidence).*

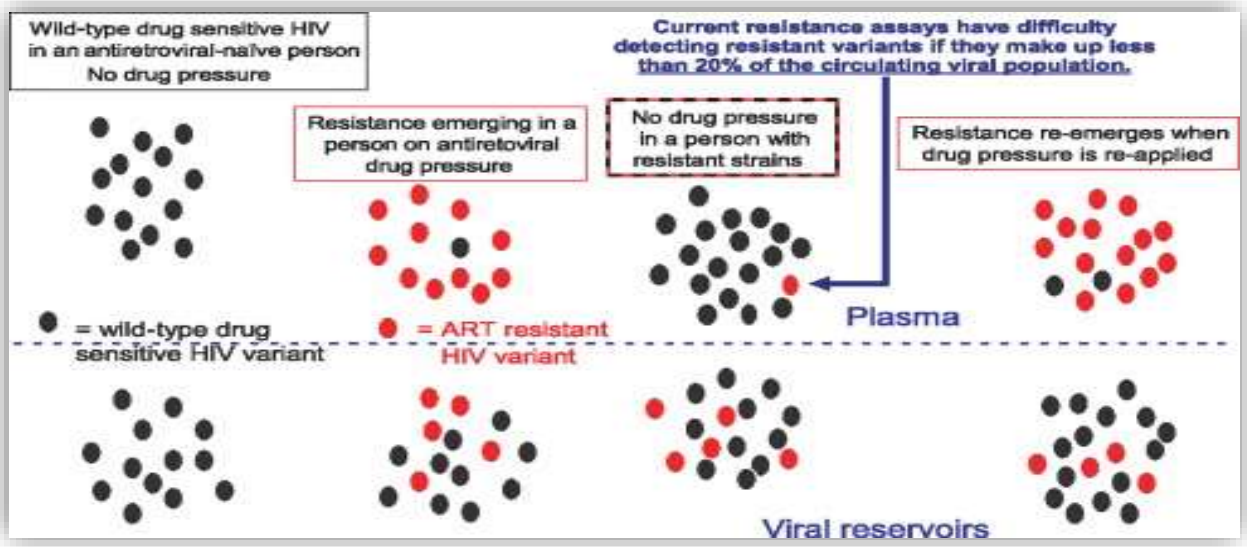
Consensus statement

In countries in which the prevalence of pretreatment HIV drug resistance to NNRTIs among people initiating first-line ART is equal to or greater than 10%, NNRTI-based ART should be avoided.

Viral mutasyonların gelişimi

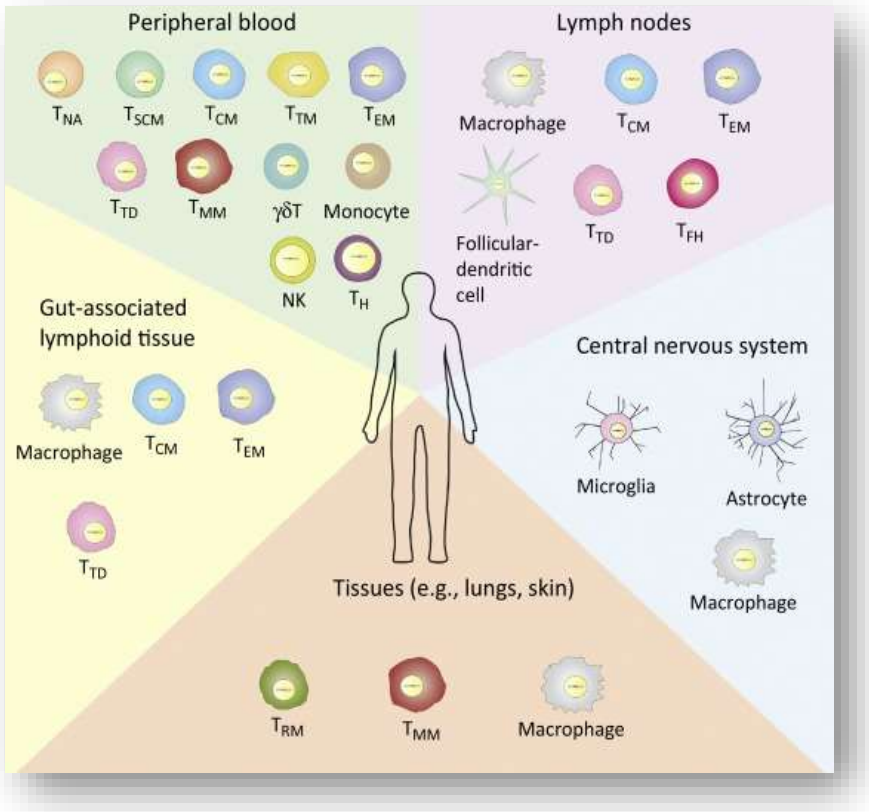
Terapötik ilaç seviyelerinin elde edilememesi/ sürdürülmemesi HIV replikasyonunun devamına ve dirençli virüslerin ortaya çıkmasına neden olabilir





- İlaç baskısı olduğunda dominant olan dirençli virüs varyantı, ilaç kesildiğinde minör varyant haline gelebilir.
- Azınlık populasyonun dirençli olduğu ilaç kullanıldığında varyant çoğalmaya devam eder, direnç tespit edilebilir duruma gelir
- Viral rezervuarlar ise ART'den etkilenmez ve immün sistemden kaçar.

HIV rezervuarları



RETROVIRAL LATENCY

- Çoğu hastada plazmada HIV RNA saptanmasa da viral replikasyonun devam etmekte

- Dokularda HIV DNA ve CD+4 T lenfositlerden proviral DNA (+)

Gunthard HF et al.Evolution of envelope sequences of human immunodeficiency virus type 1 in cellular reservoirs in the setting of potent antiviral therapy. *J Virol*, 1999; 73:9404-12.

Pomerantz RJ.Reservoirs of HIV-1: The main obstacles to viral eradication. Clin Infect Dis. 2002; 34:91-7.

Direnç gelişimi

Virüse bağlı nedenler

- Rekombinasyon yeteneği ↑ varyant oluşumunda çeşitlilik (quacispecies)
- Replikasyon kapasitesi ↑ (10 milyar virion/gün)
- RT enzimi nedenli hatalı sentez ve düzeltememe (1/10000)
- Mutasyon sıklığı (günde 20 milyon) ↑ ↑
- Dirençli varyantın rölatif dayanıklığı
- Latent rezervuarların varlığı (PBMC, lenf nodu, AC, SSS)

Antiviral ajanın hedef proteinini kodlayan gende *mutasyon*

Hastaya bağlı nedenler

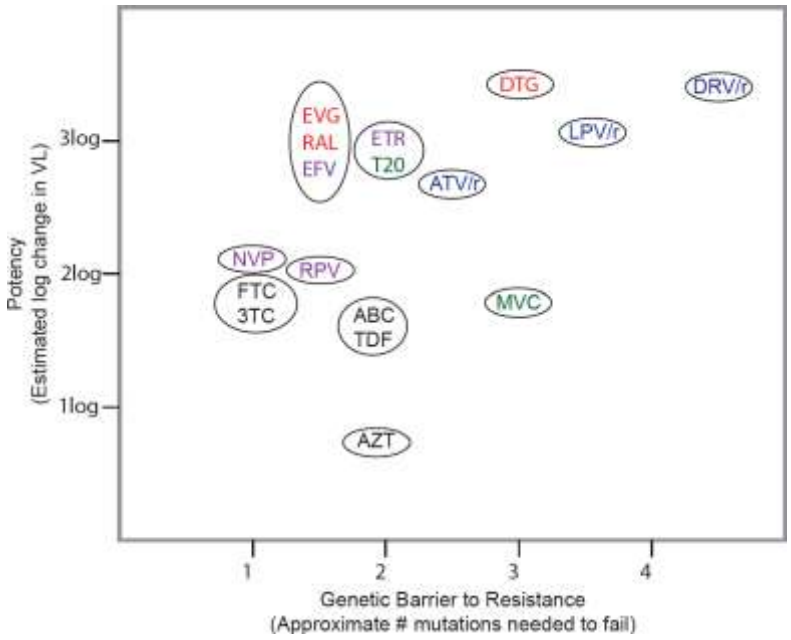
- İlaç rejimine uyum (ilacı bırakma, düzensiz kullanım)
- İlacı tolere edebilme

Antivirale bağlı nedenler

- İlacın replikasyonu tamamen engelleyebilmesi
- Farmakokinetik
- İlacın sağlanabilirliği
- **Genetik bariyer**

Ho et al. 1995; Wei wt al. 1995; Coffin 1995

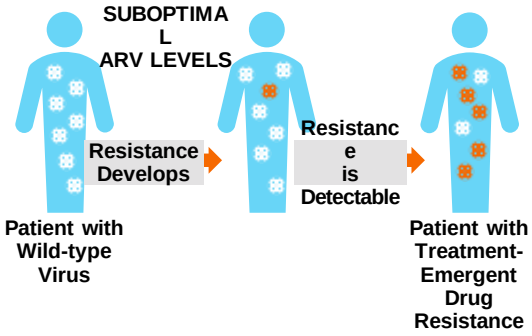
Genetik Bariyer:
Direnç için gerekli mutasyon sayısı, Direncin gelişme kolaylığı



- **Düşük genetik bariyer:** (1 mutasyonla bile direnç)
  - NRTI: 3TC, FTC
  - NNRTI: NVP, EFV, delavirin
  - Füzyon inhibitörü: enfuvirtid
- **Orta derecede bariyer:**
  - NNRTI: etravirin
  - NRTI: ddI, TAF ve ABC
- **Yüksek genetik bariyer:**
  - Ritonavir ile güçlendirilmiş PI
  - NRTI: AZT, d4T
  - İntegraz inhibitörleri: DTG, BIC

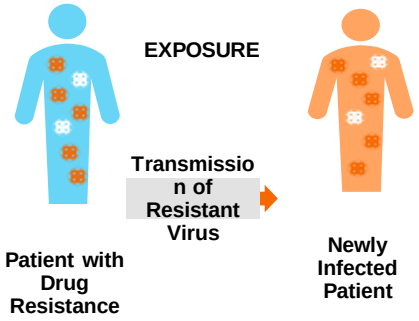
Clutter, D.S et al. (2016)., 46, 292-307

Antiretroviral İlaç Direnci



Tedavi İlişkili (Acquired, Kazanılmış)

- ART alan ve suboptimal ilaç düzeyine sahip hastalarda görülür **(Örn. Düşük tedavi uyumu)**
- Dirençli suşların ortaya çıkması ART'ye yanıtın azalması ve virolojik başarısızlığa neden olabilir
- **En sık tip**



Aktarılmış (Transmitted)

- İlaca dirençli mutasyona sahip virüsle enfeksiyon
- Mutasyonun yarattığı viral fitnesstaki azalma nedeniyle ART yokluğunda birçok dirençli suş, vahşi tip HIV tarafından domine olabilir veya vahşi tipe geri dönüşebilir
  - Mutant suşlar kronik enfekte hastalarda tespit edilemeyebilir seviyede olabilir (ancak yine de arşivlenebilir)
  - ART kullanımı mutant suşun yeniden ortaya çıkmasına ve ardından virolojik başarısızlığa neden olabilir
  - **En önemli tip**

NYSDOH AIDS Institute. HIV Resistance Assays. June 2016 (Updated Feb 2020). https://www.hivguidelines.org/antiretroviral-therapy/hiv-resistance-assays/ 2. Clutter, D et al. Infect Genet Evol 2016; 46:292-307

Aktarılabilen direnç (TDR)

