Interview

Nanomedicine and the fight against HIV/AIDS

Ahead of the 4th Annual Meeting of the American Society of Nanomedicine, this collection of interviews brings together experts from the fields of nanomedicine and HIV/AIDS treatment. Professor André Nel gives us a general introduction and update on the nanomedicine field and how he hopes it will progress. Professor Susan Swindells describes the current challenges faced in the clinic for HIV/AIDS treatment. Professor Tatiana Bronich explains the research efforts being undertaken by the nanomedicine community for the treatment of microbial infections and HIV/AIDS specifically. Finally, Professor Howard Gendelman looks to the future and assesses the potential and challenges of nanomedicine approaches for HIV eradication.

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Professor André Nel (UCLA, CA, USA)

■ What are the most promising nanomedicine approaches to traditional disease therapies that are currently being developed?

The biggest promise in diagnostics is the development of highly sensitive biomarkers of disease at the femtomol and attomol detection levels, with the additional ability to miniaturize the instrumentation to provide lab-on-chip approaches that will begin to replace methods such as PCR or ELISA. Multiplexing portable devices that can give instantaneous readouts of biomarkers of disease at the bedside or in the home is emerging for monitoring disease and point-of-care diagnostics. There has been a consistent improvement of nanocarriers that deliver drugs, including those designed to treat cancer. While we are observing that polymers and polymeric micelles are taking their place alongside liposomes, we are also starting to see the emergence of multifunctional nanocarriers of inorganic or hybrid composition. It is also becoming clear that high-throughput methods to elucidate the interaction of nanoparticles and nanocarriers with various nano–bio interfaces can be used to speed up the rate of discovery and provide design principles for making new pharmaceuticals. Nanotechnology is also being widely applied for antibacterial effects in the hospital environment and is also poised to make an impact on the treatment of infectious disease. Utilizing the unique capabilities of nanomaterials to provide antigen delivery, adjuvant effects and immune modulation, we are also beginning to observe the impact of nanotechnology on vaccine development.

■ What are you currently working on in your laboratory?

The research in my own and affiliated groups at UCLA (Center for Environmental Implications of Nanotechnology, UCLA Center for Nanobiology, UCLA NanoMedicine Working Group, UCLA Nanomachine Center; CA, USA), is in three broad research areas. The first area is focused on more basic discovery at the nano–bio interface [1–3]. The goal is to elucidate the structure–activity relationships that reveal how the unique physicochemical properties of engineered nanomaterials can be used for manipulating biological nanoscale systems or for safer and improved design [1,4,5]. This interfacial and nanoparticle colloid science is utilized in rapid-throughput discovery platforms to screen for nanomaterial hazards or for adapting nanocarrier properties to enhance pharmacokinetics (PK), pharmacodynamics (PD) and drug efficacy [5,6]. The second major area is the development of nanocarriers for the delivery of cancer chemotherapeutic agents, for example, for pancreatic and lung cancers. We frequently use a multifunctional mesoporous silica delivery system to perform nanocarrier design [7]. An example of a recent accomplishment is the development of a nanocarrier that targets the dense dysplastic stroma in pancreas carcinoma, thereby allowing the egress of a second wave of nanoparticles that carry gemcitabine [8]. In addition to being

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able to package a variety of hydrophilic and hydrophobic drugs, the mesoporous silica nanoparticles can also be fitted with a polymer to assist their biodistribution, or fitted with nanovalves that provide on-demand delivery [3,6]. We also alternate the use of mesoporous nanoparticles with complementary delivery platforms (e.g., liposomes and polymers) where necessary to make use of a comprehensive nanotechnology toolbox for a clinical problem-driven design. The third area of research is the development of a comprehensive nanomaterial safety platform, in which we use compositional and combinatorial nanomaterial libraries, toxicological injury pathways, high-throughput screening and computational modeling to survey large groups of nanomaterials to provide hazard ranking, structure–activity analysis, safer by design strategies, and a risk translation platform for regulatory purposes [5,9,10]. This information is also used to improve therapeutic nanocarrier design.

■ **What are the most important challenges that should be addressed by the nanomedicine community?**

Now that the hype is over, it is time to show that the futuristic visions for nanomedicine can be reduced to marketable products that can solve real-world medical problems. Because the rapid development of nanoscience has outstripped the level of understanding of what this science could do for medicine, most physicians are still ignorant about the practical use of nanotechnology. Therefore, a key challenge is to inform and invite physicians to bring their problems to multidisciplinary scientific teams in which physicians, materials scientists, physicists and chemists work together to treat disease. A massive toolbox that can be implemented to solve biomedical problems has gone untapped because of a lack of clinical awareness. We see too often that a potential nanocarrier or imaging agent is being developed without taking into consideration the biological obstacles and pharmaceutical challenges. For instance, we frequently hear about the use of active and passive delivery of nanocarriers to treat cancer, but do not hear enough about the diverse challenges presented by complicated cancer cell biology and heterogeneity of the tumor environment. It is not practical to assume that nanocarriers will readily make it to the cancer site when complexity such as a dysplastic stroma, pericyte coverage of vasculature or a high interstitial pressure may interfere in carrier access. If one moves beyond the cell to intact organisms and humans, a big challenge becomes how to study the nanomaterial in a more complex biological environment to address its PK, processing, fate and dosimetry. This discovery is also important from the perspective of the safety of nanomaterials based on nanoscale considerations. In addition, it is important to consider whether the introduction of nanoscale properties along with other pharmaceutical principles generate new levels of hazards not included in traditional methods of drug safety assessment.

