Cancer vaccine collaboration

Biotechnology companies MedImmune and Cerus have agreed to develop and commercialize a novel therapeutic vaccine designed to treat an array of different cancers.

"Cerus's therapeutic vaccine technology greatly complements MedImmune's existing program targeting the EphA2 protein in cancer," says Peter Kiener, vice president of research at MedImmune. "Because EphA2 is overexpressed by many types of human cancers, we believe Cerus's technology may be employed to develop a vaccine that can stimulate the immune system to attack cancerous cells expressing EphA2."

At the annual meeting of the American Association for Cancer Research in April, the collaborators presented preclinical results demonstrating the EphA2 vaccine technology's effectiveness in suppressing the growth of certain tumors. The results demonstrated that 80% of treated mice survived for more than 43 days after tumor implantation, while untreated controls had a median survival time of approximately 20 days.

Previous research indicates that EphA2 antibodies selectively destroy tumor cells while minimizing damage to normal cells. Additionally, the highest levels of EphA2 were found on the most aggressive cancer cells, which was consistent with data linking EphA2 with clinical features of metastasis. "This research demonstrated the potential to develop potent therapeutic vaccines utilizing our proprietary Listeria vaccine platform in combination with cancer antigens," says Stephen T. Isaacs, president and chief executive officer of Cerus.

Under the companies' agreement, Cerus will participate in developing the therapeutic vaccine and receive upfront and development funding, as well as milestone payments and royalties on future product sales. MedImmune is responsible for clinical testing, manufacturing, and commercialization of any product resulting from the collaboration.

Medimmune formed this partnership on the heels of dissolving another one—its collaboration with Wyeth for the nasal flu vaccine FluMist, approved by the FDA in 2003, and a second-generation liquid formulation called CAIV-T. The decision was reached after disappointing FluMist sales this past winter.

—KIMBERLY S. CLEAVES

Stem cell savings

A new type of bank account is now available. But you're not likely to hear about it from your financial planner.

Southern California-based life sciences company NeoStem has opened the first commercial adult stem cell bank. It is currently collecting stem cells on a "predisease basis."

"This capability simply hasn't existed before," says NeoStem CEO Denis Rodgerson. "Adult stem cell banking is an 'early-resort' therapy. Banking now provides a form of insurance against conditions that may occur down the road."

Although there is some consensus among scientists that embryonic cells will provide the most effective form of stem cell therapy, adult stem cells invite less political and moral controversy and fewer restrictions on use.

Retransplantation of autologous stem cells is an established procedure to repopulate bone marrow cells following chemotherapy. Adult stem cells have also been used experimentally to repair heart damage and treat autoimmune diseases.

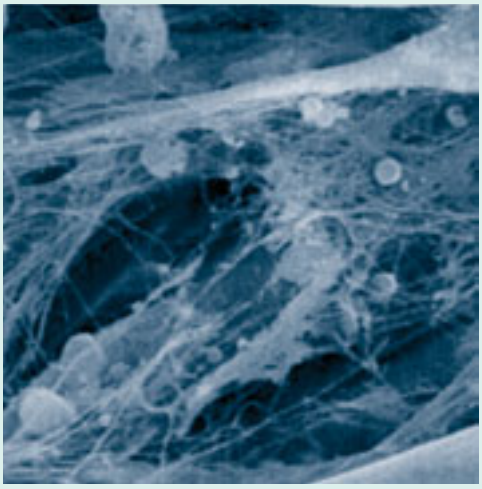
"Using one's own stem cells significantly reduces the cost of treatment, shortens hospital stays, and eliminates the threat of rejection that often develops using someone else's cells," the company explains. Banking ensures the availability of autologous stem cells for the donor.

NeoStem envisions bank customers might use their "deposits" to help treat conditions ranging from heart disease to stroke to neurological disorders, as well as various cancers.

"Especially for cancer patients in remission, adult stem cell banking offers an important measure of control," Rodgerson says.