- En sık NRTI>NNRTI>PI>INSTI
- Naive hastalarda ABD %11-24.5, Avrupa'da %6-11 arasında ve genelde stabil
- Almanya'da %9.2 (RESINA, n=2078)
- İngiltere, 2014-2016 yılları arasında (n=655) PI %2.4, NRTI %3.4, NNRTI %2.9
- ABD'de 2014-18 arasında 50747 hastada %18.9 TDR; PI %4.2, NRTI %7, NNRTI %12
- Çin'de %2.2; PI %0.3, NRTI %0.9, NNRTI %0.9
- Macaristan MSM %20.7; PI %0.6, NRTI %9.5, NNRTI %1.2
- İngiltere: %8; ileri yaş ve non-B tip enfeksiyonda risk yüksek
- Portekiz NRTI %6.8, NNRTI %8.9; Subtip B ve düşük viral yük TDR riskini artırmakta

Sapozhnikov j et al. HIV Medicine 2017; 18:756-63.
Tostevin et al. HIV Med 2017; 18:204-13
Vella S, et al.Clin Infect Dis 2005, 41: 239-246
Wensing AM, et al. J Infect Dis 2005; 192: 958-966
Weinstock HS, et al. J Infect Dis. 2005; 189: 2174-2180.
Oette et al. Intervirology 2012; 55:154-159.
Chen et al. BMC Infect Dis 2012; 12: 382
Mbisa JL aet al. J Antimicr Chemother 2020; 75: 3311-18.
Mc Clung et al. Clin Infect Dis. 2021; 27:ciab583.
Günthard HF, CID 2019: International Antiviral Society-USA Panel.
Jun Lan et. Al Virol J . 2021 Sep 6;18(1):181.
Eva Ay et al. J Glob Antimicrob Resist. 2020 Mar:20:124-130
Richardson D. Et al. Int J STD AIDS 2021;32(2):199-201
Pingarillo M et al. Viruses. 2020; 30;12(11):1238.

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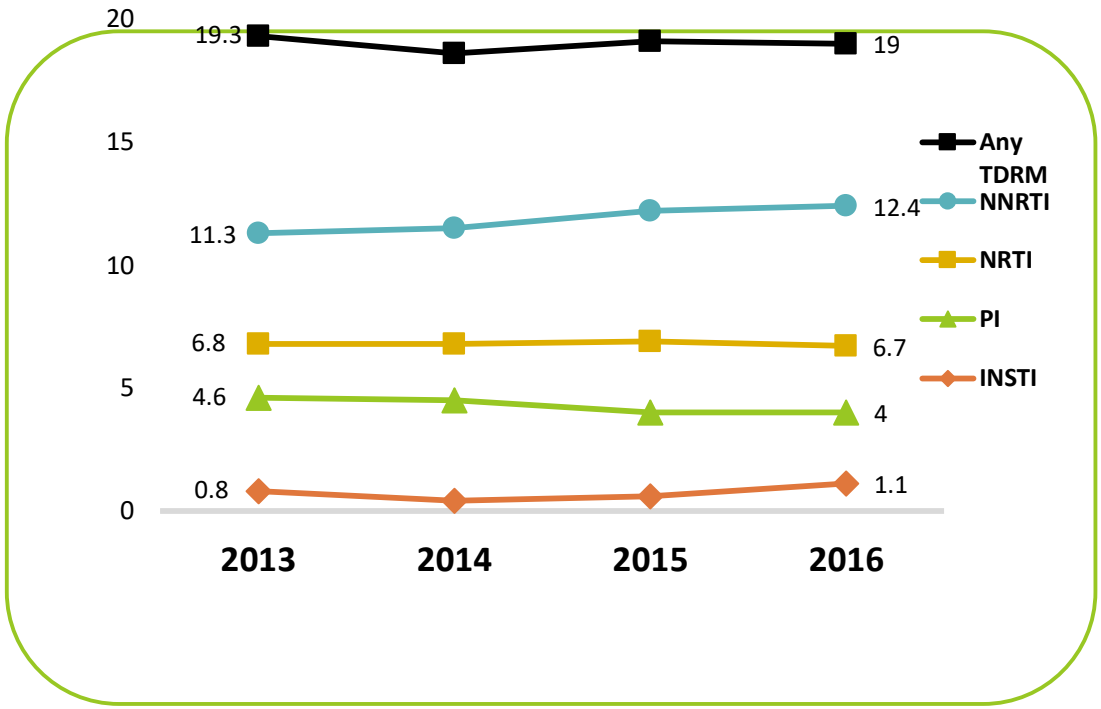
ARV sınıfına göre TDR yaygınlığı

2013–2016 (N=36,288), ABD



En sık NNRTI ve NRTI direnci ,

PI direnci daha az yaygın ve INSTI direnci nadir

McClung RP et al. Conference on Retroviruses and Opportunistic Infections 2019. Seattle, WA. Poster 526; 2. DHHS. Guidelines for the Use of Antiretroviral Agents in HIV-1-Infected Adults and Adolescents. https://aidsinfo.nih.gov/guidelines/archive/adult-and-adolescent-guidelines. June 2021

ABD ve Fransa'da aktarılan direnç

CDC: 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%

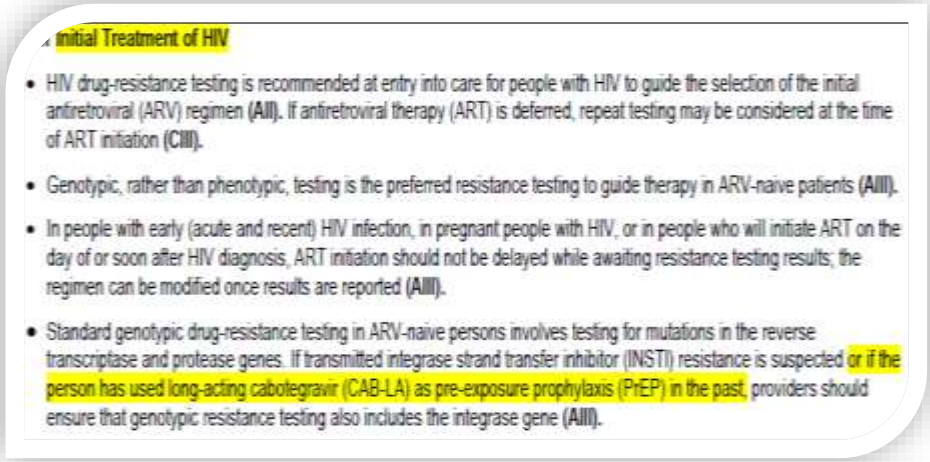
ANRS Survey: TDR from Patients (*N*=799) with Primary HIV Infection in France (2010-2013)

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

- TDR risk faktörü: MSM ve B subtip
- TDR mutasyon prevalansı ABD'dehafif artışta, Fransa'da stabil
- ABD'de NNRTI TDR en sık tip

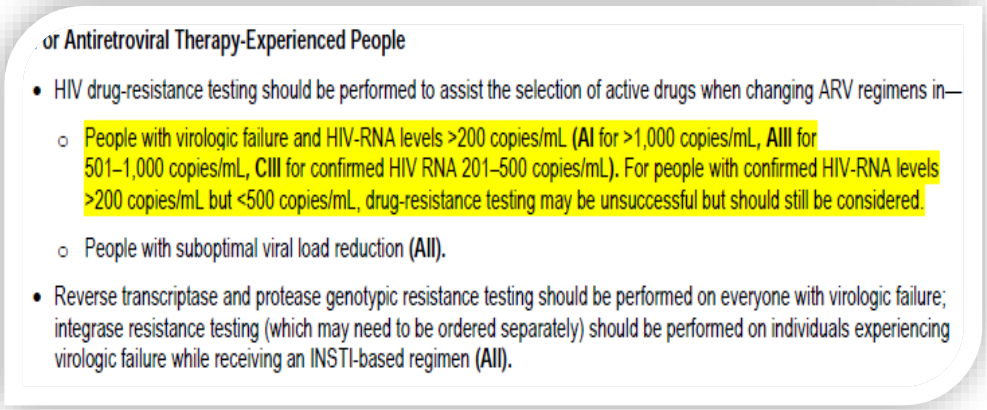
Banez-Ocfemia C, et al. CROI; 2014 Boston. #579
Chaix M, et al. CROI 2014; Boston. #582

Direnç testleri ne zaman yapılmalı?



Initial Treatment of HIV

- HIV drug-resistance testing is recommended at entry into care for people with HIV to guide the selection of the initial antiretroviral (ARV) regimen **(AII)**. If antiretroviral therapy (ART) is deferred, repeat testing may be considered at the time of ART initiation **(CIII)**.
- Genotypic, rather than phenotypic, testing is the preferred resistance testing to guide therapy in ARV-naive patients **(AIII)**.
- In people with early (acute and recent) HIV infection, in pregnant people with HIV, or in people who will initiate ART on the day of or soon after HIV diagnosis, ART initiation should not be delayed while awaiting resistance testing results; the regimen can be modified once results are reported **(AIII)**.
- Standard genotypic drug-resistance testing in ARV-naive persons involves testing for mutations in the reverse transcriptase and protease genes. If transmitted integrase strand transfer inhibitor (INSTI) resistance is suspected or if the person has used long-acting cabotegravir (CAB-LA) as pre-exposure prophylaxis (PrEP) in the past, providers should ensure that genotypic resistance testing also includes the integrase gene **(AIII)**.



or Antiretroviral Therapy-Experienced People

- HIV drug-resistance testing should be performed to assist the selection of active drugs when changing ARV regimens in—
  - People with virologic failure and HIV-RNA levels >200 copies/mL **(AI** for >1,000 copies/mL, **AIII** for 501–1,000 copies/mL, **CIII** for confirmed HIV RNA 201–500 copies/mL). For people with confirmed HIV-RNA levels >200 copies/mL but <500 copies/mL, drug-resistance testing may be unsuccessful but should still be considered.
  - People with suboptimal viral load reduction **(AII)**.
- Reverse transcriptase and protease genotypic resistance testing should be performed on everyone with virologic failure; integrase resistance testing (which may need to be ordered separately) should be performed on individuals experiencing virologic failure while receiving an INSTI-based regimen **(AII)**.

ART naive bireyde:

PR-RT

* Erken (Akut-recent) enfeksiyonda
- Gebe

PR-RT-INSTI

- TDR INSTI şüphesi varsa, PrEP- CAB-LA kullanım öyküsü ----INSTI DR

Tedavi deneyimli bireyde:

PR-RT

- Virolojik failure (HIV RNA >200 kopya)
- Suboptimal viral supresyon

PR-RT-INSTI

* INSTI ted alanlarda virolojik failure

Virolojik failure durumunda direnç testi;

* ART alımına devam ederken ya da ART bırakılmışsa 4 hafta içinde yapılmalı
* *4 haftadan sonra da tedavi için bazı bilgiler elde edilebilir ancak drug-selective baskı olmadığı için mutasyonlar izlenemeyebilir*

Guidelines for the Use of Antiretroviral Agents in Adults and Adolescents with HIV, Sept 2022

Direnç testlerinin klinik kullanımı

- Direnç testleri tedavi kararlarını belirlemek için önemli araçlardır
  - RNA genotipleme çoğu klinik durumda genellikle tercih edilen testtir
  - HIV RNA genotipleme ile kaçırılan direnç Proviral DNA genotipleme ile saptanabilir
- RNA genotipleme çoğu klinik durumda genellikle tercih edilen testtir. Sadece tespit edilebilir viral yük varlığında ***yapılabilir*****
- Virolojik baskılanma sağlanmış ve sonrasında LLV (<200 kopya/mL) bireylerde Proviral DNA genotipleme-PBMC DNA faydalı olabilir. Direnç testi denenebilir. **Viral yükden bağımsız ancak dirençli populasyon saptama oranı daha düşük??**
- Proviral DNA genotipik analizi "arşivlenmiş" veya sirkülasyonsuz viral direnç hakkında bilgi sağlayabilir.