■ **What more needs to be done to ensure the clinical translation of promising nanostrategies for disease therapy?**

A big hurdle is to relay information about nanoscience and nanomedicine to healthcare providers in a format they can understand and implement. This includes a need for education of healthcare providers to allow them to bring practical problems to nanotechnology experts. Indeed, it is always important to think about nanotherapeutics from the perspective of clinical translation. Currently, there is an explosion of production, and synthesis of a large number of smart nanomaterials for which there is no practical outlet because the science is far ahead of the implementation. This may lead to the impression that nanotechnology is engaged in developing gimmicks. Nanomedicine should become more practical to include concepts such as the rapid and easy synthesis to allow manufacturing and good control of the colloidal stability of nanocarriers to make systemic drug delivery possible. The carriers being developed for drug delivery should have a high loading capacity and systemic distribution properties to allow favorable PK and therapeutic efficacy. The material should degrade into nontoxic components and there should be no premature release of the drug. The entire production process should be scalable to make the product marketable. Where possible, smart and iterative design approaches (e.g., through

the use of high-content screening) should be used to reduce the number of animal studies. It is also important to address the safety assessment and design.

■ What areas of the field are you most excited about?

From a personal perspective, I'm most excited about developing drug carrying nanocarriers for cancer treatment, as well as using engineered nanomaterials to provide immune modulation and vaccine development. Two current areas of nanocancer research in my laboratory are the development of nanocarriers for treatment of lung and pancreatic cancers. Here nanotechnology can play a unique role. For example, the desmoplastic stroma that surrounds pancreatic cancer plays a big role in the resistance to cancer drugs and therefore needs to be considered as a first step toward the development of nanotherapeutics. We are in the process of developing a dual wave nanotherapeutic approach, in which the first wave of nanoparticles delivers a drug that interferes in stromal obstruction of vascular fenestrations, while the second wave of nanoparticles will deliver gemcitabine once the stromal obstacle is removed. A similar, engineered approach can be used to treat lung cancer, where a key challenge of a potentially breakthrough synergistic drug combination is to reduce the toxicity of one of the drugs by a dual drug delivery system using optimized ratiometric drug design. For vaccine development, we have synthesized engineered aluminium oxyhydroxide nanorods that quantitatively improve dendritic cell activation over aluminium, and are being used to develop better adjuvants. In summary, I am excited by my own research that uses a versatile nanotechnology toolbox to bring an engineered approach to medicine by introducing biophysicochemical principles and interfaces.

■ How do you see the field progressing in the next 5 years?

- Medical diagnostics: orders of magnitude increase in sensitivity, selectivity and multiplexing function will improve point-of-care diagnosis and treatment. These capabilities will allow clinicians to track and treat disease much earlier than with conventional tools. Nanodiagnostic tools will become one of the backbones of point-of-care delivery by 2020, making the transition from remote laboratories to hospitals and then eventually the home;
- Nanotherapeutics: challenges such as PK, biodistribution, targeting, tissue penetration and loading capacity will be overcome, and major nanotherapeutics will be adopted by the industry. A large percentage of all drugs will use nanoenabled principles by 2020. Many of these will be for difficult to treat cancers like pancreatic cancer, liver cancer, ovarian cancer and glioblastoma, where patient prognosis is poor with current therapies;
- Stem cells: nanoenabled stem cell-based therapies will be in widespread use by 2020. Nanobiology and nanomedicine will aid in the understanding and control of stem cell differentiation and the transition of stem cells to widespread medicinal application;
- Tissue engineering: nanotechnology strategies will rapidly enable the growth of clinically relevant tissue regeneration and repair strategies.

■ Have you seen any recent exciting advances in antimicrobial therapies with nanomedicines? Do you believe there is potential for HIV/AIDS prevention & long-term treatment with nanomedicines?

There is considerable interest in the antibacterial effects of metal and metal oxide nanoparticles, as well as carbon nanotubes for infectious disease or biofouling applications. From the perspective of nanomedicine, the development of nanoantibiotics constitutes an exciting advance. This could take the form of metal and metal oxide nanoparticles that are surface functionalized (e.g., cationic) to treat bacterial biofilms. Metal and metal oxide nanoparticles induce bactericidal effects by the generation of reactive oxygen species and membrane damage, which differs from the mechanisms of action of traditional antibiotics. Therefore, applications may emerge in which the unique properties of these materials are combined with antibiotics to

find a new way of treating drug-resistant bacteria by making use of nanomaterial properties that cannot be subjugated by genetic mutations. Nanocarriers can also be used for intracellular antibiotic delivery in diseases such as tuberculosis and other intracellular infectious disease agents. Finally, in the area of HIV therapy, the delivery of biocides by nanoparticles or hydrogels can be used to prevent mucosal infection.

