

## HIV-1 drug resistance during the transition from NNRTI- to INSTI-based therapy in Thailand: prevalence, resistance-associated mutations, and antiretroviral

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## ABSTRACT

**Background:** Antiretroviral therapy (ART) effectively suppresses HIV replication and reduces HIV-related morbidity and mortality; however, HIV drug resistance (HIVDR) remains a major challenge to long-term treatment success.

**Objectives:** This study investigated the prevalence of HIVDR, resistance-associated mutations (RAMs), antiretroviral susceptibility profiles, and factors associated with HIVDR among HIV-infected patients receiving ART at Queen Savang Vadhana Memorial Hospital, Thailand.

**Materials and methods:** A retrospective study was conducted among 261 HIV-infected patients with HIV RNA levels >1,000 copies/mL who underwent HIVDR testing between January 2020 and December 2024. Drug resistance was determined using next-generation sequencing (NGS)-based assay simultaneously analyzes the protease (codons 1-99), reverse transcriptase (codons 1-376), and integrase (codons 1-288) regions of the HIV-1 pol gene. Variant-calling thresholds were 20% was applied to samples with HIV RNA  $\geq$ 1,000 copies/mL, 5% was used for samples with HIV RNA  $\geq$ 15,000 copies/mL, and resistance interpreted using the Stanford HIV Drug Resistance Database (HIVdb version 9.4).

**Results:** Among 261 patients, 169 (64.8%) harbored genotypic drug resistance mutations. CRF01\_AE was the predominant HIV-1 subtype (93.1%). Dual resistance to nucleoside reverse transcriptase inhibitors (NRTIs) and non-nucleoside reverse transcriptase inhibitors (NNRTIs) was the most common resistance pattern (49.7%), followed by NNRTI-only resistance (24.3%). NNRTI resistance was the most prevalent resistance class (85.8%), followed by NRTI (65.1%), protease inhibitor (PI) (17.8%), and integrase strand transfer inhibitor (INSTI) resistance (4.1%). M184V and K103N were the most frequently detected NRTI- and NNRTI-associated mutations, respectively. Resistance to nevirapine, efavirenz, and abacavir was common, whereas susceptibility to darunavir/ritonavir, dolutegravir, and bictegravir remained high. Patients with CD4 counts <100 cells/ $\mu$ L and ART duration of 5-10 years were more likely to harbor drug-resistant HIV strains, whereas second-line ART was associated with lower odds of HIVDR.

**Conclusion:** Combined NRTI-NNRTI resistance remained the predominant HIVDR pattern during 2020-2024. Although INSTI-associated resistance emerged following the implementation of INSTI-based therapy in Thailand, its prevalence remained low, supporting the continued effectiveness of dolutegravir-based regimens. Continuous HIVDR surveillance remains essential to optimize long-term treatment outcomes.

## Introduction

Human immunodeficiency virus (HIV) infection remains a major global public health challenge. According to the World Health Organization (WHO), an estimated 40.8 million people were living with HIV worldwide in 2024, with approximately 1.3 million new infections and 630,000 AIDS-related deaths. Despite these challenges, substantial progress has been achieved through the expansion of antiretroviral therapy (ART), with 31.6 million people receiving treatment globally.<sup>1</sup> Thailand has made considerable progress in HIV prevention and treatment through the implementation of universal access to ART. In 2023, an estimated 474,675 individuals were receiving ART, while 9,083 new HIV infections and 12,072 AIDS-related deaths were reported nationwide.<sup>2</sup> Since 2013, Thailand has achieved reductions of approximately 37% in new HIV infections and 57% in AIDS-related deaths, reflecting the success of national HIV treatment programs.<sup>2</sup> Current Thai and international treatment guidelines recommend initiating ART as early as possible following HIV diagnosis, regardless of CD4 cell count, with the goal of achieving sustained viral suppression and maintaining plasma HIV RNA levels below 50 copies/mL<sup>3,4</sup>

Antiretroviral agents are classified into several major

drug classes, including nucleoside/nucleotide reverse transcriptase inhibitors (NRTIs), non-nucleoside reverse transcriptase inhibitors (NNRTIs), protease inhibitors (PIs), integrase strand transfer inhibitors (INSTIs), entry inhibitors, capsid inhibitors, and pharmacokinetic enhancers.<sup>5</sup> Since 2021, Thailand has adopted dolutegravir (DTG)-based regimens as the preferred first-line therapy, consisting of two NRTIs combined with DTG because of its high efficacy, favorable tolerability, and high genetic barrier to resistance.<sup>3,4</sup> Prior to initiating abacavir-containing regimens, screening for the HLA-B\*57:01 allele is recommended to reduce the risk of severe hypersensitivity reactions. During treatment, plasma HIV RNA monitoring remains the standard approach for assessing treatment response and identifying virologic failure.<sup>3,4</sup>

Despite the effectiveness of ART, HIV drug resistance (HIVDR) continues to threaten long-term treatment success. Acquired HIVDR results from the accumulation of viral genetic mutations that reduce antiretroviral susceptibility and compromise virologic suppression. Persistent viral replication under drug-selective pressure may lead to the emergence of resistance-associated mutations (RAMs), treatment failure, and limitations in future therapeutic options. HIVDR can be assessed using genotypic, phenotypic,

or virtual phenotypic resistance testing, with genotypic resistance testing (GRT) being the most widely used approach in clinical practice.<sup>6</sup> Although several studies have reported HIVDR patterns in Thailand, limited data are available regarding acquired HIVDR, RAMs, antiretroviral susceptibility profiles, and factors associated with HIVDR during the transition from NNRTI-based to INSTI-based first-line therapy. Understanding contemporary resistance patterns is essential for optimizing treatment strategies and monitoring the long-term effectiveness of current ART regimens.

Therefore, this study investigated the prevalence of HIVDR, RAMs, antiretroviral susceptibility profiles, and factors associated with HIVDR among HIV-infected patients receiving ART who underwent HIVDR testing at Queen Savang Vadhana Memorial Hospital, Thailand. In addition, this study provides real-world evidence on acquired HIVDR during Thailand's transition from NNRTI-based to INSTI-based first-line therapy.

