



Resistance To Antiretroviral Therapy In People With HIV

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Abstract: Human Immunodeficiency Virus (HIV) is a virus that damages the immune system, and has RNA genetic material which is converted by the reverse transcriptase enzyme into DNA. This research aims to determine the existence of resistance by identifying mutations related to drug resistance in HIV-1, especially in the genes encoding the PR and RT enzymes which are the targets of ARVs. This research uses the observational method. Research data analysis was carried out in the form of descriptive data analysis of the output of each formula based on the results of HIV measurements. This study contains a series of reviews that focus on the topic and incidence and possibility of HIV drug resistance (ART) in PLHIV/PLWHA. The main and important thing in detecting the possibility of resistance to ARV therapy is by examining the genotype and phenotype. Standardization between laboratories for drug resistance studies through the use of various methods, especially to identify mixed bases that cause estimated resistance mutations. Additionally, sequencing of protease, RT, and/or integrase is used to identify the clinical significance of DRM. There are two genetic mechanisms of NRTI resistance, namely: (1) discriminative mutations that activate RT to differentiate between the dideoxy-NRTI chain terminator and the cell's own dNTP; and (2) unblocking mutations that facilitate phosphorylytic excision of NRTI-triphosphate from viral DNA. Blocking mutations are also referred to as thymidine analogue mutations (TAMs). ARV resistance can be detected by examining the virus genotype which aims to determine the occurrence of mutations in one of the virus codons compared to ARV-sensitive wild type HIV-1 and by in vitro phenotyping, where this method takes quite a long time and is usually focused on finding a regimen new drug.

Keywords: ARV; CD4; HIV; INSTI; NNRTI; NRTI; PI; Viral load.

Introduction

The HIV/AIDS control program aims to stop the AIDS epidemic with the specific objectives of: reducing or eliminating new HIV infections; reduce to eliminate deaths caused by conditions related to AIDS; and eliminating discrimination against people living with HIV AIDS (PLWHA) (Stover, et al., 2016). WHO targets reducing the number of new HIV infections by 500,000 per year by 2020. To help achieve this goal, WHO in 2016 recommended that all PLHIV/PLWHA should start ART as soon as possible after diagnosis and that pre-exposure prophylaxis (PrEP) should be considered for people who are at high risk of HIV infection (WHO,

2016). While successful implementation of these recommendations will reduce the number of new HIV infections, the dramatic increase in ARV use will likely increase the prevalence of acquired drug resistance (ADR) in treating individuals and transmitted drug resistance (TDR) in newly infected individuals (Daskalopoulou, et al., 2014). In Indonesia, cases of HIV and AIDS reached 519,158 people in the period January to June 2022, and around 1,188 children in Indonesia were found to be HIV positive (Gustawan, et al., 2022). The number of cases in men is 75% and women are 25%. Over the last 12 years, a total of 12,553 children under the age of 14 were found to be infected with HIV (Sadarang, et al., 2022).

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Human Immunodeficiency Virus (HIV) is a virus that damages the body's immune system, and has RNA genetic material which is converted by the reverse transcriptase enzyme into DNA (Kapila, et al., 2016). HIV has genetic material in the form of two positive single-strand RNAs which code for 10 types of genes (gag, pol, env, tat, rev, nef, vif, vpr, vpu, and tev) which code for 19 proteins (Allabidi, 2014). The gag, pol, and env genes are the main genes that are often used as a basis for classifying HIV genotypes and subtypes. The gag gene functions to regulate the process of viral replication and encodes structural proteins, as well as the pol gene which functions to code for enzymes needed for virus replication (transcriptase, integrase, and protease), while the env gene functions to regulate the formation of the envelope (membrane glycoprotein) of the HIV virus (Nakano, et al., 2022). Other genes also function to regulate the HIV transcription process (Ne, et al., 2018).

HIV is classified into two genotypes, namely HIV-1 and HIV -2. HIV -1 is divided into 11 subtypes, circulating recombinant form (CRF). The group of HIV-1 consists of three groups (groups M, N, and O). Apart from that, HIV-1 has 9 subtypes, namely subtypes A, B, C, D, F, G, H, J, K. Under certain circumstances, 2 viruses from different subtypes can meet in the body cells of an infected person and their genetic material will mixed together to form a new hybrid virus. Although some types of new viruses do not survive long, they can infect more than one person and are called Circulating Recombinant Forms (CRF) and to date 34 CRFs have been discovered. For example, CRF01_AE is a mixture of subtype A and subtype E. This subtype is most often found in the Southeast Asia region.

Subtype B is mostly found in sufferers who have homosexual contact and inject drug users. Meanwhile, subtypes C and CRFO 1_ AE are often found in heterosexual sufferers, so it is suspected that there is a relationship between the method of transmission and the type of HIV subtype that infects the sufferer.

HIV sufferers need antiretroviral treatment to reduce the number of viruses so that they do not enter the AIDS stage, while AIDS sufferers need treatment to prevent opportunistic infections. (Indonesian Ministry of Health, 2014). In accordance with WHO recommendations, the clinical protocol for HIV treatment for the initiation of universal ART is administered no later than 14 days after diagnosis, regardless of the clinical stage of the disease or CD4 count (Koenig, et al., 2017). ARV therapy has been proven to improve the quality of life of PLWHA, but it can also cause mutations in the HIV-1 virus. Mutations that arise due to ARV therapy are related to viral resistance to the ARV. Resistance is associated with high viral load values, low CD4 values, and adherence to

HIV/AIDS therapy. PLWHA who experience ARV resistance tend to have difficulty reducing the number of viruses.

