HHS Public Access

Author manuscript

Adv Ther (Weinh). Author manuscript; available in PMC 2020 August 07.

Published in final edited form as:

Adv Ther (Weinh). 2018 October ; 1(6): . doi:10.1002/adtp.201800054.

Pharmaceutical Approaches to HIV Treatment and Prevention

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Abstract

Human immunodeficiency virus (HIV) infection continues to pose a major infectious disease threat worldwide. It is characterized by the depletion of $CD4^+$ T cells, persistent immune activation, and increased susceptibility to secondary infections. Advances in the development of antiretroviral drugs and combination antiretroviral therapy have resulted in a remarkable reduction in HIV-associated morbidity and mortality. Antiretroviral therapy (ART) leads to effective suppression of HIV replication with partial recovery of host immune system and has successfully transformed HIV infection from a fatal disease to a chronic condition. Additionally, antiretroviral drugs have shown promise for prevention in HIV pre-exposure prophylaxis and treatment as prevention. However, ART is unable to cure HIV. Other limitations include drug–drug interactions,

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Conflict of Interest
The authors declare no conflict of interest.

drug resistance, cytotoxic side effects, cost, and adherence. Alternative treatment options are being investigated to overcome these challenges including discovery of new molecules with increased anti-viral activity and development of easily administrable drug formulations. In light of the difficulties associated with current HIV treatment measures, and in the continuing absence of a cure, the prevention of new infections has also arisen as a prominent goal among efforts to curtail the worldwide HIV pandemic. In this review, the authors summarize currently available anti-HIV drugs and their combinations for treatment, new molecules under clinical development and prevention methods, and discuss drug delivery formats as well as associated challenges and alternative approaches for the future.

Keywords

antiretroviral drugs; drug delivery; HIV prevention; HIV treatment; pre exposure prophylaxis

1. Introduction

According to the World Health Organization, an estimated 37 million people were living with HIV-1 (hereafter referred to as “HIV”) infection worldwide in 2016 and around half of HIV-positive people are not aware of their status. There are about 2 million new HIV infections occurring annually. Only about 19 million HIV-infected people are currently receiving antiretroviral therapy (ART)[1] and the decline in the incidence of new HIV infections is not optimum. This can be attributed to inadequate access to ART in economically disadvantaged developing countries, lack of adherence or regular clinical monitoring during therapy, and incomplete outreach to communities. The prevalence of HIV is higher in the regions with pronounced gen der imbalance and increases risk of HIV infection among women and their children.[2] Other individuals at high risk of HIV infection worldwide include sex workers, intravenous drug users, transgender people, prisoners, and men who have sex with men (MSM).[3] In the United States, there were 1.1 million people living with HIV infection and 39 000 new HIV infections reported in 2016. A disproportionate increase in HIV infection is found among minority underserved populations including young African Americans and among people aged 13–29.[4]

Since the first isolation of HIV over 30 years ago, there has been considerable knowledge gained about the molecular structure and characteristics of the virus, its replication cycle, and pathogenic mechanisms contributing to the development of acquired immune deficiency syndrome (AIDS).[5] The major driver of viral pathogenesis is the ability of HIV to infect and kill $CD4^+$ T cells expressing co-receptor CC-chemokine receptor-5 (CCR5) or CXC receptor-4 (CXCR4) causing $CD4^+$ T cell depletion, which results in the impairment of the host immune system and its functions.[6] Following entry of the virus into the target host cell, the single-stranded HIV RNA genome is reverse transcribed into complementary DNA by reverse transcriptase (RT), transported to the nucleus, and integrated into the host genomic DNA (Figure 1). The integrated provirus can be further transcribed into viral RNA copies and translated to viral proteins. Viral particles are assembled and released from the infected cell and are positioned to infect new target cells including $CD4^+$ T cells.[7] Highly active antiretroviral therapy (HAART) which consists of combination antiretroviral therapy

(cART) can effectively suppress HIV replication by targeting multiple specific steps in the viral replication cycle (fusion, reverse transcription, integration, and/or maturation).[8] Advances in HIV research have dramatically improved our understanding of HIV pathogenesis and led to successful efforts in enhancing HIV treatment and prevention resulting in a reduced number of AIDS-related deaths. In the last 2 years, the number of people on HAART has increased by 30%, totaling 19.0 million.[9] However, several limitations in HAART prevent complete eradication of HIV. While HAART suppresses the virus, latent viral reservoirs in lymphoid tissues remain hidden from the immune system and are not eradicated.[10] Inter-ruption of HAART results in a rapid rebound of HIV from latent viral reservoirs.[11] The requirement for lifelong administration of HAART presents a constant challenge for financial affordability and patient compliance and management of secondary effects. New approaches are needed for developing less toxic antiretroviral drugs (ARVs) and therapeutic regimens consisting of decreased dosage and frequency.

In this review, we present current and cutting-edge HIV prevention and therapeutic regimens, as well as the advantages and limitations of the various approaches and delivery formats. We provide brief historical background on successes and failures in HIV treatment and prevention, as well as a summary of future directions in treatment and prevention efforts aimed at diminishing the HIV pandemic.

2. HIV Treatment

2.1. Challenges in Current Treatment

Current HIV treatment relies heavily on the use of a combination of ARVs, which effectively reduces AIDS-associated morbidity and prolongs lifespan of HIV-infected patients. There are more than 25 ARVs available that encompass multiple different mechanisms of viral inhibition (Table 1) and target different steps in the HIV replication cycle (Figure 1).[12] These anti-HIV drugs can be divided in six groups based on their mechanism of action[13] (Table 1).

Success of HIV treatment and management has been guided by the availability of cART that uses a combination of two nucleoside/nucleotide reverse transcriptase inhibitors (RTIs) and a drug with different mechanism of action.[15] In 2006, the FDA approved the first “one-pill-daily” tablet Atripla that combined efavirenz (600 mg), emtricitabine (200 mg), and tenofovir disoproxil fumarate (TDF, 300 mg).[16] Atripla has been used as a first line HIV treatment for years until the U.S. Department of Health and Human Services (DHHS) made some changes in 2015 and recommended an alternative treatment with reduced side effects. Current, first line therapeutic regimen includes Stribild (single tablet FDA approved in 2012 as a combination of elvitegravir [EVG, 150 mg]/cobicistat [150 mg]/emtricitabine [200 mg]/TDF [300 mg]),[17] Triumeq (FDA approved in 2014) that combines abacavir (600 mg)/dolutegravir (50 mg)/lamivudine (300 mg) in a single once-daily tablet,[18] and Genvoya (FDA approved in 2015) with EVG (150 mg), cobicistat (150 mg), emtricitabine (200 mg), and tenofovir alafenamide (TAF, 10 mg).[19] How-ever, ART is unable to fully restore immune health in all HIV infected patients. Researchers from Denmark and France have reported that overall life expectancy has significantly increased for HIV^+ patients receiving HAART, but it was still lower compared to the HIV-seronegative population.[20] Despite

suppressive cART, HIV-infected patients may have incomplete recovery of $CD4^+$ T cell numbers and incomplete resolution of chronic immune activation and experience an increased prevalence of non AIDS diseases such as cancer, osteoporosis, and cardiovascular diseases.[21]

Drug toxicity and the development of drug resistance are major limitations of cART. ARVs usually target either viral or cellular proteins. While drugs directly acting on viral proteins are more specific and have lower toxicity, drug resistance can emerge. Drugs targeting host cellular proteins have a broader spectrum of action and might be less likely to promote development of drug resistance, but tend to have higher toxicity compared to other drugs.[22] The International AIDS Society-USA (IAS-USA) publishes an update on drug resistance mutations in HIV for HIV clinicians.[23] Identification of new viral mutations every year demonstrates limitations of cART. Common side effects include metabolic and liver disorders, muscular dystrophy, peripheral neuropathy,[24] and neurocognitive impairment.[25] Other side effects include constipation and fever that may contribute to the reduced patient compliance and viral rebound. cART can also lead to undesirable drug–drug interactions that might reduce its efficacy. A combination of nevirapine and saquinavir (SQV) presents an example of adverse drug–drug interactions, where nevirapine induced liver cytochrome p450 increasing metabolization and elimination of SQV.[26]

One of the major challenges in ART is the dosing route, as most ARVs have poor solubility and bioavailability. Orally applied solid dosage forms are the most common way to administer ARVs (Figure 2) although they suffer from significant hepatic first-pass effects, and variable absorption and degradation due to enzymes and extreme pH conditions in the gastrointestinal tract, leading to low bioavailability. Frequent dosing (at least once daily) is required as a result of the short half-life of some ARVs, which may cause reduced patient compliance.[27] Furthermore, ARVs may not reliably reach high levels in tissues through the lymphatic system or in the brain across the blood–brain barrier (BBB).[28] Thus, conventional ART fails to target the HIV anatomical (i.e., lymphatic system, central nervous system [CNS], reproductive tract, liver, and lungs) and cellular (i.e., $CD4^+$ T lymphocytes and monocytes, etc.) reservoirs and increases the risk of relapse.[29] Better drug delivery systems and development of new drug molecules with high anti-viral potency and longer half-life would enhance the success of HIV treatment.

2.2. New Drugs in Clinical Development

New drug development is focused on minimizing drugassociated toxicity and reducing drug resistance profiles either within the existing ARV classes or with novel molecules with different mechanisms of action, including entry/fusion inhibitors and maturation inhibitors.[15] There are several newdrugs that belong to the previously described ARV classes. Doravirine is a NNRTI that is currently in Phase III clinical evaluation as a single-drug tablet and as part of a doravirine/lamivudine/tenofovir combination tablet.[30] Despite doravirine resistance observed for viruses with the K103N and Y181C mutations in RT, the drug has lower toxicity than efavirenz.[31] Cabotegravir (CAB) is an integrase inhibitor that is in Phase IIb for HIV treatment and Phase IIb/III for HIV prevention. It was reported as orally bioactive with a long halflife.[32] Fostemsavir (BMS-663068) belongs to a relatively newer

class known as small-molecule attachment inhibitors (AIs) that bind to gp120, stabilizing a conformation of the viral protein that is unable to recognize host cell CD4 receptors, thus preventing viral entry into the cell; the drug is in Phase III clinical assessment as a later-line therapeutic for heavily treatment experienced (HTE) patients with multidrug-resistant HIV.[33]

For some of these new drugs, long-acting (LA) formulations to be administered as injections are being investigated to improve problems with low drug adherence. The combination of LA CAB and LA rilpivirine (RPV), a non-nucleoside RTI, as intramuscular (IM) injections has progressed to Phase III clinical trials for treatment of HIV-1-infected adults. The most recent study is ATLAS 2M, following up ATLAS, which seeks to compare the efficacy and safety of the combination of CAB LA and RPV LA injections at 4 and 8 week intervals over a 48 week timespan. The estimated date to complete this study is 2022.[34]

Broadly neutralizing antibodies (bNAbs), such as 3BNC117 and VRC01, bind to the HIV envelope (Env) and are among the new candidates for HIV treatment, since they can bind and eliminate infectious viral particles and infected cells. This is also discussed in the context of HIV prevention in Sections 3.1 and 3.2.3. These antibodies (Abs) have been advanced to Phase 1 trials and showed significant HIV suppression, however, there are concerns regarding the development of resistance.[35] Though still in pre-clinical development and testing phase, other potent bNAbs and especially combinations thereof (which will help to combat the selection of strains that are resistant to single bNAbs), are envisioned as future options in HIV treatment (also see Section 3.2.2–*Microbicide Candidates* for the application of bNAbs in HIV prevention).[36] BMS-936559, an anti-PD-L1 antibody, has been studied in Phase I trials and reported to enhance HIV-1–specific immunity in healthy HIV-1-infected subjects.[37] Pro-140 (developed by CytoDyn) is also an antibody that belongs to the entry and fusion inhibitor class and is currently in Phase IIb/III safety and efficacy trials (since August 2016), administered as a weekly subcutaneous injection of 350 mg. Pro-140 acts by binding to the CCR5 co-receptor on the surface of immune cells and blocking HIV's ability to infect target cells. Designated a "fast track" product candidate by the FDA, pending success of an ongoing clinical trial, Pro-140 may become the second FDA-approved monoclonal antibody (mAb) therapeutic for HIV,[38] joining the recently approved anti-CD4 mAb ibalizumab (brand name Trogarzo).[39] Bispecific antibodies have also been designed to have two arms that attach to both the target and the effector cells. Bispecific T-cell engagers (BiTEs) and dual-affinity re-targeting (DART) were developed for cancer treatment then redesigned to tackle HIV-1 treatment. HIV-specific BiTEs and DARTs have shown promising results in vitro, however more research is required for evaluation of safety and efficacy of the molecules.[40]

2.3. Delivery Strategies for Anti-HIV Molecules

Current HIV treatments have several shortcomings such as drug resistance, drug–drug interactions, and biological barriers that prevent drug access to potential target tissues. Other drug-related challenges include low solubility, low bioavailability, premature elimination, short shelf life, and off-target side effects. Furthermore, plasma drug concentrations in therapeutic dose ranges are difficult to meet due to the short half-life of these drugs.[41]

Phar-maceutical enhancers (boosters) like ritonavir (RTV) and cobicistat (Tybost, FDA approved in 2012) have been used to increase the bioavailability of PIs by inhibiting cytochrome P450 (CYP) 3A enzymes.[42] However, cobicistat interacts with many drugs and can cause life threatening side effects. In addition, there are still questions regarding whether the boosting mechanism itself causes serious long-term side effects.[43]

These issues are being resolved either by discovering new active molecules or targets for HIV treatment, or by improving the delivery of currently available molecules. Several novel drug delivery systems have been tested for delivering anti-HIV agents in order to address the series of concerns:[44]

- Increase bioavailability
- Decrease metabolization/elimination
- Reduce undesired side effects
- Improvestabilityandshelflife of drug molecules
- Reduce dosing frequency and improve patient compliance
- Maintain therapeutic drug level and prevent fluctuation
- Improve drug penetration to CNS
- Target cells selectively

In this section, we discuss potential targets for anti-HIV drugs, drug targeting strategies, and drug delivery systems that have been studied to enhance HIV treatment.