The findings provide real-world evidence on acquired HIVDR among patients with virologic failure and may help inform clinical decision-making for the management of virologic failure and selection of second- and third-line antiretroviral regimens.

## Materials and methods

### Study design and population

A retrospective descriptive study was conducted among HIV-infected individuals receiving ART at Queen Savang Vadhana Memorial Hospital, Thailand, between January 2020 and December 2024. Patients with HIV RNA levels >1,000 copies/mL who underwent HIVDR testing were eligible for inclusion.

The inclusion criteria were:

- 1) HIV-infected patients receiving ART,
- 2) HIV RNA levels >1,000 copies/mL,
- 3) availability of HIV GRT results, and
- 4) complete demographic and clinical data, including sex, age, CD4 cell count, HIV RNA level, duration of ART, ART regimen, and genotypic resistance results.

Duplicate HIVDR test submissions were excluded. All eligible patients (N=261) had complete data for the study variables and were included in the analyses.

This study was approved by the Human Research Ethics Committee of Queen Savang Vadhana Memorial Hospital (COA No. 005/2568; IRB No. 008/2568; approved on March 10, 2025).

### HIV viral load testing

Plasma HIV RNA levels were determined using the Cobas® 6800 System (Roche Molecular Systems, Branchburg, NJ, USA). This fully automated real-time reverse transcription polymerase chain reaction (RT-PCR) assay employs a dual-target strategy that amplifies highly conserved regions of the HIV-1 genome, including the gag gene and the long terminal repeat (LTR) region. The assay has a lower limit of quantification

(LLOQ) of 20 copies/mL.

### Genotypic resistance analysis

GRT was performed at the Virology and Molecular Biology Unit, Faculty of Medicine Ramathibodi Hospital, Bangkok, Thailand, using the Sentosa® SQ HIV-1 Genotyping Assay v2.0 (Vela Diagnostics, Singapore). This next-generation sequencing (NGS)-based assay simultaneously analyzes the protease (codons 1-99), reverse transcriptase (codons 1-376), and integrase (codons 1-288) regions of the HIV-1 pol gene and supports detection of HIV-1 subtype and RAMs. The assay incorporates an integrated workflow consisting of automated RNA extraction, library preparation, semi-conductor-based sequencing, and bioinformatic analysis. Samples with HIV RNA levels >1,000 copies/mL were considered suitable for sequencing. The overall sequencing success rate during the study period was 97.4%.

Variant-calling thresholds were determined by HIV RNA level. A threshold of 20% was applied to samples with HIV RNA ≥1,000 copies/mL for detection of major variants, whereas a threshold of 5% was used for samples with HIV RNA ≥15,000 copies/mL to identify minority variants. Quality control criteria included a minimum sequencing coverage depth of 1,000× and contamination assessment using the system control (SC).<sup>7</sup>

Genotypic resistance was interpreted using the Stanford HIV Drug Resistance Database (HIVdb) algorithm version 9.4.<sup>8</sup> Drug susceptibility results were categorized as susceptible, potential low-level resistance, low-level resistance, intermediate resistance, or high-level resistance.

### Drug resistance mutations

RAMs were identified according to the 2025 update of the International Antiviral Society-USA (IAS-USA) drug resistance mutation list.<sup>9</sup>

### Data collection and Interpretation

Data on HIV RNA levels, HIVDR profiles, and RAMs were retrieved from the laboratory information system, the National AIDS Program database, and the hospital information system. Demographic and clinical variables, including sex, age, CD4 cell count, HIV RNA level, duration of ART, ART regimen, HIV-1 subtype, and resistance profiles, were collected and evaluated for completeness. We included only patients with complete datasets in the final analysis. All data were anonymized prior to analysis and recorded in a secure study database.

For this study, genotypic drug resistance was defined as the presence of low-, intermediate-, or high-level resistance to at least one antiretroviral agent according to the HIVdb interpretation algorithm. We categorized continuous variables using clinically relevant cut-off values to facilitate statistical analysis and interpretation.

### Statistical analysis

Continuous variables were summarized as medians and interquartile ranges (IQRs), whereas categorical variables were presented as frequencies, percentages, and 95% confidence intervals (CIs) for key proportions. We used descriptive statistics to summarize demographic, clinical, virological, and resistance-related characteristics. The Wilson score method was used to calculate 95% confidence intervals (CIs) for key proportions. Temporal differences in resistance frequencies and other comparisons between categorical variables were assessed using the chi-square test or Fisher's exact test, as appropriate. Associations between clinical variables and HIVDR were assessed using univariate logistic regression analysis, and results were reported as odds ratios (ORs) with 95% confidence intervals (CIs). Comparisons between categorical variables were performed using the chi-square test or Fisher's exact test, as appropriate. No missing data were identified for the variables included in the statistical analyses; therefore, no imputation was required.

All statistical analyses were performed using GraphPad Prism version 10 (GraphPad Software, San Diego, CA, USA). A two-sided  $p < 0.05$  was considered statistically significant.

