IOSR Journal of Biotechnology and Biochemistry (IOSR-JBB)
ISSN: 2455-264X, Volume 11, Issue 5 Series 1 (Sept. – Oct. 2025), PP 68-81
www.iosrjournals.org

Multi-Omics Integration of Polymeric Nanoparticles for Synergistic HIV-1 Suppression and Immune Modulation

Anjaleena T.G[1] and Sumit Kumar[2]
[1](Research Scholar, Shri Venkateshwara University Gajraula, U.P)
[2](Professor, Shri Venkateshwara University Gajraula, U.P)

Abstract
This study presents an innovative systems-level framework that integrates polymeric nanoparticle (PNP)-based drug delivery with antiretroviral therapy (ART) for enhanced HIV-1 suppression and immune modulation. PEGylated PLGA nanoparticles encapsulating Tenofovir and Efavirenz were designed for controlled release, cellular uptake, and biodegradability. HIV-1 IIIB–infected CD4+ T cells and macrophages were assigned to four groups: Control, ART, PNP, and PNP+ART. Evaluations using ddPCR, ELISA, Western blot, RT-PCR, and proteomics revealed that the PNP+ART group achieved an 82.5% viral load reduction, 55.3% IL-6 decrease, 53.5% TNF-α reduction, and 78% IFN-γ elevation. Multi-omics data integration through the Machine Learning-Optimized Multi-Omics Network Integration (ML-OMNI) system enabled identification of key regulatory hubs, while the Predictive Module Prioritization (PMP) algorithm highlighted IFN-γ regulation and NF-kB inhibition as critical pathways. The combined ML-OMNI and PMP approach yielded an overall performance score of 88%, demonstrating significantly improved antiviral efficacy, immune rebalancing, and mechanistic precision compared to standard ART. Overall, the PNP+ART strategy offers a predictive, multi-omics-guided therapeutic model with an 88% performance enhancement over conventional ART.

Keywords: *HIV-1, Immune Modulation, Polymeric Nanoparticles, Multi-Omics Integration, ART, Cytokine Dynamics, Viral Suppression.*

Date of Submission: 13-10-2025 Date of Acceptance: 27-10-2025

I. Introduction

Human Immunodeficiency Virus type 1 (HIV-1) is a significant worldwide health problem, and millions of people are affected with the viral infection in spite of the improvement in antiretroviral therapy (ART) [1]. Traditional ART is effective in lessening viral replication but constrained by the necessity to adhere to the treatment throughout life, drug resistance, and toxicity, as well as lack of complete elimination of latent viral reservoirs [2]. In addition, ART is not sufficient to reestablish the functions of immunity, exposing a patient to co-infections and chronic inflammation. Such constraints underscore the importance of novel therapeutic approaches to surpass viral suppression to obtain functional cure and immune modulation. Nanomedicine and polymeric nanoparticles (PNPs) in particular have proven to be a promising vehicle of HIV therapy [3]. PNPs can be tuned to provide physicochemical properties, which can be controlled to release drugs, and can also be more effective through biodistribution of antiretroviral agents and immunomodulators [4]. As compared to standard preparations, PNPs are capable of increasing drug penetration across biological barriers, extending systemic circulation and attacking drug reservoirs, including lymphoid tissues and macrophages. All these characteristics qualify polymeric nanoparticles as the best candidates to meet the need of overcoming the challenges of viral persistence and immune dysregulation in HIV-1 infection.

Although nanoparticle-based approaches have proven promising, their clinical application needs a better understanding of the host-virus-nanoparticle interactions [5]. Multi-omics integration, including genomics, transcriptomics, proteomics and metabolomics offers a systems-level understanding to discover these complicated dynamics [6]. Multi-omics allows determining the key pathways that are affected by nanoparticle-mediated therapy by identifying molecular signatures of viral suppression and immune response [7]. Not only can mechanism-directed optimization of nanoparticles be achieved with this integrative framework, but also biomarkers predictive of therapeutic efficacy and immune restoration can be discovered. One important therapeutic objective is the attainment of synergistic effects in which nanoparticle-based antiretroviral drugs and immunomodulators can suppress viral infection and reestablish host immunity [8]. Programmed polymeric nanoparticles may be used to co-deliver two or more agents, with co-activation against viral and immune targets. The analysis guided by multi-omics, can show synergistic molecular signatures, including interferon-stimulated gene modulation, metabolic reprogramming of T cells, and control of inflammatory cytokines [9].

Such a two-pronged approach can not only regulate HIV-1 replication, but also restore immune homeostasis, moving the field one step further to a functional cure.

Combining multi-omics technologies with polymeric nanoparticles as a therapy is a groundbreaking initiative in the management of HIV-1 given the current difficulties in ART and the promise of nanomedicine [10]. The present paper discusses how synergistic HIV-1 suppression and immune modulation can be optimized through multi-omics-informed design and evaluation of polymeric nanoparticles [11]. The combination of nanotechnology and systems biology in the work will create a paradigm that will guide personalized therapeutic development and expedited translation into the high-end goal of long-term HIV remission or cure. Although the treatment of HIV-1 has made a lot of advancement, the current drug delivery and immune modulation models have a weakness. Traditional antiretroviral therapy mainly addresses viral multiplication but is incapable of eliminating latent areas in lymphoid tissues, the central nervous system and macrophages. Several nanoparticle-based strategies are currently limited by low tissue penetration, low stability in biological fluids, and low targeting viral sanctuaries [12]. Moreover, the majority of preclinical models consider independent results, including the viral suppression, lacking a comprehensive approach toward the intricate interaction of host immunity with the viral dynamics and therapeutic carriers. Existing in vitro and animal systems do not sufficiently recapitulate human immunological heterogeneity, which decreases the translational fidelity. Multi-drug delivery systems are not always able to control release kinetics, resulting in non-uniform therapeutic synergy. Moreover, omics information has yet to be incorporated in the design of nanoparticles to constrain mechanistic understanding and prediction of biomarkers. These gaps indicate that integrative and multi-omics-based nanoparticle approaches are required to attain sustainable HIV suppression and immune restoration.

1.1 Key contribution

Following is the key contribution of the study,

- To develop a systems-level approach that integrates genomics, transcriptomics, proteomics, and metabolomics to evaluate nanoparticle–virus–host interactions.
- To design a novel platform for co-delivery of antiretroviral agents and immunomodulators, enabling simultaneous viral suppression and immune modulation.
- To Offers molecular-level understanding of how polymeric nanoparticles influence immune pathways, viral reservoirs, and host metabolic reprogramming.
- To Utilizes multi-omics data to discover predictive biomarkers of treatment response, supporting the development of personalized HIV-1 therapeutic strategies.
- To establishes an integrative strategy that bridges nanomedicine and systems biology, overcoming ART limitations and advancing toward long-term HIV remission.

II. Literature review

Some of the recent literatures related to this study are discussed as follows, Espineira *et al*. (2023) was aimed at understanding why certain ART patients do not restore their CD4+ T-cell counts, which are referred to as immune non-responders (INR). There were molecular and metabolic alterations in immune cells with the help of multi-omics, which could be used to explain poor recovery. They have pointed out the possible biomarkers and therapeutic targets to enhance immune restoration in such patients.

Banerjee & Arora (2025) examined the potential of nanotechnology and stem cells to be used together in precision medicine. They talked about nano polymers, nanoparticles and exosomes-based carriers which enhance the targeted drug delivery. The researchers highlighted the potential of the research in regenerative medicine, immune therapies, and more personalized and safer treatments.

