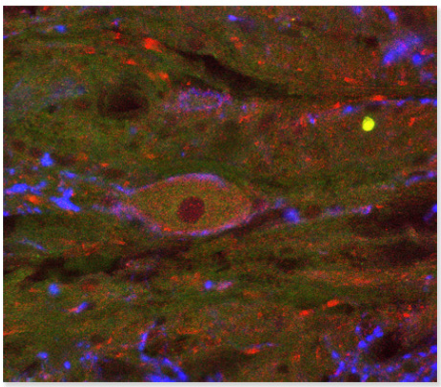
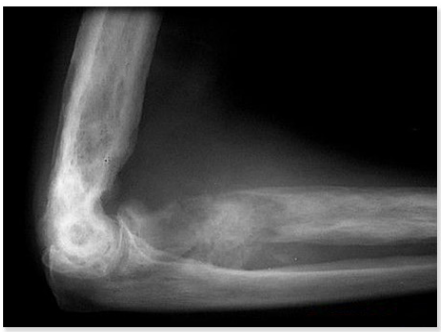
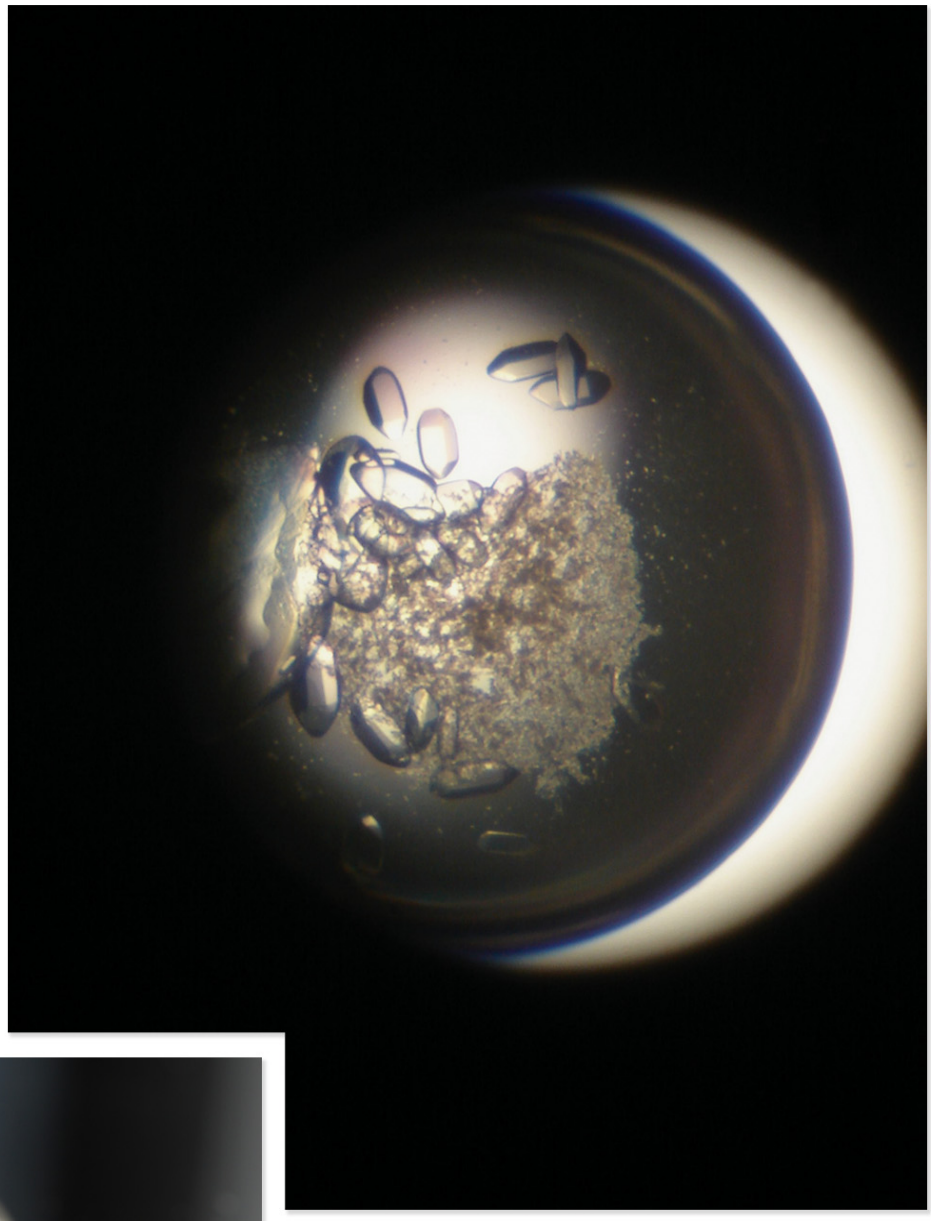
Fusion

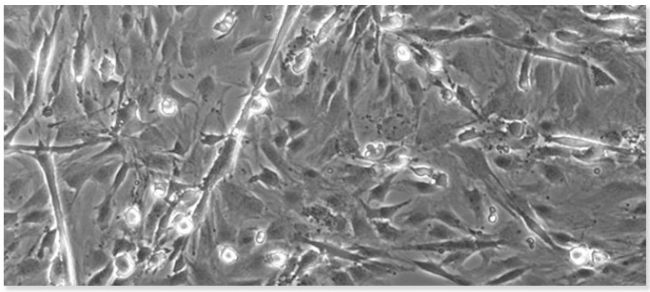
William H. Beaumont Research Honor Society at
The George Washington University School of Medicine and Health Sciences

Spring 2007









A Student-run Scientific Journal Serving The George Washington University Medical Center

inside this *issue*. . .

Fusion is a publication of The George Washington University Medical Center's William H. Beaumont Society. This research journal is published by students in collaboration with the Office of the Dean, Office of Health Research, Compliance and Technology Transfer, and Office of Communications and Marketing.

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From the *Editors* . . .

At The George Washington University School of Medicine and Health Sciences, we are fortunate to have exceptional research opportunities available to us in diverse and exciting fields. Some of the projects medical students have undertaken involve basic science research, others involve clinical research and health policy, and several include international medical experiences. In recent years, the development of Track Programs and the number of fellowships offered by the Office of Student Opportunities have dramatically increased these worthwhile opportunities.

By establishing a scientific medical student journal named *Fusion*, which we hope will be published annually, we aim to capture the excitement of the many research-related endeavors of our peers and give members of the broader scientific and medical community an opportunity to learn about the comprehensive and multi-layered experiences of GW medical students. We are hopeful that *Fusion* will entice current and future students to engage in the many research opportunities available and share their experiences.

Responses to our call for submissions to *Fusion* exceeded our expectations. We received 22 submissions, demonstrating the pressing need and desire of our peers for an opportunity to write about and share their work. Our decision to solicit submissions from members of both the Classes of 2009 and 2010 acknowledges our understanding that the number of GW students who enter with previous research experience is rapidly increasing and that the work they have undertaken is valuable to us. Members of the Student Editorial Board, consisting of elected officers of the William Beaumont Research Honor Society, reviewed each submission selected for publication. Responsibility for the content of each article, however, remains with its author.

We thank W. Scott Schroth, MD, MPH, and James Scott, MD, FACEP, in the Office of the Deans; Anne Hirshfield, PhD, in the Office of Research; Linda Dent, Thom Kohout, Debbie Goldstein and Abby Vogel in the Office of Marketing and Communications; and Rachel Mazzotta and Chistopher D'Avella in the Office of Student Opportunities for providing guidance, generous support and resources to make this publication possible.

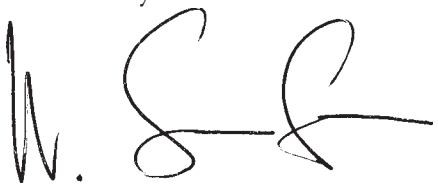
Thank you,

from the *dean's office. . .*

Many would say that the opportunity to practice medicine is, by itself, a sufficiently noble and rewarding career. However, the real beauty of the field is that it offers one so many possible avenues to contribute to the health of individuals and society. Not only are there a multitude of different clinical specialties, but also, one may contribute through research, education, administration and community service. Most importantly, all of these contributions can be carried forward as scholarly disciplines, adding not only to the health of people, but also to the advancement of medicine as a field.

This new journal, conceived and carried forward by students dedicated to scholarly investigation, is a testament to the array of talents and interests among our diverse and accomplished student body. I commend both the students who have organized this journal and all of those who have contributed their work to its pages.

