Received: 16 March 2021 | Accepted: 23 March 2021

DOI: 10.1002/prp2.776

REVIEW

Nanoparticle delivery system, highly active antiretroviral therapy, and testicular morphology: The role of stereology

Edwin Coleridge S. Naidu[1] | Samuel Oluwaseun Olojede[1] | Sodiq Kolawole Lawal[1] | Carmen Olivia Rennie[1] | Onyemaechi Okpara Azu[1,2]

[1]Discipline of Clinical Anatomy, School of Laboratory Medicine & Medical Sciences, Nelson R Mandela School of Medicine, University of KwaZulu-Natal, Durban, South Africa

[2]Department of Anatomy, School of Medicine, University of Namibia, Windhoek, Namibia

Correspondence
Edwin Coleridge Stephen Naidu, Discipline of Clinical Anatomy, School of Laboratory Medicine & Medical Sciences, Nelson R Mandela School of Medicine, University of KwaZulu-Natal, 719 Umbilo Road, Durban, South Africa.
Email: naidue@ukzn.ac.za, naiduecs@gmail.com

Funding information
The authors declare that there is no external funding or financial aid for this minireview.

Abstract

The conjugation of nanoparticles (NPs) with antiretroviral drugs is a drug delivery approach with great potential for managing HIV infections. Despite their promise, recent studies have highlighted the toxic effects of nanoparticles on testicular tissue and their impact on sperm morphology. This review explores the role of stereological techniques in assessing the testicular morphology in highly active antiretroviral therapy (HAART) when a nanoparticle drug delivery system is used. Also, NPs penetration and pharmacokinetics concerning the testicular tissue and blood–testis barrier form the vital part of this review. More so, various classes of NPs employed in biomedical and clinical research to deliver antiretroviral drugs were thoroughly discussed. In addition, considerations for minimizing nanoparticle-drugs toxicity, ensuring enhanced permeability of nanoparticles, maximizing drug efficacy, ensuring adequate bioavailability, and formulation of HAART-NPs fabrication are well discussed.

KEYWORDS
blood–testis barrier, highly active retroviral therapy, nanoparticles, spermatogenesis, stereology, testis

1 | INTRODUCTION

The existence of a protective "sanctuary site" in the testis, which limits its drug concentration or sequestration, has been suggested.[1-3] This sanctuary site is typified by the testis, an essential compartmentalized reproductive organ within the scrotum. It is encapsulated in the outermost to the innermost thick layers of connective tissue capsules, tunica vaginalis, tunica albuginea, and tunica vasculosa.[4] Septa from the tunica albuginea partition the testis into different lobules. Each of these lobules contains seminiferous tubules that are approximately 200 μm in diameter, with a total length of ~600 m that contributes to about 60 percent of the total volume of the testis.[5,6]

Abbreviations: ABC, ATP binding cassette transporters; AJ, adherens junction; ARVDs, antiretroviral drugs; BCRP, breast cancer resistance protein; BTB, blood–testis barrier; CBNPs, ceramic-based nanoparticles; CD4 Count, Cluster of Differentiation 4 count; CD68$^+$, Cluster of Differentiation 68; CNPs, carbon-based nanoparticles; EMA, European Medical Agency; EPR, enhanced permeability and retention; ES, ectoplasmic specialization; FDA, Food & Drug Administration; GJ, gap junction; GNPs, gold nanoparticles; HAART, highly active antiretroviral therapy; HIV, human immunodeficiency virus; LBNPs, lipid-based nanoparticles; LNE, lipid nanoemulsions; MNPs, metal nanoparticles; NLC, nanostructured lipid carriers; NNRTIs, non-nucleoside reverse transcriptase inhibitors; NPs, nanoparticles; NRTIs, nucleoside reverse transcriptase inhibitors; PLGA, polylactic-co-glycolic acid; PLGA/MMT, poly (d, l-lactide-co-glycolide)/montmorillonite polymeric nanoparticles; PLGA-b-PEG-COOH, carboxy-terminated poly (D, L- lactide-co-glycolide)-block-poly (ethyleneglycol); PNPs, polymeric nanoparticles; SCNPs, semiconductor nanoparticles; SLN, solid lipid nanoparticles; TBC, tubulobulbar complex; TJ, tight junction.

Spermatogenesis occurs in this guarded region of the seminiferous tubules[7] that is compartmentalized by the blood–testis barrier (BTB). This compartmentalization presents a boundary between the area of spermatogenic cells and the vascular environment,[8] which provides an enabling environment for spermatogenesis.

The testis has been described as an organ that may harbor human immunodeficiency virus 1 (HIV-1).[9,10] Several studies have reported adverse effects of antiretroviral drugs (ARVDs) on the reproductive parameters, suggesting that appropriate consideration has not been given to the overall effects of highly active antiretroviral therapy on the testis. Deleterious effects of highly active antiretroviral therapy on sperm motility continue to add to the debate.[11] On the one hand, studies have reported no changes in semen parameters of the HIV-1 patients undergoing highly active antiretroviral therapy (HAART).[12] On the other hand, changes in semen parameters of HIV-1 patients under HAART have been suggested,[13] with likely adverse effects of HAART on the reproductive organs.[14] This includes a significant reduction in total sperm motility in the groups of animals treated by HAART, with lamivudine, nevirapine, and zidovudine.[15,16] Together these reports have established that HAART penetrates the seminiferous tubules but in reduced quantities because of the blood–testis barrier (BTB). However, HAART has been effective at improving Cell of Differentiation 4 (CD4) counts, suppressing viral replication, and viral load to undetectable levels in many patients.[17]

The BTB that partitions the seminiferous tubules and the vascular compartment of the testis significantly reduces the uptake of ARVDs into the testis. This reduction reflects the action of the breast cancer resistance protein (BCRP) and efflux transporters P-glycoprotein (P-gp) that together block and restrict the penetration of ARVDs.[17-20] However, difficulty in penetration of BTB can be overcome by loading ARVDs with nanoparticles. A few studies have reported penetration of nano formulated ARVDs across the BTB. Accumulation of lopinavir, ritonavir, and efavirenz coupled with poly (lactic-co-glycolic acid) (PLGA) nanoparticles have been shown in peripheral blood mononuclear cells in mice testis for 28 days without cytotoxicity.[21]

2 | NANOPARTICLE PENETRATION IN HAART FROM BENCH TO CLINIC

Despite the technological advancement in medical diagnosis and treatment, the toll of infectious and noncommunicable diseases is still high. There is a need for simple, inexpensive, rapid, and sensitive point-of-care diagnostic tools and drug therapies with reduced toxicity and side effects to minimize mortality.[22] Nanoparticles with unique properties are being incorporated into many products as the horizon, and commercial interest in nanomedicine is broadening. Over 500 consumer products in the market claim to contain elements of nanoparticles and more are still emerging.[23,24] This uncontrolled use tends to increase human exposure to nanomaterials.

Significant Statement

This minireview demonstrates that the route of administration, drug dosage, duration of treatment, drug transporters are all essential factors in minimizing toxicity and maximizing drug efficacy and bioavailability when a nanoparticle is used to deliver antiretroviral drugs. To reduce drug toxicity while achieving maximum drug efficacy, stereological quantification of cell and cell types, and morphology of the target organs can be utilized.

Characterization protocols, predictive toxicities, and hazard capabilities of nanodevices and nanomaterials need to be validated.[25] Nanomaterials have been attractive for technology development in the basic sciences and have been used in medicine. Nanotechnology synthesis and the use of the ultramicroscopic particles invisible to the unaided eye are not a latter-day invention[26] from which nanomedicine arose. This area embraces an increasing number of miniaturized technology platforms as they are adopted in biomedicine to solve medical problems.[27] It has the potential to completely shape, direct, and change the future of medical treatments over the next decade.

2.1 | Classes of nanoparticles

Nanoparticles (NPs) are materials in the order of approximately 100 nm, similar to the size of HIV particles.[28] There are several routes by which NPs can enter the organs and bloodstream, with inhalation being one of the more accessible routes. A large number of NPs are safe with beneficial effects,however, cases of toxicities have also been documented for some NPs.[29] Nanoparticles are classified into different categories based on their properties and diverse application. These include metal nanoparticles (MNPs), semiconductor nanoparticles (SCNPs), ceramic-based nanoparticles (CBNPs), polymeric nanoparticles (PNPs), carbon-based nanoparticles (CNPs), and lipid-based nanoparticles (LBNPs) (Figure 1).

Metal nanoparticles have received significant attention possessing optical and electrical characteristics with clinical and medical applications.[30] Their absorption and storage of a large number of electrons, quantum detention ability, large area energies, and large surface area to volume ratio are the characteristics that have made silver, gold, zinc, cadmium, platinum, copper, and iron popular for use in the synthesis of nanoparticles.[31] Owing to their physicochemical attributes,[32] MNPs derived from silver, gold, and copper are being developed as drug carriers for use in the diagnosis, treatment, and bioimaging.

Semiconductor nanoparticles are derivatives of elements, compounds, or a combination of two or more elements that appear in groups IV and VI in the periodic table between metals and nonmetals.

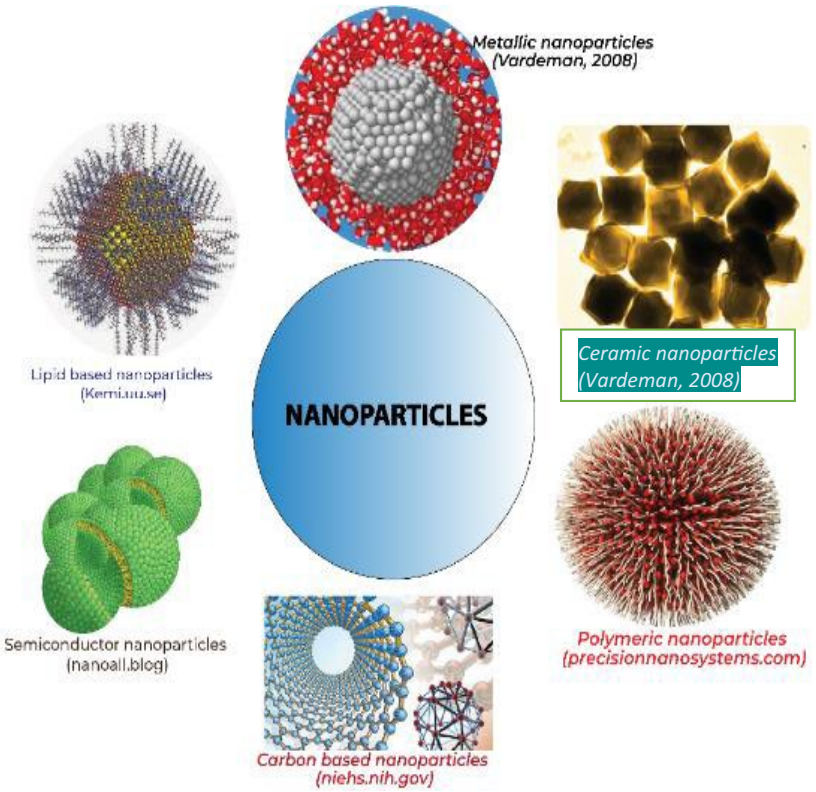

FIGURE 1 Different types of nanoparticles. This figure depicts different types of nanoparticples (Metalic, ceramic, polymeric, carbon-based, Semiconductor and Lipid-based nanoparticles)

Semiconductor nanoparticles, such as silicon (SiNPs), germanium (GeNPs), tin (SnNPs), selenium (SeNPs), tellurium (TeNPs), zinc oxide (ZnO), zinc sulfide (ZnS), cadmium sulfide (CdS), cadmium selenide (CdSe), and gallium nitride (GaN) are used in the area of electrical, optical, electronics, and fiber networks.[33]

Ceramic-based nanoparticles are inorganic nonmetal solids of different forms; amorphous, porous, and polycrystalline.[34] These NPs are used in medical imaging, photo catalyzes, and photodegradation of dyes.[35] Also, CBNPs such as titanium dioxide (TiO_2) and aluminum oxide (Al_2O_3) has been widely used in the manufacturing of nano delivery systems,[36] with silica, albumin, and iron oxide being employed in drug delivery systems.[37,38]

Graphite, graphene, nanodiamonds, carbon nanotubes, and Buckminsterfullerene (C_{60}) are the most widely employed CNPs.[39] Some of these CNPs can form carbon nanotubes,[40] including graphene[41] that have been used in therapeutic and drug delivery systems or as cellular labeling agents.[40-42] The therapeutic application of Buckminsterfullerene, as an anti-HIV agent, has been reported.[43]

Polymeric nanoparticles (PNPs) are organic-based NPs with solid mass wrapped within a particle.[44] Polymers are distinct because of their huge molecular structures, crystallization performance, long-chain involvement, and glass transition.[45] Application of carboxy-terminated poly(D,L-lactic-co-glycolide)-block-poly (ethylene glycol) (PLGA-b-PEG-COOH) and poly(D,L-lactide-co-glycolide)/montmorillonite (PLGA/MMT) PNPs are used in drug delivery systems.[46,47]

Lipid-based nanoparticles (LBNPs) contain functional lipids that make them ultimately tolerated and degraded to a nontoxic precipitate. Over a decade, LBNPs such as ethosomes, lipid nanoemulsions (LNE), liposomes, transfersomes, solid lipid nanoparticles (SLNs), and niosomes have received broad attention for the effectiveness and safety in drug delivery systems.[48,49] Furthermore, high thermal stability, ease of prepare, biocompatibility, large-scale preparation, cost-effectiveness, biodegradability, and robust loading capacity are the advantages of LBNPs.[50,51]

2.2 | Application of nanoparticles in medicine

The emerging field of nanotechnology may change the contemporary treatment modality of HIV by enhancing the delivery of highly active antiretroviral drugs to the intended organs and their effectiveness.[52-56] This novel direction has been credited to the application of various NPs, with the ability to penetrate the blood–testis barrier.[57] Several studies have described the intracellular drug delivery system using NPs: receptor-mediated phagocytosis of nanocarriers, passive diffusion of free drugs, nonspecific phagocytosis of nanocarriers, and pinocytosis process of nanocarrier uptake as pivotal mechanisms of action.

