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Nanotechnology-based systems for the treatment and prevention of HIV/AIDS☆

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ABSTRACT

The HIV/AIDS pandemic is an increasing global burden with devastating health-related and socioeconomic effects. The widespread use of antiretroviral therapy has dramatically improved life quality and expectancy of infected individuals, but limitations of currently available drug regimens and dosage forms, alongside with the extraordinary adapting capacity of the virus, have impaired further success. Alongside, circumventing the escalating number of new infections can only be attained with effective and practical preventative strategies. Recent advances in the field of drug delivery are providing evidence that engineered nanosystems may contribute importantly for the enhancement of current antiretroviral therapy. Additionally, groundwork is also being carried out in the field nanotechnology-based systems for developing preventative solutions for HIV transmission. This manuscript reviews recent advances in the field of nanotechnology-based systems for the treatment and prevention of HIV/AIDS. Particular attention is given to antiretroviral drug targeting to HIV reservoirs and the usefulness of nanosystems for developing topical microbicides and vaccines.

Contents

Abbreviations: AcLDL, acetylated low-density lipoprotein; AF, amniotic fluid; AF/BP, amniotic fluid/blood plasma concentration ration (determined at birth); AIDS, acquired immune deficiency syndrome; $AUC_{0-24\ h}$, area under the curve (0–24 h); b.i.d., twice daily; BBB, blood-brain barrier; BCS, biopharmaceutics classification system; BMEC, brain-microvascular endothelial cells; CBP, cord blood plasma; CBP/BP, cord blood plasma/blood plasma concentration ration (determined at birth); CNS, central nervous system; CSF, cerebrospinal fluid; CSF/BP, cerebrospinal fluid/blood plasma concentration ratio; DCs, dendritic cells; DC-SIGN, dendritic cell-specific intercellular adhesion molecule-grabbing non-integrin; EMF, electromagnetic field; FGT/BP, female genital tract/blood plasma concentration ratio; fMLF, *N*-formyl-methionyl-leucyl-phenylalanine; GALT, gut-associated lymphoid tissues; HAART, highly active antiretroviral therapy; HIV, human immunodeficiency virus; HJV, hemagglutinating virus of Japan (Sendai virus); HLA-DR, human leukocyte antigen DR-1; IC_{50}, 50% inhibitory concentration; ID, intradermal; IM, intramuscular; IN, intranasal; IP, intraperitoneal; ISCOM, immunostimulating complex; IVag, intravaginal; LCs, Langerhans cells; LN/BP, lymph node/blood plasma concentration ratio; NNRTI, non-nucleoside reverse transcriptase inhibitors; NRTIs, nucleoside reverse transcriptase inhibitors; NtRTI, nucleotide reverse transcriptase inhibitors; PEG, poly(ethylene glycol); PEG-PLA, PEGylated-poly(L-lactide); PEI, poly(ethyleneimine); PEO–PCL, poly(ethylene oxide)-modified poly(epsilon-caprolactone); PHCA, polyhexylcyanoacrylate; PIs, protease inhibitors; PLA, poly(L-lactide); PLGA, poly(D,L-lactide-*co*-glycolide); PMBCs, peripheral blood mononuclear cells; PPI, poly(propyleneimine); RANTES, regulated on activation normal T cell expressed and secreted chemokine; RES, reticulo-endothelial system; RTIs, reverse transcriptase inhibitors; SC, subcutaneous; SHIV, simian human immunodeficiency virus; SIV, simian immunodeficiency virus; SLNs, solid lipid nanoparticles; Sm/BP, semen/blood plasma concentration ratio; t.i.d., three times a day; $t_{1/2}$, plasma half-life; TAT peptide, HIV-1 trans-activating transcriptor peptide; Th1, type 1 T helper cells; Th2, type 2 T helper cells.

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1. Introduction

In the last quarter of a century human immunodeficiency virus infection and acquired immune deficiency syndrome (HIV/AIDS) became an increasing global health, social, and economical concern. By 2007, it was estimated that the total number of people infected by HIV accounted for around 33 million, while another 25 million more have already died since the first reported cases in 1981 [1]. Whereas the infection has a worldwide distribution, there is clearly a disproportionate impact of HIV/AIDS on sub-Saharan African countries, accounting this region for 67% of all people living with HIV. Other important regions affected by HIV include the Caribbean, Latin America, and South and Southeast Asia. Heterosexual transmission is the most common route of viral entry in these developing nations. This fact is resulting in important socioeconomic, family, and public health burdens that compromise the convergence of these regions with developed countries [2,3]. While the number of infected people is steadily increasing each year, most recent available reports from UNAIDS show a slight decrease in the pandemic since the beginning of the 21st century due to the decrease of new infections (2.7 million in 2007 vs. 3.0 million in 2001) [1]. These figures can be explained by the expanding access to antiretroviral drugs, especially in resource-limited settings, which has not only increased lifespan, but also the quality of life of HIV infected people. Indeed, in the absence of an effective cure, prevention and access to antiretroviral therapy are the best options to affect the HIV pandemic [4]. However, current strategies for providing universal access to prevention and treatment, and their field applicability are not enough, urging the search for new and improved options [1].

Although of undeniable importance, antiretroviral therapy has been limited by several factors, such as its inherent toxicity, insufficient efficacy, and drug resistance. The development and recent approval of innovative or improved drugs has managed to minimize some of this issues but the remarkable ability of HIV to resist the new therapeutic options has limited success. Alongside, inadequate physical–chemical properties of most of these antiretroviral drugs (e.g. poor solubility, permeability, and stability) impair optimal absorption, biodistribution, and sustained antiretroviral effect, thus contributing to poor clinical outcome. In order to solve these problems, several new and improved delivery systems and dosage form have been proposed in the literature [5,6]. Particularly, several nanotechnology-based delivery systems have been developed in order to improve HIV therapy, namely polymeric nanoparticles, solid lipid nanoparticles (SLNs), liposomes, nanoemulsions, dendrimers, and drug conjugates (e.g. with low-density lipoproteins or peptides) [6–10]. Although these wide range of systems share their submicron dimensions (from a few nanometers up to 1 micrometer), they differ in physical–chemical properties, biological behavior, preparation methods, or even characterization methodologies [11–13]. For more detailed information about these features, readers are referred to specialized literature in the field. Contrasting with the time and effort dedicated to the investigation of new treatment options, the interest in nanotechnology-based systems for the prevention of HIV/AIDS has been slim and mainly focused on the development of vaccines and microbicides. Conversely, the field of microbicides in particular has seen recent thrilling progresses, namely because of the advanced state in the development pipeline of VivaGel® (Starpharma Pty Ltd., Australia), a dendrimer-based microbicide gel, which captured the attention of the scientific and medical communities to the potentialities of nanotechnology-based microbicides. Hence, the scope of this manuscript is to review recent developments in nanotechnology-based systems specifically designed and developed for the treatment and prevention of HIV/AIDS, with particular emphasis focused on specific individual examples of significant interest. Further, we discuss new prospects and future directions for advancing in the field. Specific crucial topics in the HIV pharmacotherapy are discussed more briefly (e.g., CNS targeting) as other articles of the present issue address them in more detail.

2. HIV basics

Since the identification of the causative agent of AIDS in 1983, efforts to understand the biology of HIV have been impressing and the acquired knowledge contributed valuably to the development of currently available therapeutic and preventative strategies [14]. Undoubtedly, knowledge of the transmission process and pathogenesis of HIV infection is essential to provide important insights towards the development of new and better treatment options, as well as endow researchers with important opportunities to develop novel preventative measures [15]. Therefore the following section is dedicated to briefly address some of the most important aspects of the biology of HIV related to the development of nanotechnology-based systems for the treatment and prevention of HIV/AIDS.

2.1. The virus

HIV is a lentivirus of the family Retroviridae, mostly known for being the causative agent of AIDS [16]. This virus can be seen as a biological nanostructure (around 100–150 nm), composed by a host-derived membrane, a nucleocapsid and genetic material in the form of RNA containing three structural genes. These genes code for important group-specific antigens (gag gene), essential viral enzymes such as reverse transcriptase, integrase and protease (pol gene), and the two glycoproteins present in the outer viral membrane, gp120 and gp41, which are responsible for recognizing the CD4 receptor and the CCR5 or CXCR4 co-receptors of the host cell membrane, and for virus/cell fusion, respectively (env gene). As a consequence of constant transcription errors, these viral structures present high polymorphism

which leads to mutation, thus constituting a major source of antiretroviral-resistance development [16].

Two different types of HIV, HIV-1 and HIV-2, are known to cause infection and disease in humans [17]. Among other differences, HIV-2 is associated with slower progression to immunodeficiency and is less efficiently transmitted [18]. HIV-2 is also much less prevalent than HIV-1, being HIV-2 mostly found in individuals from West Africa, India, and, to a more limited extent, Portugal and former Portuguese African colonies [19]. For the purpose of this review, presented data refers mainly to HIV-1 unless otherwise stated.

2.2. HIV/host cell interaction

Human cells expressing the cell-surface protein CD4 may be productively infected by HIV. These include macrophages, T cells and dendritic cells (DCs) [20]. HIV-1 life cycle is complex and is dependent upon several viral and host factors. In order to infect target cells HIV requires the attachment of its envelope with the cell membrane by means of the interaction of gp120 (envelope glycoprotein) with the cell-surface receptor CD4. Once this bond is established, gp120 undergoes a conformational change that facilitates its binding to one of two chemokine co-receptor molecules, CXCR4 or CCR5 [16]. The gp120 interaction with CD4 and co-receptors is vital for viral binding to human cells but subsequent interaction of gp41 with a cellular fusion receptor is responsible for the fusion of HIV with the cell. R5 viruses, i.e. virus presenting preferential CCR5-expressing cell tropism, are responsible for most HIV new infections [21]. After HIV/cell fusion, the viral core containing RNA, reverse transcriptase, and integrase are released inside the cell cytoplasm. After core disassembly, the viral RNA is reverse transcribed into DNA by the viral reverse transcriptase and migrates to the cell nucleus, where it is inserted in the host chromosomal DNA by the viral integrase. At this point cell infection is irreversible, being the cell now capable of producing virions [16].

2.3. HIV transmission

HIV is mostly transmitted by vaginal or anal sexual intercourse [22]. Other means also contribute significantly to the spread of the infection, namely by transfusion of contaminated blood products, sharing of contaminated needles among injected drug users, and transmission from mother-to-child during pregnancy, labor or breast-feeding. When considering vaginal sexual intercourse, research data supports that HIV transmission occurs due to viral penetration of the vaginal and cervical mucosa [23–25]. Upon sexual intercourse, HIV may be deposited in the vaginal lumen either as free virions or cell-associated, being macrophages the primary transmission carriers for HIV present in vaginal and seminal secretions [26].

In the genital mucosa, the immune population consists mainly of dendritic cells (DCs), macrophages, T cells and B cells present in the lamina propria [23,27]. All these cells can potentially express either CXCR4 or CCR5 co-receptors, therefore being susceptible to HIV infection. Unlike all other cells, DCs are also capable of binding to HIV gp120 without membrane fusion occurs, which facilitates viral transport to secondary lymphoid organs and allows enhancing viral infectivity of T cells. This fact is associated with the presence of a dendritic cell-specific HIV-1 binding protein, DC-SIGN (dendritic cell-specific intercellular adhesion molecule-grabbing non-integrin) [28]. In addition to the immune cell population present alongside the lamina propria, immature DCs, termed Langerhans cells (LCs), are abundant in the cervicovaginal epithelium, acting as antigen-presenting cells and playing a key role in the viral infection [23]. Dendritic processes of LCs (not of DCs) are able to extend to the vaginal lumen and collect antigens, such as HIV, mediate transmission of the virus across intact genital epithelium and present it to susceptible cells in the underlying tissues, which support initial HIV replication (local amplification) [29,30]. Also, infected/HIV-bearing LCs and, generally, all DCs can readily migrate to T cell-rich lymph nodes. This ability to transport virions across the epithelium and their strategic positioning in the epithelium makes these cells important contributors to vaginal HIV transmission [27,31]. Additionally, HIV access to sub-epithelium tissues may be facilitated by the presence of thinned or damaged epithelium [32,33].

Rectal transmission is also a major form of viral transmission. The presence of a single columnar epithelial lining makes the rectum and terminal colon a straightforward route for HIV infection. The presence of M cells in these tissues is thought to guarantee constant lumen sampling of antigens that are presented to sub-epithelial lymphocytes and macrophages [34]. While still considering both vaginal and anal sexual intercourse, penile transmission is not only a possibility but an important contribution to HIV cases worldwide. Viral invasion occurs mostly through the inner foreskin and the penile urethra, which are covered by a poorly keratinized, thin squamous epithelium or by a thinly stratified columnar epithelium, respectively [27]. Both these sites harbor HIV-susceptible cells that play similar parts in viral spread as those discussed above for vaginal transmission [35].

2.4. HIV pathogenesis

After initial infection and local amplification at the mucosal site, infected cells migrate to regional lymph nodes, leading to a mild initial viral amplification in naïve T cells [4]. The viral infection is then quickly disseminated by T cells to lymphoid organs, particularly the gut-associated lymphoid tissues (GALT), spleen, and bone marrow, being accompanied by a burst in the viral load (acute infection) [36]. During the acute and early stages of infection the gastrointestinal tract is particularly affected by the virus, leading to a dramatic loss of CD4+ and CD8+ T cells which never quite recover completely and remain despite antiretroviral treatment [37,38]. Individuals during this acute phase pose an increased risk for sexual transmission as result of high blood and genital viral load, with clear implications in the prevention of HIV transmission [39]. CD4+ cell levels recover soon after; in the case of CD8+ cells there is a rise followed by a rapid recovery of normal levels. Levels of the virus are then down regulated in response to the intense immune response, but never completely depleted, resulting in clinical latent and asymptomatic infection [40]. During latency, the virus persists particularly in extra-vascular tissues, lymph node dendritic cells and resting CD4+ memory cells. This state can evolve to a symptomatic clinical stage (usually several years after the initial infection), designated by AIDS, which is characterized by decreased CD4+ T cell counts and rising viral load [16]. With the progression of infection, HIV genetic diversity increases noticeably due to intense error-prone reverse transcription and evolutionary pressure to evade the immune system [34]. This new heterogeneous population dramatically increases the generation of viruses resistant to cellular and humoral immune response, and represents a major challenge in the development of therapy and preventative strategies.

2.5. HIV reservoir sites

Even if current antiretroviral therapy is able to reduce the viral load to undetectable levels, HIV is able to persist in the human body, namely in several reservoir sites. These may be defined as cellular or anatomical locations where a replication-form of the virus is persistently harbored with more stable kinetic properties than in the main pool of actively replicating virus [41]. Reservoir sites are able to protect the virus from biological elimination pathways, immune response and/or antiretroviral drugs, making it impossible to eradicate the virus and achieve a cure with currently available therapy [41]. The difficulties that constitute the reservoir sites are different when considering cellular or anatomical sites. Generally, cellular reservoirs are able to sustain HIV infection by allowing its

residence in a physical state capable of surviving for prolonged periods despite otherwise therapeutic levels of antiretroviral drugs. In the case of anatomical reservoir sites, the problem is mainly to achieve and sustain adequate levels of antiretroviral agents within these spaces [42].

2.5.1. Cellular reservoir sites

Several cell types have been proposed as potential reservoirs; however, only some are clearly recognized as so: macrophages, resting CD4+ T cells, and follicular dendritic cells (i.e., dendritic-like cells present in the lymph nodes) [41,42]. Among these, macrophages constitute one of the most important viral reservoirs outside the bloodstream and are able to transport HIV into the central nervous system, allowing this anatomical site to become infected [43]. These cells infection by HIV-1 can be productive but noncytopathic, allowing it to carry and produce the virus for prolonged periods [44]. Most important probably, is macrophages' role in later stages of infection. When CD4+ T cells are largely depleted, these cells, that otherwise are irrelevant as source of HIV particles, constitute a key cell population in maintaining the HIV replication cycle [42].

2.5.2. Anatomical reservoir sites

Main anatomical reservoir sites of HIV include the lymphoid organs (particularly the spleen, lymph nodes, and GALT) and the central nervous system (CNS) [41,42]. Other potential sites have also been reported as possible reservoirs, namely the testicles and the female genital tract. The importance of lymphoid organs is directly related with their role in the circulation and production of lymphocytes and the abundant presence of HIV-susceptible immune cells, namely those able to constitute reservoirs as discussed above. Undoubtedly, the CNS is of particular importance for HIV persistence and a serious challenge for effective antiretroviral therapy [42]. Infection of the CNS may result in a variety of neurocognitive disorders, namely HIV-associated dementia, accompanied by several structural alterations at the microscopic and macroscopic levels [45]. The virus accesses the nervous tissue by means of monocytes/macrophages, taking advantage of the natural turnover of these cells in the CNS. In the nervous tissue, lymphocytes, particularly CD4+, are rare, being macrophages and macrophages-related microglial cells the primary targets in brain infection [45]. Poor penetration of antiretroviral in the CNS due to insufficient blood-brain barrier (BBB) permeation is a matter of concern, resulting in suboptimal drug levels that allow continuous replication of HIV [42].

3. Current treatment and prevention of HIV/AIDS

3.1. Treatment options: practice and limitations

First antiretroviral drugs were introduced in therapy in the late 1980s and early 1990s. After fast development of antiretroviral resistance in individuals treated with single drug regimens, the concept of highly active antiretroviral therapy (HAART) was introduced in the late 1990s, comprising the intense use of combination drug regimens [46]. Widespread use of HAART dramatically increased life expectancy and its quality, shifting AIDS from a rapid-progressing to a chronic disease [47–49]. Undoubtedly, antiretroviral treatment is currently the best option for prolonged and maximal viral suppression, and preservation of the immune system after HIV infection onset [50]. There are now around 30 individual drugs and fixed-dose combinations available to treat HIV infection. Currently used antiretroviral drug classes include reverse transcriptase inhibitors (RTIs), protease inhibitors (PIs), entry inhibitors (CCR5 antagonists and fusion inhibitors), and integrase inhibitors (Table 1).

Meanwhile, other antiretroviral drugs belonging to these and new classes are being actively developed [86]. The choice between this variety of drugs and drug regimens is not easy and depends of multiple variables related to drug pharmacological and toxicological properties, therapy costs, disease staging and progression, drug-resistance status and patient characteristics [50].

Even if HAART regimens present considerable anti-HIV activity, several factors frequently compromise its success. To begin with, current therapy is not able to provide a cure mainly because of HIV's ability to persist in latency state in cellular and anatomical reservoir sites. Beside this fact, problems of current antiretroviral therapy also include prolonged treatment periods with drugs possessing important adverse effects, poor drug-regimen compliance, drug resistance, drug–drug interactions, poor drug pharmacokinetics (see Table 1), viral levels rebound after therapy cessation, and costs.

