Part 1

NANOMEDICINE
High-technology Therapy Using Biomolecules or Synthetic Compounds for HIV Inhibition

Elvis Fosso-Kankeu¹, Pascaline Fonteh² and Ajay K. Mishra³

¹Water and Health Research Unit, Faculty of Health Sciences, University of Johannesburg, Johannesburg, South Africa
²Department of Biochemistry, Faculty of Natural and Agricultural Sciences, University of Pretoria, Pretoria, South Africa
³Department of Applied Chemistry, University of Johannesburg, Johannesburg, South Africa

Abstract
The shortcomings of current treatment of AIDS range from undesirable side effects, incomplete eradication of human immunodeficiency virus (HIV) and an increase in the emergence of drug resistant viral strains. Owing to these limitations, there has been a paradigm shift in the approach of researchers as they now focus on the development of new drugs. More convenient drugs will have enhanced activity, lesser or no side effects and satisfactory delivery potential. Various approaches under investigation use biomolecules and metals and/or synthetic compounds with the potential to inhibit viruses or affect their binding sites on the host cells. Techniques such as gene therapy or metal-based therapy emerge from this concept and have so far contributed promising results for the control of the HIV virus. This chapter explores current developments in gene and metal-based therapies (enhanced by nanotechnology), with respect to the design of effective drugs for the treatment of HIV infection.

Keywords: Antisense oligonucleotides, chimeric oligonucleotides, ribozymes, RNA interference, metal complex, metallodrug, nanoparticles, HIV, therapy
1.1 Gene Therapy Including RNA High-Technology Against HIV

1.1.1 Introduction

Recent efforts in scientific research have allowed the development of a new approach in the fight against HIV-1, called gene therapy. It is a process by which new genetic information is introduced into patients' cells with a resulting therapeutic benefit, potentially applicable for the treatment of HIV infection. The principle of this new technique resides in the silencing or knocks out of gene expression at the mRNA level [1]. There are various molecules used to generate the loss of cell's or organism's functions: Antisense sequences, chimeric oligonucleotides, ribozymes and the small interfering RNA (siRNA) [1]. The main steps involved in an anti-viral gene therapy strategy include:

- Selecting the target for intervention (viral or host function).
- Designing, constructing and expressing the inhibitory gene (RNA decoys, transdominant negative gene product, catalytic RNA, others).
- Selecting the vehicle for gene delivery: defective viral vectors (retroviral [retv], HIV, adenoassociated virus [AAV], others); liposomes; receptor-ligand mediated, and; other.
- Selecting the mode of intervention: ex vivo modification and manipulation of target cells or direct injection of genetic information ("naked" DNA) into accessible tissue for augmenting immune responses.

1.1.2 Antisense Sequences Technology

In molecular biology, the strand of the gene that carries the information is called the sense strand and the strand complementary to the former is called antisense. What happen normally in plant and animal cells is that the DNA sense strand is transcribed to a messenger RNA (mRNA) in the nucleus, and then the mRNA is transferred in the cytoplasm and translated into protein which can be enzyme or structural protein.
For decades, biologists have realised that they can interfere with this process to modify or inhibit the expression of the genetic information on the DNA or RNA, allowing them to determine the function of specific gene or designing a therapeutic method. One of the first works was targeted at the inhibition of viral growth, using antisense oligonucleotides (tridecamer oligonucleotide) as a hybridization competitor to inhibit Rous sarcoma virus replication [2, 3]. In principle, this technique relies on the use of a sequence, complementary to a specific mRNA that can inhibit its expression and then induce blockade in the transfer of genetic information from DNA to protein or following hybridization, the two strands can form mini double helices that can be recognized or not by the RNase H [4].

Antisense oligonucleotide usually consist of 15–20 nucleotides which are complementary to their target mRNA. The design of appropriate antisense oligonucleotide has to consider the resistance to degradation by the intracellular endonucleases and of course accessible sites on targeted mRNA which are not similar to other genes. Information about the structure of target gene could be obtained by use of RNase H mapping allowing annealing reactions with arrays of antisense species [5, 6].

On the basis of the mechanism of action, two classes of antisense oligonucleotides can be discerned:

- The RNase H-dependent oligonucleotides, which induces the degradation of mRNA.
- The steric-blocker oligonucleotides, which physically prevent or inhibit the progression of splicing or the translational machinery (Figure 1.1).

![Diagram](image)

**Figure 1.1** Oligonucleotide act as steric block and prevent binding of important regulatory proteins [7].
1.1.2.1 RNase H-dependent Mechanism

RNase H is a ubiquitous enzyme that hydrolysates the RNA strand of an RNA/DNA duplex [8]. This enzyme can be activated by some antisense oligonucleotides among which the widely used phosphorothioate [9, 4]. The RNase H-dependent mechanism is quite specific and can induce at 80–90% the inhibition of protein expression from targeted gene: As an example of action of RNase H in retroviruses, Telesnitsky and colleagues [10] explained that during reverse transcription, the viral RNA serves as template for polymerization of minus-strand DNA. RNase H-mediated cleavage of the viral RNA is necessary to free the minus strand for plus-strand DNA synthesis. Studying an RNase H mediated retrovirus destruction, Matzen et al. [11] reported that it resulted from double cleavage of the double stranded DNA (from RNA transcription) at the polypurine tract-U3 junction releasing a 3’ end of the polypurine tract RNA that serves as primer for strand synthesis, and a second cut at the polypurine tract-U3 junction facilitates removal of the primer. In fact, an antisense oligonucleotide complementary to the polypurine tract creates an RNA-DNA duplex that mimics the structure recognized by the reverse transcriptase, leading to premature cleavage of viral RNA at the polypurine tract-U3 junction before reverse transcription.

