Evaluation and Management of Virologic Failure

This is a PDF version of the following document:
Module 3: Antiretroviral Therapy
Lesson 5: Evaluation and Management of Virologic Failure

You can always find the most up-to-date version of this document at https://www.hiv.uw.edu/go/antiretroviral-therapy/evaluation-management-virologic-failure/core-concept/all.

Background

Among persons with HIV who are not taking antiretroviral therapy, HIV replicates at an extraordinarily high rate, typically producing billions of virions daily.[1] During the reverse transcription step of the HIV replication process, mutations occur at a high rate, primarily 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.

Development of Drug Resistance with Suboptimal Adherence

If a person with HIV takes antiretroviral therapy with consistent medication adherence and maintains suppressed HIV RNA levels, HIV replication remains insufficient for these mutations to occur at a significant frequency. In contrast, with inadequate adherence, viral strains that develop mutations conferring resistance to the antiretroviral regimen will gain a fitness advantage and eventually emerge as the dominant quasispecies (Figure 2)**.** The establishment 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] 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. Although transmitted drug resistance is less relevant in the integrase strand transfer inhibitor (INSTI) era, it is still important for clinicians to understand the importance of drug resistance testing prior to starting antiretroviral therapy.

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 are complex, and clinicians should have a low threshold in seeking 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 of HIV drug resistance testing, the interpretation of drug resistance test results, and strategies for managing virologic failure.

Definition of Terms Related to Virologic Responses

In the Adult and Adolescent ARV 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 defined as less than50 copies/mL) (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 less than 200 copies/mL (Figure 7).

Causes of Virologic Failure

Multiple factors can contribute to 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 antiretroviral therapy and has become more rare in the INSTI era.

Categories of Factors Related to Virologic Failure

The Adult and Adolescent ARV Guidelines list the following three groups of factors that most often contribute to virologic failure: (1) HIV-related factors, (2) antiretroviral regimen-related factors, and (3) social and adherence-related factors (Table 1).[8]

False Elevation in HIV RNA Levels with Plasma Preparation Tubes

Several 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, HIV may adhere to the surface of 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 possible virologic failure. The following section outlines the recommended approach in the Adult and Adolescent ARV 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] Studies on adherence have been conflicting, with some studies showing a correlation between viral blips and adherence, whereas others have not.[16,20] 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 possible drug interactions, and recheck the HIV RNA level 2–4 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).[21,22] Most experts do not alter lab monitoring intervals or make any other changes based on HIV RNA levels detectable in this range. In addition, most experts agree that, for all intents and purposes, individuals with HIV RNA levels detectable below the limit of quantification should be managed the same as those with an undetectable HIV RNA level.

Low-Level Viremia (HIV RNA Detectable but

HIV Drug Resistance Assays

Two types of assays are most often used to evaluate HIV drug resistance: conventional HIV-1 genotypic drug resistance assays and HIV-1 proviral DNA (archived) genotypic assays.[7] The conventional genotypic drug resistance assay analyzes HIV RNA in plasma, whereas the proviral DNA (archived) genotypic assay analyzes cell-associated proviral HIV DNA (Figure 9).[7,26] A third type of test is available, the HIV drug resistance phenotypic assay, but this test is not recommended for routine use and is rarely ordered in clinical practice now.[7] The sensitivity of all of these assays has some limitations, since they use techniques that detect the majority of HIV-1 quasispecies (present in at least 80% of the HIV in the sample). The conventional genotypic drug resistance assay usually requires a plasma HIV-1 RNA level of at least 500 copies/mL for the test to be performed, but it can be considered, and sometimes works, if the HIV-1 RNA level is 200-499 copies/mL.[7] In contrast, the proviral (archived) HIV drug resistance testing can be used with undetectable plasma HIV RNA levels or very low plasma HIV RNA levels.

Conventional Genotype Testing

The most commonly used HIV-1 drug-resistance test in clinical practice is the conventional HIV RNA-1 genotypic assay, which involves multiple steps, including (1) transcribing HIV-1 RNA into DNA, (2) amplifying and sequencing newly transcribed HIV-1 DNA, (3) comparing HIV-1 gene sequences to known HIV-1 wild-type gene sequences, and (4) determining what amino acid alterations will result from specific DNA mutations (Figure 10).[26] The standard HIV drug-resistance genotypic assays used by commercial laboratories sequence the region of the polymerase (*pol*) gene that encodes HIV-1 protease and HIV reverse transcriptase enzymes.[26] The *pol* gene also encodes the HIV integrase enzyme, but most standard tests do not provide HIV integrase resistance data. Thus, testing for integrase resistance usually requires a separate request. No commercially available capsid genotype test is currently available for assessing capsid inhibitor resistance. Some research laboratories can perform genotypic analysis of the HIV *gag* gene, which encodes the capsid protein, and the envelope (*env*) gene, which encodes the HIV gp120 and gp41 proteins.[27,28,29,30] The genotypic drug resistance assays typically require a plasma HIV-1 RNA level of at least 200 copies/mL for the test to be performed. Drug resistance testing for HIV-2 is available in the United States only in a few selected laboratories.

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, which is integrated into the host cell DNA (Figure 11) .[31,32] 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 between HIV RNA and DNA genotypes in viremic patients, whereas others found incomplete information from DNA genotypes when compared with cumulative drug resistance mutation data from previous drug resistance genotypes.[33,34,35,36] 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 regarding switching regimens to optimize antiretroviral therapy.[34,37] The overall clinical utility and optimal use of this test in clinical practice remain unclear.

HIV Drug Resistance Phenotype Assay

The phenotype assay is performed on a blood sample using polymerase chain reaction (PCR) amplification of reverse transcriptase, protease, and possibly the envelope genes; the process relies on the patient's dominant circulating HIV strain. 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. 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 (Figure 12). In practice, phenotype resistance testing has become rare because it is more expensive and has a longer turnaround time than genotype testing. The antiretroviral drug is tested on a patient's HIV isolate and a laboratory reference (wild-type strain).

Indications for HIV Drug-Resistance Testing

For individuals receiving antiretroviral therapy who may have HIV drug resistance contributing to virologic failure, HIV drug resistance plays an essential role in determining if drug resistance has developed and, if so, it will inform the determination of an appropriate regimen based on the resistance mutations. As summarized in the following discussion, the Adult and Adolescent ARV Guidelines provide specific recommendations for drug resistance testing in cases of virologic failure and incomplete virologic responses.[7] Use of resistance testing with acute HIV, at entry into care, and during pregnancy is discussed in other lessons.

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] In addition, integrase resistance testing, including for integrase, if 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 to less than 50 copies/mL.[38] Nearly all individuals with excellent adherence and no baseline resistance will achieve an HIV RNA level of less than 50 copies/mL by 24 weeks after initiation of antiretroviral therapy, and this is typically achieved by 12 weeks with an INSTI-anchored regimen.[7,8] Occasionally, such as with a very high baseline HIV RNA level, virologic suppression may take longer than 12–24 weeks to achieve. An individual who starts taking antiretroviral therapy and has two consecutive plasma HIV RNA levels greater than 200 copies/mL after 24 weeks is considered to have an incomplete virologic response.[8] In most circumstances, an incomplete virologic response suggests missed doses of the prescribed therapy or baseline HIV resistance to one or more of the medications in the initial regimen.[7] Thus, for persons with an incomplete virologic response, HIV genotypic drug resistance testing is recommended.[7] In addition, if an individual reports excellent 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. In both of these situations, resistance testing should include testing for integrase resistance if the person is taking an INSTI-anchored regimen.

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, the sensitivity of the resistance assay 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 outpaces 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.[39,40,41] 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.[39]

Interpretation of Drug-Resistance Assays

Interpreting Drug-Resistance Genotype

The genotype assay generates HIV DNA sequence data, which is used to generate reporting of 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. 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. In addition, most drug resistance genotype reports provide an interpretation of the impact of drug resistance mutations and classify resistance results into three categories: no evidence of resistance, low-level resistance, or high-level resistance. Further, 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.[42,43,44,45]

Interpreting Drug-Resistance Phenotype

A phenotypic resistance assay evaluates HIV susceptibility to antiretroviral agents by directly measuring the viability of the predominant HIV strain in the presence of antiretroviral medications.[26] For each antiretroviral agent tested, the phenotype report provides an IC_{50} or IC_{90} value, a value representing the drug concentration required to inhibit HIV replication 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 the wild-type virus to generate a fold change; the fold change represents relative resistance of the patient's HIV to the antiretroviral medication. 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 15).

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 resistance mutations that were 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 via a drop-down menu and then view the genotype analysis. Options for input include mutations involving reverse transcriptase, protease, and integrase.

- National HIV Clinician Consultation Center: HIV/AIDS Management: This free service offers clinicians consultation with an HIV expert and the consultation can involve 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 844-275-6222.
- International AIDS Society-USA (IAS-USA): Drug Resistance Mutation Figures: The IAS-USA regularly publishes a concise, up-to-date compendium of HIV drug resistance mutations and their impact on each antiretroviral medication. 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.[46] 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.[47] 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 or (2) excision (primer unblocking) of the antiretroviral medication from the HIV DNA strand (Figure 16).[48,49,50,51,52]

- **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.[49] These mutations are often referred to as discriminatory mutations.[49] 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).[49,50,53,54]
- **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 the addition of any cellular deoxynucleotides to the elongating strand of the primer (primer blocking).[49] Therefore, if the NRTI is excised from the primer, the net effect is referred to as primer unblocking. Characteristic mutations that occur via primer unblocking include the M41L, D67N, K70R, L210W, T215Y/F, and K219Q/E.[49,50,53]

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.[55] The M184I and M184V mutations develop via the discriminatory resistance pathway.[49,51] The M184V mutation causes high-level resistance to emtricitabine and lamivudine, intermediate resistance to islatravir, low-level resistance to abacavir, and enhanced susceptibility to tenofovir alafenamide, tenofovir DF, and zidovudine.[56,57]

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 17).[42,53,56,58,59,60] 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) the type-II pattern consisting of D67N, K70R, T215F, and K219Q/E.[53,61,62,63,64] 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.[53,65] 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 included tenofovir DF, tenofovir alafenamide, or abacavir, but not a thymidine analog (e.g., stavudine or zidovudine).[66,67] 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.[68] 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 and islatravir.[53,57] 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 hypersusceptibility to islatravir and zidovudine.[66,69] With abacavir resistance, the M184V typically precedes the K65R.[70] In clinical trials and clinical practice, it is uncommon to observe the K65R mutation in conjunction with multiple TAMs.[66,67]

L74V Mutation

The L74V mutation was first identified in patients receiving didanosine or abacavir monotherapy; this mutation alone causes intermediate-level resistance to abacavir. The L74V mutation, in combination with M184V, has been observed in patients treated with abacavir plus lamivudine, or didanosine plus lamivudine.[53] 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.[71]

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.[72,73] 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.[53,72,74] 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. [49]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).[49]

Lamivudine and Emtricitabine

In clinical trials involving combination antiretroviral therapy, the M184V mutation is among the most common mutations to develop with initial virologic failure then the regimen includes lamivudine or emtricitabine.[75] One early study showed that patients treated with lamivudine monotherapy developed the M184V mutation and virologic failure within 4 weeks of starting lamivudine.[76] 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.[76] 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.[77,78] 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.[79] 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.[80]

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).[42,53,56,58,59,60] 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.[81]

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 HIV reverse transcriptase, causing functional changes in the enzyme.[46,47] 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).[39,50] 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 or by preventing adequate access of the NNRTI to the binding pocket (Figure 18).[47,50] 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 with other agents, such as certain INSTIs (dolutegravir and bictegravir) or boosted protease inhibitors, the NNRTIs have a relatively low barrier to the development of drug resistance.[39,82]

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.[83,84] 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 19), or (2) a minor pathway that involves selection of V108I and L234I mutations.[85,86] Clinical experience with cross-resistance for doravirine and other NNRTIs is limited, but the emergence of resistance to doravirine may not impact all NNRTIs.[86] 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.[85,87] 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.[88] 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.[39,89]

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 NNRTIs, 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.[90] 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).[90] 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.[42]

Nevirapine

Although nevirapine is rarely used in clinical practice now, prior receipt of nevirapine may be important if NNRTI drug resistance occurred while taking nevirapine. Patients treated with nevirapine monotherapy most commonly develop the Y181C mutation.[82] In about 20% of patients who develop virologic failure on a nevirapine-based regimen, the mutations V106A/M or Y181C/I initially emerge.[91] 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.[39,91]

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.[92] 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.[80,92,93,95] Less often, patients taking rilpivirine develop a K101E mutation, which typically causes intermediate-level rilpivirine resistance.[96]

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.[33,97,98,99,100]
- **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, and etravirine.[92] If the E138K occurs with an M184V mutation, it increases the likelihood of virologic failure on a rilpivirine-based regimen.
- **Y181C**: The Y181C mutation causes intermediate-level resistance to efavirenz, etravirine, and rilpivirine and potential low-level resistance to doravirine.[97,101,102,103,104]
- **G190E**: The G190E mutation is problematic in that it causes high-level resistance to doravirine, efavirenz, and rilpivirine, as well as intermediate-level resistance to etravirine, essentially leaving no good options in the NNRTI class.[105]

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).[100] Data from multiple studies suggest the K103N mutation has minimal impact on viral fitness and thus there is no benefit in 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.[106]

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.[107] 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 20).[108,109,110] 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.[111,112] 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 reduction in 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 a higher barrier than elvitegravir or raltegravir.[112] 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 (R263K, S153Y, S147G, H51Y, or Q146L) or multiple mutations (Q148R/K along with secondary mutations).[113] 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.[113] 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.[114,115,116] 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.[117] A number of secondary and minor mutations also emerged, including G140A/G/S, E138E/K, and L74I.[117]

Dolutegravir

Dolutegravir, similar to bictegravir, has a high genetic barrier to resistance, which is higher than the barrier with cabotegravir, elvitegravir, or raltegravir.[108,112] Although integrase resistance infrequently develops in a person taking a regimen with dolutegravir, when it does occur, R263K is the most frequently identified mutation.[118,119,120] Other commonly identified major mutations include the N155H, and N155H plus E92Q.[121,122] 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.[123,124,125] 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.[118,119,126,127] 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.[128] The R263K mutation causes intermediate-level resistance to dolutegravir, bictegravir, and cabotegravir, as well as low-level resistance to raltegravir.[129] 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.[130] In persons who develop resistance to raltegravir or elvitegravir, 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.[42,131] The N155H mutation, followed by the A49P, L68F, T97A, E138K, and L234V can also lead to dolutegravir resistance.[132] If integrase mutations are present that cause low- 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.[133] The E92Q mutation is the most common initial mutation to arise with elvitegravir failure, followed in frequency by N155H and Q148H/K/R.[108] The E92Q mutation alone reduces elvitegravir susceptibility by more than 20-fold and reduces raltegravir susceptibility by 5-fold.[42] The T66I mutation causes an approximately 10-fold reduction in elvitegravir susceptibility, but it does not significantly impact the susceptibility to raltegravir or dolutegravir. When resistance to raltegravir develops, high-level cross-resistance with elvitegravir usually occurs.[130,134,135] 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.[109,136,137,138] 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 21).[109,110,137,138] 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.[139] Importantly, the accessory mutations G140S and T97A restore viral fitness to the mutated virus, whereas E92Q does not.[139] Development of drug resistance to raltegravir generally translates to high-level cross-resistance with elvitegravir.[130,134,135] 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.[140] 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; inhibition of HIV protease can impact multiple enzymes generated during the normal cleavage process.[141] Multiple protease mutations are required to significantly impact the virologic response to a PI boosted with either cobicistat or ritonavir (Figure 22).[42,53] In general, PIs boosted with either cobicistat or ritonavir have medium or high potency and a medium or high genetic barrier to resistance.[49,142] Among the commonly used boosted PIs, darunavir has the highest barrier to resistance, followed by lopinavir and then by atazanavir.[49,142] 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.[42,53,141] 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.[53]

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.[42] 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.[80,143] 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.[42] One study suggested the L76V mutation, with or without M46I, generates atazanavir hypersusceptibility when no additional PI mutations are present.[144]

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 previously developed virologic failure and resistance while taking amprenavir or fosamprenavir, they are likely to have developed cross-resistance to darunavir, which is not the case with prior 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.[145,146,147] 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.[42,148,149,150] Diminished virologic response to darunavir generally requires at least three darunavir resistance-associated mutations to emerge.[151] Major mutations include I47V, I50V, I54M, L76V, and I84V.[42] 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.[152,153,154]

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.[155,156,157] Accordingly, lopinavir boosted with ritonavir is considered to have a high genetic barrier to resistance.[49] Major mutations associated with lopinavir resistance include V32I, I47V/A, L76V, and V82A/F/T/S; there are also 13 identified minor mutations.[155,156,158] The combination of lopinavir-ritonavir requires accumulation of at least three lopinavir-associated mutations for a reduced virologic response to lopinavir-ritonavir.[49,142] 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 three PI mutations also increases resistance to lopinavir-ritonavir.[42,144] Although lopinavir-ritonavir resistance infrequently develops in virologic failure in treatment-naïve persons, mutations can emerge in persons who previously failed other protease inhibitors.[159] In addition, significant cross-resistance exists with lopinavir-ritonavir and atazanavir.[160]

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.[161,162] Available data suggest that development of resistance to fostemsavir does not cause cross-resistance to other entry inhibitors (ibalizumab, maraviroc, or enfuvirtide).[163] 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 reduce the number of 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.[164] 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 (Figure 23).[164,165] 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 or a *de novo* tropism switch caused by multiple mutations in the HIV gp120 V3 region [42,165] 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.[27,28] 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.[164,165]
- **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 fully elucidated, but likely involves enhanced affinity of HIV gp120 binding to the N-terminal domain region of the CCR5 coreceptor.[53,164,166,167] 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.[164]

Evaluation of Resistance with CCR5 Antagonists

Because the 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. A change in tropism (e,g., CCR5 changing to CXCR4) indicates resistance to maraviroc.
- **Genotypic Tropism Assay**: In contrast, the HIV-1 tropism genotype is performed by sequencing the V3-coding region of the *env* gene, which is known to be the principal determinant of coreceptor usage; with this method, the 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.[168]

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, 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, but it is no longer manufactured. Enfuvirtide is a synthetic 36-amino-acid peptide that mimics amino acids 127-162 in the HR2 region of HIV-1 gp41 envelope protein (Figure 24).[169,170] The enfuvirtide peptide binds to the HR1 region, preventing the normal folding interaction between HR1 and HR2. Resistance to enfuvirtide is associated primarily with mutations in the HR1 region of the HIV gp41 *env* gene; these mutations correspond with the site on the HR1 region where enfuvirtide binds.

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 host cell nucleus, and reassembly of the viral capsid core.[171] 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.[171] 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.[172] In the phase 3 trial of lenacapavir for individuals with extensive treatment experience, eight participants developed virologic failure with evidence of lenacapavir-associated capsid resistance substitutions; six developed M66I (including one with M66I and N74D), one developed Q67H with K70R, and one developed K70H.[29] All of these mutations significantly reduced lenacapavir activity, especially the M66I combined 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 such as the Q67H alter the hexamer proteins in a way that interferes with lenacapavir binding (a closed-to-open conformational change of the proteins prevents lenacapavir binding).[30] Other mutations, such as 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.[173] Experience with lenacapavir resistance is evolving, and currently there are no commercially available capsid resistance assays.

General Approach to Management of Virologic Failure

Management of virologic failure can be challenging, and expert consultation is advised. The Adult and Adolescent ARV Guidelines outline appropriate steps to take when virologic failure is suspected or documented, including assessing adherence and medication tolerability as well as evaluating possible pharmacokinetic issues (e.g., drug-drug or drug-food interactions). In addition, these guidelines outline recommendations based on whether the individual is failing a first or subsequent regimen.[8] The following summarizes several additional points outlined in the guidelines for managing virologic failure.[8]

- If an individual develops virologic failure while inconsistently taking (or after stopping) a regimen that has a relatively high barrier to resistance, such as a regimen with bictegravir, dolutegravir, or boosted darunavir, resistance testing should be ordered, but the regimen can usually be continued (or restarted) with close virological monitoring while resistance results are pending.
- If virologic failure occurs with evidence of drug resistance mutations, the goal is to construct a new regimen (often referred to as a “salvage” regimen) that will reduce the individual’s HIV RNA levels to less than 50 copies/mL. In order to accomplish this, the new regimen should include at minimum two fully active antiretrovirals—if at least one of the medications in the regimen has a relatively high barrier to resistance, such as bictegravir, dolutegravir, or boosted darunavir. If the existing regimen does not have any medications considered to have a high genetic barrier to resistance, the new regimen should include a minimum of three fully active antiretrovirals.
- There are some antiretrovirals that are typically only used after virologic failure to help create a salvage regimen (e.g., lenacapavir, ibalizumab, fostemsavir, and maraviroc). None of these medications are considered to have a high genetic barrier to resistance.
- 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.

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.
- Resistance mutations can develop as a result of suboptimal virologic suppression (due to a variety of adherence-related or antiretroviral regimen-related causes) or it can occur via the acquisition of drug-resistant HIV at the time of initial infection.
- Drug resistance has been documented in all FDA-approved antiretroviral medications, with signature mutations associated with particular drugs.
- Testing for antiretroviral resistance mutations is recommended in persons with virologic failure and with incomplete response to initial therapy.
- Conventional genotypic assays are most often used to assess for HIV drug resistance in plasma HIV RNA. Proviral (archived) genotypes are available to assess proviral HIV DNA that is integrated into host cell DNA. Currently, there are no commercially available tests to assess for lenacapavir (capsid inhibitor) resistance.
- Conventional genotypic drug 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. Proviral genotypic drug resistance tests can be performed in persons with very low or undetectable HIV RNA levels.
- 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.
- 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.

Citations

1. Perelson AS, Neumann AU, Markowitz M, Leonard JM, Ho DD. HIV-1 dynamics in vivo: virion clearance rate, infected cell life-span, and viral generation time. Science. 1996;271:1582-6.
[PubMed Abstract] -

2. O'Neil PK, Sun G, Yu H, Ron Y, Dougherty JP, Preston BD. Mutational analysis of HIV-1 long terminal repeats to explore the relative contribution of reverse transcriptase and RNA polymerase II to viral mutagenesis. J Biol Chem. 2002;277:38053-61.
[PubMed Abstract] -

3. 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. Laboratory testing: plasma HIV-1 RNA (viral load) and CD4 count monitoring. September 25, 2025.
[HIV.gov] -

4. Wheeler WH, Ziebell RA, Zabina H, et al. Prevalence of transmitted drug resistance associated mutations and HIV-1 subtypes in new HIV-1 diagnoses, U.S.-2006. AIDS. 2010;24:1203-12.
[PubMed Abstract] -

5. Jain V, Liegler T, Vittinghoff E, et al. Transmitted drug resistance in persons with acute/early HIV-1 in San Francisco, 2002-2009. PLoS One. 2010;5:e15510.
[PubMed Abstract] -

6. Poon AF, Aldous JL, Mathews WC, et al. Transmitted drug resistance in the CFAR network of integrated clinical systems cohort: prevalence and effects on pre-therapy CD4 and viral load. PLoS One. 2011;6:e21189.
[PubMed Abstract] -

7. 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. Laboratory testing: drug-resistance testing. September 24, 2024.
[HIV.gov] -

8. 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 people with HIV and antiretroviral therapy experience: Virologic failure. September 12, 2024.
[HIV.gov] -

9. Kraft CS, Binongo JN, Burd EM, et al. Successful use of Plasma Preparation Tubes™ (PPTs) in the COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 test. J Clin Virol. 2013;57:77-9.
[PubMed Abstract] -

10. Stosor V, Palella FJ Jr, Berzins B, et al. Transient viremia in HIV-infected patients and use of plasma preparation tubes. Clin Infect Dis. 2005;41:1671-4.
[PubMed Abstract] -

11. Rebeiro PF, Kheshti A, Bebawy SS, et al. Increased detectability of plasma HIV-1 RNA after introduction of a new assay and altered specimen-processing procedures. Clin Infect Dis. 2008;47:1354-7.
[PubMed Abstract] -

12. Kran AM, Jonassen TØ, Sannes M, et al. Overestimation of human immunodeficiency virus type 1 load caused by the presence of cells in plasma from plasma preparation tubes. J Clin Microbiol.

