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REVIEW

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Nanomedicine(s) under the Microscope

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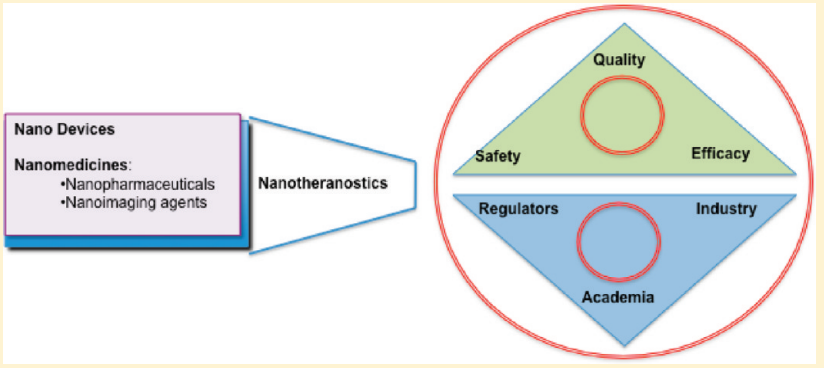
ABSTRACT:



Depending on the context, nanotechnologies developed as nanomedicines (nanosized therapeutics and imaging agents) are presented as either a remarkable technological revolution already capable of delivering new diagnostics, treatments for unmanageable diseases, and opportunities for tissue repair or highly dangerous nanoparticles, nanorobots, or nanoelectronic devices that will wreak havoc in the body. The truth lies firmly between these two extremes. Rational design of "nanomedicines" began almost half a century ago, and >40 products have completed the complex journey from lab to routine clinical use. Here we critically review both nanomedicines in clinical use and emerging nanosized drugs, drug delivery systems, imaging agents, and theranostics with unique properties that promise much for the future. Key factors relevant to the design of practical nanomedicines and the regulatory mechanisms designed to ensure safe and timely realization of healthcare benefits are discussed.

KEYWORDS: nanomedicine, nanopharmaceutical, nanoimaging agent, theranostic, regulation, cancer

INTRODUCTION

The past decade has seen many publications dedicated to "nanomedicine". This literature is however peppered with inaccuracies. Some seem unaware of the historical background and in their enthusiasm overexaggerate (hype) the potential benefits, giving the impression that their proposed new technologies are already reality today. Others express disproportionate concern as to the possible risks. Objective nanomedicinista (nanomedicine researchers) understand that the truth lies firmly between these two extremes and that the risk−benefit−promise of each technology should be clearly presented on its own merits. The sudden convergence of so many scientific disciplines for the first time exploring the frontiers of nanoscale science relating to biomedical nanotechnologies is responsible for the good (unprecedented opportunity for invention), the bad (lack of appreciation of the scientific state of the art), and the ugly (overexaggeration of potential benefits of "nanomedicine" to a degree that is both unprofessional and unfair to those who desperately seek the solace of remedies for life threatening and debilitating diseases). Every month, articles claim novel/superior designer nanosized therapeutics, imaging agents, theranostics, and also nanomaterials to promote tissue repair. Most are, as yet, far from first in patient clinical trials, and many will never arrive there. At a moment when "in biomedical research, multidisciplinary collaboration has become mandatory",[1] and converging interests in the nanosciences are creating so many new opportunities, it is essential that "all parties involved in debates about scientific and medical discoveries must remember not only the incremental nature of scientific truth but a broader responsibility imposed by the public interest".[2,3] Linking generations and converging scientific disciplines is always problematic. Greater awareness of, and/or greater acknowledgment of, the historical lessons learned can only accelerate realization of the undoubted potential of nanomedicines, and use of robust scientific methodology is needed to ensure conclusions are secure—essential in the healthcare sector.

Using recent consensus reports, and objective scientific literature as a broader reference, here we seek to demystify the terminology and place modern "nanomedicines" under the

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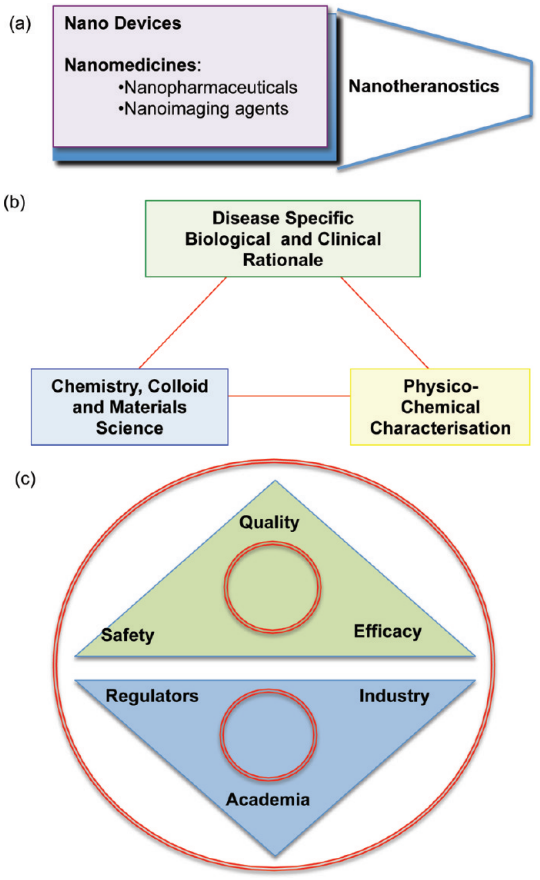

Figure 1. Harnessing multidisciplinarity: A successful template for design and transfer of nanopharmaceuticals into clinical use. Panel a shows the relationship between the nanotechnologies under development for medical use highlighting the complex regulatory boundaries. The need for partnership between the core scientific disciplines (panel b) and academia, industry, and regulation (panel c) to ensure translation and "Quality by Design"[5] is also shown.

microscope in an attempt to define the current status of technologies and their clinical relevance. With a critical eye, the history is briefly overviewed, the key issues for design and characterization of those exciting new technologies emerging today are discussed, and the regulatory scenario helping society ensure timely and safe realization of the benefits is also briefly described.[4] Successful first generation nanomedicines were born from a multidisciplinary approach, and their realization to clinical use was orchestrated by visionary champions "conducting the orchestra" from the interdisciplinary interface. Research and development has encompassed a sound biological/clinical rationale, innovative chemistry/materials science, and innovative methodologies for physicochemical and biological characterization to ensure optimization of properties relevant to the clinical setting (Figure 1). The partnership between academia, industry, and regulatory agencies is fundamentally important if healthcare benefits are to be realized, and it relies on scientific excellence throughout the whole process—exemplified by the global credo of medicines development "Quality by Design".[5]

TERMINOLOGY AND HISTORICAL BACKGROUND

The Field of Nanomedicine. It is not surprising with the convergence of so many scientific disciplines that there are many interpretations of the terms "nanobiotechnology", "nanomedicine", and "nanomaterial". It has even been noted that nanomedicine is not nanotechnology. To quote, "nanomedicine belongs to the area of cell biology and not nanotechnology because the field of nanotechnology deals only with the science and technology of entities dominated by surface atoms".[6] It is true that early "nanomedicine" texts simply reviewed topics that could be considered cell biology and biochemistry,[7] but this comment has limited vision as it does not fairly represent all the activities (top-down, bottom-up) that have produced a fantastic array of carefully engineered (designed) nanotechnologies, each with unique properties. As consensus definitions are now well established, they are explained below.

The field of "nanomedicine" is distinguishable as it uniquely focuses on medically related, patient-centric nanotechnologies. The broader field of "nanobiotechnology" encompasses underpinning scientific research investigating fundamental cellular mechanisms such as molecular forces, molecular motors, and cellular electrochemical phenomena, and these processes are often probed using nonhuman (plant and animal) models. After much deliberation, the European Science Foundation's (ESF) Forward Look Nanomedicine[8] defined nanomedicine via consensus conference in the following simple way:

> Nanomedicine uses nano-sized tools for the diagnosis, prevention and treatment of disease and to gain increased understanding of the complex underlying patho-physiology of disease. The ultimate goal is improved quality-of-life.

This is a useful definition as it encompasses the three main nanotechnology areas being developed for healthcare applications:

- Diagnostics, sensors and surgical tools that are used *outside the patient*.
- Innovative imaging agents and monitoring technologies that can be used for diagnostic and sensing applications; *from cells to patients*.
- Innovative technologies and biomaterials (sometimes combined with cell therapy) that are used for drug delivery, for tissue engineering, and to promote tissue repair. Some applications require only *ex vivo* manipulations, but most *require patient administration* via any one of the number different routes (e.g., topical, oral, parenteral, pulmonary, surgical implantation etc).

The European Commission's (EC) Joint Research Centre Report "Nanomedicine: Drivers for development and possible impacts" is also a comprehensive information source[9] that additionally observes the following:

- Nanoparticles for medical applications are defined as particles with a size between 1 and 1000 nm (a common interpretation in pharmaceutical sciences).
- Biochips are classified as nanotechnology only if they include nanoscale components.
- Polymer therapeutics are classified as nanomedicine.

Largely for purposes of safety regulation there is an ongoing global debate as to what really constitutes "nanomaterial"? Many core academic and industrial/regulatory sectors suggest size thresholds and/or material characteristics relevant to their own interests. The complexities of this debate are beyond the scope of this review; however, we concur with the opinion that none of the popular size thresholds (e.g., 1−100 nm) can be scientifically justified in the context of a broad definition that adequately captures *all nanomaterials*.[10] Moreover, it is important to re-emphasize that nanosized objects fabricated by "top-down"

miniaturization/engineering techniques or "bottom-up" colloidal, synthetic, or supramolecular chemistry techniques have equal importance in the context of innovative nanomedicines.

Nanomedicines and Theranostics . This review specifically focuses on the current state of the art of "nanomedicines"—an overall term that includes nanopharmaceuticals, nanoimaging agents, and theranostics (see Figure 1). The ESF Forward Look noted that "nanopharmaceuticals can be developed either as drug delivery systems or biologically active drug products", with the caveat that the term encompasses "nanometre size scale complex systems, consisting of at least two components, one of which is the active ingredient".[8] Nanomedicines within the whole range of nanoscale size have well-established historical importance (see refs 11−13 for examples). Arguably this terminology is imperfect as it fails to include those nanosized drug crystals[14] and polymers (including dendrimers) that are used as therapeutic agents or sequestrants (e.g., phosphate or cholesterol binding) in their own right.[15] Although they are single component agents, they are clearly complex, engineered nanomaterials that should be considered as nanomedicines, so for completeness we include them here. The proposed terminology also excludes nanosized naturally occurring macrmolecules such as proteins and antibodies. In this case, exclusion is probably appropriate owing to the well-established industrial/Regulatory Authority development path for biotech products (including those "engineered" recombinantly).[16] Thus we only include antibody conjugates of drugs or radioisotopes (i.e., they comprise several components) as nanomedicines in this overview. Combination of therapeutic and diagnostic capabilities into a single construct has given birth to the term "theranostic", which is exemplified by radiolabeled therapeutic anticancer antibodies that achieve both patient imaging and radiotherapy.[17]

THE PRESENT: NANOMEDICINES TODAY

The Genealogy. Application of benefits of colloid (nano) science can be traced back millennia (e.g., the Lycurgus cup). Early pioneers in the modern era include Faraday (who, in the 19th century, recognized the importance of "ruby" colloidal gold[18]) and Ilya Metchnikov and Paul Ehrlich, who jointly received the Nobel Prize for medicine in 1908. Metchnikov contributed much to the appreciation of phagocytosis,[19] and Ehrlich championed the concepts of cell-specific diagnostics and cell-targeted therapies.[20] He coined the phrase "magic bullet" and gave us immunotherapeutics and the first synthetic low molecular weight chemical entities. The 20th century also saw the birth of synthetic polymer chemistry[21] and, during its second half, the pioneering research that inspired the first nanomedicines to enter routine clinical use two decades later (Table 1).

Those who witnessed the birth of these first nanomedicines remember the considerable scepticism, from other scientists and industry alike, as to their practicality for industrial manufacture, clinical potential ("small molecules cure diseases") and economic viability. Some of the landmark technologies, and some of their "champions" so often overlooked in modern "nano" reviews, include liposomes (Bangham, Gregoriadis, Papahadjopoulos, Barenholz),[22−26] nanoparticles and nanocapsules (Speiser, Couvreur, Kreuter),[27−29] DNA−drug complexes (De Duve, Trouet),[30] polymer−drug conjugates (Ringsdorf, Duncan, Kopecek, Spreafico),[31−33] polymer−protein conjugates (Davis),[34] antibody−drug conjugates (Wilchek, Arnon, Sela),[35] albumin−drug conjugates (Trouet),[36] and block copolymer micelles (Ringsdorf, Kataoka, Kabanov).[37−39] The second half of the 20th century also saw iron oxide nanoparticles emerge as clinically applied parenteral iron infusion solutions used to treat anemia[40,41] (from the 1930s) and as magnetic resonance imaging (MRI) imaging agents[42,43] (from the 1990s). These efforts laid the foundations for the small paramagentic iron oxide (SPION)-based approaches emerging today.[44] Furthermore, silver (antimicrobial agents[45]) and gold (amelioration of arthritis[46]) have also a long history of therapeutic use.

It is important to underline that each of the first nanomedicine classes (Figure 2) has distinct physicochemical features and they typically fall into distinct size ranges in the nanoscale, e.g., liposomes (80−200 nm), nanoparticles (20−1000 nm), polymer therapeutics (5−25 nm), block copolymer micelles (50−200 nm), gold nanoparticles (5−50 nm), and nanosized drug crystals (100−1000 nm). Many constructs also include carefully designed linking chemistry (for stability and/or triggered release). Most authors ignore the potential toxicity of this linking chemistry and resultant metabolites. There is an increasing tendency to call *all* nanosized medicines (including liposomes, polymer−drug conjugates, etc.) "nanoparticles".[47,48] This is neither scientifically accurate (nanoparticles constitute a specific class of nanomedicines) nor helpful as it disregards the unique features of each class that have been tailored to optimize their performance—very misleading in a Regulatory setting. (A simple analogy: though they are all forms of transport, a car is neither a boat nor a plane, and it would be inappropriate to describe it as such.)

Early scientific literature and the product information leaflets (PILs) relating to first generation products provide a rich source of background information including the paths taken for structure optimization, definition of product and formulation specifications, description of validated methods developed for characterization, and, not least, the therapeutic indication (often this is a specific subset of patients suffering the target disease). The clinical toxicities/side effects seen are also well documented with details of the percentage of patients experiencing them. Moreover, mandatory postmarket surveillance is obliged to review safety and efficacy in the population as a whole. This is undertaken to identify early trends in ethnic or age-related variations in toxicity/efficacy and/or any emerging evidence of drug−drug interactions. Despite the fact that millions of patients have been treated with first generation nanomedicines, the clinical trial literature is rarely cited in scientific articles. This is not just a missed opportunity, as it is essential to guide improved design of next generation nanomedicines, but if proposing new clinical trials it is unethical to disregard past clinical experience. Clinical observations can (i) indicate the likely preclinical−clinical correlation of the *in vitro*/*in vivo* experimentation used to select an optimal candidate; (ii) help identify the most appropriate (subset of) patients for entry into clinical trial, starting dose, and dosing protocol; and (iii) suggest appropriate early biomarkers that can be used to monitor safety and/or efficacy. For certain nanomedicines there is already a well-documented opportunity for patient individualization. The anticancer paclitaxel−polyglutamic acid (PGA) conjugate Opaxio[49] provides a good example. It was designed for cathepsin B activation, but phase III clinical studies indicated increased survival in women patients with NSCLC that had been treated with Opaxio but not men. A correlation between estrogen levels and cathepsin B activity has subsequently been reported,[50] and ongoing phase III clinical trials are evaluating activity in women

Table 1. First Generation Marketed Nanomedicines and Nano-Imaging Agents

product name	technology	indication	route of admin	info source
		Superparamagnetic Iron Oxide Imaging Nanoparticles (SPIONS)		
Feridex (ferumoxide)	SPION dextran coating	liver imaging	iv	Bayer Healthcare Pharmaceuticals
Endorem (ferumoxide)	SPION dextran coating	liver imaging	iv	AMAG Pharmaceuticals/Guerbet SA[a]
Gastromark (ferumoxsil)	SPION silicone coating	GI imaging	oral suspension	AMAG Pharmaceuticals[a]
Lumirem (ferumoxsil)	SPION silicone coating	GI imaging	oral suspension	AMAG Pharmaceuticals[a]
Sinerem (ferumoxtran), same as Combidex	ultrasmall particles (USPION)	lymph node imaging	infusion	Guerbet withdrew MAA for Sinerem in 2007 AMAG Pharmaceuticals (phase III)
Resovist	SPIONs	small liver lesions	iv	Bayer Healthcare Pharmaceuticals
		Iron Oxide Supplements		
Venofer	iron oxide, sucrose	anemia	iv	Fresenius[a]
Ferrlecit	iron oxide gluconate	anemia	iv	Sanofi-Aventis[a]
Cosmofer	iron oxide, dextran	anemia	iv	GRY-Pharma[a]
various "generic" formulations	iron oxide nanoparticles	anemia	iv	reviewed in refs 508, 509
		Drug "Nanocrystal" Technologies		
Rapamune (sirolimus)	NanoCrystal Elan	immunosuppressive	oral suspension and tablets	Pfizer/Wyeth[a]
Emend (aprepitant)	NanoCrystal Elan	antiemetic	oral capsules or iv	Merck[a]
Tricor (fenofibrate)	NanoCrystal Elan	hypercholesterolemia	oral tablets	Abbott[a]
Megace ES (megestrol)	NanoCrystal Elan	antianoretic	oral suspension	Par Pharm. Co[a]
Triglide (fenofibrate)	IDD-P Skyepharma	hypercholesterolemia	oral tablets	Sciele Pharma Inc.[a]
		Liposomes and Lipidic Products		
Ambisome	liposomal amphotericin B	fungal infections	iv	Astellas Pharma/Gilead[a]
Abelcet	lipidic amphotericin B	fungal infections	iv	Sigma-Tau Pharmaceutical[a]
Amphocil	lipidic amphotericin B	fungal infections	iv	various manufacturers
Doxil/Caelyx	PEGylated liposomal doxorubicin	cancer—various	iv	Johnson & Johnson/Schering-Plough[a]
Daunoxome	liposomal daunorubicin	cancer advanced HIV-associated Kaposi's sarcoma	iv	Gilead Sciences Inc.[a]
Myocet	liposomal doxorubicin	cancer—breast cancer	iv	Cephalon[a]
Mepact (mifamurtide)	muramyl tripeptide phosphatidyl-ethanolamine (MTP-PE)	cancer—osteosarcoma; orphan designation in EU	iv	IDM Pharma SAS[a]
Visudyne	liposome/lipidic verteporfrin	age related macular degeneration (AMD)—photodynamic therapy	iv	Novartis[a]
Depocyt	liposomal (nonconcentric vesicles) cytarabine	cancer	intrathecal	Pacira Pharmaceuticals[a]
Depodur	liposomal morphine	pain relief		EKR Therapeutics Inc.[a]
		Polymer Therapeutics		
		Polymer—Protein Conjugates		
Zinostatin stimalmer	styrene maleic anhydride-neocarzinostatin (SMANCS)	cancer—hepatocellular carcinoma	local admin via hepatic artery infusion	Yamanouchi[a] Japan

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Table 1. Continued

product name	technology	indication	route of admin	info source
Oncaspar	PEG-asparaginase	cancer—acute lymphocytic leukemia (ALL)	iv/im	Enzon[a]
Peg-intron	PEG-interferon alpha 2b	hepatitis C	sc	Schering-Plough[a]
Pegasys	PEG-interferon alpha 2a	hepatitis C	sc	Roche[a]
Neulasta	PEG-hrGCSF	chemotherapy-induced neutropenia	sc	Amgen[a]
Adagen	PEG-adenosine deaminase	severe combined immune deficiency syndrome	im	Enzon[a]
Somavert	PEG-HGH antagonist	acromegalia	sc	Pfizer[a]
Mircera	PEG-EPO (polyethylene glycol-epoetin beta)	treatment of anemia associated with chronic kidney disease i	iv/sc	Roche[a]
Cimzia (certolizumab pegol)	PEG-anti-TNF Fab	rheumatoid arthritis, Crohn's disease	sc	UCB[a]
		Polymer—Aptamer Conjugate		
Macugen	PEG-aptamer (apatanib)	AMD	intravitrous	OSI-Eyetech[a]
		Polymeric Drugs		
Copaxone	Glu, Ala, Tyr copolymer	multiple sclerosis	sc	Teva[a]
Renagel	phosphate binding polymer	end stage renal failure	oral	Genzyme (Daiichi licensed)[a]
Welchol	cholesterol binding polymer	type 2 diabetes	oral	Genzyme[a]
		Nanoparticle		
Abraxane	albumin-paclitaxel nanoparticles	cancer—breast cancer	iv	Abraxis (Celgene)[a]
		Antibody—Drug or —Radiotherapy (Theranostic) Conjugate		
Zevalin (ibritumomab tiuxetan)	^{90}Y-ibritumomab tiuxetan	cancer—indolent form of non-Hodgkin lymphoma (NHL) based on rituximab	iv	Spectrum Pharmaceuticals[a]
BEXXAR (tositumomab)	^{131}I-tositumomab	cancer—CD20 +ve follicular, NHL refractory to rituximab	iv	Bexxar (FDA approval June 2003);[a] on Feb 13, 2003, EMA orphan designation EU/ 3/03/136
Mylotarg (gemtuzumab ozogamicin)	anti-CD33 antibody-calicheamicin	cancer—acute myeloid leukemia (AML)	iv	Wyeth/Pfizer[a] (withdrawn 2010)

[a] Marketed. To note there are also a number of marketed cosmetics involving liposomes, lipid nanoparticles, nanocapsules, and TiO_2 nanoparticles in sunscreens (reviewed in ref 9).

