This issue of UT Discovers focuses on health. The University of Toledo merged with the Medical University of Ohio only six short years ago. It is natural that health and health issues would be the focus of a medical college, but you might be surprised at the disciplines and faculty interested in examining the many facets of topics associated with a focus on health. We offer only a small sampling here.

Research at UT is conducted at multiple levels from the lab to the clinic to data analysis.

As you read this issue, you will learn about discoveries that contribute to knowledge about cancer cell movement and death, fatigue, headache and stroke, pediatric drugs, drug abuse, diabetes treatment, cell function, and ethics to name a few topics.

UT researchers are dynamic, curious, excited and very individual. This issue introduces you to these people in the lab and outside the lab. They have interests ranging from extreme sports, music, hiking and cycling to gardening, reading and beading.

We hope you enjoy learning about the cutting-edge research our scholars are conducting and also what they do to relax after a long day in the lab. If you have any questions about this work or would like additional information, I would be happy to make inquiries for you.

James P. Trempe
Vice President for Research
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Jeffrey Blumer is trying to change that picture by closely examining both existing drugs and new drugs to determine their effectiveness in infants and children. As a clinical pharmacologist and toxicologist, in addition to being a pediatric intensive care physician, Blumer wants drugs that will be used in children to be tested in children. Only in this manner can they be labeled to indicate evidence-based dosing and provide a safety profile for infants and children.

Since the dosage for many drugs is weight based, doctors often prescribe lower doses for infants and children. “But children are not just little adults,” says Blumer. He comments that doctors have only recently come to this realization.

Blumer studies drugs because, as a pediatrician, drugs and their wide range of uses are essential tools of his trade. Although Blumer’s specialty is pediatric pharmacology and toxicology he works closely with those who have expertise in specific areas: neurology, anesthesia, psychiatry, to name a few. With the number of drugs on the market today, he must be careful in selecting the one to study. He says he uses two approaches: First is to study the drugs used most commonly. This is particularly important because most parents do not understand that off-label use of these drugs is a guessing game and that doctors are really experimenting on their children without permission. Second is to keep abreast of developments in the pharmaceutical industry. If a drug that might have usefulness is under development, he and his colleagues can help get it tested in children and it will then become available with dosing information and a known safety profile.

Dose changes can often be counterintuitive. For example, an adult dose of the antibiotic amoxicillin may be 20/10 mg a day. However, an effective dose for children may be 90 mg/kg a day because of differences in body composition, kidney function and metabolism. Blumer’s work with antibiotics concluded that the dosing used for 40 years was incorrect. By using a target that was not a patient, he found that an antibiotic needs to be present at the site of infection for at least half the dosing interval (time between doses) and at concentrations four times the amount needed to kill the bacteria. By measuring the concentration of a drug over time, researchers can determine what doses are required.
for effectiveness. “But this is difficult in children,” Blumer comments, “because children are constantly changing. So the dose has to change as well as the time interval, which depends on the drug.”

“People tend to be preoccupied with safety issues,” Blumer remarks, “but in pediatric patients, the biggest issue is whether the drug does anything at all.”

The issue of effectiveness has been most pronounced with sleep problems, Blumer says. “This is a huge issue for families and can lead to divorce and children up for adoption.” Sleep aids generate more than $2 billion a year for pharmaceutical companies. When the drug company Sanofi wanted to get the information necessary to label Ambien for children, it called on Blumer. In a randomized, controlled, double-blind trial, he found that Ambien does not work at all for children ages 6 to 18. Effectiveness is also a challenge with mental health issues. Antidepressants used for decades in adults do not work in children. Additionally, Blumer says these drugs have significant side effects with no hope of benefit. “When we prescribe drugs that don’t work,” comments Blumer, “we are tipping the balance of benefit and risk far in the direction of risk. If drugs had a sharp edge and could draw blood, we would have had regulatory legislation a lot sooner.”

Over the past 25 years, Blumer has been responsible for designing the trials for testing probably 60 percent of all drugs that are now labeled for use in infants and children. “Originally no one knew how to design a clinical trial for children,” he says and notes that there are special problems associated with this kind of effort. With adults, a researcher can ask about unusual side effects—headache? blurry vision? muscle ache? You can’t elicit that information from an infant or even a 3-year-old. Children who participate in clinical trials are also not normal and healthy as adults are in trials. These children are sick, so a major challenge was developing minimally invasive protocols and tests that allowed obtaining the necessary information. For example, using an indwelling catheter instead of sticking a child to draw multiple blood samples became a standard tool. Adult clinical trials obtain informed consent from the subject. That is not the case with children. Parents give consent, but the children must give assent. If a child says, “No way, no how,” that is it. But Blumer notes that some children are very sophisticated and are anxious to participate. He also remarks that the things that upset children are not what one might expect—an ECG or throat swabs can be deal breakers, but blood draws usually are not.

Blumer and his colleagues also had to learn how to engage families. Many are motivated, but adjustments still have to be made to schedules. With parents at work and children in school, researchers need to have late afternoon or evening hours and weekend options.

Drugs are the tools Blumer uses; he stresses their necessity. The key to using these tools is to use them safely and effectively. Blumer has spent his professional life learning about the drugs he uses. He remarks that, in the fall of 2012, legislation was passed that requires drugs used in children to be tested in children. After years of working with Congress, presidents and cabinets, he considers this legislation a crowning success.

This research has been funded by the National Institutes of Health, the Ohio Department of Health and several pharmaceutical companies: Merck Sharp Dohme; Cerexa, Inc.; Bristol-Myers Squibb; Ftaa Therapeutics, Inc.; Aventis Pharmaceuticals, Inc.; Abbott, Reckel Laboratory; Taro; Taiji Research & Development Center, Inc.; Cubist Pharmaceuticals, Inc.; Bopy Healthcare; GlaxoSmithKline, Amgen, Inc., and Novo Nordisk.

Jeffrey Blumer is chair and professor in the Department of Pediatrics. He received his doctorate from Northwestern University and his MD from Case Western Reserve University, where he also did postdoctoral studies. Dr. Blumer serves on several national and state boards and is regularly invited to give lectures both nationally and internationally as a recognized expert in pediatric infectious diseases and pharmacology. His current research interests include pediatric clinical pharmacology and toxicology, genetic control of drug toxicity, biochemical genetics of acute childhood leukemia, and biochemical and molecular mechanisms of mutagenesis.
As a child, Amanda Bryant-Friedrich liked to work on the farm with her father. One day when she was about nine, a drum of chemicals—probably herbicides—exploded and sprayed all over her. It was the first time she had heard the term ‘chemistry’ and became fascinated, deciding then and there to become a chemist. When her mother became ill and was on one drug after another, she decided she would make drugs. “Do I make drugs now? No,” Bryant-Friedrich says. But, following that early childhood decision, she does do chemistry.

Bryant-Friedrich says she is a medical and biological chemist. She determined early that just making drugs would not lead to the answers she wanted, so her research today focuses on understanding how diseases start—how the things we are exposed to every day promote disease. Her particular focus is on cancer etiology.

As a synthetic organic chemist, Bryant-Friedrich makes molecules. The molecules she makes allow her to study the mechanisms that lead to the development of cancer. Those mechanisms, she explains, have to do with oxidation—the process by which a molecule either picks up an oxygen atom or releases an electron, thereby creating a free radical. Radicals are reactive compounds that form through natural body processes and react with DNA, proteins and lipids. When the radical reacts with DNA, they cause mutations in the molecule that can sometimes be repaired and sometimes not. If not, the mutation can persist and create genetic changes that lead to many diseases, cancer in particular.

Some mutations are harmless; some are not, explains Bryant-Friedrich. She wants to know which mutations lead to cancer and which don’t. Furthermore, she asks how. Using her expertise in organic synthesis, she makes molecules that modify nucleic acids and allow her to study mutations and how they form. She explains that every student in her lab is required to make a new molecule and, using an automated system of DNA synthesis, make pieces of DNA that are like the DNA in a living cell but with the modifications in specific places. By selecting DNA sequences known to be related to cancer development, she and her students can then generate certain species of DNA under controlled conditions.

But how do they know when they are looking at the right species of DNA? Using sophisticated machinery such as mass spectrometry, chromatography, nuclear magnetic resonance and biochemical processes, the researchers can determine exactly what chemical modifications occur in the DNA. That information tells them a lot about the structure of the damaged DNA and if the damage can be repaired. “If we know what components of the cell react with each other and what reactions lead to mutations, we can develop ways of intervening or even stopping the process from happening in the first place,” Bryant-Friedrich says.

