Development And Evaluation of Nanosuspension Using an Antiretroviral Drug for Enhanced Oral Delivery

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***Abstract*— Antiretroviral therapy remains the cornerstone in the management of Human Immunodeficiency Virus (HIV) infection; however, the therapeutic success of many antiretroviral drugs is significantly limited by their poor aqueous solubility, low oral bioavailability, and high inter- and intra-patient variability in absorption. A large proportion of antiretroviral agents belong to the Biopharmaceutical Classification System (BCS) class II and IV, where dissolution-limited absorption and extensive first-pass metabolism result in suboptimal plasma drug concentrations. These challenges necessitate the development of advanced drug delivery systems capable of enhancing oral absorption while maintaining drug stability and patient compliance. Nanosuspension technology has emerged as a promising and versatile approach to overcome solubility-related limitations associated with conventional oral formulations of antiretroviral drugs. Nanosuspensions are colloidal dispersions of pure drug particles in the nanometer size range, stabilized by suitable surfactants or polymers. Reduction of drug particle size to the nanometric scale significantly increases the surface area, leading to enhanced dissolution rate, improved saturation solubility, and rapid drug release in gastrointestinal fluids. This improvement in dissolution behavior ultimately translates into enhanced oral bioavailability and reduced dose requirements. Unlike other nanocarrier systems, nanosuspensions do not require complex carriers, making them particularly suitable for high drug-loading and scalable manufacturing.This review comprehensively discusses the formulation development and evaluation of nanosuspensions for antiretroviral drug delivery. Various preparation techniques, including top-down approaches such as high-pressure homogenization and media milling, bottom-up methods like antisolvent precipitation, and combination techniques, are critically analyzed with respect to their advantages, limitations, and industrial applicability. The role of formulation components such as stabilizers, surfactants, and solvents in achieving physical stability and optimal particle characteristics is also highlighted.**

***Index Terms*— Nanosuspension; Antiretroviral drugs; Oral bioavailability enhancement; Nanotechnology-based drug delivery; Poorly water-soluble drugs; Particle size reduction; Dissolution rate enhancement; HIV therapy; Biopharmaceutical Classification System (BCS); Oral drug delivery systems**

I. INTRODUCTION

Human Immunodeficiency Virus (HIV) infection continues to be a major global health challenge despite significant progress in antiretroviral therapy (ART). The introduction of antiretroviral drugs has dramatically improved the life expectancy and quality of life of HIV-infected patients by effectively suppressing viral replication and reducing disease progression. However, the clinical efficacy of many antiretroviral agents is often compromised due to formulation-related challenges such as poor aqueous solubility, low and variable oral bioavailability, extensive first-pass metabolism, and high dosing frequency. These limitations frequently result in inconsistent plasma drug levels, reduced therapeutic response, and poor patient adherence, especially in long-term HIV management. Oral drug delivery remains the most preferred route for antiretroviral therapy due to its convenience, non-invasiveness, and suitability for chronic administration. Nevertheless, a large number of antiretroviral drugs fall under the Biopharmaceutical Classification System (BCS) Class II and Class IV categories, characterised by low solubility and/or low permeability. Poor aqueous solubility leads to dissolution-limited absorption in the gastrointestinal tract, which significantly affects oral bioavailability. Consequently, higher doses are often required to achieve therapeutic plasma concentrations, increasing the risk of adverse effects and drug-drug interactions.To overcome these challenges, various formulation strategies have been explored, including