Drug resistance is the most common cause of antiretroviral treatment failure and has been described for virtually every antiretroviral drug currently used in therapy [87]. An important contributing factor for the emergence of HIV/AIDS therapy resistance is the inability to attain effective and/or sustained drug levels with currently used formulations and drug-schedules, thus contributing to ineffective viral suppression (even if at undetectable levels by contemporary assays), particularly in reservoir sites. For example, PIs are substrate for the efflux cellular membrane transporter P-glycoprotein, which is able to mediate unidirectional transport of these drugs to the cell exterior [88]. The presence of this membrane transporter in macrophages and endothelial cells of the BBB explains the poor concentrations achieved by PIs in these reservoir sites. Conversely, incomplete absorption of some PIs when administered by the oral route can be partially explained, alongside with their poor aqueous solubility, by the presence of this transporter in intestinal epithelial cells [88,89]. Poor placental penetration of PIs, which may have important clinical implications in mother-to-child transmission, is also justified by the presence of high levels of P-glycoprotein in this tissue [75]. In the particular case of RTIs, these drugs need to be activated into their tri-phosphorylated form inside cells in order to be active; this step may be compromised and thus result in poor intracellular concentration of active molecules [90]. In addition, the administration of phosphorylated NRTIs and NtRTIs, which could circumvent the phosphorylation step, is not feasible for conventional dosage forms as these activated molecules cannot permeate efficiently cell membranes and undergo rapid degradation. Such issues are important hurdles that need to be resolved in order to optimize the activity of antiretroviral drugs and avoid drug resistance [91].

The use of multi-drug regimens, each of them often possessing considerable toxicity, is also one of the most problematic issues that may delay therapy initiation or determine its interruption [92]. The type, severity and frequency of clinical adverse events are variable and dependent on individual drugs, drug regimens and patients [93,94]. The problem of drug toxicity is even more dramatic if considering that for effective viral suppression it is essential a nearly perfect compliance of drug regimens for long periods, often chronically, with interruption of drug treatment frequently resulting in increased morbidity and mortality [95,96]. Also, interactions between antiretroviral drugs or with other drugs are frequent and highly complex, this fact being relevant for treatment course management [52].

Affordability of antiretroviral drugs is an increasingly huge burden for developed countries and an unattainable goal for developing ones. However, efforts have been made in the last decade by governmental and non-governmental institutions, as well as by several pharmaceutical companies, to widen the number of persons who may be treated for HIV/AIDS, particularly in resource-poor locations, setting several ambitious programs and action plans [19,97]. Even if partially successful, the universal access to antiretroviral therapy is still a mirage. The price of antiretrovirals is particularly high for drugs recently approved while older drugs are generally more affordable. An interesting approach for reducing overall costs with antiretroviral therapy would be to increase older drugs therapeutic lifespan (i.e. before treatment-compromising adverse effects or drug resistance occurs) by improving their delivery.

Table 1
Selected currently approved antiretroviral drugs.

Generic names (abbreviations) [Brand name, manufacturer][a]	FDA approval	Usual adult dosage regimens[b]	Pharmacokinetic features
Nucleoside reverse transcriptase inhibitors (NRTIs)			
Zidovudine (AZT; ZDV) [*Retrovir®, GlaxoSmithKline*]	March 1987	200 mg, t.i.d. (oral) OR 300 mg, b.i.d. (oral)	Short mean $t_{1/2}$ (1.1 h) [51,52]
Didanosine (ddI) [*Videx®, Bristol-Myers Squibb*]	October 1991	400 mg, daily (oral; delayed-release capsules)	Short $t_{1/2}$ (1–2 h); CSF/BP = ~0.21; CBP/BP = 0.38 [52–55]
Zalcitabine (ddC)[c] [*HIVID®, Roche*]	June 1992	0.75 mg, t.i.d. (oral)	Short mean $t_{1/2}$ (1.2 h); CSF/BP = 0.09–0.37 [56]
Stavudine (d4T) [*Zerit®, Bristol-Myers Squibb*]	June 1994	40 mg, b.i.d. (oral)	Short $t_{1/2}$ (0.9–1.2 h); CSF/BP = 0.16–0.40; FGT/BP < 0.1; concentrates in AF (AF/BP = 6.45) [55,57–59]
Lamivudine (3TC) [*Epivir®, GlaxoSmithKline*]	November 1995	150 mg, daily (oral) OR 300 mg, daily (oral)	Short $t_{1/2}$ (2.5 h); CSF/BP = ~0.06 [60]; concentrates in AF (AF/BP = ~4–12) [52,55,60–62]
Abacavir (ABC) [*Ziagen®, GlaxoSmithKline*]	December 1998	300 mg, b.i.d. (oral) OR 600 mg, b.i.d. (oral)	Short $t_{1/2}$ (0.8–1.5 h); CSF/BP = ~0.21[52,63]
Emtricitabine (FTC) [*Emtriva®, Gilead Sciences*]	July 2003	200 mg, daily (oral capsule) OR 240 mg, daily (oral solution)	CSF/BP = 0.43 [64]
Nucleotide reverse transcriptase inhibitors (NtRTIs)			
Tenofovir (TDV; PMPA) [*Viread®, Gilead Sciences*]	October 2001	300 mg, daily (oral)	Undetectable in CSF [65]
Non-nucleoside reverse transcriptase inhibitors (NNRTIs)			
Nevirapine (NVP) [*Viramune®, Boehringer Ingelheim*]	June 1996	200 mg, daily (for 14 days; oral), then 200 mg, b.i.d. (oral)	Sm/BP = 0.6 [66]
Efavirenz (EFV) [*Sustiva®, Bristol-Myers Squibb*]	September 1998	600 mg, daily (oral)	CSF/BP = 0.003-0.01 due to high plasma protein bound (~99%); FGT/BP = 0.01–0.1 [59,67,68]
Etravirine (TMC125) [*Intelence®, Tibotec*]	January 2008	200 mg, b.i.d. (oral)	Total oral bioavailability undetermined [69]
Protease inhibitors (PIs)			
Saquinavir (SQV) [*Invirase®, Roche*]	December 1995	1000 mg, b.i.d. (oral)	Oral bioavailability dependent of formulation (4-5% for Invirase®); CSF/BP = ~0.001 due to high plasma protein bound (~97%); CBP = 0.04; AF/BP = 0.02 [70–72]
Indinavir (IDV) [*Crixivan®, Merck*]	March 1996	800 mg, b.i.d. (oral) OR 800 mg, t.i.d. (oral)	Short $t_{1/2}$ (2 h); CSF/BP = ~0.17; Cell/blood plasma concentration ratio = 0.51; CBP/BP = 0.01 [52,73–75]
Ritonavir (RTV) [*Norvir®, Abbot Laboratories*]	March 1996	300 mg, b.i.d. (initial dose; oral) up to 600 mg, b.i.d. (in increments of 100 mg every 2 to 3 days; oral)	Short $t_{1/2}$ (3 h); CSF/BP = ~0.002 due to high plasma protein bound (98–99%); AF/BP = 0.02; negligible CBP levels [52,71,75,76]
Nelfinavir (NFV) [*Viracept®, Agouron Pharmaceuticals*]	March 1997	1250 mg, b.i.d. (oral) OR 750 mg, t.i.d. (oral)	Undetectable in CSF; Sm/BP = 0.08; FGT/BP = 0.05; CBP/BP = 0.24; AF/BP = 0.14 [68,72,73,75,77]
Lopinavir (ABT-378)[d]	September 2000	–	Rapidly metabolized when not associated to RTV; Sm/BP = 0.07; FGT/BP = 0.03; LN/BP = 0.21; CBP/BP = 0.22; AF/BP = 0.08 [68,72,73,78]

Atazanavir (ATV) [*Reyataz®, Bristol-Myers Squibb*]	June 2003	300 mg, daily (oral) OR 400 mg, daily (oral)	CSF/BP = 0.002-0.014 [79]
Fosamprenavir (fAPV) [*Lexiva®, GlaxoSmithKline*]	October 2003	700 mg, b.i.d. (oral) OR 1400 mg, daily (oral) OR 1400 mg, b.i.d. (oral)	Sm/BP = 0.10-0.39; CBP/BP = 0.27 [75,80]
Tipranavir (TPV) [*Aptivus®, Boehringer Ingelheim*]	June 2005	500 mg, b.i.d. (oral)	Low oral bioavailability because of low solubility [81]
Darunavir (DRV) [*Prezista®, Tibotec*]	June 2006	600 mg, b.i.d. (oral) OR 800 mg, daily (oral)	CSF/BP = 0.01 [82]
Entry inhibitors			
Fusion inhibitors			
Enfuvirtide (T20) [*Fuzeon®, Roche & Trimeris*]	March 2003	90 mg, b.i.d. (subcutaneous)	Needs to be injected; undetectable in CSF [83]
CCR5 antagonists			
Maraviroc (UK-427,857) [*Selzentry®, Pfizer*]	August 2007	150 mg, b.i.d. (oral) OR 300 mg, b.i.d. (oral) OR 600 mg, b.i.d. (oral)	CSF/BP = 0.1 (data from rats) [84]
Integrase inhibitors			
Raltegravir (MK-0518) [*Isentress®, Merck*]	October 2007	400 mg, b.i.d. (oral)	Undetermined oral bioavailability; short initial $t_{1/2}$ (1 h) [85]
Fixed-dose combination antiretroviral drugs			
Lamivudine/Zidovudine [*Combivir®, GlaxoSmithKline*]	September 1997	150 mg/300 mg, b.i.d. (oral)	*See individual drugs*
Lopinavir/Ritonavir (rLPV) [*Kaletra®, Abbott Laboratories*]	September 2000	400 mg/100 mg, b.i.d. (oral) OR 500 mg/125 mg, b.i.d. (oral) OR 533 mg/133 mg, b.i.d. (oral)	*See individual drugs*
Abacavir/Lamivudine/Zidovudine [*Trizivir®, GlaxoSmithKline*]	November 2000	300 mg/150 mg/300 mg, b.i.d. (oral)	*See individual drugs*
Abacavir/Lamivudine [*Epzicom®, GlaxoSmithKline*]	August 2004	600 mg/300 mg, daily (oral)	*See individual drugs*
Emtricitabine/Tenofovir [*Truvada®, Gilead Sciences*]	August 2004	200 mg/300 mg, daily (oral)	*See individual drugs*
Efavirenz/Emtricitabine/Tenofovir [*Atripla®, Bristol-Myers Squibb & Gilead Sciences*]	July 2006	600 mg/200 mg/300 mg, daily (oral)	*See individual drugs*

Abbreviations: AF, amniotic fluid; AF/BP, amniotic fluid/blood plasma concentration ration (determined at birth); b.i.d., twice daily; CBP, cord blood plasma; CBP/BP, cord blood plasma/blood plasma concentration ration (determined at birth); CSF, cerebrospinal fluid; CSF/BP, cerebrospinal fluid/blood plasma concentration ratio; FGT/BP, female genital tract/blood plasma concentration ratio; LN/BP, lymph node/blood plasma concentration ratio; Sm/BP, semen/blood plasma concentration ratio; $t_{1/2}$, plasma half-life; t.i.d., three times a day.

[a] The use of trade names is for product identification only and does not imply any kind of endorsement (only the original brand names are presented).

[b] Dependent on product characteristics, co-administered drugs, regimen specificities, or patients previous treatments.

[c] Discontinued since December 31, 2006.

[d] Only available in combination with ritonavir.

3.2. Preventative strategies

Since no curative therapy is available, prevention is a cornerstone in the battle against HIV/AIDS, particularly for stopping sexual heterosexual HIV transmission [1]. However, the only currently recommended prevention measures include sexual abstinence and condom use; even if highly effective, their applicability in the real world is far from being perfect. Thus, the continuous search for more effective and feasible prevention measures led in recent years to the research and development of innovative options, particularly in the field of vaccines [98] and microbicides [99].

4. Nanotechnology-based systems for HIV/AIDS treatment

4.1. Rationale for nanotechnology-based systems

The basic concept behind the use of nanotechnology-based systems for antiretroviral drug delivery is related with the modulation of pharmacokinetics of incorporated molecules. With this association, the properties that govern drug absorption, distribution, and elimination while in the human body are determined not by the drug properties, rather by the nanosystems physical–chemical properties, particularly surface exposed molecules and electric charge, and its size [100].

General properties of nanosystems that favor their use in antiretroviral drug delivery are well known and include versatility (virtually all drugs may be encapsulated), good toxicity profile (depending on used excipients), possibility of drug-release modulation, high drug payloads, relative low cost, easiness to produce and possible scale-up to mass production scale [7,8]. Their ability to incorporate, protect and/or promote the absorption of non-orally administrable anti-HIV drugs, namely mono- or oligonucleotides [101,102], is of importance to improve the bioavailability of several molecules [103–105]. Once bioavailable, protection of incorporated drugs from metabolism is a favorable feature of nanosystems, allowing prolonged drug residence in the human body, thus reducing needed doses and prolonging time between administrations.

The use of nanoparticulate systems for antiretroviral drug delivery may be particularly advantageous for targeted delivery, namely to cells or organs that are directly implicated in HIV/AIDS [7,8]. This can be achieved either by passive or active targeting. Passive targeting is based in the inherent properties of different nanosystems, namely size, particle shape, and surface charge, which can modulate its bioavailability, biodistribution and/or targeting; in the case of active targeting, nanotechnology-based systems are conveniently modified, most commonly by surface attachment of specific ligands that are able to recognize target cells or sites, and/or escape bioelimination processes [106]. One important limitation of many current antiretroviral drugs is their unavailability to circumvent efflux pumps (particularly P-glycoprotein) that are present, for instance, in the membrane of several HIV-target cells and BBB endothelium. Nanoparticulate systems' ability to escape these bioelimination processes is an added advantage in order to avoid this particular resistance mechanism to drug delivery, namely to the CNS [107]. Thus, increasing the amount of available antiretroviral drugs and its residence time at target sites allow thinking about dose reduction and, consequently, simpler but improved regimens with less adverse effects and increased compliance.

Also, the possibility of incorporating different antiretroviral drugs in the same delivery system and modulate their release individually has been shown possible [108]. This fact may contribute to simplify drug administration schedules, being an important objective towards the reduction of antiretroviral drug administration errors. Nanosystems seem to be able to reduce antiretroviral drugs toxicity, namely at the cellular level, providing that rigorous selection of materials and adequate preparation techniques are assured [109,110]. Even if drug uptake is increased when encapsulated in nanocarriers, cell toxicity seems to be diminished, probably due to the slow-release properties of these systems. This possibility is particularly interesting taking in consideration the well-known toxicity associated with anti-HIV therapy. However, the effects of the possible bioaccumulation of nanosystems components have not been fully addressed and may pose a point of concern for prolonged used. Lastly, nanosystems may allow obtaining antiretroviral medicines with adequate shelf-life, even if this issue has not yet been fully explored. Formulation should be carefully performed and long-term stability studied since antiretroviral drug-loaded nanotechnology-based systems may undergo several physical–chemical changes that can potentially impair efficacy and safety [111,112,113].

4.2. Intracellular delivery

The involvement of several immune cells in the pathogenesis of HIV/AIDS led to the development of nanotechnology-based systems able to target these cells. Particularly, the macrophage has been the focus of most studies, with first reports of these efforts being tracked down to the beginning of the 1990s. Macrophages are well recognized phagocytic cells of the reticulo-endothelial system (RES) and one of the main responsible for the uptake and clearance of administered drug-loaded nanoparticles. Once opsonization and endocytosis occur, nanoparticles are incorporated in an endolysosome, being degraded; however, the ability of various nanoparticles to escape the endolysomal compartment allows incorporated drugs to be delivered to the cytoplasm and, eventually, to the nucleus [114]. Indeed, the ability of nanosystems to release its content after cellular uptake is essential and not always straightforward as highlighted by Dinauer et al. for nanoparticulate protamine–antisense oligonucleotide complexes [115]. Pioneer work by Schäfer et al. found that the physical–chemical properties of nanoparticles, in particular their composition, surface characteristics and size, influence the rate of uptake by macrophages [116]. Most importantly, results obtained by this group for several polymethylmethacrylate-based and albumin-based nanoparticles loaded with zidovudine, highlighted the enhanced nanoparticle uptake by macrophages, particularly when these cells were infected by HIV (up to 60% more than for uninfected macrophages). Additional studies showed that albumin-based and polyhexylcyanoacrylate-based nanoparticles (PHCA nanoparticles) loaded with zidovudine and zalcitabine were effective in the prevention of HIV infection of monocytes/macrophages *in vitro* (known to better reflect HIV-1 infection of macrophages than other commonly used macrophage cell lines), again due to increased uptake of the drugs by these HIV-susceptible cells [117]. Further *in vitro* work by this group showed that PHCA nanoparticles were effective for increasing phagocytosis and antiviral activity of saquinavir and zalcitabine in HIV-infected monocytes/macrophages cultures [118]. Results combined substantiated the possibility of improve intracellular pharmacokinetic profile of antiretroviral drugs in HIV-infected macrophages while lowering dosages needed to allow antiretroviral drugs to be effective, thus potentially augmenting their therapeutic window.

More recently, Shah and Amiji [119] developed poly(ethylene oxide)-modified poly(epsilon-caprolactone) (PEO–PCL) nanoparticles loaded with radiolabeled [^{3}H]-saquinavir and evaluated its *in vitro* uptake by monocytes/macrophages (human THP-1 cell line). Although PEO modification is known to confer hydrophilic properties to PCL particles, which can compromise phagocytosis by steric effect, results showed that cellular uptake of [^{3}H]-saquinavir was still about 10-fold higher than for the free drug. Microscopic observations confirmed qualitatively that a significantly high percentage of nanoparticles were internalized in monocytes/macrophages (Fig. 1). Also, longer intracellular drug residence was achieved, presumably due to the slow drug release provided by the nanosystem's polymeric matrix. Taken together, the data suggest that PEO–PCL nanoparticles can target macrophages without potentially compromising prolonged drug residence *in vivo*. Similar results were obtained by Mainardes et al. for zidovudine-loaded

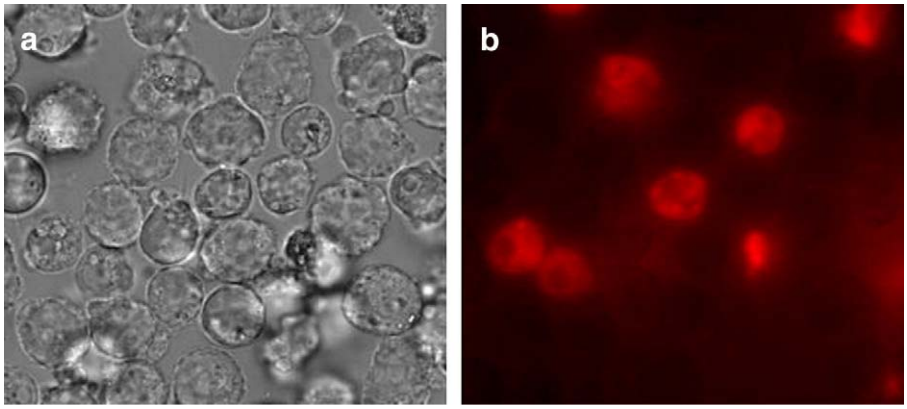

Fig. 1. Uptake of rhodamine 123-loaded PEO–PCL nanoparticles by THP-1 monocyte/macrophage cells, after 2 h of incubation at 37 °C. (a) Bright field image showing intact THP-1 cells and (b) matching fluorescent image showing the cellular uptake of rhodamine-123 labeled nanoparticles (in red). Both images were acquired at 40× magnification. Reproduced from Ref. [119], Copyright (2006), with kind permission from Springer Science + Business Media, Inc.