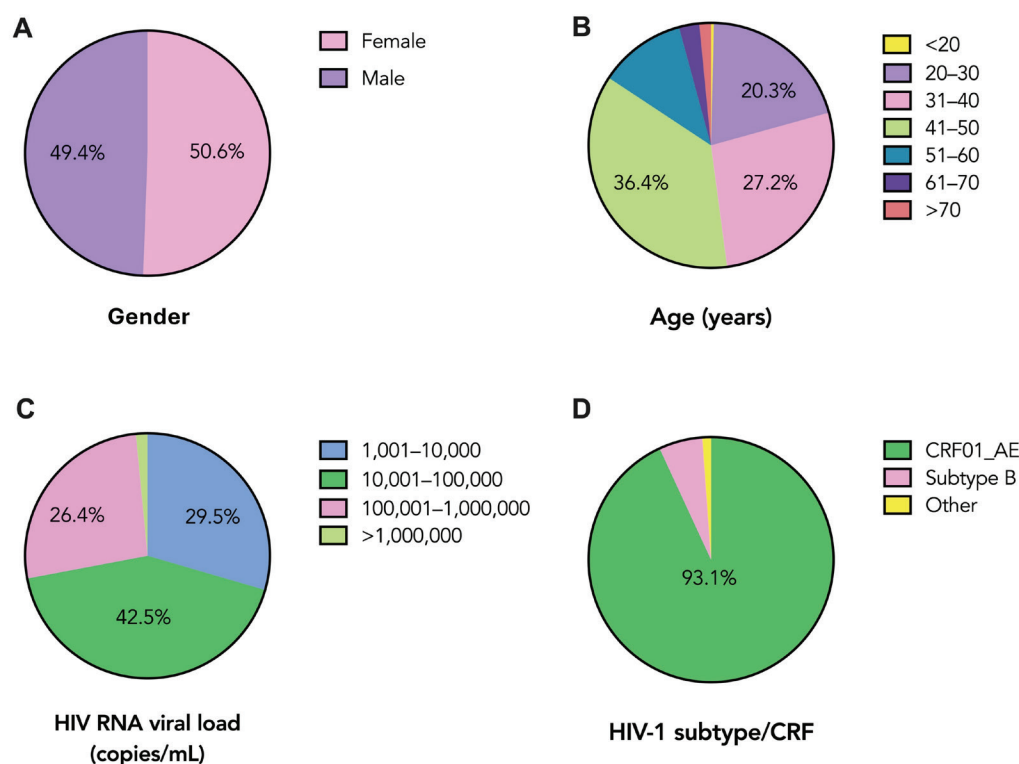
### Results

#### Study population and baseline characteristics

Between January 2020 and December 2024, a total of 7,311 HIV-infected patients underwent HIV RNA testing at Queen Savang Vadhana Memorial Hospital. Of these, 261 patients with HIV RNA levels  $>1,000$  copies/mL were referred for HIVDR testing and included in the study. The study population consisted of 132 females (50.6%) and 129 males (49.4%) (Figure 1A). Most participants were working-age adults, with the highest proportion in the 41-50 years age group (36.4%) (Figure 1B). The median age was 41 years (IQR, 33-47 years; range, 12-75 years).

Among patients undergoing HIVDR testing, the majority had HIV RNA levels between 10,001 and 100,000 copies/mL (42.5%), followed by 1,001-10,000 copies/mL (29.5%), 100,001-1,000,000 copies/mL (26.4%), and exceeding 1,000,000 copies/mL (1.5%) (Figure 1C). The median HIV RNA level was 25,900 copies/mL (IQR, 5,690-117,000 copies/mL; range, 1,010-2,170,000 copies/mL).

CRF01\_AE was the predominant HIV-1 subtype (93.1%), followed by subtype B (5.7%). Less frequently identified variants included subtype A1, CRF01\_AE/CRF28\_BF, and CRF58\_01B/B (0.4% of each) (Figure 1D). HLA-B\*57:01 screening was performed in 201 patients, of whom five (2.5%) tested positive for the allele.



**Figure 1.** Characteristics of the study population (N=261). A: gender, B: age, C: HIV RNA viral load, D: HIV-1 subtype/ circulating recombinant form (CRF). Values are presented as percentages. For HIV-1 subtype/CRF, Other includes subtype A1, CRF01\_AE/CRF28\_BF, and CRF58\_01B/B.

### Distribution of HIVDR patterns

Among the 261 patients who underwent HIVDR testing, 169 (64.8%, 95% CI, 58.8-70.3%) were found to harbor genotypic drug resistance mutations. The distribution of resistance patterns is shown in Table 1. Dual resistance to NRTIs and NNRTIs was the predominant resistance pattern, accounting for 84 cases (49.7%). Resistance to NNRTIs alone was the second most common pattern, observed in 41 patients (24.3%). In contrast, resistance confined to a single drug class was relatively uncommon, with NRTI-only, PI-only,

and INSTI-only resistance detected in 4.1%, 4.7%, and 1.2% of patients, respectively. Multiclass resistance involving three antiretroviral drug (ARV) classes was less frequent. Resistance to NRTIs, NNRTIs, and PIs was identified in 10 patients (5.9%), whereas resistance involving NRTIs, NNRTIs, and INSTIs was observed in only two patients (1.2%). Overall, resistance to reverse transcriptase inhibitors, particularly combined NRTI-NNRTI resistance, represented the dominant HIVDR pattern during 2020-2024 (Table 1).

**Table 1.** Distribution of ARV resistance patterns among HIV-infected patients with detected drug resistance, 2020-2024 (N=169).

