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Evaluation and Management of Virologic Failure

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Module 3: Antiretroviral Therapy
Lesson 5: Evaluation and Management of Virologic Failure

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Introduction

Development of Antiretroviral Drug Resistance

Among persons with HIV who are not taking antiretroviral therapy, HIV replicates at an extraordinarily high rate, typically producing billions of virions daily.[1] At the reverse transcription step during the HIV replication process, mutations occur at a high rate, predominantly because HIV reverse transcriptase fails to correct erroneously incorporated nucleotides (Figure 1).[2] These nucleotide sequence changes generate amino acid substitutions during translation that can alter the reverse transcriptase, protease, or integrase enzymes, as well as changes in capsid and envelope proteins. If a person with HIV takes antiretroviral therapy with good adherence and maintains suppressed HIV RNA levels, HIV replication remains insufficient for these mutations to occur at a significant frequency; with inadequate adherence, however, those viral strains that develop mutations with resistance to the antiretroviral regimen will have a fitness advantage, and will eventually become the dominate quasispecies (Figure 2)**.** The emergence of dominant resistant strains of HIV can result in a suboptimal response to antiretroviral therapy and virologic failure; this type of drug resistance is referred to as acquired resistance (as opposed to transmitted resistance, which is acquired at the time of initial infection because it is already present in the strain of HIV transmitted from the source individual).[3]

Transmission and Natural History of Drug-Resistant HIV

In high-income countries, such as the United States, acquisition of drug-resistant HIV occurs in an estimated 10 to 17% of new HIV infections.[4,5,6,7] In the absence of antiretroviral therapy, several outcomes may occur following such transmission: (1) back-mutation of the strains to naturally occurring “wild-type” strains that do not contain the mutation, (2) overgrowth of the strains by wild-type HIV, (3) persistence of the strains at low levels if they do not impair viral fitness, and (4) existence of strains that contain a mixture of some persistent mutations and some mutations that have reverted to wild-type HIV. If antiretroviral therapy is administered in any of these scenarios, the drug-resistant strains may become dominant if they confer resistance to the specific antiretroviral agents used, potentially causing virologic failure. The most frequently transmitted resistance mutations are non-nucleoside reverse transcriptase inhibitor (NNRTI)-associated mutations; transmission of integrase inhibitor-associated mutations remains rare. Thus, transmitted drug resistance is less relevant in the integrase strand transfer inhibitor (INSTI) era but is still important for clinicians to recognize.

Management of HIV Drug Resistance and Virologic Failure

Detection of virologic failure and testing for HIV resistance to antiretroviral medications is an important component of the clinical care of persons with HIV. Resistance assays can assist the clinician in selecting a

maximally effective antiretroviral regimen. Clinicians who care for individuals with HIV should have a general understanding of evaluating HIV drug resistance and managing virologic failure. Interpretation of resistance assay results and management of virologic failure is complex, and clinicians should have a low threshold to seek expert advice in this situation.[8] In addition, it is important for clinicians to identify more complex resistance scenarios that require expert consultation. This Topic Review will address the approach to patients with detectable HIV RNA levels, the role for HIV drug resistance testing, the interpretation of drug resistance test results, and strategies for managing virologic failure.

Definition of Terms Related to Virologic Responses

Virologic Responses

In the Adult and Adolescent ART Guidelines, the following definitions are used to characterize and define different virologic responses to antiretroviral therapy:[8]

- **Virologic Suppression**: A response to antiretroviral therapy with an HIV RNA level below the lower level of detection of available assays (typically less than 20 to 50 copies/mL, depending on the assay used) (Figure 3).
- **Virologic Failure:** The inability to achieve or maintain HIV RNA levels less than 200 copies/mL.
- **Incomplete Virologic Response**: Failure to suppress HIV RNA to undetectable levels after 24 weeks on an antiretroviral regimen, as documented by two consecutive HIV RNA levels greater than or equal to 200 copies/mL in a person who has not previously achieved virologic suppression on the same antiretroviral regimen (Figure 4).
- **Virologic Rebound**: Confirmed HIV RNA level greater than or equal to 200 copies/mL after achieving virologic suppression on antiretroviral therapy (Figure 5).
- **Virologic Blip**: After achieving virologic suppression, a single detectable HIV RNA level (usually less than 200 copies/mL) followed by a return to virologic suppression (Figure 6).
- **Low-Level Viremia**: Persistent HIV RNA levels above the level of detection of the assay but below 200 copies/mL (Figure 7).

Causes of Virologic Failure

Causes of Virologic Failure

Multiple different factors can play a role in the development of virologic failure.[8] Cohort studies and clinical experience have shown that suboptimal adherence frequently plays a major role in the development of drug resistance and virologic failure.[8] In some instances, individuals may acquire drug-resistant HIV, which can increase the likelihood of virologic failure, depending on the specific acquired mutations and the antiretroviral regimen chosen (this was much more common when NNRTI agents were used as part of first-line ART and has become more rare in the INSTI era). The Adult and Adolescent ART Guidelines list the following three groups of factors that most often contribute to virologic failure: (1) patient/adherence-related factors, (2) HIV-related factors, and (3) antiretroviral regimen-related factors (Table 1).[8]

False Elevation in HIV RNA Levels with Plasma Preparation Tubes

Multiple studies have documented factitious transient or low-level viremia that may result from the use of plasma preparation tubes, which likely does not represent virologic failure or replication-competent viremia.[9,10,11,12,13] The false elevation in HIV RNA levels is thought to result from retained cellular material following the normal specimen processing that separates plasma from whole blood; specifically, some of the cellular material may have fragments of peripheral blood mononuclear cells that are infected with HIV and that contain proviral HIV DNA, which subsequently gets amplified during the quantitative HIV RNA assay.[13] In addition, after the initial separation process of the blood specimen, some residual platelets may remain that have HIV adherent to the platelet surface. This plasma preparation-associated false elevation in HIV RNA levels appears to occur more frequently when a freezing step is included. One group has described a solution to this problem that involves implementing a second centrifugation step.[9]

Approach to Detectable HIV RNA Levels

New detectable HIV RNA levels may or may not indicate virologic failure, and several factors must be considered when evaluating for possible virologic failure. The following section outlines the recommended approach in the Adult and Adolescent ART Guidelines for different scenarios of detectable HIV RNA levels.[8]

Intermittent or Transient Viremia (Virologic Blips)

Most available data suggest that isolated blips in HIV RNA levels do not correlate with HIV drug resistance or virologic failure.[14,15,16,17,18] Some studies have shown that virologic blips more often occur in patients with high baseline HIV RNA and low baseline CD4 cell count (less than 350 cells/mm^3).[19] One study showed an association between the magnitude of the blip and virologic failure, with blips greater than 500 copies/mL associated with an increased risk of virologic rebound.[20] Studies on adherence have been conflicting, with some studies showing a correlation between viral blips and adherence, whereas others have not.[16,21] Experts do not recommend making any antiretroviral regimen changes based on an isolated virologic blip, especially with an HIV RNA blip less than 200 copies/mL. In this situation, it is reasonable to review adherence, assess for possible drug interactions, and recheck the HIV RNA level approximately 4 weeks after the viral blip.

HIV RNA Detectable but Below the Limit of Quantitation

In some instances, HIV RNA can be detected in a sample, but the amount of virus is so low that an accurate count of the HIV RNA cannot be determined; this situation is typically referred to as detectable below the limit of quantitation and is sometimes called “very low-level viremia” (Figure 8). Individuals in this situation are generally considered to have well-controlled HIV and do not require any change in antiretroviral therapy based on this very low-level viremia (most commercial assays have a cutoff in the range of 20 to 50 copies/mL for reporting quantitative HIV RNA levels).[22,23] Most experts do not alter lab monitoring intervals or make any other changes based on HIV RNA levels detectable in this range.

Low-Level Viremia (HIV RNA Detectable but Less than 200 copies/mL)

Low-level viremia is defined as a persistent detectable HIV RNA level above the lower limit of detection of the assay (usually 20 to 50 copies/mL) but less than 200 copies/mL.[8] Low-level viremia may be due to suboptimal adherence, drug interactions, drug-food interactions, or rarely, may be an early sign of virologic failure. Persistent low-level viremia in the setting of excellent adherence with a potent antiretroviral regimen may be caused by the laboratory detection of replication-incompetent HIV or from large clones of HIV-infected cells (so called “repliclones”) that are producing HIV and are not fully controlled with antiretroviral therapy.[24] In both instances, however, low-level viremia usually does not lead to the development of drug resistance. When HIV RNA levels are less than 200 copies/mL, standard genotypic drug resistance testing usually cannot be performed. The Adult and Adolescent ART Guidelines recommend that persons with low-level viremia continue the same antiretroviral regimen, but undergo monitoring with HIV RNA levels every 3 months.[8,23] Additional approaches used by some experts include (1) obtaining a proviral DNA genotype, especially if the past antiretroviral history and resistance history are unknown and unavailable, and/or (2) switching the antiretroviral therapy to a regimen with a high barrier to resistance if the low-level viremia occurred on a regimen with a relatively low barrier to resistance.

HIV RNA Level between 200 and 1,000 copies/mL

In persons taking antiretroviral therapy, persistent HIV RNA levels above 200 copies/mL is considered virologic failure and is often associated with the emergence and evolution of new drug resistance.[8,25,26] The emergence of resistance mutations typically occurs more rapidly in antiretroviral medications that have a lower genetic barrier to resistance. Virologic failure may also occur in the absence of new resistance mutations in the setting of very poor adherence; although this scenario is defined as virologic

failure, virologic suppression may be possible to achieve on the same antiretroviral regimen with excellent subsequent adherence. If virologic failure occurs and the viral load is greater than 200 copies/mL or greater, genotypic drug resistance testing should be requested, although the ability of laboratories to successfully perform resistance testing at a level between 200 and 500 copies/mL level of viremia is not always reliable and testing may be unsuccessful.[7] If the drug resistance genotype is successfully performed, the subsequent management should be based on the test results, and if new resistance is identified, the subsequent regimen should contain at least two fully active antiretroviral medications, including one with a high genetic barrier to resistance.[8] In the situation where the genotypic drug resistance testing is not successful, clinicians may consider requesting proviral DNA resistance testing or making an empiric change to the antiretroviral regimen; management of these individuals is complex and may benefit from expert consultation.

HIV RNA Level Greater than 1,000 copies/mL and Drug Resistance Identified

For persons with HIV RNA levels greater than 1,000 copies/mL, a drug-resistance genotype can reliably be performed. If new or old drug resistance mutations are identified that are relevant to the regimen the individual is taking, then a prompt change in the antiretroviral regimen is warranted and the choice of the new regimen should incorporate expert consultation.[8] Some experts would also immediately change the regimen once the elevated HIV RNA is identified. The decision to immediately change the regimen or wait until the genotype results return is complex and should be informed by expert consultation.

HIV RNA Level Greater than 1,000 copies/mL and Drug Resistance Not Identified

For persons with HIV RNA levels greater than 1,000 copies/mL, a drug resistance genotype can reliably be performed. Individuals with an HIV RNA level greater than 1,000 copies/mL and no drug resistance mutations identified are likely to have very poor adherence as the major factor for virologic failure.[8] Further, persons with dramatic increases in HIV RNA levels to well above 1,000 copies/mL who do not show any drug resistance mutations typically have completely stopped taking all antiretroviral medications or are rarely taking their medications. Concurrent development of a dramatic increase in HIV RNA level and the absence of drug resistance mutations typically only occur when there is a lack of substantial drug pressure.

HIV Drug Resistance Assays

Types of Drug Resistance Assays

Two basic types of drug resistance assays are currently available to assess HIV resistance to antiretroviral agents: genotypic assays and phenotypic assays.[27] The sensitivity of either the genotype or phenotype for detecting minority HIV populations is limited. More recently, some investigators have employed newer techniques, such as allele-specific PCR, single-genome, and ultra-deep sequencing, to assess the role of minority HIV variants that harbor drug resistance but are not detectable by current standard genotypic or phenotypic assays; these tests are not currently commercially available but have contributed to our understanding of HIV resistance.[28] A newer HIV genotypic drug resistance assay (GenoSure Archive) that analyzes archived proviral HIV DNA in peripheral blood mononuclear cells (PBMCs) is commercially available for use in patients with undetectable or very low HIV RNA levels.

Conventional Genotype Testing

The most commonly used HIV drug-resistance test in clinical practice is the conventional HIV RNA genotypic assay, which involves multiple steps: (1) polymerase chain reaction (PCR)-amplification of HIV RNA circulating in the patient's plasma; (2) direct sequencing of these amplified regions; (3) comparison of the patient's gene sequences to known HIV wild-type gene sequences; and (4) determining the corresponding amino acid alterations that would result from the specific DNA mutations identified (Figure 9).[27] The standard HIV drug-resistance genotypic assays used by commercial laboratories sequence the region of the polymerase (*pol*) gene that encodes for HIV protease and HIV reverse transcriptase enzymes.[27] Of note, the *pol* gene also encodes& the HIV integrase enzyme, but most standard tests do not provide information on this region of the *pol* gene that encodes HIV integrase. Thus, testing for integrase resistance usually requires a separate request (in addition to the standard genotype). Some labs, especially for research purposes, can perform genotypic analysis of the HIV *gag* gene (encodes capsid protein) and the envelope (*env*) gene (which encodes the HIV gp120 and gp41 proteins.[29,30,31,32] Most commercial HIV drug-resistance genotypic assays detect the majority of HIV quasispecies (present in at least 80% of the HIV in the sample). If a person has stopped taking their antiretroviral medication for longer than 4 weeks prior to the genotype testing, the assay may not detect mutations in some strains that have back mutated to wild-type HIV because of the absence of drug pressure.

DNA Genotyping

The HIV DNA drug resistance genotype test—often referred to as a proviral DNA genotype, archive DNA genotype, or peripheral blood mononuclear cell (PBMC) genotype—evaluates cell-associated proviral HIV DNA, either as integrated proviral DNA (Figure 10) or unintegrated cytoplasmic HIV DNA (Figure 11).[33,34] The HIV DNA drug resistance genotype can be performed with very low or even undetectable HIV RNA levels, but the assay requires the use of whole blood (not plasma) samples and immediate freezing of the whole blood sample without performing centrifugation. One study reported a high level of concordance of HIV RNA and DNA genotype in viremic patients,[35] whereas others found incomplete information from the DNA genotype when compared with cumulative drug resistance mutation data from previous drug resistance genotypes.[36,37,38] Therefore, the use of standard HIV RNA drug genotyping (taking into account cumulative data from past RNA resistance tests when available) remains the preferred test for decision-making in persons with virologic failure. Nevertheless, in some individuals with low or fully suppressed HIV RNA levels, historical HIV drug resistance data may not be available, and proviral DNA genotyping may inform decisions about switching antiretroviral therapy.[37,39] The overall clinical utility and optimal use of this test in clinical practice remains unclear.

HIV Drug Resistance Phenotype Assay

The phenotype assay is performed on a blood sample using PCR amplification of reverse transcriptase,

protease, and possibly the envelope genes; the process relies on the patient's dominant circulating strain of HIV. The amplified HIV genes are then inserted into a laboratory HIV strain from which these genes have been deleted, generating large numbers of recombinant HIV clones. These clones are then tested for drug susceptibility to antiretroviral agents using automated assays. In practice, phenotype resistance testing has become rare because it is more expensive and has a longer turnaround time than genotype testing.

Indications for HIV Drug-Resistance Testing

Based on existing evidence and expert opinion, the Adult and Adolescent ART Guidelines recommend drug resistance testing in situations as summarized in the following discussion (Figure 12).[7]

Acute HIV Infection

In the setting of acute or early HIV, obtaining HIV drug resistance genotypic testing is recommended, regardless of whether antiretroviral therapy is immediately started.[7,40] Obtaining a resistance test in the setting of acute or early HIV provides the greatest likelihood of detecting transmitted drug-resistant HIV. For persons with acute HIV, initiation of antiretroviral therapy should not be delayed while awaiting resistance testing results; modification of the antiretroviral regimen can be made, if needed, when the results become available.[7] If a person has acute HIV and antiretroviral therapy is deferred, the guidelines suggest considering repeat resistance testing prior to initiating antiretroviral therapy, since the person may acquire drug-resistant HIV in the interim.[7]

Chronic HIV at Entry Into Care

Drug resistance genotypic testing is recommended in the setting of chronic HIV at the time a person enters into HIV medical care.[7] Multiple studies, when taken together, estimate that 10 to 17% of antiretroviral-naïve persons with HIV have evidence of resistance to at least one antiretroviral medication.[6,7,41,42] Several studies performed prior to the widespread use of INSTIs as the preferred anchor drug showed that baseline drug-resistant HIV can negatively impact response to antiretroviral therapy if using a regimen that did not include an INSTI.[43,44] The recommended standard baseline drug resistance genotype for treatment-naïve individuals evaluates for mutations in the reverse transcriptase and protease genes. Because transmitted integrase resistance remains uncommon, baseline integrase resistance testing is recommended only if there is clinical suspicion of transmitted integrase resistance, such as transmission from a partner known or suspected to have integrase resistance, or if the individual diagnosed with HIV has a history of receiving long-acting cabotegravir as preexposure prophylaxis (PrEP).[7] If a person with chronic HIV enters medical care and antiretroviral therapy is deferred, a repeat resistance test should be considered prior to initiating antiretroviral therapy, since drug-resistant HIV could be acquired in the interim.[7]

Virologic Failure

For persons with HIV who develop virologic failure and have an HIV RNA greater than 200 copies/mL, genotypic HIV drug resistance testing should be ordered, but at levels between 200 and 500 copies/mL, the resistance test may be unsuccessful due to inadequate viral amplification.[7] If a person develops virologic failure while taking an INSTI-based regimen, integrase resistance testing should also be ordered (note that adding integrase testing may require a separate order or request since most standard HIV genotypic drug resistance tests do not include integrase).[7] Ideally, HIV drug resistance testing should be performed while the person with HIV is still taking the failing antiretroviral regimen or within 4 weeks of discontinuation of antiretroviral therapy.[7] Nevertheless, in some situations, performing drug resistance testing within 4 weeks of stopping antiretroviral therapy is not possible; in these situations, resistance testing should still be performed since some mutations may still be detected.[7] In addition, the guidelines recommend testing for resistance, including integrase, for an individual who has developed virologic failure with prior use of long-acting, injectable cabotegravir and rilpivirine, regardless of the amount of time that has passed since the last injection, since these injectable agents have very long half-lives.[7]

Incomplete Virologic Response

After initiation of antiretroviral therapy, the virologic goal is to lower HIV RNA levels below the limit of detection. For nearly all individuals, this should be achieved by 24 weeks after initiation of antiretroviral therapy, especially when the antiretroviral regimen includes an INSTI.[7,8] Occasionally, such as with a very

high baseline HIV RNA level, virologic suppression may take longer than 24 weeks to achieve. In most circumstances, suboptimal suppression of HIV RNA levels after 24 weeks of therapy suggests baseline HIV drug resistance. Thus, HIV genotypic drug resistance testing is recommended in persons with suboptimal suppression of HIV RNA levels after initiating antiretroviral therapy (HIV RNA level greater than 200 copies/mL after 24 weeks following initiation of therapy).[7] In addition, if an individual reports good adherence but does not achieve a 1 to 2 log reduction in HIV RNA level after 4 to 8 weeks of treatment, most experts would promptly perform HIV drug resistance testing, including testing for integrase resistance if the person is taking an INSTI.

Pregnancy

Drug resistance testing is recommended for all pregnant women with HIV who are antiretroviral-naïve and have an HIV RNA level above the threshold for resistance testing (200 copies/mL).[45] In this setting, initiation of antiretroviral therapy should not be delayed while awaiting resistance testing results; modification of the antiretroviral regimen can be made, if needed, when the results become available.[7] Resistance testing is also indicated before modifying antiretroviral therapy regimens for pregnant women with HIV who have detectable HIV RNA levels (above 200 copies/mL) upon entry to prenatal care while taking antiretroviral therapy or who have a suboptimal virologic response to a new regimen started during pregnancy.[45]

After Discontinuation of Antiretroviral Therapy

For individuals who have discontinued antiretroviral therapy for more than 4 weeks, drug resistance testing may provide useful information if mutations are identified.[7] In this situation, resistance assay sensitivity is reduced, and mutations may be present but not detected due to overgrowth of wild-type HIV.[7] When HIV drug resistance develops in response to antiretroviral therapy, populations of wild-type and resistant strains of HIV typically coexist, but upon discontinuation of antiretroviral therapy, replication of wild-type strains outpace the growth of most drug-resistant strains, and the resistant strains will likely become a minority species in the overall viral population (Figure 13). Because currently available resistance assays do not reliably identify strains of HIV that constitute less than 20% of the overall viral population, the "minority resistant" strains often evade detection by resistance assays in persons who discontinue therapy.[46,47,48] Although drug-resistant strains may not be evident on resistance testing in this situation, they can quickly become dominant if the patient reinitiates antiretroviral therapy that includes medications to which they had previously developed resistance.[46]

Interpretation of Drug-Resistance Assays

Interpreting Drug-Resistance Genotype

The genotype assay generates HIV DNA sequence data, which allows for the direct inference and reporting of data related to HIV amino acid sequences (Figure 14). Specific HIV DNA mutations can result in amino acid changes that impact the activity of an antiretroviral medication. The genotype report provides specific information regarding the amino acids in the patient's HIV sample that deviate from those found in wild-type HIV strains (Figure 15). The shorthand convention used in a genotype report lists the wild-type amino acid, followed by the position of that amino acid in the protein, followed by the substituted amino acid that confers resistance; for example, the K103N mutation occurs as a result of a mutation that causes replacement of the amino acid lysine (K) by asparagine (N) at amino acid position 103 in the reverse transcriptase protein (Figure 16). In addition, most drug resistance genotype reports provide an interpretation of the impact of drug resistance mutations on antiretroviral medications. Most genotype interpretation results classify drug susceptibility in one of three categories: no evidence of resistance, low-level resistance, or high-level resistance. In addition, some mutations can result in viral hypersusceptibility to medications, as is the case with the M184V reverse transcriptase mutation that enhances virologic response to tenofovir DF, tenofovir alafenamide, and zidovudine.[49,50,51,52]

Interpreting Drug-Resistance Phenotype

A phenotypic resistance assay evaluates the susceptibility of HIV to antiretroviral agents by directly measuring the viability of the predominant strain of HIV in the presence of antiretroviral medications.[27] For each antiretroviral agent tested, the phenotype report provides an IC_{50} or IC_{90} value—this value represents the drug concentration required to inhibit the replication of HIV by 50% (for IC_{50}) or 90% (for IC_{90}). The IC_{50} (or IC_{90}) of the patient's sample is divided by a reference IC_{50} (or IC_{90}) value from wild-type virus to generate a "fold change" value; the fold change represents relative resistance of the patient's HIV to the antiretroviral medication (Figure 17). Based on the fold change observed when testing the patient's HIV against an antiretroviral medication, the patient's HIV can be considered either susceptible, resistant, or hypersusceptible to the medication tested (Figure 18).

Relevance of Past Drug-Resistance Tests

Clinicians should regard antiretroviral resistance as cumulative drug resistance that has developed in an individual with HIV, recognizing that a recent standard resistance assay may not detect all the resistance mutations that may have been detected in an earlier resistance test. Resistance results should always be documented for future consideration. When selecting a new antiretroviral regimen, clinicians should incorporate data from all past resistance tests performed for the patient.

Resources for Interpreting Drug Resistance Testing

The goal with both initial and salvage antiretroviral therapy is to fully suppress HIV RNA levels. If HIV drug resistance has been identified, this should be accounted for when constructing the antiretroviral regimen. Accurate interpretation of the HIV drug resistance data is essential when selecting this regimen. In general, in the setting of significant drug resistance present on an HIV drug resistance test, consultation with an HIV expert is recommended. In addition, given the complexity associated with the interpretation of HIV drug resistance assays, expert clinical consultation is advised for clinicians who do not have significant experience in interpreting HIV drug resistance tests. The following are excellent resources for help with resistance issues.

- Stanford University HIV Drug Resistance Database: This free website provides comprehensive information on HIV drug resistance. Most importantly, the site features a Genotypic Resistance Interpretation Algorithm that allows the user to input specific genotypic mutations from a patient's sample in a drop-down menu and then view the analysis of the genotype. Options for input include

mutations involving reverse transcriptase, protease, and integrase.

- National HIV Clinician Consultation Center: HIV/AIDS Management: This free service offers clinicians expert consultation service for any aspect of HIV clinical care, including evaluation and management of virologic failure and interpretation of HIV drug resistance genotype tests. The phone number is 800-933-3413.
- International AIDS Society-USA (IAS-USA): Drug Resistance Mutation Figures: The IAS-USA regularly publishes a concise and updated compendium of HIV drug resistance mutations and the impact of each of these mutations on antiretroviral medications. The HIV drug resistance mutation figures and the accompanying notes provide an excellent visual overview of the current state of knowledge about relevant HIV drug resistance mutations.