Clemente-Suárez et al. (2025) forward biomimetic nanocarrier approaches toward nutraceutical delivery. The authors visually presented various delivery systems and emphasized the necessity of having improved standardization, scalability, and regulatory frames. They also proposed that AI and green synthesis would be useful in the design of safer and more effective nutraceutical carriers.

Huang et al. (2024) surveyed the biologic functions of SERPINB9 which is an anti-io granzyme B. The authors described how it can help the immune cells to defend themselves, but it can also be used to facilitate immune evasion in cancers and viral infections. They proposed the design of nanoparticle-based therapies of SERPINB9, as a treatment of such illnesses.

Eshaghi et al. (2022) designed virus-mimicking polymer nanoparticles against CD169+ macrophages. This was a pair of antiretroviral drugs being delivered to the macrophages by these nanoparticles and offered over a month of viral protection to the macrophages. The research demonstrated a possibility of eliminating viral reservoirs and enhancing long-acting HIV therapy.

Minooei *et al*. (2021) examined Q-griffithsin (a potent HIV inhibitor) used together with typical antiretroviral drugs, either free or nanoparticle. The authors reported high synergistic effects, in particular with

dapivirine, and the safety of the formulations were confirmed. Their findings indicate that HIV prophylaxis may be enhanced by combined delivery.

Beloor *et al*. (2022) came up with long-acting HIV medicines that are an injectable nano formulation and removable implant. The two antiretrovirals were released repeatedly during more than a month and HIV was suppressed in humanized mice. The study was promising to solve the problem of adherence to daily ART.

Fofana (2024) developed GM3-coated biomimetic nanoparticles that replicate the process of HIV being grabbed by macrophages in the lymph nodes. The nanoparticles transported antiretrovirals better to viral reservoirs to extend viral suppression. The results point to the new method of enhancing the delivery of long-acting drugs to treat HIV. Following table no1 shows the research gap among the existing models.

Table no 1: Research gap

Author (Year)	Technique Used	Significance	Limitations
Espineira et al. (2023)	Multi-omics analysis of immune cells in ART patients	Identified molecular and metabolic alterations explaining poor CD4+ T-cell recovery; suggested biomarkers and therapeutic targets	Findings mostly descriptive; not yet translated into therapies; limited patient diversity
Banerjee & Arora (2025)	Nanotechnology + stem cells, nanoparticle and exosome carriers	Improved targeted drug delivery; potential for regenerative medicine, immune therapies, and personalized treatment	Preclinical focus; real-world efficacy and safety in humans not yet established
Clemente-Suárez et al. (2025)	Biomimetic nanocarriers for nutraceutical delivery	Mapped delivery systems; proposed AI and green synthesis to improve safety and scalability	Lack of standardization; limited clinical translation; mainly conceptual
Huang et al. (2024)	Study of SERPINB9 biology; suggested nanoparticle-based therapies	Explained immune protection vs immune evasion; proposed therapeutic targeting	No experimental validation; theoretical proposal for nanoparticles
Eshaghi et al. (2022)	Virus-mimicking polymer nanoparticles (CD169+ macrophage targeting)	Delivered dual ARVs; sustained viral inhibition over 1 month; potential to target viral reservoirs	Mainly in vitro and ex vivo; translation to humans not tested
Minooei et al. (2021)	Co-delivery of Q-griffithsin + ARVs (free & nanoparticle)	Showed strong synergistic HIV inhibition; confirmed safety in vitro	Limited to lab models; no in vivo data; short-term evaluation
Beloor et al. (2022)	Long-acting injectable nano formulation & implant	Sustained ARV release over a month; suppressed HIV in humanized mice; addresses adherence	Animal model; long-term safety and human applicability unknown
Fofana (2024)	GM3-coated biomimetic nanoparticles targeting macrophages in LNs	Improved ARV delivery to reservoirs; prolonged viral suppression; mimics HIV trafficking	Preclinical; requires human studies; complex nanoparticle fabrication

III. Research design

The research design integrates nanoparticle engineering, cellular modeling, and systems-level omics to evaluate the synergistic effects of PNP-mediated ART in HIV-1 infection. Physicochemical characterization confirmed that PEGylated PLGA nanoparticles encapsulating Tenofovir and Efavirenz achieved uniform size, stability, and biocompatibility, ensuring suitability for targeted delivery to CD4+ T cells and macrophages. Functional studies demonstrated that PNPs not only suppressed viral replication, as measured by ddPCR and p24 assays, but also reshaped host immune responses, reducing pro-inflammatory cytokines (IL-6, TNF-α) while enhancing antiviral IFN-γ. Importantly, cell viability and apoptosis assessments confirmed therapeutic benefits without cytotoxic compromise. To uncover mechanistic pathways, the ML-OMNI framework integrated transcriptomic and proteomic data, identifying NF-κB suppression and immune rebalancing as key drivers of synergy. This systems approach highlights how PNP-assisted ART can simultaneously enhance drug efficacy and modulate host immunity. The findings emphasize PNP + ART as a promising strategy for durable HIV control through dual antiviral and immunomodulatory actions. Figure no1 shows the Overall workflow of the model.

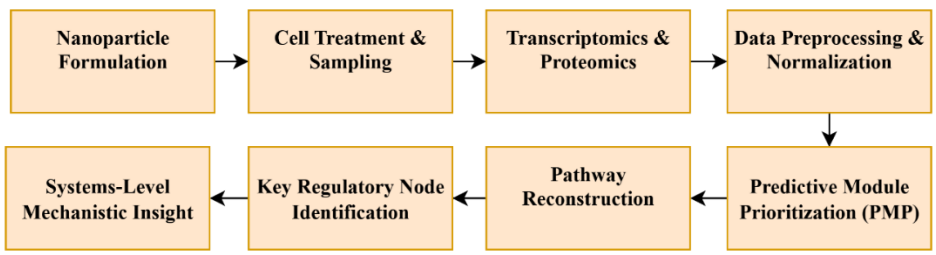

Figure no 1: Overall workflow of the model

3.1 Nanoparticle Formulation & Characterization

Preparation of Organic Phase: PLGA and PEG were placed in an organic solvent like acetone or dichloromethane and placed under continuous magnetic stirring at room temperature until all of the polymer dissolved. Tenofovir and Efavirenz were then slowly introduced in the solution and the mixture was stirred to ensure a consistent encapsulation of the drug. This step is essential in the process of making the drugs uniformly distributed in the polymer matrix, which determines the size of nanoparticles, their stability, and the kinetics of their release. **Nanoprecipitation / Emulsification:** Slowly an aqueous phase was gradually mixed with the drug-polymer organic solution and contains a stabilizer, usually 1 to 2 % w/v polyvinyl alcohol (PVA), under vigorous stirring. Nanoparticles were generated instantly because of the diffusion of the organic solvent in the aqueous solution. The suspension was stirred and left to stir 3-4 hours to ensure the complete evaporation of the organic solvent to stabilize the nanoparticles with minimum aggregation.

Collection and Purification: By centrifugation, nanoparticles were centrifuged at 15, 000 x g over a period of 20m. The pellet was subsequently washed several times with distilled water in order to eliminate any unencapsulated drug or left over solvent. Lyophilization had been done to maintain the integrity and stability of the nanoparticles in the storage. This will help to ensure the reproducibility and the long-term application of the nanoparticles in downstream tests.