2009;47:2170-4.
[PubMed Abstract] -

13. Wan H, Seth A, Rainen L, Fernandes H. Coamplification of HIV-1 proviral DNA and viral RNA in assays used for quantification of HIV-1 RNA. J Clin Microbiol. 2010;48:2186-90.
[PubMed Abstract] -

14. Havlir DV, Bassett R, Levitan D, et al. Prevalence and predictive value of intermittent viremia with combination hiv therapy. JAMA. 2001;286:171-9.
[PubMed Abstract] -

15. Nettles RE, Kieffer TL, Kwon P, et al. Intermittent HIV-1 viremia (Blips) and drug resistance in patients receiving HAART. JAMA. 2005;293:817-29.
[PubMed Abstract] -

16. Podsadecki TJ, Vrijens BC, Tousset EP, Rode RA, Hanna GJ. Decreased adherence to antiretroviral therapy observed prior to transient human immunodeficiency virus type 1 viremia. J Infect Dis. 2007;196:1773-8.
[PubMed Abstract] -

17. Sklar PA, Ward DJ, Baker RK, et al. Prevalence and clinical correlates of HIV viremia ('blips') in patients with previous suppression below the limits of quantification. AIDS. 2002;16:2035-41.
[PubMed Abstract] -

18. Taiwo B, Bosch RJ. More reasons to reexamine the definition of viral blip during antiretroviral therapy. J Infect Dis. 2012;205:1189-91.
[PubMed Abstract] -

19. Farmer A, Wang X, Ganesan A, et al. Factors associated with HIV viral load "blips" and the relationship between self-reported adherence and efavirenz blood levels on blip occurrence: a case-control study. AIDS Res Ther. 2016;13:16.
[PubMed Abstract] -

20. Miller LG, Golin CE, Liu H, et al. No evidence of an association between transient HIV viremia ("Blips") and lower adherence to the antiretroviral medication regimen. J Infect Dis. 2004;189:1487-96.
[PubMed Abstract] -

21. Gandhi RT, Deeks SG. Plasma HIV-1 RNA levels during antiretroviral therapy: how low is low enough? Clin Infect Dis. 2012;54:733-5.
[PubMed Abstract] -

22. Ryscavage P, Kelly S, Li JZ, Harrigan PR, Taiwo B. Significance and clinical management of persistent low-level viremia and very-low-level viremia in HIV-1-infected patients. Antimicrob Agents Chemother. 2014;58:3585-98.
[PubMed Abstract] -

23. Halvas EK, Joseph KW, Brandt LD, et al. HIV-1 viremia not suppressible by antiretroviral therapy can originate from large T cell clones producing infectious virus. J Clin Invest. 2020;130:5847-57.
[PubMed Abstract] -

24. Wirden M, Todesco E, Valantin MA, et al. Low-level HIV-1 viraemia in patients on HAART: risk factors and management in clinical practice. J Antimicrob Chemother. 2015;70:2347-53.
[PubMed Abstract] -

25. Swenson LC, Min JE, Woods CK, et al. HIV drug resistance detected during low-level viraemia is associated with subsequent virologic failure. AIDS. 2014;28:1125-34.
[PubMed Abstract] -

26. Hanna GJ, D'Aquila RT. Clinical use of genotypic and phenotypic drug resistance testing to monitor antiretroviral chemotherapy. Clin Infect Dis. 2001;32:774-82.
[PubMed Abstract] -

27. Swenson LC, Dong WW, Mo T, et al. Use of cellular HIV DNA to predict virologic response to maraviroc: performance of population-based and deep sequencing. Clin Infect Dis. 2013;56:1659-66.
[PubMed Abstract] -

28. Swenson LC, Chui CK, Brumme CJ, et al. Genotypic analysis of the V3 region of HIV from virologic nonresponders to maraviroc-containing regimens reveals distinct patterns of failure. Antimicrob Agents Chemother. 2013;57:6122-30.
[PubMed Abstract] -

29. Segal-Maurer S, DeJesus E, Stellbrink HJ, et al. Capsid Inhibition with Lenacapavir in Multidrug-Resistant HIV-1 Infection. N Engl J Med. 2022;386:1793-1803.
[PubMed Abstract] -

30. Bester SM, Adu-Ampratwum D, Annamalai AS, et al. Structural and Mechanistic Bases of Viral Resistance to HIV-1 Capsid Inhibitor Lenacapavir. mBio. 2022;13:e0180422.
[PubMed Abstract] -

31. Turriziani O, Andreoni M, Antonelli G. Resistant viral variants in cellular reservoirs of human immunodeficiency virus infection. Clin Microbiol Infect. 2010;16:1518-24.
[PubMed Abstract] -

32. Devereux HL, Loveday C, Youle M, Sabin CA, Burke A, Johnson M. Substantial correlation between HIV type 1 drug-associated resistance mutations in plasma and peripheral blood mononuclear cells in treatment-experienced patients. AIDS Res Hum Retroviruses. 2000;16:1025-30.
[PubMed Abstract] -

33. Delaugerre C, Braun J, Charreau I, et al. Comparison of resistance mutation patterns in historical plasma HIV RNA genotypes with those in current proviral HIV DNA genotypes among extensively treated patients with suppressed replication. HIV Med. 2012;13:517-25.
[PubMed Abstract] -

34. Lambert-Niclot S, Allavena C, Grude M, et al. Usefulness of an HIV DNA resistance genotypic test in patients who are candidates for a switch to the rilpivirine/emtricitabine/tenofovir disoproxil fumarate combination. J Antimicrob Chemother. 2016;71:2248-51.
[PubMed Abstract] -

35. Wirden M, Soulie C, Valantin MA, et al. Historical HIV-RNA resistance test results are more informative than proviral DNA genotyping in cases of suppressed or residual viraemia. J Antimicrob Chemother. 2011;66:709-12.
[PubMed Abstract] -

36. Derache A, Shin HS, Balamane M, et al. HIV drug resistance mutations in proviral DNA from a community treatment program. PLoS One. 2015;10:e0117430.
[PubMed Abstract] -

37. Porter DP, Toma J, Tan Y, et al. Clinical Outcomes of Virologically-Suppressed Patients with Pre-existing

HIV-1 Drug Resistance Mutations Switching to Rilpivirine/Emtricitabine/Tenofovir Disoproxil Fumarate in the SPIRIT Study. HIV Clin Trials. 2016;17:29-37.
[PubMed Abstract] -

38. 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. Laboratory testing: laboratory testing for initial assessment and monitoring of people with HIV. September 25, 2025.
[HIV.gov] -

39. Deeks SG, Wrin T, Liegler T, et al. Virologic and immunologic consequences of discontinuing combination antiretroviral-drug therapy in HIV-infected patients with detectable viremia. N Engl J Med. 2001;344:472-80.
[PubMed Abstract] -

40. Devereux HL, Youle M, Johnson MA, Loveday C. Rapid decline in detectability of HIV-1 drug resistance mutations after stopping therapy. AIDS. 1999;13:F123-7.
[PubMed Abstract] -

41. Verhofstede C, Wanzeele FV, Van Der Gucht B, De Cabooter N, Plum J. Interruption of reverse transcriptase inhibitors or a switch from reverse transcriptase to protease inhibitors resulted in a fast reappearance of virus strains with a reverse transcriptase inhibitor-sensitive genotype. AIDS. 1999;13:2541-6.
[PubMed Abstract] -

42. Wensing AM, Calvez V, Günthard HF, et al. 2017 Update of the Drug Resistance Mutations in HIV-1. Top Antivir Med. 2017;24:132-3.
[PubMed Abstract] -

43. Gallant JE. The M184V mutation: what it does, how to prevent it, and what to do with it when it's there. AIDS Read. 2006;16:556-9.
[PubMed Abstract] -

44. Turner D, Brenner BG, Routy JP, Petrella M, Wainberg MA. Rationale for maintenance of the M184v resistance mutation in human immunodeficiency virus type 1 reverse transcriptase in treatment experienced patients. New Microbiol. 2004;27(2 Suppl 1):31-9.
[PubMed Abstract] -

45. Wolf K, Walter H, Beerenwinkel N, et al. Tenofovir resistance and resensitization. Antimicrob Agents Chemother. 2003;47:3478-84.
[PubMed Abstract] -

46. Schauer G, Leuba S, Sluis-Cremer N. Biophysical Insights into the Inhibitory Mechanism of Non-Nucleoside HIV-1 Reverse Transcriptase Inhibitors. Biomolecules. 2013;3:889-904.
[PubMed Abstract] -

47. Clavel F, Hance AJ. HIV drug resistance. N Engl J Med. 2004;350:1023-35.
[PubMed Abstract] -

48. Tu X, Das K, Han Q, et al. Structural basis of HIV-1 resistance to AZT by excision. Nat Struct Mol Biol. 2010;17:1202-9.
[PubMed Abstract] -

49. Tang MW, Shafer RW. HIV-1 antiretroviral resistance: scientific principles and clinical applications.

Drugs. 2012;72:e1-25.
[PubMed Abstract] -

50. Singh K, Marchand B, Kirby KA, Michailidis E, Sarafianos SG. Structural Aspects of Drug Resistance and Inhibition of HIV-1 Reverse Transcriptase. Viruses. 2010;2:606-638.
[PubMed Abstract] -

51. Deval J, Courcambeck J, Selmi B, Boretto J, Canard B. Structural determinants and molecular mechanisms for the resistance of HIV-1 RT to nucleoside analogues. Curr Drug Metab. 2004;5:305-16.
[PubMed Abstract] -

52. Meyer PR, Matsuura SE, Mian AM, So AG, Scott WA. A mechanism of AZT resistance: an increase in nucleotide-dependent primer unblocking by mutant HIV-1 reverse transcriptase. Mol Cell. 1999;4:35-43.
[PubMed Abstract] -

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

54. Sluis-Cremer N, Wainberg MA, Schinazi RF. Resistance to reverse transcriptase inhibitors used in the treatment and prevention of HIV-1 infection. Future Microbiol. 2015;10:1773-82.
[PubMed Abstract] -

55. Keulen W, Back NK, van Wijk A, Boucher CA, Berkhout B. Initial appearance of the 184Ile variant in lamivudine-treated patients is caused by the mutational bias of human immunodeficiency virus type 1 reverse transcriptase. J Virol. 1997;71:3346-50.
[PubMed Abstract] -

56. Whitcomb JM, Parkin NT, Chappey C, Hellmann NS, Petropoulos CJ. Broad nucleoside reverse-transcriptase inhibitor cross-resistance in human immunodeficiency virus type 1 clinical isolates. J Infect Dis. 2003;188:992-1000.
[PubMed Abstract] -

57. Lai MT, Feng M, Xu M, et al. Doravirine and Islatravir Have Complementary Resistance Profiles and Create a Combination with a High Barrier to Resistance. Antimicrob Agents Chemother. 2022;66:e0222321.
[PubMed Abstract] -

58. Marcelin AG, Delaugerre C, Wirden M, et al. Thymidine analogue reverse transcriptase inhibitors resistance mutations profiles and association to other nucleoside reverse transcriptase inhibitors resistance mutations observed in the context of virological failure. J Med Virol. 2004;72:162-5.
[PubMed Abstract] -

59. Miller MD. K65R, TAMs and tenofovir. AIDS Rev. 2004;6:22-33.
[PubMed Abstract] -

60. Miller V, Larder BA. Mutational patterns in the HIV genome and cross-resistance following nucleoside and nucleotide analogue drug exposure. Antivir Ther. 2001;6 Suppl 3:25-44.
[PubMed Abstract] -

61. Beerenwinkel N, Däumer M, Sing T, et al. Estimating HIV evolutionary pathways and the genetic barrier to drug resistance. J Infect Dis. 2005;191:1953-60.
[PubMed Abstract] -

62. Hu Z, Giguel F, Hatano H, Reid P, Lu J, Kuritzkes DR. Fitness comparison of thymidine analog resistance pathways in human immunodeficiency virus type 1. J Virol. 2006;80:7020-7. [PubMed Abstract] -

63. Lengauer T, Sing T. Bioinformatics-assisted anti-HIV therapy. Nat Rev Microbiol. 2006;4:790-7. [PubMed Abstract] -

64. Sluis-Cremer N, Sheen CW, Zelina S, Torres PS, Parikh UM, Mellors JW. Molecular mechanism by which the K70E mutation in human immunodeficiency virus type 1 reverse transcriptase confers resistance to nucleoside reverse transcriptase inhibitors. Antimicrob Agents Chemother. 2007;51:48-53. [PubMed Abstract] -

65. Melikian GL, Rhee SY, Taylor J, et al. Standardized comparison of the relative impacts of HIV-1 reverse transcriptase (RT) mutations on nucleoside RT inhibitor susceptibility. Antimicrob Agents Chemother. 2012;56:2305-13. [PubMed Abstract] -

66. Parikh UM, Bacheler L, Koontz D, Mellors JW. The K65R mutation in human immunodeficiency virus type 1 reverse transcriptase exhibits bidirectional phenotypic antagonism with thymidine analog mutations. J Virol. 2006;80:4971-7. [PubMed Abstract] -

67. Parikh UM, Zelina S, Sluis-Cremer N, Mellors JW. Molecular mechanisms of bidirectional antagonism between K65R and thymidine analog mutations in HIV-1 reverse transcriptase. AIDS. 2007;21:1405-14. [PubMed Abstract] -

68. Gallant JE, Rodriguez AE, Weinberg WG, et al. Early virologic nonresponse to tenofovir, abacavir, and lamivudine in HIV-infected antiretroviral-naive subjects. J Infect Dis. 2005;192:1921-30. [PubMed Abstract] -

69. White KL, Margot NA, Wrin T, Petropoulos CJ, Miller MD, Naeger LK. Molecular mechanisms of resistance to human immunodeficiency virus type 1 with reverse transcriptase mutations K65R and K65R+M184V and their effects on enzyme function and viral replication capacity. Antimicrob Agents Chemother. 2002;46:3437-46. [PubMed Abstract] -

70. Moyle G. Resistance and cross-resistance to abacavir. HIV Med. 2001;2:154-62. [PubMed Abstract] -

71. Wang J, Li D, Bambara RA, Yang H, Dykes C. L74V increases the reverse transcriptase content of HIV-1 virions with non-nucleoside reverse transcriptase drug-resistant mutations L100I+K103N and K101E+G190S, which results in increased fitness. J Gen Virol. 2013;94:1597-607. [PubMed Abstract] -

72. White KL, Chen JM, Margot NA, et al. Molecular mechanisms of tenofovir resistance conferred by human immunodeficiency virus type 1 reverse transcriptase containing a diserine insertion after residue 69 and multiple thymidine analog-associated mutations. Antimicrob Agents Chemother. 2004;48:992-1003. [PubMed Abstract] -

73. Winters MA, Coolley KL, Girard YA, et al. A 6-basepair insert in the reverse transcriptase gene of human immunodeficiency virus type 1 confers resistance to multiple nucleoside inhibitors. J Clin

Invest. 1998;102:1769-75.
[PubMed Abstract] -

74. Scherrer AU, von Wyl V, Götte M, et al. Polymorphic mutations associated with the emergence of the multinucleoside/tide resistance mutations 69 insertion and Q151M. J Acquir Immune Defic Syndr. 2012;59:105-12.
[PubMed Abstract] -

75. Gupta R, Hill A, Sawyer AW, Pillay D. Emergence of drug resistance in HIV type 1-infected patients after receipt of first-line highly active antiretroviral therapy: a systematic review of clinical trials. Clin Infect Dis. 2008;47:712-22.
[PubMed Abstract] -

76. Eron JJ, Benoit SL, Jemsek J, et al. Treatment with lamivudine, zidovudine, or both in HIV-positive patients with 200 to 500 CD4+ cells per cubic millimeter. North American HIV Working Party. N Engl J Med. 1995;333:1662-9.
[PubMed Abstract] -

77. Castagna A, Danise A, Menzo S, et al. Lamivudine monotherapy in HIV-1-infected patients harbouring a lamivudine-resistant virus: a randomized pilot study (E-184V study). AIDS. 2006;20:795-803.
[PubMed Abstract] -

78. Petrella M, Wainberg MA. Might the M184V substitution in HIV-1 RT confer clinical benefit? AIDS Rev. 2002;4:224-32.
[PubMed Abstract] -

79. Trignetti M, Sing T, Svicher V, et al. Dynamics of NRTI resistance mutations during therapy interruption. AIDS Res Hum Retroviruses. 2009;25:57-64.
[PubMed Abstract] -

80. Kulkarni R, Babaoglu K, Lansdon EB, et al. The HIV-1 reverse transcriptase M184I mutation enhances the E138K-associated resistance to rilpivirine and decreases viral fitness. J Acquir Immune Defic Syndr. 2012;59:47-54.
[PubMed Abstract] -

81. Ross L, Parkin N, Chappey C, et al. Phenotypic impact of HIV reverse transcriptase M184I/V mutations in combination with single thymidine analog mutations on nucleoside reverse transcriptase inhibitor resistance. AIDS. 2004 Aug;18:1691-6.
[PubMed Abstract] -

82. Richman DD, Havlir D, Corbeil J, et al. Nevirapine resistance mutations of human immunodeficiency virus type 1 selected during therapy. J Virol. 1994;68:1660-6.
[PubMed Abstract] -

83. Molina JM, Squires K, Sax PE, et al. Doravirine versus ritonavir-boosted darunavir in antiretroviral-naive adults with HIV-1 (DRIVE-FORWARD): 48-week results of a randomised, double-blind, phase 3, non-inferiority trial. Lancet HIV. 2018;5:e211-e220.
[PubMed Abstract] -

84. Orkin C, Squires KE, Molina JM, et al. Doravirine/Lamivudine/Tenofovir Disoproxil Fumarate is Non-inferior to Efavirenz/Emtricitabine/Tenofovir Disoproxil Fumarate in Treatment-naive Adults With Human Immunodeficiency Virus-1 Infection: Week 48 Results of the DRIVE-AHEAD Trial. Clin Infect Dis. 2019;68:535-44.
[PubMed Abstract] -

85. Colombier MA, Molina JM. Doravirine: a review. Curr Opin HIV AIDS. 2018;13:308-314.
[PubMed Abstract] -

86. 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.
[PubMed Abstract] -

87. Feng M, Sachs NA, Xu M, et al. Doravirine Suppresses Common Nonnucleoside Reverse Transcriptase Inhibitor-Associated Mutants at Clinically Relevant Concentrations. Antimicrob Agents Chemother. 2016;60:2241-7.
[PubMed Abstract] -

88. Riddler SA, Haubrich R, DiRienzo AG, et al. Class-sparing regimens for initial treatment of HIV-1 infection. N Engl J Med. 2008;358:2095-106.
[PubMed Abstract] -

89. Bacheler L, Jeffrey S, Hanna G, et al. Genotypic correlates of phenotypic resistance to efavirenz in virus isolates from patients failing nonnucleoside reverse transcriptase inhibitor therapy. J Virol. 2001;75:4999-5008.
[PubMed Abstract] -

90. Vingerhoets J, Tambuyzer L, Azijn H, et al. Resistance profile of etravirine: combined analysis of baseline genotypic and phenotypic data from the randomized, controlled Phase III clinical studies. AIDS. 2010;24:503-14.
[PubMed Abstract] -

91. Delaugerre C, Rohban R, Simon A, et al. Resistance profile and cross-resistance of HIV-1 among patients failing a non-nucleoside reverse transcriptase inhibitor-containing regimen. J Med Virol. 2001;65:445-8.
[PubMed Abstract] -

92. Rimsky L, Van Eygen V, Hoogstoel A, et al. 96-Week resistance analyses of rilpivirine in treatment-naive, HIV-1-infected adults from the ECHO and THRIVE Phase III trials. Antivir Ther. 2013;18:967-77.
[PubMed Abstract] -

93. Rossotti R, Fonte L, Meini G, Maggiolo F, Zazzi M, Rusconi S. Rilpivirine resistance and the dangerous liaisons with substitutions at position 184 among patients infected with HIV-1: analysis from a national drug-resistance database (ARCA). J Med Virol. 2014;86:1459-66.
[PubMed Abstract] -

94. Xu HT, Colby-Germinario SP, Asahchop EL, et al. Effect of mutations at position E138 in HIV-1 reverse transcriptase and their interactions with the M184I mutation on defining patterns of resistance to nonnucleoside reverse transcriptase inhibitors rilpivirine and etravirine. Antimicrob Agents Chemother. 2013;57:3100-9.
[PubMed Abstract] -

95. Xu HT, Colby-Germinario SP, Huang W, et al. Role of the K101E substitution in HIV-1 reverse transcriptase in resistance to rilpivirine and other nonnucleoside reverse transcriptase inhibitors. Antimicrob Agents Chemother. 2013;57:5649-57.
[PubMed Abstract] -

96. Sluis-Cremer N. The emerging profile of cross-resistance among the nonnucleoside HIV-1 reverse

transcriptase inhibitors. Viruses. 2014;6:2960-73.
[PubMed Abstract] -

97. Rokx C, Verbon A, Rijnders BJ. Successful switch to rilpivirine/tenofovir/emtricitabine in HIV-1-infected patients with an isolated K103N mutation acquired during prior nonnucleoside reverse transcriptase inhibitor therapy. HIV Med. 2014;15:611-4.
[PubMed Abstract] -

98. Gallien S, Charreau I, Nere ML, et al. Archived HIV-1 DNA resistance mutations to rilpivirine and etravirine in successfully treated HIV-1-infected individuals pre-exposed to efavirenz or nevirapine. J Antimicrob Chemother. 2015;70:562-5.
[PubMed Abstract] -

99. Deeks SG. International perspectives on antiretroviral resistance. Nonnucleoside reverse transcriptase inhibitor resistance. J Acquir Immune Defic Syndr. 2001;26 Suppl 1:S25-33.
[PubMed Abstract] -

100. Antinori A, Zaccarelli M, Cingolani A, et al. Cross-resistance among nonnucleoside reverse transcriptase inhibitors limits recycling efavirenz after nevirapine failure. AIDS Res Hum Retroviruses. 2002;18:835-8.
[PubMed Abstract] -

101. Casado JL, Moreno A, Hertogs K, Dronda F, Moreno S. Extent and importance of cross-resistance to efavirenz after nevirapine failure. AIDS Res Hum Retroviruses. 2002;18:771-5.
[PubMed Abstract] -

102. Hanna GJ, Johnson VA, Kuritzkes DR, et al. Patterns of resistance mutations selected by treatment of human immunodeficiency virus type 1 infection with zidovudine, didanosine, and nevirapine. J Infect Dis. 2000;181:904-11.
[PubMed Abstract] -

103. Shulman NS, Zolopa AR, Passaro DJ, et al. Efavirenz- and adefovir dipivoxil-based salvage therapy in highly treatment-experienced patients: clinical and genotypic predictors of virologic response. J Acquir Immune Defic Syndr. 2000;23:221-6.
[PubMed Abstract] -

104. Melikian GL, Rhee SY, Varghese V, et al. Non-nucleoside reverse transcriptase inhibitor (NNRTI) cross-resistance: implications for preclinical evaluation of novel NNRTIs and clinical genotypic resistance testing. J Antimicrob Chemother. 2014;69:12-20.
[PubMed Abstract] -

105. Xu HT, Asahchop EL, Oliveira M, et al. Compensation by the E138K mutation in HIV-1 reverse transcriptase for deficits in viral replication capacity and enzyme processivity associated with the M184I/V mutations. J Virol. 2011;85:11300-8.
[PubMed Abstract] -

106. Pommier Y, Johnson AA, Marchand C. Integrase inhibitors to treat HIV/AIDS. Nat Rev Drug Discov. 2005;4:236-48.
[PubMed Abstract] -

107. Hurt CB, Sebastian J, Hicks CB, Eron JJ. Resistance to HIV integrase strand transfer inhibitors among clinical specimens in the United States, 2009-2012. Clin Infect Dis. 2014;58:423-31.
[PubMed Abstract] -

108. Malet I, Delelis O, Valantin MA, et al. Mutations associated with failure of raltegravir treatment affect integrase sensitivity to the inhibitor in vitro. Antimicrob Agents Chemother. 2008;52:1351-8. [PubMed Abstract] -

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

110. Smith SJ, Zhao XZ, Burke TR Jr, Hughes SH. Efficacies of Cabotegravir and Bictegravir against drug-resistant HIV-1 integrase mutants. Retrovirology. 2018;15:37. [PubMed Abstract] -

111. Zhao AV, Crutchley RD, Guduru RC, Ton K, Lam T, Min AC. A clinical review of HIV integrase strand transfer inhibitors (INSTIs) for the prevention and treatment of HIV-1 infection. Retrovirology. 2022;19:22. [PubMed Abstract] -

112. Oliveira M, Ibanescu RI, Anstett K, et al. Selective resistance profiles emerging in patient-derived clinical isolates with cabotegravir, bictegravir, dolutegravir, and elvitegravir. Retrovirology. 2018;15:56. [PubMed Abstract] -

113. Orkin C, Arasteh K, Górgolas Hernández-Mora M, et al. Long-Acting Cabotegravir and Rilpivirine after Oral Induction for HIV-1 Infection. N Engl J Med. 2020;382:1124-35. [PubMed Abstract] -

114. Overton ET, Richmond G, Rizzardini G, et al. Long-acting cabotegravir and rilpivirine dosed every 2 months in adults with HIV-1 infection (ATLAS-2M), 48-week results: a randomised, multicentre, open-label, phase 3b, non-inferiority study. Lancet. 2021;396:1994-2005. [PubMed Abstract] -

115. Swindells S, Andrade-Villanueva JF, Richmond GJ, et al. Long-Acting Cabotegravir and Rilpivirine for Maintenance of HIV-1 Suppression. N Engl J Med. 2020;382:1112-23. [PubMed Abstract] -