2.3.1. Targeting Molecules for Drug Delivery—Drug targeting is a relatively a new approach to help optimize the therapeutic index of a drug (efficacy window) by localizing the drug to the site of action. Targeting can help with reducing dosing and related toxicity while increasing the efficacy of the drug. HIV infects immune cells such as macrophages, dendritic cells (DCs), and $CD4^+$ T cells[27b] and these cells should be targeted by HIV therapies.[45]

Targeting anti-HIV drugs to the brain is also a major goal since HIV can migrate and localize in the CNS. The BBB limits ARV delivery to brain due to epithelial tight junctions and the efflux transporters on its surface. Neuronal tissue has bradykinin type II (B2) receptors that are important targets for drug delivery to the CNS. One of the most popular approaches to deliver drugs to the brain involves the use of nanoparticles that can cross the BBB via endocytosis/phagocytosis, coupled with their ability to evade clearance through the efflux systems. Also, the transferrin receptor is an important target to deliver drugs to the CNS since it is highly expressed in the BBB endothelium.[46] Furthermore, the efficiency of nanoparticles in delivering drugs to the BBB can be enhanced with an electromagnetic field, however long-term effects of change in permeability may be detrimental.[47]

Another approach is to target virus (guest) moieties such as envelope glycoproteins gp41 and gp120 regions that are responsible for binding to the $CD4^+$ T cells. Targeting these proteins increases the specificity of the therapeutic approach, but may drive the high mutation rate of

HIV creating additional challenges.[48] Following the viral infection, envelope gp120 is exposed on the surface of HIV-infected cells, creating a favorable target to deliver drugs specifically to infected cells. Functionalized liposomes and nanoparticles have been studied for targeting HIV proteins and they were reported to bind gp120.[49] Aptamers were suggested as alternatives to the Abs in order to bind specifically to the target molecules, since they are easier to formulate with their smaller structures.[50] Similar to gp120, gp41 is also a potential target moiety on HIV-infected cells; hence, there have been several studies using anti-gp41antibodies to target HIV-infected cells.[51] Radioactive labeled gp41 Abs were used to target HIV-infected cells in vivo and results showed that the radioactive molecule killed the targeted cells and significantly reduced the HIV load.[52]

The alternative approach to guest targeting is to seek out the host cells, which include HIV-infected cells with unique cellular markers. The most relevant host markers so far are CD4, chemokine receptors (CXCR4 and CCR5), leukocyte function–associated antigen (LFA-1), human leukocyte antigen—antigen D related (HLA-DR), DC-specific ICAM-3-grabbing nonintegrin (DC-SIGN), tuftsin, and carbohydrate binding antigens.[27b] The CD4 and chemokine receptors are expressed on macrophages, T lymphocytes, monocytes, and DCs, and play a role in HIV entry into cells and are utilized for targeting infected cells.[27b] LFA-1 is present in macrophages, neutrophils, and T and B lymphocytes and its expression is increased during HIV infection. Anti-LFA-1 had a double benefit, as it inhibited viral replication while specifically targeting HIV-infected cells.[27b] The HLA-DR is a major histocompatibility complex (MHC) class II protein that is expressed in DCs, macrophages, and B cells. It is an attractive target since it serves as an identifying marker on HIV reservoir cells and attracts $CD4^+$ T helper cells.[53] Liposomes and other nanocarriers have been used to target HLA-DR delivering ARVs to HIV-infected cells and reduce viral spreading.[54] DC-SIGN is a C-type lectin that binds HIV and is one of the major targets for prevention as well as treatment.[55] Cyclodextrin-based glycoconjugates showed high affinity for DC-SIGN and prevented binding to gp120.[56] There are several sugar moieties on the surface of HIV-infected cells that can be targeted by “carbohydrate binding agents.” Mannan and mannose receptors are among the receptors on monocytes and macrophages and may be potential targets for prophylactic protection or to increase the uptake efficiency of anti-HIV drugs. Mannosyl and galactosyl receptors on macrophages have been used for drug targeting via surface modified nanoparticles. Mannosylated gelatin nanoparticles were used for didanosine delivery and showed that ex vivo macrophage uptake was significantly higher when compared to free drug and uncoated nanoparticles.[57] Tuftsin is an immunoglobulin G derivative that binds and activates macrophages and DCs and is a potential target. However, tuftsin targeting is still unexplored— other than reports of tuftsin-dendrimer conjugates for efavirenz delivery.[58] Transferrin, low-density lipoprotein, and aptamers are some of the other potential targets that are subjects of studies for HIV treatment. Increased phagocytic activity of macrophages during HIV infection was also used for passive targeting of drugs such as indinavir, zidovudine, and didanosine to infected cells.[59] Furthermore, RNA interference (RNAi) technology is being developed to silence newly identified targets.[60]

Pharmaceutical scientists have been working on a variety of novel drug delivery systems for delivering anti-HIV molecules to the target moieties that were discussed above. We focus on examples of these carriers in Section 2.3.2.

2.3.2. Novel Drug Delivery Systems for Anti-HIV Molecules—Novel drug carrier systems like microparticles, nanoparticles, liposomes, dendrimers, implants, and drug reservoirs have been studied to overcome the challenges of conventional HIV treatment and prevention. Different delivery routes including longacting injectable formulations were also considered due to the shortcomings of oral delivery of anti-HIV drugs.[61] Sustained transdermal drug delivery can improve bioavailability by overcoming the issues like intestinal absorption, the hepatic first-pass effect, and drug metabolism in the gastrointestinal (GI) track. Percutaneous absorption of ARVs has also been studied and reported to be a promising administration route.[44a,62] A schematic of anti-HIV delivery approaches is presented in Figure 3.

Microparticle carriers have been used for taste masking or controlled drug delivery of ARVs, to reduce dosing frequency and maintain drug plasma levels.[63] Bioadhesive microparticles were designed to prolong retention time of the drugs in the gastrointestinal track and increase drug absorption.[64] Bioadhesive and pH sensitive microparticles have also been studied for vaginal delivery of microbicides for HIV prevention.[65] However there are also some limitations related to the microparticles, such as aggregation and difficulties in handling and storage.[66] Microparticle carriers that have been tested for ARVs are summarized in Table 2.

Nanoparticle carriers are solid colloidal particles that range between 1 to 1 000 nm in size and are able to encapsulate drugs. *Solid lipid nanoparticles (SLNs)* are also nano-sized particulate carriers that consist of biodegradable/biocompatible lipids (such as cetyl palmitate and myristic acid salts) that are solid at physiological temperatures.[81] Both types of nanoparticles have large surface areas that help increase the biological half-lives of drug molecules and reduce dosing frequency. In addition, nanoparticles can encapsulate significant amounts of drugs and improve drug release kinetics,[82] can be internalized by lymphocytes due to their small size and serve as drug depots,[83] and have surfaces that can be modified to target specific cells to increase efficacy and reduce side effects.[84] Nanosystems such as nanoparticles, SLNs, nanosuspensions, dendrimers, and liposomes can help drugs cross the BBB, enter the lymphatic system, or internalize in cells and improve anti-HIV drug delivery efficiency. Advantages of nanoparticles include reproducibility in processing, multiple drug loading (combination therapy), and drug stability.[85] Certain types of nanoparticles can also reverse drug resistance, such as overcoming P-glycoprotein effux in drug-resistant tumors.[86] Most nanoparticle studies have been aimed at targeting cells of the mononuclear phagocytic system or brain, however, several studies have focused on increasing bioavailability of the drugs. We summarize studies that used nanoparticles to improve HIV treatment and the reported outcomes in Table 3.

Dendrimeric systems have also been explored for anti-HIV drug delivery. Dendrimers are synthetic monodisperse macromolecules with unique tree-like architectures that present several functional end groups for modifications and drug loading.[110] These structures are also called “nanocontainers” since they entrap molecules like a box. Due to their favorable properties, they have been used for controlled release of ARVs as well as targeting by surface modifications. Dendrimers themselves have also been reported as anti-HIV

therapeutic agents.[111] Studies using dendrimers for HIV treatment are summarized in Table 4.

Liposomes are phospholipid-based vesicular carriers (80–100 nm) with a unique structure that can encapsulate both hydrophilic and hydrophobic drugs. Liposomes are easy to surface modify to improve properties or targeting to a specific cell or tissue. Also, PEG-conjugated liposomes are able to avoid recognition by the reticuloendothelial system.[82] Despite disadvantages like poor stability and low encapsulation efficiency, liposomes have been investigated extensively for sustained delivery and targeting of ARVs; various drugs, prodrugs, and conjugates have been encapsulated and tested in vitro and in vivo. Examples of these studies are summarized in Table 5.

Biological carriers are used for drug delivery and represent a novel approach and consist of viral vectors, erythrocytes, and macrophages.[44b] HIV-infected macrophages reside in tissues that may not be fully penetrant for ARVs. Based on this idea, a macrophage-based carrier system for indinavir was described.[133] The numbers of HIV-infected cells in peripheral blood, liver, lymph nodes, and spleen were reduced in HIV-1-infected humanized mice. A similar carrier system with the incorporation of Lipoid E80 was prepared and used for sustained indinavir release from macrophages.[134] Chimeric RNA nanoparticles with gp120 binding aptamer showed specific binding to the HIV gp120 expressing cells and internalization to block viral infectivity.[135] In addition, recombinant adeno-associated viral (rAAV) vectors are being actively investigated and developed as vehicles of gene therapy, wherein the rAAV is essentially a protein-based nanoparticle capable of delivering DNA either systemically or to specifically targeted cells (such as skeletal muscle), with consequent expression of a therapeutic gene product.[136] rAAVs are in the developmental and pre-clinical testing phase for gene transfer of anti-HIV bNAbs, with the goal of achieving stable long-term transgene expression and secretion of bNAbs, both for HIV treatment and prevention purposes.[137] If successful, rAAV-mediated antibody gene transfer could eliminate the need for repeated passive infusions of bNAbs in HIV treatment and prevention, by creating a constant, long-term source of therapeutic levels of potent bNAbs in the human body.

Other novel approaches: In a recent study, albumin-polymerdrug conjugates have been designed to prolong drug residence time and increase lymphatic accumulation of the ARV drugs. Albumin was conjugated to *N*-(2-hydroxypropyl) methacrylamide (PHPMA) and the conjugate was combined with azidothymidine and lamivudine through copolymerization. The results of in vivo studies showed that albumin-PHPMA-ARV conjugates are able to deliver potent drugs and drug combinations with an increased biodistribution profile.[138] Another study evaluated transdermal films composed of ethyl cellulose and HPMC for delivery of a potent NNRTI IQP-0410 to prevent extensive first-pass metabolism the drug was subject to.[139] The films were found promising car-riers based on in vitro studies, however no in vivo studies were conducted to show the efficacy of the system.

Despite decades of research, discovery of new targets and studies on numerous novel formulations, none of these approaches has yet reached the clinic. However some of these approaches have already demonstrated success in in vivo animal experiments and are

currently being further investigated in animal models prior to entering clinical trials.[140] As the search goes on for an alternative treatment for better life quality, there has been a substantial new investment in HIV *cure* research from governments, foundations, and industry in the past decade. Clearly there are still challenges ahead and much work is needed but if the scientific success of targeting HIV latency can be brought to clinic effectively, an HIV cure may replace lifelong ART.[141]

2.4. Targeting HIV Latency

As noted, cART effectively suppresses the virus but does not eradicate it. In particular, HIV provirus exists in so-called reservoirs, where it reactivates from latency if treatment is suspended, allowing viral rebound.[142] Latency upon HIV infection has been linked to several causes, including insufficient or mutated HIV Tat.[143]

The main latent viral reservoir is generally believed to reside in resting memory $CD4^+$ T cells.[144] However, T follicular helper cells (T_{fh}) have also been proposed as a major celular reservoir,[145] and non-T cell reservoirs have also been suggested.[146] The cells comprising the reservoir can be found in many areas of the body, particularly the peripheral blood, lymph nodes, and the gut.[147] An additional subject of debate is whether low-level replication continues during cART,[148] leading to the possibility that both latency and low-level replication contribute to HIV persistence.

