

Ion-Exchange Membrane Technology Targets Antisense Production Challenge



“Mustang Q” 1-L process module.

An emerging family of biotech drugs promises to revolutionize the treatment of life-threatening diseases. “Antisense” drugs work at the molecular level by binding to messenger RNA—interrupting the process by which disease-related proteins are produced. Antisense drugs have been shown to inhibit the production of faulty proteins responsible for cancer, AIDS-related afflictions and cardiovascular diseases. And because they are highly-targeted therapies, antisense drugs should minimize patient side effects.

However, as companies now work to move many of these drugs forward from development and clinical trials into production, they face a number of challenges, one of which is the need to quickly and effectively purify large quantities of the antisense oligonucleotide active ingredient from very chemically similar production byproducts. Already, there are more than 25 antisense drugs in clinical trials, and

significant bottlenecks may occur in full-scale manufacturing if traditional purification methods are relied upon.

To help address this challenge, Pall Corporation (East Hills, NY) is proposing a novel new purification technology. According to Ajay Lajmi, PhD, senior research scientist with Pall Corp., the company’s “Mustang Q” ion-exchange

membrane chromatography technology can provide a tenfold increase in antisense drug production speeds compared to conventional column chromatography.

The first step in the preparation of an antisense drug is the construction of the oligonucleotide active ingredient using automated DNA synthesizers. As explained by Lajmi, this process poses a special challenge since, as the antisense molecule is synthesized, a number of impurities are also created that are difficult to separate from it due to their molecular similarity.

Antisense oligonucleotides are synthesized by linking nucleotides, one at a time, to build a complementary sequence to the targeted messenger RNA (mRNA) strand. However, since all of the individual synthetic reactions don’t go to 100% completion, a fraction of the building block molecules don’t become attached, resulting in incomplete oligonucleotide molecules. These are called “failure sequences.” So, for

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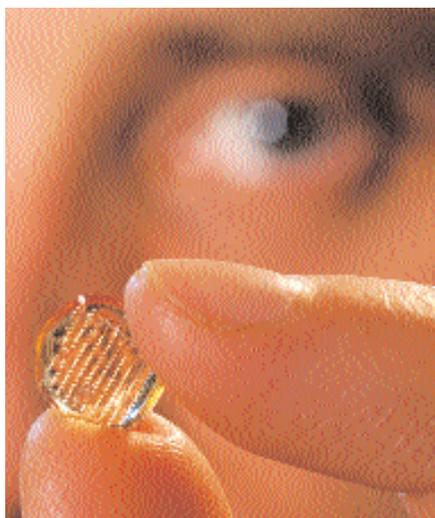
Microneedles Open New Doors for Transdermal Drug Delivery

They may be painful, but hypodermic needles have long been a reliable drug delivery method. However, recent advances in biotech-based pharmaceuticals have made the centimeter-size of traditional needles out-of-scale with the nano-sized active ingredients they must now transport. To address this issue, researchers at the Georgia Institute of Technology (Georgia Tech, Atlanta, GA) are developing a new, microscopic injectable drug delivery method that can target specific layers of the skin.

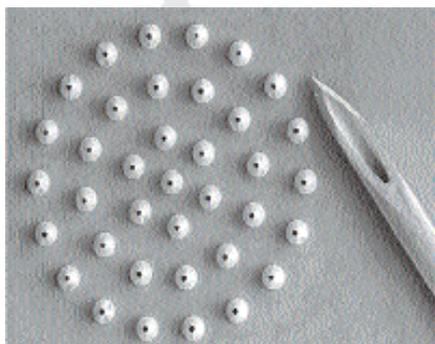
The novel dual-delivery method combines the advantages of hypodermic syringes and transdermal patches. Composed of dozens to hundreds of hollow microneedles, a 1–2-cm² transdermal patch is applied to the skin to increase its permeability. An array of microneedles that are 100–1000 μm in length poke through the top layers of skin and allow micron-scale drugs to pass into the body. Rapid delivery could be achieved by coupling the microneedles with an electrically controlled micropump that delivers medications at prescribed times. The pump would include an interface that allows patients or healthcare providers to control the amount of drug delivered. Because the needles are too small to stimulate nerve endings, patients wouldn’t feel any pain when a microneedle injection is performed.