116. Landovitz RJ, Donnell D, Clement ME, et al. N Engl J Med. 2021;385:595-608. [PubMed Abstract] -

117. Lübke N, Jensen B, Hüttig F, et al. Failure of Dolutegravir First-Line ART with Selection of Virus Carrying R263K and G118R. N Engl J Med. 2019;381:887-9. [PubMed Abstract] -

118. Pena MJ, Chueca N, D'Avolio A, Zarzalejos JM, Garcia F. Virological Failure in HIV to Triple Therapy With Dolutegravir-Based Firstline Treatment: Rare but Possible. Open Forum Infect Dis. 2019;6:ofy332. [PubMed Abstract] -

119. Cardoso M, Baptista T, Diogo I, et al. Two cases of dolutegravir failure with R263K mutation. AIDS. 2018;32:2639-40. [PubMed Abstract] -

120. Cahn P, Pozniak AL, Mingrone H, et al. Dolutegravir versus raltegravir in antiretroviral-experienced, integrase-inhibitor-naive adults with HIV: week 48 results from the randomised, double-blind, non-inferiority SAILING study. Lancet. 2013;382:700-8. [PubMed Abstract] -

121. Wijting IEA, Lungu C, Rijnders BJA, et al. HIV-1 Resistance Dynamics in Patients With Virologic Failure to Dolutegravir Maintenance Monotherapy. J Infect Dis. 2018;218:688-97.
[PubMed Abstract] -

122. Brenner BG, Wainberg MA. Clinical benefit of dolutegravir in HIV-1 management related to the high genetic barrier to drug resistance. Virus Res. 2017;239:1-9.
[PubMed Abstract] -

123. Wainberg MA, Han YS. HIV-1 resistance to dolutegravir: update and new insights. J Virus Erad. 2015;1:13-6.
[PubMed Abstract] -

124. Wainberg MA, Han YS. Will drug resistance against dolutegravir in initial therapy ever occur? Front Pharmacol. 2015;6:90.
[PubMed Abstract] -

125. Cevik M, Orkin C, Sax PE. Emergent Resistance to Dolutegravir Among INSTI-Naïve Patients on First-line or Second-line Antiretroviral Therapy: A Review of Published Cases. Open Forum Infect Dis. 2020;7:ofaa202.
[PubMed Abstract] -

126. Fulcher JA, Du Y, Zhang TH, Sun R, Landovitz RJ. Emergence of Integrase Resistance Mutations During Initial Therapy Containing Dolutegravir. Clin Infect Dis. 2018;67:791-4.
[PubMed Abstract] -

127. Anstett K, Mesplede T, Oliveira M, Cutillas V, Wainberg MA. Dolutegravir resistance mutation R263K cannot coexist in combination with many classical integrase inhibitor resistance substitutions. J Virol. 2015;89:4681-4.
[PubMed Abstract] -

128. Pham HT, Labrie L, Wijting IEA, et al. The S230R Integrase Substitution Associated With Virus Load Rebound During Dolutegravir Monotherapy Confers Low-Level Resistance to Integrase Strand-Transfer Inhibitors. J Infect Dis. 2018;218:698-706.
[PubMed Abstract] -

129. Fourati S, Charpentier C, Amiel C, et al. Cross-resistance to elvitegravir and dolutegravir in 502 patients failing on raltegravir: a French national study of raltegravir-experienced HIV-1-infected patients. J Antimicrob Chemother. 2015;70:1507-12.
[PubMed Abstract] -

130. Castagna A, Maggiolo F, Penco G, et al. Dolutegravir in antiretroviral-experienced patients with raltegravir- and/or elvitegravir-resistant HIV-1: 24-week results of the phase III VIKING-3 study. J Infect Dis. 2014;210:354-62.
[PubMed Abstract] -

131. Hardy I, Brenner B, Quashie P, et al. Evolution of a novel pathway leading to dolutegravir resistance in a patient harbouring N155H and multiclass drug resistance. J Antimicrob Chemother. 2015;70:405-11.
[PubMed Abstract] -

132. Goethals O, Clayton R, Van Ginderen M, et al. Resistance mutations in human immunodeficiency virus type 1 integrase selected with elvitegravir confer reduced susceptibility to a wide range of integrase inhibitors. J Virol. 2008;82:10366-74.
[PubMed Abstract] -

133. Garrido C, Villacian J, Zahonero N, et al. Broad phenotypic cross-resistance to elvitegravir in HIV-infected patients failing on raltegravir-containing regimens. Antimicrob Agents Chemother. 2012;56:2873-8.
[PubMed Abstract] -

134. Van Wesenbeeck L, Rondelez E, Feyaerts M, et al. Cross-resistance profile determination of two second-generation HIV-1 integrase inhibitors using a panel of recombinant viruses derived from raltegravir-treated clinical isolates. Antimicrob Agents Chemother. 2011;55:321-5.
[PubMed Abstract] -

135. Delelis O, Thierry S, Subra F, et al. Impact of Y143 HIV-1 integrase mutations on resistance to raltegravir in vitro and in vivo. Antimicrob Agents Chemother. 2010;54:491-501.
[PubMed Abstract] -

136. Malet I, Gimferrer Arriaga L, Artese A, et al. New raltegravir resistance pathways induce broad cross-resistance to all currently used integrase inhibitors. J Antimicrob Chemother. 2014;69:2118-22.
[PubMed Abstract] -

137. Malet I, Thierry E, Wirden M, et al. Combination of two pathways involved in raltegravir resistance confers dolutegravir resistance. J Antimicrob Chemother. 2015;70:2870-80.
[PubMed Abstract] -

138. Blanco JL, Varghese V, Rhee SY, Gatell JM, Shafer RW. HIV-1 integrase inhibitor resistance and its clinical implications. J Infect Dis. 2011;203:1204-14.
[PubMed Abstract] -

139. 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.
[PubMed Abstract] -

140. Rabi SA, Laird GM, Durand CM, et al. Multi-step inhibition explains HIV-1 protease inhibitor pharmacodynamics and resistance. J Clin Invest. 2013;123:3848-60.
[PubMed Abstract] -

141. Clutter DS, Jordan MR, Bertagnolio S, Shafer RW. HIV-1 drug resistance and resistance testing. Infect Genet Evol. 2016;46:292-307.
[PubMed Abstract] -

142. Molina JM, Clotet B, van Lunzen J, et al. Once-daily dolutegravir is superior to once-daily darunavir/ritonavir in treatment-naïve HIV-1-positive individuals: 96 week results from FLAMINGO. J Int AIDS Soc. 2014;17:19490.
[PubMed Abstract] -

143. Young TP, Parkin NT, Stawiski E, et al. Prevalence, mutation patterns, and effects on protease inhibitor susceptibility of the L76V mutation in HIV-1 protease. Antimicrob Agents Chemother. 2010;54:4903-6.
[PubMed Abstract] -

144. Lambert-Niclot S, George EC, Pozniak A, et al. Antiretroviral resistance at virological failure in the NEAT 001/ANRS 143 trial: raltegravir plus darunavir/ritonavir or tenofovir/emtricitabine plus darunavir/ritonavir as first-line ART. J Antimicrob Chemother. 2016;71:1056-62.
[PubMed Abstract] -

145. Lathouwers E, De Meyer S, Dierynck I, et al. Virological characterization of patients failing

darunavir/ritonavir or lopinavir/ritonavir treatment in the ARTEMIS study: 96-week analysis. Antivir Ther. 2011;16:99-108.
[PubMed Abstract] -

146. Lathouwers E, Gupta S, Haddad M, Paquet A, de Meyer S, Baugh B. Trends in darunavir resistance-associated mutations and phenotypic resistance in commercially tested United States clinical samples between 2006 and 2012. AIDS Res Hum Retroviruses. 2015;31:628-35.
[PubMed Abstract] -

147. De Meyer S, Azijn H, Surleraux D, et al. TMC114, a novel human immunodeficiency virus type 1 protease inhibitor active against protease inhibitor-resistant viruses, including a broad range of clinical isolates. Antimicrob Agents Chemother. 2005;49:2314-21.
[PubMed Abstract] -

148. de Meyer S, Vangeneugden T, van Baelen B, et al. Resistance profile of darunavir: combined 24-week results from the POWER trials. AIDS Res Hum Retroviruses. 2008;24:379-88.
[PubMed Abstract] -

149. Mitsuya Y, Liu TF, Rhee SY, Fessel WJ, Shafer RW. Prevalence of darunavir resistance-associated mutations: patterns of occurrence and association with past treatment. J Infect Dis. 2007;196:1177-9.
[PubMed Abstract] -

150. Pozniak A, Opravil M, Beatty G, Hill A, de Béthune MP, Lefebvre E. Effect of baseline viral susceptibility on response to darunavir/ritonavir versus control protease inhibitors in treatment-experienced HIV type 1-infected patients: POWER 1 and 2. AIDS Res Hum Retroviruses. 2008;24:1275-80.
[PubMed Abstract] -

151. Lathouwers E, De La Rosa G, Van de Casteele T, et al. Virological analysis of once-daily and twice-daily darunavir/ritonavir in the ODIN trial of treatment-experienced patients. Antivir Ther. 2013;18:289-300.
[PubMed Abstract] -

152. De Meyer SM, Spinosa-Guzman S, Vangeneugden TJ, de Béthune MP, Miralles GD. Efficacy of once-daily darunavir/ritonavir 800/100 mg in HIV-infected, treatment-experienced patients with no baseline resistance-associated mutations to darunavir. J Acquir Immune Defic Syndr. 2008;49:179-82.
[PubMed Abstract] -

153. Cahn P, Fourie J, Grinsztein B et al. Week 48 analysis of once-daily vs. twice daily darunavir/ritonavir in treatment-experienced HIV-1-infected patients. AIDS 2011; 25:929-39.
[PubMed Abstract] -

154. Kempf DJ, Isaacson JD, King MS, et al. Analysis of the virological response with respect to baseline viral phenotype and genotype in protease inhibitor-experienced HIV-1-infected patients receiving lopinavir/ritonavir therapy. Antivir Ther. 2002;7:165-74.
[PubMed Abstract] -

155. Kempf DJ, Isaacson JD, King MS, et al. Identification of genotypic changes in human immunodeficiency virus protease that correlate with reduced susceptibility to the protease inhibitor lopinavir among viral isolates from protease inhibitor-experienced patients. J Virol. 2001;75:7462-9.
[PubMed Abstract] -

156. Molina JM, Andrade-Villanueva J, Echevarria J, et al. Once-daily atazanavir/ritonavir versus twice-daily lopinavir/ritonavir, each in combination with tenofovir and emtricitabine, for management of antiretroviral-naive HIV-1-infected patients: 48 week efficacy and safety results of the CASTLE study. Lancet. 2008;372(9639):646-55.

[PubMed Abstract] -

157. Mo H, King MS, King K, Molla A, Brun S, Kempf DJ. Selection of resistance in protease inhibitor-experienced, human immunodeficiency virus type 1-infected subjects failing lopinavir- and ritonavir-based therapy: mutation patterns and baseline correlates. J Virol. 2005;79:3329-38.
[PubMed Abstract] -

158. Chandwani A, Shuter J. Lopinavir/ritonavir in the treatment of HIV-1 infection: a review. Ther Clin Risk Manag. 2008;4:1023-33.
[PubMed Abstract] -

159. Paulsen D, Liao Q, Fusco G, St Clair M, Shaefer M, Ross L. Genotypic and phenotypic cross-resistance patterns to lopinavir and amprenavir in protease inhibitor-experienced patients with HIV viremia. AIDS Res Hum Retroviruses. 2002;18:1011-9.
[PubMed Abstract] -

160. Gartland M, Cahn P, DeJesus E, et al. Week 96 Genotypic and Phenotypic Results of the Fostemsavir Phase 3 BRIGHTE Study in Heavily Treatment-Experienced Adults Living with Multidrug-Resistant HIV-1. Antimicrob Agents Chemother. 2022;66:e0175121.
[PubMed Abstract] -

161. Lataillade M, Zhou N, Joshi SR, et al. Viral Drug Resistance Through 48 Weeks, in a Phase 2b, Randomized, Controlled Trial of the HIV-1 Attachment Inhibitor Prodrug, Fostemsavir. J Acquir Immune Defic Syndr. 2018;77:299-307.
[PubMed Abstract] -

162. Rose R, Gartland M, Li Z, et al. Clinical evidence for a lack of cross-resistance between temsavir and ibalizumab or maraviroc. AIDS. 2022;36:11-18.
[PubMed Abstract] -

163. Woollard SM, Kanmogne GD. Maraviroc: a review of its use in HIV infection and beyond. Drug Des Devel Ther. 2015;9:5447-68.
[PubMed Abstract] -

164. Westby M, Lewis M, Whitcomb J, et al. Emergence of CXCR4-using human immunodeficiency virus type 1 (HIV-1) variants in a minority of HIV-1-infected patients following treatment with the CCR5 antagonist maraviroc is from a pretreatment CXCR4-using virus reservoir. J Virol. 2006;80:4909-20.
[PubMed Abstract] -

165. Trkola A, Kuhmann SE, Strizki JM, et al. HIV-1 escape from a small molecule, CCR5-specific entry inhibitor does not involve CXCR4 use. Proc Natl Acad Sci U S A. 2002;99:395-400.
[PubMed Abstract] -

166. Roche M, Salimi H, Duncan R, et al. A common mechanism of clinical HIV-1 resistance to the CCR5 antagonist maraviroc despite divergent resistance levels and lack of common gp120 resistance mutations. Retrovirology. 2013;10:43.
[PubMed Abstract] -

167. 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. Laboratory testing: co-receptor tropism assays. October 25, 2018.
[HIV.gov] -

168. Esté JA, Telenti A. HIV entry inhibitors. Lancet. 2007;370:81-8.

[PubMed Abstract] -

169. Kilby JM, Eron JJ. Novel therapies based on mechanisms of HIV-1 cell entry. N Engl J Med. 2003;348:2228-38.
[PubMed Abstract] -

170. Bester SM, Wei G, Zhao H, et al. Structural and mechanistic bases for a potent HIV-1 capsid inhibitor. Science. 2020;370:360-4.
[PubMed Abstract] -

171. Margot N, Vanderveen L, Naik V, et al. Phenotypic resistance to lenacapavir and monotherapy efficacy in a proof-of-concept clinical study. J Antimicrob Chemother. 2022;77:989-95.
[PubMed Abstract] -

172. Margot N, Ram R, Rhee M, Callebaut C. Absence of Lenacapavir (GS-6207) Phenotypic Resistance in HIV Gag Cleavage Site Mutants and in Isolates with Resistance to Existing Drug Classes. Antimicrob Agents Chemother. 2021;65(3):e02057-20.
[PubMed Abstract] -

References

- Deeks SG, Lu J, Hoh R, et al. Interruption of enfuvirtide in HIV-1 infected adults with incomplete viral suppression on an enfuvirtide-based regimen. J Infect Dis. 2007;195:387-91.
[PubMed Abstract] -

- Eron JJ, Clotet B, Durant J, et al. Safety and efficacy of dolutegravir in treatment-experienced subjects with raltegravir-resistant HIV type 1 infection: 24-week results of the VIKING Study. J Infect Dis. 2013;207:740-8.
[PubMed Abstract] -

- Gonzalez-Serna A, Min JE, Woods C, et al. Performance of HIV-1 drug resistance testing at low-level viremia and its ability to predict future virologic outcomes and viral evolution in treatment-naive individuals. Clin Infect Dis. 2014;58:1165-73.
[PubMed Abstract] -

- Grennan JT, Loutfy MR, Su D, et al. Magnitude of virologic blips is associated with a higher risk for virologic rebound in HIV-infected individuals: a recurrent events analysis. J Infect Dis. 2012;205:1230-8.
[PubMed Abstract] -

- Hu Z, Kuritzkes DR. Interaction of reverse transcriptase (RT) mutations conferring resistance to lamivudine and etravirine: effects on fitness and RT activity of human immunodeficiency virus type 1. J Virol. 2011;85:11309-14.
[PubMed Abstract] -

- Jiang X, Feyertag F, Meehan CJ, et al. Characterizing the Diverse Mutational Pathways Associated with R5-Tropic Maraviroc Resistance: HIV-1 That Uses the Drug-Bound CCR5 Coreceptor. J Virol. 2015;89:11457-72.
[PubMed Abstract] -

- Letendre SL, Mills AM, Tashima KT, et al. ING116070: a study of the pharmacokinetics and antiviral activity of dolutegravir in cerebrospinal fluid in HIV-1-infected, antiretroviral therapy-naive subjects. Clin Infect Dis. 2014;59:1032-7.

[PubMed Abstract] -

- Ly JK, Margot NA, MacArthur HL, Hung M, Miller MD, White KL. The balance between NRTI discrimination and excision drives the susceptibility of HIV-1 RT mutants K65R, M184V and K65r+M184V. Antivir Chem Chemother. 2007;18:307-16.
[PubMed Abstract] -

- Margot NA, Liu Y, Miller MD, Callebaut C. High resistance barrier to tenofovir alafenamide is driven by higher loading of tenofovir diphosphate into target cells compared to tenofovir disoproxil fumarate. Antiviral Res. 2016;132:50-58.
[PubMed Abstract] -

- Munir S, Thierry E, Malet I, et al. G118R and F121Y mutations identified in patients failing raltegravir treatment confer dolutegravir resistance. J Antimicrob Chemother. 2015;70:739-49.
[PubMed Abstract] -

- Pace CS, Fordyce MW, Franco D, Kao CY, Seaman MS, Ho DD. Anti-CD4 monoclonal antibody ibalizumab exhibits breadth and potency against HIV-1, with natural resistance mediated by the loss of a V5 glycan in envelope. J Acquir Immune Defic Syndr. 2013;62:1-9.
[PubMed Abstract] -

- Roche M, Borm K, Flynn JK, Lewin SR, Churchill MJ, Gorry PR. Molecular Gymnastics: Mechanisms of HIV-1 Resistance to CCR5 Antagonists and Impact on Virus Phenotypes. Curr Top Med Chem. 2016;16:1091-106.
[PubMed Abstract] -

- Singhroy DN, Wainberg MA, Mesplède T. Combination of the R263K and M184I/V resistance substitutions against dolutegravir and lamivudine decreases HIV replicative capacity. Antimicrob Agents Chemother. 2015;59:2882-5.
[PubMed Abstract] -

- Smith SJ, Pauly GT, Akram A, et al. Rilpivirine and Doravirine Have Complementary Efficacies Against NNRTI-Resistant HIV-1 Mutants. J Acquir Immune Defic Syndr. 2016;72:485-91.
[PubMed Abstract] -

- Tambuyzer L, Vingerhoets J, Azijn H, et al. Characterization of genotypic and phenotypic changes in HIV-1-infected patients with virologic failure on an etravirine-containing regimen in the DUET-1 and DUET-2 clinical studies. AIDS Res Hum Retroviruses. 2010;26:1197-205.
[PubMed Abstract] -

- Vingerhoets J, Nijs S, Tambuyzer L, Hoogstoel A, Anderson D, Picchio G. Similar predictions of etravirine sensitivity regardless of genotypic testing method used: comparison of available scoring systems. Antivir Ther. 2012;17:1571-9.
[PubMed Abstract] -

- Wainberg MA, Zaharatos GJ, Brenner BG. Development of antiretroviral drug resistance. N Engl J Med. 2011;365:637-46.
[PubMed Abstract] -

