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Nanomedicines: addressing the scientific and regulatory gap

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Nanomedicine is the application of nanotechnology to the discipline of medicine: the use of nanoscale materials for the diagnosis, monitoring, control, prevention, and treatment of disease. Nanomedicine holds tremendous promise to revolutionize medicine across disciplines and specialties, but this promise has yet to be fully realized. Beyond the typical complications associated with drug development, the fundamentally different and novel physical and chemical properties of some nanomaterials compared to materials on a larger scale (i.e., their bulk counterparts) can create a unique set of opportunities as well as safety concerns, which have only begun to be explored. As the research community continues to investigate nanomedicines, their efficacy, and the associated safety issues, it is critical to work to close the scientific and regulatory gaps to assure that nanomedicine drives the next generation of biomedical innovation.

Keywords: nanomedicine; nanosimilars; nanopharmaceuticals; regulation; patents; nanotechnology; non-biologic complex drug (NBCD); follow-on biologics; biosimilars; immunogenicity; pharmacokinetics; pharmacodynamics; Abbreviated New Drug Application (ANDA); bioequivalence

Nanomedicine: the nexus of medical research and nanotechnology

Nanomedicine refers to any application of nanomaterials for medical purposes, ranging from diagnostic to therapeutic applications. Although a consensus definition has not been reached by the various scientific and international regulatory bodies, a construct is loosely classified as a nanomedicine if it has at least one dimension in the nanoscale range (i.e., measured in nanometers, up to 1000 nm) and exhibits properties dependent upon those dimensions. Putative medical applications of nanotechnology include drug delivery and targeting, imaging, surgery, and tissue engineering—all being applied to such diverse medical specialties as oncology, immunology, osteopathy, and urology.

The New York Academy of Sciences and Teva Pharmaceutical Industries, Ltd. (Petah Tivka, Israel) held a conference at the Academy on November 21, 2013 to discuss recent topics in the area of nanomedicine. The meeting, "Nanomedicines: Addressing the Scientific and Regulatory Gap," gathered experts from academia, the pharmaceutical industry, nanomedical societies, and federal regulatory bodies to discuss the history and the current state of nanomedical research and development; to emphasize the critical lessons learned from past medical applications of nanotechnology, both successful and unsuccessful; and to highlight the gaps in both knowledge and regulatory oversight that must be addressed to advance the broad fields of nanomedicine and nanopharma. In this publication, the following terms are used interchangeably:

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nanodrugs, nanotherapeutics, nanomedicines, and nanopharmaceuticals.

Keynote address: the current state of nanomedicine

Sally Tinkle (IDA/Science and Technology Policy Institute) delivered a keynote address on the evolving roles of government and scientists in filling the scientific and regulatory gaps in nanomedicine. Nanoscience and nanotechnology focus on the understanding and control of matter at the nanoscale, including application-specific top-down or bottom-up design and engineering of nanoscale materials, systems, and devices. Nanotechnology integrates knowledge from physics, mathematics, materials and pharmaceutical science, chemistry, biology, and engineering. Medical research encompasses primarily human biology and bioengineering, research tools and methods, therapeutics, and diagnostics. Nanomedicine lies at the intersection of these many disciplines, bringing the power of advanced materials with novel, size-dependent properties to the maintenance, diagnosis, and restoration of human health through the development of innovative medical products.

Complex scientific and economic factors shape the U.S. science and technology (S&T) enterprise, and have done so for nanotechnology and nanomedicine. Research challenges include the measurement of physical, chemical, and functional properties; synthesis, reproducibility, and scale-up; and *in vivo* assessment, tracking, and imaging of materials with nanoscale size or features. Nanotechnology and nanomedicine have been affected by shifts in the research enterprise that include multidisciplinary team science; the changing value of data, information, and knowledge; the requirement for transparency and data sharing; and the disruption of funding streams. Economic realities and the global marketplace have added to this mix the challenges of internationally distributed research collaborations and manufacturing facilities and the race to capitalize on nanotechnology for commercial benefit in faltering economies.

According to Tinkle, the regulatory communities are challenged by a confusion of definitions for nanomaterials and nanotechnology-enabled products and devices and by limited standard nomenclature and reference materials. Within the context of an increasingly complex nanomedicine product pipeline (mainly nanopharmaceuticals), regulators grapple with the application of existing regulations to nanoscale materials and devices with complex and frequently novel properties. The data and information needed for fundamental research, product development, safety assessment, regulation, and policy are acquired on differing timelines and timetables. This is evident, for example, in the mismatch between the rate at which products are developed and the rate at which data for policy and regulation are produced. Products are developed but not released to market until safety testing is completed; however, regulations cannot be developed until a weight of evidence and a convergence of data support conclusions on safety. This timeline mismatch, coupled with the complex scientific and economic factors that influence the S&T enterprise and the accelerated rate at which new technologies now move from the laboratory to the marketplace, has reshaped the research business model, the regulatory process, and the roles of its principal participants, the scientist and the federal government.

Scientists no longer work primarily within the dual roles of researcher and teacher. There is increased emphasis on collaboration through centers, consortia, and networks, making management skills more necessary and time intensive. The multidisciplinary nature of data, the vast amounts of data generated by high-throughput, large data–content experiments, and the requirements for data organization, mining, and sharing require informatics and data management skills. There is increasing pressure on academic scientists to become entrepreneurs and innovators and to transfer fundamental research to products and to develop start-up companies. As start-ups become successful, scientists become businesspersons responsible for employees, infrastructure, and profit. Reverberating through all of these roles is the increased awareness of public and product safety—the scientist as socially responsible ethicist. The question of what is possible to do with technology now intersects with questions about what is desirable for medical research and the human condition and what is viable in the marketplace.

The federal research enterprise is also responding to changes in the S&T ecosystem and the global economy. Federal efforts to organize nanotechnology under the National Nanotechnology Initiative (NNI) have proven useful where they align with existing agency missions and operating procedures,

and in certain areas and under certain conditions provide momentum and rationale for change. Technology is emphasized as a driver for jobs and the economy, and federal research agencies have revised center and network funding mechanisms to propel the translation of fundamental research to technology transfer and product development. Federal big-data sets, such as weather and astronomical data, were released for public use, with others under consideration, and better stakeholder access to federal research facilities has been devised. Regulatory agencies are actively evaluating the application of their authorities to nanomedicine, and efforts continue to make this a more open, transparent, and consistent process.

It is against this backdrop—utilizing precisely engineered, nanotechnology-enabled solutions to the challenges of drug delivery, imaging, and diagnostics—that nanomedicine leads the next generation of biomedical advances.

Characterization and safety of nanomedicines: lessons learned from the NCL

Nanomaterials used in medicine are becoming increasingly sophisticated. Researchers have developed techniques to create complex multifunctional conjugates. Investigators now routinely attach coatings, targeting molecules, drugs, prodrugs, tracking moieties, and imaging agents to nanoparticle platforms. As developers engineer and test this next generation of sophisticated nanomedicines, they must develop methods to characterize the nanomedicines' physical and chemical properties (including size, morphology, charge, purity, etc.) and techniques to characterize performance (including protein binding, cellular uptake, drug release, and metabolism). Of course, these aspects are inextricably linked, since physical and chemical properties contribute to a nanomaterial's biocompatibility and performance.

Scott E. McNeil (Nanotechnology Characterization Laboratory (NCL), Frederick National Laboratory for Cancer Research) described research conducted at the NCL and its implications for the characterization of nanomedicines. The NCL was launched by the National Cancer Institute (NCI) in 2004 to accelerate the pace at which cancer nanomedicines get into clinics. The NCL is a partnership among the NCI, the U.S. Food and Drug Administration (FDA), and the National Institute of Standards and Technology (NIST). The NCL conducts translational studies to help nanomedicine developers bridge the so-called "valley of death" between academic research and clinical trials. The NCL has collaborated with more than 80 companies and academic labs, and after 9 years of running such studies on some 300 nanomaterial formulations, has helped put seven medicines into clinical trials. In doing so, the lab has accumulated knowledge about what approaches are effective in characterizing the properties of nanomedicines. The NCL works with developers and regulators to align advances in materials science with clinical standards and to assist with standards and methods development.

As part of its assay cascade of scientific tests for nanomedicines, the NCL characterizes nanoparticles' physical attributes, their *in vitro* biological properties, and their *in vivo* compatibility using animal models. The NCL also looks at trends across nanoparticle platforms and parameters that are critical to nanoparticle biocompatibility and develops assays for the preclinical characterization of nanoparticles. The NCL has developed more than 40 protocols that rigorously characterize nanoparticle physicochemical properties, as well as *in vitro* immunological and cytotoxic characteristics and absorption, distribution, metabolism, excretion, and toxicity (ADME/Tox) profiles in nonhuman animal models. These assays have undergone extensive in-house validation and are subjected to regular revision to ensure applicability to a variety of nanomaterials.