Figure 1a. Standard resistance assays analyze viral RNA in plasma[1]

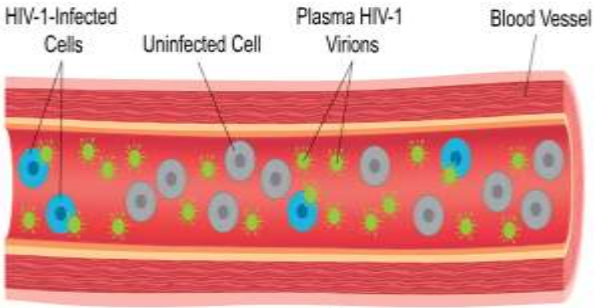
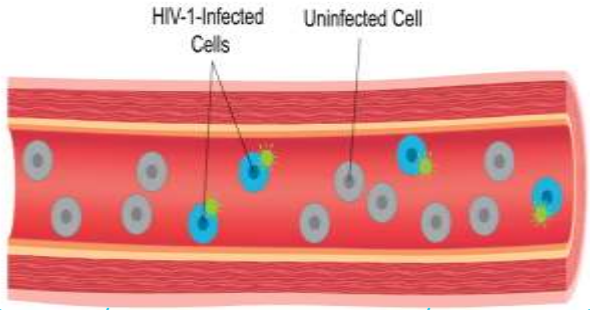


Figure 1b. Proviral DNA analyzes archived proviral DNA embedded in host cells during virus replication[1]



Monogram Biosciences. GenoSure Archive. https://www.monogrambio.com/hiv-tests/suppression-management/genosure-archive
DHHS. Guidelines for the Use of Antiretroviral Agents in Adults and Adolescents Living with HIV, June 2021. Available at: http://aidsinfo.nih.gov/guidelines
NYSDOH AIDS Institute. HIV Resistance Assays. June 2016 (Updated Feb 2020). https://www.hivguidelines.org/antiretroviral-therapy/hiv-resistance-assays/

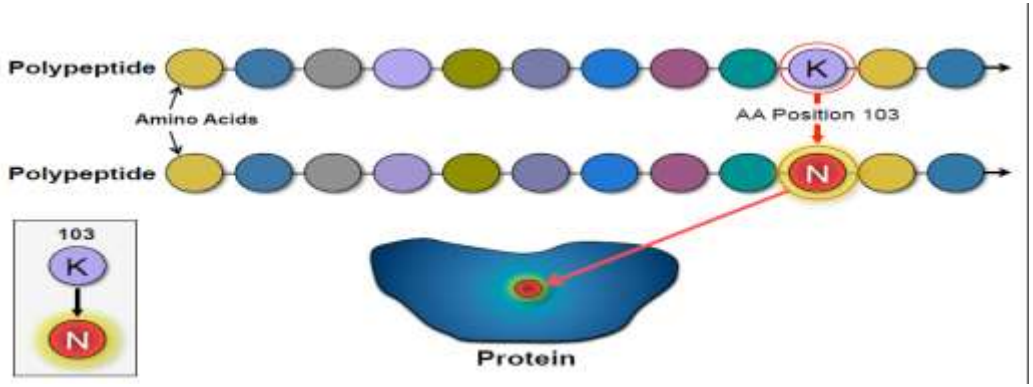
Direnç testlerinin klinik kullanımı

- Tedavi seçeneklerini sınırlar ve tedavi sonuçlarını etkiler
- Duyarlılık ≠klinik etkinlik
- Direnç testi halen kullanılmakta olan ilaç rejimine direnci yansıtır
- Yeni bir ART rejimi oluşturulurken, önceki ve mevcut tüm ilaç direnci test sonuçları ve ilaç geçmişi göz önünde bulundurulmalıdır.
- Tedavinin herhangi bir aşamasında kullanılan ilaca direnç varsa, yeni yapılan direnç testinde ilaca duyarlı olsa bile ilaç tekrar kullanılmamalıdır.

DHHS, AIDSinfo. Pharmacokinetics. Updated March 2019. 2.
Aarnoutse RE et al. Drugs. 2003;63:741-753 3. Luber AD. *MedGenMed*. 2005;7:69 4. Zdanowicz MM. *Am J Pharm Educ*. 2006;70:100; DHHS. Drug Resistance. Published January 2019; 6. Clutter DS et al. *Inf Gen Evol.* 2016;46:292-307. 7. DHHS. Guidelines for the Use of Antiretroviral Agents in Adults and Adolescents Living with HIV, June 2021 8. NYSDOH AIDS Institute. HIV Resistance Assays. June 2016 (Updated Feb 2020). https://www.hivguidelines.org/antiretroviral-therapy/hiv-resistance-assays/

Direnç nasıl okunur?

Referans sekansa göre nükleik asit diziliminde değişimler



Kodon

Silent Mutation

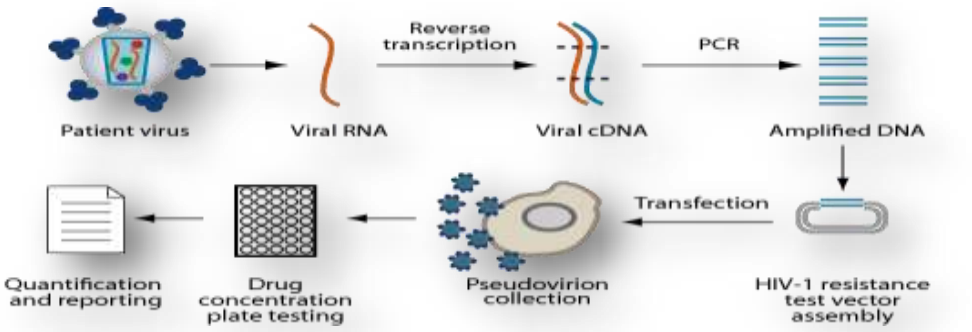
AAA GAC AGT → AAA AAC AGC

Lys (K)	Asp (D)	Ser (S)	Lys (K)	Asn (N)	Ser (S)

İlaç direnci saptama yöntemleri

Fenotipik duyarlılık testi

- **Bir virüsün farklı ARV konsantrasyonlarında büyüme yeteneğini ölçer**
- Hastanın virüsünün IC50'si ilaca duyarlı (vahşi tip) bir referans virüsün IC50'si ile karşılaştırılır ve farkı tanımlanır
- **Virüs inhibisyonu için gerekli ilaç miktarı ölçülür** Replikasyonu %50 (IC50) inhibe eden ilaç konsantrasyonu belirlenir ve referans HIV suşu sonuçları ile karşılaştırılır



*** Fenotipik testler teknik olarak genotipik testlerden daha karmaşık, emek yoğun ve pahalı**

*** Bilinen/şüphelenilen karmaşık ilaç direnci mutasyon paterni tespitinde genotipik testlere eklenebilir**

1. DHHS. Guidelines for the Use of Antiretroviral Agents in Adults and Adolescents Living with HIV, June 2021. Available at: http://aidsinfo.nih.gov/guidelines
2. NYSDOH AIDS Institute. HIV Resistance Assays. June 2016 (Updated Feb 2020). https://www.hivguidelines.org/antiretroviral-therapy/hiv-resistance-assays/

İlaç direnci saptama yöntemleri

Genotipik duyarlılık testi

I. **Sekans temelli testler:** Sanger, NGS

II. **Nokta mutasyon temelli testler:** allel spesifik PCR, oligonükleotid ligation assay,…

- Fenotipik metoda göre hızlı (1-2 hafta)
- Göreceli olarak daha ucuz ($100 - $400)
- Teknik gereksinim daha az
- Subtip belirlenebilir

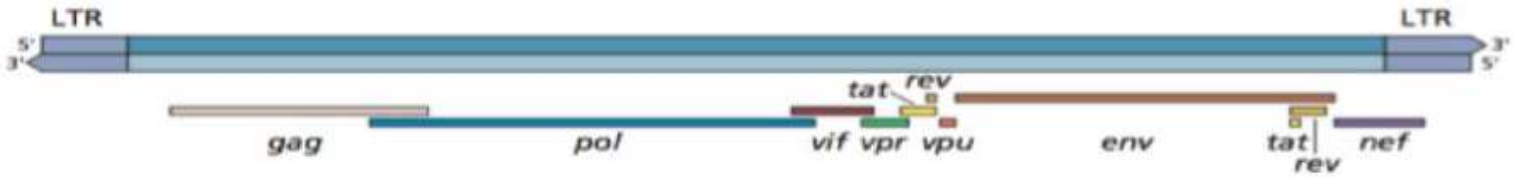
- Duyarlılık indirekt olarak ölçülür
- Testlerin yorumlanması zor
- Tüm mutasyon paternleri bilinmiyor, öz. yeni ilaçlar
- Mixture tespitinde subjektivite
- Viral yük >500 kopya/ml üzerinde ise sonuçlar iyi
- **%20 altında minör populasyonlarda mutasyonların tespitinde iyi değil**

Sanger sekanslama

- **Altın standart**
- Genomdaki bölgeleri sekanslama için zincir sonlandırma metodu kullanılır.
- Elde edilen sekans HIV DB kullanılarak yorumlanır
- **Viral mutantlar total virüs miktarının >%20 ise saptanabilir**
- Ticari kitler mevcut: Abbott, ABL
- In-house testler

NGS

- Sekanslama: Ligasyonla ya da sentezle
- Duyarlılık yüksek, hızlı
- **%5 altındaki varyantlar tespit edilebilir….Minör populasyonların analizi klinik olarak anlamı?, hatalı direnç sonucu nedenli ilaç değişimi?**
- Biyoinformatik bilgi gerekli, yorumlama zor
- Geliştirilmesi gerekli