“All the things we are looking at have to do with oxidation.”
According to the National Institutes of Health, diabetes affects more than 20 million Americans.

Diabetes is caused by too little insulin, a resistance to insulin, or both. The primary function of insulin, a protein produced by the pancreas, is to move sugar from the bloodstream into cells where it is used for energy. People with diabetes have high blood sugar because their pancreas makes insufficient insulin or the body does not properly respond to insulin. The build-up of glucose in the blood can affect the kidneys, heart, eyes, and nerves. Diabetes, as related to secondary complications, is commonly an underlying cause of heart attacks and strokes. If your Aunt Sally has diabetes, she must monitor her blood glucose levels closely; she does this by pricking her finger and using a small device to measure the sugar content of a drop of blood.

What if Aunt Sally did not have to stick her finger every day—or twice a day? Brent Cameron has been working for several years on developing noninvasive glucose sensors using optical technologies. Being an engineer, Cameron knew that liquids bend light at different angles depending on the refractive index of the fluid, which also varies with the concentration of substances in that liquid (think of looking at a penny lying at the bottom of a pool—you can see it, but the perception of its actual position is distorted). Moreover, based on another principle, glucose can rotate the electric field of light—a phenomenon commonly referred to as optical activity. Each of these principles are just two ways of measuring glucose concentration noninvasively using light.

The eye contains a clear fluid called the aqueous humor, which is a blood filtrate. It is contained in a chamber located between the cornea and the lens. Because glucose is the source of energy for cells in the body and is transported by the blood; it is also present in the aqueous humor at nearly the same concentration as in the bloodstream. Cameron thought that if he could assess the interaction of glucose with light in the aqueous humor, he could noninvasively monitor glucose concentration in the body.

Cameron and his colleagues found that if they pass a pulsed laser beam through the aqueous humor of the eye, they could measure the optical activity of glucose and therefore determine its concentration. In this case, because the light travels through the eye in a horizontal direction,
process using a high-resolution camera.

“The idea is to monitor how the iris appears to change shape as the concentration of glucose in the aqueous humor changes,” Cameron says. “The iris is distinct in every individual—like a fingerprint. Because glucose dissolved in solution changes the optical refractive index, a sequence of images of the iris can yield a measure of the glucose concentration.”

The real trick, however, was creating the algorithm that processes very small variations in the pixelated images of the iris. Although Cameron has been working on this technique for several years, the first formal report on its feasibility appeared in 2008; and it is now headed for clinical trials in the near future.

His experience with developing novel methods to detect the presence of minute concentrations of substances has now led Cameron to explore biomarker detection. This research uses a technique known as surface plasmon resonance (SPR) sensing, which uses biomolecular recognition units to detect a specific target. What does this mean? Cameron explains: If you want to detect a biomolecule in a liquid or gas, you can identify a DNA sequence that will attract that molecule and bind it to an extremely thin nanometer scale noble metal surface (e.g., gold, silver, or platinum).

These surfaces exhibit optical plasmonic properties which can be exploited for sensing purposes. For example, the surface can be coupled to a light source and detector. The light source, when reflected or scattered off this surface under certain conditions, will excite plasmons within the metal. The conditions for which energy transfer occurs will change depending on the amount of target bound to the attached DNA on the metal surface. Therefore, monitoring the condition(s) under which energy transfer occurs can provide highly sensitive measurements at ultralow concentration levels. In addition, Cameron has worked on developing methods for specific DNA selection, which allows this technique to be customized to almost any target imaginable.

The performance of this sensor system is comparable to large, expensive laboratory equipment such as mass spectrometers and liquid gas chromatography, notes Cameron, except the sensor itself is about the size of a quarter. Further advantages are that it is mobile, inexpensive, and does the analysis in real-time. Most current biomarker work is in the discovery area, he says, but the future of biomarker research is in sensors, which will allow for their widespread use in healthcare.

This new sensor has implications in clinical diagnosis, security concerns, and individualized medicine. The next generation of biomarker sensors will improve health, tailor therapies, and analyze drug tolerances and effectiveness. Cameron, his colleagues, and students are opening doors and moving into new and previously unimagined worlds.

BRENT CAMERON

A stent is a small tubing usually made of metal that is inserted into a constricted artery or other anatomical passageway to facilitate the flow of some fluid.

Most people hear about coronary stents that increase blood flow to or from the heart. But there are also carotid stents to increase flow to the brain, renal stents to increase blood flow to the kidneys, and various other types of stents inserted to open or repair a natural conduit.

Having spent time conducting vascular research at Harvard University under Andrew Selwyn and Mark Creager, Christopher Cooper came to the University of Toledo to work with Mark Burket, who was doing research on the safety and efficacy of renal artery stents. But, with a more global view and a long-range goal of determining how best to manage patients with blocked kidney arteries, Cooper says it became apparent to him that he needed to do more than study renal stents.

After writing several grant proposals, Cooper says he finally managed to get one off the ground. The first randomized clinical trial of glycoprotein inhibitors and an embolic protection device, this study examined their efficacy in treating renal artery constriction. Glycoprotein inhibitors are chemicals that prevent platelets from binding together, platelets being the components of the blood responsible for clotting. An embolic protective device is a cone-shaped mesh inserted into an artery to trap debris released by other treatments. “But the interaction between the device and the drug was a surprise,” Cooper comments. Patients treated with both were much more likely to have an improvement in kidney function.

At about the same time he was working on the glycoprotein inhibitor study, he received funding for a study entitled “Cardiovascular Outcomes for Renal Atherosclerotic Lesions,” nicknamed CORAL. “This is a pivotal trial,” notes Cooper, “and will provide data for many years.” UT is the coordinating center for more than 100 clinical study sites on five continents. The study is blinded and will help answer some basic questions.

Brett Cameron is a professor in the Department of Biomedical Engineering at Texas A&M University. Cameron received his PhD in Biomedical Engineering from Texas A&M University in 2000. On arriving at UT he founded the Biomedical Optical Laboratory, the focus of which is on the development of innovative and novel medical devices based on the use of optical techniques. Research in his lab has developed advanced modeling techniques for applications such as physiological glycemic forecasting for diabetes and critical care diagnostics. Cameron is also affiliated with the Center for Diabetes and Endocrine Research in the College of Medicine.

To Stent—or Not
The last patient visit was in September 2012 with the first data analysis reports being issued in Fall 2013. Cooper explains that for years science and physicians thought that the best treatment for narrowing of the kidney artery was surgery to bypass the constriction. But surgery is risky as 10 percent of patients who undergo surgery die. Then balloon angioplasty arrived. Physicians thought it worked as well as surgery, and it did for a while, but the narrowing returns. When the stent came on the scene, some studies suggested that it is better than medication alone and prevents a remarrowing of the artery. Cooper says that, at the end of the day, CORAL will provide answers to three primary questions: (1) Do stents provide additional benefit beyond medications; (2) if so, what groups of patients benefit; and (3) what is the best, safest way to do the procedure?

Cooper says he is particularly excited about a new research project he is working on in collaboration with Bina Joe (in the Department of Medicine and now Dean of the School of Medicine at Marshall University). This study will examine the relationship of platelet activation and inflammation to kidney injury. The research will combine human studies and the creation of new transgenic animal models that will shed light on the mechanisms that lead to kidney injury. “The first of these knockout rats have been born,” he adds, “and DNA analysis demonstrates that the genes were removed.”

An advantage of the interdisciplinary work and large trials such as the ones Cooper has led is that they provide data for other scientists. He says that Jiang Tian, in the Department of Medicine has recently received NIH funding to use stored blood from the CORAL study to look at novel cardiotonic steroids. “These compounds are naturally occurring substances that may be implicated in hypertension, congestive heart failure, diabetes, and end-stage renal disease. Cooper comments that a spin-off of these large clinical trials is the development of datasets that provide an opportunity for graduate students in mathematics and statistics to work with large, robust datasets. He is particularly enthusiastic about his work with Don White and Paul Hewitt in the Mathematics and Statistics Department on Main Campus, and their students. Presenting his work in multidisciplinary colloquia opens doors to interdisciplinary inquiry and collaboration, broadens horizons and can lead to productive new insights.

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Christopher Cooper is professor and chair of the Department of Medicine. He received his MD from the University of Cincinnati and completed a residency and fellowship at Brigham and Women’s Hospital at Harvard University. He has successfully combined clinical practice and research, focusing on research that will produce better treatment for patients suffering from renal artery disease. When not working, Cooper relaxes with his wife and children.

