SUGAR BIOTECH SUCCESS: Aranesp, an improved version of an existing anemia-fighting drug, has been on the market for almost a year. Two sugar chains added to the original drug molecule give Aranesp longer staying power in the body.

Sweet medicines

Sugars play critical roles in many cellular functions and in disease. Study of those activities lags behind research into genes and proteins but is beginning to heat up. The discoveries promise to yield a new generation of drug therapies

By Thomas Maeder

RANDI BEREZ

Now that the human genome has been deciphered, much of

the fanfare surrounding it has transferred to the proteome, the full complement of proteins made from the genetic "blueprints" stored in our cells. Proteins, after all, carry out most of the work in the body, and an understanding of how they behave, the press releases say, should translate into a font of ideas for curing all manner of ills. Yet living cells are more than genes and proteins. Two other major classes of molecules—carbohydrates (simple and complex sugars) and lipids (fats)—play profound roles in the body as well. These substances, too, need to be considered if scientists are to truly understand how the human machine operates and how to correct its maladies.

Sugars in particular perform an astonishing range of jobs. Once regarded mainly as energy-yielding molecules (glucose and glycogen) and as structural elements, they are now known to combine with proteins and fats on cell surfaces and, so situated, to influence cell-to-cell communication, the functioning of the immune system, the ability of various infectious agents to make us sick, and the progression of cancer. They also help to distinguish one cell from another and to direct the trafficking of mobile cells throughout the body, among other tasks. So ubiquitous are these molecules that cells appear to other cells and to the immune system as sugarcoated.

Recognizing the importance of sugars in health and disease, increasing numbers of researchers in academia and the biotechnology industry have recently stepped up efforts to learn the details of their structures and activities and to translate those findings into new therapeutic agents. These pioneers have also gained support from the federal government. In October 2001 the National Institutes of Health awarded a five-year, $34-million "glue" grant to the Consortium for Functional Glycomics, a group of 54 investigators around the world who aim to coordinate and facilitate research in the area, such as by developing a library of synthetic sugar chains and a structural database available to all. The grant, says James C. Paulson of the Scripps Research Institute in La Jolla, Calif., the consortium's principal investigator, is "a vote of confidence" in the field.

Overview/*Sugars*

- Sugars modify many proteins and fats on cell surfaces and participate in such biological processes as immunity and cell-to-cell communication. They also play a part in a range of diseases, from viral infections to cancer.
- Scientists are finally overcoming the obstacles impeding efforts to decipher the structures of complex sugars and to synthesize sugars for use in research and as drugs.
- The advances are leading to new medicines for a variety of ills.

Clearing Roadblocks

THE WORDS "functional glycomics" in the consortium's title announce that the research complements more ballyhooed efforts to catalogue human genes and proteins (genomics and proteomics), decipher their functions and open broad new fields of applied biology. The term "glycomics" derives from "glycobiology," which Raymond A. Dwek of the University of Oxford coined in 1988. Until then, carbohydrate research was spoken of as the science of oligosaccharides (chains of sugars), vocabulary that lay interviewers and even some scientists had trouble pronouncing. In chemistry, the prefix "glyco" refers to sweetness or sugar.