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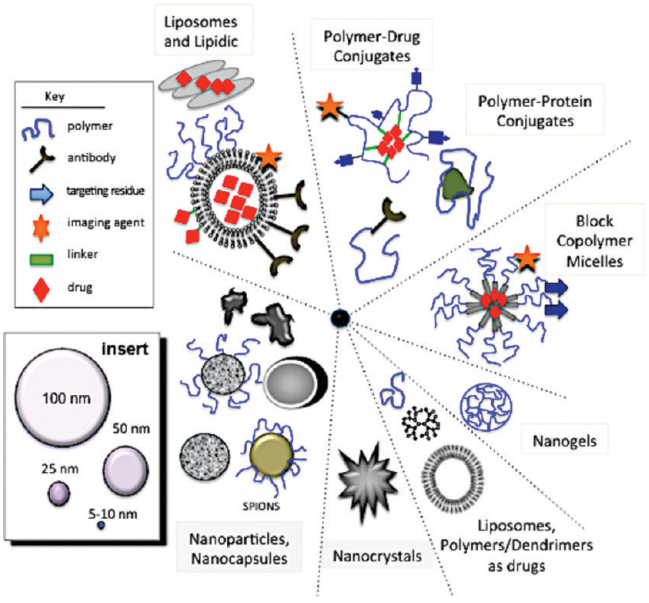

Figure 2. Schematic showing the main classes of first generation nanomedicines in clinical trial and routine clinical use. The inset gives an idea of the relative sizes of nanomedicines as the cartoons in each panel are not drawn to scale. For example, liposomes, nanocrystals, and some polymeric nanoparticles are ≥100 nm, and some polymeric nanoparticles, polymer conjugates, and dendrimers are in the range 5−25 nm.

(NSCLC) who require baseline estradiol levels >25 pg/mL to enroll.[51] However, to note a recent study, patients with metastatic prostate cancer whose disease had progressed with hormone therapy and who were treated with low dose transdermal estradiol in combination with Opaxio did not show any therapeutic benefit.[52]

Products in the Market and Clinical Trial. Modern nanomedicines fit into three groups. The first group consists of first generation nanomedicines that have already entered routine clinical use (Table 1), and they include both "blockbuster" drugs (>1 billion US$ annual revenue) and certain products (e.g., specific liposomes, PEGylated proteins, polymeric drugs) that are of such an age that they will soon begin to appear as "generics" (discussed briefly in the section Translating Nanomedicines to Practice). Figure 2 shows schematically each class of nanomedicines in the market or clinical development. Second, there are an increasing number of nanomedicines, mostly born in the 1980s/90s, in clinical development (examples given in Table 2). Finally, the third group consists of those innovative 21st century nanotechnologies, mostly still embryonic, that may have the potential to enter clinical development and may bring the hoped for new paradigm to diagnosis and/or therapy (The Future: Nanomedicines of Tomorrow?).

Whether nanomedicines are being developed as inherently active drug substances, as drug and/or imaging agent delivery systems, or as theranostics (Tables 1 and 2), it has become clear that the most effective candidates arise from rational design (see refs 53−72 for specific examples) rather than a "make it and screen it" approach. This fact continues to create the most discord between the basic disciplines such as polymer chemistry, materials science, and engineering that are pushing the technology frontiers in order to discover a nanotechnology that may one day be "good for something" and those, especially biologists, pharmaceutical scientists, and clinicians, who prefer to use a reiterative, rational design to optimize a specific candidate for a specific purpose from the outset. It has been wisely observed that "the art of medicine" is "perspiration, inspiration, and the 10-year rule",[73] and this opinion leads to the conclusion that quick and successful future translation of emerging nanomedicines is most likely to arise from the established cornerstones of "Quality by Design"[5] elaborated for the first generation products and not by starting afresh. From the very beginning there is a need for an appreciation of the "target product profile" and the current therapeutic state of the art relating to that specific indication coupled with clearly predefined stop−go points while benchmarking the new technology (using validated methodology) throughout the early R & D phases. Although it is impossible to review all fundamentals of "advanced drug delivery" here, it is evident that many nanomedicinista are new to pharmaceutical development and thus unaware of the key issues. Thus the fundamental lessons learned during the development of the products listed in Tables 1 and 2 are discussed here.

New Drugs or Improved Drug Delivery? Very few pharmaceuticals exhibit unique specificity for their pharmacological target, and the pharmacokinetic−pharmacodynamic profile is often suboptimal. Today two distinct approaches are commonly taken in the quest for improvement. First the search for a unique pharmacological target(s) within a diseased cell and new drug design. Following elaboration of the human genome[74] there has been exponential growth in interest in development of target-oriented low molecular weight chemical entities[75] and target-oriented macromolecular therapeutics including antibodies,[16] proteins, peptides, aptamers and oligonucleotides (e.g., gene and siRNA therapy).[76] The resultant so-called molecular/targeted medicines have seen some successes, but overall the clinical benefit has been much more modest than was predicted a decade ago.[77]

Some nanomedicines (Tables 1 and 2) have been developed as novel "drugs", for example, Copaxone (a random copolymer of 4 amino acids which is used to treat muscular sclerosis[78]), the oral polymeric sequestrants Renagel (binds phosphate and is used to treat chronic kidney disease[79]) and Welchol (binds cholesterol and helps to lower blood sugar and low density lipoprotein (LDL) cholesterol[80]), and the multivalent lysine-based dendrimer product VivaGel currently under clinical development as a vaginal virucide (topical administration). Not all clinical trials involving such polymeric drugs have, however, been successful. For example, despite showing promise in preclinical and early clinical studies, Tolevamer, a styrene-derived polymer designed to treat *Clostridium difficile*-associated diarrhea (CDAD), did not meet its noninferiority end points in a phase III clinical trial when tested in direct comparison to metronidazole and vancomycin.[81] Although the polymer was initially shown to bind *C. difficile* toxins A and B *in vitro* and prevent cytopathic effects in Vero cells *in vitro*,[82] subsequent studies using an *in vitro* human gut model confirmed the lack of activity that was consistent with the clinical outcome.[83] The emergence of multiple drug resistant "superbugs", coupled with the fact that antibiotics can have limitations including poor efficacy, side effects, and the potential for acquired resistance, supports continuing efforts to identify novel antimicrobial nanomedicines.[84] Nevertheless, it is clear that those preclinical models used to predict their potency/activity must be carefully optimized with the specific clinical setting in mind.

The alternative to new drug design is the development of a drug delivery "vehicle"[68] able to guide a bioactive more precisely

Table 2. Examples of Nanomedicines and Nanoimaging Agents in Clinical Development

product name	technology	indication	route of admin	stage	info source
		Superparamagnetic Iron Oxide Nanoparticles			
Nano-Cancer	iron oxide nanoparticles	cancer—glioblastoma	local, brain	phase II	Magforce Nanotechnologies AG[a]
		Drug Nanocrystal Technologies			
CPSI-2364; Semapimod (guanylhydrazone)	nanocrystals	TNF-a inhibitor (also IL-1, IL-6 and nitric oxide)	iv	phase II	Cytokine PharmaSciences Inc.
Paxceed (paclitaxel)	nanocrystals, micellar	arthritis—anti-inflammatory	iv	phase III	Angiotech
Theralux (thymectacin)	NanoCrystal Elan	anticancer for PDT	iv	phase II	Kiadis Pharma
		Liposomes and Lipidic Products			
ONCO TCS	vincristine sulfate	cancer—NHL	iv	phase II/III	Inex/Enzon
LipoPlatin	cisplatin	cancer—NSCLC and pancreatic	iv	phase II/III	Regulon Inc.
CPX-351 (combination therapy)	both cytarabine and daunorubicin	cancer—newly diagnosed AML and first relapse AML	iv	phase II	Celator Pharmaceuticals
CPX-1 (combination therapy)	both irinotecan HCl and floxuridine	cancer—colorectal cancer		phase II	Celator Pharmaceuticals
Sarcodoxome	doxorubicin	cancer—advanced soft tissue sacrcoma in patients >65 y	iv	phase I/II	GP Pharma
SLIT cisplatin	cisplatin	cancer—lung	aerosol	phase II	Transave
LEP-ETU (Neolipid technology)	paclitaxel	cancer—various	iv	phase I	NeoPharm Inc.
LE-DT (Neolipid technology)	docetaxel	cancer—various	iv	phase I	NeoPharm Inc.
L-Annamycin	annamycin	cancer—ALL/AML	iv	phase I/II	Callisto Pharmaceuticals Inc.
ThermoDox	temperature sensitive liposomal doxorubicin + local radio frequency ablation	cancer—hepatocellular carcinoma	iv	phase III	Celsion
LiPlaCis (Liplasome technology)	liposomes designed for PLA_2 degradation containing a platinate	cancer—various	iv	phase I stopped, unaccaptable toxicity[b]	LiPlasome Pharma
Atragen	*t*-retinoic acid	cancer—acute pro-AML and advanced renal cancer	iv	phase II	Aronex
		Polymer Therapeutics			
		Polymer—Protein Conjugates			
ADI-PEG 20	PEG-arginine deaminase	cancer—hepatocellular carcinoma, melanoma	iv	phase I/II	Phoenix Pharmalogics-Polaris Group
Uricase-PEG 20	PEG-uricase	elevated uric acid associated with tumor lysis syndrome	iv	phase I	EnzymeRx-Polaris Group
Hemospan MP4OX	PEG—hemoglobin	delivery of O_2 in postsurgery and trauma patients	iv	phase I/II	Sangart
CDP 791	PEG-anti VEGFR-2 Fab	cancer—NSCLC	iv	phase II	UCB Pharma
		Polymer—Aptamer Conjugates (Reviewed in Ref 371*)*			
ARC1779	PEG-antiplatelet-binding function of von Willebrand Factor	thrombotic microangiopathies	iv	phase II	Archemix

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Table 2. Continued

product name	technology	indication	route of admin	stage	info source
E10030	PEG-anti-PDGF aptamer combination with Lucentis	AMD	local intravitreal	phase II	Ophthotech
		Polymeric Drugs			
AMG 223	phosphate binding polymer	hyperphosphatemia in CKD patients on hemodialysis	oral	phase II	Amgen
VivaGel	lysine-based dendrimer	microbiocide	topical	phase II/III	StarPharma
		Polymeric Drug Conjugates			
CT-2103; Xyotax; Opaxio	poly glutamic acid (PGA)−paclitaxel	cancer—NSCLC, ovarian, various other cancers and combinations	iv	phase III, phase III	Cell Therapeutics Inc.
Prolindac	HPMA-copolymer-DACH platinate	cancer—melanoma, ovarian	iv	phase II	Access Pharmaceuticals
PEG-SN38	multiarm PEG-camptothecan derivative	cancer—several	iv	phase II	Enzon Inc.
NKTR-118	PEG-naloxone	opioid-induced constipation	oral	phase II	Nektar
NKTR-102	PEG-irinotecan	cancer—metastatic breast	iv	phase II	Nektar
NKTR-105	PEG-docetaxel	cancer—various	iv	phase I	Nektar
XMT-1001 (Fleximer technology)	polyacetal-camptothecin conjugate	cancer—various	iv	phase I	Mersana (phase I/II)
		Micelles			
SP1049C Biotransport	doxorubicin block copolymer micelle	cancer—upper GI, NSCLC colorectal	iv	phase I/II	Supratek Pharma Inc.
NK 105 (NanoCarrier technology)	paclitaxel block copolymer micelle	cancer—stomach	iv	phase II	NanoCarrier Co.-Nippon Kayaku Co
NK-6004, Nanoplatin (NanoCarrier technology)	cisplatin block copolymer micelle	cancer	iv	phase I/II	NanoCarrier Co.-TOUDAI TLO Ltd./
NC-4016 (NanoCarrier technology)	oxaliplatin block copolymer micelle	cancer	iv	phase I	NanoCarrier Co. TOUDAI TLO Ltd./
		Nanoparticles			
IT-101 (Cyclosert technology assembled into a nanoparticle)	polymer-conjugated cyclodextrin-camptothecin	cancer	iv	phase I	Cerulean Pharma Inc.
CALAA 01 (Cyclosert technology assembled into a nanoparticle)	polymer-conjugated cyclodextrin-siRNA	cancer	iv	phase I	Calando Pharmaceuticals
ABI-008 (nab technology)	albumin nanoparticles containing docetaxel	cancer—hormone refractory prostate cancer	iv	phase II	Abraxis Biosciences/Celgene
ABI-009 (nab technology)	albumin nanoparticles containing rapamycin	cancer—mTOR Inhibitor (solid tumors)	iv	phase I	Abraxis Biosciences/Celgene
ABI-011 (nab-5404)	albumin nanoparticles	cancer VDA and topoisomerase (solid tumors)		phase II	Abraxis Biosciences/Celgene
Aurimune (CYT-6091)	PEGylated colloidal gold bound TNF	cancer	iv	phase I	Cytimmune Therapeutics
Auroshell	gold coated silica	cancer—refractory head and neck	iv	phase I	Nanospectra Biosciences

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Table 2. Continued

product name	technology	indication	route of admin	stage	info source
BA-003 (Transdrug)	polyisohexylcyano-acrylate nanoparticles containing doxorubicin	cancer—hepatocarcinoma	intra-arterial	phase II stopped due to toxicity[c]	BioAlliance, EMA Orphan Drug Status (EU/ 3/04/229) granted on Oct 21, 2004
		Antibody−Drug Conjugates (Reviewed in Ref 16)			
Glembatumumab vedotin (CDX-011)	human mAb to GPnMB - auristatin conjugate	cancer—metastatic breast and melanoma	iv	phase II	Celldex Therapeutics
Trastuzumab emtansine (trastuzumab-DM1)	humanized mAb to HER2-maytansine (DM1) conjugate	cancer—HER2 +ve metastatic breastiv cancer		phase II/III	Roche-Genentech-Chugai
		Others			
NPI 32101	silver nanocrystals	antibacterial (atopic dermatitis)	topical		Nucryst Pharmaceuticals Corp. (phase II)

[a] Regulatory approval of the Nano-Cancer therapy (CE European conformity marking, as a medical device) received in June 2010; medicinal product still under clinical trials.[214] [b] see ref 510. [c] July 16th, 2008 Bioalliance Pharma http://www.biospace.com/news_story.aspx?NewsEntityId=103438.

to its desired location of action (i.e., drug targeting) and/or control the release to ensure that an optimal concentration is maintained at the therapeutic target over a desired time frame. Historically, drug delivery systems were usually developed to improve performance of an established drug (better formulation, better route of administration, and/or improved therapeutic index), or to provide a product line extension (for economic benefit). Early technologies were macro- or microsized (e.g., the contraceptive Norplant;[85] Gliadel treatment for glioma;[86] Oros osmotically controlled oral drug delivery technology[87]). They were frequently designed to improve the bioavailability or sustain the release of orally administered low molecular weight drugs, or the bioavailability of drugs administered by the transdermal, nasal, or pulmonary routes. Patient convenience continues to make these routes attractive. Together with biodegradable polymeric implants and microparticles used for parenteral sustained release of peptides (Zoladex and Leupron Depot), such drug delivery technologies still contribute most to pharmaceutical industry activity in the drug delivery sector. The oral route is currently the largest sector (52%) of a global drug delivery market (anticipated to be ~200 billion US $ by 2014[88]), but the parenteral, implantable, and inhalation technology sectors are growing fastest and are predicted to outstrip oral technologies soon.[89] Nanomedicines are foreseen to play an important role here.

Although there are some oral (e.g., nanosized drug crystal formulations and polymeric sequestrants, see Tables 1 and 2) and topical (nanocrystalline silver) nanomedicine products, many first generation nanopharmaceuticals were designed for parenteral administration. As risk is more justifiable where there is a potential beneficial treatment for a life-threatening disease, often the first nanomedicine in a class was developed as an anticancer agent (e.g., Doxil,[90] Abraxane,[91] Oncaspar[92]). The ability of nanosized particles to translocate across both external biological barriers (e.g., gastrointestinal (GI) tract and lung) (e.g., refs 93 and 94) and internal barriers (e.g., blood brain barrier (BBB)[95,96]) has been well-known for several decades, but the process is very inefficient in terms of percentage of dose transferred and thus has been difficult to harness into practical to use products.[97] Even with the aid of transient physical disruption of the blood brain barrier (BBB) by osmotic opening[98] to promote polymeric transfer or the use of dendrimers that have demonstrated exceptionally high transcytosis rates *in vitro*,[99,100] maximum transfer *in vivo* is typically <1% dose.

