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Proof of concept for next-generation nanoparticle drugs in humans

The results from a human trial of a nanoparticle-based cancer drug developed by BIND Biosciences of Cambridge, Massachusetts, provide positive evidence for the progress of a new generation of nanomedicines in the clinic. The phase 1 clinical study of patients with a variety of advanced and/or metastatic cancers published online in April (*Sci. Transl. Med.* 4, 128ra39, 2012), a collaboration between BIND and the Brigham and Women's Hospital, Dana-Farber Cancer Institute and Harvard Medical School, demonstrates the ability of BIND-014, a polylactide (PLA)-based nanoparticle bearing the cytotoxic drug docetaxel (Doxetaxel, Taxotere) to target solid tumors bearing prostate-specific membrane antigen (PSMA). Other nanoparticle-based approaches from Cambridge, Massachusetts-based Alnylam Pharmaceuticals and Warren, New Jersey-based joint venture MidaSol (Table 1) are also delivering promising early-stage data in hypercholesterolemia and diabetes, respectively. These human studies may provide the first proof of concept that new nanoparticle-

based modalities can optimize the pharmacological and therapeutic profiles of existing drugs. Current clinical-stage programs, however, will require a great deal more human data, and a lot more investment, before they can be viewed as a mainstream proposition for drug developers.

Thus far, the contributions of nanotechnology—loosely defined as modalities operating in the sub-100-nm range—to drug delivery have been limited to a handful of first-generation products, notably the cancer drugs Doxil (liposome-encapsulated doxorubicin, approved in 1995) and Abraxane (albumin-stabilized, nanoparticle-based paclitaxel, approved in 2005), which racked up sales of \$402 million and \$386 million in 2011, respectively. However, New Brunswick, New Jersey-based Johnson & Johnson (J&J) manufacturing problems with Doxil have led to substantial supply interruptions, illustrating at least some of the difficulties with manufacturing first-generation nanoparticles. Scott Minick, former president of Sequus Pharmaceuticals (now part of J&J), the original developer of Doxil, recalls that when the drug was first approved, around one batch in every three had to be discarded.

Minick, who is now CEO of BIND Biosciences, has encountered no such problems with the company's lead molecule BIND-014. "I think we started with a far more robust technology platform. Liposomes are a fairly fragile technology," he says. One big advantage of the latest generation of drug-carrying nanoparticles is improved scalability and consistency in terms of manufacturing. Better precision and control at the target level is another. "Doxil never had the targeting ligand that BIND-014 has; nor did it have controlled release," he says.

"The thing it had in common was the long circulation time."

The current generation of nanoparticles varies widely in size, chemical composition, surface charge, tissue tropism and sensitivity to external activators (e.g., light or temperature). A typical structure contains a hydrophobic, drug-bearing inner core, surrounded by a hydrophilic corona, which is decorated with targeting ligands. "The community has learned how to build these features in without annihilating other functions. That was a big issue early on in the field," says Mark Davis, professor of chemical engineering at California Institute of Technology, in Pasadena, California, and founder of Insert Therapeutics and Calando Pharmaceuticals. The 'rules' for understanding how nanoparticles interact with different organs are starting to emerge, he says, although for now, most of the available evidence has come from animal models. "There's virtually no human data on this."

BIND-014 is based on BIND Biosciences' Accurin platform, originally developed by nanomedicine pioneer Robert Langer, of the Massachusetts Institute of Technology (MIT), in Cambridge, Massachusetts, and Omid Farokhzad, of Harvard Medical School, in Boston. It consists of a hydrophobic core, based on polylactide polymers encapsulating docetaxel, surrounded by a hydrophilic corona of polyethylene glycol (PEG) bound to a small molecule, S-2-[3-[5-amino-1-carboxypentyl]-ureido]-pentanedioic acid, which targets PSMA, an antigen that is overexpressed in most solid tumors.

BIND-014 was not constructed *de novo*, according to rational design criteria, but was selected from a combinatorial library containing around 100 different nanoparticle variations, all based on the same basic building blocks, which have a long history of clinical use. "That's really enabled BIND to do what they've done very quickly because there are so many potential variables, such as release rate, degradation rate, size, surface characteristics, etcetera, that have to be explored simultaneously," Langer says.