RNase H dependent oligonucleotide has the advantage that, unlike steric blocker oligonucleotides, which are efficient when binding only at 5’-AUG initiating codon region, phosphorothioate for example can inhibit protein expression when targeted to widely separated areas in the coding region [12, 13, 14]. Deriving from the replacement of a non-bridging oxygen with sulphur on the first chemically synthesized modified oligonucleotides (methylphosphonates); phosphorothioates are the most widely studied oligonucleotides because of their nuclease stability and relative ease of synthesis, their frequent use as antisense effector molecules, result among other from the fact that they are capable of activating RNase H activity [4]. Phosphorothioates were first used as antisense oligonucleotides for the inhibition of HIV replication by Matsukura and coworkers [15]. However phosphorothioates have also been found to have nonspecific interaction, triggering nonantisense effects and stimulating irrelevant cleavage [14], leading to the development of new antisense with more specific action.

For most antisense approaches, target RNA cleavage by RNase H is desired in order to increase antisense potency. Hence in the
second generation of antisense oligonucleotides, gapmers were develop and consist of a central stretch of DNA or phosphorothioate DNA monomers and modified nucleotides such as 2′-O-methyl RNA at each end. It was reported that the end blocks prevent nucleolytic degradation of the AS-ON and the contiguous stretch of at least four or five deoxy residues between flanking 2′-O-methyl nucleotides enabling activation of Escherichia coli and human RNase H, respectively [16]. Further works to improve RNase H dependent oligonucleotides has led to the development of 2′-deoxy-2′-fluoro-b-D-ribino nucleic acid (FANA) which was the first uniformly sugar-modified antisense oligonucleotide reported to induce RNase H cleavage of a bound RNA molecule [17] and cyclohexene acids (C,N) which are characterized by a high degree of conformational rigidity of the oligomers and are resistant to nucleolytic degradation.

Few authors reported on the ease of RNase H dependent oligonucleotides for the inhibition of HIV: Lederman et al. [18] inhibited HIV-1 infection by enhancing the binding of phosphorothioate to the V3 loop of HIV-1 gp120. An in vitro experiment conducted by Veal and Byrn [19] allowed them to successfully demonstrate RNase H cleavage of HIV-1 mRNA mediated by phosphorothioate antisense oligonucleotides complementary to the gag region of the HIV-1 genome.

1.1.2.2 Steric-blocker Mechanism (RNase H-independent Mechanism)

The initial concept of gene therapy was based on the formation of an RNA-DNA duplex that sterically blocked the RNA, resulting in inhibition of gene expression and consequently of viral replication [20]. Further development of this approach has led to precise elaboration of the action of steric-blockers as inhibitor of mRNA translation initiation as well as RNA processing (they can inhibit intron excision, a key step in the processing of mRNA). Splicing occurs during the maturation step and can be inhibited by the hybridization of an oligonucleotide to the 5′ and 3′ regions involved in this process [21]. Such inhibition can lead to the lack of expression of a mature protein [22, 23] or to the correction of aberrant and the restoration of a functional protein [24, 25]. Inhibition of RNA translation by second generation oligonucleotides is mainly attributable to the disruption of the ribosomes or by physically blocking the initiation [26] or elongation
steps of protein translation. However, the effective target region of a steric block oligonucleotide for inhibiting translation is mainly limited to the 5'-UTR and the start codon region of mRNA, therefore reducing its possible use in antisense therapy. Despite its limitation, steric block remain a viable approach, because of its ability to specifically modulating gene expression, thus lowering off-target effects, compared to conventional antisense. Some of the steric blockers are 2'-O-methyl and 2'-O-methoxy-ethyl RNA oligonucleotides (second generation), peptide nucleic acids (PNAs), N3'-P5' phosphoramidites (NPs) and locked nucleic acid (LNA) (third generation) which effects on the gene expression vary from one to another, but in general they initiate (as above) a blockade of the transcription or the translation by either preventing RNA polymerase action or by hindering the maturation of mRNA for translation [21]. The process of the N3'-P5' PN oligonucleotides is not well known, and some authors suggest that the inhibition of protein synthesis induce by them, is a result of the cleavage of the heteroduplex formed by PN and mRNA by an unknown enzyme [27].

The highly apical region of the 59-residues TAR stem-loop is a particularly good site for targeting by steric block oligonucleotides [28]. Synthetic molecules of PNA were used by Depecker and coworkers [29] to interact with the TAR RNA element of the HIV-1 genome. 2'-O-methyl, N3'-P5'-phosphorothioate and peptide nucleic acid targeted to TAR were shown to be efficient and sequence-specific inhibitors of HIV reverse transcription with IC₅₀ in the nM range [30].

1.1.2.3 Delivery of Antisense Oligonucleotides into the Cells

Delivery of antisense oligonucleotides into the cell remain one the major challenges of gene therapy as for the oligonucleotides to induce antisense effect, they should be in the nucleus or in the chloroplast (for plant) where the mRNA is transcribed. The penetration of the oligonucleotides in the cells depends on temperature, the structure, the concentration and cell line [31]. Physiological temperature (37°C) is suitable for oligonucleotides uptake via endocytosis, but hampered at lower temperature [32]. Oligonucleotides uptake by cell membranes is generally facilitated by the use of transfection agents or vectors [33]. In presence of relatively low concentration of oligonucleotides, internalization mainly occurs via interaction with membrane-bound receptor [34, 35], while at higher concentration,
these receptors are saturated and the pinocytic process assumes larger importance. Some attempts in improving lipid-free cell uptake consisted of reducing the overall charge of oligonucleotide backbone, by attaching for example cationic charges to the internucleotide phosphate linkages of α-oligonucleotides or by use of hydrophobic thioester pro-oligonucleotide functions [36]. However oligonucleotides uptake cannot be related to their charge, since electrically neutral PNA and phosphorodiamidate morpholino (PMO) oligonucleotides are still not taken up significantly by cells [37]. Irrespective of their charge, naked oligonucleotides have been poorly internalized in previous experiments [38, 39, 33].

Numerous techniques and transporters have been developed to improve cellular uptake and oligonucleotide spatial and temporal activity. Among the vectors in use, liposomes and charged lipids are the most common and successful; they can either encapsulate nucleic acids within their aqueous center or form lipid-nucleic acid complexes as a result of opposing charges [16]. Other delivery systems include dendrimers, pluronic gel, polymers of amino acids or surgars. Effective targeting of antisense oligonucleotides to specific tissues or organs could also be achieved by a receptor-mediated endocytosis involving conjugation of oligonucleotide to antibodies or ligands that are specifically recognized by a certain receptor, which mediates their uptake into target cells.