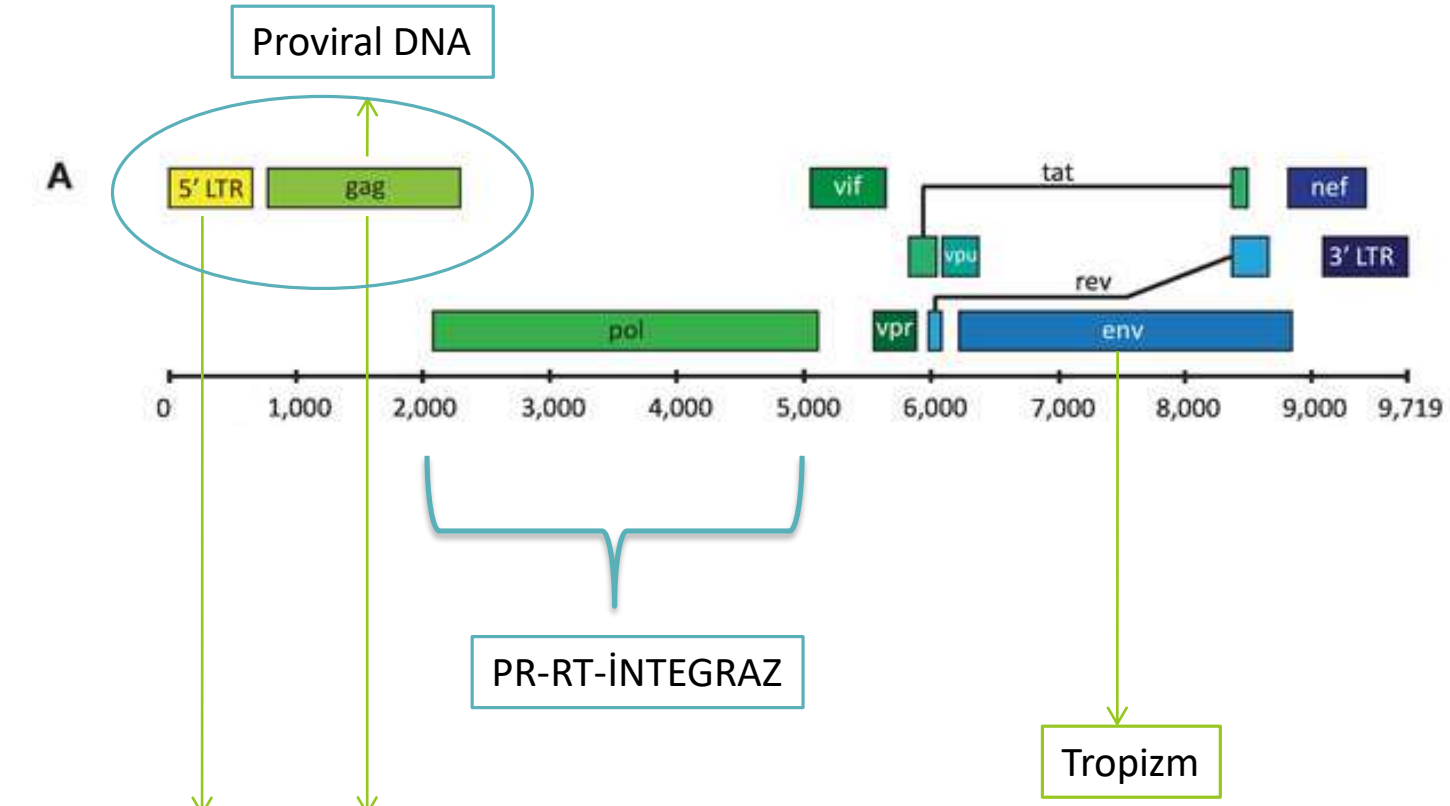


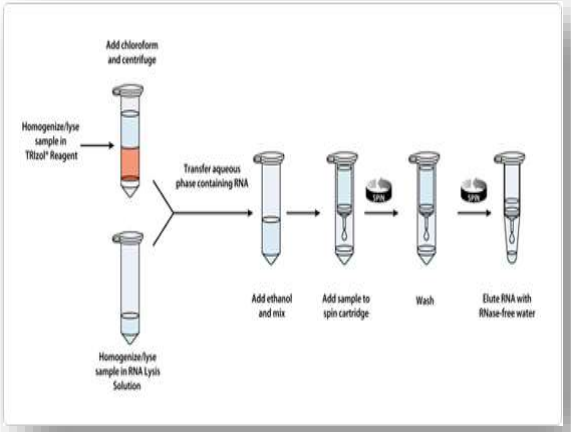
Sanger vs NGS

- Akut HIV-1 enfeksiyonlarının %70-90'ı tek bir virüs varyantı ile gelişir
- %10-30'unda düşük oranlarda azınlıkta olan varyantlar aktarılır
- Nadiren DRM taşıyan azınlık varyantlar ilaç almamış akut enfekte vakalarda bulunabilir.
- **Akut enfeksiyonda DRM taşıyan azınlık varyantların tedaviyi negatif etkilediği belirlenmemiş**
- **Bazı çalışmalarda kronik enfekte ve NNRTI (EFV, NEV) alanlarda tedavi başarısında azalma izlenmiştir. NGS??**

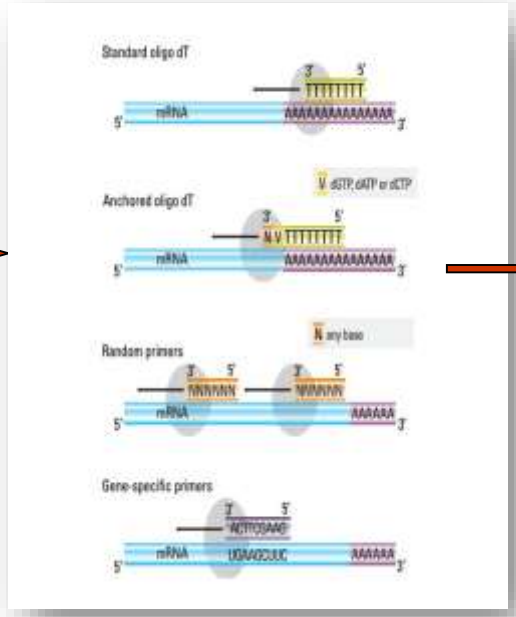
Abrahams MR et al. J Virol 2009 83 3556-67.
Cozzi-Lepri A et al. J Antimicrob Chemother 2015 70 930-40.
Gianella S et al. J Virol 2011; 85. 8359-67.
Metzner KJ et al. J Infect Dis 2010; 201: 1063-71.
Mrtzner KJ et al. AIDS 2014; 28. 2231-9.
Li JZ, et al. JAMA 2011; 305: 1327-35.

Viral genom

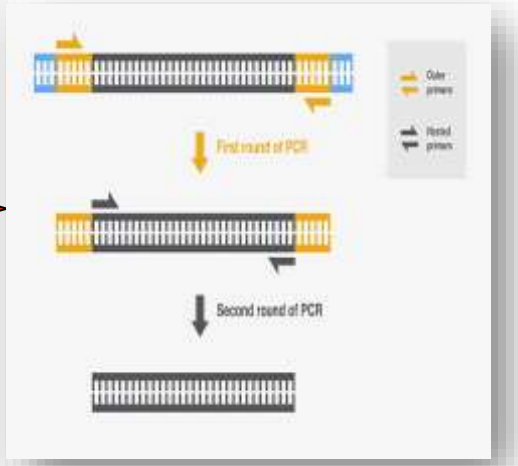

Proviral DNA
A
5' LTR
gag
vif
tat
nef
vpu
3' LTR
rev
pol
vpr
env
0
1,000
2,000
3,000
4,000
5,000
6,000
7,000
8,000
9,000
9,719
PR-RT-İNTEGRAZ
Tropizm
Ticari viral RNA tespit eden sistemlerde hedef bölge



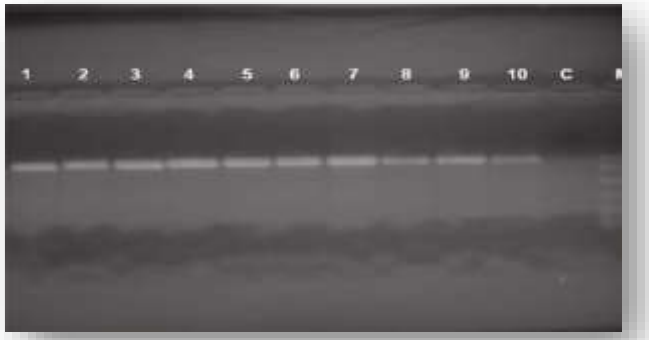
Viral RNA izolasyonu



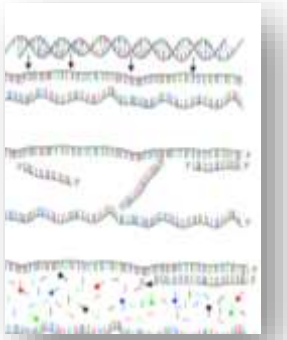
cDNA sentezi +PCR



Nested PCR

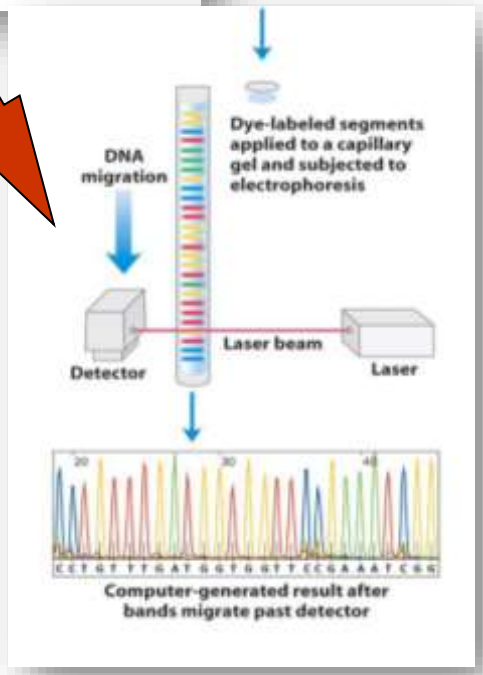


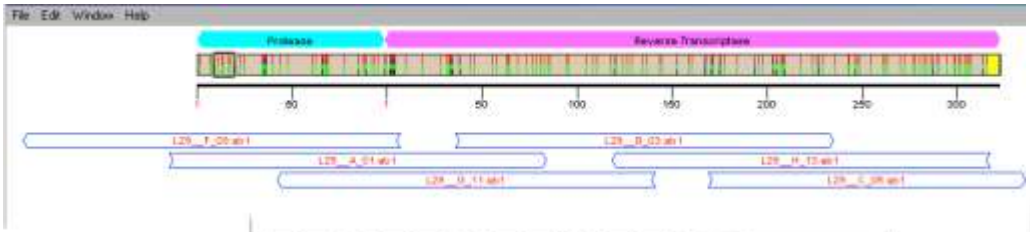
PCR clean-up

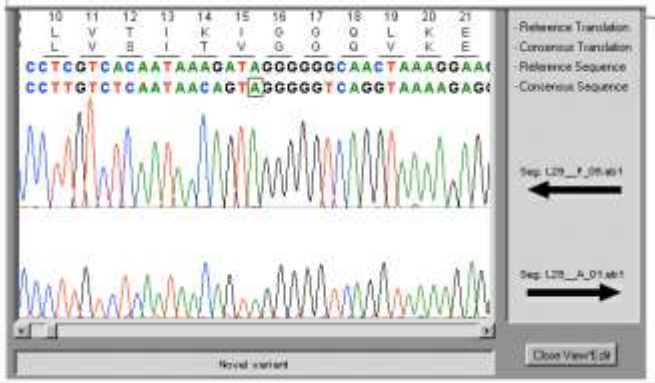


Cycle-sequencing

Sekans clean-up







Sekans dizilimi…..Fasta dosyası

DİRENÇ YORUMLAMA KAYNAKLARI

Kurala dayalı (Rule-based)

1. ANRS, France
2. Rega (Belgium)
3. HIV Genotypic Resistance Algorithm Deutschland
4. HIV dbStanford University,USA

Yapay zekaya dayalı (Machine-learning)

İlaç direnç raporlarını nasıl yorumlamalıyız?

Stanford University
HIV DRUG RESISTANCE DATABASE
A curated public database to represent, store and analyze HIV drug resistance data.

HIVdb Program Report

Genotypic Resistance Interpretation Algorithm

Sierra version 2.2.9 (last updated on 2018-09-13)
HIVdb version 8.6.1 (last updated on 2018-07-18)

Sequence 337-18 SHSARU97

Sequence Summary

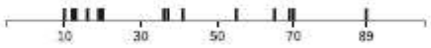
Sequence includes PR:	codons 1 - 99
Sequence includes RT:	codons 1 - 323
Subtype:	⊖ C (7.11%)

- AF443091: **Botswana (2000); C (7.11%); best match**
- AY713415: Somalia (1989); C (7.11%)
- AY586540: Cuba (1999); CRF18_cpx (7.27%)
- AB286851: Ghana (2003); CRF06_cpx (7.27%)
- KC870044: China (2009); C (CRF57_BC, 7.35%)
- EU839600: Haiti (2005); B (7.35%)
- AF286227: South Africa (1997); C (7.35%)
- AY967806: China (1998); C (7.35%)
- AY444811: United States (2000); A + G (CRF02_AG, 7.42%)
- HQ026634: Korea, Republic of (2004); A + G (CRF02_AG, 7.42%)

PR SDRMs:	None
RT SDRMs:	None

Sequence Quality Assessment

PR



RT

Drug Resistance Interpretation: PR

PI Major Resistance Mutations:	None
PI Accessory Resistance Mutations:	None
Other Mutations:	L10I, T12K, I13V, G16E, L19I, K20I, M36I, N37T, R41K, K55R, E65D, H69K, K70R, L89M

Protease Inhibitors

atazanavir/r (ATV/r)	Susceptible
darunavir/r (DRV/r)	Susceptible
lopinavir/r (LPV/r)	Susceptible

PR Comments

Other

- **L10I/V** are polymorphic, PI-selected accessory mutations that increase the replication of viruses with other PI-resistance mutations.
- **K20I** is the consensus amino acid in subtype G and CRF02_AG. In subtypes B and C, **K20I** is a PI-selected accessory mutation that reduces NFV susceptibility.