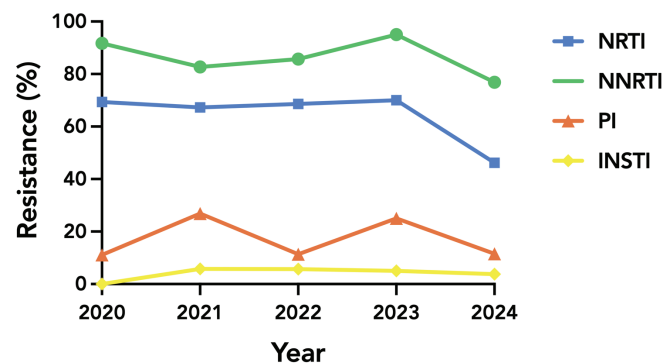
| Resistance pattern      | 2020<br>(N=36) | 2021<br>(N=52) | 2022<br>(N=35) | 2023<br>(N=20) | 2024<br>(N=26) | Total<br>N (%) |
|-------------------------|----------------|----------------|----------------|----------------|----------------|----------------|
| NRTIs only              | 1 (2.8)        | 2 (3.8)        | 2 (5.7)        | 0              | 2 (7.7)        | 7 (4.1)        |
| NNRTIs only             | 10 (27.8)      | 9 (17.3)       | 7 (20.0)       | 4 (20.0)       | 11 (42.3)      | 41 (24.3)      |
| NRTIs + NNRTIs          | 21 (58.3)      | 24 (46.2)      | 20 (57.1)      | 10 (50.0)      | 9 (34.6)       | 84 (49.7)      |
| PIs only                | 1 (2.8)        | 1 (1.9)        | 3 (8.6)        | 1 (5.0)        | 2 (7.7)        | 8 (4.7)        |
| INSTIs only             | 0              | 1 (1.9)        | 0              | 0              | 1 (3.8)        | 2 (1.2)        |
| NRTIs + PIs             | 1 (2.8)        | 5 (9.6)        | 0              | 0              | 1 (3.8)        | 7 (4.1)        |
| NNRTIs + PIs            | 0              | 4 (7.7)        | 0              | 1 (5.0)        | 0              | 5 (3.0)        |
| NRTIs + NNRTIs + PIs    | 2 (5.6)        | 4 (7.7)        | 1 (2.9)        | 3 (15.0)       | 0              | 10 (5.9)       |
| NNRTIs + INSTIs         | 0              | 2 (3.8)        | 1 (2.9)        | 0              | 0              | 3 (1.8)        |
| NRTIs + NNRTIs + INSTIs | 0              | 0              | 1 (2.9)        | 1 (5.0)        | 0              | 2 (1.2)        |

**Note:** NRTIs: nucleoside reverse transcriptase inhibitors, NNRTIs: non-nucleoside reverse transcriptase inhibitors, PIs: protease inhibitors, INSTIs: integrase strand transfer inhibitors.

### Temporal trends in antiretroviral drug class resistance

When resistance patterns were classified according to ARV class, NNRTI resistance was the most prevalent (85.8%; 95% CI, 79.7-90.3%), followed by NRTI resistance (65.1%, 95% CI, 57.6-71.9%), PI resistance (17.8%, 95% CI, 12.7-24.2%), and INSTI resistance (4.1%, 95% CI, 2.0-8.3%) (Figure 1). Throughout the study period, resistance patterns

remained relatively stable, with persistently high rates of NNRTI resistance, moderate rates of NRTI resistance, lower rates of PI resistance, and infrequent INSTI resistance. INSTI resistance was first detected in 2021 and remained uncommon thereafter. No statistically significant temporal changes were observed in resistance to NRTIs ( $p=0.296$ ), NNRTIs ( $p=0.372$ ), PIs ( $p=0.296$ ), or INSTIs ( $p=0.674$ ) (Figure 2).



**Figure 2.** Temporal trends in resistance to antiretroviral (ARV) drug classes among HIV-infected patients with detected drug resistance, 2020-2024 (N=169). Values represent the percentage of resistant cases with resistance to each ARV class by year. Sample sizes were 36 (2020), 52 (2021), 35 (2022), 20 (2023), and 26 (2024), respectively. No statistically significant temporal changes were observed for NRTI, NNRTI, PI, or INSTI resistance (chi-square test or Fisher's exact test, as appropriate).

### Resistance-associated mutations

RAMs were most frequently identified in the reverse transcriptase region, particularly among NRTI- and NNRTI-associated mutations (Table 2). Among NRTI-associated mutations, M184V was the most prevalent mutation (36.1%), followed by D67N (13.6%), K70R (13.0%), and K65R (11.2%). Several thymidine analog mutations (TAMs), including M41L,

D67N/G, K70R/E, T215Y/F, and K219Q/E, were also commonly observed. Among NNRTI-associated mutations, K103N was predominant, occurring in 58 patients (34.3%), followed by Y181C (23.7%), V179I (22.5%), G190A (15.4%), and H221Y (13.6%). Additional frequently detected mutations included P225H (13.0%), V108I (12.4%), K101E (10.7%), and Y188L (9.5%).

**Table 2.** Major HIVDR- associated mutations identified among patients with genotypic resistance (N=169).

| NRTI mutations     |           | NNRTI mutations                   |           | PI mutations                 |          | INSTI mutations     |         |
|--------------------|-----------|-----------------------------------|-----------|------------------------------|----------|---------------------|---------|
| Mutation           | N (%)     | Mutation                          | N (%)     | Mutation                     | N (%)    | Mutation            | N (%)   |
| M184V              | 61 (36.1) | K103N                             | 58 (34.3) | M46I                         | 11 (6.5) | G163R               | 5 (3.0) |
| D67N               | 23 (13.6) | Y181C                             | 40 (23.7) | I54V                         | 8 (4.7)  | G118R, N155H, R263K | 2 (1.2) |
| K70R               | 22 (13.0) | V179I                             | 38 (22.5) | V82A                         | 6 (3.6)  | N155T               | 1 (0.6) |
| K65R               | 19 (11.2) | G190A                             | 26 (15.4) | L76V                         | 4 (2.4)  |                     |         |
| M41L               | 16 (9.5)  | H221Y                             | 23 (13.6) | I84V                         | 3 (1.8)  |                     |         |
| K219E, T215Y       | 11 (6.5)  | P225H                             | 22 (13.0) | I47V, M46L                   | 2 (1.2)  |                     |         |
| K219Q, V151I       | 10 (5.9)  | V108I                             | 21 (12.4) | I50L, I50V, V32I, V82S, V82T | 1 (0.6)  |                     |         |
| M184I              | 8 (4.7)   | K101E                             | 18 (10.7) |                              |          |                     |         |
| L74V, T215F, Y115F | 7 (4.1)   | Y188L                             | 16 (9.5)  |                              |          |                     |         |
| K70E, L74I         | 6 (3.6)   | V106I                             | 13 (7.7)  |                              |          |                     |         |
| D67G               | 5 (3.0)   | E138A                             | 10 (5.9)  |                              |          |                     |         |
| L74M               | 3 (1.8)   | L100I, V179D                      | 8 (4.7)   |                              |          |                     |         |
| K70Q, Q151M        | 2 (1.2)   | V179E                             | 6 (3.6)   |                              |          |                     |         |
| K65N               | 1 (0.6)   | V179T                             | 5 (3.0)   |                              |          |                     |         |
|                    |           | E138G, E138K, K101P, M230L        | 4 (2.4)   |                              |          |                     |         |
|                    |           | E138Q, G190S, K103S, V106M, Y181I | 3 (1.8)   |                              |          |                     |         |
|                    |           | Y181V                             | 2 (1.2)   |                              |          |                     |         |
|                    |           | G190E, V106A, Y188H               | 1 (0.6)   |                              |          |                     |         |

**Note:** NRTI: nucleoside reverse transcriptase inhibitor, NNRTI: non-nucleoside reverse transcriptase inhibitor, PI: protease inhibitor, INSTI: integrase strand transfer inhibitor.

