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In silico drug repurposing approach to predict most effective HAART for HIV drug resistance variants prevalent in the Indian HIV-positive population

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Abstract

HIV epidemics still exist as a major global public health burden, especially in middle- and low-income countries. Given the lack of approved vaccines, antiretroviral therapy (ART) remains the primary approach to reduce the mortality and morbidity linked to this disease. Effective treatment for HIV-1 requires the simultaneous administration of multiple drugs. However, the virus can show resistance to antiretroviral drugs, resulting in treatment failure. Therefore, this study focused on assessing the prevalence of mutations within the Indian HIV-positive population. After assessing the data, we intended to identify the most effective highly active ART (HAART) regimens for individuals with drug-resistant variants. Furthermore, our analysis revealed a spectrum of HIV mutations, with varying effects on protein stability. The significance of this analysis lies in its potential to optimize HAART selection for HIV-positive individuals by accounting for both prevalence and stability-altering mutations. By considering mutation effects on protein stability, we can modify treatment regimens, increasing the likelihood of therapy success and diminishing the risk of resistance. Moreover, this study contributes to the broader field of drug repurposing, offering insights into the rational design of antiretroviral therapies.

Keywords

HIV drug resistance. HAART. Drug resistance mutations. Reverse transcriptase. Protease.

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Introduction

HIV is a pathogen that targets and compromises the immune system of the body, particularly by affecting the white blood cells. The advanced stage of the disease is known as AIDS. HIV infection is considered a public health threat with a serious impact on different aspects of people's health and lives[1]. In 1981, the first case of HIV infection was reported by the Center for Disease Control, and in 1983 it was given the name of AIDS. In India, AIDS was first identified in a female sex worker in Chennai in 1986[2,3]. As of 2023, there are 25 lakh HIV-positive individuals living in India, however, the latest 2023-2024 HIV estimates highlight the overall progress in AIDS response at the national level, especially the improvement in declining mortality levels.

HIV is a virus that is grouped within the family Retroviridae, and the genus Lentivirus. On the basis of its genetic makeup and antigens, it is divided into two types; HIV1 and HIV2. The HIV retrovirus genome is made up of two copies of single-strand positive sense RNA. The virus contains three structural genes *gag, pol,* and *env* and five non-structural proteins *tat, rev, nef, vif,* and *vpr* present in both HIV 1 and HIV2. Other than these, HIV 1 contains *vpu* and HIV 2 contains *vpx*, both differ in their genome organization[4,5]. Given the infectious and contagious nature of HIV, effective treatment is of paramount importance, however, at present, there is no effective vaccine or cure for HIV infection. Therefore, the focus has primarily been on the development and implementation of antiretroviral therapies to manage and mitigate the impact of HIV on affected individuals. There are approximately 25 antiretroviral drugs, categorized into six classes, approved for the treatment of HIV infection. In India, the national program offers first-line treatment as a combination of two nucleoside reverse transcriptase inhibitors (NRTIs), and one non-NRTI (NNRTI)[6]. Furthermore, protease inhibitors are provided as a second-line treatment option when the first-line regimen fails[7]. Moreover, the World Health Organization (WHO) recommends regular monitoring of drug resistance, helping in decisions on when to switch to different therapies if the first-line treatment is not working[8,9]. In case of therapy failure, another combination of antiretroviral agents should be substituted with a more appropriate one.