Consequently, some researchers are now saying that the focus needs to change from fighting primary tumors to fighting and developing therapies for metastatic disease. All cells have a cytoskeleton made up of proteins that give a cell support and shape. Some cells have a quite rigid cytoskeleton, others are very flexible. Cancer cells have a rigid but dynamic cytoskeleton and are capable of changing from rigid to flexible on the fly, explains Kate Eisenmann. This ability to change from a rigid structure to an amorphous, amoeboid one is critical in driving some forms of metastasis and is known to occur in glioblastomas and breast, prostate, and colon cancers.

As a postdoc, Eisenmann was working on characterizing proteins that build the cytoskeleton of cancer cells. The idea at the time was that the proteins responsible for building cellular structures called filopodia, which act as sensors for motile cells, went haywire. When she started to manipulate the proteins, she saw the filopodia being destroyed rather than being built. She thought she had made a terrible mistake and ran the experiment another 10 times. When cells die, they create membrane bubbles that break off—a process called blebbing; but these cells were not dying, Eisenmann says. So she spent hours in...
structures to “crawl” and emitting energy because it uses hydrostatic force. “It is actually thought that the squeeze comes up against a hole too small to pass through,” she says. Mouse data suggest that these amoeboid-type cells cause more tumors to form. Moreover, these morphological changes occur in every cancer cell tested so far. “It is a conserved mechanism,” she adds, “present in every type of developing cell. It is a normal process that cancer cells have hijacked.”

Cancer cells can secrete enzymes that allow them to forge their way through tissue that is too rigidly woven for them to squeeze through in their amoeboid form. If a flexible cell comes up against a hole too small to squeeze through, it needs to change on the fly into the more rigid form. “It is actually thought that the squeeze method of moving is more energy efficient because it uses hydrostatic pressure, whereas using internal structures to ‘crawl’ and emitting enzymes to dissolve tissue require expending much more energy,” notes Eisenmann. The need for both methods of locomotion explains why cancer cells must be able to change their internal structures.

Eisenmann and her laboratory co-workers have identified a specific protein that is present in normal cells but missing in most metastatic cancer cells. “This protein is very likely a tumor suppressor,” she explains, adding that her hypotheses have been substantiated in a recent paper. This paper analyzed primary and metastatic breast cancer tumors and found that the protein Eisenmann has identified is lost in 80 percent of metastatic tumors and 40 percent of primary tumors. “This reinforces the idea that our protein is a normal tumor suppressor. Take it away and the cells go crazy,” she says. Moreover, when the protein is removed from cancer cells, we get more tumors. The cell is stuck in its amoeboid form.

Using advanced technological methods, Eisenmann says she is now able to track protein activity in real time. “A new biosensor will allow us to monitor the activity of the protein that activates the switch from rigid to flexible and back,” she says. And not only will they be able to see when the protein turns on, they will find out where in the cell it initiates the shape-changing signaling cascade.

“But what induces these cells to move in the first place?” asks Eisenmann. Cells exist in a microenvironment, surrounded by other cells. Cells also signal each other by releasing various proteins and chemicals that other cells sense. So what is the signal that cancer cells receive that induces them to move or to change shape? And where does that signal (or signals) come from? Eisenmann says she thinks the local microenvironment may be the culprit—that the fibroblasts, which are cells that are responsible for the extracellular matrix and play a critical role in wound healing, are secreting a protein that the cancer cells detect. This protein, called a chemokine, is a normal substance that can induce an immune response at the site of an infection and also control the migration of cells. In cancer, the cells have taken a normal process and hijacked and amplified it.

Eisenmann’s work has raised other questions—such as what causes the cells to lose the chunk of DNA that codes for the protein she has described. “A whole piece of DNA on chromosome 13 is missing in the migrating cancer cells,” she notes. “Something is going on there that we don’t understand. But that is someone else’s quest.” Her focus, her niche, is on the mechanisms of cell migration and invasion—what happens at the molecular level to induce these changes. A thorough understanding of cell migration and invasion will help in diagnosis and therapy of various disease states as well as shed light on embryonic development, growth and cytoskeletal remodeling in many cell types.

**KATHRYN EISEMANN**

Kathryn (Katie) Eisenmann is an assistant professor of biochemistry and cancer biology. She received her Ph.D. from the University of Missouri, St. Louis, and was a Postdoctoral Fellow at the Laboratory of Cell Structure & Signal Integration, Van Andel Research Institute before coming to UT. She has funding from the National Institutes of Health. Her research on the mechanisms of cytoskeleton restructuring has appeared in major professional journals and has even been controversial in some areas, causing a bit of ferment. Eisenmann participates in extreme sports and is shown here in a Warrior Dash race.
gerally improves a person's quality of life. Interestingly enough," adds Elmer, "another drug called rasagiline, which was originally developed for treating Alzheimer's symptoms, actually works better in improving memory and executive function in Parkinson's patients."

One of the major advances in treating Parkinson's symptoms was the development of L-dopa, which gets absorbed by the nerve cell and converted into dopamine. However, oral medications always have peaks and troughs in the bloodstream and the brain, Elmer explains. "These peaks and troughs are not the normal physiology of the brain, so most symptomatic therapies focus on making the dopamine effects more constant and stable. One of these methods is to use chemicals called dopamine agonists, which are chemicals that bind to dopamine receptors and mimic dopamine. Some are once-daily formulations, and one is a skin patch that gives a stable dose more in line with what the brain itself does with dopamine," he says.

New medications, such as adenosine and adrenergic receptor antagonists, work through completely different pathways, separate from dopamine receptors, coming along, Elmer says. "Adenosine and adrenergic compounds modify the activity of nerve cells and has been shown to treat symptoms and possibly slow progression of Parkinson's disease in animals. "The biggest challenge is finding optimal individual medication regimens," notes Elmer. "Not every patient responds well or can tolerate all meds."

In a concerted effort to improve the quality of life for Parkinson's disease patients, Elmer has developed a cross-disciplinary approach as a whole physician, nurse, psychologists, pharmacists and therapists cooperate in treating Parkinson's. In particular, his work with Michelle Masterson, Chair of the Department of Rehabilitation Sciences, indicates a positive impact from individualized, dedicated therapies. "We have learned so much about how the brain adapts," he comments. A series of therapies and exercises—exaggerated movements held for lengthy periods—seem to activate and reprogram areas of the brain not previously involved in motor control. He adds that newer brain scans can image brain function and pinpoint dopamine levels in the brain. "In selected individuals, it helps to know what we are really dealing with. Studies using these brain scans indicate that, in some cases, medications for bipolar disorder can actually cause Parkinson's symptoms. This knowledge permits us to change their bipolar medications and relieve the symptoms."

The most exciting development in treating movement disorders is the prospect of diagnosing the disease even before symptoms occur. "Recent research indicates that Parkinson's doesn't start just when symptoms appear but many years before," Elmer says, "when people start to lose their sense of smell, have REM sleep behavior disorders, bowel problems, blood pressure control issues and other seemingly unrelated problems. These small symptoms precede the onset of full-blown Parkinson's. He explains that the pathology spreads from nerve cell to nerve cell, starting in olfactory and intestinal cells, eventually working its way to dopamine neurons in the brain. "We can now look at pre-motor symptoms and intervene at a much earlier stage before most of the damage to dopamine neurons has occurred," Elmer says. "Knowledge of this progression makes the treatment of the disease earlier but actually prevent the onset of typical Parkinson's symptoms."

Elmer says we have now gone full circle to again concentrate on research that seeks to slow disease progression rather than just treating the symptoms. Drugs have failed to radically slow the disease if used only after symptoms are manifest. "But if we can use them before symptoms appear, they might work beyond our wildest dreams," he adds. "What would be needed is a simple and inexpensive test that is easy to administer. The hope is to eventually halt the disease in its tracks. Elmer thinks the same process is possibly true for Alzheimer's—that the same kind of early detection and prognosis could slow progression or possibly prevent development of the disease."
To live and reproduce, many bacteria require a host—a plant or animal—and most are quite particular about their living quarters, having evolved reciprocal arrangements over the millennia. Bacteria don’t have brains, and they don’t “think,” but it is reasonable to assume that they don’t want to kill their host, as that would kill them—or at least prevent replication and persistence. However, when they manage to find themselves in unfamiliar or hostile territory, they fight back.