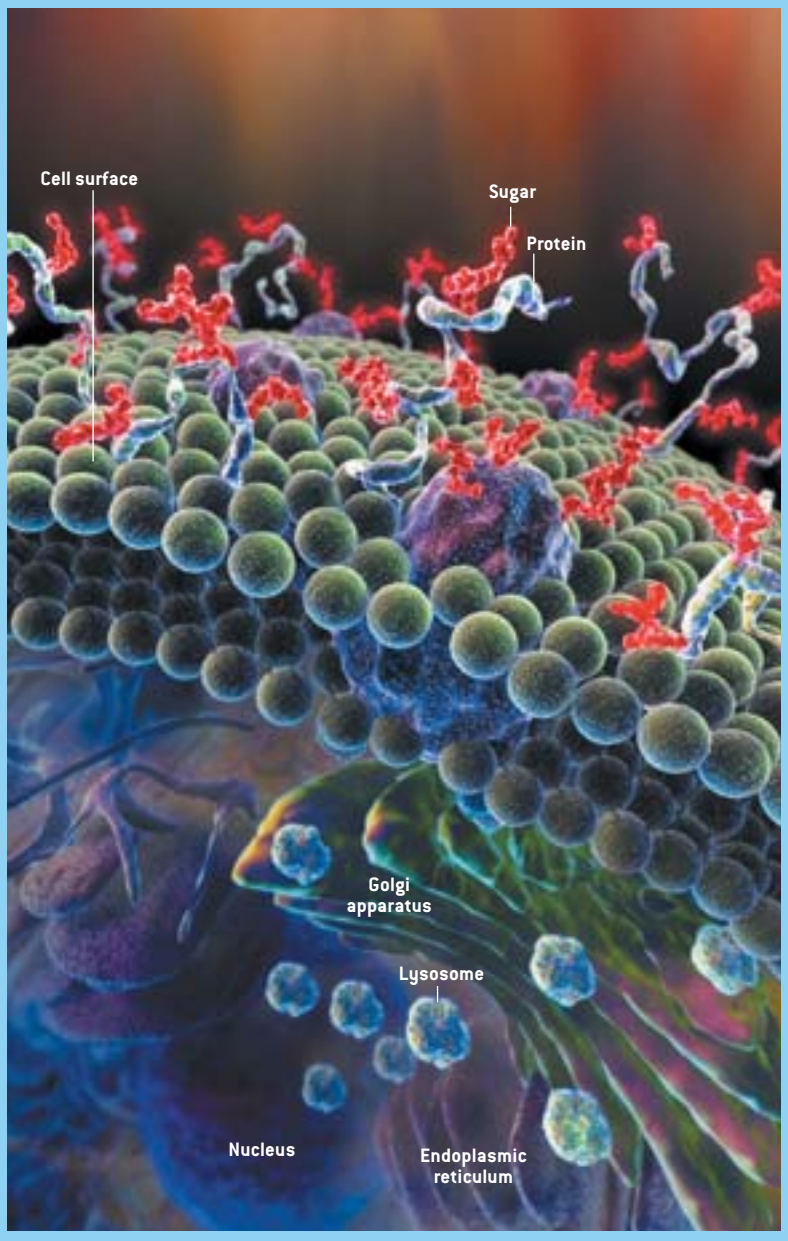
It is easy to see why observers might feel daunted by all the terms that carbohydrate researchers throw around. Simple sugars—such as glucose and sucrose (table sugar), which consist of some carbon atoms, oxygen and hydrogen—are often referred to as monosaccharides, disaccharides and so on, depending on how many sugar units they contain. The term "oligosaccharide" typically refers to larger chains, whereas *really* big molecules are called polysaccharides. And molecules formed by the pairing of carbohydrates with proteins or fats are known as glycoconjugates or, more specifically, as glycoproteins and glycolipids. And that's just Sugar 101.

Scientists of the past did not neglect sugars from lack of interest. They were stymied by a dearth of tools for deciphering the structure of complex versions and for synthesizing such molecules readily, reproducibly and in the amounts needed for study or for formulation as drugs.

The problems stemmed, in large part, from the extraordinary structural variability of sugars. The four nucleotides that make up DNA, and the 20 common amino acids that form proteins, link together in linear fashion like beads on a string, always joined by the same chemical connection. In contrast, the roughly 10

Glyco Drugs at Work

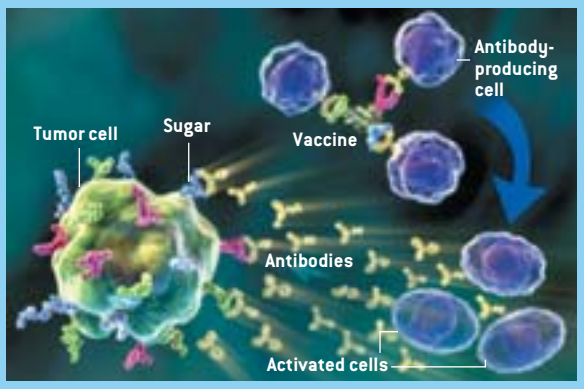
SUGARS DECORATE many proteins and lipids (fats) on the surfaces of cells (*below*). Cells add the sugars through enzymatic reactions carried out in compartments called the endoplasmic reticulum and the Golgi apparatus, and they break down sugared molecules (glycoconjugates) in structures known as lysosomes. The figures at right and bottom depict some of the therapeutic ideas that have emerged from insights into the structure, function and processing of carbohydrates in the body.