There are intracellular drug delivery systems that may employ a combination of more than one mechanism. The drug may be broken down, leading to an ineffective treatment when the NP is released within the lysosome. However, effective treatment can be achieved when drugs are released within the cytosol.[58-60] Testis $CD68^+$ macrophages are indulgent to immunodeficiency virus-1 infection and aid replication of the virus without affecting testosterone secretion.[61]

The previous study has indicated that the degree to which antiretroviral drugs can penetrate anatomical compartments, anatomical

sanctuary regions, and viral reservoir sites is based on the changing interaction between metabolism, drug efflux, and influx. These have been attributed to unproductive viral suppression, viral persistence, and the virus's resistance to anti-viral drugs.[62] There are increasing pieces of evidence that ATP-binding cassette transporters (ABC) are one of the essential factors that impede the entrance of drugs into the testes,moreover, studies have demonstrated that testes could retard the entrance of antiretroviral drugs and act as a harbor for HIV-1, thereby causing persistent HIV-1 infections and subsequent drug resistance.[9]

A previous study revealed that creating an equilibrium between the efficacy, safety, permissibility, and administration of antiretroviral drugs are essential factors that require maximum attention in achieving a good outcome in the management of HIV infections.[63] However, aspersions have been cast on these antiretroviral drugs, especially the nucleoside reverse transcriptase inhibitors (NRTIs) and non-nucleoside reverse transcriptase inhibitors (NNRTIs), owing to reported toxicities, side effects, and adverse effects.[2] Several studies have linked highly active antiretroviral therapy (HAART), especially nucleoside reverse transcriptase inhibitors (NRTI) and protease inhibitors (PI), to insulin resistance and possible cause of diabetes mellitus in HIV-infected persons.[64-66] In the same vein, gastrointestinal disorder and lipodystrophy syndrome have been described.[67] Another profound adverse effect documented is cardiovascular and liver toxicities[68,69] and severe hyperbilirubinemia.[70] These adverse effects have resulted in drug noncompliance with the patients, changes in therapeutic modalities, and discontinuation of treatment.[71]

Tissue-specific drug-targeted methods have proven to boost the drug's effectiveness at a low dose while reducing adverse effects by controlling the bio-distribution of the drug in nonspecific tissues.[72-74] Suppression of the viral load to an undetectable level and minimizing antiretroviral drugs' toxicity without affecting the therapeutic concentration has been described as the primary goal in the management of HIV infection. In this regard, the usefulness of antiretroviral drug-loaded NPs has received considerable attention. Notably, Ochekpe et al[75] have described the application of nanotechnology to HIV therapy as a core area in drug delivery systems that addresses the issue of bioavailability, tissue distribution, drug level imbalance, and minimizing of toxic effects of common antiretroviral drugs.[75] Likewise, antiretroviral drug-loaded NPs have been delineated as a drug delivery system that ensures an improvement in side-effects of antiretroviral drugs.[76]

Nanoparticle-loaded drugs hold a promising future in nanotherapeutics because of their ability to penetrate biological membranes.[52] Nanomaterials have received a wide range of interest, and applications have increased in drug delivery systems to reduce drug adverse effects and toxicities. Priority has been placed on synthesized NPs to achieve a wide range of applications in the field of nanomedicine. Still, not all nanoparticles can be used in this regard due to the regulations of the Royal Society and Royal Academy of Engineering.[77] Different characteristics that make these NPs highly applicable include their capacity to absorb and pick up other molecules, their quantum characteristics, and more substantial surface to mass ratio, which proved to be larger than other particles. This larger surface primarily enables NPs to adsorb, bind, and pick up other substances such as proteins and drugs.[78]

The method of NP synthesis also plays a role in the toxicity of antiretroviral drug-loaded NPs. There are two approaches to manufacturing NPs; one in which bulk products are curtailed, which is known as the top-down approach, and one in which materials are combined to form larger particles known as the bottom-up approach.[79] Physical, chemical, and green synthesis of NPs have previously been discussed. Green synthesis methods have received a wide range of attention than physical and chemical processes because of their natural stabilizing and reducing abilities. Consequently, there is more interest in the biosynthesis method of NPs employing microorganisms nowadays.[80]

To maintain environmentally safe procedures, the use of chemicals that usually come with hazards should be abolished, whereas green synthesis processes that present biological methods, irradiation methods, polysaccharides, and blended-valence polyoxometalates should be embraced. Moreover, the green synthesis offers enormous benefits compared to procedures that require chemicals linked to ecological hazards should be embraced. Choosing a solvent and environmentally safe stabilizing and reducing agents free of hazards must receive special consideration during the green manufacturing of NPs.[81,82] The previous study has revealed that steady release and effective therapeutic drug delivery of NPs and materials depend on their synthesis method.[83]

To date, only a few studies have investigated the penetration of antiretroviral drug-loaded NPs or HAART through the blood–testis barrier. The previous research has reported the distribution and accumulation of nano-coupled antiretroviral drugs such as lopinavir, ritonavir, and efavirenz-loaded poly lactic-co-glycolic acid nanoparticles in the testes of mice.[21] This result indicates the need to utilize NPs for delivering antiretroviral drugs into the male reproductive system.

There have been tremendous efforts to formulate HAART NPs against a wide range of HIV-1 strains, but the issue of toxicity resulting in DNA damage has been reported.[52] Few NPs, such as polymeric, liposomes, silver/gold, have been reported to enhance the delivery of antiretroviral drugs effectively to combat or treat HIV infection.[84] Ritonavir, lopinavir, and efavirenz coupled with PLGA NPs[21] and dapivirine coupled with poly(ε-caprolactone) NPs[85] have been reported to be in the preclinical stage amongst other nano formulated HAARTs.

3 | PHARMACOKINETICS OF NANOPARTICLES IN RELATION TO TESTICULAR TISSUE AND BLOOD–TESTIS BARRIER

The pharmacokinetics (absorption, distribution, metabolism, and excretion) of NPs largely rely on their physiochemical characteristics.[86]

Nanoparticles presented in solid or liquid forms also penetrate the barriers as well as associated physical, biological, and chemical processes of the tract,consequently, altering and transforming their pharmacological and toxicological properties.[87] Importantly, particle size is an essential factor,the smaller the particle size, the more effective its disease curative effects. As a result, NPs synthesized drugs have higher penetration, proper absorption, more extensive distribution, better metabolism, and greater bioavailability compared to drugs of the same size.[88]

Nanoparticles are administered in various ways, including oral, percutaneous, pulmonary, nasal, and injection.[89] Following administration, NPs are absorbed into the circulatory system and get excreted *via* feces or other means.[90] The mucosal lining and epithelial tissue of the gastrointestinal tract have been identified as primary barriers to the absorption of nano-synthesized drugs. Previous studies show NPs to be absorbed through intestinal enterocytes.[91] More so, the Peyer's patches at the small intestine wall are the site of absorption for NPs within the range of 50–200 mm.[92]

Several animal studies have documented different types of NP absorption; through the skin,[93] by the skin through lymph nodes and the lymphatic system,[94] through the olfactory region (nasal), which goes straight to the central nervous system, a perfect choice for crossing the BTB[95] (Figure 2). Furthermore, the inhalation method whereby NPs are absorbed through alveoli has been reported to be one of the best methods because of the larger surface area of alveoli which permits easy ingress of NPs to the lymphatic and blood circulation system.[94,95] Additionally, when considering drug response and bioavailability, various injectable methods of NPs should be employed.[96]

The significant benefit of the biodistribution of NPs is the ability to determine the likely mechanism of action of NPs.[97] After absorption, effective NP distribution depends on the composition, size, morphology, surface charge, and coating effects. Based on composition, there was reported evidence of a greater affinity of mesoporous silica NPs to the lungs than polymeric NPs to the liver.[97] Regarding the size, for NPs to bypass the liver hepatocytes, it must be smaller. The justification for this is based on the reduced blood circulation period due to bigger particles being taken up by the spleen and the liver.[98,99]

Coating NPs with starch-like materials such as dextran, polyethylene, and other coating materials predominantly intensifies the biodistribution of the NPs.[100] Degraded products of biodegradable NPs are simply metabolized,[101] whereas the metabolism of metal NPs such as silica, silver, iron oxide, and gold is intricate. For instance, a previous study reported that a quantum dot NP remained in the body for two years.[94] Further, one of the brain's supporting cells, the astrocytes, has been identified as the site of metabolism for iron oxide.[102]

There are several elimination methods of NPs and drugs, but the primary process is renal excretion, which is a multiplex method that involves glomerular filtration and tubular secretion.[103] Another method is elimination through feces or urine.[104] Interestingly, there has been a significant link between drug pharmacokinetics and drug transporters located at the junctions of the BTB. A recent study reported the drug transporters that are in different regions and junctions of the BTB are the determinants of the number of drugs and chemical agents that enter the testis under healthy and disease conditions.[105,106] The BTB is a unique blood barrier in the body because of additional and specialized barriers. Besides the tight junction (TJ) and gap junction (GJ) that are also found in other barriers, the BTB also contains the adherens junction (AJ), ectoplasmic specialization (ES), desmosome, hemidesmosome, and tubulobulbar complex (TBC).[107,108]

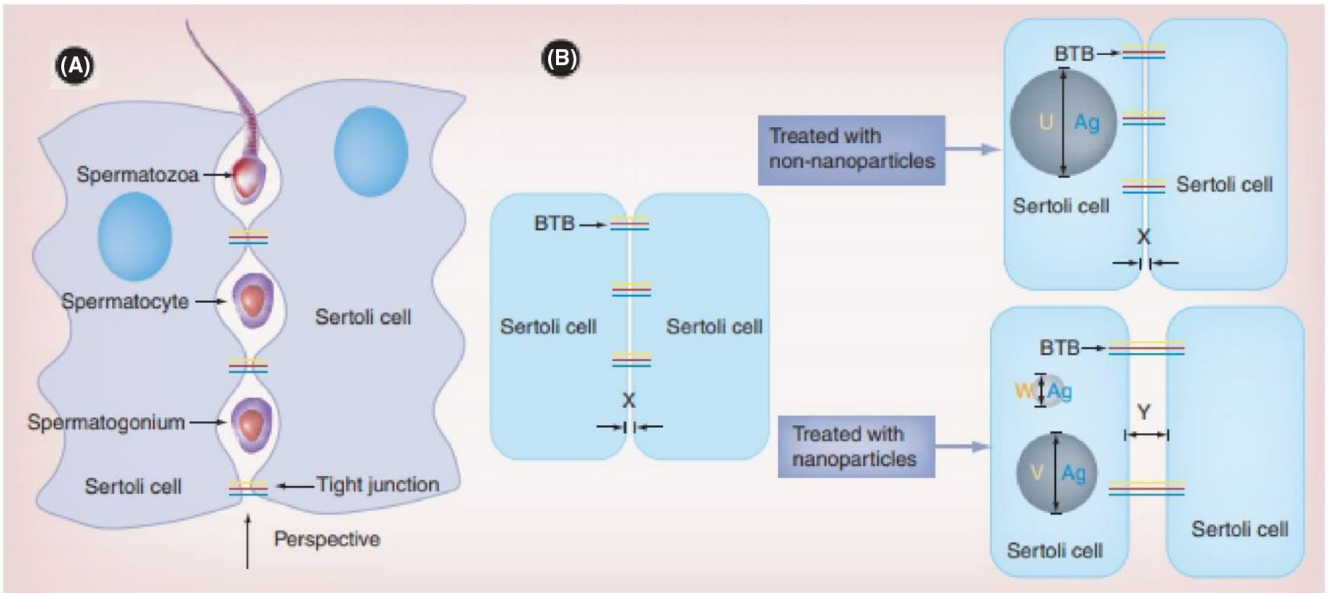

FIGURE 2 The Blood-Testis Barrier and the Nanoparticle penetration[114]
The hypothesis of how nano-Ag penetrates the blood-testis barrier. (A) The outline of part of the seminiferous tubule. (B) only depicts the Sertoli cells which contact with the basal lamina viewed from the outside of seminiferous tubules to the inside of seminiferous tubules.

The BTB separates the adluminal and basal compartments of seminiferous tubules so that sperm production can occur in the apical compartment posterior to the BTB without any interference. A previous study has reported the immunomodulatory function of the BTB based on preventing the formation of molecules or abnormal antibodies that may hinder sperm formation.[109] The BTB provides an enabling and healthy environment for sperm production.[110]

This barrier is made up primarily of the tight junctions between the Sertoli-Sertoli cells and actin-formed adherens junctions and a cytoskeleton-based junction, majorly the intermediate filament-forming desmosome junctions. Out of the three compartments in the substance of seminiferous tubules (basal, luminal, and adluminal), the BTB formed the demarcation between the adluminal and basal compartments. These compartments have been reported to be essential for the development of sex cells and protect them from NPs, foreign bodies, hormonal imbalances, toxins, and infectious diseases to perpetuate their reproductive function.[111]

The seminiferous tubules, which is the testis' functional unit, are surrounded by myoid cells, a contractile cell that propels the mature sperms from secretion, the seminiferous tubule into the epididymis, whereby it will mature. Nevertheless, the BTB starts with Sertoli cells, an epithelium covering the innermost part of the seminiferous tubule that functions to anchor and supply necessary nutrients during sperm formation.[112-114]

Nanoparticles in the real sense and hypothetically cannot pass through the BTB. Still, some studies on animal models revealed that some characteristics of NPs allow penetration through the BTB, whereas larger particles do not penetrate[115] (Figure 2). Wang et al[116] reported that NPs could penetrate the BTB.[116] A similar study that examined silver NPs revealed that small-sized NPs possess the capacity to penetrate the BTB, whereas large-sized NPs do not.[117]

There have been contradictory reports on the ability of NPs to reach the testis and alter spermatogenesis. In a microscopic study, NPs were not found in the testis.[118] Another study reported the ability of NPs to reach the testis,[119] penetrate the BTB, and alter the process of sperm production.[120] In a different study, the nontoxic effects of NPs on spermatogenesis were documented.[121] Importantly, results from previous studies revealed that a small quantity of NPs is getting to the substance of testis irrespective of the method of administration[115,122,123,124] (Figure 2). The unique characteristics of the BTB and NPs may complicate the conventional way of evaluating cytotoxicity and the effectiveness of NPs.[125]

Although significant progress has been made on NP penetration across the testicular tissue. However, specific concerns have yet to be addressed regarding employing NP drug delivery systems in basic and clinical research, such as NP toxicity[126] and the type and properties of NPs to be used. A previous study by Papageorgiou et al.[127] reported that properties of NPs, such as surface features, crystalline properties, size, and chemical constituents, determine the toxicity profile of these NPs. The synthesis route is another issue that must be addressed when employing NPs in drug delivery systems. The biogenic bottom-up synthesis method has been viewed as a better method because of its viability and lack of toxicity, as reported by recent studies.[128] The process of loading NPs with drugs must also be addressed when using NPs in drug delivery systems. Addressing the penetration of NPs in each of the junctions that constitute the BTB requires thorough investigation.