Bind Biosciences

BIND's Accurins. These drug-bearing nanoparticles are decorated with ligands that recognize disease-associated cell surface proteins or receptors. The first such molecule, BIND-014, has now proceeded through human phase 1 testing.

IN brief

First plant-made biologic approved

Carrot cell bioreactors

The US Food and Drug Administration in May approved Eleyso (taliglucerase alfa), an enzyme produced in genetically engineered carrot cells, for treating type 1 Gaucher's disease. This is the first plant-made drug approved

by the regulators, and for Israeli company Protalix BioTherapeutics of Carmiel, it is the first product made in their ProCellEx protein expression system to reach the market. The plant cell platform produces recombinant proteins with a glycan and amino acid structure similar to naturally produced human counterparts. Some 10,000 patients worldwide have Gaucher's, a rare genetic disorder in which individuals fail to produce the enzyme glucocerebrosidase. Eleyso, a recombinant form of human glucocerebrosidase, which is injectable, replaces that enzyme and thus prevents lipids from accumulating in organs and tissues, eventually damaging the liver and spleen, leading to low red blood cell and platelet counts, and bone problems. Protalix and US partner Pfizer of New York are pricing Eleyso at a 25% discount from Genzyme's Cerezyme (imiglucerase), the market leader, a price they hope will persuade clinicians and patients to switch. They are also touting this platform for its dependability, pointing to a year-long spate of supply disruptions for comparable enzymes made by Genzyme, wholly owned by Paris-based Sanofi, to treat Gaucher's disease and Fabry's disease (*Nat. Biotechnol.* **28**, 994, 2010). Pfizer and partner are attempting to position Eleyso as the most reliable product on the market launching a "supply continuity program," whereby a 24 months' supply is maintained. They have also developed several programs to help individuals with Gaucher's disease to afford treatments with this new product. It may be too late, however, to gain a significant market share. A year ago, at the height of the supply disruptions affecting Genzyme's enzyme production, the Protalix-Pfizer product stood to gain a majority share, but regulatory delays in approving the plant-based product meant that that opportunity was lost. In a separate development earlier this year, Cambridge, Massachusetts-based Genzyme, a Sanofi company, announced four-year data from a phase 2 clinical trial for an orally administered version of its treatment for type 1 Gaucher's disease, eliglustat tartrate. Ireland-based Shire also has a competing injectable product for treating Gaucher's disease, called Vpriv (velaglucerase alfa).

Jeffrey L Fox

As well as protecting the drug from degradation—docetaxel, in its native form, is quickly broken down after administration—the nanoparticle needs to evade patients' immune responses. PEGylation, already widely used for stabilizing biologic drugs, is the most commonly used method. "It really creates this hydration shell around the particle. In essence, it looks like water in some ways," says Jeff Hrkach, senior vice president of pharmaceutical sciences at BIND. Early observations of patients with advanced or metastatic cancers indicate responses at dose levels of 30 mg/m², well below the traditional 75 mg/m² dose used for solvent-based docetaxel. At the other end of the spectrum, BIND-014 does not appear to be hampered by the same dose-limiting toxicities associated with traditional docetaxel therapy. "We're seeing effects you're not expected to see at any dose without killing people," Minick says. The dose-escalation trial is ongoing. "As soon as we have a dose, we intend to start our phase 2 study," he says.

The BIND-014 results are blazing a trail for the new generation of nanoparticle approaches. Elsewhere, the application of nanoparticles to the delivery of short-interfering RNA (siRNA) therapies is at an earlier stage proposition, for multiple reasons. "Some of the challenges include targeting; [but] you also have to maintain activity; you have to ensure there are no off-target effects; and you want to keep the siRNA dose as low as possible," Langer says. Nevertheless, Alnylam reported on April 19 the successful knockdown of the gene encoding pro-protein convertase subtilisin/kexin type 9 (PCSK9), an emerging target for managing high cholesterol levels, with a single dose of the siRNA drug ALN-PCS (*Nat. Biotechnol.* **30**, 302–304, 2012). The siRNA molecule is packaged in a cationic lipid nanoparticle (termed MC3) comprising *N*-[(methoxy poly(ethylene glycol)₂₀₀₀)carbamoyl]-1,2-dimyristyloxypropyl-3-amine (PEG-C-DMA), dipalmitoylphosphatidylcholine and synthetic cholesterol (US provisional patent application 61/185,800 2009) to which Cambridge-based Alnylam and its partner Vancouver, British Columbia-based AlCana Technologies have an exclusive license. (It is also the subject of a legal dispute with their erstwhile partner Tekmira Pharmaceuticals, of Burnaby, British Columbia.) MC3 exploits an uptake mechanism based on a liver cell receptor called apolipoprotein E.