Naturally, HIV-1 has a high mutation rate, namely 1 nucleotide in each replication cycle. Other research shows that HIV-1 exposed to zidovudine (AZT) experienced an increase in mutations 7.6 times in 1 replication cycle, and 3.4 times when exposed to lamivudine (3TC). In general, the principles of drug resistance are the same in all populations. Differences lie between low and middle income countries in the management of therapy. In developing countries like Indonesia, ART resistance testing is not routinely recommended. Understanding the principles and mechanisms of ART therapy is important to improve surveillance management, therapy delivery algorithms and case management.

Method

This research uses the observational method. Research data analysis was carried out in the form of descriptive data analysis of the output of each formula based on the results of HIV measurements. This study contains a series of reviews that focus on the topic and incidence and possibility of HIV drug resistance (ART) in PLHIV/PLWHA. The discussion was carried out through a literature study regarding the incidence of ARV resistance.

Result and Discussion

HIV drug resistance (ART)

Resistance to ARVs consists of 2 types, namely: primary resistance which occurs in naïve patients (not yet receiving therapy) and secondary resistance in patients who are undergoing ARV therapy. The most common and frequently used method for resistance detection testing is genotypic studies, namely by comparing gene sequences isolated from patient samples with wild type HIV-1 sequences that are sensitive to ARVs (Megasari, 2020). Resistance mutations themselves are differences in the wild type consensus subtype B, causing major and minor resistance (Kantor and Katzenstein, 2004).

Major resistance mutations can substantially reduce sensitivity to ARVs while minor mutations increase the replication ability of viruses that have major mutations. In most studies, detection of genotypic mutation resistance to ARVs often occurs (Dugyu T. et al., 2020). Administer ARVs to sufferers (PLHIV/PLWHA) no later than 14 days after diagnosis. The first line regimen is combination or alternative ART. If the main treatment component of choice is not suitable for PLWHA, it can be changed to alternative treatment. For example, in

pregnant female patients with low CD4⁺ (≤ 50 cells/ μ L), PLWHA with comorbid neurocognitive disorders, chronic kidney disease, cardiovascular disease, or chronic hepatitis, alternative ART regimens can be considered (Tekin, et al., 2021).

ARV resistance, especially in the NRTI, NNRTI and protease inhibitor groups, is caused by persistent inhibition of the HIV-1 PR and RT enzymes. As a result, mutations will occur in the PR and RT genes in the pol region which play a role in coding for the PR and RT enzymes. This mutation aims to maintain HIV-1's ability to produce enzymatic proteins that are important for the continuity of its life cycle (Clutter et al, 2016).

There are two types of approaches to identify HIV-1 resistance to ARVs: (1) Genotypic examination, which is carried out by comparing gene sequences, generally protease and reverse transcriptase, from the patient's HIV-1 to the wild type HIV-1 sequence which is sensitive to ARVs (Vella and Palmisano, 2005); (2) Phenotypic examination, which is carried out by testing the patient's HIV-1 sensitivity to ARVs in vitro, then compared with the sensitivity of wild type HIV-1.

Sensitivity to ARVs is reported based on changes in inhibitory concentration (IC 50). Phenotypic examination can generally only be carried out to determine resistance to PR and RT genes, because it relies heavily on available commercial kits. This makes genotypic examination a better approach or method for identifying HIV-1 resistance to ARVs (Vella and Palmisano, 2005).

In genotypic studies, the first stage of mutation interpretation is to list the amino acid differences between the sequence in the sample and the reference wildtype sequence (subtype B consensus sequence). Mutations were defined as differences to the wildtype subtype B consensus sequence, and were classified as major and minor mutations. Major mutations or primary mutations are mutations that can substantially reduce sensitivity to ARVs. Minor mutations or accessory mutations are mutations that do not have a substantial effect on the phenotype of the virus. Minor mutations can only increase the viral fitness or replication ability of viruses that have major mutations, resulting in a greater reduction in sensitivity to ARVs. The second stage is to use the list of identified mutations to estimate sensitivity to specific ARVs (Clutter et al., 2016).

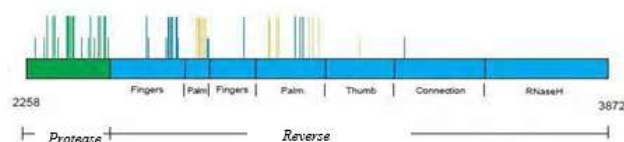


Figure 1. Schematic diagram depicting the distribution of mutations related to drug resistance in the PR and RT genes of HIV-1 (Brown et al., 2000)

Figure 1 shows the distribution of drug-resistance-related mutations in the HIV-1 PT and RT genes. Mutations that cause resistance to one or more drugs are represented by high lines. Accessory mutations that cause resistance only in the presence of another mutation are represented by shorter lines. Mutations related to resistance to protease inhibitors are depicted by green lines, resistance to NRTI is depicted by blue, while resistance to NNRTI is depicted by brownish yellow (Brown et al., 2000). Subtype B is mostly found in sufferers who have had homosexual contact and groups of injecting drug users. Meanwhile, subtype C and CRFO 1_ AE are often found in heterosexual sufferers. So it is suspected that there is a relationship between the method of transmission and the type of HIV subtype that infects the sufferer.