PI-associated mutations were less common. The most frequently detected PI-associated mutation was M46I (6.5%), followed by I54V (4.7%), V82A (3.6%), and L76V (2.4%). INSTI-associated mutations were uncommon overall. G163R was the most frequently identified INSTI-accessory mutation (3.0%), while major INSTI resistance mutations, including G118R, N155H, and R263K, were 1.2% each. These findings are consistent with the low prevalence of INSTI resistance observed in the study population.

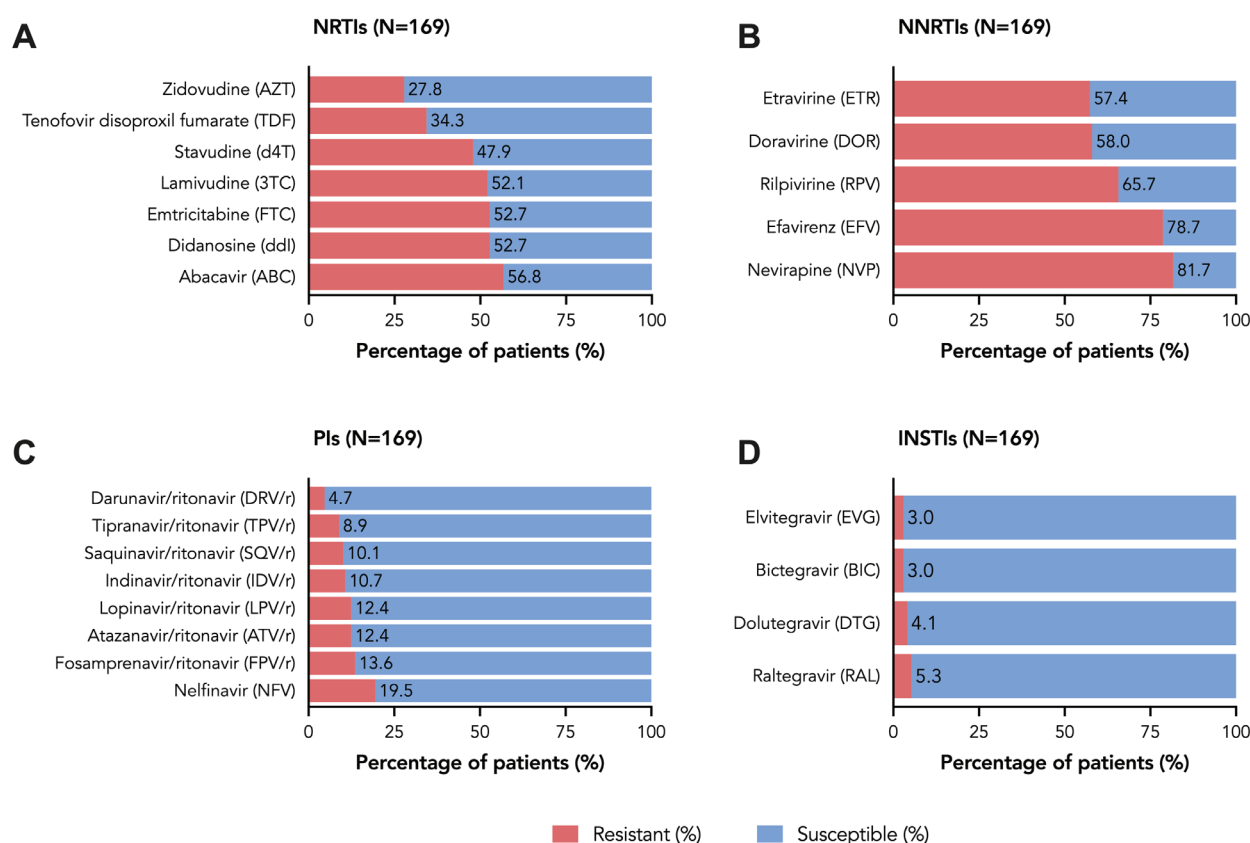
### Antiretroviral susceptibility profiles

Figure 3 summarizes antiretroviral susceptibility profiles among patients with genotypic drug resistance. Among NRTIs, the highest resistance rates were observed for abacavir (ABC) (56.8%), followed by didanosine (ddI) and emtricitabine (FTC) (52.7% each), and lamivudine (3TC) (52.1%). In contrast, resistance to tenofovir disoproxil fumarate (TDF) (34.3%) and zidovudine (AZT) (27.8%) was comparatively lower

(Figure 3A). Among NNRTIs, nevirapine (NVP) exhibited the highest resistance rate (81.7%), followed by efavirenz (EFV) (78.7%) and rilpivirine (RPV) (65.7%). Resistance to doravirine (DOR) 58.0% and etravirine (ETR) (57.4%) was also common (Figure 3B).

Overall resistance to PIs remained relatively low. Nelfinavir (NFV) demonstrated the highest resistance rate (19.5%), whereas darunavir/ritonavir (DRV/r)

showed the lowest resistance rate (4.7%) and the highest susceptibility (95.3%) (Figure 3C). INSTI resistance was uncommon. Raltegravir (RAL) exhibited the highest resistance (5.3%), followed by dolutegravir (DTG) (4.1%), and bictegravir (BIC) and elvitegravir (EVG) were detected in 3.0% of patients. Overall susceptibility to INSTIs remained above 94% for all agents evaluated (Figure 3D).



