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Antiretroviral Resistance and Resistance Testing

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Learning Objectives

- Discuss how antiretroviral resistance develops
- Review the available methods and indications for antiretroviral resistance testing
- Understand how resistance tests are interpreted

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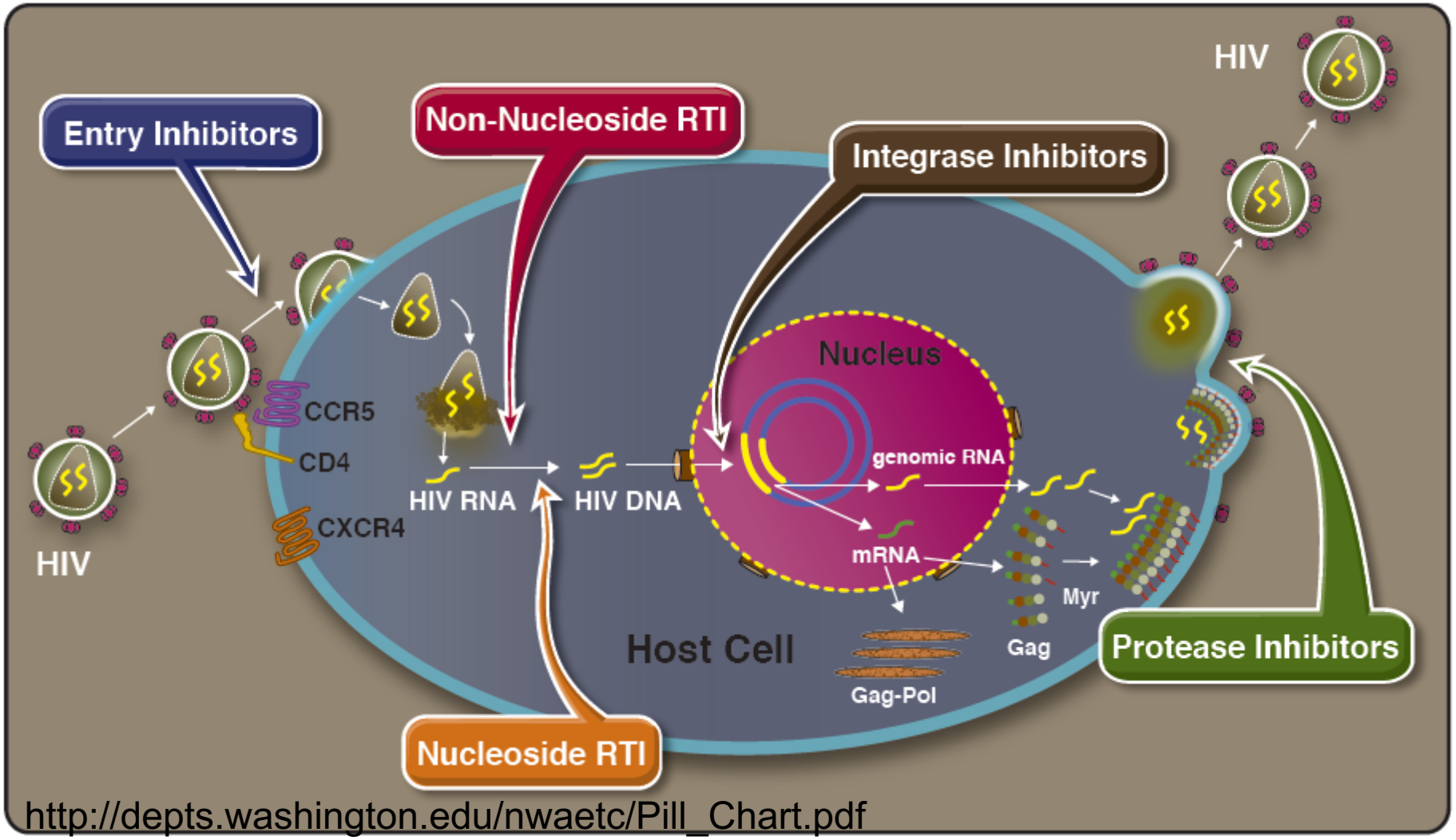
HIV Life Cycle



http://depts.washington.edu/nwaetc/Pill_Chart.pdf

Antiretroviral Medications

Nucleoside Reverse Transcriptase Inhibitors (NRTIs)

Abacavir (ABC) (Ziagen®)
Didanosine (ddI) (Videx®)
Emtricitabine (FTC) (Emtriva®)
Lamivudine (3TC) (Epivir®)
Stavudine (d4T) (Zerit®)
Tenofovir (TDF) (Viread®)
~~Zalcitabine (ddC) (Hivid®)~~ *withdrawn 2005*
Zidovudine (ZDV, AZT) (Retrovir®)
3TC/ABC (Epzicom®)
3TC/ABC/ZDV (Trizivir®)
3TC/ZDV (Combivir®)
FTC/TDF (Truvada®)
FTC/TAF (Descovy®)

Non-nucleoside Reverse Transcriptase Inhibitors (NNRTIs)

Delavirdine (DLV) (Rescriptor®)
Efavirenz (EFV) (Sustiva®)
Etravirine (ETR) (Intellence®)
Nevirapine (NVP) (Viramune®)
Rilipvirine (RPV) (Edurant®)

