

CHAPTER 12

The Challenge of Regulating Nanomedicine: Key Issues

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12.1 Introduction

There is no shortage of excitement, exuberance, hype and misinformation when it comes to anything “nano”. Many claim it to be the next industrial revolution, some purport that it has already permeated virtually every sector of our economy while others dismiss it as nothing new but a repackaging of old concepts and technologies with a new label.

In reality, nanotechnology is the natural continuation of the miniaturization of materials and biomedicine. One can now say with relative certainty that research and development (R&D) is in full swing with novel nanomedical products starting to arrive, or at least inching their way, into the marketplace. Everyone agrees that this decade has witnessed relatively more advances and product development in nanomedicine than years prior.

Too often though, companies, academia and policymakers exaggerate basic R&D in nanotechnology as potentially revolutionary advances and

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claim these early-stage discoveries as confirmation of downstream novel products and applications to come. Many have desperately tagged or thrown around the “nano” prefix to suit their purpose, whether it is for federal research funding, patent approval of supposedly novel technologies, raising venture capital funds, running for office or seeking publication of their manuscript. All of this is happening while thousands of over-the-counter products containing silver nanoparticles, titanium dioxide and carbon nanoparticles continue to stream into the marketplace without adequate safety testing, labeling or regulatory review.

Nanoproducts and applications do continue to evolve and play a pivotal role in various industry segments, spurring new directions in research, patents, commercialization and technology transfer. In spite of anemic product development in the 1980s and 1990s, patent filings and patent grants have continued unabated.¹⁻³ It is no secret that nanopatents of dubious scope and breadth continue to be granted by patent offices around the world. In fact, since the early 1980s, “patent prospectors” have been on a global quest for “nanopatent land grabs”. Universities have jumped into the fray with a clear intention of patenting as much “nano” as they can. As indicated above, academia, start-ups and established companies often continue to exaggerate basic research or project potential downstream applications based upon early-stage discoveries. Venture funding has mostly shied away in recent years, although industry–university alliances have continued to gel. Wall Street’s interest in nanotechnology has been somewhat mixed over the years, from cautionary involvement to generally shying away, partly due to the definitional and regulatory issues.

12.2 Defining “Nano”: A Problem for Regulators?[†]

How should regulators define nanotechnology in the context of pharma? What does nanoscale drug delivery mean? Are all engineered nanotherapeutics unique purely due to a specific size range? Is there a regulatory or legally plausible definition of nanotech from a pharma perspective with respect to size? Does a specific or unique size range of nanotherapeutics impart nanotoxicity?

All these questions are related to the definition of the prefix “nano.” In fact, this definitional issue, or lack thereof, continues to be one of the most significant problems shared by regulators, policy-makers, drug companies, patent practitioners, and legal professionals.⁴⁻¹⁰ In particular, regulatory agencies and governmental entities such as the US Food and Drug Administration (FDA), the European Medicines Agency (EMA), Environmental Protection Agency, Centers for Disease Control and Prevention, National Institute for Occupational Safety and Health and the US Patent and Trademark Office are grappling with this critical issue. Clearly, the need for an internationally agreed definition for key terms like nanotechnology,

[†]This section is adapted, with permission, from ref. 16.

nanoscience, nanomedicine, nanobiotechnology, nanodrug, nanotherapeutic, nanopharmaceutical and nanomaterial has gained urgency. This is essential for harmonized regulatory governance, accurate patent searching and prosecution, standardization of procedures, assays and manufacturing, quality control, discussion of ethical issues, safety assessment and much more.

In this chapter, we use the following terms interchangeably: nanomedicine, nanodrug, nanotherapeutic and nanopharmaceutical. Similarly, we use these terms interchangeably: nano, nanotech and nanotechnology.

So, what does the prefix “nano” refer to? Any term with this prefix is broad in scope. Consider the widely used terms nanotechnology, nanomedicine and nanopharmaceutical, all of which are misnomers/misleading because they do not refer to a single technology. The terms nanotechnology and nanomedicine refer to interdisciplinary areas that draw from the interplay among numerous materials, products and applications from several technical and scientific fields. In other words, “nano” is an umbrella term/prefix encompassing several technical/scientific fields, processes and properties at the nano/micro scale.⁴⁻¹³

Clearly, there is confusion over the definition of nanotechnology. Moreover, due to a lack of any standard nomenclature, various inconsistent definitions have sprung up over the years.⁴⁻¹⁶ For instance, nanotechnology has been *inaccurately* defined by the US National Nanotechnology Initiative (NNI)[‡] since the 1990s as “the understanding and control of matter at the nanoscale, at dimensions between approximately 1 and 100 nanometers, where unique phenomena enable novel applications...”. Some definitions increase the upper limit to 200 nm or 300 nm or even 1000 nm. Some definitions omit a lower range. Others refer to sizes in one, two or three dimensions while others require a size plus special/unique property or *vice versa*.¹⁴ Given this backdrop, Dr Bawa proposed the following definition of nanotechnology in 2007, one that is unconstrained by an arbitrary size limitation:^{15,16} “The design, characterization, production and application of structures, devices, and systems by controlled manipulation of size and shape at the nanometer scale (atomic, molecular, and macromolecular scale) that produces structures, devices and systems with at least one novel/superior characteristic or property.” This definition has three key features. First, the definition recognizes that the properties and performance of the synthetic, engineered *structures, devices and systems* are inherently rooted in their nanoscale dimensions. Second, the focus of this flexible definition is on “technology” that has commercial potential from a consumer perspective, not “nanoscience” or basic R&D in a laboratory setting that may

[‡]The NNI is the US government’s interagency program for coordinating, planning and managing R&D in nanoscale science, engineering, technology and related efforts across 25 agencies and programs. The NNI has been regularly reviewed by the President’s Council of Advisors on Science and Technology since the council was designated in 2004.

lack commercial implication. Third, the *structures, devices and systems* that result from or incorporate nano must be *novel/superior* compared to their bulk, conventional counterparts. Fourth, the concept of *controlled manipulation* (as compared to “self-assembly”) is critical here.

One of the major impacts of nanomedicine is taking place in the context of drug delivery. Novel nanodrugs[§] and nanocarriers are being developed that address fundamental problems of traditional drugs, ranging from poor water solubility, unacceptable toxicity profiles, poor bioavailability, solubility issues, physical/chemical instability and a lack of target specificity. Additionally, *via* tagging with targeting ligands, these nanodrug formulations can serve as innovative drug delivery systems for enhanced cellular uptake of therapeutics into tissues of interest (site-specific delivery). As a result, nanodrugs are being developed to allow delivery of active agents more efficaciously to the patient while minimizing side effects, improving drug stability *in vivo* and increasing blood circulation time. There are a number of FDA-approved, commercialized first-generation nanodrugs for intravenous and non-intravenous delivery. Some of these are completely novel while others are redesigned variations of earlier versions (Figure 12.1). The first-generation nanodrugs mainly address single challenges such as targeted delivery while the second and third generations currently in development can offer two or more functions together (*e.g.*, delivery and imaging) or overcome multiple physiological barriers to deliver their therapeutic payloads. However, most nanodrugs are still in their infancy, being at the pre-clinical development stage or in clinical trials. As these nanodrugs move out of the laboratory and into the clinic, governmental regulatory agencies such as the FDA and EMA continue to struggle to encourage their development while imposing some sort of order in light of regulatory and safety concerns. If a size limit must be imposed on the definition of “nano”, then an upper limit of 1000 nm (1 μm) may be most appropriate, at least regarding a discussion on nanodrugs. It appears that the FDA in 2014 finally expanded the upper limit to 1000 nm (from 100 nm), albeit unofficially.[¶]

[§]There is no formal definition for a nanoscale drug delivery system (nanodrug product). Dr Bawa¹⁶ defines it as being: (1) a formulation, often colloidal, containing therapeutic particles (nanoparticles) ranging in size from 1 to 1000 nm; and (2) either (a) the carrier(s) is/are the therapeutic (*i.e.*, a conventional therapeutic agent is absent) or (b) the therapeutic is directly coupled (functionalized, solubilized, entrapped, coated, *etc.*) to a carrier. The FDA defines the term “colloid for regulatory purposes as a chemical system composed of a continuous medium (continuous phase) throughout which are distributed small particles, 1 to 1000 nm in size (disperse phase), that do not settle out under the influence of gravity; the particles may be in emulsion or in suspension.”