solid dispersions, lipid-based delivery systems, self-emulsifying drug delivery systems, polymeric nanoparticles, and liposomes. Although these approaches have shown potential, many suffer from limitations such as low drug loading, formulation complexity, stability issues, and high production costs. In this context, nanosuspension technology has emerged as a simple yet highly effective approach for enhancing the oral delivery of poorly water-soluble antiretroviral drugs without altering their chemical structure.Nanosuspensions are colloidal dispersions consisting of pure drug particles in the nanometer size range, typically below 1000 nm, stabilized by suitable surfactants or polymers. The fundamental principle of nanosuspension technology is particle size reduction, which leads to a significant increase in surface area and, consequently, an enhanced dissolution rate as described by the Noyes–Whitney equation. Additionally, nanosizing increases saturation solubility, improves wettability, and promotes rapid drug dissolution in gastrointestinal fluids, thereby enhancing oral absorption and bioavailability.The application of nanosuspension-based drug delivery systems is particularly advantageous for antiretroviral drugs due to their high dose requirements and solubility-limited absorption. By improving dissolution and absorption characteristics, nanosuspensions can reduce dose frequency, minimize gastrointestinal side effects, and improve patient compliance. Moreover, nanosuspensions offer flexibility in formulation, as they can be further processed into solid dosage forms such as tablets, capsules, and pellets, making them suitable for large-scale pharmaceutical manufacturing.In recent years, extensive research has been conducted on the development and evaluation of nanosuspensions using various antiretroviral drugs, demonstrating significant improvements in in-vitro dissolution behavior and in-vivo bioavailability compared to conventional formulations. However, formulation challenges such as physical stability, particle aggregation, and scalability remain critical considerations. Therefore, a comprehensive understanding of formulation strategies, preparation methods, and evaluation parameters is essential for the successful development of nanosuspension-based oral delivery systems.This review aims to provide a detailed overview of the development and evaluation of nanosuspensions using antiretroviral drugs for enhanced oral delivery. It highlights the formulation approaches, preparation techniques, critical evaluation parameters, advantages, challenges, and future prospects of nanosuspension technology in the context of antiretroviral therapy.Human Immunodeficiency Virus (HIV) infection is a chronic viral disease that primarily targets the immune system, leading to progressive immunodeficiency if left untreated. Antiretroviral drugs are specifically designed to inhibit different stages of the HIV replication cycle and form the backbone of HIV therapy. Understanding the mechanism of HIV replication and the sites of action of antiretroviral drugs is essential for the rational design and development of effective drug delivery systems. However, despite their potent antiviral activity, the clinical performance of many antiretroviral drugs is limited due to poor oral bioavailability, which necessitates the development of advanced formulation approaches such as nanosuspension technology.

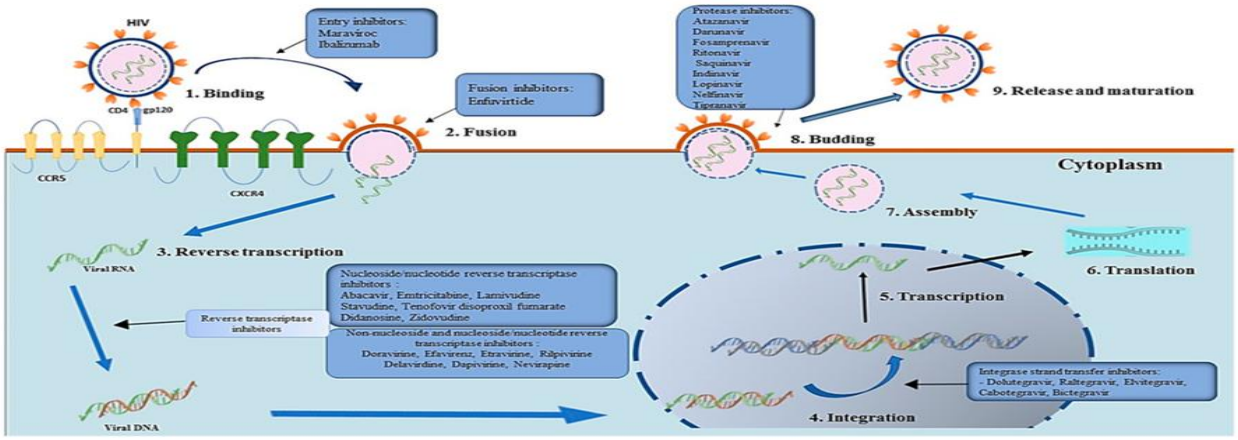

Figure 1: Mechanism of action of antiretroviral drugs at different stages of the HIV life cycle