In a study by Zain, et al (2016), 89.1% of PLHIV in Malaysia experienced virological failure due to at least resistance to 1 type of NRTI, NNRTI or PI therapy. The results of the research also showed that the majority of ARV resistance occurred in NRTI and NNRTI (85%) and 15% in PI, while the level of codon resistance in each type of therapy was: minor PI at codon A71V/AT (16%), NRTI at codon 8184V (31.8%) and NNRTI at codon K103N (24%). Codon 8184 is located in the conserved region of the RT coding gene so that resistance occurs during 3TC, FTC and ABC therapy.

In NVP and EFP therapy, mutations often occur in the K103N codon (37.8%) and Y181C codon (23.7%), while in PI administration, mutations occur in the M46I, 154IV and V82A/T codons. The study also showed that 30 PLHIV children in Malaysia experienced therapy failure, the majority of PLHIV children (89.1%) had at least 1 type of mutation that was associated with clinical resistance to PI, NRTI/NNRTI therapy.

Megasari (2019), obtained results in his research that there was major resistance in naive PLHIV which fell into the high resistance category >15%, namely in the K310N, G190A, K219Q and E138A/6 genes which were associated with ART resistance from the NNRTI group. This resistance indicates that first-line therapy is inadequate.

Mechanisms of HIV drug resistance (ART)

Several polymorphic and non-polymorphic mutations have been identified in the HIV-1 integrase gene, some of which are the Y143C/H/R, Q148H/K/R, and N155H/S genes (Homat et al., 2018). In HIV-1, genetic variability results from high rates of HIV-1 reverse transcriptase (RT) processing errors, recombination when more than one viral variant infects the same cell, and the accumulation of proviral variants during infection (Abram et al., 2010). Although most HIV-1 infections are initiated by a single viral variant (Keele et al., 2008), countless variants (quasispecies)

associated with the initial transmitted virus emerge within weeks of infection (Perelson & Ribeiro, 2013).

Selection of ART-resistant variants depends on the extent to which viral replication continues during incomplete therapy resulting in the acquisition of a drug resistance mutation (DRM), and the effect of DRM on drug sensitivity and viral replication (Perelson & Ribeiro, 2013). For some ARVs, multiple DRMs are necessary to reduce sensitivity, whereas for others, a single DRM is sufficient. The number of DRMs required and the effect of each virally active DRM contribute to the ARV genetic barrier to resistance.

There is essentially no cross-resistance between drug classes. Viruses that are highly resistant to drugs in one ARV class are very sensitive to ARVs from a class that is not used (Larder, 1994). In contrast, significant cross-resistance within drug classes is common as most DRMs reduce sensitivity to multiple ARVs of the same class (Melikian et al., 2014). However, there is an important exception that some DRMs increase sensitivity to other ARVs of the same class (Whitcomb et al., 2002). Therefore, knowledge of the cross-resistance profile of ARVs is very important when using more than one drug from ARVs.

Most ART regimens used for first-line therapy are successful in completely blocking HIV-1 from replicating and have a genetic barrier to resistance. As a result, most cases of drug resistance arise from non-adherence to taking medication (Chi et al., 2007). Lower rates of HIV resistance have also been linked to therapy monitoring programs where early detection of virological flare provides the opportunity for adherence counseling or regimen modification as necessary (Charest et al., 2014).

Kridaningsih, et al (2021) explained that most ARV-resistant HIV is HIV-1 subtype CRF01_AE. In addition, the M184V mutation motif was found in all respondents who experienced resistance to NRTIs which could cause high-level resistance to lamivudine and emtricitabine. The mutation also confers low-level cross-resistance to abacavir and possibly didanosine, but increases susceptibility to tenofovir and zidovudine. Pathogenetically, the M184V mutation causes the virus to weaken and can be associated with a lower number of viruses compared to the wild type.

Bonura, et al (2010), reported that the highest resistance was in HIV-1 subtype B (61.2%) and there were at least 10.2% who were resistant to 1 type of drug with an average of 2% resistance mutations to NRTI administration and 8.1% against NNRTI. Resistance occurs in the M41L and T215D genes which cause resistance to zidovudine (FTC), abacavir (ABC), didanosine and TNF. Apart from that, there is also a mutation in the E1386 gene which causes resistance to efavirenz (EFV) and nevirapine (NVP). The research was

also continued with sequencing using NGS which identified resistance to at least 1 type of drug. The highest resistance occurred when giving NNRTI therapy (12.2%) and the lowest when giving PI (4%).

ARV Resistance Test

The main and important thing in detecting possible resistance to ARV therapy is by examining the genotype and phenotype (Zain, et al., 2016). In vitro phenotypic susceptibility testing by measuring ARV susceptibility in cell culture. Susceptibility is usually reported as the ARV concentration that inhibits HIV-1 replication by 50% (IC₅₀). The IC₅₀ of the patient's virus is compared with that of the drug-sensitive reference strain, and expressed as a ratio. Due to cost and long turnaround time, phenotypic resistance testing is usually reserved for development drugs, drug resistance studies, or complex clinical cases.