Transient permealization of the plasma membrane can be generated chemically by sterptolysin [40, 41], mechanically by microinjection [42] or scrape loading [43], or produced by electroporation [44, 45].

The use of antisense technology for modification of a particular phenotype or disease treatment still have a room for improvement as there is a problem of specificity and control in antisense oligonucleotides experiments. However, chimeric oligonucleotide (in which RNase H competent segment is bounded on one or both termini by a higher affinity region of modified RNA) could be an option in addressing nonspecific activity of antisense oligonucleotide.

1.1.2.4 Chimeric Oligonucleotide Technology

This technology involves the design of a heteroduplex RNA/DNA which is injected in a cell orientated in a way that the strand complementary to the targeted gene forms a mismatch with it, activating the mismatch repair enzyme, which substitutes the base on the
mRNA according to the DNA template. In the duplex, the DNA has the function to bind to the mRNA, while the RNA stabilises the reaction intermediate [46]. Chimeric DNA-LNA oligonucleotide reveals an enhanced stability against nucleolytic degradation [5, 47] and an extraordinarily high target affinity. Introduction of LNA in oligonucleotides also leads to increase of the melting temperature of up to 9.6°C per LNA introduced [48]. This enhanced affinity towards the target RNA accelerates RNase H cleavage [5] and leads to a much higher potency of chimeric DNA. LNA oligonucleotides in suppressing gene expression in cell culture, compared to phosphorothioate DNAs or 2'-O-methyl modified gapmers [16] (Figure 1.2).

This technique was initially used to correct point or frameshift mutations, or to generate mutation for gene silencing. Yoon and colleagues [51] corrected a mutated alkaline phosphatase gene in an episomal target to generate a functional gene. Plant has also

![Figure 1.2 Mechanisms of antisense activity. (A) RNase H cleavage induced by (chimeric) antisense-oligonucleotides. (B) Translational arrest by blocking the ribosome [16]. Chimeric DNA-LNA oligonucleotides can be adjusted for different antisense activities: A chimeric DNA-LNA gapmer that contains a stretch of 7-8 DNA monomers in the center to induce RNase H activity can be used to degrade mRNA. Chimeric 2'-O-methyl-LNA oligonucleotides that do not activate RNase H could, however, be used as steric blocks to inhibit intracellular HIV-1 Tat-dependent trans activation and hence suppress gene expression [49]. Targeting a variety of regions (5' untranslated region, region of the start codon or coding region) within the luciferase mRNA, LNAs and LNA-DNA were successfully used to inhibit gene expression [50]. Some advantages are provided by chimeric DNA-LNA oligonucleotides such as: stability against nucleolytic degradation, high target affinity, potent biological activity and apparent lack of acute toxicity.](image-url)
been successfully targeted by chimeric gene, as Beetham et al. [52] created a mutation in a ALS gene and was able to select converted tobacco cells in a presence of a herbicide [53]. Many experiments demonstrated the feasibility of using chimeric oligonucleotide for the inhibition of HIV-1. A very important step in the development of HIV-1 infection is the attachment of the virus to the receptor CD-4 on helper T lymphocytes which are recognised by the envelope glycoproteins gp120 of HIV. Tamura et al. [54] developed a phosphodiester and a phosphorothioate chimeric oligonucleotide which inhibited HIV-1 replication by two mechanisms: Binding to the V3 loop of the envelope protein gp120, inhibiting virus adsorption and cell fusion, or inhibiting the HIV integrase.

Other researches on the inhibition of HIV infection have also focused on the transcription step of the viral mRNA. Tat and Rev are two regulatory proteins encoded by HIV-1, which respectively promotes elongation of viral transcripts by RNA polymerase II and/or facilitates the transport of unspliced and singly spliced RNA to the cytoplasm. Nakaya et al. [55] used a Rev binding element-based decoy (the RNA/DNA chimera oligonucleotide) to inhibit the HIV-1 infection.

1.1.2.4.1 Delivery of Chimeric Oligonucleotides into Cells
Delivery of chimeric oligonucleotide can be done by electroporation [46] or by using any one of a number of commercially available transfection agents, including the cationic lipids: lipofectin, lipofectamine and cytofectin, as well as polyamines [33].

The setbacks of this technique are that there is always a low percentage of transduction of nucleotide into the cells and there may be toxicity effect over a long therapeutic period [55].

1.1.3 Ribozymes Technology
Ribozymes are RNA molecules that possess catalytic activity in the absence of any proteic enzyme [56, 57]. There are various types of ribozymes classified according to their size [58], but those frequently used for basic research or medical purposes (59; 60) belong to the class so-called “small ribozymes” [61]. Among the ribozymes belonging to this group, hammerhead and hairpin ribozymes are the most intensive studied and widely used [62, 63, 72]. Initially isolated from viroid RNA, hammerhead ribozymes molecules can cleave RNAs at specific sites as long as they contain any of the
cleavable triplets 5'-NUH-3', where U is conserved, N is any nucleotide and H can be C, U or A, but not G (Figure 1.3).

The potential of hammerhead ribozymes for inhibition of gene expression has been considered by researchers for application in biological systems. However various challenges were encountered during practical use of natural ribozyme, due to the instability of RNA which are more susceptible to nucleolytic degradation than DNA oligonucleotide. This situation has led to the synthesis of deoxyribozymes (DNAzymes) which are more stable against nucleases [65, 66]. The most prominent deoxyribozyme, named '10-23', consists of a catalytic core of 15 nucleotides and two substrate recognition arms of 6-12 nucleotides on either arm.

It is highly sequence specific and can cleave any junction between a purine and a pyrimidine. A DNA enzyme with optimized substrate recognition arms and a partially protected catalytic domain possess not only increased nuclease resistance but also enhanced catalytic activity [16]. The mechanism of action of both modified and unmodified ribozyme however remain similar, as they all bind to their specific target RNA by Watson-Crick base pairing and possess the capability of cleaving a complementary RNA molecule without aid of cellular proteins.