Mark Wooten and Jason Huntley both study some rather nasty bacteria that can be lethal and are highly resistant to antibiotics. These bacteria are always found in the environment, Wooten explains, but when they become airborne—aerosolized—they become the ultimate weapon. “Something changes when they are airborne and enter the lungs,” he adds. “They suddenly become super-virulent. We want to find out what changes, what makes them so virulent, and what we can do to make an effective vaccine.”

**THE RESEARCHERS EXPLAIN THAT SOMETHING ON THE SURFACE OF THE BACTERIA WOULD BE THE BEST TARGET SINCE NEUTRALIZING THAT PROTEIN WOULD PREVENT THE BACTERIA FROM DOING ANY HARM. BUT TRYING TO FIGURE OUT WHAT THAT MIGHT BE AND MAKING THE RIGHT ANTIBODIES ARE PROBLEMATIC. WE ARE FINDING OUT THAT WHAT THE BUG MAKES IN THE TEST TUBE IS NOT THE SAME AS WHAT IT DOES IN A MAMMALIAN BODY, WOOTEN COMMENTS.**

Wooten is working with *Burkholderia pseudomallei* and *B. mallei*, while Huntley is working with an organism named *Franciscella tularensis*. These organisms live in different environments throughout their life cycles and make what they need for survival in each particular environment. *Burkholderia* does fine living in water, Wooten says, but when it finds itself in a human host and faces immune system cells, it turns on a whole new set of proteins for protection. He likens bacterial protective systems to a military deployment—you don’t take Arctic gear to the Tunisian desert; you only take what you need for that particular environment.

When dangerous bacteria enter the body, the immune system springs into action to control the infection. It was once thought that macrophages were the critical element, but recent results on *Burkholderia* indicate that the neutrophil comes in to do the actual killing and can enlist the aid of other cells in the immune system. However, the bacteria can adapt quickly to counter the efforts of the immune system—they can change their surface structure and even hide in different places in the body. On the other hand, Huntley says that *Franciscella* actually infects neutrophils and uses them as a place to hide from the immune system.

Huntley explains that it is the surface proteins (antigens) of the bacteria that mediate virulence. But these are the very proteins that, because they are on the surface, are the molecules targeted by immune cells. He says that *Franciscella* has about 2,000 proteins. Showing a host all the antigens at once could be a recipe for suicide, but continually changing the number and amount of surface proteins confuses the immune system. But which surface proteins to target for new vaccines?

Huntley and his colleagues have developed a method of purifying just the surface proteins from the bacteria. They have identified about 50 of the most abundant. “But there are probably another 50 unidentified surface proteins,” he adds. When they put the bacteria into a host, those unidentified proteins become more plentiful. So he is trying to identify these unknowns and also looking at them in host organs to see which are the most important in mediating the disease state. It turns out that the proteins expressed are different if the bacteria are in the lungs than when they are in the liver, spleen, intestine or kidneys. Huntley has come up with a group of five vaccines made with some of these surface proteins, and one of them protects mice from pulmonary tularemia.

Huntley and Wooten, although working with different organisms, are working towards the same goal—describing how bacteria respond to host attacks and developing effective new vaccines. Once they understand the host-pathogen interaction, developing effective vaccines should be easier. Furthermore, developing vaccines for these two deadly pathogens can lead to methods for quickly developing vaccines against related invaders.

**JASON HUNTLEY**

Jason Huntley is an assistant professor of microbiology and immunology. He received his doctorate in veterinary pathology from Iowa State University and completed postdoctoral training at the University of Texas Southwestern Medical Center before coming to UT in 2010. Huntley plays bluegrass and is a regular member of a small band.

**MARK WOOTEN**

Mark Wooten is an associate professor of microbiology and immunology. He received his PhD from the University of Mississippi Medical Center and came to UT after completing a postdoctoral position at the University of Texas College of Medicine. His research has centered on Lyme disease which he came to be native in Ohio. The spirochete *Borrelia burgdorferi*, which is responsible for Lyme disease, and *Burkholderia pseudomallei*, which causes melioidosis, are both capable of evading host defenses—an area he is fascinated with three shape-changing organisms: Wooten, shown here with his son at Zion National Park, enjoys hiking, climbing and spending time with his family.
How Do They Do That?

Viruses—from the Latin virus, meaning poison—are minute structures that infect living plants or animals. They are incapable of self-propagation and can replicate only inside a living cell by forcing the host cell to produce identical copies of the viral invader. About 5,000 individual viruses have been described, but there are many more; they are thought to be the most abundant type of biological organism on Earth.

As a graduate student, Doug Leaman worked with a family of proteins called interferons. He found them so fascinating that he has worked with them ever since. From fish to humans, cells come equipped with built-in immune responses, called the innate immune system, which mobilize to fight viral or microbial invasions. Interferons are the primary pathway used by the innate immune system to block viral infections and are produced in almost every cell in the body. "If we eliminate the interferon system by knocking out the receptor, mice become exquisitely sensitive to all viruses, even ones that normally cause no symptoms," Leaman says.

Research Leaman is particularly excited about is his investigation into a specific cell protein called RNF114, which belongs to a class of proteins called E3 ubiquitin ligases. This group of proteins is primarily involved in regulating protein stability, and Leaman’s data suggest that RNF114 is responsible for regulating the sensitivity of certain virus detection pathways. "If RNF114 is highly expressed and active," explains Leaman, "it helps to shut down or desensitize virus detection pathways.” Why would this occur? "If the proteins responsible for virus detection were active all the time, you would have constant interferon production and would always feel sick. So turning off the response in the absence of a virus is important," he adds. "We believe RNF114 plays a critical role in keeping the virus detection pathway silent in the absence of a virus.”

Leaman’s lab has successfully created a knock-out mouse model that is missing the RNF114 gene. In these mice, the end point of the virus detection pathway—interferon expression—is elevated compared to wild-type (or normal) mice. The implications are extensive, he comments. This mouse model will allow researchers to study the cumulative effects of constant interferon expression—a situation that could cause continuous inflammation and contribute to the development of autoimmune diseases such as multiple sclerosis, diabetes, lupus, Crohn’s disease and psoriasis. Leaman thinks these mice will be a good model to help understand how the inappropriate expression of proteins involved in fighting viral and other microbial infections can ultimately contribute to long-term diseases.

But Leaman is also looking at host-virus interaction from the viewpoint of the virus.
Obviously, when we get a viral infection, the virus has somehow evaded or disabled the host’s immune system defenses. He is currently working with a fish virus—viral hemorrhagic septicemia virus, VHSV for short—that is devastating to fish populations. It may be "only" a fish virus, but understanding how this virus behaves could well lead to better understanding of virus behavior in human diseases.

Laboratory studies over the past 30 years have shown that most—if not all—viruses can actively inhibit the host’s virus detection pathways. Leaman’s data suggest that this is true of VHSV infections as well. However, recognizing this is just the first step, he notes. His lab is seeking to determine which VHSV proteins are involved in shutting down the host responses, how they manage to do this, and how the virus adapts and changes in response to changes in its host. Viruses can be around and be benign, he explains, until they undergo a genetic change that makes them more virulent and thus more capable of inhibiting host responses. Work on this project is being conducted in collaboration with Carol Stepien of the UT Lake Erie Center, who is eager to map the evolution of VHSV throughout the Great Lakes. Viral genomic sequence information from Stepien’s work will be combined with the laboratory studies conducted in Leaman’s laboratory to better understand VHSV’s recent emergence in the Great Lakes.

Leaman says that they have so far identified two separate viral proteins that appear to block the host’s innate immune response—and the virus is doing this via two distinctly different mechanisms. In the first case, a viral protein is blocking cellular signaling from two directions: it is preventing the production of interferon and also preventing cells from responding to interferon’s warning signals. In the second case, the virus blocks the host cell’s RNA synthesis, which is a crucial way in which a virus can outpace the cell’s ability to thwart it.

Most viruses, like the VHSV, do inhibit the host detection system in some way. Thus, Leaman’s discoveries with this fish virus, although important in fish populations and for aquaculture, can also potentially contribute to our understanding of how other viruses cause human diseases.

Contrary to what many think, bone is a dynamic tissue that responds to many cues from other organs: progesterone, estrogen, vitamins, phosphorus, calcium, metabolic products, and other substances. Research on bone health has shown that osteoporosis—the loss of bone mass leading to thin and fragile bones—is related to a number of conditions such as aging, renal disease, inflammation, estrogen deficiency, and diabetes.