Elimination of the HIV reservoir is among the highest priorities in HIV research, since removal of this obstacle could lead to a cure. Several strategies have been proposed to eliminate the reservoir. The most studied has been the so-called “shock and kill” strategy, [144c,149] in which a drug is added to latently HIV infected cells that allows activation of transcription (the “shock”), leading to active replication of the HIV genome within the cell. In vivo, this would in turn lead to cellular responses that would allow recognition by cytotoxic immune cells, possibly with the help of additional immune activators, mediating elimination of the infected cell (the “kill”). A second strategy to eliminate the reservoir is to “lock” cells into their latently infected state so that HIV is not transcribed and never re-emerges, even if cART is suspended or stopped completely. Some recent success has been shown for this latter “locking” technique.[150] Finally, the DART strategy has shown success by covalently pairing an antigp120 antibody fragment with an immune activating antibody fragment. This molecule binds the gp120 on the surface of an infected cell and brings it into proximity to an activated immune cell for elimination. However, most DART approaches must be combined with latency-reversing agents (LRAs) because latently infected cells have little or no gp120 on their surface.[151]

Several strategies have evolved for “shock and kill,” with probably the most work being reported on histone deacetylase inhibitors (HDACi). These small molecules are effective reactivating agents, and indeed several different HDACi do activate HIV transcription in cells, including vorinostat, panobinostat, and romidepsin. However, these did not reduce the viral reservoir,[152]and it is possible that the dosages required for low toxicity limit their effectiveness to activate the cells. Other strategies are also being tested as LRAs, including agonists of protein kinase C (PKC);[153] agonists of Toll-like receptor;[154] and immune checkpoint blockade antibodies.[155] These have shown promise, and in particular it has

been shown that PKC agonists are able to reactivate cells with several different models of latency.[155] Clinical trials are ongoing with several of these.[156] Finally, HIV Trans Activator of Transcription (Tat) has been shown to reverse latency in HIV reservoirs, either added as a protein or expressed within a cell,[157] although these studies did not target Tat to particular cells; Tat is able to enter cells nonspecifically but is toxic at high concentrations.[158]

3. HIV Prevention

For HIV prevention and treatment to be effective at the population level, individuals need to adhere to the drug usage and dosing regimens in a consistent manner. This poses a great challenge, as demonstrated by the outcomes of many clinical trials that have reported low adherence as shown by low plasma or local drug concentrations. Inconsistent use lowers the apparent efficacy of the drug, which can worsen patient outcomes. There is increasing interest in how users perceive products, termed “user sensory perceptions and experiences,” and how this correlates with adherence to product use.[159] These are important considerations for improving acceptability and increasing the efficacy of products for HIV prevention. New systems are needed that are effective, affordable, and convenient to improve user consistency in HIV prevention.

3.1. HIV Vaccines

An effective HIV vaccine would alleviate the burden placed on users to properly and consistently use a product over time to maintain protection. However, despite significant efforts in the past decades, the development of a successful HIV-1 vaccine remains elusive. Correlates of immune protection for HIV vaccines have not been fully defined and variability in HIV genomes present challenges for HIV.[160] Most vaccines allow infection in the portal of entry, but the immune system subsequently neutralizes the infection before it spreads systemically. HIV integrates into the host’s genome, and can exist latently for long periods of time without being detected by the immune system. Once HIV has entered the systemic circulation, viral reservoirs are established quickly in lymphoid tissues and prohibit complete viral eradication despite long-term ART. Therefore, much of the research effort has focused on preventing HIV entry.[160b,161]

The majority of new HIV infections occur via sexual transmission[162] through mucosal tissue in the genital and rectal areas. Thus, mucosal immunity is an important consideration in rational vaccine design, as it is the first line of defense for the body. Mucosal vaccines have been explored, but thus far have not been shown to work better than systematic vaccines. It is thought that a combination of a systematic and mucosal vaccine may result in increased protection compared to either one alone.[163] Mucosa-associated lymphoid tissue (MALT) are the sites where mucosal vaccines can be administered, as this tissue is functionally connected throughout the body.[163] For instance, nasal-associated lymphoid tissue and gut-associated lymphoid tissue are potential sites for induction. However, not all sites provide the same immunity, due to unique local environments of the mucosa.[164] Both human and non-human primate (NHP) studies have identified immune correlates of mucosal protection. These correlates involve both the cellular and humoral arms of the immune

system, so the mechanism is not fully clear.[163] NHP and other models have been used to study mucosal vaccines and have shown some success. One study in sheep showed successful elicitation of high levels of antigen-specific mucosal IgA and large amounts of local antigen-reactive B cells after intramuscular injection and prolonged intravaginal administration (via ring) of 167 μg of both CN54gp140 protein and R848 adjuvant.[165] In a mouse study, they demonstrated that immunization with recombinant influenza–HIV vectors via combination intranasal and intravaginal administration resulted in localization of HIV-specific tissue-resident memory CD8 T cells in the vaginal mucosa.[166] Another study involving rhesus macaques, published in 2018, showed that combination adenovirus and protein vaccines were effective in increasing protection in intrarectal challenges.[167] After three intrarectal challenges, all the ten controls were infected, while only three and four out of ten were infected in the two respective vaccinated macaque groups. These studies show some success resulting from mucosal vaccination in non-human models. Mucosal vaccines are in the early stages of human clinical trials, which will give important information about how their safety and elicited immune response differs in humans compared to other models.

Systemic vaccines are further along in development and testing. To date, the most successful vaccine trial has been the Phase III HIV-1 vaccine trial RV144 in Thailand where a 31.2% efficacy in preventing HIV-1 infection compared to placebo was reported.[168] The vaccine was composed of a recombinant canarypox vector vaccine (ALVAC) and a recombinant gp120 subunit vaccine (AIDSVAX B/E). ALVAC was given at weeks 0 and 4. ALVAC/AIDSVAX B/E was given as two booster injections at weeks 12 and 24. Building on the partial success of this study, there is now a Phase IIb/III vaccine trial HVTN 702 in South Africa in which the vaccine regimen in RV144 has been modified. The HVTN 702 vaccine will consist of ALVAC and a two component gp120 subunit vaccine, modified to be specific for HIV-1 subtype C.[169] Additionally, the vaccine includes the adjuvant MF59, which is different from that used in RV144, to induce a greater immune response. The results from this trial are expected in 2020 or 2021.

As mentioned earlier, combining systematic vaccines with other prevention modalities could increase overall protection. We have already discussed the rationale for pairing mucosal vaccines with systematic vaccines. Along a similar line of thinking, combination with ARVs (oral pre-exposure prophylaxis [PrEP] or microbicides) could be useful. It is important to provide protection at the portal of entry to reduce acquisition of the virus. A macaque study demonstrated vaccine and microbicide combinations increase protection against transmission.[170] Two separate experiments were performed, in which either the fusion inhibitor T 1249 or CCR5 inhibitor maraviroc (MVC), applied vaginally at a partially protective concentration, was paired with a T cell–based adenovirus (Ad) vectored vaccine (Ad26/Ad5HVR48 expressing Gag-Pol-Env-Nef) delivered intramuscularly. The T-1249 experiment was the first to be done, and it was paired with a vaccine aimed primarily at reducing post-infection viral loads, thus not expected to prevent infection. This resulted in only two of the six animals in the combination vaccine and microbicide group remaining uninfected following high-dose vaginal challenge with SIVmac251. In the infected vaccinated animals, viral loads remained about tenfold lower than in the control group, with the best outcome observed with the vaccine–microbicide combination. When the vaginal MVC gel was tested in combination with a more potent Ad vaccine (Ad35/Ad26 expressing

Gag-Pol-Env) delivered intramuscularly, increased efficacy was observed against high-dose vaginal challenge with SHIV-SF162P3 with intervention efficacies of 43% and 67% for the MVC only and combination MVC plus vaccine groups, respectively. In the infected animals, post-infection viral loads were also significantly reduced with better virologic control obtained with MVC plus vaccine than with MVC only. In both experiments, the combination of vaccine and microbicide provided the best outcome.[170] In another NHP study, the combination of a partially effective dose of 1% tenofovir vaginal gel with an intranasally and intramuscularly administered Env-based vaccine was tested against 12 consecutive low-dose SHIV-SF162P3 vaginal challenges.[171] The vaccine alone provided no protection, and even resulted in a greater infection rate than the control group. The tenofovir group had a 45% and 68% reduction in risk of infection compared to the control and vaccine groups, respectively. Whereas in the combination vaccine–microbicide group 79% and 88% reduction in the per-exposure risk of infection was observed compared to the control and vaccine groups, respectively. Additionally, the protected animals from the combination group were challenged another 12 times afterward, in the absence of the microbicide. The total risk reduction over the 24 exposures for this group was 91%. These studies showed that combining a microbicide with a vaccine provides increased levels of protection compared to either alone.[170,171]

3.2. Pre-Exposure Prophylaxis

In the absence of an effective HIV vaccine, PrEP has emerged as a viable option to help curtail the spread of the virus in the global HIV/AIDS pandemic.[172] PrEP is an HIV prevention strategy wherein HIV-negative individuals ingest (*oral* PrEP), apply topically (*microbicide*), or inject (*injectable* PrEP) anti-HIV compounds *prior* to exposure to the virus. These compounds become locally or systemically distributed throughout the human body and can reduce the risk of infection, preventing viral dissemination.

3.2.1. Oral Systemic PrEP—ARV-based oral PrEP has been shown to be effective.[172a–c,173]The first FDA-approved drug for oral PrEP is Truvada, which contains two NRTIs, tenofovir and emtricitabine. Clinical trials have primarily been conducted with tenofovir alone or with tenofovir and emtricitabine combined.[172a–c] The Phase III iPrEx trial in six countries with 3 324 MSM participants concluded that the combination of TDF and emtricitabine (FTC) resulted in a 44% reduction of HIV infection rate compared to the placebo.[172a] Higher efficacy was observed in users that were more adherent. In Botswana, the Phase III clinical trial TDF2 studied the effectiveness of TDF-FTC (F/TDF) taken once daily in 1219 heterosexual men and women. It showed an efficacy of 62.2%.[172c] The Partners PrEP Phase III clinical trial in Kenya and Uganda examined the efficacy of TDF alone and F/TDF in 4747 heterosexual serodiscordant (in which only one partner has HIV) couples. TDF alone showed a 67% reduction in HIV transmission compared to the placebo, whereas, F/TDF had a 75% reduction.[172b] The FDA-approved Truvada for use in 2012.[174] The number of people taking Truvada in the United States has greatly increased between 2012 and 2016, by about six times the original number.[175]

Recently, considering the negative effects of Truvada on bone mineral density and the renal system, there has been optimism about emtricitabine combined with TAF, called F/TAF, as

an alternative. TAF has been shown to be more stable and potent, thus it requires a lower dose and has lessened side effects compared to TDF.[176] There are two ongoing clinical trials of F/TAF. One of the trials is CONRAD 137, which is Phase I and has 72 women enrolled. They are studying the pharmacological properties and bioavailability of F/TAF[177] using two different dosages, 200/10 mg and 200/25 mg, with Truvada (F/TDF) 200/300 mg as a control. The other ongoing study is the Phase III DISCOVER trial. It has 5400 enrollees from the MSM and transgender women (TGW) who have sex with men populations.[178] DIS-COVER is testing the difference in seroconversion rates between F/TDF and F/TAF.

Besides F/TDF and F/TAF, other drugs with various mechanisms of action have been investigated for oral PrEP use. Raltegravir (RAL), which is an integrase inhibitor, was shown to be effective in preventing infection in humanized mice challenged vaginally with HIV. An ongoing clinical trial (NCT03205566) is testing the capability of RAL to protect against HIV challenge ex vivo.[179] MVC, a CCR5 antagonist, was also tested in the same humanized mouse model and found to be effective in preventing infection.[180] However, results differed when tested in both NHPs and humans. In one study, macaques were challenged weekly with SHIV rectally.[181] Twenty-four hours before challenge and 2 h post-challenge, the macaques were given a 44 mg kg−1 MVC dose orally (higher than human dose, but equivalent due to faster drug elimination in small mammals). Even though rectal concentrations of MVC were much higher than the concentration needed to block SHIV in vitro, five out of six macaques became infected during the five weekly challenges. While three out of four controls became infected under the same challenge conditions. Additionally, despite high, sustained concentrations of MVC in a human trial consisting of 54 healthy people given a 300 mg MVC dose, no protection was observed against ex vivo challenge of vaginal or rectal biopsies.[182]

3.2.2. Topical PrEP—In addition to oral dosing to prevent or reduce transmission at the mucosal portal of viral entry, anti-HIV drugs have also been formulated for topical vaginal and/or rectal application.