The figure illustrates the complete life cycle of the Human Immunodeficiency Virus (HIV) along with the specific sites of action of different classes of antiretroviral drugs. The HIV replication cycle begins with the attachment of the virus to the CD4 receptor and chemokine co-receptors (CCR5 or CXCR4) present on the surface of host immune cells, a process known as viral binding. This step is followed by fusion of the viral envelope with the host cell membrane, allowing the viral RNA to enter the cytoplasm. Entry and fusion inhibitors act at this stage to prevent the virus from entering the host cell.Once inside the host cell, the viral RNA undergoes reverse transcription, where it is converted into complementary viral DNA by the enzyme reverse transcriptase. This crucial step is inhibited by nucleoside/nucleotide reverse transcriptase inhibitors and non-nucleoside reverse transcriptase inhibitors, which block viral DNA synthesis. The newly synthesized viral DNA is then transported into the nucleus and integrated into the host cell genome with the help of the enzyme integrase. Integrase strand transfer inhibitors prevent this integration process, thereby stopping further viral replication. Following integration, the viral DNA is transcribed into messenger RNA, which is subsequently translated into viral proteins. These viral components are assembled near the host cell membrane to form immature viral particles. The immature virus then buds out of the host cell, and the final maturation step occurs through the action of the viral protease enzyme. Protease inhibitors block this maturation process, resulting in the formation of non-infectious viral particles. Overall, this figure demonstrates that antiretroviral drugs act at multiple stages of the HIV life cycle to suppress viral replication. However, despite their potent antiviral activity, many antiretroviral drugs exhibit poor aqueous solubility and limited oral bioavailability, which can reduce therapeutic effectiveness. Therefore, advanced drug delivery approaches such as nanosuspension technology are required to enhance the oral absorption, bioavailability, and clinical performance of antiretroviral drugs.

II. NEED FOR NANOSUSPENSION IN ANTIRETROVIRAL DRUG DELIVERY

Despite the availability of potent antiretroviral drugs that effectively inhibit various stages of the HIV life cycle, their clinical performance is often limited by formulation-related challenges. A significant number of antiretroviral agents exhibit poor aqueous solubility, low dissolution rate, and limited permeability across the gastrointestinal membrane. As a result, these drugs show low and variable oral bioavailability, leading to inconsistent plasma drug concentrations and reduced therapeutic efficacy. To compensate for poor absorption, higher doses are frequently administered, which may increase the risk of dose-related toxicity, gastrointestinal side effects, and poor patient compliance during long-term therapy.Conventional oral dosage forms such as tablets and capsules often fail to provide adequate dissolution of poorly water-soluble antiretroviral drugs in gastrointestinal fluids. Since dissolution is the rate-limiting step for absorption in BCS Class II and IV drugs, insufficient dissolution directly affects systemic availability. Moreover, first-pass metabolism and drug efflux mechanisms further reduce the effective drug concentration reaching systemic circulation. These challenges highlight the necessity for novel drug delivery systems capable of improving solubility and dissolution without altering the chemical structure of the drug.Nanosuspension technology offers a promising solution to overcome these limitations. By reducing drug particle size to the nanometer range, nanosuspensions significantly increase the surface area available for dissolution, resulting in enhanced dissolution rate and improved saturation solubility. Improved wettability and intimate contact with gastrointestinal fluids further facilitate rapid drug release and absorption. Additionally, nanosuspensions allow high drug loading, are suitable for oral administration, and can be easily converted into solid dosage forms, making them advantageous for large-scale pharmaceutical manufacturing.Therefore, the development of nanosuspension-based delivery systems for antiretroviral drugs represents an effective strategy to enhance oral bioavailability, reduce dose requirements, minimize side effects, and improve patient compliance. This approach has gained considerable attention in recent years as a viable and scalable formulation strategy for improving the therapeutic outcomes of antiretroviral therapy.

III. PREPARATION METHODS OF NANOSUSPENSION

The preparation of nanosuspensions involves the reduction of drug particle size to the nanometer range using suitable techniques, followed by stabilization with surfactants or polymers to prevent aggregation. Selection of an appropriate preparation method depends on the physicochemical properties of the antiretroviral drug, desired particle size, scalability, and cost-effectiveness. Broadly, nanosuspension preparation methods are classified into bottom-up techniques, top-down techniques, and combination approaches.

1. Bottom-Up Techniques

Bottom-up techniques involve the formation of nanoparticles from molecular solutions through controlled precipitation or crystallization processes. In these methods, the drug is first dissolved in a suitable organic solvent and then rapidly mixed with a non-solvent, leading to supersaturation and precipitation of drug nanoparticles. Common bottom-up approaches include antisolvent precipitation and solvent evaporation methods. These techniques offer advantages such as low energy requirements and simple equipment; however, they often suffer from challenges like particle growth, agglomeration, and residual solvent presence, which may affect product stability and safety.