Standard Genotypic Resistance Testing (SGRT) involves the use of Sanger sequencing or NGS to perform protease, RT, and/or integrase sequencing to identify the clinical significance of DRM. SGRT was performed on PCR amplification products that were amplified directly from cDNA from plasma RNA. Because the virus population in an individual is usually heterogeneous, there will be more than one nucleotide in a particular position visible in the sequencing electropherogram.

Standardization between laboratories for drug resistance studies through the use of various methods, especially to identify mixed bases causing estimated resistance mutations (Woods et al., 2012). Additionally, sequencing of protease, RT, and/or integrase is used to identify the clinical significance of DRM. The lower limit of detection for SGRT is generally agreed to be around 20%. High DRM is associated with reduced response to ART especially NNRTI-based ART (Li et al., 2011).

NRTI ARV resistance mutations

There are two genetic mechanisms of NRTI resistance, namely: (1) discriminatory mutation that activates RT to differentiate between the dideoxy-NRTI chain terminator and the cell's own dNTP, thus preventing NRTI from being incorporated into viral DNA; and (2) the primer unblocks mutations that facilitate phosphorylytic excision of NRTI-triphosphate from viral DNA. Blocking mutations are also referred to as thymidine analogue mutations (TAMs) (Tang and Shafer, 2012).

The most common NRTI resistance mutation is M184V/I. M184V/I caused a high degree of decreased sensitivity (>200-fold) to the NRTI cytosine analogues 3TC and FTC. M184V/I also causes low-level resistance to abacavir (ABC). During Virologic Failure (VF) on regimens containing 3TC or FTC, M184I often appears

before resistance mutations in the M184V gene (Frost et al., 2000).

3TC and FTC were well tolerated with little side effects or toxicity. The most common mutations are mutations in the K65R, K70E/G/Q, L74V/I, Y115F genes and the Q151M mutation complex. K65R especially in TDF therapy. Resistance mutations in the K65R gene increase sensitivity to AZT. DRM most commonly occurs in patients with VF on TDF-containing regimens. Meanwhile, mutations in the L74V/I and Y115F genes mainly occur when abacavir is administered. However, L74I and Y115F also frequently occur with TDF administration, especially in areas where resistance testing is frequently performed due to VF (Skhosana et al., 2015).

Resistance mutations in the K70E/G/Q gene also occur with TDF and ABC therapy and are associated with a minimal reduction in sensitivity to NRTIs in vitro (Tenores Study, 2016). In the Q151M gene resistance mutation is a DRM that usually occurs only in heavily treated patients with prolonged VF with a combination of several accessory DRMs such as the A62V, V75I, F77L and F116Y genes which are associated with high levels of resistance to AZT and ABC and intermediate resistance to TDF, 3TC, and the FTC. Meanwhile, mutations in the M41L, D67N, K70R, L210W, T215F/Y and K219Q/E genes provide lower levels of cross-resistance to TDF and ABC (Tang and Shafer, 2012).

Some viruses that have possible thymidine analogue mutations (TAM) will cause a double amino acid insertion at position RT 69 which is referred to as T69S_SS (wild type threonine replaced with three serines). In combination with many TAMs, DRM causes high levels of resistance to AZT, ABC, and TDF, and intermediate resistance to 3TC and FTC (Masquelier et al., 2001).

Isayan, et al (2016) research, a mutation was found in the 184th amino acid, namely Methionine to Valine (M184V), which was caused by NRTI (Nucleoside Reverse Transcriptase Inhibitor) drugs, namely lamivudine and emtricitabine. This mutation also confers low-level cross-resistance to abacavir and possibly didanosine, but increases susceptibility to tenofovir and zidovudine.

Pathogenetically, the M184V mutation causes the virus to become weakened and can be associated with the lower number of this variant virus compared to the wild type.

ARV NNRTI resistance mutations

NNRTIs have a relatively low genetic barrier to resistance. There is a high level of cross-resistance in administering NNRTI therapy as a result of the mechanisms: (1) most NNRTI-resistant mutations reduce sensitivity to two or more NNRTIs (Melikian et

al., 2014); and (2) the low genetic barrier to NNRTI resistance allows the emergence of multiple independent resistant lineages to NNRTIs (Tang & Shafer, 2012).

The most common NNRTI mutations are mutations in the L100I, K101E/P, K103N/S, V106A/M, Y181C/I/V, Y188C/H/L, G190A/S/E, and M230L genes. Each of these mutations causes intermediate or high levels of phenotypic resistance to NVP, except for the resistance mutation in the Y181C/I/V gene which causes intermediate or high levels of phenotypic resistance to EFV (Melikian et al., 2014).

Although mutations in the Y181C gene reduced the incidence of EFV by only 2-fold, this was associated with a reduced response to EFV in patients with virological failure on NVP-containing regimens as a result of mutations in the K103N/S, V106A/M, Y188C/H genes, and G190A/S which causes phenotypic resistance to ETR and RPV (Melikian et al., 2014).

