

Reshaping the Future of Nanopharmaceuticals: *Ad Iudicium*

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Advances in nanoscale science and bionanoengineering are revolutionizing our understanding and ability to manipulate complex biological processes as well as the way health care is administered. These approaches and innovations, collectively termed *nanomedicine*, are on the verge of improving disease prevention, detection, treatment, and management.¹ Oncology has been a key beneficiary. First-generation liposomal-based nanopharmaceuticals for cancer treatment reached the global market years ago with outstanding therapeutic efficacies in many cases.^{2,3} Today, creative nanotechnology and nanoscience approaches to formulation design are further improving the cause, and second-generation cancer nanomedicines (e.g., albumin–drug complexes) are beginning to prove their value.^{3,4} Collectively, these nanopharmaceuticals have considerably reduced adverse drug and solvent effects as well as being passively targeted—they take advantage of the enhanced permeability and retention effect and other tumor-vasculature-specific properties to accumulate at the sites of solid tumors. Foreseeable progress in nanoparticle targeting of pathological sites is further expected since emerging classes of targeting and cell-specific ligands are currently at various stages of clinical development. These emerging classes of ligands include smaller recombinant antibody fragments, nanobodies, and engineered variants as well as ligands discovered through technologies such as phage, yeast, and ribosomal display and cell systematic evolution of ligands by exponential enrichment.^{5–7} These ligands not only show great promise for therapeutic and diagnostic interventions in their own right, but their small size, impressive affinity and target specificity make them ideal candidates for conjugation to nanoparticles. Possible

ABSTRACT We present views on the future development of biologics-based nanopharmaceuticals from a “high risk—high gain” perspective and within the context of personalized therapies. Integrated scientific, commercial, and societal aspects are addressed, and provocative combined realistic biotech, computational, and nanotech approaches for tailor-made engineering of nanopharmaceuticals are discussed.

synergistic effects between nanoparticle characteristics (size and geometry) and molecular targeting may dramatically enhance specificity of delivery, thus opening the path to new and sophisticated design solutions for clinical use. Economically, the complexity and the know-how of nano-based approaches to formulation design have the advantage of offering market exclusivity to the pharmaceutical industry and confronting generic threat better. Indeed, such technological approaches are expected to deliver a reduction in and/or prevent rapid revenue falloff for proprietary nanopharmaceuticals even after patent expiration.⁴

In parallel, the growing interest in biotechnology has already begun to transition the pharmaceutical industry to biologics research and development.^{8,9} Examples include growth factors, cytokines, and nucleic-acid-based medicines such as small interference RNA (siRNA) and microRNA (miRNA). Approximately 70% of the 150 biologics in today's market were approved in the past decade, and 11 of these reached blockbuster sales status (generating more than US\$1 billion revenue) in 2004. Today, biopharmaceuticals account for 25% of all pharmaceuticals in the development pipeline.¹⁰ An aging population and the associated increase in chronic diseases provide significant opportunities for biologic manufacturers, where the global market is projected to reach US\$185 billion by 2015.¹¹

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Cancer, cardiovascular diseases, diabetes, central nervous system disorders, chronic inflammatory conditions, allergies, autoimmune diseases, metabolic disorders, and hormone/enzyme replacement therapies are among the major therapeutic areas that are likely to see significant biotech product launches in the next 10–15 years. Increased regulatory approval rates for biotech products compared with small molecule drug candidates are also driving growth of the market and interest among the pharmaceutical industry. The demand for biotech products is greatest in complex areas where conventional pharmaceutical products have been less successful, such as in the case of multiple sclerosis.