Many of the first generation nanomedicines (Tables 1 and 2) were designed with the aims of (i) drug targeting to a diseased organ, cell, intracellular compartment (e.g., nucleus, cytosol) or recently even a subcompartment in an organelle, targeting of drug away from potential sites of toxicity (i.e., to achieve an optimal therapeutic index) and/or (ii) delivery at the required concentration and duration to maximize pharmacological benefit and minimize nonspecific toxicity. The most sophisticated systems combined both enhanced site specificity and local controlled release of the bioactive agent. Moreover, those antibodies developed to carry radioactivity for therapeutic purposes (e.g., Zevalin and Bexxar) can be viewed as the first theranostics as they enable diagnosis and therapy. Such radiolabeled antibodies illustrate well the limitations of receptor-mediated targeting in the clinical setting as typically only 0.001−0.01% of the dose localizes to the tumor in patients.[17] This raises the question, if >99% of the dose does not localize to the target, is this "targeting"?

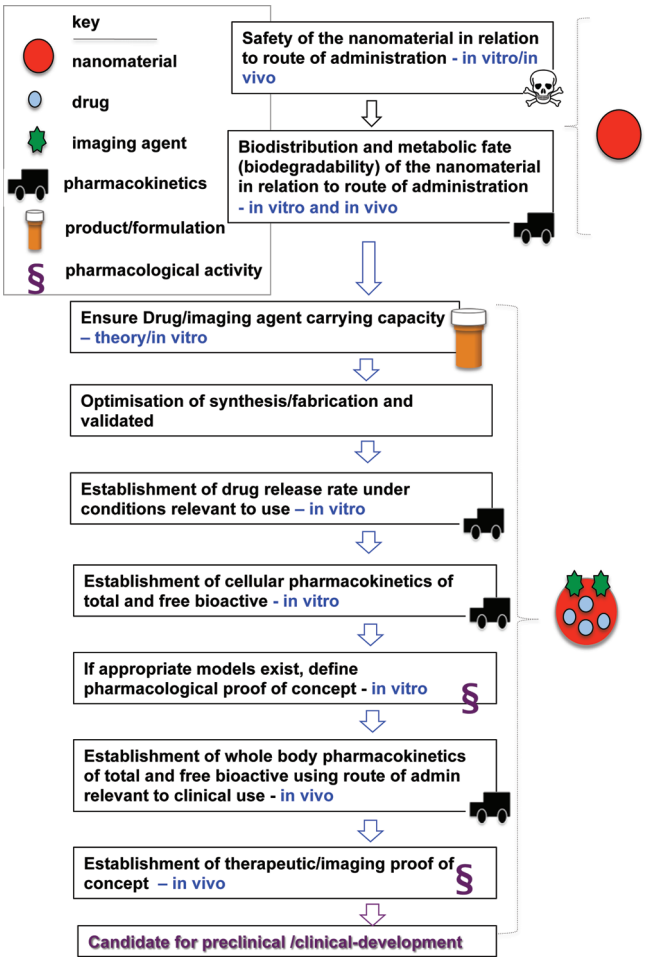

Figure 3. Summary of stop−go checkpoints for nanomedicine design, optimization, and candidate selection for preclinical development. Methodology usually used (theoretical, *in vitro*, *in vivo*) is also shown.

General Considerations for Translation. During the basic research phase each specific nanomedicine must be optimized with respect to its proposed clinical use, route of administration, likely dose, and frequency of dosing; however, there are several empirical stop−go checkpoints common to all. These are summarized in Figure 3. Although clearly a nanomedicine or nanoimaging agent will never be useful in practice without the ability to display functional (e.g., pharmacological) activity, it is evident that inability to pass the desired pharmacokinetic, safety, and product specification checkpoints will render any interesting pharmacological or imaging properties redundant.

Potency/Payload. If the nanomedicine is biologically active in its own right (drug or a sequestrant), it is essential that the dose required to exhibit pharmacological activity is low enough to allow practical formulation for patient administration; e.g., tablet size and infusion volume have limits. Similarly, when the nanomedicine is transporting a drug or imaging agent, it must be able to carry a sufficiently high payload in relation to drug potency/imaging capacity. Acceptable minimum threshold values for carrying capacity are easy to estimate theoretically at the outset, especially if composition is expressed as weight % for all components. Unfortunately this information is rarely given. For example, the drug:lipid:polymer weight ratio of a PEGylated liposome is rarely stated, and it is more common to report % drug entrapment efficiency. Although useful to indicate manufacturing efficiency (impacts on cost and potential free drug content), this value gives no appreciation of overall composition by weight (i.e., the doses to be given). The latter better highlights practicality vis-à-vis final formulation design, and potential safety and efficacy of drug and carrier. It also guides the concentration or dose range of all components needed for meaningful preclinical *in vitro* and *in vivo* safety evaluation.

The potency, structure, physicochemical properties, and mechanism of action of the bioactive component are all important to determine its suitability for incorporation into a nanomedicine. Additionally, physicochemical properties govern stability in the environments encountered following administration, very different for different administration routes. It is noted that the bioactive's whole body and intracellular biodistribution is often radically changed (purposely so) when it is administered as a nanomedicine, an obvious fact that is often overlooked.

Candidates that will not be able to meet fundamental criteria regarding potency and stability, often theoretically determinable in advance, should be eliminated at this early stage.

Safety. The toxicity/immunotoxicity of the product as a whole and all the components (they may ultimately be released by degradation/metabolism) must be considered in the context of the proposed route of administration from the beginning. The recent statement "interestingly pharmaceutical sciences are using nanoparticles to reduce toxicity and side effects of drugs and up to recently did not realize that carrier systems themselves may impose risks to the patient"[101] was not a well-informed observation. Over many decades, pharmaceutical scientists from academia and industry have studied the general toxicity, hematocompatibility, complement activation, immunotoxicology, pharmacokinetics, toxicokinetics, and metabolic fate of novel materials proposed for use as components of advanced drug delivery systems (see examples[102−108]). Moreover all the nanomedicine products entering clinical development (Tables 1 and 2) must be subjected to rigorous, often "good laboratory practice" (GLP), preclinical evaluation (for examples, see refs 109 and 110). We have recently reviewed the approaches used for preclinical safety evaluation of novel polymers and polymer therapeutics elsewhere.[111]

There are important points to make in relation to the complex, novel, and often hybrid nanomedicines emerging now. Some researchers often overexuberantly claim that their material or technology is "biocompatible" or "biodegradable" without any robust scientific experimentation (*in vitro* or *in vivo*) to back their statement. (In the context of a medicine rather than a biomaterial the term "toxicity" is more appropriate than "biocompatibility" as they have different meanings.[111]) Cytotoxicity studies often use short time frames (hours) chosen to match *in vitro* pharmacological experiments without any consideration of likely clinical pharmacokinetics (patient exposure can be hours, days, or months), and the concentration range used is too low to define an inhibitory concentration for 50% cell kill (IC_{50}). If a short incubation time and low concentration are used, how can it be stated the material tested is nontoxic? Such statements promote dogma that pervades the literature. Claims of biodegradation are rarely qualified by time frame (seconds, minutes, or years?) or the mechanism. Many natural polymers, e.g., alginates, chitosans, dextran, are poorly degraded by mammalian enzymes, and many materials actually never access the physiological compartment (maybe intracellular) where the target mammalian catabolic machinery resides. Additionally, chemical functionalization can

render a natural polymer effectively nonbiodegradable.[112] Misuse of the terms "biocompatibility" and toxicity (discussed in ref 111) is also exemplified by the frequent misuse of the term GRAS (generally recognized as safe). The FDA term GRAS is a designation given to a *specific material* (designated specification), for use at *specific doses* and via *designated routes of administration*. There is frequently failure to realize that materials approved for topical or oral administration maybe entirely *unsuitable* for parenteral use.

Of course all drugs display side effects. Even humanized monoclonal antibodies (hMabs) display an array of side effects including acute anaphylaxis and other immune reactions such as life-threatening cytokine release syndrome (reviewed in ref 113). Only 7 hMabs have come to market since the antitumor necrosis factor alpha (TNFα) antibody Humira in 2002.[16] Risk−benefit is always of paramount importance. Mylotarg, the only antibody−drug conjugate to come to market, was withdrawn in 2010 when postmarketing surveillance indicated an inadequate efficacy−side effect relationship.[114] The promising immunoconjugate BR96 (antibody targeting the Lewis-Y antigen, expressed on >75% breast cancers)−doxorubicin displayed significant toxicity and limited antitumor activity in phase II clinical trials despite excellent efficacy in mouse tumor models.[115] Such observations not only relate to the development of novel antibody−drug conjugates (see Table 2 and refs 116 and 117) but also illustrate key challenges for development of nanomedicines that use surface exposed ligands (e.g., peptides, proteins, and antibodies) to promote receptor-mediated targeting and the need for appropriate preclinical models.

Techniques used to evaluate nanomedicine safety continue to evolve, and some examples are given here. Screening often uses *in vitro* cytotoxicity testing (e.g., polymers,[102,118,119] dendrimers[120−123] and polymeric nanoparticles[124,125]) to give an early indication of the material suitability for a particular use. Microscopy (TEM/SEM and light) is also used to highlight subtle cellular changes,[119,120,126] but such techniques require careful interpretation as sometimes methodology used can introduce artifacts ("seeing is not always believing"[127]). It has been noted that when synthetic polymers and nanomaterials are administered together with noncovalently or covalently conjugated cytotoxic agents, DNA, or antigens, they can markedly alter genetically controlled responses, and this has given rise to studies designed to explore polymer genomics.[128] To note, for useful data to come from biological assays, nanomaterials must be reproducibly manufactured and well characterized.[129]

Putative parenteral nanomedicines displaying acceptable toxicity *in vitro* must then be subjected to rigorous investigation of their antigenicity, immunotoxicity, and potential to activate complement (Figure 4b). In the 1980s Rihova and colleagues pioneered *in vitro* and *in vivo* models for definition the immunotoxicology of polymeric materials[104,106] and the immunomodulatory properties of *N*-(2-hydroxypropyl)methacrylamide (HPMA) copolymer−anthracycline conjugates,[108,130] and showed that they did not activate complement.[105] Infusion reactions have

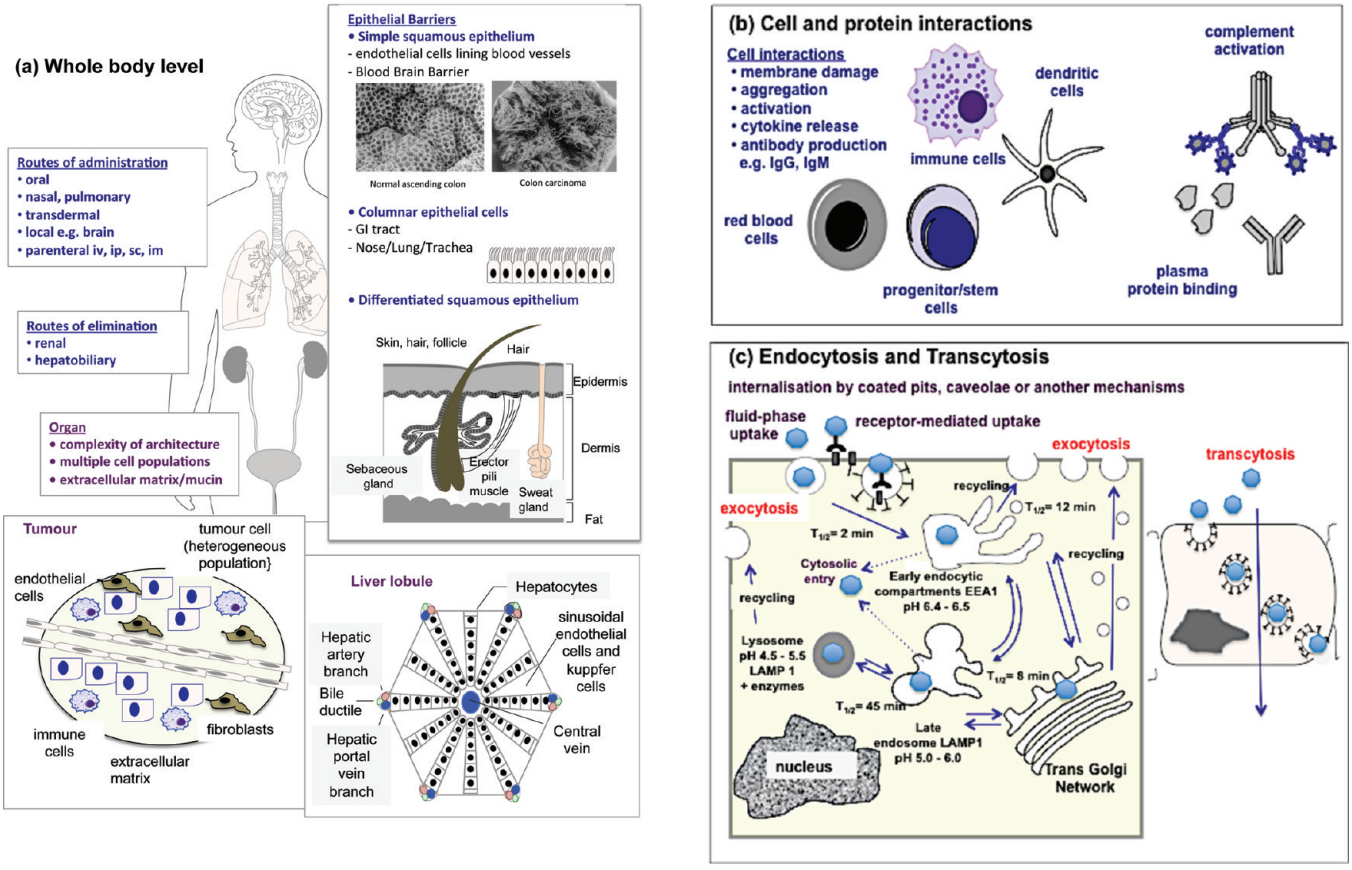

Figure 4. Illustration of pathophysiological complexity and biological barriers requiring consideration when designing nanomedicines for administration via different routes and with different pharmacological targets in mind. The scanning electron micrographs of colorectal vascular corrosion casts of normal and carcinoma vasculature shown in panel a are reprinted with permission from ref 507. Copyright 2001 Nature Publishing Group.

been observed clinically during iv administration of nanosized particles,[131] certain polymer−drug conjugates (e.g., refs 132 and 133), and PEGylated liposomes.[134] Clinically such effects have often been managed by dilution, a longer infusion time, or patient premedication, but nonetheless it is important to predict potential problems and if possible eliminate them. Over the past decade preclinical studies have documented complement activation by dendrimers,[135] liposomes,[136] PEGs,[137] and polaxamers.[138] Animal models are available to evaluate complement-mediated hypersensitivity using liposomes and other lipid based nanoparticles used for validation.[139] Szebeni has termed "complement activation-related pseudoallergy" the CARPA effect,[140,141] noting that it is essential to consider type 1 anaphylactic reactions early in the development of all nanomedicines designed for parenteral use.[142] When dealing with nanoparticles, the polymer used for surface coating can play an important role in this context.[143]

Observation that ultrafine particles are an important cause of pollution-related adverse health effects, and that novel engineered nanomaterials may be hazardous in the workplace or environment if not appropriately contained,[144,145] has led to a convergence of interests in nanotoxicology. Although the methodology used in these fields (techniques, time frames, etc.) does not always translate to the experimental design and validation needed to establish safety of nanomedicines, there is much to learn from this literature (see reviews on the toxicology of inorganic nanoparticles[146,147]). In the USA there have been efforts to establish high-throughput toxicology screening programs for identification of any potential environmental hazards of novel manufactured nanomaterials,[148] and to accelerate the development of safe nanomedicines. The US National Cancer Institute (NCI) Alliance for Nanotechnology in Cancer program[149] is well supported by the Nanotechnology Characterization Lab (NCL),[150] an agency that interfaces with NCI, the National Institute of Standards and Technology (NIST), and the Food and Drug Administration (FDA). NCL undertakes physicochemical characterization of nanomaterials as well as safety and efficacy evaluation of emerging nanomedicines, and McNeil and colleagues have already evaluated the safety of >180 different nanomaterials proposed for use as drugs, biologics, and medical devices.[151−153] A typical example of the NCL approach was the physicochemical and *in vitro* biological evaluation of polyamidoamine (PAMAM) dendrimers based on a diaminobutane (DAB).[154] Compounds tested included PAMAM G4 terminating in tris(hydroxy) (and its Magnevist complex), G4 terminating in pyrrolidinone (both have 64 surface groups with a molecular weight in the range approximately18,000−22,000 g/mol), and PAMAM G4.5 terminating in COONa (128 surface groups and molecular weight of approximately 26,000 g/mol) and its Magnevist complex. Comprehensive biological studies were undertaken to measure endotoxin contamination (rarely undertaken in research laboratories for novel nanomaterials subjected to immunotoxicity evaluation), microbial sterility, mycoplasma contamination, hemolysis, platelet aggregation, toxicity to bone marrow cells, coagulation, complement activation, interaction with plasma proteins, effect on leukocyte proliferation, nitric oxide production by macrophages, effect on chemotaxis, phagocytosis, cytokine secretion, and general cytotoxicity.[154]

Development of specific *in vitro* assays that can be validated for nanomaterials is to be applauded, but the establishment of meaningful high-throughput screening, especially in the context of safety evaluation that can be optimal for all nanomaterials, is not without challenges. For each nanomedicine it is essential to choose a specific portfolio of tests and the assays used must be carefully optimized, for example by (i) using time frames that are relevant to material's pharmacokinetics (single time point readouts can easily give false positive or false negative results), (ii) using the cell lines to which the material will most likely be exposed (primary cells may be needed, and all cells *in vivo* will be exposed to serum), and (iii) using analytical techniques only where it is known that the analyte does not interfere with the assay readout.

All nanomedicines must display an acceptable risk−benefit with respect to proposed use, and early safety studies should be used as a stop−go checkpoint to decide whether or not the technology has promise for further development toward clinical trials in the context of the proposed use.

Metabolic Fate. Rarely do authors reflect on the potential safety of each component and/or the metabolites that might arise following *in vivo* degradation/metabolism of the nanomedicine they are proposing. The dose, frequency of dosing, and clinical setting are particularly relevant factors here. Increasing complexity of hybrid technologies (see The Future: Nanomedicines of Tomorrow?) and, additionally, introduction of novel polymers and linkers can potentially create a plethora of metabolites never before seen in humans. Although information is readily available regarding the exposure limits allowed for many inorganic components of nanosized particles (e.g., gold, silver, iron, cadmium, etc.),[155] the potential long-term hazards of novel polymers used to make and/or coat nanosized particles is frequently not known.

In addition many polymers, including PEG, which are so often proposed as coatings to improve "stealth" properties or prevent aggregation, are not biodegradable. Even if lower molecular weight polymer fractions are used to facilitate renal elimination, their lack of biodegradability may limit safety. There is a particular danger of accumulation within lysosomes after high dose and/or chronic administration, and even if the material is excreted via the kidney, tubular reabsorption can be an issue for certain polymers/nanomaterials. The pathophysiological consequences of macromolecular accumulation in lysosomes are well documented in the context of the lysosomal storage diseases. These rare genetic disorders result from a missing catabolic lysosomal enzyme[156] with therapy involving enzyme replacement[157] (nanomedicines are also being explored in this role). The potential of nondegradable or poorly degradable materials to accumulate in lysosomes has been reported clinically, and the fact that PEGylated proteins can cause (sometimes transient) intracellular vacuolation in animal models[158−160] illustrates the need to carefully consider the potential effects of nonbiodegradable or slowly degrading elements on normal lysosomal function.

