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Nanotherapeutics and HIV: Four decades of infection canvass the quest for drug development using nanomedical technologies

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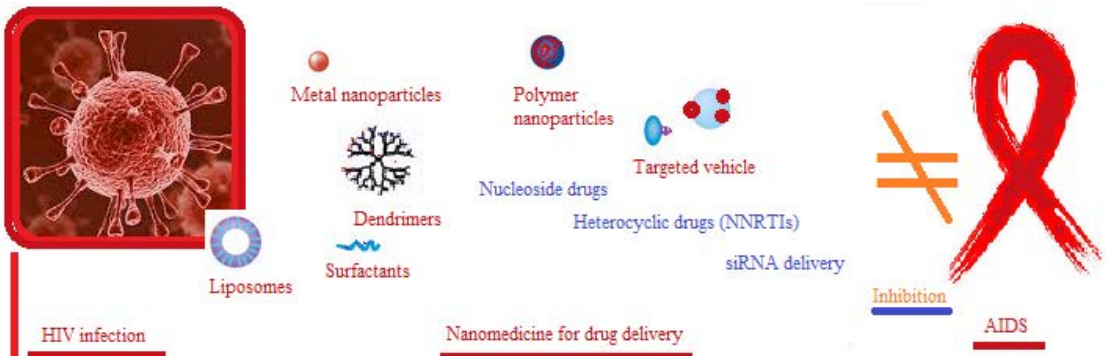
Editorial

ABSTRACT



We have seen four decades of human struggle to cure or eradicate HIV infection since the first clinical detection of HIV infection. Various developed drugs such as nucleoside reverse transcriptase inhibitors (NRTIs), non-nucleoside reverse transcriptase inhibitors (NNRTIs), protease inhibitors, integrase inhibitors, microbicides, and others have known restrictions, such as side effects and resistance development when used alone, and hidden reservoir of the virus, which have opened the gates for the involvement of nanomedicine associated systems, particularly for latent sites of HIV infection. The nanotechnological vehicles, such as liposomes, dendrimers, metal nanoparticles, polymeric nanocapsules/particles, surfactants, and targeted vehicles have become part of extensive studies for application in real settings for the delivery of NRTIs, NNRTIs, microbicides, and siRNA. The positional standing of research in quest of potential therapeutics for combating HIV infection in reference to four decades with this virus need a rational evaluation of nanotechnology to achieve a practical solution to save the lives.

Keywords: AIDS, Drug Delivery, HIV, Nanobiotechnology, Nanomedicine, siRNA delivery

INTRODUCTION

The patients with an immune system compromised disease, later designated as Aquired Immune Deficiency Syndrome (AIDS), were first observed clinically in 1981. Since then, there have been potential progress in identifying and characterization of causative virus, controlling the infection, drug development and awareness of this infection. These four decades from the first observation of this infection have witnessed active research in developing a suitable cure for the virus infection and AIDS. Though an amicable system to control the infection have been successful via different drugs and medical technologies still, a complete cure, particularly for AIDS, is still a subject of major interest and a goal for medicinal researchers.

The Human Immunodeficiency Virus (HIV), the causative agent of AIDS and first characterized in 1983, definitively linked to AIDS in 1984, is a lentivirus known for its delayed onset leading to this chronic infection. Structurally, it consists of a

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"First identification in 1981 to current prevalence in 2022 marks the four decades of known existence of HIV infection"

double-stranded RNA genome with 9,300 base pairs enclosed in protein capsid. The RNA genome contains three major portions or open reading frames known as *gag* (encodes the protein to form the structural components of the virus), *pol* (encodes polyproteins to serve as viral enzymes reverse transcriptase, integrase, and protease, all of which are required for infecting the host cells), *env* (encodes transmembrane proteins for viral binding on host cell) and six small genes that encode regulatory proteins.[1]

The spread of HIV infection is mediated through body fluids during sex, blood transfusion, and umbilical link. Since the first identification of this viral infection in New York in 1981, it has spread to all countries leading to the current known infection of over 36 million people worldwide; approximately 1.0 million people die due to AIDS-associated disease with ~ 1.8 million new infections occurring each year.[2]

HIV infection starts with the attachment of virus on CD4+ T lymphocytes, entry of virus into the cells, duplication of the genome using cellular machinery along with the synthesis of required proteins, and finally, budding out of new virions. This causes rupturing of T cells leading to a decline in their numbers; the continuous repeat of the process ultimately leads to the immune dysfunction known as AIDS.

Taking this multiplication path of virus in consideration, a number of different drugs have been approved that target the different stages of virus multiplication cycle. They include Fusion inhibitors or entry inhibitors (which target the virus binding on CCR4 receptors), reverse transcriptase inhibitors (that target the transcriptase enzyme and include nucleoside reverse transcriptase inhibitors (NRTIs) (Figure 1) and heterocyclic non-nucleoside reverese transcriptase inhibitors (NNRTIs)), protease inhibitors (PIs) (inhibits the viral proteins synthesis), fusion inhibitors (FIs) and integrase inhibitors.[3]

In general, effective therapy includes the combination of the above drugs to target multiple stages towards better effective outcomes. This combination of drugs is known as Highly Active Anti Retroviral Therapy (HAART) and mostly contains a mixture of three drugs that may include NRTIs/NNRTIs, Fusion inhibitors and or Protease inhibitors. The HAART has been highly successful in controlling the infection and was approved as standard therapy in 1996.

NRTIs are lead role players in the management of HIV, constitute an important part of HAART

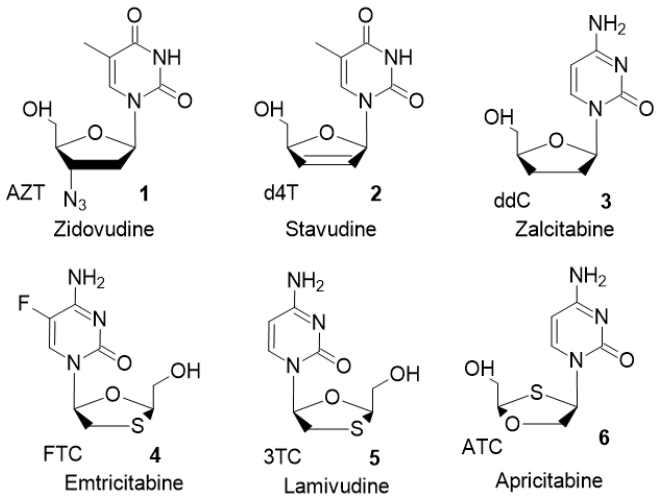

Figure 1. Structure of representative FDA-approved drugs. Structure of representative first-generation NRTIs approved for HIV treatment (Apricitabine (ATC) is a recent drug (i.e., a second-generation NRTI).

The HAART therapy for the treatment of HIV-infected individuals using a combination of three antiviral drugs approved by the FDA has been extremely effective in controlling the infection and further preventing the progression of infection to AIDS. This has attributed to decline in the AIDS-related deaths globally. Though HAART can bring the viral load to negligible (and undetectable) levels, the patients still carry the virus throughout their lifetime. This arises because of the emerged drug resistance or due to the presence of the virus in hidden reservoirs in the individuals' body where drugs cannot be delivered.[4] The dosage includes frequent administration (once or twice daily) of antiviral drugs, which leads to other associated toxicities and also the emergence of drug resistance due to non-compliance of patients to the required dosage.[5]

With the current scenario with HIV therapy, the use of medical nanotechnologies could facilitate achieving higher deliverability, crossing over of membrane barriers, and generating desired pharmacological profiles for respective drugs. The meticulous properties of respective nanosystems have been purposefully applied with different drugs for different diseases like cancer and viral infections.[6] The prodrug and nanostructured conjugates with current available HIV drugs have been extensively studied, and few have reached various stages of clinical evaluations, while final approved formulations are still treading in the stages of the clinical trials due to different inherent challenges in preclinical and clinical requirements of the respective formulation.[7]

The prodrug and nanotechnological conjugates and derivatives potentiate the promise of altering the pharmacological profiling of hydrophilic antiviral drugs along the ability to cross the blood-brain-barrier to overcome the inability of antiviral drugs to reach to hidden reservoirs of the virus in parts of the brain and lymph nodes. Table 1 summarizes some of the prodrug and nanoformulations that have been approved or are evaluated in clinical trials. The effective elimination of the virus from these viral reservoirs requires the sufficient concentration of antiviral

drugs in the central nervous system and lymphatic nodes. Furthermore, the introduction of targeted agents in nanotechnological systems enhances the ability to reach to specific sites with increased availability of antiviral drugs at hidden sites of virus and thus can lead to the required effective elimination of virus from the body.

