Poised to branch out

Although dendrimers have not yet taken the drug industry by storm, biomedical research and industrial applications of these tiny, highly branched molecules continue to grow. Vivien Marx reports.

As with any other groundbreaking experimental treatment, progress of the first dendrimer drug, VivaGel (SPL7013), through the clinic has not been entirely smooth sailing. Last October, VivaGel's manufacturer, Melbourne, Australia–based Starpharma, agreed to 'pause' the phase 1 trial of its microbicidal drug to get a better handle on toxicities and refine trial design. But the legion applications of the versatile dendrimer platform mean that dendrimer use both in biomedical research and in the chemicals and materials industries has continued to grow. Just in April, Starpharma entered into an agreement with London-based Unilever to co-develop dendrimers as imaging agents to analyze the microscopic structure of foods. Elsewhere, dendrimers are being applied as novel coatings for medical supplies (from catheters to condoms), biomimetics for sutureless wound healing, diagnostics for acute coronary syndrome, transfection reagents, drug delivery vehicles and imaging contrast agents. Ultimately, the key factors in determining the wide adoption of dendrimer technology will likely center on reducing the cost and complexity of their manufacture and confirming their biocompatibility and safety in humans.

No gold rush

Dendrimers—nanometer-scale molecular bushes of highly branched, synthetic polymers—have real-world versatility: not only can they be tailored in size, shape, solubility, charge or flexibility, but also functional end-groups can be attached to the branches, creating many reactive sites. Among the inner branches, specific protected cavities with their own microenvironments can be created for different payloads, to be released upon arrival at a specific destination. This is all possible because variations in the number of 'generations' of synthesis (**Fig. 1**) of dendrimers give researchers precise control over the size and surface charge of the molecules.

The term 'dendrimer'—based on the Greek *dendra* for tree, and *meros* for part—was first coined by Donald Tomalia, at the time a polymer chemist at Dow Chemical in Midland, Michigan. But it was chemist Fritz Vögtle and his group at the University of Bonn that were first able to synthesize dendrimers in 1978 as they tinkered with multi-armed molecules. At the time, Vögtle's university did not even have a patent office. Despite failing to lay claim to the patent landscape, Vögtle says he would not regret missing out if dendrimers were ever to set off a gold rush. It is the "dream of every scientist" to see one's work come to fruition, he says. It is a dream he and others, including Tomalia, Robert G. Denkewalter at Merck in Whitehouse Station, New Jersey, George Newkome of the University of Akron in Ohio, Jean Fréchet of the University of California, Berkeley, James Baker of the University of Michigan in Ann Arbor and many others in the field have nurtured, as the field has alternated from irrational exuberance to disappointing setback.

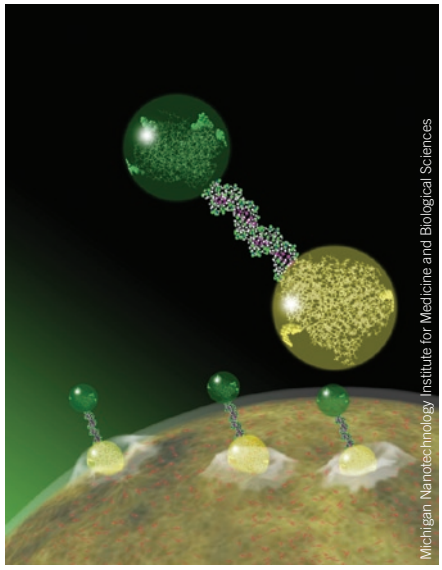

Figure 1 A conceptualization of DNA-linked dendrimers being internalized by a cell

Certainly, among all the nanomaterial platforms—fullerenes, nanotubes, quantum dots, nanowires to name a few—dendrimers appear to be at the forefront in terms of near-term potential in both the biomedical and industrial worlds[1]. But their promise has yet to materialize into commercial success, despite the fact they've been around for over 20 years. Global dendrimer revenues in 2007 were only $9.5 million, according to nanotech venture capital firm Lux Research of New York[2]. Meanwhile, dendrimer experts in the biomedical field eagerly await a success story to propel the field forward.

Viva VivaGel!