Nanomedicines should preferably be biodegradable (to safe metabolites) if proposed for use at high dose or for long-term administration. If they are nondegradable, renal and/or hepatobiliary elimination of should be confirmed at an early stage.

Pharmacokinetics. Nanomedicines have been often designed to improve bioavailability, facilitate controlled drug release, or promote drug targeting, thus it is not surprising that the most effective products have arisen from a careful, pharmacokinetically guided design. No matter how promising the potential pharmacological response, if a therapeutic cannot reach its target in the clinical setting, it will never be efficacious. Early establishment of the desired pharmacokinetic profile is essential, even *before* screening for pharmacological activity. Almost all of the pivotal

checkpoints relate to the dynamic processes that involve transport to the target (pharmacokinetics and biodistribution) or duration of action at the target (metabolism).

Whole Body Pharmacokinetics and Biodistribution. All medicines must circumvent a large number of biological barriers[161] that have evolved, on one hand, to protect the body from entry of "foreign" pathogens and chemicals, and on the other, compartmentalize the complex biochemical/cell biological pathways responsible for normal physiological homeostasis (Figure 4).

Depending on the clinical target and route of administration nanomedicine pharmacokinetics can be important at (i) the whole organism level (organ distribution) (Figure 4a), (ii) the cellular distribution within a tissue (e.g., liver and tumor as a disease target) (Figure 4a), (iii) the subcellular level (Figure 4c), and/or (iv) the suborganelle level. For external routes of administration (e.g., topical, oral, pulmonary) a nanomedicine may first need to gain access to the body (Figure 4a). Then lack of stability and/or unwanted protein or cellular interactions in the circulatory or lymphatic systems during subsequent localization to a diseased tissue/cell can frustrate this goal (Figure 4b). Circumnavigation of internal physical barriers, such as the vasculature endothelial lining or the blood brain barrier (BBB) or a complex extracellular matrix (ECM) (e.g., a tumor or arthritic joint), may also be needed (Figure 4a).

When the pharmacological target resides in the extracellular space (e.g., an enzyme substrate as is the case for some PEGylated enzymes) or is a plasma membrane-localized receptor (e.g., PEGylated cytokines) the nanomedicine itself may be able to initiate therapeutic activity on arrival there. However, more often than not the bioactive must be released from the carrier to become pharmacologically active. If a low molecular weight drug is liberated extracellularly, it can often traverse the plasma membrane to gain access to an intracellular therapeutic target, but most of the emerging macromolecular drugs (e.g., siRNA, genes, and proteins) require further help to pass the plasma or endo/lysosomal membrane barriers to gain the cytosolic access needed for their pharmacological activity (Figure 4c). Although considerable effort has been made since the 1980s to develop nanovectors for this purpose, particularly for gene delivery (reviewed in refs 162−165), success has been limited, e.g., the DermaVir Patch in clinical trial for delivery of a DNA vaccine which contains polyethyleimimine (PEI)/nanoparticle.[166] Currently a number of early clinical trials are ongoing with nanomedicines designed to deliver siRNA (e.g., polymeric nanoparticles[167] and lipidic nanoparticles[168]), but it is still too early to know if these can become viable medicines.

Cellular Pharmacokinetics. Although receiving renewed interest in the context of cytosolic macromolecular delivery,[169] the concept of hijacking the endocytic pathways for drug delivery is not new. In the 1960s Ryser and Hancock found that poly(L-lysine) (PLL) stimulates endocytosis[170] (the polycation actually acts as a "glue" increasing substrate binding to the cell surface rather than stimulating vesicle formation[171]), and in the 1970s De Duve and colleagues discussed the concept of lysosomotropic delivery[172] and proposed albumin as a drug carrier.[36,173] At that time, the rational design of synthetic polymer-based anticancer drug conjugates for lysosomotropic drug delivery also began.[31] Similar design approaches are used today by modern nanomedicines, e.g., for proteolytic activation by lysosomal enzymes (e.g., Opaxio[174] and HPMA copolymer conjugates[175]) or pH activation (reviewed in refs 176 and 177).

If a nanocarrier requires internalization and trafficking to a specific intracellular compartment before pharmacological activation, it is essential to prove the feasibility of cellular uptake, and to quantitate the internalization rate and intracellular fate. The fact that these are highly cell specific dynamic processes interfacing with exocytosis is often overlooked (Figure 4c). Material resident in the cell at any one time *does not* give a quantitative measure of uptake rate; total uptake = cell association + exocytosis + intracellular degradation. Understanding and optimization of the rate of cellular uptake and the rate and location of bioactive release is for many nanomedicines the key factor that will determine the clinical outcome. Although a variety of techniques and cell lines are being used *in vitro*, it is essential to consider the particular target cell (endocytosis is cell type- and cell cycle-dependent) and also to gain information about endocytosis and lysosomal degradation *in vivo*. For efficacy, it is essential to deliver A sufficient drug molecule dose to the pharmacological target at a rate compatible with mechanism of action, and also to have the capability to repeat dosing according to the duration of pharmacological action, rates of replenishment of target, and rate of drug metabolism. For a specific nanomedicine it is also possible to model the specific kinetics needed with respect to the safety, resistance, and efficacy implications.

Stability/Drug Release Rates. The terms "stability" and "drug release" are frequently used interchangeably, but they do have different meanings. Inadequate stability can manifest itself as premature degradation of a nanomaterial and/or dissociation of noncovalent or covalently linked surface coatings, and it can happen during formulation, during storage, and in devices used for patient administration (infusion tubing etc.). Poor stability can adversely affect safety and/or efficacy, and specific techniques must be developed to explore such phenomena. It will not necessarily influence drug liberation.

Nanopharmaceuticals either noncovalently entrap or covalently bind the active principal. The location and rate of bioactive release must be fine-tuned to ensure optimal therapeutic index. Many technologies are specifically designed to be stable in transit and release drug at an optimal rate at a particular target site (e.g., a region of the GI tract, a tumor cell or the interstitium, hepatocyte, etc.). The design of covalent linkers (reviewed in ref 178) for drug attachment to a carrier can provide additional control compared to simple entrapment (e.g., for liposomes, nanoparticles, and block copolymer micelles) although sophisticated coatings and nanomaterials design for regiospecific, time-dependent degradation can be used.

Proof of concept requires quantitative studies defining pharmacokinetics and biodistribution, stability, and drug release rates, and, where relevant, endocytosis/exocytosis and intracellular trafficking should be defined before progressing to large-scale pharmacological screening (Figure 3). Only with this knowledge is it possible to interpret the significance of the pharmacological response measured (good or bad).

Unfortunately most often investigators screen putative nanomedicines for pharmacological activity using *in vitro* cell culture and *in vivo* disease models without prior optimization of the model and/or time frame with respect to the cellular and whole body pharmacokinetics (and biodistribution). It is widely agreed (in their paper "Seven challenges for nanomedicine" Sandhai et al.[179] also emphasize it) that there is a need for *early quantitation* of pharmacokinetics and biodistribution coupled with wider use of modeling to enhance understanding of dynamic processes and guide design optimization. Clinical development

of first generation nanomedicines has advanced the techniques available for quantitation of free and bound/entrapped drug in biological fluids and tissues (e.g., using radioactivity, HPLC, atomic absorption spectroscopy (AAS), etc.[151,180−182]) and development of complementary nanoprobes for gamma camera imaging,[183−186] magnetic resonance imaging (MRI),[187−189] and positron emission tomography (PET).[190,192] Recently it has become popular to use optical imaging using near-infrared (NIR) fluorescent and luminescent tools to visualize nanomaterials inside cells, and for small animal imaging. The latter involves either injection of fluorescent probes or production of transgenic cells or species able to express fluorescent proteins when activated in a particular way. Although visually compelling, compared to gamma camera, MRI, and PET techniques these approaches can be more difficult to quantitate (with respect to the % dose administered),[193] they can have low sensitivity, and there can be problems associated with signal tissue penetration.

Fluorescence microscopy is notably prone to technical artifacts,[194] and misinterpretation is compounded when light or TEM is used to define intracellular location (e.g., it is "within endosomes or lysosomes" etc.) without any direct experimental evidence. Subcellular fractionation coupled with HPLC assay[195] or radiolabeled substrates[196−199] using validated live and/or fixed cell fluorescence microscopy (e.g., ref 200) is needed to verify fate and quantitate with time passage through intracellular compartments and more accurately localize nanomaterials to specific organelles over time. An example showing the liver lysosomal and cytosolic levels of radioactivity after administration of HPMA copolymer-[^{3}H]daunomycin-galactosamine as determined by subcellular fractionation is shown in Figure 5c.

Pharmacological Evaluation and Preclinical Development. Models and techniques for pharmacological evaluation of nanomedicine must be optimized on a disease target and technology specific basis. They must also satisfy the Regulatory Authority requirements before first in human studies are authorized. However, it is important to emphasize that (i) the traditional models used to screen the pharmacological response of low molecular weight chemical entities are not always appropriate for a nanomedicine evaluation as they can have such a different pharmacokinetic profile and (ii) pharmacological models should be used that are resistant to (rather than responsive to) existing therapies if therapeutic advantage is to be demonstrated.

TUMOR TARGETING: A CASE ANALYSIS

Cancer is a major focus for development of new drugs with ~16,000 of the ~40,000 clinical trials listed in 2009 dedicated to this topic (reviewed in ref 201). Currently ~70 cancer clinical trials are ongoing involving nanomedicines,[202] many being follow-on indications and/or combinations of first generation products. Technologies include liposomes,[203−205] polymer conjugates[176,177,206] and block copolymer micelles,[207−210] nanoparticles (e.g., Abraxane[211]), and nanosized crystals (e.g., 2-methoxyestradiol (2ME2) NanoCrystal dispersion[212]). The goals have been improved formulation, tumor targeting by local administration,[213,214] passive targeting using the enhanced permeability and retention (EPR) effect[215] (e.g., Doxil,[216] Opaxio[49]) or receptor-mediated targeting (e.g., Mylotarg,[217,218] HPMA copolymer-doxorubicin-galactose FCE28069,[132] Trastuzumab-DM1[117]). Certain products also seek to promote cytosolic delivery (e.g., cyclodextrin polymer-based nanoparticle[219]) and overcome drug resistance and reduce chemotherapy side effects,[185] as well as improve patient convenience (e.g., Neulasta[220]).

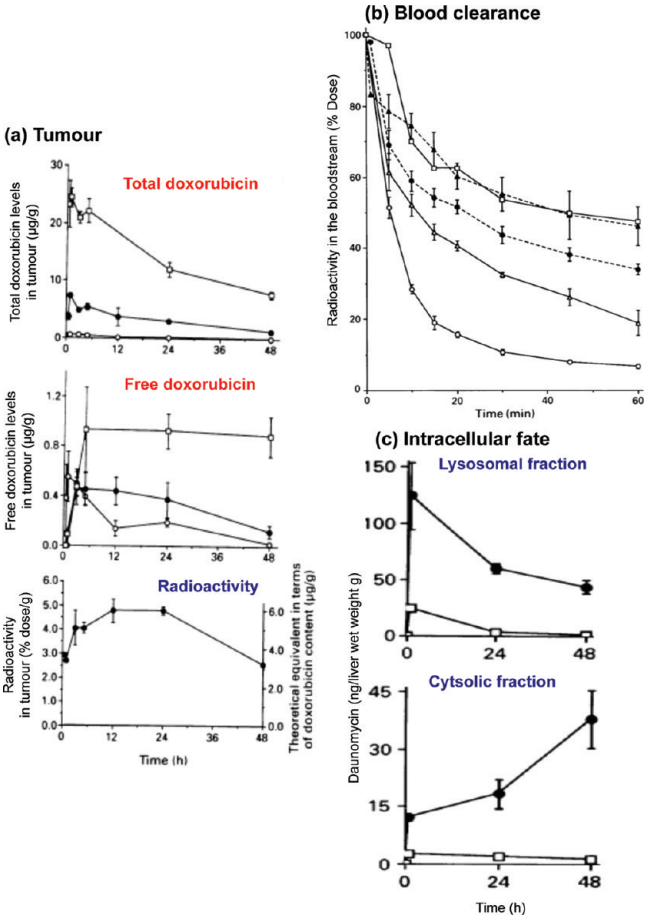

Figure 5. Preclinical studies using HPMA copolymer−doxorubicin conjugates to illustrate methodology that can be used for quantitation of the distribution of free and bound drug and the polymer backbone. Panel a shows the total and free doxorubicin (by HPLC) measured in sc B16F10 melanoma after iv administration of free doxorubicin (5 mg/kg open circle) or HPMA copolymer−doxorubicin conjugate (5 mg/kg closed circle or 18 mg/kg-open square) to mice. The levels of radioactivity detected after injection of ^{125}I-labeled (backbone) polymer conjugate (equivalent dose 5 mg/kg) in the same experimental model are also shown. Data are expressed as % dose recovered/g of tumor for radioactivity and equivalent μg of doxorubicin/g of tumor (n = 5).[180] Reprinted with permission from ref 180. Copyright 1994 Macmillan Press Ltd. Panel b shows dose-dependency of blood clearance of HPMA copolymer-doxorubicin-galactosamine in DBA2 mice. Doxorubicin-equivalent doses of 0.05 mg/kg (open circles), 0.5 mg/kg (open triangles), 5 mg/kg (closed circles) and 15.0 mg/kg (closed triangles) were used, and the blood clearance of HPMA copolymer−doxorubicin (without galactoseamine) is shown for comparison (open squares) administered at a dose of 0.05 mg (n = 3 ± SE). Effect of increasing dose on receptor saturation can be clearly seen.[271] Reprinted with permission from ref 271. Copyright 1991 Macmillan Press Ltd. Panel c shows the distribution of radioactivity in the liver lysosomal and cytosolic fractions with time after iv injection of HPMA copolymer-[^{3}H]daunomycin (n = 3 ± SE).[196] The transfer of from lysosomes to cytosol with time can be seen. Reproduced with permission from ref 196. Copyright 1991 Macmillan Press Ltd.

Emerging cancer nanomedicines has been comprehensively reviewed.[221−225] The following are key issues likely to increase the probability of successful translation:

(i) Rigorous characterization of the nanotechnology before any biological testing, with development of new methods to enable this where necessary.
(ii) Use of appropriate preclinical methodologies/tumor models for selection of lead candidates (discussed in refs 151, 202, and 226).
(iii) If tumor targeting is claimed, this should be quantitatively verified.
(iv) Use of the nanomedicine to deliver combination therapy[187,227] and/or its use in the context of combination therapy.
(v) Early consideration of nanomedicine-relevant patient biomarkers to guide selection of appropriate patients for therapy.

Passive Targeting. Tumor angiogenesis (and related biomarkers) not only provides an important target for therapy[228,229] but also creates the gateway for tumor access of nanosized medicines. Matsumura and Maeda described the EPR effect in the 1980s.[215] Initially many dismissed the phenomenon, even suggesting that the failure of anticancer antibodies was due to their size; "too big to reach the tumor cells". Since then, numerous *in vivo* studies have demonstrated passive targeting (reviewed in refs 215, 230, and 231) using polymer conjugates,[180,232−234] micelles,[235,236] liposomes,[237] and nanoparticles[238] and started to elucidate nanomedicine structure−activity relationships (e.g., refs 239 and 240) and tumor characteristics governing the magnitude of EPR-mediated targeting achievable preclinically[241,242] and in patients.[184] Typical data showing the *in vivo* EPR mediated targeting of HPMA copolymer−doxorubicin conjugates in sc B16F10 melanoma bearing mice are shown in Figure 5a. Understanding of the mechanisms of angiogenesis continues to grow,[243] and the complexity of different classes of angiogenic vessels cannot be overlooked.[244] Using the window chamber model[245] Jain and colleagues have given significant insight into the tumor characteristics that govern nanomedicine access[246] and possibilities to enhance delivery, e.g., using vascular endothelial growth factor (VEGF)[247] or controlling liposomal charge.[248] Radiotherapy augments EPR-mediated targeting of polymer conjugates *in vivo*,[249] and this is being explored clinically using Opaxio combined with radiotherapy as a treatment for esophageal cancer.[250]

In rodent tumor models the extent of passive targeting reported typically lies between <1 and ∼15% dose administered/g (reviewed in ref 224). The lower values are not dissimilar to those of chemotherapeutic agents distributing by random diffusion. A primary factor governing the extent of passive targeting achieved is initial blood clearance. A short plasma half-life ($t_{1/2}\alpha$), due to RES clearance, rapid renal elimination, and/or clearance by nontumor specific receptors, limits the dose fraction actually arriving at the tumor. Once there, the second critical factor is the extent of vascular permeability. So what is the size limit for tumor extravasation? There is no definitive answer to this question. The gaps between tumor endothelial cells vary from one tumor type to another (and with microenvironment), from one vessel type to another, and from moment to moment as the angiogenic vessel matures. The "gaps" created by angiogenesis are dynamic, ever-closing as a sliding door. They can be much larger (100 nm to 2 μm) than those reported in normal tissues, typically 2−6 nm at the tight junction, and even bigger than fenestrations of the liver sinusoid (∼150−200 nm),[251] but as a vessel matures the gaps continually narrow. Initially nanosized particles >500 nm may extravasate (indeed this has been visualized), but later only the smallest particles will escape. Clearly smaller will be better and constructs in the size range of 5−30 nm should be optimal. High early phase plasma concentration[239] and longer plasma residence time lead to greatest localization,[232,252] hence the popularity of stealth approaches such as PEGylation to prolong circulation of large particles by avoidance of RES clearance, and to increase the size of smaller constructs that would display rapid renal clearance. PEGylation also has disadvantages. Long circulation may increase normal tissue exposure leading to unexpected side effects (e.g., the hand and foot syndrome seen following Doxil administration[216]). The increased diameter can diminish extravasation and tumor penetration. Thus compromises must be made to produce an optimal pharmacokinetic−therapeutic index relationship for each technology. For any new nanomedicines it is essential to benchmark the extent of passive targeting achievable by quantitative measurement of tumor levels over time (not just at one time point) against reference standards such as free drug or other competing nanomedicines. For example, data obtained for an HPMA copolymer-platinate[253] and a PAMAM dendrimer-platinate[254] showed clear differences in kinetics with the dendrimer leaving the tumor much more rapidly.

Although it clearly has potential to aid tumor targeting, many naively claim the EPR effect as a universal gateway. This is clearly not the case. Different tumor types display significant differences in degree of vascularization and vascular permeability. Clinical studies involving nanomedicines have begun to document EPR-mediated tumor localization using gamma camera imaging[184,185] (Figure 6) or HPLC analysis of tumor tissue[255]). The percentage of dose localized to tumor in the clinical setting is modest.[255] It has also been shown that smaller tumors can display higher uptake[184] (Figure 6c), a situation that would be advantageous for localization to micrometastases. This observation correlates with results obtained in many preclinical models. Using a panel of murine and xenograft models it was shown that the tumor localization expressed as % dose/g can fall daily after implantation for some tumors[256] (probably due to the formation of a necrotic central region with increased interstitial pressure and restriction of angiogenesis to the tumor periphery) but not for others. Although the last decades have seen considerable evolution of the models used for preclinical screening of anticancer drugs per se (reviewed in refs 257 and 258), and there has been increasing sophistication of tumor mathematical modeling,[259] there is generally insufficient attention to factors that will be important to aid nanomedicine preclinical−clinical correlation. As noted by Suggit and Bibby "the value of tumor models ultimately depends on their ability to accurately predict clinical response".[257] The importance of validation of the extent of passive targeting exhibited by rodent models used for pharmacological and pharmacokinetic studies cannot be overemphasized.