The prodrug formation of the antiviral drug is the prime step to improve the bioavailability and cellular uptake of the drugs. Tenofovir (TFV) **7** and Amprenavir (APV) **10** (Figure 2) are NRTI and protease inhibitor, respectively, that are administered in their prodrug forms as the parent drug have limited bioavailability due to their high hydrophilic or lipophilic nature. TFV has a phosphonate group which makes it highly hydrophilic and thus limits its oral administration as the hydrophilic nature of TFV causes poor membrane permeability leading to negligible absorption from intestine. The prodrug form of TFV is used to mask the phosphonate group.[8] In tenofovir disoproxil fumarate (TDF) **8** prodrug, the acyloxyalkyl ester functionalization is used to mask the negatively charged phosphonate group of TFV, and thus increases the lipophilicity and oral bioavailability of the drug. In another prodrug of TFV, tenofovir alafenamide (TAF, **9),** the aryl and L-alanine isopropyl ester functionalization of TFV phosphonate group improves the lipophilicity and bioavailability. Compared to TDF (**8)** prodrug, TAF **(9)** prodrug was found to be more effective in increasing drug availability at the site of HIV infection.[9]

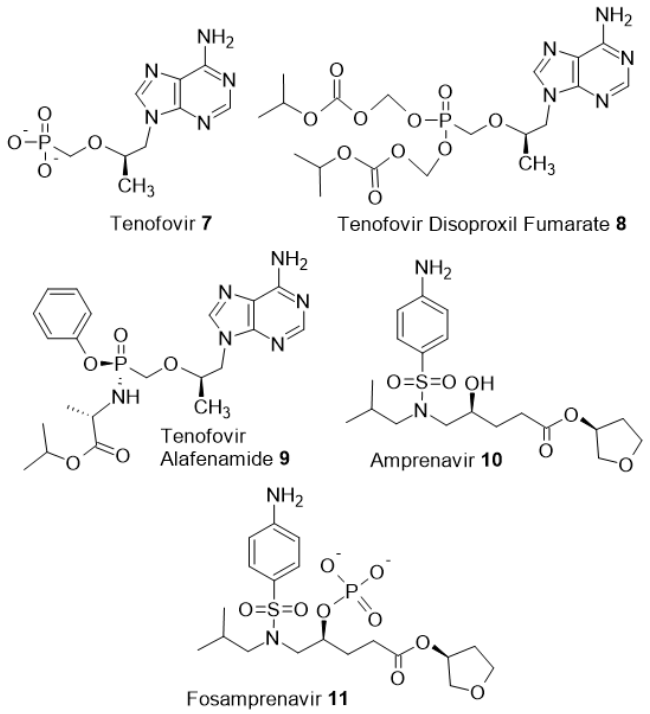

Figure 2. Chemical structures of Tenofovir (TFV), Amprenavir, and their prodrugs.

Amprenavir (**10)** has very low water solubity which compromises its oral bioavailability. It is converted into fosamprenavir (FPV) prodrug form (**11**) via the incorporation of a polar phosphate group. FPV(**11)** has higher water solubility compared to amerenavir by virtue of the presence of polar phosphate group that is hydrolyzed back to release the more membrane permeable parent drug amprenavir after oral administration. All of these three prodrugs: tenofovir disoproxil fumarate (TDF), tenofovir alafenamide (TAF), and fosamprenavir (FPV) have been approved by FDA for HIV treatment.[9]

In a similar way, NRTIs are also polar due to the presence of ribose and base structures. Because of their polar nature, the NRTIs (and also few NNRTIs) have a high rate of biodistribution and excretion. This higher rate of excretion limits their bioavailability in the serum as well as at the target site of HIV infection. The lower bioavailability along with increasing drug resistance, leads to the administration of high doses of the drug, which causes the over-burden of dosages on the patient and thus, generates pronounced associated side effects. Our group has investigated different methodologies and various prodrug forms of nucleoside-based and other anticancer drugs to improve their pharmacological profile.[10–19] Similar investigations have been conducted to circumvent the side effects of NRTIs by synthesizing different derivatives of parent nucleoside drugs or by devising various delivery vehicles to reduce the side effects and increase the availability and potency of the nucleoside-based drugs.[20–23] Alterations in the lipophilicity of NRTIs by the introduction of a fatty chain with varying degree of lipophilicity has been a priority option to improve the pharmacological profile of nucleoside drugs.[24] Besides modulation of lipophilicity profile of NRTIs, the selected fatty acids are also known to inhibit *N*-myristoyltransferase (NMT) enzyme, the main enzyme known to myristoylate various HIV proteins, such as P17 capsid protein, Pr55gag, Pr160gag-pol, p27nef in the virus-infected cells.[25] Myristoylation of proteins allows it to become more hydrophobic thus enhancing the protein localization at the cellular membrane and protein-membrane interactions. Certain heteroatom-containing myristic acid analogs such as 12-thioethyldodecanoic acid, 4-oxatetradecanoic acid, and 2-methoxydodecanoic acid exhibited to inhibit the HIV-1 replication in acutely infected T-lymphocytes.[26] 12-Thioethyldodecanoic acid was found to have EC_{50} of 9.4μM and therefore was moderately active against HIV-infected T4 lymphocytes.[26] Considering the required modulation of the lipophilicity profile of NRTIs, the 5′-*O*-fatty acyl derivatives of NRTIs were synthesized, which displayed higher cellular uptake than the parent analogs due to enhanced lipophilicity and better activity profile.[22,23,27,28,29] These ester conjugates act as bifunctional agents because once it enters the cells, the esterases hydrolyze the conjugate to release reverse transcriptase inhibitor (the nucleoside drug) and NMT inhibitor (the fatty acid). Furthermore, as the lipophilic conjugates are formed for more than one nucleoside, so it increases potency by releasing more than one NRTI drugs simultaneously thus helping to combat the drug resistance.[22,23]

Similalry some of the synthesized lipophilic derivatives of lamivudine (3TC, **5**) were found to have higher antiviral activity compared to parent 3TC.[29] The 3TC derivatives with functionalization at NH_2 (N4 of pyrimidine base) and 5′-OH (5′-O of sugar residue) with varying lengths of alkyl chain were synthesized (Figure 3). The N4- or 5′-*O*-monosubstituted

lipophilic derivatives of the 3TC nucleoside (**12-14** and **15-17**) exhibited a higher potency than parent nucleoside 3TC against cell-free HIV-1. Compared to N4 derivative, compounds with 5'-O-monosubstituted ester derivatives (**15,16,17**) were the most potent compounds (EC_{50} = 0.2−2.3 μM) as determined by viral inhibition single-round infection assays. Among 5′-*O*-substituted derivatives, 5′-*O*-myristoyl analogue (**15**) exhibited the highest anti-HIV activity (EC50 = 0.2−0.5 μM). Compound **12** (EC_{50} = 0.7 μM) was the most potent conjugate against cell-associated HIV-1 showing approximately 115-fold higher antiviral activity than 3TC (EC_{50} = 80.3 μM).[29]

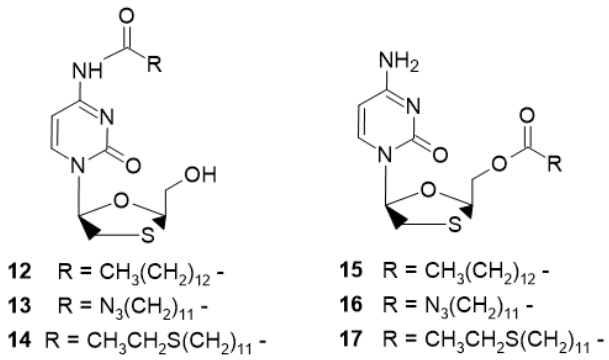

Figure 3. Chemical structures of lipophilic derivatives of N4 (**12, 13,** and **14**) and 5′-O (**15**, **16,** and **17**) substituted 3TC.

In addition to the fatty acids, other moieties such as peptides, amino acids, etc. have been explored as conjugating units to deliver more than one NRTI drug simultaneously along with moiety-mediated[21] improved drug delivery.[20,22,30] Presence of more than one nucleoside drug in a single vehicle conjugate potentiates to circumvent the drug resistance and provide a better synergistic effect of the released drugs. Prospectively, combination of lipophilic unit and amino acids on the single delivery vehicle adds better biocompatibity to the conjugate.[20] For example, the glutamic acid conjugates having mono-, di-, and trinucleoside (NRTIs) conjugates of glutamate or peptide scaffolds containing nucleoside reverse transcriptase inhibitors (NRTIs) having fatty acid as part of amino acid scaffold showed improved activity over multidrug resistance. The selected derivatives with glutamic acid scaffold are shown in Figure 4.