When Dean James Scott was appointed several years ago, he articulated an important vision for the education of our medical students. Building on a solid foundation of medical education in basic and clinical sciences, he wanted to enable students to pursue their unique interests by taking advantage of the many opportunities afforded by the School of Medicine and Health Sciences and through our close relationships with the School of Public Health and Health Services, other schools in the University and organizations throughout the District of Columbia. Indeed, GW exists in a landscape that is unique and rich with opportunity. Over the past several years, the work of many has supported this vision. The Beaumont Research Society, the Office of Student Opportunities, the Office of International Medical Programs and the Program in Medical Humanities now form a unique infrastructure to support the scholarly interests of medical students throughout their four years at GW. The contributions to *Fusion* are testament to the success of this effort, and to the curiosity and hard work of our students.



W. Scott Schroth, MD, MPH
Senior Associate Dean for Academic Affairs

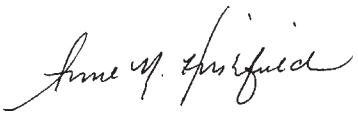
from the *research office. . .*

It is with great pleasure that I introduce to you the inaugural issue of ***Fusion***, a compilation of articles submitted by some of GW's most promising medical students. These students are members of GW's *William Beaumont Medical Research Honor Society*. The Beaumont Society was established at the GW School of Medicine in 1935 to promote student research and there are many accomplished scientists throughout the country who list membership in the society amongst their honors.

The articles in this book describe various short term research projects performed by Beaumont students during the summer of 2006. Most of these students were supported by stipends from the WT Gill Endowment which has provided our medical students with the resources to experience what biomedical research entails. In 2006, we were fortunate to be able to provide more opportunities to students due to the additional stipends made available through generous gifts from an anonymous donor, as well as from Dr. Jane Klein and Dr. Robert D. Rosenberg (Medicine '61), a graduate of our Medical School who has had a distinguished career in biomedical research. We are grateful for these generous donations that enable us to provide intensive short-term research experiences for our students. The opportunity to experience research first-hand is invaluable at this stage in these students' education as they decide whether a career as a clinician/researcher is the right path for them. In 2006, we were fortunate to be able to provide more opportunities to students due to the additional stipends made available through generous gifts from an anonymous donor, Drs. Gerald (Medicine '63) and Audrey Lazarus; Christopher Barley, MD '93; Keshav Narain, MD '92; Charles Walkoff, MD '59; Robert Rosenberg, MD '61; and Jane Klein.

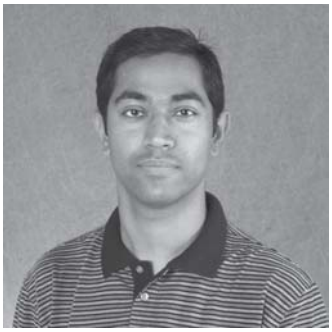
Fusion is a testament, not only to the creativity and accomplishments of our medical students, but also to the creativity and commitment of the student leaders of the Beaumont Society. I want to especially recognize Andrew Lerner, President of the Beaumont Society, and Rahul Araya, the Society's Research Journal Chair. These students conceived of the idea to produce this issue and worked tirelessly to make it happen. Remarkably, they managed to find time for this extracurricular activity while managing the demands of the second-year curriculum. Recognition also goes to the GW staff members who contributed to this effort, especially Linda Dent, *Medical Center Communications Director,* who gave generously of her time and valuable guidance, and Abby Vogel, who worked with the students with the editing and layout, along with Debbie Goldstein and Thomas Kohout, all from the *Communications and Marketing Office*. Thanks also to Sarah Hassaine, *Health Research Information Support Specialist*, for her editing and suggestions.

As you flip through the proceeding pages and see first-hand the varied interests of GW's students, we hope that you will appreciate all the hard work that our students have put forward. I'm sure their mentors would agree that these students have given back more than they received; having them in our labs and clinics greatly enriches our own growth and research endeavors.



Anne Hirshfield, PhD
Associate Vice President

Smoke-Free Children's National Medical Center Campus



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Hydrogen cyanide—used in chemical weapons. Arsenic—used in pesticides. Benzene—found in gasoline. Polonium-210—radioactive and toxic. What do these substances have in common? They are among the thousands of carcinogenic and toxic substances found in tobacco smoke.

Tobacco use is the leading preventable cause of death in the United States, and the health consequences extend beyond smokers to non-smokers involuntarily exposed to secondhand smoke (SHS).[1] In the U.S., SHS has been linked to at least 53,000 non-smoker deaths each year,[2,3] including 3,400 deaths from lung cancer, 43,000 from cardiac-related illnesses and 430 from Sudden Infant Death Syndrome (SIDS).[4]

These statistics make it imperative that hospitals be smoke free. In 1991, The Joint Commission on Accreditation of Health Organizations (JCAHO) announced a tobacco control standard for accredited American hospitals that mandated that hospital buildings be smoke free by December 31, 1993.[5] Ninety-six percent of hospitals met this JCAHO tobacco control standard.[5]

A number of hospitals throughout the country have taken this smoke-free standard further and made their entire campuses smoke free, meaning that no smoking is allowed in campus buildings or on the surrounding grounds and ramps.

The major factor behind going smoke free was recognition of the fact that healthcare facilities must promote smoking cessation and serve as role models.

The smoking policy at the Mayo Medical Center in Minnesota recognizes its obligation to its patients and the public to strongly assert the risks of tobacco use by establishing a smoke-free campus.[6] The University of Michigan Health System implemented a campus-wide smoke-free policy in February 1999 to "help people take control of their health" and "to create healthy communities."[7] Advocates of campus-wide smoking bans argue that providing a place to smoke does not support a healthcare institution's goal and mission to provide a healthier environment for patients, visitors and employees.[7]

At Children's National Medical Center (CNMC) in Washington, DC, the official smoking policy prohibits smoking on hospital premises, including all buildings and parking areas. The designated smoking area is a booth located outside the Emergency Room entrance. However, there are no clear guidelines on tobacco use on other parts of the campus, nearby grounds, ramps and adjacent sidewalks. As a result, people frequently smoke in CNMC's entranceways and on the campus grounds, despite the presence of conspicuous "No Smoking" signs.

A straightforward campus-wide smoke-free policy, if implemented at CNMC, would provide a healthier environment for patients, employees and visitors, and would further the hospital's mission statement. It could also promote smoking cessation among the hospital em-

ployees and send a strong message to the community about the harmful effects of smoking. To further this message, research is currently underway at CNMC to assess employee, patient and visitor attitudes toward making the entire campus smoke free. The results of the study will be used to develop a proposal to introduce a campus-wide smoking ban at CNMC.

CNMC is currently ranked ninth among all pediatric hospitals nationwide by *US News and World Report.*[8] It is high time that a nationally recognized healthcare institution with a mission "to excel in care, advocacy, research and institution" implement a campus-wide smoke-free policy.

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Speeding for a Solution: Ambulance Diversion and the ED Crowding Crisis

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Emergency Departments (EDs) are in a state of crisis as they struggle to manage a surging demand with insufficient resources. Emergency room visits have risen an average of 3.7 percent over the last five years,[1] making EDs the primary source of healthcare for many Americans.[2]

Consequently, EDs have become crowded and unable to readily care for such a large patient population. It is therefore necessary to find a way to moderate demand and improve the quality of patient healthcare.[3]

One method hospitals currently use to relieve crowding and limit patient outflow is through ambulance diversion. In the event that an ED feels it cannot safely accept additional patients, it can request that ambulances be diverted to an alternate hospital.

Although ambulance diversion was initially intended to be a temporary solution, it has become an increasingly routine response to the escalating ED crowding crisis. However, widespread use of this measure has had unintended consequences that compound, rather than relieve, the problem of crowding. The increased practice of diverting ambulances to less crowded hospitals has led to increased patient transport time, increased paramedic turnaround time and decreased ambulance availability.[4] Efforts are now being made to reduce diversion as a response to ED overcrowding.

Establishing policy and regulations for managing diversion has proven to be a significant challenge. Without sufficient federal attention on the issue, responsibility has fallen to states and local communities. In an investigation of major initiatives, three states and three local communities stood out in their efforts to manage ambulance diversion. Although they differed in management style, each sought to find some standardized fashion to manage and decrease diversion.

Massachusetts, New York and Arizona produced written guidelines to direct hos-

pitals regarding how to implement a diversion reduction plan. Massachusetts mandated several detailed plans and procedures for hospitals to follow, while New York and Arizona created guidelines that gave hospitals the autonomy to develop their own specific protocols. Massachusetts provided the most direction and subsequently displayed the most evidence of improvement in ambulance diversion reduction statewide.