Epidemiology of HIV in India

Although a declining trend has been observed in newly reported cases of HIV in India in past few years, there are still a significantly high number of people living with HIV (PLWH) in different regions of India[10]. According to the data released by the National AIDS Control Organization (NACO), India is the third most burdened country with a population of 25 lakh people living with HIV[10]. However, the estimated adult HIV prevalence (ages 15-49) in India has been steadily declining since the epidemic was at its highest point in the year 2000. At that time, the prevalence was estimated at 0.56%, which decreased to 0.32% by 2010 and then dropped to 0.20% in 2022. Further, in 2022, the estimated HIV prevalence recorded was 0.22% among adult males and 0.19% among adult females[10]. In addition, according to the state-wise distribution, it was reported that the north-east states of India, such as Mizoram, Nagaland, and Manipur have the highest estimated adult HIV prevalence, followed by Andhra Pradesh, Telangana, Karnataka, Meghalaya, and Delhi, with prevalence rates ranging between 0.70% and 0.30%[10]. HIV is transmitted through several key routes, including unprotected sexual contact, sharing of contaminated needles or syringes among IV drug users, transfusion of infected blood, and from mother to child during pregnancy, childbirth, or breastfeeding. Among all modes of transmission, HIV data published under the WHO global HIV program have indicated that intravenous IV drug use (IVDU) poses a significant risk of HIV transmission in the Indian population with an estimated incidence of 9.0% which is significantly higher compared to other high-risk groups, such as female sex worker (1.9%) and homosexual men (3.3%)[11]. The high-risk of HIV transmission among IVDU is primarily due to the use of contaminated needles or syringes. On the basis of geographical distribution, studies have shown that transmission of HIV infection among IV drug users is most prevalent in the north-east region of India with Mizoram at the highest risk of infection at 19.8% followed by Manipur[12]. In addition to this, unprotected sexual contact remains another significant route of HIV transmission globally including in India. The groups who are more vulnerable to receive or transmit HIV infection through unprotected sexual contact are female sex workers (FSW) and men who have sex with men (MSM). Studies have found that adolescent FSWs often have numerous sex partners, report inconsistent condom use, and are

likely to engage in high-risk sexual acts such as unprotected intercourse, with HIV prevalence of 1.9%[11]. Further, the estimated MSM population in India is 3.1 million, with approximately, 0.13 million living with HIV, indicating a significantly high prevalence of HIV infection[13]. Contrary to this, vertical, perinatal transmission and blood transfusion are found to be less prevalent modes of HIV transmission in the Indian population[10]. Moreover, the latest reports stated that the overall prevalence of blood transfusion-mediated infections in India was 1.58%, and HIV alone contributed to 0.14% of these infections[14]. However, while India has made considerable efforts in reducing the overall HIV prevalence, significant challenges still remain, particularly among high-risk groups and regions. Therefore, effective strategies and targeted interventions are essential to further mitigate the spread of HIV across the country.

Literature search to identify mutant HIV strains in the Indian population

To accomplish the purpose of finding prevailing mutations, we used a combination of web-accessible data and hence, conducted an extensive literature search and prepared a data set of mutations present in different regions of India.

In our study, we used PubMed and Google Scholar as search engines for the literature. Hence, the systematic literature search was performed according to the user guide given in PubMed. We used appropriate keywords such as “HIV drug resistant mutations” and “India”, listing a number of documents from 1988 to 2023. In addition, MEDLINE (NLM premier bibliographic database) was also used for some recent journal articles on the prevalence of HIV mutations in India. Furthermore, we searched documents by date of publication, article types like review and systematic review and meta-analysis article[3].

In silico analysis to predict drug resistance in HIV mutant strains

To assess the level of resistivity against antiretroviral drugs (NRTI, NNRTI, and PI), we used the Stanford University HIV drug resistance database (https://hivdb.stanford.edu), consisting of the HIVdb program. In the HIVdb system, each drug resistance mutation (DRM) is assigned a drug penalty or mutation score and is accompanied by a comment. The total score for a drug is calculated by adding the scores of all DRM mutations associated with resistance to that particular drug. Based on this cumulative drug score, the program generates five levels of drug resistance such as susceptible, potential low-level resistance, low-level resistance, intermediate resistance, and high-level resistance[15].

Evaluation of change in protein stability in drug-resistant HIV mutant strains

Predicting the impact of single-point genetic variations on protein stability is important for unraveling the interplay between protein structure and function. In pursuit of this objective, in our investigation, we used two bioinformatic tools, mCSM and DDMut (http://biosig.unimelb.edu.au/mcsm/, https://biosig.lab.uq.edu.au/ddmut/submit_prediction). The Cutoff Scanning Matrix (CSM) (introduced by Pires et al. in 2011) serves as a robust protein structural signature[16]. Furthermore, mCSM exhibits a remarkable capacity, not only in predicting the directional changes in protein stability and the affinity of protein-protein and protein-DNA complexes but also in providing quantitatively accurate experimental values. DDMut, on the other hand, is a fast and accurate network to predict changes in Gibbs Free Energy ($\Delta\Delta G$) upon single and multiple point mutations.

Prevalence of mutant HIV-strains prevailing in the Indian HIV-positive population

All the HIV mutation patterns and types of mutations derived from literature and databases were identified as HIV subtype C, the predominant subtype in India. Detailed information on the type of mutations with patterns from each region is provided in table 1. It has been analyzed from the table that M184V/I is the most prevalent mutation pattern among NRTI, Y181C/I/G is the most prevalent mutation pattern among NNRTI, and M46I is the most prevalent mutation pattern among protease inhibitors (PI). Furthermore, among NNRTI-DRM mutations, it has been observed that K103N and Y181C are common which leads to virological failure.