The donation process is similar to donating platelets, the company says. Blood is removed from one arm, the stem cells are extracted, and then the blood is reinjected into the other arm. The cells are preserved in multiple cryostorage compartments for use at different times.

In addition, each donor will have the option of contributing 10% of collected stem cells for research purposes. Determining how well adult stem cells can differentiate into various types of organ tissue is an important line of research NeoStem is pursuing with other institutions.



Tissue growth. Adult stem cells can be induced to form extracellular matrices for tissue engineering. (Adapted with permission from Grayson, W. L.; et al. *Biotechnol. Prog.* **2004,** *20,* 905–912.)

The company indicates that the collection and storage charge will depend on an individual's needs. It also provides assurances that, in the event its business model doesn't work out, each account will be safe. The stem cell bank is backed by a trust fund protecting the collected cells even if NeoStem goes under.

—DAVID FILMORE

Fixing defective genes

David Lawrence and colleagues at the Albert Einstein College of Medicine have developed a strategy for suppressing nonsense mutations, the cause of genetic diseases such as Duchenne muscular dystrophy and cystic fibrosis.

Nonsense mutations are errant stop codons resulting in the truncation or absence of key proteins. Researchers have found that gentamicin and related aminoglycoside antibiotics increase the levels of these critical proteins, but only in high doses, at which the drugs are toxic. Thus, the Albert Einstein researchers looked for ways to augment the efficacy of low-dose gentamicin (*J. Am. Chem. Soc.* **2004,** *126,* 5660–5661).

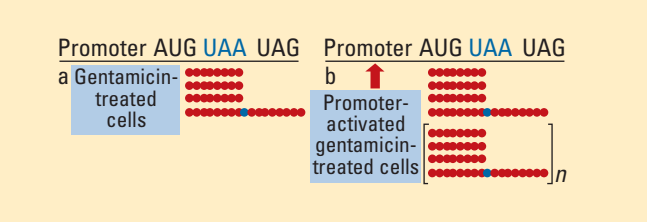
The scientists focused their efforts on ataxia telangiectasia (A-T), a rare nonsense-mutation disorder characterized by waning motor activity, premature aging, and moderate to severe immunodeficiency. They believed they could enhance gentamicin's activity by finding a drug to stimulate expression of the A-T-causing gene (*atm*). Thus, they inserted the *atm* promoter sequence into a plasmid carrying the firefly luciferase gene and transfected this construct into a human cell line. They then screened the cells with a library of almost 400 FDA-approved drugs and looked for compounds that triggered luciferase activity.

The researchers identified several compounds that activated the *atm* promoter. They tested one of these drugs, the fluorinated quinolone ofloxacin, for its ability to enhance native *atm* transcription levels in normal 293T cells and cells derived from A-T patients. They found ofloxacin stimulated *atm* expression 2.4-fold in 293T cells, but almost 6-fold in the mutant *atm* cells.

The team then examined ofloxacin and another promoter-enhancing drug, thioguanine, for their ability to augment gentamicin's effects on a luciferase construct carrying a nonsense mutation. They found that whereas the aminoglycoside alone increased luciferase activity 6- to 15-fold over that of untreated cells, the addition of ofloxacin or thioguanine to gentamicin enhanced luciferase activity 30-fold.

Lawrence's group is confident its promoter-activation strategy is applicable to analyzing nonsense mutations in general. Similarly, screening libraries of FDA-approved drugs will facilitate the application in humans of any compounds that give positive results.

—RANDALL C. WILLIS



Increase expression, increase efficacy. Although gentamicin is effective in treating nonsense mutations (UAA; a), researchers believe they can augment its activity by increasing the number of gentamicin targets (b). (Adapted with permission from Xi, B.; et al. *J. Am. Chem. Soc.* **2004,** *126,* 5660–5661.)

Hepatitis C handoffs

The U.S. hepatitis C drug market has gotten more crowded since the FDA cleared two companies in April to sell generic versions of ribavirin as part of a combination therapy to treat the virus.