According to Mark Prausnitz, associate professor in Georgia Tech’s School of Chemical and Biomolecular Engineering, a key advantage to this delivery method is that the microneedles and pump are external to the body at all times. Whereas traditional delivery

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Mark Prausnitz, a professor in Georgia Tech's School of Chemical and Biomolecular Engineering, holds an array of polymer microneedles that are approximately 1000 μm tall.



Microscopic view of an array of microneedles shown next to the tip of a typical hypodermic needle.

methods make it very difficult to interact with a drug once it enters the body, the new technology allows the timing and dosage of a drug to be adjusted while a patient is receiving the treatment. "The microneedle delivery method would be particularly effective for changing the dosage of insulin or pain medications as more or less treatment is needed," Prausnitz states.

The ability to target specific levels or depths in the skin is another unique feature of the microneedle technology. "One of the limitations of current hypodermic delivery techniques is they aren't able to localize drug in the top layers of skin," Prausnitz points out. "They affect tissue deeper down, and as a result, they cause pain, irritation, or other problems." In addition to eliminating patient discomfort, the Georgia

Tech is particularly interested in using microneedles to reach the upper, capillary-rich layers of the skin where drugs would be more readily absorbed and special dendritic immune cells reside. For this reason, the team believes that microneedle delivery of more effective vaccinations is a potential application for the technology. In addition, there are several practical advantages for vaccine microneedle delivery. "Microneedles could work well for mass vaccinations because they're cost-effective, could be applied by individuals with minimal training, and may require smaller doses of the drug," Prausnitz states.

The Georgia Tech research team anticipates that drug formulations would need to be altered for use with this delivery method. Because microneedles are significantly smaller than traditional hypodermic needles, it would be impossible to rapidly inject the same quantity of solution through a microneedle. "The most likely consequence on formulations is that they'd need to be more concentrated so that smaller doses could be used," notes Prausnitz.

Georgia Tech is also experimenting with ways to cost-effectively manufacture their microneedle technology. The research team recently fabricated molds of their silicon microneedles that can then be used to produce arrays of identical metal or polymer microneedles using a modified form of injection molding. "In many cases the molds are reusable as many as 100 times," says Prausnitz. Because the metal and polymer materials are inexpensive and the injection molding techniques don't need to be performed in a cleanroom, the single-step molding process can be easily adapted to industrial mass production. The molding technique has the potential to produce billions of microneedle arrays each year for as a little as five cents per array. To bring the technology to market, the research team licensed their intellectual property to an outside company that is in the process of performing clinical trials that will assess the ease and efficacy of microneedle-based delivery.

Kaylynn Chiarello

Taking PAT to the Next Wavelength

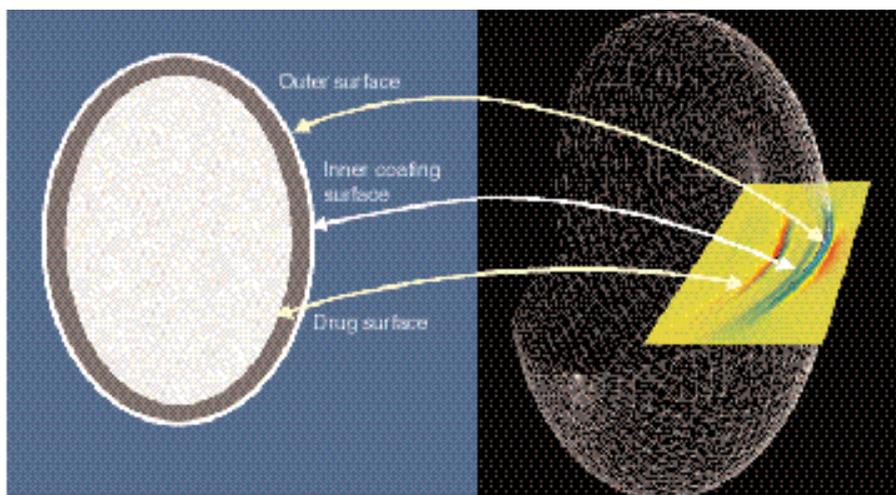
As listed on the FDA's web site, the top 10 reasons for pharmaceutical product recalls from 2000 to 2002 include subpotency and failure of a drug to dissolve properly. To deal with these and other quality issues, pharmaceutical scientists have long-used several forms of imaging, such as near-infrared (near-IR), mid-IR and, more recently, Raman spectroscopy to conduct various types of quantitative and qualitative analysis. All of these techniques, however, already have shortcomings including inadequate image resolution and the need for destructive testing. And, with the implementation the FDA's Process Analytical Technology (PAT) initiative, the demand for improved analytical measurement and analysis techniques will continue to grow.