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It is perceivable that future developments in biopharmaceuticals will increasingly be driven by advances in enabling technologies such as genomics, proteomics, pharmacogenomics, and toxicogenomics. Far greater emphasis, however, should be placed on personalized macromolecular medicines to improve human health at all levels. A panel of exciting advanced technologies is beginning to help this cause. For instance, a newly described algorithm, copy number, and expression in cancer seems to be effective in identifying drivers of tumorigenesis, the genes affected by these drivers, and their possible functions from cancer genomics analysis, thus setting provisions for precision and individualized cancer therapies.¹³ There are some limitations with this approach too; it cannot detect mutated oncogenes or tumor suppressive genes that drive tumorigenesis. Other alternative technologies may include paired-end sequencing,

next-generation (deep) sequencing and microfabrication approaches, and platform advances that result in *in vitro* cell reprogramming, modification, and selection. Through such interventions, we envisage rapid developments in cancer vaccines, vaccines for autoimmune diseases, and tailor-made treatments for cancer-based personal genomes.¹² Pertinent to realizing these goals, with the ultimate aim of optimizing individualized and population-based therapies, is a need for libraries of safe and multifunctional nanomaterials and nanoparticles that not only afford protection to biologics against the hostile *in vivo* microenvironments but further achieve controlled targeted delivery and cargo release (Figure 1).^{1,14,15} Here, a detailed understanding of dynamic behavior and interactive forces between macromolecular cargo and the nanomaterial components is still required and remains central for optimization strategies (*e.g.*, in terms of stability optimization and developing new extra- and intracellular release mechanisms). An integrated approach is also necessary, including not only a detailed mapping of “structure–activity” relationships but also concomitant extensive computational network knowledge of genomics and epigenomics of inter-individual variations to biologics responses, thus pushing the boundary of the envisaged personalized nanomedicine therapies to reality.¹⁶

High Risks, High Gains. Today, substantial developments are arising from nanomaterial structure–activity approaches that could dramatically reshape the future of nanopharmaceuticals and pave the way for individualized nanomedicine therapies. A prime example is the recent attempt in the rational design of cationic lipids for safe and effective delivery of siRNA to the liver.¹⁷ Cationic lipoplexes and polyplexes efficiently condense nucleic acids and act as powerful nonviral transfectants,^{18,19} but the cationic nature of the lipid or the polymeric component

often induces immune adverse reactions and cell death.^{20–22} Through a structure–activity approach and detailed understanding of molecular biophysics, Semple *et al.*¹⁷ have designed an array of ionizable cationic lipids that interacts with naturally occurring anionic phospholipids in the endosomal membrane, forming ion pairs that adopt molecular “cone” shapes, which promotes formation of inverted, nonbilayer phases (*e.g.*, hexagonal H_{II} phase). These phases do not support bilayer structure and disrupt membranes, thus aiding rapid nucleic acid release into the cytoplasmic compartment. These engineered nanomedicines were well-tolerated in both rodents and non-human primates and demonstrated efficacious therapeutic (hepatic silencing in this case).¹⁷ Clinical evaluations are currently underway with these formulations.

Unforeseen toxicity toward man-made nanomaterials that are intended to optimize biologics performance still remains a concern. Immunological reactions to nano-engineered materials are particularly notable. These may comprise induction of antibody formation against select components and pro-inflammatory reactions (*via* immune cell receptor activation and signaling), particularly through repetitive epitope presentation, chemical modification procedures, pharmacokinetic profiles, lymphocyte activation, idiosyncratic non-IgE-mediated acute anaphylactic reactions, and intrinsic immune-stimulating properties (*e.g.*, interferon response and adjuvant effects).^{21,23,24} For instance, idiosyncratic and acute anaphylactic reactions to conventional as well as PEGylated liposomes, micellar drug formulations, and a plethora of contrast agents have been well-documented and occur in certain individuals, while their molecular basis is largely neglected.^{23,25} The incidence of acute, allergic-like responses in pharmaceutical formulations and nanomedicines corresponds to approximately 80% of all immune-mediated hypersensitivity reactions