TREATMENT APPROACHES

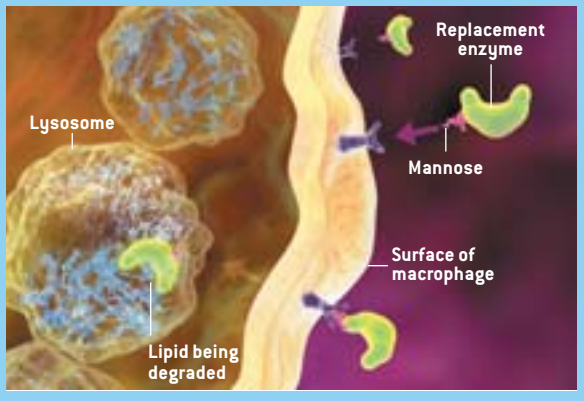
Combating Cancer

Tumor cells often display unusual versions of sugars. One proposed treatment (*below*) would incorporate those sugars in a vaccine. This vaccine would induce the immune system to produce antibodies able to recognize the selected sugars on cancer cells and would thus facilitate the cells' destruction.



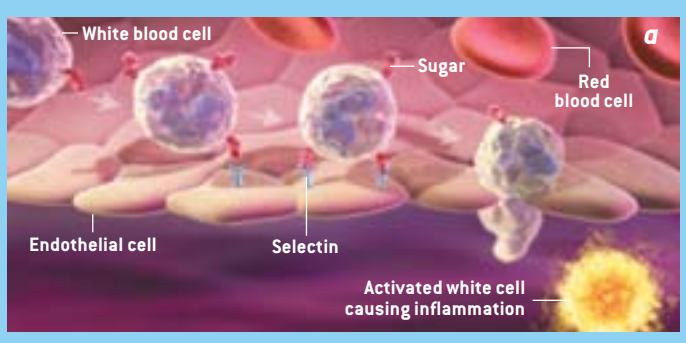
Easing Lysosomal Storage Diseases

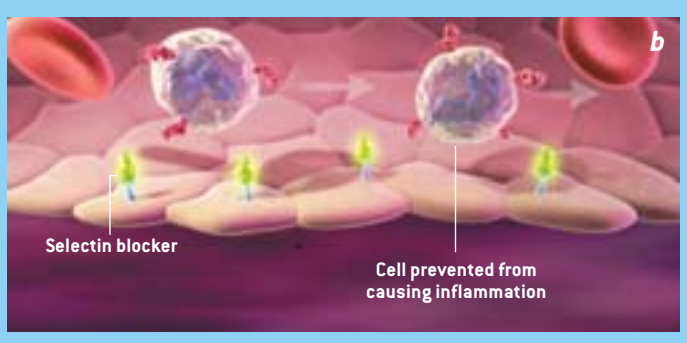
Many inherited disorders arise because some enzyme needed to break down sugar-bearing lipids in lysosomes is defective. A drug for Gaucher's disease (*below*) consists of a replacement enzyme that has been modified to display the sugar mannose, which guides the enzyme to macrophages, cells sorely affected by the lack of a functional enzyme.



Interfering with Inflammation

Inflammation occurs when white blood cells invade tissues (*below, a*). To leave the blood, the cells first bind through a sugar to molecules called selectins on the endothelial cells that line blood vessel walls. Anti-inflammatory drugs under study aim to prevent the white cells from binding to selectins (*b*).





JEFF JOHNSON *Hybrid Medical Animation*

Sugar-Taming Technologies

Technical breakthroughs are laying the groundwork for the development of new drugs that consist of or act on sugars

ADVANCES IN SEQUENCING and data processing have driven some of the most significant breakthroughs in recent biomedical science. Such advances could be especially energizing to the emerging field of glycomics. As drug developers learn more about the structure and function of complex sugars and about how to control their synthesis, they are also uncovering fresh ideas for treating disorders that involve sugars.

Straightforward sequencing of the type common with linear gene or protein sequences, in which subunits are enzymatically lopped off and identified one at a time, is impossible with huge, complex branching sugars, which require every trunk, branch and twig to be tracked. Instead Ram Sasisekharan of the Massachusetts Institute of Technology and his colleagues work simultaneously from the global toward the specific and from the particular to the more general, bracketing an answer in the middle. First they determine a target molecule's size and use a computer algorithm to generate a master list of the vast number of theoretically possible sequences, including those of each fork and branch in nonlinear structures. They then rule out many of these possibilities, for example, by running tests that reveal which monosaccharides (one-unit sugars) are present in what relative proportions or by examining the molecule's susceptibility to enzymes that cleave linkages between specific units or at particular branch points.