Nucleoside Reverse Transcriptase Inhibitor Resistance

Principles of Nucleoside Reverse Transcriptase Inhibitor Resistance

The nucleoside reverse transcriptase inhibitor (NRTI) medications block HIV reverse transcription, the process whereby HIV RNA is converted into HIV DNA.[53] Specifically, the inhibition occurs when the HIV reverse transcriptase enzyme incorporates the NRTI into the elongating HIV DNA strand, but the NRTI acts as a chain terminator due to the absence of a 3' hydroxyl group.[54] The development of NRTI resistance by HIV involves one of two biochemical mechanisms that take place in the reverse transcriptase process: (1) discrimination (decreased incorporation) of the antiretroviral medication into the elongating HIV DNA strand (Figure 19) or (2) excision (primer unblocking) of the antiretroviral medication from the HIV DNA strand (Figure 20).[55,56,57,58,59]

- **Decreased Incorporation (Discrimination)**: With one mechanism of NRTI resistance, the mutations that occur result in the reverse transcriptase enzyme preferentially selecting the human deoxynucleotides in the cell over the NRTI chain terminators, thereby creating a relative decreased incorporation of the NRTI-triphosphate into the elongating HIV DNA strand.[56] These mutations are often referred to as discriminatory mutations.[56] Examples of mutations that cause discrimination (decreased NRTI incorporation) include K65R, K70E, L74V, M184I/V, and the Q151M complex (Q151M followed by the accessory mutations A62V, V75I, F77L, and F116Y).[56,57,60,61]
- **Excision (Primer Unblocking)**: Excision mutations enhance the phosphorolytic excision of the NRTI-triphosphate that had been added to the elongation HIV RNA-DNA complex. When the NRTI is incorporated into the RNA-DNA complex, it blocks further addition of any cellular deoxynucleotides to the elongating strand of the primer (primer blocking).[56] Therefore, if the NRTI drug is excised from the primer, the net effect is referred to as primer unblocking. Characteristic mutations that occur via the primer unblocking include the M41L, D67N, K70R, L210W, T215Y/F, and K219Q/E.[56,57,60]

Specific Reverse Transcriptase Mutations that Impact NRTIs

M184I/V Mutations

The M184I/V mutations are the signature resistance mutations that can develop in persons taking lamivudine or emtricitabine. The M184I mutation typically develops prior to the M184V, but is usually rapidly replaced by the M184V, primarily because the M184I mutation causes a greater impairment in viral fitness than does the M184V.[62] The M184I and M184V mutations develop via the discriminatory resistance pathway.[56,58] The M184V mutation causes high-level resistance to emtricitabine and lamivudine, low-level resistance to abacavir, and enhanced susceptibility to tenofovir alafenamide, tenofovir DF, and zidovudine.[63]

Thymidine Analog Mutations

The thymidine analog mutations (TAMs), which include M41L, D67N, K70R, L210W, T215Y/F, and K219Q/E, develop in the setting of virologic failure with a regimen that includes either of the thymidine analogs, stavudine or zidovudine (Figure 21).[49,60,63,64,65,66] The TAM mutations tend to accumulate in one of two characteristic, overlapping patterns: (1) the type-I pattern that has M41L, L210W, and T215Y, or (2) type-II pattern consisting of D67N, K70R, T215F, and K219Q/E (Figure 22).[60,67,68,69,70]. In general, type-I TAM mutations result in higher levels of resistance to stavudine and zidovudine, as well as greater cross-resistance to abacavir, didanosine, tenofovir DF, and tenofovir alafenamide.[60,71] For persons taking a thymidine analog plus either lamivudine or emtricitabine, the development of TAMs is typically preceded by the development of an M184V mutation. Some patients develop the D67N mutation with the type-I cluster.

K65R Mutation

In clinical trials, the development of the K65R mutation primarily occurred in patients taking a non-

suppressive antiretroviral regimen that includes tenofovir DF, tenofovir alafenamide, or abacavir, but not one of the thymidine analog medications (e.g., stavudine or zidovudine).[72,73] For example, high rates (greater than 50%) of K65R mutation developed in patients treated with the outdated triple NRTI regimen of abacavir plus lamivudine plus tenofovir DF.[74] In current practice, the K65R is most often seen with a tenofovir DF or tenofovir alafenamide-based treatment regimen, but the addition to the regimen of a drug with a high genetic barrier to resistance from a class other than NRTI markedly reduces the likelihood of developing the K65R mutation. The K65R mutation has also been reported in persons who acquire HIV while receiving tenofovir DF-emtricitabine or tenofovir alafenamide-emtricitabine for HIV preexposure prophylaxis. The K65R mutation alone causes high-level resistance to tenofovir DF and tenofovir alafenamide, intermediate-level resistance to abacavir, emtricitabine, and lamivudine, and hypersusceptibility to zidovudine.[60] When the K65R mutation occurs in combination with the M184V mutation, it causes high-level resistance to abacavir, emtricitabine, and lamivudine; intermediate-level resistance to tenofovir DF and tenofovir alafenamide; and marked hypersusceptibility to zidovudine.[72,75] With abacavir resistance, the M184V typically precedes the K65R.[76] In clinical trials and clinical practice, it is uncommon to observe the K65R mutation in conjunction with multiple TAMs.[72,73]

L74V Mutation

The L74V mutation was first identified with didanosine monotherapy and abacavir monotherapy; this mutation alone causes intermediate-level resistance to abacavir. The L74V mutation, in combination with M184V, has been seen in patients treated with abacavir plus lamivudine, or didanosine plus lamivudine.[60] Overall, the L74V mutation is an uncommon NRTI mutation but is identified more often in conjunction with certain NNRTI mutation combinations, such as K101E plus G190S and L100I plus K103N.[77]

Multi-NRTI Resistance Mutations: T69 and Q151M Insertion Complexes

The multi-nucleoside resistance mutations occur relatively infrequently but may have a major impact on NRTIs. The T69-insertion mutation consists of a double amino acid (diserine) insertion between codons 69 and 70 in the reverse transcriptase enzyme.[78,79] The T69 occurs only in the setting of existing TAM-1 mutations; together, the T69-insertion and TAM-1 generate high-level resistance to all the NRTI medications, except for lamivudine and emtricitabine, which have intermediate resistance.[60,78,80] The Q151M mutation complex usually occurs with several accessory mutations (A62V, V75I, F77L, and F116Y), and these mutations, in tandem with Q151M, cause high-level resistance to abacavir, didanosine, and zidovudine, as well as intermediate resistance to emtricitabine, lamivudine, and tenofovir. [56]The Q151M mutation complex develops only in the setting of prolonged viremia while on therapy with a regimen that includes a thymidine analog (zidovudine or stavudine).[56]

Lamivudine and Emtricitabine

In clinical trials involving combination antiretroviral therapy, the M184V mutation is characteristically one of the most common mutations to develop with initial virologic failure in regimens that include lamivudine or emtricitabine.[81] One early study showed that patients treated with lamivudine monotherapy developed the M184V mutation and virologic failure within 4 weeks of starting lamivudine.[82] Even after the development of the M184V mutation, lamivudine, if continued, will maintain a 0.4 to 0.5 $\log_{10}$ decrease in HIV RNA levels, probably due to the impact this mutation has on viral fitness.[82] Several later studies suggested that continuing treatment with lamivudine in the presence of an M184V mutation may confer benefit, potentially through decreased viral fitness, hypersensitivity to several other NRTIs (tenofovir alafenamide, tenofovir DF, and zidovudine), and perhaps delayed development of mutations that impact other NRTIs.[83,84] In the absence of drug pressure from either emtricitabine or lamivudine, HIV strains with the M184V mutation often rapidly become a minority species, reflecting the fitness cost to HIV if it maintains this mutation.[85] Once the M184V mutation develops, there are no further cascading lamivudine or emtricitabine mutations that develop that would negatively impact other antiretroviral medications. The M184V mutation is not known to impact medications outside the NRTI class, but the M184I mutation augments resistance to rilpivirine when present in conjunction with the E138K mutation.[86]

Stavudine and Zidovudine

The thymidine analog mutations (TAMs), which include M41L, D67N, K70R, L210W, T215Y/F, and K219Q/E, can develop in the setting of virologic failure with a regimen that includes a thymidine analog (stavudine or zidovudine).[49,60,63,64,65,66] Although these medications are now only rarely used in clinical practice, individuals with long-standing HIV may have acquired TAMs in the past. In general, the more TAMs that are present, the more extensive the NRTI drug resistance. The presence of an M184V mutation reduces the impact of the TAM mutations to some degree, but the favorable impact of the M184V is negligible with higher numbers of TAM mutations.[87]

Non-Nucleoside Reverse Transcriptase Inhibitor Resistance

Principles of Non-Nucleoside Reverse Transcriptase Inhibitor Resistance

The NNRTIs block HIV replication via non-competitive inhibition of the HIV reverse transcriptase enzyme. The NNRTIs bind to a hydrophobic pocket of the HIV reverse transcriptase, which causes functional changes in the enzyme (Figure 23).[53,54] The NNRTI hydrophobic binding pocket region is predominantly lined by amino acid codons 98 to 108 and 179 to 190; the key amino acids that line the binding pocket are L100, K101, K103, V106, T107, V108, V179, Y181, Y188, V189, G190, F227, W229, L234, Y318 (of p66), and E138 (of p51).[46,57] Resistance to NNRTIs typically occurs as a result of one or more mutations involving amino acids that line the NNRTI binding pocket or are adjacent to the pocket; these mutations can prevent NNRTI binding either by altering the NNRTI binding site (Figure 24) or by preventing adequate access of the NNRTI to the binding pocket (Figure 25).[54,57] Although all NNRTI drugs bind to the same general region in the binding pocket, subtle differences exist in the interaction between the specific drug and the hydrophobic pocket, and there are important differences in the mutations that develop and the amount of resistance and NNRTI cross-resistance that occurs with each. Compared to other agents like boosted protease inhibitors or certain INSTIs (dolutegravir and bictegravir), the NNRTIs have a relatively lower barrier to the development of drug resistance, and NNRTI-resistant mutants can rapidly develop.[46,88]

Doravirine

Data from clinical trials that included 11 patients with virologic failure showed doravirine resistance-associated substitutions in reverse transcriptase that included one or more of the following mutations: A98G, V106I, V106A, V106M/T, V108I, E138G/K, Y188L, H221Y, P225H, F227C, F227C/R, and Y318Y/F.[89,90] Two mutation pathways of resistance have been identified with doravirine: (1) a major pathway with selection of the V106A mutation followed by the F227L or L234I mutation (Figure 26), or (2) a minor pathway that involves selection of V108I and L234I mutations.[91,92] Clinical experience with cross-resistance for doravirine and other NNRTIs is limited, but emergence of resistance to doravirine may not impact all NNRTIs.[92] Doravirine maintains excellent activity in the presence of some of the most common NNRTI mutations, including K103N, E138K, and Y181C, but varying levels of resistance can occur with other mutations, with high-level resistance to doravirine associated with the V106A; Y188L; G190E; F227C; F227L; M230L; and Y318F mutations (Figure 27).[91,93] In vitro data have shown a marked reduced susceptibility to doravirine with (1) Y188L substitution alone or in combination with K103N, or (2) V106I, V106A in combination with G190A and F227L, or (3) E138K in combination with Y181C and M230L.

Efavirenz

For individuals who develop virologic failure while taking an efavirenz-based regimen, the K103N mutation is the most common NNRTI mutation observed; three other mutations can cause primary resistance: Y188L, G190S, and G190A.[94] Although early virologic failure with efavirenz characteristically involves a single mutation, such as the K103N mutation (or sometimes dual mutations), prolonged virologic failure leads to the accumulation of multiple mutations that may also include L100I, V108I, Y181C/I, and P225H; the development of these multiple mutations compounds the level of resistance to other NNRTIs.[46,95]

Etravirine

Etravirine is a second-generation NNRTI that has a higher barrier to resistance than the first-generation NNRTIs and retains activity against most HIV strains that have developed resistance to efavirenz and/or nevirapine. Because the resistance profile is more complex than the first-generation NNRTIs (a single mutation can affect virologic response to the first-generation NNRTI's, but multiple mutations often must accumulate to reduce the activity of etravirine), a scoring rubric has been developed to help determine the impact of specific resistance mutations on susceptibility to etravirine and thus predict virologic response.[96] According to the etravirine-weighted genotypic score ("etravirine score"), a score of 0 to 2 indicates

susceptibility, 2.5 to 3.5 indicates intermediate resistance, and a score of 4 or greater indicates resistance; for example, the Y181C mutation yields a resistance weight factor of 2.5 (intermediate), whereas the G190A mutation yields a resistance weight factor of 1 (low).[96] Etravirine resistance has mostly been studied in the context of coadministration with ritonavir-boosted darunavir in the DUET studies, and the performance of the weighted scoring system has not been validated with other antiretroviral agents.[49]

Nevirapine

Patients treated with nevirapine monotherapy most commonly develop the Y181C mutation.[88] In about 20% of patients who develop virologic failure on a nevirapine-based regimen, the mutations V106A/M or Y181C/I initially emerge.[97] Among patients with early virologic failure, while taking a combination antiretroviral therapy regimen that includes nevirapine, approximately 80% develop the K103N or Y188C/L/H mutations.[46,97]

Rilpivirine

Individuals who develop virologic failure while taking rilpivirine most often develop a mutation at the E138 amino acid position (E138A/G/K/Q/R/V); among these, E138K is the most common.[98] A solitary E138 mutation is associated with intermediate-level resistance to rilpivirine, but when it occurs in conjunction with the M184I mutation, high-level resistance develops.[86,98,99,100,101] Less often, patients taking rilpivirine develop a K101E mutation, which typically causes intermediate-level rilpivirine resistance.[102]

NNRTI Cross-Resistance

The following summarizes key cross-resistance with several common and important NNRTI mutations. In general, patients who develop virologic failure while taking an efavirenz-based regimen have a better chance of responding to etravirine or doravirine in the future than those with virologic failure on a rilpivirine-based regimen. Overall, initial virologic failure with nevirapine or rilpivirine typically causes more NNRTI cross-resistance as compared to virologic failure with efavirenz. Etravirine and doravirine may retain activity in the setting of past virologic failure on an NNRTI, but assessing the activity of etravirine and doravirine following virologic failure requires the use of scoring systems, such as the Stanford Resistance Database, to assess activity in the setting of specific NNRTI mutations.

- **K103N**: The K103N mutation, which often occurs in the setting of virologic failure with an efavirenz-based regimen, confers cross-resistance to nevirapine but not to doravirine, etravirine, or rilpivirine.[36,103,104,105,106]
- **E138K**: The E138K mutation, which is the signature mutation that develops with rilpivirine-associated virologic failure, generates intermediate-level resistance to rilpivirine, and potential low-level cross-resistance to doravirine, efavirenz, etravirine, and nevirapine.[98] If the E138K occurs with an M184V mutation, it increases the likelihood of virologic failure on a rilpivirine-based regimen.
- **Y181C**: The Y181C mutation, which can occur with nevirapine failure, causes high-level resistance to nevirapine, intermediate-level resistance to efavirenz, etravirine, and rilpivirine, and potential low-level resistance to doravirine.[103,107,108,109,110]
- **G190E**: The G190E mutation is problematic in that it causes high-level resistance to doravirine, efavirenz, nevirapine, and rilpivirine, as well as intermediate level resistance to etravirine—essentially leaving no good options in the NNRTI class.[111]

Resistance to NNRTIs and Viral Fitness

Although resistance mutations have the potential to impair viral replication, most mutations in the NNRTI class appear to have little impact on viral replication (fitness).[106] Data from multiple studies suggest the K103N mutation has minimal impact on viral fitness; thus, HIV that contains the K103N mutation can exist as a highly resistant and highly fit virus. Accordingly, experts do not recommend continuing NNRTI medications

in the setting of the K103N mutation. In patients who experience virologic failure on rilpivirine and have E138K and M184I mutations, the E138K mutation appears to nullify any fitness benefit that might be achieved by maintaining the M184I mutation.[112]

Integrase Strand Transfer Inhibitor Resistance

Principles of INSTI Resistance

The integrase strand transfer inhibitors (INSTIs) interfere with the insertion of HIV DNA into host DNA.[113] The integration of HIV into host DNA is a complex process that involves multiple steps. The INSTIs exert their action by blocking the HIV integrase-mediated strand transfer of the HIV DNA into the host DNA. The HIV integrase enzyme is a 288-amino acid protein comprised of the C-terminal domain, the N-terminal domain, and the catalytic core domain; most INSTI resistance mutations occur in proximity to the integrase enzyme active site in the catalytic core domain (Figure 28).[114,115,116] Viruses can also accumulate minor or accessory integrase mutations that can raise the level of overall resistance. Note that testing for integrase resistance typically requires a separate request and order, since most standard HIV genotypic drug resistance tests do not include integrase resistance testing.[7]

Bictegravir

Available data suggest that bictegravir has a high genetic barrier to resistance, similar to dolutegravir, and this barrier is higher than with cabotegravir, elvitegravir, and raltegravir.[117,118] In addition, in vitro data have shown that bictegravir retains activity against a wide range of HIV strains with INSTI-resistance substitutions. Specifically, bictegravir retains good activity (less than 2-fold reduced susceptibility) with the following common single integrase mutations: 92Q, T97A, Y143C/R, Q148R, and N155H. Nevertheless, bictegravir is not recommended for the treatment of persons with integrase-resistant HIV, primarily because increased dosing of bictegravir is not an option (bictegravir is available only as the fixed-dose combination tablet bictegravir-tenofovir alafenamide-emtricitabine). In contrast, doses of dolutegravir can be increased in the setting of integrase resistance mutations. The combination of the mutations G140A/C/S and Q148H/R/K is often associated with more than a 2.5-fold reduced susceptibility to bictegravir, especially with an additional INSTI-resistance substitution at L74M, T97A, or E138A/K.

Cabotegravir

Cabotegravir, which is available in a long-acting, injectable formulation, may be used alone for HIV PrEP or in combination with long-acting injectable rilpivirine for HIV treatment. Cabotegravir has a lower genetic barrier to resistance than dolutegravir or bictegravir, but higher than with elvitegravir or raltegravir.[118] Integrase resistance can occur with virologic failure while taking cabotegravir and rilpivirine as treatment, or when receiving injectable cabotegravir for HIV PrEP. In a laboratory study, HIV strains that developed resistance to cabotegravir either developed solitary mutations, such as R263K, S153Y, S147G, H51Y, or Q146L, or multiple mutations (Q148R/K along with secondary mutations).[119] This study found cabotegravir to be less potent than dolutegravir or bictegravir.[119] If certain mutations develop following exposure to cabotegravir, such as the Q148 mutation combined with secondary mutations, cross-resistance to all INSTIs may be present.[119] In clinical trials of long-acting, injectable cabotegravir plus rilpivirine for HIV treatment, integrase resistance mutations detected in individuals with virologic failure included Q148Q/R, G140G/R, N155N/H, R263K, T97T/A, and E138E/K.[120,121,122] In the principal clinical trial of long-acting cabotegravir for HIV PrEP for men who have sex with men, integrase resistance emerged in 4 of 9 incident cases that had a resistance test result, and the mutations detected included the major mutations R263K in one participant and Q148R in three participants.[123] A number of secondary and minor mutations also emerged, including G140A/G/S, E138E/K, and L74I.[123]

Dolutegravir

Dolutegravir, similar to bictegravir, has a high genetic barrier to resistance, and this barrier is higher than with cabotegravir, elvitegravir, or raltegravir.[114,118] Although integrase resistance infrequently develops in a person taking a regimen with dolutegravir, when it does occur, R263K is the most frequently identified mutation.[124,125,126] Other commonly identified mutations include the S230R, N155H, and N155H plus

E92Q.[127,128] The development of dolutegravir resistance is more common in the setting of preexisting integrase resistance mutations than when dolutegravir is used as a first-line regimen.[129,130,131] When dolutegravir resistance develops with a first-line regimen, it usually involves persons with advanced HIV and/or in conjunction with drug interactions that may have affected dolutegravir levels.[124,125,132,133] The R263K mutation can coexist with certain other integrase resistance mutations, such as N155H or E92Q, but is unlikely to coexist with other integrase resistance mutations.[134] The R263K mutation causes intermediate-level resistance to dolutegravir, bictegravir, and cabotegravir, as well as low-level resistance to raltegravir.[135] The S230R mutation confers low-level resistance to dolutegravir, cabotegravir, and raltegravir (and potential low-level resistance to bictegravir).[135] The infrequent development of resistance to dolutegravir has hampered an understanding of the evolutionary pathways of resistance with primary virologic failure with dolutegravir. Most patients who have initial virologic failure with raltegravir have HIV that retains susceptibility to dolutegravir.[136] In persons failing a raltegravir- or elvitegravir-containing regimen, the accumulation of Q148H in combination with the secondary mutations G140S/A/C, L74M, and E138K/A causes a greater than 10-fold reduced HIV susceptibility to dolutegravir.[49,137] The N155H mutation, followed by the A49P, L68F, T97A, E138K, and L234V can also lead to dolutegravir resistance.[138] If integrase mutations are present that are causing low-level or intermediate-level dolutegravir resistance, most experts would increase the dolutegravir dose to 50 mg twice daily if dolutegravir is continued. In general, dolutegravir should not be used with high-level dolutegravir resistance.

Elvitegravir

The primary resistance mutations in HIV-1 integrase selected with virologic failure on elvitegravir (T66I, E92Q, and Q148R) may also confer reduced susceptibility to other integrase inhibitors.[139] The E92Q mutation is the most common initial mutation to arise with elvitegravir failure, followed in frequency by N155H and Q148H/K/R.[114] The E92Q mutation alone reduces elvitegravir susceptibility by more than 20-fold and reduces raltegravir susceptibility by 5-fold.[49] The T66I mutation causes an approximate 10-fold reduction in elvitegravir susceptibility, but it does not have significant impact on raltegravir or dolutegravir. When resistance to raltegravir develops, high-level cross-resistance with elvitegravir usually occurs.[136,140,141] If the Q148 mutation emerges, it can confer significant cross-resistance to dolutegravir and bictegravir, especially if it is associated with secondary integrase mutations.

Raltegravir

Three major resistance pathways have been identified with HIV resistance to raltegravir: N155, Q148, and Y143.[115,142,143,144] The most common raltegravir resistance pathways are: (1) Q148H plus G140S, (2) N155H plus E92Q, and (3) Y143R plus T97A; other secondary mutations can develop, and the initial N155H mutation often crosses over to the Q148 pathway (Figure 29).[115,116,143,144] Although a single major mutation reduces raltegravir susceptibility by 10-fold, the combination of Q148H plus G140S causes more than 150-fold reduced susceptibility to raltegravir and elvitegravir.[145] Importantly, the accessory mutations G140S and T97A restore viral fitness to the mutated virus, whereas E92Q does not have an impact on viral fitness.[145] In patients taking raltegravir, the emergence of integrase resistance mutations can occur, even with low-level viremia. One group analyzed patients with low-level viremia (50 to 500 copies/mL) while taking raltegravir and found 7.7% of (3 of 39) patients had developed INSTI mutations.[146] Development of drug resistance to raltegravir generally translates to high-level cross-resistance with elvitegravir.[136,140,141] In addition, patients taking raltegravir with incomplete virologic suppression who remain on raltegravir will gradually experience a progressive emergence of higher levels of drug resistance to raltegravir, eventually developing high-level, class-wide integrase resistance (Figure 30).[147] The infrequently seen G118R and F121Y raltegravir-associated mutations induce broad cross-resistance to raltegravir, elvitegravir, and dolutegravir.[143,148] The development of the Q148 mutation can cause significant cross-resistance to dolutegravir and bictegravir, especially if it is associated with secondary integrase mutations.

Protease Inhibitor Resistance

Principles of Protease Inhibitor Resistance

The HIV protease inhibitors (PIs) bind to and inhibit HIV protease, an enzyme that is only 99 amino acids in length but functions to cleave the Gag and Gag-Pol precursor proteins; the inhibition of HIV protease can impact multiple enzymes that are generated during the normal cleavage process.[149] Multiple protease mutations are required to significantly impact the virologic response to a PI boosted with either cobicistat or ritonavir (Figure 31).[49,60] In general, PIs boosted with either cobicistat or ritonavir have medium or high potency and a medium or high genetic barrier to resistance.[56,150] Among the commonly used boosted PIs, darunavir has the highest barrier to resistance, followed by lopinavir, followed by atazanavir.[56,150] Most virologic failures in persons taking a PI-containing regimen that has a dual NRTI backbone occur with resistance to the NRTIs but not the protease inhibitor, which reflects a significantly higher barrier to resistance with boosted PIs compared with the NRTIs.[49,60,149] Mutations to PIs can occur with prolonged virologic failure. Some PI mutations enhance susceptibility to one or more PIs. For example, I50L increases susceptibility to all PIs except for atazanavir, whereas L76V enhances susceptibility to atazanavir.[60]

Atazanavir

Atazanavir is infrequently used now in clinical practice, predominantly boosted with ritonavir or cobicistat. Previously, there was some use of unboosted atazanavir. The atazanavir barrier to resistance is substantially lower when unboosted, and fewer mutations are required to develop resistance than when boosted.[49] Treatment-naïve patients who have virologic failure while taking boosted atazanavir do not usually have evidence of new protease mutations at the initial time of virologic failure.[86,151] Atazanavir can select the I50L mutation, which has little impact on other PIs. Multiple major PI mutations have been associated with reduced atazanavir susceptibility, including I50L, I84V, and N88S.[49] One study suggested the L76V mutation, with or without the M46I, generates atazanavir hypersusceptibility if no additional PI mutations are present.[152]

Darunavir

Darunavir boosted with either ritonavir or cobicistat is considered to have a very high genetic barrier to resistance and is the most commonly used protease inhibitor in modern clinical practice. Although darunavir has a resistance pattern distinct from most other protease inhibitors, it shares structural similarities and resistance patterns with the older PIs amprenavir and fosamprenavir. Thus, if an individual developed virologic failure and resistance while taking amprenavir or fosamprenavir in the past, they are likely to have developed cross-resistance to darunavir, which is not the case with past virologic failure when taking other PIs. Antiretroviral-naïve patients who develop virologic failure on a ritonavir-boosted darunavir-based regimen do not generally develop major (primary) PI resistance-associated mutations.[153,154,155] Investigators have identified eleven protease mutations at 10 protease positions that have been associated with reduced HIV susceptibility to darunavir: V11I; V32I, L33F, I47V, I50V, I54L, I54M, T74P, L76V, I84V, L89V.[49,156,157,158] Diminished virologic response to darunavir generally requires at least three darunavir resistance-associated mutations to emerge.[159] Major mutations include I47V, I50V, I54M, L76V, and I84V.[49] Several studies have shown that treatment-experienced patients, including those with protease resistance, respond equally well to darunavir boosted with ritonavir when given once daily (800/100 mg) versus twice daily (600/100 mg), if no baseline darunavir resistance-associated mutations are present.[160,161,162]

Lopinavir-Ritonavir

Earlier in the HIV epidemic, lopinavir-ritonavir was frequently used in the United States for HIV treatment, including for treatment during pregnancy. It is now infrequently used, given the relatively high pill burden and poor tolerability. Persons who received it in the past and developed virologic failure may have multiple

archived PI mutations. Antiretroviral-naïve individuals who develop virologic failure while receiving two NRTIs combined with lopinavir-ritonavir do not typically have evidence of lopinavir drug resistance at the time of virologic failure.[163,164,165] Accordingly, lopinavir boosted with ritonavir is considered to have a high genetic barrier to resistance.[56] Major mutations associated with lopinavir resistance include V32I, I47V/A, L76V, V82A/F/T/S; there are 13 sites identified as minor mutations.[163,164,166] The combination of lopinavir-ritonavir requires accumulation of at least 3 lopinavir-associated mutations for a reduced virologic response to lopinavir-ritonavir.[56,150] Furthermore, certain mutations, either alone or in combination with other mutations, confer high-level resistance: I47A/V and V32I are each associated with high-level resistance, and the L76V mutation in combination with 3 PI mutations also increase resistance to lopinavir-ritonavir.[49,152] Although lopinavir-ritonavir resistance infrequently develops in virologic failure in treatment-naïve persons, mutations can emerge in persons who previously failed other protease inhibitors.[167] In addition, significant cross-resistance exists with lopinavir-ritonavir and atazanavir.[168]

Entry Inhibitor Resistance

Attachment Inhibitors (Fostemsavir)

To date, fostemsavir is the only attachment inhibitor approved for HIV treatment. Among persons who develop resistance to fostemsavir, treatment-emergent gp120 genotypic substitutions occur at 4 key sites: S375, M426, M434, and M475.[169,170] Available data suggest that development of resistance to fostemsavir does not cause cross-resistance to other entry inhibitors (ibalizumab, maraviroc, or enfuvirtide).[171] In contrast, the activity of some isolates to fostemsavir is reduced after the development of ibalizumab or maraviroc resistance; this effect has not been seen with enfuvirtide resistance.