PEGylated-poly(L-lactide) (PEG–PLA) nanoparticles [120]. These investigators observed that, although PEGylation reduced nanoparticle uptake by polymorphonuclear leucocytes in a concentration dependent fashion, even at the highest PEG/PLA ratio tested (1:1) phagocytosis was not prevented. Additionally, the presence of a hydrophilic moiety at the surface acts as a barrier to which low soluble drugs have poor affinity, thereby delaying transport from the system core to the outside, and consequently prolonging drug release.

Alongside polymeric nanoparticles, other nanosystems were studied for their ability to increase drug uptake by macrophages and enhance antiviral effects. For instance, Dou et al. observed that HIV infected monocytes/macrophages are able to uptake and retain indinavir-loaded, lipid-based nanoparticles *in vitro*, maintaining the drug concentration inside and outside the cell for at least 5 days, while silencing HIV replication [121]. Mechanism of increased uptake is not clear but appears to be related, at least partially, with particles' size and surface charge. These results seem to confirm that these nanosystems may allow prolonging drug levels when compared with the free drug, thus potentially reducing the number of needed drug administrations. Nanosystems were also shown to have the potential of intracellular delivery of labile antiretrovirals, such as phosphorylated nucleoside analogues. Vinogradov et al. tested poly(ethyleneimine) (PEI) nanogels in order to deliver zidovudine triphosphate, the active form of this NRTI, to the interior of cells [122]. The higher affinity of developed cationic nanogels for cell membranes of two breast cancer cell lines (MCF-7 and MDA-MB-231) helped their uptake, resulting in increased intracellular levels of zidovudine triphosphate. *In vitro* results obtained by Hillaireau et al. also indicate that hybrid polymeric nanocapsules of PEI and poly (*iso*-butylcyanoacrylate) may be feasible and non-cytotoxic systems to incorporate and increase macrophage uptake (10 to 30-fold) for zidovudine triphosphate [123]. These strategies demonstrate potential in overcoming antiretroviral resistance due to cellular phosphorylation impairment and open new roads to the delivery of phosphorylated nucleoside analogues.

Even if HIV-target cells, in particular macrophages, are generally able to uptake nanoparticles by non-specific phagocytosis, receptor-mediated endocytosis has been shown advantageous in increasing its specific cellular uptake. Thus, decorating the surface of nanoparticles with specific ligands is an easy way to augment the uptake by specific cells. Early reports mentioned obvious modifications of nanosystems with molecules known to be implicated in HIV infection, such as surface modified liposomes with CD4 receptor molecules or anti-CD4 immunoglobulin [124]. Even if *in vitro* results seemed promising, the feasibility of this type of modification was compromised by the variability and immunogenicity of chosen surface ligands, therefore recommending the search for other substances. For example, Couvreur et al. recently developed a new formulation approach for nucleoside analogue drugs based on the conjugation of the molecule of interest with 1,1′,2-trisnorsqualenoic acid [125,126]. This conjugation process, denominated by the authors as *squalenoylation*, renders molecules that self-organize in water by nanoprecipitation with poly(ethylene glycol) (PEG) derivatives (cholesterol-PEG or squalene-PEG), forming nanoassemblies of 100–300 nm in diameter. This group applied the innovative concept to zalcitabine and didanosine; the squalenoylation of these two drugs resulted in increased antiretroviral activity *in vitro* in human peripheral blood monocyte cultures (roughly 2-fold) when compared to the free molecules [125]. Also, the high drug loading capacity of these nanocarriers (up to 36% w/w for zalcitabine nanoassemblies of approximately 200 nm [126]) combined with an efficient surface coverage by PEG are interesting features that may increase the half-life of conjugated drugs. However, further studies are required to confirm the utility of these novel nanosystems.

Active targeting of drug-loaded nanosystems is also possible by using approaches that do not involve the use of surface modifiers, such as the development of stimuli-sensitive formulations [127]. For example, improved macrophages uptake of anti-HIV compounds was shown to be possible by using environmentally-sensitive nanosized systems. Düzgüneş et al. developed pH-sensitive liposomes in order to target several antiretroviral compounds (one antisense oligodeoxynucleotide, one ribozyme, and two acyclic nucleoside phosphonates) to macrophages and tested their ability *in vitro* to inhibit viral replication in these cells [128]. Also, liposomes were PEGgylated in order to improve liposomes circulation time in the bloodstream. Results showed increased inhibition of all tested antiretroviral molecules when compared to their free form; the ability of liposomes to be taken up by macrophages, alongside with their improved capacity to deliver content into the cytoplasm by destabilization at the acidic pH achieved in endosomes, are responsible for these outcome.

As reported above, the number of studies showing enhanced cellular uptake of antiretroviral-loaded nanosystems is significant and provides proof of the utility of this drug delivery strategy. Even so, the mechanisms supporting experimental data are not always clear, thus resulting in incomplete understanding of the role of different kinetic processes and their interactions in the final outcome. In a recent paper by Ece Gamsiz et al. [129], the mechanisms responsible for the enhanced uptake previously observed for saquinavir-loaded PEO–PCL nanoparticles [119] were investigated by developing a predictive theoretical model based in the results of various individual key processes, namely drug release from nanocarriers outside and inside the cells, free drug and drug-loaded nanocarrier uptake into cells. The developed model showed to be useful in predicting saquinavir uptake

enhancement by THP-1 human monocyte/macrophage cells when encapsulated in PEO–PCL nanoparticles, confirming the major role of nanoparticle uptake kinetics on the overall higher cellular drug concentration [129]. The authors further highlighted that even though the developed model may not be generalized for other than the specific presented conditions, it represents a rational approach for theoretical developing and understanding nanotechnology-based enhancement of antiretroviral drug delivery to macrophages, and a starting point for more advanced and generic models.

Modifications of nanocarriers for the targeted delivery of anti-HIV drugs are further discussed in another article of the present Theme Issue.

4.3. Lymphatic system delivery

The presence of wide amounts of HIV-susceptible immune cells in the lymphoid organs makes antiretroviral drug targeting to these sites of tremendous interest in HIV therapy. This strategy comprises once again targeting nanosystems to immune cell populations, particularly macrophages. The normal uptake of nanoparticles by macrophages present in the RES is indeed an important passive method for targeting this anatomical reservoir site, as early demonstrated *in vivo* by Löbenberg et al. [130,131]. In one study by this group, [^{14}C]-zidovudine-loaded PHCA nanoparticles were administered intravenously in a rat model; soon after administration, the drug was detected in the organs of the RES in concentrations above 18-fold of those for the drug aqueous solution [131]. These results were further confirmed by radioluminography experiments, which showed accumulation of zidovudine-loaded nanoparticles in macrophage rich organs, particularly the gastrointestinal tract and liver [132]. Identical effects were also observed by these investigators after oral intake [133], providing evidence that cell/organ drug targeting may also be achieved by administering drug-loaded nanoparticles through more patient-friendly routes. This possibility was backed up by Dembri et al., who studied the applicability of oral administration for zidovudine-loaded poly(*iso*-hexylcyanoacrylate) nanoparticles in rats [134]. This group observed drug accumulation in the intestinal mucosa after direct gastric administration, being the concentration of zidovudine in Peyer's patches around 4-times higher for nanoparticles than for drug solution; also, tissue concentrations (30–45 μM) were much higher than those reported for HIV IC_{50} (0.06–1.36 μM). This approach showed to be efficient in concentrating zidovudine in the gastrointestinal tract and GALT, which are important sites for HIV replication and perpetuation, as highlighted previously. Also, studied nanoparticles allowed prolonging drug release because of the slow degradation of the polymeric matrix.

Jin et al. recently proposed a series of self-assembled drug delivery systems based on amphiphilic drug–cholesteryl conjugates, presenting adequate physical–chemical characteristics (e.g. narrow submicron size distribution, prolonged shelf-life after freeze-drying, stable after heat sterilization), to be used in the parenteral administration of nucleoside analogues [135,136]. Once in water, these cholesteryl derivatives are able to self-arrange in various nanosized structures. Of particular interest, *in vivo* studies with cholesteryl–succinyl didanosine nanotubes showed preferential distribution of these drug conjugates towards the liver, spleen and lungs after intravenous injection to rats [137]. Despite favorable drug distribution to lymphoid tissues, poor *in vitro* activity against HIV due to the slow degradation of the conjugate into free didanosine may limit the applicability of these systems in the future.

Indinavir-loaded liposomes administered subcutaneously to HIV-2_{287}-infected pig-tailed macaques (*Macaca nemestrina*) were shown successful in achieving higher levels of this drug in the lymph nodes than when formulated in aqueous solution and administered by the same route [138]. This localized drug concentration increase was variable according to lymph node anatomical localization (2.5 to 32.5 lymph node/plasma ratio, contrasting to negligible lymph node levels for indinavir aqueous solution); conversely, indinavir-loaded liposomes allowed for a 10-fold reduction in peak plasma concentration and a 6-fold enhancement in plasma terminal half-life, leading to viral load reduction in lymph nodes and plasma and, in some cases, reversed the CD4+ T cell decline. Kinman et al. further optimized indinavir-loaded liposomes in order to improve lymphoid tissue localization and pharmacokinetic profile [139]. PEGylation of liposomes demonstrated to provide 6-fold higher indinavir levels in lymph nodes and enhance drug exposure in blood when compared to non-PEGylated liposomes administered subcutaneously to macaques. Additionally, antiviral results were improved. Mathematical models based on the pharmacokinetic results obtained by this previous group [138] indicate that the concomitant use of the proposed nanocarrier and conventional oral indinavir regimens could be a valuable strategy in order to prolong the utility (i.e. drug lifespan before resistance occurs) of antiretroviral drugs [140].

The applicability of active drug targeting to immune cells, as shown previously *in vitro*, is also an interesting approach for delivering antiretroviral drug to lymphoid organs [141,142]. For example, Gagné et al. used anti-HLA-DR immunoglobulin-modified liposomes to deliver indinavir to lymphoid organs, based on the fact that immune cells present on these sites (e.g. macrophages and activated CD4+ T cells) express substantial levels of the HLA-DR determinant of the major histocompatibility complex class II molecules [143]. Obtained results after subcutaneous administration to mice indicated that surface modification was successful in active drug-targeting lymphoid tissues, while maintaining anti-HIV activity of the drug *in vitro*. However, this study also alerts to the perils of specific-drug targeting by nanocarrier surface modification with potentially immunogenic molecules, as Fab′ fragments-modified liposomes were 2.3-fold less immunogenic than carriers bearing the entire IgG [143]; adequately toxicity and immunogenic studies are therefore imperative in such cases.

Based on previous *in vitro* work showing that monocytes/macrophages are able to uptake and retain indinavir-loaded, lipid-based nanoparticles [121], and the ability of these cells to cross the BBB, Dou et al. proposed that nanoparticle-loaded monocytes/macrophages could serve as "Trojan horses" for delivering antiretroviral drugs to the CNS (further discussed on Section 4.4, *Central nervous system delivery*) [144]. At the same time these researchers were pursuing this intention, *in vivo* studies in mice showed that nanoparticle-loaded monocytes/macrophages were able to distribute indinavir to lymphoid and nonlymphoid tissues in significantly therapeutic concentrations for at least 14 days, allowing to reduce the number of virus-infected cells in plasma, lymph nodes, spleen, liver, and lungs, as well as promoting CD4+ T cell protection in a HIV-1-infected humanized immunodeficient rodent model [144,145]. The complexity related with the direct handling of monocytes/macrophages may limit this technique as it is presented; however, this study represents significant evidence that the uptake by monocytes/macrophages is a crucial event for explaining the ability of nanosystems to target lymphoid organs.

Besides oral and intravenous routes, other means for antiretroviral drug administration such as transdermal delivery would be of interest [146–148]. Obtained results of *in vitro* permeability studies using rat skin indicate that these systems are able to augment skin permeation. Additionally, *in vivo* studies in rats using topically administered zidovudine-loaded liposomes showed that these systems enhanced plasma concentrations by nearly 12-fold when compared to the drug formulated as a hydrophilic ointment (expressed as $AUC_{0-24\,h}$); preferential distribution to RES organs (e.g. spleen and lymph nodes) was also observed for liposomal formulations, particularly in the case of PEGylated liposomes (up to 2-fold and 27-fold of the concentrations observed for non-PEGylated liposomes and zidovudine hydrophilic ointment, respectively) [146,147]. According to the authors, PEGylation of these systems possibly avoids interaction with components of the subcutaneous interstitium, which could cause their partial deposition in this tissue. However, further studies are required in order to assess the

significance of these results, namely if the amounts of drug required to achieve therapeutic levels can be adequately formulated in transdermal delivery systems.

In agreement with the reports presented above, several research groups developed and reported other nanocarriers, namely liposomes and SLNs, that were able to provide lymphatic system drug-targeting, presenting a consistent basis for their therapeutic use to target this important HIV-reservoir site [149–154]. Globally, these studies also highlight the safety of used nanosystems and the improved drug residence *in vivo*, suggesting that enhanced and prolonged anti-HIV suppression could be obtained using nanotechnology-based carriers.

4.4. Central nervous system delivery

Nanotechnology-based systems have been extensively studied, with particular success, to overcome the natural barriers to drug delivery posed by the CNS anatomy, histology and physiology [155,156]. Particularly, the BBB is characterized by the presence of tight cell junctions between endothelial cells of the CNS capillaries, representing an efficient obstacle to the delivery of many drugs [157]. Poor BBB penetration and/or extensive binding to plasma proteins are intrinsic characteristics of several anti-HIV drugs that act as limiting factors; however, even for drugs that penetrate sufficiently into the CNS, the presence of drug efflux pumps at the BBB, such as P-glycoprotein, can rapidly diminish drug concentrations in the nervous tissues. Consequently, the use of nanosystems should help not only achieving higher concentrations of encapsulated drugs, but also allow prolonged residence in the CNS.

Experiments in the late 1980s by Kim et al. evidenced that zalcitabine-loaded liposomes could provide an effective method to retain this antiretroviral drug in the CNS of rats [158]. However, these investigators administered developed liposomes by intraventricular injection which is not feasible for human anti-HIV therapeutics, particularly for prolonged use. Since these first observations several efforts have been made in order to understand and improve CNS delivery of antiretroviral drugs by using nanocarriers. Some insights on the mechanisms and particle properties involved in the enhanced permeability of antiretroviral-loaded nanosystems through the BBB have been gained from recent *in vitro* experiments by Kuo et al. [159–161]. This group tested the ability of different nanocarriers, namely polybutylcyanoacrylate nanoparticles, methylmethacrylate-sulfopropylmethacrylate nanoparticles, and cationic SLNs, to permeate *in vitro* models of the BBB based on brain-microvascular endothelial cells (BMEC). Various antiretroviral drugs were successfully incorporated, and their uptake by BMEC *in vitro* was enhanced by 3- to 16-fold (human BMEC) or by 8- to 20-fold (bovine BMEC), depending on encapsulated drug and nanocarrier properties. In another study, Vyas et al. developed several nanoemulsion formulations containing [^{3}H]-saquinavir and examined the drug brain uptake after oral administration to Balb/c mice [162]. Results showed higher plasma and brain concentrations of [^{3}H]-saquinavir for nanoemulsion formulations, as compared to the aqueous suspension of the drug. Also, oral results were compared to those obtained by intravenous injection of developed formulations (nanoemulsions and aqueous suspension). Combined pharmacokinetic analysis of results suggested that increased enteric absorption and brain concentrations of saquinavir could be attributed mainly to the preferential uptake of polyunsaturated essential fatty acids that compose used oils (flax-seed oil or safflower oil) and P-glycoprotein inhibition by deoxycholic acid (co-surfactant) at both sites, i.e. gastrointestinal tract and BBB [162].

As highlighted before for other HIV-reservoir sites, active drug targeting to the brain has proved to be an efficient way to enhance antiretroviral drug delivery to the brain. For instance, transferrin receptors present in the luminal membrane of brain endothelial cells have been used as preferential targets for enhanced antiretroviral drug delivery to the CNS by means of nanoparticulate systems [107]. For example, Mishra et al. developed and administered intravenously zidovudine-loaded PEGylated albumin nanoparticles to rats [163]. Further, these investigators modified these systems by anchoring transferrin molecules and compared CNS uptake of zidovudine. Biodistribution results showed an increase in brain distribution and plasmatic circulating time for both nanoparticulate systems, as compared to the free drug; however, transferrin modification enhanced significantly brain concentration (approximately by 20%), while modestly diminishing distribution to RES organs. In another study, the group of Labhasetwar tested the ability of ritonavir-loaded PLA nanoparticles conjugated with HIV-1 trans-activating transcriptor (TAT) peptide to overcome the BBB and bypass the efflux action of P-glycoprotein [164]. The ability of TAT peptide to permeate biological membranes by a receptor-mediated, endocytosis-independent and transporters-independent mechanism makes this new nanosystem of particular interest to overcome poor CNS drug penetration by most PIs and other antiretrovirals. *In vitro* and *in vivo* results from this study showed that, despite larger than the median opening of the BBB (~300 nm vs. ~8 nm), these systems are transported through the brain parenchyma (Fig. 2) without any apparent damage of the BBB integrity. Ritonavir levels in the CNS were up to 800 times higher at 2 weeks post-intravenous administration of TAT-nanoparticles to mice, compared to the drug in aqueous solution [164]. In addition to enhanced CNS drug delivery, the sustained brain levels of ritonavir provided by TAT-nanoparticles in mice expressing P-glycoprotein also makes proof that this is an attractive strategy to overcome PIs efflux from the CNS.