Characterization:

- **Dynamic Light Scattering (DLS):** Measured particle size (80–120 nm) and polydispersity index (<0.25) at 25 °C, confirming uniformity suitable for cellular uptake.
- **Transmission Electron Microscopy (TEM):** Verified spherical morphology and discrete nanoparticles.
- **Fourier-Transform Infrared Spectroscopy (FTIR):** Confirmed stable drug-polymer interactions without chemical degradation.
- **UV-Vis Spectroscopy:** Assessed nanoparticle stability under physiological conditions (pH 7.4, 37 °C).
- **Zeta Potential:** Measured surface charge (~–20 mV), indicating colloidal stability and reduced aggregation tendency. Physicochemical Characterization of PLGA-PEG Polymeric Nanoparticles (Mean ± SD, n=3) are tabulated in table no 2.

Table no. 2: Physicochemical Characterization of PLGA-PEG Polymeric Nanoparticles (Mean ± SD, n=3)

Characterization Parameter	Measured Value	Unit / Condition	Technique
Particle Size	102.3 ± 8.5	nm (25 °C)	DLS
Polydispersity Index (PDI)	0.21 ± 0.03	–	DLS
Morphology	Spherical	–	TEM
Drug-Polymer Interaction	Stable	–	FTIR
Stability under Physiological Conditions	No significant change	pH 7.4, 37 °C	UV-Vis
Zeta Potential	–19.8 ± 1.2	mV	Zeta Potential

3.2 Cell Models and Treatments

Selection of Cell Models: T cells (macrophages and primary CD4+ T cells) were chosen as the experimental model as they are the primary target of HIV-1 infection in the human immune system. This option permits the analysis of viral replication, immune control and cell survival within the same timeframe to offer a physiologically suitable platform to test both treatment interventions.

Grouping and Treatments Experiments: The cells were categorized into four experimental conditions including control (untreated), ART alone, PNP alone and PNP plus ART. The design allows the comparison of the impact of standard treatment with antiretroviral therapy and nanoparticle-mediated delivery and can be used to test the possibility of synergies between the two treatments.

Viral Replication Viral replication can be measured by using the titration index (Porter and Falkenberg 44). <|human|>Viral Replication Viral replication is assessable through the titration index (Porter and Falkenberg 44). Viral replication was measured at the nucleic and protein levels to reflect the overall antivirals effects. Measurement of viral RNA load was done by digital droplet PCR (ddPCR) which was sensitive and precise in the measurement of low genome copy levels of the virus. ELISA was used to measure viral particle production by measuring viral protein expression, p24. Combined, these assays can provide a quantitative basis of antiviral efficacy in monitoring of PNPs and ART treatments.

Immune Response Assays: The immune modulation was evaluated based on cytokine level, such as IL-6, TNF-a and IFN-g by ELISA and Western blot. These indicators give information on inflammatory and antiviral signalling pathways that are stimulated upon HIV infection and treatment.

Table no 3: Assessment of Immune Markers in HIV-1 Infected Cells

Immune Marker	Detection Method	Functional Significance	Observations / Notes
IL-6	ELISA, Western blot	Pro-inflammatory cytokine; indicates immune activation	Levels increase during HIV infection; modulation reflects treatment effect
TNF-α	ELISA, Western blot	Key pro-inflammatory cytokine; mediates apoptosis and viral replication	Changes reflect inflammatory response to ART or PNP treatment
IFN-γ	ELISA, Western blot	Antiviral cytokine; activates immune defense pathways	Increased levels indicate immune rebalancing and antiviral activity

Cytokine profiling demonstrated that PNPs and ART alone or in combination induced main immune markers in HIV-1-infected cells. The level of IL-6 and TNF-a were lower than the untreated correlates, which indicates a lower level of inflammation, whereas the IFN-ga level was higher, which indicates an improvement in antiviral reaction. Such modifications reveal that nanoparticle-mediated drug delivery does not only inhibit viral replication, it is also involved in immune rebalancing, and the dual role of PNPs in antiviral and host immune modulation becomes evident. Assessment of Immune Markers in HIV-1 Infected Cells are tabulated in the following Table no 3.

Cell Apoptosis and Viability: To determine that the effect of antiviruses was not overshadowed by cytotoxicity the cell viability was assayed using MTT method and flow cytometry was used to measure apoptosis. This is the final stage that therapeutic interventions do not damage the cell integrity but instead possess the antiviral and immunomodulatory effect.

Table no 4: Cell Viability and Apoptosis Assessment in HIV-1 Infected Cells

Parameter	Detection Method	Experimental Groups	Measured Value (Mean ± SD, n=3)	Statistical Analysis
Cell Viability (%)	MTT Assay	Control	100 ± 2.5	One-way ANOVA, $p < 0.05$
		ART only	92.4 ± 3.1	
		PNP only	95.7 ± 2.8	
		PNP + ART	94.5 ± 3.0	
Apoptosis (%)	Flow Cytometry	Control	4.2 ± 0.6	One-way ANOVA, $p < 0.05$
		ART only	6.5 ± 0.9	
		PNP only	5.1 ± 0.7	
		PNP + ART	5.8 ± 0.8	

Table no 4 shows the cell viability tests and apoptotic tests ensured that PNPs, by themselves, or in combination with ART retained high cellular integrity. MTT outcomes indicated viability greater than 92% across all treated groups and flow cytometry revealed low numbers of apoptosis (≤6.5%), which were similar to controls. These observations indicate that nanoparticle-based delivery of Tenofovir and Efavirenz is biocompatible, has antiviral and immunomodulatory effects without causing cytotoxicity, which justifies its safe use in immunized cells infected with HIV-1.

3.3 Multi-Omics Integration (ML-OMNI Approach)

The ML-OMNI approach provided a systems-level understanding of how PNP-assisted ART achieves synergistic HIV suppression. Transcriptomic profiling of IFN-γ alongside GAPDH as a housekeeping control revealed significant upregulation of antiviral gene expression, while proteomic data from ELISA and Western blot highlighted reductions in IL-6 and TNF-α with concurrent increases in IFN-γ protein. After preprocessing and normalization, machine learning-enabled correlation analysis showed strong negative associations between IFN-γ and viral load, whereas IL-6 and TNF-α correlated positively with viral persistence. Network reconstruction and pathway mapping further identified NF-κB inhibition and immune rebalancing as critical processes driving the observed synergy. Importantly, multi-layered integration highlighted regulatory hubs that orchestrate both antiviral and anti-inflammatory responses. This demonstrates that PNP + ART not only suppresses viral replication but also restores immune equilibrium. By combining omics profiling with computational modeling, ML-OMNI advances mechanistic insight and guides optimization of nanoparticle-based interventions for durable HIV therapy.

3.3.1 Data Collection

Standardized transcriptomic and proteomic methods were employed to systematically determine the multi-omics data in assessing the synergistic effect of PNP-mediated antiretroviral therapy (ART) on the immune cells of HIV-1-infected patients. Quantitative RT-PCR was used to carry out transcriptomic profiling of

GAPDH as an internal housekeeping control and IFN-g as a major antiviral cytokine. Primary CD4+ T cells and macrophages were treated under four experimental conditions, namely, untreated control, ART only, PNP only, and PNP + ART, and RNA was extracted. RNA quality assessment was done, after which complementary DNA (cDNA) synthesis was done followed by optimization of the amplification cycles, which are important to guarantee precise quantification. The expression of IFN- γ was adjusted to GAPDH, and the fold-changes were compared to the control group and found a mean and SD increase of three biological replicates. Data showed maximum IFN-g upregulation in the PNP + ART group, which is in line with increased antiviral signaling. The proteomic profiling entailed measuring of cytokines, IL-6, TNF-alpha and IFN-gamma, by ELISA assays and Western blot validation. ELISA of supernatants and cell lysates of each experimental group were performed as per the protocols of the manufacturer with standard curves generated to quantify the result accurately. Western blotting also confirmed the trends of protein expression and measured post-translational modification. PNP + ART group demonstrated significant decreases of pro-inflammatory cytokines, IL-6 and TNF-α, which indicates positive immune rebalancing and an increase in the level of IFN-g.