At the NCL, McNeil and colleagues recommend the use of multiple methods based on different principles to measure each physicochemical and performance property of a nanomedicine. Different instruments are sensitive to different effects, and using multiple methods based on different principles can provide a more complete picture of the particle population being studied. Thorough characterization data allows a developer to know what they have, which is critical since the manufacturer's specifications for nanomaterials may not always be correct. For example, carbon nanotubes often come with detailed characterization data specifying a particular average diameter, chirality, and length, but the actual product may look more like a cotton ball under an electron microscope. The NCL has tested gold nanoparticles where the supplier specified an

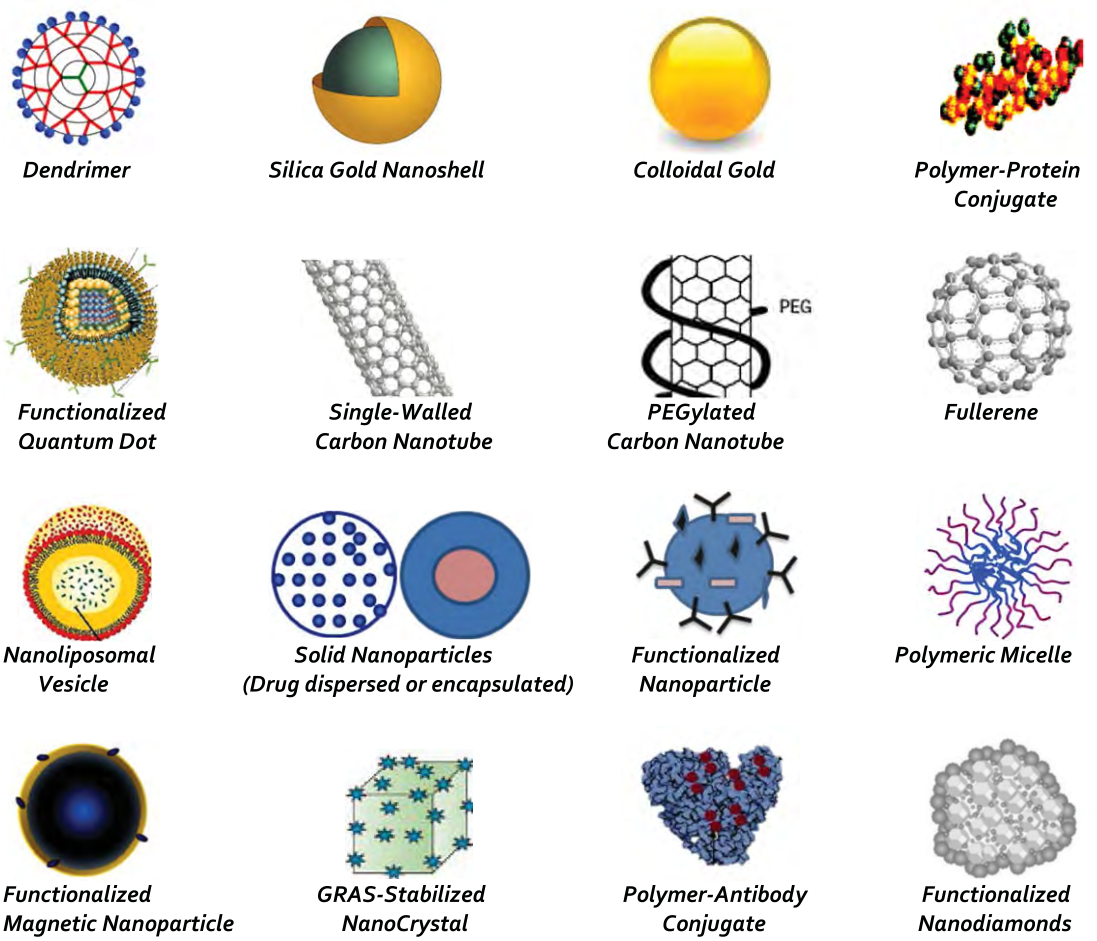

Figure 1. Nanomedicines for drug delivery. Copyright © 2014 Raj Bawa. All rights reserved.

"equivalent sphere average diameter," but the vast majority of the particles were not spherical. Rather, they were a range of shapes and sizes, including rods and trapezoidal particles.

Reliable, validated characterization data will provide a foundation for understanding nanomedicine biology and for realizing the promise of these new technologies to diagnose and treat disease. As sophisticated nanomedicines enter clinical trials, regulators are increasingly asking for physicochemical characterization data obtained through several methods and seeking a detailed understanding of how the unique properties of nanomedicines influence their biological performance. This type of thorough understanding can be achieved only through an integrated approach where physicochemical characterization informs biological performance testing and vice versa. This type of integrated testing will allow identification of scientific and regulatory gaps in nanomedicine and help developers and regulators strategize new ways to bridge those gaps.

Nanosimilars and follow-on nanosized therapeutics

Nanomedicines have been successfully used as medicinal products in clinical routine over the last several decades, frequently without taking into account either their specific nanoparticular structure or the resulting complexity of their mechanical, chemical, and pharmacological properties (Fig. 1, Table 1). Stefan Mühlebach (Vifor Pharma Ltd. and the University of Basel) discussed this *nano-character*, which has a potentially strong and important impact on the quality, efficacy, and safety of such high molecular weight,

Table 1. Selected FDA-approved nanomedicines for drug delivery

Drug name/ U.S. tradename	Therapeutic active agent and/or nanomaterial/ nanomedicine class	Delivery route	Manufacturer/ alliance/ marketer	Indication(s)	FDA approval date
Doxil® Caelyx® (in the EU)	Pegylated doxorubicin (Adriamycin)HCl liposomes/lipid NPs	Intravenous	Centocor Ortho Biotech Johnson & Johnson Schering-Plough Janssen Biotech	Metastatic ovarian or breast cancer; AIDS-related Kaposi's sarcoma	November 1995
Abraxane® (ABI-007, nab-paclitaxel, NSC-736631)	Paclitaxel (Taxol) bound albumin nanoparticles	Intravenous	Abraxis BioScience Celgene	Various cancers	January 2005
AmBisome®	Amphotericin B liposomes/lipid NPs	Intravenous	Astellas Pharma USA Gilead Ltd	Fungal infections	August 1997
Rapamune®	Nanocrystal sirolimus	Oral solution oral tablet	Wyeth Elan Corp Pfizer	Immunosuppressant for kidney transplants	September 1999
TriCor®	Nanocrystal fenofibrate	Oral tablet	Elan Corp Abbot Labs	Primary hypercholesteremia, mixed lipidemia, hypertriglyceridemia	November 2004
Emend®	Nanocrystal aprepitant	Oral capsule intravenous	Merck Elan Corp	Nausea in chemotherapy patients	March 2003
Diprivan®	propofol liposomes/lipid NPs	Intravenous	Astra Zeneca Pharma	Anesthetic	October 1989
Renagel®	Cross-linked poly(allylamine) resin (sevelamer hydrochloride)	Oral tablet	Genzyme Corp	Control of serum phosphorus in patients with chronic kidney disease on dialysis	October 1998
Triglide®	Nanocrystal Fenofibrate	Oral tablets	Skye Pharma First Horizon Sciele Pharma	Lipid disorders; reduces elevated plasma concentrations of triglycerides, LDL, and total cholesterol and raises abnormally low levels of HDL	May 2005
Myocet®	Doxorubicin-citrate liposomes/lipid NPs	Intravenous	Zeneus Pharma Sopherion Therapeutics	Cardioprotective formulation of doxorubicin used in late stage metastatic breast cancer	Approved in EU and Canada – 2000
DepoCyt®	Sustained release cytarabine liposomes/lipid NPs	Intravenous	Pacira Pharma Sigma-Tau Pharma	Lymphomatous meningitis	April 1999
DaunoXome®	Encapsulated-daunorubicin citrate liposomes/lipid NPs	Intravenous	NeXstar Pharma Gilead Sciences Ltd Galen Ltd	HIV-related Kaposi's sarcoma	April 1996
Estrasorb®	Estradiol hemihydrate micelles (emulsion)	Transdermal	Novavax Graceway	Reduction of vasomotor symptoms, such as hot flushes and night sweats, in menopausal women	October 2003
Macugen®	siRNA anti-VEGF inhibitor (PEG) aptanib sodium (polymer–aptamer conjugate)	Intravitreal	OSI Pharma	Neovascular age-related macular degeneration	December 2004