2. Top-Down Techniques

Top-down techniques involve the mechanical size reduction of coarse drug particles into nanosized particles using high-energy input. High-pressure homogenization and media milling are the most widely used top-down methods for nanosuspension preparation. In high-pressure homogenization, the drug suspension is forced through a narrow gap under high pressure, resulting in particle size reduction due to cavitation and shear forces. Media milling involves the use of milling beads to fracture drug particles through impact and attrition. Top-down techniques are highly reproducible, scalable, and suitable for industrial production; however, they require specialized equipment and high energy consumption.

3. Combination Techniques

Combination techniques integrate both bottom-up and top-down approaches to achieve better control over particle size and stability. Typically, a preliminary particle size reduction is achieved through precipitation, followed by high-pressure homogenization to obtain uniform nanosized particles. This approach minimizes the drawbacks of individual techniques and results in stable nanosuspensions with narrow particle size distribution. Combination methods are increasingly preferred for antiretroviral drug nanosuspensions due to their improved efficiency and scalability.

IV. MATERIALS AND METHODS

Methods

1. Preparation of Nanosuspension

The nanosuspension of the selected antiretroviral drug was prepared using the antisolvent precipitation technique combined with ultrasonication (or high-pressure homogenization, depending on equipment availability). Initially, the antiretroviral drug was accurately weighed and dissolved in a suitable organic solvent to form the organic phase. This drug solution was then rapidly injected into an aqueous phase containing a pre-selected stabilizer under continuous magnetic stirring, resulting in immediate precipitation of drug particles due to supersaturation. The resulting coarse suspension was subsequently subjected to probe ultrasonication for a predetermined time to reduce particle size and achieve uniform dispersion. The prepared nanosuspension was collected and stored under refrigerated conditions for further evaluation.

2. Optimization of Formulation

Formulation optimization was carried out to obtain a stable nanosuspension with minimum particle size and narrow size distribution. Various stabilizers such as Polyvinylpyrrolidone (PVP), Poloxamer, and Hydroxypropyl Methylcellulose (HPMC) were screened to study their influence on particle size, physical stability, and redispersibility of the nanosuspension. Different formulation batches were prepared by varying stabilizer type and concentration. Optimization was performed either using a Design of Experiments (DoE) approach or by a trial-and-error method, depending on the study design and available resources.

3. Characterization of Nanosuspension

The prepared nanosuspensions were characterized using the following evaluation parameters:

- Particle Size and Polydispersity Index (PDI): Particle size distribution and PDI were determined using Dynamic Light Scattering (DLS) to assess uniformity of particle size.
- Zeta Potential: Zeta potential measurements were carried out to evaluate the surface charge and physical stability of the nanosuspension.
- Drug Content (Assay): Drug content was estimated using a UV-Visible spectrophotometer or High-Performance Liquid Chromatography (HPLC) to ensure uniform drug distribution.
- Saturation Solubility Studies: Saturation solubility of the nanosuspension was compared with that of the pure drug in distilled water and suitable buffer solutions.
- In-vitro Dissolution Studies: Dissolution studies were performed using a USP dissolution apparatus Type II (paddle method) in simulated gastric fluid (SGF) and simulated intestinal fluid (SIF) to evaluate dissolution enhancement.
- Differential Scanning Calorimetry (DSC): DSC analysis was carried out to investigate changes in the crystalline or amorphous nature of the drug.
- X-ray Diffraction (XRD): XRD studies were performed to confirm reduction in crystallinity of the drug following nanosuspension formulation.
- Scanning Electron Microscopy (SEM): SEM was used to examine surface morphology and particle size of the nanosuspension.

4. Stability Studies

Stability studies were conducted in accordance with International Council for Harmonisation (ICH) guidelines. The optimized nanosuspension formulation was stored at 25 °C ± 2 °C / 60% ± 5% RH and 40 °C ± 2 °C / 75% ± 5% RH. Samples were withdrawn at predetermined intervals (0, 1, 2, and 3 months) and evaluated for particle size, PDI, zeta potential, and drug content to assess physical and chemical stability.