Postattachment Inhibitors (Ibalizumab)

Ibalizumab is currently the only postattachment inhibitor approved for clinical use for the treatment of HIV. Insufficient data exist regarding ibalizumab and drug resistance. Persons with virologic failure on ibalizumab have developed genotypic mutations in gp120 that diminish potential N-linked glycosylation sites in the V5 loop of gp120, but the significance of these findings is unknown. Resistance to other entry inhibitors (or other antiretroviral medications) does not impact the activity of ibalizumab.

CCR5 Antagonists (Maraviroc)

The C-C chemokine receptor 5 (CCR5) antagonists exert their mechanism of action by binding to the human cell CCR5 coreceptor, causing a conformational change in the coreceptor that prevents the gp120 region of R5-tropic HIV from effectively binding with the CCR5 coreceptor; the third variable region (V3) of HIV gp120 is the major determinant of viral tropism (Figure 32).[172] Maraviroc is the only CCR5 antagonist approved for use by the United States Food and Drug Administration. Resistance to maraviroc can occur by two distinct mechanisms: (1) emergence of R4-tropic HIV or (2) binding of R5-tropic HIV to CCR5 in the presence of maraviroc.[172,173] There is no evidence that increasing the dose of maraviroc will overcome either one of these types of mutations.

- **Emergence of R4-Tropic HIV**: Because maraviroc only blocks entry of R5-tropic HIV, the emergence of any type of X4-tropic virus (X-4 tropic, dual-tropic, or mixed-tropic) will allow HIV to bypass the CCR5 receptor blockade by maraviroc. The emergence of X4-tropic virus during treatment can result either from an expansion of preexisting X4-tropic virus that was not detected prior to starting the CCR5 antagonist (Figure 33) or a *de novo* tropism switch caused by multiple mutations in the HIV gp120 V3 region (Figure 34).[49,173] When a true HIV tropism shift from R5-tropic to X4-tropic occurs, it is frequently associated with multiple mutations in the HIV gp120 V3 region, including G11R, P13R, and A25K.[29,30] Most cases of virologic failure that occur in individuals taking maraviroc result from an outgrowth of X4-tropic HIV, usually from an expansion of preexisting X4-tropic virus not originally detected.[172,173]
- **Persistent CCR5 Binding in Presence of Maraviroc**: The second type of resistance to maraviroc can occur independent of a change in HIV tropism, and this involves binding of the gp120 region R5-tropic HIV to the CCR5 coreceptor in the presence of a CCR5 antagonist; the exact mechanism for how the R5-tropic HIV circumvents the effect of the CCR5 antagonist has not been completely elucidated, but likely involves enhanced affinity of HIV gp120 binding to the N-terminal domain region of the CCR5 coreceptor (Figure 35).[60,172,174,175] Resistance involving R5-tropic HIV has been associated with mutations in the HIV envelope gp120 V3 loop that alter the binding properties of HIV, but predictable and characteristic mutations are not well defined.[172]

Evaluation of Resistance with CCR5 Antagonists

Because emergence of X4-tropic virus is the most common reason for virologic failure on maraviroc, performing an HIV tropism test is recommended for all patients who develop virologic failure while taking

maraviroc. Two major methods are used to determine HIV tropism: phenotypic testing and genotypic testing.

- **Phenotypic Tropism Assay**: The phenotypic test is performed by first generating laboratory pseudoviruses that express envelope proteins (gp120 and gp41) when combined with viruses obtained from a patient sample. These replication-deficient pseudoviruses are then used to infect laboratory target cell lines that express either CCR5 or CXCR4; the HIV tropism is then determined based on the cells that become infected (CCR5, CXCR4, or both).
- **Genotypic Tropism Assay**: In contrast, the HIV-1 tropism genotype is performed by sequencing the V3-coding region of the env gene that is known to be the principal determinant of coreceptor usage; with this method, genotypic sequence of the HIV-1 V3-coding region is then used to predict the HIV-1 tropism. In general, the phenotypic tropism assay is preferred over the genotypic tropism assay.[176]

Currently, there is no recommended modality for determining resistance in R5-tropic HIV that binds to CCR5 in the presence of maraviroc. If virologic failure occurs on maraviroc and the tropism assay result shows pure R5-tropic HIV, then it can be inferred that resistance has likely occurred that is allowing the R5-tropic HIV to bind to CCR5 in the presence of maraviroc.

Fusion Inhibitors (Enfuvirtide)

Enfuvirtide is the only fusion inhibitor approved for use in the United States. Enfuvirtide is a synthetic 36-amino-acid peptide that mimics amino acids 127-162 in the HR2 region of HIV-1 gp41 (Figure 36).[177,178] Resistance to enfuvirtide is associated primarily with mutations in the HIV gp41 component of the HIV envelope; specific drug-resistant mutations have been identified in the HR1 region of the HIV gp41 *env* gene; these mutations correspond with codons 36 to 45 in the HR1 region of gp41 at the site where enfuvirtide binds. Less often, resistance is associated with mutations in the *env* gene that codes for a region of HR2 outside of amino acids 36-45 or gp120.[49,60] The mutations most often identified correspond to amino acids 36, 37, 38, 39, 40, 42, and 43.[49] A single one of these HR1 mutations is associated with about a 10-fold decreased susceptibility to enfuvirtide, which increases to 100-fold with a second mutation.[60] Enfuvirtide-resistance mutations reduce HIV replication capacity, and accessory mutations can develop that help restore viral fitness.[60] As might be expected, HIV strains that emerge with resistance will often rapidly revert to wild-type viruses soon after discontinuation of enfuvirtide.[179]

Capsid Inhibitors

Capsid inhibitors work at multiple stages in the HIV lifecycle, including interfering with capsid core disassembly, inhibiting the passage of the viral capsid core into the nucleus of the host cell, and reassembly of the viral capsid core.[180] The HIV capsid core is a conical structure made up of capsid protein hexamers (and pentamers) and contains the viral genetic material and enzymes necessary for reverse transcription.[180] The protein capsid is encoded by the HIV *gag* gene. Therefore, genotypic resistance testing for capsid inhibitors requires sequencing of the *gag* gene.

Lenacapavir

Lenacapavir is currently the only medication in the capsid inhibitor class that is approved by the FDA. At this time, there is limited experience with virologic failure and lenacapavir, but studies have identified clinically significant resistance mutations that can develop. For example, monotherapy with lenacapavir led to the capsid mutation Q67H.[181] In the phase 3 trial of lenacapavir for individuals with heavy treatment experience, 8 participants developed virologic failure with evidence of lenacapavir-associated capsid resistance substitutions; 6 developed M66I (this was the most common substitution), including one with M66I and N74D, one developed Q67H with K70R, and one developed K70H.[31] All of the mutations were found to reduce lenacapavir activity significantly (especially with the M66I and with the K70H). A study that examined the effects of certain capsid mutations within the capsid hexamers, with and without the presence of lenacapavir, found that mutations like Q67H change the hexamer proteins in a way that interferes with lenacapavir binding (a closed-to-open conformational change of the proteins prevents lenacapavir binding).[32] Other mutations, such as the N74D, alter hydrogen bonding interactions. Second-generation capsid inhibitors that are in development may overcome some of these resistance mechanisms. It has been documented that lenacapavir activity is not affected by resistance mutations in other common antiretroviral drug classes.[182] Clinical experience and research on lenacapavir resistance is still evolving, and to date, there is no commercially available capsid resistance assay.

General Approach to Management of Virologic Failure

Management of virologic failure can be challenging, and expert consultation is advised. The Adult and Adolescent ART Guidelines outline appropriate steps to take when virologic failure is suspected, including assessing adherence and medication tolerability as well as evaluating possible pharmacokinetic issues (e.g., drug-drug or drug-food interactions), and has also outlined recommendations based on whether the individual is failing a first or subsequent regimen.[8] Once resistance has developed, the goal is to construct a new regimen (often referred to as a “salvage” regimen) that will suppress the individual’s HIV RNA levels to below the threshold of detection.[8] In order to accomplish this, the new regimen should include at minimum two fully active antiretrovirals—if one of the drugs has a relatively high barrier to resistance, such as dolutegravir or boosted darunavir; otherwise, the new regimen should include a minimum of three fully active antiretrovirals.[8]. In rare cases of multi-class drug resistance, three fully active antiretrovirals or a fully suppressive antiretroviral regimen may not be possible to achieve; in these cases, expert consultation is advisable, and investigational therapies should be pursued. Given the possibility of archived or low-frequency variant resistance mutations, which are not reliably detected with standard drug resistance tests, the choice of antiretroviral medications should always be based on both past and current drug resistance test results as well as on treatment history.[8]

Therapeutic Drug Monitoring

The clinical interest in HIV antiretroviral therapeutic drug monitoring most often arises in the setting of virologic failure in a person who reports excellent adherence. In this situation, therapeutic drug monitoring may help to determine whether poor drug absorption has occurred or the reporting on adherence is not accurate. Obtaining a drug level is most often done in two situations: (1) a random drug level at a visit linked to patient-reported last dose of medication, and (2) directly observed administration of the medication in the clinic followed by a drug level at a specific time. For many of the available antiretroviral medications, however, the therapeutic threshold is uncertain, and there is no evidence that therapeutic drug monitoring improves clinical outcomes.[183] Consequently, the routine use of therapeutic drug monitoring in the management of adults with HIV is not recommended.

Summary Points

- The presence or emergence of resistant strains of HIV can lead to a suboptimal antiretroviral therapy response, virologic failure, and the need for a new antiretroviral regimen.
- Testing for antiretroviral resistance mutations is recommended at the time of entry into HIV care (including in the setting of acute HIV, if the patient presents at that time), at the time of antiretroviral therapy initiation (if not started at entry into HIV care), and in cases of virologic failure.
- Resistance mutations can be acquired through suboptimal virologic suppression (due to a variety of patient-related or antiretroviral regimen-related causes) or transmitted at the time of initial infection.
- Three types of resistance tests are available to help guide clinicians in the choice of an appropriate antiretroviral regimen: genotype, phenotype, and DNA genotype.
- Resistance tests provide the most accurate information when performed while the person with HIV is taking antiretroviral therapy, and the tests most accurately reflect resistance to the medications currently being taken. When a person stops taking antiretroviral therapy, wild-type virus tends to outgrow resistant virus, usually within 12 weeks of stopping therapy.
- Resistance testing is ideally performed within 4 weeks of stopping oral antiretroviral therapy, but should be requested for a person who has received long-acting cabotegravir plus rilpivirine in the past, regardless of the time since the last injection.
- Resistance testing should be attempted if the HIV RNA level is above 200 copies/mL, but may be unsuccessful in persons with HIV RNA levels between 200 and 500 copies/mL, due to the inability of the lab to adequately amplify HIV.
- Resistance testing has significant limitations, including the failure to detect mutant strains that constitute a minority of the individual’s population of HIV strains.
- Resistance mutations affect drugs in all six classes of FDA-approved antiretroviral medications, with signature mutations associated with particular drugs.
- Management of virologic failure can be challenging, and expert consultation is advised to help in constructing a new antiretroviral regimen, taking into account prior and current drug resistance testing results, as well as treatment history.

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[PubMed Abstract] -

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[PubMed Abstract] -

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[PubMed Abstract] -

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[PubMed Abstract] -

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[PubMed Abstract] -

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[PubMed Abstract] -

Figures

Figure 1 High Error Rate with HIV Reverse Transcription

Illustration: David Ehlert, Cognition Studio and David Spach, MD

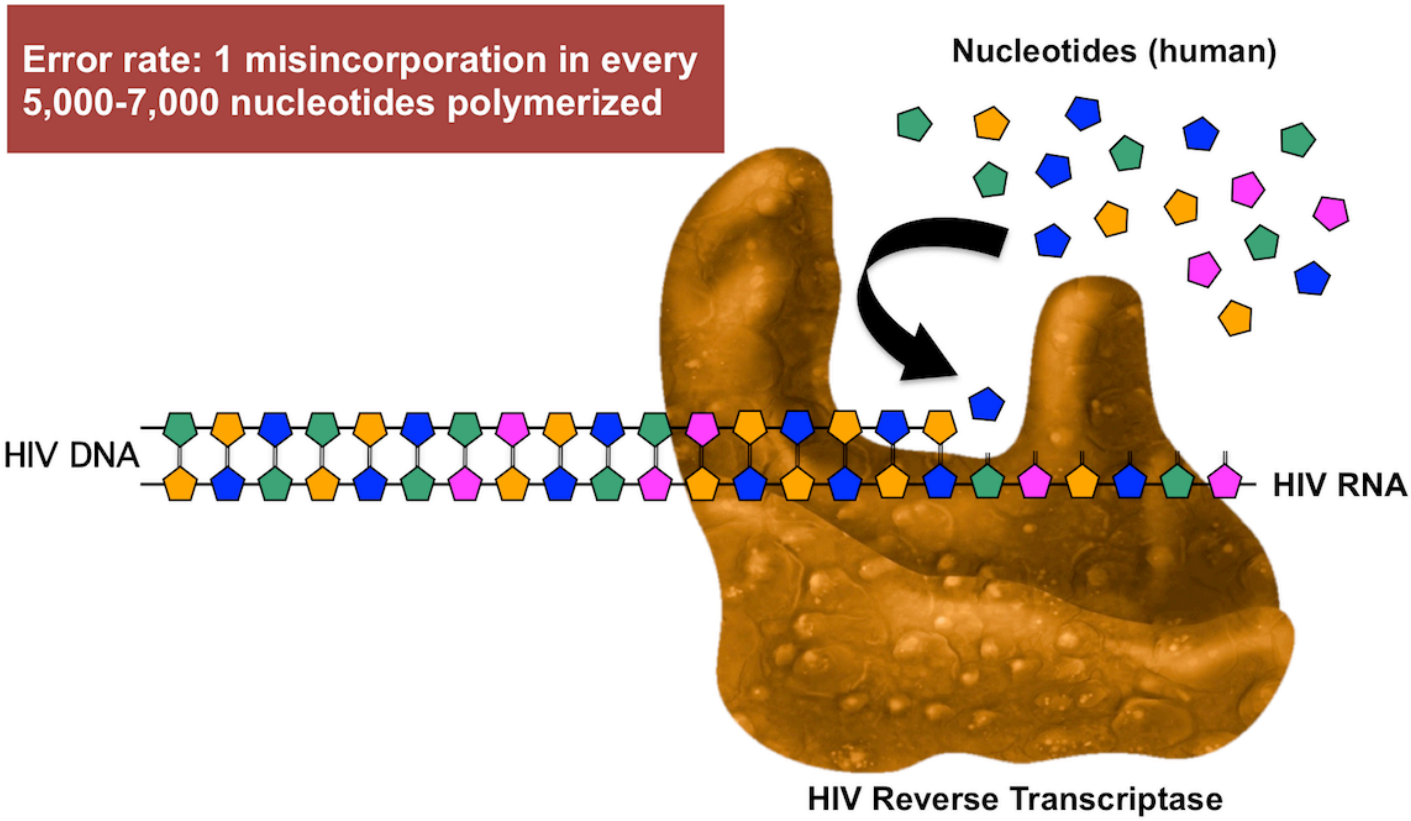


Figure 2 HIV Resistance Basic Concepts

This graphic illustrates the basic concept that with suboptimal antiretroviral therapy, as may occur with poor adherence, drug-resistant strains of HIV have a selective advantage and can emerge to become the dominant circulating strains of HIV.

Illustration: David Spach, MD

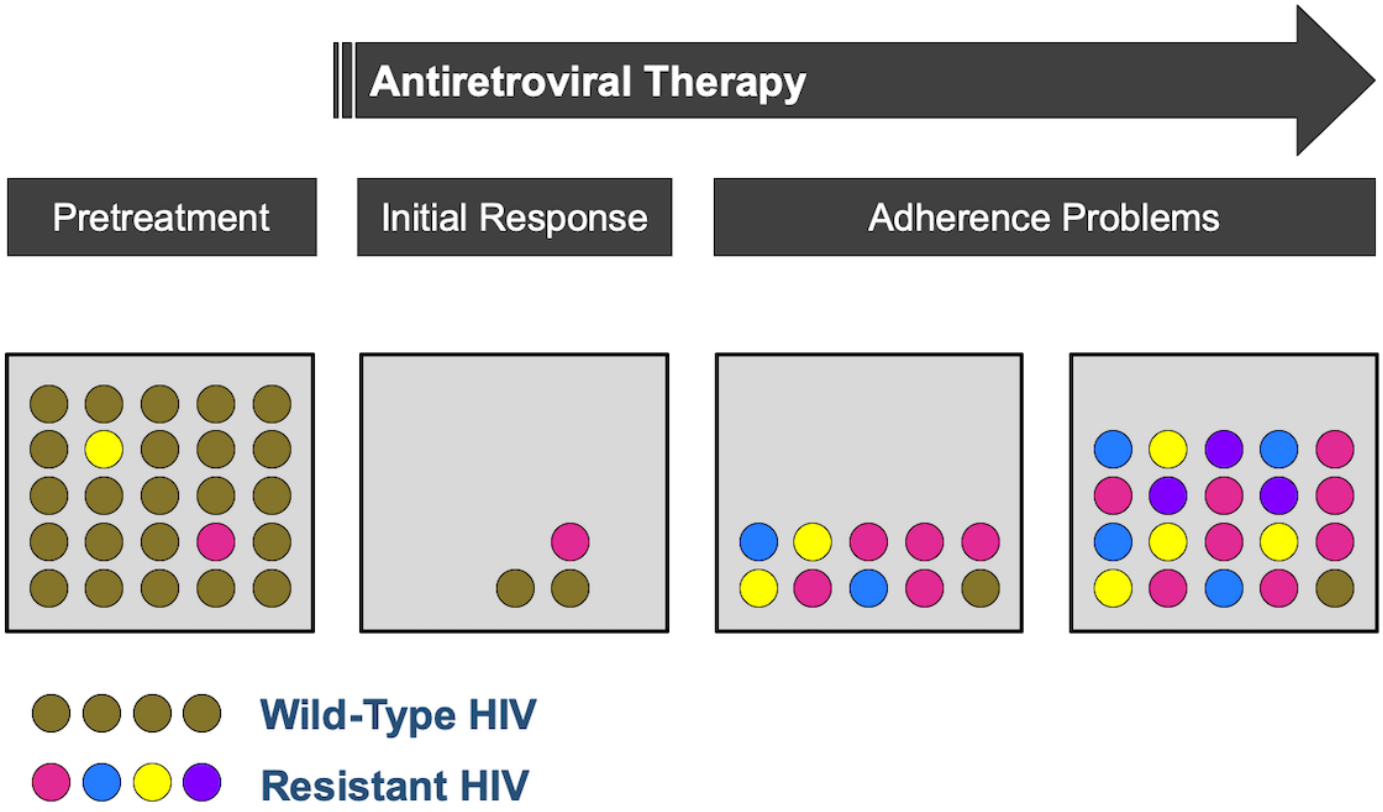


Figure 3 Virologic Suppression

Virologic suppression is defined as a confirmed HIV RNA level below the limit of assay detection (e.g., less than 48 copies/mL).

Illustration: David Spach, MD

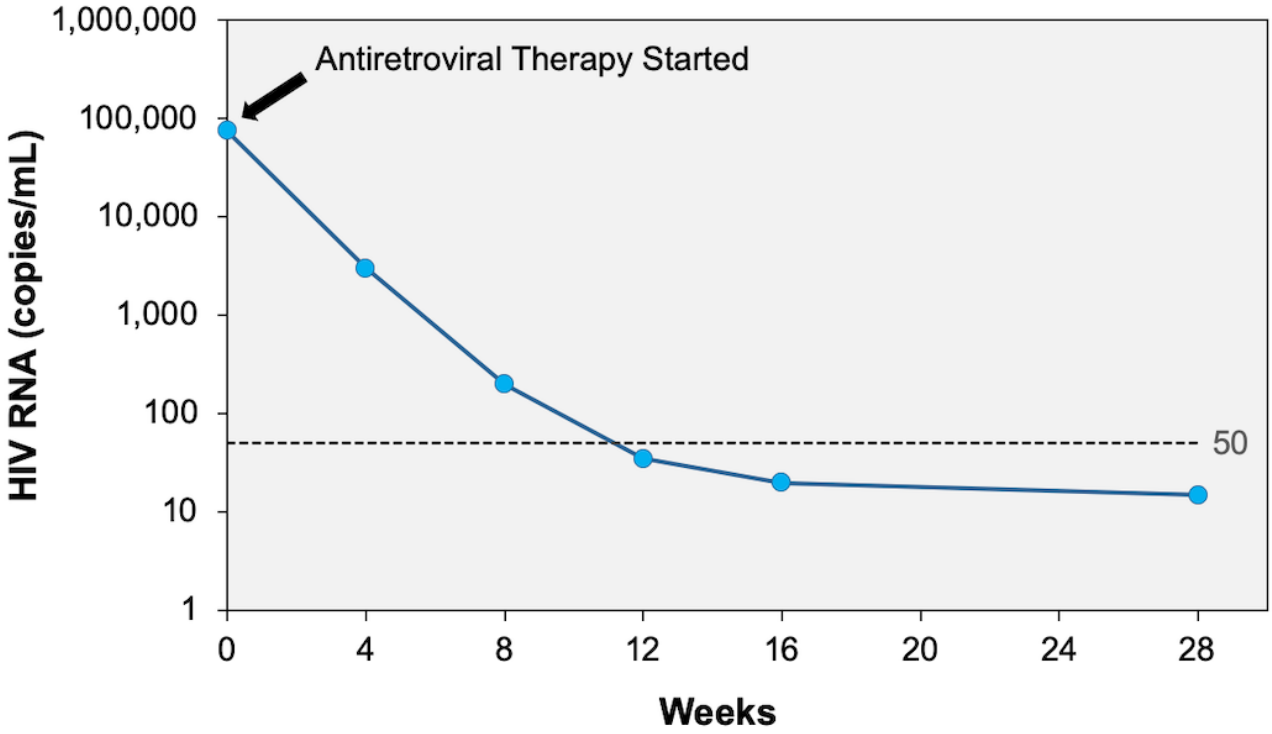


Figure 4 Incomplete Virologic Response

Incomplete virologic response is defined as two consecutive plasma HIV RNA levels greater than 200 copies/mL after 24 weeks on an ARV regimen. Note that baseline HIV RNA may affect the time course of response, and some regimens will take longer than others to suppress HIV RNA levels. Most assays can typically quantitate HIV if the plasma HIV RNA level is greater than 40 copies/mL.

Illustration: David Spach, MD

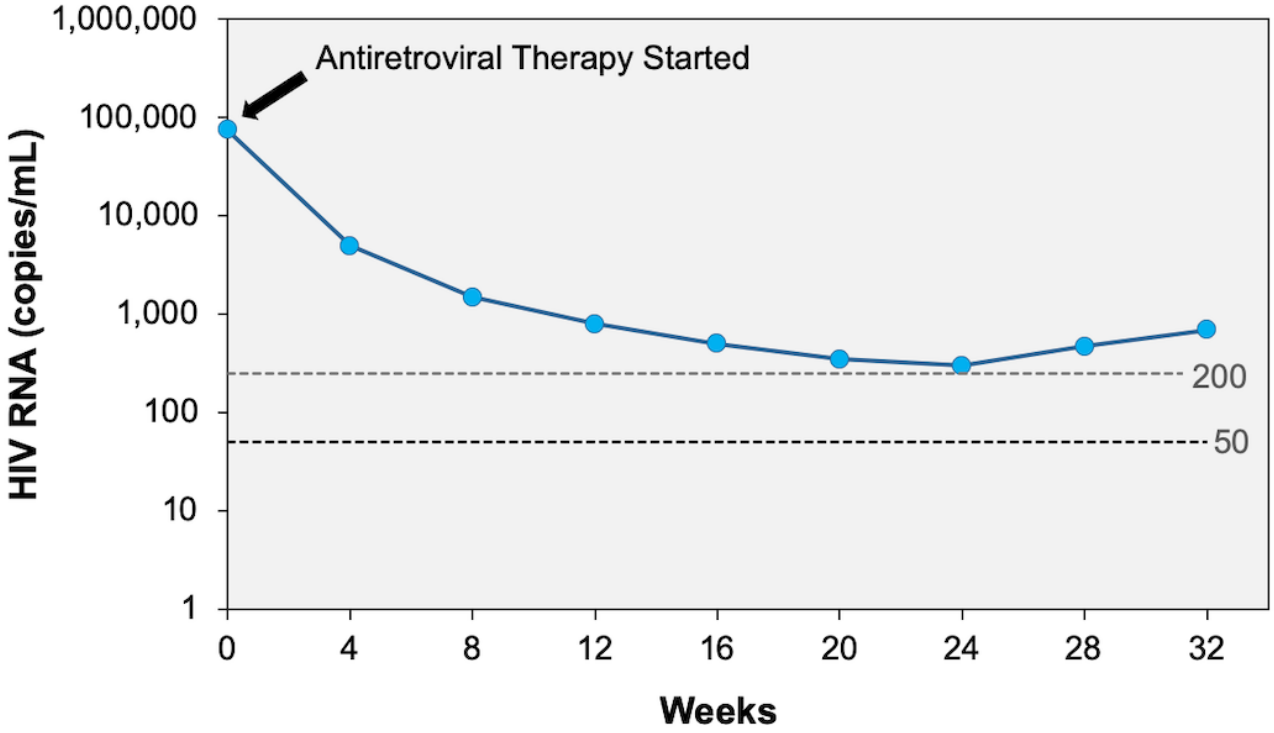


Figure 5 Virologic Rebound

Virologic rebound is defined as confirmed detectable HIV RNA (greater than 200 copies/mL) after virologic suppression.

