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HIV Drug Resistance Mutations

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ABSTRACT

ART resistance, according to WHO, is the presence of one or more mutations in HIV that reduces the ability of certain drugs or drug groups to inhibit viral replication. According to the 2019 HIV Drug Resistance Report issued by the WHO, the prevalence of Antiretroviral Therapy (ART) drug resistance is 3%-29%. The prevalence of HIV drug resistance varies by country. In developed countries, the prevalence ranges from 6.6% to 11%. There are two types of resistance to ART: primary and secondary resistance. Primary resistance reflects the acquisition of drug-resistant strains in individuals who have recently been infected and have not received therapy. Secondary resistance occurs after treatment with ART. Resistance to antiretroviral therapy, mainly NRTIs, NNRTIs, and protease inhibitors, is caused by continuous inhibition of the HIV reverse transcriptase enzyme. World Health Organization (WHO) has recommended two NRTIs plus Lopinavir or Atazanavir as a second-line regimen for individuals who have failed treatment with efavirenz or dolutegravir; two NRTIs plus Darunavir and Lopinavir plus Raltegravir are recommended as an alternative due to cost constraints and the fact that Darunavir is unstable in moderately hot conditions.

1. Introduction

World Health Organization (WHO) and United Nations Program on HIV/AIDS (UNAIDS) have set a goal to end the AIDS pandemic as a health threat by 2030 by means that 90% of people infected with HIV know they have HIV, 90% of people infected get HIV. Antiretroviral therapy and suppression of HIV were achieved in 90% of people receiving antiretroviral therapy. To achieve this target, WHO recommended that all HIV patients be given antiretroviral therapy (ART) as soon as the diagnosis is made.¹

Currently, there are five known classes of ART, namely nucleoside reverse transcriptase inhibitors (NRTIs), non-nucleoside reverse transcriptase inhibitors (NNRTIs), protease inhibitors (PIs), integrase strands transfer inhibitors (INSTI), and fusion

inhibitors. ART regimens used as first-line have a strong potential to suppress viral replication. In Indonesia, first-line therapy generally consists of two types of NRTI drugs and one type of NNRTI drug. Protease inhibitors are used for second-line therapy. Combination therapy with ART is quite potent in suppressing viral loads and HIV transmission rates compared to single therapy.²

Improved access to ART reduces the incidence of HIV infection, morbidity, and mortality. However, widespread ART therapy can lead to ART resistance. ART resistance can lead to reduced drug susceptibility, poor treatment response, and treatment failure. ART resistance can affect response to therapy and increase mortality. In addition, the management

of drug-resistant HIV patients is quite tricky and requires careful consideration to prevent further failure of therapy.³

Definition of resistance of ART

ART resistance, according to WHO, is the presence of one or more mutations in HIV that reduces the ability of certain drugs or drug groups to inhibit viral replication. One mutation can cause the HIV to become resistant to one type of antiretroviral therapy, such as lamivudine or the NNRTI class. The mutation of the virus will multiply itself so that it will increase the viral load and cause therapy failure.³

Several factors that play a role in the life cycle and replication of HIV are the causes of resistance. First, the reverse transcriptase enzyme in viruses is not selected during the replication process and is prone to errors when converting viral RNA into DNA. This enzyme can cause errors in the HIV genome every single round of replication. This means that there is one mutation for every 2000 nucleosides. Since most of these errors occur during substitution, other mutations, such as insertions or duplications, can also occur.⁴

Second, HIV has a very high replicative capacity, i.e., several million new viral particles are produced per day in untreated patients. In untreated HIV patients, the total HIV RNA is 10³-10⁵ copies/ml and can reach 10⁶ copies/ml in acute infection or other comorbidities. Since the half-life of HIV-infected cells ranges from 1 to 2 days, the HIV must infect new cells at a high rate to maintain infection. The high replication speed with the high error rate of the reverse transcriptase enzyme will create many new virus types.⁴

Epidemiology of HIV drugs resistance

According to the 2019 HIV Drug Resistance Report issued by the WHO, the prevalence of ART drug resistance is 3-29%. The prevalence of HIV drug resistance varies by country. In developed countries, the prevalence ranges from 6.6-11%. In 2008, the prevalence of HIV resistance in England was 8%, while

in Switzerland, the prevalence varied from 2.2-15.5%. In developing countries, the prevalence of HIV is above 10%, such as in South Africa, as much as 23%, in East Africa, as much as 17%, and in Latin America, as much as 11%.^{3,5}