Diabetes, a condition in which the body’s energy metabolism is impaired, has major consequences for bone health and is of particular interest to Beata Lecka-Czernik. Cells use glucose as their energy source, but first that glucose must enter the cell, a process...
within bone defect
of adipocytes (fat cells)
(bottom panel – white areas represent fat cells)
(healing) and causes accumulation of fat at the site of healing
generated with micro
3D images of bone

Lecka-Czernik notes, “but fat
in bone increases. “We don’t
have a negative side effect: they
molecules. Unfortunately, they
admit the passage of glucose
cells to “see” insulin and so
marrow. These drugs allow
blood glucose levels but have
no control-no drug
Rosiglitazone

There are some powerful
drugs, called TZDs, available
to treat Type 2 diabetes, Lecka-
Czernik says. They are very
efficient at controlling high
clears into the
bone marrow than controls. The
animals were lean and had higher
converted to brown fat, the
model in which white fat cells
convert white fat into brown fat,”
she states. White fat is for energy
storage; it is the fat responsible for
obesity and, by extension, diabetes.
Brown fat is so named because it
contains a lot of mitochondria—
the organelles that produce energy
in the cell. It is metabolically active
and capable of rapidly producing
energy. Even though it is fat, brown fat acts as a power plant and
actually burns white fat by using
the energy stored there.

Some of the new anti-diabetic
compounds enable fat cells
in the cell. It is metabolically active
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the energy stored there.
"An additional problem," Maltese notes, "is that cells that practice evasive techniques and escape from the primary tumor site are often the most resistant to apoptosis-inducing drugs." He wondered if an alternate pathway might exist that could be triggered to cause cell death by a mechanism different from apoptosis. And then—he found it.

"Cells normally test their environment by taking in fluids from the extracellular space," Maltese explains. He and his lab discovered that brain tumor cells maintained in a culture dish could be induced to indulge in this tasting exercise so extensively that the resulting vacuoles, which are enclosed cellular compartments, fill with fluid. The vacuoles then accumulate and eventually cause the cell itself to explode from what, in essence, is overdrinking. They called this new pathway methuosis, which in Greek means to drink to intoxication.

The next step was to find a drug-like chemical that could induce methuosis. "We found a class of small molecules with a specialized chemical structure that would induce methuosis," Maltese says. The prototype compound was nicknamed MIPP. Unfortunately, he adds, one of the major problems with MIPP is that it is not soluble in water. Then Maltese started working with Paul Erhardt in UT’s Center for Drug Design and Development to create a compound that was more potent than MIPP.

"By a happy confluence of events, we were introduced to an MD/PhD student—Mike Robinson—who was interested in combining cancer and chemistry research," Maltese comments. He spent a year doing chemical synthesis and came up with a related compound, MOMIPP, that is more potent and better suited for testing as an anti-cancer drug. This compound is now patented and in the public domain.

"The exciting thing is that we have been able to take glioblastoma cells that are resistant to the front-line drug, temozolomide, and kill them using MOMIPP," Maltese states. But what has transpired so far has been in the lab—in vitro and in animals. Maltese is asking how to deliver this chemical to patients in such a way that it will concentrate in the brain tumor and not affect other viable cells. 'That goal led him to look at working with nanoparticles. Nanoparticles have two distinct advantages: they can be used to package a drug that is not soluble in a water-based environment like blood, and they can be decorated with short peptides that have specific affinity for glioblastoma or other types of tumor cells.

The ultimate goal Maltese says is to concentrate the drug in the tumor and even to hunt out cells that may have escaped the primary tumor site. But the hitch to this long-range goal is that the researchers do not know what the drug is binding to. The binding site could be a protein on the surface of the cell or inside it. To find out, Maltese and his colleagues will be using trace C14 isotopes to label the drug. Analysis of cell proteins will then enable them to determine the drug target. The protein that binds to the drug may exist in some tumors but not in others, Maltese comments, and could thus serve as a biomarker to tell if a patient might benefit or not from a particular treatment.

Answers to these questions could lead to tumor-specific uptake of the drug and so minimize damage to normal tissue while the tumor cells are destroyed.
What, you ask, does the law have to do with ethics? “The law,” says Susan Martyn, “embodies moral judgments and the consensus of society. There is often no law that encompasses a new scientific or medical enterprise. But when scientific advances occur, law often is called upon to regulate science.” We see this phenomenon in the existence of institutional review boards, which came into existence in response to Nazi atrocities and the infamous Tuskegee Syphilis Study to regulate biomedical and behavioral research involving human subjects. The Atomic Energy Commission came into existence after World War II to monitor development of atomic science and technology—duties now undertaken by the Nuclear Regulatory Commission and the Energy Research and Development Administration.
Martyn is particularly interested in biomedical ethics and how the law responds to medical advances and situations. An early interest dealt with advance directives. “Most states have provisions for living wills and advance directives,” Martyn states. But, she notes, many states, including Ohio, had language and requirements that were more extensive and confusing than necessary. Martyn and her colleagues wanted any revision to include both a do not resuscitate provision and one allowing a non-related surrogate to make treatment and end-of-life decisions. “There are patients whose only or best surrogate is an adult who is not related in any way and with whom they have a significant personal relationship,” explains Martyn.

“But what happens if there is nothing in writing?” asks Martyn. In the case of Nancy Cruzan, the U.S. Supreme Court upheld a Missouri court decision that required “clear and convincing evidence” of what a patient would want before removing life support—and left that decision to the states. The issue is determining what that person would decide by examining moral and religious values and what those who knew the patient can tell us. Cruzan’s parents and friends said they had had discussions with her when her grandmother died. “Most ethics committees and courts want as much information as possible,” states Martyn. That same standard came into play in the case of Terry Schiavo in Florida, whose husband and parents disagreed on terminating life support. In both cases the courts finally agreed to remove the feeding tube, but only after being convinced the decision was truly respectful of the patient’s wishes.

“How do you make decisions for people who have never been mentally competent?”

Then Martyn asked, “How do you make decisions for people who have no need for, or need this?” She asks, “Do we allow physicians even to suggest it to patients who are sick and vulnerable?”

In keeping with her penchant for examining controversial issues, Martyn has looked at the issue of using brain dead individuals for medical research. These individuals, termed biobanks, are really cadavers whose organs are being maintained in a functional state by mechanical and artificial means. This situation presents a paradox and legal confusion, comments Martyn. To solve the associated problems, she recommends that studies of this nature be reviewed by the Institutional Review Board and embody three principal considerations to control for potential abuses: absolute certainty of the diagnosis of brain death, certification of the need and therapeutic benefit of the research, and adequate disclosure to and consent from relatives.

Martyn used the possibility of human cloning to summarize the four legal responses available for new scientific possibilities: encouragement, laissez faire, regulation and banning. “Encouragement through funding explains why there is often debate about the expenditure of money for new research,” Martyn says. She argues that the next legal response, laissez faire (no regulation, no funding) is unlikely with human cloning because it produced abuses in the history of research involving human subjects. “Marketer incentives alone invite neglect and abuse,” she notes, “and such research demands extensive regulation.” She also notes that regulation will be needed to address unique questions of consent, “parent-child” relationships, privacy and other issues that are likely to arise. As for banning, Martyn says any profit, as for organ donation, must be prohibited. “Those who wish to advance cloning must prove its safety and benefits—that it would encourage development of noble human qualities. But I still worry about trusting my duly elected representatives with the power to decide which noble qualities to foster. This means that we have to act to reverse the usual legal presumption that everything is allowed unless it is specifically prohibited.”

Martyn explains that her teaching inspires her to research topics. If she is teaching bioethics and the law, she teaches about advance directives and then wants to know more about the topic, which leads to more research in both law and medical journals. “This process means better teaching, accompanied by improved and informed scholarship. She tells of her bioethics and law course to which groups of fourth-year medical students are invited for four-week rotations. Martyn has the students study informed consent and death and dying issues. She assigns court opinions that illustrate that the courts often do not agree. One day a medical student pulled up Terri Schiavo’s CAT scan and read it for the class. “The goal,” Martyn says, “is to bring together medical and legal ethics and encourage the two professions to communicate better and trust each other more.”
Are You Tired?
People undergoing chemotherapy for cancer frequently report being fatigued—so fatigued that it affects their quality of life.

Kristi Reuille of the College of Nursing was working toward her masters as an Adult Health Clinical Nurse Specialist and was doing clinical rotations in an outpatient oncology clinic. She was intrigued by her experience with five patients who had lymphoma. “All reported the same level of fatigue,” she says, “but those who had lived through prior treatment had lower levels of distress.” This simple fact caused her to ask why and, being a nurse, how could she help cancer patients minimize their distress the first time through chemotherapy? At the same time, Reuille says her uncle was dying of lung cancer; all he wanted to do was to go home and be able to spend time with his granddaughter.