Continued

Table 1. *Continued*

Drug name/ U.S. tradename	Therapeutic active agent and/or nanomaterial/ nanomedicine class	Delivery route	Manufacturer/ alliance/ marketer	Indication(s)	FDA approval date
Abelcet®	Amphotericin B liposomes/lipid NPs	Intravenous	Sigma-Tau Pharma	Invasive fungal infections in patients who are refractory to or intolerant of conventional amphotericin B therapy	November 1995
Adagen® (pegademase bovine)	Pegylated bovine adenosine deaminase (polymer–protein conjugate)	Intravenous	Sigma-Tau Pharma Enzon	Enzyme replacement therapy for patients with severe combined immunodeficiency disease; adenosine deaminase deficiency	March 1990
PEGASYS®	Peginterferon alfa-2a (polymer–protein conjugate)	Subcutaneous	Nektar Hoffmann-La Roche	Chronic hepatitis C virus infection	October 2002
Somavert®	Pegvisomant (PEG-hGH) (polymer–protein conjugate)	Subcutaneous	Nektar Pfizer	Acromegaly	March 2003
Neulasta®	PEG-G-CSF or pegfilgrastim (covalent conjugate of recombinant methionyl human G-CSF (filgrastim) and monomethoxypolyethylene glycol) (polymer–protein conjugate)	Subcutaneous	Amgen	Febrile neutropenia	January 2002
Copaxone® (glatiramer acetate, copolymer 1)	Copolymeric mixture of L-glutamic acid, L-alanine, L-tyrosine and L-lysine (polypeptide colloidal suspension)	Subcutaneous	Teva Pharma	Relapsing–remitting multiple sclerosis	December 1996
Amphotec®	Colloidal suspension of lipid-based amphotericin B (~115 nm)	Subcutaneous	Sequus	Invasive aspergillosis patients who are refractory to or intolerant of conventional amphotericin B	November 1996
PEGINTRON®	Peginterferon alfa-2b (polymer–protein conjugate)	Subcutaneous	Enzon Schering-Plough	Chronic hepatitis C virus infection in patients with compensated liver disease; (Merck's Sylatron approved for melanoma with nodal involvement after surgical resection)	January 2001
Oncaspar® (PEG-L-asparginase)	Pegasparginase (polymer–protein conjugate)	Subcutaneous	Sigma-Tau Pharma	Lymphoblastic leukemia	February 1994

Continued

Table 1. *Continued*

Drug name/ U.S. tradename	Therapeutic active agent and/or nanomaterial/ nanomedicine class	Delivery route	Manufacturer/ alliance/ marketer	Indication(s)	FDA approval date
Epaxal® (HAVpur®, VIROHEP-A)	Hepatitis A vaccine adjuvanted with immunopotentiating reconstituted influenza virosomes (IRIV)	Intramuscular (in the deltoid muscle)	Berna Biotech Crucell	Active immunization against hepatitis A for adult and children >12 months (age may vary and depend upon the country)	Approved in Canada and elsewhere
Elestrin®	Estradiol gel (0.06%) incorporating calcium phosphate nanoparticles	Transdermal	BioSanté	Moderate to severe hot flashes in menopausal women	December 2006

NOTE: This table only lists selected FDA-approved nanomedicines. The table excludes the following (1) nanomaterials, unless they serve as the nanomedicine *per se*; (2) FDA-approved imaging and diagnostic agents; and (3) nanomedicine candidates in preclinical research, including basic research, bench science, early animal testing, etc. A nanomedicine approval by the FDA does not necessarily indicate that it is commercially available to consumers. Various factors, in addition to FDA approval, influence its commercialization.
NPs, nanoparticles; AIDS, acquired immunodeficiency syndrome; HDL, high-density lipoprotein; LDL, low-density lipoprotein; PEG, polyethylene glycol; PEG -G-CSF, pegylated granulocyte colony-stimulating factor; PEG-hGH, pegylated human growth hormone; VEGF, vascular endothelial growth factor.

polymeric products composed of structurally related components.[1] Examples include synthetic macromolecular non-biological complex drugs (NBCDs) like nanocolloidal formulations for intravenous (IV) use and innovative biological complex therapeutics like monoclonal antibodies (mABs). Such non-homomolecular therapeutics contrast in their characteristics from well-defined classical small-molecule drugs and cannot be fully identified by the physicochemical means typically used to define the quality and pharmaceutical identity of a product, as described in pharmacopoeial monographs. As a consequence, the European Directorate for the Quality of Medicines and Healthcare (EDQM), the body issuing the European Pharmacopoeia, has established a specific working group for non-biological complexes.[2] For copies, the regulatory challenge is to identify minor but clinically meaningful differences between a test and the reference product. Additional preclinical and clinical characterization data are needed to document the product profile, and as a consequence, the comparability, especially for nanoparticulate structures, between a test and reference product.[3] Whereas for the biological products and their follow-on therapeutics the biosimilarity approach has been established by the European Medicines Agency (EMA), such a defined regulatory assessment is lacking for NBCD similars of often even more complex structures.[3,4]

The generic paradigm based on comparability of the pharmaceutical properties and the bioequivalence measured in volunteers facilitates conclusions on the therapeutic equivalence of well-defined small-molecule drugs. Therapeutic equivalence is a prerequisite for interchange and (automatic) substitution between innovator and follow-on therapeutics. The successfully applied generic paradigm is, however, no longer valid if the complete pharmaceutical characterization and the comparability of bioequivalence cannot be established (Fig. 2). For biosimilars, as well as other similar therapeutics, the determination of the extent of similarity is crucial to allow an alternative use in therapeutically naive patients. The clinical differences for such intended follow-on versions of nanosized NBCDs have been published for intravenous iron nanocolloids authorized in some geographic areas by a generic approach (reviewed in Refs. 3 and 4). This shows that the extent of similarity and the summary or weight of evidence of qualitative, preclinical, and clinical data is even more demanding when it comes to interchange or substitution of the medication in pre-treated patients. That special challenge is mirrored by several EMA and FDA regulatory reflection/industrial guidance papers for follow-on nanoparticular intravenous iron NBCDs (nanosimilars) asking for expert comments to fill the regulatory science gap.[5,6] In the absence of supportive clinical data, a switch in a well-established therapy

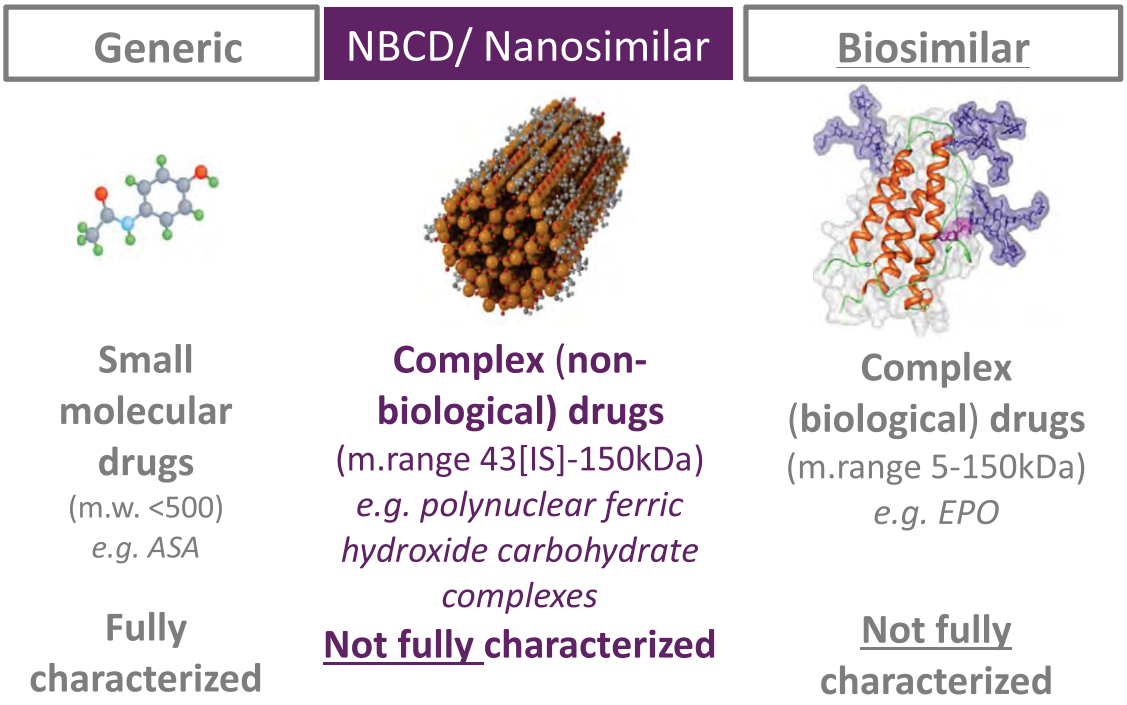

Figure 2. Categories of follow-on versions of drugs. From Ref. 7.

from the innovator to the copy product and vice versa is discouraged without assuring appropriate traceability.[2,4,7] According to Mühlebach, this substitution/interchange aspect has to be regulated by the FDA in the United States, but not by the EMA, as in Europe this decision is made by national authorities. NBCDs typically use multiple starting components and the final product represents the result of a laborious and difficult-to-control manufacturing process, which is key to determining the product's properties. If one of these components or the final products exhibits nano-dimensions or nano-characteristics, the term nanomedicine or nanosimilar applies. The need for an in-depth understanding of the physicochemical functionality at the surface of nanostructures is key and has an important impact on regulatory assessments of nanomedicines resulting from a coating process of the core structure.[1] This shell influences the distribution, tissue targeting, and clearance of such a product, which is ultimately relevant to its efficacy and safety but also to the comparability of a test and reference product. It stresses the need for full control of the critical manufacturing process, which defines the therapeutic product (Fig. 3).[7] This also indicates the importance of realizing a common terminology for a harmonized regulation approach.[3,8,19,20]

The FDA's approach to the regulation of nanotechnology products

Ritu Nalubola[a] (U.S. Food and Drug Administration) discussed elements of the FDA's approach to regulating nanomedicines. The FDA recognizes that nanotechnology is an emerging technology that has the potential to be used across the full spectrum of FDA-regulated products, including drugs, biological products, and medical devices. Over the past several years, the FDA has taken multiple steps to help ensure the responsible development of nanotechnology products.