Single Tablet Regimens

EFV/FTC/TDF (Atripla®)
RPV/FTC/TDF (Complera®)
RPV/FTC/TAF (Odefsey®)
EVG/cobi/FTC/TDF (Stribild®)
EVG/cobi/FTC/TAF (Genvoya®)
DTG/3TC/ABC (Triumeq®)

Protease Inhibitors

~~Amprenavir (APV) (Agenerase®)~~ *discontinued 2004*
Atazanavir (ATV) (Reyataz®)
Atazanavir/cobicistat (ATV/c) (Evotaz®)
Darunavir (DRV) (Prezista®)
Darunavir/cobicistat (DRV/c) (Prezcobix®)
Fosamprenavir (FPV) (Lexiva®)
Indinavir (IDV) (Crixivan®)
Lopinavir/ritonavir (LPV/r) (Kaletra®)
Nelfinavir (NFV) (Viracept®)
Ritonavir (RTV) (Norvir®)
Saquinavir (SQV) (Invirase®)
Tipranavir (TPV) (Aptivus®)

Entry Inhibitors

Enfuvirtide (ENF, T20) (Fuzeon®)
Maraviroc (MVC) (Selzentry®)

Integrase Inhibitors

Raltegravir (RAL) (Isentress®)
Elvitegravir (EVG) (Vitekta®)
Dolutegravir (DTG) (Tivicay®)

Pharmacokinetic Enhancers “Boosters”

Ritonavir (r) (Norvir®)
Cobicistat (cobi) (Tybost®)

Recommended Regimens for Treatment-Naïve Patients

2 NRTIs

Tenofovir/Emtricitabine

OR

Abacavir/Lamivudine*

*only w/ Dolutegravir

+

PROTEASE INHIBITOR
(boosted with Ritonavir)

Darunavir + Ritonavir

OR

INTEGRASE INHIBITOR

Raltegravir
Elvitegravir/cobicistat
Dolutegravir

DHHS. Guidelines for the use of antiretroviral agents in HIV-1 infected adults and adolescents. Available at http://www.aidsinfo.nih.gov/ContentFiles/AdultandAdolescentGL.pdf

How Drug Resistance Occurs

- Untreated HIV produces 10 billion new virions each day
  - Most common form of HIV is wild-type virus
  - Wild-type: Viral strain that has not mutated and is susceptible to all drugs
- High mutation rate, ~1 nucleotide mutation per replication cycle
  - Mutation: Slight change in specific section of genetic material (HIV RNA)
  - Not all mutations cause resistance

Tang MW, Shafer RW. Drugs 2012;72(9):e1-e25.

How Drug Resistance Occurs

- Resistance develops from genetic mutation of viral enzymes & proteins leading to changes in the way drugs interact with them
  - Resistance: Reduction of the sensitivity of a pathogen to a particular drug

- HIV usually becomes resistant when not totally controlled by ART

How Drug Resistance Occurs: Cross Resistance

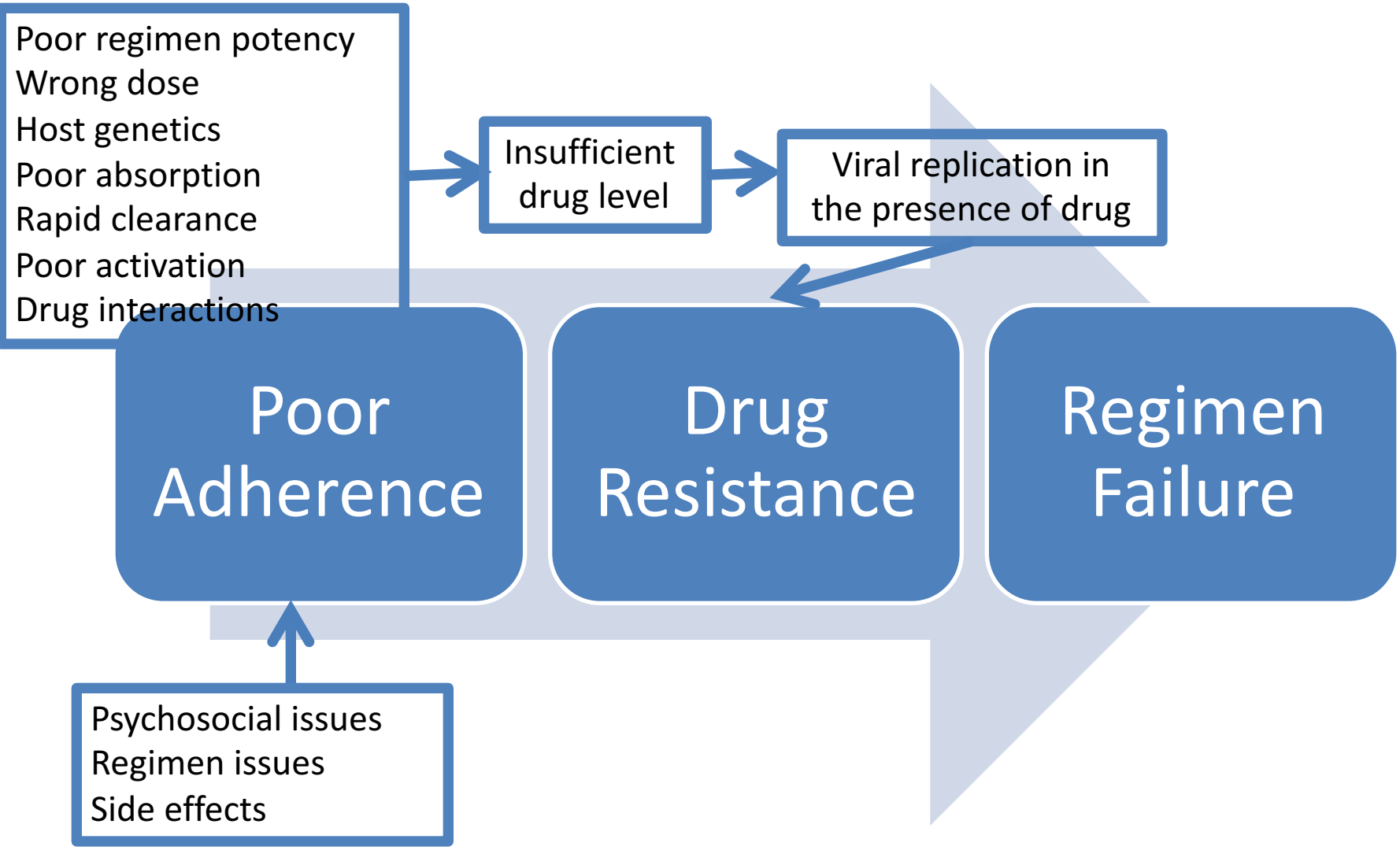
- ARV classes work at different stages of viral replication and different mutations confer resistance to each class
- High levels of cross resistance within drug classes
  - Cross resistance: Drug resistance within the same class “crosses over” from one drug to another
- No cross resistance between drug classes

