Pharm Res (2016) 33:2373–2387
DOI 10.1007/s11095-016-1958-5

EXPERT REVIEW

Nanoparticle-Based Medicines: A Review of FDA-Approved Materials and Clinical Trials to Date

Daniel Bobo[1,2,3,4] • Kye J. Robinson[4,5] • Jiaul Islam[2,4,5] • Kristofer J. Thurecht[1,2,4] • Simon R. Corrie[1,2,4,5]

Received: 19 April 2016 / Accepted: 26 May 2016 / Published online: 14 June 2016

ABSTRACT In this review we provide an up to date snapshot of nanomedicines either currently approved by the US FDA, or in the FDA clinical trials process. We define nanomedicines as therapeutic or imaging agents which comprise a nanoparticle in order to control the biodistribution, enhance the efficacy, or otherwise reduce toxicity of a drug or biologic. We identified 51 FDA-approved nanomedicines that met this definition and 77 products in clinical trials, with ~40% of trials listed in clinicaltrials.gov started in 2014 or 2015. While FDA approved materials are heavily weighted to polymeric, liposomal, and nanocrystal formulations, there is a trend towards the development of more complex materials comprising micelles, protein-based NPs, and also the emergence of a variety of inorganic and metallic particles in clinical trials. We then provide an overview of the different material categories represented in our search, highlighting nanomedicines that have either been recently approved, or are already in clinical trials. We conclude with some comments on future perspectives for nanomedicines, which we expect to include more actively-targeted materials, multi-functional materials ("theranostics") and more complicated materials that blur the boundaries of traditional material categories. A key challenge for researchers, industry, and regulators is how to classify new materials and what additional testing (e.g. safety and toxicity) is required before products become available.

KEY WORDS clinical trials · FDA · nanomedicine · nanoparticles · nanopharmaceuticals · nanotherpeutics

✉ Simon R. Corrie
simon.corrie@monash.edu

[1] Centre for Advanced Imaging, University of Queensland, St Lucia, QLD 4072, Australia

[2] Australian Institute for Bioengineering and Nanotechnology, University of Queensland, St Lucia, QLD 4072, Australia

[3] School of Medicine, Faculty of Medicine and Biomedical Sciences, University of Queensland, St Lucia, QLD 4072, Australia

[4] ARC Centre of Excellence in Convergent Bio-Nano Science and Technology, Queensland node, St Lucia 4072, QLD, Australia

[5] Department of Chemical Engineering, Monash University, Clayton, VIC 3800, Australia

ABBREVIATIONS

CHOP	Chemotherapy containing cyclophosmphamide, doxorubicin, vincristine, and prednisolone
CKD	Chronic Kidney Disease
CMC	Critical micelle concentration
cRGDY	Cyclic arginine-glycine-aspartic acid
EPR	Enhanced permeability and retention
IDE	Investigational device exemption
IND	Investigational New Drug
MTAs	Molecularly targeted agents
NABs	Albumin bound nanoparticles
NCL	Nanotechnology Characterization Laboratory
NDA	New Drug Application
NIR	Near-infrared
NP	Nanoparticle
PEG	Poly (ethylene glycol)
PLGA	Polyactide-co-glycolic acid
PPX	Poliglumex
PSMA	Prostate-specific membrane antigen
PTCL	Peripheral T-cell lymphomas

INTRODUCTION

Nanomedicine is an emerging field that combines nanotechnology with pharmaceutical and biomedical sciences, with the goal of developing drugs and imaging agents with higher efficacy and improved safety and toxicological profiles. Due to their sub-micrometer size and high surface area to volume ratio, these materials show key differences in comparison to

bulk materials, including changes in biochemical, magnetic, optical, and electronic properties (1–7). Like traditional drugs, biologics and devices, pre-market authorization is regulated by the FDA, and hence nanomedicines are subject to the usual range of pre-clinical and clinical validation (8). This review provides a snapshot of the range of materials that have been used to develop specific FDA-approved therapeutics, along with a description of key materials that are emerging through the clinical trials pipeline. This is a rapidly evolving field; in just 3 years, the number of clinical trials involving nano-sized components has increased 3-fold, based on a set of search criteria employed in a review published by Etheridge, *et al.*, in 2013 (9).

Interactions of nanomedicines with their biological surroundings (at the level of molecules, cells, organs, etc.) is dependent on a complex interplay between the controllable properties of the particles and the largely uncontrollable properties of the surrounding media. Particle size, shape, and surface chemistry are key factors that determine performance criteria, including the degree of protein adsorption, cellular uptake, biodistribution patterns, and clearance mechanisms (for a detailed review, see Nel, *et al.*, 2009 (10)). Particle size plays a key role in clearance of these materials from the body, with small particles (<10 nm) being cleared *via* the kidneys, and larger particles (>10 nm) being cleared through the liver and the mononuclear-phagocyte system (MPS) (11–14). The desired clearance mechanism can be a factor in the design of the nanomedicine; e.g. selecting small, actively targeted particles that are rapidly cleared if they are not taken up into the target organ after first-pass distribution. This might be an important factor when designing molecular imaging agents, for example, where minimal background signal is desirable. In contrast, it is typically considered favorable for drug-delivery vehicles to circulate for longer times, allowing greater accumulation in the disease site. When nanoparticles are exposed to the biological milieu, the process of non-specific protein adsorption results in the formation of a protein corona around the material. It appears practically impossible to completely avoid the formation of this protein layer (15), however its composition can be altered through the addition of low-fouling polymer coatings on the particle surface (e.g. polyethylene glycol (PEG)). Such coatings lead to the formation of hydration shells at the solid/liquid interface which minimizes the passage of protein molecules, leading to protein repulsion. The main issue associated with protein adsorption is the subsequent denaturing of the protein, leading to a signaling cascade that results in either aggregation of the nanoparticles and/or phagocytosis *via* activated macrophages. This in turn results in non-ideal biodistribution and unpredictable pharmacokinetics of the nanomaterial. While researchers are still developing universal design criteria that lead to predictable nanomedicine behavior *in vivo*, it is clear that size, shape and surface chemistry can all affect the potential for accumulation and/or cell uptake in different tissues.

We define nanomedicines to be drugs or biologics that incorporate nanoparticles (1–100 nm) in order to achieve either improved targeting, reduced toxicity, or otherwise enhanced efficacy of therapeutic or imaging agents *in vivo*. Typically, these are administered orally or intravenously, however examples of transdermal delivery also exist (e.g. Estrasorb™). Most commonly, nanoparticles are conjugated to existing drugs in order to change the pharmacokinetic and/or the pharmacodynamic properties in order to bring about these improvements. In the majority of cases, these nanoparticle/drug conjugates achieve their effects through passive targeting, which relies on non-specific accumulation in diseased tissue (usually tumors). This approach has been used to target solid tumors, since the increased permeability of blood vessels in combination with poor lymphatic drainage or transport (the so-called enhanced permeability and retention (EPR) effect) leads to accumulation of nanomedicines within the tumor microenvironment (16). Targeting specific cells that over-express certain cell-surface receptors can also be achieved by immobilizing ligands (e.g. proteins, antibodies, small molecules) to the surface of the nanoparticle, leading to active targeting which results in accumulation followed by specific uptake of the nanomedicine into the tissue of interest (e.g. tumors). To date only one of the active targeting nanomedicines, Ontak®, has been FDA approved (the exception being the antibody-drug conjugates, which do not contain bespoke nanoparticles and thus are beyond the scope of this review; for a full review see Casi & Neri, 2012 as well as Diamantis & Banerji, 2016 (17,18)).

Currently, the approval process for nanomedicines in humans is regulated by the Food and Drug Administration (FDA) in the USA, and is essentially the same as that for any other regulated drug, device or biologic (for a detailed review, see Eifler & Thaxton, 2011 (8)). The entire process is estimated to take ~10–15 years and approximately $1 billion per new drug. Following the discovery/invention of the material, the pre-clinical phase of testing usually involves animal studies to demonstrate efficacy, safety, toxicity profile, and to identify appropriate dose ranges. For nanomedicines it is becoming increasingly important to have a comprehensive understanding of the physicochemical parameters of the material, and the reproducibility and scalability of the manufacturing process. Publications from the Nanotechnology Characterization Laboratory (NCL; established by the National Cancer Institute) are available to guide this process, and researchers have the option to send material for testing and validation at the NCL according to a series of emerging protocols (19–21). However, significant research is still required to understand and predict how these materials will behave in biological

systems, including the development of new assays and readout methods that are not confounded by the presence of the nanoparticle component. The issue of nanomaterial characterization is at the heart of several guidance documents issued by the FDA in the past several years, hence it is a particularly important aspect of research and development. The efficacy, toxicity, and physicochemical properties can then be compiled into an Investigational New Drug (IND) application for FDA consideration. Upon approval of the IND, human trials can be initiated to determine the safety and efficacy of the new nanomedicine. This process is broken into Phase I (dosing, toxicity, excretion in healthy subjects), Phase II (safety, efficacy in a larger group of patients with the target illness), and Phase III (multi-center, randomized, placebo-controlled) trials, after which a New Drug Application (NDA) can be filed with the FDA requesting approval for marketing purposes. Further "post-marketing" (Phase IV) studies are also often undertaken at the request of the FDA, clinicians, or other groups, based on specific questions raised during the Phase III trials.