Figures

Figure 1 High Error Rate with HIV Reverse Transcription

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

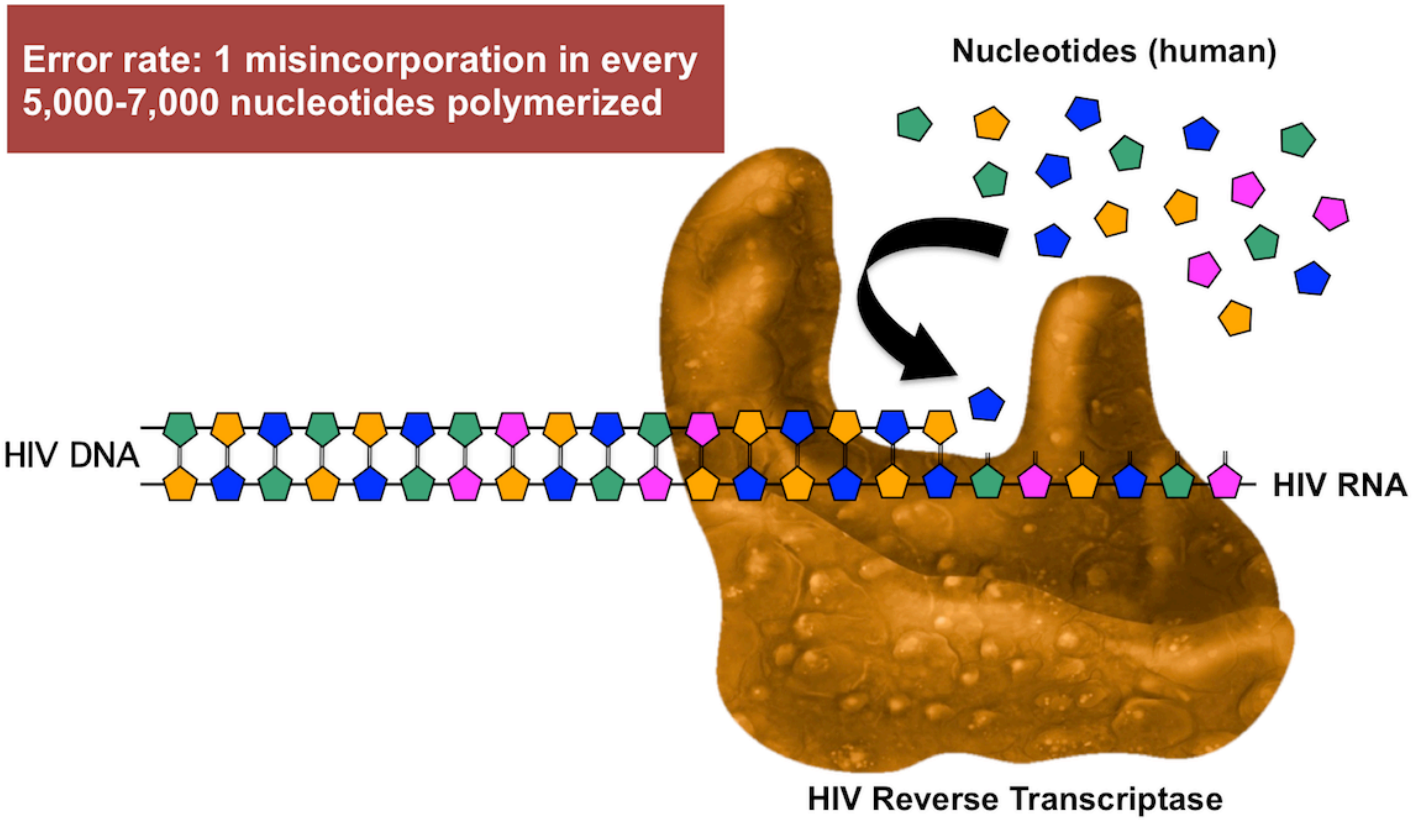


Figure 2 HIV Resistance Basic Concepts

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 H. Spach, MD

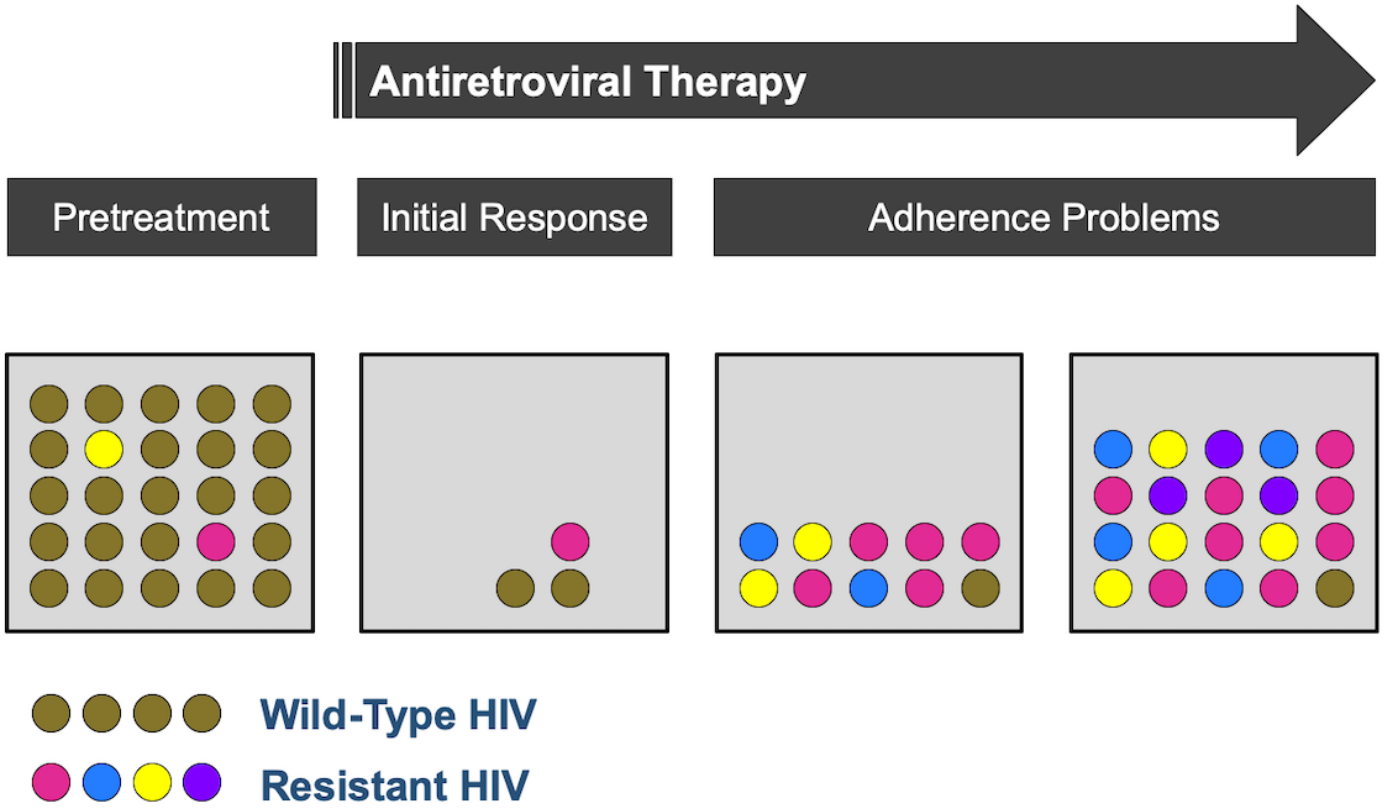


Figure 3 Virologic Suppression

Illustration: David H. Spach, MD

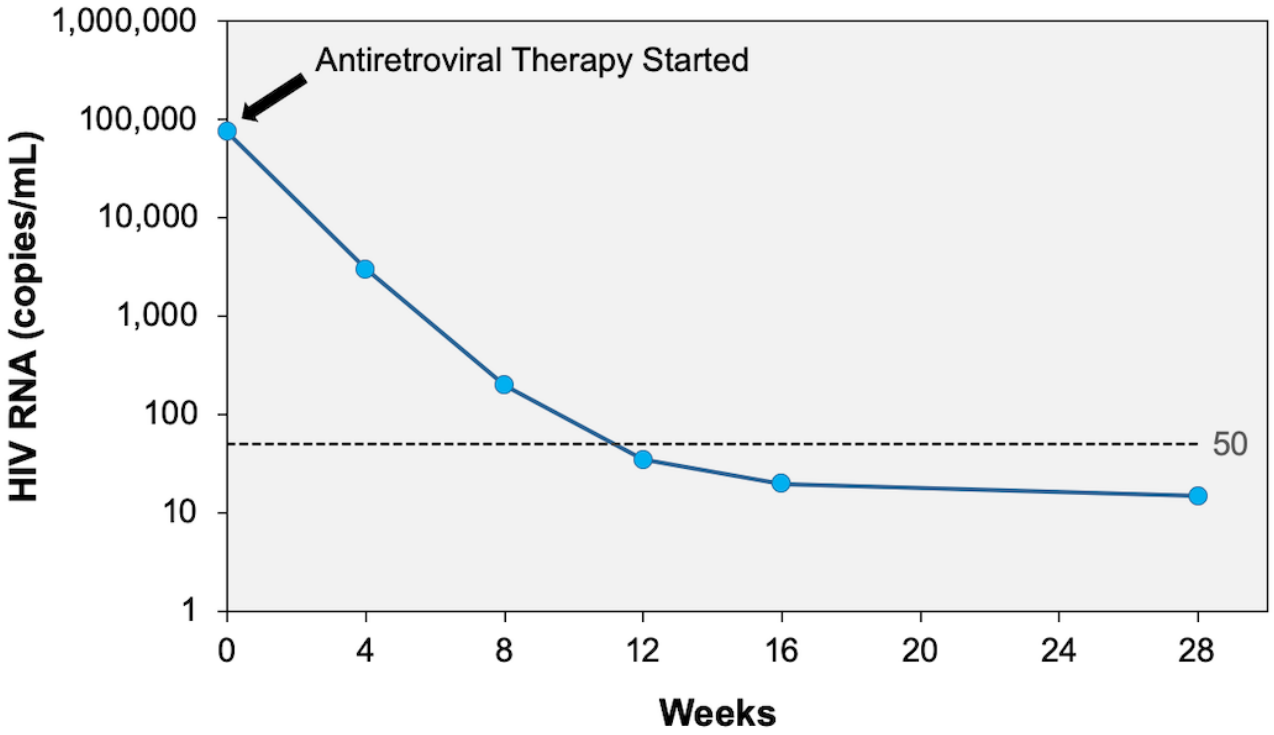


Figure 4 Incomplete Virologic Response

Illustration: David H. Spach, MD

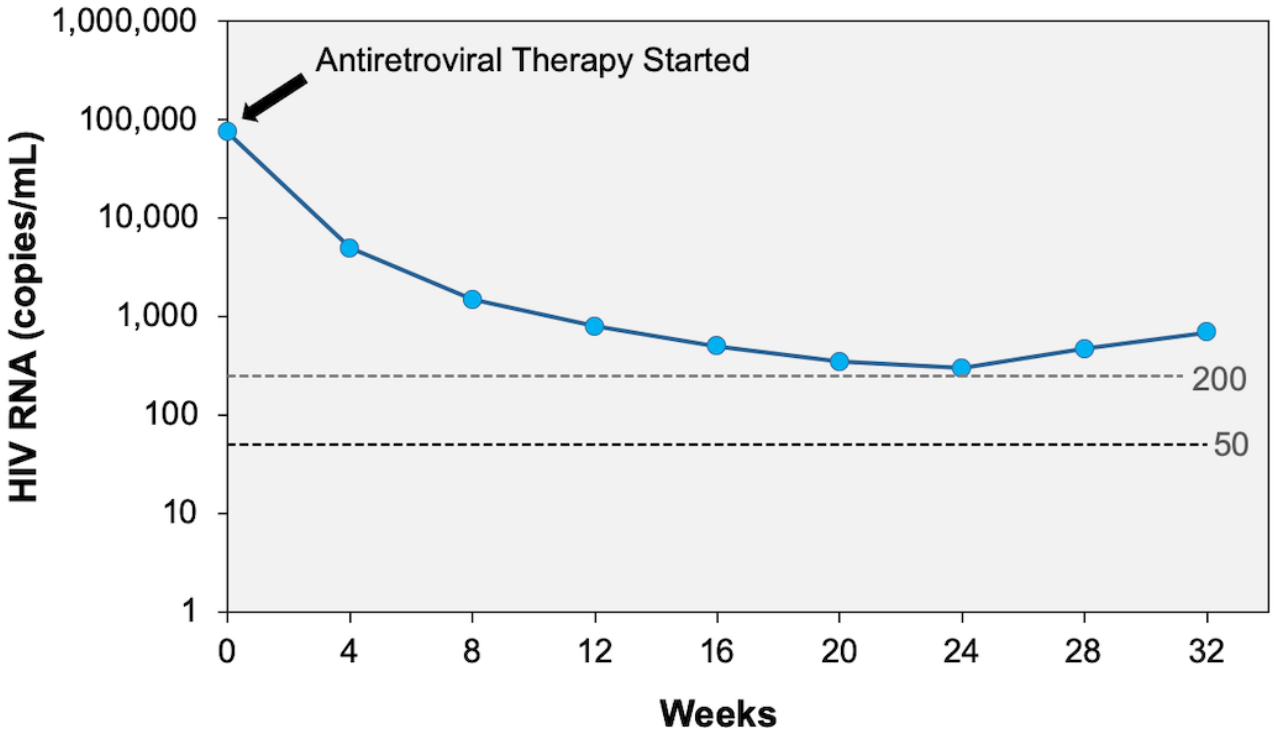


Figure 5 Virologic Rebound

Illustration: David H. Spach, MD

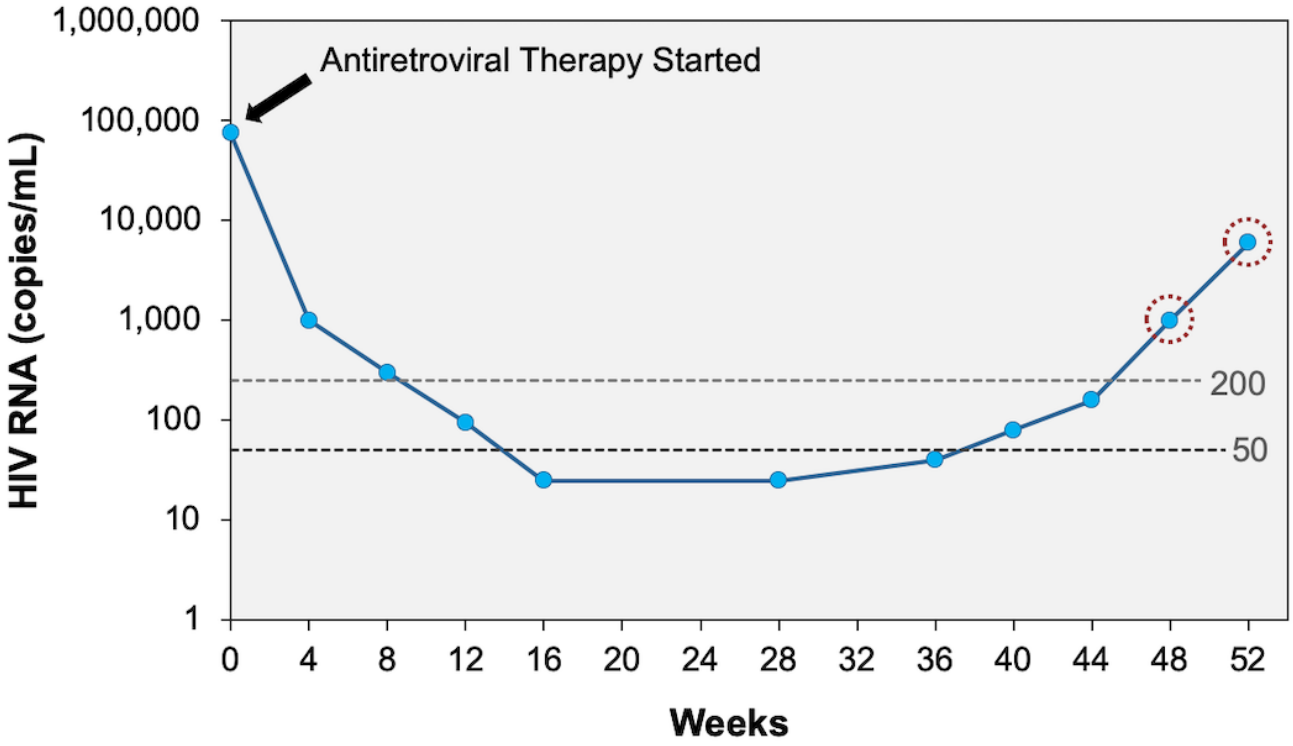


Figure 6 Virologic Blip

Illustration: David H. Spach, MD

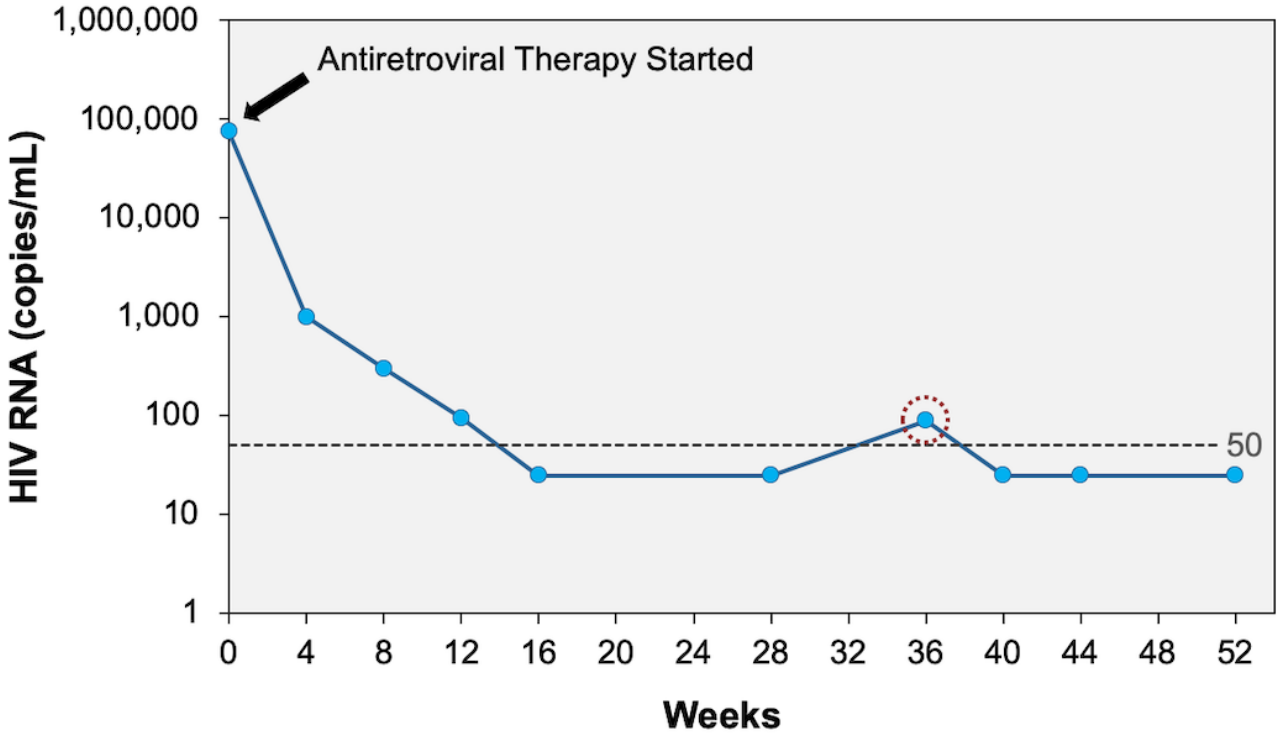


Figure 7 Low-Level Viremia

Illustration: David H. Spach, MD

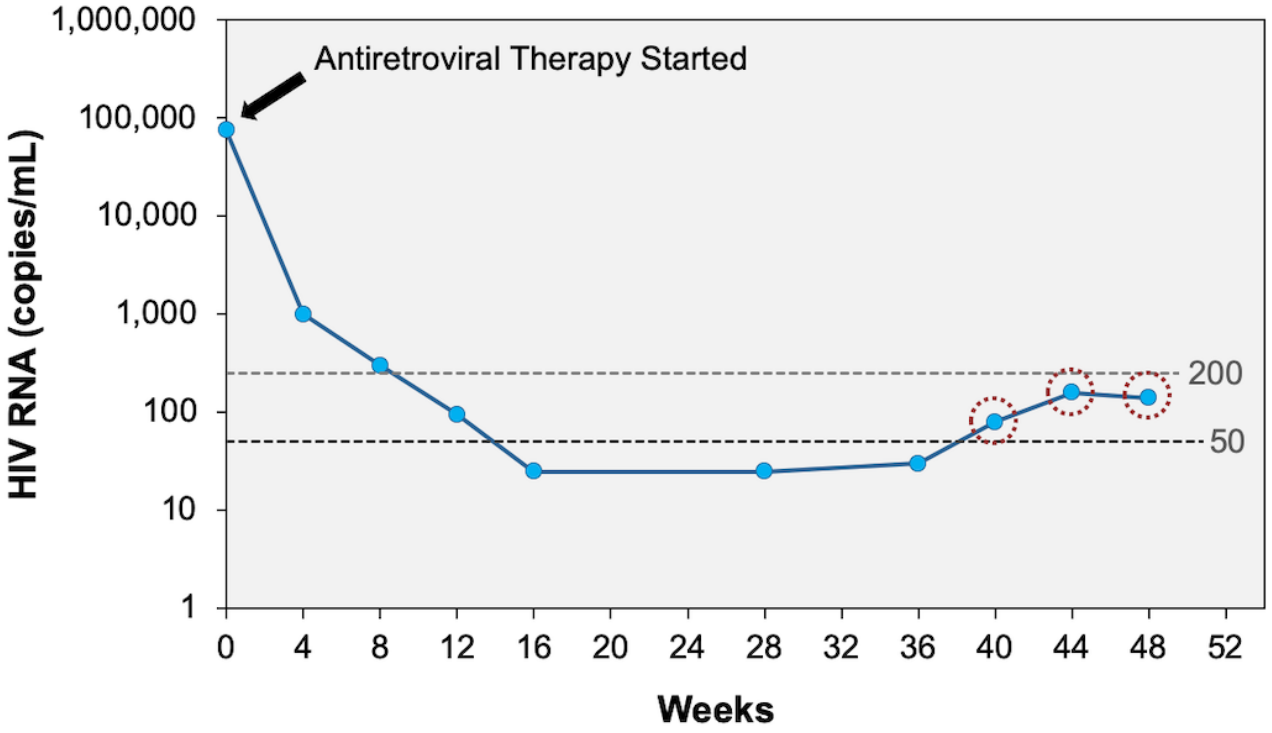


Figure 8 Detectable HIV RNA Below the Limit of Quantification

As shown, the sample for patient 1 has detectable plasma HIV-1 RNA that is at a high enough level that it can accurately be quantitated by the laboratory (55 copies/mL). In the sample from patient 2, the HIV-1 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 (the laboratory reports HIV-1 RNA was detected 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.

Illustration: David H. Spach, MD

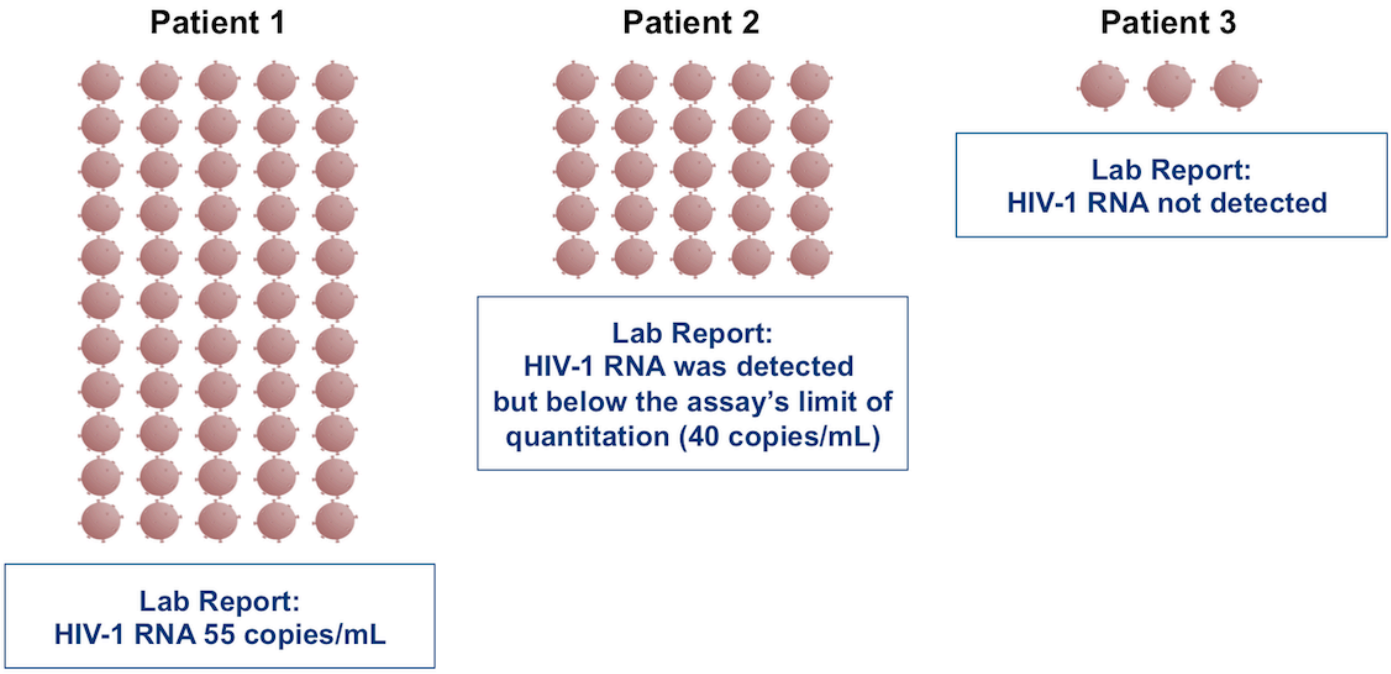


Figure 9 HIV Conventional and DNA Genotypic Drug Resistance Assays

A conventional HIV drug resistance assay (left side) 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 integrated into the host cell DNA. The HIV DNA resistance assay can be performed in patients who have undetectable plasma HIV RNA levels.

Illustration: David H. Spach, MD

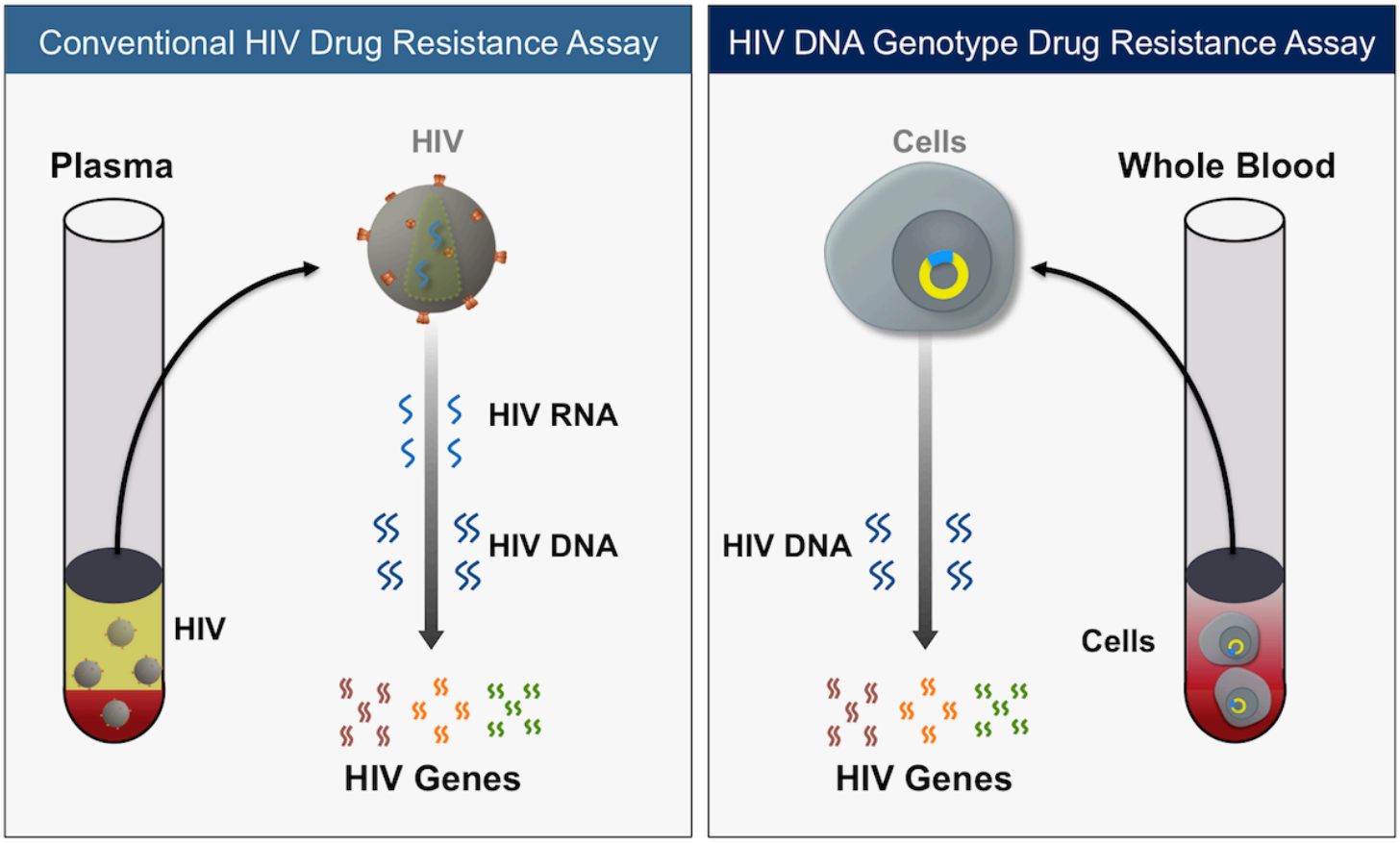


Figure 10 (Image Series) - Conventional HIV Drug Resistance Genotypic Assay (Image Series) - Figure 10 (Image Series) - Conventional HIV Drug Resistance Genotypic Assay
Image 10A: Obtaining Blood Sample from Person with HIV