How Drug Resistance Occurs: Genetic Barrier to Resistance

- Some ARVs require only one mutation to cause resistance (low genetic barrier) while others require multiple drug resistance mutations (high genetic barrier)
- Genetic barrier: Number of HIV mutations required for development of resistance to each ARV

Low Genetic Barrier	High Genetic Barrier
•Some NRTIs: Single mutation causes lamivudine or emtricitabine resistance •Most NNRTIs: Single mutation causes "cross resistance" to most drug in this class	•PIs: Require multiple mutations for resistance

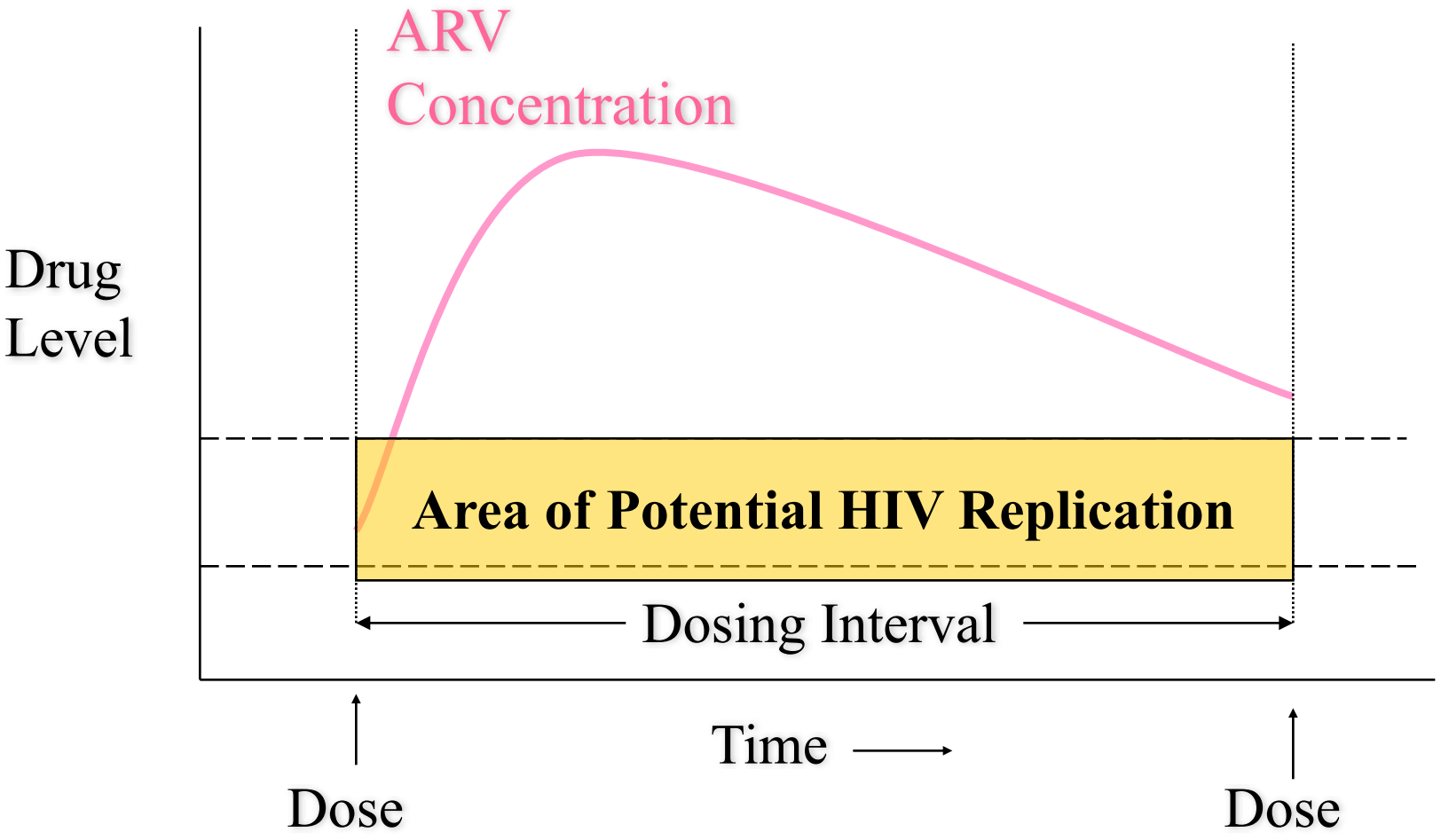
How Drug Resistance Occurs



Poor Adherence May Contribute to Drug Resistance

- Mechanism multifactorial, not clearly defined
  - Pattern of non-adherence: interruptions in therapy, missing single doses
  - ARV characteristics: class, frequency of dosing, pharmacokinetics, duration of viral suppression
  - Patient characteristics: prior ARV experience, host genetics, rate of absorption and metabolism
  - Virus characteristics: replicative rate, genetic variants

Poor Adherence May Contribute to Drug Resistance



Other Factors Causing Treatment Failure

Medication Intolerance

- Severity and duration of side effects