Illustration: David Spach, MD

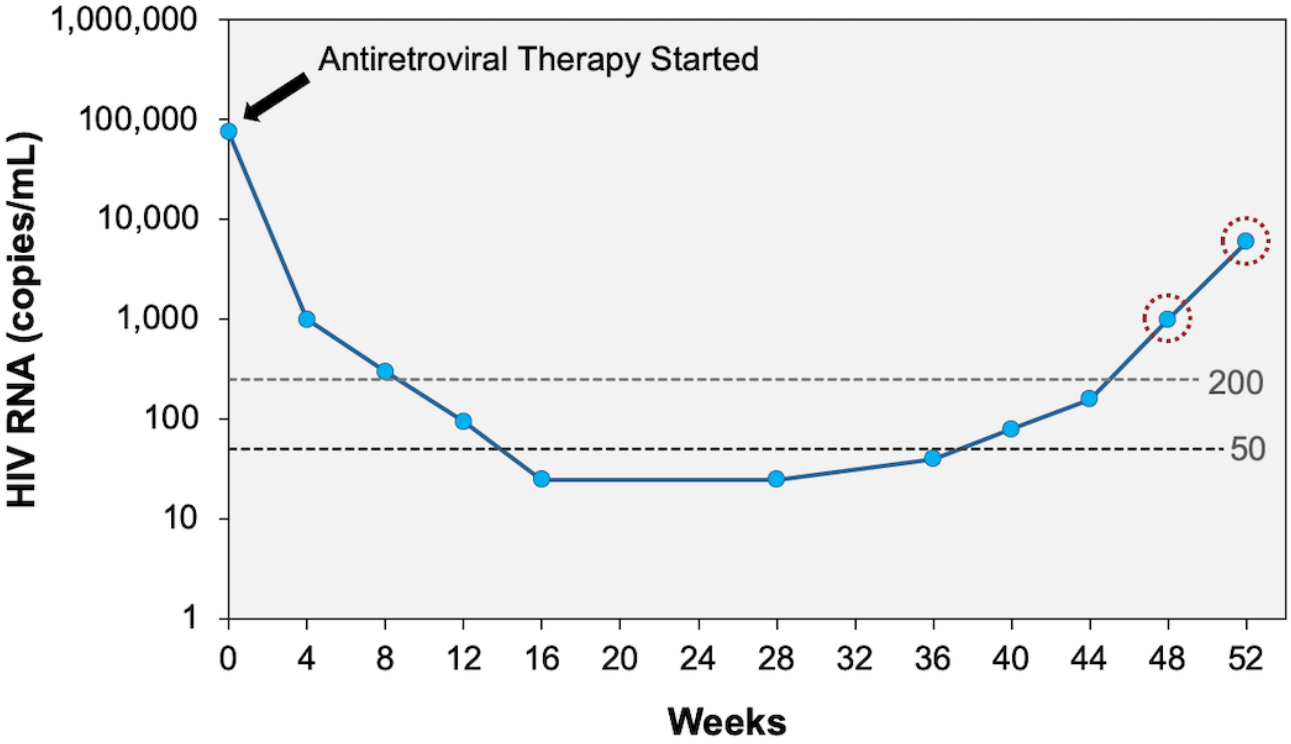


Figure 6 Virologic Blip

In an individual with suppression of HIV RNA levels, a virologic blip is defined as an isolated detectable HIV RNA level followed by return to virologic suppression.

Illustration: David Spach, MD

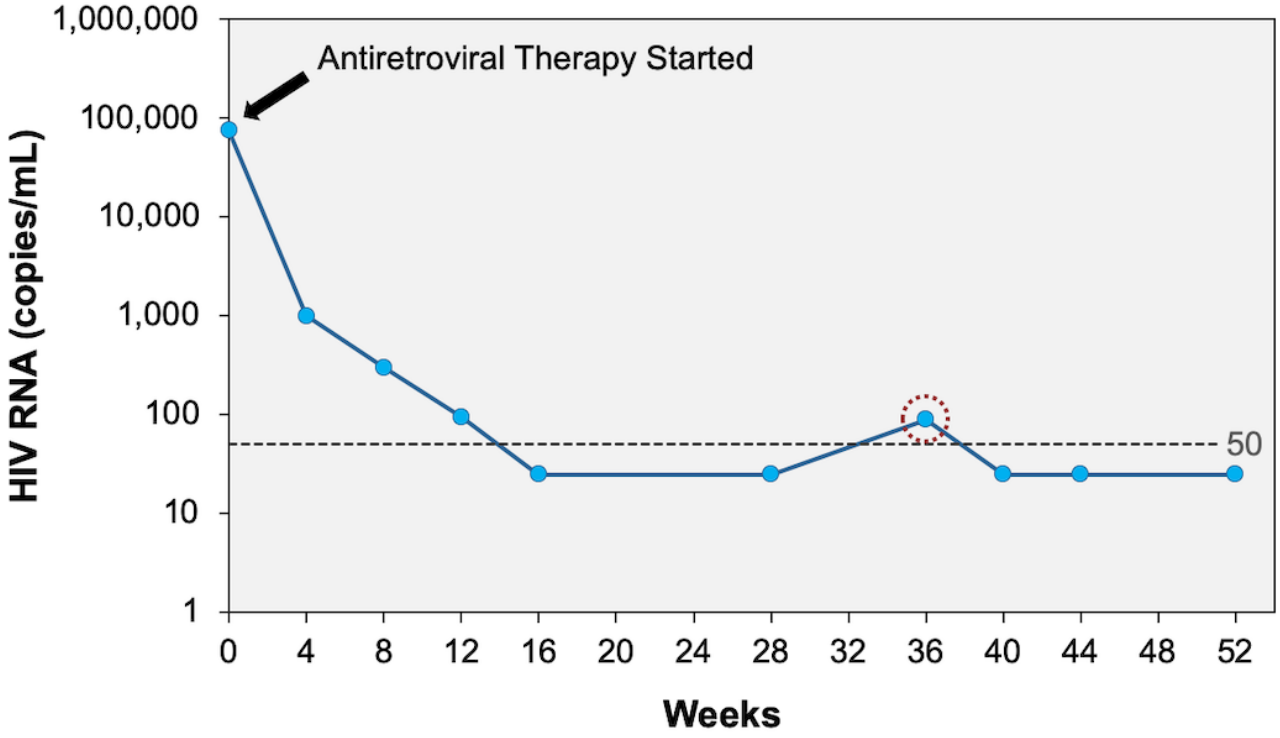


Figure 7 Low-Level Viremia

Low-level viremia is defined as HIV RNA levels above the level of detection of the assay but below 200 copies/mL

Illustration: David Spach, MD

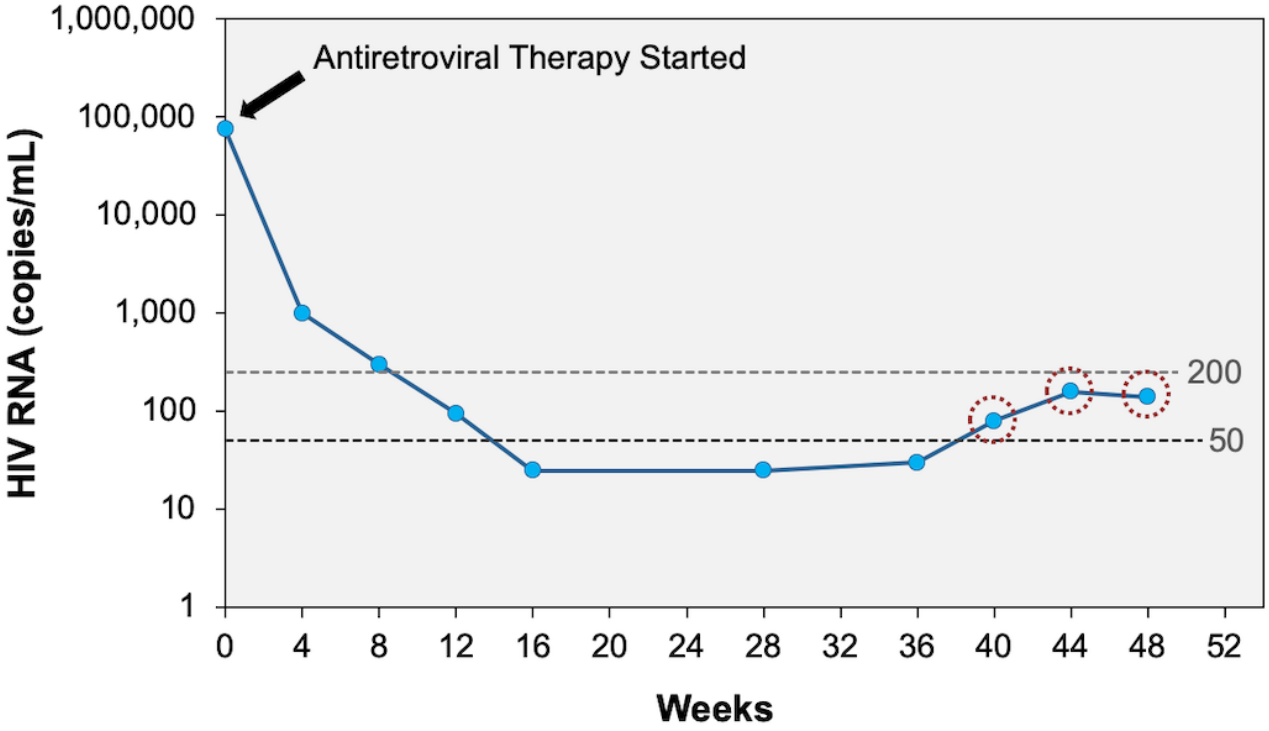


Figure 8 Detectable HIV RNA Below the Limit of Quantification

As shown in the sample from patient 2, in some individuals with HIV infection, HIV RNA is detectable in a plasma sample but the amount of HIV RNA is so low (less than 40 copies/mL) that the laboratory assay cannot accurately quantitate the HIV RNA level. In this situation, the laboratory report typically states HIV-1 RNA was detected in this sample but below the assay's limit of quantitation. This contrasts with the sample from patient 1 that corresponds with a quantitative HIV-1 RNA level since it is above 40 copies/mL. For the sample from patient 3, the HIV RNA level is extremely low and would not be detected on most standard commercial assays.

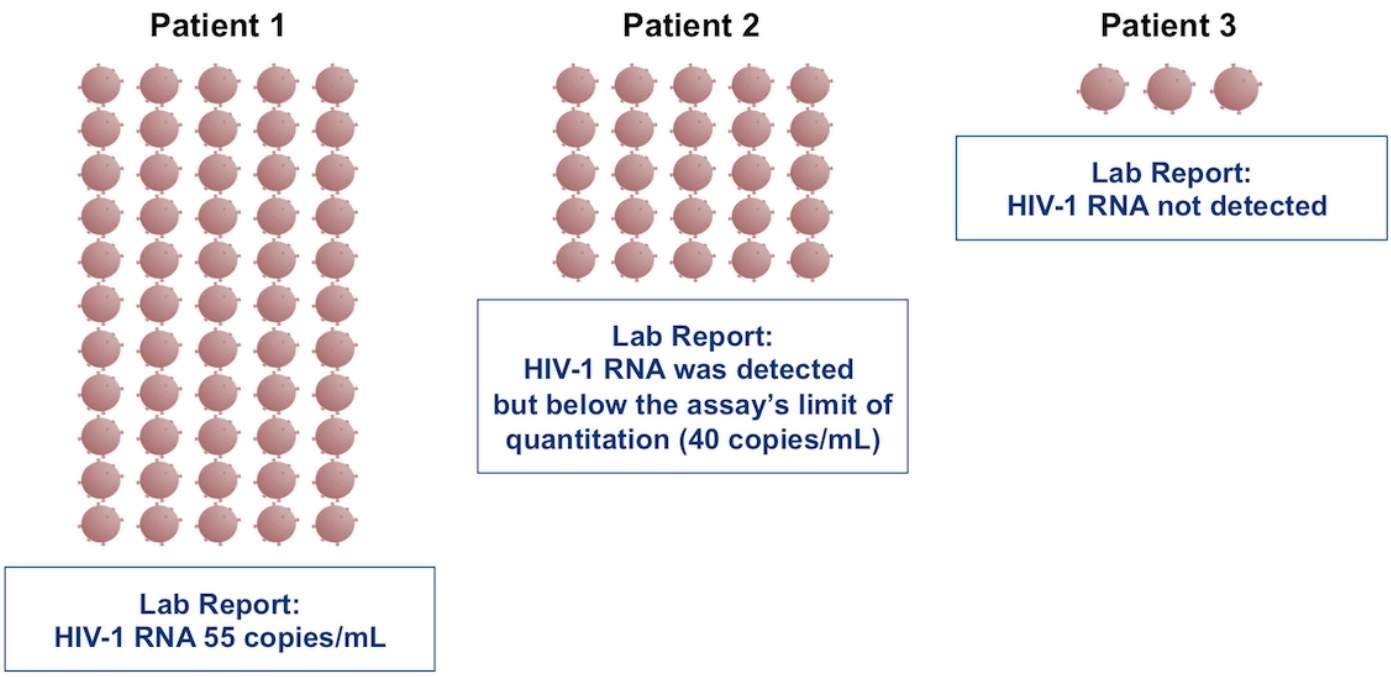


Figure 9 (Image Series) - Conventional HIV Drug Resistance Genotypic Assay (Image Series) -
Figure 9 (Image Series) - Conventional HIV Drug Resistance Genotypic Assay
Image 9A: Obtaining Blood Sample from Person with HIV

Conventional HIV drug resistance genotype testing requires obtaining a blood draw from a person with HIV and the test is run on plasma.

Illustration: David Spach, MD

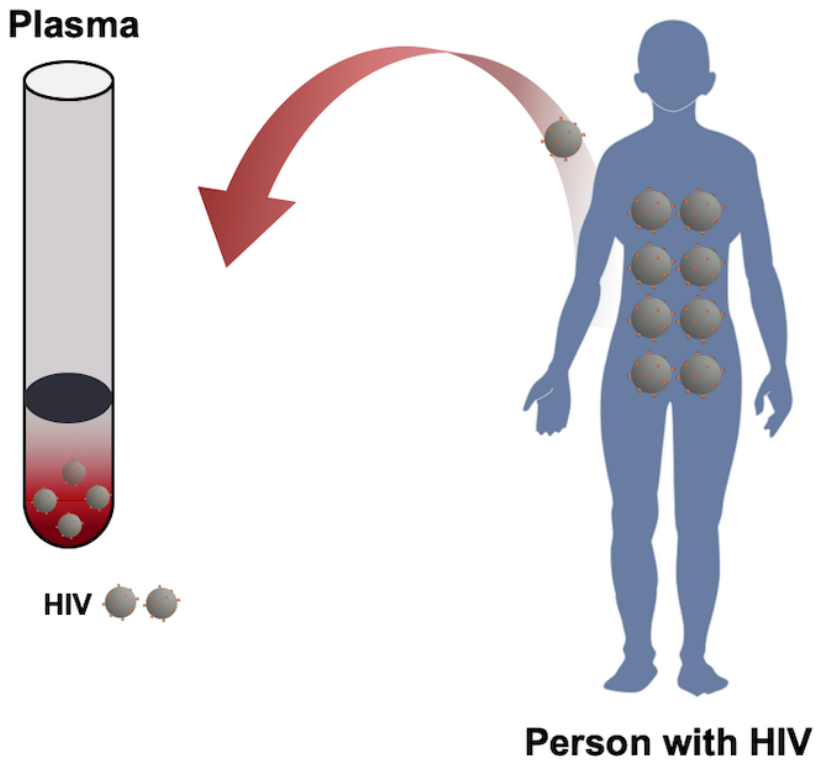


Figure 9 (Image Series) - Conventional HIV Drug Resistance Genotypic Assay
Image 9B: Steps for Isolating and Sequencing HIV DNA for Genotype

The HIV is first isolated from the plasma sample, then reverse transcribed in the laboratory to form HIV DNA. The HIV DNA sample is amplified using PCR techniques and then sequenced. Conventional HIV genotype assays routinely sequence DNA for the reverse transcriptase and protease genes. Assays are also available that can sequence the HIV integrase and envelope genes.

Illustration: David Spach, MD

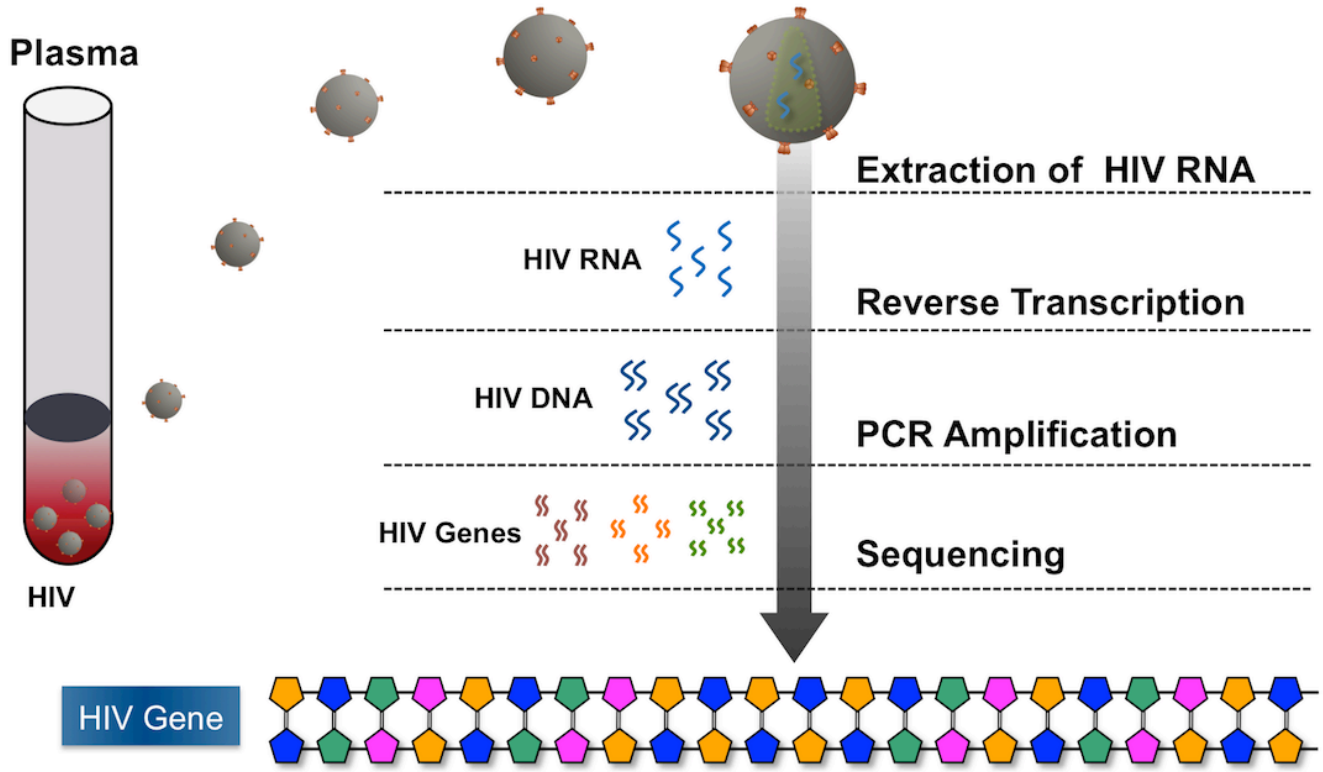


Figure 9 (Image Series) - Conventional HIV Drug Resistance Genotypic Assay
Image 9C: Normal Protein Synthesis Process

This image represents a schematic of normal HIV protein synthesis. In this process, HIV DNA is transcribed to HIV RNA, which is then translated into polypeptides that are eventually processed into proteins.

Illustration: David Spach, MD

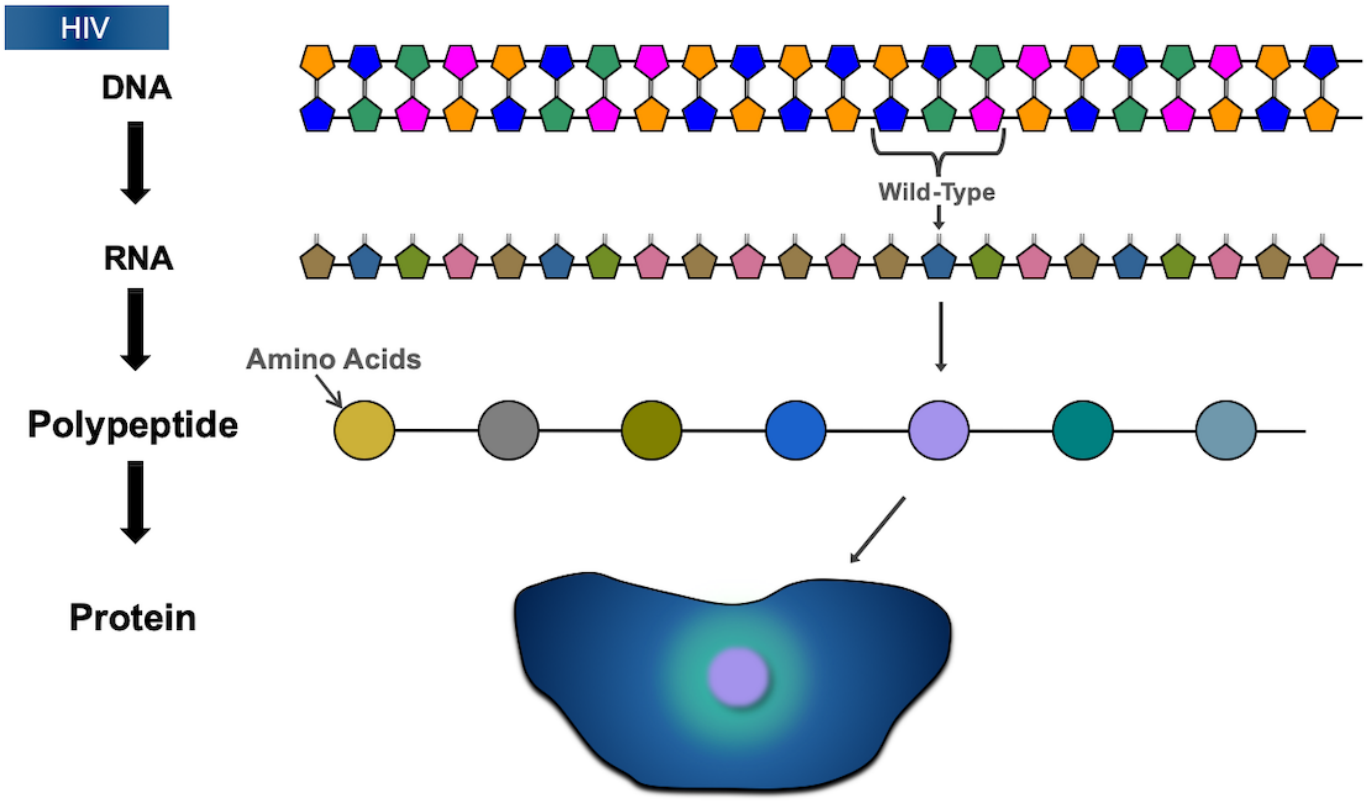


Figure 9 (Image Series) - Conventional HIV Drug Resistance Genotypic Assay
Image 9D: Mutation in HIV DNA Leading to Amino Acid Substitution

Mutations in the HIV DNA nucleotides can result in amino acid substitutions that may impact specific regions or functions of HIV proteins.

Illustration: David Spach, MD

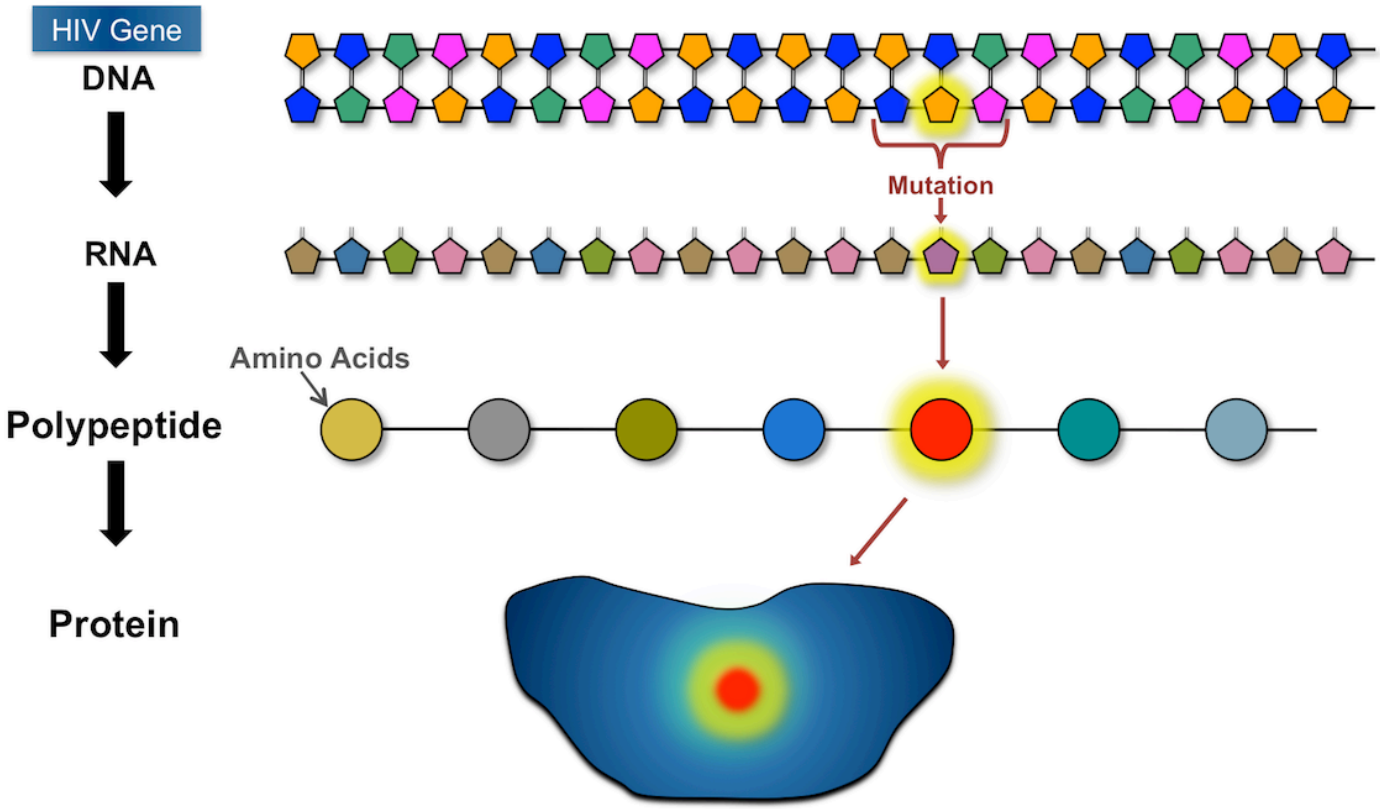


Figure 10 Proviral DNA

Proviral DNA refers to HIV DNA that has been incorporated into the host DNA.

