EXPLORE THE DISSOCUBE TECHNOLOGY FOR INHIBIT ANTI HIV-I TARGET TO CD4 – T CELL BY NANOSUSPENSION

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ABSTRACT

The human immune deficiency virus (HIV) is the causative agent of acquired immune deficiency syndrome (AIDS). According to Global HIV statistics, about 33.9 million- 43.8 million people were living with HIV in 2021. The development of effective antiretroviral drugs and the scientific accomplishments in HIV research over the past twenty-six years have been challenging. The pathogenesis of HIV disease isthe basis of a decrease in the number of CD4-T cells. These 'helper T cells are responsible for severalroles for other immune residents, and their loss leads to profound immunosuppression. The close relationship between the HIV-1 life cycle and the activation state of cells supporting viral replication results in a dynamic interaction between co-infections and HIV-1 replication in infected people. Nanosuspension is a convenient tool for the drug delivery system. Most recently discovered drugs are insoluble or poorly absorbed due to their flexible characteristics and various advantages. Dissocube is a technology for homogenization and preparation for a new form of particle size it's having, high pressure for producing new insert texture cavitation of desired particle size to form a nanosuspension. Nanotechnology suggests a unique opportunity to unite and enhance different pharmacological profiles of antiretroviral medication with more convenient drug administration and potentially better patient compliance with HIV therapy. The development of nanotechnology has introduced a new opportunity to improve the therapeutic efficiency of the dosage form. Nanomaterial's can deliver the drug to the targeted area and have fewer side effects. A Nano range of less than 100 nm can easily penetrate the cell, and sensitivity to various external and internal stimuli and immune activation is a desirable component.

Key words: Nanosuspension, AIDS, HIV, CD4- cells, Antiretroviral, NDDS

Abbreviations:

HIV- Human immune deficiency virus

AIDS - Acquired immune deficiency syndrome

TEM- Transmission Electron Microscopy

INTRODUCTION

The HIV/AIDS pandemic is an increasing global burden with damaging health-related and socioeconomic Effects. The human immune deficiency virus is a Lentivirus that causes the fatal viral illness AIDS (100- 500 nm). According to the Statistical survey of HIV, 38.4 million people globally were living with HIV in 2021, and 1.5 million people became newly infected with HIV.HIV spreads through sexual contact, organ transplants, blood transfusion, and other bodily fluids exchanges, also transmission from mother to children, especially sexual transmission. HIV-1 reaches across the world, and HIV-2 is more prominent in Africa.[1] An essential indicator of the degree of immunosuppression is the CD4+ T cell count, an important determinant of immune status and treatment outcome in HIV- infected individuals. [2] Dissocube is a technology for. homogenization, preparation for the new form of particle size. Nanosuspension have appeared as a promising drug delivery strategy for hydrophobic drugs via the parenteral route.[3,4] Nanosuspension is a very finely dispersed solid drug particle in an aqueous vehicle in which the diameter of the suspended particle is less than 1 micrometer in size and stabilized by a surfactant.[5] More than 40% of drugs are poorly soluble in water, making it difficult to prepare them in a conventional dosage form challenge is even more problematic for class medicines, which are less soluble in both aqueous and organic conditions.[6] Nanosuspension have emerged as a promising parenteral drug delivery strategy for hydrophobic drugs.[7] As a result, a decrease in particle size leads to an increase in the dissolution rate.[8] Pharmaceutical nanosuspension is an aqueous dispersion of insoluble drug particles that are Nano sized and stabilized using surfactants. [9] The reduction in the dosing frequency increases patient compliance and can be possible through nanosuspension due to its various advantages. [10]The HIV pandemic remains one of the most life and death challenges to global health and continues to be one of the leading causes of death and disability in the world for decades to come. [11]