Among the reported glutamate esters having two nucleosides, compound **19** (Figure 4), encompassing the conjugated FLT and FTC, was the most potent anti-HIV agent (EC_{50} = 0.1-0.4 μM). Compound **19** displayed superior activity compared to the individual parent nucleosides FLT and FTC whose activity EC_{50} values ranges 0.4−2.0 μM. In the dinucleoside glutamate ester derivatives, *N*-myristoylated derivatives exhibited significantly higher anti-HIV activity than the corresponding *N*-acetylated conjugates against the cell-free virus.[20] Myristoyl-Glu(3TC)-FLT (**18**, EC_{50} = 0.3-0.6 μM) and myristoyl-Glu(FTC)-FLT (**19**, EC_{50} = 0.1-0.4 μM) derivatives were the most active glutamate-dinucleoside conjugates.[30] Among trinucleoside glutamate derivatives, compound **20** comprising AZT, FLT, and 3TC (**20**, EC_{50} = 0.9-1.4μM) exhibited higher anti-HIV activity than AZT and 3TC against cell-free virus. Compound **20** also displayed higher anti-HIV activity against multidrug (IC_{50} = 5.9 nM) and NNRTI (IC_{50} = 12.9 nM) resistant viruses than parent nucleosides. Compound **20** (EC_{50} = 0.8 μM) exhibited 115-fold higher activity against cell-associated virus compared to the physical mixture containing FLT−succinate, AZT, 3TC, and glutamic acid exhibited (EC_{50} = 91.9 μM). Similarly, many additional molecules have been designed with linear and cyclic peptides for improving the biological activity profile of the NRTIs and to counter the drug resistance. Most of the these studies are at laboratory scale with molecules showing varied properties *in vitro* assays with possible scope for further applications and testing for clinical applications.

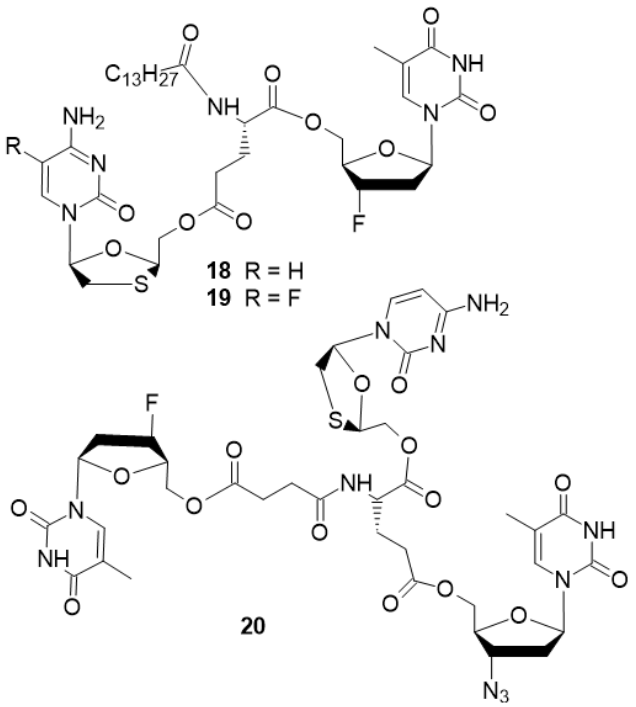

Figure 4. Chemical structures of glutamic acid nucleoside conjugates. **20** is the triad conjugate having AZT, 3TC, and FLT attached to glutamic acid succinate. Compounds **18** and **19** are dinucleoside conjugates of myristoylated glutamic acid.

In addition to anti-HIV drugs that are used systemically, the microbicides are meant for the local topical delivery or application of the anti-HIV drug at the site of exposure to prevent the spread of the virus or acquisition of infection. The nanoformulations of the anti-HIV agents can potentiate the prolonged release of the microbicide drugs at the local site for sustained prevention, enhance the cellular uptake mediated by the nanosystem deployed, improve the activity of drug due to encapsulation, and protect the drug from inactivation by the vaginal microenvironment. With these prepositions, the microbicides conjugates using gels,[31] hydrogels, liposomes, dendrimers, and polymeric nanoparticles have been developed by many research groups; however, most of the studies have been limited to laboratory scale evaluation. The dendrimer named

Liposomes and lipid-based carrier carry high potential of applicability and development of therapeutics for HIV infection

VivaGel produced with the naphthalene-3,6-disulfonate terminated polylysine has been applied to deliver anti-HIV microbicides in primates. The clinical phase I trial was completed in 2009 (Table 1),[32] however, further developments remained elusive due to other necessary requirements.[33] Similarly, maraviroc, an anti-HIV drug that binds to human chemokine receptor CCR5, incorporated in a silicone elastomer gel produced enhanced and sustained concentrations of this microbicide in the vaginal tissue in preliminary investigations.[34] The microbicides have offered a better perspective for local application of drugs; however, formulation development requires more focused advances oriented towards requirements of local delivery or microenvironment system at a localized site.

The nanocarrier-based strategies have the potential for successful application in this regimen. Nanobased formulations have accoladed their might in the development of therapeutics for different disease,[35] particularly cancer.[36,37] The potential nanocarriers for HIV management encompass polymeric nanoparticles, solid lipid nanoparticles, liposomes, dendrimers, biomacromolecules, and metal nanoparticles.[38] The polymeric nanoparticles of poly(lactide-co-glycolide) (PLGA), poly (L-lactic acid) (PLA), poly(capro-lactone) (PL), and biomacromolecule chitosan nanoparticles have been studied with different antiviral drugs, Lamivudine,[39] Zidovudine,[40] Indinavir,[41] and Tenofovir.[42] Nanogel carriers consisting of PEG- or Pluronic-PEI biodegradable networks, star PEG-PEI, or PAMAM-PEI-PEG dendritic network have shown efficient delivery of NRTIs, AZT-triphosphate and ddI-triphosphate in macrophages.[43] These nano-Gels on further decoration with vector peptides (e.g. brain-targeting ApoE peptide) displayed high delivery in the brain with the highest antiviral efficacy.[44] However, most of the above formulations showed a varied degree of uptake in different cells and most of the studies have been restricted to a laboratory scale.

Dendrimers are the hyperbranched structures that generate different types of natural and synthetic materials. They can entrap the drugs in its structure, cross the blood brain barrier, and have enhanced cellular uptake profile. The dendrimers have the ability to be used with antiviral drugs for enhanced cellular uptake and sustained release of encapsulated drugs. The mannose terminated dendrimer encapsulated with anti-HIV drug efavirenz showed sustained release up to 144 hours.[45] The stand-alone poly(amidoamine) (PAMAM) dendrimer and carbosilane dendrimer has the affinity to bind to cell receptors (gp120 and CD4), and thus dendrimer itself has anti-HIV effect by preventing the binding of the virus to cells, indicating the possibility of use as a microbicide in cervicovaginal tract applications.[46]

Lipid nanoparticles such as solid-lipid nanoparticles (SLNs), liposomes, ethosomes, niosomes, lipid nanoemulsions etc. are among the leading class of nanoparticles for application in drug delivery-related studies. Many formulations based on this class of nanoparticles are in clinical applications, particularly for anticancer therapies. The SLNs prepared from synthetic lipids in thin-film hydration exihibit good colloidal stability and can deliver drugs efficiently.[47] Assorted antiviral drugs such as Atazanavir (protease inhibitor (PI)),[48] Ritonavir (protease inhibitor),[49] Darunavir (protease inhibitor),[50] and nelfinavir.[51] encapsulated in SLNs have been observed to enhance the cellular uptake with higher anti-HIV effect on encapsulation of drug. The SLN loaded with anti-retroviral drug atazanavir sulfate showed superior oral bioavailability compared to the free drug. SLNs exhibited the lymphatic route for transportation of formulation and have better retention in the site of viral reservoirs, thus suggesting better applicability of SLNs with antiviral drugs.[52]