Professor Susan Swindells (University of Nebraska Medical Center, NE, USA)

■ What are the therapies currently widely available for HIV/AIDS?

There are 25 antiretroviral agents approved by the US FDA, and many of them are coformulated into combination tablets containing two, three or four drugs [101]. Used in combination regimens of at least three agents, currently available therapies have improved steadily over the last three decades. Newer drugs have novel mechanisms of action, improvements in potency, including against drug-resistant viruses, and better tolerability. Use of coformulated products in particular has allowed decreased pill burden and simpler dosing schedules, with several regimens only requiring one pill to be taken once daily. However, the drugs are expensive and the need for lifelong daily therapy persists, issues that present many challenges to both treating clinicians and the affected population [11].

■ How effective are these current therapies?

Contemporary antiretroviral therapy (ART) has dramatically reduced HIV-associated morbidity and mortality, and HIV disease has been transformed from a lethal illness into a chronic, manageable condition, for those with access to therapy and ability to maintain daily adherence [12]. In addition, ART has been shown to be highly effective at preventing sexual transmission to uninfected persons if the infected partner is successfully treated [13]. However, despite these tremendous gains and the effectiveness of current ART, less than a third of infected persons in the USA have successfully treated disease, as measured by plasma viremia below the level of detection for commercially available assays (typically less than 20–50 copies/ml). The reasons for this are multifactorial and include the fact that new infections continue to occur, HIV infection is undiagnosed in more than 20% of individuals, compliance to antiretroviral medicines continues to be a problem in a segment of infected people and there is a common failure to link and maintain diagnosed patients in continued care [14].

■ When do these therapies generally fail & for what reasons?

ART may fail at almost any time during treatment, although treatment failure becomes less common after years of successful viral suppression. Once an infected patient is linked into care and prescribed ART, sometimes the treatment fails because of pre-existing viral resistance, although this is avoided by resistance testing before ART is begun [15]. Poor PK, perhaps from drug–drug interactions with concomitant medications, may contribute. However, the single most common reason for treatment failure is suboptimal adherence to daily oral therapy. Predictors of poor adherence include drug and alcohol abuse, mental illness and notably depression, lack of patient education, lack of trust in the medicines and the healthcare providers who distribute them, and treatment fatigue [16].

■ What are the toxicities associated with long-term therapy of HIV/AIDS?

With many different medications available and different possible combinations, it is hard to summarize the potential adverse effects. Briefly, the most common side effects of preferred agents are skin rashes, gastrointestinal disturbances, renal and liver toxicity and for efavirenz specifically, neuropsychiatric effects such as dizziness and insomnia. Overall most of these symptoms are mild and easily managed. Certainly the side-effect profile of ART is very different today from 20, or even 10, years ago [101].

■ Which patients would benefit from long-acting antiretrovirals the most?

Depending somewhat on the dosing intervals, long-acting oral therapy would likely

be welcomed by many infected persons, as it would provide relief from the daily routine of taking their ART. However, most of the work I have seen thus far suggests long-acting ART will probably be given parenterally, which is quite a different proposition. Having said that, I can see a niche for certain patient populations – largely those with adherence difficulties. This would be most appropriate as maintenance therapy for patients already on established ART. I would be reluctant to initiate ART with long-acting formulations, in case side effects occurred. ART is also being investigated for prevention of HIV infection in high-risk persons (known as pre-exposure prophylaxis) and long-acting formulations may have a role here too [17].

■ Do you think nanomedicine approaches could aid in HIV eradication?

It is really too early to speculate much on this topic, but a clear obstacle to viral eradication using our current approach is the poor tissue penetration into viral reservoirs, such as the lymphoid tissues, brain and gut, of many ART agents, which can then promote the development and maintenance of viral reservoirs [18]. If nanomedicine approaches could improve this situation by providing better targeting of low-level restricted or latent infections and facilitate the action of drugs aimed at eliminating latent viruses, such approaches could play an important role in such efforts.

■ Can viral eradication be achieved & how do you believe it might be done?

We already have some examples of infected persons with apparent viral eradication, so this certainly appears to be achievable. Unfortunately, the procedures necessary to achieve this included such things as cytotoxic chemotherapy, radiation and bone marrow transplantation, so they are far from generalizable to others. Just the proof-of-concept that this is possible is exciting though, and has renewed enthusiasm in the scientific community and the patient population. After HIV integrates proviral DNA into the genome of CD4 lymphocytes, the virus can either enter a state of active replication, killing the cell, or a long-lived latent state [19]. As ART targets only actively replicating virus, latent cells are not affected and this helps to ensure lifelong persistence even with therapy. Current efforts to purge the reservoir include the so-called 'shock and kill' approach, which includes first activating cells to induce latent virus, then purging with antiretroviral therapy. Unfortunately, these efforts have not been successful to date and it now seems that the reservoir is much larger than was first thought [20].