Interestingly, macrophage targeting also seems to be an indirect way for enhancing CNS levels of antiretrovirals. The migration of these immune cells to the CNS, alongside with the relatively high amounts persistently present at this site, makes this strategy conceptually possible. *In vivo* results obtained by various investigators seem to backup this possibility. In one study, Kaur et al. administered subcutaneously didanosine-loaded, mannan-coated gelatin nanoparticles to rats and found 12.4 times higher concentrations of the drug in brain tissue, when compared with didanosine in aqueous solution [165]. Also, Dou et al. observed that indinavir levels in the brain of mice could be increased up to 20 times when monocytes/macrophages loaded with lipid-based nanoparticles containing the drug were intravenously administered (as compared with the drug solution) [166]. Particularly, rhodamine-labeled indinavir-loaded nanoparticles were readily observed in brain subregions with active astrogliosis, microgliosis, and neuronal loss. However, none of the reviewed studies was able to unequivocally demonstrate that macrophage-mediated transport to the CNS of antiretroviral drug-loaded nanosystems contributes significantly to increased brain levels, requiring this hypothesis further mechanistic insight.

5. Nanotechnology-based systems for HIV/AIDS prevention

5.1. Microbicides

5.1.1. Microbicides: possibilities and limitations

The simple idea of inserting topical microbicidal products in the vagina or rectum before sexual intercourse, in order to prevent sexual transmitted diseases, particularly HIV/AIDS, has gained significant support in the last decade [167]. Among other potential advantages, microbicides may be particularly important in those settings where women do not have the possibility to negotiate condom use, as these products do not require the cooperation, consent or even knowledge of male partners. First microbicides to reach clinical trial were almost exclusively tested for the prevention of vaginal HIV transmission and based on simple formulations, such as polymeric gels containing promising compounds that were shown to have significant unspecific antiviral activity *in vitro*. Nonoxynol-9 formulations were the first being tested in preventing HIV vaginal transmission [167]. Despite

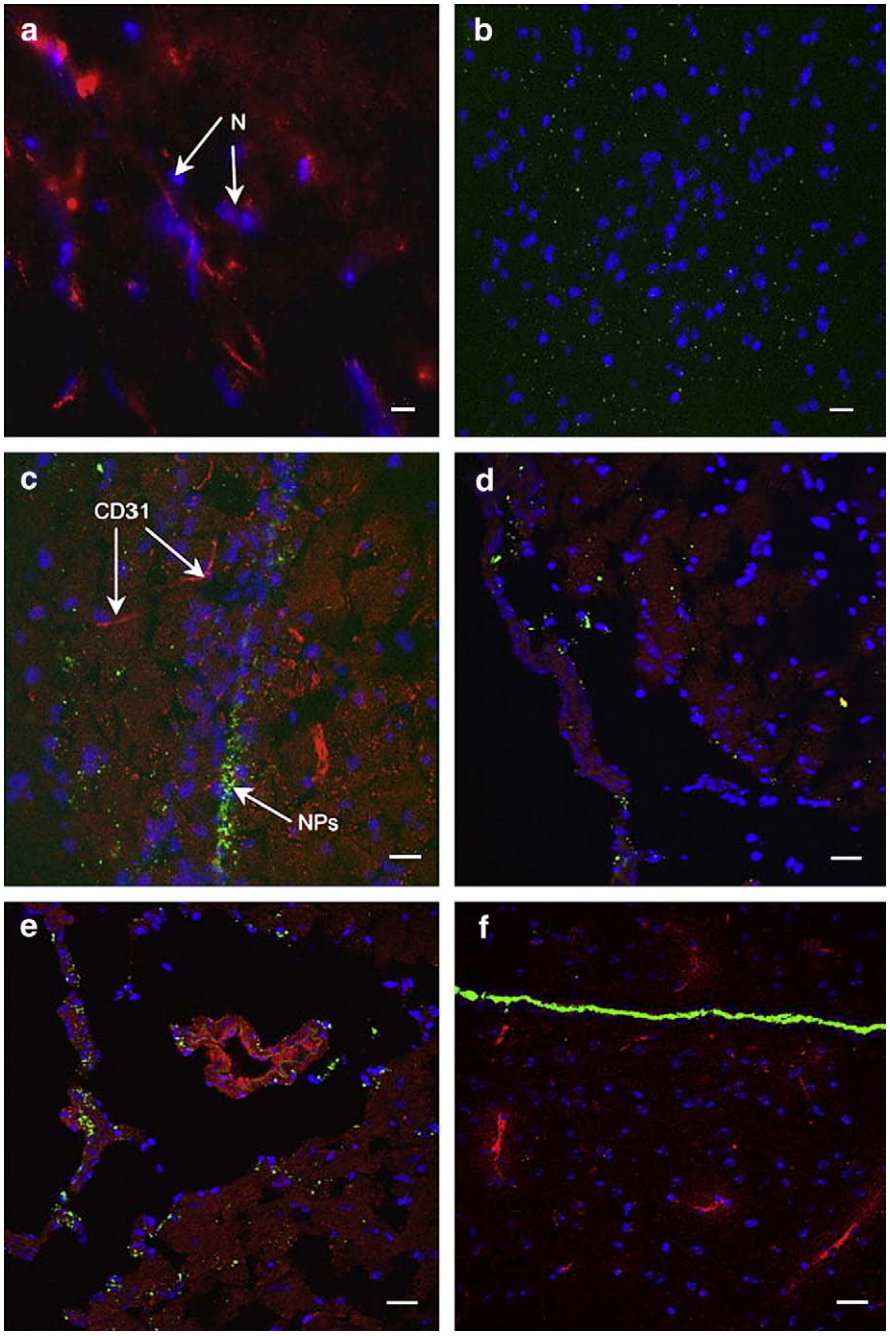

Fig. 2. Fluorescent confocal microscope imaging of mice brain sections showing the localization of non-conjugated and TAT-conjugated PLA nanoparticles loaded with 6-coumarin, 24 h after intravenous injection at a dose of 250 mg/kg (equivalent to 45 mg/kg dose of ritonavir used in the biodistribution study). (a) Endothelial cell staining with anti-CD31 antibody conjugated to AlexaFluor 568, (b) localization of TAT-nanoparticles within the cortex, (c) and (d) localization of non-conjugated nanoparticles within the ventricles and parenchyma of the brain, respectively, and (e) and (f) localization of TAT-nanoparticles within the lateral and third ventricles, respectively. All images were acquired at 40× magnification (representative data from studies in three mice per group; bar = 25 μm). Blue, red, and green are due to DAPI-staining of the nuclei (N), CD31 antibody-staining the blood capillaries (CD31), and 6-coumarin-loaded nanoparticles (NPs), respectively. Reprinted from Ref. [164], Copyright (2008), with permission from Elsevier.

initial high expectations, large clinical trials concluded that no protection was achieved; in fact, nonoxynol-9 formulations were shown to enhance HIV transmission [168]. Other candidates followed but similar frustrating results led researchers to start questioning the real value of this strategy. While waiting for the next generation of microbicides, several hypotheses have been proposed to explain these results, mostly related to drug-induced mucosal damage, inconsistent microbicide use or poor clinical trial design [169]. However, it has also been suggested that inappropriate drug formulation may be an important factor for failure [170]. The main problem of currently available products is their poor ability to provide an effective and durable drug barrier along the epithelial lining. Also, the inefficiency of current conventional formulations to provide sustained-release and deliver active molecules to target sites is an important issue that should be resolved [171,172]. Thus, innovative approaches to the vaginal delivery of microbicides, namely those based in nanosystems, are being considered to achieve effective protection.

5.1.2. Nanotechnology-based microbicides

As documented above, several antiretroviral agents have already been successfully incorporated in diverse nanocarriers, but the utilization of these systems in the formulation of vaginal microbicides

has not yet been conveniently investigated. Some properties of these nanotechnology-based systems, e.g. prolonged release of active agents and ability to penetrate epithelial linings, are important advantages that may favor their utilization in the field of microbicides. Also, their possible use for targeted and intracellular drug delivery, namely to HIV-target cells, and capability to protect antiretroviral agents from *in vivo* degradation are interesting features. In one of the first reports endorsing the possible use of nanotechnology-based systems for the development of microbicides, the groups of Baba and Akashi described polystyrene-based nanospheres bearing a lectin (concanavalin-A) on their surface and demonstrated *in vitro* their ability to capture HIV-1 virions as a result of the high affinity between concanavalin-A and viral gp120 [173]. Although these nanospheres were originally developed as mucosal vaccine adjuvants, their capacity to reduce HIV-1 infectivity led these investigators to suggest the study of nanosystems for microbicides development. An additional highlighted advantageous feature of these nanosized systems for the purpose of microbicide development was their high surface area, which allowed increasing the amount of available concanavalin-A molecules for interacting with virions [174].

Even if no further studies towards this goal have been conducted using concanavalin-A-bearing nanospheres, proof of concept was established and the potential of nanotechnology-based microbicides was recognized. The group of Penadés has been working for several years now in the development of gold glyconanoparticles, comprising sugar bio-functional gold nanoclusters of reduced dimensions [175], and is currently exploring the potential of mannose-coated gold nanoparticles as possible microbicides, namely because of their capacity to interfere with HIV-DCs binding and dissemination. As previously discussed, DCs (and Langerhans cells) play an important role in the vaginal transmission of HIV and may be an important target for the development of preventing strategies. *In vitro* results obtained by surface plasmon resonance indicate that α-1,2-mannose disaccharide-coated gold nanoparticles are able to completely inhibit DC-SIGN/gp120 binding at the level of 10 nM, contrasting with 500 μM for α-1,2-mannose disaccharide alone [176]. Also, significant *in vitro* inhibition of HIV-1 binding to DC-SIGN expressing cells and *trans*-infection of T cells by α-1,2-mannose disaccharide-coated gold nanoparticles was observed (85%–90% inhibition vs. 60–79% for the free disaccharide) [177]. These preliminary results illustrate again that the high surface area provided by nanoparticles is able to maximize the number of available molecules to interact with DC-SIGN; however, further studies are needed to fully evaluate the potential of these nanosystems to be used as microbicides.

Recently, Ham et al. studied the feasibility of poly(D,L-lactide-*co*-glycolide) (PLGA) nanoparticles to encapsulate PSC-RANTES (~8 kDa) in order to enhance vaginal epithelial penetration and drug targeting to CCR5-expressing cells, thus resolving poor drug distribution and retention issues [178]. PSC-RANTES is an analog of the natural occurring chemokine RANTES (Regulated on Activation Normal T cell Expressed and Secreted), being able to inhibit R5-tropic HIV-1 infection of T lymphocytes and monocytes by blocking CCR5 co-receptor [179]. Despite showing protection *in vitro* against HIV-1, *in vivo* experiments in the rhesus macaque (*Macaca mulatta*) SHIV model demonstrated that the needed dose of PSC-RANTES for protection was much higher than in cell models. Results obtained by Ham et al. showed that nanoparticles (257 ± 20 nm) loaded with biotinylated PSC-RANTES (used in substitution of PSC-RANTES to allow tissue localization by streptavidin-FITC staining) were able to penetrate human ectocervical tissues *in vitro* and mediate drug transfer to the deeper layers of the epithelium, immediately above the lamina propria where CCR5-bearing target cells are predominantly located (Fig. 3) [178]. Indeed, ectocervical/vaginal basal epithelium may be an optimal target site for nanoparticles containing antiretroviral agents as this site is not submitted to coital shearing felt in the vaginal lumen, which promotes leakage of current microbicide products. Moreover, the basal epithelial layer is not located too deeply in the mucosa, which may allow drug diffusion to the nearby

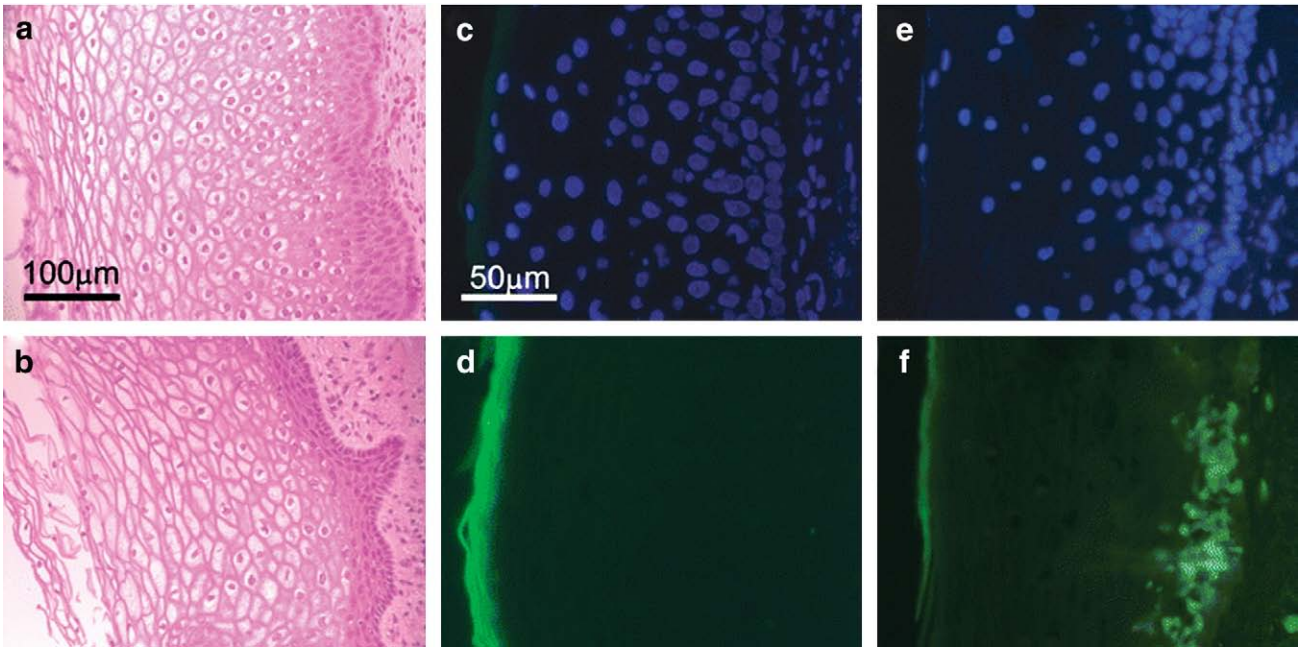

Fig. 3. Microscopic images of ectocervical tissue (a) before exposure to nanoparticles and (b) after exposure to nanoparticles indicating no significant gross morphologic changes (H&E staining). Fluorescent microscopic images (40× magnification) of ectocervical tissue treated with unformulated PSC-RANTES-biotin for 4 h with (c) DAPI staining (epithelial cells nuclei in blue) or (d) streptavidin-FITC staining (PSC-RANTES-biotin in green) revealed that unformulated PSC-RANTES-biotin remained at the superficial epithelial layer. Conversely, fluorescent microscopic images (40× magnifications) of ectocervical tissue treated with PSC-RANTES-loaded nanoparticles for 4 h with (e) DAPI staining (epithelial cells nuclei in blue) or (f) streptavidin-FITC staining (PSC-RANTES-biotin in green) showed that PSC-RANTES-biotin was able to permeate the superficial epithelium and accumulate at the basal epithelium. Reproduced from Ref. [178], Copyright (2008), with kind permission from Springer Science + Business Media, Inc.

milieu of the ectocervical/vaginal epithelium but not to nearest blood vessels of the lamina propria in considerable amounts. The group of Rohan further observed that mucosal uptake of PSC-RANTES-loaded nanoparticles was nearly 5-fold higher when compared to unformulated PSC-RANTES [178]. Also, nanosystems were able to retain the drug activity *in vitro* when compared to the unformulated form. PSC-RANTES-loaded nanoparticles presented prolonged drug release, an important feature considering that microbicide formulations should require minimal number of applications per time period while maintaining sustainable amounts of active drug *in loco*. However, these results require further *in vivo* confirmation in order to attest the real value of this interesting novel approach. Supporting these observations, other investigators have also reported interesting properties of nanoparticles for their utilization in microbicides development. For instance, *in vitro* studies have demonstrated the ability that polymeric nanoparticles have to permeate cervical mucus, depending on size, concentration and surface properties, which is known to be a significant biological barrier for particles reaching the epithelium [180,181]. Of particular interest, PEGylation seems to be an interesting strategy for enhancing nanoparticle transport through cervicovaginal fluids. Also, recent work by Saltzman et al. highlighted the favorable behavior PLGA nanoparticles have *in vivo* with the aim of developing effective microbicides, namely their safety, full and deep genital tract distribution, and tissue retention (up to one week) [182]. These researchers developed 100–300 nm PLGA nanoparticles containing small interfering RNA (siRNA) targeted against enhanced green fluorescent protein (EGFP) and observed the complete knockdown of gene expression in the genital tract of transgenic GFP mice up to around 2 weeks, after vaginal instillation. These results seem to substantiate that PLGA nanoparticles might confer prolonged action to incorporated microbicidal molecules due to sustained drug-release and intracellular drug-targeting.

The potential of other nanocarriers for vaginal administration of RANTES analogues has also been investigated by Kish-Catalone et al. [183,184]. This group developed a new synthetic analogue, designated −2 RANTES, and tested the ability of commercial paucilamellar non-phospholipidic liposomes (Novasomes 7474; Novavax, Inc., Rockville, MD) to incorporate this peptide-based molecule [183]. Loaded systems were able to release −2 RANTES *in vitro* in a dose-dependent manner over a time-frame of 30–120 min, while retaining its antiviral activity. Also, local safety studies performed in two rodent models (murine and rabbit) showed no evidence of cervicovaginal toxicity. This group has further evaluated the efficacy of −2 RANTES-loaded Novasomes in preventing infection of cynomolgus macaques (*Macaca fascicularis*) challenged vaginally with R5-tropic $SHIV_{162P3}$, after 30–45 min of the vaginal administration of 0.3 ml of Novasomes formulation [184]. Surprisingly, Novasomes, either blank or drug-loaded (0.13 mM), exhibited considerable prophylactic effect against infection (4 out of 6 animals protected in blank Novasomes and drug-loaded Novasomes groups), contrasting with the poor results of −2 RANTES in PBS solution (2/5, 0/4, and 0/4 animals protected for 0.5 mM, 0.25 mM, and 0.065 mM concentrations, respectively). Although unclear, a possible explanation for protection resides in the physical–chemical properties of these nanosystems (e.g. surfactant properties of its components), which can provide a physical barrier above the epithelium lining that is able to interact and inactivate virions. Results also highlight the importance of complete mucosal coverage provided by Novasomes for the observed protection against vaginal HIV transmission.