One example of the need for more powerful tools can be readily seen in coatings analysis. As observed by Paul Davies, PAT business development manager at Bruker Optics (Billerica, MA), "Coatings are often essential to ensuring proper dissolution, yet some coatings are only 50–100 μm apart, which makes them difficult to map." Common imaging methods such as mid-IR and near-IR spectroscopy, for example, will provide only surface-level images of a test surface. "That means you have to cut a tablet in half and then map and image the exposed surface. And, for something like material distribution analysis, you'll have to repeat the process several times," notes Davies.

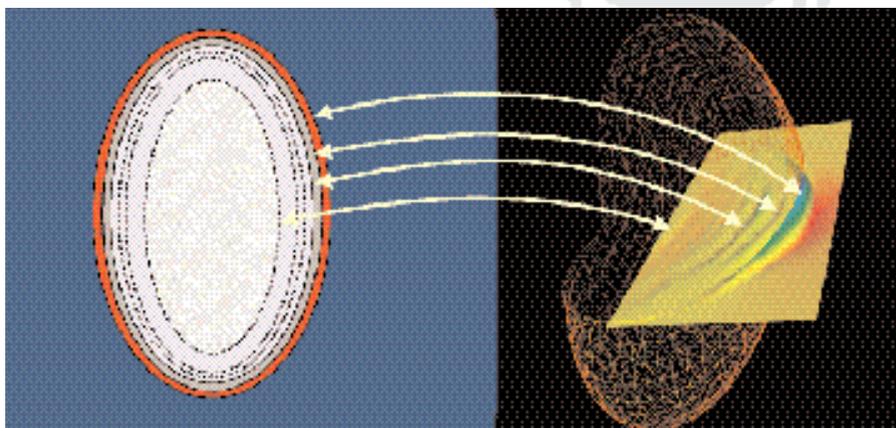
Now, however, scientists are expanding their analysis toolbox into the terahertz frequency range. Terahertz wavelength (1–0.1 mm) signals can penetrate solid objects to produce a high-resolution, three-dimensional map quickly and nondestructively. For example, the technique can be used to map a series of coatings that have been applied to a tablet. Says Davies, "Because this technology can provide images of cross sections that are only 20 μm apart, you can look at several layers of coatings very easily and quickly."

Bruker Optics (Billerica, MA) and





Terahertz spectroscopy can produce a three-dimensional map of a tablet without destroying it.



Because the instrument is capable of mapping cross sections only 20 mm apart, even complex layered coatings can be characterized.

TeraView (Cambridge, UK) have recently introduced a new spectrometer operating in the terahertz frequency range, the "TPI Spectra Series." The system works by scanning the target object with a terahertz frequency pulse signal. When the signal meets a change in density or chemical properties, there's a

measurable change in the reflective signals that are returned to the detector. By interpreting these signals, the system creates a map of the surface that shows where the density changes occur. "One pulse can be sent every 19 femtoseconds," notes Davies. "That means you can get your image very quickly."

The system images an object such as a tablet by mapping a series of vertical and horizontal cross sections without ever cutting the tablet. "When you use an approach that requires physical cutting, you're limited by how thin a slice you can make," continues Davies, "With terahertz scale imaging, you can get images of vertical cross sections that are ~75 μm apart and horizontal cross sections ~20 μm apart. This allows the system to produce a three-dimensional image." The system is also fast; in some cases, it can produce a three-dimensional map of the entire tablet in less than a minute. Because of the speed of this imaging method, it offers the potential to test a higher number of samples per batch, even at-line, without slowing down manufacturing processes.

The data provided through terahertz spectroscopy can allow formulation scientists to detect changes in content uniformity, which can indicate changes in chemical properties related to matrix stability, the concentration and distribution of active ingredients, and polymorph concentration—the occurrence of a compound in more than one crystalline form. Polymorphic characteristics frequently have an impact on various important parameters such as thermal and mechanical stability and bioavailability. "With terahertz spectroscopy, you can examine the finished product to determine the distribution of polymorphs within it," comments Davies.