In this review, we aim to provide a "snapshot" of the nanomedicines that have been FDA approved, a comparison of the different materials commonly employed, and to highlight some examples of topical nanomedicines that have been granted IND approval for clinical trials. Importantly, our definition of nanomedicines excludes several classes of closely-related drugs and biologics, including those involved in composites for bone regeneration or dental implants, immunotherapies, vaccines, and adjuvants (e.g. Gardasil®, Cervarix® and MF59®), antibody-drug conjugates (e.g. Kadcyla® and Adcetris®) and those observational trials in which endogenous particles are identified as part of a disease process (e.g. NCT02549248) (22). The data we present on clinical trials in Fig. 1 was extracted from the clinicaltrials.gov website in February 2016 using search terms centered around "nano". Compliance with US law (FDAAA 801) requires sponsors to provide entries into this database for any interventional trials conducted that involve FDA-regulated drugs, biologics, or devices, initiated under an IND or investigational device exemption (IDE) after September 27, 2007, or with a completion date after December 26, 2007 (23). It is therefore possible that trials conducted prior to 2007, or outside of the US and FDA regulatory system are not included here; however after cross-checking with the World Health Organization's International Clinical Trials Registry Platform (ICTRP) and previous reviews in this area (8,9,24–26), we confirmed that that the vast majority of trials we identified were present in both resources. A comparison between those nanomedicines currently approved and those in trials provides insights on how this field is changing and what products might be expected in the near future.

Trends in FDA-Approved Nanomedicines and Clinical Trials

Figure 1a/b shows that an average of ~13 nanomedicines have been approved for specific clinical indications per 5-year period since the mid-1990s. This includes approval for both novel materials (51 unique products) along with the use of approved materials for new clinical indications (e.g. Abraxane® has been approved for several different indications). The list is dominated by liposomal and polymeric nanoparticles, which accounted for the majority of nanomedicines approved in the 1990s. Approvals appeared to peak in the 2001–2005 time period, with a subsequent drop leading up to 2008, a trend that may be related to funding limitations associated with the global financial crisis of 2008 (27,28). The clinical trials data in Fig. 1c/d shows some interesting trends in comparison to those for FDA approvals. Firstly, the number of nanomedicines which have been granted IND approval to undertake clinical trials has increased steadily since ~2007. 2014 and 2015 were the best years to date, suggesting that the pipeline for FDA-approved products is healthy. In terms of the material categories under consideration, there are significantly more micellar, metallic and protein-based particles coming through the development process in comparison to what has previously been approved. However, this does not mean that polymeric particles and liposomes are necessarily out of favor. Indeed, most of the micelle and liposome systems being developed incorporate polymers as synthetic building blocks (29), while the protein-based systems may also have a synthetic polymer component.

Key Examples of Nanomedicines by Material Category

Polymeric Nanoparticles

Polymeric nanoparticles are perhaps the simplest form of soft-materials for nanomedicine applications owing to their facile synthesis and wide applicability across all aspects of the field. Polymer utility in enhancing therapeutic and diagnostic advantages over conventional medicines is evidenced by their prevalence in Table I, and continuing investigations in clinical trials. Indeed, two of the top 10 best-selling drugs in the US in 2013 were polymeric drugs (Copaxone® and Neulasta® (30)). Polymer nanomedines usually fall into one of two categories: (a) polymer-drug conjugates for increased drug half-life and bioavailability, and (b) degradable polymer architectures for controlled release applications. However, it should be noted that aspects of polymer chemistry are emerging in nearly all of the categories, because many of the components required (e.g. amphiphilic block copolymers) can be designed and controlled through organic synthesis methods. The polymers themselves include those that are synthetic, pseudo-synthetic or those that arise from natural sources. Their application has spanned the

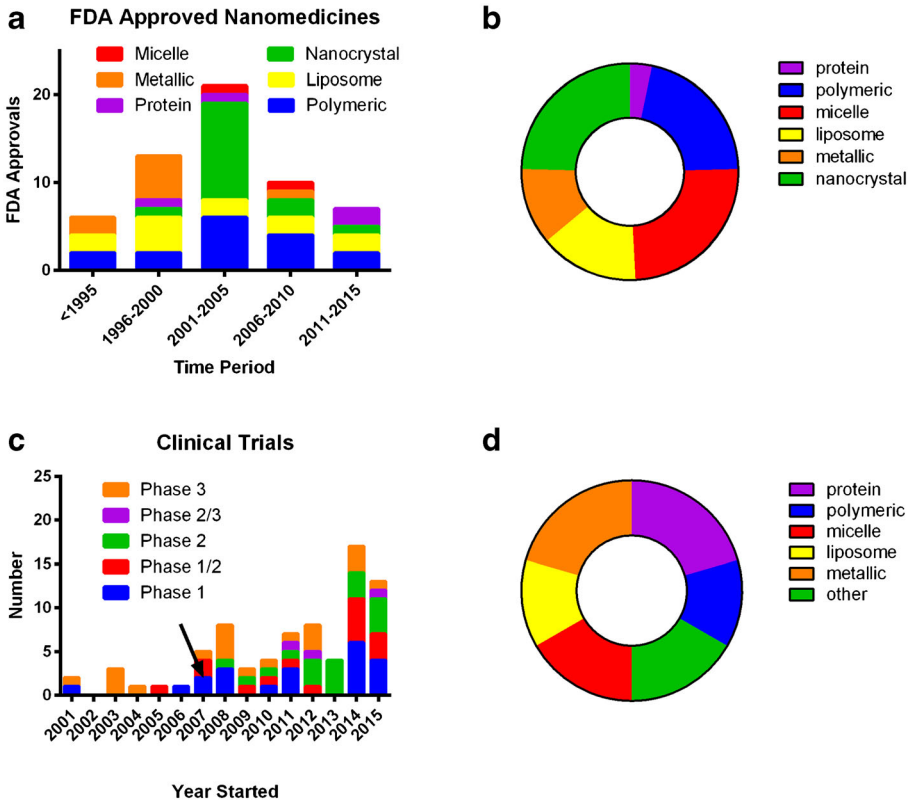

Fig. 1 Trends in the development of nanomedicines. (**a**) FDA-approved nanomedicines stratified by category; (**b**) FDA-approved nanomedicines stratified by category overall; (**c**) clinical trials identified in clinicaltrials.gov from 2001 to 2015 with arrow indicating approximate start date of US law (FDAAA 801) requiring reporting to FDA database; (**d**) nanomedicines under clinical trial investigation stratified by category overall.

full nanomaterial size-scale, from single polymer chains up to large aggregates, depending on the required therapeutic outcome.

The most basic class of polymeric nanomedicines is utilization of single polymer chains either directly as the therapeutic, or as a modifying agent for a drug or diagnostic agent. As direct drugs, there are a number of examples of both FDA-approved products and those under clinical trial. The best example is Copaxone® (glatiramer acetate), which is a random copolymer composed of L-glutamic acid, L-alanine, L-lysine and L-tyrosine (31). Initially approved in 1996, Copaxone® was a revolutionary treatment that acted as an immunomodulator in the treatment of multiple sclerosis. More frequently in terms of polymeric nanomedicines, drugs are attached to a hydrophilic polymer to increase circulation or improve biocompatibility/solubility (32). The most well-established polymer is poly (ethylene glycol) (PEG). Such systems include the extremely popular and successful drug Neulasta® (PEGylated granulocyte colony stimulating factor), which has been FDA-approved since 2002 for chemotherapy-induced neutropenia. In this case, PEGylation resulted in a significant increase in biological half-life in plasma (15–80 h compared to only 3–4 for the basic filgrastim (33)). More recently, the FDA has approved 2 new PEGylated biologic drugs. In 2014, PEGylated interferon gamma beta-1a (PEGylated IFN beta-1a; PLEGRIDY®) was approved for treatment of relapsing multiple sclerosis. Addition of PEG to the therapeutic protein improved biological half-life and exposure in comparison to the protein alone (34). In comparison to Copaxone® and other IFN-based MS treatments which are often administered daily, PLEGRIDY® can be administered every 2–4 weeks. In 2015, PEGylated anti-hemophilic factor VIII (ADYNOVATE) was approved for treatment of hemophilia A, both in terms of preventing bleeding episodes, or treating acute bleeding. Based on the increased half-life of this drug in comparison to non-PEGylated factor VIII, there is also hope that the need for less frequent administration may reduce the incidence of anti-factor VIII antibody generation, which affects up to 30% of patients (for most biologics, anti-drug antibody generation occurs in <5% of patients) and leads to reduced drug efficacy (35). There are also a large number of polymer-immobilized nanomedicines that are under Phase II or III investigation in clinical trials. By way of example, NKTR-102 is a PEGylated etirinotecan drug, that has extended into Phase III clinical trials (36). The trial showed that extended exposure of tumor cells to the topoisomerase-I inhibitor typically showed enhanced therapeutic response – this is attributed to the longer circulation of PEGylated nanomaterials. In addition to PEGylation, other hydrophiles can be utilized to increase circulation. A Polymer-drug conjugate of paclitaxel and polyglutamic acide (poliglumex (PPX)) has entered phase III trials and is showing significantly improved standard of living for patients who undergo paclitaxel therapy for non-small cell lung cancer (37). This exemplifies