**Figure 3.** Antiretroviral susceptibility and resistance profiles among patients with HIV-1 drug resistance (N=169). The proportions of patients classified as resistant or susceptible to individual antiretroviral drugs are shown according to drug class. A: nucleoside reverse transcriptase inhibitors (NRTIs), B: non-nucleoside reverse transcriptase inhibitors (NNRTIs), C: protease inhibitors (PIs), D: integrase strand transfer inhibitors (INSTIs). Bar values represent the percentage of patients with resistance to each antiretroviral drug.

### Factors associated with HIV DR

Table 3 presents factors associated with HIVDR. Patients with CD4 counts <100 cells/ $\mu$ L were significantly more likely to harbor drug-resistant HIV strains than those with CD4 counts >200 cells/ $\mu$ L (OR=2.51, 95% CI: 1.17-5.09,  $p=0.019$ ). In contrast, no significant association was observed among patients with CD4 counts of 100-200 cells/ $\mu$ L (OR=0.47, 95% CI: 0.22-1.08,  $p=0.068$ ).

Duration of ART was also associated with HIVDR. Compared with patients receiving ART for less than 5 years, those receiving ART for 5-10 years had significantly higher odds of drug resistance (OR=2.40, 95% CI: 1.27-4.36,  $p=0.007$ ). Although patients receiving ART for more than 10 years also showed increased odds of resistance, the association did not reach statistical

significance (OR=2.05, 95% CI: 0.98-4.17,  $p=0.069$ ). With respect to treatment regimen, patients receiving second-line ART had significantly lower odds of HIVDR than those receiving first-line ART (OR=0.58, 95% CI: 0.35-0.98,  $p=0.049$ ) (Table 3).

No statistically significant associations were observed between HIVDR and HIV RNA level. Compared with patients with HIV RNA levels of 1,001-10,000 copies/mL, the odds ratios were 0.55 (95% CI: 0.30-1.07,  $p=0.084$ ) for patients with HIV RNA levels of 10,001-100,000 copies/mL and 0.53 (95% CI: 0.26-1.04,  $p=0.083$ ) for those with HIV RNA levels >100,000 copies/mL. No significant associations were observed between HIVDR and sex, age group, HIV subtype, or year of testing ( $p>0.05$ ).

**Table 3.** Univariate analysis of factors associated with HIVDR among HIV-infected patients undergoing HIVDR testing (N=261).

| Variable                       | Drug resistance<br>(N=169) | No drug resistance<br>(N=92) | Crude OR<br>(95% CI)      | p value |
|--------------------------------|----------------------------|------------------------------|---------------------------|---------|
| <b>Gender</b>                  |                            |                              |                           |         |
| Female                         | 82 (48.5)                  | 50 (54.3)                    | Reference                 | -       |
| Male                           | 87 (51.5)                  | 42 (45.7)                    | 1.26 (0.7632 to 2.111)    | 0.437   |
| <b>Age group (years)</b>       |                            |                              |                           |         |
| ≤40                            | 83 (49.1)                  | 42 (45.7)                    | Reference                 | -       |
| >40                            | 86 (50.9)                  | 50 (54.3)                    | 0.87 (0.5211 to 1.441)    | 0.607   |
| <b>CD4 count (cells/μL)</b>    |                            |                              |                           |         |
| >200                           | 113 (66.9)                 | 66 (71.7)                    | Reference                 | -       |
| 100-200                        | 13 (7.7)                   | 16 (17.4)                    | 0.47 (0.2169 to 1.082)    | 0.068   |
| <100                           | 43 (25.4)                  | 10 (10.9)                    | 2.51 (1.171 to 5.090)     | 0.019*  |
| <b>HIV RNA (copies/mL)</b>     |                            |                              |                           |         |
| 1,001-10,000                   | 57 (33.7)                  | 20 (21.7)                    | Reference                 | -       |
| 10,001-100,000                 | 68 (40.2)                  | 43 (46.7)                    | 0.55 (0.2997 to 1.070)    | 0.084   |
| >100,000                       | 44 (26.0)                  | 29 (31.5)                    | 0.53 (0.2598 to 1.043)    | 0.083   |
| <b>Duration of ART (years)</b> |                            |                              |                           |         |
| <5                             | 32 (18.9)                  | 32 (34.8)                    | Reference                 | -       |
| 5-10                           | 96 (56.8)                  | 40 (43.5)                    | 2.40 (1.272 to 4.356)     | 0.007*  |
| >10                            | 41 (24.3)                  | 20 (21.7)                    | 2.05 (0.9840 to 4.167)    | 0.069   |
| <b>ART regimen</b>             |                            |                              |                           |         |
| First-line ART                 | 105 (62.1)                 | 45 (48.9)                    | Reference                 | -       |
| Second-line ART                | 64 (37.9)                  | 47 (51.1)                    | 0.5836 (0.3466 to 0.9751) | 0.049*  |
| <b>HIV subtype</b>             |                            |                              |                           |         |
| CRF01_AE                       | 155 (91.7)                 | 88 (95.7)                    | Reference                 | -       |
| Non-CRF01_AE                   | 14 (8.3)                   | 4 (4.3)                      | 1.987 (0.6635 to 5.674)   | 0.310   |
| <b>Year of testing</b>         |                            |                              |                           |         |
| 2020-2022                      | 123 (72.8)                 | 57 (72.8)                    | Reference                 | -       |
| 2023-2024                      | 46 (27.2)                  | 35 (27.2)                    | 0.6091 (0.3584 to 1.046)  | 0.093   |

**Note:** OR: odds ratio, CI: confidence interval, ART: antiretroviral therapy. Odds ratios were calculated using univariate logistic regression. First-line ART included NNRTI-based and standard INSTI-based regimens, whereas second-line ART included boosted PI-containing and salvage regimens. \* $p < 0.05$  was considered statistically significant.