Liposomes are lipid nanostructures with ball-like lipid layer structure (similar to phospholipid bilayer of cell membrane structure), excellent cellular uptake properties, easy synthesis, easy surface modification with targeting/carrier molecules, and ability to enclose the drug to keep the drug protected during transportation.[53,54] Their robust drug delivery applicability is accoladed by the presence of different drug-liposome formulations in the market (Doxil, Depocyt, Visudyne, DipoDur, DaunoXome, Ambisome etc.).[55] The liposomes have also been studied with different anti-HIV drugs. The liposome-based carrier system has high potential to deliver the drugs to macrophages (one of HIV-infected cells) because the macrophages consider liposomes as foreign particles and engulfs them. This is one way of directing the delivery of drugs to target sites.[56] Different anti-HIV drugs azidothymidine (zidovudine, AZT), stavudine (2, 3-didehydro-2,3-dideoxythymidine, d4T), and indinavir have been evaluated with encapsulation into surface-modified liposome for targeted delivery of the drugs.[57] The indinavir encapsulated in the dipalmitoyl phosphatidylcholine (DPPC) bilayer immunoliposomes showed much higher uptake in different tissues than in naked drugs only.[57] The storage and biological stability of liposomes can be enhanced by using the poly(ethylene glycol) (PEG) on liposome surface or with chitosan or hydroxyapatite coating that will provide better applicability of liposomes.[58] The PEGylated liposomes loaded with anti-HIV saquinavir drug showed better colloidal stability and enhanced cellular uptake of the drug-liposome formulation compared to the free drug.[59] The etravirine drug loaded on the lipidic nanocarrier showed better uptake and accumulation in different body organs, such as lymph nodes, liver, ovary, and brain, compared to the free drug. This selected enhanced uptake in organs would be beneficial in the eradication of HIV infection from respective organs.[60] The lipid nanocarriers loaded with the HIV-fusion inhibitor peptide E1P47 were applied as vaginal microbicide since the formulation has sustained release of fusion inhibitor peptide;[61] its sustained release profile was much better from lipid formulation compared to similar release from synthetic PLGA polymer. The vaginal tissue have better retention of liposomal vesicals.[61] Based on these laboratory scale studies, the liposomes-based anti-HIV drug formulations

have shown superior potential towards the development of clinical applicable nanodrug formulation, however, efforts with thorough and concise evaluation are needed in future studies.

Other lipid nanoparticles like lipid nanosuspensions, niosomes (self-assembled vesicular structures of non-ionic surfactants), and ethiosomes (ethanolic liposomes with polyols chains) have been studied with different anti-HIV drugs for better cellular uptake; however, varied reasons limited their evaluation only at experimental level.[62]

The long-acting nanoformulations are constructed by converting the drugs in protected structures, such as solid drug nanoparticles, drug nanocrystals, and sometimes prodrugs. The long-acting nanoformulation of anti-HIV drugs rilpivirine, a NNRTI, and cabotegravir, an HIV integrase inhibitor, was approved by Health Canada under brand name Cabenuva™ for use once in a month administration by intramuscular route.[63] After completing the clinical trials, the drug formulation Cabenuva has been approved by the FDA. Cabotegravir/Rilpivirine is, therefore a recent FDA-approved combinational injectable drug to treat HIV composed of long-acting formulation of cabotegravir (second-generation integrase strand transfer inhibitor) and rilpivirine (NNRTI) (Figure 5).[64] The outcomes of clinical trials (ATLAS-2M) study indicated a good efficacy as well as the safety profile of Cabenuva™ regimen for injection every 8 weeks, offering hope for the reduced dosing for the management of HIV infection. Thus, the success of this long-acting formulation can potentiate future research with other drug combinations.[64]

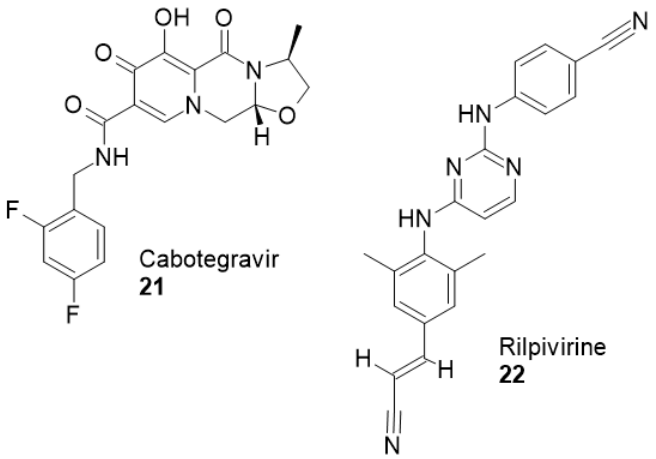

Figure 5. Chemical strucutres of Cabotegravir and Rilpivirine, the drug components of long-acting formulation Cabenuva™.

The nanosuspensions of the NNRTI efavirenz and the protease inhibitor lopinavir are currently in Phase I clinical trials.[65] A porous solid drug particle, surrounded by a matrix of stabilizers, is obtained by freeze-drying the frozen emulsion of drugs. The obtained nanocrystals have high drug loading (70%) and are suitable for sustained release of drugs.[66]

Metal nanoparticles constitute the widest variety of nanosized particles accessible from different metals and metal salts (oxides, sulfides), which find applications in diverse fields including nanobiotechnology, nanocatalysis,[67] materials sciences,[68,69] among others. The nanoparticles of silver and gold are leading metal nanoparticles for explorations in different fields due to their easy synthesis by well-established methods, tunability of properties, and easy surface functionalization with organic compounds to alter their surface properties and biocompatibility. The inherent properties of silver nanoparticles as antibacterials led to their extensive explorations for applications in various infectious diseases[70] and their functionalization with natural and synthetic products to enhance the effect.[71] The silver nanoparticles have also been investigated for anti-HIV effect. The silver nanoparticles synthesized deploying natural plant extractives have demonstrated the inhibitory effect on reverse transcriptase and protease enzymes.[72,73] In an *in vitro* vaginal topical study, the poly(vinylpyrrolidone) (PVP) coated silver nanoparticles have shown the HIV transmission inhibition.[74]

The surface plasmon exhibited by gold nanoparticles assists in the tracking of nanoparticles and detection of nanoparticle-drug conjugate. Because of the easy surface functionalization of gold nanoparticles with drugs, biocompatible and/or targeting molecules makes these nanoparticles suitable for drug delivery applications. The delivery of anti-HIV drugs using gold nanoparticles with or without surface modifications has been explored extensively. The gold nanoparticles coated with glucose and conjugated with anti-HIV drugs lamivudine and abacavir through thiol linkage exhibited well-tuned pH dependent release of drugs.[75] The gold nanoparticles coated with manno-glycosaccharides have shown the ability to bind with the lectins present on the surface of macrophages and thus inhibit the HIV infection by preventing the dendrite-mediated binding of the virus to the macrophage cell surface.[76] The gold nanoparticles coated with cyclic and linear peptides have been demonstrated for enhanced cellular delivery of anti-HIV drugs lamivudine (3TC) and emtricitabine (FTC) (Figure 1).[77] The cyclic peptide c[KW]$_5$ coated gold nanoparticles showed localization in the nucleus while linear peptide l(KW)$_5$-AuNPs delivered the lamivudine drug in the cytoplasm.[77] Inclusion of cell-penetrating cyclic peptide further potentiates the drug delivery using gold nanoparticles.[78]

Metal-organic frameworks (MOFs) are emerging as promising drug delivery vehicles due to their higher amount of drug loading capacity, biocompatibility, and biodegradability.[79] The anti-HIV drug azidothymidine triphosphate (AZT-TP) loaded up to 24% onto the nanoMOFs made up of iron trimesate.[80] The AZT-TP loaded nanoMOFs efficiently penetrated and released the drug inside HIV-infected cells comparatively at a very high concentration compared to free drug.[80] The magnetic nanoparticles help in controlled release of the drug in response to external stimuli of the applied magnetic field.[81] These nanoparticles also can be tracked under magnetic resonance imaging. Many superparamagnetic iron nanoparticle and other paramagnetic metals serve as drug delivery vehicles for cargo molecules. In a recent study, the magentic nanoparticles having dendrion with carboxylate surface functionalization have been

evaluated for entrapping of HIV-1 strains towards easy and rapid diagnosis of virus.[82]

The NRTIs are important drugs in the therapeutics development for HIV treatment; however, the development of long-acting formulations of NRTIs is still lacking; none has reached clinical trials yet. The NRTIs have hydrophilic structures, and there is variations of chemical properties of nanosuspensions of NRTIs in the varied biological microenvironment. Thus, there is still a need to explore other delivery nanosystems that can suitably incorporate and release the drugs. We have used cell-penetrating peptides (CPPs) to encapsulate and enhance the delivery of a number of NRTIs, such as 3TC, FTC, and d4T, in different cell lines.[83–87] More studies are required to determine whether the encapsulation and improved delivery can be translated to long acting effect or enhanced anti-HIV activity.

Table 1. List of successful nanoconjugates for HIV (clinical stages/ approved).