In addition, mutations in the E138K/G/Q/A gene are the most common mutations that appear in patients receiving RPV (Rinsky et al., 2012). Although mutations in the E138K gene minimally reduce RPV susceptibility, mutations in this gene are one of the most common DRMs occurring in patients treated with first-line regimens containing RPV.

In research by Hotma, et al, 2018, resistance mutations in the K103N gene were found in the majority of respondents who experienced resistance to NNRTI (NonNucleoside Reverse Transcriptase Inhibitor). This mutation causes high-level resistance to nevirapine, intermediate or high-level resistance to efavirenz, and is not associated with reduced susceptibility to etravirine and/or rilpivirine. There is an unusual occurrence of "cross talk" between NRTI and NNRTI resistance-associated mutations. Patients who fail tenofovir/emtricitabine/rilpivirine therapy often have a resistance mutation in the M184I gene, and not in the M184V gene (which is a more common mutation) in combination with a resistance mutation in the E138K gene.

PI ARV resistance mutations

Lopinavir/ritonavir (LPV/r), atazanavir/ritonavir (ATV/r), and darunavir/ritonavir (DRV/r) are the three most commonly used PI therapies. ATV/r and DRV/r are the two types of PI ARVs that are most often recommended but have the possibility of resistance. LPV/r is widely used in second-line ARV regimens (de Meyer et al., 2008; King et al., 2007). LPV/r and DRV/r therapy have also been shown to be effective in maintaining virological suppression in patients routinely on ARVs (Collins et al., 2016). ATV/r therapy has a lower genetic barrier to resistance than LPV/r and DRV/r, this is due to: (1) fewer mutations required for

reduced in vitro sensitivity; (2) smaller reductions in in vitro sensitivity are required to increase clinical virologic failure rates (Monogram Biosciences, 2016); and (3) ATV/r monotherapy is more effective than LPV/r and DRV/r for regimen simplification (Castagna et al., 2014).

DRM on PI therapy developed significantly less in patients receiving initial LPV/r, ATV/r, or DRV/r-containing regimens compared with DRM on NRTI and NNRTI therapy in patients receiving NRTI/NNRTI therapy regimens (Dolling et al., 2018).

The concentration range of ARV PIs is low to allow viral replication but high enough to exert selective drug pressure, which may lead to a reduction in the risk of resistance associated with PIs (Siliciano and Siliciano, 2013). Indeed, the majority of patients without DRM on PI therapy who experience virological failure (VF) experience virological suppression with increased therapy adherence (Lopez-Cortes et al., 2016).

Mutations in the gag matrix (MA) and gp41 cytoplasmic domains (gp41-CD) contribute to PI resistance but are not consistently responsible for the PI resistance that has been identified (Sutherland et al., 2014). Although PI-associated DRM rarely develops in naïve patients receiving one of the currently recommended PI-boosted therapies, the history of use of unboosted PIs in sufferers in high-income countries has led to the evolution of many highly PI-resistant viruses (Rhee et al., 2016).

The DRM with the greatest impact on sensitivity to ARV PI therapy is mainly due to the occurrence of resistance mutations in the V32I, G48V/M, I50V/L, V82A/T/L/F/S/C/M, and I84V/A/C genes. In addition, several mutations in enzymes including M46I/L and I54V/M/L/T/S/A, and in the core enzymes L33F, L76V, and N88S also significantly reduce the sensitivity of sufferers to ARV administration. Most PI-resistant viruses also require one or more additional protease mutations and one or more mutations in the compensatory gag cleavage site (Fun et al., 2012).

The occurrence of several accessory mutations in the protease enzyme contributes to a high genetic barrier to resistance to Protease Inhibitor (PI) administration. DRM in ATV therapy causes high levels of PI resistance. Each resistance mutation that occurs in the protease enzyme can reduce sensitivity to two or more types of PI therapy and provide the possibility of complex cross-resistance to PI administration (Tang and Shafer, 2012). This especially occurs in DRM at codon positions 50, 54 and 82 (Rhee et al., 2010).

Due to its high genetic barrier to resistance, DRV/r and LPV/r therapy is often used in patients who experience therapy failure as an alternative (Kempf et al., 2002; King et al., 2007).

INSTI ARV resistance mutation

Globally, the occurrence of resistance mutations to INSTI therapy tends to be low due to low resistance in the integrase gene region (Dugyu T. et al., 2020). DRM is usually associated with >150-fold reduced sensitivity to the ARV raltegravir (RAL). Although individual DRMs are associated with much lower levels of phenotypic resistance, it is likely that high levels of resistance may occur.

Most of the occurrences of resistance to INSTI therapy, especially in RAL therapy, occur in patients who received first-line regimens containing RAL and from patients who received RAL therapy late. In addition, along with resistance mutations to RAL, the majority of INSTI resistance events are related to resistance to elvitegravir (EVG) administration derived from viral isolates obtained during first-line EVG-containing regimens.

There are two types of non-polymorphic DRM in resistance to INSTI therapy. Resistance to the T66I/K and S147G genes occurs more commonly with EVG administration and causes a lower level of cross-resistance to RAL administration (Abram et al., 2013). In some patients with virological failure, the INSTI therapy given is rarely switched to another INSTI (Huang et al., 2013). In contrast to RAL and EVG therapy, some DRM showed clinically significant resistance to dolutegravir (DTG). Most of the data on the genetic mechanisms of DTG resistance were obtained from patients who had experience with RAL therapy (Castagna et al., 2019).