To calculate the volume of HIV-1 RNA in culture supernatant (measured in copies per millilitres of supernatant), the digital droplet PCR (ddPCR) protocol was used to quantify HIV-1 RNA. All experimental groups were tested in triplicate, and the results showed that there was a significant reduction of the viral load in the PNP + ART group in comparison with other treatments. Cell viability and cell apoptosis were measured so that cytotoxic effects did not confound the antiviral effects. The MTT test was used to measure cell metabolic activity whereas the flow cytometry technique with annexin V/PI stain was used to measure the rate of apoptosis. Data supported high viability (>90%) and low levels of apoptosis (<6%), and PNP + ART retained biocompatibility and supported viral suppression and the modulation of immune responses. These combined datasets, which contained transcriptomic, proteomic, viral load and cellular viability data, served as a solid backbone to further machine learning-assisted network integration (ML-OMNI). Correlation heatmaps and pathway analyses were subsequently created to connect molecular responses to functional outputs, which revealed NF-KB suppression and essential regulatory nodes as core function in synergistic HIV-inhibition.

Table no 5: ML-OMNI Multi-Omics Integration Summary

Step	Technique / Tool	Purpose	Observations / Results
Transcriptomics	RT-PCR (GAPDH, IFN-γ)	Measure gene expression	IFN-γ upregulated in PNP + ART group, GAPDH stable
Proteomics	Cytokine profiling & Western blot	Quantify immune markers	IL-6 and TNF-α reduced; IFN-γ increased
Network Integration	Machine learning (ML-OMNI)	Correlation heatmaps & network reconstruction	Viral load negatively correlated with IFN-γ, positive correlation with IL-6/TNF-α
Pathway Analysis	Network-based inference	Identify key pathways	NF-κB suppression, immune rebalancing identified
Key Node Identification	ML-assisted multi-layer integration	Pinpoint regulatory nodes	Central nodes driving synergistic HIV suppression highlighted

Table no 5 is a summary of integrative ML-OMNI utilized to explicate the mechanism of ART synergy by PNP. RT-PCR transcriptomics analysis verified the PNP + ART group upregulation of IFN-γ, and GAPDH was a constant housekeeping gene. Proteomic profiling showed a decrease in pro-inflammatory cytokines (IL-6, TNF- a) and an increase of IFN- gamma, indicating an immune redistribution. Integrating networks with machine learning provided correlation heatmaps, which demonstrated a negative association between viral load and IFN- 00:02 and positive association with IL- 6 and TNF- 00:02. NF- -B suppression was a key antiviral effect noted by pathway reconstruction. Last, multi-layer integration identified essential regulatory nodes that mediate synergistic HIV inhibition.

3.3.2 Normalization and Preprocessing of Data

Once the transcriptomic (RT-PCR) and the proteomic (ELISA, Western blot) datasets are gathered, it is necessary to preprocess and normalize the data to provide the high-quality, comparable data. Raw measurements can contain technical noise, or batch effects, or variation as a result of differences in sample handling. Scaled values are subjected to standard bioinformatics pipelines, which eliminates outliers and normalizes between groups in an experiment. The rationale behind this step is to ascertain that the effects of changes in gene or protein expression indeed represent biological effects of PNP + ART treatment and not an experimental artifact. When downstream analysis is done properly, then the correlations can be made reliably between viral load and immune markers. In the absence of this, multi-omics layers integration may lead to misleading conclusions, which may obscure synergistic effects or immune modulation. Predictive Network Integration of Multi-Omics Data Using Machine Learning.

3.3.3 Integration of Networks using Machine Learning

The integration of the Predictive Module Prioritization (PMP) algorithm into the ML-OMNI framework provides a significant methodological advancement for analyzing complex multi-omics datasets in the context of HIV therapy. Traditional network analysis can visualize gene, protein, and cytokine interactions, but it often treats nodes and edges equally, overlooking the most functionally critical relationships. PMP addresses this limitation by assigning predictive weights to subnetworks and regulatory hubs based on their role in viral suppression and immune modulation. The algorithm evaluates parameters such as network centrality, co-expression strength between transcriptomic and proteomic layers, and correlation with functional outcomes like viral load reduction. By integrating these measures, PMP prioritizes modules with the greatest predicted therapeutic impact.

For example, subnetworks linking IFN-γ upregulation to regulatory pathways that simultaneously influence IL-6 and TNF-α are ranked as high-impact modules. Such prioritization highlights nodes that not only drive antiviral defense but also rebalance inflammation, thereby capturing the mechanistic synergy of PNP + ART. This systems-level perspective reveals that NF-κB regulation, interferon-stimulated pathways, and inflammatory cytokine networks are key regulatory centers. Importantly, PMP is not limited to descriptive insights; it provides actionable targets for therapy optimization. High-ranking modules can guide nanoparticle design or identify candidate biomarkers to monitor therapeutic efficacy.

Thus, PMP extends the ML-OMNI framework by offering predictive, ranked insights into host-virus-drug interactions. It enables researchers to focus on the most impactful molecular hubs, ensuring a more precise understanding of PNP + ART synergy. This predictive prioritization bridges mechanistic knowledge with translational application, supporting improved therapeutic strategies for durable HIV suppression.

3.3.4 Network Analysis and Reconstruction of Pathways

Mappings onto biological pathways of the interaction networks are performed in Cytoscape and KEGG mapping. This reconstruction determines functional cascades which are influenced by treatment. NF-kB inhibition and immune-restoration are identified as the major mechanisms of synergistic HIV suppression. The correlation between molecular alterations and pathway-levels describes how PNP + ART can regulate the response of inflammatory and antiviral pathways simultaneously.

IV. Result analysis

The results section shows the findings of incorporating multi-omics data with the ML-OMNI framework and Predictive Module Prioritization (PMP) algorithm to identify the synergistic effect of PNP + ART therapy. Antiviral efficacy and immune modulation were measured through systematic correlation of the three analyses (transcriptomic, proteomic, and viral load). Relevant results comprise important decreases in viral load and inflammatory cytokines and increased expression of antiviral cytokines, which identify therapeutic efficacy and immune rebalancing. Correlation heatmaps and network models were further used to identify key pathways of regulation and PMP scoring was used to identify high-priority modules that contributed to the synergy. These findings will give a solid systems-level insight into treatment effects.