4 | NANOPARTICLES/ NANOFORMULATIONS USED IN CLINICAL RESEARCH

To date, only a few of these NPs have been approved for clinical use, whereas many are still in the pipeline of getting approval. Some of the NPs used in treating cancer, iron-replacement, bacterial and fungal treatments have been approved by the Food & Drug Administration (FDA) and the European Medical Agency (EMA). The important NPs used in clinical diagnosis and therapeutics are classified into two categories, namely, organic NPs, which include liposomal NPs, protein-based NPs, and polymeric NPs, and inorganic NPs, which include metal and metal oxide NPs.[129]

The inorganic NPs have been successful in preclinical research. Iron oxide NPs have been developed and approved to treat anemia and imaging applications.[130,131] Organic NPs, such as liposomes, have been an enormous success and have also been developed into vaccines, anesthetics, and fungal treatments.[1,132]

Nanoparticles have been successfully employed with anti-cancer drugs to ensure the effective management of cancer. Doxorubicin, an anti-cancer drug loaded with pegylated liposomal HCl (CAELYX/ Doxil) was formulated and employed in metastatic breast cancer phase III clinical research.[71] In another clinical research on heart disease, gold NPs were able to deliver drugs to telomerase and consequently alter cancer cells' proliferation.[133] In recent experimental research on the treatment of heart disease, it was evident that gold NPs loaded with Levosimendan (Simdax) and gold NPs with size 30 nm exhibited remarkable cardioprotective results in doxorubicin-induced heart failure rats, considerably better than rats treated with Levosimendan (Simdax) alone.[134]

Liposomes were the first nanoformulations approved by the FDA for clinical trials. The liposomal formulations such as doxorubicin and amphoterin B approval started in the mid-1990.[135] Recently, Onivyde (liposomal irinotecan) was approved as a second-line treatment for metastatic pancreatic cancer. Marqibo (liposomal vincristine) was also recently approved for the treatment of pancreatic cancer, multiple sclerosis, fungi infections, and respiratory distress syndrome.[37,136] There is clear evidence that liposomal formulations have become clinically stable and improved in nanotechnology,therefore, nanomedicines' evolution remains relevant.[37]

Polymer nanoformulations such as Coagulation factor IX (Rebinyn) and Antihemophilic factor VIII (Adynovate) have also been investigated and approved for the treatment of hemophilia due to their more excellent protein stability and long half-life.[137] Recently, Oncaspar (pegaspagase) was approved for the treatment of conditions such as chronic gut, hepatitis, multiple sclerosis,

prostate cancer, among others. Protein nanoformulations such as Abraxane (albumin-bound paclitaxel) and Ontak (denileukin diftitox) have recently been approved to treat breast cancer, pancreatic cancer, and cutaneous T-cell lymphoma due to their more excellent stability, increased delivery to the tumor, and targeted T-cell specificity and lysosomal escape.[137] The FDA receives new nanoformulations for clinical investigations yearly, and many have been approved for clinical use.[137] As of October 2017, 56 clinical trials nanoformulations have been received or are in the inactive stage (Clinical Trials.gov).

Iron oxide nano-drugs such as Venofer Ferleccit have been studied extensively in the clinical trial phase. The FDA has approved them as an indication for iron replacement therapies.[37] However, iron oxide nanoformulations used as a contrast enhancer reagent for magnetic resonance imaging are still in the clinical trial stage.[138]

Several NPs have been clinically proven for the treatment of HIV/AIDS. The DermaVir patch was employed for immunotherapy of HIV/AIDS after being proven safe and tolerable in preclinical and phase I clinical trials and have consequently progressed to stage II trials.[139] An L-lysine dendrimer is in phase I/II trials.[64,141,142] Silver NPs,[143,144] dendrimers,[145,146] gold NPs,[147] and PGLA NPs[148] are all in preclinical trials.

The first long-acting regimen of antiretroviral drugs, cabotegravir, and rilpivirine, has been approved to treat HIV.[149] Recently, the role of nanoformulation of the long-acting injectable cabotegravir and rilpivirine on the treatment of HIV infection has been reported. Emphasis was placed on its advantages, such as reducing the number of drugs, minimizing drug-associated toxicity, reducing adverse drug effects, and treatment simplification.[150] In another study, myristoylated cabotegravir prodrug was formed, and this crystal was formulated into nanoparticles. The nano myristoylated cabotegravir (NMCAB) that was fabricated has proven to improve biodistribution and viral clearance profiles in mice.[151]

There is no unanimous consensus about toxicity or risks in most NPs used for clinical trials or nanomaterials developed for commercial purposes. Although biomedical researchers have made tremendous efforts to investigate the toxicological profile of these NPs, the results have not been convincing enough. Recently, organ toxicities of NPs have been highly documented. Previously, a study that examined toxicity in mice following chronic oral administration of CeO_2 NPs, testis impairment, sperm DNA damage,[152] sperm malformation, asthenospermia,[153] and reduction in testicular cytology was reported.[154] Nephrotoxicity,[155] chronic cardiac toxicity,[126] and other organ toxicities of NPs are summarized in Table 3.

5 | TOXICITY PROFILE OF ANTIRETROVIRAL DRUGS/HIGHLY ACTIVE ANTIRETROVIRAL THERAPY AND NANOPARTICLES

Diverse works of literature have documented different toxicities of antiretroviral drugs ranging from mild to severe adverse effects on major organs and systems of the body. Consequently, the World Health Organisation has revealed that it has now become more difficult to distinguish the adverse effects of antiretroviral drugs from common complications of HIV infection.[156,157] Despite numerous beneficial effects of HAART, research has unveiled toxicities, side effects, and even clinical adverse events.[16] HAART is associated with clinical adverse effects such as hyperglycemia, gastrointestinal, and lipodystrophy symptoms.[158]

The combination of different antiretroviral agents (HIV-HAART) exposes the entire body to multiple doses at high doses, resulting in enormous side effects, limiting the therapeutic effect, or resulting in toxicity. Numerous adverse effects of antiretroviral drugs on the organs and systems of the body have been documented; suppression of bone marrow, which would later result in thrombocytopenia and anemia, has been linked to the zidovudine, azidothymidine, tenofovir disproxil fumarate, efavirenz, lamivudine, and stavudine.[157,159] The previous study has reported peripheral neuropathy, lactic acidosis, hyperlipidemia, and insulin resistance as adverse effects of stavudine,[160-162] which corresponds with other reports in Table 1. Renal dysfunction and nephrotoxicity have been linked with Nevirapine, Efavirenz, Stavudine, and Indinavir,[163,164] as shown in Table 1.

Despite the growing knowledge on the effects of HAART on male reproduction, there are contradictory findings concerning real sperm functional tests.[16] Onanuga et al. (2018) reported a severe histological alteration of the seminiferous tubule in the experimental animals subjected to diabetes and HAART, although it is not clear whether the alteration was caused by drug-induced diabetes.[204] Other studies have reported toxicity and adverse effects of antiretroviral drugs (Table 1), antiretroviral drugs coupled with NPs (Table 2), and toxicity of these nanomaterials on several organs (Table 3). Nevertheless, the NP drug delivery system has improved the efficiency of the delivery of antiretroviral drugs (such as Saquinavir)[205,206] as well as a combination of different antiretroviral drugs.[207] Gold and silver have been reported to have antiviral properties against a wide range of HIV-1 strains but posed high toxicity issues resulting in DNA damage and cellular apoptosis.[52] Studies have shown that antiretroviral drug-loaded NPs or nanocarriers achieve adequate drug distribution to specific sites in the body[208] and recognize HIV-infected cells and can deliver multiple therapeutic doses, thereby increasing drug efficacy.[59,85]

Recently, toxicity has been reported in some of the antiretroviral drug-loaded NPs, as shown in Table 2. Madugulla et al[209] reported a significant decrease in litter size through the oral administration of lactoferrin NPs,however, there was no significant difference in the litter size and postnatal development of the same drugs administered through the vaginal route. This result could suggest that the toxicity of NP-loaded drugs may depend on the route of administration. Additionally, Ogunwuyi et al[212] reported that antiretroviral drug-loaded NPs (Nevirapine, Raltegravir, Zidovudine, and Lamivudine) are effective in the inhibition of HIV-1 infection in CEM T cells and PBMCs but are toxic at higher concentrations.[212]

TABLE 1 Toxicity profile of non-nano antiretroviral drugs

S/N	ARDS	Studies	Toxic effects
1.	Nevirapine	Guidelines for the use of antiretroviral agents in HIV-1-infected adults and adolescents	Hepatic necrosis[165]
		Safety profile of nevirapine, a nonnucleoside reverse transcriptase inhibitor for the treatment of human immunodeficiency virus infection	Hypersensitivity[166]
		Limitations to treatment safety and efficacy: adverse effects of antiretroviral agents	Renal dysfunction[167]
2.	Efavirenz	A randomized cross-over study to compare raltegravir and efavirenz	Persistent and troubling neuropsychiatric symptoms[168]
		A phase IV, double-blind, multicenter, randomized, placebo-controlled, pilot study to assess the feasibility of switching individuals receiving efavirenz with continuing central nervous system adverse events to etravirine	[169]
		Neuropsychiatric side effects of efavirenz therapy	[170]
		Acute Liver Toxicity due to Efavirenz/Emtricitabine/Tenofovir	Hepatotoxicity[171]
		CYP2B6 haplotype and biological factors responsible for hepatotoxicity in HIV-infected patients receiving efavirenz-based antiretroviral therapy	[172]
		EFV/FTC/TDF-associated hepatotoxicity: a case report and review	[173]
		Hepatotoxicity in patients prescribed efavirenz or nevirapine	Teratogenicity[174]
		Periconceptional exposure to efavirenz and neural tube defects	[175]
		Myelomeningocele in a child with intrauterine exposure to efavirenz	
3.	Raltegravir	Severe rhabdomyolysis associated with raltegravir use	Skeletal muscle toxicity, Rhabdomyolysis, and Elevated serum creatine kinase (CK)[176]
4.	Zidovudine, or azidothymidine	Antiretroviral Therapy for HIV Infection in Infants and Children: Towards Universal Access	Anemia, neutropenia and, more rarely, thrombocytopenia,[157]
		Management of the Adverse Effects of Antiretroviral Therapy and Medication Adherence	
		Tenofovir DF, emtricitabine, and efavirenz versus zidovudine, lamivudine, and efavirenz for HIV	Bone marrow suppression[177,178]
		Improvement of dyslipidemia in patients switching from stavudine to tenofovir: preliminary results	Hyperlipidemia[179,180]
		Lipid levels and changes in body fat distribution in treatment-naive, HIV-1-Infected adults treated with rilpivirine or Efavirenz for 96 weeks in the ECHO and THRIVE trials	
		Mechanisms of zidovudine-induced mitochondrial toxicity and myopathy	Myopathy[181]
5.	Didanosine (ddI)	Antiretroviral Therapy for HIV Infection in Infants and Children: Towards Universal Access:	Lactic acidosis, hepatic toxicity, pancreatitis and peripheral neuropath, Mitochondrial dysfunction[157]
		Incidence of pancreatitis in HIV-infected patients: comment on findings in EuroSIDA cohort	Pancreatitis [182]
		Didanosine. An update on its antiviral activity, pharmacokinetic properties and therapeutic efficacy in the management of HIV disease	[183]
6.	Stavudine (d4 T)	Antiretroviral Therapy for HIV Infection in Infants and Children: Towards Universal Access	Hyperlipidemia, hyperglycemia, insulin resistance, diabetes mellitus, osteopenia, osteoporosis and osteonecrosis. lactic acidosis, hepatic toxicity, pancreatitis and peripheral neuropath, Mitochondrial dysfunction[157]
		HIV drug stavudine (Zerit, d4 T) and symptoms mimicking Guillain–Barré syndrome	Neuromuscular weakness[184]

(Continues)

TABLE 1 (Continued)

7.	Stavudine and didanosine combination	Neurological and psychiatric adverse effects of antiretroviral drugs	Peripheral neuropathy[185]
		The risk of developing peripheral neuropathy induced by nucleoside reverse transcriptase inhibitors decreases over time: evidence from the Delta trial	[186]
		Improvement of dyslipidemia in patients switching from stavudine to tenofovir: preliminary results	Hyperlipidemia[179]
		Lipid levels and changes in body fat distribution in treatment-naive, HIV-1-Infected adults treated with rilpivirine or Efavirenz for 96 weeks in the ECHO and THRIVE trials	[180]
8.	Abacavir	Use of nucleoside reverse transcriptase inhibitors and risk of myocardial infarction in HIV-infected patients	Myocardial infarction[187]
		Abacavir and risk of myocardial infarction in HIV-infected patients on highly active antiretroviral therapy: a population-based nationwide cohort study	[188]
		Cardiovascular risks associated with abacavir and tenofovir exposure in HIV-infected persons	[189]
9.	Tenofovir disoproxil fumarate (Tenofovir DF)	Clinical practice guideline for the management of chronic kidney disease in patients infected with HIV: 2014 update by the HIV Medicine Association of the Infectious Diseases Society of America	Nephrotoxicity[163]
		Drug-induced acute interstitial nephritis mimicking acute tubular necrosis after initiation of tenofovir-containing antiretroviral therapy in patient with HIV-1 infection	Interstitial nephritis[190]
10.	Tenofovir alafenamide	Tenofovir alafenamide versus tenofovir disoproxil fumarate, Page 23/60 coformulated with elvitegravir, cobicistat, and emtricitabine, for initial treatment of HIV-1 infection: two randomized, double-blind, phase 3, non-inferiority trials	Increase in lipid parameters (total cholesterol and HDL)[191]
11.	Dolutegravir	Dolutegravir plus abacavir-lamivudine for the treatment of HIV-1 infection	Insomnia[192]
		Dolutegravir: a next-generation integrase inhibitor for treatment of HIV infection	Myopathy[193]
12.	Rilpvirine	Rilpivirine versus efavirenz-based single-tablet regimens in treatment-naive adults: week 96 efficacy and safety from a randomized phase 3b study	Neuropsychiatric side effects, depression and insomnia[194]
		Neurological and psychiatric tolerability of rilpivirine (TMC278) versus efavirenz in treatment-naïve, HIV-1-infected patients at 48 weeks	[195]
13.	Atazanavir	In vitro inhibition of UDP glucuronosyltransferases by atazanavir and other HIV protease inhibitors and the relationship of this property to in vivo bilirubin glucuronidation	Hyperbilirubinemia[196]
		Urolithiasis in HIV-positive patients treated with atazanavir	Nephrolithiasis[197]
		Complicated atazanavir-associated cholelithiasis: a report of 14 cases	Cholelithiasis[198]
14.	Indinavir	Crystalluria and urinary tract abnormalities associated with indinavir	Nephrotoxicity, kidney stone[199]
15.	Lopinavir-Ritonavir	Lopinavir/ritonavir: a review of its use in the management of HIV infection	Alcohol in liquid formulation[200]
16.	Tipranavir/ritonavir	Intracranial hemorrhage and liver-associated deaths associated with tipranavir/ritonavir: review of cases from the FDA's Adverse Event Reporting System	Intracranial hemorrhage, Hepatotoxicity[201]
17.	Protease Inhibitors	HIV protease inhibitors activate the unfolded protein response in macrophages: implication for atherosclerosis and cardiovascular disease	Insulin resistance, Atherosclerosis, cardiovascular disease[202]
18.	Maraviroc	Hepatic safety of maraviroc in patients with HIV-1 and hepatitis C and/or B virus: 144-week results from a randomized, placebo-controlled trial	Hepatotoxicity[203]

This table delineates scribes the toxicity profile of non-nano antiretroviral drugs and the recent studies on non-nano antiretroviral drugs with their various toxic effects on organ profiles.

