Nanomedicine for Infectious Disease Applications: Innovation towards Broad-Spectrum Treatment of Viral Infections

*Joshua A. Jackman, Jaywon Lee, and Nam-Joon Cho**

***N**anomedicine enables unique diagnostic and therapeutic capabilities to tackle problems in clinical medicine. As multifunctional agents with programmable properties, nanomedicines are poised to revolutionize treatment strategies. This promise is especially evident for infectious disease applications, for which the continual emergence, re-emergence, and evolution of pathogens has proven difficult to counter by conventional approaches. Herein, a conceptual framework is presented that envisions possible routes for the development of nanomedicines as superior broad-spectrum antiviral agents against enveloped viruses. With lipid membranes playing a critical role in the life cycle of medically important enveloped viruses including HIV, influenza, and Ebola, cellular and viral membrane interfaces are ideal elements to incorporate into broad-spectrum antiviral strategies. Examples are presented that demonstrate how nanomedicine strategies inspired by lipid membranes enable a wide range of targeting opportunities to gain control of critical stages in the virus life cycle through either direct or indirect approaches involving membrane interfaces. The capabilities can be realized by enabling new inhibitory functions or improving the function of existing drugs through nanotechnology-enabled solutions. With these exciting opportunities, due attention is also given to the clinical translation of nanomedicines for infectious disease applications, especially as pharmaceutical drug-discovery pipelines demand new routes of innovation.*

J. A. Jackman, Prof. N. J. Cho
School of Materials Science and Engineering
and Centre for Biomimetic Sensor Science
Nanyang Technological University
50 Nanyang Drive, 637553 Singapore
E-mail: njcho@ntu.edu.sg
Prof. N. J. Cho
School of Chemical and Biomedical Engineering
Nanyang Technological University
62 Nanyang Drive, 637459 Singapore
Prof. J. W. Lee
College of Business
Korea Advanced Institute of Science and Technology
85 Hoegiro, Seoul 130–722, Korea

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1. Introduction

Nanomedicine represents an emerging frontier in clinical medicine that harnesses our increasingly sophisticated control over nanomaterials design and fabrication to yield novel solutions to human health challenges.[1] In the nanoscale regime (typically defined as one dimension of less than 300 nm), materials may exhibit unique optical, electrical, and mechanical properties.[2] The most mature and widely explored area of nanomedicine lies in cancer therapy, with nanomaterials demonstrating enhanced permeability and retention that facilitates drug delivery to angiogenic tissues. Other fields of clinical medicine are similarly poised to benefit from nanomedicine, although applications generally remain

in the developmental stage. This potential is particularly evident for infectious diseases, which are one of the leading causes of global mortality (≈20%) and are responsible for some of the most serious public health crises in the world.[3] Indeed, there is growing recognition that the continual emergence and re-emergence of infectious diseases has exacerbated classical, single-pathogen targeting strategies, and new approaches are needed. Nanomedicine strategies have been increasingly adopted to deal with bacterial and fungal infections in clinical settings, however, applications against viral infections remain sparse and exploratory.[4] Herein, we present a conceptual framework which envisions new possible classes of nanomedicines that eclipse present therapeutic capabilities for combating viruses, one of the most prolific classes of infectious pathogens. Antiviral medicine as a whole encompasses great breadth and no single account or concept can take into account all of the ongoing research in the field or the challenges that lie ahead. The main objective here is to present a new vantage point looking at how nanomedicine strategies inspired by lipid membranes may form the basis for improved antiviral therapies.

Viruses are obligate intracellular parasites that consist of infectious virion particles, which are typically between 50 and 1000 nm in diameter. Viruses lack independent replicative capacity, and must instead hijack the replication machinery of infected cells. Hence, there is an intimate relationship between viruses and infected cells, and a core element underlying this relationship is lipid membranes which are involved in mediating virus infection and replication. The importance of lipid membranes is especially true for enveloped viruses, which include HIV, hepatitis C (HCV), influenza, and Ebola because these viruses possess envelope coats—lipid bilayers surrounding virus particles—that are derived from host cell membranes. At present, direct-acting antiviral drugs primarily target specific viral proteins, and this approach is setback by the rapid emergence of drug-resistant virus strains due to error-prone virus genome replication. On the other hand, targeting the virion envelope is therapeutically attractive because its lipid components originate from host cell membranes and there is a significantly higher barrier to the development of drug-resistant virus strains, opening the door to broad-spectrum antiviral strategies.