San Diego, Sacramento and Syracuse substantially reduced ambulance diversion through voluntary hospital and county collaboration. San Diego and Sacramento produced detailed protocols that focused on hospital operational improvements to reduce crowding and hence decrease the need for diversion, while Syracuse put minimal restrictions on hospitals, allowing them to develop their own strategies.

These community collaborations showed significantly greater reduction in diversion hours than those reported in statewide initiatives. With smaller areas and fewer stakeholders (e.g., hospitals), communities were able to closely monitor and maintain progress through consistent commitment and cooperation.

Ambulance diversion is a flawed solution to ED crowding that requires urgent attention on a national level. Without a national standard in place, some states and communities have worked to create their own protocols, resulting in improvements as well as inconsistencies throughout the country. The time has come for federal guidance in creating a unified, proven standard for states and local communities to use. The federal government must take responsibility for this problem by developing national evidence-based diversion reduction policies and protocols. Implementing proven solutions through a national effort will help states and communities achieve consistency and increase the likelihood of nationwide success.

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Impact of Medicare Part D on Prescription Drug Coverage for Patients with Chronic Conditions

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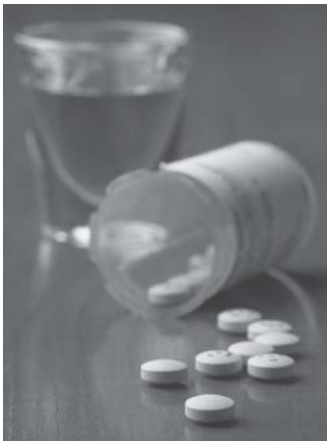
President Bush enacted the Medicare Prescription Drug, Improvement, and Modernization Act of 2003 in December 2003. At its core was Medicare Part D, a voluntary benefit intended to decrease prescription drug costs for Medicare enrollees.

Starting in 2004, Medicare enrollees could purchase Medicare drug discount cards to buy prescriptions at a discount. This program ended on January 1, 2006, when the full Medicare Part D drug benefit began for those who enrolled by November 15, 2005.

Rather than having one uniform plan for all Medicare Part D enrollees, there were a number of plans provided by various private companies that differed in types of cost sharing and drug formularies. Medicare beneficiaries had to determine which of the many plans was best for them and enroll in Part D independently.[1] Some enrollees were previously enrolled in drug plans that provided more cost savings than the Part D plans to which they switched.[2,3] Determining whether to enroll in Part D and choosing a specific plan may have been confusing to many Medicare beneficiaries.[4]



Because the emergency department is a critical access site, it was a useful location to study prescription drug coverage as part of care. Study participants were recruited from patients presenting to The George Wash-

ington University Hospital Emergency Department with chronic conditions, regardless of their primary complaint. The study population included insured (private/HMO), Medicaid, Medicare and uninsured patients. Participants were given a primary survey with basic demographic questions and questions to evaluate barriers to care, including access to prescription medications. Univariate analysis was used to assess the relationship between the outcomes of interest and main demographic variables.

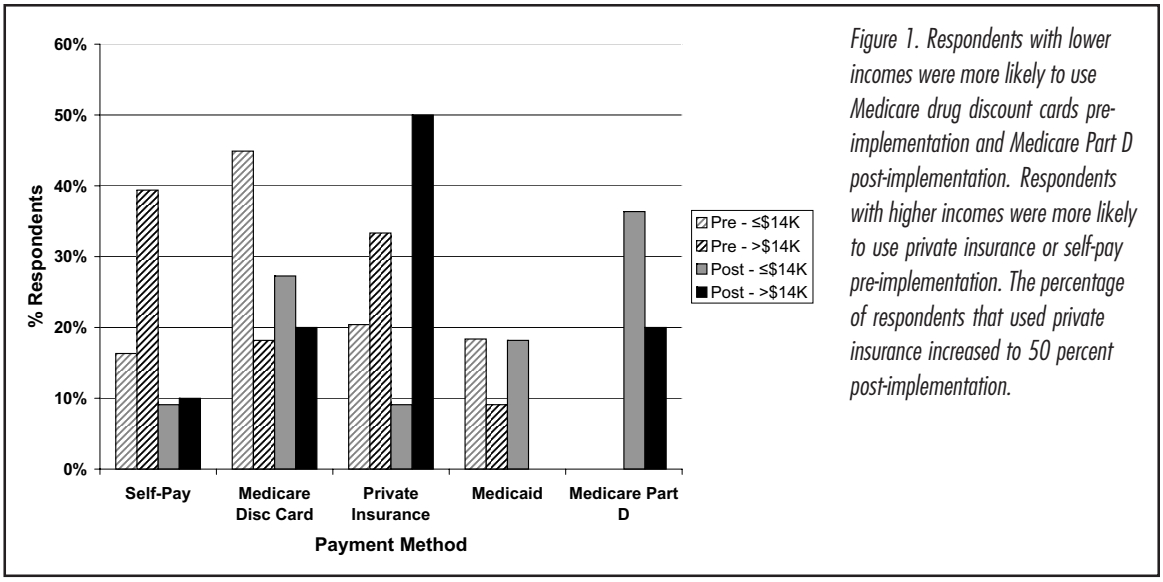


Figure 1. Respondents with lower incomes were more likely to use Medicare drug discount cards pre-implementation and Medicare Part D post-implementation. Respondents with higher incomes were more likely to use private insurance or self-pay pre-implementation. The percentage of respondents that used private insurance increased to 50 percent post-implementation.

A total of 862 surveys were collected between March 1, 2004 and August 1, 2006, and a pilot cross-sectional analysis of the Medicare subpopulation was performed. There were 152 Medicare respondents pre-implementation of Part D and 38 respondents post-implementation. Methods of payment for prescriptions were compared by income and out-of-pocket (OOP) costs.

Comparing insurance type to income, respondents with a salary of less than $14,000 reported using Medicare drug discount cards pre-implementation of Part D more than any other type of payment (Figure 1). After implementation, the majority of respondents in the same income bracket reported using Medicare Part D to obtain prescription medications than other payment method. The majority of respondents with a salary greater than $14,000 used private insurance or self-paid for prescriptions.

Analysis of OOP costs showed that respondents not enrolled in Part D were more likely to have increased OOP costs of more than $50 per month post-implementation (Figure 2). Respondents using Medicaid for prescriptions pre-Part D were dually eligible Medicaid-Medicare enrollees and were least likely to have high OOP costs. Post-implementation, the percentage of Part D respondents spending more than $50 per month was less than for the other insurance categories, but higher than for Medicaid pre-Part D. Medicare beneficiaries not using Part D post-implementation were asked why they didn't enroll and the most common response was "never heard of it" (66 percent).

In order to better determine the implications of Part D, a larger sample is needed. However, preliminary results of the cross-sectional analysis indicate that Medicare recipients may not fully understand the benefit of Part D, despite government efforts to increase enrollment.

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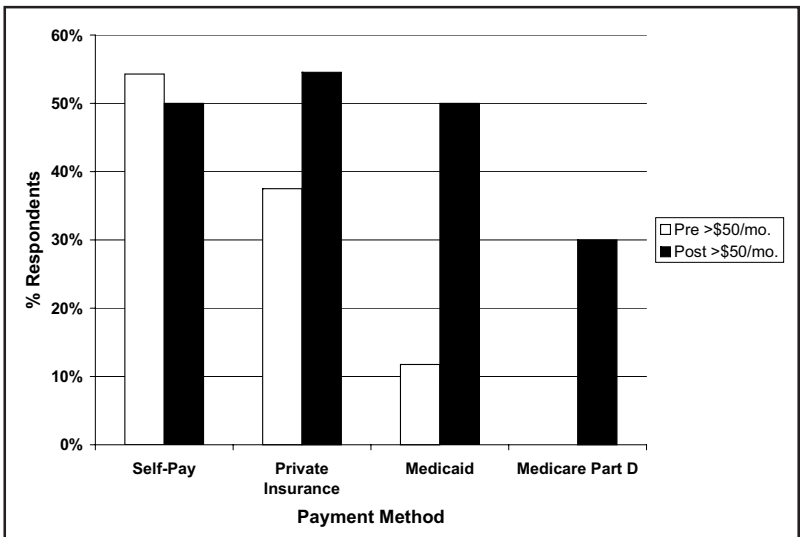

Figure 2. Respondents not enrolled in Part D were more likely to have OOP costs of more than $50 per month. Respondents using Medicaid for prescriptions pre-Part D were dually eligible Medicaid-Medicare enrollees and were least likely to have high OOP costs. Post-implementation, the percentage of Part D respondents spending more than $50 per month was less than for the other insurance categories, but higher than pre-implementation Medicaid.