In Indonesia, data on the prevalence of ART is not available, but several studies in various regions have researched ART resistance. Kusumadiningrum A et al. (2019) studied ART resistance at CiptoMangunkusumo Hospital in 11 patients who experienced virological failure after six months. The most common mutations found were M184V, K103N, and Y181C. These mutations are expected to occur when patients are given first-line ART, namely the lamivudine (NRTI) and nevirapine or efavirenz (NNRTI) regimens.^{3,5} Hutapea H et al. (2018) conducted a study of ART resistance in Papua and found the prevalence of ART resistance was 5.16%. The M184V mutation was found in all respondents who were resistant to NRTIs. The K103N mutation was found in most respondents who were resistant to NNRTIs. However, in this study, the causes of resistance to ART were not explained.⁶

Identifying HIV resistance

There are two types of resistance to ART: primary and secondary resistance. Primary resistance reflects the acquisition of drug-resistant strains in individuals who have recently been infected and have not received therapy. Secondary resistance occurs after treatment with ART. Resistance to antiretroviral therapy, mainly NRTIs, NNRTIs, and protease inhibitors, is caused by continuous inhibition of the HIV-1 reverse transcriptase enzyme. As a result, there will be mutations in the PR and RT genes in the pol region that play a role in encoding PR and RT enzymes. The mutation aims to maintain the ability of HIV-1 to produce enzymatic proteins that are essential for the survival of its life cycle.⁷

There are two approaches to identifying HIV-1 resistance to ART. The first approach is genotyping, which compares gene sequences, generally proteases and reverse transcriptases, from HIV-1 patients to

HIV-wild-type sequences sensitive to antiretroviral therapy. The second approach is to examine the phenotype by testing the patient's HIV-1 sensitivity to antiretroviral therapy in vitro and then comparing it with the sensitivity of wild-type HIV-1. Sensitivity and susceptibility to ART were reported based on changes in inhibitory concentration (IC 50). In general, phenotyping can only determine resistance to PR and RT genes because it depends on available commercial kits. This makes genotyping a better approach or method to identify HIV-1 resistance to ART.⁷ Studies related to drug resistance mutations still tends to be limited to HIV-1 subtype B isolates. However, it is assumed that mutations occurring in subtype B isolates will produce the same phenotypic effect in all HIV-1 subtypes. Several studies showed that mutations in HIV-1 subtype B also occurred in non-B subtypes exposed to antiretroviral drugs.⁷

Mechanism of action and drug resistance

Nucleoside reverse transcriptase inhibitors (NRTI)

HIV has an envelope that contains RNA as genetic material and produces the reverse enzyme transcriptase. Reverse transcriptase is what converts

RNA into DNA polymerase by copying the HIV viral RNA genome into DNA strands. The DNA formed will become double-strand DNA and will be inserted into the human genome through the activity of the integrase enzyme. In the formation of new viral proteins, the HIV protease activity system is activated so that the HIV functions and causes infection.

Resistance to NRTIs occurs through two mechanisms. The first is the mutation of the residue remaining from the NRTI fusion when it enters the DNA strand. While mutations occur in the active site of reverse transcriptase, several other mutations have occurred in the proximal part of reverse transcriptase, which acts as a catalyst and can cause structural changes in the enzyme that interfere with the drug's binding to the active drug site. While mutations in the thymidine analogue affect AZT and stavudine, other mutations are observed in the other analogues. Levels of resistance occurred to the cytosine analogue of lamivudine, and the M184V mutation was found. Whereas in guanosine analogue abacavir resistance, concurrent mutations were found, such as M184V and L74V.¹⁰

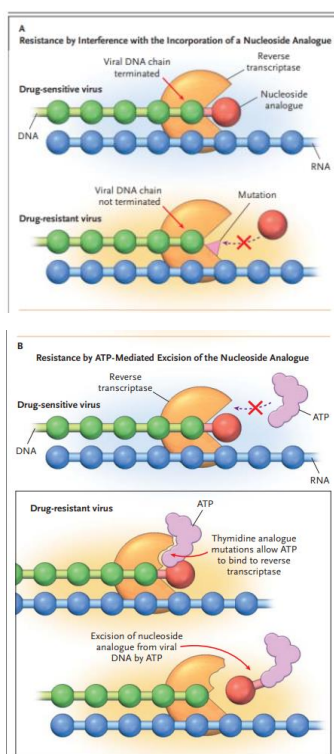


Figure 1. Resistance NRTI A) incorporation B) excision.