[a]This section, provided by Dr. Ritu Nalubola of the U.S. Food and Drug Administration, reflects Dr. Nalubola's presentation at the meeting, "Nanomedicines: Addressing the Scientific and Regulatory Gap," held on November 21, 2013. No other portion of this article should be construed to represent the opinions of Dr. Nalubola or the views or policies of the FDA.

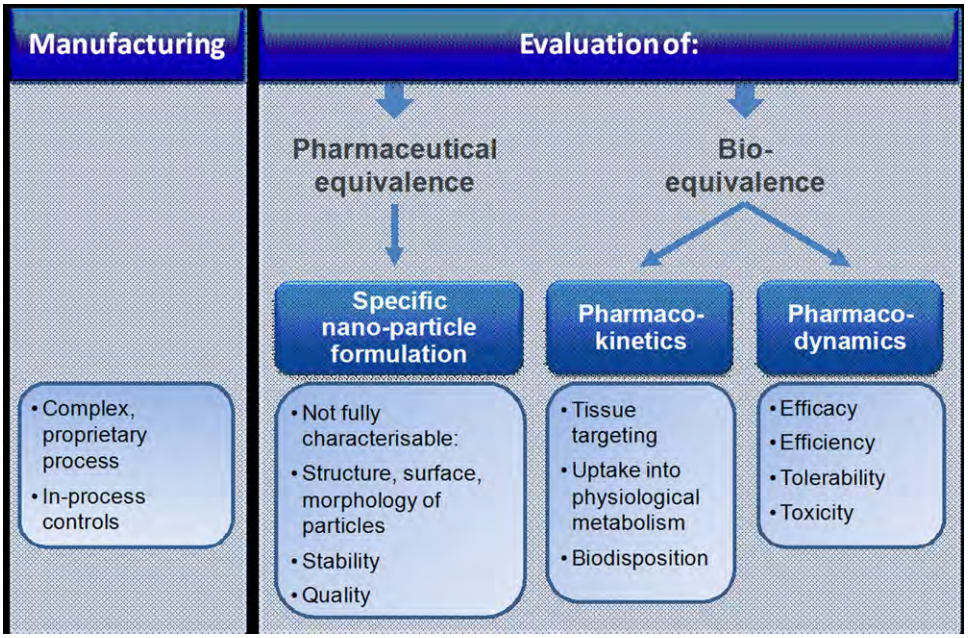

Figure 3. **Full control of the manufacturing process for NBCDs and their follow-ons to define the therapeutic product's (nanoparticular) profile. From Ref. 7.**

In a policy statement articulating its approach,[9] the FDA noted that it does not categorically judge all products involving the application of nanotechnology to be either inherently benign or harmful. The FDA continues to regulate nanotechnology products under its existing statutory authorities in accordance with the specific legal standards applicable to each type of product under its jurisdiction. The FDA believes that this regulatory policy allows for tailored approaches that adhere to applicable legal frameworks and reflect the characteristics of specific products or product classes and evolving technology and scientific understanding. The FDA intends to ensure transparent and predictable regulatory pathways grounded in the best available science.

Nanomaterials can have unique physical or chemical properties that potentially offer great promise, but these same properties may also merit further examination to determine if they affect product safety or other product attributes. The FDA has invested in a nanotechnology regulatory science program to help address key scientific gaps in knowledge, methods, and the tools necessary for regulatory assessments of nanotechnology products.[10]

Nalubola and colleagues consider their current frameworks for safety assessment sufficiently robust and flexible to be appropriate for a variety of materials, including nanomaterials. Regardless of whether a product contains nanomaterials, the FDA asks relevant questions concerning product safety in order to ensure that the product meets statutory and regulatory requirements for safety. Industry remains responsible for ensuring that its products meet all applicable requirements, and the FDA continues to offer technical advice and guidance, as needed, to help industry meet its obligations.

In 2011, the FDA issued a draft guidance for industry entitled "Considering Whether an FDA-Regulated Product Involves the Application of Nanotechnology" to present its current thinking on nanotechnology and to seek public comment.[11] As noted in that draft guidance, the FDA has not adopted a regulatory definition of nanotechnology or related terms. In determining whether an FDA-regulated product involves the use of nanotechnology, the FDA asks (1) whether an engineered material or end product has at least one dimension in the nanoscale range (approximately 1–100 nm); or (2) whether an engineered material or end product exhibits properties or phenomena, including physical or chemical properties or biological effects, that

Table 2. Comparison of nanomaterial-related descriptions

FDA's Points to Consider	Health Canada Working Definition of Nanomaterial[a]	European Commission Recommendation on the Definition of Nanomaterial[b]
– Whether an engineered material or end product has at least one dimension in the nanoscale range (approximately 1 nm to 100 nm); or – Whether an engineered material or end product exhibits properties or phenomena, including physical or chemical properties or biological effects, that are attributable to its dimension(s), even if these dimensions fall outside the nanoscale range, up to one micrometer	Any manufactured substance or product and any component material, ingredient, device, or structure if: a. It is at or within the nanoscale in at least one external dimension, or has internal or surface structure at the nanoscale, or; b. It is smaller or larger than the nanoscale in all dimensions and exhibits one or more nanoscale properties/phenomena i. The term "nanoscale" means 1 to 100 nanometres, inclusive; ii. The term "nanoscale properties/phenomena" means properties which are attributable to size and their effects; these properties are distinguishable from the chemical or physical properties of individual atoms, individual molecules and bulk material; and, iii. The term "manufactured" includes engineering processes and the control of matter.	A natural, incidental or manufactured material containing particles, in an unbound state or as an aggregate or as an agglomerate and where, for 50% or more of the particles in the number size distribution, one or more external dimensions is in the size range 1 nm–100 nm. In specific cases and where warranted by concerns for the environment, health, safety or competitiveness the number size distribution threshold of 50% may be replaced by a threshold between 1 and 50%

[a]Policy Statement on Health Canada's Working Definition for Nanomaterial. Available online at: http://www.hc-sc.gc.ca/sr-sr/pubs/nano/pol-eng.php
[b]Commission Recommendation 2011/696/EU, OJ L 275, 20.10.2011. Available online at: http://ec.europa.eu/environment/chemicals/nanotech/pdf/commission_recommendation.pdf

are attributable to its dimensions, even if these dimensions fall outside the nanoscale range, up to 1 μm. Table 2 provides a comparison of the FDA's Points to Consider and the definitions proposed by Health Canada and the European Commission. In 2012, the FDA issued two additional draft guidances for industry to address technical issues related to the use of nanotechnology in cosmetic products and in food substances.[12,13] Nalubola and colleagues are currently working to finalize these three draft guidances, taking into account public comments received.

The FDA's Center for Drug Evaluation and Research provided an update on its ongoing work related to the use of nanotechnology in drug products to the Advisory Committee for Pharmaceutical Science and Clinical Pharmacology in August 2012.[14] As explained in this update, the FDA evaluated its existing regulatory review processes for drug products and determined that current procedures are adequate to identify and address the potential risks associated with the use of nanomaterials in drug products. Certain areas were identified for improvement, including the need for increased regulatory science research and the training of review staff. The FDA also established specific policies and procedures for use by internal reviewers of human drug[15] and animal drug submissions.[16]

As noted in the draft guidance documents, the FDA encourages industry to consult early with the agency to address questions related to the safety, effectiveness, or regulatory status of nanotechnology

products. These early consultations afford an opportunity to clarify the manufacturer's obligations and discuss methodologies and data needed to meet those obligations.

The FDA also continues to collaborate with relevant domestic and international counterparts on regulatory science and policy issues, including through the NNI, the Emerging Technologies Interagency Policy Coordination Committee, and international regulatory cooperation forums.

Nanopharmaceuticals in the post-blockbuster world: critical patent issues

Rapid advances and product development in nanomedicine are in full swing as it continues to influence the pharmaceutical, device, and biotechnology industries.[17,18] The most robust sector within nanomedicine is nanotech-enabled drug delivery (Table 1, Fig. 1), although bottlenecks persist (Table 3). Currently, the field is poised at a critical juncture, as numerous market forces and drivers are dictating a change in the pharmaceutical industry's quest for discovering, developing, and delivering novel therapeutics owing to numerous challenges ranging from revenue losses caused by patent expirations on blockbusters to enhanced regulatory oversight to an ever-increasing challenge from generic manufacturers. In the process, these diverse forces are altering the drug landscape. Clearly, new ground rules and competitive business strategies are needed in this post-blockbuster drug era. As a result, pharmaceutical companies are partly turning to miniaturization and nanotechnology to enhance or supplement drug target discovery and drug development.