HIV sufferers who have mutations in the Q148 gene in combination with E138 and G140 mutations have an increased risk of VF. A number of INSTI accessory DRMs can also increase the risk of resistance to DTG, although in a low percentage. Virological failure in resistance to DTG, especially in DRM with a combination of Q148 gene resistance mutations.

INSTI resistance mutations were not found in treatment-naïve HIV-infected patients. However, in patients who had been treated with ARV F121Y, Y143R, Q148R, and E157Q were detected as the main resistance mutations associated with raltegravir and elvitegravir. The overall prevalence of INSTI resistance mutations in ARV-experienced patients was 6.6%.

Since the beginning, dolutegravir has attracted quite a lot of attention as a breath of fresh air for the treatment of HIV sufferers, which is considered to have very good effectiveness in suppressing the virus. Dolutegravir has been proven to have a high viral suppressive effect with milder side effects and fewer drug interactions, but the possibility of resistance must remain a concern. Sayan, et al (2016) research shows that naturally occurring mutations or substitutions that can cause primary resistance to INSTIs are not routinely found. According to The SPEARD Program of Europe,

no INSTI-resistant variants were found circulating in Europe before the introduction of INSTI therapy. Therefore, INSTI resistance testing before initiation of first-line therapy may not be necessary. However, INSTI resistance testing may be performed before changing therapy, as the major resistance mutations of RAL and EVG (F121Y, Y143R, Q148R, and E157Q) are likely to occur (Hotma et al., 2018).

INSTI resistance testing can be an integral part of the management of HIV-1 infection, and a viable second-line therapy regimen option. Therefore, INSTI may be a powerful option for initial or replacement ARV therapy in HIV-1-infected individuals. In this study, it was found that what causes resistance to Integrase Inhibitor class drugs is not the drugs in this class, but the NRTI, NNRTI and PI drugs. This is interesting because as we know, the drug resistance should be due to exposure to the drug class itself. However, in this study, the Integration Inhibitor drug was resistant because the patient had been given drugs from other groups due to gene mutations caused by these drugs (Sayan et al., 2016).

HIV subtypes

HIV-1 is classified into three main groups, namely M (main), N (non-M non-O), and O (outlier). Group M is the group most associated with the global HIV infection pandemic. Group O is often found in western Central Africa and Europe, while group N is very rare and has only been identified in Africa (Hemelaar et al., 2006).

Group M, the most widely circulating group, is divided into subtypes based on their gene sequences. HIV-1 subtypes, which are also referred to as clades, are HIV-1 strains that are phylogenetically related, having more or less the same genetic distance from one another (Hemelaar et al., 2006). Group M is divided into nine subtypes, namely A, B, C, D, F, G, H, J and K, and additional subtypes in the form of circulating recombinant forms (CRF) and unique recombinant forms (URF). Subtype A is then classified into four sub-subtypes, namely sub-subtypes A1 to A4, while subtype F is classified into two sub-subtypes, F1 and F2 (Hemelaar et al., 2006).

HIV-1 is a virus with very high genetic variability and is the result of a high level of mutation and recombination rate of the reverse transcriptase enzyme, coupled with a fast rate of viral replication. In one replication cycle, reverse transcription can produce one substitution and seven to thirty crossovers in one HIV-1 genome (Robertson et al., 2000; Tebit et al., 2007; Taylor et al., 2008). The high genetic variability of HIV-1 is shown by the existence of genetic variation within one HIV-1 subtype which ranges from 8% to 17%, and genetic variation between subtypes can reach 17% to 35% (Fischer et al., 2021).

Effect of HIV Subtype on HIV disease progression

Nakawa et al. (2016) conducted research using Concerted Action on SeroConversion to AIDS and Death in Europe (CASCADE) data. The study included 3,364 seroconverter individuals. Individuals infected with subtype CRF01_AE had a higher number of CD4 T lymphocytes at the time of seroconversion, followed by subtype B and subtype C. The decline in the number of CD4 T lymphocytes was slower in subtypes A and CRF02_AG compared with subtype B.

The study conducted by Kiwanuka et al. (2004) in Africa, showed that individuals infected with subtype D had more rapid progression to AIDS and AIDS-related death compared with individuals infected with subtypes A, C, and the recombinant form. Research conducted by Easterbrook et al. (2010) in the United Kingdom, showed that individuals infected with subtype D experienced a more rapid decrease in the number of CD4 T lymphocytes compared to individuals infected with subtypes A, B, C, and CRF01_AG. Subtype D also has a higher level of virological rebound (>400 copies/mL) after six months of ARV therapy than subtypes A, B, and C.

A cohort study of commercial sex workers in West Africa showed that the rate of progression to AIDS was up to eight times faster in subtypes C, D, and G than in subtype A. Several studies conducted in Southeast Asia showed an association between the most common subtypes of HIV-1 widely circulating, namely CRF01_AE, with the speed of disease progression. Patients infected with subtype CRF01_AE experienced a more rapid decrease in the number of CD4 T lymphocytes compared to subtypes B, CRF33_01B, CRF34_01B, and G (Netanya et al., 2011).