(Continues)

TABLE 2 Toxicity profile of antiretroviral drugs loaded nanoparticles

S/N	ARVDS loaded NPS	Studies	Toxicities/activities
1.	ARV loaded lactoferrin nanoparticles	Evaluation of the reproductive toxicity of antiretroviral drug loaded lactoferrin nanoparticles	Significant decrease in litter size[209]
2.	Dapivirine-loaded nanoparticles	Polymeric nanoparticles affect the intracellular delivery, antiretroviral activity and cytotoxicity of the microbicide drug candidate dapivirine	Improved antiviral activity compared to free drug[85]
3.	Poly-(lactic-co-glycolic acid) (PLGA) nanoparticles (NPs) containing ritonavir (RTV), lopinavir (LPV), and efavirenz (EFV)	Combination antiretroviral drugs in PLGA nanoparticle for HIV-1.	No significantly cytotoxicity[21]
4.	Poly(alkylcyanoacrylate) saquinavir loaded nanoparticles	Formulation and cytotoxicity of combined cyclodextrin poly(alkylcyanoacrylate) nanoparticles on Caco-2 cells monolayers intended for oral administration of saquinavir	Decreased cyctotoxicity[210]
5.	Poly (lactic-co-glycolic acid) zidovudine-lamivudine nanoparticles	Formulation and in vitro evaluation of zidovudine-lamivudine nanoparticles	Acute toxicity to animal cells was not detected[211]
6.	Poly-(dl-lactide-coglycolic acid; PLGA) containing efavirenz (EFV) and boosted lopinavir (lopinavir/ritonavir; LPV/r)	Polymeric nanoparticles containing combination antiretroviral drugs for HIV type 1 treatment	No cytotoxicity seen for 28 days of treatment[207]
7.	ARV (zidovudine, lamivudine, nevirapine, and raltegravir)-loaded PMM-based nanoparticles	Antiretroviral Drugs-Loaded Nanoparticles Fabricated by Dispersion Polymerization with Potential for HIV/AIDS Treatment	CEM cells and PBMCs culture toxicity at higher concentration (CC_{50} = 42 Mm[212]
8.	Raltegravir gold nanoparticle and penetration into the brain in vivo without toxicity	Gold nanoparticles to improve HIV drug delivery	No neurotoxicity found[213]

This table depicts the different nanomaterials, organ toxicities, and the recent studies of nanomaterials with their various toxic effects on organ profiles.

6 | INTERPLAY BETWEEN NANOMEDICINE: ACHIEVING DRUG EFFICACY, ADEQUATE BIOAVAILABILITY, AND BALANCING TOXICITY

Nanomedicine plays a crucial role in achieving biological barrier penetration and drug delivery efficacy while balancing toxicity owing to their physicochemical properties. The contemporary method of loading antiretroviral drugs with NPs has previously been reported to reduce adverse side effects of antiretroviral drugs as well as required dosage, which lessens the drug resistance and ensures drug potency.[52]

Premature release of the drug has been described as an impediment to intracellular and systemic diseases and infections.[225] Moreover, steady and sustained drug delivery has been stated as an essential feature for retaining adequate concentrations of drugs within the beneficial range,[226] which alleviates the likelihood of drug resistance.

Nanoparticles have been viewed as a tool to achieve increased drug efficacy with decreased potential toxicities owing to their ability to be kept in the body for a more extended period than traditional modalities,[227] which aids steady and sustained delivery. It is, therefore, necessary to consider ways to increase drug efficacy while decreasing potential toxicities and the tendency of drug resistance. The study by Cauchetier et al[228] described directing the nanoformulation to the specific site, thereby boosting drug efficacy.

Several mechanisms by which NPs lessen the toxicity of drugs have been reported. Nanoparticles can work as a substitute for the harmful solubilizing medium when administering hydrophobic agents.[229-231] Additionally, enhanced permeability and retention (EPR) ability has been described as another mechanism by which NPs reduce the toxicity of drugs.[232-234] Previous studies have also documented the ability of NPs to enhance absorption, distribution, metabolism, and elimination of drugs by reducing the toxicity of drugs that have build-up at the site of action. Moreover, boosting the curative effect of drugs by accelerating intracellular delivery and sustenance of retention period both in the systematic circulation and inside the cell are also recorded as other means by which NPs reduce the toxicity of drug.[235,236]

In achieving drug efficacy and balancing toxicity, biological barriers are the determinant of the size-dependent biodistribution of NPs within tissue, organs, and surrounding fluid. A study indicated NPs penetrating ability to be a function of size. Hence, an increase in the size of NPs will bring about a decrease in barrier permeability.[237] To achieve good penetration and avoid excessive accumulation that may

TABLE 3 Nanomaterials and organ toxicities

S/N	Nanomaterial	Study	Organ toxicity	References
1.	Gold nanoparticles	Reversible cardiac hypertrophy induced by PEG-coated gold nanoparticles in mice	Chronic cardiac toxicity	[126]
		Application of gold nanoparticles in biomedical and drug delivery		
		Cytotoxic effects of gold nanoparticles exposure employing in vitro animal cell culture system as part of nanobiosafety	Spleen, Lung	[214,215]
2.	Carbon nanoparticles (CNP)	A comparison of dispersing media for various engineered carbon nanoparticles	Largest CNP agglomerates in lung	[216]
3.	Zinc oxide (ZnO) nanoparticles (NPs)	Relating cytotoxicity, zinc ions, and reactive oxygen in ZnO nanoparticle-exposed human immune cells	Cytotoxicity	[217]
4.	Silver nanoparticles	In vitro toxicity of nanoparticles in BRL3A rat liver cells	Cytotoxic effects on $HepG_2$ cell line and primary liver cells of mice	[218]
5	ZnO nanoparticles	Zinc oxide nanoparticles cause nephrotoxicity and kidney metabolism alterations in rats	Nephrotoxicity (mitochondria and cell membrane impairment in rat kidney)	[219]
6.	Titania (TiO2) nanoparticles	Cytotoxic and genotoxic impact of TiO2 nanoparticles on A549 cells	Cytotoxic and genotoxic impact on a cell line representative of human lung	[220]
7	Mn_2O_3 nanoparticle	Toxic effects of Mn_2O_3 nanoparticles on rat testis and sex hormone	Reduction in testicular cytology	[154]
8.	Titanium oxide nanoparticles	Unraveling the neurotoxicity of titanium dioxide nanoparticles: Focusing on molecular mechanisms	Neurotoxicity	[155]
9.	Silica nanoparticles	Silica nanoparticles induce neurodegeneration-like changes in behavior, neuropathology, and affect synapse through mapk activation	Neurodegeneration disorders	[221]
10.	Polyethylene glycol (PEG)	Assessment of PEG on polymeric particles surface, a key step in drug carrier translation	Immunotoxicity	[222]
		Subchronic toxicity and immunotoxicity of MeO-PEG-poly (D, L-lactic-co-glycolic acid)-PEG-OMe triblock copolymer nanoparticles delivered intravenously into rats		[223]
11.	Cerium oxide nanoparticles	SF-1 mediates reproductive toxicity induced by Cerium oxide nanoparticles in male mice	Testis impairment and sperm DNA damage	[152]
12	Anatase TiO_2 nanoparticles (NPs)	Toxic effects of anatase titanium dioxide nanoparticles on spermatogenesis and testicles in male mice	Sperm malformation and Spherospermia	[153]
13	Iron oxide nanoparticles (FeNP	Effects of iron oxide nanoparticles on mouse sperm parameters and testicular tissue	Reduction in testicular interstitial tissue volume, Reduction in the sperm parameters	[224]

This table describes the different nanomaterials, organ toxicities, and the recent studies on nanomaterials with their various toxic effects on organ profiles.

lead to toxicity, the size of NPs should be more than 10 nm,[238,239] and 20 nm or less to achieve the most significant permeability or penetration.[240-242] Activation of the complement system and accumulation of NPs in the spleen and liver resulted from the administration of NPs with a diameter of more than 200 nm[238,243,245] have been documented.

Conversely, considering HIV infection, substantial accumulation of NPs in the macrophages, which also serves as a sanctuary region for HIV, may likely present a therapeutic advantage. More significant accumulation within the macrophages may worsen cell physiological activities.[244] Nevertheless, enhancement of safety by reducing the dosage, adverse effects, and boosting biodistribution to the infected cells are pivotal to the invention of nanomedicine.[52,244] A previous study revealed that NPs reduce the toxicity of primary hydrophobic therapeutic agents such as antiretroviral drugs by boosting their solubility and strengthening their stability, shielding them from non-specific regions.[52]

A recent study shows that antiretroviral drugs' efficacy depends on the distribution and sustenance of adequate dosage at the

specific site for the recommended period.[208] Several studies have reported that loading antiretroviral drugs with NPs appears to be a breakthrough in ensuring drug efficacy at reduced doses. Recently, a finding delineated the great translational prospects of antiretroviral drug-loaded NPs to aid drug compliance and reduce viral resistance based on its sustained delivery system and targeted efficacy with little toxicity.[246]

Interestingly, owing to emerging shreds of evidence and their physicochemical characteristics, antiretroviral drugs can be separately loaded with particles of nano-size to effectively combat HIV infection. Recent findings have accredited 50-double curtailment in the 50 percent inhibitory concentration to the HIV inhibitory ability of antiretroviral drug-loaded NPs, as well as a 50-fold improvement in antiviral effects when compared to free antiretroviral drugs, which establishes the effectiveness and activities of antiretroviral drug-loaded NPs compared to free antiretroviral drugs.[247]

However, with the advent of NPs coupled with antiretroviral drugs, a few studies have documented their toxicity and adverse effects, suggesting that NPs could not completely eradicate the issue of toxicity. This suggestion indicates that structural architecture and morphometric assessment of specific organs or tissues should be considered in formulating the NPs.

Therefore, time of release, duration in the body, route of administration, biological barriers, drug transporters, and delivery methods all play a crucial role in achieving drug efficacy and adequate bioavailability.

7 | STEREOLOGICAL CONSIDERATION

Design-based stereology has been reported to be a useful tool because of its application to different organs. More so, it has been described as an appropriate tool to assess the precise morphological and morphometric parameters.[248] Design-based stereology can be utilized to extrapolate two-dimensional objects to three-dimensional objects concerning advanced stochastic and statistical information. Furthermore, a three-dimensional profile has been regarded as an integral feature of stereology and quantification devices. Hence, incorporating stereological techniques with 3D radiological procedures such as volume electron microscopy, small computed tomography, and confocal microscopy would analyze the broad sample size and give a perfect resolution.[249]

Stereology has been widely applied in morphological and morphometric research. It is a combination of quantitative and comparative approaches that utilize lines, points, numbers, length, area, volume, and planes to evaluate three-dimensional indices.[250,251]

This method has been widely employed in neuro research,[250,252] quantifying the microarchitecture of the kidney.[253,254] Stereology has been employed to quantify the liver macrophages and hepatocytes,[254-256] also to assess the human lung pathologies [257] as well as testicular morphological and morphometric parameters.[258,259]

Previously, issues regarding penetration of the BTB and distribution of antiretroviral drugs to the viral sanctuary sites and the effect of the antiretroviral drugs on testicular morphology have received significant attention.[2] A recent study has demonstrated the adverse effects of HAART on reproductive parameters employing qualitative histopathological methods and morphometric analyses and revealed that HAART causes detrimental histopathological changes in the testes leading to tubular atrophy with altered morphometric parameters.[260]

However, very few stereological approaches have been recorded in assessing the adverse effects of antiretroviral drugs on reproductive indices. A recent review suggested that the stereological method is applicable in evaluating changes in testicular morphological parameters, volume estimation, biological reference spaces, and resulting damage on endocrine organs from the way they appear in two dimensions to three dimensions following an altered distribution of highly active antiretroviral therapy.[2]

Testis presents an additional biological barrier that exists between the seminiferous tubules and vascular compartment, consequently favoring the tenacity of viral replication. There have also been reports of a decrease in antiretroviral drugs' penetration through the BTB attributed to both the breast cancer resistance protein and efflux transporters P-glycoprotein.[98,199] The application of NPs in drug delivery has offered new hope in treating HIV infections by enhancing antiretroviral drugs' penetration through the BTB and improving therapeutic efficacy.[52,57]

Over the years, pathologists have depended on the two-dimensional method to assess cell profile and cell numbers, but recently, research has proven that this method seems biased, assumption-based, and insensitive. Likewise, literature has reported limited sensitivity in detecting cell numbers based on qualitative analysis. Furthermore, quantitative data derived from an interpretation of the two-dimensional morphometric analysis method are usually assumption-based, inaccurate estimations. They are not the true reflection of the sample size and numbers. This fact is based on the literature that revealed that the resulting profiles are one dimension less than the actual when different objects of one, two, or three dimensions are subjected to a two-dimensional section plane.[250,262] This finding implies that the two-dimension surface would produce a one-dimensional profile, and a three-dimension profile produces a two-dimensional shape.

In the same vein, for precise changes in cell number and structure to be appreciated and well defined, a sensitive qualitative evaluation such as a stereological method is required.[263] Stereological methods provide an experimental and technically reasonable way of getting a concise and correct qualitative assessment of morphological changes in the tissue obtained from the histological sections. Besides, where other qualitative analyses discover changes in tissue morphology at 25%–40%, though depending on tissue type, the stereological method picks it up earlier.[263]

Although wide attention has been given to applying the stereological method in quantifying testicular parameters, few studies have been done on the stereological quantification of testicular parameters of rats under antiretroviral drugs,[260,264,265]. To date, very few articles have documented the stereological approach in antiretroviral drug-loaded nanoparticles. In a recent study, a stereological

method was used to investigate the toxicity profile of Tenofovir and Tenofovir nanoparticles on the liver and the kidney of experimental rats. This finding shows accurate stereological assessment, as there were no significant changes in the kidney's morphological parameters and that of control rats in both stereological approach, Renal function test, Liver function test, and cell count.[266]

The blood–testis barrier is unique. Aside from the tight junction (TJ) and gap junction (GJ) that are also found in other barriers, the BTB also contains the adherens junction (AJ), ectoplasmic specialization (ES), desmosome, hemidesmosome, and tubulobulbar complex (TBC)[107,108] which could be considered in the formulation of drugs loaded with NPs. Therefore, it is imperative to consider employing a stereological approach in describing abnormalities of testicular morphology, quantitative estimation of antiretroviral drugs reaching seminiferous tubules, and toxicity evaluation of NPs loaded with antiretroviral drugs in the nanocarrier formulation of HAART (Figure 3).