Even if they arrive within the tumor, nanomedicines are faced with additional obstacles. These include vascular heterogeneity, slow blood flow, the high interstitial pressure within necrotic tumors, and the complex ECM that impedes access to tumor cells distant from the vasculature (reviewed in ref 241). The tumor tissue also contains many different cell types (Figure 4a). Larger nanosized particles display limited tumor penetration evidenced by the fact that liposomes reside close to blood vessels following

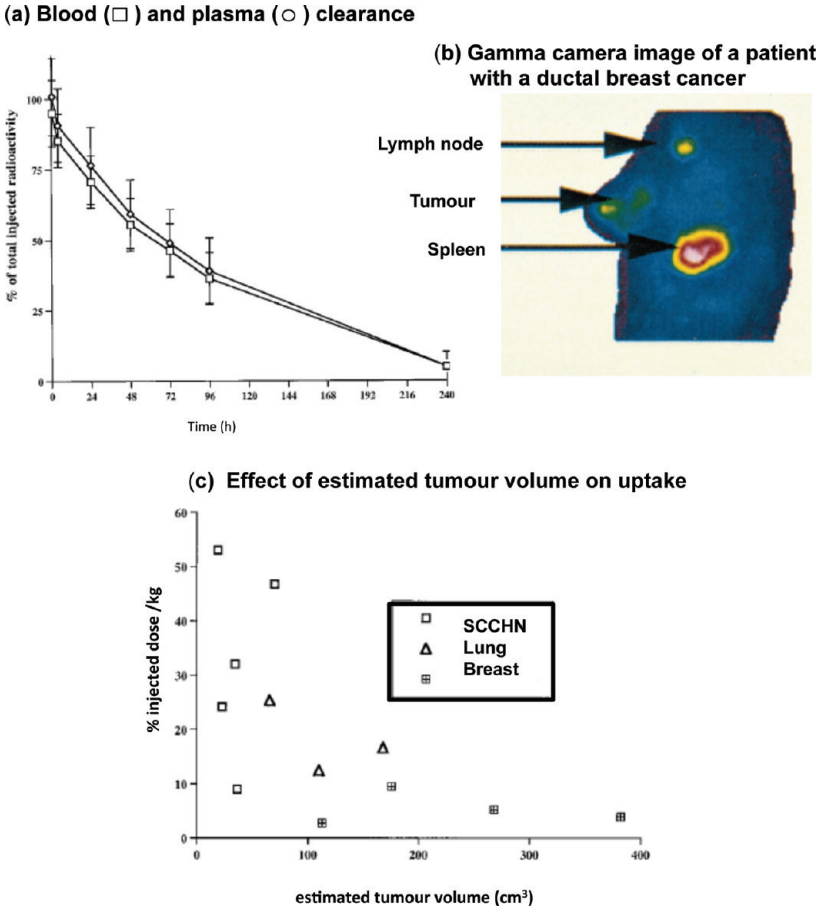

Figure 6. Pharmacokinetics and tumor imaging of ^{111}In-DTPA-labeled PEGylated liposomes: (a) blood and plasma clearance in 17 patients with advanced solid cancers; (b) gamma camera images (72 h) of a patient with ductal breast cancer; (c) effect of estimated tumor volume on liposomal uptake. Adapted and reprinted with permission from ref 184. Copyright 2001 American Association for Cancer Research.

extravasation[246]). Experiments with dendrimers, proteins and polymer−drug conjugates in the same *in vivo* model underline the fact that chemistry and architecture also influence rate of entry/efflux.[254,232] This might be due to extracellular matrix interactions and/or the fact that macromolecules with linear, semiflexible structure diffuse more efficiently in the interstitium than a rigid spherical molecule of similar size.[260] To aid passage through the tumor extracellular matrix (EM) recent studies have successfully used coadministration of enzymes, e.g., type 1 collagenase[261] and hyaluronidase,[262] to reduce tumor interstitial fluid pressure. The latter can also increase tumor uptake of liposomal doxorubicin without altering microvascular pressure. It is interesting to consider how to use this approach in a clinical setting.

Finally, it should be noted that passive targeting due to leaky vasculature could also be used to facilitate localized nanomedicine targeting in other inflammatory diseases, e.g., arthritis and infection.[263,264]

Receptor-Mediated Targeting. It is frequently stated that "targeted delivery will revolutionize cancer treatment" (e.g., ref 265), and almost all cartoons illustrating putative anticancer nanomedicines include "a ligand" for tumor-specific delivery. Rarely are the biological barriers acknowledged (discussed above) which more often than not threaten the practicality of such approaches *in vivo*. Despite many decades of effort and many interesting concepts, clinically receptor-mediated targeting of cancer has had little success. The withdrawal of Mylotarg leaves no targeted drug product currently in the market, although there is anticipation that the antibody−drug conjugates in clinical development may do better. Receptor-mediated targeting is easy to demonstrate *in vitro*, but it is exceptionally difficult to demonstrate *in vivo* and even more so in patients. As mentioned earlier, even antibody-targeted radiotherapy localizes well below 0.01% of the dose administered to the patient's tumor (reviewed in ref 17).

Receptors that have been explored clinically for drug and/or radiotherapy targeting include the epidermal growth factor receptor (EGFR) (e.g., antihuman epidermal growth factor receptor 2 HER2),[117] somatostatin (reviewed in ref 266), and folate receptors (FR) (reviewed in ref 267). Due to their increased density on tumor cells, transferrin receptors have also been popular,[167] and combinatorial phage display techniques have identified peptides with potential to "address" specifically tumor vasculature (reviewed in ref 268). Preliminary clinical imaging of angiogenesis has been undertaken.[269] Targeted nanomedicines face the challenges discussed above for passive targeting, but there are new ones: in transit they must escape the target receptors also present on normal tissues; tumor cells display heterogeneity in receptor expression; there is a tumor "binding site barrier" (interaction with receptors present on cells closest to the vasculature restricts further penetration); and also there is the possibility of receptor saturation with consequent loss

of targeting efficiency. Receptor expression can also vary according to stage of disease and tumor microenvironment. These factors make patient selection for therapy and choice of dose to be administered even more important, e.g., the antibody conjugate Trastuzumab-DM1 is only given to HER2 +ve breast cancer patients.[117] Similarly, as not all cancers express FR, FR-targeted imaging agents are being used to identify those patients most likely to respond to folate-targeted therapies.[267]

Efficient liver-specific (hepatocyte) targeting (this is organ-specific delivery that relies on the bystander effect to increase the probability of efficacy) was demonstrated clinically using HPMA copolymer−doxorubicin conjugates containing additionally galactosamine to target the asialoglycoprotein receptor of hepatocytes and hepatoma.[132] Patient SPECT gamma camera imaging indicated that this conjugate achieved liver targeting of 15−20% dose after 24 h. The majority of radioactivity was associated with normal liver (16.9%, 24 h) with lower accumulation in hepatic tumor (3.2% dose). This is not surprising, as hepatoma cells tend to lose the asialoglycoprotein receptor as the disease progresses. Nevertheless the doxorubicin concentration in hepatoma was estimated to be 12−50-fold higher than could be achieved by administration of free doxorubicin. Specific physiological transport mechanisms may also aid translocation into the tumor by endothelial cell transcytosis (Figure 4c). It has been suggested[211] that the albumin-paclitaxel nanoparticle Abraxane elicits improved tumor targeting due to interaction with the albumin-binding protein SPARC (secreted protein, acidic and rich in cysteine) which promotes gp60 and caveolae-mediated endothelial transcytosis. Preliminary evidence that SPARC expression in head and neck cancer patients correlates with response to therapy supports this theory.[270]

For novel nanomedicines it is essential to verify that targeting does actually occur *in vivo*. Many ligand-directed nanomedicines use improved pharmacological activity, but not evidence of pharmacokinetic targeting, to support the notion of targeting. If receptor interaction is involved, it is essential to document the dose- and time-dependency of targeting to guide protocol design for *in vivo* pharmacological evaluation, and also for first in human studies. Early evaluation of the dose-dependency of HPMA copolymer-doxorubicin-galactose targeting in rats showed that receptor saturation occurred at really low doses when conjugate was given by bolus iv injection[271] (Figure 5b). Rarely are issues such as receptor density or receptor saturation discussed in the context of a phase I clinical protocol design. Typically a protocol is used to assess the maximum tolerated dose (MTD) with respect to the chemotherapy delivered, rather than to achieve optimal the receptor occupancy required for targeting without saturation. The merits of concurrent patient imaging in early clinical trials is evident.

Using validated tumor models early, quantitative pharmacokinetic/biodistribution studies are essential to identify those systems that have the best chance of arriving at tumor tissue and identify accumulation at sites where unacceptable off target toxicity might occur. It is important to benchmark new technologies against each other as well as the lead clinically used (nano)medicine to demonstrate improvement.

Preclinical Anticancer Models and Translational Challenges. Many classical *in vitro* and *in vivo* models used to screen anticancer drugs have limitations for evaluation of nanomedicines because of their very different cellular and whole body pharmacokinetics from the low molecular weight drugs they often carry. For example, *in vitro* cytotoxicity screening methods such as the 60-cell line "NCI COMPARE" analysis[257,258] severely disadvantage a nanomedicine that has been designed for lysosomotropic drug delivery. (This is due to the differences in cellular pharmacokinetics of free drug (rapid entry in minutes) and those systems that must gain entry via endocytosis over much longer times followed by slow intracellular release of bioactive drug.) Although a low molecular weight cytotoxic agent diffuses across the plasma membrane in minutes to access an intracellular pharmacological target, the nanomedicine will first be slowly internalized by endocytosis, the bioactive drug will be released slowly in the lysosome (this process can be ongoing for hours to days), and only then will it be able to access the cytosol. Moreover, *in vitro* cytotoxicity testing is complicated as all nanomedicines inevitably contain (albeit at a low level) free drug, and also many release drug into the incubation medium during the incubation period. This results in multiple factors (plus any toxicity of the carrier) contributing to the measured cytotoxicity outcome. Often the least stable or most contaminated products fare best in such *in vitro* assays even though they are poor candidates for *in vivo* evaluation. As discussed, *in vivo* tumor models used to evaluate pharmacological activity must be validated in terms of the rate limiting steps relevant to nanomedicine activity, e.g., vascular permeability, changes in vascular permeability with time after implantation, endocytosis rates, presence of activating enzymes, etc. McNeil and colleagues of the NCL have recently discussed many of the key issues relating to *in vivo* study design, the need for validated analytical methods able to determine stability *in vivo*, and also methodology for determination of the first in human dose to be used.[153]

Nanomedicine Biomarkers. Even though there has been global effort to improve preclinical anticancer drug screening, this has still not translated into an increased success rate. Only 5% of drugs that enter clinical trial progress to marketing approval.[272] There is a universally agreed need to indentify key indicators (biomarkers) for efficacy and safety, although the potential of such biomarkers to accelerate medicines development is widely debated.[273,274] Nevertheless there is a distinct lack of appreciation of *nanomedicine-specific biomarkers*. As for all medicines, it is important to know if a patient has the pharmacological target, markers for toxicity, markers indicative of a likelihood of acquired resistance and/or drug−drug interactions. However, due to their particular pharmacokinetics, required conditions for drug release, etc., there are additional biomarkers that would enhance the probability of acceptable efficacy/safety of nanomedicines. Examples of potential anticancer nanomedicine biomarkers are listed in Table 3. In addition to the indicators relating to active and/or passive targeting,[275] it is important to carefully consider potential tumor-specific abnormalities in endocytic internalization or trafficking pathways,[276] levels of the activating enzymes (e.g., ref 277) or microenvironment (e.g., ref 278) as these issues can be required for appropriate triggering of therapeutic activity.

It is noted that nanomedicine biomarkers should also be carefully considered with respect to each technology and the specific therapeutic indication proposed.

■ THE FUTURE: NANOMEDICINES OF TOMORROW?

Innovative nanoscience in concert with increased knowledge arising from genomics and proteomics research brings exciting novel opportunities for nanomedicine development. There is a real chance to harness modern molecular medicines (too many

Table 3. Potential patient biomarkers for nanomedicines. Combining gene profiling and functional imaging to improve patient selection for therapy

potential markers	patients to include (√) exclude (×)	importance	ref
	General		
presence of pharmacological target biomarkers (clusters)	√	as for all drugs	273, 274
absence of resistance markers	√	as for all drugs	511
markers showing probability of normal tissue toxicity	×	as for all drugs	512; 513[a]
	Nanomedicine-Specific		
evidence of tumor EPR-mediated tumor targeting	√	all nanomedicines claiming to target tumors by EPR	*b*
evidence of normal tissue exposure due to enhanced vascular permeability—likely to cause normal tissue toxicity	×	all long circulating nanomedicines	*b*
presence of receptors for receptor-mediated targeting	√	all nanomedicines designed for receptor-mediated targeting	*b*
evidence of transport mechanisms, e.g., SPARC	√	Abraxane predictor of activity?	270
presence of activating enzyme, e.g., cathepsin B/blood estradiol surrogate	√	Opaxio predictor of activity?	50, 51
activating conditions, enzymatic pH, reducing environment	√	all nanomedicines claiming these conditions for drug release	277, 424[c]
functioning endocytosis/trafficking	√	all nanomedicines requiring endocytic uptake	276
early evidence of response, e.g., functional PET	√/×	all antitumor nanomedicines	e.g., 439
potential normal tissue toxicity due to nanomedicine pharmacokinetics in a clinical setting	√/×	e.g., unwanted targeting of a potent anticancer agent due to increased vascular permeability at a site of infection, inflammation, brain metastases	*b*

[a] Example, renal toxicity. [b] Patient imaging required. Potential probes are discussed throughout the text. [c] Discussed throughout the text.

new nanomedicines still use very "old" drugs as the bioactive) and capitalize on nanomedicine-relevant biomarkers to improve patient therapy. If, and only if, we can capitalize on the background so far discussed, it will be possible accelerate transfer from lab to patient. Too many publications still begin with a phrase "widely used for biomedical applications" whereas in most cases the system has not even entered clinical trial (or been tested *in vivo*), and even some first generation technologies that are in clinical development are not yet products approved for routine human use. Investigators need to understand and articulate the differences between a lab experiment and a medicine if society is to reap the benefits. Here emerging opportunities are critically reviewed discussing unique features and challenges for translation. Emerging insights regarding structure−activity relationships and opportunities for improved design are also briefly discussed.

Emerging Materials. Many nanotechnologies have been proposed for use as nanomedicines, and Figure 7 shows some of the most interesting structures. There is a need to identify a *specific application* where a unique added advantage and appropriate safety profile can be demonstrated. Additionally, each technology must also be amenable to reproducible, large-scale manufacture and validated characterization of all components (in the presence of the others) (the trick is to "keep it simple") and must be cost-effective.

Fullerenes, Carbon Nanotubes, and Nanohorns. It is 25 years since the discovery of fullerenes (reviewed in ref 279) for which Kroto, Curl, and Smalley were awarded the chemistry Nobel Prize in 1996. Since then, the C_{60} "buckminsterfullerene" and other fullerenes have been proposed as bioactive agents (e.g., anti-HIV protease inhibitors and quenchers of reactive oxygen species (ROS) for treatment of Alzheimer's disease), for drug delivery, as imaging agents, and as radioprotectants (reviewed in refs 280−283). There is much debate however regarding potential toxicity (e.g., refs 284 and 285), a discussion complicated by the wide variety of fullerene structures and surface modifications.[280] Functionalized amphiphilic fullerenes can also form spherical vesicles called "buckysomes" (100−150 nm)[286] (Figure 7a) and are proposed as hybrid materials with dendrimers. The fullerenol toxicity toward renal proximal tubule cells was recently reported *in vitro*,[287] important to note for those fullerenes cleared renally. Carbon nanotubes are byproducts of fullerenes created by direct current arc discharge.[288] Their unique geometry and electrochemical, thermal, and spectroscopic properties have resulted in proposed use as drug carriers, as imaging agents, for gene delivery, and as hybrid theranostics. Again their toxicity has been widely discussed,[289] not least because their physical form draws comparison with carcinogenic asbestos fibers. Some have optimistically championed biomedical use, but it is not clear whether risk−benefit will ever justify clinical development for many proposed applications. The need to avoid hype with respect to carbon nanotubes and avoid "unrealistic expectations that may prove to be counter-productive to the development of the field overall" has been wisely noted.[290]

Although individual carbon nanotubes have a diameter in the small nano range (typically ∼4 nm), structure can vary significantly. Both single walled carbon nanotubes (SWCNT) (Figure 7b) and multiwalled carbon nanotubes (MWCNT) have been described with length from nm to μm. They can be dispersed single nanotubes or aggregates of nanotube bundles. To enhance dispersion in physiologically relevant solutions the surface is often modified by noncovalent adsorption (e.g., PEG-lipids) or covalent functionalization (e.g., carboxylation). Studies on the whole body and cellular pharmacokinetics of carbon nanotubes revealed several interesting phenomena. Some suggest that carbon nanotubes can "pierce" the cell membrane and translocate directly into the cytosol.[291] If this were proven with efficient

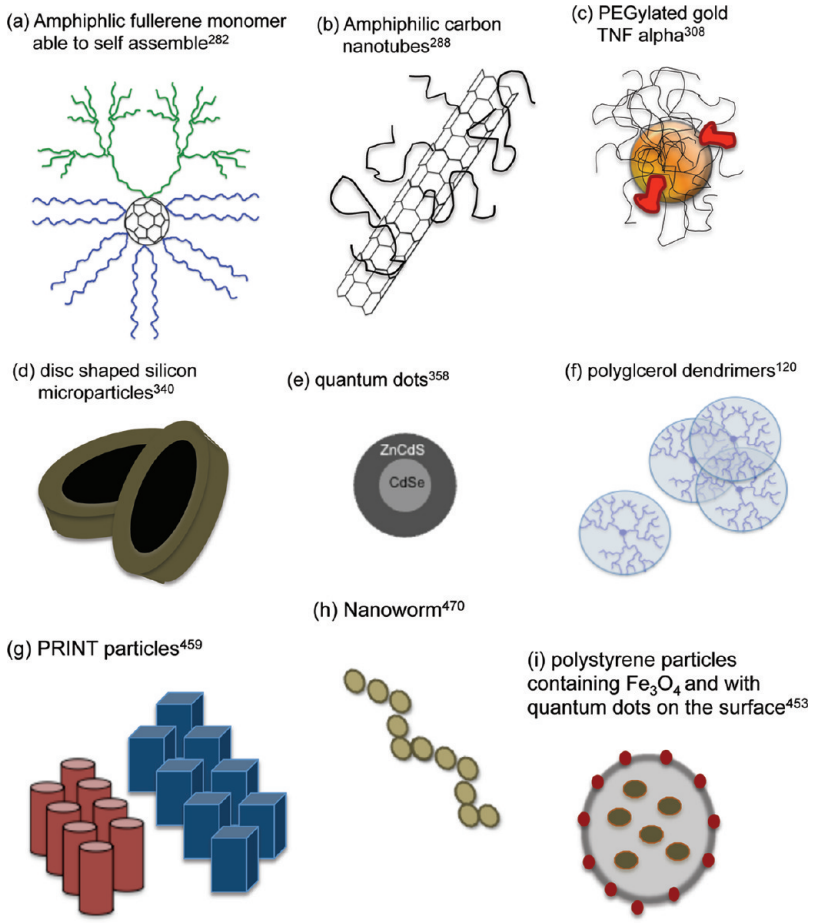

Figure 7. Emerging nanomaterials. The next nanomedicines?

uptake, it could bring a new option for intracellular drug delivery. MWCNTs have also been proposed as a means of electropermeabilization of cells[292] or, when placed at the tip of a glass pipet and filled with magnetic nanoparticles (to enable remote maneuvering), as a "cellular endoscope" to interrogate the intracellular environment and gain access to organelles without disrupting the cell.[293] In contrast, SWCNTs absorbed via the GI seem to be lysosomotropic (although they also appear to enter mitochondria at higher doses) leading to the conclusion that uptake occurs by endocytosis.[283] This is consistent with theoretical calculations that found the energy requirement for nanotube insertion into a phospholipid bilayer so high[294] that uptake by an energy-dependent mechanism like endocytosis was thought most likely. A review of uptake mechanisms concluded that the physicochemical characteristics of carbon nanotubes (e.g., aggregation, length, and functionalization) govern their cell uptake mechanism.[295] Phagocytosis predominates for larger aggregates and single dispersed nanotubes (>1 μm in length), endocytosis for nanotubes forming supramolecular structures <1 μm, with diffusion for the smaller, well-dispersed nanotubes. There is need for quantitation of time-dependent uptake and subcellular distribution studies with carbon nanotubes in different cell types to verify the universality of these conclusions.