In silico analysis of different antiretroviral drugs across a range of specific mutant strains

After analyzing the number of prevailing mutations of HIV-positive in the Indian population, we then accustomed the Stanford HIV drug resistance database[38]. A total of 123 mutations were observed from the literature, out of which 30 mutations were of NRTI,

Table 1. List of mutant HIV strains more prevalent in the Indian HIV population

References	HIV resistance mutations		
	NRTI	**NNRTI**	**PI**
17	L74V, K219N, K219R	Y181	None
18	M41L, K65R, D67N, K70R, L74I, V75MV, Y115F, M184V, L210W, T215Y, K219Q	V90I, A98AG, K101E, K103N, V106IMV, V108I, Y181C, Y188L, G190A, H221Y	None
19	M184\M, Q151M, K65R, N69S, M184V, V111I	None	I50L
7	None	K101E, K103N, Y188C/Y	N83D, V82L
20	None	None	H69K, I93L, I15V, L19I, I13V, K14R, G16E, K20R/I, K70R, I72V, V82I, E35D, R41K, L63D, L89M, M36I
21	M184V, T215Y, M41L	V108I, H221Y	M46I, I47V
22	None	K101E, Y181C, G190A	None
23	None	Y181C, Y188L, K103N, G190A, V106M, P225H, M230L, K101E/K, V106M, L1001	None
24	M41L, E44D, M184V, T215Y, K219R	V90I, A98G, K103N, K103R, V106M, V108I, E138A, Y181C, G190A	None
25	None	None	D30N, M46I (major) T12S, I15V, L19I, M36I, S37N, R41K, D60N, L63T, E65K, G68E, H69K, I72V, G86E, L89M, I93L, G94K, T12S, I15V, L19I, M36I, S37N, R41K, L63P, H69K, T74S, V771, L63P (minor)
26	None	Y181C, K103N and Y188C	None
27	None	K103N, Y181C, and G190A	V82A, L90M, M46I
28	None	V106M, K103N	None
29	K65R, M184V, K219Q, K65R, Y115F and T215F, M41L, D67N, T69D, K70G/R, Y115F, T215N, K219R/Q	Y188L, Y181C, K103N, G190A and Y188L, A98G, K103N, V179D/E, Y181C, G190A/R, K101E, V106A/M, V108I, Y181F, H221Y and M230I	I54V, L76V and I84V, M46L
30	M184V	None	K20R, M36I, and H69K, D30N, L63P, A71E, A71V, I13V, L10V, K45I, K45R
6	M184V	I135T, I135M, E138A, R211K	None
31	M184V	None	None
32	V75I, M184V	A98G, K101E/P, K103N/S, V106M, V108I, Y181C/I, Y188L G190A/S/E, F227L, M230L	M46I, G48V I54V G73S, V82A I84V L90M

(*Continues*)

Table 1. List of mutant HIV strains more prevalent in the Indian HIV population *(continued)*

References	HIV resistance mutations		
	NRTI	**NNRTI**	**PI**
33	M184V/I, K65R,	K103N, V90I, A98G, K101E, V106A/M, V108I, E138A/K/Q, V179D/T, Y181C/I, G190A/E/S, H221Y	L90M, L89M, V77I, L63P, H69K/R/Q, M36I, K20I/M/R/T, G16E, L10V/I
34	M184V, M184V/MV/I/IM, E44D/DE/A/K, L74V, V75M, V118I, T69D/DN, K65R, Q151M	Y181C, K103N, G190A, Y181C/CY/I/V, K103N/KN/R, K101E/EK/Q/KQ, G190A/AG, V108I, A98G, V106M/MV, V90I, Y188L, E138K/EK	None
35	None	K101E	None
36	None	E138A and L210LS	None
37	T69N, T69S, M41L, M184V, T215Y, D67N, F116A, G333DE, K219R,	A98G, V19A	V82A, L10I, A71V

31 were falling under NNRTI, and 62 were of protease inhibitor gene. We have demonstrated the levels of resistance (intermediate and high levels) of all these mutations for specific drugs through using the above-mentioned database (Stanford HIV database) (Table 2). It depicted that high-level NRTI drug resistance was observed for didanosine followed by stavudine, abacavir, and zidovudine. The intermediate drug resistance level observed was for zidovudine, stavudine, and abacavir followed by FTC, 3TC, and TDF. On the other hand, in the case of NNRTI, an intermediate level of resistance was seen for RPV, NVP, ETR, and EFV (Table 2). In addition, as compared to NRTI and NNRTI, PI-associated mutations showed significantly less resistance toward specific drugs. A high level of resistance was seen for drugs ATV/r, FPV/r, IDV/r, NFV, and SQV/r but not for DRV/r, LPV/r, and TPV/r. However, various mutations showed intermediate levels of resistance for all these drugs except DRV/r.