Apart from being related to infection progression, subtype is also related to transmission efficiency. Associated with vertical transmission, subtype C transmission is higher than subtypes A and D. Heterosexual transmission is higher in subtype A than subtype D (Kiwanuka et al., 2009), while heterosexual transmission in the form of vaginal shedding occurs higher in subtype C than in subtype A and D (Kiwanuka et al., 2009).

Transmission of HIV (ARV) drug resistance

In most studies, more than 70-80% of patients with virological failure caused acquired ARV resistance (ADR) (Clutter, et al., 2016). The M184V/I gene resistance mutation is usually the initial NRTI ART DRM for regimens containing cytidine analogues (3TC or FTC). Among patients receiving NNRTI-containing ART regimens, one or more NNRTI DRMs were also commonly seen early in virologic failure. Other NRTI DRMs usually arise after mutations in the M184V/I and NNRTI genes (Rhee, et al., 2015).

DRM on INSTI therapy can develop since initial treatment, but DRM on PI therapy is rare. Resistance is detected more frequently in patients with infrequent viral load testing during therapy monitoring because VF is usually detected later, after accumulation of DRM (Gupta et al., 2009). Viral load testing that is rarely carried out by HIV sufferers can jeopardize the success of administering second-line therapy, especially administering NNRTI and INSTI-based regimens.

ADR patterns in VF vary according to regimen and level of patient compliance. In all recommended first-line regimens containing cytidine analogues, resistance mutations in the M184V gene are the most common in DRM (Rhee et al., 2015). The subsequent development of NRTI therapy DRM relies on administration of a second NRTI in the therapy regimen (Rhee et al., 2015).

When administering Abacavir therapy, resistance mutations mainly occur in the L74V/I, Y115F genes, and less frequently in the K65R gene the occurrence of DRM on NNRTI regimen therapy is earlier and more frequent with NNRTI-based regimens and occurs widely in the majority of patients with VF (Molina et al., 2021). In high and middle income countries, resistance mutations occur in the K103N gene followed by Y181C, G190A/S, Y188L, and V106M which are the most common mutations causing DRM in NNRTI regimens (Rhee et al., 2015). After failure of first-line NNRTI-based regimens containing EFV or NVP, cross-resistance to second-generation NNRTIs in RPV and ETR therapy is common and associated with virological failure/VF (Theys et al., 2015).

In patients who have never received a PI antiretroviral regimen (naïve sufferers), DRM often occurs in approximately 10% or less and occurs in patients who fail PI-based therapy (Barber et al., 2012). Most patients with VF on PI-based regimens without PI DRM will achieve virological suppression resulting in a reduction in HIV viral load simply by improving adherence (Zheng et al., 2014).

In patients with VF on first-line Raltegravir (RAL) or Elvitegravir (EVG)-based regimens, resistance to INSTI therapy is not uncommon (Kulkarni et al., 2019). However, on the contrary, resistance to INSTI-based treatment is rarely found in patients given first-line Dolutegravir (DTG)-based regimens (Raffi et al., 2013).

Standard genotypic resistance testing (SGRT) is recommended in patients with VF when the viral load is >1000 copies/mL (Raffi et al., 2013), and should be considered when the viral load is >50 copies/mL (Gonzalez-Serna et al., 2014). In patients with VF, the ability to detect ADRs is much higher and SGRT is performed while the patient is on a failed regimen or within four weeks of stopping ARV therapy (Gonzalez-Serna et al., 2014). Genotypic testing can indicate whether VF is primarily caused by therapy

nonadherence and can also be used to help design second-line therapy when ADR is the cause of VF.

Patients who experience VF without DRM can often be treated successfully with ARV therapy adherence counseling alone (Bonner et al., 2013). In contrast, patients with VF who have experienced DRM on a first-line regimen usually require a change in ARV therapy. In high-income countries where SGRT is routinely performed in the setting of VF, second-line regimens should include at least two active therapy regimens.

Management and care of patients with ADR who fail to use second-line ARV regimens requires expert guidance and special treatment. With complex resistance patterns, phenotypic testing is useful for determining the activity of various ARVs against viruses in infected cells (Gonzalez-Serna et al., 2014). ARV regimens in patients who have experienced multiple VFs and who have multiclass resistant viruses tend to be complex in treatment and require multiple ARVs that have partial residual activity.

Relationship between CD4 levels and progression of HIV-1 infection

CD4 cells play a role in providing support to B cells to form antibodies and help augment the cellular immune response to antigens (WHO, 2014). In primary infection, the CD4 count decreases between 20% and 40%, accompanied by a decrease in the qualitative function of CD4 cells. (WHO, 2007). After going through the primary infection stage, infected individuals remain asymptomatic for years. During this period, the immune system is still competent to carry out immune surveillance and prevent most infections. A quantitative decrease in the number of T lymphocyte cells indicates a decrease in immune capacity (WHO, 2014).

WHO classifies the level of immune suppression (immunosuppression) based on the number of CD4 T lymphocytes, as shown in Table 1. Apart from being an indicator of the ability of the immune system, estimation of the number of CD4 cells is also used to monitor the response to ART along with checking the HIV viral load (WHO, 2014).