Starpharma hopes one of those success stories could be VivaGel (**Table 1**). This drug is a topical, vaginal microbicide for preventing transmission of HIV and herpes simplex virus (HSV), the cause of genital herpes. In VivaGel, the dendrimer is not used to deliver a drug, the dendrimer is the drug. The product is a water-based gel formulation of a lysine-based dendrimer, with naphthalene disulfonic acid surface groups and a polyanionic outer surface and end groups that presumably bind to the proteins on the outer surfaces of either virus, blocking their cellular uptake. *In vitro* and animal studies have shown that this mechanism thwarts both HIV and HSV-2. The drug was fast tracked by the US Food and Drug Administration in January 2006.

As a microbicidal product, VivaGel could be particularly timely after the failure of the current crop of HIV vaccines in the clinic. Indeed, microbicides are a useful alternative line of attack against HIV/AIDS, according to the University of Pittsburgh Medical Center's Ian McGowan, who is the principal investigator of the Microbicide Trials Network—one of several microbicide trial networks established in 2006 by the Division of AIDS of the National Institute of Allergy and Infectious Diseases.

But VivaGel has also raised some toxicity flags in humans. After Starpharma launched human studies of VivaGel a year ago, the trial was paused only three months later. "We were seeing genital adverse events of low-grade severity," says McGowan, such as genital irritation and discharge. These events are "incredibly common" in phase 1 microbicide studies, he adds. After all, "you are asking women to put an unusual product in their vagina and then ask them how they feel about it."

The trial is now being amended to include a so-called universal placebo, a gel that will allow better comparison across microbicide trials. McGowan could not provide a timeline for the changed protocol or a date for when the trials would be resumed.

Jackie Fairley, Starpharma's CEO, says the pause is "disappointing," but that it was a decision mutually agreed upon and not equivalent to either a trial suspension or a halt. She adds that data from a second VivaGel phase 1 trial in San Francisco and Kenya in young women who administered VivaGel to themselves twice daily showed no severe adverse reactions. The dendrimer was not systemically absorbed—it was not found in the bloodstream of any of the trial participants—and similar percentages of women from the experimental and control groups showed localized genital irritation.

Table 1 Starpharma pipeline/research partnerships

Developer	Product	Dendrimer type
SSL International (London; makers of Durex) and undisclosed partner	VivaGel microbicide for prevention of HIV and HSV-2 transmission; condom coating	Polylysine
Stiefel Laboratories (Research Triangle Park, North Carolina)	Cancer drug delivery; dermatological drug delivery	Polylysine
Internal program	ADME engineering for Starpharma internal protein drug candidates	Polylysine
Undisclosed partnership/projects	Enhanced solubilization of drug	Polylysine and PEA
Siemens	Stratus CS assay device	PAMAM
Baker Heart Research Institute/ NCI	X-ray angiography contrast agent and magnetic resonance imaging contrast agent	Polylysine
Qiagen (Venlo, The Netherlands)	Gene transfection reagents	PAMAM
EMD/Merck (Madison, Wisconsin)	Small interfering RNA/DNA transfection reagents	PEA and other dendrimers

Toxicology concerns recede

Thus far, toxicology hasn't appeared to be a major concern for the dendrimer field. On the basis of data from animal experiments and cell culture work—most of which has looked at basic polyamidoamine (PAMAM) or 'Starburst' dendrimers—the immunological properties look "very promising," according to Marina Dobrovolskaia, an immunologist at the National Cancer Institute's (NCI's) Nanotechnology Characterization Laboratory (NCL). NCL is operated by US government contractor Science Applications International located in San Diego and provides services in characterizing toxicology, chemistry and the immunological impact of nanomaterials.

Overall, immunological data on dendrimers is still accumulating, but some rules are starting to emerge, even if it is impossible to make generalizations for the therapeutic class as a whole, she says. Positively charged dendrimers induce blood clots[3]. In addition, according to Dobrovolskaia, cationic platforms are more toxic due to their interaction with the cell surface. They also call the immune system to action more than anionic or neutral species, she says. By chemically capping surface amines, however, dendrimer toxicity can be reduced to the point at which dendrimers with <40% surface amines do not react with cells *in vitro*, she adds.

In the two animal studies carried out with dendrimers so far, researchers "could not detect any anti-dendrimer–specific immune response, which is a promising trend." Dobrovolskaia cautions, however, that the final proof of safety must await clinical studies.