Microbicide Candidates.: A wide range of compounds have been proposed and tested as potential microbicides to prevent HIV infection at sites of mucosal transmission. Although some of the earliest microbicides failed to show adequate protection in humans,[183] there are many promising candidates currently in the pre-clinical study stage and in clinical trials.[184] Micro bicides fall into several different categories including: vaginal milieu (pH) protectors, surfactants, polyanionic polymers, small molecules (entry inhibitors), RTIs, integrase inhibitors (IIs), protease inhibitors (PIs), proteins, and peptides. Vaginal milieu protectors, surfactants, and polyanions are collectively termed “nonspecific agents” given their mechanisms of action in preventing viral transmission: milieu (pH) protectors help to maintain normal vaginal acidity (pH [2264] 5), creating an unfavorable environment for HIV infection,[185] and serving to help destroy bacterial species that cause bacterial vaginosis (dysbiosis); vaginal dysbiosis is associated with increased HIV transmission susceptibility in women.[186] Polyanions (negatively charged) interact with the positively charged V3 loop of HIV-1 gp120, disrupting its interaction with cell receptors,[187] while surfactants solubilize the membranes of bacteria and enveloped viruses (such as HIV), rendering them inactive.

[184d] However, in the earlier stages of vaginal microbicide research, numerous clinical trials (including through the Phase III stage) were conducted with these nonspecific agents, demonstrating either a lack of efficacy in preventing viral transmission[184b] or, in the case of the spermicidal surfactant Nonoxynol-9 (N-9), actual *increased* risk of HIV infection in users due to inflammatory responses in the cervicovaginal mucosa, including ulcer formation.[188]

Following the lack of success with nonspecific agents, the focus shifted to the development of formulations containing ARV-based compounds as microbicides. RTIs such as tenofovir (and its prodrug form, TDF) and dapivirine (DPV) have emerged at the forefront of successful microbicidal device formulation and development.[184b] In addition, the small molecule HIV entry inhibitor, MVC, is also seeing increased inclusion in microbicide devices. Although MVC on its own has shown limited efficacy in pre-clinical studies[189] it is a promising component in combinatorial strategies.[190] Other small molecule entry inhibitors in development include BMS-378806 and its derivatives, which act by binding gp120 and preventing a conformational change that is necessary for its interaction with CD4.[191] Other small-molecule ARVs in development as microbicides include the NNRTI UC781,[192] several protease inhibitors (PIs, including SQV, darunavir [DRV], lopinavir [LPV], and RTV)[193] and several integrase inhibitors (IIs, including RAL, EVG, and L-870 812).[194] Presentation of clinical studies involving these compounds is given in the proceeding sections for the particular formulation formats in which they have been tested.

In addition to small-molecule compounds, biomolecular (protein and peptide) microbicides have also made progress through the microbicide development pipeline.[184d] These particular microbicide candidates typically function as entry inhibitors by binding to Env or to HIV co-receptors. These biomolecular microbicides typically exhibit low systemic absorption, low toxicity, and reduced side effects.[195] Carbohydrate binding proteins (CBPs, also known as “lectins”) such as scytovirin, cyanovirin, and griffithsin, recognize glycans on the surface of HIV-1 gp120 and are endowed with remarkable anti-HIV activity against a broad range of subtypes and strains.[196] Among these lectins, the homodimeric protein Griffithsin (“Grft”), derived from the red algae *Griffithsia* sp., has emerged as a potent (at subnanomolar concentrations) and broad-spectrum anti-viral microbicidal candidate[197] that can be produced inexpensively in large quantities.[198]

Other prime candidate protein microbicides include analogs of the chemokine RANTES that potently block CCR5, the primary co-receptor used by HIV to enter and infect human cells.[199] Like Griffithsin, 5P12-RANTES (5P12R) can also be produced inexpensively in large quantities,[200] and has a favorable stability profile over a wide pH range and at elevated temperatures for certain periods of time, as well as demonstrated stability in the presence of both human semen and cervicovaginal lavage.[201] Peptides that mimic HIV gp41 and act as potent fusion inhibitors of the virus, have found use in HIV treatment (such as T-20/Enfuvirtide, see Table 1 above) and several are also being investigated for use as microbicides, including RC-101, T20, T1249, C34, and L’644.[202] It is also noteworthy that chimerization of both 5P12R and Grft, with C-type peptide fusion inhibitors has produced microbicidal candidates with even greater potency and breadth of viral strains inhibited than that exhibited by any single “parent” component,[203] demonstrating the amenability of

biomolecular candidates to even greater optimization as microbicides. In addition, some monoclonal antibodies (mAbs) isolated from HIV-positive individuals have demonstrated potent and broadly neutralizing activities against HIV, that have led to their inclusion and development as microbicide candidates, including antibodies such as: b12, 2F5, 2G12, 4E10 (these four are “firstgeneration” bNAbs)[204] and VRC01, 3BNC117, and 10–1074 (all “second-generation” antibodies), for instance.[205] In addition to VRC01, 3BNC117, and 10–1074, other generations of more potent (compared to “first-generation”) bNAbs have been discovered in recent years (including the PG and PGT series[204,206]) and combinations of these have been proven to be remarkably potent and broadly acting.[36b,207] These can be envisioned as possible options in HIV prevention or treatment. Many mAbs can now be expressed via recombinant DNA modes and produced in large quantities.[208] The status of pertinent pre-clinical studies or clinical trials for these biomolecular microbicides is given below under the relevant delivery formats in which they have been evaluated.

Topical Vaginal and Rectal Microbicidal Delivery Systems.: Equally important as the microbicidal agents themselves is consideration of the vehicles by which they will be delivered. Important properties of a delivery system include: microbicide stability within the format, dispersal of the compound from the system, duration of drug release and dosage, impact of the formulation on the mucosal environment and vice versa for mucosal safety, as well as user acceptability of the format. Below, we describe some of the main delivery systems studied to date for topical PrEP and also highlight some of the benefits and limitations of each format (also summarized in Table 6).

Vaginal and Rectal Gels.: The first and earliest microbicides to be evaluated were formulated as gels intended for daily use. Indeed, semi-solid gels have been the most common and prolific formulation format for delivery of HIV microbicides.[210] These gels are semi-solid polymeric matrix formulations generally composed of cellulose derivatives (such as hydroxyethylcellulose [HEC] or cross-linked polyacrylic acid [Carbopol] polymers), with the active compound usually being dispersed throughout the interstitial liquid phase.[211] Topical vaginal (or rectal) gels have the advantages of being easily administered by the user (though an applicator may be required), low cost of production, rapid dispersal of the active compound to coat the mucosal compartment (conferring protection either immediately or within a few hours of application), and attainment of higher localized drug concentrations than those achieved through systemic approaches. On the other hand, limitations include the vulnerability of active compounds in semi-solid formulations to extreme conditions of temperature and humidity, and the necessity for pre and/or peri-coital dosing; the self-cleansing and flushing nature of the orifices in question results in rapid removal of the active compound from the site of application, thus necessitating more frequent application of the microbicide. Another significant disadvantage of conventional gels is the leakage and potential discomfort for the user.[159b,209a,b]

Following several failed clinical trials involving gel formulations of “nonspecific agent” microbicides, the first positive results in microbicide clinical trials came from the NRTI tenofovir formulated as a 1% gel in HEC. The CAPRISA-004 (Center for the AIDS Program of Research in South Africa) Phase IIb efficacy trial was conducted from May, 2007 to

March, 2010 among women (ages 18–40) in South Africa with a dosing regimen called "BAT24"—one application of the gel within 12 h *B*efore intercourse, followed by a second dose within 12 h *A*fter, with no more than *T*wo doses in 24 h.[212] The tenofovir gel used in this study reduced HIV infection by 39% overall, and by 54% in women with higher adherence (>80%) to the dosing regimen. Contemporary to CAPRISA-004, another Phase IIb safety and efficacy trial was conducted in sub-Saharan Africa, sponsored by the Microbicide Trials Network (MTN-003)—VOICE (Vaginal and Oral Interventions to Control the Epidemic)—which comprised arms evaluating the efficacy of oral PrEP (Truvada) and of 1% tenofovir gel (with a once daily coitally independent dosing). The results of this trial concluded that the interventions were ineffective against HIV infection due to poor adherence among participants; in fact, the gel arm of the study was prematurely terminated in 2011 due to futility.[213] A Phase III safety and efficacy trial, FACTS-001 (Follow-on African Consortium for Tenofovir Studies), commenced in 2011 in South Africa to evaluate the 1% tenofovir gel with the same "BAT24" dosing regimen as had been employed in CAPRISA-004; the FACTS-001 study concluded that participants were not using the microbicide gel, but that there was correlation between higher user adherence to the dosing regimen and stronger protection against the virus.[214] Thus, user adherence to dosing regimens (and increasing this adherence through better microbicide delivery device design) is of critical importance in the success and efficacy of these microbicides.

In addition, a 1% tenofovir gel has also been evaluated in clinical studies for rectal application. Topical rectal microbicides were initially based upon formulations for the vaginal tract. However, the rate of progress in this particular field is expected to increase due to recognition of fundamental physicochemical differences between the vagina and rectum, with consequent re-formulations specifically for the rectal tract.[215] A topical 1% tenofovir gel for-mulated for rectal application has successfully passed through Phase I safety and pharmacokinetics evaluations in the United States (the MTN-007 and CHARM-01 trials), [216] as well as an extended safety Phase II trial (MTN-017) among international participants.[217] The NNRTI UC781 formulated as a gel has been successfully evaluated in a Phase I safety and acceptability trial as a rectal microbicide (RMP-01/MTN-006),[218] and UC781 micro-bicide gel also retained anti-HIV activity in cervicovaginal lavage fluids collected from women in a Phase I clinical safety trial.[192] Poor solubility and stability issues of the ARV DPV stymied early efforts in its formulation and development as a topical gel, however, micronization of the active compound has allowed its successful formulation in this particular delivery format including its progression into Phase II clinical trials.[209e] Importantly, both UC781 and DPV were abandoned as orally administered drugs for HIV treatment due to their poor systemic absorption, and thus as microbicides, would be orthogonal to drugs currently used in HAART.

Due to the short retention time of gel formulations at the site of application, efforts have been made to increase formula retention and drug residence times through addition of mucoadhesive polymers such as chitosan, carrageenan, or sodium alginate[219]. More recent developments also include expansion of the gel delivery format to include biomolecular microbicides, such as the lectin Griffithsin, the CCR5-targeted HIV entry inhibitor 5P12 RANTES, and anti-HIV bNAbs including VRC01, to name a few. Following successful pre-clinical studies in animal models,[220]a Phase I clinical trial (sponsored by the Population

Council) is currently recruiting participants for safety and pharmacokinetics evaluation of a vaginal Griffithsin gel in women (ClinicalTrials.gov Identifier: NCT02875119, with expected completion date in late 2018), and evaluations of a rectal Griffithsin gel are ongoing as part of PREVENT ("*Pre*-exposure prevention of *v*iral *ent*ry"), an integrated pre-clinical/clinical program. The proteinbased HIV entry inhibitor 5P12-RANTES (5P12R) has recently undergone further successful pre-clinical evaluation as a vaginal gel in sheep[221] and has also shown stability in human rectal lavage.[222] According to the Mintaka Foundation, human clinical trials involving the 5P12-RANTES microbicide are expected to begin soon in Geneva, Switzerland. In addition to these examples, pre-clinical studies of the gel-formulated bNAb VRC01 demonstrated protection against HIV vaginal challenge in a humanized mouse model.[205] There has also been a Phase 1 clinical safety and pharmacokinetics trial of a gel (MABGEL) containing a cocktail of three different bNAbs (2F5, 4E10, and 2G12), demonstrating the safety of a daily dosing regimen for a duration of 12 days, as well as potentially sufficient concentrations of bNAbs to block HIV infection.[223]