- Resolution: Symptomatic treatment (e.g., antiemetics, antidiarrheals)

Pharmacokinetic Issues

- ARV fasting/food requirements
- GI symptoms (vomiting, diarrhea) cause malabsorption
- Concomitant medications/ dietary supplements cause adverse drug interactions
- Resolution: Perform thorough med review

Mechanisms for ART Resistance

- Transmitted resistance: Infected with resistant strain of HIV at baseline (6-16% of newly diagnosed patients in US are infected with resistant virus[1])
- Spontaneous resistance: HIV develops mutations easily and becomes resistant (75% of patients on ART with detectable viral load have at least 1 major resistance mutation[2])
- Once HIV develops resistance to a medication it will stay resistant forever

1. DHHS. Guidelines for the use of antiretroviral agents in HIV-1 infected adults and adolescents. Available at http://www.aidsinfo.nih.gov/ContentFiles/AdultandAdolescentGL.pdf
2. Richman DD, et al. AIDS. 2004;18:1393-1401.

Resistance and Treatment Failure

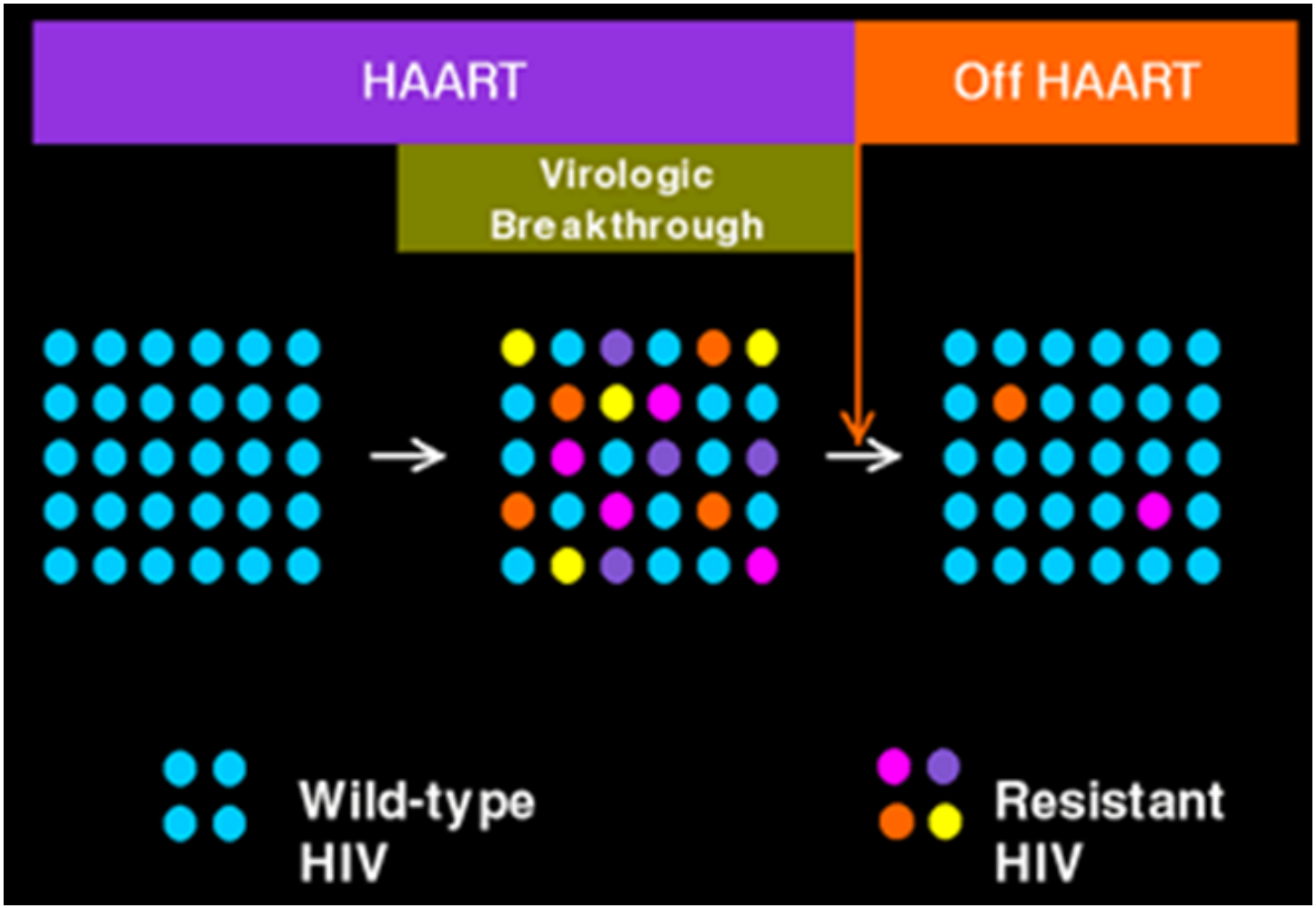
- Treatment failure: Client on ART and viral load rises sharply
  - May indicate HIV has grown resistant to one or more medications
  - May indicate other issues (*e.g.*, adherence, drug interactions, med access, intolerability)