Nanosystem	Drug used	Formulation	Stage
Prodrug	Tenofovir	tenofovir disoproxil fumarate	In clinics – FDA approved
Prodrug	Tenofovir	tenofovir alafenamide	In clinics – FDA approved
Prodrug	Amprenavir	fosamprenavir	In clinics – FDA approved
Gel	Microbicides	VivaGel	Phase I trials
NanoCrystals	Cabotegravir + rilpivirine	Cabenuva™	Approved by FDA and Health Canada
Nanocrystals	Efavirenz and lopinavir	Solid nanoparticles of drugs	Phase I

The small interfering RNAs (siRNAs) hold the capacity to inhibit the viral replication in the host cells as these are small fragments of noncoding RNA that mediate the genetic material replication inhibition or stop the central dogma to inhibit the protein synthesis. This phenomenon, known as gene silencing using siRNA, have high specificity and is advantageous in targeting siRNA for treating any disease that requires genetic regulation. The initial analysis of genetic material and subsequent designing of specific sequence of RNA for targeted action have potential in therapy of different disease like cancer, diabetes, brain disease and viral diseases etc. The siRNAs have also been evaluated for the development of HIV therapeutics. The major problem with the siRNA is their delivery to target site due to highly polar nature of the RNA fragments (phosphate backbone). A number of different vehicles such as lentiviral vehicles and non-viral nanomaterials such as liposomes, lipids nanoparticles, carbon nanotubes,[88,89] metal nanoparticles and other carriers have been studied for the delivery of siRNA. The viral vehicles has associated immune response which hinder their clinical application while non-viral nanomaterials vehicles has loading and continuous release related issues, however, the continuous efforts have led to the successful clinical applications as evident by marketed formulations such as (1) Patisiran is the lipid nanoparticles formulation of the first RNAi based drug approved in 2018 for treatment of hereditary transthyretin mediated amyloidosis (hTTR),[90] which acts by inhibition of translation of protein from TTR mRNA; (2) Givosiran is the siRNA conjugated to Alnylam's Enhanced stabilization Chemistry N-acetylgalactosamine (ESC-GalNAc) residue meant for the treatment of acute hepatic porphyria;[91] (3) Lumasiran is siRNA conjugated to ESC-GalNAc for treatment of Hyperoxalurea type 1;[92] (4) Inclisiran is the recent siRNA drug conjugated to GalNAc for the treatment of atherosclerotic cardiovascular disease.[93]

In case of siRNA for HIV, different delivery vehicles may be required to target the different organs as the virus remain located in different organs including its hidden reservoirs. The siRNA designing is further complicated by the different strains of the virus (present in different countries) along with generated mutations during the lifecycle or propagation of virus from host to host. The siRNA design as well as delivery vehicle selection makes it a challenging task for application in HIV therapy.[94]

The 'block and lock' strategy is meant to inactivate the virus by inhibiting the essential parts/proteins involved in transcription cycle. By targeting the key transcriptional steps, this strategy might provide a functional cure by keeping the virus in permanently latent stage.[95] Currently, the most studied therapeutic of "block and lock" is a Tat-inhibitor, didehydro-cortistatin A (dCA).[96] As the Tat protein is essential unit to support the RNA polymerase II and its inhibition with dCA have shown promising results with significant inhibition of virus replication.[97] Another class of inhibitors include the small molecules based Integrins (LEDGINs) that inhibit integrase required for the integration of chromatins at active transcription sites and have given promising results.[98] Here, the siRNA comes in prominent role due to their higher specificity and ability to target different stages of transcription components.[99] This is transcriptional gene silencing (TGS) where siRNA inhibit the generation of new virions via inhibition of transcription. The siRNA can be designed to target the viral RNA or host mRNA that are necessary for viral replication.[100] Though, this transcriptional gene silencing (TGS) using siRNA promises efficient output in 'block and lock' inhibition, its delivery at target site remains a challenge for efficient application as the siRNA are rapidly cleared from circulation as well as not easily taken up by cells.[101] Besides lentiviruses, all other synthetic nanocarriers such as liposomes, polymer nanoparticles, nanocapsules, siRNA-polymer conjugates, gold nanoparticles, glycogen nanocarrier and magnetic nanocarriers have extensively been studied for delivery of siRNA for HIV therapy.[94] Among all these, the liposomes are presumed and have been most potent carriers for siRNA delivery as a number of formulations containing siRNA with liposomes or lipid nanoparticles are

clinical stages of evaluation for different diseases.[90,94] The liposomes and other carrier subjected for anti-HIV activity with siRNA have remained in infancy. Thorough evaluation and development of delivery vehicles with siRNA with consistent effort in future would bring the desired therapeutics for HIV.

FUTURE PERSPECTIVE

There are currently potent anti-HIV drugs targeting different events in the life cycle of viruses, such as fusion, reverse transcription, integration, and protein synthesis. Many of these drugs are currently used in different combination cocktails, such as HIV integrase strand transfer inhibitor (INSTI) (e.g. dolutegravir, bictegravir) or NNRTI (e.g. rilpivirine, etravirine, doravirine,) plus two or more NRTIs (e.g. tenofovir, lamivudine, emtricitabine,). and have been able to control the HIV infection in patients and have transformed the HIV infection as a lethal disease into a more manageable chronic one. There is still a problem associated with the adherence to daily oral drug use and the prevention of drug-resistant viral strains. Furthermore, there are issues related to drug-drug interactions that may occur because many patients take other medications. Thus, there is a lot of interest in developing long-acting formulations. The success of FDA-approved long-acting cabotegravir and rilpivirine has revolutionized the field since patients need to have only six intramuscular injections per year instead of 365 daily pill. Another recent success story is the development of long-acting islatravir, an NRTI with a dosing interval of one year or more. It is envisioned that by using prodrug approaches and different nanoformulation strategies, several more long-acting antiretroviral will become available. There is a potential for implant and patch formulation that would exempt patients from oral administration and provide a long-term effect. Many implant formulations need the use of polymers. The therapeutic plasma level of drug needs to be maintained to avoid viral replication rebound. Furthermore, long-acting cabotegravir/rilpivirine is an injectable formulation and needs to be kept at 2-8 °C. Therfore future formultion should avoid painful modes of administration. For the implants, there is a need for surgical procedures to remove and replace implants. Thus, there is still need to develop more optimized formulations to maximize the therapeutic and prophylactic potential of long-acting agents. Prodrug and nanoformulation strategies have the potential to circumvent some of these problems.

CONCLUSION

The specific chemical and pharmacological profile of anti-HIV drugs, such as NRTIs, NNRTIs, PIs, and FIs play a critical role in their bioavailability in blood serum and at the targeted site of infection. Though HAART has been quite successful in eradicating of HIV infection, the drugs used in this combination therapy, however, could not eliminate the virus completely from the body. Their low bioavailability and increased drug resistance potentially require the use of alternate systems, including nanomedicine for a better outcome. Modulation of these drugs profile with nanostructured systems and concepts, although extensively explored, have seen limited success in clinical applications. The prodrugs of selected antiviral drugs are in clinical use, but the rest of the nanostructured systems are either in clinical trial stages or require more robust investigations to make them applicable in clinics for anti-HIV therapy.

Nanomedicine comprising medical nanostructures and nanoformulations have the potential to reach to the hidden reservoirs of the virus or circumvent the challenges to cross membrane barriers (like blood-brain-barrier), and considering this capability, various systems such as liposomes, solid lipid nanoparticles, metal nanoparticles, dendrimers, gels, micelles, and other nanomaterials have been evaluated for possible development as an applicable vehicle for anti-HIV therapy. With these efforts, few conjugates or nanosystems have reached to the clinical trial stages, while others warrant more detailed evaluation. The nanoconjugate systems have the potential to be successful in clinical trials, and applicable nanosystems may reach to clinics in near future, however, research efforts in this field must focus on the efficient deliverable drug-nanosystem conjugate having acceptable biocompatibility and appropriate treatment outcome.

AUTHOR CONTRIBUTION

BSC, KP conceptualized and composed the article contents. NS, P, PB, RSV contributed in writing, improving and editing the text of manuscript. All authors have read and approved the final article.

CONFLICT OF INTEREST

The authors do not have any academic, financial or other conflict of interest for publication of this article.