The second mechanism occurs through a reverse transcriptase mutation to cleave the phosphorolytic binding of the 3' end of the viral DNA chain from the leading site. This mutation causes ATP or pyrophosphate, which has a high concentration in the cell, to bind to the active site of the nucleoside analogue. The high energy of ATP or pyrophosphate will attack the bond between the drug and DNA. Thus, the drug will be released, and its effect will be reduced. Pyrophosphorolysis usually occurs with zidovudine and stavudine. Mutations of this type occur in thymidine analogue mutations (TAM), such as M41I, D67N, K70R, L210W, T215Y, and K219Q/E. Mutations in TAM also mean resistance to all NRTIs except lamivudine and emtricitabine, but cross-resistance can also occur. Two resistance mechanisms, namely discrimination, and excision can also influence each other. For example, the M184V1 mutations in lamivudine and emtricitabine are discriminatory, but viruses containing M184V/I also affect TAM, such as zidovudine.¹⁰

The low adherence of the HIV viral reverse transcriptase and the high rate of replication cause mutations every 10⁴ per cycle. The mutant virus

occurs due to replication errors and continuous production of CD4 cells containing proviral DNA. Mutations occurring in K65R, T69D, Q151M, and M184V/I caused an alteration in reverse transcriptase binding with little change in the dNTP substrate. This mutation occurs close to the substrate attachment site for deoxynucleoside triphosphates (dNTP).¹⁰

Non-nucleoside reverse transcriptase inhibitor (NNRTI)

The structure of this class of drugs is different from NRTIs. NNRTIs bind to sites close to the reverse transcriptase catalyst and alter the enzyme's ability to change the structure, preventing polymerization. The side effects of NNRTIs are generally lower than that of nucleoside analogues. NNRTIs have never been used as monotherapy because they quickly cause resistance. Efavirenz are the recommended first-line NNRTIs. Although efavirenz has relatively high effectiveness, resistance to efavirenz often causes virological failure. The most common mutations were mutations in K103N, L74V, and L1001. Resistance to nevirapine usually results from mutations in Y188C, K130N, G190A, and V106A.^{11,12}

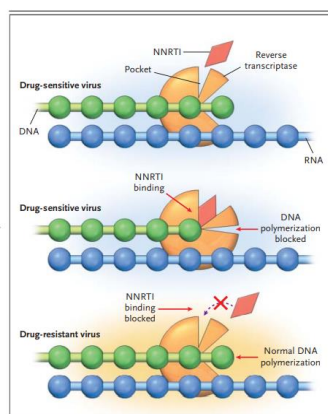


Figure 2. Resistance NNRTI10.

Protease inhibitor

Not all viral particles are functional or infectious until they reach maturation. Viral maturation is the cleavage of viral proteins by protease enzymes. This enzyme consists of a symmetrical arrangement of dimeric chains with the core bound to protein

peptides. Protease inhibitors work by binding to the enzyme catalyst site with a high affinity so that it will block the activity of the enzyme. Inhibition of the HIV protease enzyme still allows the formation of viral particles to be released by host cells but in immature and inactive forms.¹⁴

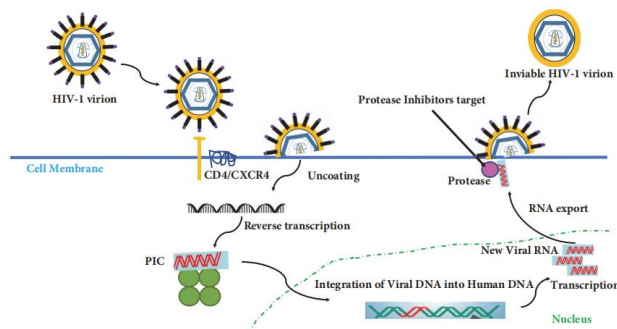


FIGURE 2. Schematic of HIV protease inhibition.

Figure 3. Resistance protease inhibitor.¹⁴

Resistance to protease inhibitors occurs through the mutation of amino acids located proximal to the catalytic site of the drug. Replacement of amino acids with protease enzymes changes the enzyme's affinity for binding. The geometry of the catalyst section will change and expand due to these mutations. Mutations in this strain have a significant impact on the binding of the drug to the endogenous peptide catalytic moiety.¹⁴

Integrase strand transfer inhibitor (INSTI)

Integrase is a specific enzyme that functions in viral replication. The enzyme integrase mediates the insertion of viral DNA into the host chromosome. This process begins when the double strand of viral DNA

meets endonucleolytic enzymes in the cytoplasm and is carried to the cell nucleus by the integrase enzyme as a preintegration complex. Then the integrase will insert the viral DNA into the host DNA. This is known as strand transfer. The iteration process will end with proviral DNA ligation. Examples of INSTI drugs are dolutegravir and elvitegravir.¹³ First-generation INSTIs such as raltegravir and elvitegravir have a low probability of developing resistance and cross-resistance. According to one study, resistance to raltegravir occurred in N155H, Q148R/H, and Y143R/C. In some cases, the primary mutation is replaced by several mutations, thereby increasing resistance.¹³