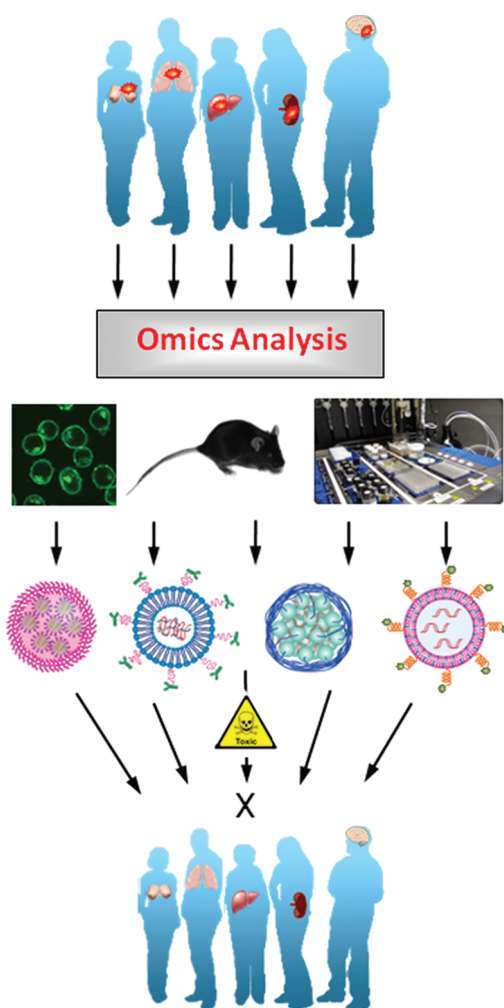


Figure 1. Schematic illustration of a personalized approach for future nanopharmaceuticals. Individuals with different diseases (e.g., breast, lung, liver, kidneys, and brain tumors) will be screened by genomics, proteomics, and glycomics technologies. Next, tailor-made nanocarriers will be designed carrying appropriate biologics-based therapeutic payloads fit with optimized triggered-release mechanisms. Design efforts are placed on a structure–activity relationship in the context of the biological target and nanocarrier immunotoxicity profiling through an *omics* approach for patient selection and personalized treatment.

and translates to over 20 000 fatalities every year in the United States alone.²⁶ Such outcomes impose further health and financial burdens and may complicate the development phase of new nanomedicine initiatives. Computation-based immunogenomic knowledge, however, could salvage this and help immensely toward a mechanistic approach. It remains essential to unravel the molecular basis of serious adverse immune responses at both humoral and cellular levels through structure–activity approaches. If this can be put within the framework of population genetics, then it will ultimately

provide significant gains for human health and treatment selection. Structure–function approaches are beginning to thrive, at least in relation to mechanistic issues that exert immune attack on long-circulating nanoparticles. For instance, a recent initiative demonstrated that changing the conformation of adsorbed block copolymer on nanosphere surfaces with the aim of circumventing the body's defense system unexpectedly triggered the immune system differently.²⁶ These studies are flagging difficulties and current limitations in surface engineering with synthetic polymers

and highlighting the need for better approaches. We believe that detailed profiling, fingerprinting, and computational analysis (including DNA and RNA sequencing) are required to establish relationships between physicochemical characteristics of a typical nanocarrier and adverse population-based immunological responses. These efforts may further lead to the design of *in vitro* precision assays to predict individuals at risk and ultimately may provide global guidelines, not only for safe nanoparticle dosing regimens but also for the design and nanoengineering of immunologically safe nanocarriers applicable to wider site-specific drug delivery. It is of the highest importance to map out such nanomaterial characteristics because small changes in surface chemistry, morphology, and charge may affect immunological responses differently in different populations. Indeed, poor understanding of material characteristics is often related to opposing reports of the safety of many nanomaterials.²⁴

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Other parallel recent developments that could help this cause include computational, quantitative structure–activity relationships that predict the toxicity of various metal

TABLE 1. Expected Global Nanopharmaceutical Market (Based on a 10 Year Development Cycle)^a

nanopharmaceutical	2020 (US\$ billions)	2025 (US\$ billions)	targets
protein-based	14 ± 7	28 ± 14	cancer/inflammatory/CNS
nucleic-acid-based	7 ± 3	14 ± 7	cancer/inflammatory/CNS
small-molecule-based	3 ± 3	6 ± 3	cancer/inflammatory/CNS/cardiovascular/infections

^a Does not include diagnostic nanoparticles/theranostics (e.g., magnetic and luminescence-based optical contrast agents) for *in vivo* imaging (cell tracking, anatomical, functional) and/or combined therapy (e.g., hyperthermia). Source: Roadmaps in Nanomedicine, Toward 2020; Joint European Commission/ETP Nanomedicine Expert Report 2009.