Table I List of FDA-Approved Nanomedicines Stratified by Material Category

Name	Material Description	Nanoparticle Advantage	Indication(s)	Year(s) approved
Polymer Nanoparticles – synthetic polymer particles combined with drugs or biologics				
Adagen®/pegademase bovine (Sigma-Tau Pharmaceuticals)	PEGylated adenosine deaminase enzyme	Improve circulation time and decreased immunogenicity	Severe combined immunodeficiency disease (SCID)	1990
Cimzia®/certolizumab pegol (UCB)	PEGylated antibody fragment (Certolizumab)	Improved circulation time and greater stability *in vivo*.	Crohn's disease Rheumatoid arthritis Psoriatic Arthritis Ankylosing Spondylitis	2008 2009 2013 2013
Copaxone®/Glatopa (Teva)	Random copolymer of L-glutamate, L-alanine, L-lysine and L-tyrosine	Large amino-acid based polymer with controlled molecular weight and clearance characteristics	Multiple Sclerosis (MS)	1996
Eligard® (Tolmar)	Leuprolide acetate and polymer (PLGH (poly (DL-Lactide-co-glycolide))	Controlled delivery of payload with longer circulation time	Prostate Cancer	2002
Macugen®/Pegaptanib (Bausch & Lomb)	PEGylated anti-VEGF aptamer (vascular endothelial growth factor) aptamer	Improved stability of aptamer as a result of PEGylation	Macular degeneration, neovacular age-related	2004
Mircera®/Methoxy polyethylene glycol-epoetin beta (Hoffman-La Roche)	Chemically synthesized ESA (erythropoiesis-stimulating agent)	Improved stability of aptamer as a result of PEGylation	Anemia associated with chronic kidney disease	2007
Neulasta®/pegfilgrastim (Amgen)	PEGylated GCSF protein	Improved stability of protein through PEGylation	Neutropenia, Chemotherapy induced	2002
Pegasys® (Genentech)	PEGylated IFN alpha-2a protein	Improved stability of protein through PEGylation	Hepatitis B; Hepatitis C	2002
PegIntron® (Merck)	PEGylated IFN alpha-2b protein	Improved stability of protein through PEGylation	Hepatitis C	2001
Renagel®[sevelamer hydrochloride]/ Renagel®[sevelamer carbonate] (Sanofi)	Poly(allylamine hydrochloride)	Increase circulation and therapeutic delivery	Chronic kidney disease	2000
Somavert®/pegvisomant (Pfizer)	PEGylated HGH receptor antagonist	Improved stability of protein through PEGylation	Acromegaly	2003
Oncaspar®/pegaspargase (Enzon Pharmaceuticals)	Polymer-protein conjugate (PEGylated L-asparaginase)	Improved stability of protein through PEGylation	Acute lymphoblastic leukemia	1994
Krystexxa®/pegloticase (Horizon)	Polymer-protein conjugate (PEGylated porcine-like uricase)	Improved stability of protein through PEGylation; introduction of unique mammalian protein	Chronic gout	2010
Plegridy® (Biogen)	Polymer-protein conjugate (PEGylated IFN beta-1a)	Improved stability of protein through PEGylation	Multple Sclerosis	2014
ADYNOVATE (Baxalta)	Polymer-protein conjugate (PEGylated factor VIII)	Improved stability of protein through PEGylation	Hemophilia	2015
Liposome formulations combined with drugs or biologics				
DaunoXome® (Galen)	Liposomal Daunorubicin	Increased delivery to tumour site; lower systemic toxicity arising from side-effects	Karposi's Sarcoma	1996
DepoCyt© (Sigma-Tau)	Liposomal Cytarabine	Increased delivery to tumour site; lower systemic toxicity arising from side-effects	Lymphomatous meningitis	1996
Marqibo® (Onco TCS)	Liposomal Vincristine	Increased delivery to tumour site; lower systemic toxicity arising from side-effects	Acute Lymphoblastic Leukemia	2012
Onivyde® (Merrimack)	Liposomal Irinotecan	Increased delivery to tumour site; lower systemic toxicity arising from side-effects	Pancreatic Cancer	2015
AmBisome® (Gilead Sciences)	Liposomal Amphotericin B	Reduced nephrotoxicity	Fungal/protozoal infections	1997
	Liposomal Morphine sulphate	Extended release	Analgesia (post-operative)	2004

Table I (continued)

Name	Material Description	Nanoparticle Advantage	Indication(s)	Year(s) approved
DepoDur® (Pacira Pharmaceuticals)				
Visudyne® (Bausch and Lomb)	Liposomal Verteporfin	Increased delivery to site of diseased vessels; photosensitive release	Macular degeneration, wet age-related; myopia; ocular histoplasmosis	2000
Doxil®/Caelyx™ (Janssen)	Liposomal doxorubicin	Improved delivery to site of disease; decrease in systemic toxicity of free drug.	Karposi's Sarcoma; Ovarian cancer; multiple myeloma	1995 2005 2008
Abelcet® (Sigma-tau)	Liposomal Amphotericin B lipid complex	Reduced toxicity	Fungal infections	1995
Curosurf®/Poractant alpha (Chiesei farmaceutici)	Liposome-proteins SP-B and SP-C	Increased delivery for smaller volume; reduced doxicity	pulmonary surfactant for Respiratory Distress Syndrome	1999
Micellar nanoparticles combined with drugs or biologics				
Estrasorb™ (Novavax)	Micellar Estradiol	Controlled delivery of therapeutic	Menopausal therapy	2003
Protein nanoparticles combined with drugs or biologics				
Abraxane®/ABI-007 (Celgene)	Albumin-bound paclitaxel nanoparticles	Improved solubility; improved delivery to tumor	Breast cancer NSCLC Pancreatic cancer	2005 2012 2013
Ontak® (Eisai Inc)	Engineered Protein combining IL-2 and diphtheria toxin	Targeted T-cell specificity; lysosomal escape	Cutaneous T-Cell Lymphoma	1999
Nanocrystals				
Emend® (Merck)	Aprepitant	Surface area allows faster absorption and increases bioavailability	Antiemetic	2003
Tricor® (Lupin Atlantis)	Fenofibrate	Increases bioavailability simplifies administration	Hyperlipidemia	2004
Rapamune® (Wyeth Pharmaceuticals)	Sirolimus	Increased bioavalibility	Immunosuppresent	2000
Megace ES® (Par Pharmaceuticals)	Megestrol acetate	Reduced dosing	Anti-anorexic	2001
Avinza® (Pfizer)	Morphine sulfate	Increased drug loading and bioavailability; extended release	Psychostimulant	2002 (2015)
Focalin XR® (Novartis)	Dexamethyl-phenidate HCl	Increased drug loading and bioavailability	Psychostimulant	2005
Ritalin LA® (Novartis)	Metyhlphenidate HCl	Increased drug loading and bioavailability	Psychostimulant	2002
Zanaflex® (Acorda)	Tizanidine HCl	Increased drug loading and bioavailability	Muscle relaxant	2002
Vitoss® (Stryker)	Calcium phosphate	Mimics bone structure allowing cell adhesion and growth	Bone substitute	2003
Ostim® (Heraseus Kulzer)	Hydroxyapatite	Mimics bone structure allowing cell adhesion and growth	Bone substitute	2004
OsSatura® (IsoTis Orthobiologics)	Hydroxyapatite	Mimics bone structure allowing cell adhesion and growth	Bone substitute	2003
NanOss® (Rti Surgical)	Hydroxyapatite	Mimics bone structure allowing cell adhesion and growth	Bone substitute	2005
EquivaBone® (Zimmer Biomet)	Hydroxyapatite	Mimics bone structure	Bone substitute	2009
Invega® Sustenna® (Janssen Pharms)	Paliperidone Palmitate	Allows slow release of injectable low solubility drug	Schizophrenia Schizoaffective Disorder	2009 2014
Ryanodex® (Eagle Pharmaceuticals)	Dantrolene sodium	Faster administration at higher dses	Malignant hypothermia	2014
Inorganic and metallic nanoparticles				
Nanotherm® (MagForce)	Iron oxide		Glioblastoma	2010

Table I (continued)

Name	Material Description	Nanoparticle Advantage	Indication(s)	Year(s) approved
		Allows cell uptake and introduces superparamagnetism		
Feraheme™/ferumoxytol (AMAG pharmaceuticals)	Ferumoxytol SPION with polyglucose sorbitol carboxymethylether	Magnetite suspension allows for prolonged steady release, decreasing number of doses	Deficiency anemiairon deficiency in chronic kidney disease (CKD)	2009
Venofer® (Luitpold Pharmaceuticals)	Iron sucrose	Allows increased dose	iron deficiency in chronic kidney disease (CKD)	2000
Ferrlecit® (Sanofi Avertis)	Sodium ferric gluconate	Allows increased dose	iron deficiency in chronic kidney disease (CKD)	1999
INFeD® (Sanofi Avertis)	Iron dextran (low MW)	Allows increased dose	iron deficiency in chronic kidney disease (CKD)	1957
DexIron®/Dexferrum® (Sanofi Avertis)	Iron dextran (high MW)	Allows increased dose	iron deficiency in chronic kidney disease (CKD)	1957
Feridex®/Endorem® (AMAG pharmaceuticals)	SPION coated with dextran	Superparamagnetic character	Imaging agent	1996 (2008)
GastroMARK™; umirem® (AMAG pharmaceuticals)	SPION coated with silicone	Superparamagnetic character	Imaging agent	2001 (2009)

the fact that while PEGylated polymers have been most extensively utilized in nanomedicine, other examples of hydrophiles can be equally successful in improving therapeutic outcomes.