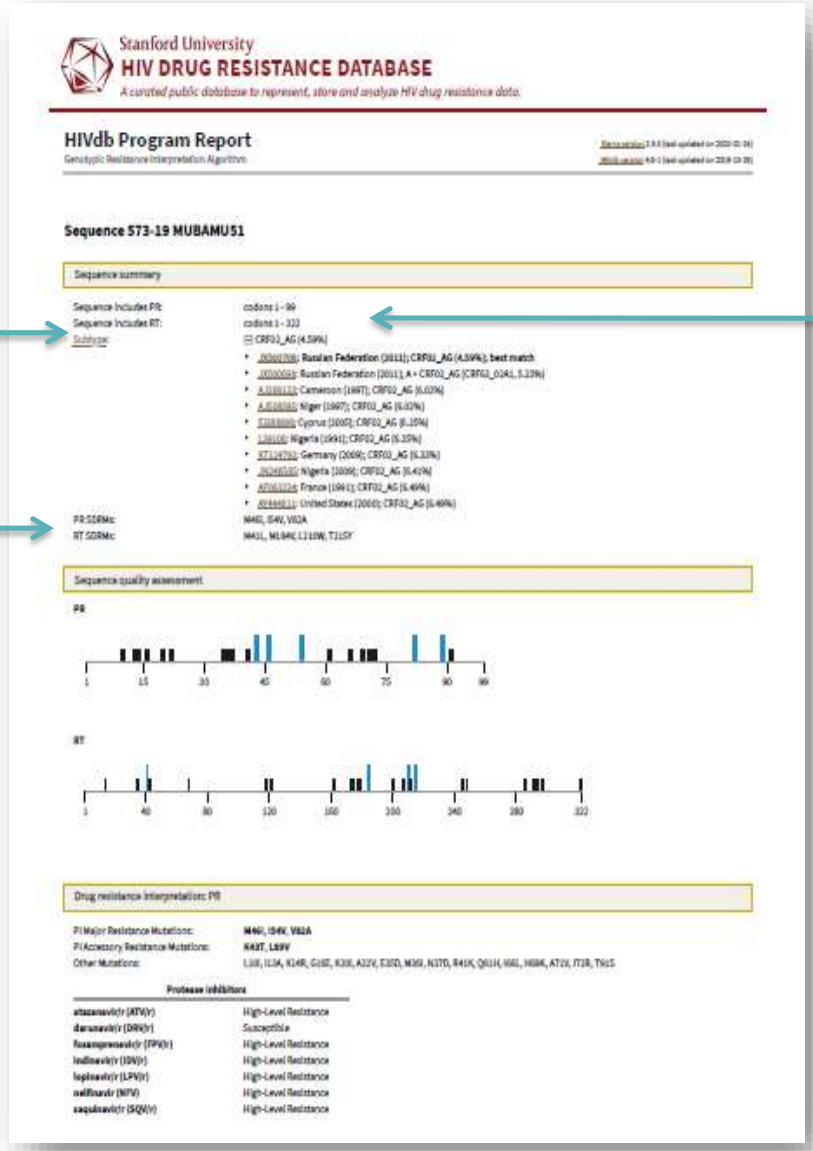
Mutation Scoring: PR

PI	ATV/r	DRV/r	LPV/r
Total	0	0	0

Drug Resistance Interpretation: RT

NRTI Resistance Mutations:	None
NNRTI Resistance Mutations:	None
Other Mutations:	E6D, V35T, T39A, E40D, V60I, V118I, K122P, S162C, K173A, Q174K, D177E, T200A, Q207E, R211K, V245Q, A272P, K277R, E291D, V292I, I293V

Nucleoside Reverse Transcriptase Inhibitors


Stanford University
HIV DRUG RESISTANCE DATABASE
A curated public database to represent, store and analyze HIV drug resistance data.

HIVdb Program Report
Genotypic Resistance Interpretation Algorithm

Sequence 573-19 MUBAMU51

Sequence summary

Sequence includes PR: codons 1 - 99
Sequence includes RT: codons 1 - 322
Subtype: CRF02_AG (4.59%)

Sequence quality assessment

PR
RT

Drug resistance interpretation: PR

PI Major Resistance Mutations: M46I, I54V, V82A
PI Accessory Resistance Mutations: K43T, L89V

Protease inhibitors	
atazanavir/r (ATV/r)	High-Level Resistance
darunavir/r (DRV/r)	Susceptible
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

3. Virüsün subtipi

4. PR ve RT DR varlığı

1. İncelenen bölgeler
(PR için: 10-93 aa
RT için: 41-238 aa olmalı)

2. Sekans kalitesi değerlendirilir

HIVdb Program Report

Genotypic Resistance Interpretation Algorithm

Sierra version 2.2.8 (last updated on 2018-09-13)

HIVdb version 8.6.1 (last updated on 2018-07-18)

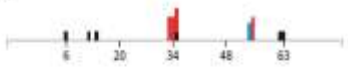
Sequence PATIENT ID X

Sequence Summary

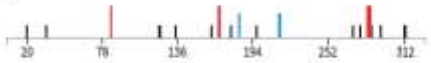
Sequence includes PR:	codons 1 - 94
Sequence includes RT:	codons 1 - 331
Subtype:	⊞ B (5.58%)
PR SDRMs:	I54M
RT SDRMs:	M184V, T215C

Sequence Quality Assessment

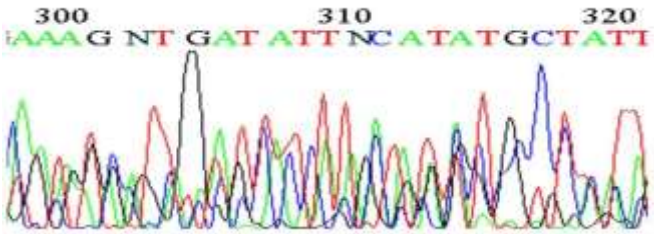
PR



RT



- **Severe warning:** There are 2 stop codons in RT: L168*, G285*HQY.
- **Warning:** The PR sequence had 5 amino acids trimmed from its 3'-end due to poor quality.
- **Warning:** The PR gene has a frameshift: PR35ins1bp_K.
- **Warning:** The RT gene has a frameshift: RT169ins1bp_A.
- **Warning:** There are 5 unusual mutations in RT: Q8SL, E169L, L283M, R284T, G285*HQY.
- **Note:** There are 3 unusual mutations in PR: L33S, E34EDHQ, K55AGRT.



HIVdb Program Report

Genotypic Resistance Interpretation Algorithm

Sierra version 2.2.8 (last updated on 2018-09-13)

HIVdb version 8.6.1 (last updated on 2018-07-18)

Sequence PATIENT ID X

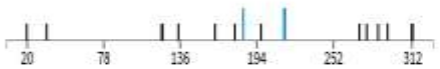
Sequence Summary

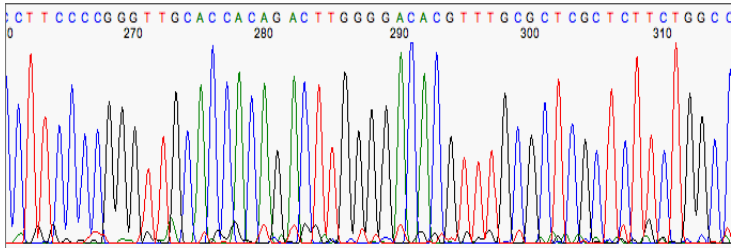
Sequence includes PR:	codons 1 - 99
Sequence includes RT:	codons 1 - 331
Subtype:	⊞ B (4.57%)
PR SDRMs:	None
RT SDRMs:	M184V, T215C

Sequence Quality Assessment

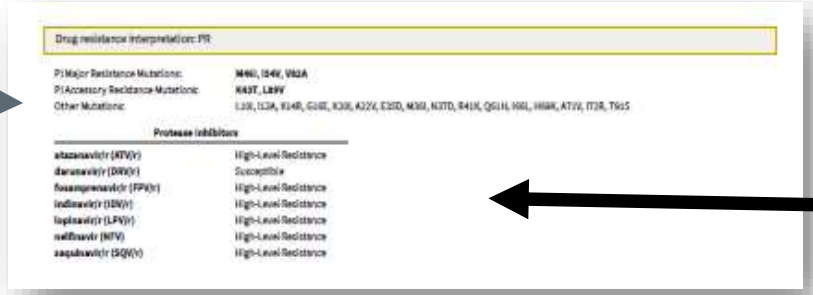
PR

RT





5. Proteaz direnci varlığı



Drug resistance interpretation: PR

PI Major Resistance Mutations:	M46I, I54V, V82A
PI Accessory Resistance Mutations:	K43T, L89V
Other Mutations:	L10I, I13A, K14R, G16E, K20I, A22V, E35D, M36I, N37D, R41K, Q61H, H69L, H69K, AT3V, IT3R, TN15

Protease Inhibitors	
atazanavir/r (ATV/r)	High-Level Resistance
darunavir/r (DRV/r)	Susceptible
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

6. PI İlaç duyarlılık sonucu

tipranavir/r (TPV/r) Intermediate Resistance

PR comments

PI Major

- M46I/L are relatively non-polymorphic PI-selected mutations. In combination with other PI-resistance mutations, they are associated with reduced susceptibility to each of the PIs except DRV.
- I54V is a non-polymorphic PI-selected mutation that contributes reduced susceptibility to each of the PIs except DRV.
- V82A is a non-polymorphic mutation selected primarily by IDV and LPV. It reduces susceptibility to these PIs and contributes cross-resistance to each of the remaining PIs except DRV and TPV.

PI Accessory

- K43T is a non-polymorphic PI-selected accessory mutation. K43T is included in the Boehringer-Ingelheim TPV genotypic susceptibility score.
- L89V is a non-polymorphic PI-selected accessory mutation that contributes reduced susceptibility to FPV, DRV, NFV, and IDV. L89V is included in the Tibotec DRV genotypic susceptibility score. L89T is a rare non-polymorphic PI-selected mutation that has not been well studied.

Other

- L10I/V are polymorphic, PI-selected accessory mutations that increase the replication of viruses with other PI-resistance mutations.
- K20I is the consensus amino acid in subtype G and CRF02_AG. In subtypes B and C, K20I is a PI-selected accessory mutation that reduces NFV susceptibility.
- AT3V/T are polymorphic, PI-selected accessory mutations that increase the replication of viruses with other PI-resistance mutations.