Early studies reported rapid renal clearance of iv injected SWCNT (e.g., ref 296). Although this might seem counterintuitive given their length, the high aspect ratio would allow passage of the narrow end through glomerular pores. Renal tubule reabsorption might again be a toxicological concern (cf. fullerenes). Others have reported RES clearance of pristine carbon nanotubes and materials modified with surfactant.[290,296] Biodistribution of PEGylated SWCNT ± RDG (~1−5 nm diameter, length ~100−300 nm) after iv injection was quantified over 3 months using the intrinsic Raman signal[297] or radiolabeled PEG-DOTA-^{64}Cu probes for PET analysis.[298] Liver uptake was seen (diminished by PEGylation)[297,298] followed by clearance within 2 months. Renal and hepatobiliary elimination were deemed responsible, but, as neutrophil myeloperoxidase catalyzes SWCNT degradation,[299] perhaps a degradation pathway also plays a role. The SWCNT modified with PEG_{5400} and RGD showed higher tumor uptake (10−15% injected dose/g) than those without the targeting ligand (3−4% injected dose/g)[298] thus giving potential for tumor targeting.

A variety of carbon based architectures and hybrid structures (e.g., "carbon nanohorns" derived from SWCNTs tubes of diameter between 2 and 5 nm aggregated to give a spherical form that resembles a "sea urchin"[300]) have also been described. The unique Raman signature, high optical absorption, and potential for photoacoustic imaging present interesting opportunities for imaging and/or theranostic applications,[290,301] but it remains to be seen if any of these carbon nanotubes can be applied safely in a clinical setting.

Inorganic Nanosized Particles. The unique electronic, optical, and magnetic properties of metals (gold and silver), metal oxides (e.g., iron oxide, silicon dioxide, etc.), and quantum dots (QDs)

(e.g., cadmium sulfide and cadmium selenide) have heightened interest in their use in nanomedicine. Many of these materials raise toxicological concerns,[146] but pre-clinical experience with some suggests that judicious choice of administration route and application may facilitate practical clinical development.

Gold. Techniques for preparation and characterization of gold nanoparticles and nanorods have been reviewed.[302] Since the 1970s monodisperse colloidal gold (5 −150 nm) has become a standard cytochemical tool. Conjugation to proteins and lectins allows receptor localization, and radioactive colloidal gold enables quantitation of endocytosis (e.g., refs 303 and 304). Gold-based reagents are markers for lateral flow immunoassays. In the clinical setting, the doses used and the safety and efficacy of intramuscular (im) administration of gold salts as a treatment for rheumatoid arthritis are well documented.[305−307] Biodistribution of gold particles (10−250 nm) in rats (24 h) measured using inductively coupled plasma mass spectrometry (ICP-MS) showed uptake in liver and spleen for all sizes.[308] Hollow gold nanoparticles (sometimes called nanoshells) are particularly interesting as they can be activated by a NIR laser for photoablation of tumor tissue.[309] First pilot clinical studies with AuroLase therapy are ongoing in refractory head and neck cancer patients given a single dose of AuroShell nanoparticles followed by 1 or more laser treatments. The AuroLase therapy approach is a complex system from a Regulatory viewpoint as the treatment involves 3 different components: near-infrared laser source, an interstitial fiber optic probe for laser energy delivery to tumor tissue, and the new "medicine", the AuroShell nanoparticles.

PEGylated gold particles (27 nm) covalently linked to rhTNFα (CYT-6091) are also in early clinical trial as an anticancer agent[310] (Figure 7c). In phase I CYT-6091 was injected iv to patients (given prophylactic antipyretics and H2 blockers) using a dose based on the rhTNFα content. It was found that CYT-6091 could be administered at 3-fold higher dose than free rhTNFα, and tumor biopsies (24 h) showed the presence of gold nanoparticles. This complex, multicomponent nanomedicine was carefully studied preclinically. After injection the peak blood concentrations of gold and gold bound rhTNFα were similar, but the gold had a much slower rate of clearance indicating dissociation and/or metabolism of rhTNFα.[151] As TNFα increases vascular permeability, and TNFα-gold nanoparticles do alter permeability in tumor tissue,[311] there are exciting possibilities to enhance EPR-mediated targeting using this system. Moreover the relatively small size of gold nanoparticles (typically 10−50 nm) is advantageous compared to many new anticancer nanomedicines.

Preclinical studies with gold nanoshells also look promising. After iv injection of gold nanoshells breast cancer stem cells normally more resistant to radiation therapy are sensitized following local hyperthermia.[312] If transferable, this would be extremely important clinically. Gold nanoparticles have been conjugated to tumor-targeting ligands, e.g., melanocyte stimulating hormone (MSH)-targeted PEG stabilized hollow gold nanospheres (~44 nm) that have demonstrated photothermal ablation of melanoma *in vivo* as measured by [^{18}F]fluorodeoxyglucose PET.[313] A combined antihypoxic and vascular disrupting strategy based on a gold nanoshell vascular focused hyperthermia has been reported.[314] Folate receptor-targeted hollow gold nanospheres carrying anti-NF-κB siRNA showed NIR laser−induced siRNA delivery as evidenced by NF-κB downregulation and irinotecan chemosensitization,[315] and bombesin-targeting of gold nanoparticles administered intraperitoneally (ip) are also under investigation.[316] With a different clinical objective in mind, VEGF-nanogold (13 nm) administered by intra-articular injection led to an improvement of collagen-induced arthritis in rats.[317]

Silver. Silver is an established topical antimicrobial agent used to aid wound healing (the pros and cons have been recently reviewed[318]), and it is also under evaluation as a treatment for atopic dermatitis.[319] Recent clinical studies comparing nanocrystalline silver with cadexomer iodine in patients with chronic leg ulcers concluded that nanocrystalline silver led to quicker healing during the first 2 weeks.[320] Although many commercially available silver-containing dressings claim broad-spectrum bactericidal activity, "nanocrystalline silver" was shown to be essential for *Staphylococcus aureus* activity, suggesting that this form is crucial for successful therapy.[321] However, it is clear that silver is generally cytotoxic[322] and the window between normal tissue toxicity and bactericidal activity can be narrow. Great care must be taken when developing silver for other applications, e.g., an oral nanocrystalline silver suspension (NPI 32101) has recently been proposed as a treatment for GI tract associated inflammatory diseases, and it was reported to suppress colonic inflammation in *in vivo* ulcerative colitis models.[323]

Iron Oxide Nanoparticles (SPIONS). SPIONs are typically composed of a magnetic particle core (usually magnetite, Fe_3O_4) coated with polymers such as dextran to give a final diameter of 20−150 nm. They are well-established MRI imaging agents (reviewed in Table 1 and refs 44, 324, and 325), and the propensity for phagocytosis gives negative imaging. There is considerable interest in the further development of SPIONs (single agents or components of hybrid materials) due to the potential for external manipulation using magnets, for multimodal imaging, for triggered drug delivery, and for their use in hyperthermia induced tumor ablation. Careful manufacture with optimization of size, surface functionalization, and optimized coatings is needed for each specific application.[326] SPIONs are widely considered as tumor-targeted imaging agents, e.g., urokinase plasminogen activator (uPA)-targeted nanoparticles target breast cancer cells *in vitro* and *in vivo*.[327] A major challenge is RES avoidance.[328] Hybrid systems incorporating SPIONs (typically diameter 5−10 nm) include micelles,[329] liposomes,[330] and gold nanoshells[331] and are being proposed to improve biodistribution, but these complex multicomponent systems have other challenges, e.g., reproducible manufacture, characterization, and safety. Nevertheless the rewards of success would be great.

Use of magnetic fields to target magnetic particles carrying anticancer drugs (ferrofluids) was proposed 20 years ago. Preliminary phase I studies used magnetic particles carrying adsorbed epirubicin infused iv over 15 min into a vein located contralateral to the tumor. Then a magnetic field was applied to tumor tissue for 45 min.[332] A pharmacokinetic advantage was claimed with targeting in ~50% of patients,[332] but, although viewed as promising, the significance of the study was questioned by Gallo and Hafeli;[333] to quote, "to further advance this drug targeting strategy, a quantitative examination of the mechanisms that control distribution of magnetic particles to tumors and an understanding of how to optimise the associated factors are needed". Such careful experimental design is challenging, but it will be needed if these approaches are to enter routine clinical use.

Jordan and colleagues pioneered the use of iron oxide (Fe_3O_4) nanoparticles (~12 nm) with an aminosilane coating for thermal ablation of tumors.[214,334] Particles are injected directly into

tumor tissue, which is then exposed to an alternating magnetic field with the aim of thermal destruction or sensitization to radiotherapy. A phase II study in brain cancer (glioblastoma multiforme) investigated the effect of magnetic heating of intratumorally administered iron oxide nanoparticles combined with fractionated stereotactic radiotherapy. Only the tumor size on entry correlated with overall survival, but it was concluded that thermotherapy combined with a reduced radiation dose is safe and effective and leads to a longer time to first recurrence compared to conventional therapy in this disease. It was recently announced[335] that this approach has "received European regulatory approval for its Nano-Cancer® therapy", "and that the approved medical devices fulfill all requirements with regard to quality, safety and medical efficacy." Similar to the gold nanoshell Aurolase system this is a complex clinical procedure involving several steps, a device for local injection of the iron oxide (Fe_3O_4) nanoparticles, a device for application of a localized alternating magnetic field, and the iron oxide (Fe_3O_4) nanoparticles themselves as a "novel" therapeutic agent. Another pilot clinical study has explored the use of similar iron oxide nanoparticles and hyperthermia to treat minimally invasive prostate cancer.[336] This kind of localized therapy can be very important for treatment of primary tumors and/or debulking prior to surgical removal of a large tumor; however it should be noted that it could not be used to treat disseminated metastatic disease (e.g., breast, prostate, colon cancers) responsible for most cancer-related mortality.

Although SPIONs are safely used for clinical MRI imaging, it is important to reconsider carefully potential toxicity in each clinical application as the dose used, frequency of dosing and surface functionalization may be very different. Few papers discuss potential toxicity,[337] and this is disappointing as the adverse effects of iron overload and iron induced free radical generation are well documented (e.g., iron-induced neurotoxicity[338]), as is the effect of different coatings.[339]

Silicon-Based Nanoparticles. Silica nanoparticles are well-known as enzyme and antibody supports for immunoassays[340] and assemblies with gold, QDs and fullerenes, etc. for non-nanomedicine applications. The fact that mesoporous silicon can be produced in a variety of sizes and shapes and has a high surface area and tunable pore structure (2−20 nm hexagonal channels or cubic pores) is creating significant interest in its use in nanomedicine (reviewed in ref 341). Although the toxicology of crystalline silica is well-known,[147] there has been little investigation of the toxicological properties of mesoporous (amorphous)/mesocaged silica and surface functionalized silicates (e.g., refs 342−344). A recent single and repeated dose toxicity study involving mesoporous hollow silica nanoparticles injected iv to mice showed accumulation in the liver and spleen and suggested low toxicity.[345] Ferrari and colleagues have proposed a multistage delivery system based on mesoporous silicon. Initially large, disk-shaped microparticles (~3 μm with pores of ~25 nm) (Figure 7d) were used to incorporate QDs and SWCNTs for proof of concept.[342] Then SPIONs[346] and other MRI imaging agents (Magnevist, gadofullerenes, and gadonanotubes)[347] were entrapped, and the colocalization of the silicon and SPIONs in spleen, liver, and lungs was demonstrated after iv injection to mice. One disadvantage of these particles is their very large size, which will restrict their tissue access. When the biodistribution of uncoated silica spherical beads (700 nm to 3 μm) and uncoated nonspherical silicon-based particles with quasi-hemispherical, cylindrical, and disk shapes were compared in tumor bearing mice, differences were seen according to size and shape.[348] It has been suggested that "disc-like", cylindrical and hemispherical silicon particles may outperform spherical particles when it comes to evading uptake by phagocytic cells, flowing through capillaries, and firmly adhering to the walls of blood vessels. As they will not extravasate well, there could be advantages for targeting within the vasculature but not for tumor targeting.

Smaller luminescent porous silicon nanoparticles (LPSiNPs ~126 nm) including an NIR probe to monitor biodistribution and degradation[349] were shown to accumulate in the liver and spleen after iv injection. They disappear within 4 weeks, and this was attributed to degradation to soluble silicic acid. PEGylation is being explored as a means of tuning the degradation rate of silicon.[350,351] Stoddart and colleagues have taken a different approach. Their mesoporous silica nanoparticles have surface-bound rotaxanes (encircled by cucurbit[6]uril or α-cyclodextrin rings) designed for reductive or pH triggered degradation . This chemistry acts as a gateway ("nanostoppers" or "nanovalves") for drug release,[344,351,352]and the field of molecular/supramolecular switches is reviewed in ref 353. Organically modified silica (ORMOSIL) nanoparticles (20−25 nm) conjugated with NIR fluorophores and radiolabeled with [^{124}I]iodide for optical and PET imaging[354] also accumulated in liver and spleen thus diminishing the opportunity for tumor drug delivery or tumor imaging.

For each specific silicon-based system there is a need to determine *in vivo* biodistribution, safety, and efficacy. Both nonporous and porous silica nanoparticles are hemolytic in a size-dependent manner.[355] Although mesoporous silica was less hemolytic, the pore structure was critical in determining hemolysis.[355] Also small SiO_2 particles incubated with human HaCaT cells cause a widespread epigenomic response, e.g., hypoacetylation of methyltransferases and DNA-binding domain proteins[356] indicating that subtle biochemical and toxicogenomic effects of silicon must be carefully considered. Perhaps as mesoporous silica has shown the potential to improve the solubility of poorly soluble drugs, control drug release rates[357] and aid permeation across the GI barrier,[358] oral administration could provide an alternative opportunity for nanomedicine application.

Quantum Dots. Like carbon nanotubes and silicon nanoparticles, the semiconductor nanocrystals known as QDs (1−100 nm) are among the most widely investigated new biomedical nanomaterials. They provide novel imaging labels (many excellent reviews e.g., refs 359 and 360) for tumor imaging[361,362] and for use in theranostics. Typically they are made from cadmium selenide (CdSe) with a surface coating of ZnS or CdS to protect against photo-oxidation and improve the fluorescence quantum yield (Figure 7e) (for full review of the chemistry, see ref 360). A variety of additional surface coatings (e.g., with hydrophobic or electrostatic interaction and/or PEGylation) have been used to minimize aggregation, to introduce targeting ligands, and to improve biodistribution/elimination. Coatings also stabilize against loss of signal in acid pH and salt solutions. Appealing properties of QDs include the ability to fine-tune the fluorescence emission color by varying composition, size, shape, solvent, and stability against photobleaching. However, on−off fluorescence ("blinking") can be a disadvantage for detection.

Most studies have explored QDs as *in vitro* tools (e.g., for cell tracking, immunolabeling, and FRET[359]), but there are aspirations for *in vivo* nanomedicine applications. However, a significant problem is the well-documented toxicity of heavy metals (reviewed in ref 363). Successful use will depend on

identifying the window where the dose used is high enough for signal detection without toxicity. Maysinger and colleagues have contributed significantly to our understanding of intracellular fate and possible long-term cellular impact of QDs,[364,365] and they have shown that size, charge, and surface coating/functionalization influence both subcellular distribution and toxicity.[366]

Relatively few studies have quantified QD biodistribution after systemic administration. CdSe/ZnS mercaptoundecanoic acid-coated QDs, lysine-cross-linked QDs, and bovine serum albumin (BSA)-conjugated QDs were predominantly cleared by the RES after iv injection to rats coupled with some localization to kidneys.[367] In contrast CdSe (ZnCdS) core shell QDs (~5.5 nm) with a zwitterionic cysteine coating predominantly showed kidney elimination.[368] QDs that localize to tumors probably do so by the EPR effect,[361] but coupling GPI (protein to target prostate-specific membrane antigen) and cRGD (cyclic peptide to target the integrin $\alpha v\beta 3$ receptor) to the small zwitterionic QDs mentioned above[369] led to increased tumor targeting as demonstrated by NIR imaging although % dose/g was not given. Rapid renal elimination was also seen (>65% dose by 4 h), together with high levels in the liver and kidneys. It will be essential to find a therapeutic niche where QD uniqueness can be exploited in an appropriate risk−benefit setting.[301,370]

Polymer Conjugates, Micelles and Polymeric Nanoparticles. Polymer therapeutics are among the most successful nanomedicines (Tables 1 and 2 and shown schematically in Figure 2) (reviewed in refs 176, 177, and 206−208). PEGylated proteins, antibodies, and most recently aptamers have been particularly successful, and several PEG-aptamers are undergoing clinical evaluation as treatments for age-related macular degeneration, cancer, diabetic nephropathy, and coronary disease (reviewed in ref 371). Block copolymer micelles (e.g., the PEG-poly(aspartate) block copolymers[38]) incorporating drug by either chemical conjugation or physical entrapment (20−100 nm) are undergoing phase I/II studies as anticancer agents, e.g., micelles containing cisplatin (NC-6004),[372,373] oxaliplatin (NC-4106), and paclitaxel (NK-105)[374,375] (reviewed in ref 376). In a phase II study in patients with advanced gastric cancer after failure of first-line chemotherapy[375] NK105 (150 mg paclitaxel-equiv/m^2 every 3 weeks) there were 2 complete responses and 12 partial responses in 56 evaluable patients with good tolerability.