Beyond just extending the circulation time of established drugs, polymeric nanoparticles can be developed based on hydrophobic materials that facilitate controlled release of the therapeutic. This is achieved by using slowly-degradable functionality that subsequently leads to kinetically-driven release of the drug. A long established polymer nanoparticle that has had significant success is based upon incorporation of leuprolide (a testosterone inhibiting drug) into a polylactide-co-glycolic acid (PLGA) nanoparticle. This drug is sold under the tradename Eligard® and is indicated as an effective treatment for the symptoms of prostate cancer (38). PLGA is a well-established degradable polymer that slowly decomposes into the constituent monomeric units over controlled time-courses. Another chemotherapeutic, Camptothecin (a DNA topoisomerase I inhibitor), has been encapsulated in cyclodextrin-PEG copolymers to form nanoparticles ~20–50 nm in diameter (39). These nanoparticles (named CRLX101 by licensee Cerulean Pharma Inc.) are administered by intravenous injection and utilize the so-called enhanced permeability and retention effect (EPR) that relies on leaky vasculature in tumors to increase accumulation of the drug molecules at the target of interest. CRLX101 has achieved phase I/II trials in patients with rectal, ovarian, tubal and peritoneal cancer and is an example of classical nanomedicine therapeutics utilizing biocompatible polymeric nanoparticles.

Polymeric Micelles

Polymeric micelles consist of self-assembled polymeric amphiphiles tailored for controlled delivery of hydrophobic drugs. Through careful design of the hydrophobic/hydrophilic balance in the amphiphile, the size and morphology of the assembled micelles can be controlled. The internal core is hydrophobic and can be used to encapsulate poorly water soluble drugs, whereas the exterior surface is polar enough to allow dissolution in aqueous solution. The use of block copolymers as the amphiphiles has led to lower critical micelle concentration (CMC) and thus higher stability in comparison to traditional surfactant-based micelles (40). To date, a traditional micellar formulation of estradiol (Estrasorb™) is the only FDA-approved micelle, indicated as a topical treatment for moderate to severe vasomotor symptoms of menopause. Transdermal delivery avoids first pass metabolism and also gastrointestinal side-effects, leading to stable serum levels for 8–14 days (41). Beyond this example, a number of micelle formulations are in late-stage clinical trials.

Intravenously-administered micellar systems are emerging with notable performances in clinical trials. The first of these that will be discussed is BIND-014 which has received significant attention for its potential as a prostate cancer therapeutic and is a commercial entity of the company, Bind Therapeutics. Docetaxel, an anti-mitotic chemotherapeutic agent, is incorporated into the core of core-shell polymeric micelles which contain a degradable and hydrophobic polymeric core and hydrophilic PEG shell. Upon assembly, the hydrophobic docetaxel is incorporated into the core of the micelle. In the case of prostate cancer trials, these micelles are

targeted to prostate-specific membrane antigen (PSMA) which is a well-defined protein marker on the surface of many prostate cancer cells (42). Recently, there has been a move away from cytotoxic cancer drugs towards molecularly targeted agents (MTAs) that have had little attention in the nanomedicine field. While highly efficacious, these drugs are associated with serious toxicity profiles on their own, and therefore the combination with nanoparticles may help to reduce toxicity and allow higher dosing (43). Next generation BIND micelles that incorporate kinase inhibitors as future drug therapeutics have been used to investigate efficacy against metastatic colon cancers, showing promising results in pre-clinical trials. In a similar strategy to that utilized by the BIND system, micellar nanoparticles have also been utilized to deliver docetaxel to various solid tumors using CriPec® particles through the company, Cristal Therapeutics (44). Here, degradable lactate-based hydrophobic blocks were linked to PEG-based hydrophilic blocks and the resulting therapeutic was incorporated through a degradable linker into the hydrophobic block. The authors demonstrated complete remission of breast cancer tumors in rodents owing to the favorable biodistribution profile of this therapeutic. One final micellar complex worthy of mention is the transferrin-targeting cyclodextrin-based micellar system for delivering siRNA directly to tumor cells. This is an important study as it clearly highlights how polymeric micelles can be used to deliver biologically-sensitive molecules, such as gene therapeutics. CALAA-01, a PEG-stabilized micelle developed by Calando Pharmaceuticals and regarded as the first targeted nanoparticle study in humans, entraps siRNA against the M2 subunit of ribonucleotide reductase (45). The polymeric micelles act not only to protect the siRNA against enzymatic degradation (by RNAses in the blood), but also increases accumulation and provides a cellular transport mechanism of action for the gene therapeutic. Such trials are expected to increase the utility of gene therapies such as siRNA in future applications. Combined, these approaches exemplify the robust and broad applicability of polymeric micelles, and new products are eagerly anticipated in the near future.

Liposomal Nanoparticles

The utilization of liposomal platforms for drug delivery has had a significant impact on pharmacology. Approved drugs with high toxicity or low bioavailability benefit from the stabilizing nature and improved biodistribution of liposomes in circulation. First described in 1965, the simplest form of liposome is a phospholipid bilayer surrounding an aqueous core (46).

There have been many improvements to manufacturing and loading of these systems allowing delivery of hydrophobic and hydrophilic compounds ranging from small weak bases to large macromolecules. Liposomes arguably are the most easily synthesized class of nanoparticle that can integrate established targeting ligands into already approved liposome drug carriers to create new potential combinations to improve therapeutic delivery. They are self-assembling and have amphipathic domains surrounding an aqueous core that chemically allows for the rapid integration of multiple molecules with different physico-chemical properties. Advances have facilitated active targeting by conjugating cell surface receptor ligands to the liposome surface. These materials are currently in various phases of clinical trials (47).

Liposomes became the first nanomedicines in FDA clinical trials. Starting with approval of liposomal formulations of doxorubicin and Amphotericin B in mid-1990s there has been a growing number of trials and approvals using liposomal delivery (48). The most recently approved liposomal drug carrier is Onivyde® (liposomal irinotecan), a topoisomerase I inhibitor approved as a second line treatment for metastatic pancreatic cancer. There are still very few options for these patients and no consensus of care has been established. Patients given Onivyde® in addition to flurouracil and folinic acid were shown to have a 6.1 month median survival vs 4.2 months with the addition of the liposomal irinotecan formulation (49,50). Like all of the approved liposomal systems before it, Onivyde® is based on passive targeting.

In terms of intravenous delivery, classical liposomes typically exhibite short circulating half-lives due to rapid clearance. In general, the lipid bilayer structure of liposomes results in recognition by the immune system and subsequent clearance from circulation by macrophages. Clearance has been minimized by PEGylation of the liposome surface. PEGylated liposomal doxorubicin (Doxil®), the first example of a liposomal nanoparticle drug, was proven effective in the reduction of cardiotoxic side effects of doxorubicin treatment. Doxil® has been approved for Karposi's sarcoma, ovarian cancer, and multiple myeloma, as well as for metastatic breast cancer (51). In comparison to free doxorubicin (DOX), the PEGylated liposome resulted in 4 to 16 fold enhancement of drug levels in malignancies (52). The PEGylated liposomal carrier was quickly adopted for the delivery of other drugs such as Ambisome®, an amphotericin B to treat fungal infections, and Visudyne®, delivering verteporphin for wet macular degeneration (53,54). These and many of the other approved liposomes rely strictly on passive targeting which successfully increases distribution to diseased tissue.

More complex liposomal systems are also in the clinical trial phase. A multifunctional particle in advanced clinical trials is Thermodox®. It is a liposome bound doxorubicin similar to Doxil®, but is formulated with thermally sensitive lipids that degrade the bilayer when exposed to high heat. The coupling of this chemotherapeutic with radiofrequency thermal ablation of tumor cells allows for site-specific release of the drug (55). A dual targeting example in preclinical use is the

combination of cyclic arginine-glycine-aspartic acid (RGD) peptide and transferrin (TF). Cyclic RGD has been previously used to increase tumor cell uptake and TF is a potential ligand to enable delivery across the blood–brain barrier. These have been added to a liposome to establish RGD/TF-LP, a "cascade" delivery system. When combined with paclitaxel, RGD/TF-LP forms a complex new system that may precisely target gliomas in the brain, which is by its anatomical nature difficult to access (56). Liposomal drug delivery has become a clinical staple and it is clear that with improvements in nanotechnology, they will continue to evolve into next generation nanomedicines into the future.

Protein Nanoparticles

Protein nanoparticles span a number of different nanomedicine classes, from drugs conjugated to endogenous protein carriers to engineered proteins where the active therapeutic is the protein itself, and to combined complex platforms that rely on protein motifs for targeted therapeutic delivery. Early protein nanoparticles sought to use the natural properties of protein circulating in serum, allowing dissolution and transport of drug compounds in blood during circulation. This approach consisted of natural protein combined with known drugs in order to reduce toxicity. Abraxane® is an early example of protein-drug conjugation. Approved by the FDA in 2005, Abraxane® is albumin-bound paclitaxel in particle form designed to eliminate the need for the toxic solvent, Cremophor, normally required for paclitaxel delivery (57). The 130 nm human serum albumin protein-bound paclitaxel particles improved infusion time and eliminated the need for co-administration of powerful antihistamines or dexamethasone in order to prevent immunoreaction to the Cremophor solvent (polyethoxylated castor oil). Beyond the initial goal of reducing toxicity, further study of Abraxane® has found improved pharmacokinetics and enhanced tumor inhibition when compared to the Cremophor-based therapy due to enhanced endothelial binding and transcytosis of the nanoparticle (58). Abraxane® has exemplified protein-drug nanoparticles as excellent nanomaterials for improving toxicity and passive delivery to a desired target. As such, several albumin bound nanoparticles (NABs) have been entered into clinical trials seeking to improve the therapeutic efficacy of other drugs. Examples include NAB-docetaxel, NAB-rapamycin, and NAB-heat shock protein inhibitor. Since its approval in 2005, there has been a shift from unmodified protein to the utilization of more highly engineered particle complexes in order to gain active targeting functionality.