"Once you have the exhaustive tool kit, it's not that complicated," says Ganesh Venkataraman of M.I.T. Each successive constraint shrinks and refines the originally unwieldy universe of possibilities into something a little more manageable. "You go back to the database, put in the answers and eliminate everything that doesn't satisfy [the constraints]. It's like those puzzles where seven people are at a table, and you have clues about who does or does not sit next to whom and have to figure out the seating arrangement."

The reciprocal problem of constructing sugars has similarly enjoyed significant progress only recently. Proteins are read from a genetic "blueprint" that can be used to generate limitless copies. No blueprint exists for sugars. Different enzymes must operate in series to build complex sugar chains (oligosaccharides). When the needed enzymes are available in nature, they can be used to link specific building blocks efficiently and in the desired orientation. But if scientists do not have such enzymes, they have to find alternative, more laborious ways to construct the structures.

M.I.T.'s Peter H. Seeberger and his co-workers have developed a method of oligosaccharide assembly analogous to an approach devised by R. Bruce Merrifield of the Rockefeller University for protein synthesis. Sugars join at sites where they have an OH (hydroxyl) group. So the scientists begin by anchoring one monosaccharide to a polymer bead and masking all the hydroxyl groups except the one meant to form a link. Then they expose the first sugar to a second, partly masked one and allow the two to interact. Next they unmask a new OH site and repeat the process, adding one new sugar at a time. Most linkages and branches can now be made very reliably, although the process is not yet as simple as the routine automated synthesis of peptides and DNA molecules. The largest sugars produced in Seeberger's laboratory to date are 12 units long and take 16 hours to make. Fortunately, a good number of important sugars, including those that help to distinguish one cell surface from another, fall within this range. Longer molecules can be cobbled together from smaller modular units.

RAM SASISEKHARAN sits by a sophisticated sugar-sequencing unit. After enzymes chop up a complex sugar, advanced high-pressure liquid-chromatography equipment (*on cart*) sorts the resulting fragments, and a mass spectrometer (*right*) characterizes the building blocks in the separated pieces. Computers analyze the results from both procedures to arrive at the full sequence of simple sugars in the complex molecule.

An alternative, "one-pot" synthetic method requires more careful advance planning but has simpler execution. A single reaction chamber is filled with all the needed ingredients at once, and a preprogrammed reaction sequence is determined by the degree of reactivity of differently protected sugars. The most reactive form bonds first and the least reactive last, and thus the order of reaction strengths determines the sequence of the final molecule.

Glycomics researchers are also perfecting methods for learning about the various functions of a sugar. Often this effort involves producing animals that have a defective or missing sugar—say, by genetically altering the biological pathways involved in sugar synthesis or by delivering abnormal monosaccharides that inhibit sugar-processing enzymes or disrupt interactions between normal sugars and other kinds of molecules. By observing what goes wrong when a sugar is faulty or missing, researchers obtain clues to the molecule's usual activities.

"Sugars used to be a nuisance, because the technology to understand them wasn't there," Sasisekharan says. Now they are considered an opportunity. —*T.M.*

RANDI BEREZ

(depending on who's counting) simple sugars common in mammalian carbohydrates can join with one another at many different points and can form intricate branching structures. Moreover, two linked units do not always orient in the same way: sometimes a building block will point up relative to the other unit, and sometimes it will point down. The four nucleotides in the DNA "alphabet" can combine to produce 256 different four-unit structures, and the 20 amino acids in proteins can yield about 16,000 four-unit configurations. But the simplest sugars in the body can theoretically assemble into more than 15 million four-component arrangements. Although not all these combinations occur in nature, the possibilities remain overwhelming.

Determining the sequences of the building blocks in complex sugars and producing such sugars remain challenging, but scientists have devised ingenious methods that make these tasks more feasible [*see box on opposite page*]. Progress in glycomics, even more than in genomics, will be driven by advances in molecular sequencing technology and bioinformatics (the cyber-methods that bring order to massive amounts of sequence data).