Improving Medical School Curricula: Examining Basic Science Lectures and Assessments

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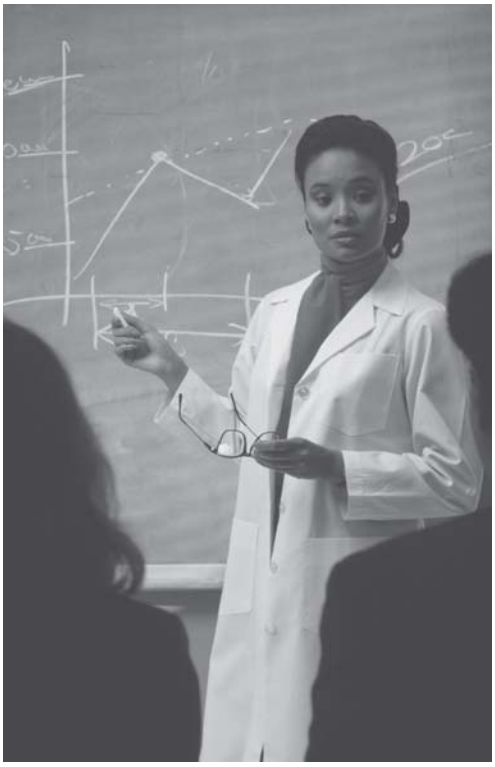
I entered medical school interested in pursuing a career in both medicine and medical education. As a participant in the Medical Education Track, I have been able to pursue this professional goal.

This past summer, I crossed the border into Canadian territory and had the opportunity to work on two separate projects focused on two major areas of basic science medical curriculum: basic science lectures and the quality of basic science assessments. This article provides a brief history of medical school curriculum and a synopsis of the two projects on which I worked at McGill University in Montreal, Quebec, Canada.

Since the publication of the Flexner Report in 1910,[1] medical schools in the United States and Canada have divided their curricula into separate biomedical science and clinical educations. The basic science curriculum is didactic—requiring students to sit through lectures organized by course directors and deans and then later assessed by in-house examinations to hold students accountable for the information presented to them. These two processes govern how medical educators organize their curricula and dictate how students learn and prepare to succeed in medical school courses. Throughout the last century, the traditional views of medical curricula have been challenged.

About 30 years ago, McMaster University in Hamilton, Ontario, Canada developed a problem-based learning curriculum that has evolved and been adopted by many American and Canadian medical schools. It was met with relatively insignificant success; medical schools are now attempting to implement an organ-system approach to their basic science curricula. This approach to learning medicine investigates each organ system by integrating normal structure and function with pathological processes and pharmacological intervention. Lastly, lectures have become less didactic and more student centered, and examinations emphasize clinical application and analysis. Unfortunately, this reform has been slow moving and narrow reaching.



At McGill University, my first project focused on how students perceive basic science lectures and what value they place on lectures in their medical education. Jim Brawer, PhD, a McGill medical educator and a medical education researcher, was inspired to pursue this project after a recent mandate by the Licensing Committee on Medical Education to reduce lecturing hours. The value of lecture has been a controversial topic in higher education and has been negatively reviewed by the popular press and various educational journals. However, never before have research studies asked medical students their opinions about the value of lecture and the role that it plays in learning medicine. Through questionnaires, medical students

were asked their opinions about basic science lectures.

Responses were categorized into themes and rated based on frequency. Of 150 students in the class, 50 completed questionnaires were submitted. Students' views on lecture were generally positive, and the following themes were uncovered. Lectures:

- Provided focus or emphasis on the material
- Provided an overview or "big picture" of course
- Explained or resolved difficulties in the notes or other readings
- Reinforced learning by hearing and seeing the information
- Provided depth, insights and examples not in readings
- Allowed for a time-efficient way to learn
- Provided exposure to and interaction with experts or role models
- Encouraged structure and discipline
- Soothed anxiety about the material.

Limited to one medical school and only one-third of one class of students, the results of this preliminary study showed that students value lecture in ways that cannot be substituted by any other teaching methodology. However, if the lecture is valuable, one might wonder how other teaching methodologies can complement the value that is inherent to the lecture. A research paper on this topic is in preparation.

My second project focused on the quality of medical school examinations. While this project was not part of my original summer project goals, my curiosity and passion for methodologies in medical testing prodded me to initiate several discussions with Centre director Yvonne Steinert, PhD, about a potential project. With her support and mentorship, I started to formulate a literature review on medical school examinations. I contacted experts in the field of medical test development and became inspired to present my findings to the faculty of The George Washington University School of Medicine and Health Sciences (SMHS). Among the group of test experts, I was able to communicate with Susan Case, PhD, and David Swanson, PhD, psychometricians and inventors of the United States Medical Licensing Examination (USMLE) Step examinations (previously the National Board of Medical Examiners 1-3). They provided important focus points and wisdom from their experiences working with medical school faculty on test development. The following is an abstract from my analysis:[2]

Results of this preliminary study showed that students value lecture in ways that cannot be substituted by any other teaching methodology.

Medical school faculty in U.S. medical schools design in-house assessments that often consist of 50 or more multiple-choice questions (MCQs). The quality of medical school MCQs is undoubtedly dependent on the level of questions that are asked. However, a common problem with writing in-house examinations is that many MCQs tend to be knowledge-based questions, therefore promoting memorization without analytical thinking.[3] Because students tend to tailor their studies appropriately to meet the expectations of their assessments, MCQs can play a critical role in learning.[4] Medical school MCQs must go beyond knowledge and offer a greater proportion of higher-level questions that emphasize application of knowledge, analysis, integration and synthesis. The USMLE Step examinations are well constructed and reviewed such that they offer a variety of question types and test a wide range of cognitive skills.[3] Thus, medical faculty must learn from the USMLE and construct examinations that allow students to learn at a higher taxonomic level. Furthermore, if low-quality examinations are administered to students, then students will be less prepared to perform well on other assessments of higher-order thinking, like the USMLE.

Jozefowicz *et al*'s paper includes an excerpt from the 2002 report on in-house medical school examinations.[5] Within their discussion, the authors state, "Faculty responsible for writing examinations should be trained; writing good examination questions is a skill that can be learned…a committee should review, critique and approve the content and format of the final draft of the examination."[5]

I hope to share what I have learned from the wealth of existing medical education research with the medical faculty and deans at the GW SMHS to help implement changes to basic science assessments.

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Equivalency of Cancer Detection in Analog Mammography versus Digitized Analog Mammography

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Screen-film mammography, the traditional x-ray and film variety used for decades, is the gold standard for breast cancer screening. However, sensitivity for breast cancer detection is only 85 percent, and therefore, 15 percent of screened cancers are overlooked.[1]

Despite the imperfect nature of current mammography, it has been credited with reducing the mortality rate of breast cancer.[2] Yearly mammograms after age 50 are important because prognosis is best when the cancer is detected early.

The goal of this study was to establish equivalency between traditional screen-film mammography and digitized analog mammography. A digitized analog mammogram is a traditional screen-film mammogram that has been digitized for evaluation on a softcopy workstation, or computer with a high-resolution monitor specifically designed for radiology applications.

Since there are advantages to using the digital medium over film, this study was both necessary and timely. One benefit of digitized mammography is that image settings, such as contrast and zoom, can be adjusted. Other advantages include the ability to store and transmit images in an electronic format. This prevents losing films and facilitates consultation with other radiologists who may be off site.

Vivek Patil studies an analog mammogram on traditional x-ray film.

In this study conducted at The George Washington University Medical Center, the equivalency of analog mammography and digitized analog mammography was assessed with respect to cancer detection rates. If both viewing conditions, analog and digitized analog, detected cancer at the same rates, Radiology departments would be able to experience the benefits of digital images without spending the large sums of money required for conversion to full-field digital mammography. Full-field digital mammography allows images to be captured directly on the computer without the intermediate film step required for digitized analog mammography.

Approval from the Institutional Review Board was obtained in March 2006 for this study involving 200 patients. Informed consent was waived because this study was retrospective and only used medical records. Mammograms were obtained from the Breast Care Center at The George Washington University Hospital in Washington, DC. Cases

were digitized with TotalLook (Icad, Inc., Nashua, NH) and viewed on a softcopy workstation (SecurView, Hologic, Inc., Bedford, MA), complete with two high-resolution 21-inch grayscale monitors.