7. PI ile ilgili yorumlar, uyarılar

Mutation scoring: PR

PI	ATV/r	DRV/r	FPV/r	IDV/r	LPV/r	NFV	SQV/r	TPV/r
M46I	10	0	10	10	10	10	10	5
M46I + V82A	10	0	10	10	10	10	10	0
I54V	15	0	30	15	15	30	15	20
I54V + V82A	10	0	30	30	30	30	30	0
V82A	15	0	15	30	30	30	15	0
L89V	0	5	30	5	0	30	0	0
K43T	0	0	0	0	0	30	0	30
Total	60	5	85	80	75	120	60	55

8. RT direnci varlığı

Drug resistance interpretation: RT

NRTI Resistance Mutations:	M41L, M184V, L210W, T215Y
NNRTI Resistance Mutations:	None
Other Mutations:	P14E, V35T, K43N, S68G, V118I, D123Y, K122E, G162A, K173R, Q174R, D177E, V179I, T200A, Q207A, R211K, V245Q, E248D, T286A, E286D, V292I, D83V, P294T, E297R, S321T

Nucleoside Reverse Transcriptase Inhibitors		Non-nucleoside Reverse Transcriptase Inhibitors	
abacavir (ABC)	High-Level Resistance	dorasirine (DOR)	Susceptible
zidovudine (AZT)	High-Level Resistance	efavirenz (EFV)	Susceptible
stavudine (D4T)	High-Level Resistance	etravirine (ETR)	Susceptible
didanosine (DDI)	High-Level Resistance	nevirapine (NVP)	Susceptible
emtricitabine (FTC)	High-Level Resistance	rilpivirine (RPV)	Susceptible
lamivudine (3TC)	High-Level Resistance		
tenofovir (TDF)	Intermediate Resistance		

9. NRTI/NNRTI İlaç duyarlılık sonuçları

RT comments

NRTI

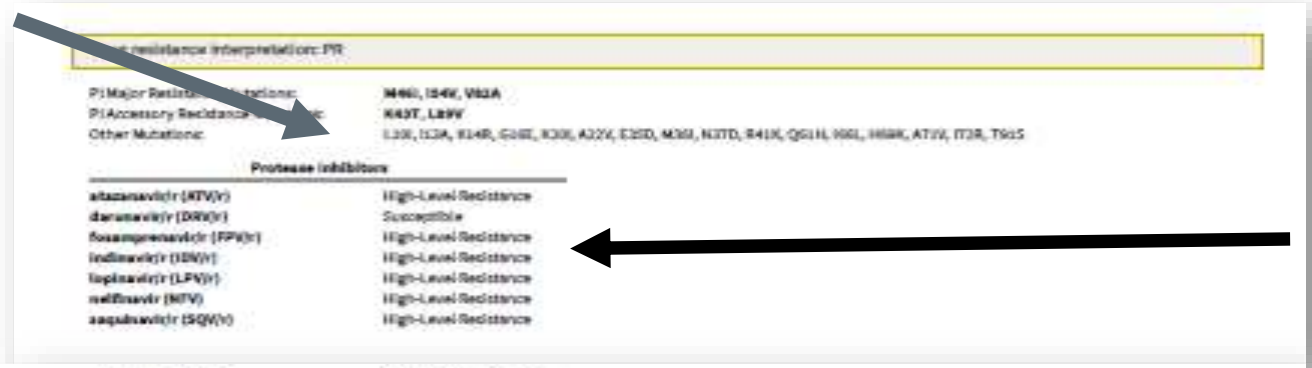
- M41L is a TAM that usually occurs with T215Y. In combination, M41L plus T215Y confer intermediate / high-level resistance to AZT and d4T and contribute to reduced ddI, ABC and TDF susceptibility.
- M184V/I cause high-level in vitro resistance to 3TC and FTC and low-level resistance to ddI and ABC. However, M184V/I are not contraindications to continued treatment with 3TC or FTC because they increase susceptibility to AZT, TDF and d4T and are associated with clinically significant reductions in HIV-1 replication.
- L210W is a TAM that usually occurs in combination with M41L and T215Y. The combination of M41L, L210W and T215Y causes high-level resistance to AZT and d4T and intermediate to high-level resistance to ddI, ABC and TDF.
- T215Y is a TAM that causes intermediate/high-level resistance to AZT and d4T, low-level resistance to ddI, and potentially low-level resistance to ABC and TDF.

Other

- S68G is a polymorphic mutation that is often selected in combination with M41L. It partially restores the replication defect associated with M41L.
- V118I is a polymorphic accessory NRTI-resistance mutation that often occurs in combination with multiple TAMs.

10. Yorumlar, uyarılar

5. Proteaz direnci varlığı



Drug resistance interpretations: PR

PI Major Resistance Mutations:	M46I, I54V, V82A
PI Accessory Resistance Mutations:	K43T, L89V
Other Mutations:	L10I, I13A, K14R, G16E, K20I, A22V, E35D, M36I, N37D, R41K, Q61H, I66L, H69K, A71V, I72R, T91S

Protease Inhibitors	
atazanavir/r (ATV/r)	High-Level Resistance
darunavir/r (DRV/r)	Susceptible
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)	Intermediate Resistance

6. PI İlaç duyarlılık sonucu

PR comments

PI Major

- M46I/L are relatively non-polymorphic PI-selected mutations. In combination with other PI-resistance mutations, they are associated with reduced susceptibility to each of the PIs except DRV.
- I54V is a non-polymorphic PI-selected mutation that contributes reduced susceptibility to each of the PIs except DRV.
- V82A is a non-polymorphic mutation selected primarily by IDV and LPV. It reduces susceptibility to these PIs and contributes cross-resistance to each of the remaining PIs except DRV and TPV.

PI Accessory

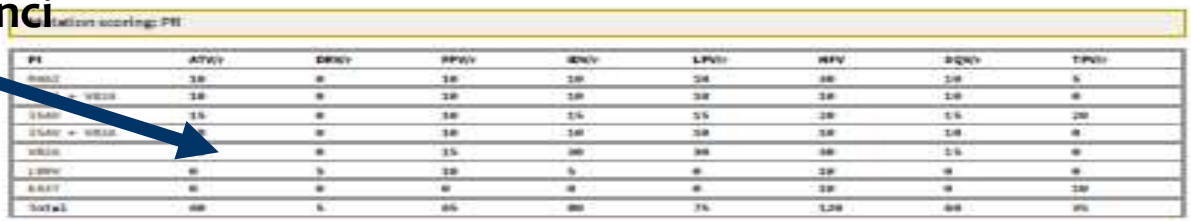
- K43T is a non-polymorphic PI-selected accessory mutation. K43T is included in the Boehringer-Ingelheim TPV genotypic susceptibility score.
- L89V is a non-polymorphic PI-selected accessory mutation that contributes reduced susceptibility to FPV, DRV, NFV, and IDV. L89V is included in the Tibotec DRV genotypic susceptibility score. L89T is a rare non-polymorphic PI-selected mutation that has not been well studied.

Other

- L10I/V are polymorphic, PI-selected accessory mutations that increase the replication of viruses with other PI-resistance mutations.
- K20I is the consensus amino acid in subtype G and CRF02_AG. In subtypes B and C, K20I is a PI-selected accessory mutation that reduces NFV susceptibility.
- A71I/T are polymorphic, PI-selected accessory mutations that increase the replication of viruses with other PI-resistance mutations.

7. PI ile ilgili yorumlar, uyarılar

8. RT direnci varlığı



Mutation scoring: PR

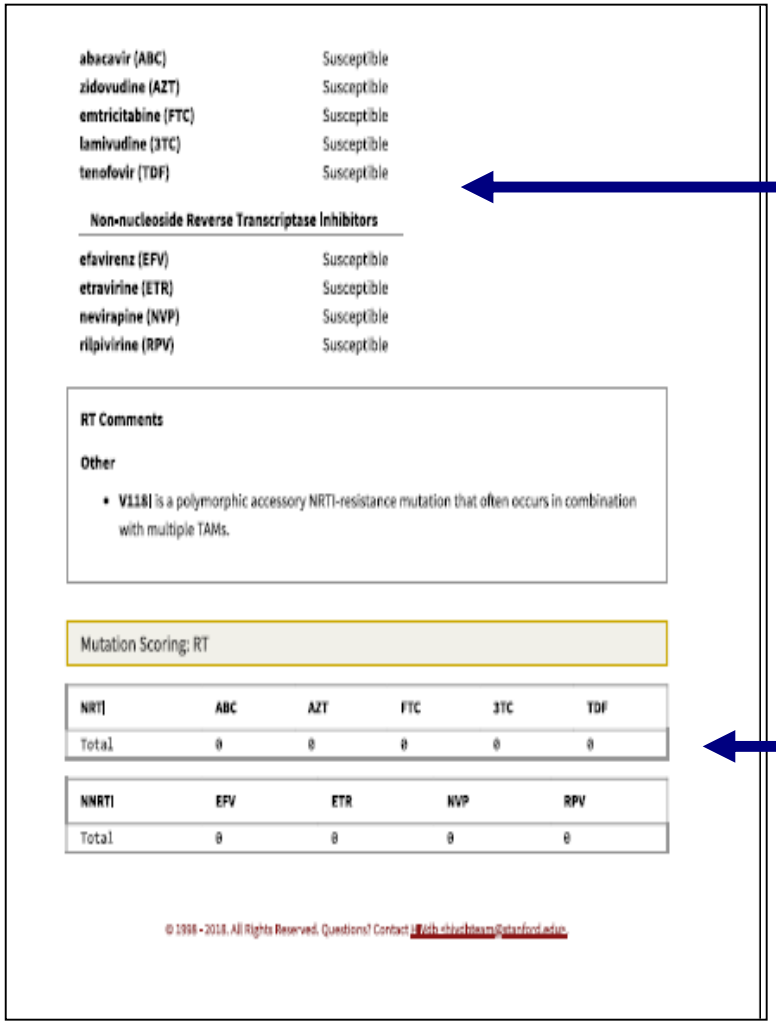
PI	ATV/r	DRV/r	FPV/r	IDV/r	LPV/r	NFV	SQV/r	TPV/r
M46I/L	10	0	10	10	10	30	10	5
M46I/L + V82A	10	0	10	10	10	10	10	0
I54V	15	0	10	15	15	20	15	20
I54V + V82A	10	0	10	10	10	10	10	0
V82A	0	0	15	30	30	10	15	0
L89V	0	5	10	5	0	10	0	0
K43T	0	0	0	0	0	10	0	10
Total	60	5	65	80	75	120	60	35

9. NRTI/NNRTI İlaç duyarlılık sonuçları

Drug resistance interpretations: RT

NRTI Resistance Mutations:	M41L, M184V, L210W, T215Y
KNRTI Resistance Mutations:	None
Other Mutations:	P14E, V35T, R40K, S68G, V118I, D123Y, K122E, S162A, K173R, Q174K, D177E, V179I, T200A, Q207A, R211K, V245Q, E248D, T286A, E286G, V292I, D headV, P294T, E297G, S321T

Nucleoside Reverse Transcriptase Inhibitors		Non-nucleoside Reverse Transcriptase Inhibitors	
abacavir (ABC)	High-Level Resistance	dorasviine (DOR)	Susceptible
zidovudine (AZT)	High-Level Resistance	efavirenz (EFV)	Susceptible
stavudine (D4T)	High-Level Resistance	etravirine (ETR)	Susceptible
didanosine (DDI)	High-Level Resistance	nevirapine (NVP)	Susceptible
emtricitabine (FTC)	High-Level Resistance	rilpivirine (RPV)	Susceptible
lamivudine (3TC)	High-Level Resistance		
tenofovir (TDF)	Intermediate Resistance		



abacavir (ABC)	Susceptible
zidovudine (AZT)	Susceptible
emtricitabine (FTC)	Susceptible
lamivudine (3TC)	Susceptible
tenofovir (TDF)	Susceptible

Non-nucleoside Reverse Transcriptase Inhibitors

efavirenz (EFV)	Susceptible
etravirine (ETR)	Susceptible
nevirapine (NVP)	Susceptible
rilpivirine (RPV)	Susceptible

RT Comments

Other

- **V118I** is a polymorphic accessory NRTI-resistance mutation that often occurs in combination with multiple TAMs.