Prediction of protein stability change upon mutations

To comprehend the effects of mutations on gene expression, regulation, and protein function, particularly concerning changes in protein stability, we focused on two well-established structure-based tools, developed in the Biosig laboratory; DDMut, and mCSM. To commence this analysis, we examined specific mutations and quantified their impact on protein stability[39,40]. For instance, we assessed mutations (M184V/I, K65R, Y181C, G190M, K103N, L90M, and I84V) associated with a high level of resistance to specific inhibitors. The prediction scores are summarized in table 3 representing changes in protein stability. While the majority of mutations were destabilizing (K103N, K65R, Y181C, M184V, M184I, G190A, L90M, and I84V), there were some which showed stabilizing effect. In addition, DDMut gave opposite results to that of mCSM, and three of the mutations were found to show stabilizing (K65R, M184I, M184V).

Discussion

Mutations that are resistant to anti-retroviral drugs have detrimental effects on the effectiveness of highly active antiretroviral therapy (HAART), impacting both individuals who have previously undergone antiretroviral therapy (ART) and treatment-naive patients. The prevalence of drug-resistant mutations has emerged as a significant concern[25]. Hence, antiretroviral drug resistance is one of the crucial factors that decide the success of antiretroviral treatment. In our analysis, we aimed to summarize the most prevalent mutations in the Indian population through a literature search and effective combination of antiretroviral drugs against different

Table 2. *In silico* analysis of HIV resistant mutations prevailing in Indian population

Drugs	Intermediate level	High-level resistance
	NRTI	
Abacavir (ABC)	L74V, K65R,	Q151M, Y115F,
Zidovudine (AZT)	K70R, T215YFN,	Q151M,
Stavudine (D4T)	T215YFN, V75M, V75MV	K65R, Q151M,
Didanosine (DDI)	T69D	L74V, K65R, Q151M, L74I
Emtricitabine (FTC)	K65R	M184VI
Lamivudine (3TC)	K65R	M184VI
Tenofovir (TDF)	K65R	None
	NNRTI	
Doravirie (DOR)	None	V106IMV, V106M, Y188L, M230L, F227L
Efavirenz (EFV)	G190A, Y181C, M230L	K103N, V106IMV, Y188C, V106M, L100I, Y188L
Etravirine (ETR)	Y181C, L100I, M230L	None
Nevirapine (NVP)	K101E, A98AG, M230I	Y181C, G190A, K103N, V106IMV, Y188C, F227L, V106M, L100I, Y181C, Y188L, M230L
Rilpivirine (RV)	K101E, E138AKQ, Y181C, M230I, E138K/EK	L100I, Y188L, M230L
	PI	
Atazanavir/r (ATV/r)	G48V	I50L, I84V
Darunavir/r (DRV/r)	None	None
Fosamprenavir/ (FPV/r)	L90M	I84V, L76V
Indinavir/r (IDV/r)	V82A, L76V	I84V
Lopinavir/r (LPV/r)	V82A, I84V, L76V	None
Nelfinavir (NFV)	M46I, G48V, M46I, V82A	D30N, L90M, I84V
Saquinavir/r (SQV/r)	L90M	G48V, I84V
Tipranavir/r (TPV/r)	V82L, I84V	None

NRTI: 3TC: lamivudine; ABC: abacavir; AZT: ziduvudine; D4T: stavudine; DDI: didanosine; FTC: emticitabine; TDF: tenofovir; 3TC: lamivudine; IDV: indinavir; DOR: doravirie; EFV: efavirenz; ETR: etravirine; NVP: nevirapine; RPV: rilpivirine; ATV: atazanavir; LPV: lopinavir; DRV: darunavir; FPV: fosamprenavir; NFV: nelfinavir; SQV: saquinavir; TPV: tipranavir.