Illustration: David H. Spach, MD

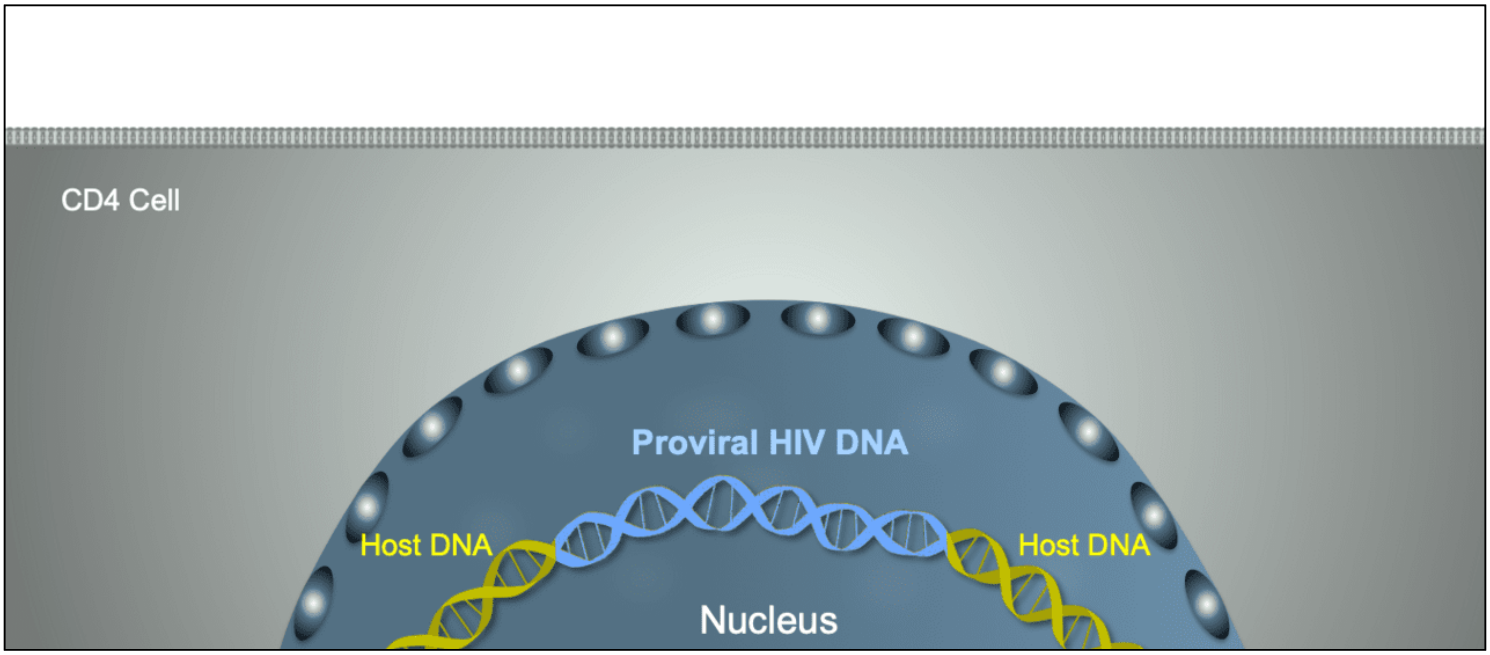


Figure 11 HIV DNA Genotypic Drug Resistance Assay

In contrast to a conventional HIV drug resistance assay, which is performed on a plasma sample and typically requires HIV RNA levels of at least 500 copies/mL or more, an HIV DNA drug resistance assay is performed on whole blood and it detects proviral DNA that is incorporated into host DNA in cells infected with HIV. The HIV DNA resistance assay can be performed in patients who have undetectable plasma HIV RNA levels.

Illustration: David Spach, MDD

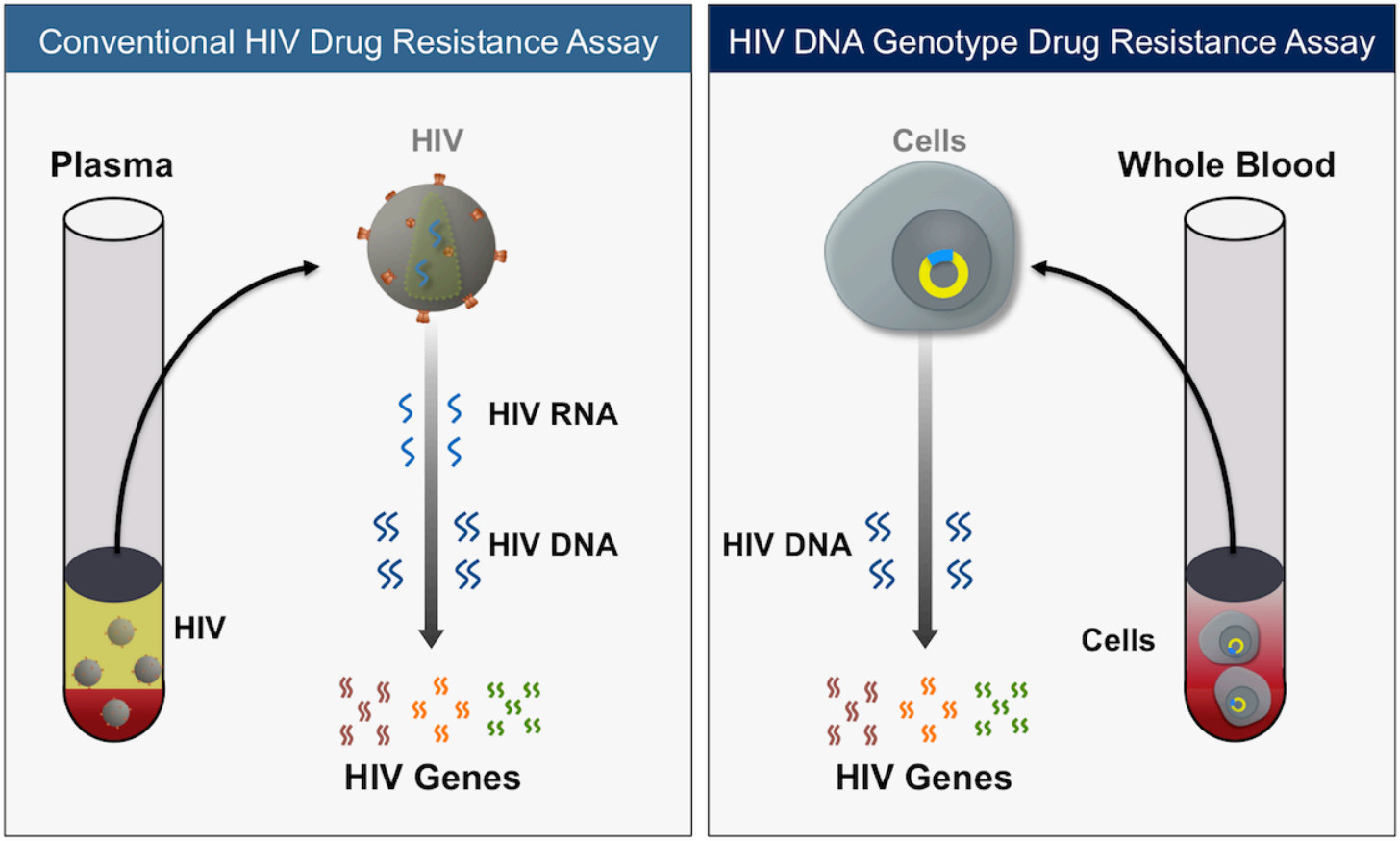


Figure 12 Indication for HIV Drug Resistance Testing

The Adult and Adolescent ARV Guidelines recommend performing routine HIV drug resistance testing in the situations shown in this figure.

Source: Panel on Antiretroviral Guidelines for Adults and Adolescents. Guidelines for the Use of Antiretroviral Agents in Adults and Adolescents Living with HIV. Department of Health and Human Services. Laboratory testing: drug-resistance testing. September 21, 2022.

Clinical Setting for Ordering HIV Drug Resistance Testing
Acute HIV
Entry into care regardless of timing for starting ARV therapy
Virologic failure and HIV RNA >200 copies/mL*
Patients with suboptimal HIV RNA suppression
Pregnant women with HIV

*HIV-RNA levels >200 copies/mL but <500 copies/mL, drug-resistance testing may be unsuccessful but should still be considered.

Figure 13 Reemergence of Wild Type HIV After Stopping Antiretroviral Therapy

In situations where HIV drug resistance has developed while an individual is taking antiretroviral therapy, the discontinuation of the antiretroviral therapy regimen will remove the selective pressure on HIV and some drug-resistant mutants may back mutate to wild-type HIV. In addition, in this situation, wild-type HIV may have greater fitness than mutated strains and thus growth of wild-type strains may outpace drug-resistant strains. Accordingly, it is optimal to obtain resistance testing while the patient is on antiretroviral therapy or promptly after discontinuation.

Illustration: David Spach, MD

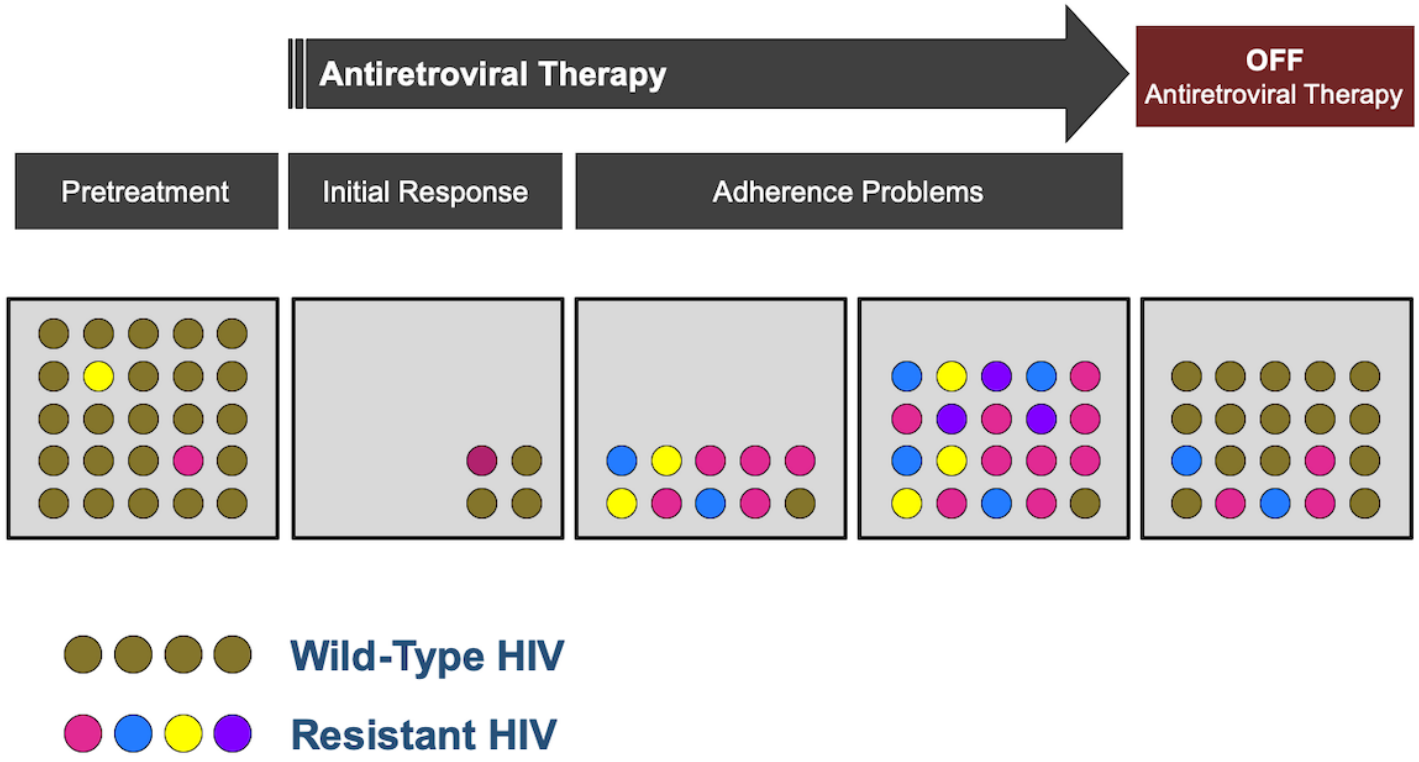


Figure 14 HIV DNA Mutations Resulting in Amino Acid Changes

Mutations in the DNA sequence are analyzed to predict amino acid substitutions in the HIV polypeptide.

Illustration: David Spach, MD

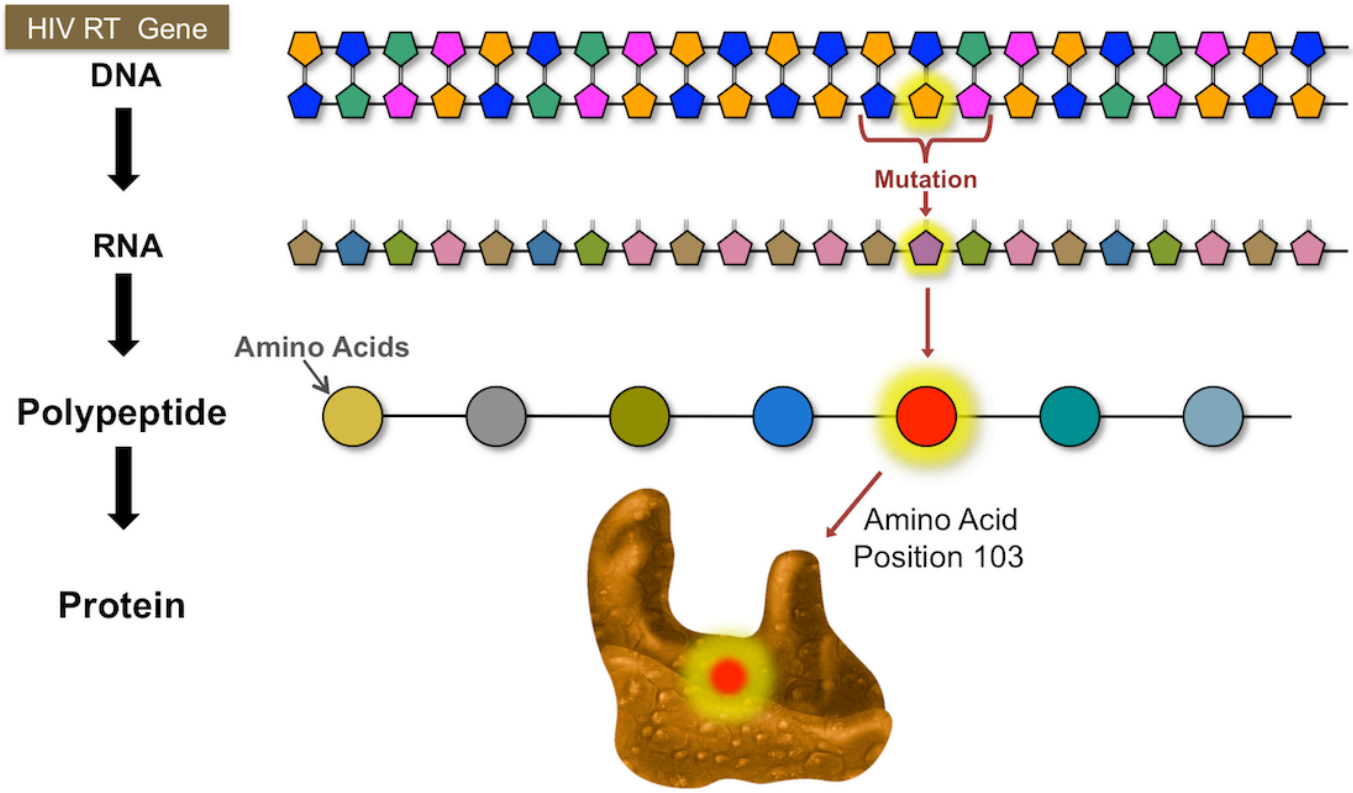


Figure 15 Amino Acid Substitution at Position 103

In this example, the amino acid lysine (K) has been replaced by asparagine (N) at amino acid position 103 in the reverse transcriptase protein. The amino acid position 103 is located in the outer rim of pocket where NNRTIs bind.

Illustration: David Spach, MD

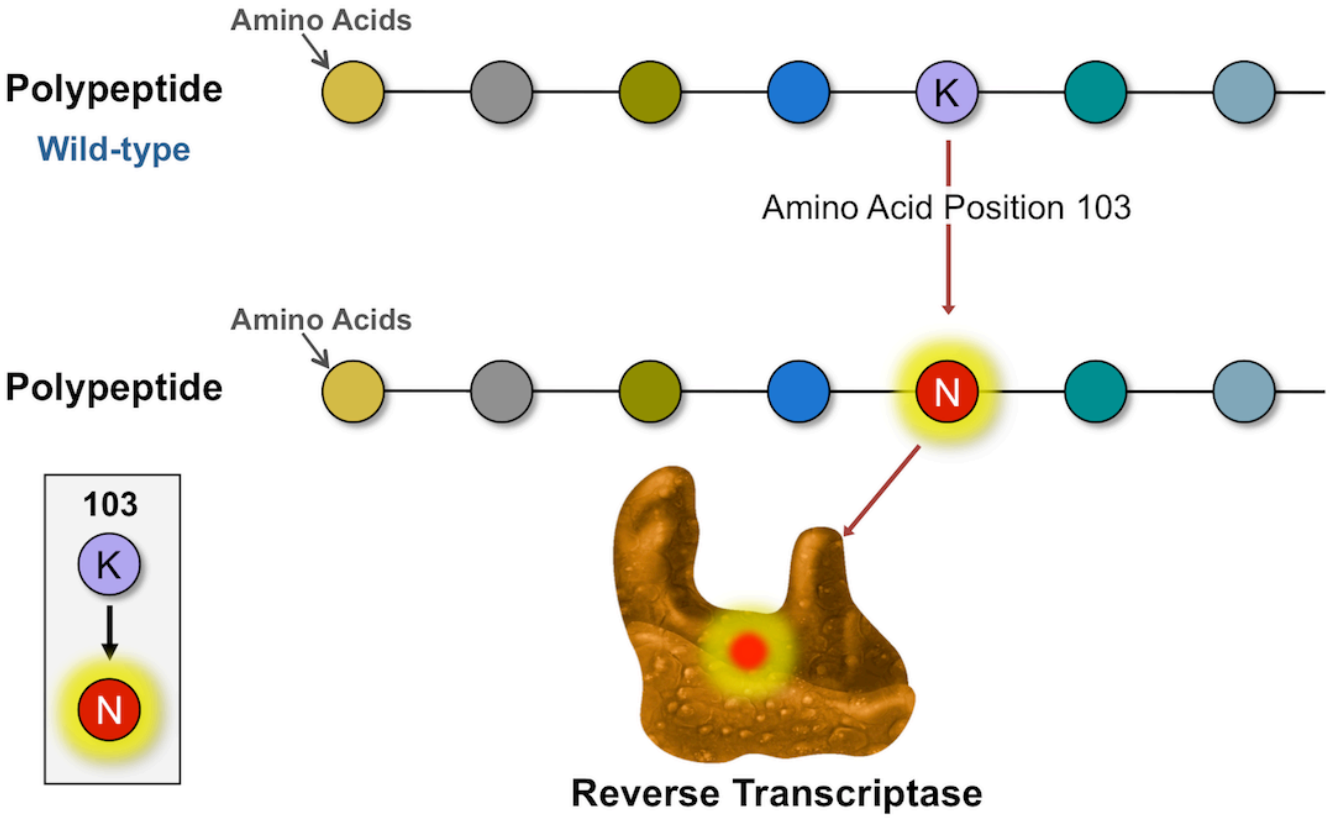


Figure 16 Interpretation of HIV Drug Resistance Genotype Report

The HIV genotype provides information based on the inferred amino acid substitutions predicted by the HIV DNA sequence and these substitutions are compared with wild-type HIV amino acid sequences. The genotype resistance report lists the wild-type amino acid, followed by the position of this amino acid, followed by the amino acid that has replaced the wild-type amino acid at the position listed.

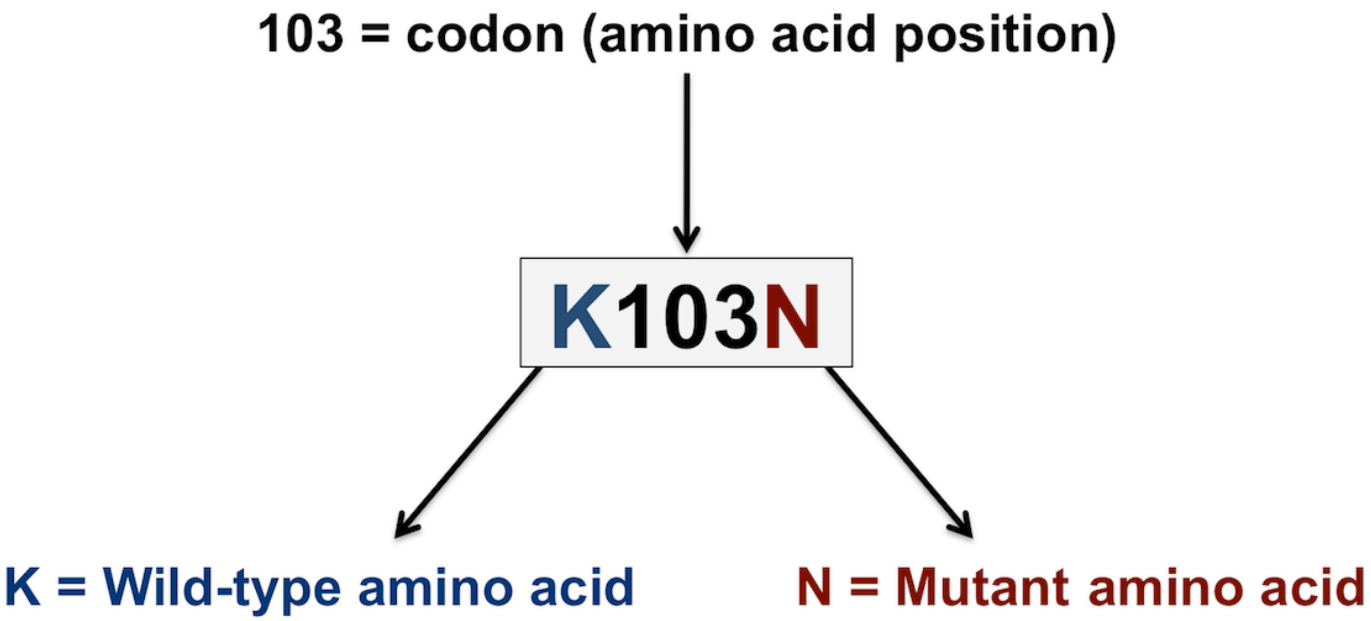


Figure 17 Method for Calculating Level of Phenotypic Resistance

This graph shows the method for calculating the level of phenotypic resistance of a single antiretroviral medication. The antiretroviral drug is tested on a patient's HIV isolate and a laboratory reference (wild-type strain). The IC50 represents the concentration of the antiretroviral drug required to cause 50% inhibition of HIV replication. The fold change is calculated by dividing the IC50 of the patient's isolate by the IC50 of the wild-type laboratory strain. As shown, as the curve shifts to the right, a higher concentration of drug would be required to inhibit HIV replication and thus the strain of HIV would be more resistant. The further the curve shifts to the right (for the patient's HIV strain tested), the greater the level of resistance.