Ontak®, (Denileukin Diftitox) is an example of an approved engineered fusion protein combining cytotoxic molecules with targeting proteins. It was initially designed to treat the aggressive form of non-Hodgkin's Peripheral T-cell Lymphomas (PTCL). Ontak®, approved in 2008, is an IL-2 receptor antagonist designed to direct the cytocidal action of diphtheria toxin to cells that overexpress the IL-2 receptor on T-cells. In this case diphtheria toxin may be considered the cytotoxic drug and the engineered IL-2 the delivery platform. IL-2R-targeted therapy appears to be an effective treatment option for PTCL patients. Combination therapy with Ontak® and CHOP (the traditional first-line chemotherapy for the disease) has an overall survival rate of 63.3% as opposed to 32–35% with CHOP alone (59,60). Ontak® is not myelosuppressive, nor is it associated with significant organ toxicity. Ontak® represented one of the first actively targeted proteinaceous nanoparticles, but is still a singular protein system. It could be used for a range of hematological tumors, many of which overexpress IL-2R (61).

Rexin-G® is a targeted gene therapy system in phase I/II trials. Its active targeting relies on a collagen-binding peptide from human von Willebrand factor (vWF). This protein normally induces platelet aggregation in the instance of vascular injury. In Rexin-G®, vWF serves to enhance particle deposition by guiding the whole particle into a tumor where exposed collagen is often found (62). In contrast to previous protein nanoparticles, Rexin-G® is a mixed system that is based on the murine leukemia virus. The von Willenbrand factor derived binding motif is expressed in the modified viral envelope for particle delivery. The proteinaceous envelop is responsible for nanoparticle accumulation and ultimate transfection of a cytotoxic *cyclin G1* gene. This therapeutic is in phase I/II trials. Leveraging the body's platelet activation system by using von Willebrand factor as a form of targeting general disease states is an interesting shift away from non-targeted protein nanoparticles and the cell-specific receptor targeting used in antibody-drug conjugates. As opposed to receptor-specific targeting, Rexin-G® is targeted against the general disease state characteristics found in tumor environments. Avoiding reliance on a specific receptor may avoid the confounding effects of mutation and adaptation. Rexin-G®'s proponents have stated this general targeting of invasive cancer characteristics has improved delivery of the genetic payload to where it is needed while reducing target selection of normal tissues and tumor adaptation (63).

Inorganic Nanoparticles

Inorganic nanoparticles are a well-studied field, with a large number of inorganic platforms being investigated for therapeutic and imaging treatments. For the purposes of this review, we use the term inorganic nanoparticles to cover both metallic and metal oxide materials. Iron oxide nanoparticles have undergone a number of clinical trials. Despite EU approval of several iron oxide nanoparticles, to date only three particles have completed FDA approval (Feraheme®, Feridex®, and GastroMARK™); two of which have been later withdrawn from the market. Beyond these, there are

examples of metallic particles and unique oxides coming through the trial pipeline with applications in both therapeutic and imaging applications (theranostics).

Iron oxide nanoparticles have an emerging research profile as contrast enhancement reagents for magnetic resonance imaging, however the majority of FDA-approved materials are indicated as iron replacement therapies. There are several approved compounds (Venofer®, Ferrlecit®, INFed®, Dexferrum® and Feraheme®), which are employed to treat anemia related to chronic kidney disease (CKD). Each of these is composed of an iron oxide core, coated with hydrophilic polymers (e.g. dextran, sucrose), which provide slow dissolution of the iron following intravenous injection. This allows rapid administration of large doses without increasing free iron in the blood to a level which causes toxicity (64). Interestingly, ferumoxytol (Feraheme®) is also under investigation as an imaging agent (65). Despite a number of similar superparamagnetic iron oxide nanoparticles (SPIONs) being granted FDA approval, they have since been discontinued for reasons that remain unclear (66). In 2010 there was EU-wide regulatory approval for Nanotherm™, which consists of aminosilane-coated SPIONs designed for tumor therapy (glioblastoma) using local tissue hyperthermia (67). Nanotherm™ is in late stage clinical trials in the US, and FDA approval is pending. Here, the "magnetic fluid" is injected directly into the tumor, and then an alternating magnetic field applicator is used to selectively heat the particles, resulting in local heating of the tumor environment (temperatures reach 40–45°C), leading to programmed and non-programmed cell death. In a recent clinical trial, treatment of glioblastoma with this magnetic nanomedicine was associated with increased overall survival of up to 12 months (68).

Gold has been utilized as a nanomedicine in clinical trials due to its unique combination of optical properties, thermal properties, and tunable size, shape, and surface chemistry (69). Tuning the size of the particles on the nanoscale allows a plasmonic characteristic to be established and augmented (70). Although well represented in a range of research fields, there are few examples of gold nanoparticles being actively investigated in clinical trials, and there are no gold-based nanomedicines that have been approved to date by the FDA. Previous uses of colloidal gold in treatment of arthritis have been largely surpassed by more effective drugs with fewer side effects (71). This is likely due to the influence of gold nanoparticles on cell function (72) owing to their affinity for DNA. Different changes in gene expression have been shown for acute and chronic exposure to gold nanoparticles (73). Particles placed into clinical trials recently have incorporated a biocompatible coating. In one trial CYT-6091 (recombinant human tumor necrosis factor (rhTNF) bound to colloidal gold) was studied for solid tumor treatment (Phase I) (74). Here, the rhTNF was attached to gold nanoparticles using a PEG linker which doubled as a biocompatible antifouling layer. During this study, Libutti *et al.* found that the dose of rhTNF administered after immobilization to gold could be 3 times higher than native rhTNF without causing toxic effects. The polyethylene glycol layer also decreased uptake by the MPS and aided in accumulation in tumor masses *via* the EPR effect. In another example a silica coating was employed as an alternative anti-fouling approach to PEG (75). These silica-coated gold particles were conjugated to iron oxide particles in order to control particle biodistribution *via* the modulation of exterior magnetic fields. This allowed the localized ablation of plaque in a blood vessel using only an injection and externally applied magnetic fields and light. These particles were also administered with protein-targeted microbubbles or stem cells to aid delivery.

Developed by Nanobiotix, NBTXR3 is a novel radio enhancer utilizing a high electron density metal oxide (hafnium oxide) nanoparticle to increase radiotherapy efficacy without increasing the surrounding tissue dose (76). Incorporation of a high electron density material maximizes x-ray interactions producing a larger number of excited electrons and in turn forming more reactive radical species (77). Developed for injection into a tumor site these particles are designed to undergo cell uptake. Uptake is increased by tuning the particle size and surface properties (50 nm diameter and negative zeta potential), which prevents leakage to surrounding tissue while maximizing the local cellular damage when particles are irradiated (76). Once again, antifouling coatings are usually employed to stabilize the nanoparticles in biological environments. Entering phase I clinical trials in 2011 NBTXR3 has since reached phase II/III for treatment of soft tissue sarcoma due for completion towards the end of 2016. Phase I trials have also begun for indications including head and neck cancer and have been completed for rectal cancer in conjunction with PharmaEngine under the name of PEP503.

Cornell dots (C-dots) are inorganic silica nanoparticles designed for fluorescence imaging applications. Under development by the Wiesner group at Cornell University these particles entered into phase I clinical trials in 2014. Designed for lymph node mapping in cancer patients these particles comprise a targeting moiety, an antifouling polymer layer, and an internal silica core labelled with a near-infrared (NIR) fluorescent dye. Cyclic arginine–glycine–aspartic acid peptide (cRGDY; targeting agent for tumor and tumor endothelial cells) was conjugated to the PEG-coated surface of the Cy5.5 labelled C-dots. The particle design leads to a nanoparticle that is 20–30 times brighter and more stable than the constituent dye in solution. A first in human trial in five patients recently indicated favorable PK/distribution profiles and safety as a tumor imaging agent, paving the way for other trials in the near future (78).

Crystalline Nanoparticles

Crystalline nanomedicines are unique because the nanoparticles themselves are composed of 100% drug compound. The increased surface area for dissolution, owing to the nanoscale dimensions, leads to increased dissolution velocity and also increased saturation solubility. This last point is particularly important – at the nanoscale, saturation solubility increases with decreasing particle size, leading to enhanced driving forces for diffusion-based mass transfer through biological structures (e.g. walls of the gastro-intestinal tract). The production of crystalline nanoparticles has been applied to both organic drugs as well as inorganic materials. (79,80). The solubility issues related to a number of drug compounds have been "rescued" by conversion into nanocrystals and are marketed for a range of indications. Inorganic crystalline nanoparticles approved by the FDA are limited to hydroxyapatite and calcium phosphate for use as bone graft substitutes. Synthesis methods include either "top-down" diminution approaches (including milling and homogenization) which are often employed for the organic compounds, and "bottom-up" precipitation methods that are more commonly applied to the inorganics. The milling approach developed by Elan Nanosystems was employed to produce the first three FDA-approved nanocrystals – Rapamune®, Tricor®, and Emend®, and is expected to be broadly applicable to a range of drugs that suffer from solubility issues, which is an estimated 70–90% of potential drug compounds (80,81).

Rapamune (Wyeth Pharmaceuticals) is a milled organic nanocrystalline drug and was the first of its kind to be approved by the FDA in 2000. Containing the active component sirolimus, a bacterial derived 914 Da macrocyclic immunosuppressant (82), Rapamune is used to prevent organ rejection following transplantation, specifically for kidneys. The nanocrystalline nature of the formulation provides the poorly soluble drug a constant extended release profile well suited to ongoing indications. The milling technology that produces these nanocrystals as well as others including Emend®, Tricor® and Megace ES® involves a pearl/ball mill able to shear particles apart during agitation. A dispersion media as well as stabilizing agents are also combined with the drug to facilitate milling. This technology has been shown flexible in terms of administration as demonstrated by the variety of formulations including oral suspension, tablet and intramuscular injection. Following the approval of Rapmune several other approved nanocrystalline drug formulations have been produced in this manner, the last of which was approved in 2009.