The goal of this concept article is to outline a few emerging approaches for developing and refining new classes of nanomedicines with antiviral activity against enveloped viruses. The approaches discussed herein are motivated by the possibilities afforded by lipid membranes—a ubiquitous element in the virus life cycle—as inspiration for antiviral strategies. By taking into account the diverse roles in which lipid membranes facilitate virus infection, replication, and morphogenesis, several targeting strategies are discussed. Compared to more traditional classes of antiviral drugs, nanomedicines demonstrate unique possibilities for targeting viruses as demonstrated by discrete individual examples. Successful implementation of nanomedicines for broad-spectrum antiviral applications will ultimately require integrating knowledge and experiences from clinical medicine, engineering, nanotechnology, business innovation, and related fields.

Joshua Jackman is a Nanyang President's Graduate Scholar and PhD candidate in the group of Prof. N.J. Cho at the School of Materials Science and Engineering, Nanyang Technological University. He received his BS degree in Chemistry from the University of Florida and has received fellowships from the Arnold and Mabel Beckman Foundation and the National Science Foundation. His research interests lie at the interface of materials science and biotechnology, with an aim towards infectious disease diagnostics and therapeutics.

Jaywon Lee is an Assistant Professor in the College of Business at the Korea Advanced Institute of Science and Technology (KAIST). He earned his BS in Business Administration at the University of California, Berkeley and a PhD in Accounting from Columbia University. His research interests include science and technology innovation, biomedical innovation, science commercialization, business development and R&D, and enterprise valuation. Prior to his academic career, he worked in the accounting and financial industries.

Nam-Joon Cho is Nanyang Associate Professor in the School of Materials Science and Engineering at Nanyang Technological University and Deputy Director of the Nanyang Institute of Technology in Health and Medicine. A recipient of the NRF Fellowship in Singapore, he received his PhD in Chemical Engineering from Stanford University and completed a postdoctoral fellowship at the Stanford University School of Medicine. His research focuses on engineering artificial lipid membrane and tissue platforms to probe biological systems, and to translate scientific knowledge into enhanced therapeutic and drug-delivery options that more effectively target infectious diseases, inflammatory disorders, and cancer.

2. Targeting Lipid Membranes in the Virus Life Cycle

Despite the wide variance in enveloped viruses, there are also many common trends. The roles of lipid membranes in the virus life cycle demonstrate striking similarities across many viruses. Here, we describe important steps in the virus life cycle in the context of developing targeting strategies for nanomedicines.

2.1. Infection

Virus infection is initiated by virion attachment to receptors on host cell surfaces (**Figure 1**a).[5] Following attachment, a variety of nonendocytic and endocytic routes may be utilized for internalization depending on the virus. For enveloped viruses, a critical step is eventually membrane fusion between viral and cell