REFERENCES AND NOTES

1. J.A.G. Briggs, J.D. Riches, B. Glass, et al. Structure and assembly of immature HIV. *Proc. Natl. Acad. Sci. U. S. A.* **2009**, 106 (27), 11090–11095.
2. J. Singh, B.S. Chhikara. Comparative global epidemiology of HIV infections and status of current progress in treatment. *Chem. Biol. Lett.* **2014**, 1 (1), 14–32.
3. B.S. Chhikara, B. Rathi. Organic molecular drugs designing for inhibition of cellular multiplication biocycle of virus for anti-HIV therapy development: A recent advances review. *Adv. Org. Chem. Lett.* **2015**, 2 (1), 1–14.
4. C. V. Fletcher, K. Staskus, S.W. Wietgrefe, et al. Persistent HIV-1 replication is associated with lower antiretroviral drug concentrations in lymphatic tissues. *Proc. Natl. Acad. Sci. U. S. A.* **2014**, 111 (6), 2307–2312.
5. C. Solas, A. Lafeuillade, P. Halfon, et al. Discrepancies between protease inhibitor concentrations and viral load in reservoirs and sanctuary sites in human immunodeficiency virus-infected patients. *Antimicrob. Agents Chemother*. **2003**, 47 (1), 238–243.
6. B.S. Chhikara, R. Kumar, Poonam, P. Bazard, R.S. Varma. Viral infection mitigations using advanced nanomaterials and tools: lessons from SARS-CoV-2 for future prospective interventions. *J. Mater. Nanosci.* **2021**, 8 (2), 64–82.
7. U. Roy, J. Rodríguez, P. Barber, et al. The potential of HIV-1 nanotherapeutics: From in vitro studies to clinical trials. *Nanomedicine* **2015**, 10 (24), 3597–3609.
8. A. Qureshi, L.A. Ouattara, N.S. El-Sayed, et al. Synthesis and evaluation of anti-HIV activity of mono- and di-substituted phosphonamidate conjugates of Tenofovir. *Molecules* **2022**, 27 (14), 4447.

9. P.E. Sax, D. Wohl, M.T. Yin, et al. Tenofovir alafenamide versus tenofovir disoproxil fumarate, coformulated with elvitegravir, cobicistat, and emtricitabine, for initial treatment of HIV-1 infection: Two randomised, double-blind, phase 3, non-inferiority trials. *Lancet* **2015**, 385 (9987), 2606–2615.
10. B.S. Chhikara, D. Mandal, K. Parang. Synthesis, anticancer activities, and cellular uptake studies of lipophilic derivatives of doxorubicin succinate. *J. Med. Chem.* **2012**, 55 (4), 1500–1510.
11. B.S. Chhikara, N.S. Jean, D. Mandal, et al. Fatty acyl amide derivatives of doxorubicin: Synthesis and in vitro anticancer activities. *Eur. J. Med. Chem.* **2011**, 46 (6), 2037–2042.
12. A. Nasrolahi Shirazi, R. Tiwari, B.S. Chhikara, D. Mandal, K. Parang. Design and biological evaluation of cell-penetrating peptide-doxorubicin conjugates as prodrugs. *Mol. Pharm.* **2013**, 10 (2), 488–499.
13. B.S. Chhikara, D. Mandal, K. Parang. Synthesis and evaluation of fatty acyl ester derivatives of cytarabine as anti-leukemia agents. *Eur. J. Med. Chem.* **2010**, 45 (10), 4601–4608.
14. B.S. Chhikara, B. Rathi, K. Parang. Critical evaluation of pharmaceutical rational design of Nano-Delivery systems for Doxorubicin in Cancer therapy. *J. Mater. Nanosci.* **2019**, 6 (2), 47–66.
15. B.S. Chhikara, K. Parang. Development of cytarabine prodrugs and delivery systems for leukemia treatment. *Expert Opin. Drug Deliv.* **2010**, 7 (12), 1399–1414.
16. K. Zoghebi, H.M. Aliabadi, R.K. Tiwari, K. Parang. [(WR)8WKβA]-Doxorubicin Conjugate: A Delivery System to Overcome Multi-Drug Resistance against Doxorubicin. *Cells* **2022**, 11 (2), 301.
17. S. Mozaffari, D. Salehi, P. Mahdipoor, et al. Design and application of hybrid cyclic-linear peptide-doxorubicin conjugates as a strategy to overcome doxorubicin resistance and toxicity. *Eur. J. Med. Chem.* **2021**, 226, 113836.
18. S. Darwish, N. Sadeghiani, S. Fong, et al. Synthesis and antiproliferative activities of doxorubicin thiol conjugates and doxorubicin-SS-cyclic peptide. *Eur. J. Med. Chem.* **2019**, 161, 594–606.
19. S.E. Park, K. Shamloo, T.A. Kristedja, et al. EDB-FN Targeted Peptide–Drug Conjugates for Use against Prostate Cancer. *Int. J. Mol. Sci*. **2019**, 20 (13), 3291.
20. H.K. Agarwal, B.S. Chhikara, M. Quiterio, G.F. Doncel, K. Parang. Synthesis and anti-HIV activities of glutamate and peptide conjugates of nucleoside reverse transcriptase inhibitors. *J. Med. Chem.* **2012**, 55 (6), 2672–2687.
21. H.K. Agarwal, K.W. Buckheit, R.W. Buckheit, K. Parang. Synthesis and anti-HIV activities of symmetrical dicarboxylate esters of dinucleoside reverse transcriptase inhibitors. *Bioorganic Med. Chem. Lett.* **2012**, 22 (17), 5451–5454.
22. H.K. Agarwal, B.S. Chhikara, G.F. Doncel, K. Parang. Synthesis and anti-HIV activities of unsymmetrical long chain dicarboxylate esters of dinucleoside reverse transcriptase inhibitors. *Bioorganic Med. Chem. Lett.* **2017**, 27 (9), 1934–1937.
23. H.K. Agarwal, B.S. Chhikara, G. Ye, et al. Synthesis and Biological Evaluation of 5′-O-Fatty Acyl Ester Derivatives of 3′-Fluoro-2′,3′-dideoxythymidine as Potential Anti-HIV Microbicides. *Molecules* **2022**, 27 (10), 3352.
24. J.A. Arnott, S.L. Planey. The influence of lipophilicity in drug discovery and design. *Expert Opin. Drug Discov.* **2012**, 7 (10), 863–875.
25. C.A. Langner, J.K. Lodge, S.J. Travis, et al. 4-Oxatetradecanoic acid is fungicidal for Cryptococcus neoformans and inhibits replication of human immunodeficiency virus I. *J. Biol. Chem.* **1992**, 267 (24), 17159–17169.
26. K. Parang, L.I. Wiebe, E.E. Knaus, et al. In vitro antiviral activities of myristic acid analogs against human immunodeficiency and hepatitis B viruses. *Antiviral Res.* **1997**, 34 (3), 75–90.
27. K. Parang, E.E. Knaus, L.I. Wiebe. Synthesis, in vitro anti-HIV activity, and biological stability of 5'- O-myristoyl analogue derivatives of 3'-fluoro-2',3'dideoxythymidine (FLT) as potential bifunctional prodrugs of FLT. *Nucleosides and Nucleotides* **1998**, 17 (6), 987–1008.
28. K. Parang, L.I. Wiebe, E.E. Knaus. Synthesis, in vitro anti-human immunodeficiency virus structure-activity relationships and biological stability of 5'-O-myristoyl analogue derivatives of 3'-azido-2',3'-dideoxythymidine (AZT) as potential prodrugs. *Antivir. Chem. Chemother.* **1998**, 9 (4), 311–323.
29. H.K. Agarwal, B.S. Chhikara, M.J. Hanley, et al. Synthesis and biological evaluation of fatty acyl ester derivatives of (-)-2′,3′-dideoxy-3′-thiacytidine. *J. Med. Chem.* **2012**, 55 (10), 4861–4871.
30. H.K. Agarwal, B.S. Chhikara, S. Bhavaraju, et al. Emtricitabine prodrugs with improved anti-hiv activity and cellular uptake. *Mol. Pharm.* **2013**, 10 (2), 467–476.
31. B.S. Chhikara, R. Tiwari, K. Parang. N-Myristoylglutamic Acid Derivative of 3'-Fluoro-3'-Deoxythymidine as an Organogel. *Tetrahedron Lett.* **2012**, 53 (39), 5335–5337.
32. T.D. McCarthy, P. Karellas, S.A. Henderson, et al. Dendrimers as drugs: Discovery and preclinical and clinical development of dendrimer-based microbicides for HIV and STI prevention. *Mol. Pharm.* **2005**, 2 (4), 312–318.
33. U.S. National Library of Medicine. Retention and duration of activity of SPL7013 (VivaGel®) after Vaginal dosing: NCT00740584. *ClinicalTrials.gov* **2012**.
34. C.J. Forbes, D. Lowry, L. Geer, et al. Non-aqueous silicone elastomer gels as a vaginal microbicide delivery system for the HIV-1 entry inhibitor maraviroc. *J. Control. Release* **2011**, 156 (2), 161–169.
35. R. Kumar, B.S. Chhikara, K. Gulia, M. Chhillar. Review of nanotheranostics for molecular mechanisms underlying psychiatric disorders and commensurate nanotherapeutics for neuropsychiatry: The mind knockout. *Nanotheranostics* **2021**, 5 (3), 288–308.
36. B.S. Chhikara. Current trends in nanomedicine and nanobiotechnology research. *J. Mater. Nanosci.* **2017**, 4 (1), 19–24.
37. B.S. Chhikara, R. Kumar, B. Rathi, S. Krishnamoorthy, A. Kumar. Prospects of Applied Nanomedicine: potential clinical and (bio)medical interventions via nanoscale research advances. *J. Mater. Nanosci.* **2016**, 3 (2), 50–56.
38. D. Soundararajan, L.N. Ramana, P. Shankaran, U.M. Krishnan. Nanoparticle-based strategies to target HIV-infected cells. *Colloids Surfaces B Biointerfaces* **2022**, 213, 112405.
39. B.K. Patel, R.H. Parikh, N. Patel. Targeted delivery of mannosylated-PLGA nanoparticles of antiretroviral drug to brain. *Int. J. Nanomedicine* **2018**, 13, 97–100.
40. R.M. Mainardes, N.M. Khalil, M.P.D. Gremião. Intranasal delivery of zidovudine by PLA and PLA-PEG blend nanoparticles. *Int. J. Pharm.* **2010**, 395 (1–2), 266–271.
41. M. Kurd, S.S. Malvajerd, S. Rezaee, M. Hamidi, K. Derakhshandeh. Oral delivery of indinavir using mPEG-PCL nanoparticles: Preparation, optimization, cellular uptake, transport and pharmacokinetic evaluation. *Artif. Cells, Nanomedicine Biotechnol.* **2019**, 47 (1), 2123–2133.
42. J. Meng, T.F. Sturgis, B.B.C. Youan. Engineering tenofovir loaded chitosan nanoparticles to maximize microbicide mucoadhesion. *Eur. J. Pharm. Sci.* **2011**, 44 (1–2), 57–67.
43. S. V. Vinogradov, L.Y. Poluektova, E. Makarov, T. Gerson, M.T. Senanayake. Nano-NRTIs: Efficient inhibitors of HIV type-1 in macrophages with a reduced mitochondrial toxicity. *Antivir. Chem. Chemother.* **2010**, 21 (1), 1–14.
44. G. T., M. E., S. T.H., et al. Nano-NRTIs demonstrate low neurotoxicity and high antiviral activity against HIV infection in the brain. *Nanomedicine Nanotechnology, Biol. Med.* **2014**, 10 (1), 177–185.
45. T. Dutta, H.B. Agashe, M. Garg, et al. Poly (propyleneimine) dendrimer based nanocontainers for targeting of efavirenz to human monocytes/macrophages in vitro. *J. Drug Target.* **2007**, 15 (1), 89–98.
46. D. Sepúlveda-Crespo, R. Lorente, M. Leal, et al. Synergistic activity profile of carbosilane dendrimer G2-STE16 in combination with other dendrimers and antiretrovirals as topical anti-HIV-1 microbicide. *Nanomedicine Nanotechnology, Biol. Med.* **2014**, 10 (3), 609–618.
47. F. Veider, Z.B. Akkuş-Dağdeviren, P. K noll, A. Bernkop-schnürch. Design of nanostructured lipid carriers and solid lipid nanoparticles for enhanced cellular uptake. *Int. J. Pharm.* **2022**, 122014.
48. N. Chattopadhyay, J. Zastre, H.-L. Wong, X.Y. Wu, R. Bendayan. Solid Lipid Nanoparticles Enhance the Delivery of the HIV Protease Inhibitor,