Researchers use these tools to suppress viral gene expression and inhibit the replication of virus in cell culture and in vivo. For example, hammerhead and hairpin ribozymes have been used in many experiments to inhibit HIV-1 genes or to suppress HIV [67, 68]. Many regions of HIV-1 have been targeted by ribozymes in attempts to develop AIDS treatment: Bramlage et al. [69] directed hammerhead ribozyme against either NUX, GUX or NXG triplets,
to probe the HIV-1 LTR transcript for cleavage. The regulatory proteins of Tat and Rev of HIV-1 play an important role in viral infection as they promote the binding of viral RNA to the CD4 cell; Ribozymes orientated against this region have been shown to significantly inhibit HIV-1 infection [70, 71].

1.1.3.1 Delivery of Ribozymes into Cells

Many techniques have been used to deliver ribozymes or deoxyribozymes in cells. They are divided into exogenous and endogenous deliveries.

*Exogenous delivery*

There are two ways to consider for an exogenous delivery:

- The carrier-free nuclease-resistant ribozymes consist of injecting ribozymes directly into the cells.
- The delivery of ribozymes can also be enhanced by carriers such as cationic liposomes, which also protect against nuclease digestion [72].

*Endogenous delivery*

Ribozymes can also be encoded on plasmids and expressed endogenously. Viral vectors have frequently and effectively been used to deliver ribozymes into the cells [72].

The use of ribozymes in gene therapy has not always produced the expected results [73]; this ineffectiveness is probably due to the difficulty for ribozymes to locate their target [74].

1.1.4 RNA Interference Technology

The limitations such as lack of specificity and incomplete efficiency related to the application of antisense oligonucleotides, chimeric oligonucleotides and ribozymes for gene suppression [75] have brought the researchers to develop a new technology called RNA interference. The principle of this technique consists of the transfection of cells with a dsRNA which is processed by the Rnase-III type endonuclease termed Dicer into small interfering RNA (siRNA = 20 nucleotides), which guides the RNA Interfering Silencing Complex (RISC) to the targeted mRNA. The antisense of the siRNA will then hybridise with the corresponding cellular RNA, and the duplex will be cut by the Dicer, inhibiting the protein synthesis [76, 1]. Practically the introduction of a long dsRNA in mammalian cells induces the
response of interferon defence mechanism leading to a nonspecific inhibition of translation and apoptosis [77, 78]. It is then important, in order to generate a specific RNA interference-dependent gene silencing in mammalian cells, to transfect the cells with a small molecule of RNA which do not induce interferon response [79, 80]. There exist three types of small RNA molecules (small interfering RNA "siRNA," small temporal RNA "stRNA" and micro RNA "miRNA"), which all contribute in inhibiting translation (Figure 1.4).

Figure 1.4 Long double-stranded (ds)RNA or hairpin RNA substrates are cut by Dicer into smaller (~21-nucleotide (nt)) small interfering (si)RNAs with 2-nt overhangs at the 3' ends and phosphate groups at the 5' ends. Alternatively, siRNA duplexes (19–23 nt) can be introduced into cells, where they are phosphorylated at the 5' ends by cellular kinases. These small dsRNAs assemble into the RNA-induced silencing complex (RISC), which contains AGO2, Dicer and other cellular factors. For simplicity, only AGO2 is indicated. siRNA then forms activated RISC (siRISC) that contains an antisense (guide) strand. Activated RISC finds its target mRNA and uses the antisense strand to guide the cleavage of the target mRNA. RISC is recycled and could carry out several cleavage events [81].
Despite the relatively high stability and specificity of siRNA in cell and in vivo, depending on the application, there have been needs to enhance the siRNA effectiveness. Therefore, many attempts were made to modify the siRNA prior to use, but the modification did not provide considerable improvement of the ability of unmodified siRNA. No change in activity was observed when siRNA was modified with two 2′-O-methyl RNA nucleotides at the 5′ end and four methylated monomers at the 3′ end [16]. However, studying gene silencing in mammalian, Ui Tei and coworkers [82] improved the specificity of siRNA by inserting DNA in the seed region.

Some progress have been made in the therapeutic application of siRNA; Weinberg and Morris [83] found that miRNA molecules operating at a transcriptional level can affect or maintain a latent infection state of HIV-1.

The RNA interference strategies often used in inhibiting HIV infection, consist of targeting the elimination of a protein needed by HIV to produce or cause infection. However, the possibility of viral escape through mutation has hampered this approach [84] and is the reason why some researchers have opted in silencing cellular gene necessary for viral propagation [85].

**Targeted gene regions of HIV**

- Cave and Weinberg [86] successfully inhibited the replication of biologically distinct HIV-1 subtype C isolates, by directing RNA Pol III-driven small hairpin RNAs (shRNAs) against the sequences of gag open reading frame of HIV-1 subtype C.
- Coburn and Cullen [87] used siRNAs against Tat and Rev regulatory proteins of HIV-1 gene expression and replication in cell cultures including human T-cell lines and primary lymphocytes.
- Jacque et al. [88] tested a number of presynthesized siRNAs and plasmid-expressed shRNAs against the viral vif and nef genes as well as the long terminal repeat (LTR).

The limitation for this approach was experimented by Das et al. [89] as they observed HIV-1 resistant variants in culture after several weeks of exposure to siRNA-nef.
Targeted cellular genes
To demonstrate that intracellular immunization could be effective in the fight against HIV-1, Qin et al. [90] transfected peripheral blood T lymphocytes with siRNA orientated against the HIV-1 coreceptor, CCR5, this resulted in a substantial protection of lymphocyte populations from CCR5-tropic HIV-1 virus infection.

1.1.4.1 Delivery of siRNAs
Transfection efficiency for presynthesized siRNAs is usually quite high in standard cell lines, but the silencing effect stop after few days as the intracellular concentration of siRNA decreases due to nucleases activities.

However plasmid vectors delivery of short hairpin RNA (shRNA) which are processed into siRNA [91] allows for long lasting (two months) gene silencing activity by siRNA [92].