Films were digitized and a multi-reader multi-case receiver operating characteristic (ROC) study was conducted to assess equivalency of analog and digitized analog mammograms for cancer detection. Each radiologist reader was required to read the same 200 cases on traditional film and softcopy workstation. Each radiologist chose the lighting conditions and a magnifying glass was provided. All identifiable patient information was hidden except for age. In order to prevent radiologists from remembering specific mammograms, a minimum of four weeks elapsed between the two reading conditions and the sequence in which the cases were presented was randomized. Five readers and 200 cases were used to obtain sufficient statistical power.

To determine whether a radiologist detected a cancer correctly, the Breast Imaging Reporting and Data System (BIRADS) developed by the American College of Radiology was used. The BIRADS score and location were collected for each case. BIRADS scores range from one (negative finding) to five (highly suggestive of malignancy). True positives were defined as an accurate BIRADS assignment with accompanying accurate lesion localization.

Fifty analog-detected and biopsy-proven cancer cases were included in the study. Only malignant lesions that were ductal carcinoma *in situ* (DCIS) or invasive carcinoma one centimeter or less were used in the study. These small cancer sizes were used because equivalency between the two reading conditions would be obvious if large lesions were used. To dilute the study set, 150 non-cancer cases were included.

The results of the study are not yet available because the study is still underway. The results of the study may have a profound impact on the field of mammography for years to come. In addition, valuable experience was gained planning and conducting this research, which led to a strong interest in a career in Radiology—a field that utilizes modern technology and clinical knowledge.

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Benefit of Intra-Articular Corticosteroid Injection Under Fluoroscopic Guidance for Subtalar Arthritis in Juvenile Idiopathic Arthritis

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Nearly 300,000 children in the United States have a form of arthritis. The most prevalent form of juvenile arthritis is juvenile idiopathic arthritis (JIA), which affects approximately 50,000 children in the U.S. Symptoms can be acute, lasting for weeks to months, or they can be chronic, lasting for months to years.

Periods of quiescence may be punctuated by bouts of recurrence, in some cases throughout a lifetime. Even if the disease "burns out," which frequently occurs before adulthood, resulting joint changes may cause permanent disfigurement, pain and/or disability.

Children with acute JIA have swollen, painful joints that may be stiff and difficult to move. Chronic inflammation can cause significant morbidity, including spontaneous joint fusion or debilitating pain,

requiring arthrodesis,[1,2] the surgical fusion of a joint with the goal of pain relief. Chronic pain, spontaneous subtalar fusion or arthrodesis can lead to an altered foot movement, or gait, with undue stresses placed on other weight-bearing joints, resulting in further morbidity.[1,3] Treating inflammation in the acute phase should reduce synovial thickening and alterations in the architecture of the cartilage and underlying bone, better preserving joint function throughout life.

Intra-articular corticosteroid injections are frequently used and a proven therapy for a variety of joints in children with JIA.[4,5] Although involvement of the subtalar joint in the foot is common in JIA, intra-articular treatment of this joint is rarely attempted due to its anatomic complexity and resulting technical difficulty in accessing the joint without image guidance (Figure 1).

The purpose of this study was to use fluoroscopically guided intra-articular subtalar steroid injection in JIA patients with acute symptoms to more accurately access the joint and relieve pain and increase foot movement.

Fluoroscopically guided subtalar joint injections were performed in 38 children (mean age 6.7 years). Medical records were reviewed retrospectively and clinical improvement was evaluated by the degree of eversion and inversion foot movements.

Image-guided subtalar joint injections were safely and successfully achieved with positive confirmation of intra-articular steroid delivery in 100 percent of the JIA patients. Improved range of motion was seen in 34 of 38 subjects (89 percent) and 44 percent of the subjects returned to a normal range of motion. Regardless of the elapsed time between injection and examination, clinical improvement was defined as increased foot eversion and inversion at the follow-up visit. Resolution was defined clinically as normal foot eversion and inversion without clinical subtalar symptoms. The mean duration of improvement was found to be 1.1±0.9 years. Notably, patients treated within one year of onset of subtalar symptoms were more likely to improve than patients treated more than one year after subtalar symptoms were first diagnosed (p=0.04).

This pilot work supports an image-guided approach to early treatment of joints affected by inflammatory arthritis, such as the subtalar joint that is difficult to access by palpation alone. Once subtalar involvement is identified, prompt referral for treatment improves the probability of clinical success. Image-guided fluoroscopy provides an accurate way to treat the disease locally in its earliest stage. Early treatment may help avoid or delay development of chronic and irreversible joint changes and the associated pain and loss of function.

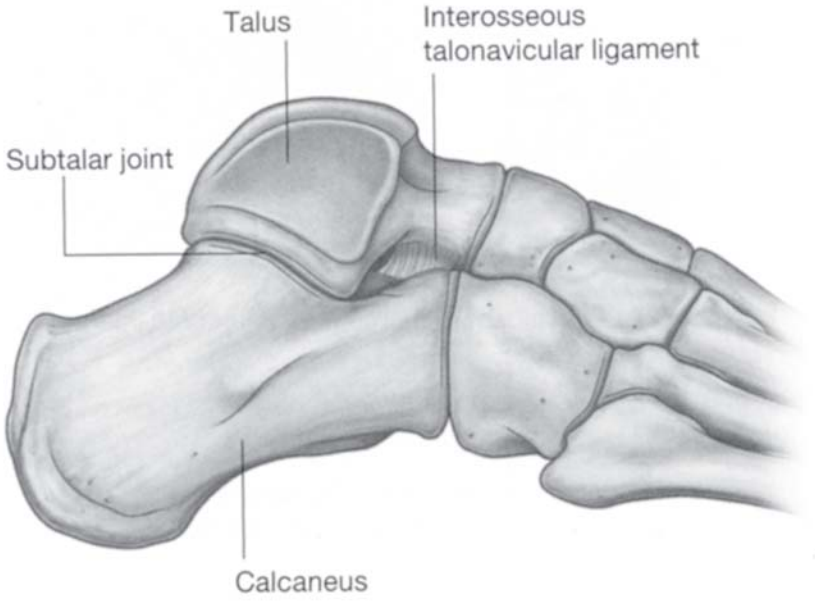

Figure 1. Diagram of foot showing the complexity of the location of the subtalar (Talocalcaneal) joint. Intra-articular treatment of this joint is rarely attempted due to the technical difficulty in accessing the joint (Courtesy of Drake RL, Vogl, W, Mitchell A. Moore K, Dalley A. Gray's Anatomy for Students. *Philadelphia: Elsevier; 2005).*

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Paget's Sarcoma: A Retrospective Review

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The purpose of this project was to contribute to a nationwide study aimed at developing new research and treatment methods to help individuals afflicted with Paget's sarcoma.

Paget's sarcoma is a rare form of bone cancer that develops in individuals who are in the advanced stages of Paget's disease—a disease characterized by abnormal and excessive remodeling of bone. In response to the excessive osteoclastic bone resorption, osteoblasts arrive and fill in the areas of resorption with new bone.

Due to the rapid activity of osteoclasts, the osteoblasts overreact and make excess bone that is abnormally large and chaotic. The resulting bone is less dense and prone to deformities and pathologic fractures. The disease can affect one bone (monostotic) or multiple bones (polyostotic). In the early stages of the disease, the affected bone becomes tender and appears enlarged upon x-ray (see Figure 1).

A small percentage of patients with Paget's disease develop Paget's sarcoma. This usually occurs in individuals who have been affected by polyostotic Paget's disease for many years. Patients with Paget's sarcoma present with severe, unremitting pain, a palpable mass around the involved bone and often with a pathologic fracture. Paget's sarcoma is a malignant bone tumor that carries an extremely poor prognosis.[1]

One of the primary reasons for the high mortality rate is that Paget's sarcoma mainly affects individuals over 65 years old. In addition to the poor health of the affected age group, the tumors are extremely aggressive and prone to pulmonary metastases due to the increased vascularity of the affected bones.

This retrospective study

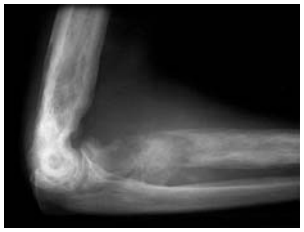

Figure 1. Paget's sarcoma of the humerus and radius[1]

consisted of 16 patients afflicted with Paget's sarcoma. The average age of cancer onset was 67.5 (range: 52–91) years old. Surprisingly, there were three patients diagnosed with Paget's sarcoma in their early 50s. In the sample population, 75 percent of the patients were male. The malignant tumor affected only one bone in nearly all of the patients. However, in two individuals, two different bones possessed the sarcomatous tumor. One of these patients possessed the cancer in the femur and pelvis, while the other patient was affected in the humerus and pelvis. Overall, the most frequently affected bone, in seven patients, was the humerus. Other afflicted sites included the pelvis (four patients), femur (four patients), radius (one patient) and thoracic vertebral body (one patient).