Vaginal Tablets and Films.: While gels were initially the pharmaceutical dosage form of choice for topical microbicides, due to certain drawbacks of this particular format, other dosage forms also emerged as viable candidates, including vaginal tablets and films. Vaginal tablets offer advantages of precise dosing, easy storage, handling, and application, as well as low cost and stability under different environmental conditions.[210] Conventional fast-dissolving tablets have been formulated containing a number of the same ARV-type microbicides as have been formulated in gels.[224] As in the case of gels, efforts have also been made to prolong the retention time of vaginal tablets and their contained active compounds at the site of application, through inclusion of mucoadhesive polymers in the formulation;[219c] inclusion of a combination of different polymers (hydroxypropylmethyl cellulose and chitosan) has helped to create a tenofovir-releasing tablet that remains adhered to the vaginal mucosa for 96 h, with sustained release of the drug for 72 h.[225] Another more recent development in vaginal tablets and an example of a multipurpose prevention technology (MPT), is the design of a multi-layered tablet capable of simultaneously releasing (at independent rates) the NNRTI DPV, the contraceptive hormone levonorgestrel, and the anti-herpes simplex 2 (HSV-2) drug, acyclovir.[226]

Vaginal films are small, thin sheets of water-soluble polymers that dissolve when placed in contact with the vaginal mucosa, quickly releasing the active compound. Like the tablet format, vaginal films also provide consistent dosage, ease of storage, handling, and application, low unit cost, and improved stability of actives under more extreme temperature and humidity conditions;[227] one drawback of the film format is low overall mass, limiting the amount of an active compound that can be loaded into a single dose. Vaginal quick-dissolve films are usually composed of cellulose derivatives or polyvinyl alcohol.[210] As with other dosage forms, ARVs have also been incorporated into films, including the NNRTI DPV;[228] this film was the subject of a Phase I safety and pharmacokinetics trial (FAME-02) demonstrating safety of the device, but produced much lower effective concentrations of the drug in situ relative to what can be achieved by dapivirine in the gel format.[209e] Polymeric vaginal films containing different dual combinations of ARVs

(tenofovir, MVC, and DPV) are also under development; these cellulose/polyvinyl alcohol–based films have demonstrated stability of the contained active compounds for 12 months at ambient temperature.[229]

In the production of conventional vaginal films, once an active compound has been incorporated into the film polymer base, the drying process may occur at room temperature or under vacuum, allowing inclusion of more labile types of drugs in this format, including biomolecules. Developments include formulation of peptide RC-101 (a retrocyclin analog that targets gp41) in a polyvinyl–alcohol vaginal film,[202b] and also formulation of the bNAb VRC01 combined with the anti-HSV mAb HSV8-N into a vaginal film; this latter device will be evaluated in a Phase I safety and pharmacokinetics clinical trial that is currently recruiting participants, with anticipated trial completion later in 2018.[230] In addition to conventional film formulations, development has begun more recently of vaginal and rectal suppository films composed of silk fibroin (SF) from the *Bombyx mori* silkworm; encapsulation of protein-based microbicides in the SF film format has demonstrated outstanding stabilization of the active compounds (protein microbicides Griffithsin [Grft], 5P12 RANTES [5P12R], and their chimerized variants Grft-linker-C37 and 5P12R-linker-C37), as well as the capacity for sustained release (over the course of 1 month) of Griffithsin.[231] Note that silk fibroin is a highly versatile substance for therapeutic delivery that can also be processed and fashioned into other delivery formats than films, including nanoparticles and electrospun fibers (Table 6), injectable hydrogels and microspheres, implantable tubes and porous scaffolds, and microneedles for transdermal delivery of therapeutics.[232]

Vaginal Rings.: In contrast to vaginal gels, tablets, and films, vaginal rings are insertable devices intended to be worn continuously and are capable of providing sustained and controlled release of an active (microbicidal) compound over the course of several weeks or months, and are thus coitally independent. Early development of vaginal ring technology (beginning in the 1970s) focused primarily on delivery of steroidal compounds for contraception and hormone replacement therapy.[233] Conven-tional vaginal rings (VRs) are comprised of hydrophobic elastomeric polymers such as silicone (polydimethylsiloxane, PDMS) or poly(ethylene-vinyl acetate) copolymer (PEVA), with the simplest VR designs either containing the drug homogeneously dispersed throughout the matrix system, or else located in a drugloaded central core that is covered by a non-medicated polymer membrane.[234]

Sustained release of a microbicide from a silicone elastomer VR was first demonstrated for the first-generation nonionic surfactant-type compound Nonoxynol-9 (N9),[235] however, further development of this device was abandoned following results of clinical evaluations of the gel-formulated active compound that revealed the unsuitability of N9 for mucosal use. Subsequent development of VRs focused on incorporation of ARVs, such as the NNRTI DPV[236] and the NRTI tenofovir.[237] Importantly, conven-tional VRs typically require high (130–190 °C) processing temperatures to create polymeric elastomer devices; fortunately, many of the ARVs examined thus far (including MVC, tenofovir, and NNRTIs UC781 and DPV) have been sufficiently stable to survive temperatures of ≈170 °C for several minutes while the drug is being incorporated into the polymer melt.[234a]

Thus far, DPV is the only candidate microbicide that has been tested in humans—successfully through the Phase III clinical trial stage–in the vaginal ring delivery format. Two Phase III clinical trials were conducted among women (ages 18–45) in subSaharan Africa, involving once-monthly placement of a ring containing 25 mg of DPV, and completed in 2015: “The Ring Study” (IPM-027, sponsored by the International Partnership for Microbicides) and “ASPIRE” (“A Study to Prevent Infection with a Ring for Extended use,” also known as MTN-020 and conducted by the Microbicide Trials Network). The Ring Study (IPM-027) was successful, reporting a 31% reduction in HIV acquisition among participants.[238] The larger ASPIRE study reported an overall 21% reduction in viral transmission, as well as a significant difference in adherence and accompanying protection between age groups, with an adherence rate of over 70% and a 56% protection rate among women older than 21, and low adherence and no protection conferred in the 18–21-year-old group of women.[239] Building upon the results of the ASPIRE trial, in 2016 the NIH announced plans to move forward with an *o*pen label extension study of the DPV ring, “MTN-025–HOPE” (*H*IV *O*pen label *P*revention *E*xtension), wherein ASPIRE participants have been offered the opportunity to continue ring use until the trial’s completion date later in 2018; the study will collect data on safety and user adherence and acceptability of the DPV ring. A similar open-label extension trial for participants of “The Ring Study,” named “DREAM” (IPM-032), has also begun in subSaharan Africa. Preliminary results from the Phase 3b “HOPE” trial (presented at the 2018 Conference on Retroviruses and Opportunistic Infections [CROI]) indicate that nearly 90% of participants are using the monthly ring at least some of the time, suggesting that adherence within the HOPE trial is roughly 16% higher than it was in the earlier ASPIRE trial, and the incidence rate of HIV-1 infection among consistent users has been half that expected for ring non-users.[240] Final results from the HOPE trial are expected in 2019. Similar preliminary results have likewise been reported for the “DREAM” trial.[241]

In addition to single microbicide component VRs, combination ARV microbicidal rings are in current development and testing, including a segmented polyurethane ring for combined delivery of the NNRTI DPV and NRTI tenofovir,[242] a silicone elastomer ring containing DPV and small-molecule CCR5-targeted HIV entry inhibitor, MVC,[243] and a matrix-type silicone elastomer ring containing DPV and the protease inhibitor (PI) DRV.[244] The DPV/MVC (25 mg DPV 100 mg MVC) combination ring was the subject of a Phase I safety and pharmacokinetics evaluation in the United States (MTN-013/IPM-026 trial); though the devices tested proved safe and effective inhibitory concentrations of the DPV component were released, it was determined that insufficient amounts of the MVC component were released, requiring further development and improvement of this device prior to any future reevaluation.[245] It is noteworthy that MTN-013/IPM-026 was the first clinical trial of a vaginal microbicide containing two ARVs, and each possessing a different mechanism of HIV interference. Following the combinatorial drug approach employed in HAART,[246] modern and next-generation microbicide candidates and formulations are likely to increasingly contain combinations of compounds that inhibit HIV via different mechanisms and/or at different stages of the viral lifecycle, with activity against a broader range of HIV isolates and less likelihood of favoring the selection and development of drugresistant strains.[190,247]

Additional VR delivery systems are in development and testing for release of biologic microbicides. Due to factors including permeability constraints and the lack of thermal stability of the active at typical processing temperatures required during device manufacture, conventional VRs are not useful for the delivery of biomacromolecules such as proteins, peptides, and nucleic acids.[234b] Such factors have led to the development of novel ring designs, including complex multi-component systems in which the polymer ring form serves as a platform or a holder, for alternative solid dosage forms that are inserted as rods/pods into the ring base; the rods/pods are often directly compressed tablets of lyophilized biomolecule packed with various excipients, and may be coated with semi-permeable polymer, such as polylactic acid (PLA).[234a] It should be noted that this type of processing of the active compound does not involve high temperatures, and the combination of excipients and freeze–drying can serve to stabilize the active component against degradation.[248] The drug pods/inserts are incorporated into the elastomeric polymer rings and the drug is released through a small delivery window in the ring, with release rates of the active being determined by the window diameter, the total number of pods inserted into the ring, etc. Additionally, while the ring form acts as a retainer for the inserts, other microbicides (such as ARVs) could also be loaded into and released from this portion of the device. At present, VRs-releasing protein-based microbicides are still in the pre-clinical and development phases, however, promising results include demonstration of sustained release of two different anti-HIV microbicidal peptides (T-1249 and “JNJ”) over 28 days from a silicone elastomer ring with the actives packed in rod inserts,[249] and also sustained release of the bNAb 2F5 over many days from the insert vaginal ring (“InVR”) design.[250] More recently, sustained controlled release over 21 days of the bNAb VRC01 was demonstrated from intravaginal rings of the pod design (e.g., “pod-IVRs”) in a macaque model, with release rates of up to several milligrams bNAb per day.[251]

Nanoparticles and Electrospun Fibers.: Nanoparticle (NP) delivery systems for microbicides involve attachment of anti-viral molecules to particles made of inorganic material (i.e., a noble metal, such as silver or gold) or comprised of a wide array of cross-linked, biodegradable, and biocompatible polymers, such as polycaprolactone (PCL) and poly(lactic-co-glycolic acid) (PLGA).[252] Advantages of this type of delivery system include protection and stabilization of the active compound(s) against degradation, enhanced solubilization of the attached drugs, and due to their small size, the ability to penetrate through cervicovaginal mucous to reach HIV susceptible sites, as well as internalization in cells to allow intracellular drug delivery.[253] The focus on polymeric nanoparticles as microbicide delivery vehicles is rather recent, but one of the earlier examples was the formulation of PSC-RANTES (an anti-HIV protein and derivative of chemokine RANTES) in co-polymer PLGA nanoparticles, which exhibited sustained release of bioactive PSC-RANTES in vitro over 30 days. It was also shown that the tissue uptake of PSC-RANTES in nanoparticles in ex vivo ectocervical explants was fivefold greater than that of unformulated PSC RANTES, which became distributed and remained only at the superficial epithelial layer, in contrast to the deeper permeation afforded by the NP formulation.[254] Several ARVs have also been formulated into NPs and incorporated into gel and film dosage formats.[255] Several other pertinent examples of nanodelivery systems for HIV microbicides have been detailed in recent reviews.[253,256]

Like nanoparticles, electrospun fibers (EFs) are also a relatively new proposed platform for delivery of HIV microbicides. EFs are polymer fibers produced by electrospinning, in which electrostatic forces are applied to polymer solutions (such as polyglycolic acid, PLA, PCL, or PLGA) to produce dry spun fibers of the polymer.[257] Fibers can be loaded with drug during the spinning process, and the release of drug can be controlled through degradation and erosion of the polymer with subsequent diffu sion of the active compound; the composition and preparation of fibers can be tuned to effect desired release kinetics, including more sustained release (ranging from hours to weeks).[258] Several small-molecule ARV drugs have been formulated in electrospun fibers and evaluated in in vitro assays.[259] Recently, the biomolecular microbicide Griffithsin has also been developed in this format as Griffithsin-coated PLGA electrospun fibers; the Grft-EFs demonstrated efficacy against HIV and were shown to be non-toxic to vaginal epithelial cells in vitro.[260]

Live Microbicides.: An intriguing area of development in anti HIV microbicide delivery systems is the live microbicide approach, wherein commensal bacterial species (such as *Lactobacil lus* or *Bifidobacterium* genera, which are common among human vaginal microbiota[261]) are genetically engineered to produce and secrete biomolecular microbicides, such as anti-HIV proteins or peptides. When administered orally or topically, these agents of mucosal drug delivery work by colonization of the intended host compartment with expression and secretion of therapeutics in situ, thereby potentially providing protection for days, weeks, months, or even longer.[262]