Table 3. Predicted effect of HIV-drug resistant mutation over stability of viral protein

Mutations	(ΔΔG) (mCSM) (Kcal/mol)	(ΔΔG) (DDMut) (Kcal/mol)	Outcome (mCSM)	Outcome (DDMut)
K65R	−0.168	0.03	Destabilizing	Stabilizing
K103N	−0.921	−0.98	Destabilizing	Destabilizing
Y181C	−1.383	−0.61	Destabilizing	Destabilizing
M184I	−1.499	0.02	Destabilizing	Stabilizing
M184V	−0.248	0.05	Destabilizing	Stabilizing
G190A	−0.805	−0.11	Destabilizing	Destabilizing
L90M	−1.337	−0.92	Destabilizing	Destabilizing
I84V	−1.542	−0.83	Destabilizing	Destabilizing

The stability scores calculated using mCSM and DDMut are represented as changes in Gibbs Free Energy (ΔΔG). A range of ΔΔG scores is used to categorize the impact of mutations on protein stability. Mutations with $\Delta\Delta G \leq -1$ kcal/mol are classified as Destabilizing and mutations with $\Delta\Delta G \geq 1$ kcal/mol are classified as Stabilizing.

mutations using *in silico* approach. In addition, we also focused on understanding the changes in the protein stability and their structure upon mutations.

After the extensive literature search, we found that the most prevalent mutations observed in the studies confer high-level resistance to NRTIs especially (M184V

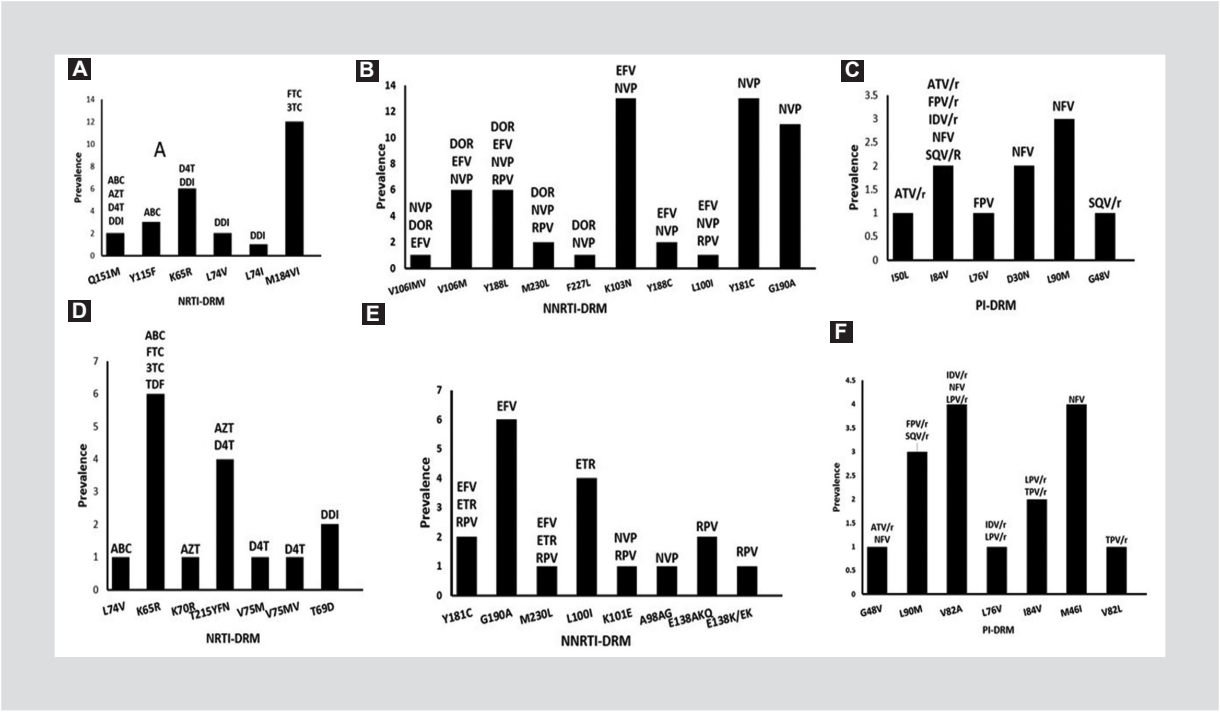

Figure 1. **A-C:** *a graphical representation of the prevalence of drug resistance mutations showing high level;* **D-F:** *intermediate level of resistance against highly active antiretroviral therapy regime viz., nucleoside reverse transcriptase inhibitors (NRTI), non-NRTI, and protease inhibitor.*