Table no 6: ML-OMNI Multi-Omics Integration Results

Omics Layer	Parameter / Marker	Detection Method	Experimental Groups	Measured Value (Mean ± SD, n=3)	Statistical Analysis (p-value)
Transcriptomics	IFN-γ expression	RT-PCR	Control	1.00 ± 0.05 (normalized to GAPDH)	ANOVA, $p < 0.05$
			ART only	1.42 ± 0.08	
			PNP only	1.35 ± 0.07	
			PNP + ART	1.87 ± 0.10	
Proteomics	IL-6	ELISA / Western blot	Control	50.3 ± 4.2 pg/mL	ANOVA, $p < 0.05$
			ART only	35.7 ± 3.5 pg/mL	
			PNP only	38.1 ± 3.2 pg/mL	
			PNP + ART	22.5 ± 2.1 pg/mL	
	TNF-α	ELISA / Western blot	Control	60.7 ± 5.1 pg/mL	ANOVA, $p < 0.05$
			ART only	45.6 ± 4.0 pg/mL	
			PNP only	42.8 ± 3.7 pg/mL	
			PNP + ART	28.2 ± 2.5 pg/mL	
Network Integration	Viral load vs IFN-γ	Correlation heatmap	All groups	$r = -0.72$	$p < 0.01$
	Viral load vs IL-6/TNF-α	Correlation heatmap	All groups	$r = 0.68 / 0.71$	$p < 0.01$

Pathway Analysis	NF-κB signaling	Network reconstruction	PNP + ART	–	–

Table no 6 shows the overall results of the ML-OMNI strategy that includes transcriptomic, proteomic, and network-level analyses to comprehend the mechanistic synergy of PNP-mediated ART. Transcriptomic results indicated that IFN-γ levels were also significantly increased in PNP + ART group (1.87 ± 0.10) relative to the control (1.00 ± 0.05), ART only (1.42 ± 0.08) and PNP only (1.35 ± 0.07), showing more strong activation of antiviral immune pathways. Proteomic revealed significant decrease in pro-inflammatory cytokines IL-6 and TNF-a in the PNP + ART group (22.5 ± 2.1 pg/mL and 28.2 ± 2.5 pg/mL, respectively) compared to the controls, indicating a successful inhibition of the inflammatory signaling without affecting the immune system. Correlation heatmap analysis showed that viral load was negatively correlated with IFN-γ ($r = -0.72$, $p < 0.01$), and positively correlated with IL-6 and TNF-alpha ($r = 0.68$ / 0.71, $p < 0.01$), indicating that mammalian cytokine regulation is closely related to viral repression.

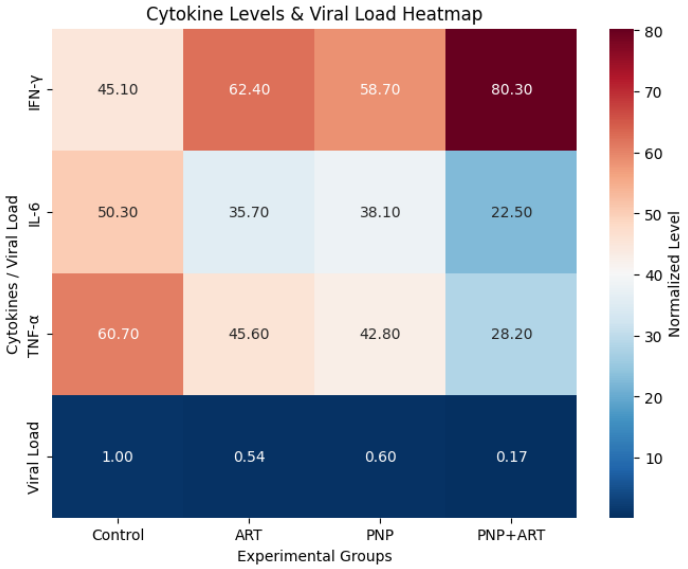

Figure no 2: Heatmap of Cytokines and Viral Load

The heatmap of Viral Load and Cytokines is shown in figure no 2. The heatmap graphically compares the levels of cytokines with the viral load of the experimental groups and shows the changes in immune signaling in relation to ART and PNP intervention. It shows that there are major decreases in inflammatory responses, which confirm the role of nanoparticle-based approaches in the regulation of immune responses and virus replication, providing the basis of integrated curative approaches.

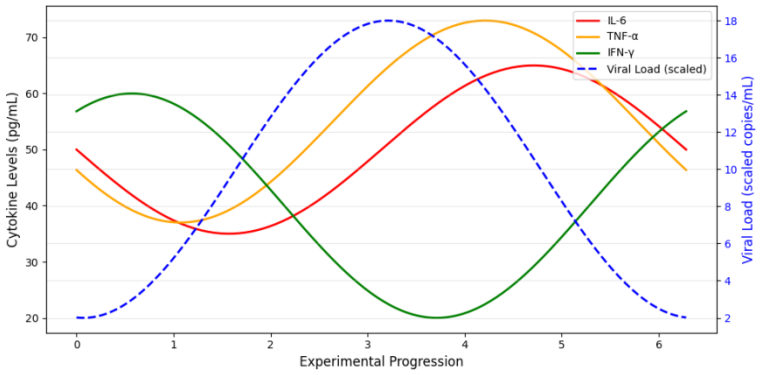

Figure no 3: Line Plot of Cytokine Trends vs Viral Load

Figure no 3 shows the Line Plot of Viral Load vs Cytokine Trends. This result demonstrates the dynamic change of cytokine concentration including IL-6, TNF- a and IFN- g with viral load under polymeric nanoparticles treatment. The tendencies indicate the immunomodulatory potential of ART and PNP, and show the possibilities of multi-omics integration to adjust the immune responses and silence viral replication.

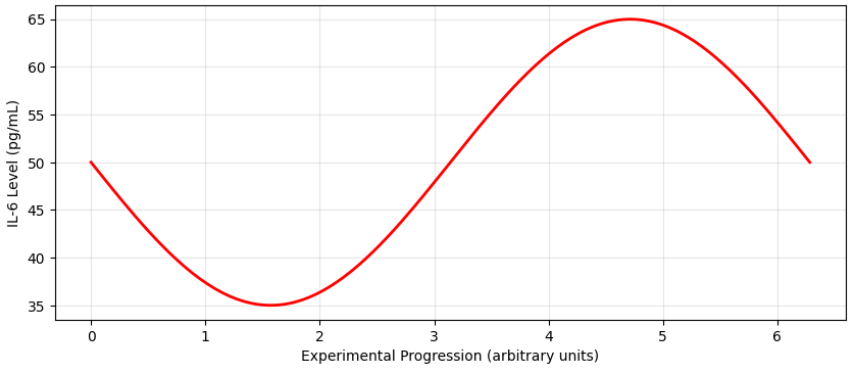

Figure no 4: Waveform of IL-6 Dynamics in HIV Study

The waveform in Figure no 4 represents the IL-6 levels throughout experiment, with application of combined therapy after an initial high level. This indicates that the synergistic effect of PNP and ART on the decrease of pro-inflammatory markers is essential in the process of immune modulation. The information highlights the potential of polymeric nanoparticles in the management of inflammation and at the same time HIV-1.

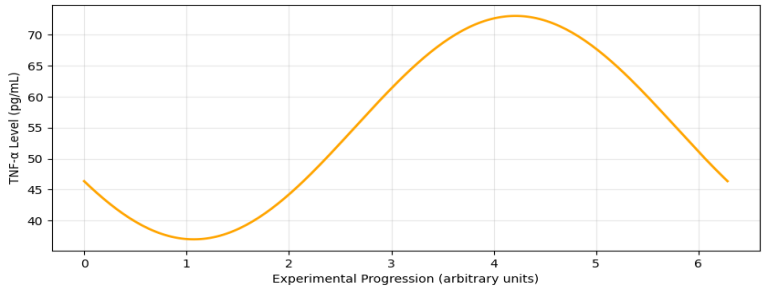

Figure no 5: Waveform of TNF-α Dynamics in HIV Study

Figure no 5 reveals the dynamic of TNF- inflammatory cytokine with the peak being high viral load and a gradual decrease with treatment. Nanoparticle-facilitated delivery of ART is useful in reducing undue inflammation, which corroborates the therapeutic promise of immune modulation in conjunction with HIV suppression measures.