[¶]Even the FDA, which has not adopted any “official” regulatory definition, appears to now be emphasizing a loose definition for products that involve or employ nanotechnology in the context of pharma that either: (1) have at least one dimension in the 1–100 nm range; or (2) are up to 1000 nm, provided the novel/unique properties or phenomenon exhibited are attributable to these dimensions outside of 100 nm.

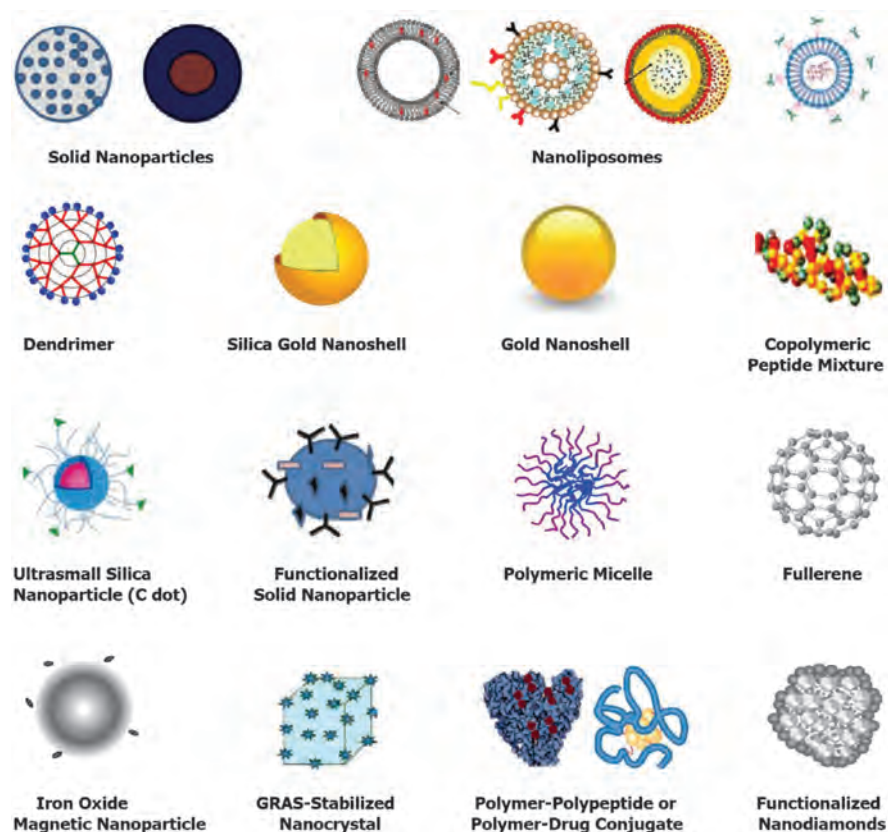


Figure 12.1 Schematic illustrations of nanoscale drug delivery system platforms (nanotherapeutics or nanodrug products). Shown are nanoparticles (NPs) used in drug delivery that are either approved, are in preclinical development or are in clinical trials. They are generally considered as first or second generation multifunctional engineered NPs, generally ranging in diameter from a few nanometers to a micron. Active biotargeting is frequently achieved by conjugating ligands (antibodies, peptides, aptamers, folate, hyaluronic acid) tagged to the NP surface *via* spacers or linkers like PEG. NPs such as carbon nanotubes and quantum dots, although extensively advertised for drug delivery, are specifically excluded from the list as this author considers them commercially unfeasible for drug delivery. Non-engineered antibodies and naturally occurring NPs are also excluded. Antibody-drug conjugates (ADCs) are encompassed by the illustration labelled “Polymer-Polypeptide or Polymer-Drug Conjugate.” This list of NPs is not meant to be exhaustive, the illustrations are not meant to reflect three dimensional shape or configuration and the NPs are not drawn to scale. Abbreviations: NPs: nanoparticles; PEG: polyethylene glycol; GRAS: generally recognized as safe; C dot: Cornell dot; ADCs: antibody-drug conjugates. Copyright © 2016 Raj Bawa. All rights reserved. The copyright holder permits unrestricted use, distribution and reproduction of this figure (plus legend) in any medium, provided the original author and source are clearly and properly credited. Reproduction without proper attribution constitutes copyright infringement.

12.3 Lessons Learned from Doxil[®]: The First FDA-approved Nanodrug

Doxil[®] was the first FDA-approved nanodrug. The success of Doxil[®] largely stemmed from it being a nanoliposome. The development of Doxil[®] is reviewed elsewhere^{17,18} and also in this book. In this chapter, we deal with two aspects of the development of Doxil[®]: can it be classified as a nanodrug and what are the special requirements for approval of generic Doxil[®] by the FDA and/or EMA? To answer the first question we have to go back to 1959, when the new discipline of nanotechnology was first mentioned, although not by name, by Nobelist Richard Feynman. He highlighted the importance of scaling issues, suggesting that certain very small assemblies have different physical behavior from large ones, to a large extent due to their very large surface-to-volume ratios and their small sizes.^{||} This imparts unique properties to most nano-assemblies that low molecular weight molecules and larger assemblies generally lack. Given this backdrop, we suggest that nanodrugs based on nanoliposomes (such as Doxil[®]) meet the above criteria and can be classified as nanodrugs primarily for the following two reasons:

- (1) Biological reason (nano-anatomy): nanoscale particulates take advantage of the unique porous vasculature that is the Achilles' heel of cancer and inflammation, as it enables "passive targeting" of ≤ 100 nm particulates to the tumor and inflamed tissues. This effect is termed the enhanced permeability and retention (EPR) effect.¹⁹⁻²¹ This selection is a decision process made by the biology/physiology/anatomy related to the disease. Neither low molecular weight drugs, nor drugs associated with particles in the micro range can take advantage of the EPR effect.
- (2) Nano-related reasons:
 - (a) For liposome fabrication, the bottom-up approach is used. In the presence of water, there is a self-assembly from single lipid molecules to particulate assemblies. The lipid bilayer width is 5 nm and many of the assemblies are < 100 nm in diameter, structures that fall under the nano classification.
 - (b) Nanovolume provides the nanoliposomes unique properties of highly efficient, stable drug loading and controlled release profile. The nanovolume enables the efficient remote loading driven by transmembrane ion and/or pH gradients, thereby achieving very high drug levels per liposome. Therefore, these nanoliposomes can bring sufficient drug load to the tumor or inflamed tissue in spite of their nanovolume. This nanovolume also enhances drug release by the ammonia present in the tumor due to the unique metabolic pathway of glutaminolysis.¹⁸ This is demonstrated by the fact that in such a nanoliposome, the shift between internal pH 5.0

^{||}As a drug particle is granulated (nanonized) into smaller particles, the total surface area of the smaller particles become much greater relative to their volume (*i.e.*, an enormously increased surface-area-to-volume ratio).

and pH 6.0 requires the movement of >10 protons. Finally, the high concentration of active pharmaceutical ingredient (API) at the intraliposome aqueous phase in the presence of a suitable counterion results in intraliposome reversible precipitation and/or crystallization which helps to stabilize drug loading during storage and circulation in the blood of the animal or human. This effect enables liposomes to reach the diseased tissue loaded with drug.

Doxil[®] is a successful anticancer drug, especially given its worldwide use for the treatment of ovarian cancer. So far, more than 500 000 patients have been treated with Doxil[®] worldwide. However, in spite of its extended use in the more than 4 years since the last Doxil[®]-related patent expired²² and in spite of major efforts by many companies around the globe to try to produce it and obtain regulatory approval, there is only one FDA-approved “generic Doxil[®]” available. This formulation is Lipodox[®], which was approved by the FDA in 2014** (not approved yet by the EMA). Why are there not many generic Doxil[®]-like products approved by the FDA, despite enormous efforts by many companies to get approval? The answer lies in the FDA requirements for the approval of generic Doxil[®]. In fact, these requirements can also serve as a good example to the tough and broad spectrum of requirements of FDA for the approval of similar nanodrugs.