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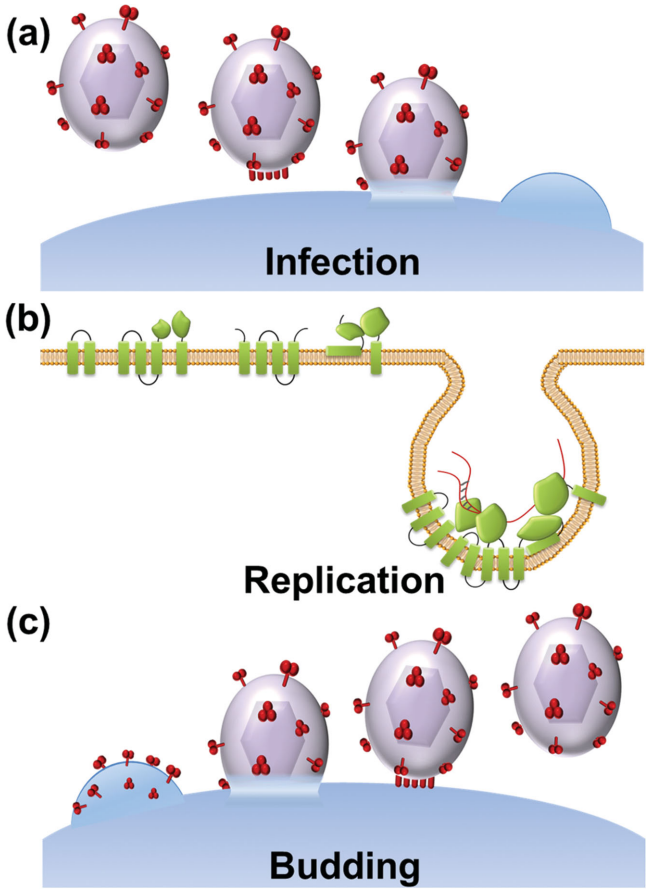

Figure 1. Role of Lipid Membranes in the Virus Life Cycle. (a) Virus infection occurs on host cell surfaces. Virus attachment to cell surface receptors is a critical step and typically initiated by envelope proteins (red spots) attaching to receptors on the cell surface. (b) Viral genome replication occurs in close association with host intracellular membranes. Viral proteins (depicted in green) assemble into multiprotein replication complexes that generate new copies of the viral genome. (c) Many viruses bud from host cell membranes forming a lipid envelope coating that surrounds virus particles.

membranes. Conformational changes in envelope proteins are triggered by cellular or environmental factors and provide the necessary driving force for membrane fusion. Two key factors strongly influence this process, the density of envelope proteins and the virion lipid composition. The protein density is important because synchronous conformational changes must occur in order to provide sufficient energy. In addition, depending on its shape, a lipid may favor positive or negative membrane curvature.[6] During the fusion event, there is an intermediate hemifusion state that has net negative curvature and this state carries an energetic cost. Hence, there must be sufficient energy to pass through the intermediate state. From this perspective, inhibiting virus infection may arise from blocking virus attachment to cell surface receptors or preventing membrane fusion.

2.2. Genome Replication

Many viruses conduct viral genome replication in association with intracellular membranes (Figure 1b).[7] In addition to structural proteins found in mature virions, the virus genome also encodes nonstructural proteins critical for viral genome replication inside host cells, yet absent from virions. A collection of nonstructural proteins forms a replicase complex, which carries out viral genome synthesis. Depending on the particular virus type, this complex forms in conjunction with membranes from various organelles (e.g., endoplasmic reticulum, mitochondria) and induces membrane alterations, including membrane invaginations and vesicle clustering. Although the specifics are not well understood, these rearrangements are important because they help sequester high local concentrations of nonstructural proteins and other necessary factors. For some viruses, it is understood that membrane alterations can be induced by specific individual types of nonstructural protein alone, while other viruses may require more than one type of protein. Cellular factors are also involved in mediating formation of the membrane-associated replicase complex, including specific proteins and phospholipids that may recruit specific components and/or stabilize the complexes. Above all, it is important to emphasize that membrane association is a key and necessary part of viral genome replication for many viruses. Accordingly, blocking membrane association of certain nonstructural proteins can inhibit formation of the replicase complex.

2.3. Virion Budding

Host cell membranes are intrinsically linked to the properties of enveloped virions (Figure 1c). This connection reflects evolutionary adaptations to increase viral spread by extending survival of host cells.[8] When nonenveloped viruses exit host cells, membrane lysis is required and the cell dies. By contrast, enveloped viruses bud from host cell membranes allowing the host cell to survive and continue producing virions. Virus budding can occur on plasma membranes, which directly leads to extracellular release, or it otherwise takes place on intracellular membranes. In either case, budding is preceded by membrane association of viral envelope proteins which induces membrane curvature leading to the budding process. Together with the envelope proteins, other viral core materials are localized to the membrane and the budding process results in the formation of enveloped virions. The lipid composition of the envelope depends on the membrane of origin and also the mechanism of budding. For some viruses, budding is proposed to occur from raft-like microdomains and the viral envelope can have significant differences in lipid composition compared to the parent cell membrane on the whole. As the viral envelope is critical for infection, lysing the membrane or modifying its properties are potential antiviral strategies.