Raj Bawa (Bawa Biotech LLC and Rensselaer Polytechnic Institute) discussed critical issues related to patent strategy, FDA regulation, and commercialization of nanopharmaceuticals. Advances in nanomedicine and the FDA system for governing it are inevitably intertwined, argued Bawa. Hence, clear regulatory and safety guidelines from the FDA are critical to any commercialization effort pertaining to nanopharmaceuticals.[19–21] Similarly, securing valid, defensible patent protection from the U.S. Patent and Trademark Office (PTO) is essential (Table 4).[19–21] However, since the early 1990s, "patent prospectors" have been staking their claims and have been on a quest for a sort of "nanopatent land grab." The PTO classifies U.S. nanopatents into Class 977, and they number fewer than 10,000 to date. However, these numbers and the classification system they are based on is clearly inadequate, argued Bawa, because it is based on the ill-conceived NNI definition of nanotechnology that limits all nanostructures and nanoproducts to a sub-nanometer range.[17–21] Therefore, he concluded, these numbers are an underestimate

Table 3. Bottlenecks to commercialization of nanomedicines

- lack of standard "nano" nomenclature: imprecise definition for nanomedicines
- lack of precise control over nanoparticle manufacturing parameters and control assays
- currently used compounds/components for nanodrug synthesis often pose problems for large scale good manufacturing (cGMP) production
- lack of quality control: issues pertaining to separation of undesired nanostructures (byproducts, catalysts, starting materials) during manufacturing
- scalability complexities: enhancing the production rate to increase yield
- reproducibility issues: control of particle size distribution and mass
- high fabrication costs
- lack of rational pre-clinical characterization strategies via multiple techniques
- biocompatibility, biodistribution and toxicity issues: lack of knowledge regarding the interaction between nanoparticles and biosurfaces/tissues
- consumer confidence: public's general reluctance to embrace innovative medical technologies without clearer safety or regulatory guidelines
- relative scarcity of venture funds
- ethical issues and societal issues are hyped up by the media
- big pharma's continued reluctance to seriously invest in nanomedicine
- patent review delays, patent thickets, and issuance of invalid patents by the U.S. Patent and Trademark Office
- regulatory uncertainty and confusion due to "baby steps" undertaken by U.S. Food and Drug Administration: a lack of clear regulatory/safety guidelines

Table 4. Legal requirements to obtain a U.S. patent[54]

U.S. patent statute	Brief description of statute
35 USC § 102 Novelty requirement	Invention must be novel (i.e., sufficiently new and unlike anything that has been previously patented, marketed, practiced, publicized, or published.
35 USC § 103 Nonobviousness requirement	Invention must be nonobvious to a person with knowledge in the field related to the invention, meaning that the person would not automatically arrive at the present invention from a review of existing ones (i.e., trivial variations that are readily apparent to a person with knowledge in the field related to the invention cannot be patented).
35 USC § 101 Utility requirement	Invention must have utility (i.e., the invention has some use and it actually works or accomplishes a useful task).
35 USC § 112(1) Written description requirement	Invention must be adequately described to the public to demonstrate "possession" of the invention at the time of filing.
35 USC § 112(1) Enablement requirement, part I	Invention must enable a person with knowledge in the field related to the invention to make/carry out the invention without "undue experimentation" (i.e., to make the claimed product or carry out the claimed process).
35 USC § 112(1) Enablement requirement, part II	Invention must enable a person with knowledge in the field related to the invention to use the invention.
35 USC § 112(2) Clarity requirement	Invention must be described in clear, unambiguous, and definite terms.
35 USC § 112(2) Best mode requirement	Invention must set forth the best mode of making or using the invention, contemplated by the inventor at the time of filing of the patent application.

and miss the majority of patents that are nanotech related (out of ~8 million total issued U.S. patents); they simply represent a starting point. Scientifically, the shortfalls with this definition, especially for nanomedicine, are well documented.[20] In fact, the confusion and ambiguity surrounding the definitions of nanotechnology, nanomedicine, and other "nano" terms continues to be one of the most significant problems facing regulators, policy makers, researchers, drug companies, and patent practitioners.[17–21]

Another significant issue pertaining to nanopatents is the lack of a universal nano-nomenclature, whereby distinct terms frequently refer to identical or similar nanostructures or nanomaterials. In addition, contrary to U.S. patent law fundamentals, the PTO has continued to issue multiple nanopatents on the same inventions. This situation initially developed because the search tools and commercial databases that were being used by patent examiners at the PTO were not well suited to search most of the early nanotech-related prior art that resided in scientific publications instead of patents. In addition, the U.S. patent examiners generally lacked expertise and training with respect to the emerging fields of nanotechnology and nanomedicine.

As a result of these limitations, in certain sectors of nanotech, "patent thickets" exist today that could stifle commercialization efforts in future. The classic example is the issuance of multiple, foundational U.S. patents on carbon nanotubes, where a classical patent thicket exists today.[22] It is hoped that such U.S. patents will expire before widespread commercialization so that there will be little or no need for litigation, otherwise such patent thickets could stifle commercialization efforts. These limitations become more serious given the fact that the PTO continues to experience budgetary issues, skyrocketing patent pendency, quality concerns, and a relatively high attrition of experienced examiners.

Maybe it is time, suggested Bawa, to seriously contemplate governmental action under the U.S. Bayh–Dole Act of 1980, whereby an imposition of compulsory licensing or exercise of march-in rights is considered for certain U.S. patents. Some experts have even urged creation of an open-source type process to rectify the erroneous issuance of some of these basic, upstream nanopatents so that downstream

development and commercialization of nanopharmaceuticals is not stifled.

In the end, governmental policies, regulatory clarity, valid patent protection, venture activity, and public confidence are all critical to nanomedicine commercialization.

Addressing the regulatory gap of nanosimilars

During a panel discussion on the global regulation of nanomedicines and the potential to harmonize guidelines, Gerrit Borchard (School of Pharmaceutical Sciences Geneva–Lausanne) argued that the variety of innovative and existing nanomedicines is too complex and difficult to address to allow the harmonization of even partially complete guidelines. A more successful approach could be to harmonize regulatory assessment proposals on follow-on versions of established nanoparticular therapeutics like most actual representatives of NBCDs, also referred to as nanosimilars. Such NBCDs are defined as synthetic (non-biologic), large-molecular nanoparticulate complexes in which the entire formulation represents the pharmaceutically active ingredient. Many classes of parenteral nanomedicines, such as liposomes, iron carbohydrates, and glatiromoids, fall under this definition. Common to all NBCDs is the failure to fully characterize the NBCD by physicochemical means (Fig. 2). The U.S. Pharmacopeial Convention (USP) includes a monograph on iron sucrose injection, and several FDA draft guidance documents on NBCDs (e.g., liposomal doxorubicin[23] and iron sucrose[6]) exist. The FDA draft guidance on iron sucrose recommends extensive *in vitro* characterization studies to demonstrate bioequivalence between the originator and generic products, and recommend particle size and distribution between originator and generic products to meet population bioequivalence criteria.

Special consideration has to be paid to the sameness in physicochemical properties and the qualitative and quantitative equivalence between the test and reference product in their relevant components. This includes stoichiometric ratio, iron core characterization, composition and surface properties of the carbohydrate shell, and the labile iron determination under physiologically relevant conditions. Clinical analyses required would include (1) single-dose (100 mg diluted and administered over 5 min), randomized, parallel bioequivalence studies; (2) measurements of total iron and transferrin-bound iron; (3) bioequivalence (90% CI) based on the maximum value of the difference in concentration between total iron and transferrin-bound iron over all time points measured; and (4) difference in AUC between total iron and transferrin-bound iron.

The Committee for Medicinal Products for Human Use (CHMP) of the European Medicines Agency (EMA)[5] suggested non-clinical comparability studies including assessment of iron distribution in plasma, the reticuloendothelial system (RES), and target tissues in suitable animal models, realizing that sampling in serum alone is insufficient to determine bioequivalence between an originator and a follow-on product. In addition, the "Leiden proposal" of the joint working party on NBCDs demanded comparison of safety, minimally in rodents, once pharmaceutical quality is assured.[4] This example, argued Borchard, shows that a pure generic approach to show bioequivalence for NBCD is not sufficient.

In 2011, the FDA approved the first generic sodium ferric gluconate iron complex Nulecit™, an alternative to Ferrlecit® for the treatment of iron deficiency in chronic kidney disease (CKD) patients. In 2013, the FDA issued a solicitation to evaluate the therapeutic equivalence of the generic sodium ferric gluconate iron complex Nulecit and Ferrlecit. This study is part of the FDA's post-market surveillance that can help address potential concerns regarding the quality of generic iron complex products and supports the agency's review standards.[24,27] Several assays, beyond simple physicochemical characterization, have been suggested to be used in this approach, including *in vitro* phagocytosis assays, *in vivo* uptake of labile iron leakage, iron distribution studies in tissues (pre-clinical species), and non-transferrin–bound iron (NTBI) formation and comparison of levels in hemodialysis patients.

Acknowledging the challenges associated with the assurance of NBCD products, the EDQM (http://www.edqm.eu/medias/fichiers/edqm_brochure_2013.pdf),[25] a council of the Europe Directorate, established a non-biological complex (NBC) working party in 2011, following an initiative by the Swiss agency for therapeutic products, Swissmedic. The working party has the task to elaborate European Pharmacopoeia (Ph. Eur.) monographs on non-biological complexes (e.g., nanoparticle

solutions, such as iron sucrose concentrated solution) allocated to the working group by the European Pharmacopoeia Commission. Working party members are representatives from European academia, industry, and regulatory authorities.