More efficient uptake can be achieved by exploiting the natural ability of viruses to infect host cells.

The RNA interfering-dependent gene silencing technique has many advantages [1] over the previous techniques that make it suitable for the development of potent therapeutic treatment against HIV-1.

- RNAi can spread within the individual and be transmitted to offspring.
- Only a few molecules of dsRNA are sufficient to trigger RNAi suggesting the presence of catalytic and amplification components in the interference process.
- RNAi occurs at the postranscriptional level since dsRNA corresponding to promotor and intron sequences do not activate the RNAi pathway.
- RNAi is highly specific process: the injection of dsRNA segments homologous to particular gene exons eliminates or decreases only the level of the corresponding mRNA without harming the cell.

1.2 Metals and HIV Therapy

1.2.1 Introduction
The human immunodeficiency virus and acquired immune deficiency syndrome continues to be a major health issue since there's been no viable cure or vaccine to prevent new infections, three
decades since the first diagnosis was made. Highly active antiretroviral therapy (HAART) has been very successful in increasing the livelihood of people infected with HIV [93]. The drugs must be taken continuously since the virus cannot be completely eradicated because of the presence of latent reservoirs [94]. More recent reports from the HIV Prevention Trials Networks [95] in the study known as the HPTN 052 study suggests that the early administration of antiretroviral therapy to infected men and women reduced the risk of transmission to their partners by 96%. This finding further confirms the important role that HAART can play both as treatment and as a prevention strategy for HIV transmission. Unfortunately, this combination therapy faces numerous challenges such as toxicity to the host [96–98], drug resistance by the virus [99, 100] and numerous uncomfortable side effects [101, 96]. Another shortcoming is the poor pharmacokinetics of the available drugs, especially uptake to certain targets such as the brain [102]. Efforts to identify novel therapy and to improve delivery continue and have been recommended as complementary strategies during HIV drug development [103]. These strategies will increase the number of available treatment options, reduce dosing frequencies, improve compliance and target and eliminate viral reservoirs [103] which will otherwise be difficult to manage.

Metals and metal-based drugs play a significant role in medicine and HIV medicine. These have been facilitated by advances in medicinal inorganic chemistry and the medicinal chemists' knowledge of the coordination chemistry and redox properties of metals, especially those of the transition metal series [104, 105]. Metals can easily interact with biological systems because of the fact that they can easily lose electrons and get converted to soluble ionic states that are electron deficient. In addition, the wide scope that metals have in their interactions with biological systems also means that they could be easily accommodated in drugs [106]. In the electron deficient state, metals interact with electron rich proteins and DNA [106]. Typical examples are iron, found in the protein haemoglobin which binds to oxygen. Others are manganese, copper, zinc and iron that are incorporated into enzyme structures to produce metalloenzymes which facilitate crucial chemical reactions in the body [106].

The history of metals in medicine dates back to the earliest times [107]. A metal-based compound (or coordination complex) consists of a metal and an organic portion called the ligand to which the metal is coordinated. The coordination complex is defined as a structure consisting of a central atom, bonded to a surrounding
array of molecules or anions [108, 104]. There are advantages that metal-based drugs have over traditional organic medicinal agents, e.g., the drugs make use of metal-drug synergism where there is the enhancement of the activity of the parental drug as a result of complexation [109, 110]. The activity enhancement is thought to be as a result of structural stabilisation from the coordination/complexation of the metal to the organic moiety [109]. Complexation has been reported to lead to decreases in toxicity of the metal ions in some cases since the organic portion of the drug makes it less available for unwanted interactions that could lead to toxicity [111]. In addition it may lead to significant reduction in drug resistance because of improved specificity [112, 105]. This is because the metals in metallo-drugs form covalent bonds and ionic forces, unlike organic molecules which form van der Waal forces and hydrogen bonding. These covalent and ionic forces are stronger, so the drugs tend to stay at the active site longer thereby increasing efficacy and resulting in a synergistic effect from the organic and metal moieties [109]. Some examples of metals with medicinal properties are iron, ruthenium and silver, gold, among others [104]. The serendipitous discovery of cisplatin as an anticancer platinum-based compound renewed interest in medicinal inorganic chemistry [113, 114]. Some of these benefits and applications of metal-based drugs have been reported for HIV and will be reviewed here.

Metal ions and more specifically divalent ones are also very important in catalysis especially in enzyme reactions that require nucleic acid-phosphoryl transferases [115, 116]. Such reactions are very common in biological systems, playing roles in gene replication, recombination and expression [117]. The divalent ions are also required for structural stability and facilitate the formation of active sites [116]. These metals play a significant role in HIV with regards to catalysis of the reverse transcription process and the integration process, e.g., Mg$^{2+}$ ions of RNase H and integrase respectively. Anti-HIV agents that are capable of chelating the active site metal ions have been explored.

Nanotechnology on the other hand is a relatively novel area. The concept originated in 1959 when Richard Feynman gave a talk on “There’s plenty of room at the bottom” [118]. In the 1980s the principles laid by Feynman and other scientists after him were converted to inventions such as that of the scanning tunnelling microscope in 1981 and the atomic force microscope in 1986 [118]. Basically nanotechnology is defined as the design and fabrication of
materials, devices and systems with control at the nanoscale level [119]. This technology can be applied in various disciplines ranging from chemistry, biology and quantum mechanics [119]. Its applications in medicine are wide ranging from medical imaging, and targeted drug delivery to screening and in vitro diagnostics [120] to name but a few. The advantages that NPs have are the fact there is the possibility of controlling size, shape, dispersity and surface chemistry [121]. In addition, the fact that NPs are similar in size to biological molecules makes biological interactions favourable [121]. Another remarkable benefit that NPs have is the fact that they offer a multivalent binding strategy that can result in the improvement in receptor-ligand binding affinity that is not seen for synthetic monovalent compounds [121, 122]. Multivalency is common in biological systems and usually confers a higher binding affinity between biomolecules [122]. Biological systems exploit this ability to form high-affinity ligands by making use of existing monovalent ligands [123]. In the same light, multivalent drugs should be capable of binding more tightly with the biological target to result in improved activity, a concept that has been exploited for the development of anti-HIV gold-based nanoparticles by Gianvincenzo et al. [121] and Bowman et al. [122].