The 80-nm ALN-PCS nanoparticle is able to gain access to the liver because of the presence of 'fenestrated' or discontinuous endothelium in the organ, which ordinarily

enables it to take up large lipoprotein particles called chylomicrons. It has long been known that tumors, because of their leaky vasculature, are also similarly permeable to nanoparticles, although this strategy is not generally applicable to all cancers or all stages of cancer. "That is a transient phenomenon in a transient phase of the tumor," says Mauro Ferrari, CEO of the Methodist Hospital Research Institute in Houston, Texas, and the co-founder of several nanotechnology firms. Other tumor therapies can influence this feature as well. "If you give a patient anti-angiogenic therapy, vascular leakiness is the first thing to go," he says. Ferrari is developing a multi-stage delivery technology, analogous to a multi-stage rocket—the starting point is a 600–800nm 'mothership' made from biodegradable nanoporous silicon—which sequentially sheds different components as it nears its target. It could reach the clinic as early as next year.

Efforts to target siRNA nanoparticles to organs other than the liver are less advanced, however. "The heart is not engineered to take up chylomicrons—you need a smaller particle to access that kind of tissue," says Alnylam CEO John Maraganore. "That work is going to need novel materials—and will also greatly benefit from targeting as well." Endosomal release of siRNA, once it is transported across the cell membrane, remains another challenge, which also requires novel materials, he notes. Alnylam is working with several partners on these efforts, including Daniel Anderson and Langer at MIT, with whom it developed a combinatorial library containing 1,536 structurally distinct nanoparticles (*Proc. Natl. Acad. USA* **108**, 12996–13001, 2011).

Alnylam is also working with a Vancouver-based firm, Precision Nanosystems, which is integrating microfluidics technology with a 'bottom-up' self-assembly approach, to develop a stable and scalable population of 1-palmitoyl, 2-oleoyl phosphatidylcholine, cholesterol and the triglyceride triolein nanoparticles in the 20-nm range to target extravascular tissues (*Langmuir* **28**, 3633–3640, 2012). Its NanoAssemblr platform is based on the work of scientific founder Pieter Cullis at the University of British Columbia, in Vancouver. "We can manipulate the mixing conditions to drive the self-assembly and the order of self-assembly," says co-founder and COO Euan Ramsay. "The key [to] this is very, very rapid and controlled mixing." Precision's desktop instrument is currently in testing before commercial release, which is slated for next year, says CEO and