Table 1. CD4 count and its relationship to immune suppression for adolescents and adults

Not significant immunosuppression	>500 sel/mm ³
Mild immunosuppression	350 sel/mm ³ – 499 sel/mm ³
Advanced immunosuppression	200 sel/mm ³ – 349 sel/mm ³

Several cases showed sufferers (PLHIV) who experienced difficulty in increasing their CD4 value to 350 cells/ul. There were 2 respondents whose CD4 value was above 350 cells/ul, but were infected with ARV-

susceptible HIV and had a viral load of more than 10,000 copies/ml blood. This is associated with the respondent's level of compliance which is considered low (>30%). A compliance level below 30% results in insufficient exposure to ARV viruses (Sayan, et al., 2016).

HIV (ARV) drug resistance surveillance

Between 2013 and 2018 WHO recommended monitoring adherence to ARV therapy among newly infected populations (Bennett et al., 2019). WHO recommended surveillance methods are limited to naïve sufferers (not yet receiving ARVs) who may be newly infected (Bennett et al., 2009). In 2015, WHO revised its recommendations and prioritized monitoring for drug resistance among all populations initiating ART, regardless of duration of infection or prior ARV exposure.

Implementation of the WHO Consolidated HIV Guidelines recommends initiation of ART for all stages of sufferers and increasing PrEP administration is expected to lead to a reduction in HIV incidence. However, among infected patients a high proportion are infected primarily with ARV-resistant strains and will be at risk of ADR. To address this problem and ensure coordinated action to monitor and respond to possible resistance to ARV administration, WHO developed a Global Action Plan on HIV ARV Resistance (WHO, 2016)

Therapy failure as a result of ARV resistance

The presence of mutations in HIV-1 can cause the emergence of drug-resistant strains which can affect the effectiveness of the ARVs given. In conditions where resistance to ARVs occurs, the drugs given will not be able to control HIV-1 replication effectively, so therapy failure can occur.

Therapy failure is a condition that needs to be suspected if the expected therapeutic response does not occur after starting therapy for at least 6 months with high compliance. Therapy failure is determined based on three criteria, namely clinical, immunological and virological criteria. In conditions where CD4 count and/or viral load cannot be checked, a diagnosis of therapy failure can be made based on clinical symptoms alone (Ministry of Health of the Republic of Indonesia, 2012).

Therapy failure according to WHO criteria is as follows: (RI Ministry of Health, 2022):

1. Clinical failure:

Clinical failure was defined as the emergence of opportunistic infections of the stage 4 group after a minimum of 6 months of ARV therapy. Some diseases included in clinical stage 3 (pulmonary TB, severe bacterial infections) can be an indication of therapy failure.

2. Immunological Failure

Immunological failure is the failure to achieve and maintain an adequate CD4 count, even though there has been a reduction/suppression of the viral load. In adult patients, immunological failure is said to occur if the CD4 count is <250 cells/mm³ after clinical failure or persistent CD4 <100 cells/mm³.

3. Virological failure

The main goal of ARV treatment is viral suppression, so that the viral load becomes very low and cannot be detected. Viral load measurements can reflect the number of HIV viral particles per milliliter (ml) of blood. Viral load testing is the gold standard for monitoring treatment. Low viral load levels indicate that the treatment given to the patient is working effectively. Generally, the viral load is undetectable if the amount of HIV-1 RNA in plasma reaches less than 50 copies/mL (UNAIDS, 2016). It is declared a virological failure if the viral load test results are >1000 copies/mL based on 2 consecutive examinations with an interval of 3 months, with support for good adherence after a minimum of 6 months of ARV treatment. (RI Ministry of Health, 2022)

High viral load levels indicate non-compliance with treatment or the possibility of viral resistance to the medication being given. In the study by Dugyu et al (2016), virological failure was detected in patients with a viral load of 56,567 DNA copies/ml and was associated with patient adherence to therapy. Resistance mutations are associated with virological failure. When administering INSTI therapy such as dolutegravir (DTG), there is also a chance that resistance mutations will occur, especially in the Q148K gene, which can cause high levels of virological failure. Meanwhile, resistance mutations are closely related to virological failure in NRTI type ART, especially thymidine analog mutations (TAM).

Conclusion

ARV resistance, especially in the NRTI, NNRTI and protease inhibitor groups, which is caused by persistent inhibition of the HIV-1 PR and RT enzymes. As a result, mutations will occur in the PR and RT genes in the pol region which play a role in coding for the PR and RT enzymes. Mechanisms of drug resistance mostly result from non-compliance with taking medication. Yet most ART regimens used for first-line therapy are successful enough to completely block HIV-1 from replicating and have a genetic barrier to resistance. ARV resistance can be detected by examining the virus genotype which aims to determine the occurrence of mutations in one of the virus codons compared to ARV-sensitive wild type HIV-1 and by in vitro phenotyping, where this method takes quite a long time and is usually

focused on finding a regimen new drug WHO revises recommendations and prioritizes monitoring for drug resistance among all populations initiating ART, regardless of duration of infection or prior ARV exposure. Therapeutic failure is greater due to resistance mutations associated with virological failure.

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Author Contributions

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Conflicts of Interest

We have no conflict of interest.

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