8 | CONCLUSION AND FUTURE PERSPECTIVES

While early studies on the effects of antiretroviral drugs on sperm (and testicular tissue) were derived from rodent models, there are now emerging new data (also in top high impact journals) revealing the diverse impact of antiretroviral drugs on the testes in humans.[267-269] Besides, there are now issues related to sperm defects[269,270] and viral replication and drug resistance[271,272] on the rise in HIV patients under antiretroviral treatment. These issues are partly attributed to the low drug concentration in the sanctuary sites with insufficient delivery to confer a competitive advantage in combating viral replication and achieving therapeutic efficacy.[273] The literature has also reported adverse and toxic effects of these antiretroviral drugs or HAART because the entire body is exposed to multiple drugs at high doses. Therefore, it is necessary to explore means of achieving targeted delivery to anatomical sanctuary sites (including the testes) nanotechnology. Nevertheless, reducing the viral load to improve the quality of life of HIV-infected patients has been the cornerstone in the management of HIV infection. Nano-delivery systems have become the appropriate means for efficient delivery of drugs to these sanctuary sites to combat viral replication, rebound, and adverse effects of antiretroviral drugs on testicular morphology.[273]

Therefore, nanomedicine has given a temporary breakthrough in this regard. Nanoparticles are now relevant in drug delivery because of their ability to penetrate the so-called "anatomical sanctuary sites" such as the brain and the testis, which have previously been reported to be challenging to penetrate, especially for antiretroviral drugs or HAART. This advancement in nanomedicine enables antiretroviral drug-loaded nanoparticles to deliver a substantial quality of antiretroviral drugs to these sanctuary sites. However, some researchers have documented different adverse effects and toxicities of NPs on organs of the body, ranging from the testis, brain, kidney, liver, spleen, lung, and on various biochemical parameters. Still, little information is available on the toxicological evaluation and mechanism of toxicity of antiretroviral drug-loaded nanoparticles. Additionally, it is becoming difficult to differentiate HIV infection complications, antiretroviral drug adverse effects, and nanoparticle toxicities. In light of this, future research on the morphology of the specific organ of study in formulating the antiretroviral drug-loaded nanoparticles to reduce the toxicity profile while achieving drug delivery efficacy should be conducted. More studies are also needed to substantiate the causes of toxicity in antiretroviral drug-loaded nanoparticles and fully understand their mechanism of toxicity. Imperatively, an animal experiment should be set up to evaluate the toxicity of testicular morphology and BTB in the nano delivery of antiretroviral drugs using a stereological approach.

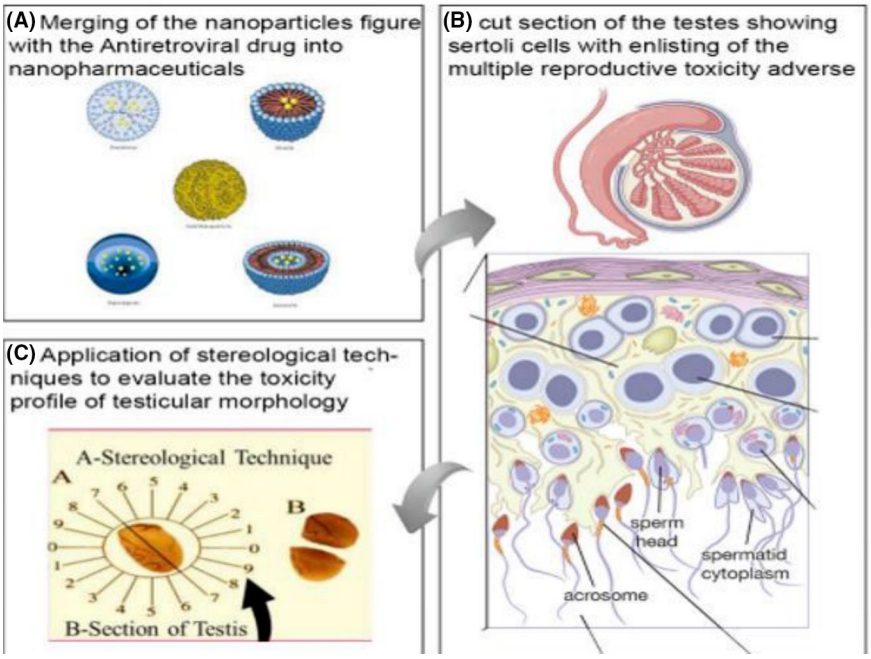

FIGURE 3 Stereological method on assessment of toxicity profile of testicular morphology in nano-delivery of highly active antiretroviral therapy. This figure describes the stereological evaluation of the testicular tissue when a nano-delivery system is employed to deliver antiretroviral drugs through blood-testis barrier. (A) Loading of antiretroviral drugs with nanoparticles. (B) Delivery of nanoparticle-loaded antiretroviral drugs through blood-testis barrier to reach testis. (C) Stereological approach in assessment of toxicity of testicular morphology

ACKNOWLEDGMENT

The authors appreciate Professor Roshila Moodley and Dr. Oseni Shina for their editing services

DISCLOSURE

The authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS

Naidu ECS, Olojede SO, Azu OO, Lawal SK, and Rennie CO conducted experiments, performed data analysis, participated in research design, and wrote or contributed to the writing of the manuscript.

DATA AVAILABILITY STATEMENT

Not applicable.

ORCID

Edwin Coleridge S. Naidu https://orcid.org/0000-0002-2691-4017
Samuel Oluwaseun Olojede https://orcid.org/0000-0002-1000-7412
Sodiq Kolawole Lawal https://orcid.org/0000-0002-8598-4058
Carmen Olivia Rennie https://orcid.org/0000-0003-2414-6293
Onyemaechi Okpara Azu https://orcid.org/0000-0002-5886-3701

REFERENCES

1. Anselmo AC, Mitragotri S. An overview of the clinical and commercial impact of drug delivery systems. *J Controlled Release*. 2014;190:15-28.
2. Azu OO. Highly active antiretroviral therapy (HAART) and testicular morphology: current status and a case for a stereologic approach. *J Androl*. 2012;33:1130-1142.
3. Miller SR, Cherrington NJ. Transepithelial transport across the blood-testis barrier. *Reproduction*. 2018;156(6):R187-R194. https://doi.org/10.1530/REP-18-0338. PMID: 30328342; PMCID: PMC6437009.
4. Brooks JD. *Anatomy of the Lower Urinary Tract and Male Genitalia*. Campbell-Walsh Urology; 2007.
5. Komeya M, Sato T, Ogawa T. In vitro spermatogenesis: a century-long research journey, still halfway around. *Reprod Med Biol*. 2018;17:407-420.
6. Nakata H, Sonomura T, Iseki S. Three-dimensional analysis of seminiferous tubules and spermatogenic waves in mice. *Reproduction*. 2017;154:569-579.
7. Modules ST. Module name. US National Institutes of Health, National Cancer Institute. Day Month Year (of access)< https://training. seer. cancer. gov. 2009.
8. Mital P, Hinton BT, Dufour JM. The blood-testis and blood-epididymis barriers are more than just their tight junctions. *Biol Reprod*. 2011;84:851-858.
9. Huang Y, Hoque MT, Jenabian MA, et al. Antiretroviral drug transporters and metabolic enzymes in human testicular tissue: potential contribution to HIV-1 sanctuary site. *J Antimicrob Chemother*. 2016;71:1954-1965.
10. Nickle DC, Jensen MA, Shriner D, et al. Evolutionary indicators of human immunodeficiency virus type 1 reservoirs and compartments. *J Virol*. 2003;77:5540-5546.
11. Van Leeuwen E, Wit FW, Repping S, et al. Effects of antiretroviral therapy on semen quality. *AIDS*. 2008;22:637-642.
12. Krieger JN, Coombs RW, Collier AC, et al. Fertility parameters in men infected with human immunodeficiency virus. *J Infect Dis*. 1991;164:464-469.
13. Bujan L, Hollander L, Coudert M, et al. Safety and efficacy of sperm washing in HIV-1-serodiscordant couples where the male is infected: results from the European CREAThE network. *AIDS*. 2007;21:1909-1914.
14. Awodele O, Popoola T, Idowu O, Bashua B, Awolola N, Okunowo W. Investigations into the risk of reproductive toxicity following exposure to highly active antiretroviral drugs in rodents. *Tokai J Exp Clin Med*. 2018;43:54-63.
15. Ogedengbe OO, Jegede AI, Onanuga IO, et al. Coconut oil extract mitigates testicular injury following adjuvant treatment with antiretroviral drugs. *Toxicol Res*. 2016;32:317.
16. Oyeyipo IP, Skosana BT, Everson FP, Strijdom H, Du Plessis SS. Highly active antiretroviral therapy alters sperm parameters and testicular antioxidant status in diet-induced obese rats. *Toxicol Res*. 2018;34:41.
17. Lori F, Calarota S, Lisziewicz J. Nanochemistry-based immunotherapy for HIV-1. *Curr Med Chem*. 2007;14:1911-1919.
18. Coombs RW, Lockhart D, Ross SO, et al. Lower genitourinary tract sources of seminal HIV. *JAIDS*. 2006;41:430-438.
19. Mogharabi, M., Abdollahi, M. Faramarzi, M.A. Toxicity of nanomaterials; an undermined issue. *DARU J Pharm Sci* 2014;22(59) 1-4.
20. Destache CJ, Belgum T, Christensen K, Shibata A, Sharma A, Dash A. Combination antiretroviral drugs in PLGA nanoparticle for HIV-1. *BMC Infect Dis*. 2009;9:198.
21. Mital P, Hinton BT, Dufour JM. The blood-testis and blood-epididymis barriers are more than just their tight junctions. *Biol Reprod*. 2011;84:851-858.
22. Cushen M, Kerry J, Morris M, Cruz-Romero M, Cummins E. Nanotechnologies in the food industry–recent developments, risks, and regulation. *Trends Food Sci Technol*. 2012;24:30-46.
23. Shah LK, Amiji MM. Intracellular delivery of saquinavir in biodegradable polymeric nanoparticles for HIV/AIDS. *Pharm Res*. 2006;23:2638-2645.
24. Jerónimo A, Baza MB, Río I, et al. Factors associated with seminal impairment in HIV-infected men under antiretroviral therapy. *Hum Reprod*. 2017;32(2):265-271.
25. Tibbals H. *Medical nanotechnology and nanomedicine*. Boca Raton, FL, USA: CRC Press; 2011.
26. Reibold M, Paufler P, Levin A, Kochmann W, Pätzke N, Meyer DJ. Materials: carbon nanotubes in an ancient Damascus sabre. 2006;444:286.
27. Buzea C, Pacheco II, Robbie K. Nanomaterials and nanoparticles: sources and toxicity. *Biointerphases*. 2007;2:MR17-MR71.
28. Gnach A, Lipinski T, Bednarkiewicz A, Rybka J, Capobianco JA. Upconverting nanoparticles: assessing the toxicity. *Chem Soc Rev*. 2015;44:1561-1584.
29. Dreaden EC, Alkilany AM, Huang X, Murphy CJ, El-Sayed MA. The golden age: gold nanoparticles for biomedicine. *Chem Soc Rev*. 2012;41:2740-2779.
30. Charitidis CA, Georgiou P, Koklioti MA, Trompeta AF, Markakis V. Manufacturing nanomaterials: from research to industry. *Manuf Rev*. 2014;1:11.
31. Mei W, Wu Q. Applications of metal nanoparticles in medicine/metal nanoparticles as anticancer agents. *Metal Nanoparticles*. 2018, pp.169-190.
32. Sahu MK. Semiconductor nanoparticles theory and applications. *Int J Appl Eng Res*. 2019;14:491-494.
33. Sigmund W, Yuh J, Park H, et al. Processing and structure relationships in the electrospinning of ceramic fiber systems. *J Am Ceram Soc*. 2006;89:395-407.
34. Thomas CS, Kumar Mishra P, Talegaonkar S. Ceramic nanoparticles: fabrication methods and applications in drug delivery. *Curr Pharm Des*. 2015;21:6165-6188.