About this same time, she came across the Common Sense Model of self-regulation of health and illness originally developed by psychologist Howard Leventhal. Reuille explains that this model involves processing on two levels: the cognitive (beliefs and thoughts) and the affective (emotions). More and more, she notes, cancer is viewed as a chronic illness because people are living longer and being treated longer—sometimes multiple times. So it seemed reasonable to Reuille that she adapt the Common Sense Model to her interest in the fatigue resulting from cancer treatment.

“Beliefs about an experience affect how people treat themselves,” Reuille says, “and the whole concept is fascinating.” This representation can apply to a disease, treatments or symptoms. Reuille is looking at the model as it applies to symptoms. She explains: “People who have never been treated for cancer think of fatigue as something akin to being down with the flu or something like that. But with cancer treatments, they don’t feel better. But with cancer treatments, they don’t feel better.” The result, she says, is a deconditioning, and people don’t balance activity with rest.

Thus, Reuille is examining how individuals represent fatigue to themselves and what coping strategies they apply to fatigue.

Moreover, Reuille says she is looking at her modified model of the fatigue experience in patients with cancer in the context of activity and what people do to balance activity with rest. But she comments that she needs a measure and is working on incorporating additional methods into her research. One technique is to use an instrument called an actigraph, which is worn like a watch and tracks the intensity of activity. This will allow her to collect real-time activity data and correlate it with fatigue. She is also considering asking her study subjects to keep a diary of fatigue and distress.

So far she reports that the proposed instrument, called the Cancer Treatment-Related Fatigue Representation Scale, or CTRFRep, is behaving the way it should and has good construct validity. But Reuille wants to make it considerably more parsimonious—right now it has 56 questions, which is asking subjects for a considerable amount of time. “Regardless,” she adds, “people have been willing to participate.” Once she can analyze how activity is related to fatigue, she will be able to develop more effective ways of giving patients some way to manage their fatigue.

Reuille notes that a number of her colleagues have developed exercise interventions to help patients undergoing cancer treatment. “But,” she says, “there is definitely some psychology involved. There are so many factors that it is a big knot to untangle.” She comments that she is not “an exercise person,” so her techniques will tend to veer away from physicality and focus more on cognitive and behavioral interventions. Reuille says that her long-term goal is to develop interventions that will help patients reduce the distress and depression that fatigue cause.

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Thus, Reuille is examining how individuals represent fatigue to themselves and what coping strategies they apply to fatigue. To that end she has modified an existing questionnaire (the Illness Perception Questionnaire-Revised) that measures beliefs about the identity of symptoms and their controllability, timeline and consequences. These concepts are processed at the cognitive level. Because Reuille wants to correlate negative effects of fatigue with symptom reporting, she has collapsed the two tracks—cognitive and emotional—into one.

IDENTITY: Do you have other symptoms at the same time as the fatigue? Do you think these contribute to the fatigue?

CONTROLLABILITY: Do you think you can control the fatigue, either through personal actions or through treatment?

TIMELINE: Is the fatigue acute or chronic? Does it come in cycles?

CONSEQUENCES: What are the consequences of having fatigue?
A part of the brain called the prefrontal cortex is the moderator for decision making. “In addicts, that part of the brain is very active and releases, in particular, a neurotransmitter called glutamate at the slightest provocation,” says Yousef Sari, “effectively by-passing any real decision making.” He has been able to investigate the role of the glutamatergic system in the central reward regions of the brain that are involved in alcohol dependence.

Furthermore, Sari explains, a protein called glutamate transporter-1 (GLT1 for short) is present in cells called glial cells. This GLT1 can take up glutamate from extracellular spaces and sequester it, thus eliminating it from synaptic clefts. “What if we could increase the expression of GLT1?” Sari asked. That would effectively reduce the amount of glutamate available to activate neurons in central reward regions of the brain and thus decrease the craving initiated by it.

Other researchers have tested more than 1,040 FDA-approved drugs to determine which might be good at removing glutamate from the space between neuronal cells. “It turns out that a drug called ceftriaxone increases the expression of GLT1,” Sari remarks. This drug has been used to treat meningitis and is in phase III clinical trial for the treatment of Amyotrophic Lateral Sclerosis, but Sari is examining its ability to increase the amount of GLT1 and thereby decrease the amount of extracellular glutamate available to activate addictive behaviors.

The problem for Sari was how to measure the effectiveness of ceftriaxone in reducing alcohol cravings and consumption. The answer lies in using an animal model—rats that have a propensity for spirits, called alcohol-prefering rats. These rats naturally prefer drinking alcohol to plain water. After five weeks of a constant free choice of an alcoholic beverage, the rats develop alcohol dependence. Sari now injects them with ceftriaxone each day for five days and measures their alcohol consumption. After treatment with ceftriaxone, the rats drink significantly less alcohol. Not only is their consumption measurably less, Sari has found that GLT1 levels increased in naturally prefer drinking alcohol to plain water. After five weeks of a constant free choice of an alcoholic beverage, the rats develop alcohol dependence. Sari now injects them with ceftriaxone each day for five days and measures their alcohol consumption. After treatment with ceftriaxone, the rats drink significantly less alcohol. Not only is their consumption measurably less, Sari has found that GLT1 levels increased in

Sari’s lab found that even under these stressful conditions, the dependent rats consumed less alcohol after a five-day treatment with ceftriaxone than the rats that did not receive any drug.

Cocaine and methamphetamine are addictive substances that share the same neurocircuitry as alcohol, Sari remarks. Limited experiments with cocaine-addicted rats have shown that ceftriaxone also reduces relapse to cocaine-seeking behavior. The neurochemistry is different in alcohol and cocaine addiction, he says, but glutamate plays a similar role in both cases.

Now that he has shown the effectiveness of ceftriaxone in reducing addictive behaviors, he is working to create synthetic analogs. “Ceftriaxone is an antibiotic,” he explains, “and it would be advantageous to have therapeutic compounds that do not have the antibiotic form.”

**FORCED WITHDRAWAL GENERALLY MAKES AN ADDICT CONSUME EVEN MORE OF A SUBSTANCE**

There are other compounds that activate GLT1, Sari notes. He asks what other signaling pathways are active and is seeking the molecular mechanism for this series of chemical actions. Ultimately, Sari wants to develop drugs that can successfully treat alcohol addiction. He believes that a focus on the glutamatergic system as a prime candidate for mediating drug and alcohol dependence is the key to finding these drugs.
Headache and Stroke: connections revealed

Migraine is a primary headache disorder in which the headache is the problem in and of itself rather than being the result of, for example, a tumor or an infection. Although migraine is thought to originate in the brain cells (neurons and glia), the trigeminal nerve, which originates in the brainstem and receives sensory input from the face, cornea and sinuses, plays an important role in the pain. When triggered centrally or peripherally, the trigeminal nerve endings to the blood vessels in the brain covering (meninges and dura) release neurotransmitters (such as calcitonin gene related peptide, neurokinin A and Substance P). This in turn causes blood vessel inflammation and dilation, and a pain signal is carried back to the brain via the trigeminal nerve. Migraine is thus a combination of central and peripheral nervous system signals, which makes studying it complicated. Moreover, the study of migraine is complicated by a lack of a good animal model.

Migraine is a common condition and is thought to be generally benign in the period between attacks. Gretchen Tietjen is not so sure. As a vascular neurologist, Tietjen was interested in blood vessels and stroke and early in her career noticed an overlap in the population of people who get migraine and also have early strokes. “Migraine associated with stroke occurs predominantly in young women between the ages of 20 and 40,” she says. But even more intriguing was the finding that aura, a premonition of migraine that affects 25 percent of those with migraine, and puts them at even higher risk for stroke.

Hints of this association of migraine and stroke came initially from epidemiological studies, Tietjen explains, the first of which in 1975 focused on oral contraceptive pills. It was in the early 90s while Tietjen was studying biomarkers of stroke risk that a growing number of epidemiological studies verified the migraine-stroke link. “I suspected that the endothelium (the lining of the blood vessels) was involved, possibly as a consequence of migraine. This was exciting research because endothelial dysfunction is an early sign of vascular disease caused by major stroke risk factors such as hypertension, diabetes, and smoking. When the endothelial cells are activated, the result is inflammation, increased clotting, and changes in the caliber of the small vessels—all factors that occur in migraine.”