Atazanavir, by a Human Brain Endothelial Cell Line. *Pharm. Res.* **2008**, 25 (10), 2262–2271.
49. F. Javan, A. Vatanara, K. Azadmanesh, M. Nabi-Meibodi, M. Shakouri. Encapsulation of ritonavir in solid lipid nanoparticles: in-vitro anti-HIV-1 activity using lentiviral particles. *J. Pharm. Pharmacol.* **2017**, 69 (8), 1002–1009.
50. M. Bhalekar, P. Upadhaya, A. Madgulkar. Formulation and characterization of solid lipid nanoparticles for an anti-retroviral drug darunavir. *Appl. Nanosci.* **2017**, 7 (1–2), 47–57.
51. M. Kovochich, M.D. Marsden, J.A. Zack. Activation of Latent HIV Using Drug-Loaded Nanoparticles. *PLoS One* **2011**, 6 (4), e18270.
52. J. Desai, H. Thakkar. Mechanistic evaluation of lymphatic targeting efficiency of Atazanavir sulfate loaded lipid nanocarriers: In-vitro and in-vivo studies. *J. Drug Deliv. Sci. Technol.* **2022**, 68, 103090.
53. M. Alavi, M. Rai, R.S. Varma, M. Hamidi, M.R. Mozafari. Conventional and novel methods for the preparation of micro and nanoliposomes. *Micro Nano Bio Asp.* **2022**, 1 (1), 18–29.
54. P. Nakhaei, R. Margiana, D.O. Bokov, et al. Liposomes: Structure, biomedical applications, and stability parameters with emphasis on cholesterol. *Front. Bioeng. Biotechnol.* **2021**, 9, 705886.
55. T.M. Allen, P.R. Cullis. Liposomal drug delivery systems: From concept to clinical applications. *Adv. Drug Deliv. Rev.* **2013**, 65 (1), 36–48.
56. D. Iannazzo, A. Pistone, R. Romeo, S. Giofrè. Nanotechnology Approaches for Antiretroviral Drugs Delivery. *J. AIDS HIV Infect.* **2015**, 1 (2), 201.
57. J.F. Gagneé, A. Deésormeaux, S. Perron, M.J. Tremblay, M.G. Bergeron. Targeted delivery of indinavir to HIV-1 primary reservoirs with immunoliposomes. *Biochim. Biophys. Acta - Biomembr.* **2002**, 1558 (2), 198–210.
58. H. Wang, P. Zhao, X. Liang, et al. Construction of a novel cationic polymeric liposomes formed from PEgylated octadecyl-quaternized lysine modified chitosan/cholesterol for enhancing storage stability and cellular uptake efficiency. *Biotechnol. Bioeng.* **2010**, 106 (6), 952–962.
59. L.N. Ramana, S. Sharma, S. Sethuraman, U. Ranga, U.M. Krishnan. Investigation on the stability of saquinavir loaded liposomes: Implication on stealth, release characteristics and cytotoxicity. *Int. J. Pharm.* **2012**, 431 (1–2), 120–129.
60. S. Rojekar, L. Fotooh Abadi, R. Pai, et al. Multi-organ targeting of HIV-1 viral reservoirs with etravirine loaded nanostructured lipid carrier: An in-vivo proof of concept. *Eur. J. Pharm. Sci.* **2021**, 164, 105916.
61. E. Sánchez-López, A. Paús, I. Pérez-Pomeda, et al. Lipid vesicles loaded with an HIV-1 fusion inhibitor peptide as a potential microbicide. *Pharmaceutics* **2020**, 12 (6), 1–17.
62. S. Jain, A.K. Tiwary, B. Sapra, N.K. Jain. Formulation and evaluation of ethosomes for transdermal delivery of lamivudine. *AAPS PharmSciTech* **2007**, 8 (4), 249.
63. D.H. Surve, A.B. Jindal. Recent advances in long-acting nanoformulations for delivery of antiretroviral drugs. *J. Control. Release* **2020**, 324, 379–404.
64. E. Taki, F. Soleimani, A. Asadi, et al. Cabotegravir/Rilpivirine: the last FDA-approved drug to treat HIV. *Expert Rev. Anti. Infect. Ther.* **2022**, 1–13.
65. NCT02631473. PK of Efavirenz & Lopinavir Nano-formulations in Healthy Volunteers. *https://clinicaltrials.gov/show/NCT02631473* **2015**.
66. N.J. Liptrott, M. Giardiello, T.O. McDonald, S.P. Rannard, A. Owen. Assessment of interactions of efavirenz solid drug nanoparticles with human immunological and haematological systems. *J. Nanobiotechnology* **2018**, 16 (1), 22.
67. B.S. Chhikara, R.S. Varma. Nanochemistry and Nanocatalysis Science: Research advances and future perspectives. *J. Mater. Nanosci.* **2019**, 6 (1), 1–6.
68. M. Alavi, R.S. Varma. Phytosynthesis and modification of metal and metal oxide nanoparticles/nanocomposites for antibacterial and anticancer activities: Recent advances. *Sustain. Chem. Pharm.* **2021**, 21, 100412.
69. P. Makvandi, Z. Baghbantaraghdari, W. Zhou, et al. Gum polysaccharide/nanometal hybrid biocomposites in cancer diagnosis and therapy. *Biotechnol. Adv.* **2021**, 48.
70. M. Alavi, R.S. Varma. Antibacterial and wound healing activities of silver nanoparticles embedded in cellulose compared to other polysaccharides and protein polymers. *Cellulose* **2021**, 28 (13), 8295–8311.
71. J. Singh, S. Kumar, B. Rathi, K. Bhrara, B.S. Chhikara. Therapeutic analysis of Terminalia arjuna plant extracts in combinations with different metal nanoparticles. *J. Mater. Nanosci.* **2015**, 2 (1), 1–7.
72. S.D. Kumar, G. Singaravelu, S. Ajithkumar, et al. Mangrove-Mediated Green Synthesis of Silver Nanoparticles with High HIV-1 Reverse Transcriptase Inhibitory Potential. *J. Clust. Sci.* **2017**, 28 (1), 359–367.
73. C.H. Tsai, C.G. Whiteley, D.J. Lee. Interactions between HIV-1 protease, silver nanoparticles, and specific peptides. *J. Taiwan Inst. Chem. Eng.* **2019**, 103, 20–32.
74. H.H. Lara, L. Ixtepan-Turrent, E.N. Garza-Treviño, C. Rodriguez-Padilla. PVP-coated silver nanoparticles block the transmission of cell-free and cell-associated HIV-1 in human cervical culture. *J. Nanobiotechnology* **2010**, 8 (1), 15.
75. F. Chiodo, M. Marradi, J. Calvo, E. Yuste, S. Penadés. Glycosystems in nanotechnology: Gold glyconanoparticles as carrier for anti-HIV prodrugs. *Beilstein J. Org. Chem.* **2014**, 10, 1339–1346.
76. F. Compostella, O. Pitirollo, A. Silvestri, L. Polito. Glyco-gold nanoparticles: Synthesis and applications. *Beilstein J. Org. Chem.* **2017**, 13, 1008–1021.
77. A. Nasrolahi Shirazi, R.K. Tiwari, D. Oh, et al. Surface decorated gold nanoparticles by linear and cyclic peptides as molecular transporters. *Mol. Pharm.* **2013**, 10 (8), 3137–3151.
78. S.E. Park, M.I. Sajid, K. Parang, R.K. Tiwari. Cyclic cell-penetrating peptides as efficient intracellular drug delivery tools. *Mol. Pharm.* **2019**, 16 (9), 3727–3743.
79. N. Rabiee, M. Bagherzadeh, M. Jouyandeh, et al. Natural polymers decorated mof-mxene nanocarriers for co-delivery of Doxorubicin/pCRISPR. *ACS Appl. Bio Mater.* **2021**, 4 (6), 5106–5121.
80. V. Agostoni, T. Chalati, P. Horcajada, et al. Towards an Improved anti-HIV Activity of NRTI via Metal-Organic Frameworks Nanoparticles. *Adv. Healthc. Mater.* **2013**, 2 (12), 1630–1637.
81. F. Sharifianjazi, M. Irani, A. Esmaeilkhanian, et al. Polymer incorporated magnetic nanoparticles: Applications for magnetoresponsive targeted drug delivery. *Mater. Sci. Eng. B* **2021**, 272, 115358.
82. A. Barrios-Gumiel, D. Sepúlveda-Crespo, J.L. Jiménez, et al. Dendronized magnetic nanoparticles for HIV-1 capture and rapid diagnostic. *Colloids Surfaces B Biointerfaces* **2019**, 181, 360–368.
83. D. Salehi, S. Mozaffari, K. Zoghebi, et al. Amphiphilic cell-penetrating peptides containing natural and unnatural amino acids as drug delivery agents. *Cells* **2022**, 11 (7).
84. S. Khayyatnejad Shoushtari, K. Zoghebi, M.I. Sajid, R.K. Tiwari, K. Parang. Hybrid Cyclic-Linear Cell-Penetrating Peptides containing alternative positively charged and hydrophobic residues as molecular transporters. *Mol. Pharm.* **2021**, 18 (10), 3909–3919.
85. S.E. Hanna, S. Mozaffari, R.K. Tiwari, K. Parang. Comparative molecular transporter efficiency of cyclic peptides containing Tryptophan and Arginine residues. *ACS Omega* **2018**, 3 (11), 16281–16291.
86. A. Nasrolahi Shirazi, D. Oh, R.K. Tiwari, et al. Peptide amphiphile containing arginine and fatty acyl chains as molecular transporters. *Mol. Pharm.* **2013**, 10 (12), 4717–4727.
87. D. Mandal, A. Nasrolahi Shirazi, K. Parang. Cell-penetrating homochiral cyclic peptides as nuclear-targeting molecular transporters. *Angew. Chemie - Int. Ed.* **2011**, 50 (41), 9633–9637.
88. B.S. Chhikara, S.K. Misra, S. Bhattacharya. CNT loading into cationic cholesterol suspensions show improved DNA binding and serum stability and ability to internalize into cancer cells. *Nanotechnology* **2012**, 23 (6), 065101.
89. S.K. Misra, P. Moitra, B.S. Chhikara, P. Kondaiah, S. Bhattacharya. Loading of single-walled carbon nanotubes in cationic cholesterol