Laura Bush

Advanced Complex-Sugar Technology Aims For Sweet Rewards

Momenta Pharmaceuticals (Cambridge, MA) and generics manufacturer Sandoz Inc. (Princeton, NJ), a Novartis company, have formed a strategic partnership based on Momenta's structure-activity-relationship (SAR) analyses technology that allows the characterization, sequenc-

ing, and engineering of complex sugars (polysaccharides). SAR relates the structure of a molecule to a specific activity or drug property. Momenta plans to apply the technology toward improving the drug delivery properties of existing products and on developing biogenerics.

Complex sugars play a fundamental role in several biochemical functions, including cell differentiation, growth, and cell communication. Most important, several biotherapeutics are coated with sugars that regulate protein activity and stability. Through better understanding of specific sugar sequences, the chemical makeup of complex sugars (including therapeutic polysaccharide-based mixtures and the sugars that are attached to therapeutic proteins), and

the related biological activity, the delivery of proteins and small molecules across mucosal membranes can be facilitated.

"The foundation of being able to understand biology and develop drugs is SAR," says Alan Crane, CEO of Momenta. "We can do it with small molecules, we can do it with proteins and DNA, but we haven't been able to do it with sugars because the 'S' part of the SAR equation has been missing." By fully understanding the SAR, a specific set of sugars can be "teased out" to activate a mechanism for delivering large-molecule protein drugs noninvasively in a rapid, reversible, and highly mechanistic means across a mucosal membrane. In addition to its program for addressing the noninvasive delivery of proteins, Momenta has a program for engineering glycoproteins to favorably affect therapeutic properties. As Crane observes, "We can see what sugars get cleared more rapidly, which more slowly, and which get targeted to specific tissues. We can determine which sugars play which roles in activating or modulating proteins."

Momenta's SAR technology encompasses a bioinformatics framework that enables the company to enter into a computer every possible sugar that can be made by a biological system and to mathematically reduce the number of possibilities to identify one solution. A second component applies proprietary analytics, including a panel of more than 120 restriction enzymes for sequencing sugar in a manner similar to the way restriction enzymes sequence DNA. Finally, the company uses patented improvements in both mass spectrometry and in NMR analysis methods.

According to Crane, the two nearest term opportunities for its technology are improving the properties of an existing drugs and creating biogenerics of existing products. The company already has plans to begin clinical trials this year for an engineered version of

heparin, which is a complex sugar structure and anticoagulant drug. The company has "engineered out" less-desirable sequences and has "engineered in" those sequences that contribute to improvements in efficacy and toxicity. The drug is targeted specifically for acute coronary syndrome and myocardial infarction. Dubbed "M118," the designer low-molecular weight heparin is expected to enter clinical trials this year (see Figure 1).

The second strategy, the development and commercialization of a biogenerical offers particularly great

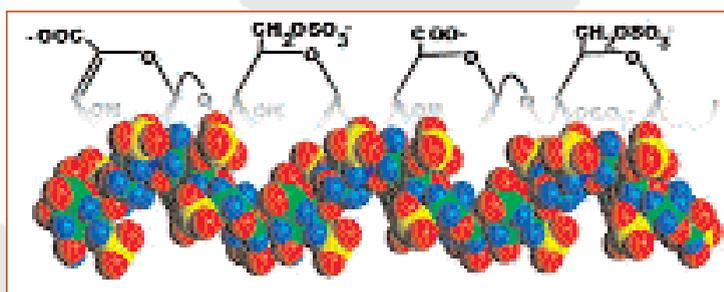


Figure 1: Fully characterized chain.

potential. In fact, projects for developing generic versions of multimillion-dollar biotech drugs brought to market in the late 1980s and early 1990s are already underway. Moreover, industry analysts have projected the worldwide sale of all generics to increase by 20% this year and by 14% per year afterward to nearly \$49 billion in 2007.

According to Crane, Momenta and Sandoz are already underway with a generic of "a very significant existing marketed product" by analyzing the product's sugar structure. "The strategy is to significantly accelerate the bringing of such a product to commercialization without having to do large-scale clinical trials," says Crane.