35. Carvalho A, Fernandes AR, Baptista PV. Nanoparticles as delivery systems in cancer therapy. 2019:257-295.
36. Bobo D, Robinson KJ, Islam J, Thurecht KJ, Corrie SR. Nanoparticle-based medicines: a review of FDA-approved materials and clinical trials to date. *Pharm Res*. 2016;33:2373-2387.
37. Chen Y, Chen H, Shi J. In vivo bio-safety evaluations and diagnostic/therapeutic applications of chemically designed mesoporous silica nanoparticles. *Adv Mater*. 2013;25:3144-3176.
38. Soppimath KS, Aminabhavi TM, Kulkarni AR, Rudzinski WE. Biodegradable polymeric nanoparticles as drug delivery devices. *J Controlled Release*. 2001;70:1-20.
39. Cha C, Shin SR, Annabi N, Dokmeci MR, Khademhosseini A. Carbon-based nanomaterials: multifunctional materials for biomedical engineering. *ACS Nano*. 2013;7:2891-2897.
40. Wang Y, Li Z, Wang J, Li J, Lin Y. Graphene and graphene oxide: biofunctionalization and applications in biotechnology. *Trends Biotechnol*. 2011;29:205-212.
41. Lien ZY, Hsu TC, Liu KK, Liao WS, Hwang KC, Chao JI. Cancer cell labeling and tracking using fluorescent and magnetic nanodiamond. *Biomaterials*. 2012;33:6172-6185.
42. Jensen AW, Wilson SR, Schuster DI. Biological applications of fullerenes. *Bioorg Med Chem*. 1996;4:767-779.
43. Rao JP, Geckeler KE. Polymer nanoparticles: preparation techniques and size-control parameters. *Prog Polym Sci*. 2011;36:887-913.
44. Nasir A, Kausar A, Younus A. A review on preparation, properties, and applications of polymeric nanoparticle-based materials. *Polymer-Plast Technol Eng*. 2015;54:325-341.
45. Cheng J, Teply BA, Sherifi I, et al. Formulation of functionalized PLGA–PEG nanoparticles for in vivo targeted drug delivery. *Biomaterials*. 2007;28:869-876.
46. Dong Y, Feng SS. Poly (d, l-lactide-co-glycolide)/montmorillonite nanoparticles for oral delivery of anticancer drugs. *Biomaterials*. 2005;26:6068-6076.
47. Chuang S-Y, Lin C-H, Huang T-H, Fang J-Y. Lipid-based nanoparticles as a potential delivery approach in the treatment of rheumatoid arthritis. *Nanomaterials*. 2018;8(1):42.
48. Kapoor B, Singh SK, Gulati M, Gupta R, Vaidya Y. Application of liposomes in treatment of rheumatoid arthritis: quo vadis. *Sci World J*. 2014;2014:1-17.
49. García-Pinel B, Porras-Alcalá C, Ortega-Rodríguez A, et al. Lipid-based nanoparticles: application and recent advances in cancer treatment. *Nanomaterials*. 2019;9(4):638.
50. Kumar R. *Lipid-based nanoparticles for drug-delivery systems*. Nanocarriers for Drug Delivery: Elsevier; 2019:249-284.
51. Mahajan SD, Aalinkeel R, Law WC, et al. Anti-HIV-1 nanotherapeutics: promises and challenges for the future. *Int J Nanomed*. 2012;7:5301.
52. Mamo T, Moseman EA, Kolishetti N, et al. Emerging nanotechnology approaches for HIV/AIDS treatment and prevention. *Nanomedicine*. 2010;5:269-285.
53. Patra JK, Das G, Fraceto LF, et al. Nano based drug delivery systems: recent developments and future prospects. *J Nanobiotechnol*. 2018;16:71. https://doi.org/10.1186/s12951-018-0392-8
54. Sagar V, Pilakka-Kanthikeel S, Pottathil R, Saxena SK, Nair M. Towards nanomedicines for neuroAIDS. *Rev. Med. Virol*. 2014;24(2):103-124.
55. Zidan AS, Spinks CB, Habib MJ, Khan MA. Formulation and transport properties of tenofovir loaded liposomes through Caco-2 cell model. *J Liposome Res*. 2013;23(4):318-326.
56. Zhang J, Liu J, Peng Q, Wang X, Li Y. Nearly monodisperse Cu2O and CuO nanospheres: preparation and applications for sensitive gas sensors. *Chem Mater*. 2006;18:867-871.
57. Chawla P, Chawla V, Maheshwari RA, Saraf S, Saraf KS. Fullerenes: from carbon to nanomedicine. *Mini Rev Med Chem*. 2010;10:662-677.
58. Kim JH, Yeom JH, Ko JJ, et al. Effective delivery of anti-miRNA DNA oligonucleotides by functionalized gold nanoparticles. *J Biotechnol*. 2011;155:287-292.
59. Kim PS, Read SW. Nanotechnology and HIV: potential applications for treatment and prevention. *Wiley Interdiscip Rev: Nanomed Nanobiotechnol*. 2010;2:693-702.
60. Roulet V, Satie AP, Ruffault A, et al. Susceptibility of human testis to human immunodeficiency virus-1 infection in situ and in vitro. *Am J Pathol*. 2006;169:2094-2103.
61. Cory TJ, Schacker TW, Stevenson M, Fletcher CV. Overcoming pharmacologic sanctuaries. *Curr Opin HIV AIDS*. 2013;8:190.
62. Kress KD. HIV update: emerging clinical evidence and a review of recommendations for the use of highly active antiretroviral therapy. *Am J Health-Syst Pharm*. 2004;61:S3-S14.
63. Avari P, Devendra S. Human immunodeficiency virus and type 2 diabetes. *London J Prim Care*. 2017;9:38-42.
64. Murata H, Hruz PW, Mueckler M. The mechanism of insulin resistance caused by HIV protease inhibitor therapy. *J Biol Chem*. 2000;275:20251-20254.
65. Schambelan M, Benson CA, Carr A, et al. Management of metabolic complications associated with antiretroviral therapy for HIV-1 infection: recommendations of an International AIDS Society-USA panel. *J Acquir Immune Defic Syndr*. 2002;31:257-275.
66. Montessori V, Press N, Harris M, Akagi L, Montaner JS. Adverse effects of antiretroviral therapy for HIV infection. *CMAJ*. 2004;170:229-238.
67. Abdelhady AM, Shugg T, Thong N, et al. Efavirenz inhibits the human ether-a-go-go related current (hERG) and induces QT interval prolongation in CYP2B6* 6* 6 allele carriers. *J Cardiovasc Electrophysiol*. 2016;27:1206-1213.
68. Jones M, Núñez M. *Liver Toxicity of Antiretroviral Drugs. Seminars in Liver Disease*. Thieme Medical Publishers; 2012:167-176.
69. Rodríguez-Nóvoa S, Martín-Carbonero L, Barreiro P, et al. Genetic factors influencing atazanavir plasma concentrations and the risk of severe hyperbilirubinemia. *AIDS*. 2007;21:41-46.
70. O'Brien ME, Clark RA, Besch CL, Myers L, Kissinger P. Patterns and correlates of discontinuation of the initial HAART regimen in an urban outpatient cohort. *JAIDS*. 2003;34:407-414.
71. Farokhzad OC, Langer R. Impact of nanotechnology on drug delivery. *ACS Nano*. 2009;3:16-20.
72. Peer D, Karp JM, Hong S, Farokhzad OC, Margalit R, Langer R. Nanocarriers as an emerging platform for cancer therapy. *Nat Nanotechnol*. 2007;2:751.
73. Wilhelm S, Tavares AJ, Dai Q, et al. Analysis of nanoparticle delivery to tumours. *Nat Rev Mater*. 2016;1:16014.
74. Ochekpe NA, Olorunfemi PO, Ngwuluka NC. Nanotechnology and drug delivery part 1: background and applications. *Trop J Pharm Res*. 2009;8(3):265-274.
75. Sanvicens N, Marco MP. Multifunctional nanoparticles–properties and prospects for their use in human medicine. *Trends Biotechnol*. 2008;26:425-433.
76. Dowling AP. Development of nanotechnologies. *Mater Today*. 2004;7:30-35.
77. De Jong WH, Borm PJ. Drug delivery and nanoparticles: applications and hazards. *Int J Nanomed*. 2008;3:133.
78. Parboosing R, Maguire GE, Govender P, Kruger HG. Nanotechnology and the treatment of HIV infection. *Viruses*. 2012;4:488-520.
79. Iravani S, Korbekandi H, Mirmohammadi SV, Zolfaghari B. Synthesis of silver nanoparticles: chemical, physical and biological methods. *Res Pharm Sci*. 2014;9:385.
80. Klaus-Joerger T, Joerger R, Olsson E, Granqvist CG. Bacteria as workers in the living factory: metal-accumulating bacteria and their potential for materials science. *Trends Biotechnol*. 2001;19:15-20.
81. Senapati S. Biosynthesis and immobilization of nanoparticles and their applications.

82. Barratt GM. Therapeutic applications of colloidal drug carriers. *Pharm Sci Technol Today*. 2000;3:163-171.
83. Iannazzo D, Pistone A, Galvagno S, et al. Synthesis and anti-HIV activity of carboxylated and drug-conjugated multi-walled carbon nanotubes. *Carbon*. 2015;1(82):548-561.
84. Das Neves J, Michiels J, Ariën KK, et al. Polymeric nanoparticles affect the intracellular delivery, antiretroviral activity, and cytotoxicity of the microbicide drug candidate dapivirine. *Pharm Res*. 2012;29:1468-1484.
85. Zhang H, Burnum KE, Luna ML, et al. Quantitative proteomics analysis of adsorbed plasma proteins classifies nanoparticles with different surface properties and size. *Proteomics*. 2011;11:4569-4577.
86. Singh AK. Engineered nanoparticles. *Chapter*. 2016;2:19-76.
87. Ravindran S, Suthar JK, Rokade R, et al. Pharmacokinetics, metabolism, distribution, and permeability of nanomedicine. *Curr Drug Metab*. 2018;19:327-334.
88. Kaur P, Garg T, Rath G, Goyal AK. In situ nasal gel drug delivery: a novel approach for brain targeting through the mucosal membrane. *Artif Cells Nanomed Biotechnol*. 2016;44:1167-1176.
89. Lai SK, Wang YY, Hanes J. Mucus-penetrating nanoparticles for drug and gene delivery to mucosal tissues. *Adv Drug Deliv Rev*. 2009;61:158-171.
90. Kohli A, Alpar H. Potential use of nanoparticles for transcutaneous vaccine delivery: effect of particle size and charge. *Int J Pharm*. 2004;275:13-17.
91. Des Rieux A, Fievez V, Garinot M, Schneider YJ, Préat V. Nanoparticles as potential oral delivery systems of proteins and vaccines: a mechanistic approach. *J Controlled Release*. 2006;116:1-27.
92. Raza K, Singh B, Singal P, Wadhwa S, Katare OP. Systematically optimized biocompatible isotretinoin-loaded solid lipid nanoparticles (SLNs) for topical treatment of acne. *Colloids Surf, B*. 2013;105:67-74.
93. Li M, Al-Jamal KT, Kostarelos K, Reineke J. Physiologically based pharmacokinetic modeling of nanoparticles. *ACS Nano*. 2010;4:6303-6317.
94. Oberdörster G, Oberdörster E, Oberdörster J. Nanotoxicology: an emerging discipline evolving from studies of ultrafine particles. *Environ Health Perspect*. 2005;113:823-839.
95. Goodman CM, Mccusker CD, Yilmaz T, Rotello VM. Toxicity of gold nanoparticles functionalized with cationic and anionic side chains. *Bioconjug Chem*. 2004;15:897-900.
96. Sa LT, De Souza AM, De Carvalho Patricio BF, et al. Biodistribution of nanoparticles: initial considerations. *J Pharm Biomed Anal*. 2012;70:602-604.
97. Ernsting MJ, Murakami M, Roy A, Li SD. Factors controlling the pharmacokinetics, biodistribution, and intratumoral penetration of nanoparticles. *J Controlled Release*. 2013;172:782-794.
98. Yuan F, Dellian M, Fukumura D, et al. Vascular permeability in a human tumor xenograft: molecular size dependence and cutoff size. *Can Res*. 1995;55:3752-3756.
99. Hsu J, Bhowmick T, Burks SR, Kao JP, Muro S. Enhancing biodistribution of therapeutic enzymes in vivo by modulating surface coating and concentration of ICAM-1-targeted nanocarriers. *J Biomed Nanotechnol*. 2014;10:345-354.
100. Mahmoudi M, Azadmanesh K, Shokrgozar MA, Journeay WS, Laurent S. Effect of nanoparticles on the cell life cycle. *Chem Rev*. 2011;111:3407-3432.
101. Hohnholt MC, Dringen R. *Uptake and Metabolism of Iron and Iron Oxide Nanoparticles in Brain Astrocytes*. Portland Press Limited; 2013.
102. Choi HS, Liu W, Misra P, et al. Renal clearance of quantum dots. *Nat Biotechnol*. 2007;25:1165.
103. Sadauskas E, Danscher G, Stoltenberg M, Vogel U, Larsen A, Wallin H. Protracted elimination of gold nanoparticles from mouse liver. *Nanomedicine*. 2009;5:162-169.
104. Kis O, Robillard K, Chan GN, Bendayan R. The complexities of antiretroviral drug-drug interactions: role of ABC and SLC transporters. *Trends Pharmacol Sci*. 2010;31:22-35.
105. Rochat B. Importance of influx and efflux systems and xenobiotic-metabolizing enzymes in intratumoral disposition of anticancer agents. *Curr Cancer Drug Targets*. 2009;9:652-674.
106. Cheng CY, Mruk DD. The blood-testis barrier and its implications for male contraception. *Pharmacol Rev*. 2012;64:16-64.
107. Yan HH, Mruk DD, Lee WM, Cheng CY. Ectoplasmic specialization: a friend or a foe of spermatogenesis? *BioEssays*. 2007;29:36-48.
108. Su L, Mruk DD, Cheng CY. Drug transporters, the blood-testis barrier, and spermatogenesis. *J Endocrinol*. 2011;208:207.
109. Dym M, Fawcett DW. The blood-testis barrier in the rat and the physiological compartmentation of the seminiferous epithelium. *Biol Reprod*. 1970;3:308-326.
110. Cheng CY, Mruk DD. Cell junction dynamics in the testis: sertoligerm cell interactions and male contraceptive development. *Physiol Rev*. 2002;82:825-874.
111. Kato R, Maeda T, Akaike T, Tamai I. Nucleoside transport at the blood-testis barrier studied with primary-cultured Sertoli cells. *J Pharmacol Exp Ther*. 2005;312:601-608.
112. Mruk DD, Cheng CY. The mammalian blood-testis barrier: its biology and regulation. *Endocr Rev*. 2015;36:564-591.
113. Palombi F, Filippini A, Chiarenza C. Cell-cell interactions in the local control of seminiferous tubule contractility. *Contraception*. 2002;65:289-291.
114. Lan Z, Yang WX. Nanoparticles and spermatogenesis: how do nanoparticles affect spermatogenesis and penetrate the blood-testis barrier. *Nanomedicine*. 2012;7:579-596.
115. Wang R, Song B, Wu J, Zhang Y, Chen A, Shao L. Potential adverse effects of nanoparticles on the reproductive system. *Int J Nanomed*. 2018;13:8487.
116. Park J, Estrada A, Schwartz JA, et al. Intra-organ biodistribution of gold nanoparticles using intrinsic two-photon-induced photoluminescence. *Lasers Surg Med*. 2010;42:630-639.
117. Leclerc L, Klein JP, Forest V, et al. Testicular biodistribution of silica-gold nanoparticles after intramuscular injection in mice. *Biomed Microdevice*. 2015;17:66.
118. Bai W, Zhang Z, Tian W, et al. Toxicity of zinc oxide nanoparticles to zebrafish embryo: a physicochemical study of toxicity mechanism. *J Nanopart Res*. 2010;12(5):1645-1654.
119. Hussein MM, Ali HA, Saadeldin IM, Ahmed MM. Quercetin alleviates zinc oxide nanoreprotoxicity in male albino rats. *J Biochem Mol Toxicol*. 2016;30:489-496.
120. Shi L, Xun W, Yue W, et al. Effect of elemental nano-selenium on feed digestibility, rumen fermentation, and purine derivatives in sheep. *Anim Feed Sci Technol*. 2011;163:136-142.
121. Geraets L, Oomen AG, Schroeter JD, Coleman VA, Cassee FR. Tissue distribution of inhaled micro-and nano-sized cerium oxide particles in rats: results from a 28-day exposure study. *Toxicol Sci*. 2012;127:463-473.
122. Morishita Y, Yoshioka Y, Satoh H, et al. Distribution and histologic effects of intravenously administered amorphous nanosilica particles in the testes of mice. *Biochem Biophys Res Comm*. 2012;420:297-301.
123. Van Der Zande M, Vandebriel RJ, Van Doren E, et al. Distribution, elimination, and toxicity of silver nanoparticles and silver ions in rats after 28-day oral exposure. *ACS Nano*. 2012;6:7427-7442.
124. McNeil SE, ed. *Characterization of Nanoparticles Intended for Drug delivery*. Springer; 2011. (Vol. 697, pp. 71-82). New York, NY: Humana press.
125. Yang C, Tian A, Li Z. Reversible cardiac hypertrophy induced by PEG-coated gold nanoparticles in mice. *Sci Rep*. 2016;6:20203.
126. Papageorgiou I, Brown C, Schins R, et al. The effect of nano-and micron-sized particles of cobalt–chromium alloy on human fibroblasts in vitro. *Biomaterials*. 2007;28(19):2946-2958.