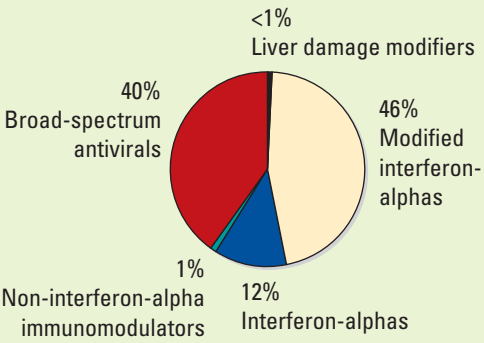
Schering-Plough has sold Rebetol, its branded version of ribavirin, since 2001. A day after the FDA approved Abbreviated New Drug Applications from Three Rivers Pharmaceuticals and Sandoz, a subsidiary of Novartis, for ribavirin, Schering-Plough announced that its generic drug-manufacturing subsidiary, Warrick Pharmaceuticals, would also produce a generic version.

Schering-Plough says it will continue to sell Rebetol as well, but product sales, which were over $600 million in 2003, will probably drop significantly. Even before the generics hit the market, the company announced that Rebetol sales had declined by 55% in the first quarter of 2004 from the previous year.

Ribavirin is not effective against hepatitis C on its own, but only in combination with the interferon-alpha protein. Currently, Roche's Pegasys—marketed alone and as a combination product with Roche's own ribavirin product Copegus—is the leading interferon product, with significantly higher sales than Schering-Plough's Peg-Intron. Both are pegylated versions of the protein, which allows it to remain active in the body for much longer.

As Schering-Plough loses share in the ribavirin market, it hopes to pick up ground in the other half of the combination, partly on the basis of research results presented in April at the European Association for the Study of the Liver annual meeting. Researchers at the University of Padova in Italy found the virological response from Peg-Intron was 76%, compared with 55% with Pegasys.

Schering-Plough also announced in April that it had submitted a New Drug Application to market Peg-Intron as a combination therapy with Rebetol in Japan. The market analysis company Decision Resources forecasts that the Japanese hepatitis C drug market will increase by more than 500% over the next decade, compared with about 300% in Europe and about 200% for the U.S. market.



2003 hepatitis C treatment breakdown. The market share of modified interferon alphas is expected to grow over the next decade. (Source: *Hepatitis C Virus;* Pharmacor Infectious Disease Study no. 69; Decision Resources, 2004.)

The current worldwide market for hepatitis C treatments is estimated at $2.9 billion.

—DAVID FILMORE

Bugs to drugs

In the name of biodefense, Democrats in the U.S. House of Representatives introduced legislation in May seeking to speed up the development of countermeasures for emerging infectious diseases.

Jim Turner (TX), ranking Democrat on the House Select Committee on Homeland Security, sponsored the Rapid Pathogen Identification to the Delivery of Cures Act, known as the RAPID Cures Act, which, he says, targets issues not dealt with in President Bush's Project BioShield legislation.

BioShield budgets $5.6 billion over 10 years to provide a guaranteed market for private sector development of vaccines against biological warfare agents such as anthrax, plague, and smallpox. Congress approved this funding during fiscal year 2004 appropriations, but the accompanying legislation describing the precise layout of the plan has yet to pass in the Senate.

"BioShield is targeted at addressing classical agents, not the laboratory-altered pathogens of the future," writes Turner in a report entitled *Beyond Anthrax: Confronting the Future Biological Weapons Threat* (*http://www.house.gov/hsc/democrats/pdf/press/040504_beyond_anthrax.pdf*), released the same day as the bill.

He warns that the current bioterrorism threat "pales in comparison" to the potential threat from the more virulent and resistant microbial strains that might be produced as biotechnology continues to advance.

"In addition," he writes, "[BioShield] relies on the current process of drug and vaccine development, which takes an average of 14 years before a new medicine is available. As a consequence, our protective biodefenses are essentially static and unmoving in the face of a threat that is highly variable and unpredictable."