Still, several obstacles must be overcome. Although most industry experts agree that the development of biogenerics is inevitable, the regulatory framework (i.e., an agreed-upon biogenerics approval mechanism) is still under debate among innovator and generics companies as well as biotech pharmaceutical organizations.

Founded two years ago, Momenta's technology derives from more than 10 years of research conducted at the

Massachusetts Institute of Technology. The company currently holds nearly 80 patents and applications that cover its approach to sequencing these sugars.

Maribel Rios

FYI

Courses slated

The University of Wisconsin—Madison, Department of Engineering Professional Development will offer a course entitled, "Tablet and Capsule Manufacturing: Introduction and Update for Competitive Organizations," 2–4 February 2004 in Las Vegas, Nevada.

The course will provide a broad foundation of processing sequences and will cover the fundamentals of solid-dose manufacturing, tablet and gelatin capsule technology, current practices and advances in equipment and technology, problem-solving approaches, and GMP standards. For more information, contact Michael F. Waxman at 608.262.2101, waxman@epd.engr.wisc.edu.



Hygienic Standards for Pharmaceutical Equipment

3-A Sanitary Standards Inc. (3-A SSI), a hygienic-standards-writing body known in the food and dairy industries, is launching a program to develop equipment standards for pharmaceutical industry applications.

The pharmaceutical standards, to be designated P3-A, will establish baseline design criteria based on principles of hygienic design and cleanability, according to Timothy R. Rugh, executive director at 3-A SSI in McLean, Virginia. "The standards will provide a performance benchmark for [pharmaceutical] equipment," says Rugh. "These standards are being developed because no comparable standards now exist."



Timothy R. Rugh

Rugh notes that part of the impetus for 3A-SSI's expansion into the pharmaceutical industry comes from pharmaceutical manufacturers themselves—who have begun asking for standards for components such as pumps, mixers, and conveyors.

"From records of 3-A standards sales, we know companies in the pharmaceutical industry have been specifying 3-A standards for current applications based on the general hygienic design requirements," says Rugh. "But because the current 3-A standards do not specifically encompass pharmaceutical applications, the new P3-A project will use our existing documents as the foundation for standards to be applied in API manufacturing."

Initial work will focus on pumps and materials, although no plan has been established for the equipment groups to follow. "Provided the drafting procedures follow smoothly and depending on the amount of re-balloting that may be required, we hope to have the first standards completed by late 2004," says

Rugh. He explains that for pumps, for example, the applicable P3-A standard will describe seals and other parts exposed to chemicals.

The P3-A standards will be developed according to the American National Standards Institute (ANSI) canvass method. Use of this method marks a change for 3-A SSI, which has traditionally used its own consensus process.

ANSI methods require 3-A SSI to issue a public notice to give interested stakeholders the opportunity to participate. The formal announcement of the standards writing had not been made as of press time, but is expected some time in December.

The P3-A project will be directed by a steering committee. Members of the steering committee will be announced shortly, says Rugh, once granted the approval of their companies. Members will be senior engineering representatives from manufacturing companies, component makers, and architectural and engineering firms.

3-A SSI provides a third-party verification program to monitor equipment conformance to its standards. Certified conformance evaluators (CCEs) conduct a physical evaluation of the equipment and its drawings and documentation as well as the manufacturing company's documented quality control procedures.

3-A SSI is a nonprofit 501 (c)(3) organization. Funding derives from authorization of the 3-A symbol, grants from founding member organizations, and from the sale of standards.

George Miller

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example, a process building a molecule composed of twenty nucleotides (20-mer), would result in 19 different short sequences remaining as impurities. As a result, a significant amount of the material produced by the solid-phase synthesizer consists of oligonucleotide impurities which must be separated from the desired molecule.

To accomplish this, the mixture must be passed through a purification system. First, ammonia is applied to "cleave" the molecular mix from the synthesizer. The ammonia is then removed by evaporation at reduced pressure, and the resultant mixture precipitated out and lyophilized.

Currently, the next step is to redissolve the compound in a mixture of water and acetonitrile or methanol solvent and load it into a reversed-phase chromatography column. The concentration of organic solvent is then gradually increased to eventually elute the desired molecule from the column. There are a number of limitations associated with this approach, however, including the cost and handling issues associated with the use of an organic solvent. The overriding concern for those planning for scale-up to large-scale production, however, is the slow throughput rate of the process.