ROLE OF NANOSUSPENSION IN HIV-1

The crucial effect of an infection by HIV is the loss of CD4+ T cells. These ‘helper T cells are responsible for several roles for other immune populations, and their loss leads to profound immunosuppression, manifested by the presence of dysfunctional B-cells, natural killer cells, and macrophages in chronically HIV-infected patients.[12] Nanotechnology suggests a unique opportunity to unite and enhance different pharmacological profiles of antiretroviral drugs with more convenient drug administration and potentially better patient adherence to HIV therapy.[13] Nanoscale devices less than 50 nanometers in diameter can easily invade most cells.[14] The particular feature of nanoscale delivery systems appears to hold the most certainty for their use in the clinical treatment and deterrence of HIV, Especially targeted delivery of antiretroviral drugs to CD4+ T cells and macrophages. By regulating the release profiles of the delivery methods, drugs are liberated over a long time and at higher effective doses to the exact targets. Nanotechnology-based targeted delivery of antiretroviral drugs to CD4+ T cells and macrophages, also delivery to lymphocytes, could assure that drugs reach a latent reservoir. [15, 16, 17]

STRUCTURE OF HIV

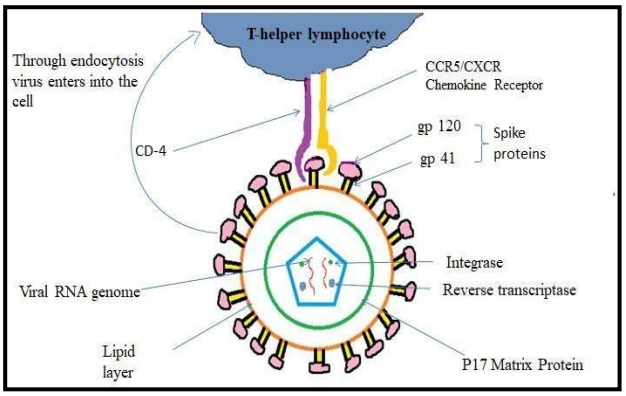


Figure 1: Structure of HIV

LIFE CYCLE OF HIV

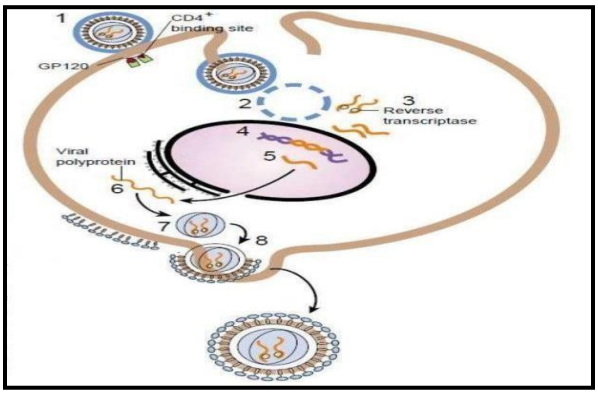
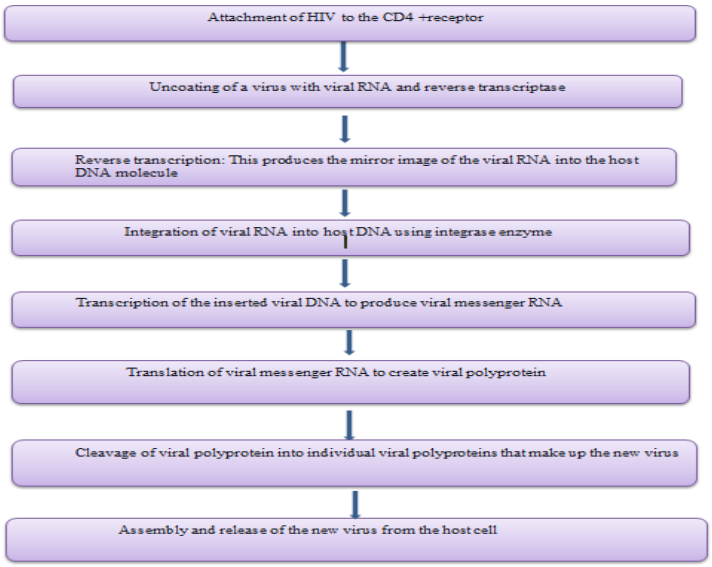


Figure 2: Life cycle of HIV

MECHANISM:


Attachment of HIV to the CD4 +receptor
Uncoating of a virus with viral RNA and reverse transcriptase
Reverse transcription: This produces the mirror image of the viral RNA into the host DNA molecule
Integration of viral RNA into host DNA using integrase enzyme
Transcription of the inserted viral DNA to produce viral messenger RNA
Translation of viral messenger RNA to create viral polyprotein
Cleavage of viral polyprotein into individual viral polyproteins that make up the new virus
Assembly and release of the new virus from the host cell

PATHOGENESIS:

The development of HIV was characterized by vulnerability to various infections early in the AIDS epidemic. It was erroneously supposed that HIV had an extended latent phase without viral replication and that its eventual reactivation to viral repetition triggered disease progression. However, sensitive viral culture and nucleic acid detection methods showed that almost all untreated patients experience continual plasma viremia. Milestone studies established that plasma. HIV-1 RNA concentration indicates the time for progression to AIDS and death that CD4 cell counts are independently predictive. Such findings have focused research on ways to achieve durable control of HIV replication. The highly effective antiretroviral agents made it possible to probe viral pathogenesis. Administering such agents disrupt the steady-state balance between virion production and clearance. Studies of treatment-naïve patients demonstrated that plasma HIV-1RNA concentrations decline by 10- to 100-fold within one week of initiating treatment with potent inhibitors of either HIV protease or reverse transcriptase. Mathematical modeling of such data shows that HIV infection is exceedingly dynamic, with daily production of an estimated 10^9virions. Approximately 99% of plasma HIV arises from recently infected CD4+ lymphocytes, which have an average of 2.2 days. The second source of the virus (presumable macrophages) decays with a life span of 2 weeks. It would be, predicted that complete inhibition of HIV replication for 2 to 3 years might allow all infected cells to be, eradicated if these were the only reservoirs for HIV. Age life span, unfortunately, there is an additional long-lived pool of resting CD4+ lymphocytes cells that harbor replication-competent HIV. Although there are relatively few such cells in the body, their average life span will be months. Approximately 99% of plasma HIV arises from recently infected CD4+ lymphocytes, which have an average of 2.2 days. The second source of the virus (presumable macrophages) decays with a life span of 2 weeks. [18]

HIV CHALLENGES

The challenges that can be associated with this chronic disease consist of requiring patient compliance for lifetime treatment, which can be challenging to adhere to lack of treatment adherence can increase the possibility of treatment failure and increase the likelihood of inventing resistant strains of the virus. Another constraint includes poor aqueous drug solubility, as this can impact the availability of the drug within the body and result in ineffective treatment of HIV. The advancement in nanotechnology and nanomedicine has provided a bright future for HIV/AIDS therapeutics. [19]

PRELIMINARY TEST FOR HIV-1[20]