## Discussion

This study investigated the prevalence of HIVDR, RAMs, and antiretroviral susceptibility profiles among HIV-infected patients with virologic failure who underwent HIVDR testing. Among 261 patients with HIV RNA levels exceeding 1,000 copies/mL, 169 (64.8%) harbored genotypic drug resistance mutations. This prevalence is comparable to previous reports from Thailand, which have documented HIVDR rates ranging from approximately 45% to 91% among patients with HIVDR testing.<sup>10-18</sup>

The demographic characteristics of the study population were consistent with previous studies conducted in Thailand. Most patients were working-age

adults, with a median age of 41 years, reflecting the aging population of people living with HIV receiving long-term ART.<sup>10</sup> CRF01\_AE was the predominant HIV-1 subtype (93.1%), consistent with its established role as the major circulating subtype in Thailand and Southeast Asia.<sup>11,16,19</sup> A small proportion of patients harbored non-CRF01\_AE subtypes, including subtype B, subtype A1, CRF01\_AE/CRF28\_BF, and CRF58\_01B/B, highlighting the ongoing genetic diversity of HIV-1 and emphasizing the importance of continued molecular surveillance. The prevalence of HLA-B\*57:01 positivity was low (2.5%), consistent with the relatively low frequency of this allele in Asian populations.<sup>10</sup> Nevertheless, routine HLA-B\*57:01 screening remains essential prior to

abacavir administration because of the risk of severe hypersensitivity reactions.

Resistance to reverse transcriptase inhibitors remained the dominant pattern of HIVDR throughout the study period. Dual resistance to NRTIs and NNRTIs was the most common resistance pattern, accounting for nearly half of all resistant cases (49.7%), followed by resistance to NNRTIs alone (24.3%). Similar findings have been reported in previous studies from Thailand and other countries.<sup>10-13,16-20</sup> The predominance of reverse transcriptase inhibitor resistance likely reflects the extensive historical use of NNRTI-based regimens as first-line ART before the introduction of DTG-based therapy. Consistent with this observation, NNRTI resistance was the most prevalent resistance class (85.8%), followed by NRTI resistance (65.1%), whereas PI resistance (17.8%) and INSTI resistance (4.1%) remained comparatively uncommon.

Although Thailand revised its national ART guidelines in 2021 to recommend DTG-based regimens as the preferred first-line treatment,<sup>3</sup> no significant temporal trends in resistance to NRTIs, NNRTIs, PIs, or INSTIs were observed during 2020-2024. NNRTI resistance remained consistently high throughout the study period, ranging from 76.9% to 95.0%, while INSTI resistance remained below 6% annually. These findings suggest that the resistance patterns observed during the study period continue to be influenced by historical exposure to NNRTI-based regimens. The persistence of high NNRTI resistance despite the transition to INSTI-based therapy is consistent with previous studies demonstrating that resistance mutations acquired during prior treatment can remain detectable for prolonged periods, even after regimen changes.<sup>10-18,20</sup> Moreover, the low prevalence of INSTI resistance supports the continued effectiveness of DTG-based regimens and is consistent with WHO recommendations that prioritize DTG-containing regimens because of their high efficacy and elevated genetic barrier to resistance.<sup>21</sup>

The resistance patterns observed in this study were further supported by the distribution of RAMs and corresponding antiretroviral susceptibility profiles. M184V was the predominant NRTI-associated mutation identified in this cohort and is well recognized for conferring high-level resistance to 3TC and FTC.<sup>20</sup> Consistent with this finding, resistance to 3TC and FTC exceeded 50% in the present study. The high prevalence of ABC resistance was likely attributable to the combined effects of M184V and other NRTI-associated mutations, including K65R, L74V, Y115F, and TAMs, which collectively reduce susceptibility to ABC. In contrast, resistance to TDF and AZT remained comparatively lower, suggesting these agents may retain activity in a proportion of patients despite NRTI resistance mutations.

Among NNRTIs, K103N was the most frequently detected mutation, followed by Y181C and V179I.

These mutations are major determinants of resistance to first-generation NNRTIs and likely contributed to the high resistance rates observed for NVP and EFV, which exceeded 75% in the present study. Resistance to RPV, DOR, and ETR was also common, indicating substantial cross-resistance within the NNRTI class. The predominance of NNRTI resistance is consistent with the historical use of NNRTI-based first-line regimens in Thailand and further explains the high frequency of dual NRTI-NNRTI resistance observed in this cohort.

Although PI-associated mutations were detected, their prevalence remained relatively low compared with mutations affecting reverse transcriptase inhibitors. M46I was the most common PI-associated mutation; however, resistance to boosted PIs remained uncommon. DRV/r demonstrated the lowest resistance rate among PIs (4.7%), supporting its well-established high genetic barrier to resistance. Similarly, resistance to INSTIs remained uncommon, with resistance rates ranging from 3.0% to 5.3% for BIC, DTG, EVG, and RAL, similar findings have been reported in previous studies from Italy.<sup>20</sup> The low prevalence of INSTI resistance, together with the limited frequency of INSTI-associated mutations such as G118R, N155H, and R263K, suggests that INSTI-based regimens continue to retain substantial activity against circulating HIV strains in this patient population. These findings support the continued use of DTG- and BIC-based regimens as key components of contemporary HIV treatment strategies.

Analysis of factors associated with HIVDR demonstrated that patients with CD4 counts <100 cells/ $\mu$ L were significantly more likely to harbor drug-resistant HIV strains than those with CD4 counts >200 cells/ $\mu$ L. This finding is consistent with previous studies reporting an association between advanced immunosuppression and the emergence of HIVDR.<sup>18,19,22</sup> Low CD4 cell counts may reflect prolonged virologic failure and ongoing viral replication under antiretroviral drug pressure, facilitating the accumulation of RAMs. In addition, severe immunosuppression may indicate delayed recognition of treatment failure and prolonged exposure to ineffective ART regimens, thereby increasing the opportunity for resistance development.