The scientific, technological, and regulatory difficulties in getting Doxil[®] generics approved can be better understood after evaluating carefully the example of FDA requirements for approval of “generic Doxil[®]”. This information is available in a 2013 FDA document.²³ The development of a generic Doxil[®] requires that certain criteria be met. Specifically, *in vitro* characterization and chemistry, manufacturing and control (CMC) levels include: (1) the same drug product composition; (2) manufactured by an active (remote) liposome drug-loading process with an ammonium sulfate gradient; and (3) on the CMC level, this means having liposomal characteristics equivalent to Doxil[®], including liposome composition, state of encapsulated drug, internal environment of the liposome, liposome size distribution, number of lamellae, grafted polyethylene glycol (PEG) at the liposome surface, electrical surface potential or charge and *in vitro* leakage rates.

The clinical requirements are deceptively simple in appearance, and include:

- a single dose,
- a two-way crossover study in ovarian cancer patients whose disease has recurred or progressed after platinum-based chemotherapy,

**In October 2011, drug shortages were such a pressing issue in the US that an executive order was issued directing the FDA to streamline the approval process for new therapeutics that could fill the voids. One of the major drugs whose supply was deficient in the US was Doxil[®], and to curb this shortage, the FDA authorized the temporary importation of Lipodox[®] in February 2012. Following this, the FDA evaluated and approved the drug formulation within a year, roughly one-third of the time it takes for an average generic to receive premarket regulatory approval. As a result, Lipodox[®] became the first generic nanodrug approved in the US.

- dose of 50 mg m⁻²,
- pharmacokinetic variables,
- free doxorubicin and liposome-encapsulated doxorubicin,
- bioequivalence based on (90% confidence interval) area under the curve (AUC) and maximum concentration (C_{\max}).

The pivotal bioequivalence study should be conducted using a test product produced by the proposed commercial-scale manufacturing process. Due to low plasma levels of free doxorubicin there is a potential for large patient-to-patient variability. As a result, overcoming this issue in the clinical trials may require a large number of patients. Other aspects of the comparison studies that cannot be ignored today are the already recognized side effects during Doxil's[®] first clinical trial:²⁴ hand-foot syndrome¹⁷ and complement activation.^{25,26}

The development of Doxil[®] required the development and validation of a large repertoire of quality control methods to deal with the chemical, biological and physicochemical characterization of a liposomal drug.²⁷ The FDA draft guidelines on liposomal drugs and on generic Doxil[®] further increases this repertoire. While the chemical aspect of the liposomal drug quality control is familiar to the drug industry, this is not the case for the unique biological assays (such as quantification of complement activation) and the unique physico-chemical methods that are a new topic to the pharma industry. The latter includes use of sophisticated equipment and methodologies such as electron microscopy (especially cryo-transmission electron microscopy or cryo-TEM), X-ray diffraction (small- and wide-angle X-ray scattering), differential scanning calorimetry and methods to determine size distribution and morphology distribution. Size and size distribution are accepted as critical attributes and can be approached using various methods.²⁷ These methods are divided into two major groups:

- (1) Methods based on measuring the diffusion coefficient from which the particle hydrodynamic radius is determined using the Stokes-Einstein equation^{27,28}. The radius obtained *via* dynamic light scattering (DLS) data includes the PEG layer as part of the hydrodynamic radius.
- (2) The second group includes methods based on image analysis of cryoTEM images, determining directly the shape and size distribution irrespective of the liposome shape. Due to lack of contrast, the radii determined by this approach do not include the liposome PEG layer. Therefore, the dimension of the PEG layer obtained by other methods such as X-ray diffraction must be added to the radii determined by the image analysis.^{28,29} For an ellipsoid such as Doxil[®], this image analysis enables the determination of the axial ratio and thereby the volume of the liposomes. When combined with X-ray diffraction data, whether the drug inside the liposome is soluble, or appears as a precipitate or as crystals can be determined.^{17,18,30}

Among the first group of methods DLS is the most commonly used and widely accepted approach.²⁷ Our recent results²⁸ provide more information on PEGylated liposomal doxorubicin (PLD) size measurements and size distribution based on both DLS and image analysis using cryo-TEM.

12.4 Baby Steps Lead to Regulatory Uncertainty: The FDA as an Example

The consequences of the overregulation of nanomedicine are potentially severe, ranging from unnecessary barriers to commercialization to inadvertent harm to the public and the environment. However, clearly there exists a need for creating regulatory guidelines that follow a science-based approach and are responsive to shifting risks. In other words, a fine balance needs to be struck, one that is flexible enough to be modified as technological innovation proceeds. A balance is always desired between promoting the development of nanomedicine and exercising effective and timely oversight when warranted. After all, laws and regulations always try to keep up with scientific advances in emerging technologies and if they lag too far, as we believe in this case, that is when things become murky, commercialization stifles, public health is negatively impacted and venture recedes.

The “baby steps” that the FDA has undertaken over the past decade or so have generally contributed to regulatory uncertainty.⁵ Although this federal agency has held public hearings, posted information on its website, presented at various conferences, created a task force to address nanotech, published a position paper in *Science* and released draft guidance documents, in our opinion it has failed to provide anything concrete with respect to assays, testing, data requirements or practical guidelines. As a result, the pharma and device industries, drug and patent lawyers, business and venture communities, policymakers and the public are left to dig up whatever information they can find on nanoregulation or on marketed products that may incorporate nanomaterials or involve nanotech. Currently, there are few reliable means to identify marketed “nano-containing” products, and consumers are unable to pinpoint which ones may be toxic. This is obviously not very comforting for the consumer, given that the FDA is an agency tasked with protecting public health. In fact, statements that summarized the state of affairs at the FDA few years ago are still applicable today in 2016.⁵

Nanoproducts, whether they are a drug, device, biologic, or combination of any of these, are creating challenges for the FDA regulators as they struggle to accumulate data and formulate testing criteria to ensure the development of safe and efficacious nanoproducts. Emerging

technologies are particularly problematic for regulatory agencies like the FDA, given the independent nature, slow response rate, significant inertia of governmental agencies. Currently, major global regulatory systems, bodies and regimes regarding nanomedicines are not fully mature, hampered in part by a lack of specific protocols for preclinical development and characterization. Additionally, in spite of numerous harmonization talks and meetings, there is lack of consensus on procedures, assays and protocols to be employed during pre-clinical development and characterization of nanomedicines. On the other hand, there is a rise of diverse nano-specific regulatory arrangements and systems, contributing to a dense global nanotechnology regulatory landscape, full of gaps and devoid of central coordination. Moreover, governmental regulators often lack technical and scientific knowledge to support risk-based regulation, thereby leaving a significant regulatory void. Therefore, guidance is critically needed to provide clarity and legal certainty to all stakeholders: manufacturers, policy-makers, healthcare providers, and the consumer.

There are hundreds, if not thousands, of nanoproducts in the market for human use, but little is known of their health risks, safety data or toxicity profiles. Even less is known of nanoproducts that are released into the environment that can potentially contact humans. Then, there are nanoproducts such as cosmetics containing metallic nanoparticles and other nanomaterials flooding the market yet not subject to premarket review by the FDA. Adding to safety concerns is the fact that manufacturers are neither required to obtain any special premarket approval from the FDA (on a case-by-case basis) nor required to list nano-ingredients on product labels at this time. Meanwhile, evidence continues to mount that many (if not most) nanoproducts inherently possess novel size-based properties and toxicity profiles. FDA continues to adopt a precautionary approach to this issue in hopes of countering negative publicity. A one-size-fits-all approach to nanogovernance may not be ideal.