To study the relationship of endothelial dysfunction and migraine, Tietjen and her colleagues decided to measure blood levels of von Willebrand factor, an endothelial protein that activates platelets to clump together and stop bleeding. Too little of this protein causes increased bleeding, too much causes clotting and stroke. What they found supported their original hypothesis: the higher concentrations of von Willebrand factor indicated a role of
the endothelium in migraine and a mechanism by which migraine might related to stroke.

Tietjen was now on the hunt. “Despite growing evidence of impaired, possibly systemic, vascular reactivity in persons with migraine, biomarkers of endothelial activation have not been comprehensively studied in migraine,” she says. “Recognition of migraine as a risk factor for stroke raises questions about the role of cerebral vessels.” First in a study of young women seen at UTMC, then in a general population in the Netherlands, she and her colleagues found an increase in levels of a number of biomarkers related to endothelial dysfunction in those with migraine. One characteristic of endothelial dysfunction is a reduction in the availability of vasodilators, such as nitric oxide. Vascular inflammation is also a result of endothelial dysfunction and was elevated in migraine. Further, they found elevated levels of proteins that promote coagulation.

As she was conducting these biomarker investigations, Tietjen also conducted a study with her colleagues in dermatology that demonstrated that a condition called livedo reticularis, a mottling of the skin related to endothelial dysfunction and stroke, was more common in persons with migraine, especially young women. Thus, she has identified one more marker to add to the arsenal for predicting a predisposition for stroke.

But Tietjen isn’t satisfied. She wants to know what besides genetic factors underlie the causes of migraine. “If we can prevent migraine, we can alleviate suffering and possibly prevent at least a subset of strokes,” she remarks. To this end she organized a group of headache specialists at 11 centers in the U.S. and Canada to explore the relationship of migraine to a history of childhood abuse, a newly recognized risk factor for a variety of adult health issues (including depression, anxiety, and heart disease).

Her research consortium is finding that persons with migraine who have suffered childhood abuse are more likely to have early onset of migraine and more frequent attacks and to have other conditions, such as depression, fibromyalgia, chronic fatigue, and irritable bowel syndrome. Preliminary data suggest that this population is also more likely to smoke, have high blood pressure, diabetes, stroke, and heart attack.

“Since we had already done the biomarker studies in young woman (and controls) with migraine,” Tietjen adds, “we decided to mail the childhood abuse survey to that population.” To her amazement, she received more than an 80 percent return rate on the surveys. Analysis of the data found that adverse childhood experiences were strongly associated with migraine and also with the vascular biomarkers, particularly those of inflammation, already determined. The studies of these relationships are still in their infancy, but Tietjen and her co-workers are intent on tracking events that set in motion the development of endothelial dysfunction, which itself is a precursor to atherosclerosis and stroke.

Questions come easily to Tietjen. The answers are more difficult to find. If past history is any clue, she will continue to seek the answers that shed light on the connection of migraine and stroke. And being a clinician as well as a researcher, she is embarking on efforts to see if alternative, nondrug, methods of altering the body’s stress response may lessen inflammation and endothelial activation and ultimately decrease the development of vascular diseases such as stroke.
Viola and his lab identified target the pathway that allows bacteria to make four compounds Viola says the researchers in his lab have identified compounds that selectively block those essential amino acids. Bacteria, however, make all 20. “They can’t go to the neighborhood McDonald’s to get a protein burger,” he chuckles. “What if we can disrupt the pathway that makes these amino acids?” he asked. “If we can block that pathway, we can kill the organism but not harm ourselves since we don’t have that pathway.”

Viola says the researchers in his lab have identified compounds that selectively block the pathway that allows bacteria to make four of those essential amino acids. The group of compounds Viola and his lab identified target an enzyme at the beginning of the pathway that makes essential amino acids. These compounds are effective in different degrees among Gram negative and Gram positive bacteria and even work against an enzyme in fungus. (Bacteria are classified into Gram negative or Gram positive according to the construction of the cell wall and how they pick up a certain stain.) In addition, some of these compounds are more effective in some bacteria than in others. His lab is now working on improving the effectiveness and selectivity of the compounds they have identified.

The approach Viola believes to have even more promise, perhaps because it is so radical, is to damage—or sidetrack—something that makes the bacteria infectious without actually killing it. “If the bacterium does not feel threatened,” he remarks, “then it is unlikely to produce toxins. After all, its goal is to survive and reproduce. If it kills the host, then it will also die.”

It is often the toxins that bacteria produce, not the bacteria themselves, that are the agents of infection. Viola thus asked, “What triggers toxin production?” When the bacteria feel threatened, some of the compounds they make normally just for survival are transformed into signaling proteins that promote toxin formation. “If they don’t sense danger, they won’t become infectious,” Viola adds. And he notes that a secondary benefit is that the bacteria are less likely to become drug resistant because their well being is not threatened—they are still surviving.

Viola and his lab have begun to explore the changes that occur when an important metabolite becomes a signaling molecule. This metabolite is needed for the transfer of methyl groups to proteins and to DNA in a process called methylation (a methyl group is one of the simplest of organic compounds—a carbon with three hydrogen atoms). Transfer of the methyl group to a protein changes the way the protein behaves. Bacteria have figured out how to take this methylating metabolite and use it in the signaling pathway; blocking this conversion prevents the production of these signals and shuts down toxin production. “If they don’t sense danger, they won’t become infectious,” he says. “The problem is, the bacteria are less likely to become drug resistant because their well being is not threatened—they are still surviving.”

Viola seeks to prevent bacteria from performing that particular conversion. He and his lab have designed a series of compounds that resemble the methyl donor, but with a crucial difference: the structure is altered so that it cannot be converted into a signaling molecule. The problem now becomes to make these compounds more efficient in blocking the signaling pathway.

Having demonstrated the possibility of both disrupting an essential survival pathway and reducing or eliminating toxin creation in bacteria, Viola and his lab are confident that they will succeed in making more effective compounds to do the job. Once they can show the efficiency and effectiveness of the new compounds, it will be up to the pharmaceutical companies to create the new antibiotics from the lead compounds that the Viola lab is identifying.

During a fellowship in infectious diseases at the State University of New York at Buffalo, Julie Westerink studied meningitis. She spent a number of years looking for ways to create a vaccine for Neisseria meningitides, a virulent and life-threatening bacterium that is a major cause of disease and death from septicemia and bacterial meningitis in industrialized countries. This bacterium encapsulates itself in a carbohydrate capsule that resists attempts by the body’s immune system to immobilize it and remove it from circulation.

Then her world came crashing down—because of FDA approval, a conjugate vaccine arrived on the scene and the need for the peptide vaccine Westerink had been working on vanished. What to do? Since her experience was with polysaccharide capsules, she turned her attention to another organism with the same kind of self-protective coating: pneumococcus, that unsavory bacteria that can cause pneumonia.

“There is a pneumococcus vaccine that is recommended for people at high risk for getting pneumonia and for everyone over the age of 65,” Westerink says. “The problem is, this vaccine is a polysaccharide vaccine; it turns out these vaccines are very good for healthy young individuals but not so good for older people.” Too little is known about how people respond to these vaccines, she added.
Because those with HIV are at particularly high risk of getting pneumonia, Westerink wanted to find out what specifically was missing in the immune system of these individuals. At the same time, she asked if older people might have the same immune deficiency as those infected with HIV. “We need to know what kinds of cells are required to make a good immune response,” she explains, adding that B cells circulating in the blood have been identified as important elements of the immune system. But Westerink notes that B cells come in many flavors, so she is asking what kinds of B cells are present in normal individuals and how that compares to the cells present in the elderly or in those with compromised immune systems.

An intact spleen is required for immunity to polysaccharide antigens using a pneumococcus vaccine, Westerink remarks. Other researchers have shown that people with decreased or missing IgM memory cells, a particular subset of mature B cells, are at greater risk of infection with polysaccharide organisms and respond poorly to polysaccharide vaccines. These at-risk populations include those without a spleen, HIV-infected individuals, and the elderly. Westerink’s lab is finding that this hypothesis is indeed true.

In a recently completed study of healthy, young volunteers, Westerink’s lab developed a method of fluorescently labeling polysaccharide-specific B cells that enables them to identify the particular subgroup of B cells that respond to the vaccine. In unvaccinated individuals, the polysaccharide-specific B cells were primarily naïve (immature) B cells. But post-vaccination, IgM memory cells are significantly more abundant. “It is unlikely that IgM memory B cells are exclusively responsible for anti-polysaccharide antibody production,” she says, “as other B cells secrete antibodies. But our results show clearly that IgM B cells are in the first line of defense.”