5.2. Vaccines

The development of a vaccine has been a paramount strategy in the prevention of HIV infection. However, *in vivo* performance of promising vaccines has proved to be meager, a fact that may be in part attributed to poor protection and delivery of the immunogenic molecules. One disadvantage of current vaccine formulations is their content in aluminum, a commonly used adjuvant, because of its potential toxicity; alongside, instability to freezing and drying, and inconsistency in producing humoral immunity are frequent difficulties posed by aluminum-based vaccines. Hence, nanosystems have been studied mostly as vaccine adjuvants due to their safety profile, as well as for the ability to protect labile immune stimulating molecules (e.g. DNA or peptides/proteins) and enhance their immunogenicity when delivered by diverse routes (e.g. intramuscular, subcutaneous, intradermal, and intranasal) [185,186]. Various examples of nanotechnology-based vaccine candidates may be found in the literature. For instance, Locher et al. developed an anti-HIV-2 nanoparticle-based vaccine comprising 150 nm complexes of gp-140-encoding plasmid DNA with polylysine-graft imidazoleacetic acid in order to enhance immune response [187]. The proposed nanostructure was able to significantly improve specific antibody expression levels after intradermal injection to BALB/c mice, when compared to the administration of plasmid DNA alone. This enhancement is presumably related with the nanostructure ability to protect plasmid DNA by evading lysosomal degradation, therefore resulting in efficient transfection. Escape from the lysosomal compartment is presumably attributed to the polymer imidazole side chain groups [187]. The immunostimulating complex (ISCOM) is an important vaccine delivery system being studied in the clinic, presenting potent adjuvant activity [186]. It comprises ~40 nm cage-like particles produced by combining a protein antigen, cholesterol, phospholipid and the saponin adjuvant Quil A, which is derived from *Quillaja saponaria*; further, due to toxicity problems, a similar system (ISCOMATRIX®; CSL Behring, King of Prussia, PA) was obtained by using a purified fraction of Quil A. Recent studies performed in guinea pigs by Boyle et al. showed that, compared to an aluminum hydroxide-based formulation, identical immune response could be achieved for roughly 100-fold lower levels of recombinant HIV-1 gp120, when incorporated in ISCOMATRIX® [188]. These results suggest that ISCOMATRIX®-based adjuvants may represent an effective means of reducing the amount of antigen required, thus potentially reducing the final cost of vaccine products. In concordance with the above presented studies, other investigators also observed the induction of high titers of anti-HIV antibodies when different immune stimulating molecules were formulated in various nanosystems and administered systemically in animal models (see Table 2 for examples) or human subjects (e.g. MF59™ oil-in-water nanoemulsion containing recombinant gp120 derived from HIV_{SF2} [206], or HIV-1-derived TAB9 protein combined with Montanide® ISA 720 water-in-oil nanoemulsion adjuvant [207]). As highlighted for antiretroviral drug-loaded nanocarriers, nanosystems are able to bear several antigenic substances simultaneously, facilitating the development of multivalent vaccines. Lamalle-Bernard et al. developed an anti-HIV divalent candidate vaccine based in PLA nanoparticles [189]. The two selected viral antigens, p24 and gp120, were co-adsorbed to nanoparticles surface, without their structural integrity being compromised. Upon subcutaneous administration to mice (three administrations separated by 2 weeks intervals), the divalent PLA nanoparticle-based vaccine was highly immunogenic for both antigens, inducing titers of 10^6 and 10^5 for p24 and gp120 antibodies, respectively.

Even if systemically administered vaccines have been intensively investigated, they are generally ineffective for inducing antigen-specific mucosal immune responses. Thus, efforts have also been performed in order to develop anti-HIV mucosal vaccines. Bielinska et al. investigated the ability of an oil-in-water nanoemulsion (~400 nm oil droplets) loaded with recombinant gp120 to immunize mice after intranasal administration [208]. Obtained results indicated that the vaccine formulation was capable to strongly induce both systemic and mucosal immune responses, allegedly because of enhanced tissue penetration of gp120 conferred by its inclusion the nanoemulsion. An

Table 2
Selected examples of nanotechnology-based anti-HIV vaccines.

Adjuvant nanosystems	Immunogens	Animal models	Administration routes	Comments	References
ISCOM	disrupted HIV-2 virions, V3-derived synthetic peptides	Macaque (*Macaca fascicularis*)	IM	Significant immune responses were obtained	[189]
Liposomes	DNA plasmid (HIV-1 *env* and *rev* genes)	Mouse, guinea pig	ID, IM, IN, IP, SC	DNA plasmid was mixed with liposomes immediately before administration; Liposomes greatly enhanced induced immune response but IM, IN, and IP routes were more effective than ID and SC routes	[190–192]
Mannan-coated-liposomes	DNA plasmid (HIV-1 *env* and *rev* genes)	Mouse	IM	DNA plasmid was mixed with liposomes immediately before administration; significantly enhanced humoral and cellular responses were obtained (probably due to Th1-type response enhancement by mannan)	[193]
polylysine-graft imidazoleacetic acid complex	DNA plasmid (HIV-2 gp-140)	Mouse	ID	*See text for details*	[187]
Sendai virus-liposome complexes	Recombinant HIV-1 gp160	Mouse	IN	*See text for details*	[194]
SLNs	Recombinant HIV-1 Tat(1-72)	Mouse	SC	Immunogen was adsorbed to SLNs; significantly enhanced humoral and cellular responses were obtained	[195]
MF59™ oil-in-water nanoemulsion	Oligomeric HIV-1 gp140	Macaque (*Macaca mulatta*)	IN	MF59 significantly enhanced anti-HIV systemic and vaginal humoral response in previously vaccinated animals (IM)	[196]
PLA nanoparticles	Recombinant HIV-1 p24	Mouse, rabbit, macaque (*Macaca fascicularis*)	SC	Immunogen was adsorbed to nanoparticles; significantly enhanced humoral and cellular responses were obtained	[197]
PLA nanoparticles	Recombinant HIV-1 p24 and gp120	Mouse	SC	*See text for details*	[198]
concanavalin-A-bearing polystyrene nanospheres	Inactivated HIV-1 or SIV	Mouse, macaque (*Macaca mulatta*)	IN, IVag	*See text for details*	[199–202]
ISCOMATRIX®	Recombinant HIV-1 gp120	Guinea pig	SC	*See text for details*	[184]
PR8-Flu ISCOM	HIV-1_{sf2} gp120, p24, and V2 and V3 peptides	Macaque (*Macaca mulatta*)	IN, SC	Immunogens were mixed with PR8-Flu ISCOM before administration; SC administration was performed in the proximity of the internal iliac lymph nodes in order to allow targeting these immune sites; ISCOM modified with PR8 (haemagglutinin and neuraminidase subunit of Influenza A Puerto Rico/8/34) elicited strong systemic immune response only when administered by SC route	[203]
PLA nanoparticles	p24, wild-type Tat, or mutated Tat	Rabbit	SC	Immunogens were adsorbed to nanoparticles; immune response was induced but differences in induced spectrum of specificity were observed for PLA nanoparticles and MF59™ nanoemulsions	[204]
Tetanus toxoid (TT) antigen-bearing SLNs	Recombinant HIV-1 gp140	Mouse	SC, IVag	Immunogen and TT were adsorbed to SLNs; Significantly enhanced humoral and cellular responses were obtained particularly when specific toll-like receptors (TLR) ligands were co-adsorbed to SLNs (TT and TLR enhance cell uptake)	[205]

Abbreviations: ID, intradermal; IM, intramuscular; IN, intranasal; IP, intraperitoneal; IVag, intravaginal; SC, subcutaneous.

interesting approach to mucosal anti-HIV vaccine comprising fusogenic liposomes obtained by combining conventional liposomes with UV-inactivated Sendai virus (HJV-liposomes) has been developed by Sakaue et al. [194]. These nanosystems were shown to be able to deliver encapsulated molecules to the cytoplasm of immune cells due to the ability of viral hemagglutinating, neuraminidase, and fusogenic proteins to mediate cellular recognition and uptake [194,209]. After intranasal administration to mice, gp120-loaded HJV-liposomes were able to induce both systemic and mucosal Th1- and Th2-type immune responses, taking advantage of the natural ability of Sendai virus to infect immune cells at the nasal mucosa [194]. As previously referred, researchers led by Baba and Akashi developed concanavalin-A-bearing polystyrene nanospheres, which are able to capture inactivated HIV-1 virions by interacting with gp120 [175]. Upon capture of inactivated HIV-1 by nanospheres and elimination of free viral particles, these HIV-1-bearing nanospheres were administered intravaginally to mice. Results showed that these systems were able to induce local immune response, even if the mechanisms behind these observations remained unclear (possibly IgA-mediated) [210]. Additionally, when HIV-1-bearing nanospheres were administered by the intranasal route to mice, IgA-mediated immune response in the genital tract against HIV-1 was even higher than when administered intravaginally [199,202]. Combined results once again suggest that one of the main advantages of these nanospheres is the high surface area provided by the small diameter (360 to 1230 nm), which allows increasing the amount of virions available for interacting with antigen-presenting cells (e.g. dendritic cells), thus boosting the immune response [201]; also important is the ability to protect viral antigens. However, *in vivo* results obtained from a macaque model (*M. mulatta*) after intranasal immunization with inactivated SHIV-bearing nanospheres showed that only partial protection was obtained upon vaginal challenge with infectious SHIV [210]. Even if results seem promising, a main setback is that the utilization of inactivated HIV-1 vaccines may pose serious safety problems in order to be developed for human use. In concordance with discussed studies, other investigators also observed similar immune induction in animal models when using other antigens formulated as nanosystems and administered by mucosal routes (see Table 2 for examples).

Taken together, these results seem to confirm the utility of nanosystems as adjuvants for the development of anti-HIV vaccines. However, formulation of nanotechnology-based vaccines should be adequately performed as to assure that immunogenic profile of antigens is not altered, for instance, due to changes in their tridimensional structure or masking of some epitopes [204]. Therefore, it is advisable that comparison between nanotechnology-based and other adjuvant-based formulations be performed for each candidate antigen rather than on a model antigen, as the outcome in terms of qualitative differences may vary from one antigen to another.

Advanced in the development process and currently undergoing human phase II clinical trials is a nanotechnology-based topical therapeutic vaccine formulated as a patch, DermaVir (Genetic Immunity LLC, United States/Hungary). Dermavir comprises HIV plasmid DNA material encapsulated in mannosylated PEI nanoparticles, these last being capable of targeting epidermal LCs upon topical administration, as demonstrated *in vitro* [211] and *in vivo* in mice (reviewed in [212,213]). After cell recognition by mannose receptor present in LCs membrane and uptake *via* endocytosis, plasmid DNA-encoded antigens are expressed similarly to what happens upon HIV infection of these cells. Antigen-loaded LCs migrate to local lymph nodes, differentiating into DCs while in transit, and present antigens to naïve T cells, thus inducing HIV-specific cellular immune response [214,215]. *In vivo* experiments performed in chronically SIV-infected rhesus macaques undergoing antiretroviral therapy showed that DermaVir-induced cytotoxic T cell response was able to effectively inhibit SIV replication and enhance control of viral load rebound during treatment interruptions [216]. Even if currently being developed as an adjuvant therapy for patients being treated with antiretroviral drugs, this vaccine may be of interest in the future for the prevention of HIV transmission.

6. Economical feasibility

One important question to be addressed relates to the economic impact that nanotechnology-based systems for the treatment and prevention of HIV/AIDS may have. In fact, this issue can be decisive in translating experimental results into clinical development and practice, particularly considering the current inability to provide effective antiretroviral therapy to most of the infected population, namely in low-income and developing countries. To our knowledge, no relevant study about the subject has been conducted and one may only infer about it [217]. However, several important facts allow the scientific community to be optimistic. For instance, the increased lifespan and therapeutic improvement of older drugs (tendentiously cheaper than those more recently approved [94]) without the development of drug resistance or intolerable adverse effects, is predictable for nanotechnology-based delivery systems. It is usual that several promising antiretroviral molecules are discarded in preclinical phases due to poor pharmacokinetics performance; nanotechnology-based systems may help in resolving some of these troublesome issues, being able to "revive" several of these drugs. Also, the recent rising of nanotechnology-based pharmaceutical companies and the introduction of nanosystem-based drug products in the available therapeutic armamentarium will further allow cost reduction of their currently relatively expensive formulation scale-up and production (even if its development is cheaper than that expected for new active molecules); innovative nanosystems are also expected to allow the enhancement of effective patent protection in a rapidly increasing pharmaceutical market segment [218,219]. Economical aspects of preventative strategies seem even more complex, particularly in the field of microbicides. It is true that expected acceptable prices of US $1 or less per application for microbicides based on conventional dosage forms are not realistic for nanotechnology-based products [220]; however, predictable reduction of the number of needed application, and enhanced efficacy and acceptability may endorse its economic viability. Additionally, potential market dimension (US$0.9–1.8 billion), and public agencies and non-governmental organizations financial support are strong arguments for the development of nanotechnology-based products [221]. Finally and more importantly, indirect savings of hindering the HIV/AIDS pandemic are expected to assure economic return [222].

7. Conclusions

The introduction of nanotechnology in the field of drug delivery opened exciting perspectives towards the development of new therapeutic options for the treatment of several devastating and complex diseases (e. cancer and infectious diseases), culminating in the last decade with the market release of more than a few nanopharmaceuticals [219,223]. In this manuscript we confirmed the ability of nanotechnology-based systems to provide a rationale approach for anti-HIV therapy and prevention. Adding to previous work in the field, new developments in nanocarrier systems for antiretroviral drugs are consolidating this strategy as a particularly interesting approach towards the improvement of HIV/AIDS treatment. Even if not providing a way to cure HIV/AIDS, nanotechnology-based systems may improve drug therapy in infected patients as demonstrate by *in vitro* and animal *in vivo* studies. Various nanosystems have been shown the ability to improve antiretroviral activity of several drugs, while reducing their toxicity and potentially simplifying drug regimens. Also, these systems can provide higher and prolonged drug levels in known reservoir sites for HIV, which can result in better viral suppression and potentially longer time until the

emergence of viral resistance. Even so, some particular aspects of these nanosystems still need to be fully assessed in order to open road towards human clinical trials and market launch of the first anti-HIV nanotechnology-based medicines. Apart from the exciting prospect of approval of VivaGel®, most of the relevance and applicability of nanotechnology-based systems in the field of microbicides are yet in its infancy and much work is still required. However, several helpful objectives towards the development of effective microbicides may be possible to achieve using drug nanocarriers, namely specific drug-targeting to local immune cells or different layers of the mucosa, increased local drug residence, lower toxicity, stimulus-sensitive drug release (e.g. variations in vaginal pH during sexual intercourse), or cellular uptake. Also, other nanostructures that have not yet been studied for this purpose, such as cyclodextrin-drug complexes, nanogels, and SLNs, are starting to be considered in the formulation of novel microbicides, although substantial results are still to be published. Hence, developments are expected soon. Lastly, experimental proof appears to support the utilization of nanosystems as adjuvants in the formulation of anti-HIV vaccines, which may be an interesting strategy to improve both efficacy and safety of currently used formulations.