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 H. Spach, MD

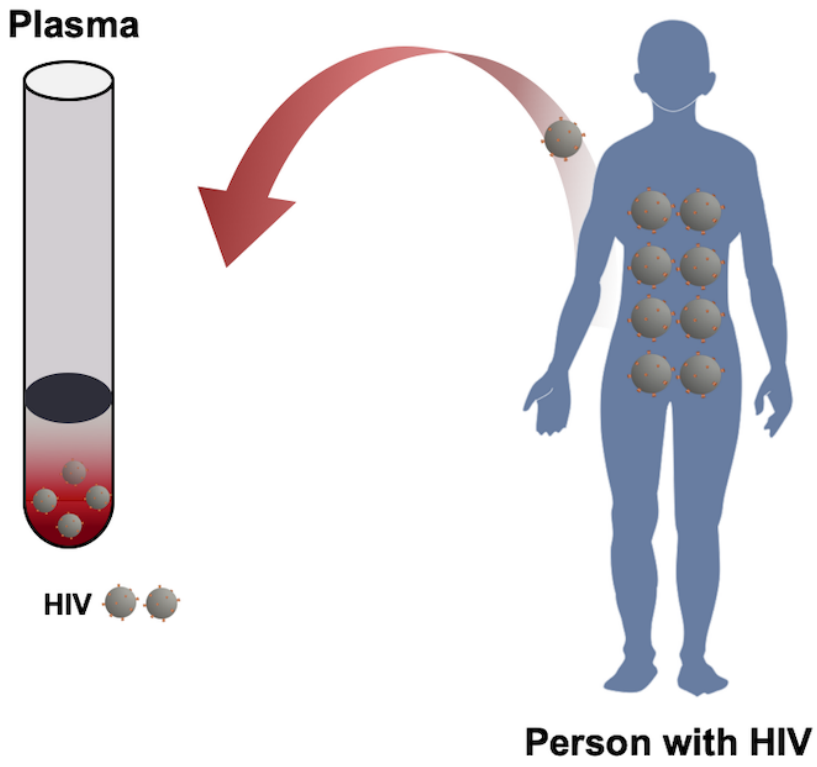


Figure 10 (Image Series) - Conventional HIV Drug Resistance Genotypic Assay
Image 10B: Steps for Isolating and Sequencing HIV DNA for Genotype

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 H. Spach, MD

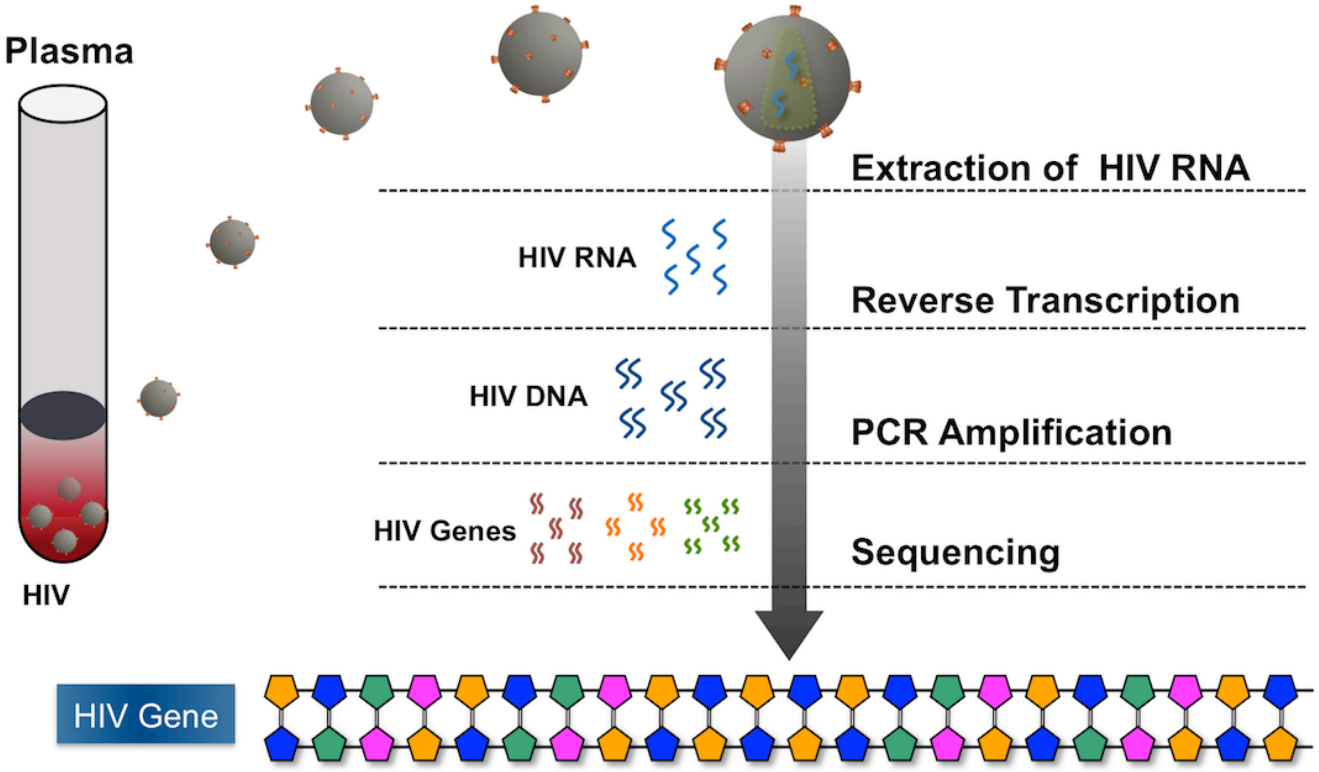


Figure 10 (Image Series) - Conventional HIV Drug Resistance Genotypic Assay
Image 10C: Normal Protein Synthesis Process

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 H. Spach, MD

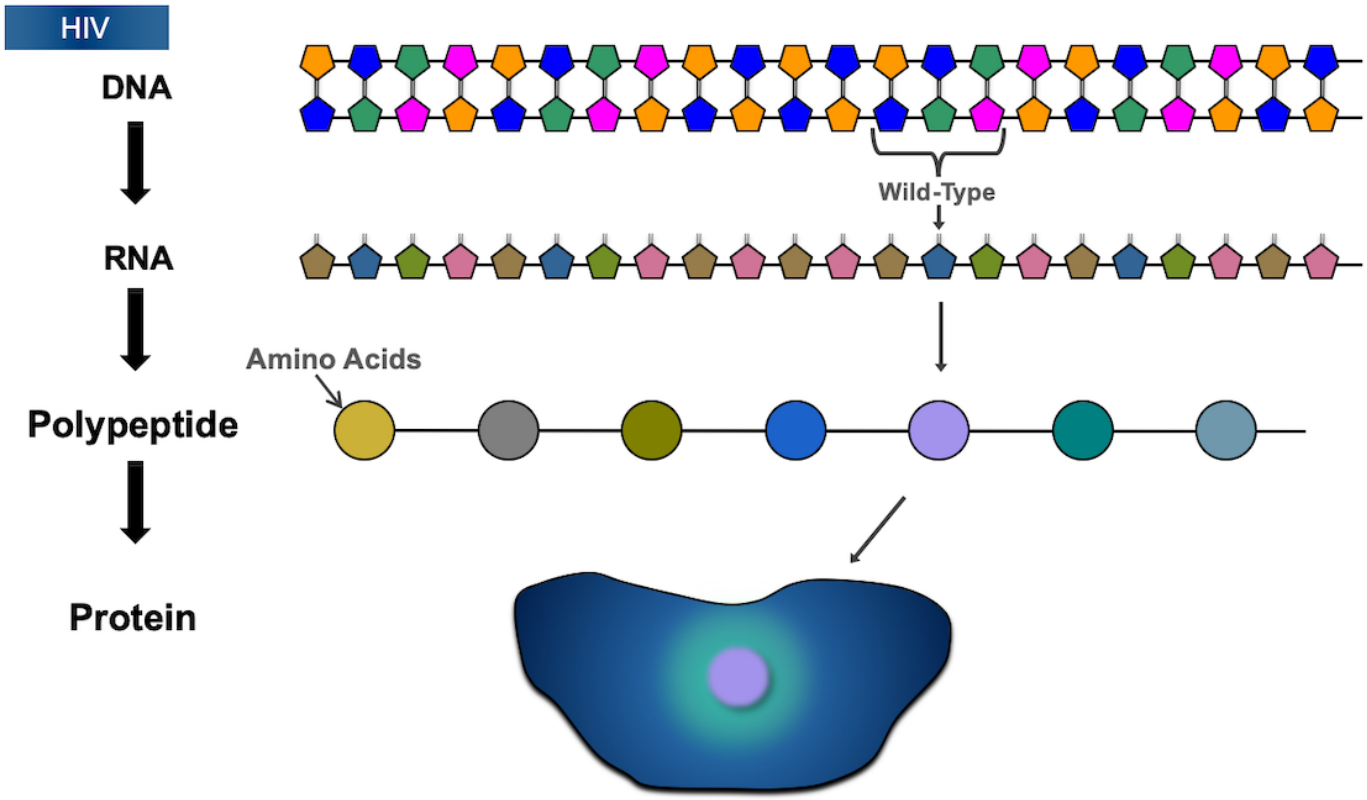


Figure 10 (Image Series) - Conventional HIV Drug Resistance Genotypic Assay
Image 10D: Mutation in HIV DNA Leading to Amino Acid Substitution

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

Illustration: David H. Spach, MD

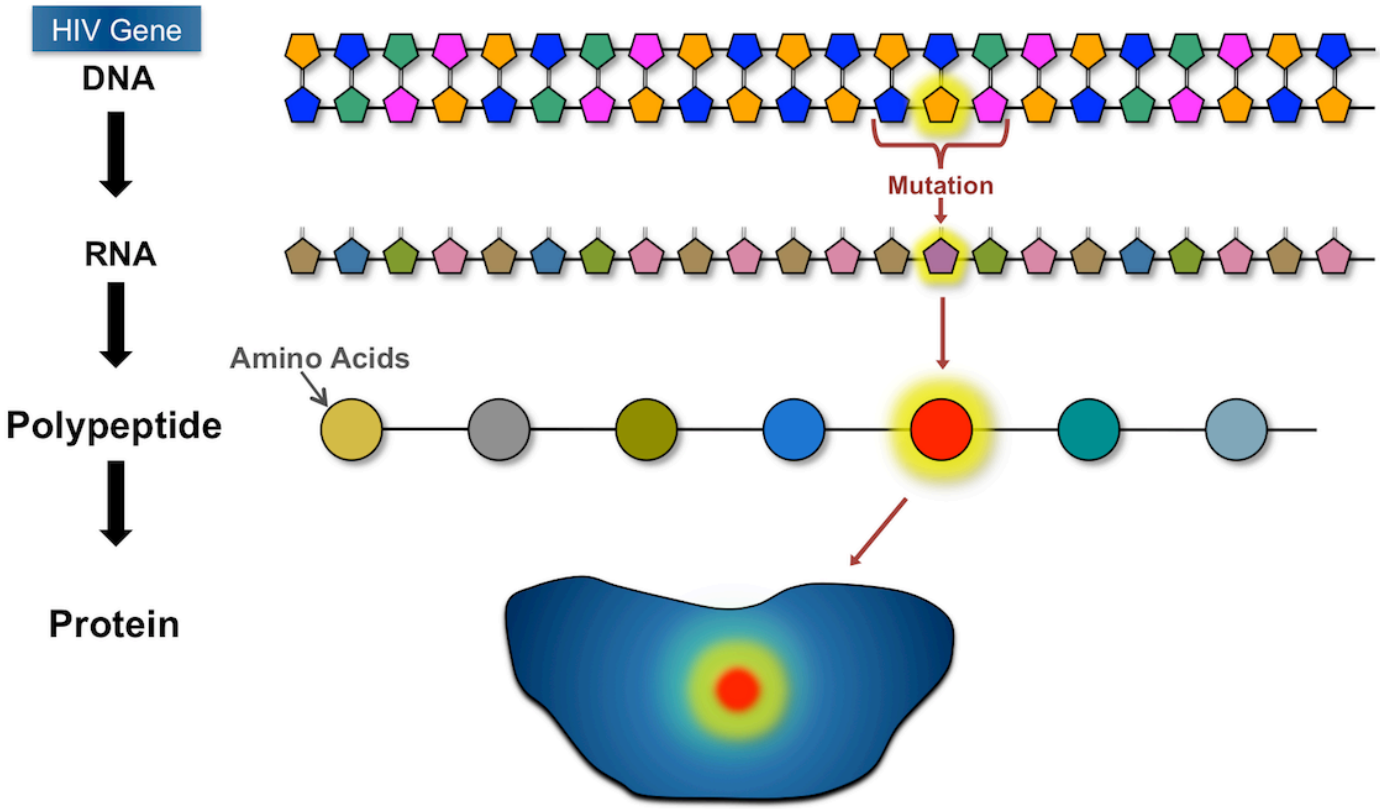


Figure 11 Proviral DNA

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

Illustration: David H. Spach, MD

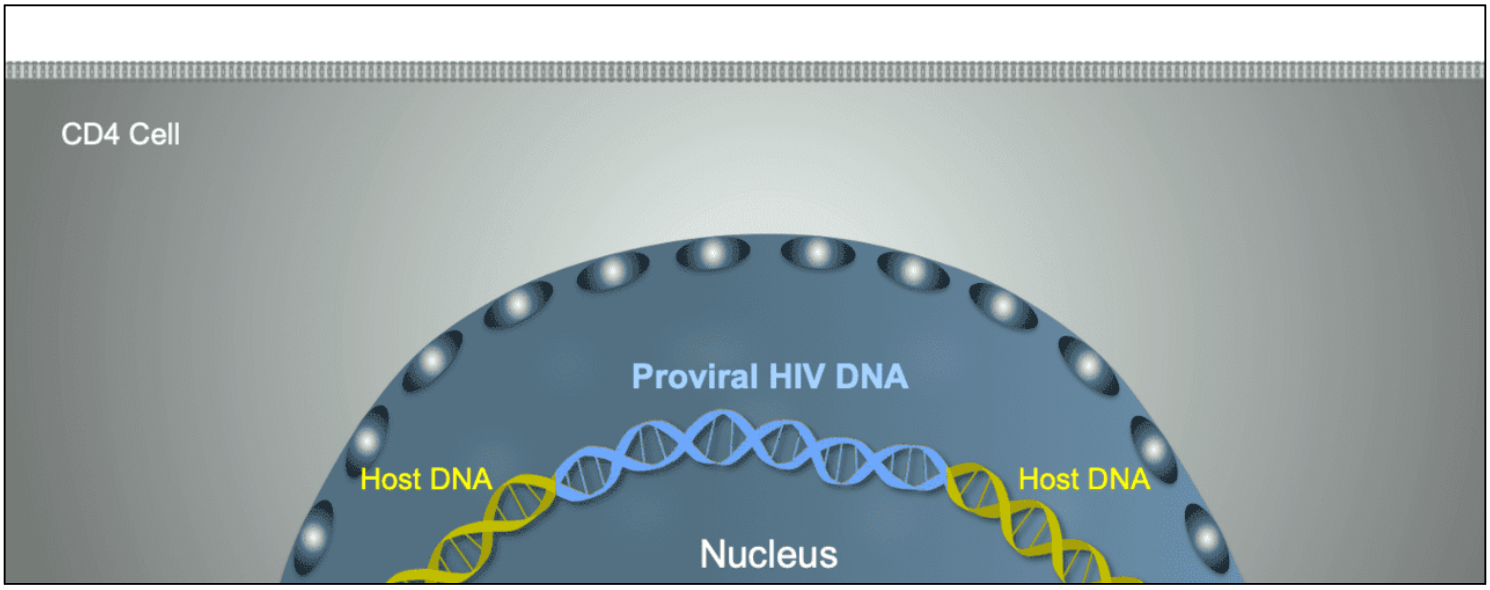


Figure 12 Method for Calculating Level of Phenotypic Resistance

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.

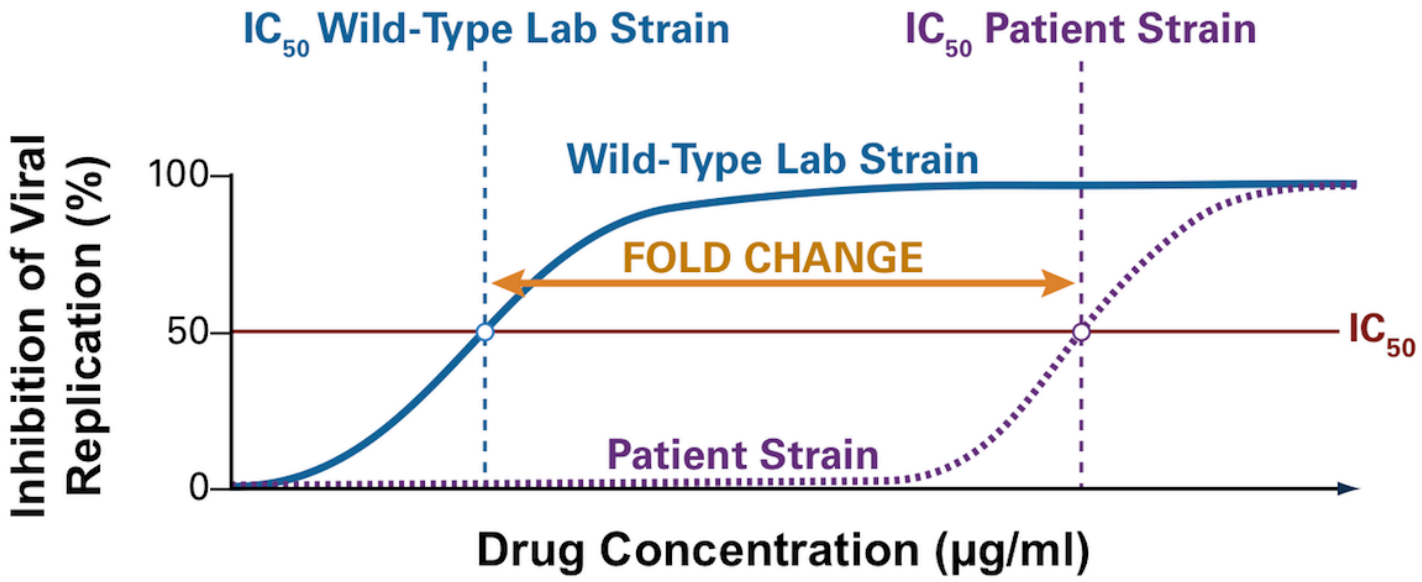


Figure 13 Reemergence of Wild-Type HIV After Stopping Antiretroviral Therapy

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, the 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 H. Spach, MD

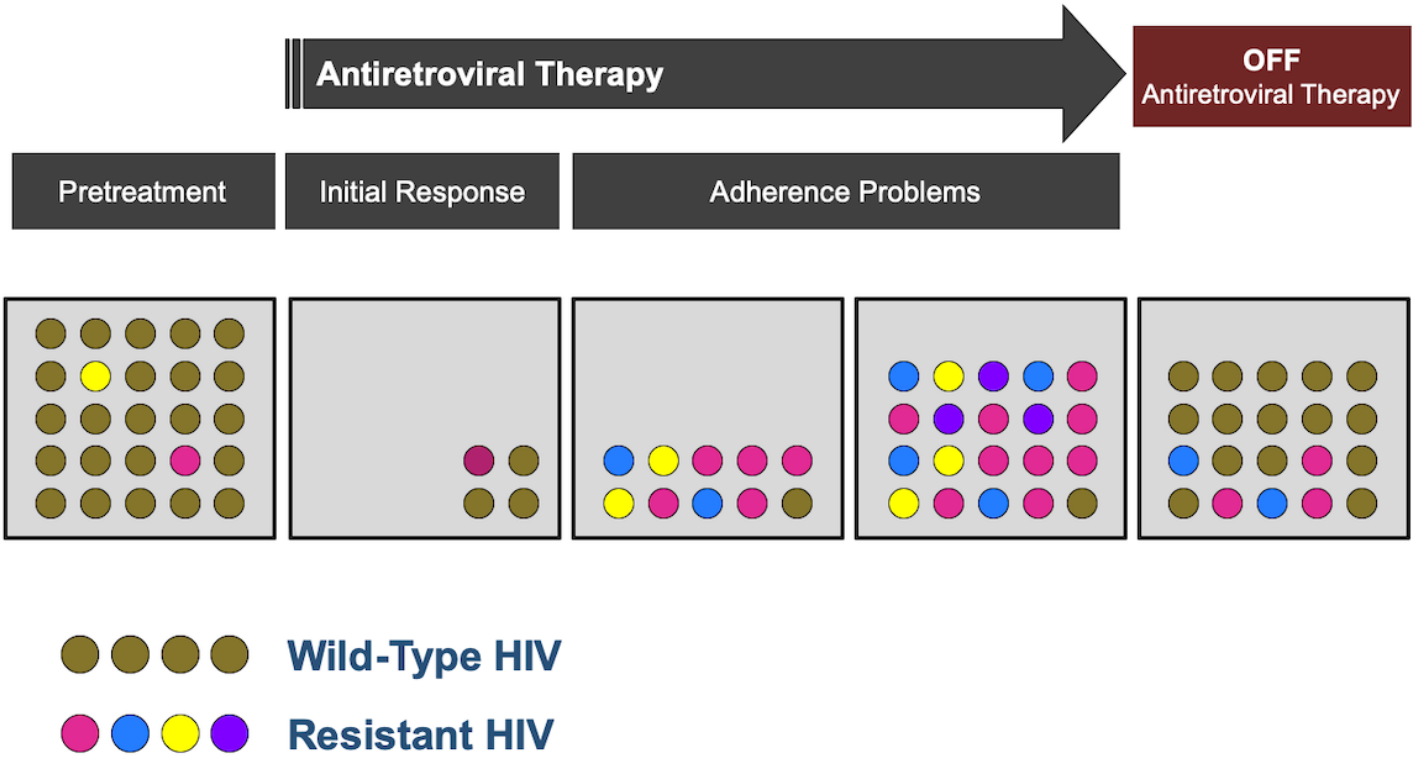


Figure 14 (Image Series) - Interpreting an HIV Drug Resistance Genotypic Assay (Image Series) - Figure 14 (Image Series) - Interpreting an HIV Drug Resistance Genotypic Assay
Image 14A: HIV DNA Mutations Resulting in Amino Acid Changes

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

Illustration: David H. Spach, MD

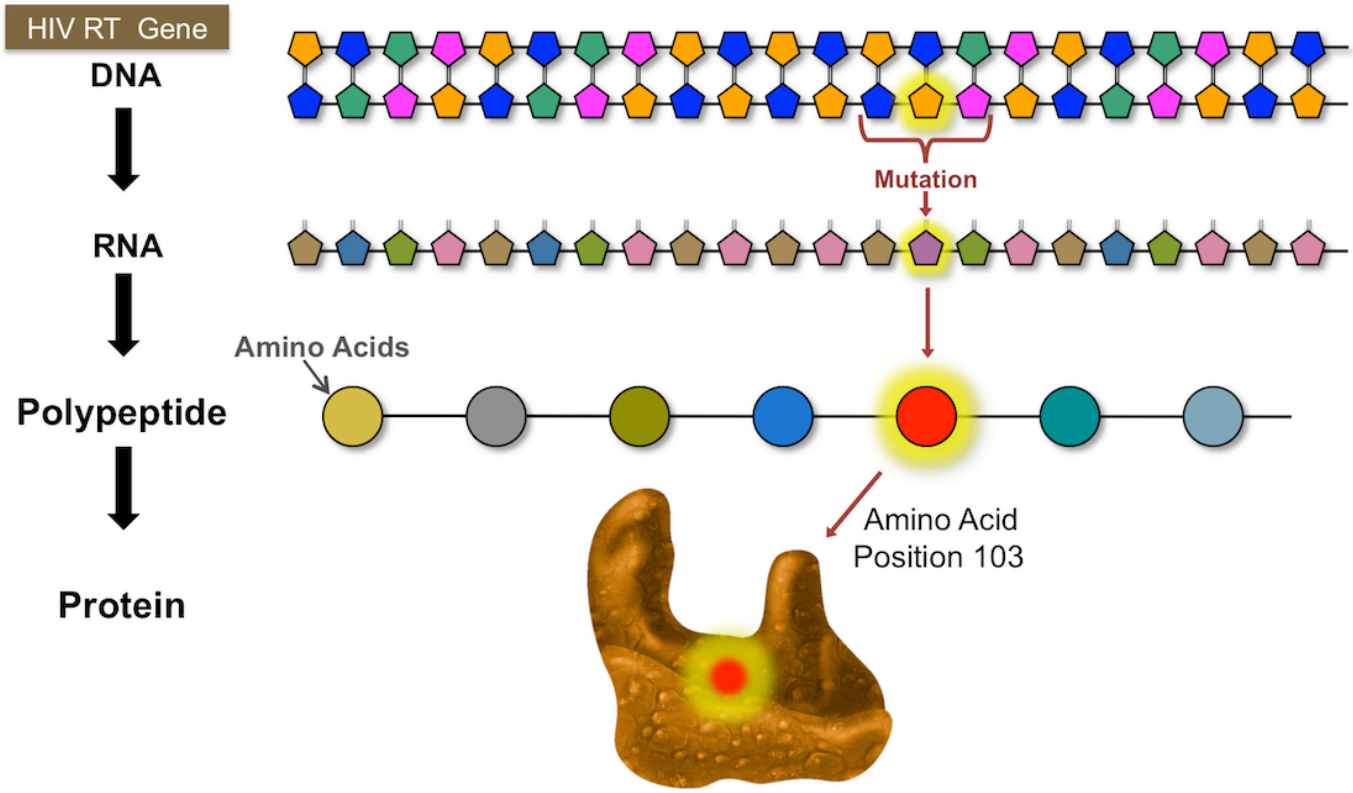


Figure 14 (Image Series) - Interpreting an HIV Drug Resistance Genotypic Assay
Image 14B: Amino Acid Substitution at Position 103