■ Often the public's perception of nanotechnology is quite negative, do you believe there is interest from the public in nanotechnology approaches to treat HIV/AIDS?

I can only speak from my own experience, which is based on a survey we administered to our clinic patients [21]. When asked their level of interest in long-acting parenteral nanoformulated antiretroviral therapy, the majority of respondents indicated that they definitely or probably would try it. Longer dosing intervals attracted greater interest than shorter intervals, and patients who reported missed doses and those who were intravenous drug users indicated increased interest. These groups may represent those who would benefit most from this strategy to optimize adherence to therapy.

Professor Tatiana Bronich (University of Nebraska Medical Center, NE, USA)

■ What have been the major advances in nanomedicine in the last 10 years for the treatment of microbial infections?

In the past decade, numerous nanotechnology-based platforms were developed and then exploited for the prevention, detection and treatment of microbial infections. These demonstrated that liposomes, polymeric nanoparticles, dendrimers and solid lipid nanoparticles improve the solubility of poorly water-soluble drugs, prolong the systemic circulation lifetime, and facilitate the drug delivery to the site of infection [22]. Currently, the most successful technology involves liposomes with several such drug formulations already on the market or in clinical development. Liposomal amphotericin B (AmBisome®, Astellas Pharma US, Inc, IL, USA) has been widely used

in the clinic to treat fungal infections and notably in immune compromised patients with enhanced therapeutic efficacy, limited side effects and significantly reduced treatment length [23]. Virosomes (reconstituted virus liposomes) have been approved for delivery of surface antigens derived from the hepatitis A or influenza virus (Epaxal® and Inflexal® V, Crucell, Leiden, The Netherlands) [24]. The toolbox of promising antimicrobial agents has been recently extended by development of novel biodegradable antimicrobial polymers based on amphiphilic cationic polycarbonates. These polymers and their nanoassemblies demonstrated broad-spectrum activity against bacterial and fungal infections with demonstrated abilities to disrupt biofilms [25]. Since these agents do not have a specific molecular target as do conventional antibiotic drugs and act as microbial walls/membranes destabilizers, they can prevent the development of pathogen resistance. Significant advances were made in developing nanomedicines for the treatment and prevention of HIV/AIDS [26,27]. Of particular significance are the efforts in development of injectable long-acting antiretroviral drug nanoformulations that can enhance patient adherence to therapy while minimizing drug toxicities [28,29]. Several surface-modified nanomedicines targeting HIV-susceptible cells have shown remarkable ability to increase drug uptake and enhance antiviral effects. A similar approach is also promising for delivering antiretroviral drugs to lymphoid organs. The most advanced application of nanotechnology for immunotherapy of HIV/AIDS is the DermaVir, topical therapeutic vaccine developed by Genetic Immunity (Budapest, Hungary) [30]. Safety, immunogenicity and preliminary efficacy of DermaVir have been clinically demonstrated in HIV-infected human subjects. There has also been progress in development of nanotechnology-based microbicides. As an example, dendrimer-based nanomedicine mixed in carbomer gel (VivaGel®; Starpharma, Melbourne, Australia) is being developed as a vaginal microbicide gel to prevent the transmission of genital herpes and HIV [31]. Initial human trials have shown VivaGel to be safe and well tolerated, and additional clinical studies are ongoing. Moreover, recent findings suggest that mucus-penetrating nanoparticles can improve epithelial distribution and retention times of vaginally administered drug, and, therefore, may significantly improve prevention of sexually transmitted infections [32].

■ **What are the most promising nanomedicine approaches that could be developed in this area?**

The current use of highly active ART regimens that are comprised of multiple drugs to simultaneously hit a number of different pharmacological targets has tremendously improved the treatment of HIV infection and patient's quality of life. However, lifelong treatment is needed because the virus quickly re-emerges from latently infected cells if treatment is stopped. Poor drug regimen adherence during chronic treatment, long-term drug toxicities and appearance of drug resistance often limit the success of the therapy. Long-acting nanoformulations developed to optimize and regulate bioavailability and tissue distribution of antiretroviral drugs, and extend their half-life, thus reducing dosing frequency and minimizing the difficulties with adherence, have a great promise to overcome the limitations of current therapeutic modalities, improve treatment efficacy and tolerability for the patient [17]. Indeed, recent studies in a rodent model have demonstrated that weekly dosing of nanosuspensions of atazanavir and ritonavir (nanoART) maintains therapeutic plasma levels of the drugs at the steady state [29]. As a result, nanoART treatment of HIV-1-infected humanized mice was associated with significantly decreased viral loads. Furthermore, significant concentrations of drug were also detected in various tissues, including liver, spleen and lungs as well as in draining lymph nodes adjacent to injection site for a prolonged period of time post-intramuscular administration. These important findings suggest that prolonged drug release from intracellular depots in tissues may be responsible for the sustained and enhanced PK profile produced by nanosuspensions. Proof-of-concept studies in rats and dogs showed that the non-nucleoside reverse transcriptase inhibitor rilpivirine (TMC278) formulated as a 200-nm nanosuspension could act as long-acting formulations,

releasing rilpivirine up to 3 months after a single intramuscular administration [28]. Currently, another long-acting parenteral formulation of an investigational drug, the integrase inhibitor S/GSK1265744, is undergoing Phase I repeat dose-escalation study in healthy subjects to determine the safety, tolerability and PK profile of intramuscular and subcutaneous injections [102]. Additionally, solid drug nanoparticles are also being explored as an option for oral delivery in HIV/AIDS [33].