A prime example of this approach has been the successful engineering of a vaginal *Lactobacillus jensenii* strain expressing the anti-HIV lectin Cyanovirin (CV-N).[263] Vaginal inoculation of macaques with this engineered strain resulted in colonization and secretion of CV-N for at least 6 weeks after administration and produced a 63% reduction in infection upon repeated vaginal challenge with SHIV; the presence of the engineered *Lacto bacilli* and continued secretion of CV-N evidenced no induction of inflammation in the animals.[264] A subsequent study evaluating the safety and toxicity of this live microbicide in macaques recapitulated its safety and also suggested that the *Lactobacilli* them selves may *positively* impact the vaginal mucosal environment.[265] This engineered *Lactobacillus* strain has also been evaluated in an in vitro human vaginal and cervical cell colonization model; similar to the macaque model, bioengineered *L. jensenii* was able to deliver bioactive microbicidal CV-N protein without inducing cellular toxicity or alterations in levels of biomarkers of inflammation.[266] Thus far, an engineered strain (a.k.a. Muco-Cept, Osel Inc.) of *L. jensenii* expressing modified CV-N (mCV-N) has been formulated as rapidly disintegrating vaginal tablets that support sustained vaginal colonization comparable to that observed when macaques were inoculated with freshly prepared culture; vaginal tablet administration resulted in colonization in 66% of macaques at 14 days post-dosing and 83% after 21 days, and the prototype tablets showed stability and potency of the active compound for about 1 month at 37 °C and for about 1 year at 25 °C.[267] It is also interesting to note that, when formulated as a yogurt product and fed to macaques, engineered *L. jensenii* CV-N expression could be detected in rectal lavage specimens up to 7 days after cessation of feeding, and the viral replication levels were reduced by 20-fold in rectal biopsies challenged ex vivo with SHIV. However, complete inhibition of viral infection and

replication was not achieved and no animals were colonized by the recombinant bacteria,[268] possibly owing to the fact that *L. jensenii* is not a prolific native species in the GI tract,[269] whereas it is so in the vagina.[270]

Other developments in the field of live microbicides include engineering of *L. jensenii* to express and secrete the anti-HIV chemokine protein RANTES as well as C1C5-RANTES, a variant that acts as a CCR5 co-receptor antagonist and lacks proinflammatory activity; both *Lactobacilli*-expressed proteins were shown in vitro to inhibit HIV infection of $CD4^+$ T cells and macrophages.[271] *L. jensenii* has also been successfully engineered to secrete or display two-domain CD4 (2D-CD4) proteins that have demonstrated inhibition of virus infectivity of HeLa cells.[272] *Escherichia coli* have also been engineered to express a fusion peptide of the single-chain variable fragment (scFv) of the VRC01 bNAb (scFv–VRC01) together with the autotransporter β-barrel domain of IgAP gene from *Neisseria gonorrhoeae*, thus enabling display of the Ab on the surface of the bacterium; the engineered bacteria were demonstrated to capture HIV particles via surface binding and inhibit HIV infection in cell culture.[273] Most recently, *L. jensenii* was engineered to express and secrete broadly neutralizing single chain and single-domain antibodies (dAbs); these bacterially secreted biotherapeutics demonstrated effective inhibition of infection in single-round TZM-bl assays against a broad range of viral strains.[274] Although microbicidesecreting probiotics are still controversial, and though this type of anti-HIV delivery strategy will require long-term clinical data clearly demonstrating safety and efficacy, the concept of live microbicides is gaining publicity and acceptance.[184e] If successful, these next-generation probiotics have the potential to serve as a sustained, self-replicating delivery system of biomolecular microbicides to combat infection by HIV-1 and potentially other enveloped viruses.

In addition to bacterial strains that have been genetically modified to express and deliver protein microbicides, it is noteworthy that some native (non-engineered) bacterial strains themselves also exhibit inherent microbicidal activity against HIV and other sexually transmitted viruses.[186b,275] In a recent study, six different *Lactobacillus* strains (including two different strains each of *Lactobacillus crispatus*, *Lactobacillus gasseri*, and *Lactobacillus vagi nalis*) were shown to inhibit HIV-1 replication in human tissues ex vivo; mechanistically, this inhibition was attributed to a combination of milieu acidification and l-lactic acid production, as well as the ability of *Lactobacilli* to actually adsorb HIV-1 onto their surfaces, thus decreasing the total number of free virions in the system.[209j] Thus, administration of probiotic *Lactobacilli*—either topically or orally (as orally administered probiotics have been shown to reach the vagina in human studies)[276] could po-tentially constitute an effective adjunct to other current microbicides. Indeed, addressing and amending the vaginal microbiome may even prove to be a necessary antecedent to the efficacious function of certain existent microbicides—as in the case of recently published work done within the context of the CAPRISA 004 trial, demonstrating that vaginal bacteria modulated the efficacy of the microbicide tenofovir gel, with detectable mucosal levels of tenofovir being much lower in women whose vaginal microbiota were not *Lactobacillus* dominated.[277]

3.2.3. Long-Acting Injectable PrEP—In contrast to other PrEP modalities such as daily oral pills or topical gels, requiring frequent administration by the user, longacting (LA)

injectable PrEP offers the advantage of reduced dosing frequency (i.e., once every 2 months) with the attendant potential to increase user adherence and thus, overall efficacy in preventing HIV transmission. All LA injectable anti-HIV agents currently in development require parenteral administration—or, injections delivered via subcutaneous (SC), intramuscular (IM), or intravenous (IV) routes, and are intended to provide sustained drug levels in fluids (serum, plasma) and relevant (vaginal, rectal, penile) tissues.[278] Limitations of this approach include differential acceptability of (potentially painful) needle injections by diverse populations, as well as issues surrounding administration of injections (and the requirement for clinical visits and infrastructure), particularly for those given via the IV or IM routes.[278]

Several long-acting ARV candidates are currently under development as injectables for HIV prevention, all with common attributes of high potency (requiring lower drug doses for efficacy) and favorable pharmacokinetics including relatively long plasma half-lives in the injectable form[279] In addition to its development for HIV treatment,[32] the investigational integrase inhibitor drug CAB is in late-stage clinical development as an every-other-monthly injection for HIV prevention. Formulated as a long-acting injectable nanosuspension ("CAB LA"),[280] CAB is entering into two Phase III clinical trials (HPTN-083 and HPTN-084) to evaluate the safety and efficacy of 600 mg gluteal IM injections administered every 8 weeks, with the first two injections given 4 weeks apart; completion of the studies and final results are expected in 2022. Aside from administration via traditional parenteral routes, there are also LA PrEP delivery methods in pre-clinical development that include biodegradable implants (which would not require terminal device removal) as well as those that are refillable.[281] For instance, the NRTI TAF was formulated in a thin-film polymer device (TFPD) as a biodegradable subcutaneous implant, with a thin-film PCL membrane to control drug release from a reservoir; in vitro studies of the prototypes demonstrated successful linear drug release at 1.2 mg per day (a potentially therapeutically relevant dosage) for up to 90 days.[281a]

In addition to long-acting injectable small-molecule ARVs, the injection of potent, bNAbs is an effective protective measure against HIV infection. This is one option for prevention, albeit expensive. Compared to the cost of the oral PrEP drug combination Truvada (and particularly with recent FDA approval of a generic version), the cost of biologics such as bNAbs greatly exceeds this amount, and though expression in plant-based systems[208] holds the promise of reducing production costs of these biologics, consideration of the sheer volume of individuals currently infected with HIV and needing treatment (19 million worldwide) and the tens of millions more at risk of HIV acquisition and needing PrEP, suggests that these biologics are unlikely to become a globally widespread, mainstream component of anti-HIV efforts. In the absence of an effective vaccine, injecting Abs gives a person almost immediate protection, but for limited periods of time. Still, the protection afforded by these injections requires less frequent dosing (as compared to a daily oral pill). In the absence of an effective vaccine, injecting Abs gives a person almost immediate protection, but for limited periods of time. At present, in addition to the requirement of a cold chain for transport and storage, these Abs (such as VRC01) are primarily being admin istered intravenously, which tends to necessitate certain clinical infrastructure and visits by

the user to clinical personnel for dosing (as opposed to the convenience of self-administered IM or SC injections).[279]

Different Abs have been tested in vitro and in vivo for efficacy and safety. An early study showed the anti-V3 domain Ab, Cβ1, protected in vivo when administered intravenously in chimpanzee challenge studies.[282] In another study, they compared the bNAbs VRC01, 10E8, and PG9, which target different regions of gp120, and an anti-CD4 Ab, 2D5, in a mucosal challenge of rhesus macaques with SHIV. VRC01, 10E8, and PG9 bind to the CD4 binding site (CD4bs) in gp120, MPER, and V1V2 glycan regions, respectively. All of the anti-gp120 antibodies were completely protected in the challenge, whereas the anti-CD4 Ab did not provide effective protection. This study suggested that targeting the HIV Env was preferable over targeting the cell surface receptor CD4.[283]

Injection of anti-HIV bNAbs prior to challenge has proven effective in preventing HIV infection. It is of interest to see how long this protection lasts after the injection. One study tested four bNAbs, VRC01, VRC01-LS (containing the mutations M428L and N343S in the Fc domain of VRC01), 3BNC117, and 10–1074, against the clade B SHIV AD8 strain. All of these Abs target the CD4bs on gp120, except 10–1074, which targets the N332 glycan on gp120. Macaques were given a single injection of one of the Abs at a concentration of 20 mg kg^{-1} 1 week prior to intrarectal challenge and were subsequently challenged each week thereafter until the macaque became infected. Control animals became infected in a median of three challenges. In contrast, VRC01, VRC01-LS, 3BNC117, and 10–1074 injected animals became infected after a median of 8, 14.5, 13, and 12.5 challenges, respectively.[284] This study is one of the pieces of evidence show-ing that injection of bNAbs is effective in preventing HIV infection for a significant length of time. In addition, the strategy of introducing mutations in the Fc domains of therapeutic Ab candidates has produced versions of the Abs (such as the VRC01 LS variant) with extended in vivo serum half-lives, potentially expanding the duration of immunoprophylactic coverage provided by these agents.[285] The combination of extended in vivo half-lives of bNAbs, coupled with greater potency (as achieved by the administration of bNAb cocktails rather than single agents) could enable the development of Ab-based HIV prevention products with less frequent dosing requirements (e.g., once every 6 months) that could be administered by subcutaneous injection.[279] The SI route of administration could potentially facilitate self-injections by users via a conventional syringe/needle (as used for routine injections in diabetes treatment), and also invites the possibility of painless delivery via patches of microneedles, which are micron-width needles fabricated from silicon or polymers into arrays that are capable of piercing the skin without stimulating proprioceptive nerves (e.g., averting pain associated with typical large needle insertions) and can deliver a wide array of therapeutics;[286] this new micro-technology is already in early clinical development for dermal vaccine delivery.[287]

VRC01 seems especially promising as an injectable Ab. Structurally, VRC01 somewhat mimics CD4 in binding to the conserved region of the CD4 binding site of gp120, as found with a crystal structure. However, VRC01 binds both CD4-bound and non-CD4-bound conformations (in the absence of CD4) of gp120 with high affinity, unlike CD4 and most other Abs against this region (with the notable exception of b12).[288] VRC01 is entering

into two international Phase 2b clinical trials, HVTN 703/HPTN 081 and HVTN 704/HPTN 085, known as the AMP (Ab-mediated prevention) Studies, in which it will be administered intravenously every 8 weeks (over the course of 72 weeks total) to test its safety and efficacy in reducing HIV transmission.[289] Though single bNAb agents are currently being tested in human clinical trials, it is envisioned that *combinations* of bNAbs (with different epitope specificities against gp120) will prove to have the greatest breadth and potency in preventing HIV transmission.[207,290]

4. Future Directions in HIV Prevention and Treatment

HIV prevention efforts include the use of many drugs and delivery systems. Because users have varying preferences, access to a multitude of product options may improve user consistency and compliance. Multiple products can be also used simultaneously for more comprehensive protection against HIV. Individual product improvement will also help to increase the efficacy of the products and user acceptability.

Regarding HIV vaccine development, the unique combination of characteristics of HIV continues to be a barrier to successful vaccine design. There was some success with the RV144 clinical trial, which demonstrated 31.2% efficacy,[168] and upon which follow-up trials are being pursued, including Phase IIb/III vaccine trial HVTN 702.[169] The knowledge gained from these subsequent trials will broaden our understanding of what contributes to the efficacy of an HIV vaccine. Potent bNAbs against Env will continue to help guide development efforts, however, many combinations of antigens over time still need to be screened in order to optimize the speed and potency of the immune response. Mucosal vaccines are also being investigated further to potentially provide greater protection at the portals of entry. Additionally, combining vaccines with microbicides has been shown to increase protection more than by solely the sum of their efficacies in NHP studies.[170,171] This is an area that will continue to be explored to achieve greater prevention. In the interim, direct injections of long-acting, potent, bNAbs (whose formation, a successful vaccine would illicit) are a promising development in HIV prevention that is rapidly progressing through clinical phase trials.