- Preliminary test was performed in laboratory with sample HIV test as follows:-

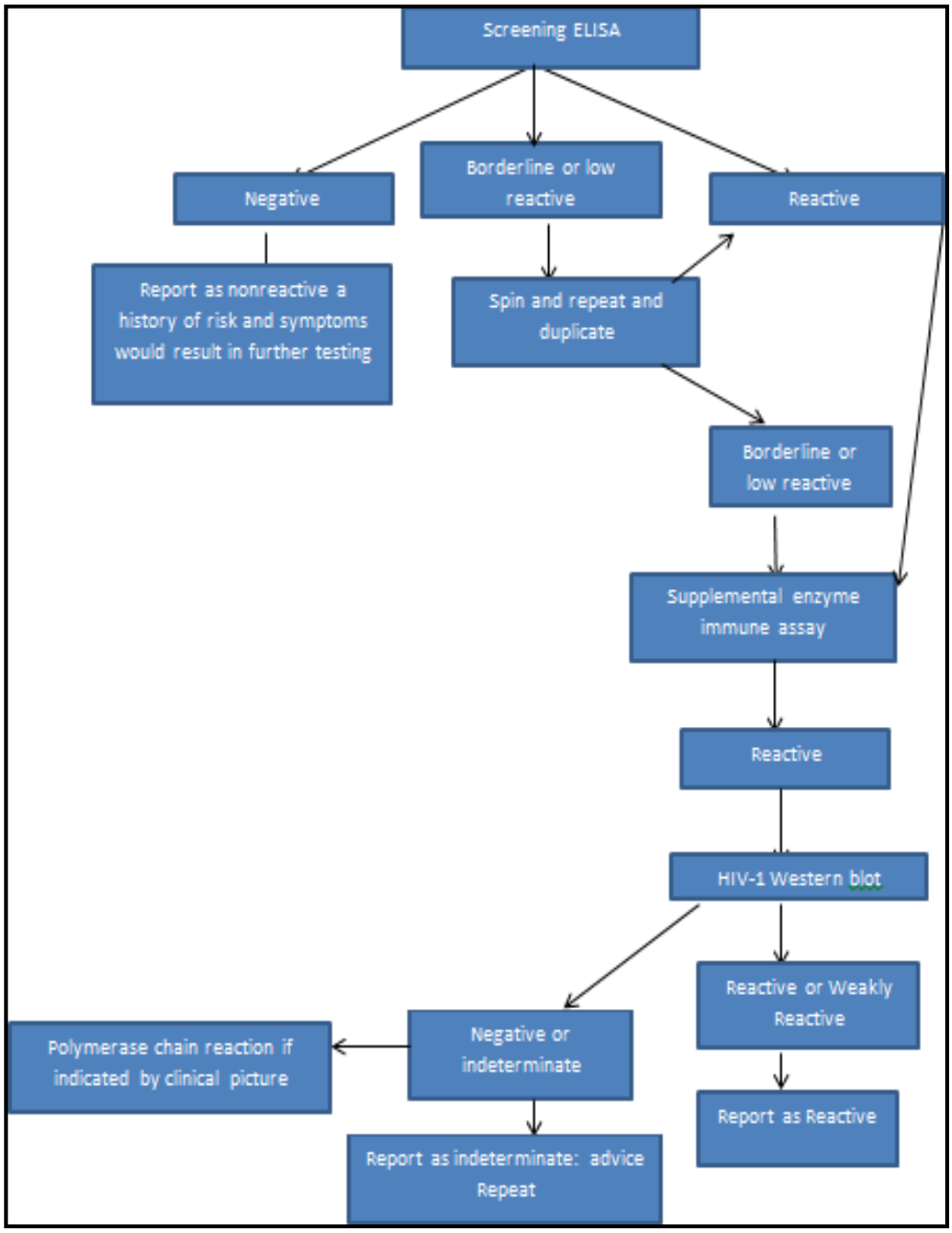


Figure 3: Sample HIV laboratory testing algorithm and flow chart

MECHANISM OF ACTION OF DRUG

Dolutegravir inhibits HIV integrase by binding to the HIV integrase active site and blocking the strand transfer step of DNA integration. It is necessary for the HIV replication cycle. Dolutegravir demonstrates activity at Nano-molar levels to HIV-1 of various strains and subtypes. If class resistance is absent, then we can minimize the interaction by adjusting dose. [19, 21, 22]

EXCIPIENTS FOR NANOSUSPENSION

1. Stabilizer- Wet the drug particle thoroughly; to prevent Ostwald ripening and the cluster of nanosuspension, provide steric or ionic barriers.eg.Lecithins,polaxomer188,Polysorbate.
2. 2.Surfactants- surfactants are incorporated to improve the dispersion by reducing the interfacialtension and also act as wetting and deflocculation agents .eg.Tween 80.
3. Cosufactants- The choice of a co-surfactant is critical when using micro-emulsions to develop nanosuspensions. Cosurfactants significantly influence phase behavior.eg: Ethanol, Transcutanol, Glycofurol.
4. Other additives- Designated according to the essential of the route of administration of the properties of drug moiety.eg cryooprotectants ,Buffers[23]