The Ph. Eur. is the official pharmacopoeia in Europe, mandatory in 37 European member states and the European Union.[25] The U.S. and Japanese pharmacopoeias are involved as observers to achieve global harmonization of pharmacopoeial standards. The pharmacopoeia defines legally binding quality standards for all medicinal products in its member states (i.e., raw materials, preparations, dosage forms, container compliance with requirements).[26] Legislation foresees a mechanism to provide the pharmacopoeia authority with information on the quality of products on the market, which ensures that monographs are routinely updated to reflect the state of the art. The European Pharmacopoeia is complemented by national pharmacopoeias for products of interest to only one member state.

Doxil®, the first FDA-approved nano-drug: experience gained and lessons learned

Yechezkel Barenholz (Hebrew University–Hadassah Medical School) discussed the development of the first FDA-approved nanodrug, the anticancer drug Doxil (pegylated liposomal doxorubicin (PLD); Caelyx® in Europe), which today, almost 20 years later, is still extensively used and remains the gold standard of injectable nano-drugs and drug delivery systems.[27–30]

Doxil is based on three unrelated principles: (1) prolonged drug circulation time and avoidance of the RES due to the use of pegylated nanoliposomes;[27,30,31] (2) high and stable remote loading of doxorubicin driven by a transmembrane ammonium sulfate gradient,[32–34] which also allows for drug release at the tumor (Silverman and Barenholz, unpublished data); and (3) having the liposome lipid bilayer in a liquid-ordered phase composed of the high-T_m (53 °C) fully hydrogenated soy phosphatidylcholine together with cholesterol and PEG-DSPE.[27] This lipid composition helps to maintain the transmembrane ammonium gradient and the excellent drug retention during storage and *in vivo* in human plasma, and to achieve a zero-order slow drug release at the tumor site.[27,34] Owing to the enhanced permeability and retention (EPR) effect,[35,36] Doxil is passively targeted to tumors,[31] and its doxorubicin is released and becomes available to tumor cells by factors related to the unique tumor microenvironment and metabolism (Silverman and Barenholz, unpublished data). Doxil's success stems from the lessons learned from the lack of success in clinical trials of a previous liposomal doxorubicin formulation developed by Gabizon and Barenholz, which was based on 200–500 nm oligolamellar negatively charged liposomes (DOX-OLV), in which the positively charged doxorubicin was passively loaded by electrostatic interaction (reviewed in Ref. 37). DOX-OLV was designed to treat liver cancer. In spite of being efficacious in mouse tumor models, the DOX-OLV formulation failed in human clinical trials due to a dilution-induced release of most of the drug immediately after its infusion.[37,38] This phenomenon was not observed in mice, where the dilution upon intravenous administration is 300-fold less than in humans. In addition, imaging studies revealed that, in humans, the empty liposomes reach the RES rather than the liver tumor target. The experience gained from the DOX-OLV studies in mice and humans led to applying a better understanding of the crosstalk between physicochemical, nano-technological, and biological principles, which resulted in the successful development of Doxil.

However, in spite of the considerable success of Doxil's large and continuously growing sales worldwide, more than 3 years after the last Doxil-related patent expired,[39] there is only one FDA-approved generic Doxil available, although many companies are trying to develop generics and obtain approval. The one current generic is Lipodox, which was approved by the FDA (but not yet by the EMA) in February 2013. Lipodox has replaced the original product Doxil/Caelyx, whose availability was very limited for the last 2 years and recently stopped production entirely.

The scientific, technological, and regulatory difficulties in obtaining approval for Doxil generics can be best understood from the FDA requirements for approval of Doxil generics, as seen in the FDA "Draft Guidance on Paclitaxel"[23] and reviewed by Jiang and colleagues.[40] The development of a generic equivalent to Doxil requires *in vitro* characterization and a chemistry, manufacturing, and controls (CMC) level assuring the generic has the same drug product

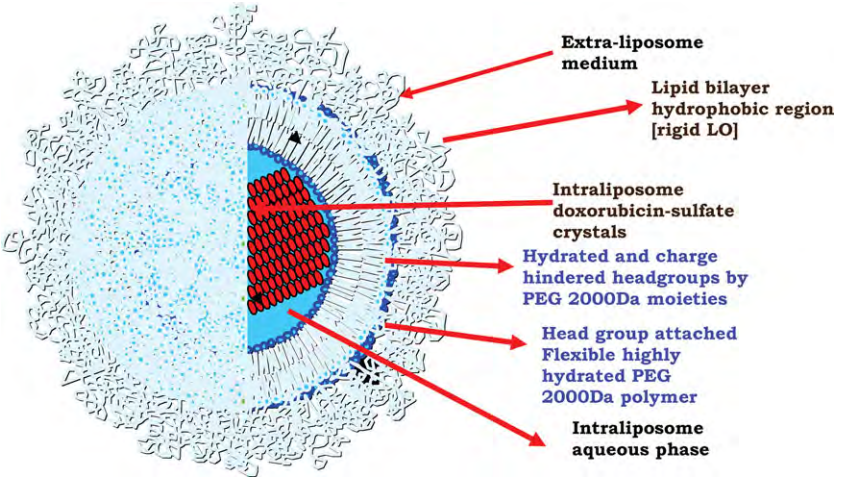

Figure 4. A cartoon of a Doxil–pegylated nano (< 100 nm) unilamellar liposome. The cartoon is based on cryo-TEM, SAXS, WAXS, DLS, compressibility, and doxorubicin absorbance and fluorescence. From Ref. 27.

composition, is manufactured by an active (remote) liposome drug-loading process with an ammonium sulfate gradient, and shares liposome characteristics with Doxil at the CMC level including liposome composition, state of encapsulated drug, internal environment of liposome, liposome size distribution, number of lamellae, grafted PEG at the liposome surface, electrical surface potential or charge, and *in vitro* leakage rates. The clinical requirements include (1) a single-dose, two-way crossover study in ovarian cancer patients whose disease has recurred or progressed after platinum-based chemotherapy; (2) a dose of 50 mg/m^2; (3) bioequivalence based on 90% CI; and (4) a pivotal bioequivalence study conducted using test product produced by the proposed commercial-scale manufacturing process (however, due to the low free doxorubicin plasma level and a large patient-to-patient variability, such a study becomes a challenge and may require a large number of patients).

Other aspects of the comparability studies that cannot be ignored are the side effects recognized at Doxil's first clinical trial: hand and foot syndrome and complement activation.[41,42]

Doxil development required developing and validating a large repertoire of QC methods to deal with the chemical, biological, and physicochemical characterization of a liposomal drug.[43] According to Barenholz, the FDA guideline drafts on liposomal drugs and on generic Doxil further increase this repertoire. While quality control for the chemical aspect of the liposomal drug is familiar to the pharmaceutical industry, this is not the case for the unique biological assays (such as quantification of complement activation) and unique physicochemical assays. The latter include the use of sophisticated equipment and methodologies such as electron microscopy (especially cryo-transmission electron microscopy, X-ray diffraction (SAXS and WAXS), differential-scanning calorimetry (DSC), and methods to determine size distribution (such as dynamic light scattering (DLS)).

Figures 4 and 5 demonstrate the complexity of measuring size distribution and structure of nanodrugs such as Doxil (Caelyx). The Caelyx cryo-TEM (Fig. 5) reveals the prolate ellipsoid shape and the long doxorubicin sulfate crystal whose presence and structural details were confirmed by small angle X-ray diffraction measurements (Ref. 44 and Schilt, Barenholz, and Raviv, unpublished data). However, the cryo-TEM does not show the PEG layer, and therefore size measurement based on cryo-TEM is misleading. DLS measures the diffusion coefficients of the liposomes from which hydrodynamic radius (which includes the PEG layer) can be determined;[44] however DLS assumes a spherical shape for the liposomes, which is not the case for Doxil (Fig. 4). X-ray diffraction studies enable determination of the PEG layer dimension (Ref. 45 and Schilt, Barenholz, and Raviv, unpublished data). This example demonstrates the need to use a broad spectrum of physical approaches and methods that complement

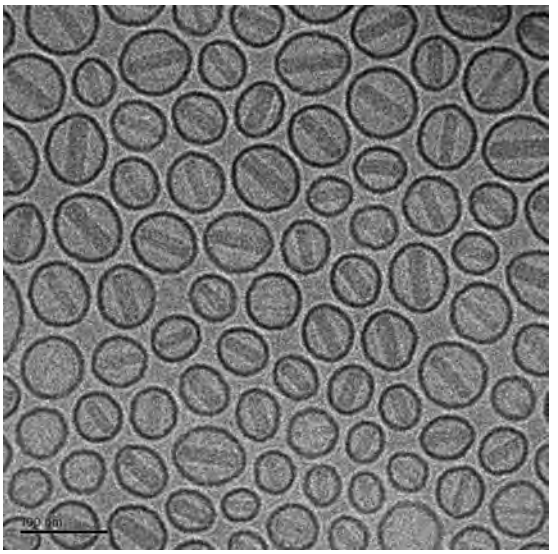

Figure 5. Cryo-TEM of Caelyx batch 101371803. Scale bar, 100 nm.

each other, such as DLS, X-ray diffraction, and cryo-TEM.