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References

[1] UNAIDS, Report on the Global AIDS Epidemic, United Nations, Geneva, Switzerland, 2008.
[2] Department of Economic and Social Affairs/Population Division, United Nations, The Impact of AIDS, United Nations Publications, New York, NY, 2004.
[3] J. Stover, L. Bollinger, The economic impact of AIDS, in: O. Wusu (Ed.), Politics and Economics of Africa, vol. 6, Nova Science Publishers, New York, NY, 2006, pp. 1–15.
[4] V. Simon, D.D. Ho, Q. Abdool Karim, HIV/AIDS epidemiology, pathogenesis, prevention, and treatment, Lancet 368 (2006) 489–504.
[5] H. Von Briesen, P. Ramge, J. Kreuter, Controlled release of antiretroviral drugs, AIDS Rev. 2 (2000) 31–38.
[6] E. Ojewole, I. Mackraj, P. Naidoo, T. Govender, Exploring the use of novel drug delivery systems for antiretroviral drugs, Eur. J. Pharm. Biopharm. 70 (2008) 697–710.
[7] T.K. Vyas, L. Shah, M.M. Amiji, Nanoparticulate drug carriers for delivery of HIV/AIDS therapy to viral reservoir sites, Expert Opin. Drug Deliv. 3 (2006) 613–628.
[8] A. Shahiwala, M.M. Amiji, Nanotechnology-based delivery systems in HIV/AIDS therapy, Future HIV Ther. 1 (2007) 49–59.
[9] J.M. Lanao, E. Briones, C.I. Colino, Recent advances in delivery systems for anti-HIV1 therapy, J. Drug Target. 15 (2007) 21–36.
[10] T. Govender, E. Ojewole, P. Naidoo, I. Mackraj, Polymeric nanoparticles for enhancing antiretroviral drug therapy, Drug Deliv. 15 (2008) 493–501.
[11] D.F. Chowdhury, Pharmaceutical nanosystems: manufacture, characterization, and safety, in: S.C. Gad (Ed.), Pharmaceutical Manufacturing Handbook: Production and Processes, Wiley, Hoboken, NJ, 2008, pp. 1289–1325.
[12] V. Torchilin, Nanoparticulates as Drug Carriers, Imperial College Press, London, 2006.
[13] V. Torchilin, Multifunctional Pharmaceutical Nanocarriers, Springer, New York, NY, 2008.
[14] J.A. Levy, HIV pathogenesis: 25 years of progress and persistent challenges, AIDS 23 (2009) 147–160.
[15] A.S. Fauci, Pathogenesis of HIV disease: opportunities for new prevention interventions, Clin. Infect. Dis. 45 (2007) S206–S212.
[16] J.A. Levy, HIV and the pathogenesis of AIDS, 3rd ed.American Society of Microbiology Press, Washington, DC, 2007.
[17] M.M. Thomson, R. Najera, Molecular epidemiology of HIV-1 variants in the global AIDS pandemic: an update, AIDS Rev. 7 (2005) 210–224.
[18] T.I. de Silva, M. Cotten, S.L. Rowland-Jones, HIV-2: the forgotten AIDS virus, Trends Microbiol. 16 (2008) 588–595.
[19] M.S. Cohen, N. Hellmann, J.A. Levy, K. DeCock, J. Lange, The spread, treatment, and prevention of HIV-1: evolution of a global pandemic, J. Clin. Invest. 118 (2008) 1244–1254.
[20] A.T. Haase, Population biology of HIV-1 infection: viral and CD4+ T cell demographics and dynamics in lymphatic tissues, Annu. Rev. Immunol. 17 (1999) 625–656.
[21] R.I. Connor, K.E. Sheridan, D. Ceradini, S. Choe, N.R. Landau, Change in coreceptor use correlates with disease progression in HIV-1–infected individuals, J. Exp. Med. 185 (1997) 621–628.
[22] F.E. McCutchan, Global epidemiology of HIV, J. Med. Virol. 78 (2006) S7–S12.
[23] C.J. Miller, R.J. Shattock, Target cells in vaginal HIV transmission, Microbes Infect. 5 (2003) 59–67.
[24] C.J. Miller, N.J. Alexander, P. Vogel, J. Anderson, P.A. Marx, Mechanism of genital transmission of SIV: a hypothesis based on transmission studies and the location of SIV in the genital tract of chronically infected female rhesus macaques, J. Med. Primatol. 21 (1992) 64–68.
[25] R.A. Royce, A. Sena, W. Cates Jr., M.S. Cohen, Sexual transmission of HIV, N. Engl. J. Med. 336 (1997) 1072–1078.
[26] K.B. Collins, B.K. Patterson, G.J. Naus, D.V. Landers, P. Gupta, Development of an in vitro organ culture model to study transmission of HIV-1 in the female genital tract, Nat. Med. 6 (2000) 475–479.
[27] F. Hladik, M.J. McElrath, Setting the stage: host invasion by HIV, Nat. Rev., Immunol. 8 (2008) 447–457.
[28] T.B.H. Geijtenbeek, D.S. Kwon, R. Torensma, S.J. Van Vliet, G.C.F. Van Duijnhoven, J. Middel, I.L.M.H.A. Cornelissen, H.S.L.M. Nottet, V.N. KewalRamani, D.R. Littman, C.G. Figdor, Y. Van Kooyk, DC-SIGN, a dendritic cell-specific HIV-1-binding protein that enhances trans-infection of T cells, Cell 100 (2000) 587–597.
[29] B. Poonia, X. Wang, R.S. Veazey, Distribution of simian immunodeficiency virus target cells in vaginal tissues of normal rhesus macaques: implications for virus transmission, J. Reprod. Immunol. 72 (2006) 74–84.
[30] J. Wilkinson, A.L. Cunningham, Mucosal transmission of HIV-1: first stop dendritic cells, Curr. Drug Targets 7 (2006) 1563–1569.
[31] L.E. Soto-Ramirez, B. Renjifo, M.F. McLane, R. Marlink, C. O'Hara, R. Sutthent, C. Wasi, P. Vithayasai, V. Vithayasai, C. Apichartpiyakul, P. Auewarakul, V. Peña Cruz, D.S. Chui, R. Osathanondh, K. Mayer, T.H. Lee, M. Essex, HIV-1 Langerhans' cell tropism associated with heterosexual transmission of HIV, Science 271 (1996) 1291–1293.
[32] S.R. Galvin, M.S. Cohen, The role of sexually transmitted diseases in HIV transmission, Nat. Rev. Microbiol. 2 (2004) 33–42.
[33] P.H. Kilmarx, K. Limpakarnjanarat, S. Supawitkul, S. Korattana, N.L. Young, B.S. Parekh, R.A. Respess, T.D. Mastro, M.E. St Louis, Mucosal disruption due to use of a widely-distributed commercial vaginal product: potential to facilitate HIV transmission, AIDS 12 (1998) 767–773.
[34] A.A. Lackner, R.S. Veazey, Current concepts in AIDS pathogenesis: insights from the SIV/macaque model, Annu. Rev. Med. 58 (2007) 461–476.
[35] S.G. McCoombe, R.V. Short, Potential HIV-1 target cells in the human penis, AIDS 20 (2006) 1491–1495.
[36] S. Mehandru, M.A. Poles, K. Tenner-Racz, A. Horowitz, A. Hurley, C. Hogan, D. Boden, P. Racz, M. Markowitz, Primary HIV-1 infection is associated with preferential depletion of CD4+ T lymphocytes from effector sites in the gastrointestinal tract, J. Exp. Med. 200 (2004) 761–770.
[37] M. Guadalupe, E. Reay, S. Sankaran, T. Prindiville, J. Flamm, A. McNeil, S. Dandekar, Severe CD4+ T-cell depletion in gut lymphoid tissue during primary human immunodeficiency virus type 1 infection and substantial delay in restoration following highly active antiretroviral therapy, J. Virol. 77 (2003) 11708–11717.
[38] J.M. Brenchley, D.C. Douek, HIV infection and the gastrointestinal immune system, Mucosal Immunol. 1 (2008) 23–30.
[39] T.C. Quinn, M.J. Wawer, N. Sewankambo, D. Serwadda, C. Li, F. Wabwire-Mangen, M. O. Meehan, T. Lutalo, R.H. Gray, Viral load and heterosexual transmission of human immunodeficiency virus type 1. Rakai Project Study Group, N. Engl. J. Med. 342 (2000) 921–929.
[40] C.D. Pilcher, J.J. Eron Jr., S. Galvin, C. Gay, M.S. Cohen, Acute HIV revisited: new opportunities for treatment and prevention, J. Clin. Invest. 113 (2004) 937–945.
[41] J.N. Blankson, D. Persaud, R.F. Siliciano, The challenge of viral reservoirs in HIV-1 infection, Annu. Rev. Med. 53 (2002) 557–593.
[42] L.K. Schrager, M.P. D'Souza, Cellular and anatomical reservoirs of HIV-1 in patients receiving potent antiretroviral combination therapy, JAMA 280 (1998) 67–71.
[43] S. Venzke, O.T. Keppler, Role of macrophages in HIV infection and persistence, Expert Rev. Clin. Immunol. 2 (2006) 613–626.
[44] C.A. Carter, L.S. Ehrlich, Cell biology of HIV-1 infection of macrophages, Annu. Rev. Microbiol. 62 (2008) 425–443.
[45] F. González-Scarano, J. Martín-García, The neuropathogenesis of AIDS, Nat. Rev. Immunol. 5 (2005) 69–81.
[46] G. Barbaro, A. Scozzafava, A. Mastrolorenzo, C.T. Supuran, Highly active antiretroviral therapy: current state of the art, new agents and their pharmacological interactions useful for improving therapeutic outcome, Curr. Pharm. Des. 11 (2005) 1805–1843.
[47] A. Babiker, S. Darby, D. De Angelis, D. Ewart, K. Porter, V. Beral, J. Darbyshire, N. Day, N. Gill, Time from HIV-1 seroconversion to AIDS and death before widespread use of highly-active antiretroviral therapy: a collaborative re-analysis, Lancet 355 (2000) 1131–1137.
[48] C.J. Gill, J.L. Griffith, D. Jacobson, S. Skinner, S.L. Gorbach, I.B. Wilson, Relationship of HIV viral loads, CD4 counts, and HAART use to health-related quality of life, J. Acquir. Immune Defic. Syndr. 30 (2002) 485–492.
[49] K. Porter, A. Babiker, K. Bhaskaran, J. Darbyshire, P. Pezzotti, A.S. Walker, Determinants of survival following HIV-1 seroconversion after the introduction of HAART, Lancet 362 (2003) 1267–1274.
[50] S.M. Hammer, J.J. Eron Jr., P. Reiss, R.T. Schooley, M.A. Thompson, S. Walmsley, P. Cahn, M.A. Fischl, J.M. Gatell, M.S. Hirsch, D.M. Jacobsen, J.S. Montaner, D.D. Richman, P.G. Yeni, P.A. Volberding, Antiretroviral treatment of adult HIV infection: 2008 recommendations of the International AIDS Society—USA panel, JAMA 300 (2008) (2008) 555–570.
[51] M.R. Blum, S.H. Liao, S.S. Good, P. de Miranda, Pharmacokinetics and bioavailability of zidovudine in humans, Am. J. Med. 85 (1988) 189–194.
[52] M. Barry, F. Mulcahy, C. Merry, S. Gibbons, D. Back, Pharmacokinetics and potential interactions amongst antiretroviral agents used to treat patients with HIV infection, Clin. Pharmacokinet. 36 (1999) 289–304.

[53] N.R. Hartman, R. Yarchoan, J.M. Pluda, R.V. Thomas, K.S. Marczyk, S. Broder, D.G. Johns, Pharmacokinetics of 2′, 3′-dideoxyadenosine and 2′, 3′-dideoxyinosine in patients with severe human immunodeficiency virus infection, Clin. Pharmacol. Ther. 47 (1990) 647–654.
[54] M.J. Shelton, A.M. O'Donnell, G.D. Morse, Didanosine, Ann. Pharmacother. 26 (5) (1992) 660–670.
[55] H. Chappuy, J.M. Treluyer, V. Jullien, J. Dimet, E. Rey, M. Fouche, G. Firtion, G. Pons, L. Mandelbrot, Maternal-fetal transfer and amniotic fluid accumulation of nucleoside analogue reverse transcriptase inhibitors in human immunodeficiency virus-infected pregnant women, Antimicrob. Agents Chemother. 48 (2004) 4332–4336.
[56] R. Yarchoan, C.F. Perno, R.V. Thomas, R.W. Klecker, J.P. Allain, R.J. Wills, N. McAtee, M.A. Fischl, R. Dubinsky, M.C. McNeely, H. Mitsuya, J.M. Pluda, T.J. Lawley, M. Leuther, B. Safai, J.M. Collins, C.E. Myers, S. Broder, Phase I studies of 2′, 3′-dideoxycytidine in severe human immunodeficiency virus infection as a single agent and alternating with zidovudine (AZT), Lancet 1 (1988) 76–81.
[57] S.J. Haworth, B. Christofalo, R.D. Anderson, L.M. Dunkle, A single-dose study to assess the penetration of stavudine into human cerebrospinal fluid in adults, J. Acquir. Immune Defic. Syndr. Hum. Retrovirol. 17 (1998) 235–238.
[58] A.P. Lea, D. Faulds, Stavudine: a review of its pharmacodynamic and pharmacokinetic properties and clinical potential in HIV infection, Drugs 51 (1996) 846–864.
[59] J.B. Dumond, R.F. Yeh, K.B. Patterson, A.H. Corbett, B.H. Jung, N.L. Rezk, A.S. Bridges, P.W. Stewart, M.S. Cohen, A.D. Kashuba, Antiretroviral drug exposure in the female genital tract: implications for oral pre- and post-exposure prophylaxis, AIDS 21 (2007) 1899–1907.
[60] R. van Leeuwen, C. Katlama, V. Kitchen, C.A. Boucher, R. Tubiana, M. McBride, D. Ingrand, J. Weber, A. Hill, H. McDade, et al., Evaluation of safety and efficacy of 3TC (lamivudine) in patients with asymptomatic or mildly symptomatic human immunodeficiency virus infection: a phase I/II study, J. Infect. Dis. 171 (1995) 1166–1171.
[61] L. Mandelbrot, G. Peytavin, G. Firtion, R. Farinotti, Maternal-fetal transfer and amniotic fluid accumulation of lamivudine in human immunodeficiency virus-infected pregnant women, Am. J. Obstet. Gynecol. 184 (2001) 153–158.
[62] C. Bennetto-Hood, Y.J. Bryson, A. Stek, J.R. King, M. Mirochnick, E.P. Acosta, Zidovudine, lamivudine, and nelfinavir concentrations in amniotic fluid and maternal serum, HIV Clin. Trials 10 (2009) 41–47.
[63] R.H. Foster, D. Faulds, Abacavir, Drugs 55 (1998) 729–736.
[64] B. Best, S. Letendre, E. Capparelli, R. Ellis, S. Rossi, P. Koopmans, I. Grant, Efavirenz and emtricitabine concentrations consistently exceed wild-type IC_{50} in cerebrospinal fluid: CHARTER findings, 16th Conference on Retrovirus and Opportunistic Infections, Montréal, Canada, 2009.
[65] C. Anthonypillai, J.E. Gibbs, S.A. Thomas, The distribution of the anti-HIV drug, tenofovir (PMPA), into the brain, CSF and choroid plexuses, Cerebrospinal Fluid Res. 3 (2006) 1.
[66] S. Taylor, R.P. van Heeswijk, R.M. Hoetelmans, J. Workman, S.M. Drake, D.J. White, D. Pillay, Concentrations of nevirapine, lamivudine and stavudine in semen of HIV-1-infected men, AIDS 14 (2000) 1979–1984.
[67] P.F. Smith, R. DiCenzo, G.D. Morse, Clinical pharmacokinetics of non-nucleoside reverse transcriptase inhibitors, Clin. Pharmacokinet. 40 (2001) 893–905.
[68] A. Kwara, A. Delong, N. Rezk, J. Hogan, H. Burtwell, S. Chapman, C.C. Moreira, J. Kurpewski, J. Ingersoll, A.M. Caliendo, A. Kashuba, S. Cu-Uvin, Antiretroviral drug concentrations and HIV RNA in the genital tract of HIV-infected women receiving long-term highly active antiretroviral therapy, Clin. Infect. Dis. 46 (2008) 719–725.
[69] E.D. Deeks, G.M. Keating, Etravirine, Drugs 68 (2008) 2357–2372.
[70] S. Vella, M. Floridia, Saquinavir: clinical pharmacology and efficacy, Clin. Pharmacokinet. 34 (1998) 189–201.
[71] S. Kravcik, K. Gallicano, V. Roth, S. Cassol, N. Hawley-Foss, A. Badley, D.W. Cameron, Cerebrospinal fluid HIV RNA and drug levels with combination ritonavir and saquinavir, J. Acquir. Immune Defic. Syndr. 21 (1999) 371–375.
[72] A. Gingelmaier, M. Kurowski, R. Kastner, J. Eberle, I. Mylonas, B.H. Belohradsky, K. Friese, T.A. Grubert, Placental transfer and pharmacokinetics of lopinavir and other protease inhibitors in combination with nevirapine at delivery, AIDS 20 (2006) 1737–1743.
[73] C. Solas, A. Lafeuillade, P. Halfon, S. Chadapaud, G. Hittinger, B. Lacarelle, Discrepancies between protease inhibitor concentrations and viral load in reservoirs and sanctuary sites in human immunodeficiency virus-infected patients, Antimicrob. Agents Chemother. 47 (2003) 238–243.
[74] S.H. Khoo, P.G. Hoggard, I. Williams, E.R. Meaden, P. Newton, E.G. Wilkins, A. Smith, J.F. Tjia, J. Lloyd, K. Jones, N. Beeching, P. Carey, B. Peters, D.J. Back, Intracellular accumulation of human immunodeficiency virus protease inhibitors, Antimicrob. Agents Chemother. 46 (2002) 3228–3235.
[75] H. Chappuy, J.M. Treluyer, E. Rey, J. Dimet, M. Fouche, G. Firtion, G. Pons, L. Mandelbrot, Maternal–fetal transfer and amniotic fluid accumulation of protease inhibitors in pregnant women who are infected with human immunodeficiency virus, Am. J. Obstet. Gynecol. 191 (2004) 558–562.
[76] R.F. Yeh, N.L. Rezk, A.D. Kashuba, J.B. Dumond, H.L. Tappouni, H.C. Tien, Y.C. Chen, M. Vourvahis, A.L. Horton, S.A. Fiscus, K.B. Patterson, Genital tract, cord blood, and amniotic fluid exposures of seven antiretroviral drugs during and after pregnancy in HIV-1 infected women. Antimicrob. Agents Chemother. 53 (2009) 2367–2374.
[77] F. Aweeka, A. Jayewardene, S. Staprans, S.E. Bellibas, B. Kearney, P. Lizak, T. Novakovic-Agopian, R.W. Price, Failure to detect nelfinavir in the cerebrospinal fluid of HIV-1-infected patients with and without AIDS dementia complex, J. Acquir. Immune Defic. Syndr. Hum. Retrovirol. 20 (1999) 39–43.
[78] G.N. Kumar, V.K. Jayanti, M.K. Johnson, J. Uchic, S. Thomas, R.D. Lee, B.A. Grabowski, H.L. Sham, D.J. Kempf, J.F. Denissen, K.C. Marsh, E. Sun, S.A. Roberts, Metabolism and disposition of the HIV-1 protease inhibitor lopinavir (ABT-378) given in combination with ritonavir in rats, dogs, and humans, Pharm. Res. 21 (2004) 1622–1630.
[79] B.M. Best, S.L. Letendre, E. Brigid, D.B. Clifford, A.C. Collier, B.B. Gelman, J.C. McArthur, J.A. McCutchan, D.M. Simpson, R. Ellis, E.V. Capparelli, I. Grant, Low atazanavir concentrations in cerebrospinal fluid, AIDS 23 (2009) 83–87.
[80] A. Pereira, J. Eron, J. Dunn, J. Gerber, R. Tidwell, K. Kenney, S. Fiscus, J.-P. Sommadossi, R. Tung, R. Gulick, R. Murphy, Analysis of amprenavir (APV)/3TC/ZDV in blood and seminal plasma: penetration of APV into the male genital tract, 7th Conference on Retroviruses and Opportunistic Infections, San Francisco, 2000.
[81] K.F. Croom, S.J. Keam, Tipranavir: a ritonavir-boosted protease inhibitor, Drugs 65 (2005) 1669–1677.
[82] A. Yilmaz, A. Izadkhashti, R.W. Price, P.W. Mallon, M. De Meulder, P. Timmerman, M. Gisslen, Darunavir concentrations in cerebrospinal fluid and blood in HIV-1-infected individuals, AIDS Res. Hum. Retrovir. 25 (2009) 457–461.
[83] R.W. Price, R. Parham, J.L. Kroll, S.A. Wring, B. Baker, J. Sailstad, R. Hoh, T. Liegler, S. Spudich, D.R. Kuritzkes, S.G. Deeks, Enfuvirtide cerebrospinal fluid (CSF) pharmacokinetics and potential use in defining CSF HIV-1 origin, Antivir. Ther. 13 (2008) 369–374.
[84] D.K. Walker, S.J. Bowers, R.J. Mitchell, M.J. Potchoiba, C.M. Schroeder, H.F. Small, Preclinical assessment of the distribution of maraviroc to potential human immunodeficiency virus (HIV) sanctuary sites in the central nervous system (CNS) and gut-associated lymphoid tissue (GALT), Xenobiotica 38 (2008) 1330–1339.
[85] J. Cocohoba, B.J. Dong, Raltegravir: the first HIV integrase inhibitor, Clin. Ther. 30 (2008) 1747–1765.
[86] E. De Clercq, The design of drugs for HIV and HCV, Nat. Rev. Drug Discov. 6 (2007) 1001–1018.
[87] V.A. Johnson, F. Brun-Vezinet, B. Clotet, H.F. Gunthard, D.R. Kuritzkes, D. Pillay, J.M. Schapiro, D.D. Richman, Update of the drug resistance mutations in HIV-1, Top. HIV Med. 16 (2008) 138–145.
[88] R.B. Kim, M.F. Fromm, C. Wandel, B. Leake, A.J. Wood, D.M. Roden, G.R. Wilkinson, The drug transporter P-glycoprotein limits oral absorption and brain entry of HIV-1 protease inhibitors, J. Clin. Invest. 101 (1998) 289–294.
[89] B.J. Aungst, P-glycoprotein, secretory transport, and other barriers to the oral delivery of anti-HIV drugs, Adv. Drug Deliv. Rev. 39 (1999) 105–116.
[90] M. Kukhanova, A. Krayevsky, W. Prusoff, Y.C. Cheng, Design of anti-HIV compounds: from nucleoside to nucleoside 5′-triphosphate analogs. Problems and perspectives, Curr. Pharm. Des. 6 (2000) 585–598.
[91] M. von Kleist, W. Huisinga, Pharmacokinetic-pharmacodynamic relationship of NRTIs and its connection to viral escape: an example based on zidovudine, Eur. J. Pharm. Sci. 36 (2009) 532–543.
[92] C.N. Tymchuk, J.S. Currier, The safety of antiretroviral drugs, Expert Opin. Drug Saf. 7 (2008) 1–4.
[93] A. Carr, Toxicity of antiretroviral therapy and implications for drug development, Nat. Rev. Drug Discov. 2 (2003) 624–634.
[94] No authors, Drugs for HIV infection, Treat. Guidel. Med. Lett. (2009) 11–22.
[95] C. Danel, R. Moh, A. Minga, A. Anzian, O. Ba-Gomis, C. Kanga, G. Nzunetu, D. Gabillard, F. Rouet, S. Sorho, M.L. Chaix, S. Eholie, H. Menan, D. Sauvageot, E. Bissagnene, R. Salamon, X. Anglaret, CD4-guided structured antiretroviral treatment interruption strategy in HIV-infected adults in West Africa (Trivacan ANRS 1269 trial): a randomised trial, Lancet 367 (2006) 1981–1989.
[96] W.M. El-Sadr, J.D. Lundgren, J.D. Neaton, F. Gordin, D. Abrams, R.C. Arduino, A. Babiker, W. Burman, N. Clumeck, C.J. Cohen, D. Cohn, D. Cooper, J. Darbyshire, S. Emery, G. Fatkenheuer, B. Gazzard, B. Grund, J. Hoy, K. Klingman, M. Losso, N. Markowitz, J. Neuhaus, A. Phillips, C. Rappoport, CD4+ count-guided interruption of antiretroviral treatment, N. Engl. J. Med. 355 (2006) 2283–2296.
[97] B. Ojikutu, C. Jack, G. Ramjee, Provision of antiretroviral therapy in South Africa: unique challenges and remaining obstacles, J. Infect. Dis. 196 (2007) S523–S527.
[98] D.H. Barouch, Challenges in the development of an HIV-1 vaccine, Nature 455 (2008) 613–619.
[99] M.M. Lederman, R.E. Offord, O. Hartley, Microbicides and other topical strategies to prevent vaginal transmission of HIV, Nat. Rev. Immunol. 6 (2006) 371–382.
[100] S.D. Li, L. Huang, Pharmacokinetics and biodistribution of nanoparticles, Mol. Pharm. 5 (2008) 496–504.
[101] H. Hillaireau, T. Le Doan, M. Besnard, H. Chacun, J. Janin, P. Couvreur, Encapsulation of antiviral nucleotide analogues azidothymidine-triphosphate and cidofovir in poly(iso-butylcyanoacrylate) nanocapsules, Int. J. Pharm. 324 (2006) 37–42.
[102] H. Hillaireau, T. Le Doan, H. Chacun, J. Janin, P. Couvreur, Encapsulation of mono- and oligo-nucleotides into aqueous-core nanocapsules in presence of various water-soluble polymers, Int. J. Pharm. 331 (2007) 148–152.
[103] F. De Jaeghere, E. Allémann, F. Kubel, B. Galli, R. Cozens, E. Doelker, R. Gurny, Oral bioavailability of a poorly water soluble HIV-1 protease inhibitor incorporated into pH-sensitive particles: effect of the particle size and nutritional state, J. Control. Release 68 (2000) 291–298.
[104] H. Boudad, P. Legrand, M. Appel, M. Coconnier, G. Ponchel, Formulation and cytotoxicity of combined cyclodextrin poly(alkylcyanoacrylate) nanoparticles on Caco-2 cells monolayers intended for oral administration of saquinavir, STP Pharma. Sci. 11 (2001) 369–375.
[105] J.C. Leroux, R.M. Cozens, J.L. Roesel, B. Galli, E. Doelker, R. Gurny, pH-sensitive nanoparticles: an effective means to improve the oral delivery of HIV-1 protease inhibitors in dogs, Pharm. Res. 13 (1996) 485–487.
[106] R. Langer, Drug delivery and targeting, Nature 392 (6679 Suppl.) (1998) 5–10.
[107] J. Kreuter, Nanoparticulate systems for brain delivery of drugs, Adv. Drug Deliv. Rev. 47 (2001) 65–81.