3. Nanomedicine Strategies

3.1. Decoy Receptors of Virus Infection

In general, virus infection is initiated by virion attachment to cell surface receptors through envelope proteins. The membrane-bound cell receptors are typically proteins or

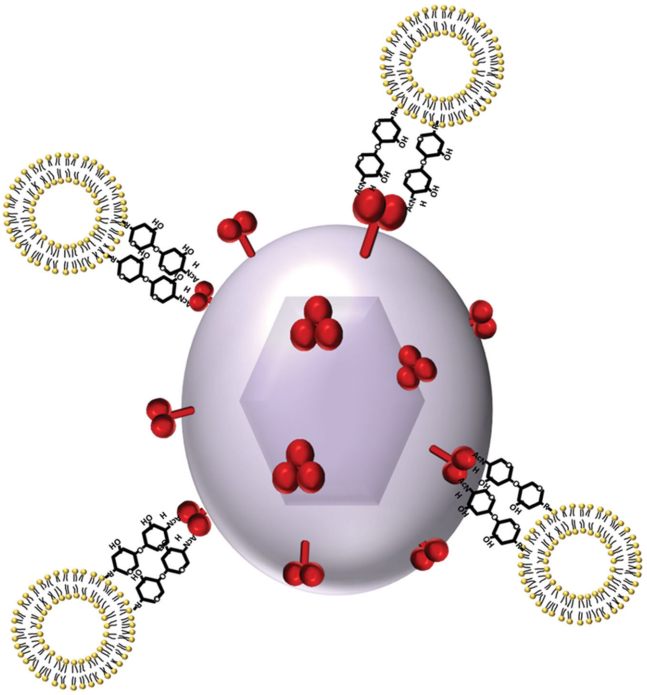

Figure 2. Nanoparticle Decoys Block Virus Infection. Nanoparticles functionalized with host cell surface receptor mimics can competitively bind to viral proteins on the envelope surface, thereby preventing the viruses from attaching to host cells. In this example, liposomal nanoparticles are functionalized with glycolipids which mimic cell surface glycans.

glycans, and developing strategies to block virus attachment by using decoy receptors is a promising antiviral approach. In particular, decoy receptors—solubilized forms of natural receptors added exogenously—can competitively bind to virions and prevent infection (**Figure 2**). In order to achieve this goal, the presentation and structure of decoy receptors must be carefully taken into account and multivalency plays an important role in virus-receptor interactions because the monovalent interactions are often weak, especially those involving glycans.[9] In many cases, generic sialic receptors have been employed with various nanoparticle systems, while more recent work has taken advantage of longer-chain representations of the fine glycan structure in fluidic configurations. Hendrik et al.[10] reported the development of liposomal decoys to capture influenza virus and delay disease progression through virus pretreatment. The liposomes contained a glycolipid, which mimics key architectural elements of a glycan receptor found on human lung cell surfaces. The liposomes demonstrated strong binding capacity for influenza virions, and competitively inhibited influenza virus infection. Extending the scope of this platform, a follow-up study[11] reported the development of decoy liposomes targeting a broader spectrum of viruses, including respiratory syncytial virus (RSV) and herpes simplex virus (HSV). In this case, the constituent glycolipid presented heparin sulfate, which is another known receptor for many types of viruses. It was critical to use a shortened, functional equivalent of heparin sulfate because the full-length version displays anti-coagulant activity. Gold nanoparticles have also been functionalized with a heparin sulfate mimic in order to block HSV infection. Interestingly, it was observed that the gold nanoparticles are also internalized by cells, however, cell uptake did not increase cell permissivity to infection and did not confer intracellular antiviral activity beyond infection blocking.[12] In another recent report, it was shown that the extent of influenza inhibition depended on the size of sialic acid-functionalized gold nanoparticles,[13] leading to a general model to optimize the design of decoy receptor systems based on the combination of steric shielding and multivalency effects.[14,15] Taken together, these examples highlight the possibilities afforded by utilizing liposomes and other nanoparticle systems as vehicles to display multivalent configurations of engineered decoy receptors in order to selectively and potently inhibit virus infection.