Better Already

AT THE SIMPLEST LEVEL, better understanding and control of sugars can improve existing therapies. Heparin, an anticoagulant sugar chain administered to prevent blood clots from forming during surgery, is the most conspicuous example. It is among the top-selling drugs in the world and has been used since the mid-1930s. Yet most commercial preparations, extracted from pig intestinal lining, are a heterogeneous and poorly characterized mix of compounds between 200 and 250 monosaccharide units long. Heparin's potency and potential for unwanted side effects vary not only from manufacturer to manufacturer but from one lot to the next, so that it must be empirically checked on a batch-by-batch basis.

Pharmaceutical makers today sell smaller, low-molecular-weight versions of the heparin molecule that, trimmed of many parts not needed for the drug's activity, produce fewer side effects. But as with the larger molecule, the manufacturers have difficulty making homogeneous batches. In 2000 Ram Sasisekharan and his colleagues at the Massachusetts Institute of Technology applied tools they developed to decipher the sequence of heparin's entire active site, the region responsible for the compound's biological activity. This information is now guiding efforts to synthesize potent low-molecular-weight heparins more reliably and to tailor their pharmacological properties for specific applications.

Enhanced control of sugars should likewise improve the effectiveness of proteins made by recombinant DNA technology. To work effectively, certain therapeutic proteins must have particular sugars attached to them at precise spots. Current technology is not always up to the task. Take the recombinant drug erythropoietin, delivered to stimulate red blood cell production in patients who have anemia or who are undergoing kidney dialysis. For years one company, Amgen, discarded 80 percent of the drug it generated because of inadequate glycosylation, which results in too rapid clearing from the blood. Then the company found a way to add two extra sugars to those normally found on erythropoietin. This newer version, sold as Aranesp, stays in the blood much longer than the original drug and thus requires less frequent dosing.

Beyond improving existing drugs, pharmaceutical developers are studying sugars to develop innovative therapies for a variety of disorders. Sometimes these treatments might consist of sugars or glycoconjugates themselves; other times they might consist of molecules that influence interactions between sugars and other molecules, including interactions with enzymes (biological catalysts) that control the synthesis or breakdown of sugar-bearing molecules.

Scotching Infections

A NUMBER OF investigators are taking aim at infectious diseases, an arena in which sugar-related drugs have already had some dramatic success. A sterling representative is the vaccine that targets *Hemophilus influenzae* type b (Hib). This vaccine has freed much of the world from the sometimes deadly meningitis caused by Hib. By presenting a sugar from the bacterium to the immune system, the vaccine primes the system to destroy the microbe swiftly once it enters the body. An early version consisting of just a sugar chain from Hib proved disappointing. But highly effective glycoconjugate preparations, in which the sugar is joined to a protein that boosts immune responsiveness, have been available since the late 1980s. Other glycoconjugate vaccines for infectious diseases—including one meant to ward off hard-to-treat *Staphylococcus aureus* infections in certain hospitalized patients—are under study.

Various disease-causing organisms, or pathogens, use carbohydrates to recognize and interact with their preferred host cells, and both existing and proposed drugs enlist sugars or sugar mimics to block such contact. The influenza virus, for example, enters the cells it infects by first docking with a sugar (sialic acid) that protrudes from glycoproteins on the cell surface. Attachment to the sugar essentially turns a key that opens cell "doors," freeing the virus to penetrate cells and to replicate within them. When newly formed viruses then bud from the cell, they can be trapped by the same sugar and must de-

A number of investigators are taking aim at infectious diseases, an arena in which sugar-related drugs have already had some dramatic success.

ploy an enzyme called neuraminidase to snip the sugar and free themselves. Two marketed drugs, Tamiflu and Relenza, shorten the duration of the flu by binding tightly to the enzyme's active site, thereby preventing it from acting on sialic acid. With the neuraminidase enzyme shackled, the virus has difficulty spreading to and infecting other cells.

In the case of the influenza virus, the drug essentially outcompetes the true sugar, winning access to the enzyme and inhibiting its activity—a phenomenon known as competitive inhibition. Competitive inhibition by synthetic analogues of problem sugars might fight other infectious diseases as well. Notably, the bacterium *Helicobacter pylori,* which causes stomach ulcers and inflammation, gains a foothold in the body by attaching to a sugar on the surface of the cells that line the stomach. And the bacterium *Shigella dysenteriae,* which causes deadly diarrheal epidemics, produces a toxin that binds to a sugar on intestinal cells. Sugar mimics that act as decoys, binding to *H. pylori* or to the *S. dysenteriae* toxin in ways that prevent docking with cells, are showing promise in laboratory tests.