It is well established that certain nanoproducts marketed for direct and indirect human consumption may be unsafe.^{31,32} These products could present unexpected human toxicity effects due to various factors, such as an increased reactivity compared to their “bulk” counterparts and an increased potential to traverse biological barriers/membranes to reach or accumulate in tissues.^{33,34} Furthermore, there are concerns about the occupational and environmental risks associated with the manufacture and disposal of nanoproducts.^{35,36} Not all nanoscale materials are created equal: some may be toxic and their toxicities dependent upon various factors that are material-specific (charge, polarity and chemical residues) and/or geometry-specific (size, shape and nanoscale features). Although nanotoxicity is complex, it is universally recognized that nanoscale products and particles *often* have fundamentally properties distinct from their larger bulk

counterparts.^{3-7,11,15,16} In other words, “nano” does not just mean that a nanoproduct is merely smaller, but that there is a possibility that the product’s “nanocharacter” may render it unsafe. Basically, it cannot be presumed that a nanoproduct will be safe or even “bioequivalent” to its larger bulk counterpart. Since the FDA position is that it only regulates nanoproducts and not nanotechnology *per se*,³⁷ overlooking potential toxicological risks of a technology poses serious public health concerns.

In 2012, the FDA commissioner summarized in general terms the FDA’s so-called “broadly inclusive initial approach” with respect to nanogovernance in a policy paper published in *Science*³⁸:

[The] FDA does not categorically judge all products containing nanomaterials or otherwise involving the application of nanotechnology as intrinsically benign or harmful. As with other emerging technologies, advances in both basic and applied nanotechnology science may be unpredictable, rapid, and unevenly distributed across product applications and risk management tools. Therefore, the optimal regulatory approach is iterative, adaptive, and flexible It is iterative by developing and delivering incremental components of a regulatory system, such as guidances specific to product areas, each as warranted and when ready. It is adaptive by providing a mechanism, within statutory constraints, to change the rules, presumptions, or pathways for these regulatory components, in light of new information gained from research or from experience in regulating earlier products. And it is flexible by using all available means, ranging from workshops to consultations to guidances to rules, in order to match the burden of regulation to its need.

However, in spite of public and industry concerns, as of March 2016, no clear guidelines or regulations have been proposed by or expected from the FDA. The “broadly inclusive initial approach” referenced above by the FDA Commissioner needs to be expanded into practical, science-based regulatory guidelines that can be depended upon by industry and consumers alike. Obviously, not every nanoproduct should be regulated, but more is clearly expected from the FDA than guidance documents that are in draft format, broad lectures that fail to identify key regulatory issues or policy papers that are often short on specifics. In this regard, although the FDA has made important strides, especially in the Hamburg era, numerous challenges continue to confront the agency as important unanswered questions linger regarding nanoregulation (Box 12.1).

Size changes within the nanoscale range and the potential unpredictability arising therefrom are likely to add complexity to the FDA review process. The traditional product-by-product regulatory model that the FDA currently employs may not be effective for all nanoproducts, because it may be difficult to put them into one of the available traditional classifications (*i.e.*, drug, device, biological or combination product). However, in many cases, the FDA may view nanoproducts as technologically overlapping

Box 12.1 Key questions for regulators: balancing public health and encouraging nanomedicine⁵.

- Many products that the FDA and EMA see during their review utilize nanotechnologies or contain nanomaterials, some lack disclosure of this while others identify them as such. Should these products be regulated? If so, how and to what degree?
- It is likely that some marketed nanoproducts (*e.g.*, sunscreens containing zinc oxide and titanium dioxide) warrant safety labeling to alert the unsuspecting consumer. Are most nanomaterials used in nanoproducts inherently toxic?
- Are advances in nanoscience and nanomedicine proceeding too fast for meaningful review to take place? Can regulations truly tame the vastness encompassed by “nano”? For example, should a different set of principles apply for regulating cosmetics versus cancer nanomedicines?
- How much harmonization of drug regulation can reasonably be expected between the US and the rest of the world? Should pharmacoeconomic data be required prior to commercialization of nanomedicines to demonstrate both social and economic added value in comparison to “conventional” established treatments?
- It appears that regulatory agencies are pushing industry to provide product-specific data for areas like cosmetics, where the FDA lacks statutory premarket review authority. Are such voluntary industry measures transparent and meaningful?
- Is the FDA’s so-called “broadly inclusive approach” of considering whether products contain nanomaterials or involve nanotechnology sufficient?
- Can the safety and efficacy of complex follow-on nanotherapeutics (nanosimilar products) ever be assured without a full slate of clinical trials?
- Have the FDA and EMA kept pace with emerging advances in nanotech R&D with respect to predicting, defining, measuring and monitoring potential “nanotoxicities”?
- Globally, should there be a wider coordinated effort on the part of regulatory agencies to review, amend or create nanoregulations where appropriate and warranted? Who in addition to regulatory agencies like the FDA and EMA be given the key responsibility to regulate nanomedical products for human use?
- Can nanomedicine, as applied to public health, be solely regulated under existing regulations and laws?
- Are new regulations needed for all regulated products containing nanomaterials or involving nanotechnology or should they be limited to only a subset of products containing nanomaterials?

- Have delayed and uncoordinated efforts hurt venture and commercialization activities in the US and Europe?
- The FDA has unofficially embraced the inaccurate definition of nanotechnology proposed by the NNI. What is the “official” position of the FDA regarding the definition of nanotechnology, nanoscale, nanotherapeutic, nanodrug, nanomaterial and nanomedicine?
- Industry and stakeholders fully understand that regulatory agencies cannot formulate generalized guidelines, assay protocols or tests for all nanomedicines or nanoproducts. Nevertheless, regulatory agencies should provide selective guidelines, at least on a case-by-case basis (instead of nonbinding, unofficial “draft guidelines” or “position papers”), for industry to rely upon in determining whether their nanoformulations or nanodrug products might be subject to regulatory examination beyond what is typical for small-molecule drugs?

(miniaturization will blur distinctions between different categories) from a review perspective, and therefore consider them as highly integrated nanomedical combination products. These complexities are likely to pose additional challenges and review issues for the FDA.^{5,39}

The FDA’s ability to regulate nanoproducts effectively will depend largely on the category into which the product seeking approval falls. However, as alluded to above, certain therapeutics are “combination products”, which consist of two or more regulated components (drug, biological or device) that are physically, chemically or otherwise combined or mixed to produce a single entity.^{5,39,40} In such cases, the FDA determines the “primary mode of action” (PMOA) of the product, which is “the single mode of action of a combination product that provides the most important therapeutic action”. This process is frequently imprecise, as it is not always possible to clearly elucidate a combination product’s PMOA. Determining which framework will apply to any combination product is the task of the Office of Combination Products (OCP).

Obviously, the OCP will be the first office within the FDA to review many nanoproducts. The OCP makes its assignments on a case-by-case basis depending on the PMOA. But again, this process is frequently imprecise as it is not always possible to clearly elucidate a combination product’s PMOA, often because at the time of an investigational application it is not clear which mode of action provides the most important therapeutic action, or the product has two different equally critical modes of action. It is quite possible that some nanoproducts will blur the distinction between mechanical and chemical action at the nanoscale or that they may be both therapeutic and diagnostic in operation. In fact, such spanning of regulatory boundaries between the various categories often results in inconsistency^{5,39,40}

Another key issue is the limited information currently available correlating the physicochemical properties of nanoscale materials with risks, and lack of validated preclinical screens and animal models for the assessment of nanomaterials. In addition, the toxicity issues surrounding many nanoscale materials may not be fully apparent until they are widely used and their exposure fully felt by a diverse population. Hence, some sort of post-market tracking or a surveillance system must be adopted or legislated to assist in product recalls. None of this has happened yet, in spite of serious signals that toxicity may be more widespread than is apparent. Although toxicological testing for health risks of nanoparticles and nano-enabled products is not currently a complete science,⁴¹ it is crucial to monitor their unique properties (if any) that may lead to serious adverse effects and toxicity. It is essential that long-term testing of nanoscale materials and nano-products is in place to allow safety testing. Box 12.2 lists recommendations for regulatory agencies to consider as they tackle the regulatory framework for nanomedicine.

Another critical issue is an important one: How does the FDA currently approve nanoscale therapeutics? Are unique nano-enabled properties assessed? Are these products reviewed on a case-by-case basis? It is clear that some nanomaterial-containing formulations (“nanoformulations”) are indeed new chemical entities (NCEs). When warranted, nanoversions of active ingredients should be treated by the FDA as NCEs. This will ensure that drugs and biologicals that have been previously approved by the FDA but later modified as nanoversions will undergo a new and rigorous round of safety testing/clinical trials in order to obtain regulatory approval.