TECHNIQUES FOR PREPARATION OF NANOSUSPENSION

1. High-pressure homogenization- It is the most widely used method for designing Nanosuspensions of many poorly aqueous soluble drugs. It applies three steps. First, the drug powders are distributed in a stabilizer solution to form a presuspension, and then the presuspension is homogenized in a high-pressure homogenizer at low pressure for pre- milling and finally homogenized at high pressure for roughly 20,000 cycles until the nanosuspensions of the expected size are formed, It is anticipated that the higher the homogenization pressure, the lower the particle size obtained.[24]

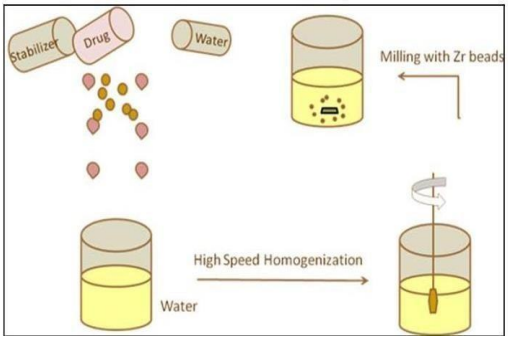


Figure 4: Preparation of Nanosuspension by High Pressure Homogenization

2. Emulsification-Solvent Evaporation Technique: This method applies to prepare a solution for the drug followed by its emulsification in another liquid that is non-solvent for the drug Evaporation of the solvent leads to precipitation of the drug, crystal growth and particle could be controlled by creating high shear forces using a high-speed stirrer. [25]

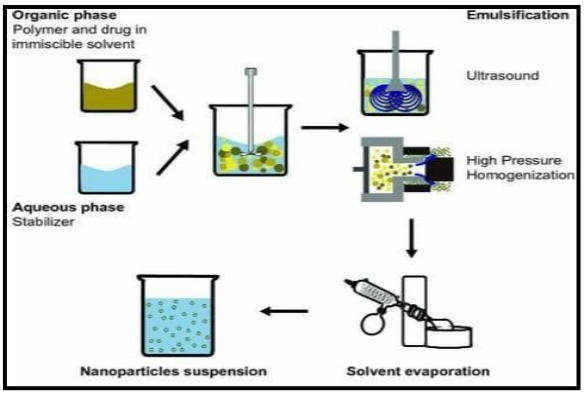


Figure 5: Emulsification Solvent Evaporation Technique

DISSOCUBE: Dissocube is one type of instrument based on homogenization principle it is popularized by R.H Muller used as piston gap type High pressure homogenization in 1999. [26]

CHARACTERIZATION OF NANOSUSPENSION

Mean particle size and size distribution: The mean particle size and the width of particle size distribution are called the polydispersity index. Particle size and polydispersity index(PI) determine the saturation solubility, dissolution velocity, and anatomical performance.(PI) can be determined by photon correlation spectroscopy. (PI) the value ranges from 0.1 to 0.25, indicating a satisfactorily narrow size distribution if the PI value is exceeding than 0.5, representing a very broads distribution

Transmission Electron Microscopy (TEM) One drop of the nanosuspension of the promising batch was placed on a carbon-coated grid (3mm) & was allowed to dry. The sample was loaded in TEM using horizontal sample holder image wear taken using appropriate magnification up to 1600. As high as 200 kV acceleration voltages allow the Tecani 20,Holland.

Zeta Potential: The zeta potential of the promising batch was measured using the Zetasizer Nano series Nano-ZS (Malvern, Malvern Instruments, U.K.). The sample was diluted 10 times with distilled water.

Drug Content: Nanosuspension equivalent to 10 mg of the drug was taken in a 100 ml volumetric flask and diluted up to 100 ml with methanol. The absorbance of the resulting solution was measured at 260 nm and drug content was calculated. [27]

Entrapment efficiency: The nanoparticles existed separated from the dispersion by centrifugation at 22,000 rpm for 25 min. The supernatant obtained after centrifugation was

properly diluted and analyzed for free diazepam by UV-Visible spectrophotometer atprecise nanometers.