127. Parveen K, Banse V, Ledwani L. Green synthesis of nanoparticles: their advantages and disadvantages. In *AIP conference proceedings*. Vol. 1724, No. 1. Melville, NY: AIP Publishing LLC; 2016:0200482016.
128. Anselmo AC, Mitragotri S. Nanoparticles in the clinic. *Bioeng Transl Med*. 2016;1:10-29.
129. Anselmo AC, Mitragotri S. A review of clinical translation of inorganic nanoparticles. *AAPS J*. 2015;17:1041-1054.
130. Huang HC, Barua S, Sharma G, Dey SK, Rege K. Inorganic nanoparticles for cancer imaging and therapy. *J Controlled Release*. 2011;155:344-357.
131. Torchilin VP. Multifunctional, stimuli-sensitive nanoparticulate systems for drug delivery. *Nat Rev Drug Discovery*. 2014;13:813.
132. Zhang J, Ma A, Shang L. Conjugating existing clinical drugs with gold nanoparticles for better treatment of heart diseases. *Front Physiol*. 2018;9:642.
133. Spivak MY, Bubnov RV, Yemets IM, Lazarenko LM, Tymoshok NO, Ulberg ZR. Development and testing of gold nanoparticles for drug delivery and treatment of heart failure: a theranostic potential for PPP cardiology. *EPMA J*. 2013;4:20.
134. Vaage J, Mayhew E, Lasic D, Martin F. Therapy of primary and metastatic mouse mammary carcinomas with doxorubicin encapsulated in long-circulating liposomes. *Int J Cancer*. 1992;51:942-948.
135. Caster JM, Patel AN, Zhang T, Wang A. Investigational nanomedicines in 2016: a review of nanotherapeutics currently undergoing clinical trials. *Wiley Interdiscip Rev: Nanomed Nanobiotechnol*. 2017;9:e1416.
136. Food and Drug Administration. Novel drug approvals for 2017. 2017.
137. Havel HA. Where are the nanodrugs? An industry perspective on development of drug products containing nanomaterials. *AAPS J*. 2016;18:1351-1353.
138. Lori F, Calarota S, Lisziewicz J. Nanochemistry-based immunotherapy for HIV-1. *Curr Med Chem*. 2007;14:1911-1919.
139. Jiang YH, Emau P, Cairns JS, et al. SPL7013 gel as a topical microbicide for prevention of vaginal transmission of SHIV89. 6P in macaques. *AIDS Res Hum Retroviruses*. 2005;21:207-213.
140. Mccarthy TD, Karellas P, Henderson SA, et al. Dendrimers as drugs: discovery and preclinical and clinical development of dendrimer-based microbicides for HIV and STI prevention. *Mol Pharm*. 2005;2:312-318.
141. Rupp R, Rosenthal SL, Stanberry LR. VivaGel™(SPL7013 Gel): a candidate dendrimer–microbicide for the prevention of HIV and HSV infection. *Int J Nanomed*. 2007;2:561.
142. Elechiguerra JL, Burt JL, Morones JR, et al. Interaction of silver nanoparticles with HIV-1. *J Nanobiotechnol*. 2005;3:6.
143. Sun RW, Chen R, Chung NP, Ho CM, Lin CL, Che CM. Silver nanoparticles fabricated in Hepes buffer exhibit cytoprotective activities toward HIV-1 infected cells. *Chem Commun*. 2005;5059-5061.
144. Blanzat M, Turrin CO, Aubertin AM, et al. Dendritic catanionic assemblies: in vitro anti-HIV activity of phosphorus-containing dendrimers bearing Galβ1cer analogues. *ChemBioChem*. 2005;6:2207-2213.
145. Wang JX, Wen LX, Wang ZH, Chen JF. Immobilization of silver on hollow silica nanospheres and nanotubes and their antibacterial effects. *Mater Chem Phys*. 2006;96:90-97.
146. Bowman MC, Ballard TE, Ackerson CJ, Feldheim DL, Margolis DM, Melander C. Inhibition of HIV fusion with multivalent gold nanoparticles. *J Am Chem Soc*. 2008;130:6896-6897.
147. Ham AS, Cost MR, Sassi AB, Dezzutti CS, Rohan LC. Targeted delivery of PSC-RANTES for HIV-1 prevention using biodegradable nanoparticles. *Pharm Res*. 2009;26:502-511.
148. US Food and Drug Administration. FDA approves first extended-release, injectable drug regimen for adults living with HIV. Published January 21, 2021. https://www.fda.gov/news-events/press-announcements/fda-approves-first-extended-release-injectable-drug-regimen-adults-living-hiv. Accessed February 2, 2021.
149. Rial-Crestelo D, Pinto-Martínez A, Pulido F. Cabotegravir and rilpivirine for the treatment of HIV. *Expert Rev Anti Infect Ther*. 2020;18(5):393-404. https://doi.org/10.1080/14787210.2020.1736561. Epub 2020 Mar 12 PMID: 32164474.
150. Zhou T, Su H, Dash P, et al. Creation of a nanoformulated cabotegravir prodrug with improved antiretroviral profiles. *Biomaterials*. 2018;151:53-65. https://doi.org/10.1016/j.biomaterials.2017.10.023
151. Qin F, Shen T, Li J, et al. SF-1 mediates reproductive toxicity induced by Cerium oxide nanoparticles in male mice. *J Nanobiotechnol*. 2019;17:41.
152. Song G, Lin L, Liu L, et al. Toxic effects of anatase titanium dioxide nanoparticles on spermatogenesis and testicles in male mice. *Pol J Environ Stud*. 2017;26(6):2739-2745.
153. Negahdary M, Arefian Z, Dastjerdi HA, Ajdary M. Toxic effects of Mn2O3 nanoparticles on rat testis and sex hormone. *J Nat Sci Biol Med*. 2015;6:335.
154. Song B, Zhang Y, Liu J, Feng X, Zhou T, Shao L. Unraveling the neurotoxicity of titanium dioxide nanoparticles: focusing on molecular mechanisms. *Beilstein J Nanotechnol*. 2016;7:645-654.
155. World Health Organization. *Antiretroviral Therapy of HIV Infection in Infants and Children: Towards Universal Access: Recommendations for a Public Health Approach-2010 Revision*. World Health Organization; 2010, Geneva.
156. WHO. *Antiretroviral Therapy of HIV Infection in Infants and Children: Towards Universal Access: Recommendations for a Public Health Approach-2010 Revision*. World Health Organization; 2010.
157. Hagmann M. Study confirms effectiveness of antiretroviral drugs for HIV patients. *Bull World Health Organ*. 2003;81:918-919.
158. Chowta MN, Kamath P, Ramapuram JT, Shenoy KA. Evaluation of adverse drug reaction profile of drugs used as first-line antiretroviral therapy. *Interdiscip Perspect Infect Dis*. 2018;2018:1-7. https://doi.org/10.1155/2018/8095609
159. Sacktor N, Nakasujja N, Skolasky R, et al. Benefits and risks of stavudine therapy for HIV-associated neurologic complications in Uganda. *Neurology*. 2009;72:165-170.
160. Mokrzycki MH, Harris C, May H, Laut J, Palmisano J. Lactic acidosis associated with stavudine administration: a report of five cases. *Clin Infect Dis*. 2000;30:198-200.
161. Kumarasamy N, Venkatesh K, Cecelia A, et al. Gender-based differences in treatment and outcome among HIV patients in South India. *J Women's Health*. 2008;17:1471-1475.
162. Lucas GM, Ross MJ, Stock PG, et al. Clinical practice guideline for the management of chronic kidney disease in patients infected with HIV: 2014 update by the HIV Medicine Association of the Infectious Diseases Society of America. *Clin Infect Dis*. 2014;59:e96-e138.
163. Nishijima Y, Rosa L, Juodkazis S. Surface plasmon resonances in periodic and random patterns of gold nano-disks for broadband light harvesting. *Opt Express*. 2012;20:11466-11477.
164. Sungkanuparph S, Techasathit W, Utaipiboon C, et al. Thai national guidelines for antiretroviral therapy in HIV-1 infected adults and adolescents. *Asian Biomed (Res Rev News)*. 2010;4:515-528.
165. Pollard RB, Robinson P, Dransfield K. The safety profile of nevirapine, a nonnucleoside reverse transcriptase inhibitor for the treatment of human immunodeficiency virus infection. *Clin Ther*. 1998;20:1071-1092.
166. Reust CE. Common adverse effects of antiretroviral therapy for HIV disease. *Am Fam Physician*. 2011;83.
167. Nguyen A, Calmy A, Delhumeau C, et al. A randomized cross-over study to compare raltegravir and efavirenz (SWITCH-ER study). *AIDS*. 2011;25:1481-1487.
168. Waters L, Fisher M, Winston A, et al. A phase IV, double-blind, multicentre, randomized, placebo-controlled, pilot study to assess

the feasibility of switching individuals receiving efavirenz with continuing central nervous system adverse events to etravirine. *AIDS*. 2011;25:65-71.
169. Arendt G, De Nocker D, Von Giesen HJ, Nolting T. Neuropsychiatric side effects of efavirenz therapy. *Expert Opin Drug Saf*. 2007;6:147-154.
170. Patil R, Ona MA, Papafragkakis H, Carey J, Moshenyat Y, Alhaddad A, Anand S. Acute liver toxicity due to efavirenz/emtricitabine/tenofovir. *Case Rep Hepatol*. 2015;2015:1-2. https://doi.org/10.1155/2015/280353
171. Manosuthi W, Sukasem C, Lueangniyomkul A, et al. CYP2B6 haplotype and biological factors responsible for hepatotoxicity in HIV-infected patients receiving efavirenz-based antiretroviral therapy. *Int J Antimicrob Agents*. 2014;43:292-296.
172. Echenique IA, Rich JD. EFV/FTC/TDF-associated hepatotoxicity: a case report and review. *AIDS Patient Care STDs*. 2013;27:493-497.
173. De Santis M, Carducci B, De Santis L, Cavaliere AF, Straface G. Periconceptional exposure to efavirenz and neural tube defects. *Arch Intern Med*. 2002;162:355-356.
174. Fundarò C, Genovese O, Rendeli C, Tamburrini E, Salvaggio E. Myelomeningocele in a child with intrauterine exposure to efavirenz. *AIDS*. 2002;16:299-300.
175. Zembower TR, Gerzenshtein L, Coleman K, Palella Jr FJ. Severe rhabdomyolysis associated with raltegravir use. *AIDS*. 2008;22:1382-1384.
176. Gallant JE, Dejesus E, Arribas JR, et al. Tenofovir DF, emtricitabine, and efavirenz vs. zidovudine, lamivudine, and efavirenz for HIV. *N Engl J Med*. 2006;354:251-260.
177. Max B, Sherer R. Management of the adverse effects of antiretroviral therapy and medication adherence. *Clin Infect Dis*. 2000;30:S96-S116.
178. Domingo P, Labarga P, Palacios R, et al. Improvement of dyslipidemia in patients switching from stavudine to tenofovir: preliminary results. *AIDS*. 2004;18:1475-1478.
179. Tebas P, Sension M, Arribas J, et al. Lipid levels and changes in body fat distribution in treatment-naive, HIV-1–infected adults treated with rilpivirine or efavirenz for 96 weeks in the ECHO and THRIVE Trials. *Clin Infect Dis*. 2014;59:425-434.
180. Scruggs ER, Naylor AJ. Mechanisms of zidovudine-induced mitochondrial toxicity and myopathy. *Pharmacology*. 2008;82:83-88.
181. Perry CM, Balfour JA. Didanosine. *Drugs*. 1996;52:928-962.
182. Fessel J, Hurley LB. Incidence of pancreatitis in HIV-infected patients: comment on findings in EuroSIDA cohort. *AIDS*. 2008;22:145-147.
183. Wooltorton E. HIV drug stavudine (Zerit, d4T) and symptoms mimicking Guillain–Barré syndrome. *CMAJ*. 2002;166:1067.
184. Abers MS, Shandera WX, Kass JS. Neurological and psychiatric adverse effects of antiretroviral drugs. *CNS Drugs*. 2014;28:131-145.
185. Arenas-Pinto A, Bhaskaran K, Dunn D, Weller I. The risk of developing peripheral neuropathy induced by nucleoside reverse transcriptase inhibitors decreases over time: evidence from the Delta trial. *Antivir Ther*. 2008;13:289.
186. Dad DG. Use of nucleoside reverse transcriptase inhibitors and risk of myocardial infarction in HIV-infected patients enrolled in the D:A:D study: a multi-cohort collaboration. *Lancet*. 2008;371:1417-1426.
187. Obel N, Farkas D, Kronborg G, et al. Abacavir and risk of myocardial infarction in HIV-infected patients on highly active antiretroviral therapy: a population-based nationwide cohort study. *HIV Med*. 2010;11:130-136.
188. Choi AI, Vittinghoff E, Deeks SG, Weekley CC, Li Y, Shlipak MG. Cardiovascular risks associated with abacavir and tenofovir exposure in HIV-infected persons. *AIDS (London, England)*. 2011;25:1289.
189. Nishijima T, Yazaki H, Hinoshita F, et al. Drug-induced acute interstitial nephritis mimicking acute tubular necrosis after initiation of tenofovir-containing antiretroviral therapy in patients with HIV-1 infection. *Intern Med*. 2012;51:2469-2471.
190. Sax PE, Wohl D, Yin MT, et al. Tenofovir alafenamide versus tenofovir disoproxil fumarate, co-formulated with elvitegravir, cobicistat, and emtricitabine, for initial treatment of HIV-1 infection: two randomized, double-blind, phase 3, non-inferiority trials. *Lancet*. 2015;385:2606-2615.
191. Walmsley SL, Antela A, Clumeck N, et al. Dolutegravir plus abacavir–lamivudine for the treatment of HIV-1 infection. *N Engl J Med*. 2013;369:1807-1818.
192. Osterholzer DA, Goldman M. Dolutegravir: a next-generation integrase inhibitor for treatment of HIV infection. *Clin Infect Dis*. 2014;59:265-271.
193. Van Lunzen J, Antinori A, Cohen CJ, et al. Rilpivirine vs. efavirenz-based single-tablet regimens in treatment-naive adults: week 96 efficacy and safety from a randomized phase 3b study. *AIDS*. 2016;30:251-259.
194. Mills A, Antinori A, Clotet B, et al. Neurological and psychiatric tolerability of rilpivirine (TMC 278) vs. efavirenz in treatment-naïve, HIV-1-infected patients at 48 weeks. *HIV Med*. 2013;14:391-400.
195. Zhang D, Chando TJ, Everett DW, Patten CJ, Dehal SS, Humphreys WG. In vitro inhibition of UDP glucuronosyltransferases by atazanavir and other HIV protease inhibitors and the relationship of this property to in vivo bilirubin glucuronidation. *Drug Metab Dispos*. 2005;33:1729-1739.
196. Couzigou C, Daudon M, Meynard JL, et al. Urolithiasis in HIV-positive patients treated with atazanavir. *Clin Infect Dis*. 2007;45:e105-e108.
197. Rakotondravelo S, Poinsignon Y, Borsa-Lebas F, et al. Complicated atazanavir-associated cholelithiasis: a report of 14 cases. *Clin Infect Dis*. 2012;55:1270-1272.
198. Kopp JB, Miller KD, Mican JA, et al. Crystalluria and urinary tract abnormalities associated with indinavir. *Ann Intern Med*. 1997;127:119-125.
199. Perry CM, Frampton JE, Mccormack PL, Siddiqui MA, Cvetkovic RS. Nelfinavir: a review of its use in the management of HIV infection. *Drugs*. 2005;65:2209-2245.
200. Chan-Tack KM, Struble KA, Birnkrant DB. Intracranial hemorrhage and liver-associated deaths associated with tipranavir/ritonavir: review of cases from the FDA's Adverse Event Reporting System. *AIDS Patient Care STDs*. 2008;22:843-850.
201. Zhou H, Pandak WM, Lyall V, Natarajan R, Hylemon PB. HIV protease inhibitors activate the unfolded protein response in macrophages: implication for atherosclerosis and cardiovascular disease. *Mol Pharmacol*. 2005;68:690-700.
202. Rockstroh JK, Plonski F, Bansal M, et al. Hepatic safety of maraviroc in patients with HIV-1 and hepatitis C and/or B virus: 144-week results from a randomized, placebo-controlled trial. *Antivir Ther*. 2016;22(3):263-269.
203. Olasile IO, Jegede IA, Ugochukwu O, et al. Histo-morphological and seminal evaluation of testicular parameters in diabetic rats under antiretroviral therapy: interactions with Hypoxis hemerocallidea. *Iran J Basic Med Sci*. 2018;21:1322.
204. Bender AR, Von Briesen H, Kreuter J, Duncan IB, Rübsamen-Waigmann H. The efficiency of nanoparticles as a carrier system for antiviral agents in human immunodeficiency virus-infected human monocytes/macrophages in vitro. *Antimicrob Agents Chemother*. 1996;40:1467-1471.
205. Shah LK, Amiji MM. Intracellular delivery of saquinavir in biodegradable polymeric nanoparticles for HIV/AIDS. *Pharm Res*. 2006;23:2638-2645.
206. Shibata A, Mcmullen E, Pham A, et al. Polymeric nanoparticles containing combination antiretroviral drugs for HIV type 1 treatment. *AIDS Res Hum Retroviruses*. 2013;29:746-754.
207. Mallipeddi R, Rohan LC. Progress in antiretroviral drug delivery using nanotechnology. *Int J Nanomed*. 2010;5:533.