The FDA on its website highlights certain critical issues in its nanoregulatory approach that, at face value appear most appropriate.⁴² However, underneath this veneer remains the real point for nanoregulation: “attributes” of materials at the nanoscale (nanocharacter) may require unique assays/testing and premarket approval beyond what the laws on the books can currently tackle to provide safe products to the consumer. There is urgency in this regard and clear guidelines are needed, not nonbinding draft reports that are meaningless from an industry perspective. Real progress in nanoregulation is urged, not mere regulatory discussions or a listing of what will be done in future. Nanoregulation with respect to pharmaceutical products, no matter how challenging or complex, needs to be addressed transparently with proper industry and public feedback. Regulation of nanomedicine must be conducted with the overarching principle that it is science based, not politically motivated or policy based.

Sadly, the conclusions drawn a few years ago regarding the regulation of nanomedicine are no different from those faced by the industry and the public in 2016⁵:

For now, nanoproducts submitted for FDA review will continue to be subjected to an uncertain regulatory pathway. This could negatively impact venture funding, stifle research and development in nanomedicine,

Box 12.2 Recommendations for regulatory agencies regarding nanomedicine regulation.⁵

Safety and risk

- On a case-by-case basis and in conjunction with industry, identify unique safety issues associated with nanoparticles and nanomedical products. The FDA should meet its regulatory and statutory obligations by offering technical advice and guidance to industry beyond what its track record currently reflects.
- 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 prior to market launch, especially in cases (*e.g.*, cosmetics) where the FDA lacks statutory pre-market review authority.
- Correlate physicochemical properties with *in vivo* biological behavior and therapeutic outcome.
- Since there are few protocols to characterize nanomedicines at the physicochemical, biological and physiological levels, it is essential to develop a research strategy that involves adsorption, distribution, metabolism, and excretion (ADME) studies. A holistic approach to understanding ADME can be realized through the integration of mechanistic ADME data through the mathematical algorithms that underpin physiologically based pharmacokinetic (PBPK) modelling, routinely utilized to support regulatory submissions for conventional medicines in the US by the FDA and in Europe by the EMA.
- Develop toxicology tests and conduct physico-chemical characterization (PCC) studies for nanomaterials. Although complexity and diversity of nanomedicines and nanomaterials poses a problem, biocompatibility and immunotoxicity must be taken into consideration during preclinical assessment.
- Understand mass transport across biological membranes and body compartments as well as biodistribution profiles following administration via a specific delivery 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 comprehensive public databank relating to the bio-interactions and toxicity profiles of engineered nanomaterials (ENMs).

Data

- Adapt existing methodologies, as well as develop new paradigms for evaluating *in vivo* animal and clinical data pertaining to safety and efficacy of nanomedical products before and during the product life cycle.
- Develop guidance that provides specifics as to what kind of data is required at each step of the nanomedical translational process.
- Share data in a transparent and harmonized manner. Seek additional data on safety or effectiveness during premarket review process when warranted. The FDA's excessive reliance on publicly available or voluntarily submitted information, adverse-event reporting and on post-market surveillance activities may not be ideal in the case of ENMs for human use.

Standardization and Nomenclature

- Create reference classes for ENMs that are synthesized and characterized.
- Develop consensus-testing protocols to provide benchmarks for the creation of classes of nanoscale materials, both ENMs and native.
- Create uniform nomenclature for and/or working definitions of nanomaterials. Refine the current definitions of nanomaterial, nanotechnology, nanodrug, nanopharmaceutical, nanoscale and nanomedicine for regulatory purposes.
- Further explore international regulatory harmonization efforts and formal treaties with relevant stakeholders.
- In addition to governmental bodies, involve various standard-setting organizations such as the ISO and ASTM International.
- Consult and collaborate with other federal agencies in a more effective, transparent and science-based manner. The FDA's current engagement in policy dialogue with other federal agencies (*via* the Emerging Technologies Interagency Policy Coordination Committee and other forums) has not produced any important guidelines for industry. The FDA should limit the number of non-binding draft guidance and policy papers that it periodically issues.

Tools and Techniques

- Assist in developing unique tools and techniques to characterize nanoscale materials.
- Develop imaging modalities for visualizing tissue biodistribution.
- Develop mathematical and computer models for risk/benefit analysis that can monitor quality, safety and effectiveness vis-à-vis standard ENMs.

Classification scheme

- Reevaluate the current FDA classification scheme, including the Primary Mode of Action (PMOA) criteria for combination products
- Develop a classification system that is based on (a) function or (b) risk of potential harm.
- Reevaluate the system of differing legal standards for different product classes that may result in divergent regulatory outcomes for different product classes.
- Place more effort in tailoring relevant guidance governing various product-classes and address interpretation of relevant statutory/regulatory standards relative to these classes.

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and erode public acceptance of nanoproducts. The end-result of this could be a delay in or loss of commercialized nanoproducts. Whether the FDA eventually creates new regulations, tweaks existing ones, or establishes a new regulatory center to handle nanoproducts, for the time being it should at least look at nanoproducts on a case-by-case basis. The FDA should not attempt regulation of nanomedicine by applying existing statutes alone, especially where scientific evidence suggests otherwise. Incorporating nanomedicine regulation into the current regulatory scheme is unwise. Regulation of nanotech must balance innovation and R&D with the principle of ensuring maximum public health protection and safety. Regulatory oversight must evolve in concert with newer generations of nanomedical products. It is hoped that the “baby steps” that the FDA has taken in the past decade regarding nanogovernance will translate into more meaningful, flexible and science-based guidance in the near future. In the end, the long-term prognosis of nanomedicine will hinge on effective, valid nanogovernance requiring the full commitment of regulatory agencies such as the FDA, as well as the regulated community such as the manufacturing sector.

12.5 Importance of Understanding Pharmacokinetics and Distribution in Development and Regulatory Submission

The drivers for the application of nanotechnology to drug delivery are numerous and can be categorized into delivering either process-specific advantages or pharmacological benefits. Insolubility of APIs is a continuing problem for the pharmaceutical industry⁴³ and negatively impacts upon the difficulty and cost associated with drug development. As discussed earlier, nanodrugs are broad in terms of their complexity and in the benefits that

have been explicitly demonstrated to date. Examples of pharmacokinetic benefits include improved oral bioavailability,^{44,45} modified delivery profiles,⁴⁴ long-acting delivery^{46,47} and overcoming fed/fasted variation.⁴⁸ Advanced nanodrugs have also been developed that offer the potential to specifically target diseased cells and tissues by either passive or active mechanisms.⁴⁹ While nanoparticles have attracted much attention with respect to understanding whether there are new safety assessments required, it is important to recognize that for many such nanomedicines, adverse drug reactions for encapsulated APIs have been mitigated through altered distribution of the drug.⁵⁰ While it is true that certain nanomaterials trigger cellular events that may lead to unexpected safety issues (*e.g.*, within the immune system^{51,52}), the real question relates to whether existing regulatory processes are sufficiently robust as to capture them. As for any medicine, nanotechnology-enabled products require a robust assessment prior to clinical application. Pharmacokinetic and targeting benefits may have important related benefits such as the ability to overcome sub-optimal patient compliance in chronic disease or reduction in doses or pill burden required for effective management of the disease. However, by their very nature, nanomedicines behave differently to dissolved molecules and this presents a challenge for accurate determination of the likely *in vivo* behavior during the development process.

Physiologically based pharmacokinetic (PBPK) modelling is now extremely well integrated into the development process for conventional medicines. PBPK modeling is increasingly used to support decision making on whether, when and how to conduct clinical pharmacology studies in humans and frequently form part of the investigational new drug and new drug application review process for the FDA and the EMA. The FDA has a PBPK program nested within the Division of Pharmacometrics that reviews the adequacy of submitted PBPK models, facilitates the review process through *de novo* analyses, supports regulatory policy and harmonises regulatory recommendations on the use of PBPK with non-US regulatory bodies and the wider scientific community.⁵² In 2014, the EMA produced a concept paper (EMA/CHMP/211243/2014) on the qualification and reporting of PBPK modeling that highlighted that PBPK explicitly features in numerous guidelines. These include those for pharmacokinetic assessment during hepatic impairment (CPMP/EWP/2339/02), pharmacogenetic methods as applied to pharmacokinetics (EMA/CHMP/37646/2009), drug–drug interactions (CPMP/EWP/560/95/Rev. 1 Corr.*), as well as upcoming new guidelines and guideline revisions.