■ Do you think nanomedicines could be used to target viral reservoirs?

Yes, nanoscale delivery systems have great prospects for targeting sanctuary sites and eradicating infected cells from viral reservoirs. Several lipid- and polymer-based formulations have already been tested in preclinical studies and have exhibited the propensity for lymphatic system drug targeting [27].

■ How would this work? Can you update us on current work in this area?

First of all, sustained therapeutic concentrations of antiretroviral drugs that can be achieved with the use of nanomedicines over an extended time period may impede virus penetration into remote areas. Viral reservoirs within the cells of the macrophage–monocyte lineage can be directly targeted by nanomedicines because of the inherent phagocytic ability of these cells towards foreign particles. For example, it has been reported that HIV-infected macrophages were able to uptake, retain and subsequently release drug-loaded lipid- or polymer-stabilized nanoparticles, maintaining the drug concentrations inside and outside the cells for prolong periods of time while silencing HIV replication [34–36]. In addition, synthetic versatility and unprecedented possibilities for surface engineering of the nanocarriers allow for their specific targeting to the infected cells. Several recent reports have demonstrated that active targeting of nanomedicines appears to be a useful strategy to improve CNS delivery of antiretroviral drugs. Importantly, since circulating monocytes and macrophages distribute throughout the body including the secondary lymphoid tissue, testes, liver, lungs, gut and the CNS, they can be used as 'natural' vehicles for delivery of nanomedicines to sanctuary sites. Particularly, it has been shown that after intravenous administration nanoformulation-laden mononuclear phagocytes were capable of effectively delivering their drug cargo into brain tissue of the animals [37]. Furthermore, folate-coated nanosuspensions of atazanavir were engineered to maximize entrapment into macrophages *in vivo* which in turn served as 'Trojan horses' to deliver the drug to the lymphoid tissue, an important HIV sanctuary site [38].

Latently infected $CD4^+$ T cells represent a key barrier to virus eradication [39]. One of the HIV purging strategies currently being investigated is a forced activation of latently infected cells to induce virus production, allowing targeting of the cell by the immune response or therapeutics. To minimize off-target effects, HIV latency activators can be packaged into nanoparticles, either alone or in combination with an antiretroviral drug, and selectively delivered to $CD4^+$ T cells using a specific targeting moiety. The first proof-of-concept study has demonstrated that a CD4-targeted liposomal formulation of a PKC activator, bryostatin-2, and protease inhibitor nelfinavir was capable of both activating latent virus and inhibiting viral spread [40]. Therefore, there is a clear promise in the application of nanomedicines to achieve adequate concentrations of the drugs in sanctuary compartments and provide more effective therapy to treat and cure HIV/AIDS.

■ What are the current limitations of nanomedicine for HIV/AIDS treatments?

Most of the nanoformulations and approaches that have been investigated for HIV/AIDS treatments are still in the early preclinical development stages [26]. The challenges remain in achieving a delicate balance between drug release profile and stability of the nanoformulation in clinically relevant biological systems. Indeed, premature drug release can diminish the aforementioned benefits of drug incorporation into nanocarriers without significant improvements of whole-body PK, drug accumulation in the viral reservoirs and, therefore, antiviral efficacy. The safety and biological fate of nanomedicines, including

distribution, accumulation and metabolism, needs to be carefully assessed to ensure meaningful results of the biological studies. It is still unknown whether the long-term use of nanomedicines can abrogate the development of HIV drug resistance especially when nanocarriers contain a single antiretroviral drug. Current combination ART that involves multiple drugs with nonoverlapping resistance profiles is a 'gold standard' for management of HIV-infected patients. However, nanotechnology-based systems containing two or more antiviral drugs have not been developed so far.

■ What modalities can be developed to preclude or deal with such limitations?