U.S. HOUSE OF REPRESENTATIVES

Turner (D-TX) is the lead sponsor of the RAPID Cures Act.

Even though in 2002 Congress authorized broader use of investigational drugs under emergency conditions, the RAPID Cures Act asserts that after an outbreak of a new pathogen, "it will likely still take years for even an experimental treatment or vaccine to become available."

The bill would authorize $10 million for the U.S. Departments of Homeland Security, Health and Human Services, and Defense to develop in six months a comprehensive strategy to achieve "dramatic reductions in the time frame from pathogen identification to the development and emergency approval of countermeasures." It also specifically charges HHS with establishing a system for rapidly setting up clinical research programs to test treatments for emerging pathogens or toxins.

"We need a Manhattan Project to help win the war on terror," Turner says. "It is time to turn science and technology to our advantage."

—DAVID FILMORE

Pushing NMR sensitivity

Although multidimensional NMR spectroscopy has proved to be a valuable tool for structure determination and provides information about macromolecular dynamics, the technique suffers from low sensitivity and therefore requires milligram quantities of expensively labeled samples. Recently, scientists have tackled this problem using microcoil NMR probes, which function on the principle that sensitivity is roughly inversely proportional to coil diameter. Thus, they have designed new probes with a 1-mm inner diameter and a 3-mm length, such that the total flow-cell volume is only 5 µL and the NMR sample is only 1.5 µL—a 180-fold improvement over the 500-µL sample volume of standard NMR tubes.

Small change. Microcoil NMR probe and a penny. (Adapted with permission from Peti, W.; et al. *J. Am. Chem. Soc.* **2004,** *126,* 5873–5878.)

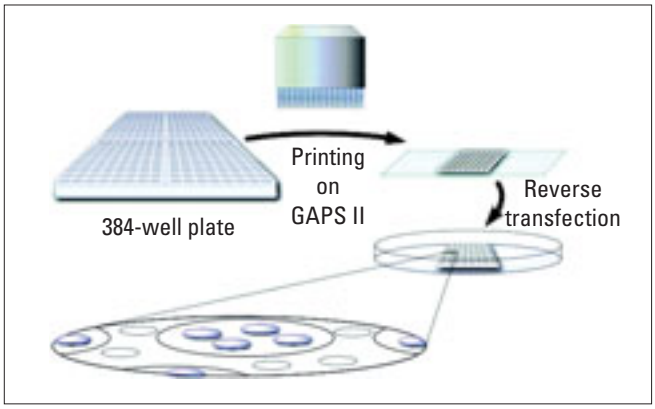
In general, researchers have only used microcoil probes to analyze small-molecule structures, but recently, Wolfgang Peti and colleagues at the Scripps Research Institute, MRM Corp., and Sequoia Sciences applied this technology to protein samples. They found that as little as 10 µg of protein in the microcoil gave spectra of resolution and intensity equal to those derived from 1 mg of protein in a full-sized NMR sample.

Using a variety of NMR pulse sequences, the researchers rapidly assigned the backbone residues of several test proteins from their spectroscopic peak patterns. Likewise, they determined they could perform experiments with the microcoils that were otherwise difficult or impossible.

For example, because of the large differences in carbon chemical shift ranges, researchers have found it difficult to correlate signals between the side-chain carbons of aliphatic (0–75 ppm) and aromatic (115–140 ppm) residues—information that is critical to understanding three-dimensional protein folding. In part, this problem is caused by the fact that traditional 5-mm NMR probe coils are not rated for the high power levels needed for pulse sequences bridging this range. With the microcoil probes, however, the researchers easily produced these spectra and could assign all of the aromatic and aliphatic side chains in a single experiment.

Thus, according to the scientists, the microcoil probes "should allow for a rapid and very detailed biophysical description of small amounts of unlabeled and labeled proteins that was, until now, impossible."