Drug researchers are pursuing a similar strategy against septic shock (an often fatal shutdown of the circulation) caused by gram-negative bacteria. (Bacteria are termed "gram-positive" or "gram-negative" based on their reaction to a particular stain.) Shock sets in when the bacteria die—frequently in response to antibiotic treatment—and release a glycolipid, lipid A, into the bloodstream, eliciting a disastrous inflammatory response. Delivery of a lipid A analogue that cannot incite a strong immune response might reduce or eliminate shock by acting as a decoy to keep immune system cells away from the real lipid A in the body. Investigators have reason to believe that such analogues could also limit bacterial replication and production of lipid A.

Almost all infectious diseases are caused by bacteria, viruses, fungi or parasites. But in some brain disorders, such as Creutzfeldt-Jakob disease (a relative of mad cow disease), misfolded proteins known as prions are thought to be the infectious agents. Research by John Collinge of St. Mary's Hospital in London suggests that the troublesome hardiness of prions has to do with improper glycosylation of the proteins, which are unusually resistant to enzymatic degradation. Deciphering the precise role of the sugars may lead to ideas for counteracting these mysterious infections.

Restoring Balance

SUGAR-BASED DRUGS could have a role in fighting an array of noninfectious disorders as well, among them conditions marked by excess inflammation. After wounding or infection, endothelial cells that line blood vessels begin to display large numbers of carbohydrate-binding proteins called selectins. Selectins on endothelial cells bind loosely to a specific carbohydrate called sialyl Lewis x on the surface of circulating white blood cells of the immune system. Like a tennis ball rolling across a strip of Velcro, the white blood cells tumble along the vessel wall and slow down enough to migrate across the wall into injured tissue, where they set about containing the threat. That response is important for preserving health but can cause illness if it becomes chronic or excessive. Substances that interfere with contact between sialyl Lewis x and selectins are now under develop-

Once and Future Therapies

A SAMPLING of the sugar-related drugs on the market or in development is listed below. Some are glycoconjugates, consisting of sugars paired with peptides (short chains of amino acids), proteins (longer sequences of amino acids) or lipids (fats).

DRUG	DESCRIPTION	MAKER	STAGE OF CLINICAL TESTING
CEREZYME (imiglucerase)	Glycolipid-degrading enzyme; compensates for the enzyme deficiency responsible for Gaucher's disease	GENZYME *Cambridge, Mass.*	On the market
VANCOCIN (vancomycin)	Glycopeptide antibiotic often used against antibiotic-resistant infections; inhibits the production of a sugary component (peptidoglycan) of the bacterial wall	ELI LILLY *Indianapolis*	On the market
VEVESCA (OGT 918)	Sugar mimic; aims to reduce the synthesis of the glycolipid that accumulates in Gaucher's disease	OXFORD GLYCOSCIENCES *Abingdon, England*	U.S. regulators are reviewing data from phase III trials (large studies of efficacy)
GMK VACCINE	Vaccine containing the sugar ganglioside GM2; designed to trigger an immune response against cancer cells bearing GM2	PROGENICS PHARMACEUTICALS *Tarrytown, N.Y.*	In phase III trials for melanoma
STAPHVAX	Vaccine containing a bacterial sugar coupled to a protein; meant to prevent hospital-acquired *Staphylococcus* infections	NABI BIOPHARMACEUTICALS *Boca Raton, Fla.*	In phase III trials for patients with kidney disease
BIMOSIAMOSE (TBC1269)	Sugar mimic; aims to stop selectins (sugar-binding molecules) on blood vessel walls from promoting inflammation	TEXAS BIOTECHNOLOGY *Houston*	In phase II (relatively small) trials for asthma and psoriasis
GCS-100	Sugar that interferes with the action of a sugar-binding protein on tumors	GLYCOGENESYS *Boston*	In phase II trials for pancreatic and colorectal cancers
GD0039 (swainsonine)	Sugar mimic that blocks production of carbohydrates important to cancer's spread in the body (metastasis)	GLYCODESIGN *Toronto*	In phase II trials for kidney cancer
PI-88	Sugar that inhibits growth factors responsible for angiogenesis (new blood vessel formation) and interferes with an enzyme involved in metastasis	PROGEN *Darra, Australia*	In phase II trials for multiple myeloma (a blood cancer); in phase I/II (safety and efficacy) trials for melanoma
UT231B	Sugar mimic that hampers the hepatitis C virus from infecting cells	UNITED THERAPEUTICS *Silver Spring, Md.*	Phase I (safety) trials have been completed

ment as potential anti-inflammatory drugs.