Duration of ART was also associated with HIVDR in the present study. Patients receiving ART for 5-10 years had significantly higher odds of HIVDR than those receiving treatment for less than 5 years, while a similar trend was observed among patients receiving ART for more than 10 years. These findings suggest that prolonged exposure to ARV increases the likelihood of accumulating RAMs, particularly among individuals with incomplete viral suppression. Similar associations between treatment duration and HIVDR have been reported in previous studies conducted in Thailand and other resource-limited settings.<sup>13-15,19</sup>

Interestingly, patients receiving second-line ART exhibited significantly lower odds of HIVDR than those receiving first-line ART. This observation may reflect

the higher genetic barrier to resistance associated with boosted PI-containing regimens commonly used in second-line therapy. Consistent with this finding, PI resistance remained relatively uncommon in the present study, whereas NNRTI resistance was highly prevalent. Together, these findings suggest that boosted PI-based regimens continue to provide durable antiviral activity despite prolonged treatment exposure and support their important role in treatment-experienced patients. No significant associations were observed between HIVDR and sex, age group, HIV subtype, HIV RNA level, or year of testing. Collectively, these findings indicate that treatment-related factors and immune status may play a greater role in the development of HIVDR than demographic characteristics in this patient population.

An important strength of this study is the use of NGS, which enabled simultaneous analysis of protease, reverse transcriptase, and integrase genes. This approach allowed comprehensive characterization of resistance mutations, HIV-1 subtypes, and INSTI-associated resistance patterns. Furthermore, NGS provides improved sensitivity for detecting low-frequency resistant variants and can reliably identify HIVDR in patients with viral loads above 1,000 copies/mL. Consequently, this study provides valuable real-world surveillance data on HIVDR patterns during Thailand's transition from NNRTI-based to INSTI-based ART.

In conclusion, resistance to reverse transcriptase inhibitors, particularly dual resistance to NRTIs and NNRTIs, remained the predominant HIVDR pattern among patients with virologic failure in this cohort. Although INSTI-associated resistance was detected, its prevalence remained low, supporting the continued effectiveness of INSTI-based regimens in Thailand. Low CD4 cell counts and prolonged ART exposure were associated with an increased likelihood of HIVDR, whereas patients receiving second-line ART demonstrated lower odds of resistance. Ongoing viral load monitoring, adherence interventions, and continuous HIVDR surveillance remain essential for optimizing treatment outcomes and limiting the emergence of ARV resistance.

### Limitations

Several limitations should be considered. Firstly, the retrospective design limited the availability of important clinical variables, particularly ART adherence, which is a major determinant of virologic failure and HIVDR. Secondly, the accuracy of the analyses depended on the completeness of routinely collected clinical data. Thirdly, drug resistance interpretation was based on the Stanford HIVdb algorithm version 9.4. As HIVdb algorithms are periodically updated, resistance classifications and mutation penalty scores may differ slightly from those generated using newer versions, potentially limiting direct comparisons with future studies. Fourthly,

selection bias may have occurred because, in accordance with the Thai national HIV treatment guidelines, HIVDR testing is recommended only for patients with virologic failure (HIV-1 RNA >1,000 copies/mL).

Therefore, the findings may not be generalizable to all people living with HIV receiving ART. Fifth, data on ART adherence were not available, precluding assessment of its association with virologic failure and HIVDR. Finally, although we performed comparative subgroup analyses and univariate logistic regression, the retrospective design precluded causal inference. Therefore, interpret the observed associations with caution.

### Practical implications

These findings support continued genotypic resistance testing and routine HIVDR surveillance to guide individualized ART during the transition to INSTI-based regimens in Thailand.

### Conclusion

This study demonstrated a high prevalence of HIVDR among patients with virologic failure undergoing HIVDR testing at a general hospital of the Thai Red Cross Society during 2020-2024. CRF01\_AE remained the predominant HIV-1 subtype, consistent with the epidemiology of HIV infection in Thailand and Southeast Asia. Resistance to reverse transcriptase inhibitors, particularly combined resistance to NRTIs and NNRTIs, remained the dominant HIVDR pattern throughout the study period. M184V and K103N were the most frequently detected RAMs and largely accounted for the high levels of resistance observed against several NRTIs and first-generation NNRTIs. Although Thailand adopted INSTI-based first-line ART in 2021, NNRTI-associated resistance remained highly prevalent, likely reflecting the long-term impact of historical exposure to NNRTI-based regimens. In contrast, resistance to PIs and INSTIs remained uncommon, and susceptibility to DRV/r, DTG, and BIC was largely preserved, supporting their high genetic barrier to resistance and continued clinical effectiveness.

Patients with severe immunosuppression (CD4 count <100 cells/ $\mu$ L) and prolonged ART exposure were more likely to harbor drug-resistant HIV strains, whereas patients receiving second-line ART exhibited lower odds of HIVDR. These findings provide valuable real-world evidence on HIVDR during Thailand's transition from NNRTI-based to INSTI-based therapy and underscore the importance of routine viral load monitoring, timely resistance testing, adherence support, and continuous HIVDR surveillance to optimize long-term treatment outcomes.

### Ethical approval

This study was approved by the Human Research Ethics Committee of Queen Savang Vadhana Memorial Hospital (COA No. 005/2568; IRB No. 008/2568; approved on March 10, 2025).

## Declaration of generative AI and AI-assisted technologies

ChatGPT (GPT-5.6, OpenAI) was used solely to assist in the design of the graphical abstract. No content generation or data interpretation was performed by AI. The authors reviewed and approved the final graphical abstract and take full responsibility for its content.

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## Conflict of interest

The authors declare no conflict of interest.

## CRediT authorship contribution statement

**Srivilai Trakulkaseamsiri:** conceptualization, methodology, performed research protocol, formal analysis, writing-original draft; review and editing, supervision, project administration, funding acquisition; **Sirirat Surinkaew:** formal analysis, visualization, writing-review and editing; **Supanida Utthapa:** investigation, data curation, visualization; **Kittipong Chumchuan:** investigation, data curation. All authors approved the final version of the manuscript and agree to be accountable for all aspects of the work.

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