Materials

- Drug: Selected antiretroviral drug such as Efavirenz, Ritonavir, or Dolutegravir, as per study design.
- Stabilizers/Polymers: Polyvinylpyrrolidone (PVP K30), Hydroxypropyl Methylcellulose (HPMC), Poloxamer 188, and Sodium Lauryl Sulfate (SLS).
- Solvents: Distilled water, ethanol, and other analytical grade solvents.
- Other Chemicals: Mannitol (used as a cryoprotectant), surfactants, and suitable buffering agents.
- All chemicals and reagents used in the study were of analytical grade.

V. RESULTS AND DISCUSSION

- The results obtained from the development and evaluation of the antiretroviral drug nanosuspension were systematically analysed and compared with those of the pure drug. The discussion focuses on the influence of formulation variables on particle size, stability, solubility, and dissolution behaviour, highlighting the potential of nanosuspension technology in enhancing oral drug delivery.

- Particle Size and Polydispersity Index (PDI)

The optimized nanosuspension formulation exhibited a significant reduction in particle size when compared to the pure drug. The particle size was found to be in the nanometer range with a narrow size distribution, as indicated by low PDI values. A lower PDI suggests uniformity of particle size and good physical stability of the nanosuspension. The reduction in particle size can be attributed to the combined effect of antisolvent precipitation and ultrasonication, which efficiently broke down drug particles and prevented aggregation through stabilizer adsorption.

- Zeta Potential

Zeta potential analysis revealed that the optimized nanosuspension possessed sufficient surface charge to maintain physical stability. The observed zeta potential values indicated effective electrostatic and steric stabilization provided by the selected stabilizers. Higher absolute zeta potential values contribute to repulsive forces between particles, thereby minimizing aggregation during storage.

- Drug Content (Assay)

Drug content analysis demonstrated uniform distribution of the antiretroviral drug within the nanosuspension. The assay values were found to be

within acceptable limits, indicating minimal drug loss during formulation and processing. This confirms the suitability of the preparation method for achieving high drug loading and content uniformity.

- Saturation Solubility Studies

Saturation solubility studies showed a marked increase in solubility of the antiretroviral drug in nanosuspension form compared to the pure drug. The enhanced solubility is primarily due to the reduced particle size, increased surface area, and improved wettability of drug particles. This improvement in saturation solubility plays a crucial role in enhancing oral bioavailability.

- In-vitro Dissolution Studies

In-vitro dissolution studies demonstrated a significantly faster and more complete drug release from the nanosuspension compared to the pure drug. The enhanced dissolution rate can be attributed to nanosizing of drug particles, which reduces diffusion distance and increases dissolution velocity. Improved dissolution behavior in both simulated gastric fluid and simulated intestinal fluid suggests that the nanosuspension formulation may provide consistent drug release throughout the gastrointestinal tract.

- Solid-State Characterization (DSC and XRD)

DSC thermograms and XRD patterns revealed changes in the crystalline nature of the drug following nanosuspension formulation. A reduction in peak intensity and slight broadening of diffraction peaks indicated partial amorphization or reduced crystallinity of the drug. These solid-state modifications contribute to improved solubility and dissolution characteristics.

- Surface Morphology (SEM)

SEM analysis confirmed the nanoscale size and uniform morphology of the drug particles in the nanosuspension. The particles appeared discrete and spherical with minimal aggregation, further supporting the effectiveness of the selected preparation and stabilization techniques.

- Stability Studies

Stability studies conducted as per ICH guidelines indicated that the optimized nanosuspension remained physically and chemically stable over the study period. No significant changes were observed in particle size, PDI, zeta potential, or drug content, confirming the robustness of the formulation.

VI. EVALUATION OF NANOSUSPENSION

The developed nanosuspension was evaluated for various physicochemical and performance parameters to assess its suitability for enhanced oral delivery of the selected antiretroviral drug.

1. Particle Size and Polydispersity Index (PDI)

Particle size and PDI were determined using Dynamic Light Scattering (DLS). Particle size plays a crucial role in dissolution rate and oral bioavailability. A low PDI value indicates uniform particle size distribution and better physical stability of the nanosuspension.

2. Zeta Potential

Zeta potential was measured to evaluate the surface charge and stability of the nanosuspension. Adequate zeta potential values indicate sufficient electrostatic or steric repulsion between particles, thereby preventing aggregation during storage.