“The implication is that we need to look at a different approach to vaccinating at-risk populations.” Since the B cells necessary for an immune response mediated by a polysaccharide vaccine are in short supply in these people, Westerink says perhaps a conjugate vaccine might work. Conjugate vaccines work quite differently than polysaccharide vaccines, using different mechanisms to activate B cells to produce immunoglobulin. Westerink’s research now seeks to define what segment of the immune system is deficient in the over-65 population so that a competent vaccine can marshal the appropriate B cell defenses to combat Streptococcus pneumonia.

The quest is to define the deficiency that research can hang the problem on. Research reported here has received support from the National Institutes of Health.
A few years ago, Zi-jian Xie was studying heart disease using the drug ouabain. Ouabain, and its relative, digoxin, has been used to treat cardiac failure by increasing contraction of the heart muscle. Ouabain also contributes to muscle growth. Muscle growth in the heart induces cardiac hypertrophy, which is a major factor in heart disease. Xie knew that ouabain was interacting with the Na/K-ATPase but was unclear how that interaction would cause heart muscle cells to grow bigger. Xie realized that the drug was, in a sense, causing the heart to work against itself and asked why—and how.

Returning to basic cell biology, Xie explains that protein kinases are enzymes that use ATP (the cell’s energy source) to power and regulate a number of cellular functions. In the presence of ouabain, protein kinases are activated and become responsible for cell growth leading to cardiac hypertrophy and heart disease. This biological process led to the next big question: How exactly do they interact? Working with graduate student Jiang Tian, Xie isolated a fragment (peptide) of the Na/K-ATPase that binds to Src and keeps it in an inactive state. Results of experiments with this new peptide will allow the researchers to pinpoint the interaction of the sodium pump with Src as well as verifying the protein-protein interaction that Xie has hypothesized all along.

On-going research to date has demonstrated that the peptide can block growth of prostate cancer in mouse animal models. Future work will examine the role of the peptide in slowing progression of renal disease and heart failure. Although the researchers developed the peptide to answer some very basic questions about how proteins govern cell activity and how they interact, they hope that their work will lead eventually to therapeutic treatments for a number of diseases.

Xie maintains that it is important for a scientist always to have an open mind and to be curious. But that curiosity needs to be transmitted to students and postdocs, who are the future of the discipline. He says further that the scientist needs to have a network of collaborators who are willing to listen and suggest new directions. Without his collaboration with Shapiro and his input as a nephrologist, Xie says he would not have considered the translational aspects of his research.
Dopamine mediates feelings of pleasure; it also is involved in movement. Serotonin is involved in sleep, learning and memory, mood and appetite. Damage to these neurotransmitters produces a variety of symptoms that can affect daily functioning.

Conventional wisdom has said that abused drugs acutely affect the release of these neurotransmitters from nerve terminals. Amphetamines are structurally similar to adrenaline and dopamine and can indirectly mimic the action of the important transmitters. Bryan Yamamoto has asked what are the long-term effects and the mechanisms that underlie the brain damage produced by these drugs. He has uncovered that the amphetamines—in particular methamphetamine and MDMA (Ecstasy)—produce neural damage through mechanisms similar to that being investigated in Parkinson’s, Alzheimer’s and Huntington’s diseases. “We think that since the mechanisms are very similar, the abusers of these drugs would suffer a similar type of pathology as that observed in Parkinson’s, Huntington’s and Alzheimer’s,” Yamamoto adds. “And they do.”

Yamamoto explains that the primary mechanisms that underlie brain damage are threefold and include cellular energy deficits, free radicals and inflammation. “Abused drugs probably simultaneously converge to affect all of these neural mechanisms and so damage the neurons,” he says.

Energy deficits occur when the mitochondria, which are the organelles that supply energy for the cell, are damaged. When the cell loses power, it fails to perform its normal functions, one of which is manufacturing neurotransmitters as well as responding to neural signals.

Free radicals damage cell membranes and inhibit neural enzymes. “Free radicals”, Yamamoto explains, “are molecules that have an unpaired electron in their outer ring. Since electrons want to be paired, this single electron will readily pair with any available electron. When this happens, the important structural and functional components of the cell, including proteins, will be damaged.”

Amphetamines—in particular methamphetamine and MDMA (Ecstasy)—produce neural damage through mechanisms similar to that being investigated in Parkinson’s, Alzheimer’s and Huntington’s diseases.

Inflammation calls in a host of immune mechanisms and mediators, some of which produce free radicals that can damage the cells’ energy factories. Inflammation itself can be damaging, but in the case of abused drugs that increase pro-inflammatory mediators in the brain, they magnify the neurotoxic effects of the amphetamines.

Yamamoto notes, “We’ve been studying the mechanisms that damage dopamine and serotonin nerve terminals for several years but we still don’t know what factor or factors in the brain initiate these mechanisms. We still haven’t decoded the mechanisms of how these abused drugs act directly on the brain to produce the effects we have observed.” So he decided to look elsewhere and at organs other than the brain. “The drugs also affect the rest of the body,” he adds, “and we focused our attention on the liver as the liver is the primary organ that metabolizes these drugs.” Once he looked at what was happening to the liver, a whole new set of doors opened, initiating a shift in the way neuroscience researchers look at the drug abuse issue.

In laboratory rats subjected to methamphetamine, Yamamoto and his lab found liver damage. “The liver is responsible for eliminating ammonia from the body,” he explains. If liver function suffers and the liver cannot perform this duty, ammonia accumulates in the body to levels that can be toxic. But one critical experiment remained to test his idea. When he and his graduate student, Laura Halpin, examined the liver of rats that were administered methamphetamine in a manner that approximates a “speed run” by methamphetamine addicts, they found that the liver was significantly damaged and that ammonia levels in the blood were abnormally high. “Then they looked at ammonia levels in the brains of these animals. Same result: ammonia levels were significantly elevated,” he adds, “If we can help the body eliminate ammonia even in the presence of frank liver damage,” he adds, “we might block the ensuing neurotoxic effects of the drugs.” Indeed, when the animals were treated with a drug that helps to eliminate ammonia, it decreased ammonia levels in the blood and brain and attenuated the damage to dopamine and serotonin neurons.

In collaboration with Nicole Northrop, an associate research scientist in his lab, the team has extended these initial findings and further hypothesized that there may also be a potential insult to the blood vessels in the brain that normally limit the passage of large, water-soluble molecules, bacteria and viruses into the brain. “We found that methamphetamine and the resultant increases in ammonia damage these brain capillaries and makes them leaky, allowing large and potentially dangerous molecules to pass through,” comments Yamamoto. Thus, the brains of amphetamine addicts may be more vulnerable to systemic diseases.

Yamamoto and his lab are now pursuing this connection between the liver and the brain that can explain at least some of the toxic effects of the abused drugs such as methamphetamine and Ecstasy. The brain damage observed after exposure to drugs of abuse may in fact be collateral damage produced initially by injury to liver cells that are normally responsible for eliminating ammonia from the body. How ammonia produces inflammation, mitochondrial damage, and free radicals, and what other organs outside the brain that might be in the direct line of fire are not known and will be investigated by Yamamoto and his colleagues.

Further research will help define just where methamphetamine starts to wreak havoc. Understanding the mechanisms that produce the damage can hopefully cultivate new ideas on how to mitigate the neurotoxicity produced by these drugs and potentially enhance our understanding of other neurodegenerative disorders. It is possible that anti-oxidants and anti-inflammatory medications such as ibuprofen could minimize the damage that occurs. The ultimate goal of Yamamoto’s research is to limit or at least minimize that damage and perhaps reverse the damage once it occurs.

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Bryan Yamamoto is professor and chair of the Department of Neuroscience at Boston University School of Medicine. After receiving his doctorate from Syracuse University, he completed a postdoctoral fellowship at the University of Colorado Health Sciences Center where he focused on Parkinson’s disease research. He went on to study neurotoxicology and neurodegenerative disorders, eventually pursuing an interest in drugs of abuse in the Department of Psychiatry at Case Western Reserve University Medical School, where he was Professor and Director of the Program in Basic and Clinical Neuroscience before moving to the Department of Pharmacology at Boston University Medical School. His interest in the toxic effects of amphetamines has been an on-going effort that has been funded continuously for more than 25 years.