Saturation solubility and dissolution velocity: The saturation solubility and dissolution velocity assists in the deduction of in vitro behavior of the formulation.
Stability: Nano-suspensions. In different stress conditions like various temperatures (15, 25, 35, 45°C), thermal cycling, and mechanical shaking, a shift in their mean particle sizecan beobserved for three months and utilized to examine the stability. [14, 27]

DRUG EXCIPIENT COMPATIBILITY STUDY

Excipients are integral components of nearly all pharmaceutical dosage forms, physical and chemical interaction between the drug and excipients, as excipients can change in drug bioavailability and stability. To develop a product that is stable and effective. Drugs and excipients must be compatible with each other. Compatibility studies are specifically dominant if the excipients are new. To test drug compatibility with various excipients use, FTIR and DSC have been widely used. [14, 27]

Table 1: The following is a list of Marketed preparation of nanosuspension [28]

Product	**Drug**	**Use**	**Company/ Individual**
RAPAMUNE®	Sirolimus	Immunosuppressant	Wyeth
EMEND® MEGACE®ES	Aprepitant Megestrol acetate	Antiemetic Appetite stimulant	Merck PAR Pharmaceutical
Triglide™	Fenofibrate	Treatment of Hypercholesterolemia	First Horizon Pharmaceutical
LA.Zanaflex Capsules™	Tizanidine Hydrochloride	To treat spasticity	Acorda
Ritalin®	Methylphenidat e Hydrochloride	Treatment of Attention Deficit Hyperactivity Disorder	Novartis
Avinza®	Morphine sulphate	To treat moderate to severe pain that lasts for more than a few days	King Pharmaceutical
Focalin® XR	Dexmethylphen i-date Hydrochloride	Treatment of Attention Deficit Hyperactivity Disorder	Novartis

Table 2: Various HIV Drugs available in the market [29,30]

Generic names	**Abbreviations**	**Brand name**	**Manufacturer**	**Date of FDA Approval**	**Half life**
Integrase inhibitor					
Dolutegravir	DTG	Tivicay® ViiV	ViiV Health care.	2013	14 hrs
Raltegravir	RAL	Isentress®	Merck	12 October 2007	9hrs
Cabotegravir	CAB	Vocabria	ViiV Health care.	20 march 2020	5-7 weaks
Reverse transcriptase inhibitor					
Didanosine	ddI	Videx®	Bristol-Myers Squibb	9 October 1991	1.3-1.6hrs
Zalcitabine	ddC	HIVID®	Roche	19 June1992	1-3hrs
Lamivudine	3TC	Epivir®	GlaxoSmithKline	17 November 1995	3-6hrs
Abacavir	ABC	Ziagen®	GlaxoSmithKline	17 December 1998	1-2hrs
Tenofovir	AZTDF	Vemlidy®	Gilead Sciences	26 October 2001	16 hrs
Stavudine	d4T	Zerit®	Bristol-Myers Squibb	24 June1994	1-1.6

Zedovudine	AZT	Retrovir ®	Glaxo Smith Kline	19 March 1987	1.1hrs
Non-nucleoside reverse transcriptase inhibitors (NNRTI)					
Efavirenz	EFV	Sustiva®	Bristol-Myers Squibb	17 September 1998	40-50hrs
Nevirapine	EFV	Viramune®	Boehringer Ingelheim	21 June 1996	25-30hrs
Etravirine	TMC125	Intelence®	Tibotec	18 January 2008	30-40hrs
Delavirdine	DLV	Rescriptor®	ViiV Health care.	4 April 1997	5.8hrs
Rilpivirine	TMC278	Edurant™	Tibotec	20 May 2011	34-55hrs
Protease inhibitors					
Saquinavir	SQV	Invirase®	Roche	7 November 1997	1.5-2hrs
Atazanavir	ATV	Reyataz®	Bristol-Myers Squibb	20 June 2003	7hrs
Indinavir	IDV	Crixivan®	Merck	13 March !996	1.2-2 hrs
Amprenavir	APV	Agenerase®	Glaxo Smith Kline	15 april 1999	7-10 hrs
Tipranavir	TPV	Aptivus®	Boehringer Ingelheim	22 June 2005	5-6 hrs
Darunavir	DRV	Pezista	Abbvie	23 June 2006	15 hrs
Ritonavir	RTV	Norvir®	Abbvie	1 March 1996	3-5 hrs
Fosamprenavir	FPV	Lexiva®	ViiV Health care.	20 October 2003	7.7 hrs