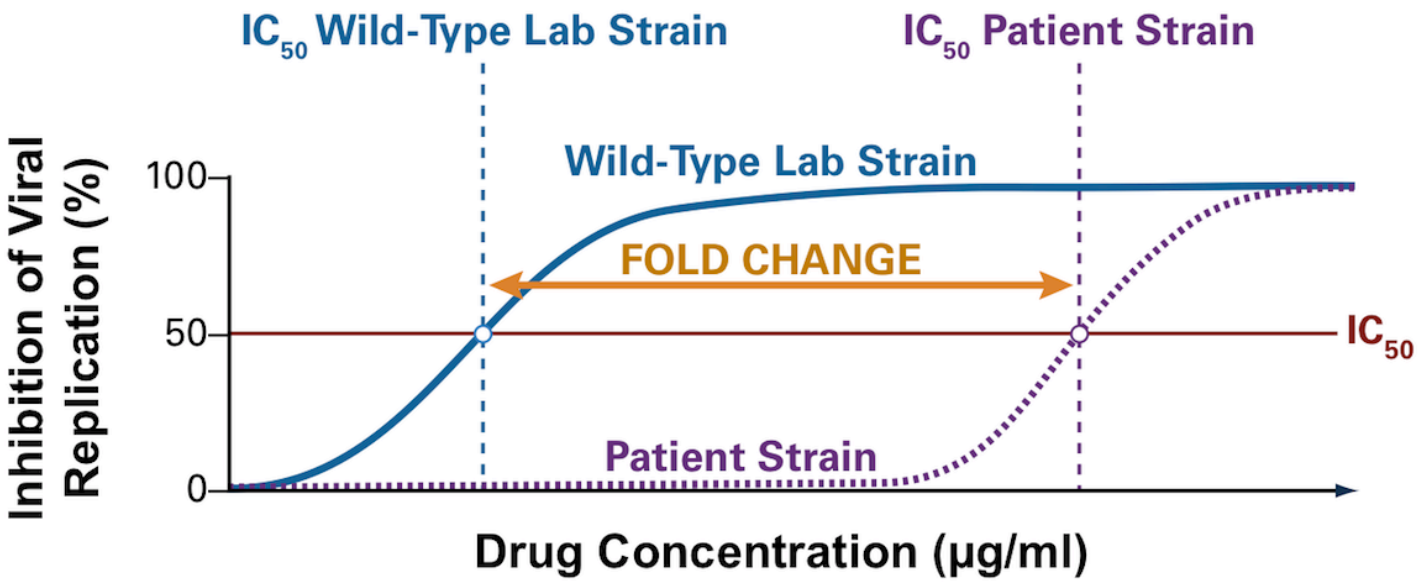


Figure 18 (Image Series) - Phenotypic Drug Susceptibility Curves (Image Series) - Figure 18 (Image Series) - Phenotypic Drug Susceptibility Curves
Image 18A: Drug-Susceptible HIV

This graph shows a phenotypic susceptibility curve comparing the effect of a single antiretroviral drug on the patient's HIV and a laboratory reference (wild-type strain). The wild-type strain is known to be susceptible to the drug tested. The graph shows a similar IC50 for both the patient and wild-type HIV and this would be interpreted that the patient's HIV is susceptible to the drug tested in this assay.

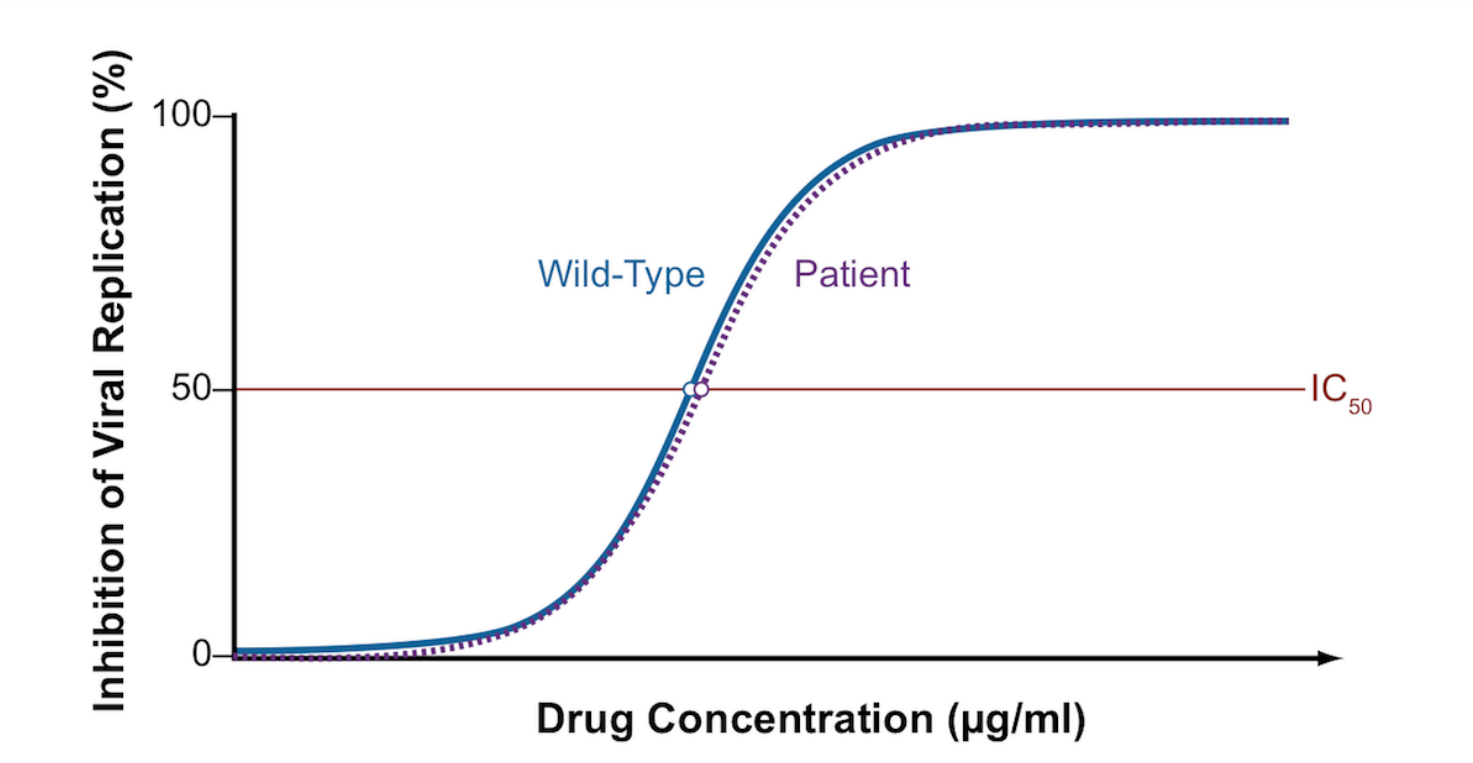


Figure 18 (Image Series) - Phenotypic Drug Susceptibility Curves
Image 18B: Drug-Resistant HIV

This graph shows a phenotypic susceptibility curve comparing the effect of a single antiretroviral drug on the patient's HIV and a laboratory reference (wild-type strain). The wild-type strain is known to be susceptible to the drug tested. The graph shows a significant shift to the right for the patient's HIV isolate compared with the wild-type strain, thus a higher concentration of drug is required to inhibit replication of the patient's HIV. Conceptually, this graph is showing the patient's HIV strain is resistant to the medication tested. In the actual phenotypic assay, the exact level of resistance is calculated by dividing the IC50 of the patient's isolate by the IC50 of the wild-type laboratory strain.

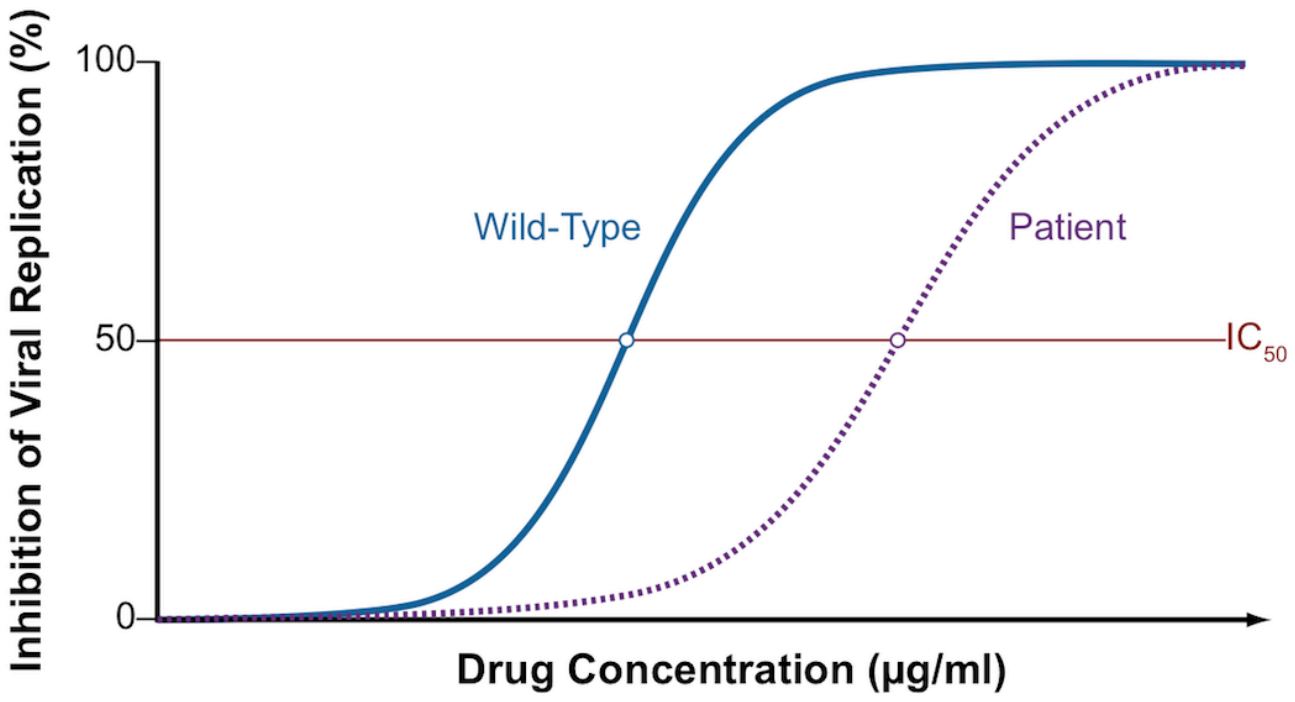


Figure 18 (Image Series) - Phenotypic Drug Susceptibility Curves
Image 18C: HIV Hypersusceptible to Drug

This graph shows a phenotypic susceptibility curve comparing the effect of a single antiretroviral drug on the patient's HIV and a laboratory reference (wild-type strain). The wild-type strain is known to be susceptible to the drug tested. The graph shows a significant shift to the left for the patient's HIV isolate compared with the wild-type strain, thus a lower concentration of drug is required to inhibit replication of the patient's HIV. Conceptually, this graph is showing the patient's HIV strain is hypersusceptible to the medication tested. In the actual phenotypic assay, the exact level of hypersusceptibility is calculated by the dividing the IC50 of the patient's isolate by the IC50of the wild-type laboratory strain.

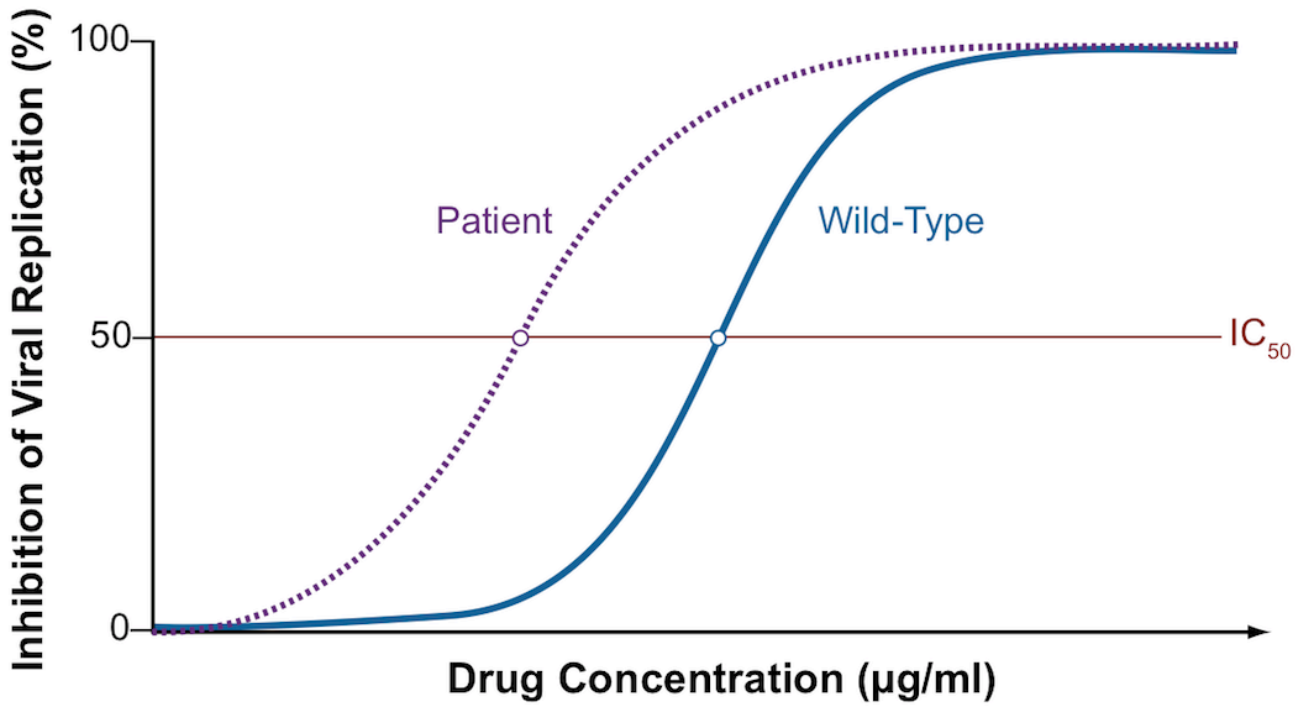


Figure 19 Discrimination (Decreased Incorporation) Mechanisms for HIV Resistance to NRTIs

Illustration: David Spach, MD

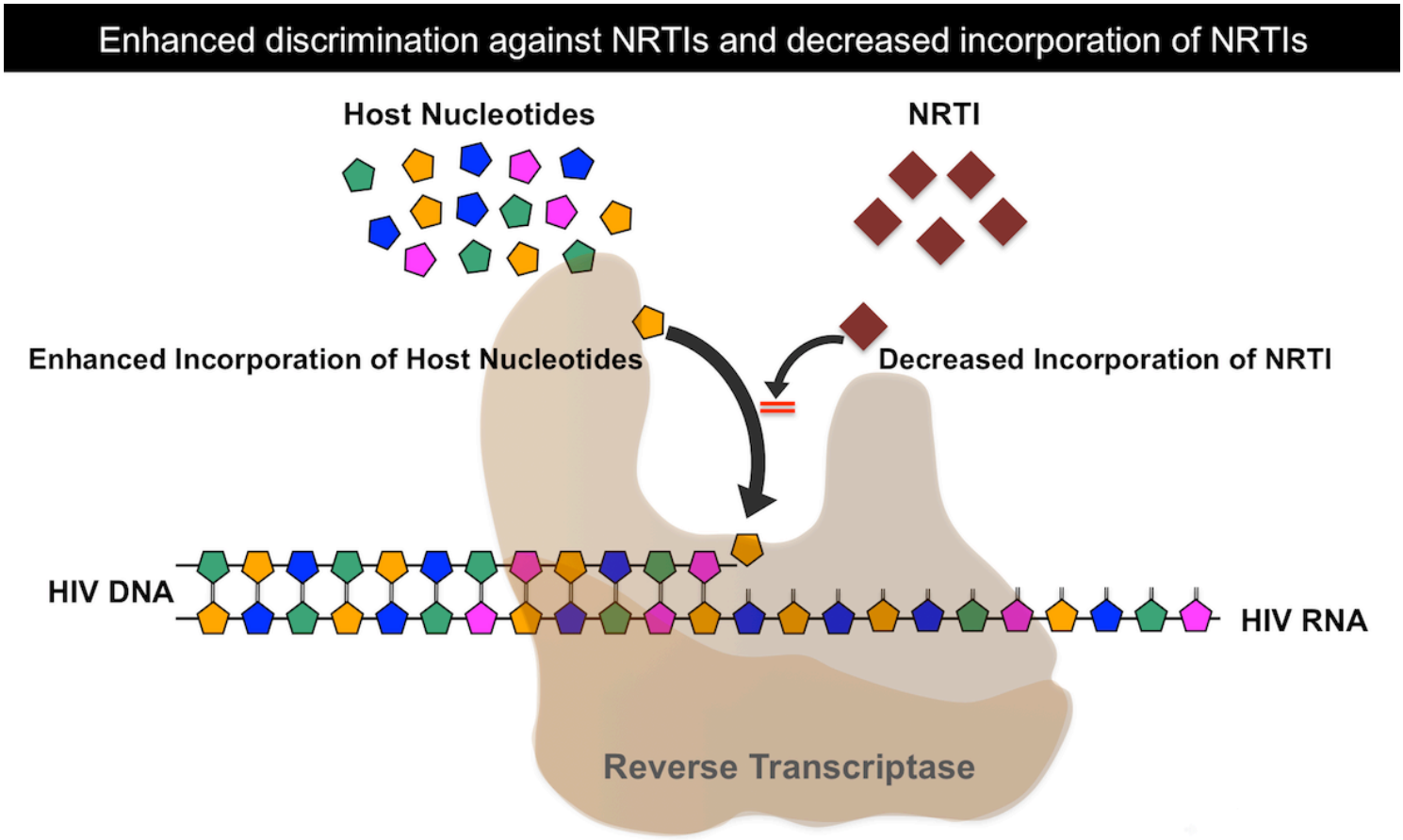


Figure 20 Excision (Primer Unblocking) Mechanisms for HIV Resistance to NRTIs

Illustration: David Spach, MD

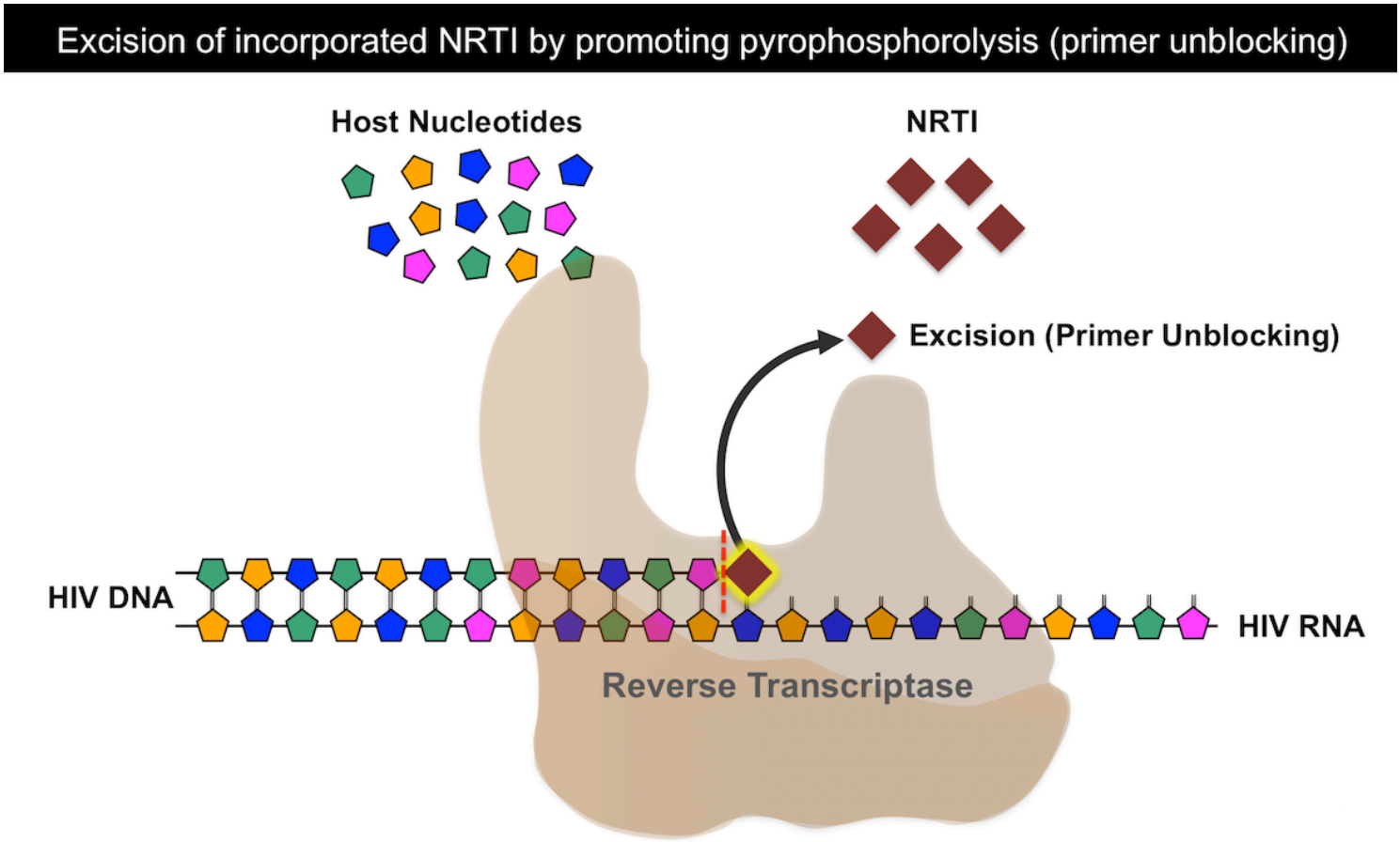
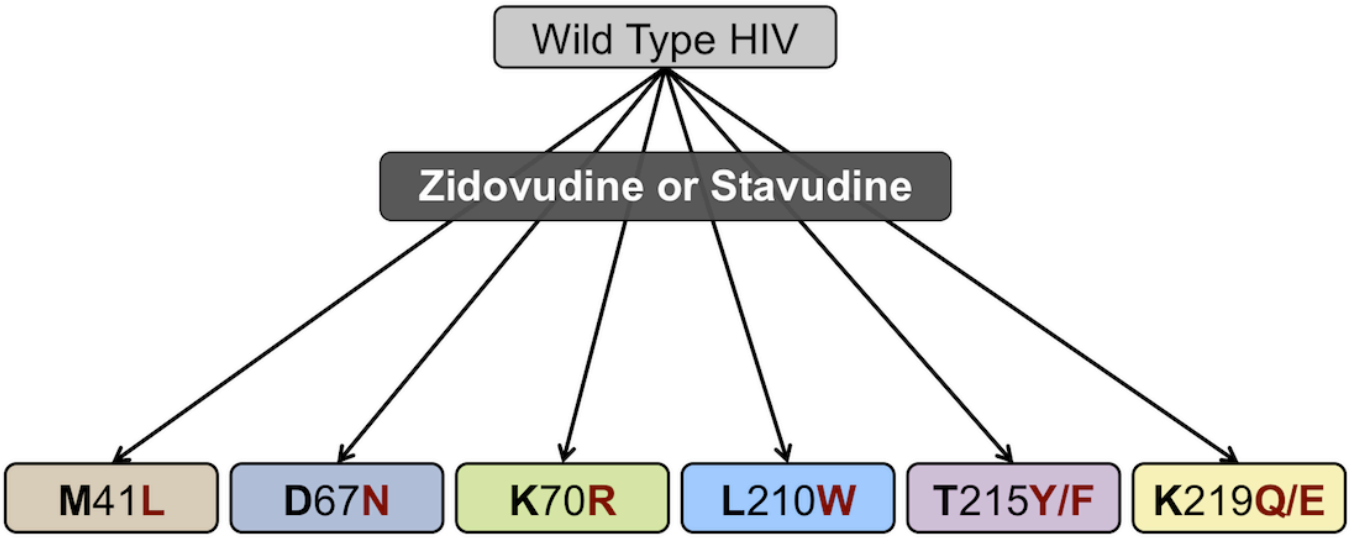


Figure 21 Thymidine Analog Mutations (TAMs)

The thymidine analog mutations arise in the setting of inadequate virologic suppression with an antiretroviral therapy regimen that contains either zidovudine or stavudine.

Source: Shafer RW, Schapiro JM. HIV-1 drug resistance mutations: an updated framework for the second decade of HAART. AIDS Rev. 2008;10:67-84.



Thymidine Analog Mutations (TAMs)

Figure 22 Thymidine Analog Mutation (TAM) Resistance Pathways

Source: Shafer RW, Schapiro JM. HIV-1 drug resistance mutations: an updated framework for the second decade of HAART. AIDS Rev. 2008;10:67-84.

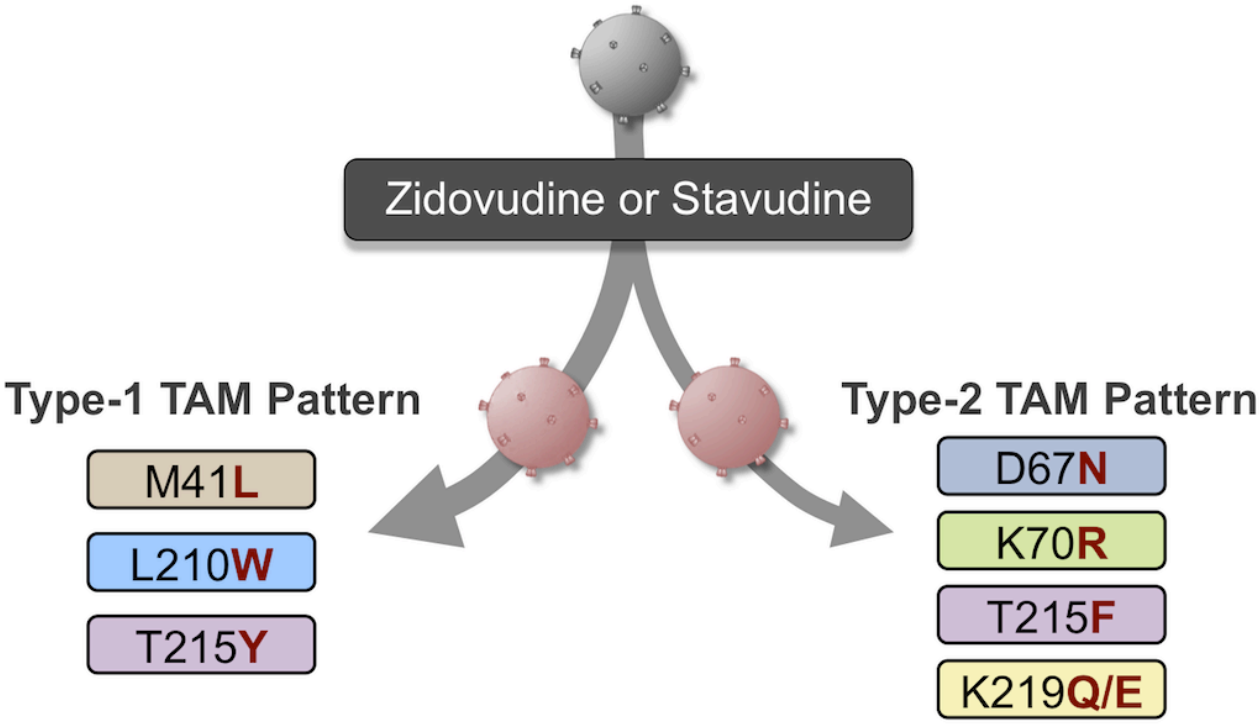


Figure 23 Interaction of NNRTI Medication with the Binding Pocket of Reverse Transcriptase

The NNRTIs exert their action by attaching to a region of the binding pocket of reverse transcriptase.