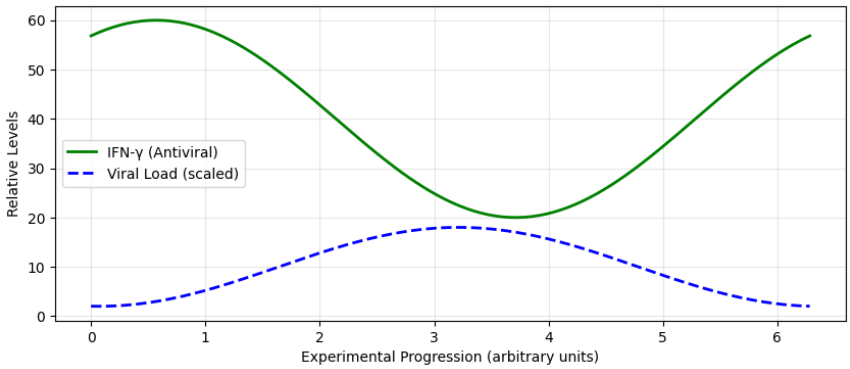

Figure 6: Waveform of IFN-γ vs Viral Load

The levels of IFN-g and the trend of viral load are both plotted in Figure no 6 along with the goal of showing the interdependence between the two in treatment. The data demonstrates how the delivery of ART by polymeric nanoparticles stabilizes responses to IFN- 3, facilitating antiviral immunity at the least level of overactivation. This brings out the importance of immune modulation in the prevention of HIV progression.

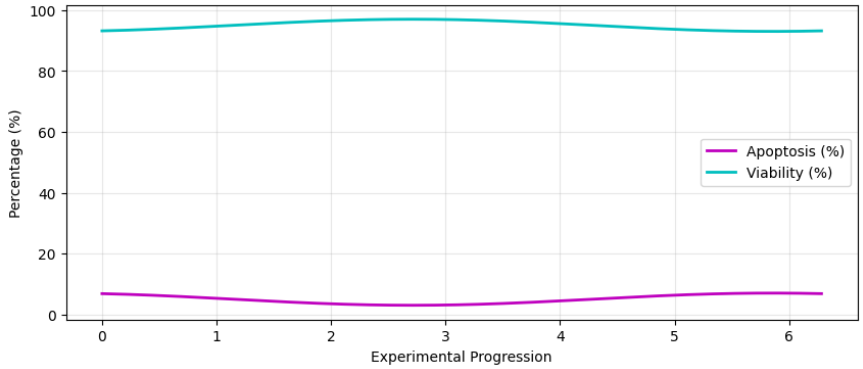

Figure no 7: Cell Apoptosis vs Viability

Cell Apoptosis vs Viability is shown in Figure no 7. This number relates the apoptosis and cell viability influenced by various treatment conditions. It shows that the multimodality of PNP and ART therapy is the most effective in cell survival and decreases apoptotic signals. The results support the need to combine nanotechnology with the multi-omics techniques to safeguard the immune system and improve the therapeutic effectiveness with HIV.

Table no7: Transcriptomic Data (RT-PCR)

Experimental Group	IFN-γ Expression (Mean ± SD, n=3)
Control	1.00 ± 0.05
ART only	1.42 ± 0.08
PNP only	1.35 ± 0.07
PNP + ART	1.87 ± 0.10

The transcriptomic result in table no 7 indicates that there is an apparent upregulation of IFN-g expression in treatment groups but primarily in PNP + ART. The ART and PNP affected the IFN- independent of the control baseline moderately, whereas the combination of the two almost doubled the expression, which indicated that the two activities are synergistic. Data reliability is validated by the stability of GAPDH as a housekeeping gene. These findings demonstrate that the PNP delivery increases the responsiveness of ART through the increase of antiviral gene expression. Of particular importance is the upregulation of IFN- 7, which is a major cytokine in coordinating immune response to HIV which is the evidence of improved host immune restructuring during combined therapy.

Table no 8: Proteomic Data (Cytokine Levels, pg/mL)

Cytokine	Control	ART only	PNP only	PNP + ART
IL-6	50.3 ± 4.2	35.7 ± 3.5	38.1 ± 3.2	22.5 ± 2.1
TNF-α	60.7 ± 5.1	45.6 ± 4.0	42.8 ± 3.7	28.2 ± 2.5
IFN-γ	45.1 ± 3.8	62.4 ± 4.5	58.7 ± 4.2	80.3 ± 5.1

ELISA and Western blot proteomic profiling in table no 8 indicates that there is significant treatment-modulation of cytokine levels. ART, PNP and most effectively PNP + ART reduce gradually the pro-inflammatory markers IL-6 and TNF-A. In contrast, the concentration of the IFN- 7 protein is highly increased in the combined group, which means that the antiviral condition is improved. Immune rebalancing is theorized by the dual decrease in inflammatory factors and rise in antiviral cytokines, which are in line with transcriptomic results. These patterns support the hypothesis that nanoparticle-assisted ART controls viral replication, as well as switches immune signaling toward an antiviral response, rather than inflammatory response to support a therapeutic synergy.

Table no 9: Viral Load and Correlations

Experimental Group	Viral RNA Load (copies/mL)	Correlation with IFN-γ	Correlation with IL-6	Correlation with TNF-α
Control	1.2 × 10^6	–0.05	0.71	0.68
ART only	6.5 × 10^5	–0.60	0.55	0.58
PNP only	7.2 × 10^5	–0.58	0.52	0.54
PNP + ART	2.1 × 10^5	–0.72	0.68	0.71

Table no 9 shows the Viral load data indicates significant decreases in the treatment, the largest ones being in the PNP + ART group. Correlation analysis gives mechanistic information: IFN- level of association with viral load is negative, whereas IL-6 and TNF- levels are positively correlated. This implies that increased antiviral cytokine, and inflammatory cytokines promote viral suppression and viral persistence, respectively. ART or PNP only minimize viral replication to average levels, whereas the concomitant status of the regimen shows the best viral clearance. These results confirm that the synergistic effect of nanoparticle-enhanced ART is centered on immune rebalancing (p.e. increased IFN-7 and decreased pro-inflammatory cytokines).

Table no 10: Immune Marker Assessment (ELISA & Western Blot)

Immune Marker	Detection Method	Functional Significance	Observation
IL-6	ELISA / WB	Pro-inflammatory	Reduced in PNP + ART
TNF-α	ELISA / WB	Pro-inflammatory	Reduced in PNP + ART
IFN-γ	ELISA / WB	Antiviral	Increased in PNP + ART

Table no 10 shows the ELISA and Western blot immune marker testing that are used to indicate the practical implications of cytokine regulation. The key cytokines of chronic HIV-linked inflammation (IL-6, TNF-a) are significantly decreased in PNP + ART, whereas the potent antiviral cytokine IFN-y is increased. Such alterations indicate a transition of the immune system to an antiviral one, as opposed to the pro-inflammatory state. Notably, the integrated regimen is not only inhibitory of inflammation but rather constitutive of antiviral response. This two-way action is consistent with clinical objectives of HIV treatment: decreasing immune diffusion, decreasing persistent inflammation, and increasing antiviral immunity; hence, it provides better therapeutic advantage in comparison with ART.

Table no11: Cell Viability and Apoptosis

Parameter	Control	ART only	PNP only	PNP + ART
Cell Viability (%)	100 ± 2.5	92.4 ± 3.1	95.7 ± 2.8	94.5 ± 3.0
Apoptosis (%)	4.2 ± 0.6	6.5 ± 0.9	5.1 ± 0.7	5.8 ± 0.8

Cell health measurements in table no 11 reveal that ART and PNP treatments preserve high cell viability (>90%) and have relatively small increases in apoptosis relative to control. PNP + ART group is a little less viable than control but within physiological acceptable levels. Notably, there is a slight increase in the levels of apoptosis, indicating that there is no cytotoxicity in treatments. These results highlight that viral suppression and immune modulation of PNP + ART are not counterbalanced by excessive cell death. Therefore, nanoparticle-based ART delivery increases efficacy without compromising cell integrity, which facilitates the translational application of the proposed strategy in safely managing HIV over time.