As an alternative to this reverse-phased chromatography step, Pall is proposing the use of its Mustang Q ion-exchange membrane chromatography. The relatively large pore size (0.8 μm) and chemical composition of the Mustang membrane significantly improves throughput rates.

The Mustang Q membrane chromatography process makes use of the fact that each of the failure sequence impurities produced during synthesis differ from each other by a single negative charge, with the largest negative charge associated with the complete antisense molecule. As a result, when a high salt (typically 1 molar NaCl) buffer is applied to the Mustang module, the antisense product molecules (which have the strongest attraction to the positive charge of the membrane) will "self-displace" the weaker-charged, shorter-sequence impurities. The NaCl

gradient is then adjusted to elute the full-length oligonucleotide. According to Lajmi, "Mustang chromatography has been shown to achieve purity levels of up to 95% while eliminating the use of an organic solvent."

In addition to throughput and purity, scalability is clearly a critical consideration for antisense product development. According to Lajmi, several companies are evaluating the Mustang Q technology for use in their antisense production processes, including a product now in Phase III clinical trials. Currently the company has 10 mL (≈ 0.3 g purification capacity), 100 mL (≈ 3.0 g) and 1 L (≈ 30 g) bed volume purification modules and is in development with a purification system that can handle from 0.5 to 1kg/injection volumes.

One antisense drug already on the market is Isis Pharmaceuticals' (Carlsbad, CA), "Vitravene," targeted for the treatment of cytomegalovirus retinitis (CMV) in AIDS patients. Vitravene is marketed by Novartis Pharmaceuticals (Basel, Switzerland).

Isis currently purifies Vitravene using a combination of both reversed-phase chromatography and ion-exchange methods (chromatography followed by ion exchange). As explained by, Doug Cole, PhD, Isis vice-president, Technical Development, the relative efficiencies of the techniques differ according to the class of impurity. "Where there are distinct charge differences between molecules, ion-exchange will work well, while in other cases, you'll get better selectivity through reversed-phase chromatography. The chromatographic 'Holy Grail' will be one that combines the best features of both."

Isis has nine additional antisense products in the pipeline ranging from Phase I to Phase III clinical trials. Among these is a second-generation antisense drug recently announced by Isis and Eli Lilly (Indianapolis, IN). In preclinical tests, the antisense drug "LY2181308" has been shown to successfully inhibit tumor growth in animal models. LY2181308 targets Survivin, a molecule that promotes cell survival. Survivin is expressed in the vast majority of cancers, where it interrupts the natural cell death cycle, but not in

normal tissue. Antitumor activity has been associated with significant reduction of Survivin expression in tumors, evidence that the drug was working through an antisense mechanism.

Isis has been working with Pall since the beginning of the Mustang Q project and has used the modules to purify a number of their developmental products. They haven't yet implemented the technology in a production setting, however.

Cole notes that ISIS may be in a unique position relative to comparing the relative benefits of reversed-phase and ion-exchange chromatography for antisense production. "The know-how relative to working up a reversed-phase system efficiently is not widespread, and we've developed our own proprietary techniques that are very fast." In fact, Cole says that their reversed-phase process is actually faster than ion exchange. He adds, however, that "you need the whole suite of technologies to make it work, and if you haven't got them, then you'll go with ion exchange."

Cole also points out another trade off between the two techniques. Though he acknowledges the drawbacks associated with the use of organic solvents, he adds that "on the other hand, given the fact that many of these drugs are parenterals, and we have excellent controls over microbiological contaminants, we like the fact that (with reversed-phase chromatography) our product (nucleic acids) is always in a medium that naturally inhibits bacterial growth. When you're doing ion exchange, you're sort of putting your product in a pure growth medium."

Regardless of the approach that companies choose for their full-scale antisense drug production, Cole observes that it's not too soon to be concerned about manufacturing capacity. "Given the number of people now in advanced development with nucleotide products, you have to look at the total available capacity and who has access to it." Three antisense drugs are now in Phase III clinical trials, one of which, the anticancer therapy "Genasense" from Genta Incorporated (Berkeley Heights, NJ), is now in NDA review.

John Haystead