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 H. Spach, MD

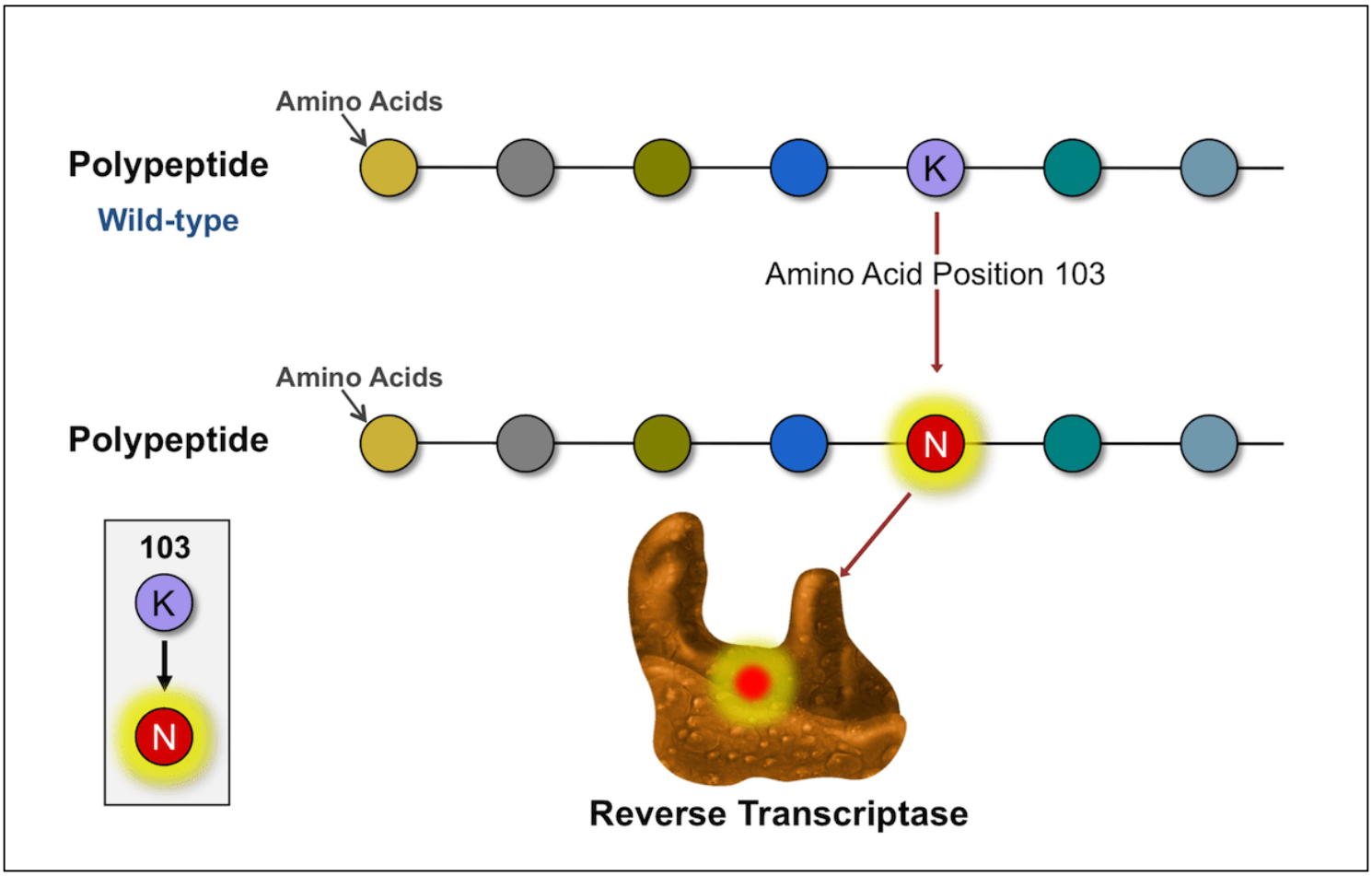


Figure 14 (Image Series) - Interpreting an HIV Drug Resistance Genotypic Assay
Image 14C: Interpretation of HIV Drug Resistance Genotype Report

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.

Illustration: David H. Spach, MD

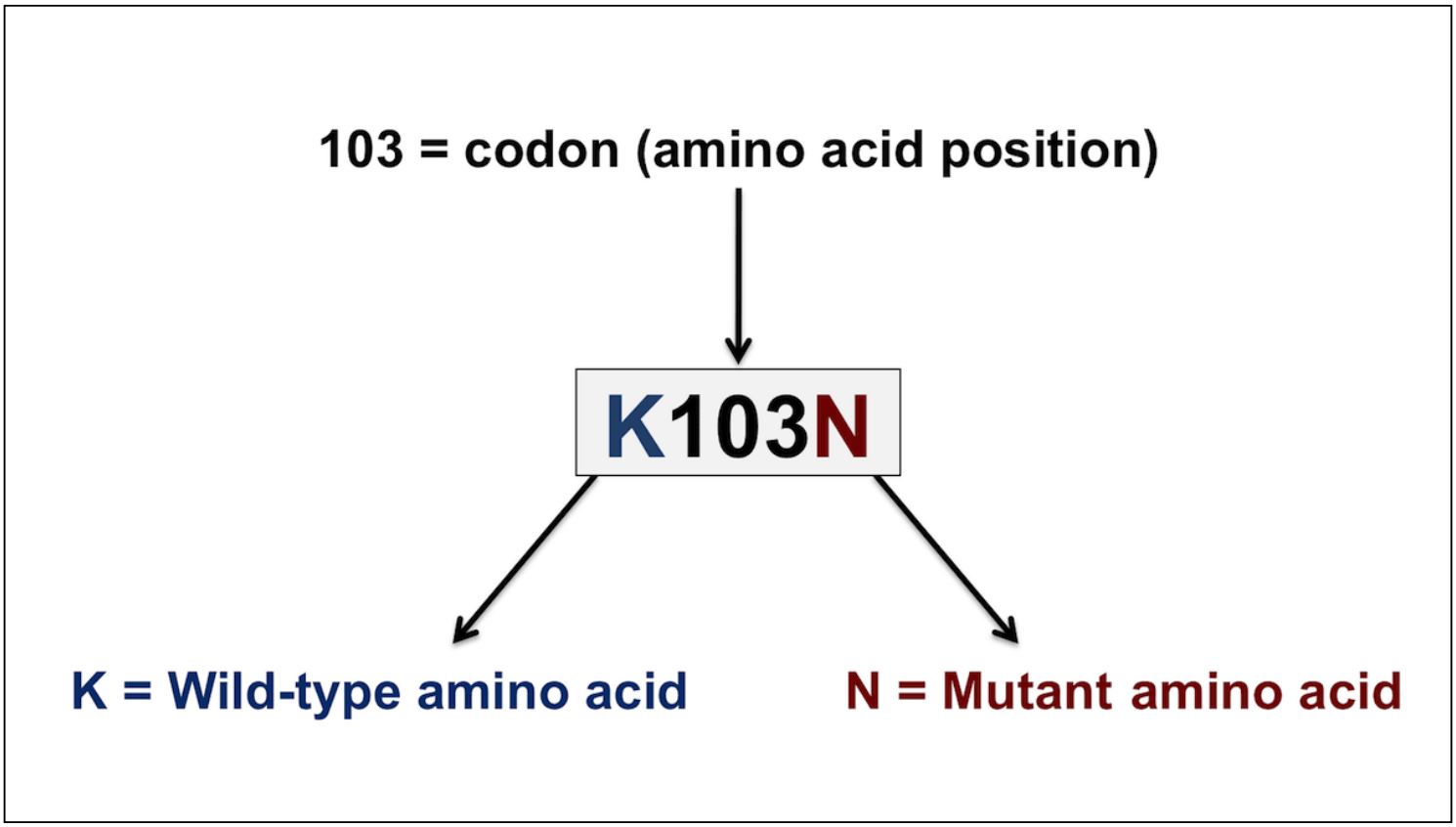


Figure 15 (Image Series) - Phenotypic Drug Susceptibility Curves (Image Series) - Figure 15 (Image Series) - Phenotypic Drug Susceptibility Curves
Image 15A: Drug-Susceptible HIV

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.

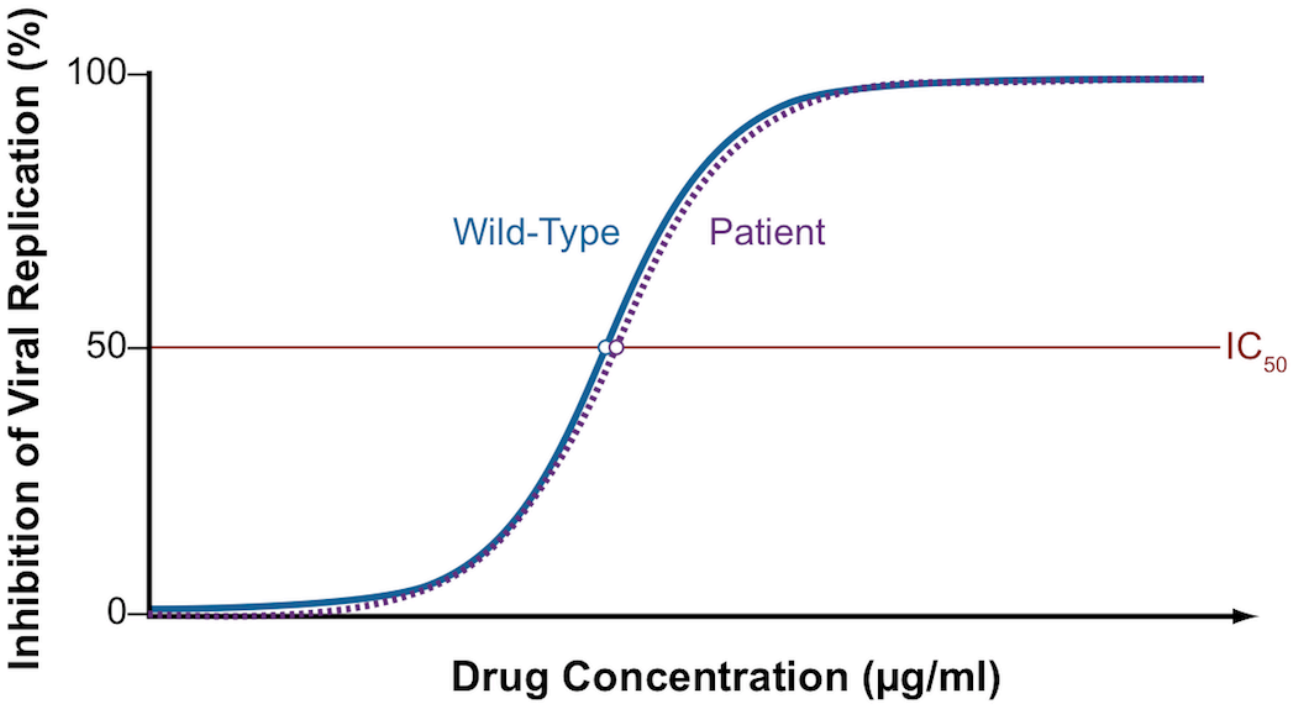


Figure 15 (Image Series) - Phenotypic Drug Susceptibility Curves
Image 15B: Drug-Resistant HIV

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. Note the 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.

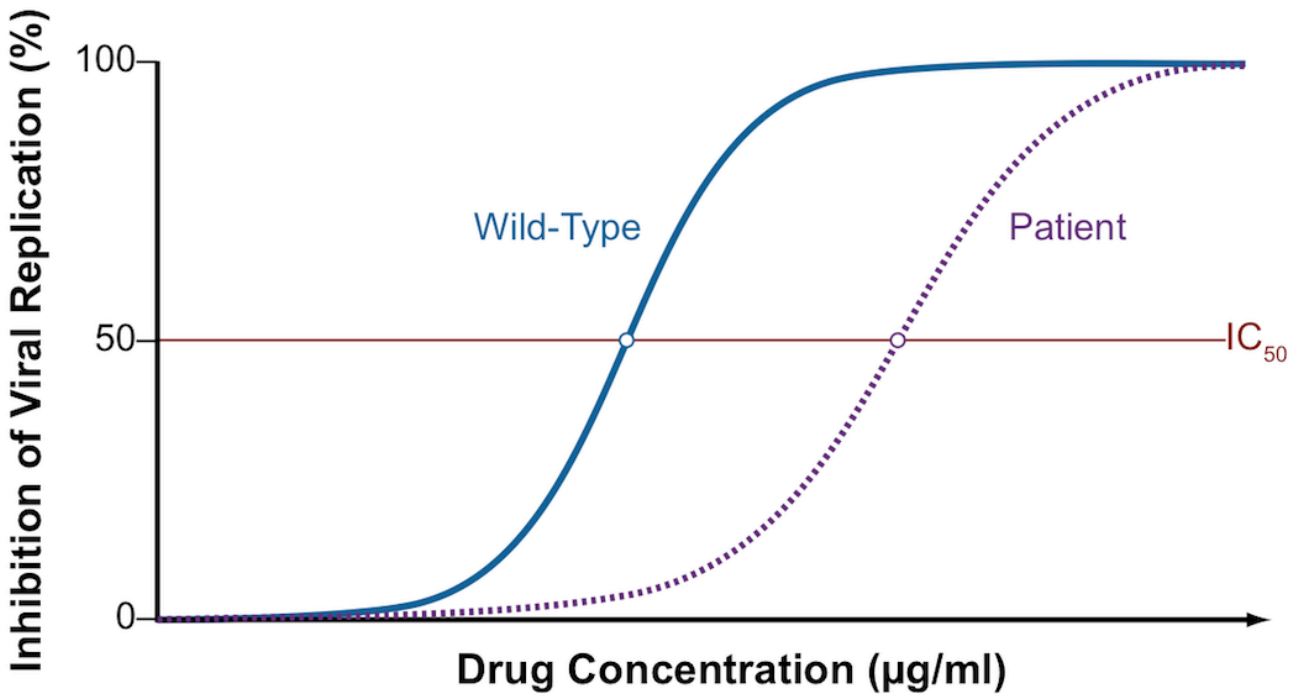


Figure 15 (Image Series) - Phenotypic Drug Susceptibility Curves
Image 15C: HIV Hypersusceptible to Drug

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 dividing the IC50 of the patient's isolate by the IC50of the wild-type laboratory strain.

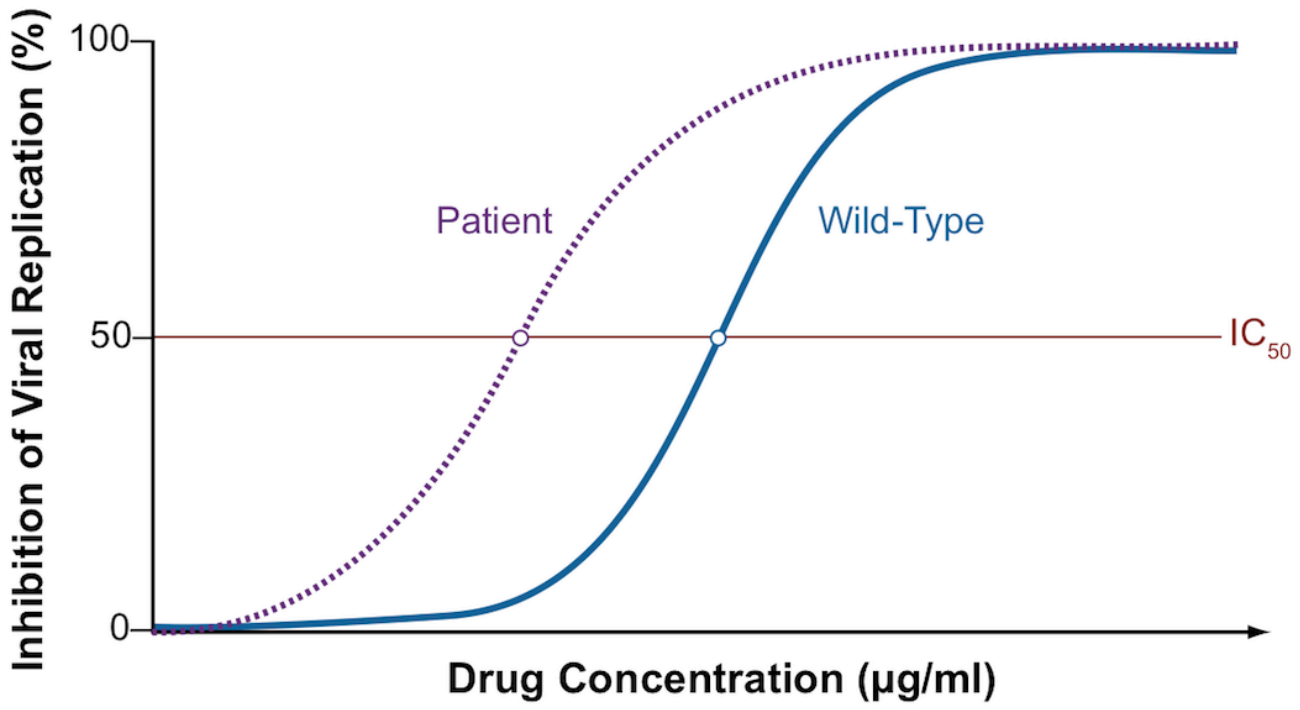


Figure 16 (Image Series) - NRTI Resistance Mechanisms (Image Series) - Figure 16 (Image Series) - NRTI Resistance Mechanisms
Image 16A: Discrimination (Decreased Incorporation) Mechanisms for HIV Resistance to NRTIs

Illustration: David H. Spach, MD

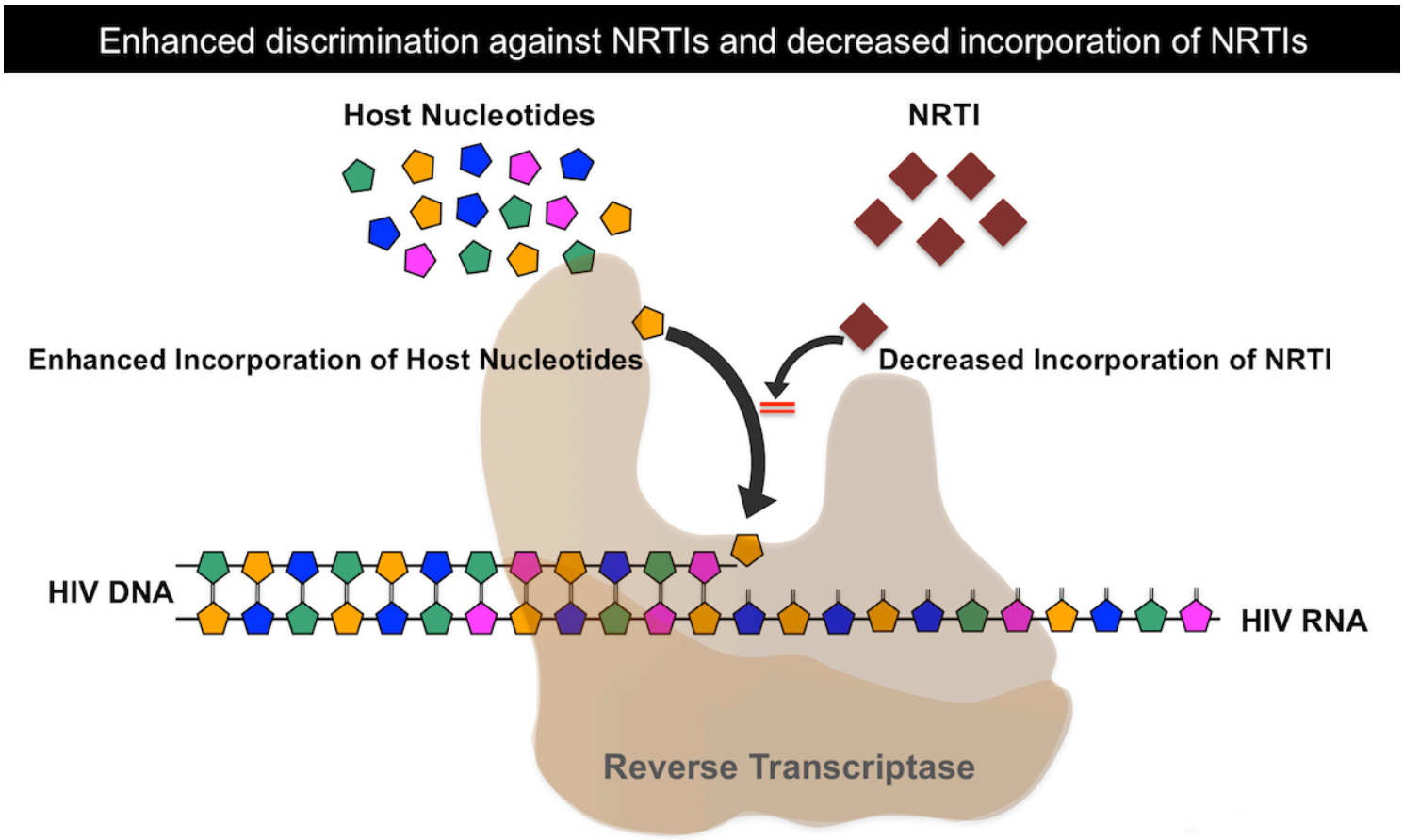


Figure 16 (Image Series) - NRTI Resistance Mechanisms
Image 16B: Excision (Primer Unblocking) Mechanisms for HIV Resistance to NRTIs

Illustration: David H. Spach, MD

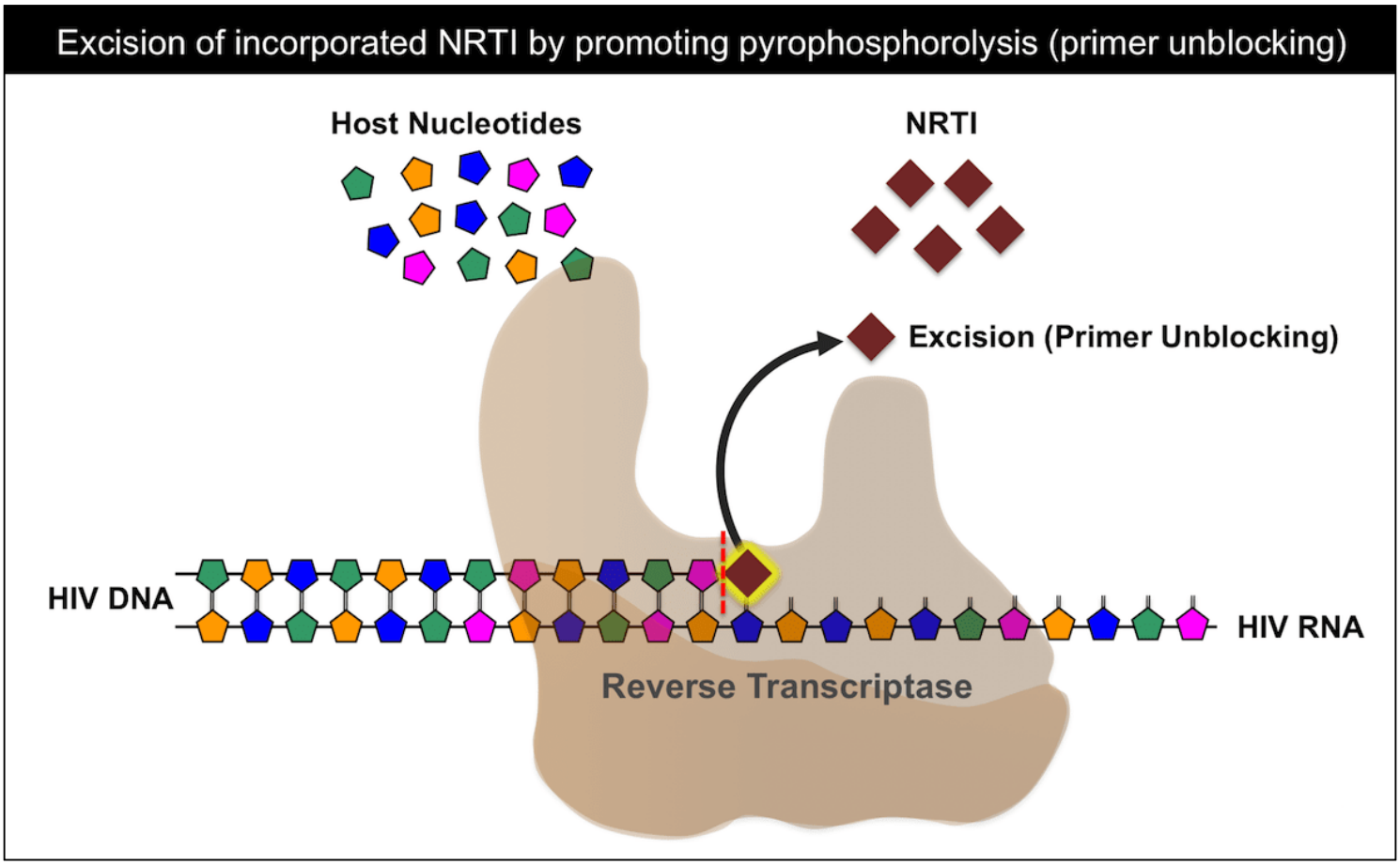


Figure 17 (Image Series) - Thymidine Analog Mutations (TAMs) and Resistance Pathways (Image Series) - Figure 17 (Image Series) - Thymidine Analog Mutations (TAMs) and Resistance Pathways
Image 17A: Thymidine Analog Mutations (TAMs)