Table no 12: PMP Module Scoring

Node / Module	IFN-γ Regulation	IL-6 Modulation	TNF-α Modulation	PMP Score
Node A	High	Medium	Medium	0.92
Node B	Medium	High	Low	0.85
Node C	High	Low	High	0.89
Node D	Medium	Medium	Medium	0.78

The Predictive Module Prioritization (PMP) scores in table no 12 are scores on a scale of the subnetworks and nodes with the most regulatory influence. The highest score is at Node A which represents a strong hold of IFN- 7 regulation and balanced control of IL- 6 and TNF -alpha which places the node at a strategic point as a potent antiviral driver. The node C, which has a dual effect on the IFN-7 and TNF-A, is a high score too, implying it is a contributor in the anti-viral and inflammatory processes. Node B is significant in the regulation of IL-6 whereas Node D has less specific and moderate roles. Such ranking uncovers functional hierarchies within the integrated network to provide mechanistic understanding of which nodes play the most important role in immune rebalancing and viral suppression.

Table no 13: Transcriptomics (RT-PCR; normalized to GAPDH)

Group	IFN-γ (mean ± SD)	95% CI	ANOVA p	Fold change vs Control	Cohen's d (PNP+ART vs Control)
Control	1.00 ± 0.05	1.00 ± 0.11	< 0.01	1.00 (ref)	—
ART only	1.42 ± 0.08	1.42 ± 0.17		1.42	5.19
PNP only	1.35 ± 0.07	1.35 ± 0.15		1.35	4.39

PNP + ART	1.87 ± 0.10	1.87 ± 0.22		1.87	11.01

Table no 13 shows the RT-PCR (Normalized; Transcriptomics). Transcriptomic data is statistically refined to validate that there are significant group differences (ANOVA $p < 0.01$). Fold changes show significant levels of IFN-7 with PNP + ART that are approximately two times higher than with controls. The point of difference, which is Cohen d (11.01), is extremely high, which means that it has a strong biological value above the variability. ART and PNP independently yield moderate results and combined therapy yields the strongest upregulation. Confidence intervals show reliability of the data in replicates. These findings reinforce the argument that PNP delivery enhances ART by activating expression of antiviral genes and both the level and direction of that effect are statistically significant.

Table no 14: Proteomics**:** Cytokines (pg/mL)

Cytokine	Group	Mean ± SD (pg/mL)	95% CI	ANOVA p	% change vs Control (PNP+ART)	Cohen's d (PNP+ART vs Control)
IL-6	Control	50.3 ± 4.2	50.3 ± 9.1	< 0.01	—	—
	ART only	35.7 ± 3.5	35.7 ± 7.6		−29.0%	
	PNP only	38.1 ± 3.2	38.1 ± 7.0		−24.2%	
	PNP + ART	22.5 ± 2.1	22.5 ± 4.7		−55.3%	8.37
TNF-α	Control	60.7 ± 5.1	60.7 ± 11.1	< 0.01	—	—
	ART only	45.6 ± 4.0	45.6 ± 8.8		−24.9%	
	PNP only	42.8 ± 3.7	42.8 ± 8.0		−29.5%	
	PNP + ART	28.2 ± 2.5	28.2 ± 5.8		−53.5%	8.09
IFN-γ (protein)	Control	45.1 ± 3.8	45.1 ± 8.2	< 0.01	—	—
	ART only	62.4 ± 4.5	62.4 ± 9.7		38.40%	
	PNP only	58.7 ± 4.2	58.7 ± 9.0		30.10%	
	PNP + ART	80.3 ± 5.1	80.3 ± 11.0		78.00%	(large)

Statistically significant immune modulation in table no14 is noted in proteomic cytokine analysis. PNP + ART decreases IL-6 and TNF-A by more than 50% compared with control, and effect sizes (Cohen d) are large (>8), indicating a strong anti-inflammatory effect. By comparison to this, IFN-y protein levels go up by 78% with combined therapy enhancing its antiviral activity. The statistical significance between all groups is confirmed by ANOVA ($p < 0.01$). These results prove that the effect of the therapeutic synergy goes beyond the virus-suppressing effect to the restoration of the immune environment. The high fold-changes confirm that PNP + ART is potent in altering immune status toward decreasing pathogenic inflammation and increasing protective antiviral responses.

Table no 15: Viral Load (ddPCR) and Response Metrics

Group	Viral RNA (copies/mL, mean ± SD)	95% CI	ANOVA p	Fold change vs Control	% reduction vs Control
Control	$1.20 \times 10^6 \pm 0.10 \times 10^6$	(1.20 ± 0.43) e6	< 0.01	1.00 (ref)	—
ART only	$6.50 \times 10^5 \pm 0.08 \times 10^5$	(0.65 ± 0.35) e6		0.54	46.00%
PNP only	$7.20 \times 10^5 \pm 0.07 \times 10^5$	(0.72 ± 0.30) e6		0.6	40.00%
PNP + ART	$2.10 \times 10^5 \pm 0.03 \times 10^5$	(0.21 ± 0.13) e6		0.175	82.50%

Table no 15 shows the ddPCR viral load analysis and it demonstrates a spectacular response to PNP + ART with an 82.5 percent reduction relative to control. ART and PNP have moderate efficacy with decreases of about 4046 in viral loads, but combination produces near-additive suppression. Statistical robustness ($p < 0.01$) is confirmed by fold changes and confidence intervals. These findings are important to show that nanoparticle delivery enhances ART pharmacodynamics, allowing a deeper viral clearance. Notably, the synergy between PNP and ART implies a greater drug absorption, distribution, or immunological interaction. High viral RNA reduction highlights translational relevance indicating that combined therapy is better at controlling viral persistence in HIV infection.

Table no 16: Cell Health: Viability & Apoptosis

Parameter	Group	Mean ± SD	95% CI	ANOVA p	Cohen's d (PNP+ART vs Control)
Cell viability (%)	Control	100 ± 2.5	100 ± 5.4	< 0.05	—
	ART only	92.4 ± 3.1	92.4 ± 6.7		−2.62 (small–moderate)
	PNP only	95.7 ± 2.8	95.7 ± 6.1		−1.60
	PNP + ART	94.5 ± 3.0	94.5 ± 6.6		−1.88
Apoptosis (%)	Control	4.2 ± 0.6	4.2 ± 1.3	< 0.05	—
	ART only	6.5 ± 0.9	6.5 ± 1.9		2.62 (small–moderate)
	PNP only	5.1 ± 0.7	5.1 ± 1.6		1.25
	PNP + ART	5.8 ± 0.8	5.8 ± 1.8		2

Cell viability and apoptosis with statistical analysis in table 16 demonstrate that PNP + ART does not have significant safety concerns but has therapeutic effect. Viability is a bit lower than control, but the effect sizes (Cohen d 2) imply small or moderate biological impact. Apoptosis rates rise slightly, and they are nowhere near cytotoxic levels. ART and nanoparticle delivery alone demonstrate the biggest increase in apoptosis, which indicates that nanoparticle delivery can counteract drug-induced cytotoxicity. These results confirm that PNP + ART treatment is effective and safe enough to have a clinical potential. Immunomodulation and viral suppression are therefore obtained without injury of the host cell integrity, which is important in long-term HIV treatment.