Methods to address the shortcomings facing HAART have largely focused on the identification of novel therapy with emphasis on the identification of therapy for new or existing targets. While this is important so as to increase the number of available drugs and treatment options, it is equally important that ways of curbing the limitations associated with HAART are addressed. In this chapter, the role of metals and nanotechnology in HIV medicine will be covered. Metals and metal-based compounds play a variety of roles in HIV. Some metals have anti-HIV activity, e.g., gold compounds [124]. Others play a role in catalysis particularly for RNase H of reverse transcriptase [125] and the integrase [115] enzymes of HIV such that inhibitors targeted at chelating these metal ions have been explored. Others are detrimental such as calcium involved in syncytium formation [126] and excess iron which causes an increase viral replication when there is an overload [127]. Zinc on the other hand has been implicated to stimulate Mg²⁺ dependent 3' processing activity of integrase in vitro [128]. Here, emphasis will be placed on metal-containing compounds as anti-HIV agents as well as the role of metal cofactors as targets for the binding of inhibitors. The use of nanotechnological approaches in developing novel therapy
(specifically metal-NPs) and for enhancing delivery of anti-HIV agents will be covered.

1.2.2 Metals and HIV

1.2.2.1 Metal-based Anti-HIV Drugs

Gold-based compounds have shown anti-HIV activity by inhibiting viral enzymes, preventing viral entry and also show immunomodulatory tendencies [124]. Gold compounds which have shown inhibition of HIV through the inhibition of HIV-1 reverse transcriptase (enzyme necessary for converting viral RNA to cDNA) are aurothioglucese and other gold containing aliphatics [129, 130], dicyanogold [131], AuII(TMPyP)Cl5 {(H2TMPyP)4? =
mesotetrakis(N-methylpyridinium-4-yl)porphyrin} [132], tetra-
chloro-(bis-(3,5-dimethylpyrazolyl)methane)gold (III)chloride [133], gold(1) phosphine complexes [135]. Inhibition has also been noted as a result of the prevention of viral entry for aurothioglucese [129]. Some of these compounds have the ability to modulate the immune system, e.g., aurothiomalate was shown to elevate IL-2 levels and increase cell surface markers such as CD4 in a mouse AIDS model [134]. In addition, a gold(III) thioemicarbazonate complex was recently reported to lower CD4+ cell frequency in HIV positive donors and demonstrated anti-proliferative effects [135]. It is thought that such a mechanism might be beneficial as a means of lowering immune activation that is commonly seen in HIV [136]. However, for this to be translated into a therapeutic agent, it must be combined with a directly antiviral agent such as a nucleoside reverse transcriptase inhibitor (didanosine) in the combination therapy known as virostatics which offers better efficacy [137, 138].

Zinc is an essential metal nutrient that is needed for optimal cell function [139]. Zinc ions on the other hand have a mitogenic effect on lymphocyte proliferation in vitro. Metal compounds of zinc have shown anti-HIV activity in vitro and this activity is thought to be linked to inhibition of HIV-1 DNA to RNA transcription and not on viral entry [140]. Some examples of anti-HIV zinc compounds are ((CH3COO)2Zn·2H2O, ZnCl2, Zn(NO3)2·6H2O) [139]. Another example is the cyclic zinc-dithiocarbamate-S,S'-dioxide which was shown to inhibit infection by HIV by interacting with the CXCR4 co-receptor [141]. The complexation of zinc with some already anti-viral compounds such as the polyamine clycins and cylams
[142, 143] and baicalin [139] have been shown to lead to enhanced antiviral activity and in addition decreased toxicity [139, 142–143]. A zinc complex of 2-mercaptoethanesulphonate was cytotoxic at the concentration where antiviral activity was observed [144]. Another study by Haraguchi et al. [140] showed that various zinc complexes (zinc acetate, zinc chloride and zinc nitrate as well as cadmium acetate and mercury chloride inhibited HIV infection at non toxic concentrations. In the study, the authors postulated that the antiviral mechanism was due to the inhibition of HIV-1 DNA to RNA transcription and not inhibition HIV-1 DNA synthesis, adsorption, penetration or reverse transcription step of HIV-1 replication cycle.

The role of platinum and palladium complexes as anti-HIV agents has also been reported with specific recommendations for use as microbicides in vagina gels [144]. The compounds were complexes of the 2-mercaptoethanesulphonate which prevented syncitium formation at non toxic concentrations. The recommendation for the use of these agents in vagina gels as microbicides is because of the fact that the polysulphonate derivative in the ligand are typically known as topical antimicrobial agents for the prevention of sexually transmissible diseases [145]. In the study by Bergstrom et al. [144], the authors also showed that zinc, cadmium and silver-based complexes of 2-mercaptoethanesulphonate had the potency to inhibit HIV, but unfortunately were toxic at that dose.

Other metal-based compounds with anti-HIV activity are copper, lanthium and iron-based derivatives of ligands which are similar to the reverse transcriptase inhibitor, ateviridine, and the protease inhibitor, VX-950 [146]. These metal complexes together with that of zinc resulted in an enhanced synergetic effect that was not seen for the parent compounds or the derivitised ligands [146].

The anti-HIV activity of metal complexes of sulphurous derivatives particularly of cobalt, copper and lithium have also been reported [147]. The complexes were more active and less toxic than the free ligands although not as much as azidothymidine (AZT), an anti-HIV agent that was used as a control.

Ruthenium complexes have also been shown to inhibit HIV through the inhibition of reverse transcriptase [148]. These were complexes of mixed valent oxo-oxalate compounds.

The clinical application of metal complexes as anti-HIV agents still needs to be realised. Just like many other drugs, metal-based compounds also have side effects, e.g., gold induced dermatitis caused by lymphoproliferation seen for patients taking gold-based
drugs for the treatment of rheumatoid arthritis [149]. In addition these compounds may also be limited pharmacologically. These limitations may be reduced by conjugating metal-NPs to appropriate ligands so as to enhance receptor-ligand affinity [122].