Illustration: David Ehlert, Cognition Studio and David H. Spach, MD

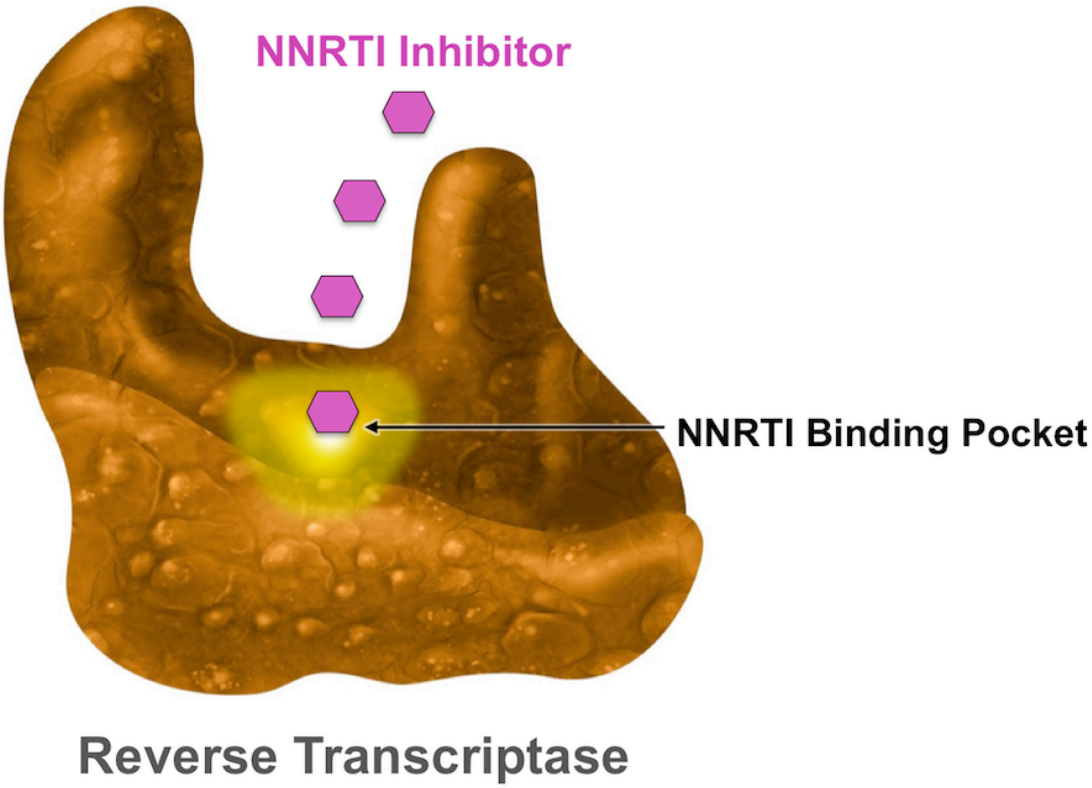


Figure 24 Resistance Mutation Causing Altered Interaction of NNRTI with Binding Pocket

Resistance to NNRTIs can result from mutations that impact amino acids surrounding the binding site thereby preventing the NNRTI from entering into the binding pocket. This is referred to as the altered binding mutation.

Illustration: David Ehlert, Cognition Studio and David H. Spach, MD

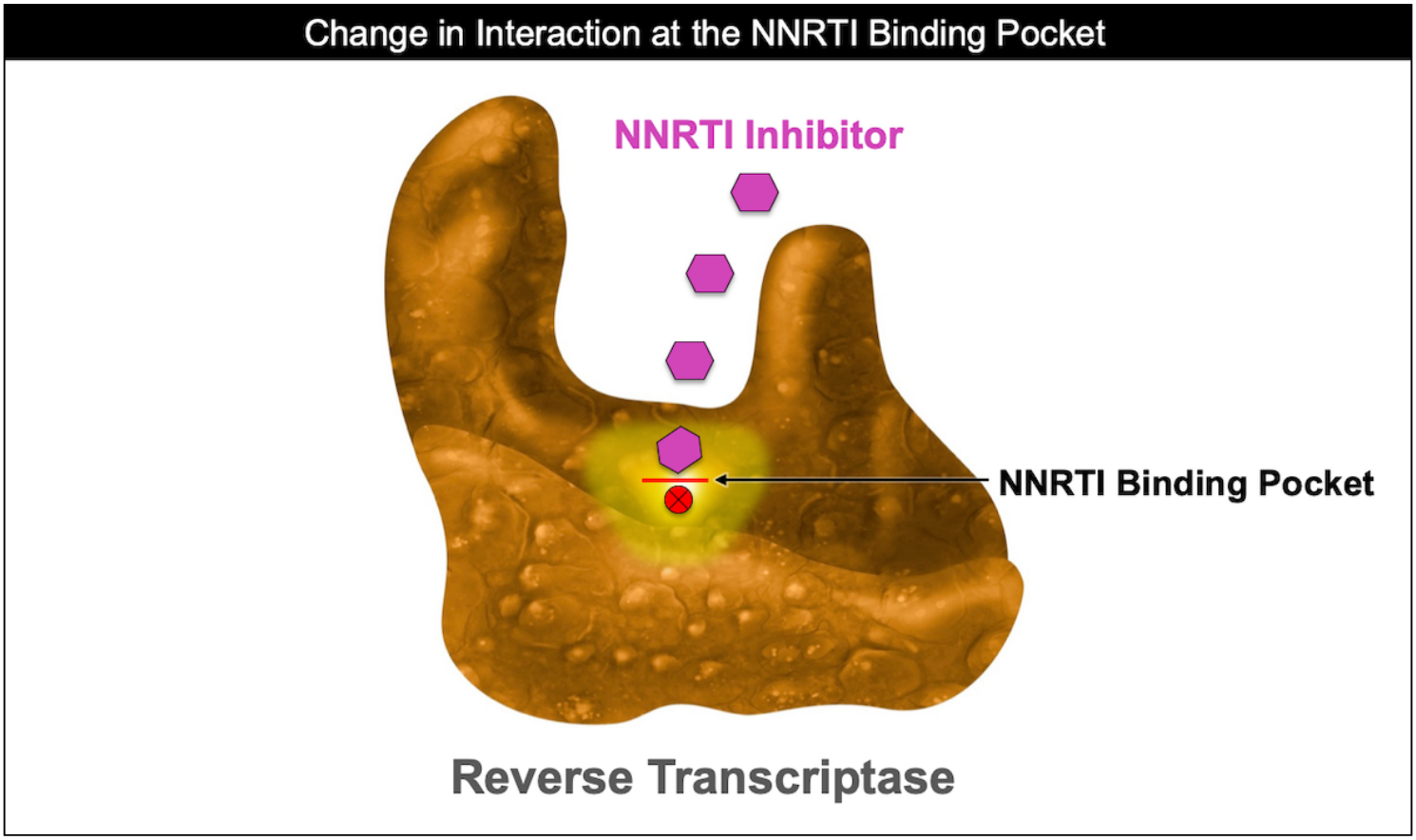


Figure 25 NNRTI Resistance Mutation Causing Reduced Access of NNRTI to Binding Pocket

Resistance to NNRTIs can result from mutations that impact amino acids surrounding the binding site thereby preventing the NNRTI from entering into the binding pocket. This is referred to as the reduced access mutation.

Illustration: David Ehlert, Cognition Studio and David H. Spach, MD

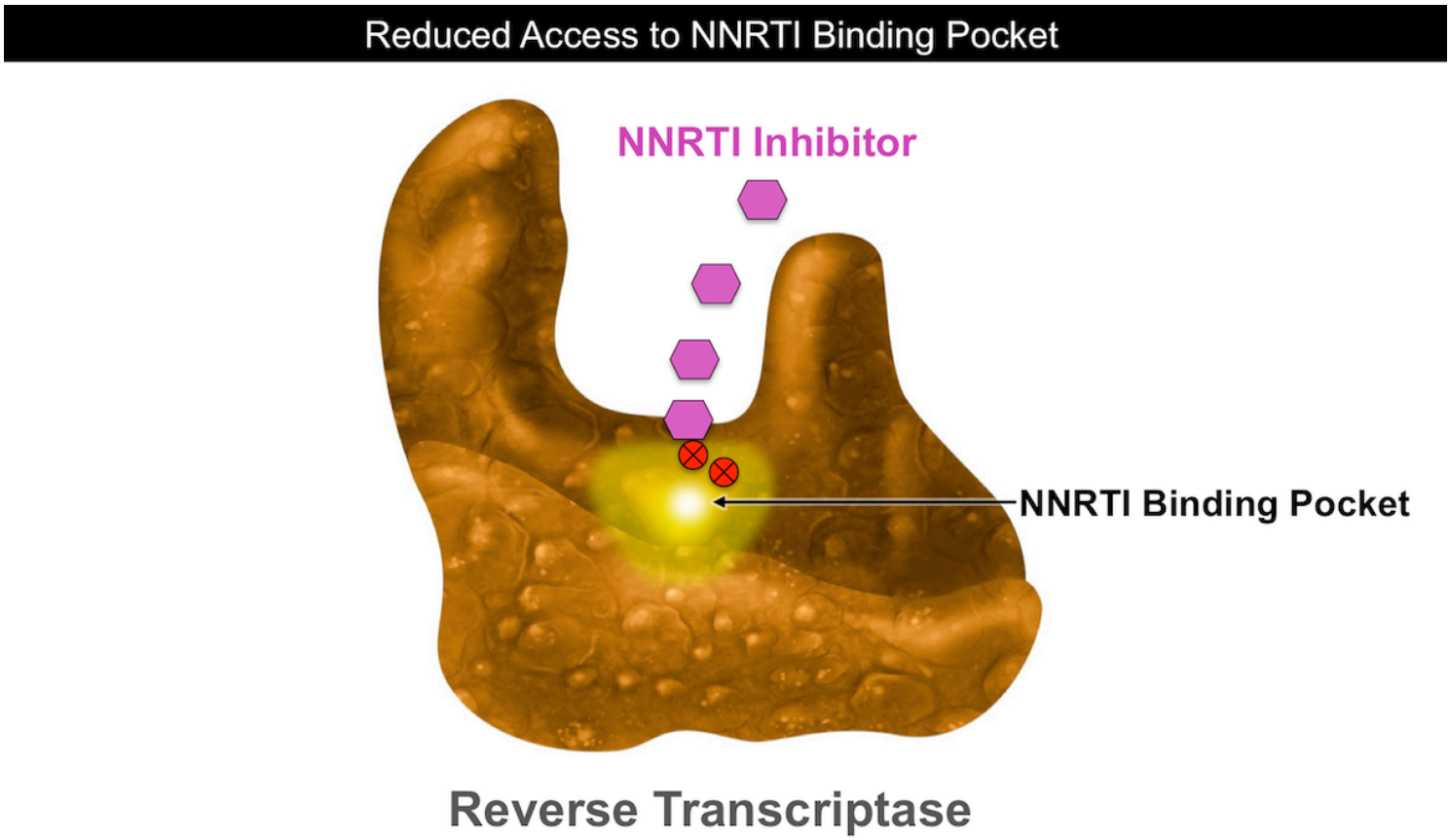


Figure 26 Doravirine Resistance Pathways

Source: Feng M, Wang D, Grobler JA, Hazuda DJ, Miller MD, Lai MT. In vitro resistance selection with doravirine (MK-1439), a novel nonnucleoside reverse transcriptase inhibitor with distinct mutation development pathways. Antimicrob Agents Chemother. 2015;59:590-8.Illustration: David H. Spach, MD

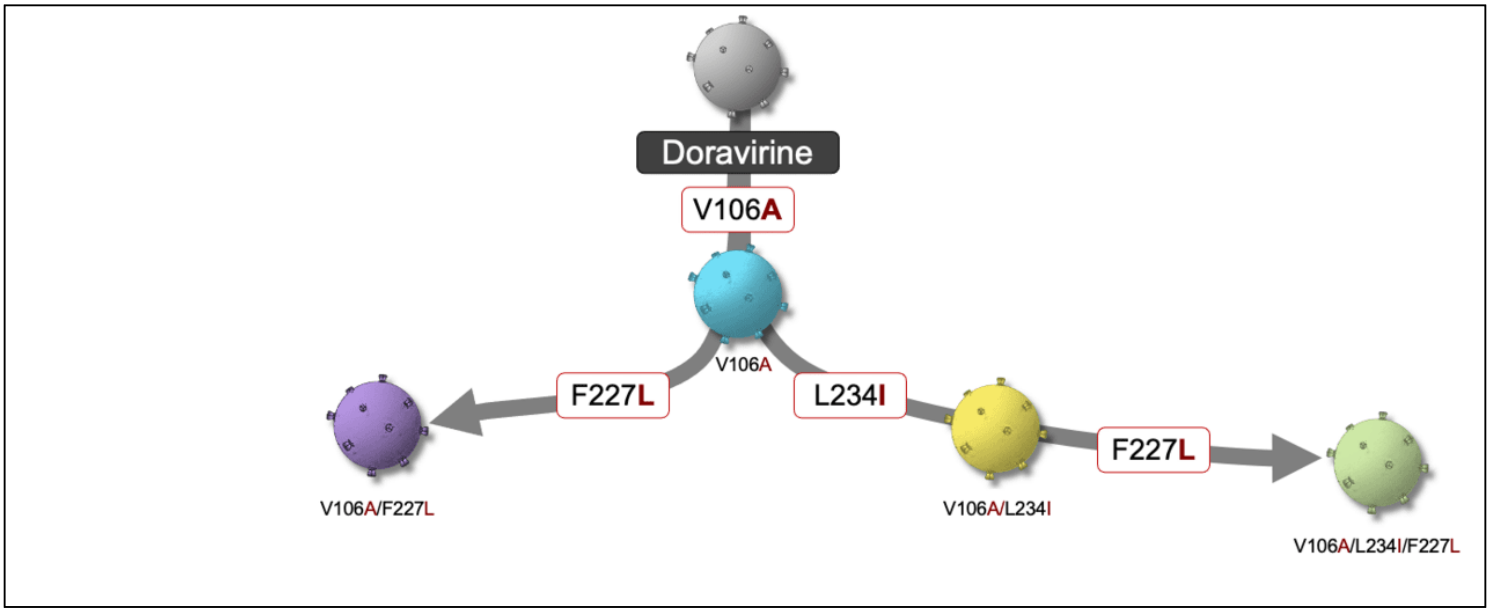


Figure 27 Impact of Reverse Transcriptase Mutations on Doravirine

Source: Stanford University HIV Drug Resistance Database Illustration: David H. Spach, MD

Susceptible to Doravirine
K103N; E138K; Y181C; G190A; H221Y

Potential Low-Level Doravirine Resistance	Low-Level Doravirine Resistance
K101E; K101P; K103N + Y181C	L100I; P225H

Intermediate-Level Doravirine Resistance
L100I + K103N; V106M

High-Level Doravirine Resistance
V106A; Y188L; G190E; F227C; F227L; M230L; Y318F

Figure 28 Major Primary Integrase Resistance Mutations

HIV integrase enzyme is a 288-amino acid enzyme comprised of three structural domains: C-terminal domain, N-terminal domain, and the catalytic core domain. Eight major primary integrase resistance mutations have been identified. Note that all of these major primary resistance mutations are located in the catalytic core domain region of the integrase enzyme.

Illustration: David Ehlert, Cognition Studio and David H. Spach, MD

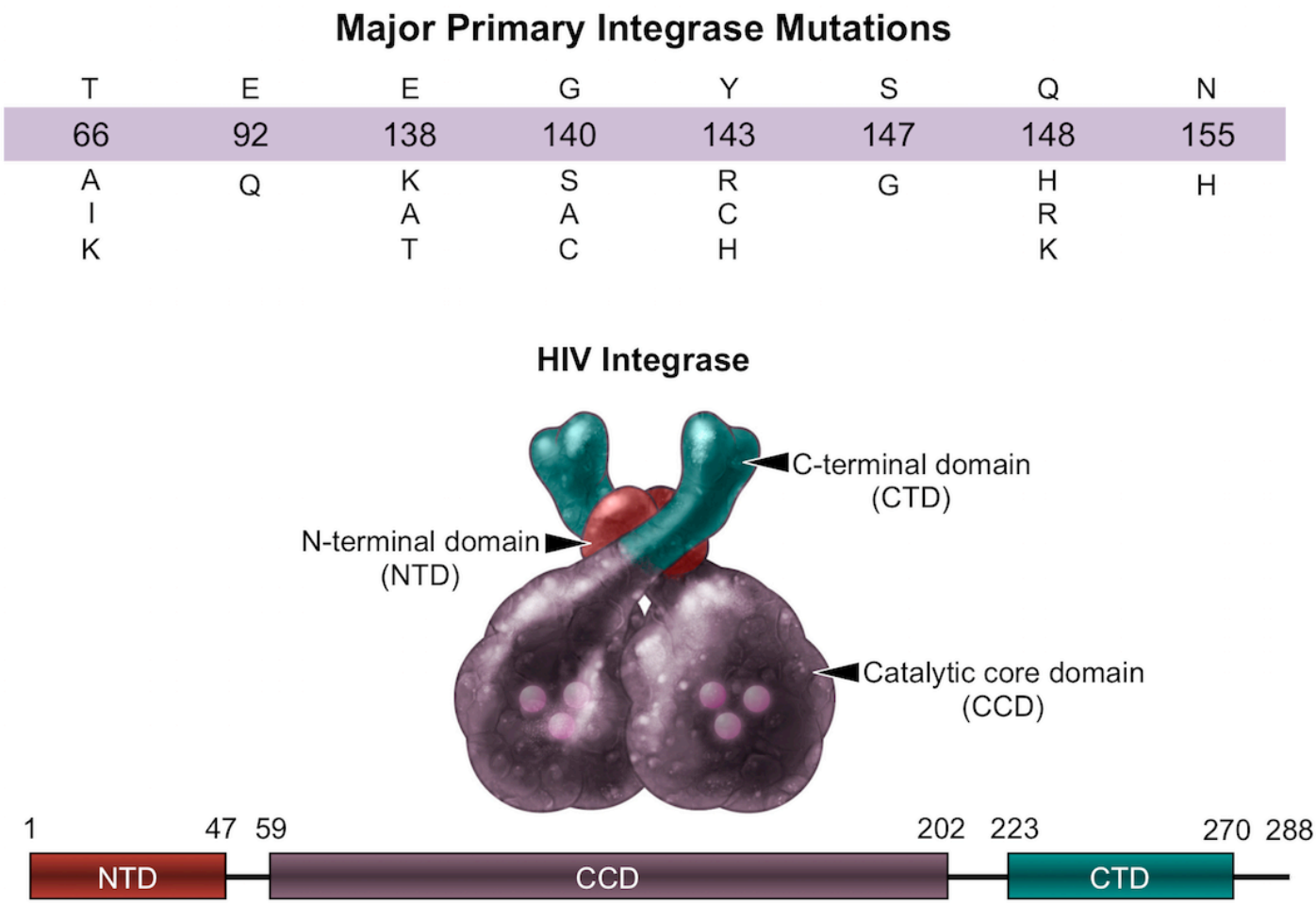


Figure 29 Raltegravir Resistance Pathways

Drug resistance to raltegravir occurs most often in one of three pathways: Q148, N155, and Y143. As shown by the relative sizes of the arrows, the Q148 pathway is the most common pathway and it has the greatest impact on raltegravir-associated resistance. The N155 pathway is the next most common pathway, but because this mutation does not impact raltegravir nearly as much as the Q148, there is frequent crossover from the N155 pathway to the Q148 pathway.

Source: Mbisa JL, Martin SA, Cane PA. Patterns of resistance development with integrase inhibitors in HIV. Infect Drug Resist. 2011;4:65-76.

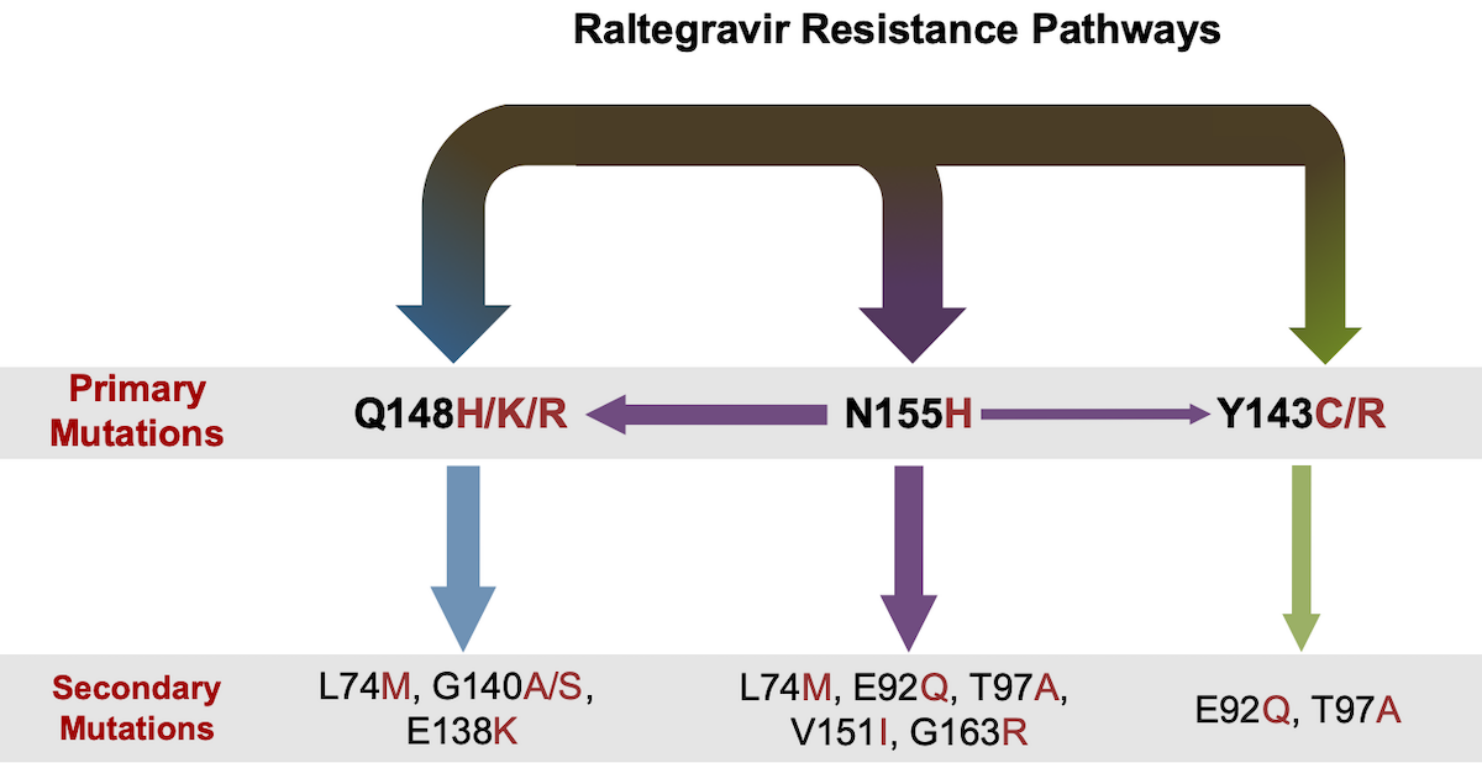


Figure 30 Evolution of Progressive Integrase Resistance during Failure with Raltegravir-Based Regimen

In this study, investigators followed 29 individuals who had incomplete virologic suppression on a regimen that contained raltegravir. With each subsequent visit, a progressive number of integrase mutations developed, translating to higher levels of resistance to raltegravir and to other integrase strand transfer inhibitors.

Source: Hatano H, Lampiris H, Fransen S, et al. Evolution of integrase resistance during failure of integrase inhibitor-based antiretroviral therapy. J Acquir Immune Defic Syndr. 2010;54:389-93.