In basic terms, PBPK modeling involves the integration of mathematical descriptors of medicine-specific and patient-specific factors in order to simulate the pharmacokinetic properties in specific scenarios. Patient-specific factors usually take the form of physiological or anatomical descriptors such as those for regional blood flow or organ mass for example, and typically include known variation in such parameters within the human population.⁵³ Traditionally, medicine-specific factors have related specifically to the

physicochemical properties of the API (*e.g.*, Log P , pK_a and fraction unbound, *etc.*) and/or *in vitro* experimental data that provide a surrogate for a pharmacological process. Examples for such *in vitro* data include the transcellular permeation across Caco-2 cells to provide a description of oral absorption of the drug, or metabolism studies in hepatocytes or microsomes to provide a measure of intrinsic clearance of the API. Accordingly, integrating such *in vitro* data with system data allows PBPK modeling to be utilized for *in vitro-in vivo* extrapolation. When the relationship between molecule physicochemical properties and pharmacological processes is understood, it is also possible to model pharmacokinetic behavior directly from the molecular descriptors of the API. However, as with any modeling approach, the robustness of the final models are only as good as the quality of the input data, and the degree of understanding of the processes regulating ADME of the medicine. While PBPK modeling for conventional small-molecule drugs (and environmental toxins) has improved remarkably over the past two decades, there are comparatively few examples of PBPK modeling for nanomedicines.^{53–59}

Transcellular permeation across Caco-2 cells is a good example to illustrate the process by which an *in vitro* observation can be validated for inclusion as a parameter in PBPK modeling. In 2002, Sun *et al.* assessed the apparent permeability (P_{app}) across Caco-2 cell monolayers for 20 commercially available drugs for which human pharmacokinetic data were available.⁶⁰ Using these data, they were able to define the mathematical relationship between Caco-2 permeability at pH 7.4 and the fraction absorbed (%) when these drugs were dosed to humans. In doing so, they provided the equation necessary for using Caco-2 cell data to model the absorption of drugs. Whether these same equations can accurately inform PBPK models for nanotechnology-enabled products remains uncertain, but the approach was recently applied to a novel solid drug nanoparticle formulation with apparent success, as qualified by observations in pre-clinical species.⁵⁷ In this case, the strategy was to improve the oral bioavailability of the API, and as such, augment a process known to occur for any orally delivered medicine be it in aqueous or nanoparticulate form. However, the specific mechanisms involved in oral bioavailability of nanoparticles may differ from those of dissolved molecules (Figure 12.2).

A conventional orally delivered API goes through the process of dissolution within the intestine, and thereafter the oral bioavailability is determined by the lipophilicity of the molecule along with its affinity for drug transporters⁶¹ and drug metabolism enzymes⁶² that may adversely impact upon its absorption. However, for various nanotechnologies, other processes have been implicated in absorption that include rate of dissolution (through high surface area to mass ratio⁶³), cellular interactions due to inherently reactive nanoparticle surfaces,⁶⁴ physical interaction of the nanoparticle within mucous,⁶⁵ paracellular permeation across the epithelium,⁶⁶ endocytic processes in M-cells and absorption through Peyer's patches.^{67,68} There are also good data to suggest that certain lipid-based nanomaterials may favor the delivery

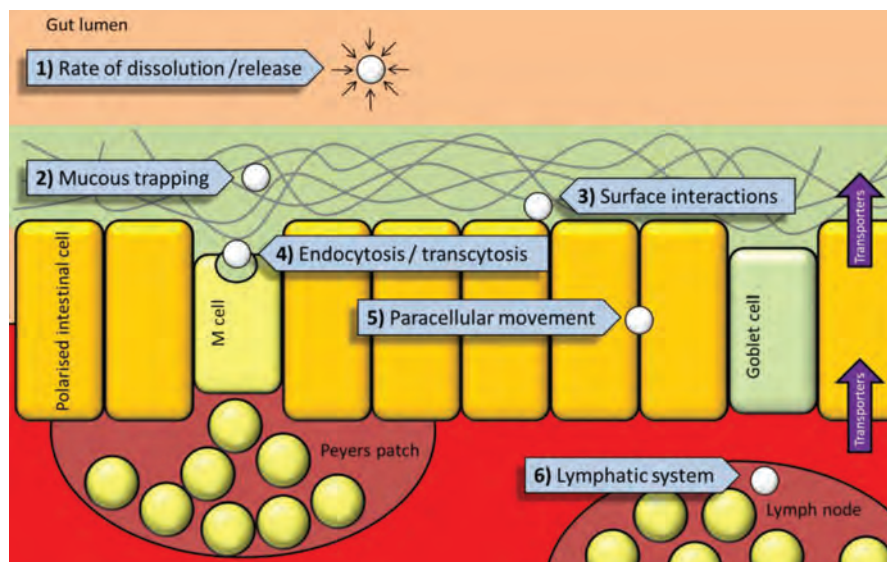


Figure 12.2 Potential mechanisms for oral drug delivery from nanotechnology-enabled products. The unique properties of nanomaterials may (1) impact the rate of dissolution; (2) result in entrapment within the mucous pores; (3) affect interactions mediated by the reactivity of the surfaces; (4) impact endocytic or transytic passage; (5) affect paracellular passage; or (6) entry to the systemic circulation *via* the lymphatics.

of drugs *via* the lymphatics.⁶⁹ Clearly, the mechanisms of oral absorption of many nanomedicines are different from those of conventional dissolved molecules and many of these nano-specific processes are unlikely to be captured using conventional Caco-2 cell monolayers. As such, there has been recent interest in developing advanced *in vitro* systems, typically involving co-culture with goblet cells (mucus-producing) and M-cells.^{70,71} While the initial validation of these new models has shown early success, they have not yet been utilized to determine the mathematical descriptors of how these processes relate to absorption in humans, which will be needed for robust PBPK modeling of oral nanomedicines. Nonetheless, one can speculate that physical descriptors of the nanomedicine such as size, surface charge or carrier material composition may have utility for informing nanomedicine PBPK alongside the physicochemical properties of the API.

For nanomedicines that enter the systemic circulation as intact particles (either directly or indirectly), many other processes dictate their distribution and clearance, and the fundamental mechanisms are still being explored in a technology-specific manner. For example, the mononuclear phagocyte system (MPS) is known to impact nanoparticle clearance and it seems likely that a better understanding of its net effect on elimination of nanomaterials will enable the development of *in vitro* tools to generate data for informing robust PBPK modelling.⁷² These are complex mechanisms to understand, since the overall effect is likely to be influenced by several processes

(*e.g.*, protein corona formation can influence MPS uptake). Nonetheless, a schematic representation of the pathway to generating robust PBPK modeling approaches for nanomaterials is shown in Figure 12.3. First it is necessary to determine specific mechanisms that underpin particular pharmacological behaviors (*e.g.*, the importance of the MPS in clearance). Once a mechanism is proven to be important *in vivo*, then it will be necessary to develop robust experimental systems to provide a quantitative measure of its contribution. Once these experimental systems have been applied across a series of nanomaterials it may be possible to determine the mathematical relationship with the *in vivo* effect (for *in vitro*–*in vivo* extrapolation) and possibly with the physical descriptors of the nanoparticle (for modeling directly from the physical attributes of the formulation).

The utility of PBPK modeling in development and regulatory submission is well recognized for conventional small-molecule drugs. However, the application of such *in silico* approaches is in its infancy for nanotechnology-enabled drug development. Central to improved modeling is the confirmation of pharmacological processes that are important in definition of the *in vivo* pharmacokinetics. As for any PBPK model, ultimate performance will need to be qualified through a series of “predict–learn–confirm” cycles that map to supporting data generated *in vitro* and in preclinical species.