Due to the rapid progression of the tumors, radical surgery needed to be performed on most of the patients. Eleven amputations and two wide surgical excisions were conducted, and three patients received radiation therapy alone. Unfortunately, most patients died from lung metastases and only two patients survived the disease. The average time from diagnosis to death was 1.75 years (range: three months to seven years).

Despite significant advances in the treatment and survival of most bone sarcomas, Paget's sarcoma remains an extremely fatal disease. The survival rate for this cancer is less than 15 percent, despite the use of aggressive surgery, radiation and chemotherapy. Hopefully future research and new treatment methods can be developed to improve the outcomes for patients with this destructive malignancy.

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fMRI Used to Define Language Organization in Epilepsy

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Medicine is stepping closer to understanding how the brain is used for language and how ordinary language organization may be reworked in patients with epilepsy. A very effective, noninvasive tool used to study the brain is a functional magnetic resonance imaging (fMRI) scanner.

fMRI uses a conventional magnetic scanner in a fashion that allows rapid images to be taken of brain activity. Each region of a subject's brain used to perform a task has a blood flow response identifiable by the scanner.

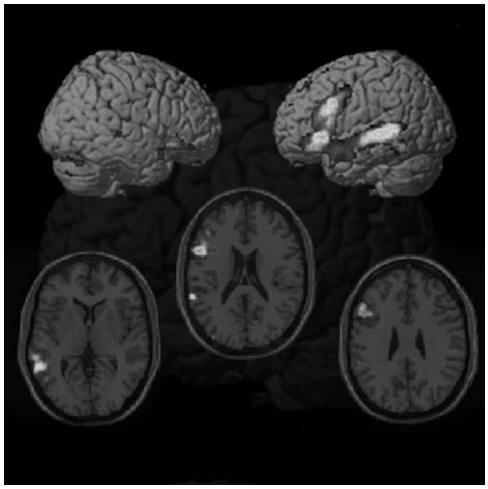

Figure 1. Sample fMRI brain activation maps of epileptic patients while performing auditory description tasks in the scanner.

This study examined scanner responses in a large sample of epilepsy patients and normal, healthy volunteers (controls) under the clinical guidance of pediatric neurologist William Gaillard, MD, of the Children's National Medical Center and the National Institutes of Health.

Each participant in the study was presented with a language-related task while in a 3 Tesla MRI scanner. Each participant was given a series of simple sentences that were true or not (e.g., "something you sit on is a chair" or "something you sit on is spaghetti"). If the sentence was true, the participant pushed a button. Tracking the performance helped ensure that the subject was concentrating on the given task while in the scanner.

Language centers traditionally reside in the left hemisphere of the brain; however, different patterns persist more commonly in epilepsy patient populations.[1] Three study groups were defined: epilepsy patients with right or bilateral language centers, epilepsy patients with left language centers and a group of controls.

This study concentrated on the temporal lobes, noting any differences between the epilepsy patients and the controls on brain activation maps (see Figure 1 for examples). The geometric distances between the points of activation in the control versus patient groups were then calculated.

This study suggested that there was reorganization intra-hemispherically and/or inter-hemispherically due to epileptic interruption of language networks. The epilepsy patients with left hemisphere language dominance used a wider region of their dominant temporal lobe to accomplish the same task as the controls. In addition, the region was significantly more posterior and superior than that of the controls. These findings may indicate the occurrence of dominant hemisphere reorganization in the wake of dominant hemisphere epilepsy. Furthermore, this study notes that pathology can also shift language activation to the right hemisphere, as shown by the epilepsy patients. Language centers of activation in these individuals were found to tightly cluster around a point in the right hemisphere temporal lobe that is otherwise active on the left side in controls.

These findings provide a clearer picture of the disturbances that epilepsy can cause. By showing differences in reorganization, researchers can better define how the brain can cope with epilepsy. Still, the exact mechanism of how epilepsy interrupts language remains unknown. However, knowing precisely what brain regions epilepsy disturbs may ultimately lead to understanding how it affects the brain, which could impact future treatments.

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Hippocampal Activation May Not Predict Memory for Auditory Events

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Together with neurologist William Gaillard, MD, and his colleagues at Children's National Medical Center in Washington, DC, studies were conducted to assess hippocampal activation, memory performance and cerebral language lateralization in children using functional magnetic resonance imaging (fMRI) and neuropsychological tests.

This research was part of a larger project undertaken to determine differences in the location of language function in the brains of children with epilepsy. This information would facilitate the surgical removal of brain tissue in children whose epileptic seizures seriously disrupted their daily activities.

Most individuals have receptive language functions isolated primarily to the temporal lobes of the left hemisphere; however, patients with injury or impaired normal function of brain tissue, as seen in stroke or epilepsy, are likely to have language functions shifted to other areas of the brain.[1] Understanding this shift is invaluable in planning neurosurgical intervention and expanding our knowledge of brain organization and plasticity.

Epilepsy is a condition in which normal signaling between neurons is disrupted, resulting in paroxysmal periods of altered consciousness and stereotyped motor activity. Prior to resecting a portion of brain tissue, a surgeon must ensure that areas crucial to the patient's normal function, such as language, are not removed.

> *Results suggest that injury to the left hippocampus may not mediate language reorganization in the temporal neocortex.*

Neuropsychological tests were administered on 42 children—21 epilepsy patients and 21 normal participants. Tests included the Wide Range Assessment of Memory and Learning (WRAML) to evaluate each child's capacity for learning and memory,[2] and tests for each child's verbal intelligence. The children then listened to a two-and-a-half minute story while undergoing fMRI. Once removed from the scanner, each child was asked a series of questions about the story to assess his or her memory performance.

Using a magnetic field to detect changes in blood flow corresponding to changes in neural activity, fMRI records brain activation when a person performs a particular task—in this case, listening to a story. After determining which side of the brain had the greatest language activation, a computer masking program was used to isolate hippocampal activation. The hippocampi, located in the medial temporal lobes of the brain, are involved in learning and memory.

This study postulated that the degree of left hippocampal activation during a verbal task would correlate with the side of language dominance. A relationship between hippocampal activation and language dominance was not found, suggesting that injury to the left hippocampus may not mediate language reorganization in the temporal neocortex, which was recently proposed as a mechanism of language reorganization. A more precise understanding of learning and memory and their relation to language function during development provides the basis for more successful outcomes with neurosurgical intervention in cases of epilepsy and other disorders.

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Comparison Study of Patients with Stages IIIC and IV Epithelial Ovarian, Fallopian Tube and Primary Peritoneal Cancers

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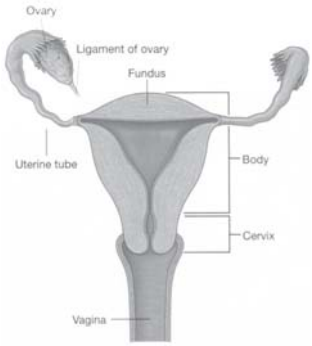
Women who develop primary epithelial, fallopian tube or peritoneal carcinoma will most likely present with advanced-stage disease.[1] Optimal cytoreduction for advanced disease varies in literature from 15 to 85 percent; however, patients who have undergone extensive upper abdominal procedures report optimal cytoreduction rates greater than 50 percent.[2,3]

The optimal primary cytoreduction rate at Memorial Sloan-Kettering Cancer Center (MSKCC) has been less than 50 percent. In these cases, upper abdominal resections were not performed and thus residual disease often involved the diaphragm, liver and/or spleen.[4,5]

In the beginning of 2001, MSKCC modified its surgical approach to patients with advanced ovarian, fallopian tube or primary peritoneal carcinoma by integrating extensive upper abdominal procedures, such as diaphragm stripping and/or resection, splenectomy, distal pancreatectomy, partial liver resection, resection of tumor from the porta hepatis and cholecystectomy. These modified surgical procedures were used at the discretion of the primary surgeon to obtain optimal residual disease status.[6] This study compared surgical events and results in patients who underwent surgery between January 1996 and December 1999 or between January 2001 and December 2004. The latter group included more extensive upper abdominal resections.