The above FDA requirements for the approval of generic Doxil, argued Barenholz, can also serve as a good example of the broad spectrum of requirements for the FDA approval of any nano-drug.

The 25 years of preclinical and clinical experience with Doxil facilitate the initiation of research into developing novel formulations and means aimed at improving the performance of PLD and similar drugs by enhancing the therapeutic efficacy (by optimizing drug release rate at the tumor) and reducing the major side effects (hand and foot syndrome and complement activation), thereby further improving the nano-drug's therapeutic index.

Using nanotechnology to change cancer care

Currently, most cancer patients first undergo surgery to remove the tumor, followed by chemotherapy and/or radiation treatment; all too often such patients present with recurring disease. Lawrence Tamarkin (CytImmune) proposed a different strategy that relies upon the unique biology of the newly formed tumor blood vessels and gold nanoparticles to deliver a potent but toxic vascular-disrupting agent directly to tumors. The underlying rationale for this strategy is based upon the now established fact that newly formed tumor blood vessels are inherently leaky, with fenestrations of 200–400 nm in size.[46] By allowing blood-borne proteins to leak into the tumor perivascular space, a physical barrier is created to systemically administered drugs, since the intratumor interstitial pressure is greater inside the tumor than outside it, leading to poor penetration of chemotherapeutics.[46]

To address this challenge, CytImmune has created a gold nanoparticle–based drug delivery system that (1) avoids immediate immune detection by binding an analog of polyethylene glycol (PEG–thiol) to the gold nanoparticles' surface, and (2) carries the potent but toxic vascular-disrupting agent tumor necrosis factor α (TNF-α).[47] When injected systemically, this 27-nm drug, termed CYT-6091, travels safely through the body, unable to exit the circulation until it reaches the tumor neovasculature, where the blood pressure forces the particles out into the tumor. The TNF-α on CYT-6091 then binds to the tumor blood vessel endothelial cells, causing them to die and breaking down the high intratumor fluid pressure.[48] By equilibrating this pressure differential, follow-on chemotherapies may penetrate the tumor more effectively, killing cancer cells better.

Clinically, dosing with 1 mg TNF-α followed by chemotherapy is only safe when administered regionally, not systemically, in the rescue of tumor-burdened limbs. With this procedure, isolated limb perfusion, patients have an 85% response rate with just one treatment.[49] This clinical success forms the rationale for treating cancer patients with CYT-6091 systemically first, followed by standard-of-care chemotherapy with the goal of achieving similar response rates. However, before a combinational study is undertaken, a single-agent CYT-6091 trial needed to be conducted in cancer patients to demonstrate the nanomedicine's safety. To that end, the first task was to create a robust, reproducible, scalable, and cost-effective manufacturing process.

This manufacturing process relies on two large vessels, one containing colloidal gold and the other a mixture of PEG–thiol and TNF-α (these two molecules do not bind to each other). The fluid from each container is drawn via a single peristaltic pump into a Y-connector, where TNF-α and PEG–thiol instantaneously and individually bind to the gold nanoparticles. The resultant nanomedicine is then ultra-filtered to concentrate the drug for dispensing and lyophilizaton. The shelf life for this product is over 3 years when stored at 4 °C.

Following IND approval, a dose-escalation Phase I clinical study was conducted at the NCI, where advanced-stage cancer patients were injected systemically twice, 2 weeks apart, with CYT-6091. The first objective was to safely reach a dose level of 1 mg of TNF-α formulated as CYT-6091, and the second objective was to determine if the gold particles trafficked to tumors, not healthy tissues and organs. Both objectives were met.[50]

A Phase II clinical trial dosing CYT-6091 before standard-of-care docetaxel treatment for patients with non-small cell lung cancer (NSCLC) is planned to determine if pretreatment with CYT-6091 improves response rates to docetaxel.

However, the ideal nanomedicine would deliver the vascular disruptor TNF-α and chemotherapy on the same gold nanoparticle. While TNF-α forms a dative covalent bond with the gold nanoparticles through available thiol groups found on cysteine residues in the TNF-α protein, small molecule therapeutics, such as paclitaxel, cannot bind, since there are no available thiol groups to bind to the gold nanoparticle. Consequently, a linker containing a distal thiol that releases native paclitaxel either by self-immolation or hydrolysis was bound to paclitaxel. With these analogs, various nanomedicine formulations were created using the same manufacturing process used for CYT-6091. Each formulation contained TNF-α, PEG–thiol, and a paclitaxel analog, all bound to the same gold nanoparticle.

This formulation, termed CYT-20000, exhibited a number of features when tested in mice. First, little to no paclitaxel was released into the circulation. Second, TNF-α in the nanomedicine formulation was essential for tumor targeting and was not dependent on TNF-α receptors on the cancer cells. Third, paclitaxel was slowly released in the tumor, suggesting that the nanomedicine behaved like a slow-release depot in the tumor. Based on these characteristics, AstraZeneca engaged CytImmune to rescue a potent but toxic small drug candidate molecule using the CYT-20000 linking chemistry and the CYT-6091 platform.

In closing, the CytImmune family of nanomedicines, built on TNF-α bound to PEGylated gold nanoparticles, relies upon naturally occurring leaky tumor blood vessels for tumor targeting and vascular disruption for improved responses to chemotherapy, leading to the conclusion that solid tumors should be treated with these nanomedicines first to reduce tumor burden *in situ*, before surgical resection.

Lessons learned from albumin-bound nanoparticles

Neil Desai (Celgene Corporation) applied the lessons learned from the successful development of Abraxane® (albumin-bound paclitaxel, *nab*-paclitaxel) to illustrate the key considerations, strategies, and major hurdles that occur during the design, manufacture, and regulatory approval process of nanomedicines. The strong motivations that drive the rapid advance in this emerging field come as a result of many potential advantages conferred by nanomedicines that differentiate them from conventional therapies, including the ability to overcome biological barriers, the effective delivery of hydrophobic drugs and biologics, specific targeting of disease sites, and improved therapeutic index.

The first protein nanotechnology-based chemotherapeutic and one of the few nanomedicines currently approved by the FDA (Table 1), Abraxane is a cremophor-free, albumin-bound nanoparticle formulation of paclitaxel with a mean particle size of approximately 130 nm that was developed to leverage the unique transport mechanisms of albumin in penetrating tumors and to eliminate the toxicities associated with the cremophor EL/ethanol vehicle used in solvent-based paclitaxel (Taxol®), resulting in an improved efficacy and safety profile.[51] Currently approved in metastatic breast cancer, non-small cell lung cancer, and metastatic pancreatic cancer, the distinct pharmacology and drug delivery of Abraxane led to differentiation of its clinical efficacy and safety from conventional paclitaxel. Desai highlighted that patients who were previously treated with or refractory to other taxanes could still respond to Abraxane. Abraxane improves clinical outcomes across multiple tumor types, including historically non-taxane–sensitive tumors such as pancreatic cancer. Previous *in vitro* results from the NCL demonstrated that conventional paclitaxel but not Abraxane results in strong complement activation through both classical and alternative pathways, which is consistent with hypersensitive reactions and anaphylaxis observed clinically with conventional paclitaxel. Despite the use of premedication, fatal reactions can still occur

with cremophor–paclitaxel, posing a serious safety issue.[52]

Desai further summarized the clinical and regulatory strategies for the development of nanomedicines, which can incorporate a novel active pharmaceutical ingredient or a previously approved drug. For the latter, developers need to deliberate whether to compete with an approved drug in the same indications or to develop new indications unapproved for the conventional drug. A nanomedicine needs to demonstrate clear advantages over an approved counterpart or existing therapies to achieve market and reimbursement success. Incorporating the latest advances in personalized medicine and molecular profiling, targeted nanomedicines can utilize additional clinical enrichment strategies directed toward the target of the carrier and/or the target of the payload. The carrier nanoparticle has the capacity to be engineered to contain different targeting moieties that home to the overabundance of their targets at the disease site, thus enhancing the delivery and accumulation of the drug. On the other hand, a nanomedicine can carry a payload that is a molecular-targeted therapeutic agent modulating a disease-specific pathway or process. It is therefore possible that a nanomedicine using a combined approach may convey additional clinical benefits by incorporating both a targeted carrier and a targeted payload.

While an innovator nanomedicine will face significant clinical development and regulatory hurdles, the emerging nanosimilars—similar or generic versions of an existing nanomedicine—pose a different set of regulatory challenges. An example of such a nanosimilar is generic Doxil (manufactured by Sun Pharma, Mumbai, India), which was approved by the FDA in February 2013 following a draft guidance for generic Doxil issued by the FDA in 2010. Regarding the regulation of nanomedicines, the FDA recognizes that each case may be different and can require specific testing and an understanding of critical aspects of physicochemical properties and structure.[53] The EMA has also paid close attention to the regulation of nanosimilars and has issued several relevant reflection papers on intravenous liposomal products, nanosized colloidal iron–based preparations, block copolymer micelles, and nanomedicine surface coatings.[1] Like the FDA, the EMA recognizes that follow-on nanomedicines (nanosimilars) need case-by-case evaluations due to the complexity of the product and that safety of nanomedicines may be different from traditional medicines. In particular, surface properties such as surface ligand orientation can govern the PK, biodistribution, stability, and intracellular fate of nanomedicines, and thus are critical for their safety and efficacy. Further, the physicochemical properties of nanomedicines are critical and sensitive to manufacturing process (i.e., the process is the product); therefore, conventional bioequivalence testing may not be enough and determining the scale of clinical data required for follow-on nanomedicines is a challenge.