suspensions significantly improves gene transfection efficiency in serum. *J. Mater. Chem.* **2012**, 22 (16), 7985–7998.
90. A. V. Kristen, S. Ajroud-Driss, I. Conceição, et al. Patisiran, an RNAi therapeutic for the treatment of hereditary transthyretin-mediated amyloidosis. *Neurodegener. Dis. Manag.* **2019**, 9 (1), 5–23.
91. M.M. Zhang, R. Bahal, T.P. Rasmussen, J.E. Manautou, X. bo Zhong. The growth of siRNA-based therapeutics: Updated clinical studies. *Biochem. Pharmacol.* **2021**, 189.
92. S.F. Garrelfs, Y. Frishberg, S.A. Hulton, et al. Lumasiran, an RNAi Therapeutic for Primary Hyperoxaluria Type 1. *N. Engl. J. Med.* **2021**, 384 (13), 1216–1226.
93. A.J. Cupido, J.J.P. Kastelein. Inclisiran for the treatment of hypercholesterolaemia: Implications and unanswered questions from the ORION trials. *Cardiovasc. Res.* **2020**, 116 (11), E136–E139.
94. E.E. Agbosu, S. Ledger, A.D. Kelleher, J. Wen, C.L. Ahlenstiel. Targeted nanocarrier delivery of RNA therapeutics to control HIV infection. *Pharmaceutics* **2022**, 14 (7), 1352.
95. C.L. Ahlenstiel, G. Symonds, S.J. Kent, A.D. Kelleher. Block and Lock HIV Cure Strategies to Control the Latent Reservoir. *Front. Cell. Infect. Microbiol.* **2020**, 10, 424.
96. G. Vansant, A. Bruggemans, J. Janssens, Z. Debyser. Block-And-Lock Strategies to Cure HIV Infection. *Viruses* **2020**, 12 (1), 84.
97. C. Li, L. Mori, S.T. Valente. The Block-And-Lock Strategy for Human Immunodeficiency Virus Cure: Lessons Learned from Didehydro-Cortistatin A. *J. Infect. Dis.* **2021**, 223, S46–S53.
98. G. Vansant, L.S. Vranckx, I. Zurnic, et al. Impact of LEDGIN treatment during virus production on residual HIV-1 transcription. *Retrovirology* **2019**, 16 (1).
99. C. Ahlenstiel, C. Mendez, S.T.H. Lim, et al. Novel RNA duplex locks HIV-1 in a latent state via chromatin-mediated transcriptional silencing. *Mol. Ther. - Nucleic Acids* **2015**, 4 (10), e261.
100. J.J.M. Eekels, B. Berkhout. Toward a durable treatment of HIV-1 infection using RNA interference. *Prog. Mol. Biol. Transl. Sci.* **2011**, 102, 141–163.
101. Y. Weng, Q. Huang, C. Li, et al. Improved nucleic acid therapy with advanced nanoscale biotechnology. *Mol. Ther. - Nucleic Acids* **2020**, 19, 581–601.