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. Illustration: David H. Spach, MD

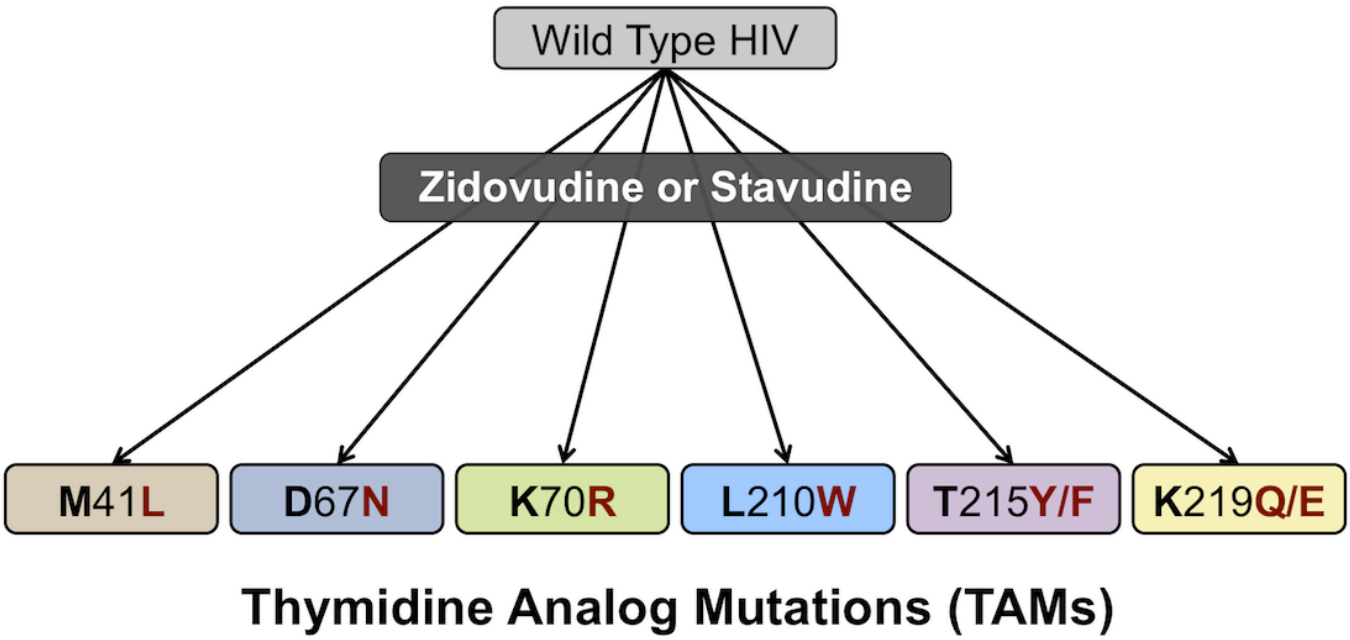


Figure 17 (Image Series) - Thymidine Analog Mutations (TAMs) and Resistance Pathways
Image 17B: Thymidine Analog Mutation (TAM) Resistance Pathways

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. Illustration: David H. Spach, MD

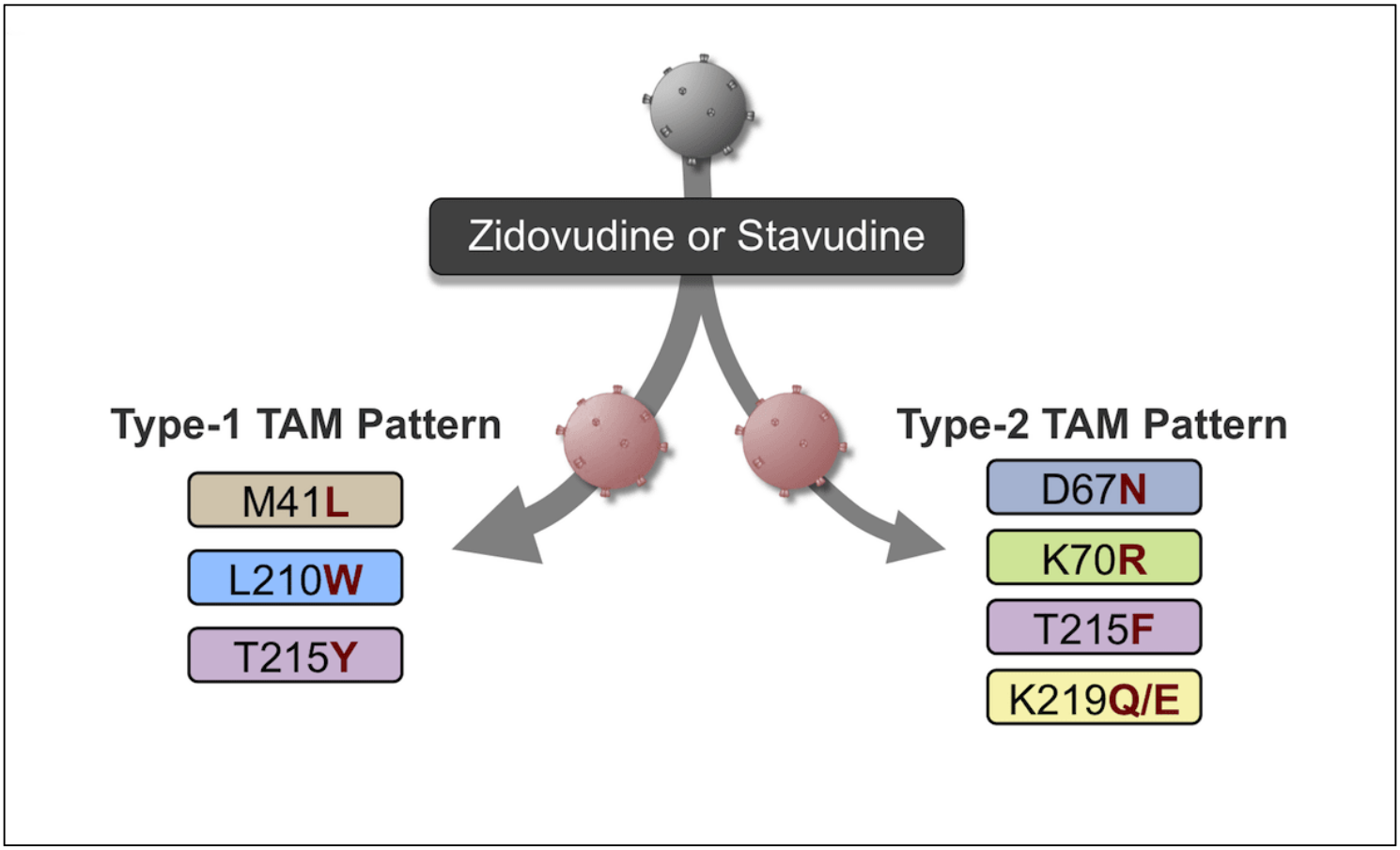


Figure 18 (Image Series) - NNRTI Resistance Mechanisms (Image Series) - Figure 18 (Image Series) - NNRTI Resistance Mechanisms
Image 18A: Altered Interaction of NNRTI with Binding Pocket

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

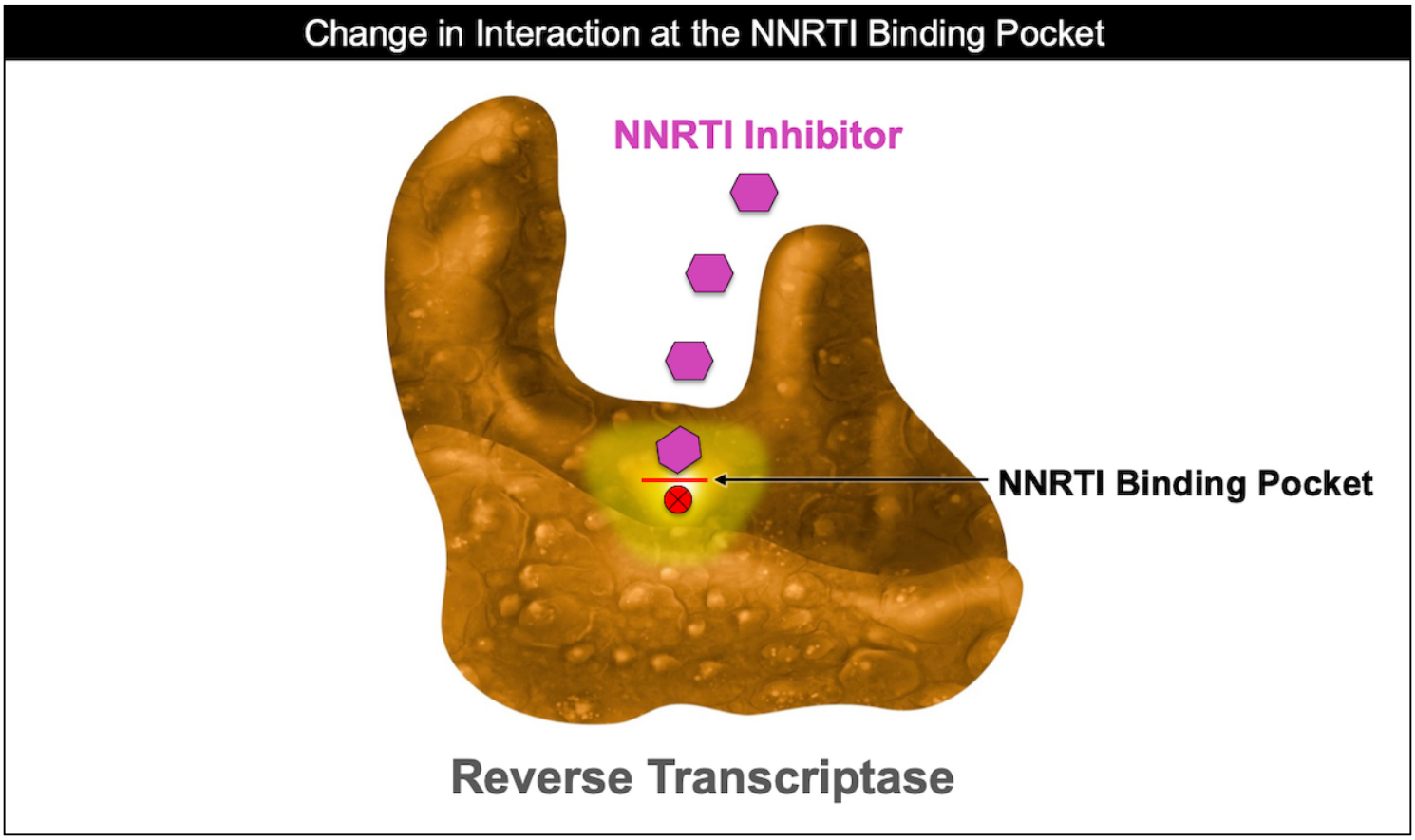


Figure 18 (Image Series) - NNRTI Resistance Mechanisms
Image 18B: Reduced Access of NNRTI to Binding Pocket

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

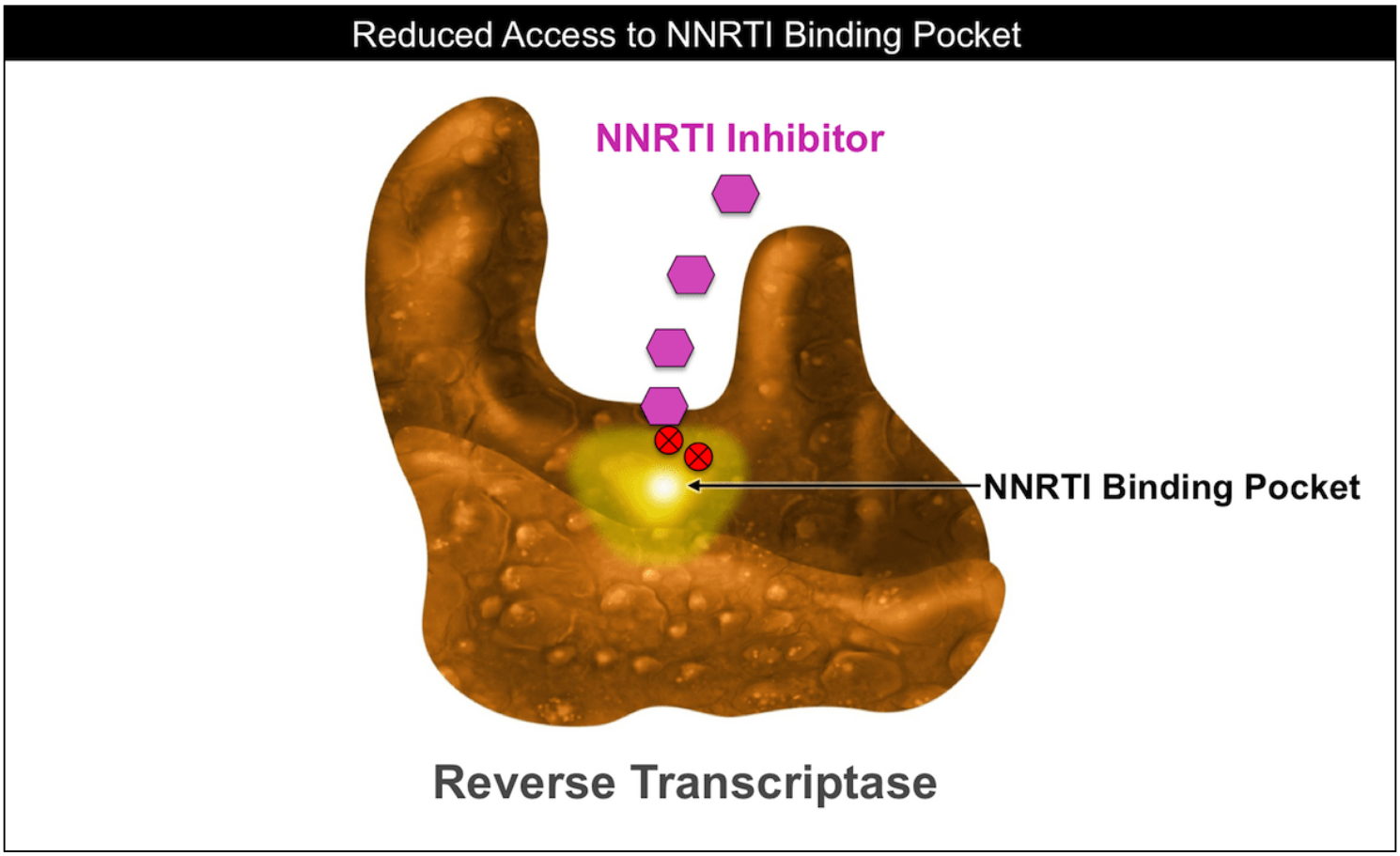


Figure 19 Doravirine Resistance Pathways

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

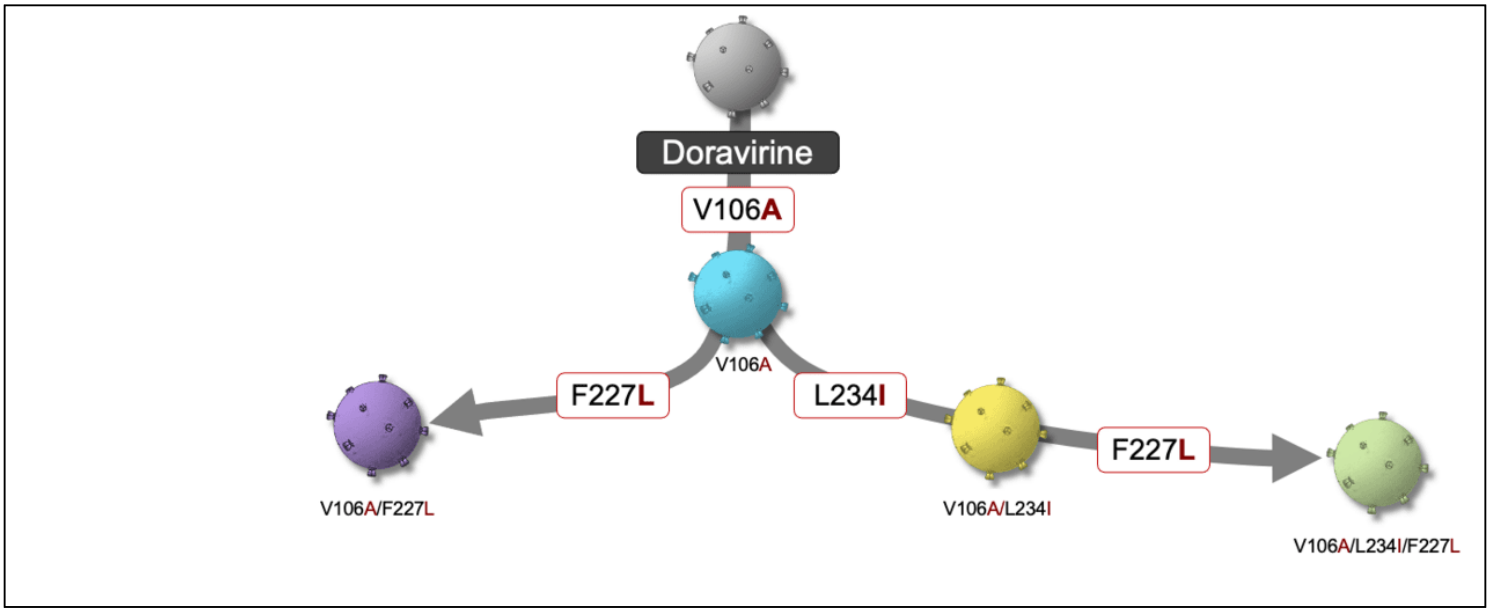


Figure 20 Major Primary Integrase Resistance Mutations

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 nearly 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

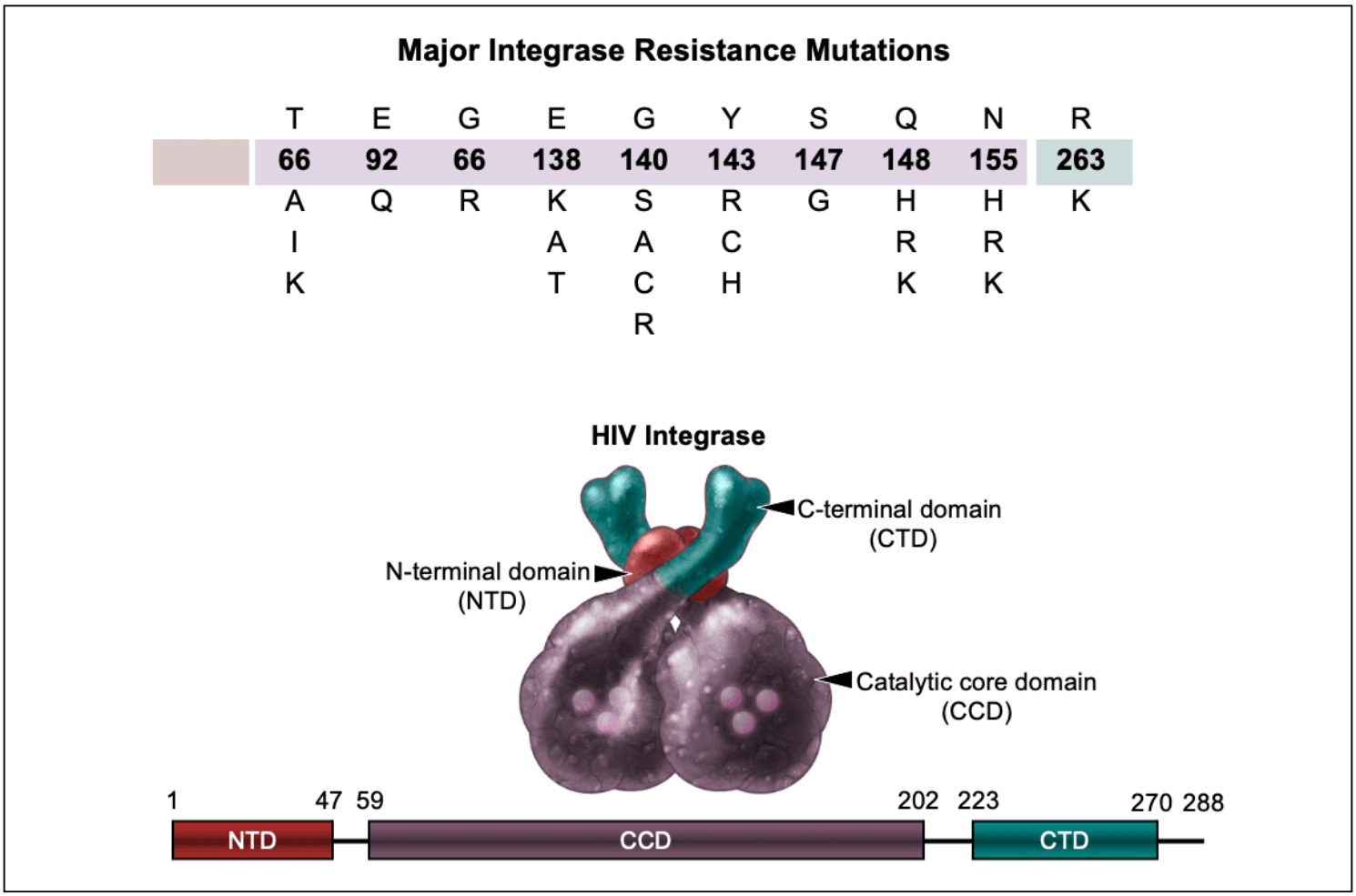


Figure 21 Raltegravir Resistance Pathways

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.