Table 1 Selected nanoparticle-based programs in clinical development

Company	Technology	Description	Lead drug	Indication	Clinical status
Cerulean Pharma (Cambridge, Massachusetts)	Cycloset	Cyclodextrin nanoparticle conjugated to camptothecin	CRLX101	Non-small cell lung carcinoma	Phase 2
Alnylam	MC3	Liver-targeting lipid nanoparticle	ALN-PCS (PCSK9 synthesis inhibitor)	Severe hypercholesterolemia	Phase 1
Bind Biosciences	Accurin	Tumor-targeted nanoparticles containing docetaxel controlled-release polymers	BIND-014	Solid tumors	Phase 1
Calando Pharmaceuticals (Pasadena, California)	Rondel	Tumor-targeting, PEGylated cyclodextrin nanoparticle	CALAA-01 siRNA targeting M2 subunit of ribonucleotide reductase	Solid tumors	Phase 1
Liquidia Technologies	PRINT	Nanoimprint lithography method for precisely controlling nanoparticle size, shape and composition	LIQ001	Seasonal influenza	Phase 1
MidaSol	MidaForm, PharmFilm	Ultrasmall gold glyconanoparticles combined with buccal drug delivery system	MidaForm insulin	Diabetes	Phase 1
Nanobiotix (Paris)	NanoXray	X-ray activated hafnium oxide crystal nanoparticles enhance tumor destruction by radiotherapy	NBTRX3	Solid tumors	Phase 1
Selecta Biosciences (Watertown, Massachusetts)	Synthetic vaccine particle	T-cell epitope encapsulated in a polylactic acid-co-glycolic acid core surrounded by a lipid monolayer and a PEG shell decorated with a B-cell epitope	SEL-068	Smoking cessation	Phase 1
Tekmira Pharmaceuticals	Lipid nanoparticle technology	Apolipoprotein B encapsulated in ionizable aminolipids, such as 1,2-dioleoyl-3-dimethylammonium propane	TKM-ApoB	High cholesterol	Phase 1
DLVR Therapeutics (Toronto, Ontario)	High-density lipoprotein-like peptide-phospholipid scaffold	Nanoparticle mimic of high-density lipoprotein	Gemcitabine and bende-	Oncology	Preclinical
Biomolecular Therapeutics (Suwon, South Korea)	lasiRNA	Second-generation asymmetric siRNA, chemically modified for direct cellular uptake	Connective tissue growth factor inhibitor	Surgical scar prevention	Preclinical

co-founder James Taylor. “What we’re trying to do is democratize the development and use of nanoparticle systems, which is otherwise solely in the domain of experts,” he says.

Liquidia Technologies, of Research Triangle Park, North Carolina, is also seeking to streamline manufacturing, by mimicking production methods from the electronics industry. It has developed a lithography process—called pattern replication in non-wetting templates (PRINT)—which employs a molding procedure rather than self-assembly to generate nanoparticles with precise size, shape and chemical composition. “It’s like a nano-muffin or a nano-ice-cube tray,” says CEO Neal Fowler. The approach, relies on fluoropolymer-based materials that are liquid at room temperature but solidify after their application to a finely etched silicon wafer. “Every one of our particles is exactly the same,” says Fowler. The approach eliminates the size distribution problem that has dogged other nanoparticle production processes. It is also readily scalable, by means of a roll-to-roll manufacturing process. “We can make many linear feet per minute of particles,” he says. The company is focusing

on vaccine development initially, although the technology is generally applicable to any therapeutic modality. It is consciously steering clear of siRNA as a payload. “We’ve made a strategic decision not to go there because it’s such a long road,” says Fowler.

Delivering insulin systemically offers no such challenges, however. MidaSol Therapeutics, a joint venture between Oxford, UK-based nanotechnology firm Midatech and Warren, New Jersey-based drug delivery specialist MonoSol Rx, has cleared its first clinical hurdle in its bid to develop a gold-based nanoparticle formulation of insulin that offers a novel delivery route. A phase 1 trial of its MidaForm insulin, which is administered in a soluble strip that can be attached to the inside of the mouth, achieved its objective of demonstrating both the safety and tolerability, and the rapid onset of activity of the gold-based glyco-nanoparticle. It exploits an osmotic gradient across the buccal mucosa and results in a rapid systemic delivery of insulin, with a peak plasma concentration time of 5–8 minutes, in marked contrast to insulin analogs or native insulin, which can take 1–2 hours to be

absorbed. “It’s an osmotic draw, an osmotic injection,” says Midatech CEO Thomas Rademacher.

The nano-based insulin, originally developed by researchers in Spain, binds, through van der Waals forces, 16 insulin monomers and results in a particle that is just 5.4 nm in diameter. It is sufficiently small to pass through the pores of the oral cavity, which are 8–9 nm wide, and enter the heavily vascularized tissue of the cheek. MonoSol has already commercialized two other drugs using its PharmFilm technology, which is based on components that have GRAS (generally recognized as safe) designation, with a fixed width and thickness. “The dose of any drug using our film is driven by dimensionality,” says MonoSol Rx CEO Mark Schobel. “The stoichiometry is very clean and very predictable.” The company completed its first trial, in 28 healthy volunteers, in Switzerland. It is seeking a partner before embarking on phase 2 studies. If MidaSol does manage to strike a deal, it could be a harbinger of more investment activity in what is still a nascent drug development niche.

Cormac Sheridan *Dublin*