3.2. Blocking of Membrane Fusion

The virion envelope plays an integral role in mediating membrane fusion that leads to the cellular entry of attached virions (**Figure 3**). Even when a virion is attached to a cellular membrane, infection can still be impeded provided membrane fusion is blocked. Several compounds have been recently discovered that interfere with the virion envelope, abrogating virus-cell fusion and leading to highly potent broad-spectrum antiviral activity.[16–18] Original findings suggested that these molecules, like lysophospholipids, induce positive curvature in the envelope which increases the activation energy for fusion. However, emerging evidence points to a common mechanism of photosensitization by hydroxylating unsaturated phospholipids, which increases membrane ordering and reduces membrane fluidity to inhibit fusion.[19–21] This mechanism helps to explain the strikingly low nanomolar IC_{50} values, and the drug candidates are particularly attractive for photodynamic therapy. Similarly, glycyrrhizin, a medicinal compound found in liquorice roots, exerts broad-spectrum antiviral activity by suppressing membrane fluidity in virion envelopes.[22] Self-assembling peptide nanotubes with non-virocidal membrane activity have also been identified that selectively inhibit hepatitis C virus particles at the stage of virus-cell fusion.[23] As such, there are promising examples of drug candidates in this class, and further exploration of nanomedicine vehicles for drug delivery options could enable higher selectivity for viral over host cell membranes.

3.3. Lysis of Virion Envelopes

Another class of envelope-targeting agents encompasses membrane-lytic agents that permeabilize virion envelopes, resulting in structural disintegration of virus particles (**Figure 4**). The most notable of these agents includes two amphipathic, α-helical peptides, denoted in the original studies as AH[24] and C5A,[25–27] respectively, which display broad-spectrum antiviral activity against enveloped viruses. Compared to antibacterial peptides (more commonly referred to as antimicrobial peptides, although this term does

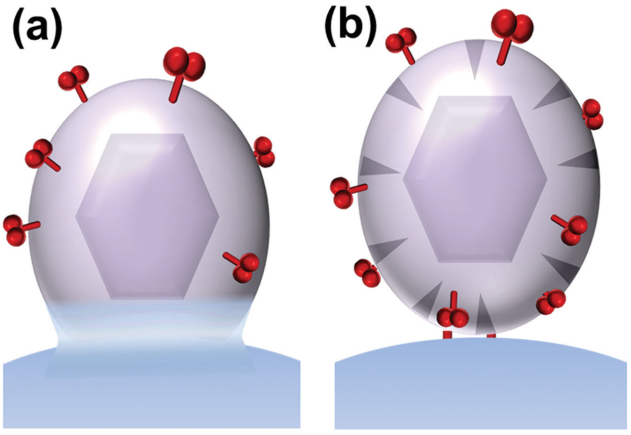

Figure 3. Approaches to Inhibit Virus-Cell Membrane Fusion. (a) Virus infection typically involves membrane fusion between enveloped viruses and host cells. (b) Certain classes of antiviral agent alter the membrane properties of virus envelopes in order to prevent membrane fusion.