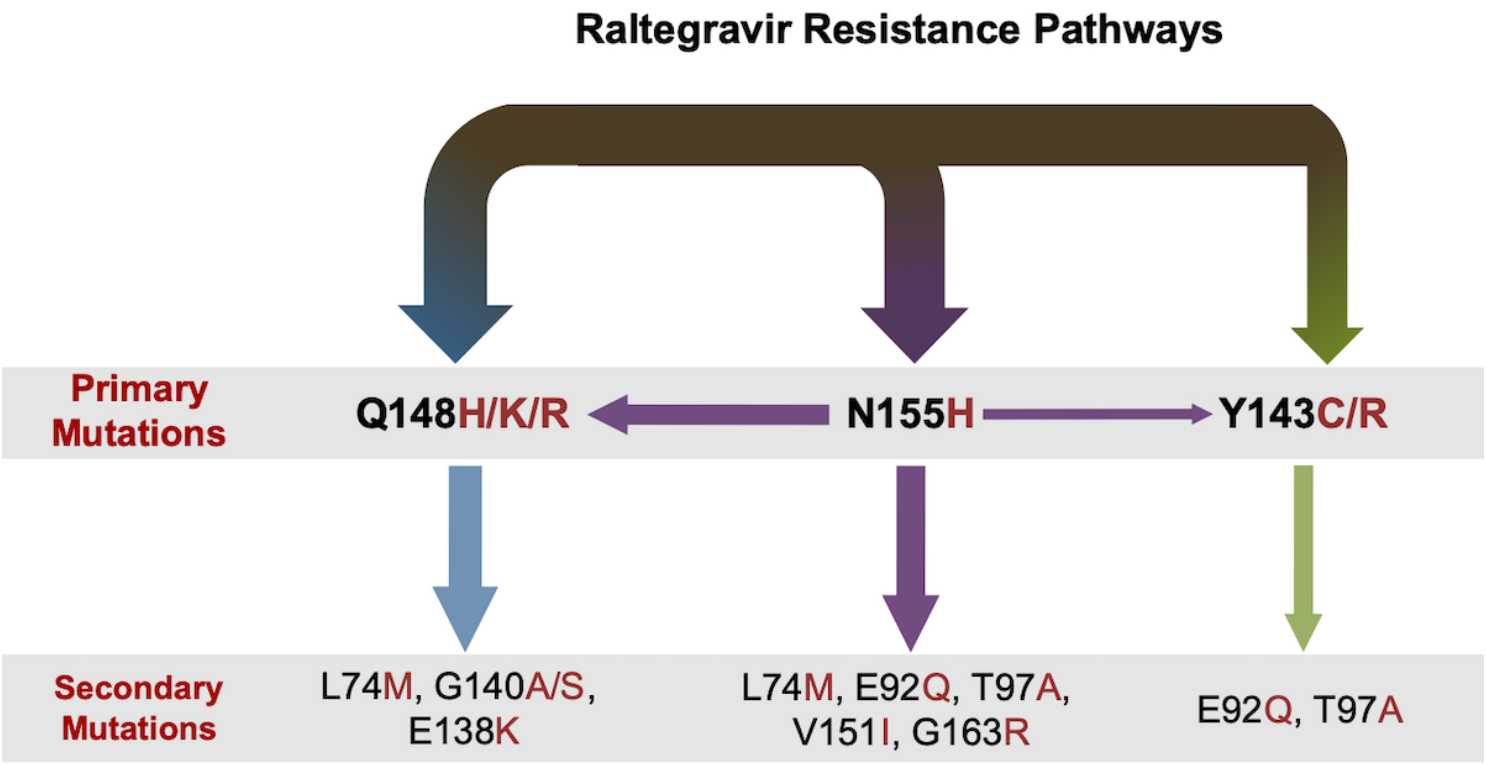


Figure 22 HIV Protease and Location of Amino Acid Resistance Mutations

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

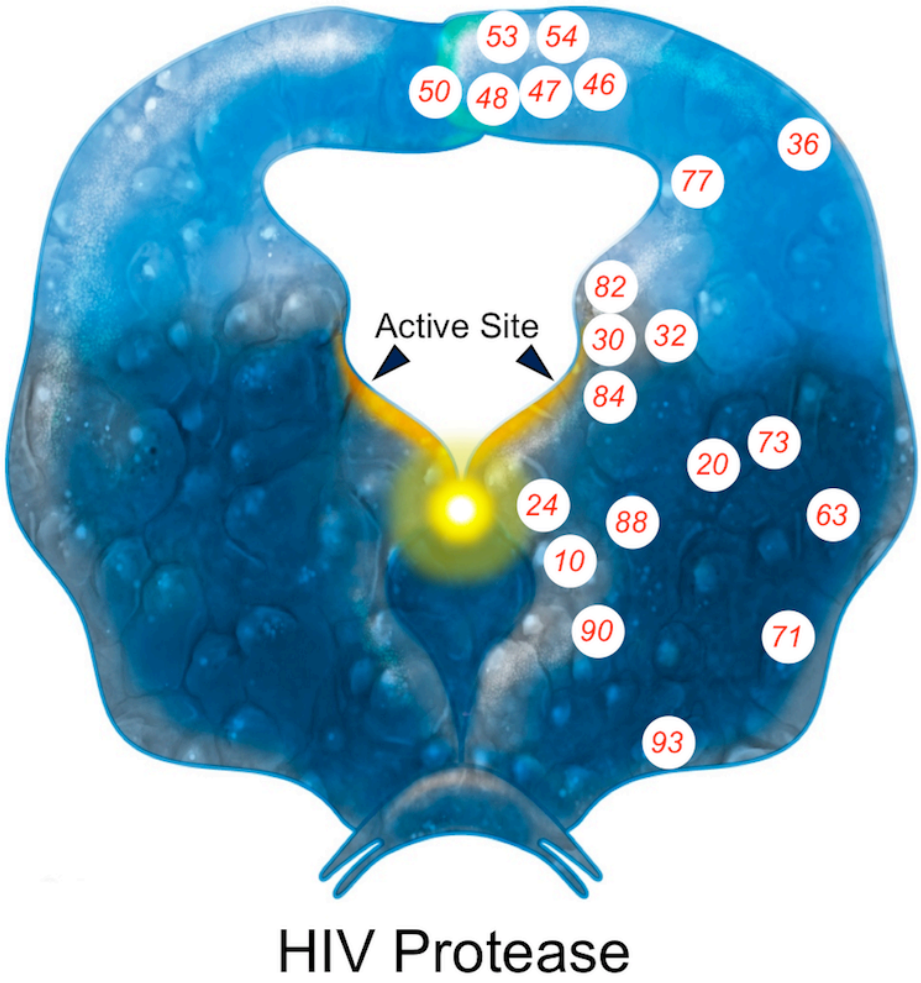


Figure 23 (Image Series) - CCR5 Receptor Antagonists and Mechanisms of Resistance (Image Series) - Figure 23 (Image Series) - CCR5 Receptor Antagonists and Mechanisms of Resistance
Image 23A: CCR5 Antagonists

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

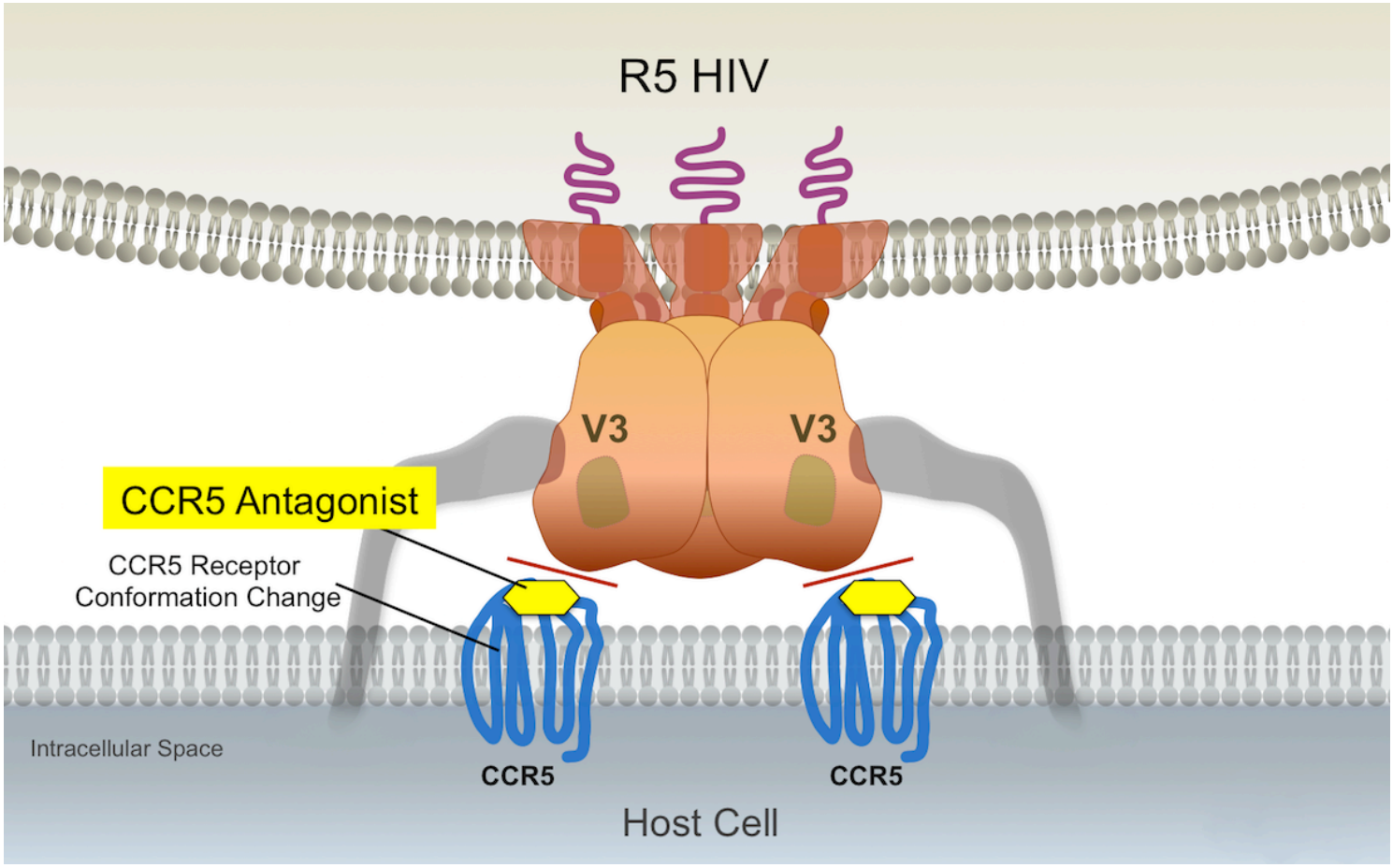


Figure 23 (Image Series) - CCR5 Receptor Antagonists and Mechanisms of Resistance
Image 23B: Emergence of Preexisting X4-Tropic Virus

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

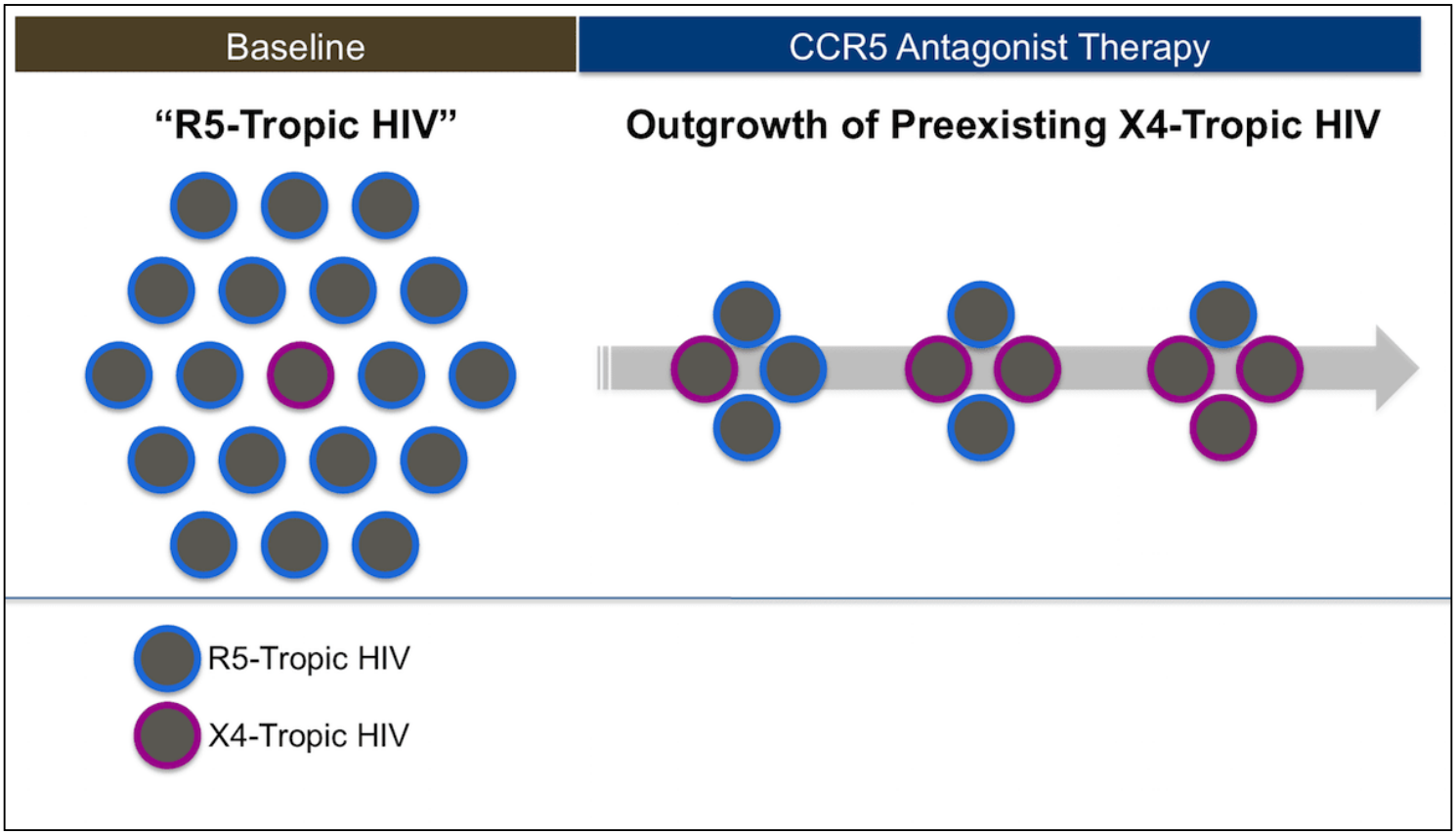


Figure 23 (Image Series) - CCR5 Receptor Antagonists and Mechanisms of Resistance
Image 23C: Emergence of New X4-Tropic Virus

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

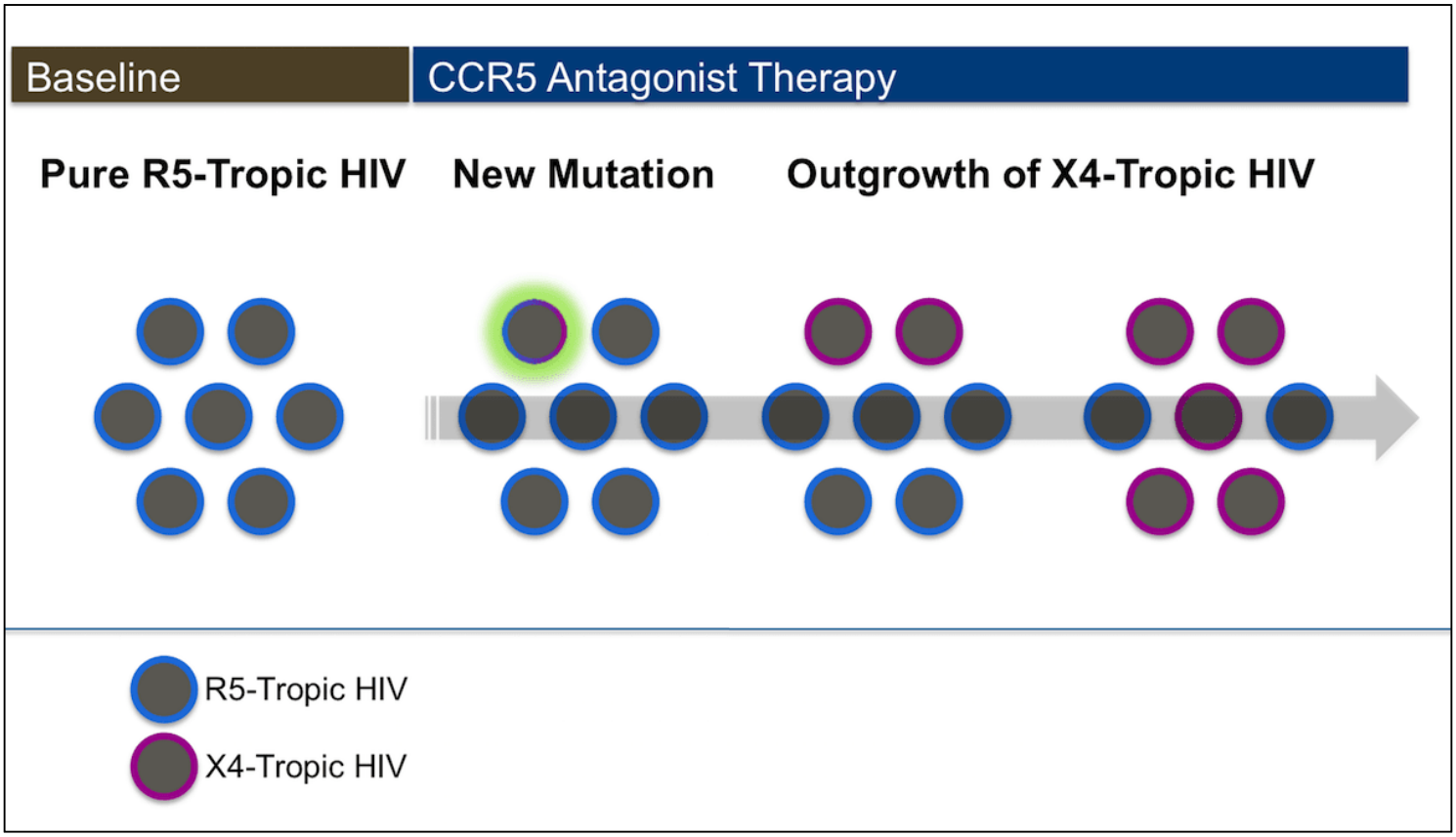


Figure 23 (Image Series) - CCR5 Receptor Antagonists and Mechanisms of Resistance
Image 23D: Resistance to CCR5 Antagonist: Binding to CCR5 in Presence of Maraviroc

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

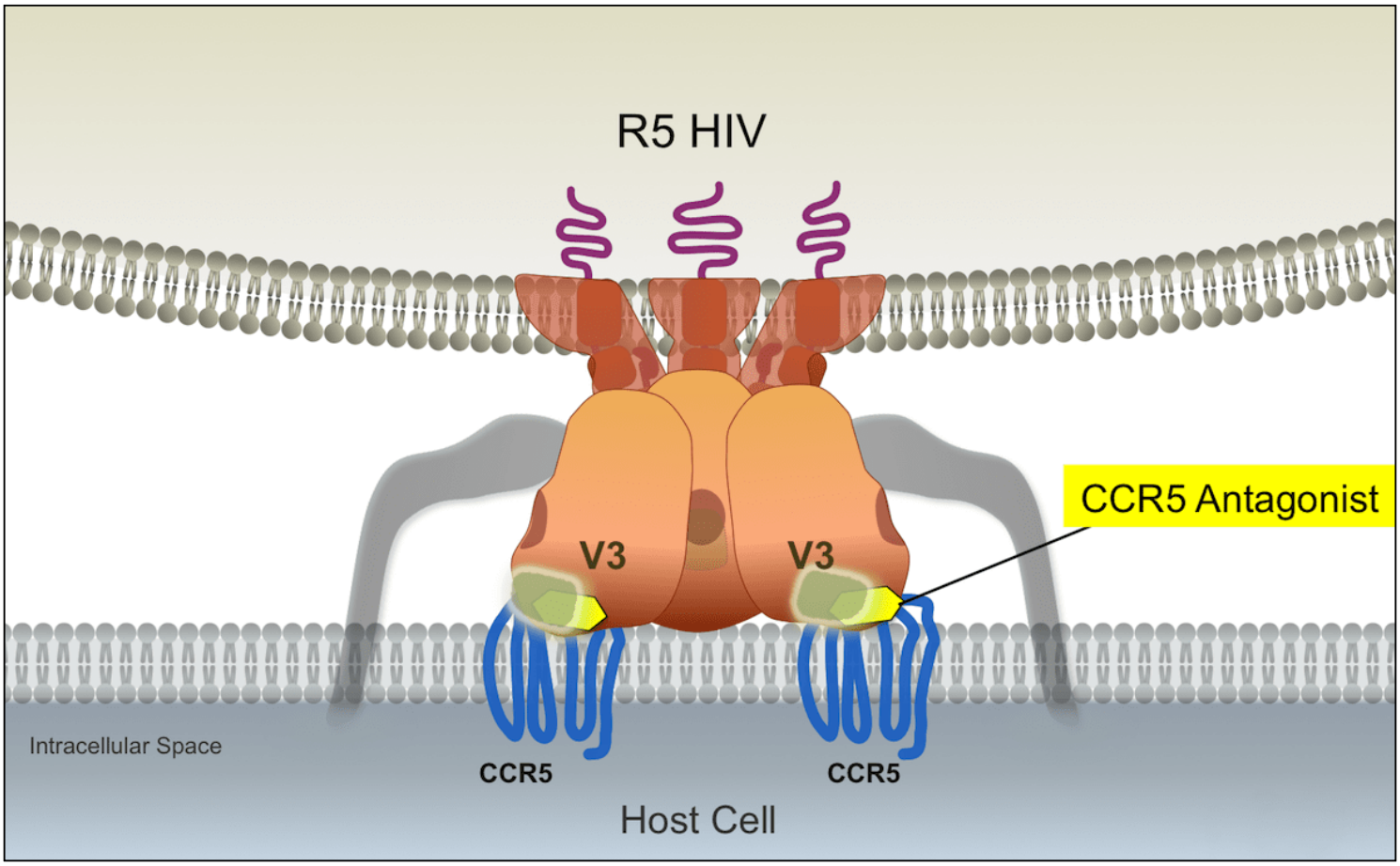


Figure 24 Enfuvirtide

The antiretroviral medication enfuvirtide is a synthetic 36-amino-acid peptide that mimics a segment of the HR2 region of the HIV-1 envelope gp41 protein. The medication binds to the corresponding HR1 region. Resistance develops from mutations in the HR1 region.

Illustration: David H. Spach, MD

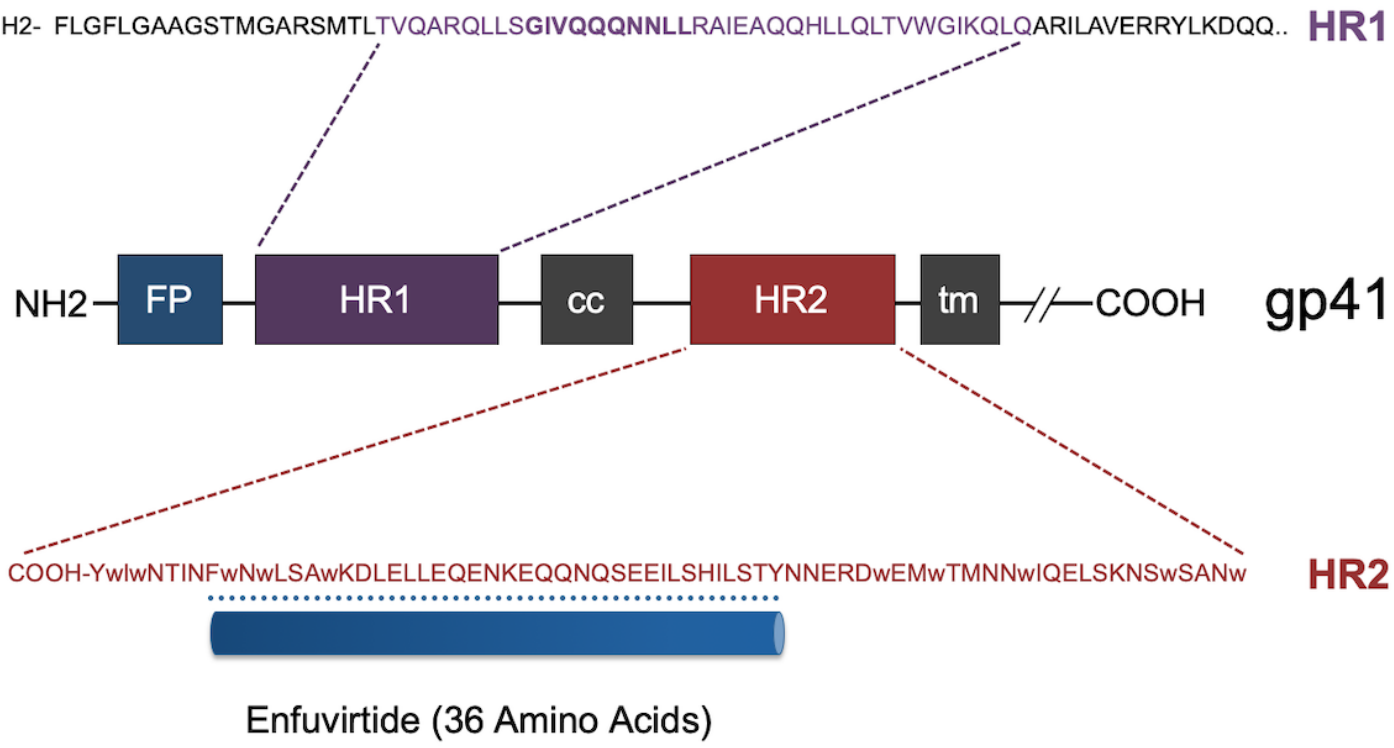


Table 1. Causes of Virologic Failure: Adult and Adolescent ARV Guidelines

Key Considerations and Recommendations
HIV-Related Factors • Presence of transmitted or acquired drug-resistant virus that may or may not be documented by current or past drug-resistance test results • Prior antiretroviral treatment failure • Innate resistance to prescribed antiretroviral drugs • Higher pre-treatment HIV-RNA levels (e.g., HIV RNA >100,00 copies/mL; some regimens may be less effective at higher levels
Antiretroviral Regimen-Related Factors • Suboptimal pharmacokinetics (PK) (e.g., variable absorption, metabolism, or penetration into reservoirs) • Suboptimal virologic potency • Low 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 ARV drugs • Adverse drug effects • High pill burden and/or dosing frequency • Prescription (prescribing or dispensing) errors
Social and Adherence-Related Factors • Active substance use, mental health disorders, or neurocognitive impairment • Unstable housing and other psychosocial factors • Missed clinic appointments • Interruption of or intermittent access to antiretroviral therapy • Cost and affordability of antiretroviral drugs, which may affect the ability to access or continue therapy

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 people with HIV and antiretroviral therapy experience: Virologic failure. September 12, 2024. [HIV.gov]