The choice of an appropriate nanomedicine platform is dictated by the specific clinical consideration and drug physicochemical properties. Furthermore, the characteristics of nanostructured materials, such as composition, size, polydispersity, shape and surface chemistry play an important role in governing biological behavior, safety and efficacy of the nanomedicines. Therefore, rational design and fine tuning of the nanocarrier properties are necessary for each particular therapeutic agent to ensure that an optimal concentration is maintained at the therapeutic target over a desired time frame. These considerations are especially important upon the design of more complex targeted nanoformulations or highly desirable multifunctional nanomedicines for the codelivery of drug combinations that can lead to reduction of resistance profiles. Furthermore, the development of formulations that will allow incorporation of conventional small-molecule drugs and newer generations of HIV-targeted biopharmaceutics, such as proteins, peptides [41], and RNA- and DNA-based therapeutics [42–44], within the same carrier may be a particularly interesting approach toward effective disease treatment. At the same time nanoformulations that contain combinations of drug and imaging agent would provide an intriguing opportunity to noninvasively monitor the biodistribution and the target site accumulation of the drug/carrier, and will be helpful for predicting therapeutic responses. Recent advances of the nanomedicine-based cancer research directed to development of chemotherapy combination delivery and visualization platforms can help HIV researchers in the search for such formulations [45,46].

■ What are the most important challenges that should be addressed by the nanomedicine community?

One important pitfall with regard to realizing the potential of antiretroviral nanomedicines is related to the availability of the animal models representative for the clinical situation. Existing nonhuman primate models of AIDS generally employ simian viruses divergent from HIV-1 and only partially mimic human disease. Limitations in terms of cost, conduct and availability to investigators further reduce their usefulness in preclinical investigations. Recently developed humanized mice with a lymphoid system of human origin generated subsequent to transplantation of human $CD34^+$ hematopoietic stem cells, or with fetal liver and thymus, have overcome many of these limitations. Indeed, humanized mice can recapitulate central steps in HIV infection, including high-titer viral dissemination, response to antiretroviral drugs, viral failure in the case of nonadherence and, very importantly, viral rebound after therapy interruption [47]. This model will be a key tool in the evaluation of novel treatment strategies and latency. Another important aspect to consider is the short- and long-term toxicity of antiretroviral nanoformulations that also requires a rigorous assessment in the relevant animal preclinical models. Finally, cost–effectiveness, the complexity and scale-up possibilities, and the batch-to-batch variance upon manufacturing are important factors that need to be well thought out upon the design of the nanomedicines for potential clinical translation.

Professor Howard E Gendelman (University of Nebraska Medical Center, NE, USA)

■ What approaches are you & others taking to develop nanomedicine-delivered antiretrovirals?

The approaches taken in formulating ART are directed towards the final goal and that is to improve patient adherence, target

viral reservoirs, reduce toxicities, provide improvements in pre- or post-exposure prophylaxis and facilitate methods aimed at viral eradication. Administration of medicines can be either oral or parenteral. Drugs that have *de novo* longer half-lives, are hydrophobic, have significant antiretroviral activities, show broad therapeutic indices and have limited toxicology would enable development of nanoformulations. Unfortunately, few if any of the currently available ART fit such a bill. The work necessitates tissue, cell and subcellular organelle targeting, polymer encasements that facilitate slow drug dissolution kinetics, evasion of clearance by the reticuloendothelial system (RES) and phagolysosomal degradation pathways while demonstrating improvements in reaching reservoirs of restricted viral infections in the gut, lymphoid tissues and CNS. Optimizing drug crystal and cocrystal formulations, testing excipients, evaluating size, shape and charge of the particles, and, when possible, sustaining the presence of intact particles in stable subcellular structures, such as recycling and late endosomes, are part of an overall approach in formulation development. Formulation approaches include choice of polymers, micellar and gel formulations, altering the drug configuration properties when plausible and using cell-based carrier systems, such as the monocyte–macrophage or dendritic cell, to traffic drugs across tissue barriers and into known sites of viral growth. Any approach must also balance the need for adequate antiretroviral drug combinations that commonly include nucleoside or nucleotide reverse transcriptase inhibitors in conjunction with integrase, protease or non-nucleoside reverse transcriptase inhibitor drugs. The facilitation in development of polymeric formulations that contain multiple drug combinations is optimal but not always feasible. Unlike cancer drugs where drug disposition in the RES is an avoidance maneuver, for HIV/AIDS this is often the site for ongoing viral replication and as such provides a novel opportunity in establishing drug depots [21,48–59].

■ How do you approach this situation?

We have approached this situation by developing cross-disciplinary systems. There are four stages for such developments. First, is making the proper choice of drugs to develop based on the chemical structure and bioavailability. With a large number of possible compounds one needs to be prudent in making choices that are likely to be developed in such measure and could be translatable for human use. The second rests in the particle synthesis. What excipients to use and why? What formulations may be applied? How to achieve optimal encasements and drug loadings? What to do with the shape, size and charge, as this can also affect cell entry and intracellular drug stability? Third, is cell–particle interaction. We have seen that relationships between monocyte–macrophages and particles with regards to nanoART entry, retention, release and antiretroviral activities can predict PK and PD in animals and, therefore, possibly in humans. Moreover, targeting of nanoART to macrophages makes biological sense in a number of separate venues. Macrophages have prodigious abilities to uptake and retain matter; macrophages contain large numbers of endosomes and produce microvesicles and exosomes in relative abundance. The cells readily secrete large numbers of bioactive molecules that affect trafficking of the cell itself; trafficking through intracellular endosomes and close contacts amongst heterogeneous cell types (e.g., the macrophage and T cell) can affect dissemination of particles amongst viral targets and tissues. To this end, our own laboratory has developed a formulation chart scoring system wherein optimal screening and uptake that follow particle–macrophage interactions are used in decision-making processes for which formulations are ultimately tested in animals. Animal testing next moves to linking PK and PD, data (performed in normal BalbC mice) to antiretroviral and immune restorative activities that are usually performed in replicate humanized mouse models for HIV/AIDS. Such testing, when appropriate, is moved to large animal investigations, including monkey testing platform evaluations [52].