Mutation Scoring: RT

NRTI	ABC	AZT	FTC	3TC	TDF
Total	0	0	0	0	0

NNRTI	EFV	ETR	NVP	RPV
Total	0	0	0	0

9. NRTI/NNRTI İlaç duyarlılık sonuçları

10. Mutasyon skor analizi

Mutation scoring: RT HIVDB 8.9-1 (2019-10-25)

NRTI	ABC	AZT	D4T	DDI	FTC	3TC	TDF
K65R	45	-15	60	60	30	30	60
M184V	15	-10	-10	10	60	60	-10
Total	60	-25	50	70	90	90	50

NNRTI	DOR	EFV	ETR	NVP	RPV
E138A	0	0	10	0	15
Total	0	0	10	0	15

Mutasyon Skorları

(1) **0–9: Duyarlı**

(2) **10–14: Olası düşük düzey direnç (PLLR):** Büyük olasılıkla tam duyarlı, fakat değerlendirilen dizide daha önce ARV ile karşılaşmış olduğuna işaret eden mutasyonlar söz konusu

(3) **15-30: Düşük düzeyde direnç (LLR)**: Suboptimal virolojik yanıt

(4) **30-59 : Orta düzeyde direnç (IR):** İlaç mutlaka kullanılmak isteniyorsa, ilacın genetik bariyerinin yüksek olması ya da fazla ilaç seçeneğinin bulunmaması gerekir.

(5) **60 ve üzeri: Yüksek düzeyde direnç**

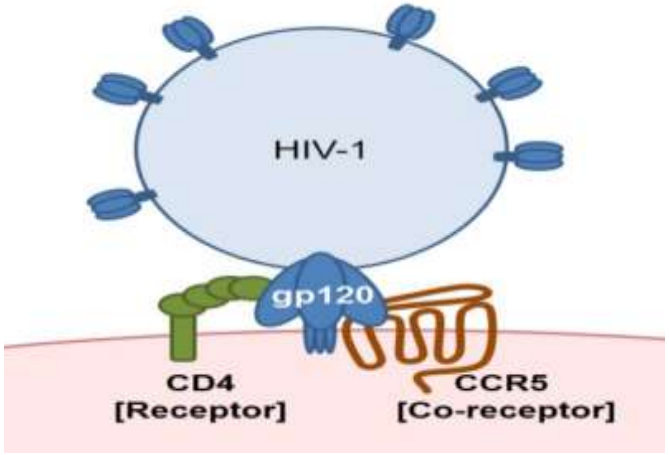
Direnç mutasyonlarının kaybolması

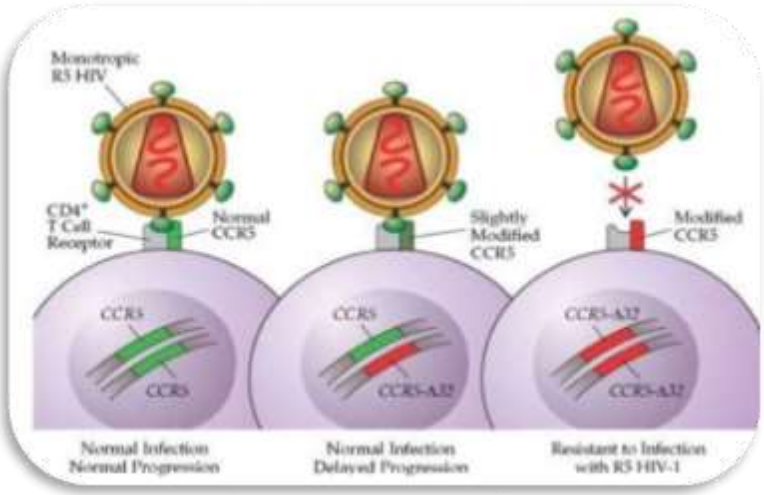
- Tedavi kesilirse
- Tedavi değiştirilirse
- Wild-type baskın hale geçerse

Minority variant halde kalırlar

Tropizm testi

- HIV gp120, CD4 ve kemokin reseptörü (CR5 ve/veya CXCR4) kompleks oluşturur ve virüs hücre içine girer.
- CCR5 reseptör antagonistleri gp120 ve CCR5 bağlanmasını engeller.
- **Test ile tedavide CCR5 reseptör antagonistinden fayda görüp göremeyeceği belirlenir**
- Gp 120 zarf glikoproteinin **V3** bölgesi sekanslanır → ***env gen bölgesi***
- Sekans sonucu virüsün R5 tropik olup olmadığını belirlenir →R5 tropik ise CCR reseptör inh fayda görür.





HIV-2 ilaç direnci

- HIV-2 ile 1 milyondan fazla vaka; Batı Afrika'ya yaşayan/göç eden kişilerde sık
- HIV-1 genomundan %50'den fazla sekans farklılığı var
- HIV-2 ile enfekte hastalarda ART ve ilaç direnci ile ilgili veri kısıtlı
- NNRTI ve füzyon inhibitörü enfuvirtide intrensik dirençlidir.
- PI , amprenavire direnç izlenen vakalar da bildirilmiştir.

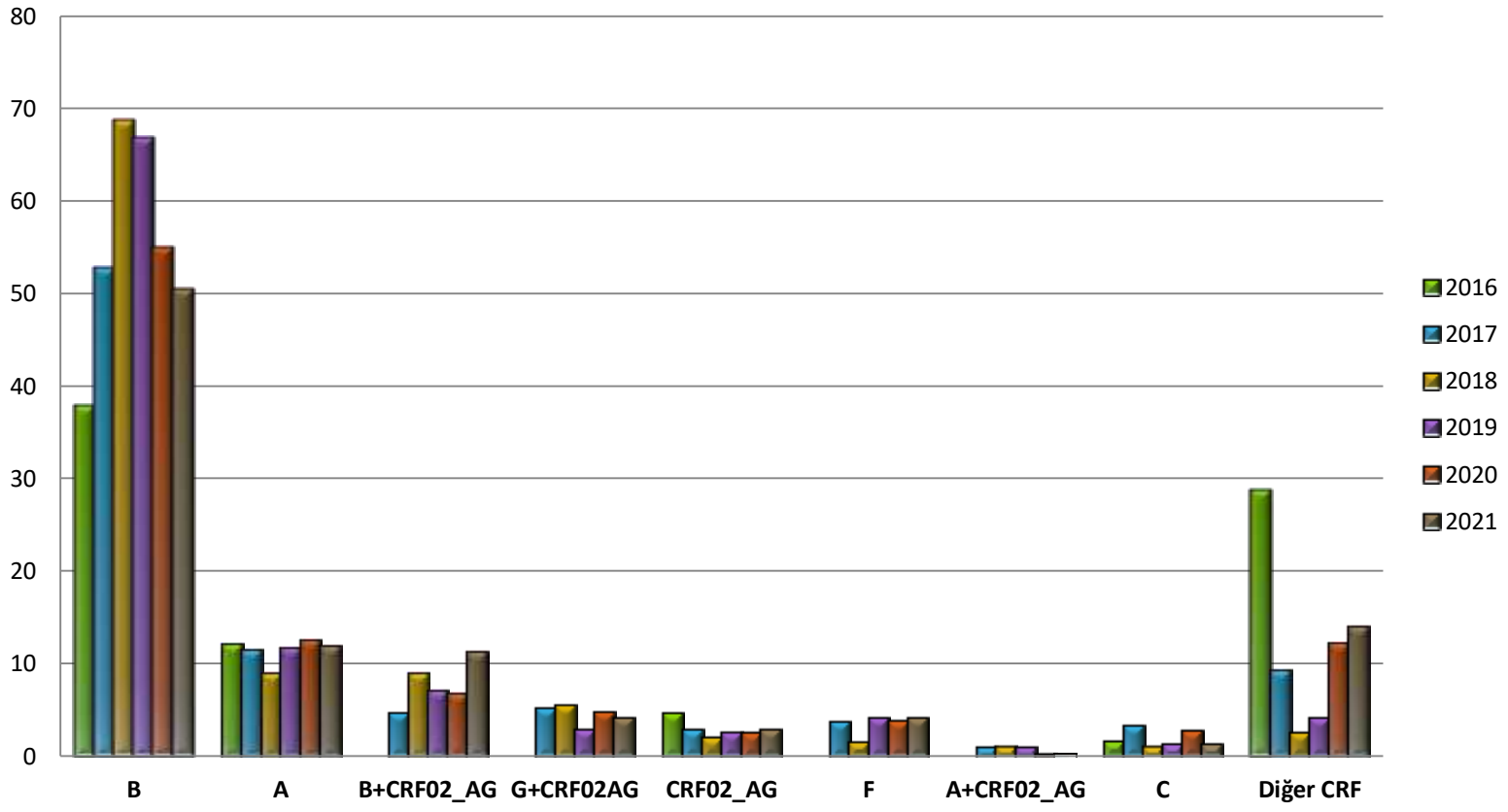
Menéndez-Arias L, et al. Antiviral Res 2014; 102:70–86.
Ekouevi DK, et al. BMC Infect Dis 2014;14:461.
Gottlieb GS, et al. Clin Infect Dis. 2009;48(4):476–483.
Fearon M. Can J Infect Dis Med Microbiol. 2005;16(1):26–30