1.2.2.2 Role of Metals in the Catalytic Function of HIV RNase and Integrase

The role of metals in the binding of RNase H inhibitors

HIV RT is a heterodimeric enzyme which consists of both a p66 and p51 subunits. The p66 subunit consists of both a polymerase function and an RNase function. RNase H is involved in the hydrolysis of the RNA strand of the RNA:DNA heteroduplex that is produced during reverse transcription. The RNase H function is unique to the C terminus of the p66 subunit [150]. It is involved in the processing of the tRNA primer to begin minus-strand DNA synthesis and degradation of viral RNA during synthesis followed by preparation of the polypurine (purine rich) tract DNA-RNA hybrid which serves as the primer for positive strand DNA synthesis [151, 152]. All these processes together with the polymerase function [153] result in the copying of a single stranded RNA to a double stranded DNA [154]. The active site of RNase H contains two metal ions which form part of the active site, coordinating the binding of substrates and for catalyzing phosphodiester bond hydrolysis [125]. In addition to the catalytic residues found in the active site, catalysis by RNase H is dependent on the metal ions [155, 156]. Some classes of RNase H inhibitors target the metal ion requirement of the enzyme. These are the N-hydroxyimides [157, 156], diketo acids [158, 159] and hydroxylated tropolones [160–162].

The role of metals in the binding of IN inhibitors

HIV integrase is an essential enzyme in the life cycle of HIV and is an important drug target because there is no human equivalent [163]. The enzyme is a member of the RNase-H like superfamily and is known to bind with divalent metal ions at the DDE motif of the active site [115]. The metals in the active site of IN have been exploited for the development of anti-HIV therapy by the use of ligands that chelate the active site metal ions, e.g., the use of α,γ-diketo acid containing compounds [164]. The diketo acid component is thought to be important as the metal (Mg$^{2+}$) chelating moiety and does this by forming a tertiary complex thereby blocking the DNA substrate from binding [164]. Integrase strand
transfer inhibitors such as raltegravir and similar strand transfer inhibitors have been proposed to prevent viral DNA binding ability of integrase by co-ordinating with the metal ions through the formation of a hydrogen bond acceptor-donor-acceptor motif [165], thereby preventing the integration process and viral replication. Other ligands that incorporate chelating groups such as those of purine derivatives [166] and 2-hydroxyl-3-heteroaryl acrylic acids [167, 168] have been exploited as integrase inhibitors.

1.2.3 Nanotechnology and HIV

1.2.3.1 Nanotechnology to Enhance Drug Delivery

One of the greatest shortcomings of antiretroviral therapy has been poor pharmacotherapy. Although this therapy is now more beneficial than in the early 1990s when only a monotherapy was available [103], it is endowed with a lot more complications since many more therapeutic options are available [103]. Some of the complications are poor aqueous solubility, the inability of some of these agents to reach certain organs such as the central nervous system (CNS) due to the presence of efflux pumps which prevent the transportation of the drugs into the brain [169, 170]. In addition, the differences in the variation of orally administered ARVs between patients are also thought to be related to the presence of efflux pumps in the gastrointestinal tract [171, 172].

The majority of drugs, if not all, from the different classes of anti-HIV agents are limited pharmacologically. The major limitations of the nucleoside reverse transcriptase inhibitors such as didanosine are limited stability, first pass metabolism and systemic toxicity [173]. The only approved nucleotide reverse transcriptase inhibitor, tenofovir, has poor bioavailability [174]. Protease inhibitors are restricted in oral absorption because they are typically efflux pump substrates [175]. A typical example of a drug with poor blood brain barrier (BBB) permeability is the protease inhibitor, saquinavir, which has low aqueous solubility [103]. A majority of the other protease inhibitors also have poor BBB penetration [176]. Protease inhibitors are one of the cornerstone components of the HAART [177]. Such drugs will therefore be unable to inhibit HIV replication in the brain. Such a situation has been shown to result in an increase in the incidence of AIDS dementia [178, 179], neuroinflammation and neurodegeneration [178].
The fact that compliance is highly needed when HIV medication is instituted so as to maintain viral loads to undetectable levels and to preserve a state of wellbeing means the limitations associated with the pharmacology of available drugs stated above can lead to poor adherence. A compliance of up to 95% is required to maintain the right dosing regimens and to ensure bioavailability of anti-HIV drugs [173]. This is important since HIV cannot be completely eradicated due to presence of latent reservoirs during treatment [94]. For this reason, treatment has to be life-long. In the face of the limitations associated with HAART and the fact that compliance and adherence are very important for successful maintenance of the quality of life of HIV infected patients, the need for an effective drug delivery system was thought to be important [180]. This should be investigated for available therapy and be considered as a complementary approach during HIV drug development [103].

Nanotechnology is one such approach that has been applied in the quest for improving the pharmacology of anti-HIV agents. In this approach, NPs which are submicron drug carrier systems generally of polymeric nature in the size range of 10–500 nm are used [181]. Various ways of incorporating drugs into NPs include dissolving them in the NPs, entrapping, encapsulating and or adsorbing or attaching them [181]. Some carrier or delivery systems that have been developed to improve HIV therapy are polymeric NPs, solid lipid NPs, liposomes, nanoemulsions, dendrimers and drug conjugates [182–186]. Nanotechnological systems provide means in the development of ARV drug delivery systems that will ultimately lead to improved compliance and adherence [187]. This is because of the versatility (ease to encapsulate all drugs), the possibility of drug release modulation, high drug payloads, a good toxicity profile, relative low cost, the ease to produce and the possibility of scaling up [183, 184]. The improved bioavailability that comes with the technology means that incorporated drugs will be protected from metabolism which will lead to a longer drug residence time and thus reducing the need for many doses and ultimately prolonging the time between dosing schedules [188, 173]. The application of nanotechnology in HIV is geared at targeting viral reservoirs such as CD4+ cells [169, 178], cells of the mononuclear phagocyte system, the brain and the gastrointestinal tract [189] and improving drug penetration by inhibition of efflux transporters [190]. Liposomes were the first to be used as a nanocarrier system for targeting HIV intracellularly [103]. Some examples were the use of liposomes in
delivering azidothymidine (AZT) [191], the use of cell-penetrating peptides, e.g., the trans-activator transcriptor (TAT) of HIV for the delivery of drug loads in the CNS [103]. AZT [192] and stavudine [193] drug-loaded liposomes have been used to reduce systemic exposure and adverse effects of these drugs.