■ What drugs are you currently studying & why?

Our research efforts started on a single class of ART and a single administration paradigm for proof-of-concept studies. First,

we used the protease inhibitor, indinavir for early proof-of-concept studies. While the drug is no longer a standard of therapy it was engaged in polymer development, PK, cell carriage and antiretroviral studies. These early evaluations demonstrated that a hydrophobic ART could be readily crystallized and incorporated into a stable formulation for both laboratory and animal testing. Second, we were able to show that while the drug is important to affect cell carriage and PK of the nanoART; the chosen excipient and the size, charge and shape of the particles also proved of significance especially linked to uptake and macrophage carriage. Whether the nanoART was homogenized, sonicated or milled proved important. Notably, the stability of the nanoART particle was linked to endosomal compartments. NanoART readily traffics through early, recycling, late and lysosomal compartments but maintains a sustained presence in the recycling endosome. Therefore, studies of a single drug, indinavir, have permitted insights into particle composition and particle–macrophage interactions. These have provided novel insights into the potential harnessing of monocyte–macrophages as cell carriers and depots for facilitating antiretroviral delivery. Third, we moved beyond indinavir as a single agent to include other protease inhibitors and those that are commonly used in the clinic. Here, ritonavir-boosted atazanavir was developed in independent and combined formulations within the laboratory. The work has now evolved significantly beyond *ex vivo* loading of monocyte-derived macrophages to assess direct parenteral subcutaneous and intramuscular injections in mice. Parallel experiments have not simply assessed PK and PD but explored antiretroviral activities in humanized mice. These boosted atazanavir particles show clear advantages from native drugs in their longer half-lives, tissue depots, diminished toxicities and ease of access in animal systems. Antiretroviral responses are also sustained and notable. However, protease inhibitors alone with or without boosting cannot be translated as a sole administration response. Therefore, we have recently expanded the classes of drugs we are studying to entry and viral integration inhibitors in parallel investigations. Such studies are now underway and open up promising new avenues with drugs of divergent physical chemical properties and antiretroviral activities [61].

■ With such a multidisciplinary effort needed, how easy is it to collaborate with polymer chemists, pharmacologists, biologists, immunologists, virologists & the pharmaceutical companies to develop products?

Arguably we are one of a few laboratories worldwide that has brought such goals to fruition. My own background in immunology and virology has merged with others in polymer chemistry and product development. Let me provide a few examples. Barrett Rabinow, a distinguished scientist at Baxter Healthcare (IL, USA) who was developing an idea of macrophage carriage of nanoART, first contacted me nearly 8 years ago. After a literature search on scientists with backgrounds in macrophage biology and immunology for a project he was developing we stumbled on one another. The idea was to target cells with nanomedicines that serve as reservoirs for virus and a carriage system for antiretroviral drugs with the aim to improve clinical outcomes. He asked if I would be interested in testing this concept in our laboratory. It sounded very intriguing. I embraced the idea and began to engage with him in a very short time period. The project was successful, demonstrated by a number of proof-of-concept findings, and within a few years the US NIH became interested. This included, most notably, the National Institute on Drug Abuse, the National Institute on Aging and the National Institute on Neurological Disorders and Stroke. Each of these institutes subsequently funded the work. When Baxter shifted direction, the pace was maintained through new relationships forged with polymer chemists including (Tatiana Bronich and Xinming Liu), toxicologists (JoEllyn McMillan), pharmaceutical experts and pharmacologists (Yazen Alnouti and Courtney Fletcher), bioimagers (Michael Boska), clinicians (Susan Swindells) and virologists (Larisa Poluektova and Howard Fox). Interactive interdisciplinary grants were begun that enabled us to bring together scientists and clinicians from diverse backgrounds

interested in such works. A company was then established with the intent of bringing drugs to the clinic for once a month or once every other month dosing as well as important relationships with more established pharmaceutical leaders who provided important insights into development and needed partnerships. All came on board. As it became clear the science had real translational value, the University of Nebraska (NE, USA) product development and venture discovery also began supporting this work in parallel efforts.

■ What are the limitations of a nanomedicine approach to deliver antiretrovirals?