mutation) and NNRTIs specifically (K103N/S and Y181C/Y/I/V mutations). Furthermore, the mutation patterns identified in our study closely resemble those observed in earlier published research conducted on subtype C[18,41,42]. Furthermore, we analyzed that the T215Y mutation showed an intermediate level of resistance to two drugs: AZT and D4T. Similar studies were found to show a higher prevalence of protease inhibitor resistance mutations among HIV-positive individuals receiving different doses of drugs[27,43]. In addition, the predominant mutations observed were M46I and V82A, aligning with similar discoveries in previous studies from India. Notably, L90M was the prevalent mutation in most patients, in contrast to D30N, similar to findings in other studies[43]. The prevalence of the level of resistance toward specific drugs is shown in figure 1.

Furthermore, one limitation of the search analysis was that the Stanford University HIV database we used did not include certain Indian mutation patterns, such as L210LS, T39A, E203DK, H208Y, D218E, K122E, V111I, T135RTVXM, L178IM, and V189D, categorizing them as other RT mutations. Similarly, mutation patterns, such as L10VI, V77I, H69KQR, I193L, I15V, L19I, M36I, R41K, L63P, L89M, G16EF, D60E, I62V, and I64M were grouped as other PR mutations. Moreover, it is anticipated that bioinformatic software tailored specifically to Indian mutation patterns will be helpful to find out mutations that are not available in the database.

After analyzing the prevalent mutations and their level of resistance toward specific drugs we intended to identify the effects of these mutations on structural and protein stability changes. Despite observing protein destabilization, we found that HIV mutant strains continue to replicate and develop resistance to specific drugs. HIV-1, known for its high genetic variability, continuously evolves, leading to resistance against existing drugs and evading host immune responses triggered by AIDS vaccine candidates. The evolution of drug resistance is a fundamental process that enables a pathogen to persist and replicate even under drug pressure. This adaptive process underscores the resilience of HIV-1 and its ability to persist even in the presence of specific drugs[44]. Through our examination of data from the Stanford database, we have gained insights into the degree of resistance, which serves as a critical step toward therapeutics, allowing a more precise selection of the combination of inhibitors[24]. Furthermore, predicting the effect of mutation over protein stability will be useful in understanding interactions of HAART drugs over drug-resistant HIV mutant strains.

Conclusion

In the ever-evolving battle against HIV, understanding mutations and drug resistance plays an important role. In our study, we aimed to assess the prevalence of HIV drug-resistant mutations in the Indian HIV-positive population and identify the most effective HAART regimens against these variants. Through an *in silico* drug repurposing approach, we were able to find different levels of resistance (intermediate and high levels) of identified mutations to various antiretroviral drugs. It was observed that both NRTI and NNRTI-associated mutations showed significant resistance to various drugs, however, PI-associated mutations were less resistant toward antiretroviral drugs. In addition, we also identified the impact of mutations on protein stability. The analysis revealed that a small number of mutations showed a stabilizing effect (K65R, M184I, and M184V) despite the fact that most of the mutations were destabilizing. Based on our findings, we suggest the implementation of active screening for resistance to available drugs in Indian HIV-positive populations for early detection. We suggest that resistance testing in HIV-infected patients should ideally be performed before the initiation of therapy to tailor the treatment for the individual to achieve optimal therapeutic outcomes. Our study can extend the knowledge of the degree of heterogeneity present in the NRTI, NNRTI, and PIs associated mutations against specific drugs, which would be invaluable in the future for combination drug development. Further, the study also highlights the importance of the *in silico* approach in designing the most effective HAART regime in a resource-limited country like India. Since, predicting prevalent drug resistance variants is a challenging task, switching to combinational drug therapy may be beneficial in the Indian population.

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Conflicts of interest

None.

Ethical disclosures

Protection of human and animal subjects. The authors declare that no experiments were performed on humans or animals for this study.

Confidentiality of data. The authors declare that no patient data appear in this article. Furthermore, they have acknowledged and followed the recommendations as per the SAGER guidelines depending on the type and nature of the study.

Right to privacy and informed consent. The authors declare that no patient data appear in this article.

Use of artificial intelligence for generating text. The authors declare that they have not used any type of generative artificial intelligence for the writing of this manuscript or for the creation of images, graphics, tables, or their corresponding captions.

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