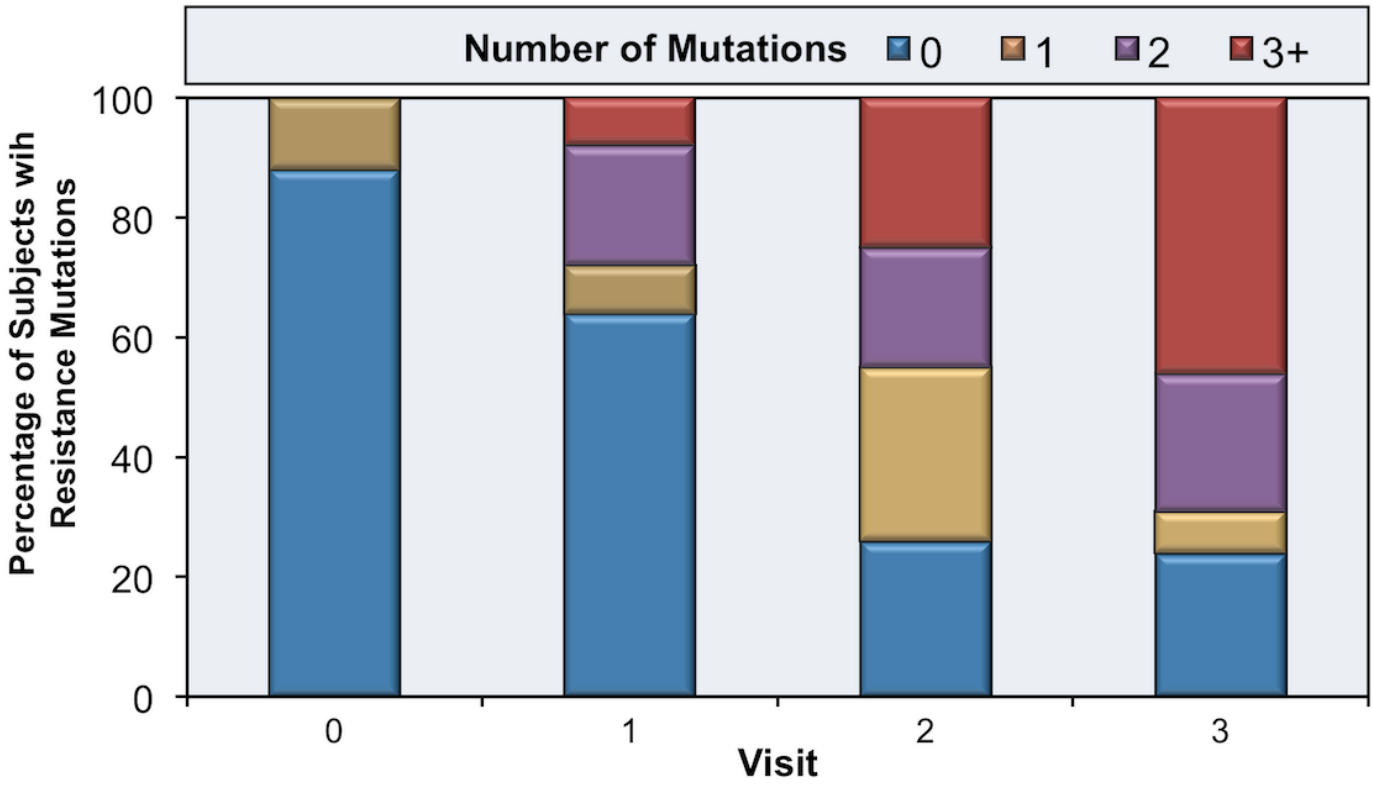


Figure 31 HIV Protease and Location of Amino Acid Resistance Mutations

Illustration: David Ehlert, Cognition Studio and David H. Spach, MD

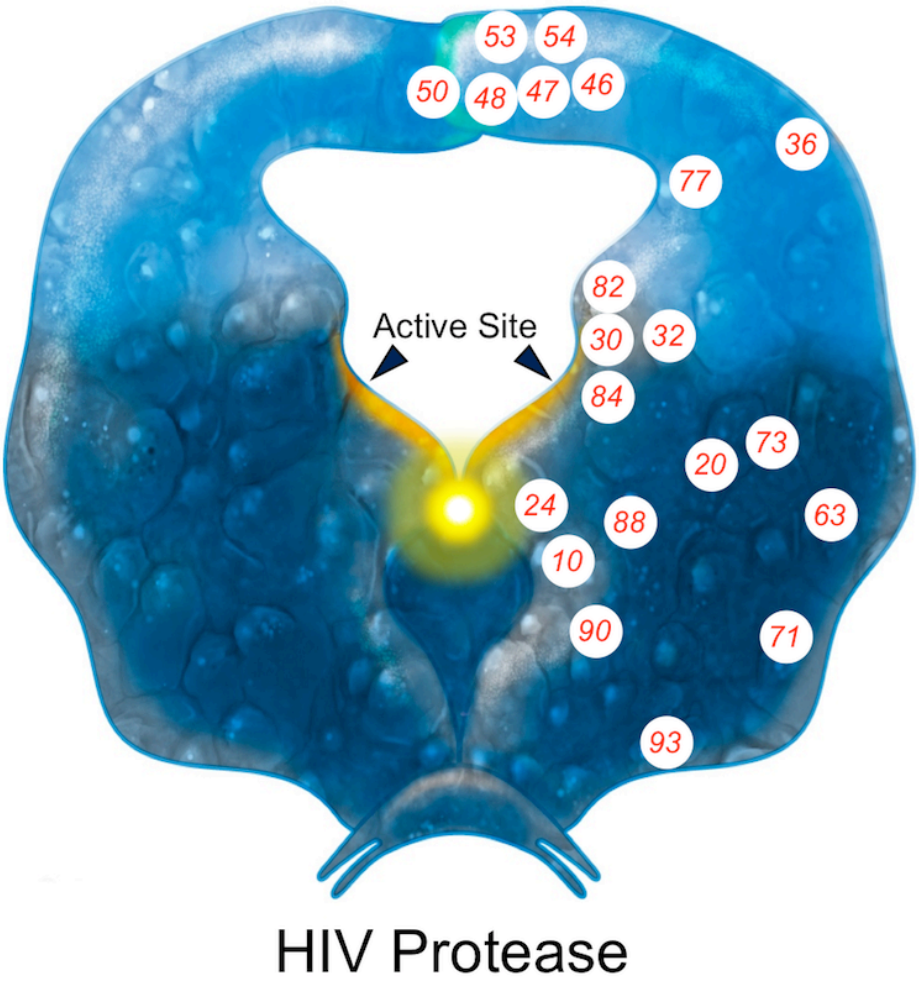


Figure 32 CCR5 Antagonists

The binding of the CCR5 antagonist maraviroc causes a conformational change in the extracellular loop region of the CCR5 coreceptor. The changes in the CCR5 coreceptor that occur do not involve significant changes in the N-terminal region of the CCR5 coreceptor.

Illustration: David H. Spach, MD

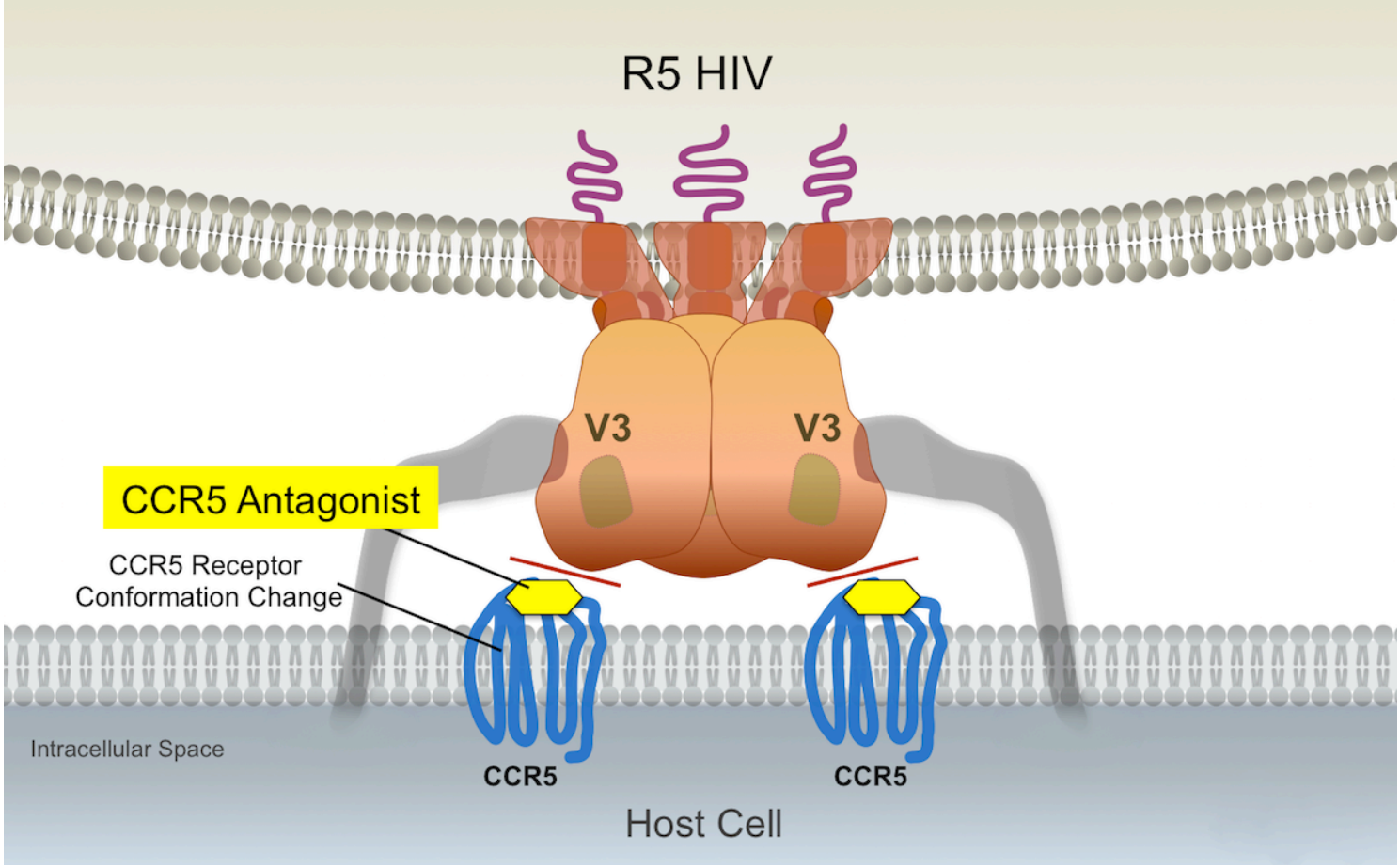


Figure 33 Emergence of Preexisting X4-Tropic Virus

This illustration shows the emergence of preexisting minority variants of X4-tropic virus that are preferentially selected out from the use of a CCR5 antagonist.

Illustration: David H. Spach, MD

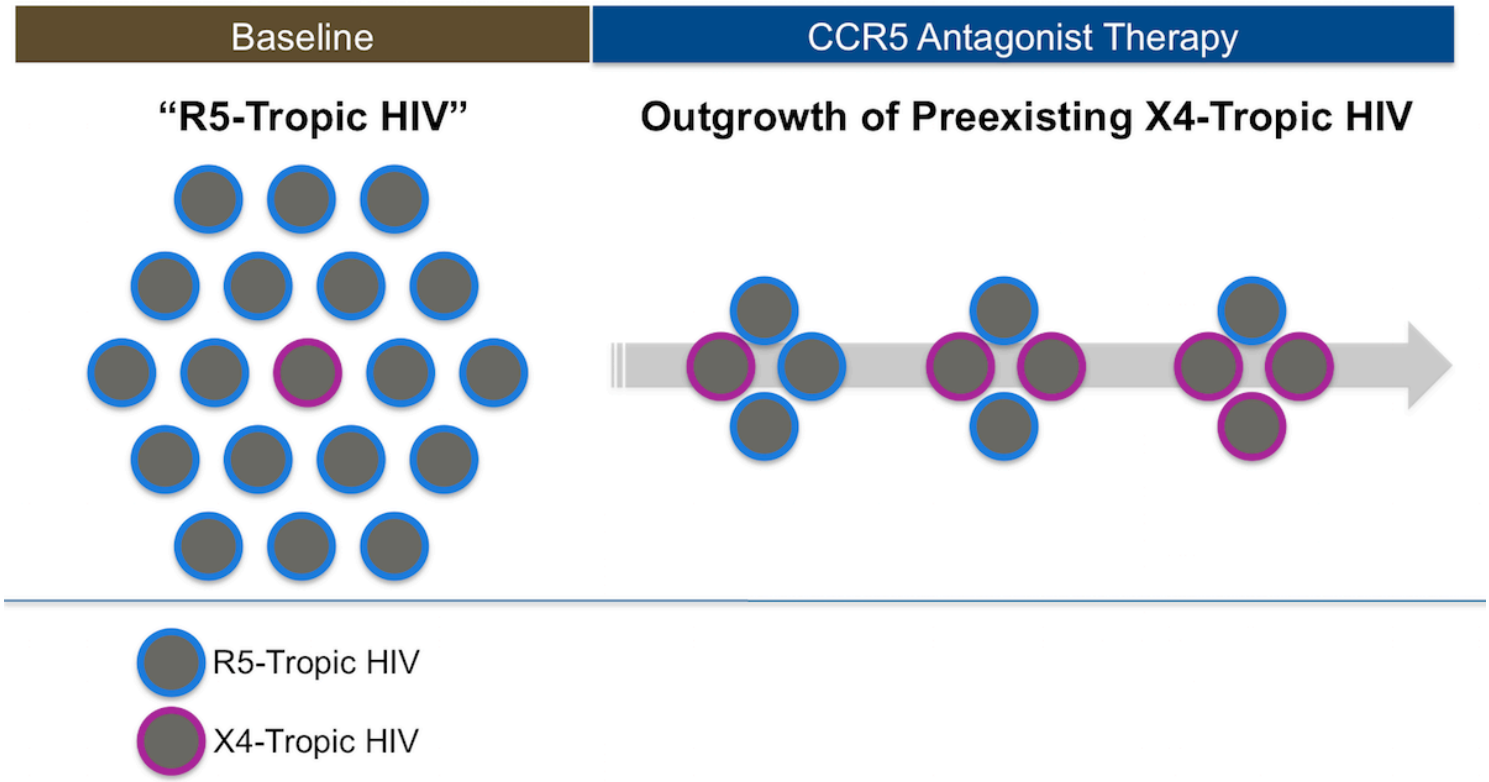


Figure 34 Emergence of New X4-Tropic Virus

This illustration shows emergence of newly formed X4-tropic HIV as a result of mutations in the HIV gp120 region. This contrasts with emergence of preexisting minority variants of X4-tropic virus.

Illustration: David H. Spach, MD

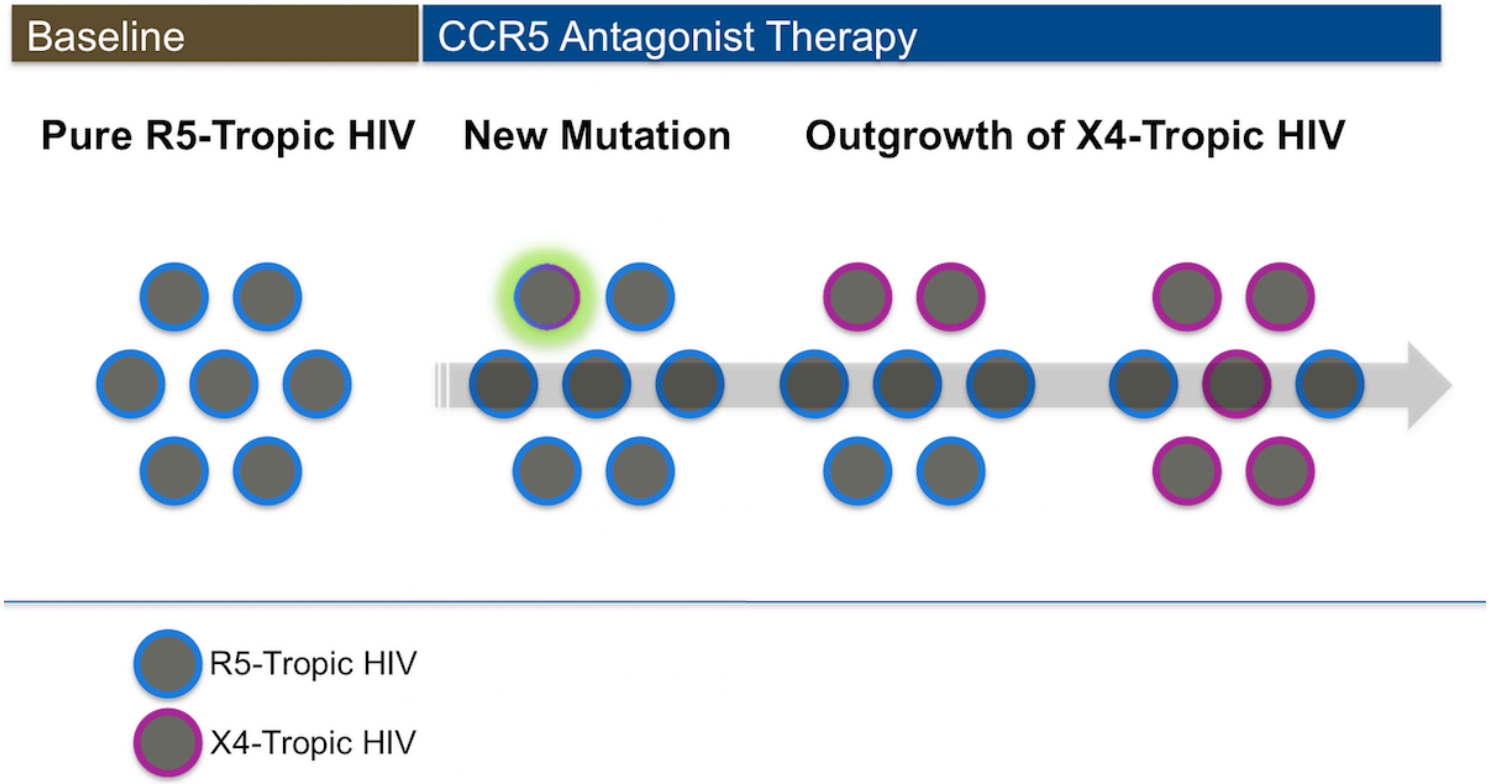


Figure 35 Resistance to CCR5 Antagonist: Binding to CCR5 in Presence of Maraviroc

Resistance to maraviroc can occur when R5-tropic HIV-1 develops mutations that facilitate the gp120-CCR5 coreceptor binding despite maraviroc attachment to the CCR5 coreceptor and receptor conformational changes. When this type of resistance occurs, the binding of HIV-1 gp120 occurs with enhanced affinity at the CCR5 N-terminal domain region.

Illustration: David H. Spach, MD

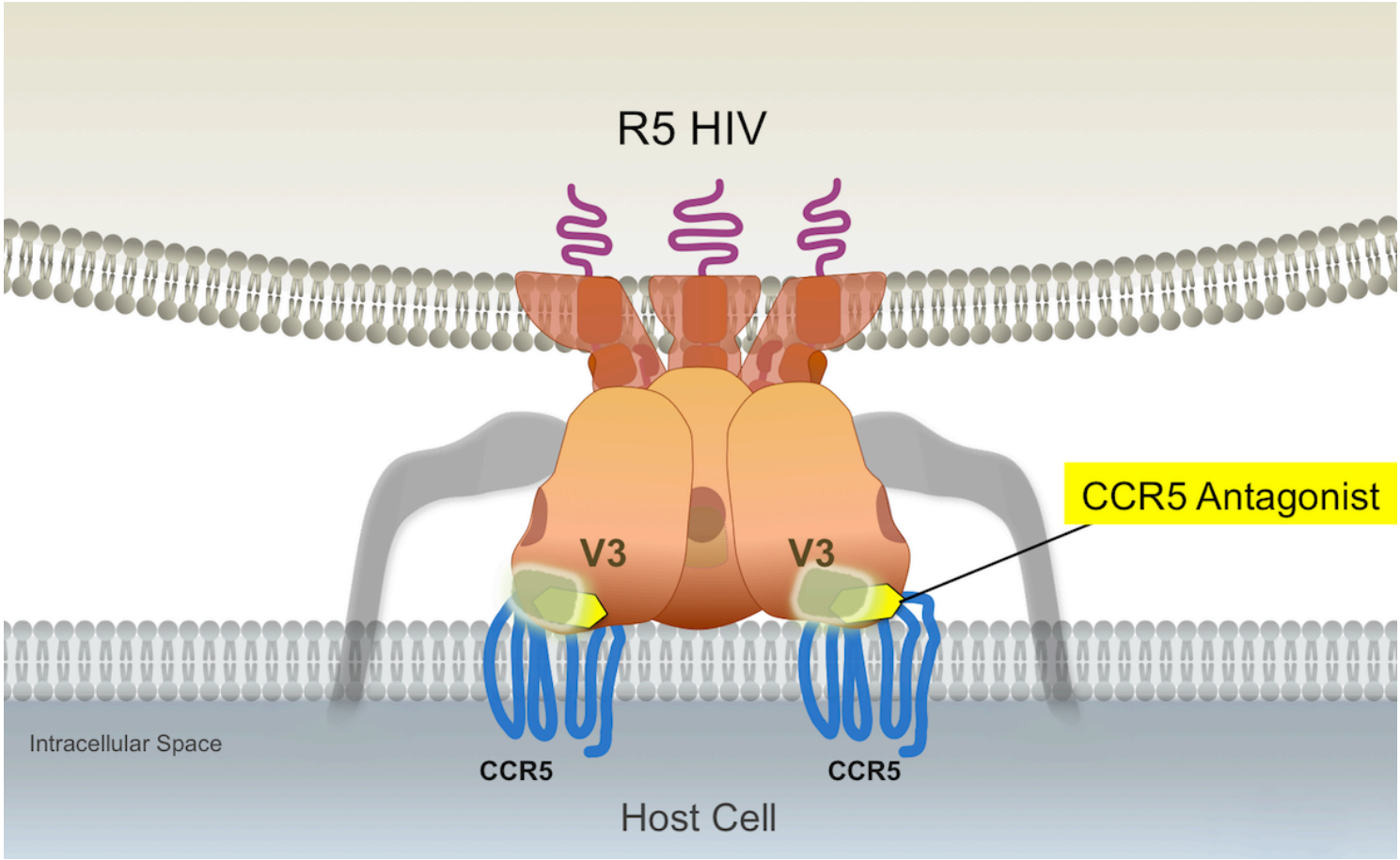


Figure 36 Enfuvirtide

The antiretroviral medication enfuvirtide is a synthetic 36-amino-acid peptide that mimics a segment of the HR2 region of HIV-1 gp41. The medication binds to the corresponding HR1 region and thus prevents the normal HR1-HR2 binding that is critical for HIV-1 to form the 6-helix bundle.

Illustration: David H. Spach, MD

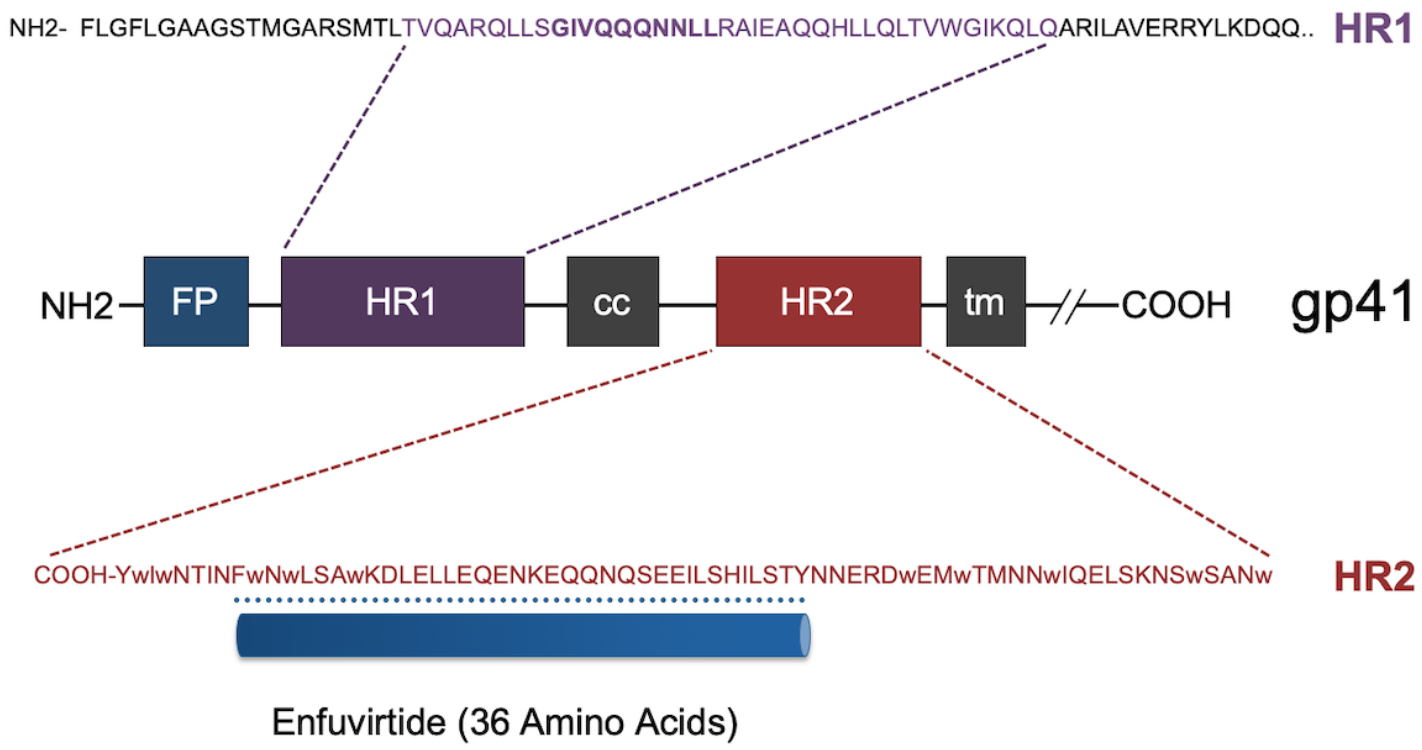


Table 1. **Guidelines for the Use of Antiretroviral Agents in Adults and Adolescents with HIV**

Causes of Virologic Failure
Patient/Adherence-Related Factors • Comorbidities that may affect adherence (e.g., active substance abuse, mental health disorders, neurocognitive impairment) • Unstable housing and other psychosocial factors • Missed clinic appointments • Interruption of or intermittent access to antiretroviral therapy • Cost and affordability of antiretroviral medications (i.e., these factors may affect the ability to access or continue therapy) • Adverse drug effects • High pill burden and/or dosing frequency
HIV-Related Factors • Presence of transmitted or acquired drug-resistant virus documented by current or past resistance test results • Prior treatment failure • Innate resistance to antiretrovirals due to viral tropism or the presence of HIV-2 infection/coinfection • Higher pretreatment HIV RNA level (some regimens may be less effective at higher levels)
Antiretroviral Regimen-Related Factors • Suboptimal pharmacokinetics (PKs) (e.g., variable absorption, metabolism, or possible penetration into reservoirs) • Suboptimal virologic potency • Low genetic barrier to resistance • Reduced efficacy due to prior exposure to suboptimal regimens (e.g., monotherapy, dual nucleoside reverse transcriptase inhibitor [NRTI] therapy, or the sequential introduction of drugs) • Food requirements • Drug-drug interactions with concomitant medications, which may reduce concentrations of the antiretroviral drugs • Prescription (prescribing or dispensing) errors

Source:

- Panel on Antiretroviral Guidelines for Adults and Adolescents. Guidelines for the use of antiretroviral agents in adults and adolescents with HIV. Department of Health and Human Services. Management of the treatment-experienced patient: virologic failure. September 21, 2022. [HIV.gov]