(Adapted from Drake RL, Vogl, W, Mitchell A. Moore K, Dalley A. Gray's Anatomy for Students. *Philadelphia: Elsevier; 2005).*

The MSKCC Gynecologic Service Database was used to compile information on all patients with stages IIIC and IV epithelial ovarian, fallopian tube and primary peritoneal carcinomas. These patients had undergone primary cytoreductive surgery at MSKCC between January 1996 and December 1999 or between January 2001 and December 2004. Patients who had undergone surgical procedures from January 2000 to December 2000 were excluded from the study because those surgeries did not routinely include the use of extensive upper abdominal surgical procedures and the surgical prototype of the service had not yet changed.[6]

Furthermore, patients were excluded from the study if they had completed neo-adjuvant chemotherapy or primary cytoreductive surgery at another institution, or if they had low malignant carcinomas, such as mucinous or carcinosarcoma histologic types. In addition, stage IIIC patients who did not present with bulky disease greater than two centimeters, but rather presented with positive lymph nodes, were further screened out.

The following information was extracted from the patients' medical records: age at diagnosis, date of primary surgery, primary site of disease, tumor grade, histology, pre-operative serum CA-125 level, platelet size, location of largest tumor mass, amount of ascites, surgical procedures performed, size of largest residual, site(s) of residual tumor, estimated blood loss, number of units of blood transfused intraoperatively, intra-operative complications, operative time, length of hospitalization, primary chemotherapy, result of chemotherapy, date of second look, outcome of second look, last follow-up and current state.

Although the data has

not yet been analyzed, the hypothesis is that patients who underwent surgery at MSKCC between January 2001 and December 2004 show improved rates of optimal primary cytoreduction and survival over patients who had surgery between January 1996 and January 1999 when extensive upper abdominal resections were not performed.

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Safe and Effective Laser Surgery on Dark Skin

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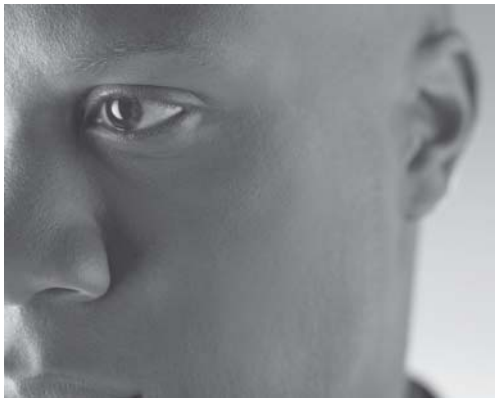
The rising popularity of cutaneous laser surgery as an accepted therapy for various skin pathologies, coupled with the diverse face of the patient population, has led to increasing demands for researchers to investigate laser treatment on darker skin tones.

United States population statistics reveal dramatically shifting demographics in the past decade, with 85 million colored individuals in the U.S. in 2000.[1]

However, most of the current literature is devoted to examining laser procedures performed on individuals with fair skin tones (skin phototypes I-II), and protocols have largely been defined on the basis of these limited patient studies. This research focuses on examining recent clinical research of laser surgery on dark skin tones to modify the standards for laser treatment of vascular-specific, pigment-specific, and ablative and non-ablative skin resurfacing.

Due to its unusually wide absorption spectrum ranging from 250-1200 nanometer (nm), melanin can be specifically targeted by all visible-light and near-infrared (NIR) dermatologic lasers currently in use. In the darkly pigmented patient, nonspecific energy absorption by relatively large quantities of melanin in the basal layer of the epidermis can increase unintended thermal injury and lead to a higher risk of unfavorable side effects, including permanent dyspigmentation, textural changes, localized degeneration and scarring. Treatment parameters, therefore, must be carefully considered when performing laser surgery on patients with darker skin.[2,3]

Although difficult, effective laser therapy in patients with darker skin can be achieved since the absorption coefficient of melanin decreases exponentially as wavelengths increase.[4-8] Laser systems generating wavelengths that are less efficiently absorbed by endogenous melanin can often provide a greater margin of safety while still achieving satisfactory results.[9] Conservative power settings should be initially employed to



Dermatologic lasers have been used for more than four decades to treat a variety of cutaneous conditions, but, until recently, have been limited to use in patients with pale skin.

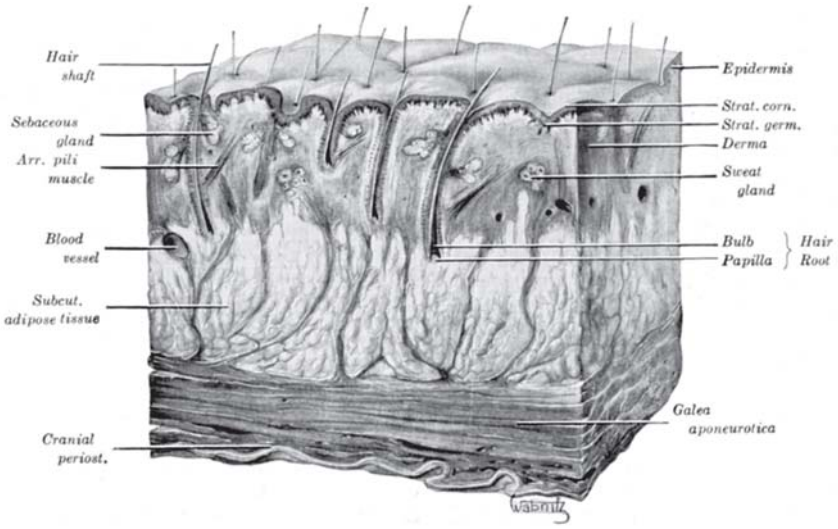
minimize the extent of collateral tissue damage. Clearly, a prudent approach to treatment is far preferable to incurring the risk of irreparable tissue destruction resulting from excessive thermal injury.

Vascular-specific laser systems include a wide array of Quality- or Q-switched, pulsed and quasi-continuous wave lasers generating green or yellow light with wavelengths ranging from 532-600 nm. Since 577 nm represents a major absorption peak of oxyhemoglobin, the 585 nm flashlamp-pumped pulsed dye laser (PDL) has proven to be the most vascular specific.

Pigment-specific laser technology generates green, red or NIR light to selectively target intracellular melanosomes, tattoo pigment or to eradicate unwanted hair by damaging follicular structures where melanin is heavily concentrated. Q-switched systems generating nanosecond (ns) pulses that are substantially shorter than the 10-100 ns thermal relaxation time of melanosomes represent the safest means for treating pigmented lesions in dark skin.[10]

Q-switched systems currently available include the 532 nm frequency-doubled Nd:YAG (neodymium-doped:yttrium aluminium garnet; Nd:$Y_3Al_5O_{12}$), 694 nm ruby, 755 nm alexandrite and 1064 nm Nd:YAG lasers. The far-infrared wavelengths generated by the alexandrite and Nd:YAG laser systems are less efficiently absorbed by epidermal melanin, which limits the extent of unwanted thermal injury to nontargeted tissues of the epidermis and upper papillary dermis, allowing for deeper dermal penetration.

Cutaneous laser resurfacing can provide an effective means for improving the appearance of diffuse dyschromia, photoinduced rhytides and atrophic scarring in patients with darker skin phototypes. Several reports document the long-term safety of the high-energy, pulsed and scanned carbon dioxide (CO_2) and short- and long-pulsed erbium:yttrium aluminum garnet (Er:YAG) lasers for the treatment of more darkly pigmented patients.[11,12]



(Courtesy of Gray, H. Anatony of the Human Body. *Philadelphia: Lea & Febiger; 1973).*

Dermatologic lasers have been used for more than four decades to treat a variety of cutaneous conditions, but, until recently, have been limited to use in patients with pale skin. This research showed that recent developments in laser technology have generated safe and effective means to treat patients with heavily pigmented skin.

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International Health Experience Shapes Future Path

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My first patient in Kenya died. On the first morning of my eight-week internship at the Shikokho Medical Clinic, I was wide eyed and ready to learn, but I could hear a boy's labored breathing before I even entered the clinic gate.

To treat what appeared to be an asthma attack, the nurses and I pumped as many bronchodilators and as much epinephrine as we could into his small body. Each injection did not significantly help his breathing as we had hoped and I felt unsure of what to do next. Without access to an emergency phone number, ambulance or viable transportation out of the village to a bigger or better equipped health facility, the boy would die. The nurses knew that. Since there was no access to another facility, the boy eventually stopped breathing.