not distinguish between viral and bacterial targets) which are a long-established class of membrane-active peptides, these antiviral peptides possess notable differences including high membrane partitioning and weak membrane charge selectivity.[28,29] AH peptide also exhibits unique membrane curvature sensing activity, with selective targeting of membrane-enclosed objects below approximately 160 nm diameter.[30,31] Thus far, C5A peptide has been explored furthest in terms of clinical potential. It offered complete protection against vaginal HIV transmission as a topically administered antiviral agent in a mouse model,[32] and could be encapsulated in a subliming matrix for controlled release and prolonged delivery.[33,34] Importantly, nanomedicine demonstrates strong potential to overcome one major challenge limiting clinical applications of peptide therapeutics, which is a short half-life. C5A peptide-polypeptide nanocomplexes with 35 nm diameter have been formulated and shown to reduce HIV load in a humanized mouse model.[35] Aside from these peptides, certain medium-chain fatty acids and monoglycerides are also known to disintegrate virion envelopes.[36] Notably, glycerol monolaurate prevented mucosal simian immunodeficiency virus transmission in a rhesus macaque model.[37] While peptides and surfactants have been the main agents explored for virucidal activity and benefit from nanotechnology-enabled formulations, several nanomaterials themselves have also been reported to destabilize enveloped viruses, including graphene oxide surfaces[38] and silver nanoparticles[39–41] as well as polymeric thin films.[42] Collectively, there are many opportunities to incorporate membrane-lytic agents into prophylactic strategies, especially topical and disinfectant applications, and further investigation of therapeutic strategies across the range of lipid envelope-targeting agents is also warranted for extracellular virion impairment.

3.4. Regulation of Host Cell Components

As parasites, viruses critically depend on host cell components for genome replication and generation of virion progeny. Depleting the host cell of important factors required for the virus life cycle is a promising therapeutic strategy that has benefited from nanomedicine tools. Iminosugars are inhibitory compounds that prevent enzymatic folding of viral proteins, thereby hindering the assembly of infectious virions. Although iminosugars are therapeutically attractive, it is difficult to achieve high serum concentrations. In order to address this problem, iminosugars can be encapsulated in liposomes enabling superior drug delivery with improved serum concentrations and potent antiviral activity against HIV[43] and dengue virus infections.[44] Endoplasmic reticulum-targeting liposomes containing polyunsaturated fatty acids have also proven successful to reduce cellular levels of cholesterol: an important building block for virion envelopes.[45] Treatment hinders virus secretion and infection, the latter resulting from production of cholesterol-deficient virions. Recently, it was also discovered that phosphoinositides mediate replication of the HCV viral genome by acting as a receptor for an important replicase complex protein.[46] It was proposed that phosphoinositides may also be involved in the genome replication of other viruses, and the ligand-receptor interaction could be blocked by competitive binding to an inhibitor. In addition, functionalized gold nanoparticles can bind to a cell surface receptor and block HIV infection.[47] Looking forward, it will also be important to understand how depletion of critical cellular factors influences host cells. In some cases, there is inherent redundancy in the functions of various cellular

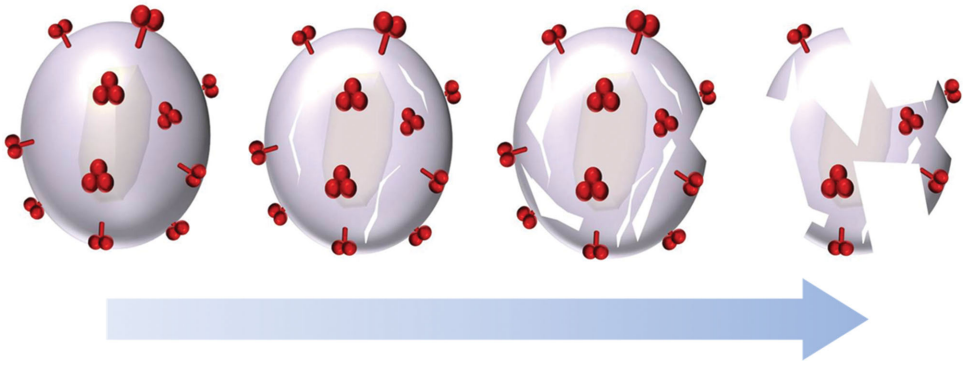

Figure 4. Degradation of Virus Envelopes by Membrane-Active Antiviral Drugs. Some agents exhibit virucidal activity and induce degradation of virus particles.

factors such that blocking one factor involved in the virus life cycle may not dramatically impair cellular function. However, the longer-term implications of the interplay of cellular factors remain to be understood in the treatment context.