Future Perspectives

The data presented in Fig. 1 on clinical trials highlights the significant expansion in clinically-validated nanomedicine-based drugs and biologics and suggests that we are likely to see an increase in the number of FDA-approved therapeutic and imaging agents over the foreseeable future. Modern approaches in protein engineering, as well as advances in polymer and inorganic chemistry have resulted in an explosion of novel nanomaterials in the academic literature; these nanomaterials often contain elements of a number of different materials such that the boundaries between the traditional material categories is blurred. This is leading to a distinct trend which evolves beyond the initial goal for nanomedicine (i.e. longer circulation times and reduced toxicities), to complex particle system designs which may allow for controlled release, active targeting of disease, and diversification of drug approaches beyond traditional chemotherapeutics (e.g. paclitaxel). Nanoparticles were introduced to decrease some of the challenges of traditional drug delivery, but come with their own difficulties and limitations that will continue to be an important area of research. *In vivo* bio-distribution and toxicity studies currently guide particle design and clinical trial candidates, but the real world use of nanomedicines need Phase IV post-marketing review after clinical application to show the full benefits and limitations of these technologies (11,20,83–86). Several nanomedicine products have undergone clinical trials only to be later withdrawn due to efficacy or safety concerns e.g. Feruglose® (NC100150) and Resovist® (87,88).

While the majority of FDA-approved nanomedicines rely on passive targeting *via* the EPR effect, there is a clear trend in emerging studies towards active targeting to further increase drug accumulation and ultimately efficacy at the disease site, while reducing toxicity in other organs. While the EPR effect enables non-specific uptake of nanomedicines, there are competing effects that are yet to be overcome. Key problems include the hyper-permeable tumor blood vessels that cause excessive fluid and drug leakage. This in turn leads to the lack of a pressure differential between the microvasculature of the tumor and the interstitial environment, effectively limiting the driving force for mass transfer into the tumor to diffusion alone. Given that diffusion decreases with increasing size of the drug or biologic, the relatively large nanoparticles may not reach the target tissue effectively (89). Beyond the EPR effect, many of the next generation nanomedicines in clinical trials employ active targeting approaches wherein the nanoparticles can bind to the surface of cells *via* affinity interactions. CALAA-01 is widely regarded as the original targeted nanomedicine under investigation, whereupon the inventors immobilized human transferrin to the particle surface to bind transferrin receptors which are upregulated on the surface of cancer cells. Beyond this approach, others including the

BIND-014 (contains specific targeting ligand for PSMA-expressing prostate cancers) and SGT-53 (90) (contains an anti-transferrin antibody fragment that interacts with transferring glycoprotein receptor over-expressed on cancer cells), both of which are in Phase 2 trials for solid tumor therapy. An ongoing challenge in the development of actively-targeted therapies is the careful balance that needs to be struck inside the tumor between the rates of drug uptake, release, and selective target binding, to encourage uniform distribution of the drug throughout the tumor (89).

Another clear trend is the move from relatively simple nanoparticles originating from the traditional material categories, to complex and multi-component materials that blur the boundaries between organic/inorganic chemistries and molecular biology. While the number of materials categorized as "polymeric nanoparticles" is likely to reduce based on the clinical trial data, in reality polymer chemistry is permeating all of the categories. Micelles and liposomes are now often synthesized using synthetic polymers, or at the very least they are coated with synthetic polymers to avoid protein adsorption. Similarly, with inorganic materials, surface coatings comprised of anti-fouling polymers is nearly a universal requirement. These complex multilayered particles include silica nanoparticles, metal and metal oxide nanoparticles, nanotubes, graphenes, dendrimers, polymers, cyclodextrins, lipids, hydrogels, and semiconductor nanocrystals, which may serve as future vehicles for delivery of new therapeutic categories such as RNA interference gene regulation mechanisms (91). A challenge here is that increased complexity may also be associated with higher costs and difficulty for scaling the process for trials and beyond. However, the development of platform technologies such as the "Accurin" particles from BIND Therapeutics is an interesting example of how this complexity can be handled. This group has effectively synthesized a library of nanoparticles based on commonly employed building blocks, which can be screened for specific traits related to drug encapsulation, *in vitro* and *in vivo* stability, controlled release, and targeting ability. This is an approach that is designed to lend itself to relatively simple scale-up, once the screening process has identified lead candidates for specific applications.

Beyond increased complexity in nanomedicine synthesis, emerging nanomedicines are also being designed to be multi-functional. The merging of therapeutic and diagnostic modalities is referred to as "theranostics" and there are a number of exciting examples of products in pre-clinical trials, but not necessarily yet in clinical trials (92). Examples of theranostic agent development can be seen across the spectrum of material categories highlighted previously. Imaging modalities most commonly employed include MRI, optical imaging (fluorescence), and ultrasound, and the therapeutic modalities usually involve photothermal therapies, or controlled delivery of chemotherapeutic or thrombolytic agents. To achieve both image enhancement as well as controllable drug delivery, development of nanomedicines that combine different traditional categories together is also apparent – e.g. the development of biocompatible polymeric agents with controlled release properties, combined with molecular imaging contrast agents to enable visualization of the treatment process (93).

While the development of exciting new nanomedicines continues, a significant challenge for both researchers, regulators, and industry, is the classification of these materials. We are not the first to identify that the simple act of gathering data on the approved and emerging nanomedicines was hampered by a lack of clear guidelines on what constitutes a nanomedicine, and how they are categorized (9). While in our study we used a number of search terms based on the "nano" prefix (including nanomedicine, nanoparticle, nanotechnology, etc.), it is possible that in some cases, these terms are specifically avoided. Furthermore, we excluded a range of material categories which others may have included (e.g. antibody drug conjugates, vaccines, etc.). This is a significant challenge, because as researchers are still learning about the "structure-function" relationships of their nanomaterials in biological systems, key material characteristics are constantly being varied and applied in different disease contexts (e.g. size, shape, charge, composition, complex architectures), resulting in an in-exhaustive list of individual candidates. This issue also complicates genuine efforts within the research community to investigate the potential toxicity of nanomedicines, because there is a significant lack of uniformity across toxicology protocols (94). With recent draft guidelines published by the FDA on the importance of nanomaterial characterization for different regulated environments, and the emergence of standard approaches for material characterization (e.g. http://ncl.cancer.gov (21)), significant effort is now being placed into developing standards for nanomaterial characterization. This is likely to lead to better information on the effects of nanomaterials in terms of medical and environmental toxicity, etc.

CONCLUSION

This up to date snapshot of nanoparticles in medicine affirms an increase in the number of FDA approvals and clinical trials utilizing nanoparticles within the past 3 years. Most of the currently approved nanomedicines consist of relatively simple nanoparticles and build on the success of well described systems and prior approved drugs, e.g. PEGlyated liposomes. There has been both a broadening in particle types and an increase in the complexity of particles within these categories over time. It is reasonable to assume that this trend will continue and the number of overall approvals will increase given that there has been a threefold increase in the number of

individually registered trials in the last 3 years within our parameters. The use and study of clinical nanoparticles has more growth potential and will continue to prove a productive and challenging field for academics, industry, clinicians, and regulators.

ACKNOWLEDGMENTS AND DISCLOSURES

We acknowledge funding from the National Health and Medical Research Council (APP1099231 KJT), the Australian Research Council (FT110100284, DP140100951 (KJT), DE130100800 (SRC)), National Breast Cancer Foundation (NC-14-037), and Centre of Excellence in Convergent BioNano Science and Technology (CE140100036 (SRC, KJT)) and thank the Ochsner Clinical School of New Orleans, LA (DPB).