Although we (R.D.) transferred the first polymer anticancer drug conjugate into clinical trial in 1994[185] and many drug conjugates have followed, this class has been slow to realize a product. Ongoing phase I−III anticancer trials include the HPMA copolymer platinate Prolindac,[377] Opaxio under evaluation for esophageal cancer[250] and ovarian cancer, a biodegradable hydrophilic polyacetal (poly(1-hydroxymethylethylene hydroxymethylformal); 70 kDa, Fleximer)−camptothecin conjugate (XMT-1001),[378] a similar polyacetal-fumagillin (antiangiogenic) conjugate (XMT-1107), and a PEG−irinotecan conjugate (NKTR-102) for which phase III trials are planned in ovarian and breast cancer.[379,380] The most important recent developments in this sector include (i) use of biodegradable polymers, (ii) polymer conjugates carrying combination therapy, e.g., endocrine therapy and chemotherapy[381] (reviewed in refs 187 and 227), and (iii) control of polymeric architecture.

Biodegradable polymers allow utilization of higher molecular weight platforms to optimize pharmacokinetics, and they are essential for treatment of diseases that require chronic administration, e.g., for tissue repair and regenerative medicine.[382−384] Polymers that degrade enzymatically or hydrolytically already in preclinical or clinical development include dextrin,[384] hydroxyethylstarch (HES),[385] polyglutamic acid,[174,383] the polyacetal Fleximer,[378] and polysialic acids. A polysialic acid−erythropoietin conjugate (ErepoXen) is in early clinical trial[386] and an insulin conjugate undergoing preclinical investigation.[387] With the exception of the branched HES, these are all linear synthetic, natural, or pseudosynthetic polymers. Dextrin and HES are approved for clinical use as a peritoneal dialysis solution and plasma expander respectively, so their safety profile and degradation mechanisms are known. However, when used as conjugates the safety of covalent linking chemistry used and/or the heterogeneous degradation products that maybe generated during storage and/or metabolism must also be carefully considered. When a HES conjugate of the iron chelator deferoxamine (HES−DFO) was evaluated clinically in healthy male volunteers (administered by iv infusion over 4 h), it was well tolerated and displayed a prolonged circulation (22−33 h) with increased urinary iron excretion.[388] A phase 1b study in transfusion-dependent patients with β-thalassemia (doses of 150−900 mg/kg) confirmed that HES−DFO at 900 mg/kg produced clinically significant urinary iron excretion. Drug-related adverse events were limited to 4 urticarial reactions, none requiring termination of the infusion.[389]

Dendrimers. The advent of dendrimer chemistry, pioneered by Tomalia in the1980s with the polyamidoamine (PAMAM) dendrimer family,[390] brought advantages of regularly branched polymer molecules of defined architecture, narrow polydispersity, and multiple surface functional groups for further modification (e.g., PEGylation, conjugation of targeting groups, and bioactive/imaging agents and hybrids with other nanomaterials). A vast array of dendrimer chemistries and hybrid dendritic architectures have since been proposed: drugs (e.g., topical microbiocides (VivaGel) evaluated clinically[391]), MRI imaging (e.g., Gadomer-17 preliminary clinical evaluation for vascular imaging[392]), boron neutron capture therapy,[393] for passive[394] and receptor-mediated tumor targeting,[395] and as nonviral vectors for gene therapy.[396] The current status of dendrimer nanomedicines is reviewed in refs 397−400. Despite continuing optimism, transfer of dendrimers into clinical trial has been slow probably due to unacceptable toxicity of some chemistries[120] (and reviewed in refs 121 and 400) and challenges of reproducible manufacture and/or validated characterization. Many studies continue to explore PAMAM dendrimers, but alternatives have emerged that are less toxic and in some cases also biodegradable.[397,401−406] The continued simplification of synthetic procedures (e.g., refs 122 and 407), and systematic study of pharmacokinetics[408] are enabling optimization of dendrimers with greater potential for parenteral use (Figure 7f).

A unique property of dendrimers is the ability to traverse biological barriers. We discovered that anionic PAMAM dendrimers exhibit exceptionally high rates of transcytosis (Figure 4c) in an *in vitro* GI model,[409] observations that were substantiated in other GI models,[410,411] endothelial barrier models,[412] and pulmonary models[413] (ligands have also been used to enhance transport across the lung). If this property can be harnessed, it offers a rare opportunity to promote drug delivery across such barriers. Preliminary studies with a dendrimer−doxorubicin conjugate showed enhanced oral bioavailability.[414] There is however a need for caution. A recent study involving PAMAM dendrimer (cationic G4) discussed a potential role in drug

delivery during pregnancy[415] by measuring human placental transport. While an interesting concept, there is a need for extreme care due to potential teratogenicity in addition to the concerns over toxicity for cationic PAMAM dendrimers to mother and/or fetus. Since the thalidomide tragedy many studies have implicated maternofetal transport and lysosomal function mechanisms in teratogenesis (e.g., ref 416). This led to use of rat embryonic tissues as a screen for factors governing endo/transcytosis of polymers (e.g., ref 417). Potential teratogenicity of novel nanomedicines is rarely discussed, but it is an essential consideration to ensure that future "accidents" do not occur.

Polymeric Nanoparticles. Anticancer polyisohexylcyanoacrylate (PIHCA) nanoparticles carrying doxorubicin were the first in class to enter phase I clinical trials.[131] A similar MTD was found for these nanoparticles compared to free drug, but some infusion reactions were also noted. PIHCA−doxorubicin overcomes multidrug resistance (MDR) *in vitro* and shows increased efficacy against hepatocellular carcinoma *in vivo*,[418] so the technology (called doxorubicin Transdrug) was also evaluated in phase I[419] and II trials[420] (35 mg/m^2 doxorubicin-equiv) in patients with advanced hepatocellular carcinoma using intrahepatic artery (IHA) administration. Although increased survival was reported, the phase II the trial was suspended due to pulmonary adverse events.[420]

Although a vast number of natural and synthetic polymer-based nanoparticles and nanocapsules have been explored (polylactide-*co*-glycolide (PLGA) and chitosan being particularly popular, e.g., for oral vaccine delivery[421]), few have progressed into clinical trial. Self-assembling cyclodextrin−polymer conjugate-based nanoparticles (30−40 nm) pioneered by Davis and colleagues[422,423] have made this transition as a camptothecin-containing nanoparticle (IT-101; reviewed in ref 422) and a transferrin targeted siRNA delivery system (reviewed in ref 423). For the latter, clinical proof of concept was recently reported in a melanoma patient.[167] Another particularly novel approach is the thioketal nanoparticles (TKN; 500−800 nm) carrying an anti-TNFα siRNA.[424] Made from poly(1,4-phenyleneacetone dimethylene thioketal), they degrade in the presence of reactive oxygen species (ROS) associated with GI inflammatory diseases. TKN administered orally to mice caused a reduction of TNFα and TNFα-mRNA levels as well as protection in an ulcerative colitis model.

Finally important to note is the emergence of template manufacturing techniques with the potential to generate monodisperse nanoparticles of specific architecture. The PRINT (particle replication in nonwetting templates) technology of DeSimone and colleagues[48,425,426] is particularly interesting. This top-down manufacturing process employs techniques more commonly used in the electronics industry to prepare nanosized particles of given shape and composition. Although the technique can be applied to a variety of materials including polymers, inorganic materials, and biologics, first clinical applications will probably be achieved using polymeric excipients already approved for clinical use. Some of the unusual shapes that can be created could provide new and interesting opportunities for control of the kinetics of drug release and also for targeting. If such methods are cost-effective, this could bring a new paradigm in process-manufacturing for the pharmaceutical industry. However, it will be interesting to see how the reproducibility of their size and complex geometry can be validated when working on the industrial scale as subtle differences may impact biological performance. The use of PRINT to create nanosized hydrogels of different architecture has enabled interesting studies on the effect of shape and deformability on pharmacokinetics and also transferrin receptor-mediated targeting[427] (Figure 7g) (discussed below).

Liposomes and Lipidic and Albumin Nanoparticles. Liposomes, lipidic and protein nanoparticles (Figure 2) became popular as their "natural" composition was seen as potentially less toxic. Liposomes (in their diverse forms, lipidic, vesicular, PEGylated, etc.) are also among the most successful nanomedicines with an expanding pipeline of products in development[428] (Tables 1 and 2). Approval of Abraxane (130 nm) as a treatment for metastatic breast cancer[270] prompted further trials in lung, pancreatic, and gastric cancers[429] and in combination with other agents such as Trastuzumab.[430,431] Potential of Abraxane in patients with advanced solid tumors and hepatic dysfunction is also under clinical evaluation. Although solid lipid nanoparticles (SLNs) have been widely explored for parental, topical, ophthalmology, and oral applications (reviewed in refs 432 and 433), transfer to medical application has been slow with the first SLN product recently introduced to market (Poland) as a topical moisturizer.[433] Exploitation of SLN interaction with apolipoprotein E to target the BBB endothelium is reviewed in refs 434 and 435.

Recent innovation includes (i) liposomes delivering combination chemotherapy, in a single vesicle;[436] (ii) liposomal composition designed for triggered release; (iii) new therapeutic indications, e.g., VGEF gene therapy using cationic liposomes administered locally as an alternative to drug eluting stents to treat coronary disease;[437,438] and (iv) development of innovative probes to aid mechanistic studies. An integrin-specific PET tracer [^{18}F]FPPRGD$_2$ synthesized from 4-nitrophenyl-2-[^{18}F]fluoropropionate conjugated to PEG$_3$-E[c(RGDyK)]$_2$ (investigational new drug 104150 in early clinical trial) has been evaluated as a probe to monitor Abraxane-induced changes in $\alpha v\beta_3$ integrin expression.[439] *In vivo* it detects an early response that precedes a decrease in tumor size and thus has potential as a clinical biomarker of Abraxane activity.

The transfer of liposomes containing two anticancer agents (Figure 8a) as a combination therapy into phase I/II trials is a landmark advance.[440,441] CPX-351 a liposome containing cytarabine and daunorubicin (ratio 5:1) has shown promising results in phase II trials in acute myeloid leukemia (AML) patients when compared to the conventional cytarabine + daunorubicin treatment.[440] CPX-1, a liposome containing irinotecan·HCl and floxuridine (ratio 1:1), is in phase II in colorectal cancer patients.[441] The final results are awaited with anticipation. If positive, this approach will set a new benchmark for future anticancer nanomedicines. Another interesting liposomal approach is a heat sensitive liposome containing doxorubicin (ThermoDox; lyso-thermosensitive liposomal doxorubicin, LTLD) undergoing pivotal phase III trials in nonresectable primary liver cancer[442] and a phase I/II study in recurrent chest wall breast cancer.[443] These liposomes are administered iv, and then local hyperthermia (39.5−42 °C) is used to release doxorubicin, producing high tumor concentrations of free drug.

Multicomponent Systems, Imaging Agents, and Theranostics. Already evident are the many hybrid multicomponent systems involving two or more components (Figure 8) even though most are far from clinical evaluation. Examples of technologies and goals include the following: polymer−drug conjugates entrapped within niosomes as a multistage drug delivery system[444,445] (Figure 8c); the mesoporous silicon multistage

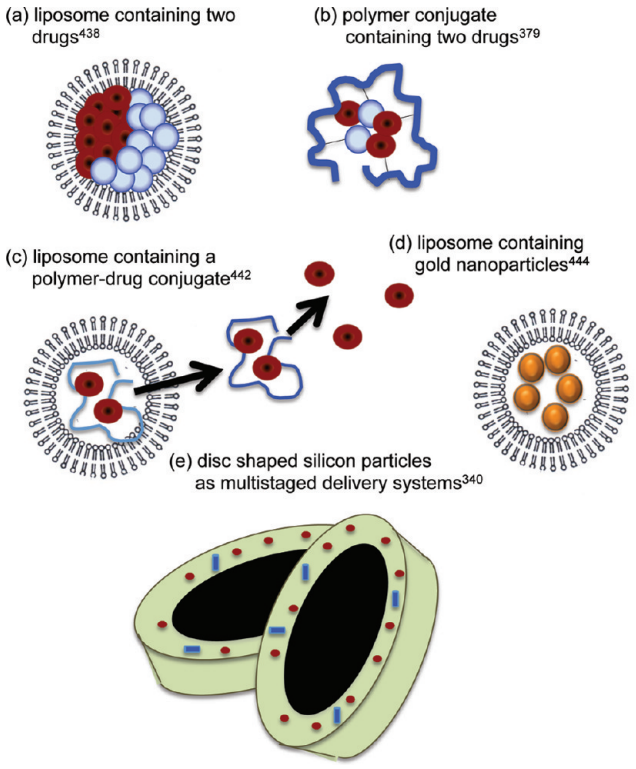

Figure 8. Nanomedicines being developed for delivery of combination therapy and/or as theranostics.

microparticles described above[342] (Figure 8e); gold particles inside liposomes as a tool for externally triggered drug release[446] (Figure 8d), and SPIONS within liposomes to facilitate magnetic targeting of tumors;[447] and dendrimer-based organic/inorganic hybrids.[448] First nanomedicine clinical imaging involved the SPION MRI[449] and dendrimer MRI[392] imaging agents, and gamma camera imaging using liposomes[184] (Figure 6), polymer conjugates,[185,186] and antibodies.[17] Techniques for clinical imaging have advanced dramatically with the introduction of SPECT/CT 3D reconstruction and PET techniques. PET isotopes are now important tools for diagnosis and therapeutic monitoring in neurodegenerative diseases, inflammation, and cancer. However, both the short half-life of PET isotopes (difficulties in probe manufacture and characterization) and scarcity of animal PET imaging facilities slowed take-up of this technique in the nanomedicine field. This is now being remedied, and PET probes have been described for polymers,[190,191] dendrimers,[450] and liposomes.[451] These will undoubtedly aid future preclinical and clinical studies.

A number of sophisticated hybrid technologies are also beginning to emerge, and Weissleder and colleagues have described important nanotechnologies applicable to atherosclerosis[452,453] and cancer.[454] For atherosclerosis magnetofluorescent nanoparticles are given by iv injection. They are taken up by macrophages within the atherosclerotic plaque and fluorescence microscopy used for NIR imaging. Irradiation is then used to kill these inflammatory cells.[453] A second approach uses dextran coated core−shell iron oxide nanoparticles to combine ^{18}F-PET and far-red optical fluorescence-mediated tomography (FMT). The nature of the manufacturing process enables interrogation of up to five molecular targets at the same time, and thus it could be particularly useful for identification of biomarker clusters. Although currently an experimental tool, there is considerable potential for patient use as a local diagnostic tool (e.g., combined with colonoscopy or endoscopy), and for preoperative PET-CT imaging to help delineate the tumor margin during surgery.[454] The field of multifunctional nanosystems for diagnosis and treatment has recently been reviewed,[455] and there is obviously enormous future potential.

Biological Rationale for Design: What's New? To aid nanomedicine optimization many past studies have attempted to correlate size with specific biological phenomena using libraries of well-defined liposomes, polymers, dendrimers, and nanoparticles. Effects of size on endocytosis, whole body pharmacokinetics, body distribution, plasma protein absorption, etc. have been documented, but it is important to emphasize (i) that well-defined materials (many nanomaterials are inherently heterogeneous) must be used for such studies and (ii) the *in vivo*/clinical relevance of the models used.

Although specific nanomaterials have distinct physicochemical properties in a narrow size range (e.g., QDs), *in vivo* biological behavior of any nanomaterial (e.g., the EPR effect discussed above) cannot be ascribed to a particular size descriptor. The *in vivo* setting is just too complex and constantly changing. Organs are composed of many different cell types, including stem cells (*in vitro* tests typically use only 1 or 2 immortalized cell lines), each cell type has unique anatomy and physiology (endocytosis is cell cycle-dependent), the extracellular and intracellular environment is ever changing, and biochemistry will respond to the prevailing conditions. Patients present additional complexity in terms of ethnic background, age, sex, and alterations in pathophysiology induced by disease. All these factors impact on the pharmacokinetics, biodistribution, and metabolism of nanomedicines and thus their performance. Such interpatient variability is rarely discussed in advance, most often being unraveled in response to observation of toxicity, or lack of efficacy, in the clinical setting. Recently, a first study described factors affecting the interpatient variation in the biodistribution of a PEGylated liposome containing a camptothecin analogue (S-CKD602).[456] S-CKD602 had a higher exposure (2.7-fold) in patients >60 years of age compared to those <60 years. Variation was also associated with prior exposure to PEGylated liposomal doxorubicin and saturable clearance.[456] Such observations have important implications for patient selection with respect to all iv nanomedicine therapies. Determination and modeling pharmacokinetics of free and entrapped drug can also further aid patient individualization.[457]

Although nanoparticle size impacts cellular pharmacokinetics (it is cell type-dependent) and whole body pharmacokinetics, it is important to emphasize that "whereas physical and chemical properties of materials may change with size, there is no scientific justification *for a single upper and lower size limit* associated with these changes that can be applied to *adequately define all nanomaterials*".[10] Biological behavior is clearly not "simply a matter of size". Surface area:volume ratio increases significantly as size decreases, a phenomenon that impacts surface reactivity and dissolution rate. Both factors can be important in relation to safety and efficacy (e.g., for gold, silver, and NanoCrystal drug particles respectively). It is well-known that other nanomaterial characteristics such as charge, hydrophobicity, surface roughness, surface modification, deformability, and architecture also impact fate depending on route of administration. Behavior is additionally complicated by protein adsorption/desorption during transit, a factor that can enhance targeting (e.g., apolipoprotein

E to BBB[434]) or promote rapid RES clearance. The pattern and kinetics of protein interaction (Figure 4b) with liposomes[458] and nanoparticles[459] have long been discussed together with its impact on biodistribution (reviewed in ref 460), but interests in nanotoxicology have rekindled research this field.[461,462] It is important to note that, after iv administration, larger particles are more prone to RES clearance, and there is a size threshold (~7 μm, it depends on deformability) for particle (or aggregate) entrapment within the narrowest capillaries in the body, i.e., the lung. This trapping often leads to claims of "lung targeting", but at worst it can cause pulmonary embolism.

New insights into the effect of shape, patterning, and deformability on cellular uptake and biodistribution are however emerging with the advent of sophisticated techniques for particle manufacture that can give monodisperse products of specific geometry and deformability.[425,426] *In vitro* studies on the endocytic properties of monodisperse, micro- and nanosized, cationic cross-linked PEG hydrogels of different shapes made using the PRINT technique led to conclusion that rod shapes with a high aspect ratio (150 nm by 450 nm) were taken up more efficiently by HeLa cells.[463] Similar conclusions were drawn in studies with polystyrene particles of different size and shape where macrophages preferred particles with the longest dimension (in the 2−3 μm range).[464] Both studies draw comparisons between the favored size and shape and the rod shaped bacteria these cells might encounter naturally. Patterning on surfaces can also influence protein adsorption and consequently cellular responses.[465] Although most relevant to cell adhesion in the context of regenerative biomaterials, these observations may also bring important lessons for nanomedicine(s) design. Interplay between size, surface curvature, and ligand presentation to receptors has indicated potential subtleties in terms of resultant cell-induced effects.[466] Using gold and silver nanoparticles (from 2 to 100 nm) linked to Herceptin, it was concluded that these factors impact receptor internalization. Although particles of all sizes altered cell signaling, the 40 and 50 nm nanoparticles had the greatest effect.