4. Roadmap to Clinical Translation

As can be seen above, there are many promising therapeutic strategies for developing nanomedicines as broad-spectrum antiviral agents. Indeed, nanomedicine agents offer the potential to increase the potency and targeting specificity of existing antiviral drugs while also serving as the basis for novel therapeutics. Liposome drug delivery systems are among the most clinically advanced nanomedicines and offer a possible nanomedicine strategy that could be incorporated into viral treatment strategies to improve the therapeutic properties of antiviral drugs. One example of this kind is the development of a liposome microbicide formulation for vaginal delivery of octylglycerol to prevent HIV transmission.[48] This move would be a natural extension of ongoing liposomal applications in cancer therapy, where a wealth of experience with manufacturing and quality control has already been accrued. There is also established precedence for treating systemic fungal infections with AmBisome, which is a liposomal formulation of amphotericin B.[49] While liposomal formulations could improve antiviral therapies, we believe that the greatest benefits of nanomedicines will ultimately be realized through enabling new functionalities and targeting opportunities with additional classes of nanomaterials as active agents or delivery vehicles. The comparable size scales of nanomedicines and viruses permit new modes of inhibition such as membrane-mimicking decoy receptors. Such strategies are particularly advantageous because viruses cannot evade them without simultaneously diminishing viral transmissibility or infectivity. In this regard, ex vivo studies have been reported that involve virus pretreatment with nanomedicine agents (e.g., gold nanoparticles[50] or administration of the nanomedicine agent to the test animal before virus inoculation.[35] It is important to extend translational research on this subject in order to explore strictly in vivo therapeutic demonstrations and to understand how nanoparticle properties can be tuned beyond antiviral activity in order to also take advantage of other attractive features, including optimal pharmacokinetics and tissue distribution of nanomedicines.

Looking forward, targeting opportunities involving lipid membranes are particularly attractive for several reasons. The lipid membrane surrounding enveloped viruses is essential for the virus life cycle, yet derived from host cells leaving it susceptible because it cannot rapidly evolve into a drug-resistant form like other viral components. Nanomedicines encompassing membrane-active compounds can either impose physical restriction on membrane curvature of the envelope or induce membrane lysis. In both cases, the virion envelope becomes functionally inactive diminishing infectivity. These options are well-suited for extracorporeal applications such as blood-cleansing in order to reduce viral titer. Similar strategies have been attempted with filtration of bloodborne pathogens by virus capture, although they require extracorporeal filters. Administration of an antiviral agent that reduces viral titer through virion lysis would be therapeutically attractive. In addition, membrane-active antiviral compounds are increasingly gaining traction as topical microbicides, with demonstrated in vivo activity. The incorporation of nanomedicine strategies such as controlled release formulations will further improve these capabilities.

With so much ongoing research directed at nanomedicines for antiviral applications, it is only a matter of time before clinical-stage antiviral nanomedicines become widely available. The conceptual basis motivating the use of nanomedicines is established, however, practical considerations will need to be addressed, including safety, regulation, and manufacturing.[51] From our viewpoint, the following questions are among those which must be answered during the development stage of a nanomedicine: i) How does a nanomedicine address the clinical problem in a superior way?; ii) Can the nanomedicine be manufactured on a sufficiently large scale?, and; iii) is there justification for the additional costs needed to produce a nanomedicine, including quality control?

Addressing these questions is important because nanomedicine approaches to infectious disease treatment represent a major shift in targeting strategies. In recent years, pharmaceutical drug discovery has been saddled by increasingly high costs and stagnant approval rates. This trend has led large pharmaceutical companies to adopt risk-averse positions in research and development, precisely at a time when new innovation approaches are sorely needed.[52] By enabling new functionalities, nanomedicines offer a compelling solution that increases the prospects for broad-spectrum therapies. Through drug discovery or repurposing (or one could rather say, multi-purposing), the development of broad-spectrum strategies would diversify business risk while leading to greater revenue potential with prorated expenses. In this regard, nanomedicines also lend unique opportunities to enhance the therapeutic potential of clinically approved drugs through one or more ways, including increased selectivity and localized delivery. Taken together, nanomedicines are poised to lead to breakthroughs in broad-spectrum antiviral treatments. Continued advancement of lead nanomedicines will strongly position them for market entry at a time when the pharmaceutical industry is looking to new possibilities for disruptive innovations.

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