REFERENCES

1. Albanese A, Tang PS, Chan WCW. The effect of nanoparticle size, shape, and surface chemistry on biological systems. Annu Rev Biomed Eng. 2012;14:1–16.
2. Chaudhuri RG, Paria S. Core/Shell nanoparticles: classes, properties, synthesis mechanisms, characterization, and applications. Chem Rev. 2012;112(4):2373–433.
3. Cimalla P, Werner T, Gaertner M, Mueller C, Walther J, Wittig D, et al. Magnetomotive imaging of iron oxide nanoparticles as cellular contrast agents for optical coherence tomography. Proc Spie. 2013;8802.
4. Dreaden EC, Alkilany AM, Huang X, Murphy CJ, El-Sayed MA. The golden age: gold nanoparticles for biomedicine. Chem Soc Rev. 2012;41(7):2740–79.
5. Elsabahy M, Wooley KL. Design of polymeric nanoparticles for biomedical delivery applications. Chem Soc Rev. 2012;41(7):2545–61.
6. Kamaly N, Xiao Z, Valencia PM, Radovic-Moreno AF, Farokhzad OC. Targeted polymeric therapeutic nanoparticles: design, development and clinical translation. Chem Soc Rev. 2012;41(7):2971–3010.
7. Tang F, Li L, Chen D. Mesoporous silica nanoparticles: synthesis, biocompatibility and drug delivery. Adv Mater. 2012;24(12):1504–34.
8. Eifler AC, Thaxton CS. Nanoparticle therapeutics: FDA approval, clinical trials, regulatory pathways, and case study. In: Hurst SJ, editor. Biomedical nanoetechnology: methods and protocols. methods in molecular biology. 7262011. p. 325–38.
9. Etheridge ML, Campbell SA, Erdman AG, Haynes CL, Wolf SM, McCullough J. The big picture on nanomedicine: the state of investigational and approved nanomedicine products. Nanomed-Nanotechnol Biology and Med. 2013;9(1):1–14.
10. Nel AE, Maedler L, Velegol D, Xia T, Hoek EMV, Somasundaran P, *et al.* Understanding biophysicochemical interactions at the nano-bio interface. Nat Mater. 2009;8(7):543–57.
11. Rolfe BE, Blakey I, Squires O, Peng H, Boase NRB, Alexander C, *et al.* Multimodal polymer nanoparticles with combined F-19 magnetic resonance and optical detection for tunable, targeted, multimodal imaging in vivo. J Am Chem Soc. 2014;136(6):2413–9.
12. Choi HS, Liu W, Misra P, Tanaka E, Zimmer JP, Ipe BI, *et al.* Renal clearance of quantum dots. Nat Biotechnol. 2007;25(10):1165–70.
13. Fox ME, Szoka FC, Frechet JMJ. Soluble polymer carriers for the treatment of cancer: the importance of molecular architecture. Acc Chem Res. 2009;42(8):1141–51.
14. Sadauskas E, Wallin H, Stoltenberg M, Vogel U, Doering P, Larsen A, *et al.* Kupffer cells are central in the removal of nanoparticles from the organism. Part Fibre Toxicol. 2007;4:10.
15. Tenzer S, Docter D, Rosfa S, Wlodarski A, Kuharev J, Rekik A, *et al.* Nanoparticle size is a critical physicochemical determinant of the human blood plasma corona: a comprehensive quantitative proteomic analysis. ACS Nano. 2011;5(9):7155–67.
16. Duncan R, Sat YN. Tumour targeting by enhanced permeability and retention (EPR) effect. Ann Oncol. 1998;9:39.
17. Casi G, Neri D. Antibody–drug conjugates: basic concepts, examples and future perspectives. J Control Release. 2012;161(2):422–8.
18. Diamantis N, Banerji U. Antibody-drug conjugates—an emerging class of cancer treatment. Br J Cancer. 2016;114(4):362–7.
19. Tinkle S, McNeil SE, Muehlebach S, Bawa R, Borchard G, Barenholz Y, *et al.* Nanomedicines: addressing the scientific and regulatory gap. Ann Reports. 2014;1313:35–56.
20. Dobrovolskaia MA. Pre-clinical immunotoxicity studies of nanotechnology-formulated drugs: challenges, considerations and strategy. J Control Release. 2015;220:571–83.
21. Nanotechnology Characterization Laboratory: National Cancer Institute US National Institues of Health; 2016 [2/16/2016]. Available from: http://ncl.cancer.gov/.
22. NCT02549248: Nanoparticles Analysis in Lung and Bronchi During Various Pulmonary Interstitial Diseases and Relationships With Their Aetiology (NANOPI) [Full text view]. Available from: ClinicalTrials.gov.
23. Registered Clinical Trial Database [Internet]. 2016 [cited 2/15/2016]. Available from: https://clinicaltrials.gov/.
24. Schutz CA, Juillerat-Jeanneret L, Mueller H, Lynch I, Riediker M, Consortium N. Therapeutic nanoparticles in clinics and under clinical evaluation. Nanomedicine-Uk. 2013;8(3):449–67.
25. Svenson S. What nanomedicine in the clinic right now really forms nanoparticles? Wiley Interdisciplinary Reviews. Nanomed Nanobiotechnol. 2014;6(2):125–35.
26. Zhang L, Gu FX, Chan JM, Wang AZ, Langer RS, Farokhzad OC. Nanoparticles in medicine: therapeutic applications and developments. Clin Pharmacol Ther. 2008;83(5):761–9.
27. Cures P. Neglected disease research and development: is the global financial crisis changing R&D. London: Policy Cures; 2011.
28. Tsoulfas G. The impact of the European financial crisis on clinical research within the European union or "when life gives you lemons, make lemonade". Hippokratia. 2012;16(1):6–10.
29. Cui JW, van Koeverden MP, Mullner M, Kempe K, Caruso F. Emerging methods for the fabrication of polymer capsules. Adv Colloid Interf Sci. 2014;207:14–31.
30. Duncan R. Polymer therapeutics: Top 10 selling pharmaceuticals - What next? J Control Release. 2014;190:371–80.
31. Johnson KP, Brooks BR, Cohen JA, Ford CC, Goldstein J, Lisak RP, *et al.* Extended use of glatiramer acetate (Copaxone) is well tolerated and maintains its clinical effect on multiple sclerosis relapse rate and degree of disability. Neurology. 1998;50(3):701–8.
32. Alconcel SNS, Baas AS, Maynard HD. FDA-approved poly(-ethylene glycol)-protein conjugate drugs. Polym Chem. 2011;2(7):1442–8.
33. Benbrook DM. Biotechnology and biopharmaceuticals: transforming proteins and genes into drugs, 2nd edition. Clinic infect Dis: Off Publ Infect DisSoc Am. 2015;60(2):331–2.
34. Hu X, Miller L, Richman S, Hitchman S, Glick G, Liu SF, *et al.* A novel PEGylated interferon Beta-1a for multiple sclerosis: safety,