208. Madugulla L, Ravula AR, Kondapi AK, Yenugu S. Evaluation of the reproductive toxicity of antiretroviral drug-loaded lactoferrin nanoparticles. *Syst Biol Reprod Med*. 2019;65:205-213.
209. Boudad H, Legrand P, Appel M, Coconnier MH, Ponchel G. Formulation and cytotoxicity of combined cyclodextrin poly (alkyl cyanoacrylate) nanoparticles on Caco-2 cells monolayers intended for oral administration of saquinavir. *STP Pharma Sci*. 2001;11:369-375.
210. Sankar V, Keerthi ML, Parmar N. Formation and in-vitro evaluation of zidovudine-lamivudine nanoparticles. *Indian J Pharm Educ Res*. 2012;46:192-196.
211. Ogunwuyi O, Kumari N, Smith KA, et al. Antiretroviral drugs-loaded nanoparticles fabricated by dispersion polymerization with potential for HIV/AIDS treatment. *Infect Dis: Res Treat*. 2016;9:IDRT. S38108.
212. Garrido C, Simpson CA, Dahl NP, et al. Gold nanoparticles to improve HIV drug delivery. *Fut Med Chem*. 2015;7:1097-1107.
213. Ambwani S, Kandpal D, Arora S, Ambwani TK. *Cytotoxic Effects of Gold Nanoparticles Exposure Employing In Vitro Animal Cell Culture System as Part of Nanobiosafety*. College Park, MA: American Institute of Physics; 2016. https://doi.org/10.1063/1.4945211
214. Daraee H, Eatemadi A, Abbasi E, Fekri Aval S, Kouhi M, Akbarzadeh A. Application of gold nanoparticles in biomedical and drug delivery. *Artif Cells Nanomed Biotechnol*. 2016;44:410-422.
215. Buford MC, Hamilton RF, Holian A. A comparison of dispersing media for various engineered carbon nanoparticles. *Part Fibre Toxicol*. 2007;4:6.
216. Shen C, James SA, De Jonge MD, Turney TW, Wright PF, Feltis BN. Relating cytotoxicity, zinc ions, and reactive oxygen in ZnO nanoparticle–exposed human immune cells. *Toxicol Sci*. 2013;136:120-130.
217. Hussain S, Hess K, Gearhart J, Geiss K, Schlager J. In vitro toxicity of nanoparticles in BRL 3A rat liver cells. *Toxicol In Vitro*. 2005;19:975-983.
218. Yan G, Huang Y, Bu Q, et al. Zinc oxide nanoparticles cause nephrotoxicity and kidney metabolism alterations in rats. *J Environ Sci Health, Part A*. 2012;47:577-588.
219. Jugan M, Barillet S, Simon-Deckers A, et al. Cytotoxic and genotoxic impact of TiO2 nanoparticles on A549 cells. *J Biomed Nanotechnol*. 2011;7:22-23.
220. You R, Ho YS, Hung CH, et al. Silica nanoparticles induce neurodegeneration-like changes in behavior, neuropathology, and affect synapse through MAPK activation. *Part Fibre Toxicol*. 2018;15:28.
221. Rabanel JM, Hildgen P, Banquy X. Assessment of PEG on polymeric particles surface, a key step in drug carrier translation. *J Controlled Release*. 2014;185:71-87.
222. Liao L, Zhang M, Liu H, et al. Subchronic toxicity and immunotoxicity of MeO-PEG-poly (D, L-lactic-co-glycolic acid)-PEG-OMe triblock copolymer nanoparticles delivered intravenously into rats. *Nanotechnology*. 2014;25:245705.
223. Mirzaeivarzeghani S, Parivar K, Abdollahifar MA, Karamian A. Effects of iron oxide nanoparticles on mouse sperm parameters and testicular tissue. *Iran J Toxicol*. 2018;12:39-44.
224. Amin R. Nanotechnology in controlling infectious disease. *Nanomed Health Dis*. 2011;167-183.
225. Ghosh P, Han G, De M, Kim CK, Rotello VM. Gold nanoparticles in delivery applications. *Adv Drug Deliv Rev*. 2008;60:1307-1315.
226. Huh AJ, Kwon YJ. "Nanoantibiotics": a new paradigm for treating infectious diseases using nanomaterials in the antibiotics resistant era. *J Controlled Release*. 2011;156:128-145.
227. Cauchetier E, Paul M, Rivollet D, Fessi H, Astier A, Deniau M. Therapeutic evaluation of free and nanocapsule-encapsulated atovaquone in the treatment of murine visceral leishmaniasis. *Ann Trop Med Parasitol*. 2003;97:259-268.
228. Celia C, Trapasso E, Locatelli M, et al. Anticancer activity of liposomal bergamot essential oil (BEO) on human neuroblastoma cells. *Colloids Surf, B*. 2013;112:548-553.
229. Norvaisas P, Ziemys A. The role of payload hydrophobicity in nanotherapeutic pharmacokinetics. *J Pharm Sci*. 2014;103:2147-2156.
230. Wolfram J, Suri K, Huang Y, et al. Evaluation of the anticancer activity of celastrol liposomes in prostate cancer cells. *J Microencapsul*. 2014;31:501-507.
231. Maeda H, Wu J, Sawa T, Matsumura Y, Hori K. Tumor vascular permeability and the EPR effect in macromolecular therapeutics: a review. *J Controlled Release*. 2000;65:271-284.
232. Matsumura Y, Maeda H. A new concept for macromolecular therapeutics in cancer chemotherapy: mechanism of tumoritropic accumulation of proteins and the antitumor agent smancs. *Can Res*. 1986;46:6387-6392.
233. Wolfram J, Zhu M, Yang Y, et al. Safety of nanoparticles in medicine. *Curr Drug Targets*. 2015;16:1671-1681.
234. Mailander V, Landfester K. Interaction of nanoparticles with cells. *Biomacromol*. 2009;10(9):2379-2400.
235. Pardakhty A, Moazeni E. Nano-niosomes in drug, vaccine, and gene delivery: a rapid overview. *Nanomed J*. 2013;1:1-12.
236. Hoshyar N, Gray S, Han H, Bao G. The effect of nanoparticle size on in vivo pharmacokinetics and cellular interaction. *Nanomedicine*. 2016;11:673-692.
237. De Barros AB, Tsourkas A, Saboury B, Cardoso VN, Alavi A. Emerging role of radiolabeled nanoparticles as an effective diagnostic technique. *EJNMMI Res*. 2012;2:39.
238. Zuckerman JE, Choi CH, Han H, Davis ME. Polycation-siRNA nanoparticles can disassemble at the kidney glomerular basement membrane. *Proc Natl Acad Sci*. 2012;109:3137-3142.
239. Albanese A, Tang PS, Chan WC. The effect of nanoparticle size, shape, and surface chemistry on biological systems. *Annu Rev Biomed Eng*. 2012;14:1-16.
240. Chauhan VP, Stylianopoulos T, Martin JD, et al. Normalization of tumour blood vessels improves the delivery of nanomedicines in a size-dependent manner. *Nat Nanotechnol*. 2012;7:383.
241. Hong H, Zhang Y, Sun J, Cai W. Molecular imaging and therapy of cancer with radiolabeled nanoparticles. *Nano Today*. 2009;4:399-413.
242. Kulkarni SA, Feng SS. Effects of particle size and surface modification on cellular uptake and biodistribution of polymeric nanoparticles for drug delivery. *Pharm Res*. 2013;30:2512-2522.
243. Curley P, Liptrott NJ, Owen A. Advances in nanomedicine drug delivery applications for HIV therapy. *Fut Sci*. 2018;4(1):FSO230.
244. Faraji AH, Wipf P. Nanoparticles in cellular drug delivery. *Bioorg Med Chem*. 2009;17:2950-2962.
245. Dash PK, Gendelman HE, Roy U, et al. Long-acting nanoformulated antiretroviral therapy elicits potent antiretroviral and neuroprotective responses in HIV-1-infected humanized mice. *AIDS (London, England)*. 2012;26:2135-2144.
246. Chaowanachan T, Krogstad E, Ball C, Woodrow KA. Drug synergy of tenofovir and nanoparticle-based antiretrovirals for HIV prophylaxis. *PLoS One*. 2013;8:e61416.
247. Tschanz S, Schneider JP, Knudsen L. Design-based stereology: planning, volumetry, and sampling are crucial steps for a successful study. *Ann Anat-Anatomischer Anzeiger*. 2014;196:3-11.
248. Knudsen L, Brandenberger C, Ochs M. Stereology as the 3D tool to quantitate lung architecture. *Histochem Cell Biol*. 2021;155:163-181. https://doi.org/10.1007/s00418-020-01927-0
249. Altunkaynak BZ, Önger ME, Altunkaynak ME, Ayranci E, Canan S. A brief introduction to stereology and sampling strategies: basic concepts of stereology. *NeuroQuantology*. 2012;10(1):31-43.
250. Kipanyula MJ, Sife AS. Global trends in application of stereology as a quantitative tool in biomedical research. *Biomed Res Int*. 2018;2018:1825697. https://doi.org/10.1155/2018/1825697

251. Napper R. Total number is important: using the dissector method in design-based stereology to understand the structure of the rodent brain. *Front Neuroanat*. 2018;12:16.
252. Madsen KM. The art of counting. *J Am Soc Nephrol*. 1999;10(5):1124-1125.
253. Marcos R, Monteiro RA, Rocha E. The use of design-based stereology to evaluate volumes and numbers in the liver: a review with practical guidelines. *J Anat*. 2012;220:303-317.
254. Catta-Preta M, Mendonca LS, Fraulob-Aquino J, Aguila MB, Mandarim-De-Lacerda CA. A critical analysis of three quantitative methods of assessment of hepatic steatosis in liver biopsies. *Virchows Arch*. 2011;459:477.
255. Santos M, Marcos R, Santos N, Malhão F, Monteiro RA, Rocha E. An unbiased stereological study on subpopulations of rat liver macrophages and on their numerical relation with the hepatocytes and stellate cells. *J Anat*. 2009;214:744-751.
256. Vasilescu DM, Phillion AB, Kinose D, et al. Comprehensive stereological assessment of the human lung using multiresolution computed tomography. *J Appl Physiol*. 2020;128(6):1604-1616.
257. Noorafshan A. Stereology as a valuable tool in the toolbox of testicular research. *Ann Anat-Anatomischer Anzeiger*. 2014;196:57-66.
258. Akosman M, Lenger Ö, Demirel HH, Akosman M, Lenger O, Demirel H. Morphological, stereological, and histometrical assessment of the testicular parameters between Holstein and Simmental bulls. *Int J Morphol*. 2013;31:1076-1080.
259. Azu O, Naidu E, Naidu J, et al. Testicular histomorphologic and stereological alterations following short-term treatment with highly active antiretroviral drugs (HAART) in an experimental animal model. *Andrology*. 2014;2:772-779.
260. Robillard KR, Chan GN, Zhang G, La Porte C, Cameron W, Bendayan R. Role of P-glycoprotein in the distribution of the HIV protease inhibitor atazanavir in the brain and male genital tract. *Antimicrob Agents Chemother*. 2014;58:1713-1722.
261. Howard V, Reed M. *Unbiased Stereology: Three-Dimensional Measurement in Microscopy*. Garland Science. Oxford, United Kingdom: BIOS Scientific Publishers; 2004.
262. Boyce RW, Dorph-Petersen KA, Lyck L, Gundersen HJ. Design-based stereology: introduction to basic concepts and practical approaches for estimation of cell number. *Toxicol Pathol*. 2010;38:1011-1025.
263. Jegede AI, Offor U, Onanuga IO, Naidu ECS, Azu OO. Effect of co-administration of Hypoxis hemerocallidea extract and antiretroviral therapy (HAART) on the histomorphology and seminal parameters in Sprague Dawley rats. *Andrologia*. 2016;49(2):e12640. https://doi.org/10.1111/and.12640
264. Ogedengbe OO, Naidu EC, Akang EN, et al. Virgin coconut oil extract mitigates testicular-induced toxicity of alcohol use in antiretroviral therapy. *Andrology*. 2018;6(4):616-626.
265. Peter AI, Naidu EC, Akang E, et al. Investigating organ toxicity profile of tenofovir and tenofovir nanoparticle on the liver and kidney: experimental animal study. *Toxicol Res*. 2018;34(3):221-229. https://doi.org/10.5487/TR.2018.34.3.221
266. Frapsauce C, Grabar S, Leruez-Ville M,,, et al. Impaired sperm motility in HIV-infected men: an unexpected adverse effect of efavirenz? *Hum Reprod*. 2015;30(8):1797-1806.
267. Jerónimo A, Baza MB, Río I, et al. Factors associated with seminal impairment in HIV-infected men under antiretroviral therapy. *Hum Reprod*. 2017;32(2):265-271.
268. Savasi V, Oneta M, Laoreti A, et al. Effects of antiretroviral therapy on sperm DNA integrity of HIV-1-infected men. *Am J Men's Health*. 2018;12(6):1835-1842.
269. Savasi V, Parisi F, Oneta M, et al. Effects of highly active antiretroviral therapy on semen parameters of a cohort of 770 HIV-1 infected men. *PLoS One*. 2019;14(2):e0212194.
270. Gupta RK, Gregson J, Parkin N, et al. HIV-1 drug resistance before initiation or re-initiation of first-line antiretroviral therapy in low-income and middle-income countries: a systematic review and meta-regression analysis. *Lancet Infect Dis*. 2018;18(3):346-355.
271. WHO, C. Global fund. HIV drug resistance report. 2017.
272. Lorenzo-Redondo R, Fryer HR, Bedford T, et al. Lorenzo-Redondo et al. reply. Re-evaluating evolution in the HIV reservoir. *Nature*. 2017;551(7681):E10. https://doi.org/10.1038/nature24635
273. Guo P, Si M, Wu D, Xue HY, Hu W, Wong HL. Incorporation of docosahexaenoic acid (DHA) enhances nanodelivery of antiretroviral across the blood-brain barrier for treatment of HIV reservoir in brain. *J Controlled Release*. 2020;328:696-709.

How to cite this article: Naidu ECS, Olojede SO, Lawal SK, Rennie CO, Azu OO. Nanoparticle delivery system, highly active antiretroviral therapy, and testicular morphology: The role of stereology. *Pharmacol Res Perspect*. 2021;9:e00776. https://doi.org/10.1002/prp2.776