[108] S.U. Choi, T. Bui, R.J. Ho, pH-dependent interactions of indinavir and lipids in nanoparticles and their ability to entrap a solute, J. Pharm. Sci. 97 (2008) 931–943.
[109] N.C. Phillips, C. Tsoukas, Liposomal encapsulation of azidothymidine results in decreased hematopoietic toxicity and enhanced activity against murine acquired immunodeficiency syndrome, Blood 79 (1992) 1137–1143.
[110] E. Kohli, H.Y. Han, A.D. Zeman, S.V. Vinogradov, Formulations of biodegradable Nanogel carriers with 5′-triphosphates of nucleoside analogs that display a reduced cytotoxicity and enhanced drug activity, J. Control. Release 121 (2007) 19–27.
[111] H. Heiati, R. Tawashi, N.C. Phillips, Drug retention and stability of solid lipid nanoparticles containing azidothymidine palmitate after autoclaving, storage and lyophilization, J. Microencapsul. 15 (1998) 173–184.
[112] Y.C. Kuo, Loading efficiency of stavudine on polybutylcyanoacrylate and methylmethacrylate-sulfopropylmethacrylate copolymer nanoparticles, Int. J. Pharm. 290 (2005) 161–172.
[113] M. Garg, T. Dutta, N.K. Jain, Stability study of stavudine-loaded O-palmitoyl-anchored carbohydrate-coated liposomes, AAPS PharmSciTech 8 (2007) Article 38.
[114] J. Panyam, V. Labhasetwar, Biodegradable nanoparticles for drug and gene delivery to cells and tissue, Adv. Drug Deliv. Rev. 55 (2003) 329–347.
[115] N. Dinauer, D. Lochmann, I. Demirhan, A. Bouazzaoui, A. Zimmer, A. Chandra, J. Kreuter, H. von Briesen, Intracellular tracking of protamine/antisense oligonucleotide nanoparticles and their inhibitory effect on HIV-1 transactivation, J. Control. Release 96 (2004) 497–507.
[116] V. Schäfer, H. von Briesen, R. Andreesen, A.M. Steffan, C. Royer, S. Troster, J. Kreuter, H. Rubsamen-Waigmann, Phagocytosis of nanoparticles by human immunodeficiency virus (HIV)-infected macrophages: a possibility for antiviral drug targeting, Pharm. Res. 9 (1992) 541–546.
[117] A. Bender, V. Schäfer, A.M. Steffan, C. Royer, J. Kreuter, H. Rubsamen-Waigmann, H. von Briesen, Inhibition of HIV in vitro by antiviral drug-targeting using nanoparticles, Res. Virol. 145 (1994) 215–220.
[118] A.R. Bender, H. von Briesen, J. Kreuter, I.B. Duncan, H. Rubsamen-Waigmann, Efficiency of nanoparticles as a carrier system for antiviral agents in human immunodeficiency virus-infected human monocytes/macrophages in vitro, Antimicrob. Agents Chemother. 40 (1996) 1467–1471.
[119] L.K. Shah, M.M. Amiji, Intracellular delivery of saquinavir in biodegradable polymeric nanoparticles for HIV/AIDS, Pharm. Res. 23 (2006) 2638–2645.
[120] R.M. Mainardes, M.P. Gremião, I.L. Brunetti, L.M. da Fonseca, N.M. Khalil, Zidovudine-loaded PLA and PLA–PEG blend nanoparticles: influence of polymer type on phagocytic uptake by polymorphonuclear cells, J. Pharm. Sci. 98 (2009) 257–267.
[121] H. Dou, J. Morehead, C.J. Destache, J.D. Kingsley, L. Shlyakhtenko, Y. Zhou, M. Chaubal, J. Werling, J. Kipp, B.E. Rabinow, H.E. Gendelman, Laboratory investigations for the morphologic, pharmacokinetic, and anti-retroviral properties of indinavir nanoparticles in human monocyte-derived macrophages, Virology 358 (2007) 148–158.
[122] S.V. Vinogradov, E. Kohli, A.D. Zeman, Cross-linked polymeric nanogel formulations of 5′-triphosphates of nucleoside analogues: role of the cellular membrane in drug release, Mol. Pharm. 2 (2005) 449–461.
[123] H. Hillaireau, T. Le Doan, M. Appel, P. Couvreur, Hybrid polymer nanocapsules enhance in vitro delivery of azidothymidine-triphosphate to macrophages, J. Control. Release 116 (2006) 346–352.
[124] D. Flasher, K. Konopka, S.M. Chamow, P. Dazin, A. Ashkenazi, E. Pretzer, N. Düzgüneş, Liposome targeting to human immunodeficiency virus type 1-infected cells via recombinant soluble CD4 and CD4 immunoadhesin (CD4-IgG), Biochim. Biophys. Acta 1194 (1994) 185–196.
[125] P. Couvreur, B. Stella, L.H. Reddy, H. Hillaireau, C. Dubernet, D. Desmaele, S. Lepetre-Mouelhi, F. Rocco, N. Dereuddre-Bosquet, P. Clayette, V. Rosilio, V. Marsaud, J.M. Renoir, L. Cattel, Squalenoyl nanomedicines as potential therapeutics, Nano Lett. 6 (2006) 2544–2548.
[126] F. Bekkara-Aounallah, R. Gref, M. Othman, L.H. Reddy, B. Pili, V. Allain, C. Bourgaux, H. Hillaireau, S. Lepêtre-Mouelhi, D. Desmaële, J. Nicolas, N. Chafi, P. Couvreur, Novel PEGylated nanoassemblies made of self-assembled squalenoyl nucleoside analogues, Adv. Funct. Mater. 18 (2008) 3715–3725.
[127] S. Ganta, H. Devalapally, A. Shahiwala, M. Amiji, A review of stimuli-responsive nanocarriers for drug and gene delivery, J. Control. Release 126 (2008) 187–204.
[128] N. Düzgüneş, S. Simões, V. Slepushkin, E. Pretzer, J.J. Rossi, E. De Clercq, V.P. Antao, M.L. Collins, M.C. Pedroso de Lima, Enhanced inhibition of HIV-1 replication in macrophages by antisense oligonucleotides, ribozymes and acyclic nucleoside phosphonate analogs delivered in pH-sensitive liposomes, Nucleosides Nucleotides Nucleic Acids 20 (2001) 515–523.
[129] D. Ece Gamsiz, L.K. Shah, H. Devalapally, M.M. Amiji, R.L. Carrier, A model predicting delivery of saquinavir in nanoparticles to human monocyte/macrophage (Mo/Mac) cells, Biotechnol. Bioeng. 101 (2008) 1072–1082.
[130] R. Löbenberg, J. Kreuter, Macrophage targeting of azidothymidine: a promising strategy for AIDS therapy, AIDS Res. Hum. Retroviruses 12 (1996) 1709–1715.
[131] R. Löbenberg, L. Araujo, H. von Briesen, E. Rodgers, J. Kreuter, Body distribution of azidothymidine bound to hexyl-cyanoacrylate nanoparticles after i.v. injection to rats, J. Control. Release 50 (1998) 21–30.
[132] R. Löbenberg, J. Maas, J. Kreuter, Improved body distribution of ^{14}C-labelled AZT bound to nanoparticles in rats determined by radioluminography, J. Drug Target. 5 (1998) 171–179.
[133] R. Löbenberg, L. Araujo, J. Kreuter, Body distribution of azidothymidine bound to nanoparticles after oral administration, Eur. J. Pharm. Biopharm. 44 (1997) 127–132.
[134] A. Dembri, M.J. Montisci, J.C. Gantier, H. Chacun, G. Ponchel, Targeting of 3′-azido 3′-deoxythymidine (AZT)-loaded poly(isohexylcyanoacrylate) nanospheres to the gastrointestinal mucosa and associated lymphoid tissues, Pharm. Res. 18 (2001) 467–473.
[135] Y. Jin, P. Ai, R. Xin, D. Chen, Morphological transformation of self-assembled nanostructures prepared from cholesteryl acyl didanosine and the optimal formulation of nanoparticulate systems: effects of solvents, acyl chain length and poloxamer 188, J. Colloid Interface Sci. 326 (2008) 275–282.
[136] Y. Jin, R. Xin, P. Ai, D. Chen, Self-assembled drug delivery systems 2. Cholesteryl derivatives of antiviral nucleoside analogues: synthesis, properties and the vesicle formation, Int. J. Pharm. 350 (2008) 330–337.
[137] Y. Jin, P. Ai, R. Xin, Y. Tian, J. Dong, D. Chen, W. Wang, Self-assembled drug delivery systems: part 3. In vitro/in vivo studies of the self-assembled nanoparticulates of cholesteryl acyl didanosine, Int. J. Pharm. 368 (2009) 207–214.
[138] L. Kinman, S.J. Brodie, C.C. Tsai, T. Bui, K. Larsen, A. Schmidt, D. Anderson, W.R. Morton, S.L. Hu, R.J. Ho, Lipid-drug association enhanced HIV-1 protease inhibitor indinavir localization in lymphoid tissues and viral load reduction: a proof of concept study in HIV-2_{287}-infected macaques, J. Acquir. Immune Defic. Syndr. 34 (2003) 387–397.
[139] L. Kinman, T. Bui, K. Larsen, C.C. Tsai, D. Anderson, W.R. Morton, S.L. Hu, R.J. Ho, Optimization of lipid–indinavir complexes for localization in lymphoid tissues of HIV-infected macaques, J. Acquir. Immune Defic. Syndr. 42 (2006) 155–161.
[140] S.J. Snedecor, S.M. Sullivan, R.J. Ho, Feasibility of weekly HIV drug delivery to enhance drug localization in lymphoid tissues based on pharmacokinetic models of lipid-associated indinavir, Pharm. Res. 23 (2006) 1750–1755.
[141] M. Garg, T. Dutta, N.K. Jain, Reduced hepatic toxicity, enhanced cellular uptake and altered pharmacokinetics of stavudine loaded galactosylated liposomes, Eur. J. Pharm. Biopharm. 67 (2007) 76–85.
[142] M. Garg, B.R. Garg, S. Jain, P. Mishra, R.K. Sharma, A.K. Mishra, T. Dutta, N.K. Jain, Radiolabeling, pharmacoscintigraphic evaluation and antiretroviral efficacy of stavudine loaded 99mTc labeled galactosylated liposomes, Eur. J. Pharm. Sci. 33 (2008) 271–281.
[143] J.F. Gagné, A. Désormeaux, S. Perron, M.J. Tremblay, M.G. Bergeron, Targeted delivery of indinavir to HIV-1 primary reservoirs with immunoliposomes, Biochim. Biophys. Acta 1558 (2002) 198–210.
[144] H. Dou, C.J. Destache, J.R. Morehead, R.L. Mosley, M.D. Boska, J. Kingsley, S. Gorantla, L. Poluektova, J.A. Nelson, M. Chaubal, J. Werling, J. Kipp, B.E. Rabinow, H.E. Gendelman, Development of a macrophage-based nanoparticle platform for antiretroviral drug delivery, Blood 108 (8) (2006) 2827–2835.
[145] S. Gorantla, H. Dou, M. Boska, C.J. Destache, J. Nelson, L. Poluektova, B.E. Rabinow, H.E. Gendelman, R.L. Mosley, Quantitative magnetic resonance and SPECT imaging for macrophage tissue migration and nanoformulated drug delivery, J. Leukoc. Biol. 80 (5) (2006) 1165–1174.
[146] S. Jain, A.K. Tiwary, N.K. Jain, Sustained and targeted delivery of an anti-HIV agent using elastic liposomal formulation: mechanism of action, Curr. Drug Deliv. 3 (2) (2006) 157–166.
[147] S. Jain, A.K. Tiwary, N.K. Jain, PEGylated elastic liposomal formulation for lymphatic targeting of zidovudine, Curr. Drug Deliv. 5 (4) (2008) 275–281.
[148] S. Jain, A.K. Tiwary, B. Sapra, N.K. Jain, Formulation and evaluation of ethosomes for transdermal delivery of lamivudine, AAPS PharmSciTech 8 (4) (2007) E111.
[149] M. Garg, A. Asthana, H.B. Agashe, G.P. Agrawal, N.K. Jain, Stavudine-loaded mannosylated liposomes: in-vitro anti-HIV-I activity, tissue distribution and pharmacokinetics, J. Pharm. Pharmacol. 58 (2006) 605–616.
[150] A. Désormeaux, P. Harvie, S. Perron, B. Makabi-Panzu, D. Beauchamp, M. Tremblay, L. Poulin, M.G. Bergeron, Antiviral efficacy, intracellular uptake and pharmacokinetics of free and liposome-encapsulated 2′, 3′-dideoxyinosine, AIDS 8 (1994) 1545–1553.
[151] P. Harvie, A. Désormeaux, N. Gagné, M. Tremblay, L. Poulin, D. Beauchamp, M.G. Bergeron, Lymphoid tissues targeting of liposome-encapsulated 2′,3′-dideoxyinosine. AIDS 9 (7) (1995) 701-707.
[152] C. Oussoren, M. Magnani, A. Fraternale, A. Casabianca, L. Chiarantini, R. Ingebrigsten, W.J. Underberg, G. Storm, Liposomes as carriers of the antiretroviral agent dideoxycytidine-5′-triphosphate, Int. J. Pharm. 180 (1999) 261–270.
[153] H. Heiati, R. Tawashi, N.C. Phillips, Solid lipid nanoparticles as drug carriers. II. Plasma stability and biodistribution of solid lipid nanoparticles containing the lipophilic prodrug 3′-azido-3′-deoxythymidine palmitate in mice, Int. J. Pharm. 174 (1998) 71–80.
[154] C.D. Kaur, M. Nahar, N.K. Jain, Lymphatic targeting of zidovudine using surface-engineered liposomes, J. Drug Target. 16 (2008) 798–805.
[155] D.J. Begley, Delivery of therapeutic agents to the central nervous system: the problems and the possibilities, Pharmacol. Ther. 104 (2004) 29–45.
[156] S.B. Tiwari, M.M. Amiji, A review of nanocarrier-based CNS delivery systems, Curr. Drug Deliv. 3 (2006) 219–232.
[157] R.J. Sawchuk, Z. Yang, Investigation of distribution, transport and uptake of anti-HIV drugs to the central nervous system, Adv. Drug Deliv. Rev. 39 (1999) 5–31.
[158] S. Kim, S. Scheerer, M.A. Geyer, S.B. Howell, Direct cerebrospinal fluid delivery of an antiretroviral agent using multivesicular liposomes, J. Infect. Dis. 162 (1990) 750–752.
[159] Y.C. Kuo, H.H. Chen, Effect of nanoparticulate polybutylcyanoacrylate and methylmethacrylate-sulfopropylmethacrylate on the permeability of zidovudine and lamivudine across the in vitro blood-brain barrier, Int. J. Pharm. 327 (2006) 160–169.
[160] Y.C. Kuo, F.L. Su, Transport of stavudine, delavirdine, and saquinavir across the blood-brain barrier by polybutylcyanoacrylate, methylmethacrylate-sulfopropylmethacrylate, and solid lipid nanoparticles, Int. J. Pharm. 340 (2007) 143–152.
[161] Y.C. Kuo, H.H. Chen, Entrapment and release of saquinavir using novel cationic solid lipid nanoparticles, Int. J. Pharm. 365 (2009) 206–213.