- Obtain resistance test immediately *before stopping or changing treatment*

Selective Pressure

- If non-effective regimen continued then resistant virus multiplies fastest
  - If ART stopped → no selective pressure → resistant virus will not replicate (archived) → wild-type virus multiplies fastest
- Selective pressure: Pressure exerted by a drug that results in a frequency increase in certain mutations in the next generation
- Resistance testing may not detect small concentrations of archived resistant strains

Reversion to Wild-Type Virus Following ART Discontinuation



Behrens C, et al. Antiretroviral Resistance Testing in the Management of HIV-Infected Patients. Northwest AETC. http://aidsetc.org/aidsetc?page=etres-display&resource=etres-9

Archived Mutations

- Archived mutations: Undetected mutations that persist after discontinuation of medication and reappear as a result of selective pressure when medication resumed
  - Archived mutations always threaten new regimen efficacy
    - Resistance testing may not identify drug-resistant mutations from past therapies for treatment-experienced patients
    - Resolution: Review client's ARV history and all prior resistance tests

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Role of Resistance Testing in Treatment Failure

- Resistance tests
  - Indicate if regimen failure due to non-adherence vs. resistance (*i.e.,* no drug resistance mutations detected may signify adherence issue)
  - Use to guide next therapy decisions
- Provisos of resistance testing
  - Requires sufficient amount of virus (viral load >500-1,000 copies/mL)
  - Detects resistance only if present in >10-20% of total virus population
  - Perform while patient is taking the failing regimen

Types of Resistance Tests

Genotype

- Detects drug resistance mutations in HIV genes
- Results in 1-2 weeks
- Cost is approximately 33%-50% of a phenotype
- When Indicated: At entry into care and in treatment failure to guide therapy decisions

Phenotype

- Measures ability of virus to grow in different ARV concentrations
- Results in 2-3 weeks
- When Indicated: Add to a genotype assay in those with known or suspected complex drug resistance patterns

Genotype Resistance Test

- Technique: Genetic code of client's virus compared to wild-type virus
- Reported as list of mutations identified in the virus sample associated with resistance
  - Mutations in HIV reverse transcriptase, protease, integrase, or envelope genes
- Includes interpretation indicating drug resistance likely correlated with mutations
- Limitation: Complex mutation pattern of multidrug resistant virus difficult to interpret

Phenotype Resistance Test

- Technique: Client's virus grown in the presence of different concentrations of ARV drugs and compared to wild-type virus
- Reported as susceptibility to each ARV drug
- Combines interaction of all mutations; more useful for complex mutation patterns
- Genotype and phenotype tests have complementary properties and may use both tests together in some circumstances (*e.g.*, highly treatment experienced patients)

Clinical Indications for Resistance Testing

Recommend

- Acute & chronic HIV infection prior to initiation of therapy
  - Determine if resistant virus transmitted
- Virologic failure during ART or suboptimal suppression of viral load after start of therapy
  - Assist in selecting new regimen and help guide treatment decisions

NOT Recommend

- After discontinuation of ART >4 weeks
  - Resistance mutations may become non-detectable minor species in the absence of selective drug pressure
- Viral load <500 copies/mL
  - Resistance assays cannot be consistently performed

DHHS. Guidelines for the use of antiretroviral agents in HIV-1 infected adults and adolescents. Available at http://www.aidsinfo.nih.gov/ContentFiles/AdultandAdolescentGL.pdf