pharmacology, and biology. J Clin Pharmacol. 2012;52(6):798–808.
35. Ing M, Gupta N, Teyssandier M, Maillere B, Pallardy M, Delignat S, *et al.* Immunogenicity of long-lasting recombinant factor VIII products. Cell Immunol. 2016;301:40–8.
36. Awada A, Garcia AA, Chan S, Jerusalem GHM, Coleman RE, Huizing MT, *et al.* Two schedules of etirinotecan pegol (NKTR-102) in patients with previously treated metastatic breast cancer: a randomised phase 2 study. Lancet Oncol. 2013;14(12):1216–25.
37. Paz-Ares L, Ross H, O'Brien M, Riviere A, Gatzemeier U, Von Pawel J, *et al.* Phase III trial comparing paclitaxel poliglumex vs docetaxel in the second-line treatment of non-small-cell lung cancer. Br J Cancer. 2008;98(10):1608–13.
38. Berges R. Eligard (R): Pharmacokinetics, effect on testosterone and PSA levels and tolerability. Eur Urol Suppl. 2005;4(5):20–5.
39. Svenson S, Wolfgang M, Hwang J, Ryan J, Eliasof S. Preclinical to clinical development of the novel camptothecin nanopharmaceutical CRLX101. J Control Release. 2011;153(1):49–55.
40. Oerlemans C, Bult W, Bos M, Storm G, Nijsen JFW, Hennink WE. Polymeric micelles in anticancer therapy: targeting, imaging and triggered release. Pharm Res-Dordr. 2010;27(12):2569–89.
41. Taylor DD, Gercel-Taylor C. MicroRNA signatures of tumor-derived exosomes as diagnostic biomarkers of ovarian cancer. Gynecol Oncol. 2008;110(1):13–21.
42. Hrkach J, Von Hoff D, Ali MM, Andrianova E, Auer J, Campbell T, et al. Preclinical development and clinical translation of a PSMA-targeted docetaxel nanoparticle with a differentiated pharmacological profile. Sci Transl Med. 2012;4(128).
43. Ashton S, Song YH, Nolan J, Cadogan E, Murray J, Odedra R, *et al.* Aurora kinase inhibitor nanoparticles target tumors with favorable therapeutic index in vivo. Sci Transl Med. 2016;8(325):325ra17–ra17.
44. Rijcken CJF, Veldhuis TFJ, Ramzi A, Meeldijk JD, van Nostrum CF, Hennink WE. Novel fast degradable thermosensitive polymeric micelles based on PEG-block-poly(N-(2-hydroxyethyl)methacrylamide-oligolactates). Biomacromolecules. 2005;6(4):2343–51.
45. Davis ME. The first targeted delivery of siRNA in humans via a self-assembling, cyclodextrin polymer-based nanoparticle: from concept to clinic. Mol Pharmaceut. 2009;6(3):659–68.
46. Bangham AD, Standish MM, Watkins JC. Diffusion of univalent ions across lamellae of swollen phospholipids. J Mol Biol. 1965;13(1):238.
47. Allen TM, Cullis PR. Liposomal drug delivery systems: from concept to clinical applications. Adv Drug Deliv Rev. 2013;65(1):36–48.
48. Vaage J, Mayhew E, Lasic D, Martin F. Therapy of primary and metastatic mouse mammary carcinomas with doxorubicin encapsulated in long circulating. Int J Cancer. 1992;51(6):942–8.
49. Saif Ur Rehman S, Lim K, Wang-Gillam A. Nanoliposomal irinotecan plus fluorouracil and folinic acid: a new treatment option in metastatic pancreatic cancer. Exp Rev Anticancer Ther. 2016:null-null.
50. Wang-Gillam A, Li C-P, Bodoky G, Dean A, Shan Y-S, Jameson G, et al. Nanoliposomal irinotecan with fluorouracil and folinic acid in metastatic pancreatic cancer after previous gemcitabine-based therapy (NAPOLI-1): a global, randomised, open-label, phase 3 trial. The Lancet 387(10018):545–57.
51. James ND, Coker RJ, Tomlinson D, Harris JR, Gompels M, Pinching AJ, *et al.* Liposomal doxorubicin (Doxil): an effective new treatment for Kaposi's sarcoma in AIDS. Clin Oncol (Royal College of Radiologists (Great Britain)). 1994;6(5):294–6.
52. Gabizon A, Catane R, Uziely B, Kaufman B, Safra T, Cohen R, *et al.* Prolonged circulation time and enhanced accumulation in malignant exudates of doxorubicin encapsulated in polyethyleneglycol coated liposomes. Cancer Res. 1994;54(4):987–92.
53. Hann IM, Prentice HG. Lipid-based amphotericin B: a review of the last 10 years of use. Int J Antimicrob Agents. 2001;17(3):161–9.
54. Arnold J, Kilmartin D, Olson J, Neville S, Robinson K, Laird A, *et al.* Verteporfin therapy of subfoveal choroidal neovascularization in age-related macular degeneration: Two-year results of a randomized clinical trial including lesions with occult with no classic choroidal neovascularization-verteporfin in photodynamic therapy report 2. Am J Ophthalmol. 2001;131(5):541–60.
55. May JP, Li S-D. Hyperthermia-induced drug targeting. Exp Opin Drug Deliv. 2013;10(4):511–27.
56. Qin L, Wang C-Z, Fan H-J, Zhang C-J, Zhang H-W, Lv M-H, *et al.* A dual-targeting liposome conjugated with transferrin and arginine-glycine-aspartic acid peptide for glioma-targeting therapy. Oncol Lett. 2014;8(5):2000–6.
57. Green MR, Manikhas GM, Orlov S, Afanasyev B, Makhson AM, Bhar P, *et al.* Abraxane((R)), a novel Cremophor((R))-free, albumin-bound particle form of paclitaxel for the treatment of advanced non-small-cell lung cancer. Ann Oncol. 2006;17(8):1263–8.
58. Desai N, Trieu V, Yao ZW, Louie L, Ci S, Yang A, *et al.* Increased antitumor activity, intratumor paclitaxel concentrations, and endothelial cell transport of Cremophor-free, albumin-bound paclitaxel, ABI-007, compared with Cremophor-based paclitaxel. Clin Cancer Res. 2006;12(4):1317–24.
59. Fuentes AC, Szwed E, Spears CD, Thaper S, Dang LH, Dang NH. Denileukin diftitox (Ontak) as maintenance therapy for peripheral T-Cell lymphomas: three cases with sustained remission. Case Pep Oncol Med. 2015;2015:123756.
60. Foss FM, Sjak-Shie N, Goy A, Jacobsen E, Advani R, Smith MR, *et al.* A multicenter phase II trial to determine the safety and efficacy of combination therapy with denileukin diftitox and cyclophosphamide, doxorubicin, vincristine and prednisone in untreated peripheral T-cell lymphoma: the CONCEPT study. Leukemia lymphoma. 2013;54(7):1373–9.
61. Foss F. Clinical experience with Denileukin Diftitox (ONTAK). Semin Oncol. 2006;33(Supplement 3):11–6.
62. Chawla SP, Chua VS, Fernandez L, Quon D, Blackwelder WC, Gordon EM, *et al.* Advanced phase I/II studies of targeted gene delivery in vivo: intravenous Rexin-G for gemcitabine-resistant metastatic pancreatic cancer. Mol Ther. 2010;18(2):435–41.
63. Gordon EM, Hall FL. Rexin-G, a targeted genetic medicine for cancer. Expert Opin Biol Ther. 2010;10(5):819–32.
64. Salah EDTA, Bakr MM, Kamel HM, Abdel KM. Magnetite nanoparticles as a single dose treatment for iron deficiency anemia. Google Patents. 2010.
65. Bashir MR, Bhatti L, Marin D, Nelson RC. Emerging applications for ferumoxytol as a contrast agent in MRI. J Magn Reson Imaging. 2015;41(4):884–98.
66. Wang Y-XJ. Current status of superparamagnetic iron oxide contrast agents for liver magnetic resonance imaging. World J Gastroenterol. 2015;21(47):13400–2.
67. Thiesen B, Jordan A. Clinical applications of magnetic nanoparticles for hyperthermia. Int J Hyperther. 2008;24(6):467–74.
68. Maier-Hauff K, Ulrich F, Nestler D, Niehoff H, Wust P, Thiesen B, *et al.* Efficacy and safety of intratumoral thermotherapy using magnetic iron-oxide nanoparticles combined with external beam radiotherapy on patients with recurrent glioblastoma multiforme. J Neuro-Oncol. 2011;103(2):317–24.
69. Kharlamov AN, Gabinsky JL. Plasmonic photothermic and stem cell therapy of atherosclerotic plaque as a novel nanotool for angioplasty and artery remodeling. Rejuvenation Res. 2012;15(2):222–30.
70. Zeng S, Yu X, Law W-C, Zhang Y, Hu R, Dinh X-Q, *et al.* Size dependence of Au NP-enhanced surface plasmon resonance based

on differential phase measurement. Sensors Actuators B Chem. 2013;176:1128–33.
71. Sanders M. A review of controlled clinical trials examining the effects of antimalarial compounds and gold compounds on radiographic progression in rheumatoid arthritis. J Rheumatol. 2000;27(2):523–9.
72. Tomic S, Dokic J, Vasilijic S, Ogrinc N, Rudolf R, Pelicon P, *et al.* Size-dependent effects of gold nanoparticles uptake on maturation and antitumor functions of human dendritic cells in vitro. PLoS ONE. 2014;9(5), e96584.
73. Qiu TA, Bozich JS, Lohse SE, Vartanian AM, Jacob LM, Meyer BM, *et al.* Gene expression as an indicator of the molecular response and toxicity in the bacterium Shewanella oneidensis and the water flea Daphnia magna exposed to functionalized gold nanoparticles. Environ Sci: Nano. 2015;2(6):615–29.
74. Libutti SK, Paciotti GF, Byrnes AA, Alexander Jr HR, Gannon WE, Walker M, *et al.* Phase I and pharmacokinetic studies of CYT-6091, a novel PEGylated colloidal gold-rhTNF nanomedicine. Clin Cancer Res. 2010;16(24):6139–49.
75. Kharlamov AN, Tyurnina AE, Veselova VS, Kovtun OP, Shur VY, Gabinsky JL. Silica-gold nanoparticles for atheroprotective management of plaques: results of the NANOM-FIM trial. Nanoscale. 2015;7(17):8003–15.
76. Marill J, Anesary NM, Zhang P, Vivet S, Borghi E, Levy L, *et al.* Hafnium oxide nanoparticles: toward an in vitro predictive biological effect? Radiat Oncol. 2014;9(1):150.
77. Pottier A, Borghi E, Levy L. New use of metals as nanosized radioenhancers. Anticancer Res. 2014;34(1B):443–53.
78. Phillips E, Penate-Medina O, Zanzonico PB, Carvajal RD, Mohan P, Ye YP, et al. Clinical translation of an ultrasmall inorganic optical-PET imaging nanoparticle probe. Sci Transl Med. 2014;6(260).
79. Junghanns J-UAH, Müller RH. Nanocrystal technology, drug delivery and clinical applications. Int J Nanomedicine. 2008;3(3):295–309.
80. Shegokar R, Müller RH. Nanocrystals: industrially feasible multifunctional formulation technology for poorly soluble actives. Int J Pharm. 2010;399(1):129–39.
81. Möschwitzer J, Müller RH. New method for the effective production of ultrafine drug nanocrystals. J Nanosci Nanotechnol. 2006;6(9–10):3145–53.
82. Sirolimus: AY 22989, NSC 226080, NSC 606698, Rapamycin, Rapamune. Drugs in R & D. 1999;1(1):100–7.
83. Almeida JP, Chen AL, Foster A, Drezek R. In vivo biodistribution of nanoparticles. Nanomedicine (London, England). 2011;6(5):815–35.
84. Alexis F, Pridgen E, Molnar LK, Farokhzad OC. Factors affecting the clearance and biodistribution of polymeric nanoparticles. Mol Pharmaceut. 2008;5(4):505–15.
85. Li S-D, Huang L. Pharmacokinetics and biodistribution of nanoparticles. Mol Pharmaceut. 2008;5(4):496–504.
86. Blanco E, Shen H, Ferrari M. Principles of nanoparticle design for overcoming biological barriers to drug delivery. Nat Biotechnol. 2015;33(9):941–51.
87. Kendall M, Lynch I. Long-term monitoring for nanomedicine implants and drugs. Nat Nanotechnol. 2016;11(3):206–10.
88. Wang Y-XJ. Superparamagnetic iron oxide based MRI contrast agents: current status of clinical application. Quant Imaging Med Surg. 2011;1(1):35–40.
89. Stylianopoulos T, Jain RK. Design considerations for nanotherapeutics in oncology. Nanomed-Nanotechnol Biol Med. 2015;11(8):1893–907.
90. Senzer N, Nemunaitis J, Nemunaitis D, Bedell C, Edelman G, Barve M, *et al.* Phase I study of a systemically delivered p53 nanoparticle in advanced solid tumors. Mol Ther. 2013;21(5):1096–103.
91. Draz MS, Fang BA, Zhang P, Hu Z, Gu S, Weng KC, *et al.* Nanoparticle-mediated systemic delivery of sirna for treatment of cancers and viral infections. Theranostics. 2014;4(9):872–92.
92. Mura S, Couvreur P. Nanotheranostics for personalized medicine. Adv Drug Deliv Rev. 2012;64(13):1394–416.
93. Thurecht KJ, Blakey I, Peng H, Squires O, Hsu S, Alexander C, *et al.* Functional hyperbranched polymers: toward targeted in vivo F-19 magnetic resonance imaging using designed macromolecules. J Am Chem Soc. 2010;132(15):5336−+.
94. Yildirimer L, Thanh NTK, Loizidou M, Seifalian AM. Toxicological considerations of clinically applicable nanoparticles. Nano Today. 2011;6(6):585–607.