12.6 Conclusions

Nanomaterial innovations are in full swing at both academic and industrial sectors as they relate to research, patents, commercial opportunities and technology transfer. However, in the nanomedicine arena, currently there are knowledge gaps relating to understanding the mechanisms that impact benefits for many technologies in terms of pharmacokinetics and ADME. Changes in both of these biological parameters are central to the achieved changes in efficacy but may also underpin new safety considerations that require additional data, assays or research tools to clarify their mechanistic basis. Importantly, a fundamental basis for the application of nanodrugs is the improvement of efficacy through a reduction in toxicity. However, as for any drug, nano-enabled products for human use require a robust, evidence-based assessment that evolves as gaps in knowledge are addressed. The evolution of the regulatory framework for nanodrugs is required to integrate a firm consideration of the needs for commercialization that is, after all, a prerequisite to patients realizing the step-change in their care. Equally, however, it is imperative that science-based regulatory guidelines mitigate the risk of inadvertent harm to patients or the environment.

As we rapidly enter the era of nanosimilars and theranostics, these are further likely to test the limits of regulatory authority, placing further strain on over-burdened regulatory agencies. Therefore, it is critical that the regulatory gaps that currently exist in nanomedicine are handled 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.

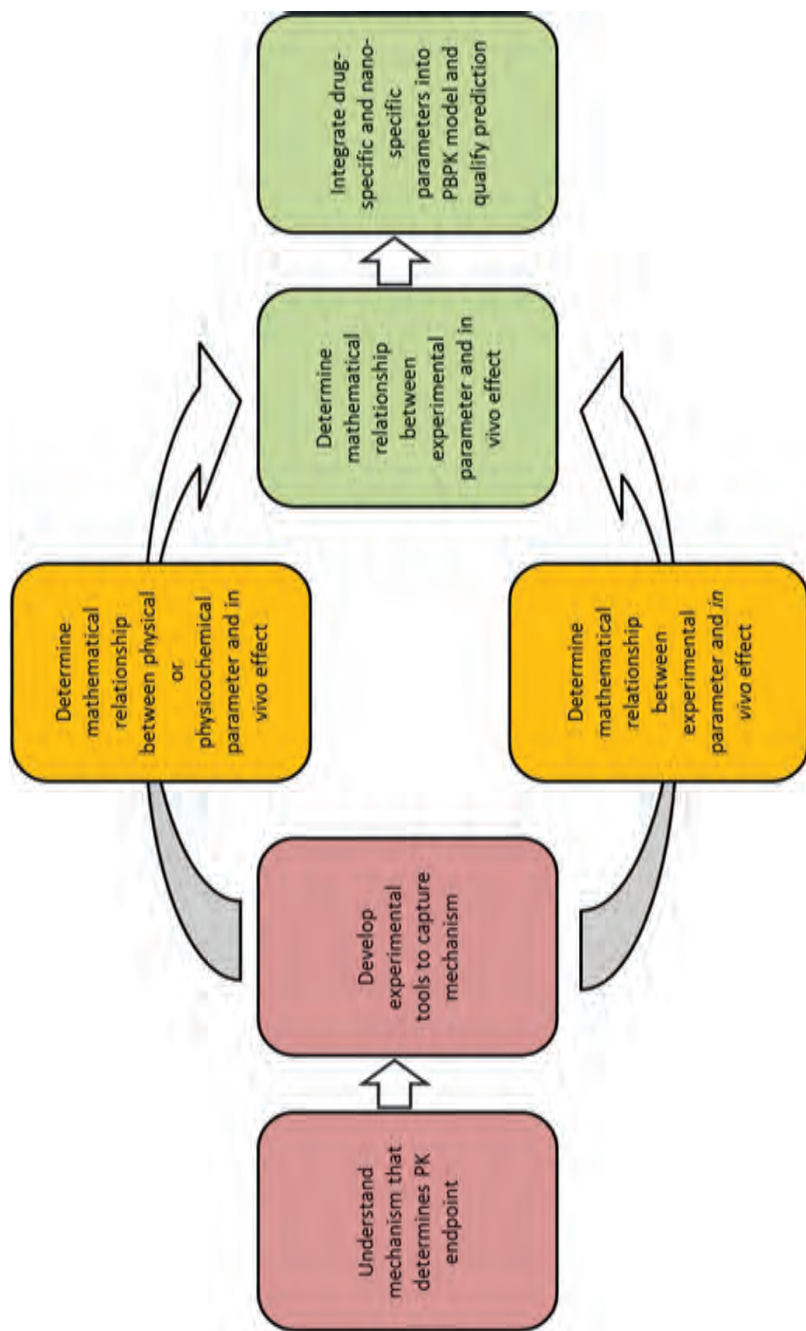


Figure 12.3 Schematic representation of a possible pathway to improved physiologically based pharmacokinetic (PBPK) modeling of nanomedicines. The fundamental processes regulating absorption, distribution, metabolism and elimination of nanomedicines first require confirmation. Development of robust *in vitro* systems may then enable clarification of how nanoparticle properties influence the pharmacological processes. Definition of the mathematical relationships will result in improved models, but additional qualification will be required through a series of “predict–learn–confirm” cycles, as is the case for conventional small-molecule drugs.

References

1. R. Bawa, *Alb. L. J. Sci. Tech.*, 2007, **17**(3), 699.
2. R. Bawa in *Bionanotechnology: Global Prospects*, ed. D. E. Reisner, CRC Press, Boca Raton, FL, 2009, p. 309.
3. R. Bawa in *Biotechnology and the Law*, ed. H. B. Wellons and E. S. Ewing, American Bar Association, Chicago, IL, 2nd edn, 2016.
4. R. Bawa, *Eur. J. Nanomed.*, 2010, **3**(1), 34.
5. R. Bawa in *Bionanotechnology: A Revolution in Biomedical Sciences and Human Health*, ed. D. Bagchi *et al.*, Wiley Blackwell, UK, 2013, p. 720.
6. H. M. Mansour, C.-W. Park and R. Bawa in *Handbook of Clinical Nanomedicine: Nanoparticles, Imaging, Therapy and Clinical Applications*, ed. R. Bawa, G. Audette and I. Rubinstein, Pan Stanford Publishing, Singapore, 2016, ch. 9, p. 233.
7. S. Tinkle, S. E. McNeil, S. Mühlebach, R. Bawa, G. Borchard, Y. Barenholz, L. Tamarkin and N. Desai, *Ann. NY Acad. Sci.*, 2014, **1313**, 35.
8. R. A. Stein, *Genetic Engineering & Biotechnology News*, 2014, Available at: <http://www.genengnews.com/insight-and-intelligence/nanotechnology-is39the-magic-bullet-becoming-reality/77900016/> (Accessed on February 14, 2016).
9. S. Fischer, *IEEE Pulse*, 2014, Available at: <http://pulse.embs.org/marc2014/regulating-nanomedicine/> (Accessed on February 14, 2016).
10. D. Johnson, 2014, Available at: <http://spectrum.ieee.org/nanoclast/at-work/innovation/nanomedicines-will-be-bigonce-they-emergefrom-regulatory-troubles> (accessed on February 14, 2016).
11. R. Bawa, S. R. Bawa and R. Mehra, in *Handbook of Clinical Nanomedicine: Law, Business, Regulation, Safety and Risk*, ed. R. Bawa, Pan Stanford Publishing, Singapore, ch. 58, p. 1291.
12. A. Siew, *PharmTech Europe*, 2014, Available at: <http://www.pharmtech.com/current-issues-nanomedicines> (Accessed on February 15, 2016).
13. M. Davenport, *Chem. Eng. News*, 2014, **92**(45), 10. Available at: <http://cen.acs.org/articles/92/i45/Closing-Gap-Generic-Nanomedicines.html> (Accessed on February 14, 2016).
14. G. Lovestam, 2010, Available at: https://ec.europa.eu/jrc/sites/default/files/jrc_reference_report_201007_nanomaterials.pdf (Accessed on February 15, 2016).
15. R. Bawa, *Nanomedicine*, 2007, **2**(3), 351.
16. R. Bawa in *Handbook of Clinical Nanomedicine: Nanoparticles, Imaging, Therapy and Clinical Applications*, ed. R. Bawa, G. Audette and I. Rubinstein, Pan Stanford Publishing, Singapore, 2016, ch. 6, p. 127.
17. Y. Barenholz, *J. Controlled Release*, 2012, **160**, 117.
18. Y. Barenholz in *Handbook of Clinical Nanomedicine: Nanoparticles, Imaging, Therapy and Clinical Applications*, ed. R. Bawa, G. Audette and I. Rubinstein, Pan Stanford Publishing, Singapore, 2016, ch. 29, p. 827.
19. Y. Matsumura and H. Maeda, *Can. Res.*, 1986, **46**, 6387.