Packing a punch

A favorable toxicological profile could augur well for one of the most touted applications of dendrimers: their use as drug and gene delivery vehicles. Already, the use of dendrimers to enhance the delivery of chemotherapies, such as cisplatin and doxorubicin, has been demonstrated in several studies. Perhaps the most prominent of these prospects is ATI-001, a potential treatment for epidermal cancers under development at Ann Arbor, Michigan–based biotech Avidimer (formerly Nanocure).

ATI-001 is an injectable PAMAM dendrimer to which four to five folic acid molecules and five to six methotrexate molecules are covalently attached, producing a targeted dendrimer (which the firm terms an 'avidimer'). As epidermal cancers commonly overexpress folic acid receptors, covalently attaching folic acid to the PAMAM dendrimer via a thiourea and amide linkage allows specific targeting of the drug payload to cancer cells. Methotrexate is a drug already approved for the treatment of skin, head and neck or lung cancer, severe psoriasis and adult rheumatoid arthritis. As it directly competes with folic acid, Larry Sternson, Avidimer's CEO, hopes that delivering methotrexate via folate acid receptors will turn out to be an effective strategy.

"It's about 6–8 months from initial clinical trials," says Sternson, who previously worked on novel drug delivery technologies at Elan (Dublin).

Thus far, the preclinical research looks promising, at least according to the NCI benchmark of showing tumor shrinkage in at least 50% of samples relative to controls. "We are absolutely meeting and exceeding the NCI standards," says Sternson. "While methotrexate may be an old poison, when attached to a dendrimer with a proprietary targeting vector, it becomes a new drug," he says. Previously a lack of specificity has limited methotrexate's use.

The technology behind this lead candidate originates from the group of University of Michigan researcher and Avidimer founder James Baker. But the underlying chemistry is a PAMAM dendrimer type first pioneered by Donald Tomalia. During much of the 1990s, it was biologist Baker who collaborated with chemist Tomalia in pushing the envelope on dendrimer technology. Together they hold much of the intellectual property (IP) behind dendrimers (**Box 1**).

Drugs and beyond

For dendrimers to capture the attention of the chemical, materials and food industries, however, the high cost and complexity of dendrimer manufacture remains a problem. Dendrimers have taken a long time to emerge because, initially, they were difficult to synthesize and extremely expensive, says Antony D'Emanuele of the University of Manchester in the UK. Jørn Bolstad Christensen of the University of Copenhagen echoes the concern. The complex chemistry can dampen popularity because not everyone intrigued by dendrimers is a synthetic chemist, he says. A good post-doc can take a few months to make his or her own material. "If you can buy it [at a reasonable cost], things become much easier," he adds.

Dendrimers have made it into catalogs and are a "steadily growing" segment within nanomaterials, says Ilya Koltover, product manager of materials science at St. Louis–based Sigma-Aldrich Corporation. For example, researchers can order 10 grams of hyperbranched polymers for around $100. Two grams of PAMAM, one of the most common dendrimers, costs around $450. "There has been good pick-up in recent years," he says, but declined to give sales figures.

How the cost factor plays out depends on the application. As industrial catalysts, for example, dendrimers may be too expensive, says Anil Patri, deputy director of the NCL. But if only about one gram of a cancer therapeutic is required, "it is commercially viable, because we don't have any other promising technologies," he says.

"It is a question of scale," Patri adds. While at Bayer in Leverkusen, Germany, chemist Harald Pielartzik (who is now in technology transfer) explored automation approaches with various academic groups. Synthesis robots and automated purification systems can handle dendrimers, he says. "For a pharmaceutical application, the yearly requirement might be a kilo; in plastics production, I have to think about how many tons I need, that makes it a little more difficult."

Cheryl Barton, head of the Chichester, UK–based consultancy Pharmavision, explains that a balance will need to be struck between cost of goods and any increase in potency associated with dendrimers. By reducing off-target effects, dendrimers might require less of the actual active ingredient. Péter Krüger, head

of Bayer's working group in nanotechnology, agrees: "If an active ingredient is extremely expensive and its effectiveness can be increased by 50% such that you need only half as much of the active ingredient…then you can pay for the so-called Trojan horse that will drive this active ingredient to its destination."