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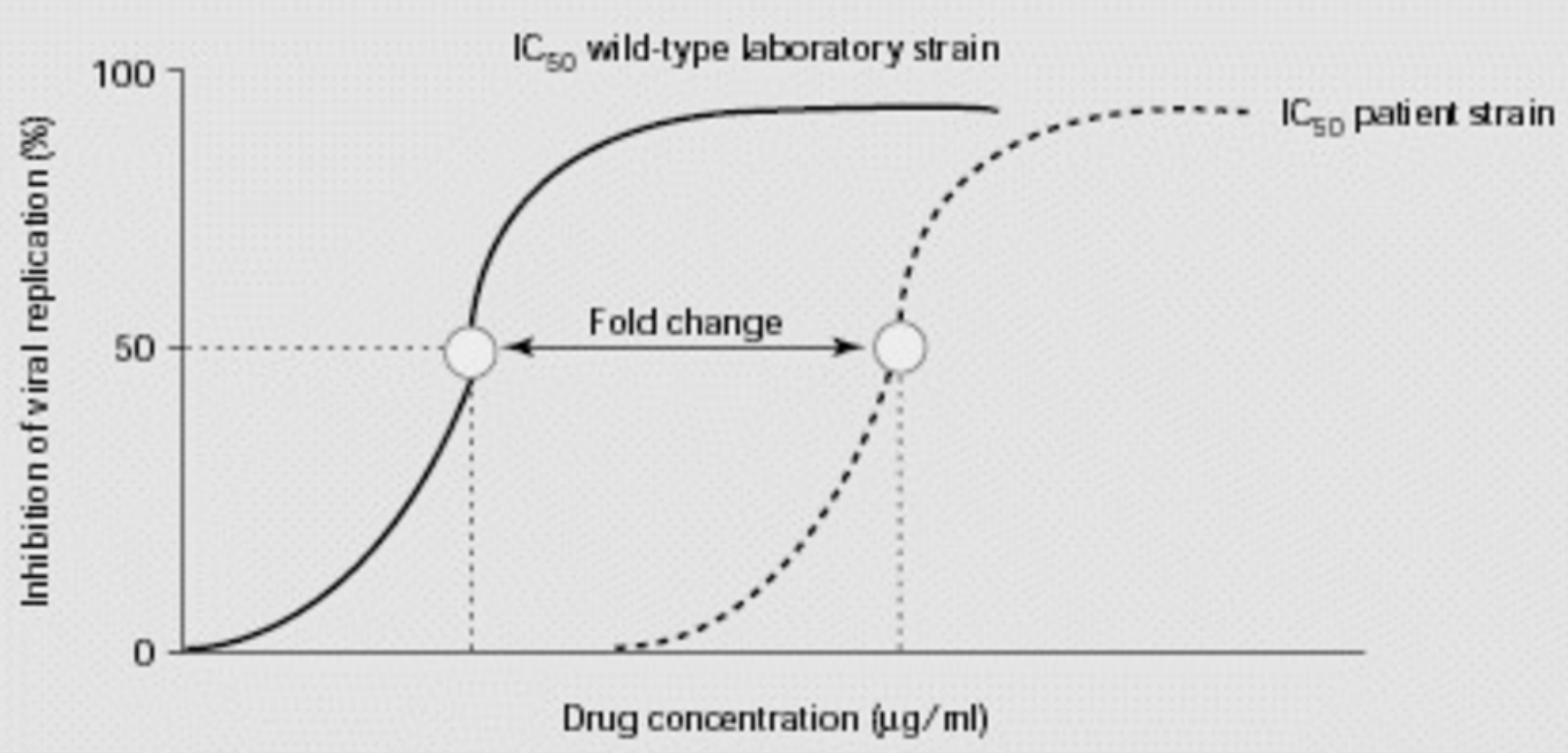
How to Interpret a Phenotype

Interpreting HIV Phenotype

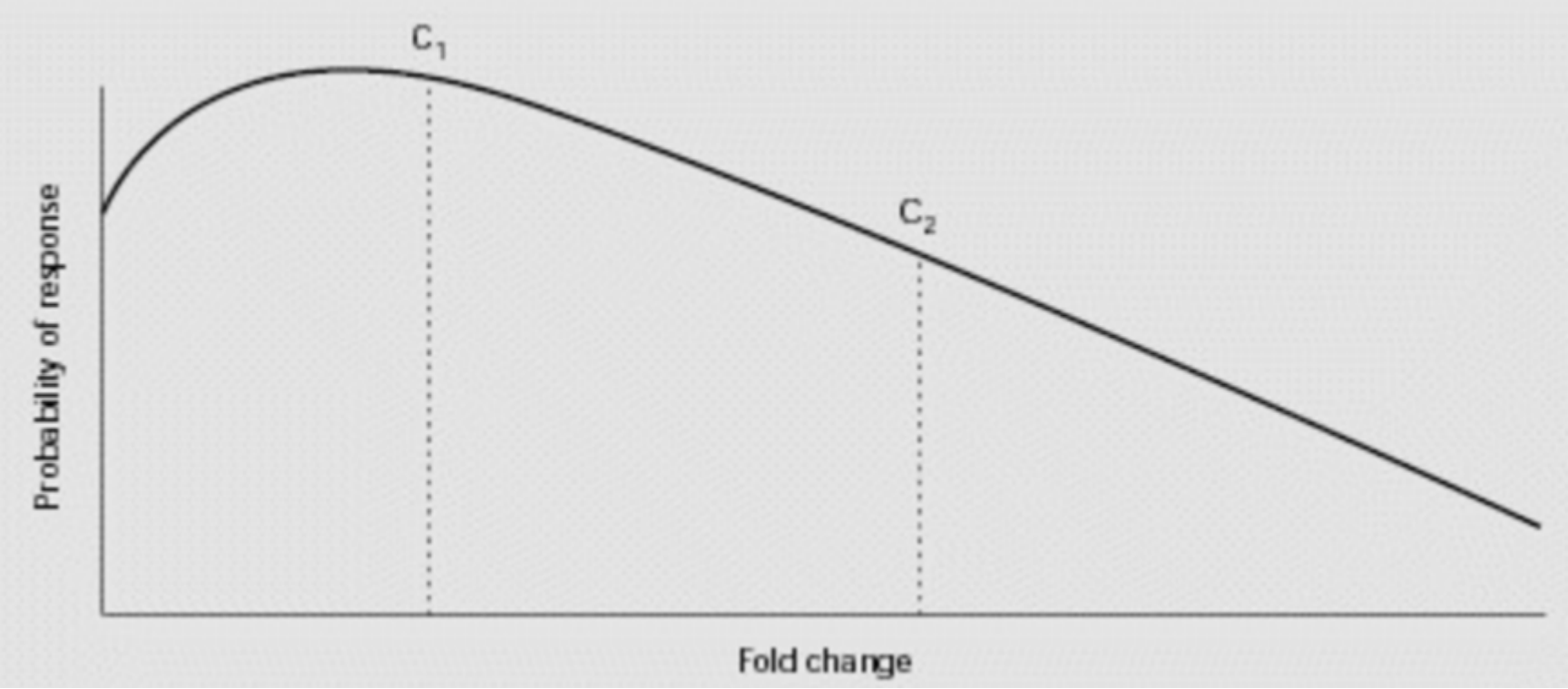
- Phenotype refers to virus growth characteristics
- Results expressed as fold-change in susceptibility compared to wild-type virus
  - Fold change: Ratio of IC_{50} of patient's virus (for specific ARV) compared with reference wild-type strain
- Interpretation of drug activity usually presented in context of clinical cutoffs
  - Clinical cutoffs: Based on patient virologic response in clinical trials