Ülkemizde durum…

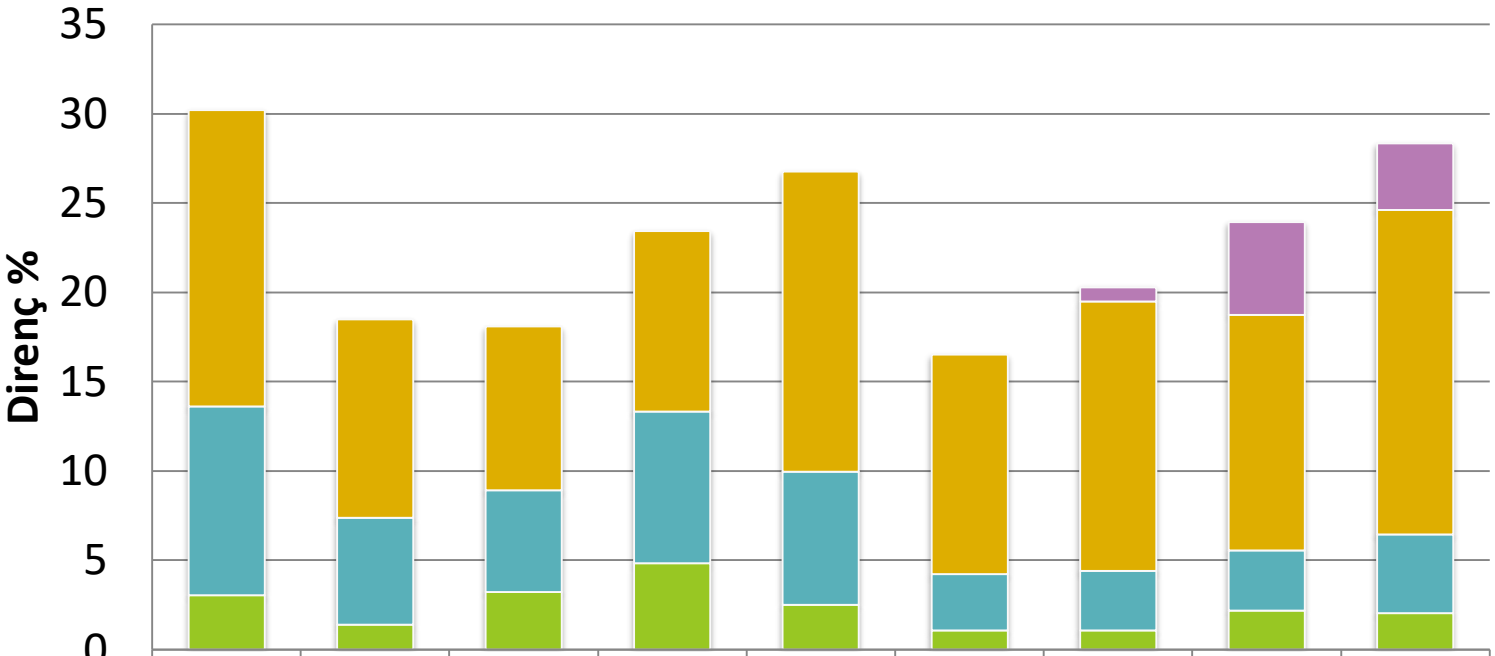
					Direnç (%)						Subtip
Çalışma	**Tedavi**	**Yıllar**	**n**	**Çalışma tipi**	**TDRM**	**NRTI**	**NNRTI**	**PI**	**INSTI**	**CAP**	
Sayan M ve ark. (1)	Naive	2009-2014	774	Çok merkezli	6.7	0.7	4.1	2.1			
Sayan M ve ark. (4)	Naive	2010-2015	1306	Çok merkezli	10.1	8.1	3.3	2.3			
İnan D ve ark. (2)	Naive	2012-2014	77	Tek merkez							B %48 CRF14__BG %39
Uluer Biçeroğlu S ve ark (3)	Naive	2008-2013	70	Tek merkez							B %44 CRF 42__BF %34
UADVHRL verileri; Yalçınkaya T ve ark. (5)	Naive	2010-2014	190	Çok merkezli		5.2	3.1	2.1			B %31 F1 %24
UADVHRL verileri	Naive	2017-2018	412	Çok merkezli	12.7	4.1	8	1.2			B %55.1 A %12.5 B+CRF29_BF %4.8
Özdemir A ve ark.	Naive	2021-2022	104	Tek merkezli	14.4	**1.92**	**4.80**	**7.69**	-	-	B %55 A %15
Yaman M ve ark.	-	2021-2024	1029	Çok merkezli		**12.4**	**22.23**	**1.12/ 1.75**	**1.12/ 4.5**		B (%54.4), A1 (%43.5)

(1) Sayan ve ark. J Int AIDS Soc. 2014; 2(17)
(2) Inan ve ark. J Int AIDS Soc. 2014; 7(4)
(3) Uluer Biçeroğlu ve ark. Mikrobiyoloji Bult. 2014; 48(3)
(4) Sayan ve ark. AIDS Res Hum Retrovirus. 2016; 32(1)
(5) Yalcinkaya ve ark. Mikrobiyoloji Bult, 2014; 48(4)
Yaman M, Gülcen BS, Özgüler K, Köksal MO, Tekol SD, İlki A. Temporal Trends in HIV-1 Subtypes and Antiretroviral Drug Resistance Mutations in Istanbul, Türkiye (2021-2024): A Next-Generation Sequencing Study. Viruses. 2025 Mar 27;17(4):478.

HIV subtiplerinin yıllar içinde değişimi(2016-2021) (n=2000)



TR Antiretroviral direnç durumu (2016-2024)



	2016	2017	2018	2019	2020	2021	2022	2023	2024
INSTI							0.81	5.2	3.71
NNRTI	16.6	11.1	9.18	10.1	16.8	12.28	15.1	13.2	18.2
NRTI	10.6	6.01	5.7	8.51	7.47	3.18	3.33	3.35	4.39
PI	3.03	1.38	3.22	4.83	2.49	1.06	1.06	2.2	2.04

INSTI: 2022: 121 olgu 2023: 882 olgu, 2024: 826 olgu

NNRTI Direnci

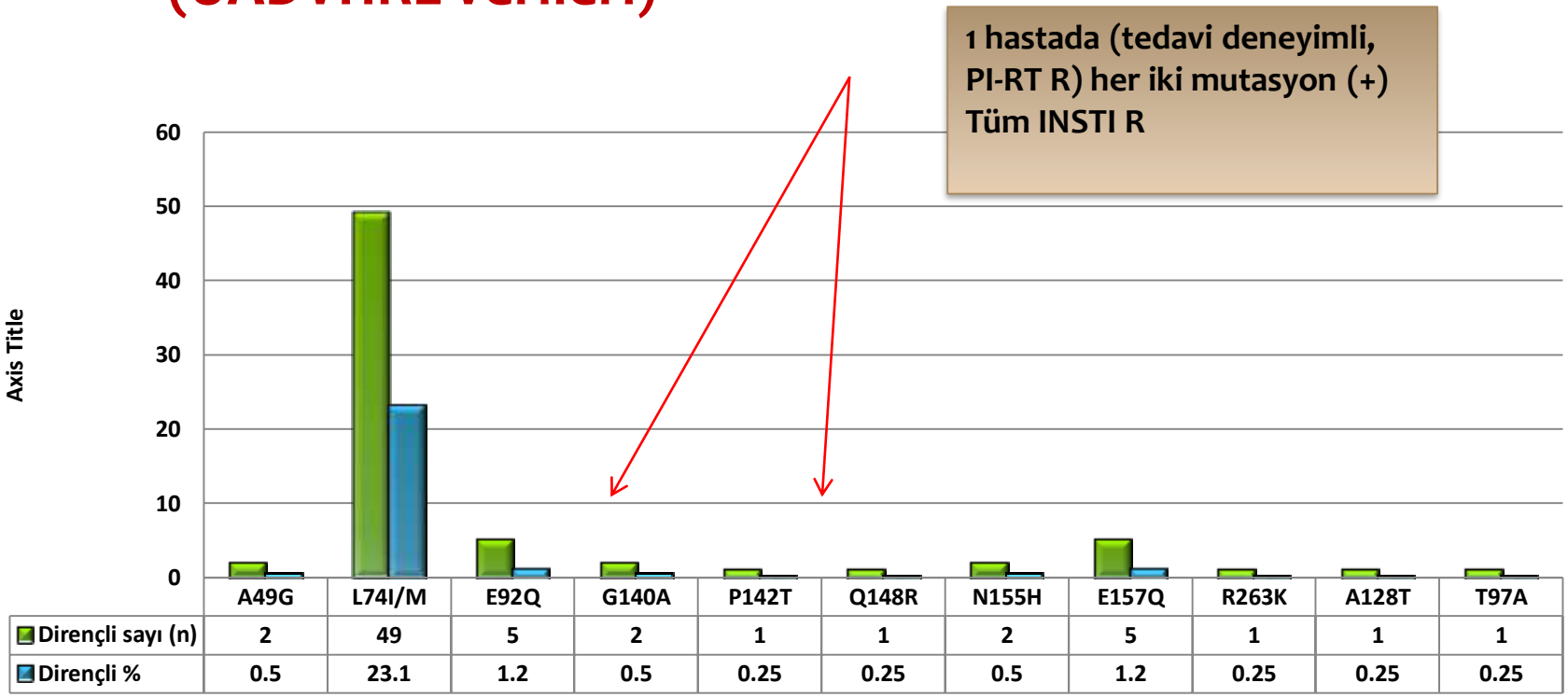
E138K/A/G/Q/R

E138K is a nonpolymorphic mutation that is selected in a high proportion of persons receiving RPV (3) and in a smaller proportion of persons receiving ETR (19). Alone it reduces RPV and ETR susceptibility about 2-fold; in combination with M184I or K101E it reduces RPV and ETR susceptibility about 3 fold (57,58,59). The combination of E138K and M184I or K101E appears sufficient to cause VF on an RPV-containing regimen (3). E138K does not appear to reduce susceptibility to NVP, EFV, or DOR (6,10).

E138A is a polymorphic mutation that ranges in prevalence from about 2% to 5% in ART-naïve persons depending on subtype. It is not known whether it is selected in NNRTI-treated persons. It reduces ETR and RPV susceptibility about 2-fold (2,6,18,57,60). In a weighted genotypic score, it was associated with a reduced virological response to treatment with an ETR-containing regimen when combined with other NNRTI-resistance mutations (8). Based on limited data, baseline E138 mutations do not appear to be associated with an increased risk of VF in persons receiving an RPV-containing regimen (61,62).

E138Q/G are nonpolymorphic accessory mutations selected by ETR (3,19,57) and occasionally by NVP and EFV. E138Q/G are associated with about 3-fold reduced NVP susceptibility and 2-fold reduced ETR and RPV susceptibility (6,57). E138R is an extremely rare 2 base-pair mutation that is selected primarily in non-subtype B viruses from NNRTI-treated persons. It appears to reduce NNRTI susceptibility slightly more than E138Q/G (2).

INSTI DRM dağılımı, 2020-21 (n=414) (UADVHRL verileri)



	A49G	L74I/M	E92Q	G140A	P142T	Q148R	N155H	E157Q	R263K	A128T	T97A
Dirençli sayı (n)	2	49	5	2	1	1	2	5	1	1	1
Dirençli %	0.5	23.1	1.2	0.5	0.25	0.25	0.5	1.2	0.25	0.25	0.25

2020 yılı: Herhangi bir PR veya RT direnci olan 86 örnek; 9 örnekte direnç (+)

2021 yılı: PR-RT direnç durumundan bağımsız; 328 örnek;

• 12 örnekte INSTR DRM; 5'i tedavi deneyimli, 7'si naive ve diğer direnç yok, acc DRM (+)

*** Dirençli olan tüm örnekler tedavi deneyimli hastalardan

2020-21 direnç: %5 (tedavi deneyimli hastalarda), TEDAVİ REJİMİ?

En sık saptanan mutasyonlar:

- PI:

M46I/L (Tüm PI etkiler; PLLR; DRV hariç)

- NRTI:

M184V, T215, M41

- NNRTI:

E138A/G, K103N (EFV, NEV HLR)

- INSTI:

T97A, E157Q, N155H, L74M

- Tüm INSTI direnci genellikle vertikal geçişli ve ted deneyimli hastalarda
- Naive hastalarda BIC DTG direnci yok ama PLLR nedeni
- **Naive hastalarda INSTI DRM yok**

	2023 (n=882)			2024 (n=826)		
	PLLR	IR	HLR	PLLR	IR	HLR
DTG	4	1	7	17	3	7
BIC	4	2	6	17	5	5

***Hepsi ted deneyimli

E138A, G140 A, Q148R (HLR)
R263K (BIC, DTG IR)

Direnç testlerinde kısıtlamalar

- Sadece tespit edilebilir viral yük varlığında ***yapılabilir*****
- ****Proviral DNA ile ART yapılabilir. Viral yükden bağımsız ancak dirençli populasyon saptama oranı daha düşük??**
- Kalite kontrol takibi sıkıntılı
- Azınlık populasyonlar tespit edilememekte, baskın suş üzerinden sonuç verilmekte
- Duyarlılık klinik etkinlikle aynı anlama gelmez

✓Primer enfeksiyonda ilk tanıda ve perinatal HIV enfekte bebekler olası TDR açısından takip edilmeli…

✓Virolojik baskılanma sağlanmış ve sonrasında LLV (<200 kopya/mL) bireylerde PBMC DNA faydalı… ART yapılmalı…

✓**InSTI TDR/PDR takibi uygun imkanlara sahip merkezlerde seçili hasta gruplarında yapılmalı…**

✓**TDR ve PDR prevalansları farklı hasta populasyonlarına göre ülke düzeyinde takip edilerek sonuçların yıllık rapor şeklinde yayınlanması planlanmaktadır.**


HIV gerçeğine
POZİTİF BAK!