The impact of nanomaterial size and shape on vascular flow, adhesion to vessel walls, and biodistribution of drug carriers has also been studied.[348,467−471] Using synthetic microvascular networks *in vitro* to study adhesion of functionalized spheres, elliptical/circular disks, and rods (1 to 20 μm) it was concluded that shape significantly affects adhesion.[467] Furthermore, stable polymer micelle assemblies (filomicelles) prepared from PEG-polyethylethylene or PEG-polycaprolactone (several μm long) remained in the circulation ~10-fold longer than an equivalent spherical particle.[468] Linear assemblies of iron oxide nanoparticles, so-called "nanoworms" (Figure 7h), display prolonged circulation times and improved tumor targeting[472] compared to single particles, and iron oxide particles combined with dendrimers, "dendriworms", have been proposed as improved tools for siRNA delivery.[473] Decuzzi, Ferrari, and colleagues[469−471] have discussed at length the importance of mathematical modeling and shape engineering for the optimization of next generation nanomedicines. Whether or not these new technologies/concepts can be transferred into clinically useful nanomedicines remains to be seen, as there is a need to carefully examine parallel factors such as protein deposition, the CARPA effect, and immunotoxicology, before the real benefits can be assessed. It is also important to note that the vast majority of nanomedicines in routine clinical use are not simple, spherical, smooth surfaced, nanosized particles as is sometimes suggested (Figure 2). Many nanomedicines are deformable (cf. polymer coated particles and the polymer/dendrimer carriers), and they can assume various shapes in physiological solutions depending on pH, salt concentration, counterion, and flow (see writings of P. G. de Gennes for inspiration[474]). The polymer coatings and adsorbed or entrapped drugs and putative targeting ligands ensure that all nanomedicines have a very complex and dynamic surface architecture.

Defining Structure−Activity Relationships: Improved Tools? To enable the definition of accurate structure−activity relationships of such complex multicomponent nanomedicines, it is essential to understand (i) architecture/conformation in physiological solutions (chemical characterization is often undertaken in organic solvent or water) and dynamic changes in different environments; (ii) rates of diffusion in typical extracellular and intracellular solutions (how fast will the system move); and (iii) passage through and/or interaction with complex bioenvironments (Figure 4).

Many of the simplified cartoons drawn in publications (including those shown in Figure 2) to illustrate the authors' vision of their idealized nanostructure are frequently too naive and/or too perfect to give an accurate impression. Often the components are not presented to scale one to another, and the conformation suggested may also be very misleading. Classical examples include the following: PEGylated liposomes where the polymer is only shown externally disposed (it can also be internally disposed); polymer−drug conjugates do not exist in solution as the Ringsdorf "washing line model" implies;[21] in aqueous solutions conjugates form unimolecular micelles with hydrophobic drugs in the interior;[176] dendrimer conformation depends on generation and pendant group chemistry (they are not all perfectly substituted small spherical particles); and polymers conjugated to surfaces (e.g., carbon nanotubes and nanoparticles) may lie on the surface rather than extend outward into solution—it depends on surface and polymer chemistry. Many structures can also dynamically change their conformation in solution depending on local salt concentration, polyelectrolyte counterion, and local pH and/or during degradation of the components. Some of the biological barriers relevant to studies on structure−activity relationships are summarized in Figure 9a.

In recent years Griffiths and colleagues[475−478] and others have pioneered the use of techniques commonly used in colloid chemistry to explore nanomedicine structure, dynamic changes, and also the interaction with complex bioenvironments (mucin, extracellular matrix, and model membranes) (see Figure 9b). In particular contrast matched small-angle neutron scattering (SANS), pulsed-gradient spin−echo NMR (PGSE-NMR), electron paramagnetic resonance, and surface tension measurements have been used. Using SANS and PGSE-NMR it was shown that a linear poly(amidoamine) (PAA) developed as an endosomolytic polymer for cytosolic protein/gene delivery had a Gaussian coil conformation of Rg of ~2 nm at pH 7.4.[475,476] The coil expanded to ~8 nm as pH was decreased. Moreover, when the same techniques were used to map coil-to-globule transition of copolymers based on a thermoresponsive cationic poly(ethyleneimine) (PEI) core grafted with different poly(*N*-isopropylacrylamides) (PNIPAMs), it was shown that changing conformation correlated with nucleic acid binding and transfection efficiency.[477]

The complexity of the bioenvironment is often overlooked when designing a nanomedicine. These examples illustrate the problems. A nanomedicine arriving in the GI tract will be

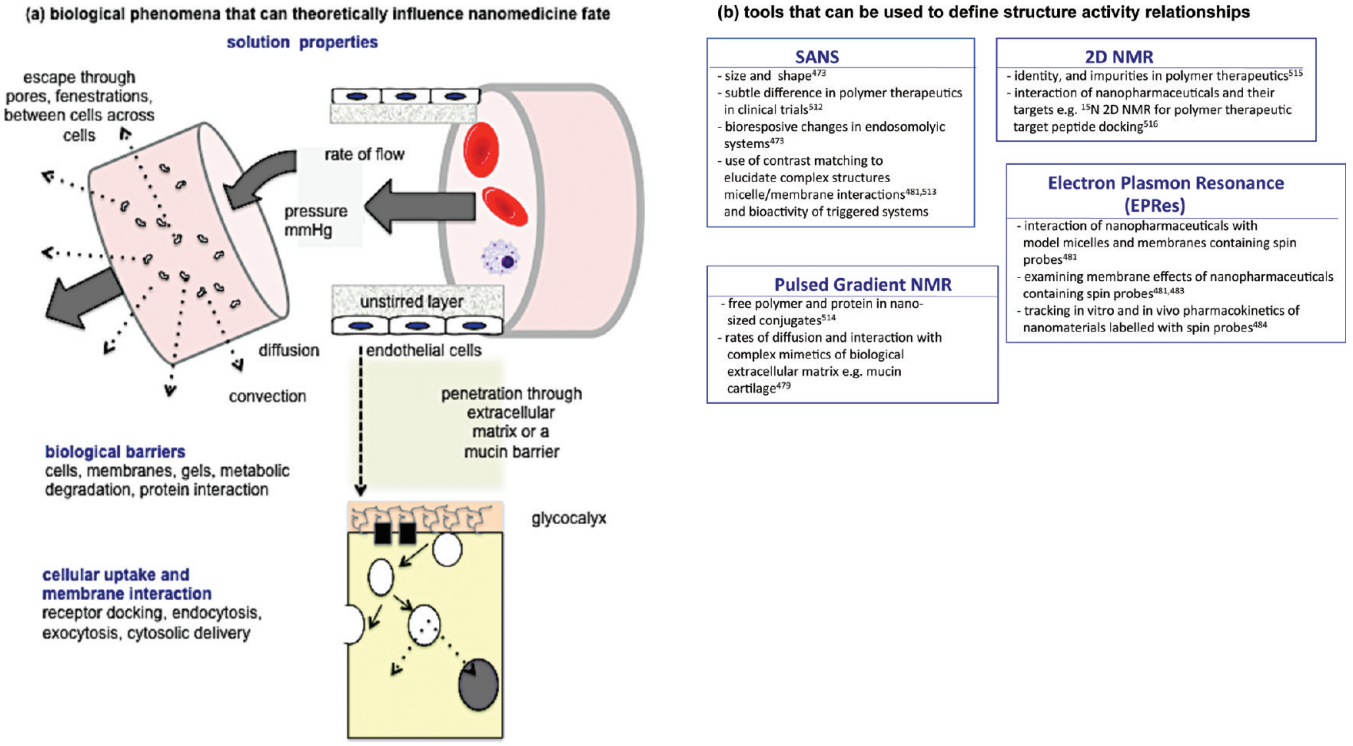

Figure 9. Schematic illustrating (a) some of the physical biological barriers that govern nanomedicine performance and (b) analytical tools that can aid the elucidation of physico-structure−activity relationships. (To note, electron plasmon resonance is usually abbreviated EPR, but to avoid confusion here, as the EPR effect terminology has been used throughout this review to describe passive tumor targeting, the abbreviation EPRes has been used for electron plasmon resonance.)

exposed to the dense mixture of the microbiota (in the colon there are $10^{11}-10^{12}$ bacteria/mL) and digesting food particles as well as the prevailing physicochemical conditions. Before even reaching the intestinal wall (enterocytes are cuboidal cells ~15 μm in height) a nano structure must percolate through the mucin layer (can be ~100 μm thick). This viscous gel is also transiting down the GI tract due to peristalsis. In the same way, when nanosized particles are propelled into the lung, they must navigate ever-narrowing airways before reaching the deep lung. The lung cells are also covered with mucin, and mucociliary clearance can move at ~80 μm/s. It is evident that this "mucin escalator" has the capacity to sweep the particles away before they penetrate the mucin and arrive close to the cell surface. A variety of techniques (reviewed in ref 478) are being used to study the transport of nanosized particles and polymers through the mucosal network including multiple-particle tracking, fluorescence recovery after photobleaching (FRAP), and also PGSE-NMR. There are many different human mucins, including cervicovaginal mucus (CVM) and cystic fibrosis sputum, and the pore size is typically in the range 200−500 nm. Diffusion of nanoparticle standards, naked or surface modified, in GI mucin[479] and cervicovaginal mucus (CVM)[480] was measured by real-time multiple particle tracking using fluorescent, carboxylated polystyrene particles (100 nm to 1 μm) that were also PEGylated. Pore size in CVM was estimated to have a diameter ~340 nm. Interestingly, in the GI model it was surprisingly found that nanoparticles (200 and 500 nm) larger than accepted pore size of human mucin (10−200 nm) could penetrate mucus if carefully PEGylated. The diffusion coefficient measure was only 4−6-fold lower than seen in water. PGSE-NMR is a new method for studying mucin and ECM penetration.[478] This technique observed lack of mucin interaction for linear and star-branched PEGs and dextrin although there was moderate decrease in their diffusion rate compared to buffer.[481] Conversely, cationic PAMAM dendrimers and hyperbranched PEI showed pH-dependent mucin interaction with a significant decrease in diffusion rate. More widespread use of such techniques would be helpful to get a more realistic insight into the likely performance of novel nanomedicines in a complex biological setting.

Design of effective nonviral nanosized vectors for reproducible and efficient cytosolic delivery of macromolecular drugs and genes remains an unmet challenge. At the intracellular level the rate limited barrier is the endosomal membrane. First studies have been reported using SANS, surface tension measurements, and electron paramagnetic resonance to study the interaction of PAAs with model micelles and liposomes prepared from lipid compositions chosen to mimic the plasma and endosomal and lysosomal membrane.[482,483] The intravacuolar pH in the target cells is a key determinant of endosomolytic efficiency of pH responsive vectors, but there have been few attempts to measure this and/or the impact of the vehicle itself on the pH environment.[484]

Recent studies have developed stable[485] and pH-responsive[486] self-assembling block copolymer micelles (40−50 nm) containing 2,2,6,6-tetramethylpiperidinyloxy (TEMPO) derivatives with an intense electron paramagnetic resonance signal as nanoprobes for the *in vivo* imaging of pH in the range 5.6−7.4. In the future it would be most helpful if such reagents could be used to image pH *in vivo* in diseased tissues and/or at the subcellular level following their endocytosis. This approach could theoretically also enable time-dependent monitoring of changes following therapeutic intervention. If successful, such probes

enable validation of novel pH biomarkers useful for improved selection of patients with the best possibility to respond to pH-activated nanomedicine therapy.

TRANSLATING NANOMEDICINES TO PRACTICE

So how will we ensure that nanomedicines actually do realize improved healthcare in the 21st century when the challenges converge from so many different directions?

- The exuberant push from the basic researcher.
- The often conservative view of the industrial sector tempered by the need to be commercially viable, and the bruises of past failures.
- The pull of society demanding safer and more efficacious new therapeutics to prolong and/or increase quality of life.
- And central to the argument, the Regulatory Agencies established to ensure acceptable risk-benefit.

Whereas nanomedicinista often accuse the industry of lack of support, the recent "Roadmaps in nanomedicine towards 2020" (a Joint EC and European Technology Platform Nanomedicine Expert Report[487]) observed that "... it has been increasingly clear to the industrial sector that an academic driven or 'laissez-faire' approach to Nanomedicine will be an inefficient process". The need to build bridges linking research and development is clear, and efforts are being made to accomplish this.[488] There is an agreed need to improve the business model enabling the Pharmaceutical sector to develop more new drugs. The number of new agents approved annually as new medicines is similar to that seen 60 years ago.[75] Failure in clinical development is the major problem, with an attrition rate in the range 86−92%,[489−491] with the late regulatory barrier accounting for less than 10% of the failures. The need for early awareness of the regulatory process is evident, and the Academic, Industrial, and Regulatory Agency partnership *must be* forged from the outset (Figure 1). A growing number of academics have working experience across these sectors, however ignorance of the rigorous process of industrial development is still widely evident.

Recommendations to nanomedicinista include (i) the need for all scientific statements to be evidenced-based, with the evidence generated using robust, validated methodology, and (ii) if the goal is a new nanomedicine, do not be afraid to learn about industrial development, clinical development, and the regulatory challenges that lie ahead at the very earliest. It can be fun and will certainly help to realize the ambition of a new therapy. Recommendations to Industry, Regulatory Agencies, and Government Committees include (i) to ensure that all advice received is evidenced-based and that scientific "experts" actually have accrued technical excellence in disciplines they profess to represent, remembering the "10-year rule",[73] and (ii) to be proactive during succession planning to ensure staff are recruited to core posts that are trained in the leading edge technical fields.

New Nanomedicine Regulation? For more than half a century Health and Medicines Regulatory Agencies across the globe have evolved legally binding procedures to ensure society has access to safe and effective medicines and devices. Although different countries and territories have specific legislative frames, there has been a significant effort to evolve procedures that are common to all via the guidelines of the International Conference on Harmonization (ICH). ICH brings together Regulators and innovative industry from the US, Europe, and Japan.[492] Medicines is a global business underpinned by manufacturing and patient use worldwide. The historical context of medicines regulation (reviewed in ref 493) and the current debate regarding the need for "nanomedicine" regulation[4,111,494,495] has been recently reviewed. Obviously the first generation nanomedicines successfully completed the journey from lab to clinic using the Regulatory procedures already in place. As for any new class of medicine, the requirements for progress into first in human clinical trial and subsequently for market approval are reviewed on a case by case basis. Regulators are currently facing the following questions:

(i) Due to the age of first generation technologies (including some liposomal and polymer therapeutics products) Regulators are defining the mechanisms needed to ensure safe introduction of follow-on (could be termed "similar" or "generic") products. Given that the manufacturers of these products do not have access to confidential information regarding the introduction of the original innovator product, and they will be using a new manufacturing site (and probably a different manufacturing process), it will be essential to ensure identical risk−benefit as the follow-on products emerge.

(ii) There is a need for Regulators to keep abreast of the fast evolving state of the art such that they can advise companies developing complex multicomponent second generation nanomedicines (see The Future: Nanomedicines of Tomorrow? for examples) regarding requirements for authorization of first in human studies and, later, the likely requirements for final product approval.

(iii) Proactive monitoring of Regulatory Policy to ensure absence of "gaps" as new technologies emerge at the borderlines, e.g., between medicines and device regulation.[496] Many emerging approaches could be viewed as a "device" a "medicine" and/or an imaging "agent". Which Regulatory path to follow? Is there a need for new guidance?

Moreover with the increasing use of polymers (natural, synthetic, dendritic) as components of nanomedicines there is a need to increase polymer chemistry expertise in general within Regulatory review panels.

So "is nanotechnology too broad to practice?"[497] The answer is certainly no. Although nanoscience, nanotechnology, and nanomedicine are immensely broad fields, and nanomaterials come in many different forms, the key to success is, on one side, having a broad field of vision, while on the other having the discipline to interrogate one specific scientific question/nanomedicine at any time. Superficial, poorly defined terminology is dangerous in any regulatory discussion. Note, as an example, the need to avoid using the term "nanoparticle" to describe well-established technology classes (e.g., liposomes and polymer therapeutics) that have been in clinical use for a long time. The "considerations when submitting nanotherapeutics to FDA/CDER for regulatory review" have very recently been discussed.[498]

Embracing Modern Tools for Development. Arguably the weakest link in preclinical experimentation is the continued failure to document dynamic processes (over time) using complex biosystems as models, i.e., a systems biology approach.[499] Deeper understanding is needed with regard to both the temporal and spatial changes in intra- and extracellular compartmentation, with additionally an appreciation of the detailed biochemical regulation mechanisms of, e.g., drug release kinetics with location and time. Quantitative techniques are needed.

To optimize first in human clinical trial protocols used for pharmacokinetic or efficacy studies, there is a need to carefully consider the pharmacokinetics of the nanomedicine. This will almost always be very different from a classical low molecular weight drug and probably also a biotech drug. Potential for issues relating to nanomedicine behavior (safety, efficacy) that could be due to age-specific (e.g., pediatric and geriatric patients) and/or disease-specific changes in biological barriers or biochemistry/metabolism should be considered. Where possible nanomedicines should be assessed using the most advanced drug development techniques available, e.g., the microdose approach that uses a very low dose of the candidate drug (<100 μg) to minimize patient risk might be useful to define nanomedicine pharmacokinetics.[500] PET, MRI, or gamma camera could also be helpful in early clinical testing to monitor biodistribution or response to therapy to give an early insight into PK−PD relationships in humans. The importance of nanomedicine-specific biomarkers to aid patient selection for therapy was discussed earlier.

Issues relating to characterization,[501] ADME studies needed for nanomedicines,[502] and Regulatory authority requirements for approval of new radiopharmaceuticals[503] have also been recently discussed. Overall, it is important to remember that "quality by design" should be applied throughout. This requires appreciation of the relationship between "the critical quality attributes (CQAs)" of a product, "the critical process parameters (CPPs)", and the clinical properties in terms of safety and efficacy.[5]

The Art of Communication. Finally, it is important to underline the need for well-balanced description of nanomedicines to members of the public, politicians and Governmental agencies—avoiding hype. This only strengthens, not weakens, the opportunities for transfer toward clinical use. There are continuing discussions regarding ethics and nanomedicines,[504] and it has been rightly noted that "one problem is that the gap between research and rhetoric makes nanotechnologies vulnerable to exaggerated claims, both about potential benefits and potential harms".[505] In the end the most important thing is that patients are given information necessary to assess therapy options and make informed consent, for example, "Most neuro-oncology patients trust their physicians to make the best decisions for them, but that does not mean they would accept subtle forms of deception. Patients prefer to have all the information necessary in order to make their own decision."[506]

CONCLUSIONS

Placing nanomedicine(s) under the microscope reveals a rich, complex, dynamic, and lively environment that is rapidly evolving moment by moment. Harnessing the undoubted potential will make a major contribution to improved healthcare in the 21st century. The key to success is true interdisciplinary collaboration based on leading edge *technical expertise* in each core discipline with equal participation of all players, an open mind, and most importantly a team ethic.

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