TABLE 2. Economic Costs of Selected Major Diseases in the United States

disease	reference year of cost analysis	direct costs (US\$ billions) ^a	indirect costs (US\$ billions) ^b	source
Alzheimer's disease	2010	direct + indirect costs = 172		Alzheimer's Association USA ^c
Parkinson's disease	2002	6.7	16.3	Huse DM <i>et al.</i> ^d
diabetes	2002	92	112.9	NIH, USA ^e
stroke	2007	41.6	21.1	NIH, USA ^e
cardiovascular disease: ^f	2009	313.8	161.6	American Heart Association ^g
heart disease		183.0	121.6	
coronary heart disease		92.8	72.6	
hypertensive disease		54.2	19.2	
heart failure		33.7	3.5	
stroke		45.9	23	

^a Costs associated with medical care (prevention, diagnosis, and treatment), continuing and terminal care, and rehabilitation. ^b Based on patients' time loss from employment (lost productivity from morbidity and mortality). ^c 2010 Alzheimer's Disease Facts and Figures; http://www.alz.org/alzheimers_disease_facts_figures.asp. ^d Burden of illness in Parkinson's disease; *Mov. Disord.* **2005**, *20*, 1449–1454. ^e Kirschstein, R. Fiscal year 2000 update. Disease-specific estimates of direct and indirect costs of illness and NIH support. ^f This category includes coronary heart disease, heart failure, part of hypertensive disease, cardiac dysrhythmias, rheumatic heart disease, cardiomyopathy, pulmonary heart disease, and other or ill-defined "heart" diseases. Totals do not add up because of rounding and overlap. ^g Heart disease and stroke statistics—2009 update (at-a-glance version); <http://www.americanheart.org/downloadable/heart/1240250946756LS-1982%20Heart%20and%20Stroke%20Update.042009.pdf>.

oxides.²³ On the basis of such computational nanotoxicology, a hypothesis that mechanistically explains differences in toxicity between individual oxides has been proposed.²⁷ Further refinements with such methodologies are expected to provide guidance for the better design of safer nanomaterials and multifunctional nanopharmaceuticals including theranostics.

Transformational Impact and Contributions to Innovation, Well-Being, and the Economy. Integration of research in nanomaterial design and nanoparticle pharmacology remains a grand challenge for controlled delivery and release of therapeutic payloads and for the molecular basis of nanomedicine toxicity at an earlier stage and through population-based design (both at cellular and immune system level).²⁸ This, however, requires multicollaborative, interdisciplinary, and innovative nanoscience, *omics* and other approaches to nanopharmaceutical formulation, but will advance broad societal goals,

from improved understanding of the material behavior at molecular and individualized levels to increased productivity through nanotechnology tool optimization, rational design, and responsible nano-based manufacturing. Ultimately, this will expand the market for many macromolecular therapeutic agents and small molecules and will form the basis for a highly profitable niche for biotechnology and pharmaceutical industries and, most importantly, for human health at all levels (Table 1). In addition, generation of biologically and environmentally safe nanoparticulate drug-delivery systems makes it possible to formulate drugs in an optimal way and for personalized therapies; this could have a significant impact on sustainability by helping to salvage a broad library of discarded compounds. The safe use of nanoparticulate delivery systems and emerging personalized nanopharmaceuticals will also create significant savings in pharmaceutical

spending, which continues to grow. The economic costs of major illnesses in the USA is shown in Table 2, and European Union countries currently spend, on average, 18% of their total expenditure for health on pharmaceuticals.²⁹ Any reduc-

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tion in spending on pharmaceuticals would allow funds to be spent on other treatments or on other healthcare facilities.

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