Phenotypic Susceptibility: Relationship Between Drug Concentration and Viral Inhibition



Gerretti AM, ed. Antiretroviral Resistance in Clinical Practice. Available from http://www.ncbi.nlm.nih.gov/books/NBK2254/

Interpreting Phenotypes: Clinical Cutoffs Differ for Each Drug



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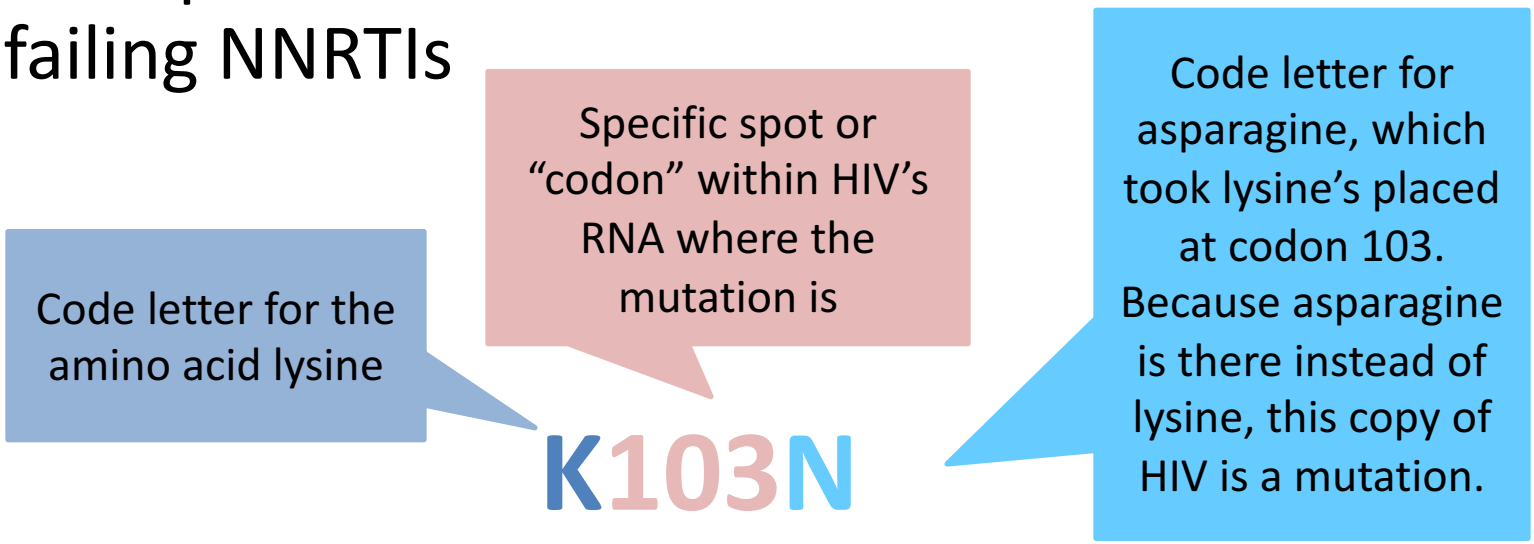
How to Interpret a Genotype

Shorthand System Used for Naming HIV Genotype Mutations

- Shorthand system used for naming HIV mutations on genotypes
- Example: K103N is a common mutation when failing NNRTIs



- K103N confers high level "cross resistance" to the NNRTIs efavirenz & nevirapine

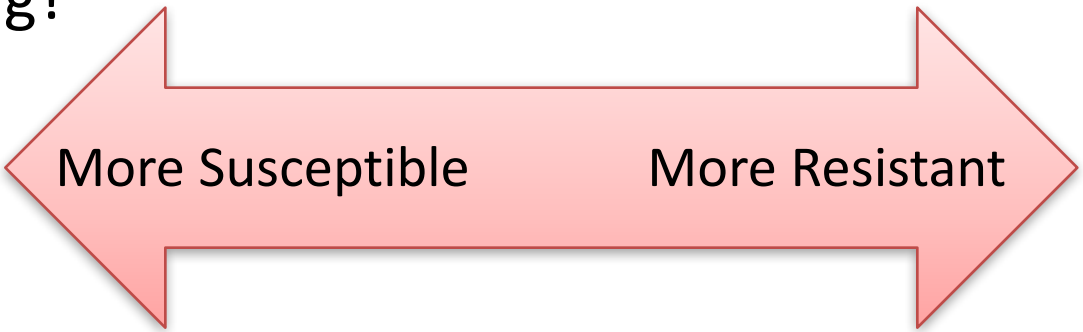
Amino acid abbreviations: A alanine, C cysteine, D aspartate, E glutamate, F phenylalanine, G glycine, H histidine, I isoleucine, K lysine, L leucine, M methionine, N asparagine, P proline, Q glutamine, R arginine, S serine, T threonine, V valine, W tryptophan, Y tyrosine