Decreasing cost of goods

In this context, there has recently been some promising news on the manufacturing front. Tomalia has now shifted his focus from PAMAM dendrimers to younger members of the dendrimer family: polylysine-based dendrimers, for example, and a newer type called Priostar (polyester-acrylate/amine PEA dendrimer). In a paper published in the *New Journal of Chemistry* last year, Tomalia and his group described the synthesis of generation-2 PEA dendrimers[4]. "This could be the architecture that really makes dendrimers a viable drug delivery mechanism," he says.

"We've standardized certain components that we are able to 'click' together, if you like, or hook together very simply and very quickly and so we basically can have a dendrimer factory with all of these parts sitting right next to an assembly line," Tomalia says. "So, if we want to assemble a dendrimer that had this feature in the core and this feature in the interior and this feature on the surface, we can start clicking these parts together."

Dendritic Nanotechnologies, a Mount Pleasant, Michigan-based subsidiary of StarPharma where Tomalia is chief technology officer, believes that PEA dendrimers could provide significant cost savings over PAMAM dendrimers, which can cost thousands of dollars per gram, eight reactions to produce and take a month to manufacture. The company claims a PEA dendrimer equivalent to a generation-5 PAMAM dendrimer can be made in three steps over five days. The key difference is that PEA dendrimers have more surface groups per generation than PAMAM dendrimers. This means that lower generation dendrimers are needed to produce equivalent polyvalency. Company literature indicates the PEA dendrimers show "enhanced thermal and hydrolytic stability, longer shelf life and enhanced solubility" compared with the older chemistries.

In March, another group at Texas A&M University in College Station announced a new synthetic route to produce dendrimers "in kilogram quantities." The synthesis revolves around the controllable reactivity of their dendrimer's triazine core—a six-membered ring of alternating nitrogen and carbon atoms functionalized with three chlorine atoms. According to Abdellatif Chouai and Eric Simanek, the first chlorine from the triazine can be displaced in minutes at 0°C, whereas displacing the third requires up to 24 hours of heating. This allows the dendrimer structure to be built up progressively. Similar to the PEA work, however, it remains unclear whether this chemistry will make dendrimer synthesis sufficiently amenable to university and industrial researchers to galvanize interest in the area.

Imaging, delivery and diagnostics

Apart from therapeutics, the other area of biomedicine where dendrimers show particular promise is imaging and diagnostics. The unique ability to control the size of a dendrimer enables delivery of an imaging (or therapeutic) payload to a particular compartment. Dendrimers up to 15 nm in size are recognized by the reticuloendothelial system, whereas 12-nm molecules remain in the circulation. Smaller sized (~3 nm) PAMAM dendrimers easily traverse vascular walls resulting in rapid perfusion throughout the body, whereas those 6 nm in diameter localize in the kidney, creating the opportunity for renal contrast agents.

These same properties make dendrimers useful for delivery of contrast agents, drugs or genes, even without a targeting agent. This is spawning a sideline of dendrimer reagents for the research community, a trend exemplified by the launch in April of a PEA dendrimer for transfecting cells developed via a research collaboration between StarPharma and EMD Biosciences of Madison, Wisconsin. Although dendrimers between 2 and 10 nm in size do appear to boost transfection in a variety of cell lines, it is not yet clear how this works.

According to NCL's Patri, it is the ability to exquisitely control dendrimer size that makes them particularly attractive for contrast agents in imaging. "If you can target and make the tumor light up more brightly when it is small, that is early detection…you can see brighter images with less of the contrast agent," he says. Animal studies indicate dendrimers manage this well, but some challenges remain including the synthesis of these complex dendrimeric contrast agents.

By attaching a targeting agent (such as an avidin, a monoclonal antibody or an aptamer) to seek out a particular target, dendrimer-based contrast agents can be made tissue specific, as has been shown by researchers at the University of Illinois and NCI[5]. In addition, dendrimer branches can hold many contrast-lending ions, creating a stronger signal, says the University of Copenhagen's Christensen.

Outside the research community, at least one contrast agent has thus far been considered for commercial development. Schering

Box 1 Locking up the IP

Dendritic Nanotechnologies' Donald Tomalia is one of the pioneers of the dendrimer field. Together with the University of Michigan's James Baker, he controls most of the IP behind dendrimers.