20. R. K. Jain, *Can. Res.*, 1986, **47**, 3039.
21. H. Maeda, G. Y. Bharate and J. Daruwalla, *Eur. J. Pharm. Biopharm.*, 2009, **71**, 409.
22. Y. Barenholz and G. Haran, 1994. *U. S. Pat. No.* 5316771.
23. Draft guidance on Doxorubicin hydrochloride. Available at: <http://www.fda.gov/downloads/Drugs/.../Guidances/UCM199635.pdf> (accessed on February 15, 2016).
24. A. Gabizon, R. Catane, B. Uziely, B. Kaufman, T. Safra, R. Cohen, F. Martin, A. Huan and Y. Barenholz, *Cancer Res.*, 1994, **54**, 987.
25. J. Szebeni, F. Muggia, A. Gabizon and Y. Barenholz, *Adv. Drug Delivery Rev.*, 2011, **63**, 1020.
26. J. Szebeni, P. Bedocs, Z. Rozsnyay, Z. Weiszhar, R. Urbanics, L. Rosivall, R. Cohen, O. Garbuzenko, G. Bathori, M. Toth, R. Bungler and Y. Barenholz, *Nanomed. Nanotechnol. Biol. Med.*, 2012, **8**, 176.
27. Y. Barenholz and S. Amselem in *Liposome Preparation and Related Technique*, ed. G. Gregoriadis, CRC Press, Boca Raton, FL, 2nd edn, 1993, vol. I, p. 527.
28. S. P. Damari, D. Shamrakov, Y. Barenholz and O. Regev, submitted.
29. Y. Schilt, T. Berman, X. Wei, Y. Barenholz and U. Raviv, 2015, *Biochim. Biophys. Acta.*, 2016, **1860**, 108.
30. D. D. Lasic, P. M. Frederik, M. C. A. Stuart, Y. Barenholz and T. J. McIntosh, *FEBS Lett.*, 1992, **312**, 255.
31. W. H. De Jong and P. J. A. Borm, *Int. J. Nanomed.*, 2008, **3**, 133.
32. N. Sanvicens and M. P. Marco, *Trends Biotechnol.*, 2008, **26**, 425.
33. W. E. Bawarski, E. Chidlowsky, D. J. Bharali and S. A. Mousa, *Nanomed. Nanotechnol. Biol. Med.*, 2008, **4**, 273.
34. M. E. Davis, Z. G. Chen and D. M. Shin, *Nat. Rev. Drug Discovery*, 2008, **7**, 771.
35. I. Linkov, F. K. Satterstrom and L. M. Corey, *Nanomed. Nanotechnol. Biol. Med.*, 2008, **4**, 167.
36. R. Gaspar, *Nanomedicine*, 2007, **2**, 143.
37. G. Mendel, *Alb. L. Rev.*, 2008, **59**, 1323.
38. M. A. Hamburg, *Science*, 2012, **336**, 299.
39. R. Bawa, *Curr. Drug Delivery*, 2011, **8**, 227.
40. S. B. Foote and R. J. Berlin, *Minn. J. L. Sci. Technol.*, 2005, **6**, 619.
41. R. Bawa, S. Melethil, W. J. Simmons and D. Harris, *SciTech. Lawyer*, 2008, **5(2)**, 10.
42. FDA's approach to regulation of nanotechnology products. Available at: <http://www.fda.gov/ScienceResearch/SpecialTopics/Nanotechnology/ucm301114.htm> (accessed on February 10, 2016).
43. B. E. Rabinow, *Nat. Rev. Drug Discovery*, 2004, **3(9)**, 785.
44. F. Haessler, F. Tracik, H. Dietrich, H. Stammer and J. Klatt, *Int. J. Clinical Pharmacol. Ther.*, 2008, **46(9)**, 466.
45. S. Weir, R. Torkin and H. R. Henney, *Curr. Med. Res. Opin.*, 2013, **29(12)**, 1627.

46. M. N. Samtani, A. Vermeulen and K. Stuyckens, *Clin. Pharmacokinet.*, 2009, **48**(9), 585.
47. G. van't Klooster *et al.*, *Antimicrob. Agents Chemother*, 2010, **54**(5), 2042.
48. B. Deschamps, N. Musaji and J. A. Gillespie, *Int. J. Nanomed.*, 2009, **4**, 185.
49. I. F. Uchegbu and A. Siew, *J. Pharm. Sci.*, 2013, **102**(2), 305.
50. M. E. O'Brien *et al.*, *Ann. Oncol.*, 2004, **15**(3), 440.
51. A. N. Ilinskaya and M. A. Dobrovolskaia, *Brit. J. Pharmacol.*, 2014, **171**(17), 3988.
52. Available at: <http://www.fda.gov/AboutFDA/CentersOffices/OfficeofMedicalProductsandTobacco/CDER/ucm365118.htm> (Accessed on February 14, 2016).
53. D. M. Moss and M. Siccardi, *Br. J. Pharmacol.*, 2010, **171**(17), 3963.
54. G. Bachler, N. von Goetz and K. Hungerbuhler, *Int. J. Nanomed.*, 2013, **8**, 3365.
55. G. Bachler, N. von Goetz and K. Hungerbuhler, *Nanotoxicol*, 2014, **1**.
56. M. Li, K. T. Al-Jamal, K. Kostarelos and J. Reineke, *ACS Nano*, 2010, **4**(11), 6303.
57. T. O. McDonald *et al.*, *Adv. Healthcare Mater.*, 2014, **3**(3), 400.
58. R. K. Rajoli, D. J. Back, S. Rannard, C. L. Freel Meyers, C. Flexner, A. Owen and M. Siccardi, *Clin. Pharmacokinet.*, 2015, **54**(6), 639–650.
59. R. S. Yang, L. W. Chang, C. S. Yang and P. Lin, *J. Nanosci. Nanotechnol.*, 2010, **10**(12), 8482.
60. D. Sun, H. Lennernas, L. S. Welage, J. L. Barnett, C. P. Landowski, D. Foster *et al.*, *Pharma. Res.*, 2002, **19**(10), 1400.
61. M. F. Fromm, *Int. J. Clin. Pharmacol. Ther.*, 2000, **38**(2), 69.
62. A. Bruyere, X. Decleves, F. Bouzom, K. Ball, C. Marques, X. Treton *et al.*, *Mol. Pharm.*, 2010, **7**(5), 1596.
63. W. Li *et al.*, *Int. J. Pharm.*, 2014, **460**(1–2), 13.
64. S. Hirsjarvi, S. Dufort, J. Gravier, I. Texier, Q. Yan, J. Bibette *et al.*, *Nanomedicine*, 2013, **9**(3), 375.
65. S. K. Lai, Y. Y. Wang and J. Hanes, *Adv. Drug Delivery Rev.*, 2009, **61**(2), 158.
66. S. Sadekar and H. Ghandehari, *Adv. Drug Delivery Rev.*, 2012, **64**(6), 571.
67. E. Gullberg *et al.*, *J. Pharm. Exper. Ther.*, 2006, **319**(2), 632.
68. J. Wang, L. Li, Y. Du, J. Sun, X. Han, C. Luo *et al.*, *Mol. Pharm.*, 2015, **12**(2), 463.
69. K. Kohli, S. Chopra, D. Dhar, S. Arora and R. K. Khar, *Drug Discovery Today*, 2010, **15**(21–22), 958.
70. A. Beduneau *et al.*, *Eur. J. Pharm. Biopharm.*, 2014, **87**(2), 290.
71. C. Schimpel *et al.*, *Mol. Pharm.*, 2014, **11**(3), 808.
72. M. A. Dobrovolskaia, P. Aggarwal, J. B. Hall and S. E. McNeil, *Mol. Pharm.*, 2008, **5**(4), 487.