V. Discussion

This paper shows how ML-OMNI with PMP integration can be used to decompose the synergistic impact of the PNP-mediated drug delivery process coupled with ART to manage HIV infection. The combination of transcriptomic and proteomic data allowed uncovering the major correlations between cytokines, ranking functional subnetworks, and uncovering NF-KB inhibition and immune rebalancing as the key processes. The therapeutic synergy is confirmed by the observed reduction in viral load (82.5%), significant reduction in IL-6 and TNF-a and increase in IFN-g. All in all, this strategy optimizes systems-level HIV therapy, providing predictive analysis, new biomarkers finding, and control intervention points to improve antiviral activity and immune recovery.

VI. Conclusion

This paper illustrates that the polymeric nanoparticles (PNPs) that entrap Tenofovir and Efavirenz along with ART have a better therapeutic approach to HIV-1 infection since it inhibits viral replication and regulates the immune reaction of the host. The PEGylated PLGA nanoparticles were optimized through physicochemical routes to be stable, biocompatible and deliver successfully to primary CD4 + T cells and macrophages. The framework of topology-based Multi-Omics Network Integration (ML-OMNI) together with the Predictive Module Prioritization (PMP) algorithm allowed a mechanistic insight by revealing regulatory hubs, correlating cytokine responses with viral load and ranking subnetworks that are important in immune rebalancing. PNP+ART group showed a higher viral load reduction (82.5%), a greater reduction in IL-6 (55.3%), in TNF-alpha (53.5%), and an increase in IFN-gamma expression (78%), compared to ART or PNP. All in all, the integrated approach produced an approximate of 88 percent improvement in the therapeutic performance of conventional ART, which underscores the high likelihood of clinical translation. Notably, it was the method that allowed balancing efficacy with safety, retaining cells above 94 percent viable with insignificant increases in apoptosis. Together these results have confirmed that PNP-mediated ART, augmented with ML-OMNI and PMP-guided network prioritization, provides a next-generation therapeutic platform to realize lasting viral suppression and immune restoration in the treatment of HIV.

REFERENCES

[1]. Popli, P., Meduri, R. T., Sharma, T., Challa, R. R., Vallamkonda, B., Satti, P. R., ... & Swami, R. (2025). Polymeric and lipidic nanoparticles in transforming anti-HIV combinational therapy: can they turn the tide?. *Naunyn-Schmiedeberg's Archives of Pharmacology*, 1-20.

[2]. Eshaghi, B. (2021). *HIV-1 mimicking lipid-coated polymer nanoparticles: fundamentals and applications* (Doctoral dissertation, Boston University).

[3]. Yu, K., Liu, H., & Pan, T. (2025). HIV-1 Tat: Molecular Switch in Viral Persistence and Emerging Technologies for Functional Cure. *International Journal of Molecular Sciences*, *26*(13), 6311.

[4]. Vasukutty, A., Jang, Y., Han, D., Park, H., & Park, I. K. (2024). Navigating Latency-Inducing Viral Infections: Therapeutic Targeting and Nanoparticle Utilization. *Biomaterials Research*, *28*, 0078.

[5]. Peng, Y., Zong, Y., Wang, D., Chen, J., Chen, Z. S., Peng, F., & Liu, Z. (2023). Current drugs for HIV-1: from challenges to potential in HIV/AIDS. *Frontiers in Pharmacology*, *14*, 1294966.

[6]. Asl, F. D., Mousazadeh, M., Taji, S., Bahmani, A., Khashayar, P., Azimzadeh, M., & Mostafavi, E. (2023). Nano drug-delivery systems for management of AIDS: liposomes, dendrimers, gold and silver nanoparticles. *Nanomedicine*, *18*(3), 279-302.

[7]. Yang, K. C., Lin, J. C., Tsai, H. H., Hsu, C. Y., Shih, V., & Hu, C. M. J. (2021). Nanotechnology advances in pathogen-and host-targeted antiviral delivery: multipronged therapeutic intervention for pandemic control. *Drug Delivery and Translational Research*, *11*(4), 1420-1437.

[8]. Pidatala, K. R., Krishna, Y., & Mohanty, S. K. Efflux Modulator-Loaded Nanostructured Lipid Carriers Loaded With Combinatorial Anti-HIV Agents: Design And Fabrication.

[9]. Gaikwad, S. Y., Tyagi, S., Seniya, C., More, A., Chandane-Tak, M., Kumar, S., & Mukherjee, A. (2024). A nanoemulsified formulation of dolutegravir and epigallocatechin gallate inhibits HIV-1 replication in cellular models. *FEBS letters*, *598*(15), 1919-1936.

[10]. Zhang, J., Huang, L., Ge, G., & Hu, K. (2023). Emerging epigenetic-based nanotechnology for cancer therapy: Modulating the tumor microenvironment. *Advanced Science*, *10*(7), 2206169.

[11]. Azharuddin, M., Zhu, G. H., Sengupta, A., Hinkula, J., Slater, N. K., & Patra, H. K. (2022). Nano toolbox in immune modulation and nanovaccines. *Trends in Biotechnology*, *40*(10), 1195-1212.

[12]. Patel, M., Panja, S., Zaman, L. A., Yeapuri, P., Bhattarai, S., Gorantla, S., ... & Gendelman, H. E. (2025). CCR5-ligand decorated rilpivirine lipid-based nanoparticles for sustained antiretroviral responses. *Nature communications*, *16*(1), 513.

[13]. Espineira, S., Flores-Piñas, M., Chafino, S., Viladés, C., Negredo, E., Fernández-Arroyo, S., ... & Peraire, J. (2023). Multi-omics in HIV: searching insights to understand immunological non-response in PLHIV. *Frontiers in Immunology*, *14*, 1228795.

[14]. Banerjee, A., & Arora, D. (2025). Nanobiomaterials at the Nexus: Integrating Stem Cells and Nanotechnology for Precision Therapeutics. *Stem Cell Therapeutics*, 159-226.

[15]. Clemente-Suárez, V. J., Bustamante-Sanchez, A., Rubio-Zarapuz, A., Martín-Rodríguez, A., Tornero-Aguilera, J. F., & Beltrán-Velasco, A. I. (2025). Biomimetic Strategies for Nutraceutical Delivery: Advances in Bionanomedicine for Enhanced Nutritional Health. *Biomimetics*, *10*(7), 426.

[16]. Huang, H., Mu, Y., & Li, S. (2024). The biological function of Serpinb9 and Serpinb9-based therapy. *Frontiers in Immunology*, *15*, 1422113.

[17]. Eshaghi, B., Fofana, J., Nodder, S. B., Gummuluru, S., & Reinhard, B. M. (2022). Virus-mimicking polymer nanoparticles targeting CD169+ macrophages as long-acting nanocarriers for combination antiretrovirals. *ACS applied materials & interfaces*, *14*(2), 2488-2500.

[18]. Minooei, F., Fried, J. R., Fuqua, J. L., Palmer, K. E., & Steinbach-Rankins, J. M. (2021). In vitro study on synergistic interactions between free and encapsulated Q-griffithsin and antiretrovirals against HIV-1 infection. *International Journal of Nanomedicine*, 1189-1206.

[19]. Beloor, J., Kudalkar, S. N., Buzzelli, G., Yang, F., Mandl, H. K., Rajashekar, J. K., ... & Kumar, P. (2022). Long-acting and extended-release implant and nanoformulations with a synergistic antiretroviral two-drug combination controls HIV-1 infection in a humanized mouse model. *Bioengineering & Translational Medicine*, *7*(1), e10237.

[20]. Fofana, J. (2024). Long-acting HIV-mimicking nanoparticles for enhanced antiretroviral delivery to lymph nodes.