Shorthand System Used for Naming HIV Genotype Mutations

- Mixture: More than one amino acid at a position
  - Components written after the position
  - Often separated by a slash
  - e.g., K103K/N denotes sequence has mixture of wild-type lysine (K) and mutant asparagine (N) at position 103

HIV Drug Resistance: Not Always All-Or-None

- Resistance testing answers two questions
  - Will the client respond to a drug in a manner comparable to a wild type virus?
  - Will the client obtain any antiviral benefit from the drug?



- Extent of resistance graded relative to wild-type (*e.g.*, low-level, intermediate, high-level)

Notable NRTI Mutations

- M184V
  - Confers high level resistance to lamivudine and emtricitabine
  - Some resistance to didanosine and abacavir
  - Restores some activity to zidovudine, stavudine, and tenofovir
  - Diminishes viral replication capacity
- K65R
  - Broad resistance to all NRTIs
  - Increases susceptibility to zidovudine
- Thymidine analog mutations (TAMs) – 41, 67, 70, 210, 215, 219
  - Decrease susceptibility to all NRTIs
  - Additive resistance with more accumulation

Notable NNRTI Mutations

- K103N
  - Most common NNRTI mutation
  - Confers resistance to efavirenz and nevirapine but not etravirine or rilpivirine
- K101P, Y181C
  - Resistance to all NNRTIs

Etravirine: Second Generation NNRTI

- Active against some NNRTI resistant viruses
  - K103N alone does not effect etravirine
- Resistance predicted using a mutation score
- Total score corresponds to chance of virologic suppression
  - 0-2: Highest response (74%)
  - 2.5-3.5: Intermediate response (52%)
  - ≥ 4: Reduced response (38%)

Weighted Mutation Score	**1**	**1.5**	**2.5**	**3**
Mutation in Reverse Transcriptase	90I, 179D, 101E, 101H, 98G, 179T, 190A	138A, 106I, 190S, 179F	101P, 100I, 181C, 230L	181I/V

Vingerhoets J, et al. AIDS 2010;24:503-514.

Notable PI Mutations

- Signature mutations for non-boosted PIs
  - D30N: nelfinavir; no cross resistance
  - I50L: unboosted ATV
  - I50V: fosamprenavir; some cross resistance to lopinavir
  - G48V: saquinavir; no cross-resistance
  - L90M: often follows unboosted PIs; causes cross resistance
- Boosted PIs (LPV/r, FPV/r, SQV/r, ATV/r, DRV/r) usually do not select for resistance if used as first PI
  - However, if first-line boosted PI failure is not addressed promptly, secondary resistance mutations can accumulate; ideally obtain phenotype to evaluate

Darunavir (DRV) Resistance Affects Dose

- PI for both treatment-naïve & treatment-experienced patients
  - Dose 800mg once daily for treatment-naïve patients
  - Dose 800 mg once daily for treatment-experienced patients if there are zero DRV resistance mutations
  - Dose 600 mg twice daily if there are ≥ 1 DRV mutation(s)
- Activity weakened by resistance mutations: V11I, V32I, I33F, I47V, I50V, I54L/M, T74P, L76V, I84V, L89V

Notable INSTI Mutations

- Raltegravir and elvitegravir are cross-resistant
  - Q148H/K/R or N155H are major mutations affecting both RAL and EVG causing high level resistance
- Dolutegravir requires several mutations to confer resistance
  - High level resistance seen with Q148H/R plus 2 or more additional INSTI mutations

Dolutegravir (DTG) Resistance Affects Dose

Adult Population	Recommended Dose
Treatment-naïve or treatment-experienced INSTI naïve	50 mg once daily
Treatment-naïve or treatment-experienced INSTI naïve when coadministered with certain UGT1A or CYP3A inducers	50 mg twice daily
INSTI-experienced with certain INSTI-associated resistance substitutions or clinically suspected INSTI resistance	50 mg twice daily

Tivicay package insert. Viiv Healthcare.
https://www.gsksource.com/pharma/content/dam/GlaxoSmithKline/US/en/Prescribing_Information/Tivicay/pdf/TIVICAY-PI-PIL.PDF

Helpful Resources on HIV Resistance

- International Antiviral Society-USA [iasusa.org]
- Stanford University HIV Drug Resistance Database [hivdb.stanford.edu]
- Clinician Consultation Center [nccc.ucsf.edu] (800) 933-3413
- Southeast AETC Partners and Training Sites [aidsetc.org/directory/regional/southeast-aetc]

Summary

- HIV resistance can be induced or transmitted
- Resistance testing available as genotypic and phenotypic assays
- Resistance testing recommended prior to ART initiation or virologic failure during ART or suboptimal suppression of viral load after start of therapy
- Resistance testing is reliable and cost-effective but must be interpreted in context and may require expert advice

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