Also, in the field of HIV prevention, the combination of multiple anti-HIV compounds is of particular interest in the microbicide development pipeline. This approach allows to broaden protection by combining different compounds that act via different mechanisms and at different stages of the viral replication cycle. This approach can potentially lower individual doses of each compound, thus reducing side effects, help provide protection against a broader range of HIV isolates including ARV-resistant mutants and decrease the likelihood of the development of drug resistance against any single compound. This combinatorial strategy applies not only to small-molecule (ARV-type) compounds, but also to protein-based microbicides; for instance, the inhibitor Griffithsin can be combined with other microbicidal proteins, as well as with small-molecule inhibitors.[291]

In addition, one particularly salient point that emerged from the many afore-mentioned clinical trials of microbicides is the importance of user acceptability and adherence to required dosing regimens.[292] Individuals must be offered a *variety* of dosage forms and

delivery formats to meet the varied needs and preferences of a diverse global population. To this end, novel dosage forms have been and will continue to be explored and developed. In addition, there is need for, and ongoing development of dosage forms with less rigorous and frequent dosing regimens for individuals. Clinical trials are increasingly expanding their scope to include examination of issues such as user adherence and accept ability (in addition to safety and efficacy evaluations of devices), and there are data to demonstrate correlation between increasing user adherence and increasing dose duration (i.e., decreased frequency of dosing or user intervention required).[293] Promising developments in this arena have included pre-clinical development and testing (in sheep) of a successful 90-day tenofovir reservoir-type intravaginal ring,[237] and in particular, an upcoming Phase I clinical trial (MTN-036/IPM-047, slated for completion later in 2018) of a DPV vaginal ring that releases effective inhibitory amounts of the drug for 90 days (e.g., ring replacement by the user would potentially only need to occur once every 3 months).

There is also an emerging trend toward the development of multipurpose prevention technologies (MPTs), which include combinations of drugs intended to simultaneously provide *dual* protection against unintended pregnancies and transmission of HIV and other STIs.[294] It is hypothesized that a device offering prevention of both HIV infection *and* unintended pregnancy may lead to a higher degree of adherence among female users as compared to microbicide-only products; also, products having the capability of preventing transmission of multiple STIs may also improve the motivation of individuals to use them, as compared to products only preventing HIV transmission. Most efforts to de velop MPTs have focused on combinations of two or more drugs rather than a single multifunctional compound. Newer developments in the MPT field include the successful development of an intravaginal ring demonstrating simultaneous and continuous release over more than 60 days, of both DPV and the contraceptive progestin levonorgestrel;[295] this promising new device entered a Phase I clinical trial (MTN-030/IPM-041) in April 2017, with results from the study expected later in 2018.

In the long-term success of HIV prevention and treatment efficacy, safety and tolerability are key and will likely be the focus of future studies. Targeting different stages of HIV infection will be investigated, since there are several targets like HLA DR, DC-SIGN, and LFA-1. Another challenge is targeting latent HIV in various reservoirs, thus LRAs are being studied to activate the viral reservoirs in order to eradicate the HIV infections completely.

Other sustained delivery options such as transdermal delivery systems and intradermal implants are being investigated to reduce the dosing frequency and improve life quality of the patients. However, these systems will require more potent drugs due to the limited drug loading capacity of these novel delivery systems. There are new ARV molecules with different mechanisms in clinical trials that might be used with these new systems. Both novel prevention and treatment options that are in early stages of testing and long-term commitment and systematic research are necessary to achieve the ultimate goal of HIV eradication.

Acknowledgements

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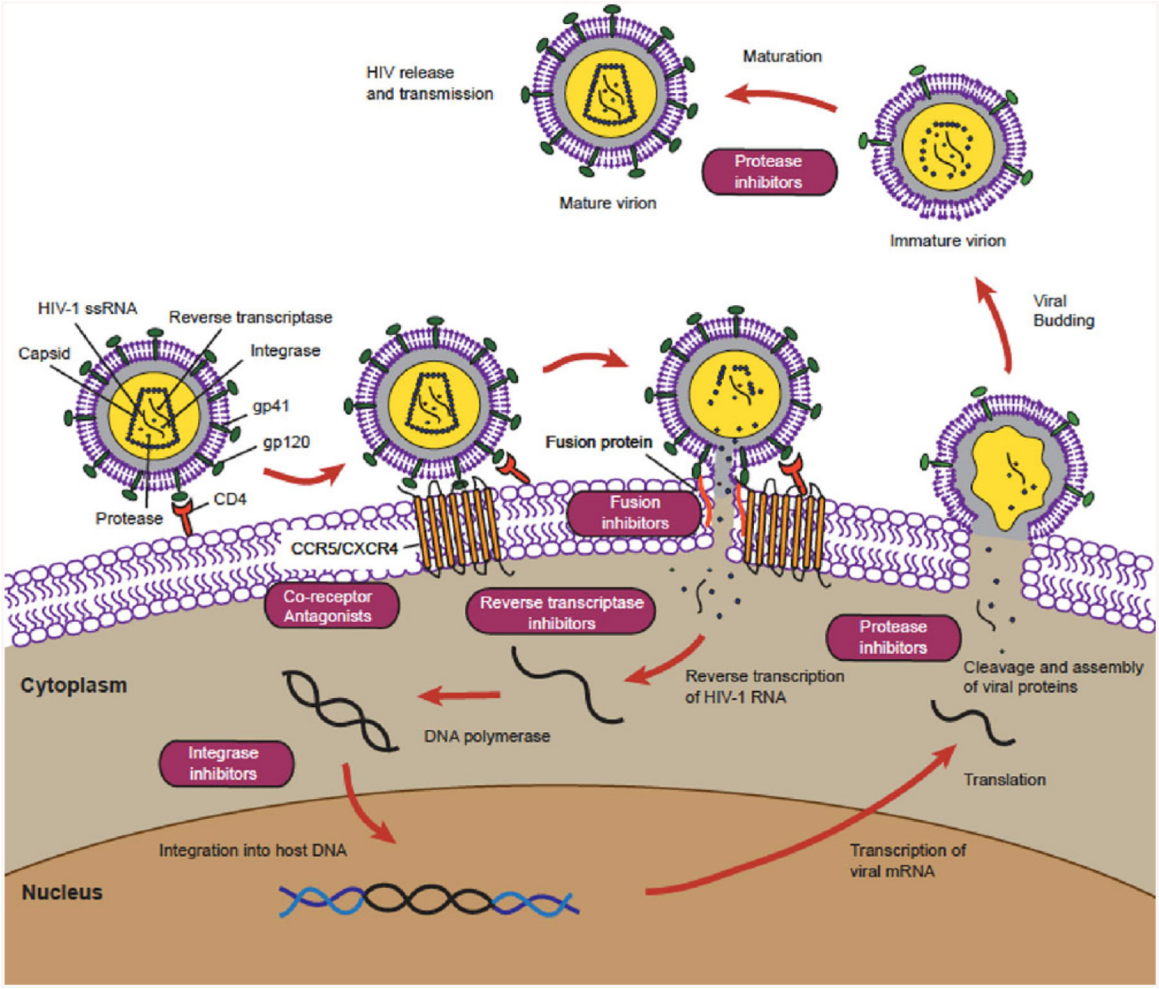

Figure 1.
ARVs target specific steps in the HIV replication cycle.

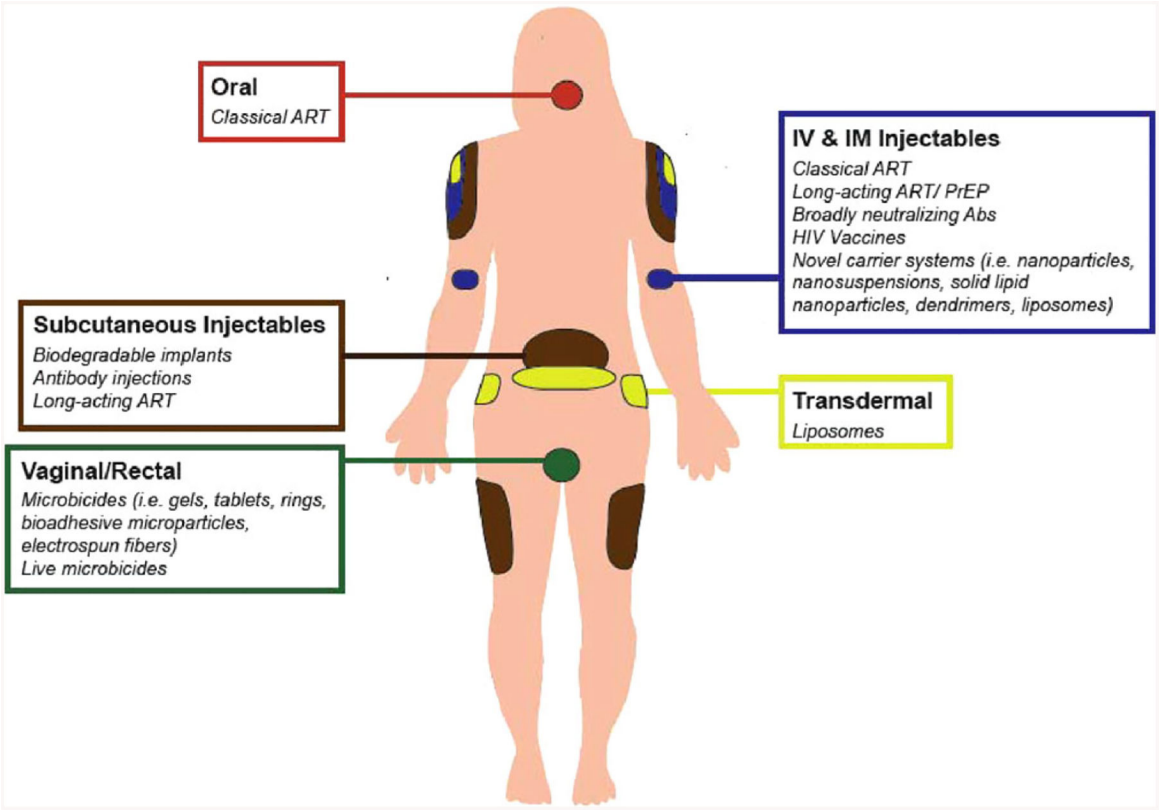

Figure 2.
Anti-HIV drug administration routes and delivery systems in the human body.

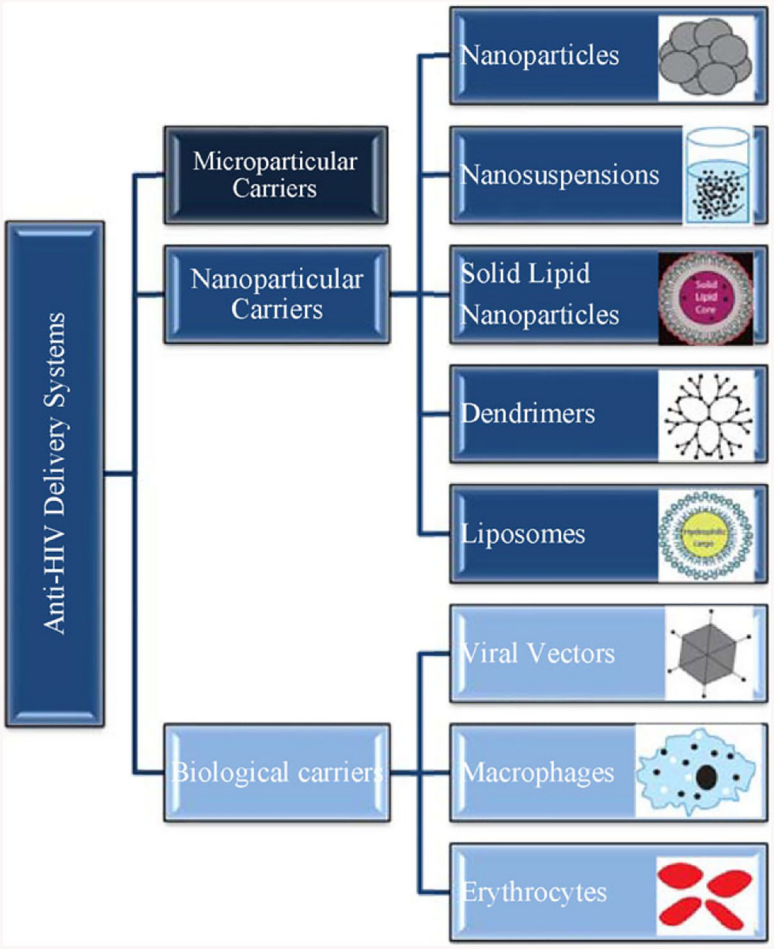

Figure 3.
Schematic of novel anti-HIV delivery systems.

Table 1.