In 1992, Tomalia left Dow Chemical to found Midland, Michigan–based Dendritech. Nine years later, when Dendritech folded, Dow granted Tomalia global licensing rights to dendrimer applications in return for waiving future royalty rights; this created the basis for another Tomalia startup, Dendritic Sciences of Mount Pleasant, Michigan. Two years later, Starpharma got together with Tomalia to form a joint venture, Dendritic Nanotechnologies, headquartered in Melbourne, Australia with production and laboratories in Central Michigan University's Center for Applied Research and Technology in Mount Pleasant.

From 1985 to 1995, Dow Chemical and Stamford, Connecticut–based Xerox were dominant dendrimer patent holders, the latter patenting the use of dendrimers in toner and ink dispersion; Bayer and DSM of Heerlen, The Netherlands, also were granted patents for the use of dendrimers in plastics manufacturing and other nano-based products. In 2005, Dow Chemical assigned its entire dendrimer IP to Dendritic Nanotechnologies in exchange for an equity stake in the firm. Then, a year later, Starpharma acquired Dendritic Nanotechnologies for $6.97 million in shares. Thus, Starpharma is currently the dominant dendrimer patent holder.

"I believe they have up to 90% of the patents," says analyst/consultant Cheryl Barton. With a total of 224 patents and applications, StarPharma is also the only company with a dendrimer in clinical trials. "As far as I know they are still out in front, nobody else is close to bringing its dendrimer along as far as they have," says Steven Rutt, IP attorney at Foley & Lardner in Washington, DC. But he still sees room to patent, stating "in an area like dendrimers, where there are so many things to tinker with, it allows you to come up with new improvements." Another dendrimer researcher, Harald Pielartzik, formerly of Bayer, agrees with Rutt: "When industry finds an application, then it's completely normal to license a technology, talk to each other, cooperate. I don't see that as a barrier."

(now part of Bayer) developed a dendrimer-based imaging agent gadomer-24, which links 24 gadomer-complexes onto the ends of the spherical branches of the dendrimer[6]. It is not yet clear, according to Krüger, whether the company will go forward with commercializing the product.

Elsewhere, it is the area of diagnostics that has provided the first marketed dendrimer product. Deerfield, Illinois–based Siemens Healthcare Diagnostics' Stratus CS is a solid-phase radial partition immunoassay technology for measuring (among other things) troponin I in whole blood samples. The assay uses a capture antibody added to a glass fiber paper linked to the PAMAM dendrimers, to which the sample and the detection antibody are added. Pratap Singh, assay development scientist in Siemens's R&D department, did the original work on the Stratus CS while at Dade Behring, which Siemens recently acquired. In their original assay development, Singh and his colleagues had tried to affix capture antibodies on the solid surface in the correct orientation for the antigen-binding sites. He had also been seeking material with a high degree of quality control in manufacture. "It was a nightmare; half of the product had to be discarded," he says.

Dendrimers, with their controlled architecture and synthesis, provided Singh with an answer. The PAMAM dendrimers optimize both presentation and functionality of the capture antibody, leading to more efficient binding of the target antigen. According to Singh, the device has also "been one of the least troublesome product lines in terms of reagent making." Pharmavision's Barton views the device as proof of the potential of dendrimer technology. "It has semi-proved itself there," she says.

Siemens would not disclose sales numbers but Singh proudly states that it is the first and only marketed dendrimer-based assay device. Intended for use in urgent care facilities, the device tells emergency room doctors whether a patient's blood contains troponin I, a tell-tale indicator of heart attack, within 15 minutes and at sensitivity levels approved by the American College of Cardiology. Now with almost 15 years of experience, he believes the future looks bright for this technology in diagnostics.

The time has come?

A final thought is that dendrimers, like other nanomaterials, may provide the biotech and big pharma sectors with new opportunities for second-generation, 'souped up' versions of existing products. According to Barton, in a climate of pharmaceutical patent expiration, new formulations using nanocrystals, nanosuspensions and nano-carriers, such as dendrimers, may be useful for extending product life cycles. By 2015, she estimates nano-enabled products could generate sales in excess of $3.4 billion driven by the uptake of innovative drug delivery systems and sales of biocompatible nanoparticle coatings (e.g., for medical devices or dental implants).

But for now, the immediate fate of dendrimers may lie with VivaGel. "If VivaGel shows it's got the goods, I am sure interest in dendrimers will increase," says Barton. The technology cannot garner enough attention until it proves its worth and gets to the clinic. "Then everyone wants to know you," she says. "Dendrimers are probably a classic of that situation."

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