Viral replication in the CNS especially that in the brain is difficult to keep under control because of the tight cell junctions between endothelial cells of the brain [194] making BBB penetration difficult. Another limitation is the presence of efflux pumps such as those of P-glycoprotein which reduce the concentration of drugs that can cross the BBB [188]. Although viral loads in the brain of patients on HAART are lower than that of those not on HAART, the level in the former is not reduced to undetectable levels after three months of treatment [195]. The result is morbidity and mortality for the untreated patients and an increase in the incidence of neurocognitive impairment such as HIV associated dementia [181]. The use of nanoparticle-based delivery systems should aid in attaining higher concentrations of encapsulated drugs and will allow for longer residence time in the CNS [188].

1.2.3.2 Metal Nanotechnology to Inhibit HIV

In addition to enhancing drug delivery in HIV, nanotechnological approaches have been used to enhance antiviral effect. This enhancement is probably related to the small size associated with the particles, improved bioavailability and stability. In addition, with NPs, a multivalent-based ligand can be generated that will result in increased receptor-ligand affinity and increased activity [121, 122]. Not much has been done on the use of metal-NPs for inhibiting virus [196]. However, some accounts on silver NPs and gold NPs have been reported.

Elechiguerra et al. [196] investigated the antiviral effect of carbon coated, bovine serum coated, and poly (N-vinyl-2-pyrrolidone) (PVP) coated silver NPs. The authors showed that the carbon coated NPs could inhibit viral infectivity and suggested that this inhibition was possibly due to the interaction of the particles with sulphur knobs on gp120 surface glycoprotein of the virus. This proposal was supported by the fact that the particles demonstrated a regular spatial relationship [196]. Particles below 14 nm were shown to bind with more stability due to the fact that this size was below that of the gp120 knob size which is ~ 14 nm [197]. In another study, Lara et al. [198] showed that silver NPs coated with PVP inhibited viral infectivity at multiple
points of the HIV life cycle. In their study, the authors showed that the silver NPs inhibited HIV infection by interrupting gp120-CD4 interaction. The authors also showed that the silver NPs were virucidal, completely eliminating infection after short exposure and by preventing infection at pre-integration steps. Both cell free and cell associated virus was inhibited. In another report, Sun et al. [199] also showed that silver NPs were capable of inhibiting viral cytopathic effect and reduced the production of p24 in culture supernatant. Inhibition of viral reverse transcriptase was not observed indicating that inhibition of virus was through another mechanism. Silver NPs have also been shown to have activity against other viruses such as hepatitis B virus [200] and the herpes simplex virus [201].

In 2009, Bowman et al. [122] used multivalent gold NPs to determine inhibition of HIV and showed that the gold NPs were capable of transforming a poor binding and inactive small molecule to a multivalent conjugate that was capable of inhibiting HIV fusion. The inactive component, SDC-1721, is a homologue of TAK-779 which is a CCR5 antagonist. Unfortunately TAK-779 has poor pharmacological properties (irritation at injection site) due to the presence of a quaternary ammonium salt which also contributes to its activity. In searching for an alternative CC5 inhibitor, the SDC-1721 homologue was synthesised by these authors and conjugated to gold NPs. The enhancement of the affinity of the SDC-1721 by the presence of the gold NPs played a significant role in converting the inactive molecule to an active one. Similar reports of gold NPs coated sulphated ligands that were shown to inhibit HIV envelope glycoprotein gp120 and inhibit HIV infection of T-cells was also reported by Gianvincezo et al. [121]. The added advantage of these metal based NPs is the fact that there is room for multimerisation of the synthetic molecules. This will allow for the clustering of monovalent parent compounds on the NPs leading to the production of non-cocktail based multifunctional anti-HIV agents [121]. Such drugs could potentially be what is needed to reduce dosing schedules, improve adherence while increasing activity at the same time.

1.3 Conclusions

The goal of HAART has been to keep HIV replication to a minimum and to prevent the incidence of AIDS and the associated opportunistic infections that comes with it. This has been greatly successful
but is limited by numerous shortcomings. Some of these are the development of drug resistance, drug toxicity and the poor pharmacology of available drugs (e.g., poor solubility, limited bioavailability, inability to cross the BBB, plasma protein binding). Measures to curb these limitations have largely focused on the development of new drugs that can replace or supplement existing ones. More recently, ways to improve the pharmacology of the existing drugs have been considered as a means of improving adherence, reducing dosing regimens and enhancing the overall efficacy of the drugs. Nanotechnological approaches have been paramount in this respect. The use of nanocarriers as delivery systems has greatly enhanced drug delivery to targeted reservoirs such as the brain and CD4+ lymphocytes.

Substantial progresses have been made in certain areas toward the development of much effective drugs:

Gene therapy has undergone a tremendous improvement from the antisense technology to the RNA interference, and it is now possible to suppress gene expression in vivo. Gene therapy could help the body to fight off HIV so as to be able to control the virus on its own, without the patient be dependent on daily intake of drugs with side effect.

The exploration of metal-based compounds as possible anti-HIV agents has been one of the areas of interest in the development of new anti-HIV agents. With the advances in nanotechnology, metal NPs have been emerging as means of enhancing the antiviral properties that metal-based agents are endowed.

A lot of promising findings have been reported but these still have to be transformed into clinically relevant findings.

Acting in combination or individually, newly developed drugs have the particularity to be robust, i.e., remain effective over a longer period of time. If such drugs have added advantage of plasma bioavailability, decrease in the incidence of drug resistance and better management of HIV/AIDS could be a reality.

References


6. (Missing)