Currently available ARVs.[14]

Mechanism of action	Drug	Oral adult dose	Brand name
Nucleoside reverse transcriptase inhibitors (NRTIs)	Abacavir (ABC)	300 mg twice daily 600 mg once daily	ZIAGEN
	Didanosine (ddI)	200 mg twice daily 400 mg once daily	VIDEX EC
	Emtricitabine (FTC)	200 mg once daily	EMTRIVA
	Lamivudine (3TC)	150 mg twice daily 300 mg once daily	EPIVIR
	Stavudine (d4T)	30 mg twice daily	ZERIT
	Tenofovir disoproxil fumarate (TDF)	300 mg once daily	VIREAD
	Tenofovir alafenamide (TAF)	10 or 25 mg once a day, as part of combination therapy	VEMLIDY, component of Descovy, Genvoya, and Biktarvy
	Zalcitabine (ddC)	0.75 mg per 8 h	HIVID
	Zidovudine (AZT)	200 mg three times daily 300 mg twice daily	RETROVIR
Non-nucleoside reverse transcriptase inhibitors (NNRTIs)	Delavirdine (DLV)	400 mg three times daily	RESCRIPTOR
	Efavirenz (EFV)	600 mg once daily	SUSTIVA
	Etravirine (TMC125)	200 mg twice daily	INTELENCE
	Nevirapine (NVP)	200 mg once daily	VIRAMUNE
	Rilpivirine (RPV)	25 mg once daily	EDURANT
	Dapivirine (DPV)	N/A—has low oral bioavailability, only used in topical PrEP	N/A (is also known as “TMC-120”)
Protease inhibitors (PIs)	Amprenavir (APV)	1200 mg twice daily	AGENERASE
	Atazanavir (ATV)	150–300 mg daily	REYATAZ
	Darunavir (DRV)	600 mg twice daily	PREZISTA
	Fosamprenavir (FOS-APV)	1400 mg twice daily	LEXIVA
	Indinavir (IDV)	800 mg per 8 h	CIRIXIVAN
	Lopinavir (LPV)	400 mg twice daily 800 mg once daily	KALETRA (In combination with Ritonavir)
	Nelfinavir (NFV)	1250 mg twice daily	VIRACEPT
	Ritonavir (RTV)	600 mg twice daily	NORVIR
	Saquinavir (SQV)	1200 mg three times daily	INVIRASE
	Tipranavir (TPV)	500 mg twice daily	APTIVUS
Fusion inhibitors (FIs; also known as entry inhibitors)	Enfuvirtide (T-20)	90 mg subcutaneous injection every 12 h	FUZEON
CCR5 antagonists (also known as entry inhibitors)	Maraviroc (MVC)	150–300 mg twice a day	SELZENTRY

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Mechanism of action	Drug	Oral adult dose	Brand name
Integrase inhibitors (IIs)	Raltegravir (RAL)	400 mg twice a day	ISENTRESS
	Dolutegravir (DTG)	50 mg once a day	TIVICAY
	Elvitegravir (EVG)	150 mg once a day, as part of combination therapy	VITEKTA, component of Stribild
	Bictegravir (BIC)	50 mg once a day, as part of combination therapy	(formerly "GS-9883"), component of Biktarvy

Table 2.

Microparticle carriers for ARV delivery.

Drug	Polymer	Results	Reference
Abacavir	Eudragit, ethyl cellulose, HPMC K4M	Controlled release	[67]
Efavirenz	HPMC, Carbopol	Enhanced dissolution rate	[68]
		Enhanced bioavailability	
Indinavir	Eudragit E100	Controlled release	[69]
		Taste masking	
Lamivudine	Chitosan	Controlled release	[70]
	Acrycoat, L30D, S100		[71]
	Cellulose acetate phthalate, ethyl cellulose		[72]
Maraviroc	Sodium alginate	Controlled release	[73]
Nelfinavir	Cellulose acetate	Controlled release	[74]
Stavudine	Ethyl cellulose	Controlled release	[75]
	Eudragit RS100	Prolonged gastric retention time	[76]
	Sodium alginate	Prolonged gastric retention time	[77]
		Enhanced bioavailability	
Zidovudine	Ethyl cellulose	Controlled release	[59b]
	HPMC		[78]
	Chitosan		[79]
	Eudragit	Prolonged gastric retention time	[80]
		Controlled release	

HPMC, hydroxypropyl methylcellulose.

Table 3.

The use of nanoparticle carriers for anti-HIV drugs.

Drug	Polymer	Results	Reference
None	Silica	Bindingto HIV-1 gp120 Targeting to the infected cell	[49b]
Amprenavir	Quantum rods	Targeting the transferrin receptor Enhanced uptake—in vitro BBB model	[87]
Atazanavir	SLNs	Increased BBB permeability	[88]
Atazanavir and Ritonavir	Nanosuspension Poloxamer 188	Neuroprotection in humanized HIV-infected animal model	[89]
Delavirdine, Stavudine, and Saquinavir	PBCA, MMA-SPM, and SLNs	Increased in vitro BBB permeability	[47]
	RMP-7 modified MMA-SPM	Increased in vitro BBB permeability	[90]
Didanosine	Mannosylated gelatin	Increased in vivo macrophage uptake Mannosyl receptor targeting	[57,91]
Dolutegravir	DTG prodrugs + Poloxamer nanosuspension	Two weeks of parenteral efficacy	[92]
Indinavir	Lipid nanoparticles	Targeting to CD4 binding peptide Increased intracellular concentration High anti-HIV activity in macaques	[93]
Lamivudine	PLA/chitosan	Sustained release Protection in gastrointestinal track (in vitro)	[94]
Lamivudine and Zidovudine	PBCA and MMA-SPM	Increased in vitro BBB permeability	[95]
Lopinavir, Ritonavir, and Tenofovir	Lipid nanoparticles	50-fold higher intracellular drug concentration in lymph nodes (in primates)	[96]
Nevirapine	PLGA	In vitro transferrin targeting Increased uptake	[97]
	Nanosuspension Serum albumin, PEG 1000, or dextran 60 surface modifications	Accumulation in brain—in vivo rat model	[98]
Ritonavir	PLA	TAT gene targeting Higher bioavailability Reduced clearance from CNS	[99]
Saquinavir	Cyclodextrins	Decreased toxicity on Caco-2 cells	[100]
	Poly(ethylene oxide) modified poly-e-caprolactone	Higher drug internalization inTHP-1 human monocyte/macrophage cell line Higher intracellular drug concentration	[101]
	Quantum rods	Targeting to transferrin receptor Enhanced uptake—in vitro BBB model Decreased HIV-1 replication—in vitro	[102]
Saquinavir and Zalcitabine	Poly(hexylcyanoacrylate)	Higher efficacy for Saquinavir (induced HIV-1 antigen production)	[103]
Zidovudine	SLN and SLN-PEG	Increased drug concentration in blood with PEGylated SLNs Decreased release rate and prolonged circulation with PEG coating	[104]
	PACA, PMMA, HSA	Uptake into macrophages isolated from HIV+ patient	[105]
	Hexylcyanoacrylate	Higher drug level in liver, blood, and brain following oral administration 18 times higher drug concentration in RES organs following IV administration	[106,107]
	PLA and PLA:PEG	Increased in vitro uptake by polymorphonuclear leukocytes	[108]

Drug	Polymer	Results	Reference
	Albumin	Targeting to transferrin receptor Increased drug amount in brain cells	[109]

BBB, blood–brain barrier; HAS, human serum albumin; IV, intravenous; MMA-SPM, methylmethacrylate-sulfopropylmethacrylate; PACA, polyalkylcyanoacrylate; PAMA, poly methylmethacrylate; PBCA, polybutylcyanoacrylate; PEG, poly(ethylene glycol); PLA, poly(lactic acid); PLGA, poly(lactic-co-glycolic acid); RES, reticuloendothelial system; TAT, “Trans-activator of transcription,” an HIV-encoded protein.

Table 4.

The use of dendrimers for anti-HIV drugs.

Drug	Dendrimer type	Results	Reference
Efavirenz	Fifth-generation PPI t-Boc-lysine Conjugated PPI Mannose-conjugated PPI	Controlled release with conjugated dendrimers Increased in vitro cellular uptake with mannose-conjugated PPI	[112]
	Tuftsin-conjugated PPI	In vitro tuftsin targeting	[58]
Lamivudine	PEGylated PAMAM Generations 4 and 5	Controlled release ofdrug	[113]
Lamivudine and Efavirenz	Mannosylated PPI	Mannose targeting Increased uptake in macrophages	[114]
Silver (nano-silver)	Anionic linear globular dendrimer	Significant ARV activity in comparison with Nevirapine	[115]
Zidovudine	Sialic acid-conjugated mannosylated PPI	Increased cellular uptake by macrophages In vivo tissue distribution shows efficiency of dual targeting	[116]

PAMAM, polyamidoamine; PEG, poly(ethylene glycol); PPI, poly (propyleneimine).

Table 5.

The use of liposomes for anti-HIV drugs.

Drug	Target	Results	Reference
Chimeric HIV entry inhibitor peptide	Two gp41 regions simultaneously	Stabilization of the peptide	[117]
Didanosine	-	Longer plasma elimination time Targeting lymph nodes and macrophage rich tissues	[118]
Didanosine glycerolipidic conjugates	-	Enhanced drug bioavailabilityAvoids hepatic first pass	[119]
Didanosine triphosphate	Fc receptor	Increased retention time	[120]
Indinavir	Anti-HLA-DR	Lower toxicity and immunogenicity in pre-clinical studies	[121]
	-	Enhanced transdermal delivery (increased in vitro permeation through human cadaver skin)	[122]
P11	Anti-gp120	Localization in HIV-1-infected cells—in vitro	[51b]
RNAi	LFA-1	Reduced viral load—in vivo	[123]
Stavudine	Mannose	Higher drug concentrations in liver, spleen, and lungs	[124]
	Galactose	Higher drug concentrations and lower toxicity in liver	[125]
Zalcitabine	-	Extended clearance time in brain tissue in rats (23 h)	[126]
	Passive targeting (anionic nature)	Rapid uptake by mice macrophages	[127]
	Galactose	In vivo higher drug accumulation and increased half-life in liver No hematological toxicity	[128]
Zidovudine	Mannose	Higher drug uptake in spleen and lymph nodes	[129]
	-	No bone marrow toxicity in mice Enhanced localization in liver, spleen, and lungs	[130]
Zidovudine 5’-triphosphate	Magnetic targeting across BBB	Increased transmigration across an in vitro BBB model	[131]
Zidovudine myristate	-	Higher concentrations in brain and longer elimination time in rats	[132]

BBB, blood–brain barrier; Fc, fragment crystallizable region; HLA-DR, human leukocyte antigen—antigen D related; LFA-1, leukocyte function–associated antigen.

Table 6.

Delivery formats for anti-HIV microbicides.[209]

Delivery format	Advantages	Limitations
Gels (semi-solid), rectal and vaginal	• Greater ease of formulation and manufacture, lower cost of production • Ease of administration by the user • Rapid dispersal of the active compound, conferring protection shortly after placement • High localized drug concentrations can be attained • Additional functionality as a lubricant for sexual intercourse	• Timing of device application is coitally dependent • Limited duration of retention at the site of application, requiring more frequent dosing • Leakage and potential user discomfort • Vulnerability of active compounds to extremes of temperatures and humidity
Vaginal tablets and films	• Rapid release of the active compound, providing protection shortly after placement • Precise, consistent dosing • Lower manufacturing costs • Ease of application by the user • Improved stability of active compounds to extreme temperatures and humidity	• Timing of device application is coitally dependent • Shorter retention time of active compounds in the site of application, requiring more frequent dosing • For films, their low overall mass limits the amount of active compound that can be incorporated into a single dose
Vaginal rings (VRs)	• Sustained release of the microbicide over weeks or months, requiring less frequent user intervention • Timing of device application is coitally independent	• Increased complexity of manufacture and production costs • User discomfort with device insertion/removal • Only useful for the vaginal (and not rectal) compartment
Nanoparticles (NPs)	• Protection and stabilization of active compounds against degradation • Enhanced solubilization of incorporated active compounds • Permeation through cervicovaginal mucus to better reach HIV susceptible sites in the epithelium	• Increased complexity of manufacture and production costs • Clinical safety and efficacy studies are still needed
Electrospun fibers (EFs)	• Dry, solid dosage form that can stabilize drugs that may be less stable in a liquid format • Base polymers used are often mucoadhesive, potentially increasing • residence time of the microbicide at the site of application	• Increased complexity of manufacture, low manufacturing production capacity at present, thus higher cost per dose • Clinical safety and efficacy studies are still needed
Live microbicides (commensals)	• Sustained release of the microbicide • over weeks, months, or longer, requiring less frequent user intervention • Timing of device application is coitally independent	• Long-term clinical data are still needed to demonstrate safety and efficacy • Limited to delivery of biologic microbicides that can be stably • expressed in bacteria

Delivery format	Advantages	Limitations
	• Lactobacilli themselves may positively impact the vaginal mucosal environment	• User concern about GMOs

GMOs, genetically modified organisms.