To illustrate the challenges and complexity of testing and regulating nanosimilars, Desai used Doxil and Abraxane as examples. FDA draft guidance for Doxil indicated that a nanosimilar drug would need to have the same drug product composition and equivalent liposome characteristics, and that the manufacturing process is critical, in addition to other attributes of pharmacokinetic bioequivalence. In contrast to Doxil, which uses well-defined phospholipids as the carrier and circulates in the body as intact liposomal particles carrying the drug, Abraxane contains a biologic component, albumin, and the nanoparticles are designed to dissociate for rapid drug release and distribution. In addition to clinical PK studies measuring unbound/total paclitaxel and particle size distribution, the FDA draft guidance for Abraxane recommends extensive *in vitro* characterization of nanoparticles, including particle morphology, size, surface potential, paclitaxel crystallinity, free and bound paclitaxel or albumin in reconstituted suspension, the nature of the bond between paclitaxel and albumin, *in vitro* release kinetics, and the oligomeric status of albumin in excipient and drug product.[53] The albumin component in Abraxane also raises the testing issues related to biologics and biosimilars, such as immunogenicity. Abraxane nanoparticles are complex three-dimensional structures with multiple components, and variations in the manufacturing process can create unintended changes in critical particle characteristics as well as product safety and efficacy profile. Therefore, additional clinical studies may be required to ensure the safety and efficacy of a nanosimilar drug, because conventional testing methods may be unable to detect such subtle changes. Desai highlighted that the urgency of this issue has been underscored by several unsuccessful attempts in the marketplace to

copy the Abraxane formulation. Alleged nanosimilars of Abraxane marketed in a non-Western country were unable to maintain the stability of nanoparticles in suspension and failed to match the Abraxane albumin-coating composition, raising serious questions of product safety and efficacy.

Finally, Desai emphasized the challenges involved in bringing a novel nanomedicine to the market by showcasing the long development history of Abraxane from early concept in 1992 to initial approval in 2005 and the failures of Tocosol (TPGS/Vitamin E formulation of paclitaxel, Sonus Pharmaceuticals) and Opaxio (paclitaxel–polyglumex, Cell Therapeutics) in Phase III clinical trials. The ultimate success or failure of a nanomedicine depends on its complexity and our knowledge about its mechanisms of transport, dissociation, pharmacokinetics, biodistribution, pharmacology, and safety, as well as clinical trial design and patient selection.

Conclusions/future perspectives

Active agents that failed as conventional formulations due to unacceptable toxicity profiles, poor bioavailability, solubility issues, or physical/chemical inability have been successfully reformulated as nanopharmaceuticals.[1,17,18] Additionally, with targeting ligands, nanomedicines can be innovative therapeutic agents for the enhancement of cellular uptake into tissues of interest. From a business perspective, nanopharmaceuticals offer the ability to extend the economic life of proprietary drugs and create additional revenue streams, thereby significantly affecting the drug commercialization landscape.

Nanopharmaceuticals offer the potential to create "magic bullets" to improve and innovate the therapeutic armory to diagnose, prevent, and treat human diseases, both emerging diseases and those that have not yet been appropriately addressed. However, the scientific and regulatory gap is large and challenging. It could be addressed most effectively in a multipronged approach combining scientific and regulatory efforts from academia, industry, and governmental bodies with a focus on patient health and safety (Table 5).

There has been a classic lifecycle management option practiced by drug companies in case of reformulation via "nano." According to Bawa, nanopharmaceuticals, whether they are small-molecule drugs, biologic drugs, or NBCDs, are usually not bioequivalent to their parent versions ("bulk counterparts"). Hence, they cannot be automatically considered as generics or follow-ons and cannot apply for FDA approval via an Abbreviated New Drug Application (ANDA) under section 505(b)(j) of the Federal Food, Drug, and Cosmetic Act.[20] Hence, a New Drug Application (NDA) under the 505 (b)(1) route may need to be filed at the FDA. If a nanopharmaceutical is bioequivalent to its parent version, an ANDA can be filed to seek regulatory approval. Therefore, when warranted, the FDA may treat nanoversions of active ingredients as new chemical entities. This will ensure that therapeutics that have been previously approved by the FDA but later modified as "nanoversions" will also undergo a new and rigorous round of safety testing before market approval.[20]

So far, lessons learned show that classical approaches for drug development and (non-) clinical assessment are partially erroneous and very costly. In addition, those approaches have delayed the progress of innovation and left safety concerns. Even nanoparticular medicines used safely for many years are heavily challenged when it comes to follow-on versions, where the classical paradigms for abbreviated authorizations are no longer valid because of the lack of full characterization of the follow-on versions and the lack of knowledge of this new class of drug products not yet accompanied by appropriate evaluation processes (Fig. 1). The complexity of the products and their non-homogenous structures defined by laborious and difficult-to-control manufacturing process involving nanomaterials on several steps is key to their profile. As a consequence, biodistribution and tissue/cell targeting is not fully understood with relation to physicochemical product characteristics. A sameness approach with therapeutic equivalence of copy products seems likely impossible to reach, and clinically meaningful differences among comparable drug products might only be detected through human exposure (Fig. 2). The somewhat vague definition of nanomedicine calls for proper and globally accepted terminology also addressing concerns and unnecessary litigations for patent-related issues. Harmonization and adaptation of regulatory requirements is urgently needed, probably starting with combined efforts from a scientifically oriented stakeholders group on a simpler and at least partially well-understood nanomedicine issue. This conference

Table 5. Recommendations for the FDA regarding nanomedicine regulation[20]

Safety and risk
- On a case-by-case basis and in conjunction with industry, identify unique safety issues associated with nanomedical products.
- Actively seek product safety data from industry where FDA statutory authority exists for pre-market review.
- Incentivize and encourage voluntary industry submissions of safety data on nanomaterials or products that incorporate nanotechnology before market launch, especially in cases (e.g., cosmetics) where the FDA lacks statutory authority for pre-market review.
- Correlate physiochemical properties with *in vivo* biological behavior and therapeutic outcome.
- Develop a research strategy that involves adsorption, distribution, metabolism, and excretion (ADME) studies.
- Develop toxicology tests and conduct physicochemical characterization (PCC) studies for nanomaterials.
- Understand mass transport across membranes and body compartments.
- Determine accurate biodistribution profiles following systemic administration via a specific route.
- Develop standards that correlate the biodistribution of various nanomaterials with safety/efficacy by using parameters such as size, surface charge, stability, surface characteristics, solubility, crystallinity, and density.
- With industry input, create a databank relating to the interactions between nanomaterials and biological systems.

Data
- Adapt existing methodologies and develop new paradigms for evaluating data pertaining to safety and efficacy of nanomedical products.
- Develop guidance that provides specifics as to what kind of data is needed.
- Share data in an internationally harmonized environment.

Standardization
- Create reference classes for nanomaterials that are synthesized and characterized.
- Develop consensus testing protocols to provide benchmarks for the creation of classes of nanoscale materials.
- Create uniform standards for and/or working definitions of nanomaterials.
- Refine the current definitions of nanomaterial, nanotechnology, nanoscale, and nanomedicine for the purpose of regulation.
- Explore international harmonization efforts and formal treaties.
- Involve standard-setting organizations such as the International Organization for Standardization (ISO) and ASTM International.
- Consult and collaborate with other federal agencies in a more effective manner.

Tools
- Assist in developing unique tools and techniques to characterize nanoscale materials.
- Develop imaging modalities for visualizing biodistribution.
- Develop mathematical and computer models for risk/benefit analysis.
- Monitor quality, safety, product liability, and effectiveness.

Classification scheme
- Reevaluate the current FDA classification scheme.
- Develop a classification scheme based on (1) function or (2) risk of potential harm.

showed, through the presentations of experts and the intense interaction with highly interested participants, the relevance and timely need for education, exchange, and common projects to direct drug innovation by nanotechnology into a benefit with low and acceptable risk for patients and improved therapy and restoration of health for society.

The toxicity of many nanoscale materials is not fully apparent, and premarket testing of nanomedicines will not detect all adverse reactions, so it is critical that long-term safety testing be conducted. Therefore, postmarket tracking or analogous surveillance systems need to be adopted to assist in recalls, toxicity data specific to nanomaterials needs to be collected, and an effective risk research strategy must be devised.

In the future, novel multifunctional nanopharmaceuticals may be designed as new generations

of drug delivery systems to target specific organs, tissues, or cells. As we rapidly enter the age of theranostics, complex combination nanoproducts will continue to be designed—these are further likely to test the limits of regulatory authority. Therefore, it is imperative that the regulatory gaps that currently exist in nanomedicine should be addressed now; this will alleviate some of the burden later. The guiding principle here should reflect a balance of innovation and R&D with public health protection.

Conflicts of interest

The authors declare no conflicts of interest. Dr. Bawa is scientifc advisor to Teva Pharmaceuticals, Inc. (Israel).

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