[162] T.K. Vyas, A. Shahiwala, M.M. Amiji, Improved oral bioavailability and brain transport of Saquinavir upon administration in novel nanoemulsion formulations, Int. J. Pharm. 347 (2008) 93–101.
[163] V. Mishra, S. Mahor, A. Rawat, P.N. Gupta, P. Dubey, K. Khatri, S.P. Vyas, Targeted brain delivery of AZT via transferrin anchored pegylated albumin nanoparticles, J. Drug Target. 14 (2006) 45–53.
[164] K.S. Rao, M.K. Reddy, J.L. Horning, V. Labhasetwar, TAT-conjugated nanoparticles for the CNS delivery of anti-HIV drugs, Biomaterials 29 (33) (2008) 4429–4438.
[165] A. Kaur, S. Jain, A.K. Tiwary, Mannan-coated gelatin nanoparticles for sustained and targeted delivery of didanosine: in vitro and in vivo evaluation, Acta Pharm. 58 (2008) 61–74.
[166] H. Dou, C.B. Grotepas, J.M. McMillan, C.J. Destache, M. Chaubal, J. Werling, J. Kipp, B. Rabinow, H.E. Gendelman, Macrophage delivery of nanoformulated antiretroviral drug to the brain in a murine model of neuroAIDS, J. Immunol. 183 (2009) 661–669.
[167] J. Balzarini, L. Van Damme, Microbicide drug candidates to prevent HIV infection, Lancet 369 (2007) 787–797.
[168] A.B. Moscicki, Vaginal microbicides: where are we and where are we going? J. Infect. Chemother. 14 (2008) 337–341.
[169] J.H. van de Wijgert, R.J. Shattock, Vaginal microbicides: moving ahead after an unexpected setback, AIDS 21 (18) (2007) 2369–2376.
[170] J. das Neves, B. Santos, T. Cunha, B. Teixeira, P. Rocha, G. Dias, Vaginal microbicides: the importance of effective distribution, retention and coating of the mucosa, AIDS 22 (2008) 908–909.
[171] J. das Neves, M.F. Bahia, Gels as vaginal drug delivery systems, Int. J. Pharm. 318 (2006) 1–14.
[172] L.C. Rohan, A.B. Sassi, Vaginal drug delivery systems for HIV prevention, AAPS J. 11 (2009) 78–87.
[173] T. Hayakawa, M. Kawamura, M. Okamoto, M. Baba, T. Niikawa, S. Takehara, T. Serizawa, M. Akashi, Concanavalin A-immobilized polystyrene nanospheres capture HIV-1 virions and gp120: potential approach towards prevention of viral transmission, J. Med. Virol. 56 (1998) 327–331.
[174] X. Wang, T. Akagi, M. Akashi, M. Baba, Development of core-corona type polymeric nanoparticles as an anti-HIV-1 vaccine, Mini-Rev. Org. Chem. 4 (1) (2007) 51–59.
[175] J.M. de la Fuente, S. Penadés, Understanding carbohydrate–carbohydrate interactions by means of glyconanotechnology, Glycoconj. J. 21 (2004) 149–163.
[176] O. Martínez-Ávila, C. Clavel, S. Penadés, M. Stefanidou, M. Hayes, R. Shattock, K. Hijazi, C. Kelly, Gold manno-nanoparticles as potential microbicides against HIV infection, TNT2007 — Trends in NanoTechnology, San Sebastian, Spain, 2007.
[177] M. Stefanidou, O. Martinez, M. Hayes, C. Clavel, C. Champion, S. Penades, R. Shattock, Evaluation of gold manno-nanoparticles in a model for DC-SIGN binding and dissemination of HIV-1, Microbicides 2006 Conference, Cape Town, South Africa, 2006.
[178] A.S. Ham, M.R. Cost, A.B. Sassi, C.S. Dezzutti, L.C. Rohan, Targeted delivery of PSC-RANTES for HIV-1 prevention using biodegradable nanoparticles, Pharm. Res. 26 (2009) 502–511.
[179] M.M. Lederman, R.S. Veazey, R. Offord, D.E. Mosier, J. Dufour, M. Mefford, M. Piatak Jr., J.D. Lifson, J.R. Salkowitz, B. Rodriguez, A. Blauvelt, O. Hartley, Prevention of vaginal SHIV transmission in rhesus macaques through inhibition of CCR5, Science 306 (5695) (2004) 485–487.
[180] S.K. Lai, D.E. O'Hanlon, S. Harrold, S.T. Man, Y.Y. Wang, R. Cone, J. Hanes, Rapid transport of large polymeric nanoparticles in fresh undiluted human mucus, Proc. Natl. Acad. Sci. U. S. A. 104 (2007) 1482–1487.
[181] Y. Cu, W.M. Saltzman, Controlled surface modification with poly(ethylene)glycol enhances diffusion of PLGA nanoparticles in human cervical mucus, Mol. Pharm. 6 (2009) 173–181.
[182] K.A. Woodrow, Y. Cu, C.J. Booth, J.K. Saucier-Sawyer, M.J. Wood, W.M. Saltzman, Intravaginal gene silencing using biodegradable polymer nanoparticles densely loaded with small-interfering RNA. Nat. Mater. 8 (2009) 526–533.
[183] T.M. Kish-Catalone, W. Lu, R.C. Gallo, A.L. DeVico, Preclinical evaluation of synthetic −2 RANTES as a candidate vaginal microbicide to target CCR5, Antimicrob. Agents Chemother. 50 (2006) 1497–1509.
[184] T. Kish-Catalone, R. Pal, J. Parrish, N. Rose, L. Hocker, L. Hudacik, M. Reitz, R. Gallo, A. Devico, Evaluation of −2 RANTES vaginal microbicide formulations in a nonhuman primate simian/human immunodeficiency virus (SHIV) challenge model, AIDS Res. Hum. Retroviruses 23 (2007) 33–42.
[185] A. Shahiwala, T.K. Vyas, M.M. Amiji, Nanocarriers for systemic and mucosal vaccine delivery, Recent Pat. Drug Deliv. Formul. 1 (2007) 1–9.
[186] L.J. Peek, C.R. Middaugh, C. Berkland, Nanotechnology in vaccine delivery, Adv. Drug Deliv. Rev. 60 (2008) 915–928.
[187] C.P. Locher, D. Putnam, R. Langer, S.A. Witt, B.M. Ashlock, J.A. Levy, Enhancement of a human immunodeficiency virus env DNA vaccine using a novel polycationic nanoparticle formulation, Immunol. Lett. 90 (2003) 67–70.
[188] J. Boyle, D. Eastman, C. Millar, S. Camuglia, J. Cox, M. Pearse, J. Good, D. Drane, The utility of ISCOMATRIX adjuvant for dose reduction of antigen for vaccines requiring antibody responses, Vaccine 25 (2007) 2541–2544.
[189] P. Putkonen, E. Bjorling, L. Akerblom, R. Thorstensson, K. Lovgren, L. Benthin, F. Chiodi, B. Morein, G. Biberfeld, E. Norrby, H. Wigzell, Long-standing protection of macaques against cell-free HIV-2 with a HIV-2 iscom vaccine, J. Acquir. Immune Defic. Syndr. 7 (1994) 551–559.
[190] N. Ishii, J. Fukushima, T. Kaneko, E. Okada, K. Tani, S.I. Tanaka, K. Hamajima, K.Q. Xin, S. Kawamoto, W. Koff, K. Nishioka, T. Yasuda, K. Okuda, Cationic liposomes are a strong adjuvant for a DNA vaccine of human immunodeficiency virus type 1, AIDS Res. Hum. Retroviruses 13 (1997) 1421–1428.
[191] E. Okada, S. Sasaki, N. Ishii, I. Aoki, T. Yasuda, K. Nishioka, J. Fukushima, J. Miyazaki, B. Wahren, K. Okuda, Intranasal immunization of a DNA vaccine with IL-12- and granulocyte-macrophage colony-stimulating factor (GM-CSF)-expressing plasmids in liposomes induces strong mucosal and cell-mediated immune responses against HIV-1 antigens, J. Immunol. 159 (1997) 3638–3647.
[192] K.Q. Xin, K. Hamajima, S. Sasaki, A. Honsho, T. Tsuji, N. Ishii, X.R. Cao, Y. Lu, J. Fukushima, P. Shapshak, S. Kawamoto, K. Okuda, Intranasal administration of human immunodeficiency virus type-1 (HIV-1) DNA vaccine with interleukin-2 expression plasmid enhances cell-mediated immunity against HIV-1, Immunology 94 (1998) 438–444.
[193] S. Toda, N. Ishii, E. Okada, K.I. Kusakabe, H. Arai, K. Hamajima, I. Gorai, K. Nishioka, K. Okuda, HIV-1-specific cell-mediated immune responses induced by DNA vaccination were enhanced by mannan-coated liposomes and inhibited by anti-interferon-gamma antibody, Immunology 92 (1997) 111–117.
[194] G. Sakaue, T. Hiroi, Y. Nakagawa, K. Someya, K. Iwatani, Y. Sawa, H. Takahashi, M. Honda, J. Kunisawa, H. Kiyono, HIV mucosal vaccine: nasal immunization with gp160-encapsulated hemagglutinating virus of Japan-liposome induces antigen-specific CTLs and neutralizing antibody responses, J. Immunol. 170 (2003) 495–502.
[195] Z. Cui, J. Patel, M. Tuzova, P. Ray, R. Phillips, J.G. Woodward, A. Nath, R.J. Mumper, Strong T cell type-1 immune responses to HIV-1 Tat (1–72) protein-coated nanoparticles, Vaccine 22 (2004) 2631–2640.
[196] M. Vajdy, M. Singh, J. Kazzaz, E. Soenawan, M. Ugozzoli, F. Zhou, I. Srivastava, Q. Bin, S. Barnett, J. Donnelly, P. Luciw, L. Adamson, D. Montefiori, D.T. O'Hagan, Mucosal and systemic anti-HIV responses in rhesus macaques following combinations of intranasal and parenteral immunizations, AIDS Res. Hum. Retroviruses 20 (2004) 1269–1281.
[197] Y. Ataman-Önal, S. Munier, A. Ganee, C. Terrat, P.Y. Durand, N. Battail, F. Martinon, R. Le Grand, M.H. Charles, T. Delair, B. Verrier, Surfactant-free anionic PLA nanoparticles coated with HIV-1 p24 protein induced enhanced cellular and humoral immune responses in various animal models, J. Control. Release 112 (2006) 175–185.
[198] D. Lamalle-Bernard, S. Munier, C. Compagnon, M.H. Charles, V.S. Kalyanaraman, T. Delair, B. Verrier, Y. Ataman-Onal, Coadsorption of HIV-1 p24 and gp120 proteins to surfactant-free anionic PLA nanoparticles preserves antigenicity and immunogenicity, J. Control. Release 115 (2006) 57–67.
[199] T. Akagi, M. Kawamura, M. Ueno, K. Hiraishi, M. Adachi, T. Serizawa, M. Akashi, M. Baba, Mucosal immunization with inactivated HIV-1-capturing nanospheres induces a significant HIV-1-specific vaginal antibody response in mice, J. Med. Virol. 69 (2) (2003) 163–172.
[200] A. Miyake, T. Akagi, Y. Enose, M. Ueno, M. Kawamura, R. Horiuchi, K. Hiraishi, M. Adachi, T. Serizawa, O. Narayan, M. Akashi, M. Baba, M. Hayami, Induction of HIV-specific antibody response and protection against vaginal SHIV transmission by intranasal immunization with inactivated SHIV-capturing nanospheres in macaques, J. Med. Virol. 73 (2004) 368–377.
[201] T. Akagi, M. Ueno, K. Hiraishi, M. Baba, M. Akashi, AIDS vaccine: intranasal immunization using inactivated HIV-1-capturing core-corona type polymeric nanospheres, J. Control. Release 109 (2005) 49–61.
[202] M. Kawamura, X. Wang, T. Uto, K. Sato, M. Ueno, T. Akagi, K. Hiraishi, T. Matsuyama, M. Akashi, M. Baba, Induction of dendritic cell-mediated immune responses against HIV-1 by antigen-capturing nanospheres in mice, J. Med. Virol. 76 (2005) 7–15.
[203] G. Koopman, W.M. Bogers, M. van Gils, W. Koornstra, S. Barnett, B. Morein, T. Lehner, J.L. Heeney, Comparison of intranasal with targeted lymph node immunization using PR8-Flu ISCOM adjuvanted HIV antigens in macaques, J. Med. Virol. 79 (2007) 474–482.
[204] C. Guillon, K. Mayol, C. Terrat, C. Compagnon, C. Primard, M.H. Charles, T. Delair, S. Munier, B. Verrier, Formulation of HIV-1 Tat and p24 antigens by PLA nanoparticles or MF59 impacts the breadth, but not the magnitude, of serum and faecal antibody responses in rabbits, Vaccine 25 (2007) 7491–7501.
[205] M.A. Arias, A. Loxley, D. Fairhurst, C. Eatmon, Y. Kim, F. Wegmann, M. Mitchnick, R. Shattock, HIV-gp140 antigen-adsorbed wax nanoparticles induce strong in vivo systemic and mucosal humoral immune responses, AIDS Vaccine 2008 Conference, Cape Town, South Africa, 2008.
[206] J.O. Kahn, F. Sinangil, J. Baenziger, N. Murcar, D. Wynne, R.L. Coleman, K.S. Steimer, C.L. Dekker, D. Chernoff, Clinical and immunologic responses to human immunodeficiency virus (HIV) type 1SF2 gp120 subunit vaccine combined with MF59 adjuvant with or without muramyl tripeptide dipalmitoyl phosphatidylethanolamine in non-HIV-infected human volunteers, J. Infect. Dis. 170 (1994) 1288–1291.
[207] H. Toledo, A. Baly, O. Castro, S. Resik, J. Laferte, F. Rolo, L. Navea, L. Lobaina, O. Cruz, J. Miguez, T. Serrano, B. Sierra, L. Perez, M.E. Ricardo, M. Dubed, A.L. Lubian, M. Blanco, J.C. Millan, A. Ortega, E. Iglesias, E. Penton, Z. Martin, J. Perez, M. Diaz, C.A. Duarte, A phase I clinical trial of a multi-epitope polypeptide TAB9 combined with Montanide ISA 720 adjuvant in non-HIV-1 infected human volunteers, Vaccine 19 (2001) 4328–4336.
[208] A.U. Bielinska, K.W. Janczak, J.J. Landers, D.M. Markovitz, D.C. Montefiori, J.R. Baker Jr., Nasal immunization with a recombinant HIV gp120 and nanoemulsion adjuvant produces Th1 polarized responses and neutralizing antibodies to primary HIV type 1 isolates, AIDS Res. Hum. Retrovir. 24 (2008) 271–281.
[209] J. Kunisawa, S. Nakagawa, T. Mayumi, Pharmacotherapy by intracellular delivery of drugs using fusogenic liposomes: application to vaccine development, Adv. Drug Deliv. Rev. 52 (2001) 177–186.
[210] M. Kawamura, T. Naito, M. Ueno, T. Akagi, K. Hiraishi, I. Takai, M. Makino, T. Serizawa, K. Sugimura, M. Akashi, M. Baba, Induction of mucosal IgA following intravaginal administration of inactivated HIV-1-capturing nanospheres in mice, J. Med. Virol. 66 (2002) 291–298.
[211] S.S. Diebold, M. Kursa, E. Wagner, M. Cotten, M. Zenke, Mannose polyethylenimine conjugates for targeted DNA delivery into dendritic cells, J. Biol. Chem. 274 (1999) 19087–19094.

[212] J. Lisziewicz, L. Kelly, F. Lori, Topical DermaVir vaccine targeting dendritic cells, Curr. Drug Deliv. 3 (2006) 83–88.
[213] F. Lori, S.A. Calarota, J. Lisziewicz, Nanochemistry-based immunotherapy for HIV-1, Curr. Med. Chem. 14 (2007) 1911–1919.
[214] J. Lisziewicz, J. Trocio, L. Whitman, G. Varga, J. Xu, N. Bakare, P. Erbacher, C. Fox, R. Woodward, P. Markham, S. Arya, J.P. Behr, F. Lori, DermaVir: a novel topical vaccine for HIV/AIDS, J. Invest. Dermatol. 124 (2005) 160–169.
[215] A.D. Cristillo, J. Lisziewicz, L. He, F. Lori, L. Galmin, J.N. Trocio, T. Unangst, L. Whitman, L. Hudacik, N. Bakare, S. Whitney, S. Restrepo, J. Suschak, M.G. Ferrari, H.K. Chung, V.S. Kalyanaraman, P. Markham, R. Pal, HIV-1 prophylactic vaccine comprised of topical DermaVir prime and protein boost elicits cellular immune responses and controls pathogenic R5 SHIV162P3, Virology 366 (2007) 197–211.
[216] J. Lisziewicz, J. Trocio, J. Xu, L. Whitman, A. Ryder, N. Bakare, M.G. Lewis, W. Wagner, A. Pistorio, S. Arya, F. Lori, Control of viral rebound through therapeutic immunization with DermaVir, AIDS 19 (2005) 35–43.
[217] S.K. Sahoo, V. Labhasetwar, Nanotech approaches to drug delivery and imaging, Drug Discov. Today 8 (2003) 1112–1120.
[218] No authors, Sizing Nanotechnology's Value Chain, Lux Research, New York, 2004.
[219] W.E. Bawarski, E. Chidlowsky, D.J. Bharali, S.A. Mousa, Emerging nanopharmaceuticals, Nanomedicine 4 (2008) 273–282.
[220] P.J. Klasse, R.J. Shattock, J.P. Moore, Which topical microbicides for blocking HIV-1 transmission will work in the real world? PLoS Med. 3 (2006) e351.
[221] Pharmaco-Economics Working Group, The Economics of Microbicide Development: a Case for Investment, Rockefeller Foundation Microbicide Initiative, New York, NY, 2002.
[222] D.P. Wilson, P.M. Coplan, Mathematical models and health economic aspects of microbicides, Curr. Opin. HIV AIDS 3 (2008) 587–592.
[223] P. Couvreur, C. Vauthier, Nanotechnology: intelligent design to treat complex disease, Pharm. Res. 23 (2006) 1417–1450.