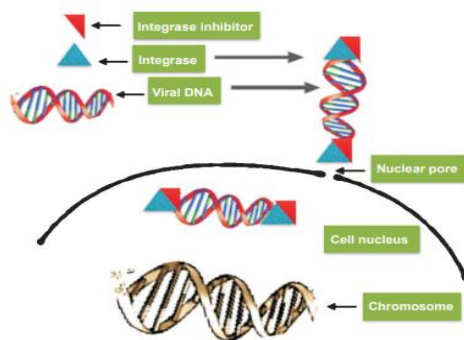


Figure 4. Resistance INSTI.¹³

Fusion inhibitor

This class of drugs works by inhibiting the entry and integration of HIV into host cells. The union of HIV and the host cell membrane is an essential step in the process of virus entry into cells. There are three steps

in entering HIV into human CD4 cells: attachment to the CD4 cell receptor, attachment to the chemokine receptor, and fusion of the viral membrane with the host cell membrane. Several viral proteins are required in this process. The protein on the surface of HIV,

namely gp120, binds to receptors on the surface of CD4 cells, and this will cause a change in the shape of gp120. This process will increase the affinity of the coreceptor and the release of the protein on the envelope, namely the gp41 protein. The gp120 protein will bind to the CCR5 or CXCR4 coreceptors on CD4

cells, and gp41 will enter the CD4 cell membrane. This results in the fusion of the viral membrane and the host CD4 cell membrane. The mechanism of fusion inhibitor resistance can occur due to changes in amino acids in the gp41 protein and coreceptors.^{8,9}

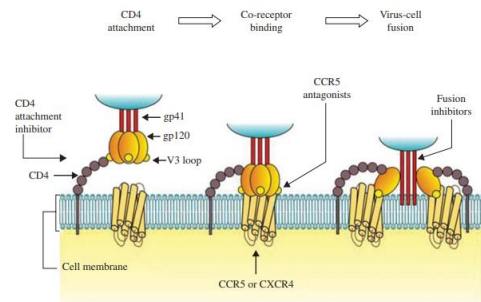


Figure 5. Fusion inhibitor.⁸

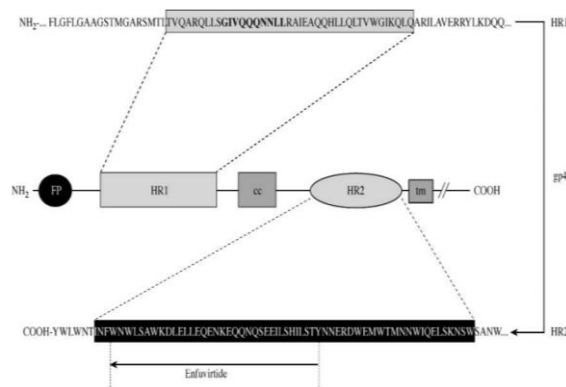


Figure 6. Resistance of enfuvirtide.⁹

Management of drug resistance

The World Health Organization (WHO) 2016 recommended two NRTIs plus Lopinavir or Atazanavir as a second-line regimen for individuals who have failed treatment with efavirenz or dolutegravir; two NRTIs plus Darunavir and Lopinavir plus Raltegravir are recommended as an alternative due to cost constraints and the fact that Darunavir is unstable in moderately hot conditions. The choice of NRTI as second-line ART depends on the type of NRTI already used in the first line. If Abacavir with Lamivudine or

Tenofovir with Lamivudine is already being used, Zidovudine and lamivudine should be used in the second line.¹⁵

Since the consensus on ART therapy was released in 2016, many studies have been carried out to explore other classes of second-line ART to find ART regimens other than NRTIs and protease inhibitors, strategies for maximizing PI doses, and the use of separate NRTI regimens. The WHO consensus in 2016 recommended the use of RAL as a second line for children and infants who experienced treatment failure with NRTIs.¹⁵

Table 1. Management antiretroviral therapy¹⁵

Population	Failing first-line regimen	Preferred second-line regimen	Alternative second-line regimen
Adults and adolescents	2 NRTI + DTG	2 NRTI + (ATV/r or lopinavir/ritonavir (LPV/r))	Darunavir/ritonavir (DRV/r) ± DTG + 1-2 NRTI (if possible consider optimization using genotyping)
	2 NRTI + EFV	2 NRTI + DTG	
Children	2 NRTI + DTG	2 NRTI + (ATV/r or LPV/r)	
	2 NRTI + LPV/r	2 NRTI + DTG	
	2 NRTI + NNRTI	2 NRTI + DTG	

2. Conclusion

ART resistance is the presence of one or more mutations in HIV that reduces the ability of certain drugs or drug groups to inhibit viral replication. World Health Organization (WHO) has recommended two NRTIs plus Lopinavir or Atazanavir as a second-line regimen for individuals who have failed treatment with efavirenz or dolutegravir; two NRTIs plus Darunavir and Lopinavir plus Raltegravir are recommended as an alternative due to cost constraints and the fact that Darunavir is unstable in moderately hot conditions.

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