Nelfinavir	NFV	Viracept®	Agouron Pharmaceuticals	14 march 1997	3.5-5 hrs
Fusion inhibitor					
Enfuvirtide	T-20	fuzeon	Roche	13 march 2003	3.8 hrs
Entry inhibitor					
Miraviroc	MVC	Selzentry	Pfizer	6 August 2007	14-18hrs

Table 3: Marketed preparation of ARV available in the market [31, 32]

Generic name	**Brand name**	**ROUTE**	**Manufacturer**	**Side effects**
Cabotegravir+Rilpivirine	Cabenuva	IM	ViiV Health care.	Hepatotoxicity, Depressive disorders
Lopinavir + Ritonavir	Kaletra®	Oral	Abbott Labs	Pancreatitis
Lamivudine+Zidovudine	Combivir®	Oral	GlaxoSmithKline	Hematologic toxicity
Abacavir + Lamivudine	Trizivir®	Oral	GlaxoSmithKline	Myocardial infarction
Tenofovir + Emtricitabine	Truvada®	Oral	Gilead Sciences	Bone Loss and Mineralization Defects
Lamivudine + tenofovird-isoproxilfumarate	Temixys®	Oral	Celltrion	Renal impairment
Enfuvirtide	Fuzeon	SC	Roche	Hypersensitivity

Table 4: The following is a Summary of nanotechnology-based treatment approaches forHIV/AIDS. [33]

Type of	**Therapeutic agent**	**Nanotechnology**	**Development stage**

therapy	**(drug or gene)**	**delivery platform**	
Antiretroviral Therapy	Rilpivirine (TMC278	Poloxamer 338/TPGS	Preclinical
	Indinavir	Liposome-laden macrophages	Preclinical
	Stavudine	Mannose- and galactose-targeted liposome	Preclinical
	Zidovudine	Mannose-targeted liposome	Preclinical
	Efavirenz	Mannose-targeted dendrimer	Preclinical
	Lamivudine	Mannose-targeted dendrimer	Preclinical
Nanomaterials	Fullerene derivatives	-	Preclinical
	Dendrimers	-	Preclinical
	Silver nanoparticles	-	Preclinical
	SDC-1721/gold nanoparticles	Gold nanoparticles	Preclinical
Gene therapy	siRNA	Peptide fusion proteins, protamine–antibody fusion proteins, dendrimers, single Walled carbon nanotubes, peptide–antibody conjugates	Preclinical
Immunotherapy	P24 protein	Poly (D,L-lactide) nanoparticles/ dendritic cells	Preclinical

Table 5: Represents integrase inhibitor available in the market [31]

Generic names	Abbreviations	Brand name	Manufacturer
Raltegravir	RAL	Isentress®	Merck
Dolutegravir	DTG	Tivicay®	ViiV Health care
Elvitegravir	EVG	Vitekta®	Gilead Sciences

DISCUSSION AND FUTURE PROSPECTIVE

The Nano medicine platform can also be a future perspective for the local or systematically injectable long-acting formulation, which will be sure to improve patient compliance. The advancement in nanotechnology and Nano medicine has provided a promising future for HIV/AIDS therapeutics. [35]

CONCLUSION

We can treat HIV with high drug potency, bioavailability and efficacy by using formulations containing nanosuspension, and particle size reduction used for increased the dissolution of poorly water-soluble drugs to enhance bioavailability in nanosuspension. For substances with a high log P value, a high melting point, and high doses of technology.as a result, a reduction in particle size leads to an expansion in the dissolution rate. Nanosuspension after downstream processing into drug products has successfully shown its impact on formulation design and management of product life cycle. Nano-sized devices with a diameter of fewer than 50 nanometers can easily enter most cells, while those with a diameter of fewer than 20 nanometers can easily exit blood arteries as they travel. Nanoscale devices can easily interact with biomolecules on the surface and inside cells due to their small size.

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