—RANDALL C. WILLIS



High-throughput, small volume. Researchers are performing RNAi studies using nucleic acid–lipid complexes to transfect mammalian cells on glass slides. (Adapted with permission from Silva, J. M.; et al. *Proc. Natl. Acad. Sci. U.S.A.* **2004,** *101,* 6548–6552.)

Spotting the difference

Researchers at Cold Spring Harbor Laboratory recently developed a cell microarray system for high-throughput RNA interference (RNAi) screening.

Although RNAi technology is used to analyze gene function in many eukaryotic organisms, its adoption in mammalian studies has been limited by difficulties in getting RNA molecules into cells. Because studying several genes usually requires several rounds of transfection, the method can be tedious and expensive.

The researchers examined whether mammalian cells grown on glass substrates could be transfected with RNA–lipid complexes, attenuating the expression of a target protein (*Proc. Natl. Acad. Sci. U.S.A.* **2004,** *101,* 6548–6552).

Initially, they studied small interfering (si) RNAs and plasmids expressing short-hairpin (sh) RNAs that target the green fluorescent protein (GFP), and used a red fluorescent protein (dsRed) as a control.

The team spotted various solutions containing different lipids, GFP-dsRed-expressing plasmid, and siRNA or shRNA onto slides, which they then exposed to cell culture. Within 24 h, they found that cells treated with control RNA molecules expressed both GFP and dsRed, whereas cells transfected with anti-GFP RNAs showed diminished GFP levels but full dsRed expression. They also found that by varying the lipid and nucleic acid composition of the spot, they could reproduce the results in different cell lines.

Furthermore, the researchers found that the shRNA slides showed no performance reduction after being stored for two months at 4 °C, while the siRNA samples were only functional up to two weeks.

The researchers then tested their method against cellular metabolic pathways, first targeting proteasomal protein degradation. They exposed cells to spots containing lipid complexes of various shRNAs and plasmids expressing modified GFP sensitive to proteasomal attack. Using control shRNAs, the researchers found that GFP expression was severely attenuated, indicating proteasomal degradation. With shRNA molecules that target protein expression involved in proteasome function, however, they found that GFP levels remained high for more than 60 h. They achieved similar results targeting cell cycle proteins.

The researchers estimate they can perform 100–500 transfections using their slides with the same amount of material required for a single transfection in a 96-well plate experiment. Likewise, they can conduct thousands of experiments in parallel on a single slide.

—RANDALL C. WILLIS

Novel angina treatment

CV Therapeutics' Ranexa (ranolazine), if approved by the FDA, would represent the first new class of angina therapy introduced in the United States in more than 25 years, according to the company.

Chronic angina affects nearly 6.6 million people in the United States, the American Heart Association reports. It is usually associated with coronary artery disease and is often marked by repeated and sometimes unpredictable attacks of chest pain.

In October 2003, CV Therapeutics received an approvable letter from the FDA regarding Ranexa's NDA (New Drug Application) for use as an anti-anginal. But the agency requested additional clinical information prior to approval.

Data from the monotherapy assessment of ranolazine in stable angina (MARISA) trials were published in an April issue of the *Journal of the American College of Cardiology.* MARISA, a Phase III, double-blind, placebo-controlled trial, consisted of 191 multinational angina patients. Patients not receiving any other angina medication were randomly selected to receive 500-, 1000-, and 1500-mg dosages of the drug or placebo twice a day. The researchers observed, at statistically significant levels, that patients taking Ranexa could exercise longer without angina pain and electrocardiographic evidence of ischemia.

Although some patients in the double-blind study were affected by dose-related adverse events including dizziness, nausea, and constipation, most elected to continue the medication in an open-label study to collect further safety information. After one year, the open-label data showed a mortality rate of 3.7% compared with an estimated 9%, for patients of similar risk.

It is unclear what additional clinical data the FDA is requesting. The time frame for a final decision on the NDA is unknown.

—KIMBERLY S. CLEAVES ■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