3. Drug Content (Assay)

Drug content was estimated using a UV-Visible spectrophotometer or High-Performance Liquid Chromatography (HPLC). This test ensures uniform distribution of the drug within the nanosuspension and confirms the accuracy of the formulation method.

4. Saturation Solubility Studies

Saturation solubility of the nanosuspension was determined and compared with that of the pure drug in distilled water and suitable buffer solutions. An increase in saturation solubility indicates the effectiveness of particle size reduction and improved wettability.

5. In-Vitro Dissolution Studies

In-vitro dissolution studies were carried out using USP dissolution apparatus Type II (paddle method) in simulated gastric fluid (SGF) and simulated intestinal fluid (SIF). The dissolution profile of the nanosuspension was compared with that of the pure drug to evaluate enhancement in dissolution rate.

6. Differential Scanning Calorimetry (DSC)

DSC analysis was performed to study the thermal behavior and crystalline or amorphous nature of the drug in the nanosuspension. Changes in melting point

or peak intensity indicate possible reduction in crystallinity.

7. X-Ray Diffraction (XRD)
XRD studies were conducted to confirm changes in the crystalline structure of the drug after nanosuspension formulation. Reduction in peak intensity suggests decreased crystallinity, contributing to improved solubility.

8. Scanning Electron Microscopy (SEM)
SEM was used to examine the surface morphology, shape, and size of the nanosuspension particles. The analysis provides visual confirmation of nanosized drug particles and uniform dispersion.

9. Stability Studies
Stability studies were conducted as per ICH guidelines to evaluate the physical and chemical stability of the optimized nanosuspension. Parameters such as particle size, PDI, zeta potential, and drug content were monitored at predetermined intervals under different storage conditions.

VII. CONCLUSION

The present review highlights the potential of nanosuspension technology as an effective approach for enhancing the oral delivery of poorly water-soluble antiretroviral drugs. Antiretroviral therapy plays a vital role in the management of HIV infection; however, the therapeutic effectiveness of many antiretroviral agents is often limited by poor aqueous solubility, low dissolution rate, and reduced oral bioavailability. These formulation-related challenges necessitate the development of advanced drug delivery systems capable of improving drug absorption and therapeutic performance. Nanosuspension-based formulations offer significant advantages by reducing drug particle size to the nanometer range, thereby increasing surface area, saturation solubility, and dissolution rate. The formulation and evaluation studies discussed in this review demonstrate that nanosuspensions can significantly enhance in-vitro dissolution behavior, improve physical stability, and potentially increase oral bioavailability of antiretroviral drugs. Additionally, nanosuspensions allow high drug loading, flexibility in formulation, and feasibility for large-scale manufacturing, making them suitable for chronic oral administration.Overall, the development of antiretroviral drug nanosuspensions represents a promising and practical strategy to overcome solubility-related limitations associated with conventional oral dosage forms. With appropriate formulation optimization and characterization, nanosuspension technology has the potential to improve therapeutic efficacy, reduce dosing frequency, and enhance patient compliance in long-term HIV treatment.

VIII. FUTURE SCOPE

Nanosuspension technology offers a promising platform for improving the oral delivery of antiretroviral drugs; however, further research and development are required to fully exploit its potential in clinical applications. Future studies may focus on optimizing nanosuspension formulations to achieve targeted delivery within the gastrointestinal tract, thereby enhancing site-specific absorption and therapeutic effectiveness. Incorporation of functional excipients and surface modification techniques may further improve drug stability and absorption characteristics. Advanced formulation strategies such as sustained-release or controlled-release nanosuspensions can be explored to reduce dosing frequency and improve patient compliance in long-term antiretroviral therapy. Additionally, combination nanosuspension formulations containing multiple antiretroviral drugs may be developed to support fixed-dose combination therapy, which is widely used in HIV treatment. In-vivo pharmacokinetic and bioavailability studies, along with clinical evaluations, are essential to establish a clear correlation between enhanced in-vitro performance and therapeutic outcomes. Furthermore, large-scale manufacturing, regulatory approval, and long-term stability studies remain critical areas for future investigation. Addressing these challenges will facilitate the translation of nanosuspension-based antiretroviral formulations from laboratory research to commercial products. Overall, continued advancements in nanosuspension technology hold significant potential for improving the effectiveness, safety, and patient adherence of antiretroviral drug therapy.

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