Researchers are also exploring several sugar-related strategies for fighting cancer. For example, malignant cells often display incomplete or abnormal sugars on their surface. Workers are therefore attempting to incorporate such sugars into therapeutic vaccines that would induce the immune system to recognize and destroy cancer cells bearing those sugars.

Sasisekharan's group at M.I.T. recently showed in mice that heparan sulfates, sugars found on normal and malignant cells, can enhance or limit cancer growth depending on how those sugars are cleaved by cellular enzymes. This discovery has led to suggestions of treating cancer by delivering the growth-slowing fragment of the sugar or by delivering some substance that would cause cancer cells themselves to produce a healthier amount of the desirable fragment.

Cancers usually kill by metastasizing: malignant cells break away from a tumor and plow through connective tissue into the bloodstream. Then they travel through the blood (or lymph) to distant tissues, where they leave the circulation and establish new tumors. One of the molecules that seem to abet such travel is a sugar-binding protein known as galectin-3, which additionally appears to facilitate metastasis by participating in angiogenesis (the formation of new blood vessels) and by helping tumor cells resist signals instructing them to kill themselves. GlycoGenesys, a Boston biotechnology company, is conducting clinical trials with a carbohydrate derived from citrus pectin that attaches to galectin-3 and basically tells tumor cells, "Do not adhere to sugar targets along your metastatic route, do not form new blood vessels, and do allow your self-destruct program to operate."

Cells produce glycoconjugates in a series of steps, during which various enzymes add or remove sugar groups. Later, enzymes in membrane-bound compartments called lysosomes break up glycolipids and glycoproteins that are no longer useful. In a heartbreaking family of ailments that includes Gaucher's and Tay-Sachs diseases, one lysosomal enzyme or another is defective, leading to a destructive buildup of glycolipids in the body. Certain of these disorders, such as Gaucher's, can be eased these days by delivery of the normal enzyme after the enzyme has been modified to display a sugar that targets it to a specific cell type. In the case of Gaucher's therapy, the sugar mannose directs the glycolipid-degrading enzyme to macrophages, which are especially sensitive to the enzyme's loss.

Enzyme therapy is expensive, however, and must be delivered intravenously, because enzymes are proteins and would be broken down by the digestive tract if taken orally. Moreover, enzymes do not cross from the blood to the brain and so cannot combat damage to nerve cells in the brain. Researchers are therefore trying to limit the glycolipid buildup in these afflictions in another way: by reducing the amount made in the first place—mainly by delivering small compounds, such as sugar mimics, able to inhibit enzymes involved in glycolipid synthesis. One such drug, developed by Oxford GlycoSciences in Abingdon, England, would be taken by mouth and has been shown in human trials to work against Gaucher's disease; the U.S. Food and Drug Administration is currently reviewing the clinical data.

Glycomics research might even lead to advances in the ability to transplant pig organs into people when human versions are in short supply. One obstacle to such cross-species, or xeno-, transplantation is that pig tissue displays a sugar not found on human tissues. That sugar would elicit a swift graft-destroying reaction by the recipient's immune system. This impediment could, in theory, be surmounted in several ways—among them, delivering sugar mimics as decoys and genetically altering pigs so that their enzymes do not give rise to the offending sugar.

Serious problems confront the development of carbohydrate-based drugs, especially ones composed of true sugars. The digestive system generally regards sugars as food, so they would have to be packaged to avoid degradation or injected. In the bloodstream, too, sugars may be broken down by enzymes, and because carbohydrates often act by binding loosely to many sites rather than by binding tightly to a few, they may need to be given in large quantities. None of these hurdles is insurmountable, however. Meanwhile a growing awareness of the roles that sugars play in the body and improved techniques for sequencing and manipulating them promise to open an entirely new dimension in therapeutics. SA

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Thomas Maeder is a science writer based in Pennsylvania.

MORE TO EXPLORE

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Consortium for Functional Genomics: **http://glycomics.scripps.edu**