There are several limitations. First, many of the drug formulations need to be administered by parenteral injection at doses sufficient to maintain therapeutic plasma levels for weeks or months. Therefore, the actual drug dose administered is considerably higher (beyond an order of magnitude) than what any infected person would see as a daily oral regiment. This can and has resulted in injection site irritation with the possibility of systemic toxicities. Second, the half-lives of the nanoART are linked to both reservoirs at the injection site (muscle and subcutaneous tissue) seen after injection and the tissue reservoir that develops. For the latter, if the particles are not taken up rapidly through the RES and contained in stable form, the particles will undergo liver metabolism and renal excretion. High levels of nanoART that might accumulate in the liver might be subjected to alterations in CYP3A4 activity and, as such, affect a myriad of drug–drug interactions. Third, inherent toxicities could arise from the particle itself, either by immune responses or impurities in the preparation itself. For the latter, it is imperative to optimize good manufacturing and laboratory practices to ensure impurity levels are greatly reduced or eliminated; this is especially critical for endotoxin contaminations. Fourth, with regard to oral drug–drug interactions, the standard drug levels operative, for example, for boosted atazanavir would not inherently be the same in what is approved for human dosing. Therefore, new paradigms need be made and developed when administering long acting combination drugs, such as atazanavir, or other boosted protease regimens. Fifth, yet another potential problem is how to remove the drug in long-acting preparations if allergic reactions do develop to the drug itself or the preparation. There are no effective means to circumvent such a potential problem or possibly a catastrophe in drug administration and pharmaceutics. These and other problems are currently being worked through prior to the more general administration of nanoART as treatments for HIV/AIDS. They have been reported and are being developed by other investigators [62,63].

■ Do you think such an approach could lead to HIV eradication?

Under the correct set of circumstances, I believe the answer is 'yes'. In what circumstances would such eradication of HIV-1 be made possible by nanomedicines? The antiretroviral drug would have to be delivered to the right tissue, the right cell and the right subcellular organelle where active viral replication is operative. It would have to be the same tissues where native or conventional ART does not reach or if it did would gain access in only a subinhibitory concentration. Such tissues would include the brain, the gut and the lymphoid reservoirs. However, bringing the drug to sites of viral growth would not be sufficient to eradicate the virus. A second step would be needed to rid the sites of latent or restricted virus. This would entail destruction of such infected cells either by the immune system after stimulation of latent infection or by facilitating the death of these cells whether they be lymphocytes, macrophages or other cell reservoirs. Notably, bringing a drug that could modulate latent infection to sites of action would be a boost. Whether this would facilitate eradication or simply lower the already low amounts of virus in such circumstances is not known. However, the possibility exists that this type of approach could be successful.

■ What else, beyond what is being researched, needs to be done for this technology to 'cure' HIV?

The main impediment for eradicating virus rests in the destruction of latent virus. Stimulation of latently infected cells is known to be incomplete no matter the drug and so far in testing all latently

infected cells cannot be stimulated in such a way that either rapid cell death ensues or secondary destruction by the immune surveillance T cells occurs. We need both better drugs and better delivery systems.

■ **Will grant agencies support such complex works when oral therapy has already proven to be effective?**

I believe grant agencies will continue to support such works, as adherence and drug accessibility remain a significant problem for HIV/AIDS care. This is ever more an issue because the pathways towards improving access to viral reservoirs by the nanomedicine drugs may make the road towards viral eradication ever more feasible.

■ **How do you think the upcoming American Society for Nanomedicine conference will help to promote nanomedicine efforts in HIV/AIDS treatment?**

The American Society for Nanomedicine meeting being held on 28–30 March 2014 at The Universities at Shady Grove Conference Center (MD, USA) will highlight the development of nanoART as it relates to viral eradication or improvements to drug compliance and accessibility. A large number of scholars will present the latest findings in this new and developing field and active discussion and scientific exchange will lead to new collaborations and sharing of ideas to speed research pursuits and outcomes. We are very excited about the meeting and see great potential for new pursuits and ideas in the nanomedicine of ART.

■ **In the next 10 years, how do you hope the field will progress?**

It is a collective hope that not one but several antiretroviral nanomedicines will be available for the ongoing clinical care of HIV/AIDS. It is our anticipation that in the next 10–20 years such pursuits will not simply lead to improved access and treatment outcomes but also to eradication for HIV infections. This may occur in a number of divergent but not mutually exclusive means. Nanomedicine affords the abilities to bring drugs to both virus-target cells and to subcellular organelles that represent sites of ongoing viral growth including stages of the HIV lifecycle. Chemical modification of all existing drugs would facilitate formation of drug crystals and improve therapeutic indices and biodistributions. Having the drugs encased in polymers would enable slow release of the drug into the circulation from tissue depots within the RES as well as in the site of injection. Drug combinations could be cocrystalized and enable a single injection or alternatively drugs could be given in oral suspensions. Targeting of the antiretroviral drugs could be used in a similar strategy to affect viral eradication or to stimulate immune clearance of already infected cells. Therefore, the directives, taken together, could not simply serve to bring medicines more easily to populations who have limited access or are poorly compliant but could improve bioavailability and treatment outcomes and increase the likelihood of the inevitable eradication of virus.

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