It was difficult not to think about the possibilities that might have existed for the boy in the U.S. with access to proper emergency care. But this was Shikokho, Kenya, a small village of about 1,500 where medical and social problems are vastly different from those in America, and, at times, seem grossly magnified. Shikokho is part of Ikolomani Division, the poorest division in the Kakamega District in the Western Province of Kenya. Kakamega is the third poorest district of more than 70 in the country and is inhabited mainly by subsistence farmers of the Luhya tribe. The estimated HIV rate for the Western Province is more than 25 percent.[1]

The developmental health issues the Shikokho community faces center on access. Shikokho is 10 kilometers (6.2 miles) away from the nearest facility that can perform emergency services, such as surgeries, or provide free HIV testing and antiretroviral treatment. Add to this that roads can only be traversed by foot and become virtually impassable after a heavy rain. The distance and conditions prevent a large sector of the population from receiving critical services.

One morning I visited St. Elizabeth's Hospital of Mukumu, the nearest facility with emergency services and a government-approved HIV testing and treatment facility. I went to inquire about the possibility of partnering with the hospital for referrals of emergency patients, including the use of the ambulance to Shikokho. Perhaps because I was a *mzungu* (Swahili for white person), the administrators agreed and immediately gave me four phone numbers for the nurses at Shikokho to call to transport a patient, including mobile phone numbers for the hospital administrator, head doctor and matron (nurse in charge). With this assistance from St. Elizabeth's Hospital, an emergency or serious health problem would not result in a death sentence for someone living in Shikokho.

During the third week of

my internship, a collaborative effort by the Kenyan Ministry of Health, the World Health Organization (WHO) and the United Nations International Children's Education Fund (UNICEF) allowed for a five-day distribution of measles vaccinations, Vitamin A and mosquito nets at all health facilities in Kenya, including the Shikokho Medical Clinic. Previously, it had been an enormous struggle to reach young women isolated at their *shambas* (farms).

This campaign gave the clinic staff, including myself, a unique opportunity to give talks about malaria and the proper use of mosquito nets to more than 500 young mothers in the community. In the Western Province, HIV prevalence is 4.8 percent, but 5.8 percent among women and 8.9 percent among pregnant women.[1] Agnes, a respected HIV-positive midwife, gave several health talks to the women on the importance of knowing one's HIV status and getting anti-retroviral treatment if HIV positive. The women responded enthusiastically and showed appreciation to the clinic staff for teaching them how to prevent disease in themselves and their families.

During the last week of my internship, the first HIV voluntary counseling and testing (VCT) day took place at the Shikokho Medical Clinic. After weeks of brainstorming how to legally perform HIV testing at the clinic, an arrangement was made with St. Elizabeth's Hospital to perform a monthly outreach at the clinic. Using bars of soap as incentive, the first VCT event enabled 53 villagers, including 12 pregnant women, to be tested, counseled and referred for treatment when necessary.

After being introduced to some of the critical health problems facing the Shikokho community during my first week, I could not have imagined some of the creative solutions that evolved in the following eight weeks. I was told by many people to move slowly and cautiously and not expect to see any results in this short amount of time. This was termed "realism." But I found it difficult to proceed *pole, pole* (slowly by slowly) when the urgency of these life-and-death situations took hold of me. Though the health problems facing Shikokho are by no means solved, I was able to be a part of many beginning stages of solutions.

Having this unique and amazing experience as a medical student opened my eyes to international health issues and how I can play a role. I have felt the drive to work in the realm of international public health for many years. This internship affirmed and solidified my desire. I discovered strengths and abilities I did not know I possessed. I found that I was driven by the needs of the community and would do almost anything in my power to address these needs. Instead of becoming disillusioned by the many obstacles, I became empowered to do whatever I could to help. I feel that my summer experience is best epitomized by former President Jimmy Carter's words, "Once we understand, we can care; and once we care, we can change."

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Compassion in Action: Bringing Medicine to the Children of Costa Rica's Ghettos

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Although Costa Rica's capital and largest city serves as the arrival and departure point for many tourists, San José is often overlooked by the many sightseers who are eager for more "exotic" attractions.

Containing approximately half of the entire country's population, the greater metropolitan area of San José consists of not only native Costa Ricans but also a large percentage (estimated at 40 percent) of Nicaraguan refugees.[1,2] They mostly live in segregated areas around the city where they struggle against discrimination for a better life. As a result of their illegal status, many Nicaraguans are denied medical care.[3] This article discusses my personal experiences working with Nicaraguans in Costa Rica through the Foundation for International Medical Relief of Children (FIMRC), an organization started by Vikram Bakhru, MD, a GW medical school alumnus who graduated in 2001. He is now in his Surgery residency in Philadelphia, but still manages FIMRC. This article discusses how such a population can help volunteers understand and appreciate the power of caring and compassion in medicine.

I met FIMRC's Medical Director, Cristian Elizondo, MD, the day after I arrived

in Costa Rica in February 2006. I expected to meet a serious middle-aged physician, but I instead came across a man not much older than myself, wearing jeans and a trendy T-shirt and joking with a friend on his cell phone. Seeing this man relieved me and his easygoing, casual attitude alleviated my previously grim and serious outlook toward this volunteer project. Dr. Elizondo's demeanor and casual way of dressing would have probably made people in the U.S. skeptical or less confident in him as a physician. But in Costa Rica, this was very appropriate. Dr. Elizondo understood that his patients felt most comfortable with him if they could look at him as a friend.

A View of "Los Pinos" (The Pines), a community outside of San José where many Nicaraguan refugees live without medical treatment and suffer from poverty, discrimination and drug and crime problems. (Courtesy of Sarah Eberhart)

A new clinic was being built by FIMRC, but it was still under construction. Over the next several days, I visited some of the "ghettos," or what the local Costa Ricans called the Nicaraguan communities around San José, and we set up an on-site clinic in one of the ghettos.

Utilizing my basic abilities in Spanish, I helped with patient charts, medication distribution and health education lessons as Dr. Elizondo examined patients. Without sophisticated equipment or tests, Dr. Elizondo was limited to practicing good medicine with his words and his hands. I did not see any life-saving procedures or any serious cases the week I was there, but the patients taught me valuable lessons and were a great testament to the foundation of compassion and caring in medicine. The genuine gratitude expressed by these people, even as I misspelled their names and made them repeat words again and again, was overwhelming. Despite several sick and upset children hanging on her, one woman kept a very patient smile on her face while I tried my best to take her children's histories. She even wanted to take the time to ask me about my life and my work in Costa Rica. Most patients were just happy someone wanted to help and care for them.

I took a second trip to Costa Rica for a week in July 2006 when the new clinic opened. An average of 20 patients visit the clinic daily and FIMRC has expanded its services to include a child psychologist and a support group for mothers suffering from psychological problems and abuse that often accompany poverty. Dr. Elizondo and everyone else at FIMRC have worked hard to give the Nicaraguan population a sense of belonging, worth and the relief that comes with knowing someone is there to help in times of need. What I found to be most memorable was that, without fancy diagnostic equipment or technology, we were able to help and bring hope to a population that goes largely ignored. With so much emphasis in the U.S. on state-of-the-art medicines and technologies, it was extremely valuable to see how a doctor's listening ear and compassion could do so much. With this lesson in mind, I hope to be able to always focus on the relationships I establish with my patients. My experience with FIMRC and the people in the Nicaraguan ghettos was eye-opening, humbling and a great way to remind me of my future goals as a medical student and beyond.

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The Uptake, Recycling and Function of Vitamin C in HT-22 Cells and its Relevance to Diabetes

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Most mammals make vitamin C in the liver with enzymes that convert glucose into vitamin C. Humans, however, lost the ability to do this. Therefore, humans must get their vitamin C from an outside source, namely from their diet.

Vitamin C is necessary for several important functions in the body and for its role as an antioxidant.[1] On the membranes of red blood cells, vitamin E has the ability to reduce peroxides and be regenerated by vitamin C. Vitamin C is also known as a water soluble, chain breaking donor antioxidant. Vitamin C has the ability to reduce oxidative stress by either slowing or preventing the oxidative progression before it even starts. It does this by directly interacting with the chain-carrying radicals. It donates either a hydrogen atom or an electron to the oxidant and is therefore able to prevent the spread of cycle chain reactions. As a powerful antioxidant, vitamin C has been shown to prevent atherosclerosis, cancer and various cerebral diseases.

Vitamin C, also known as ascorbate, enters cells through the Sodium Dependent Vitamin C Transporter 2 (SVCT2). The oxidized form, known as dehydroascorbic acid (DHA) and obtained from the loss of two electrons, enters cells through the GLUT transporter. Once DHA gets inside cells, it is immediately reduced to ascorbate via glutathione (GSH) or through thioredoxin reductase. Ascorbate's high concentration in neurons in the brain shows that the brain relies on vitamin C for its antioxidant properties. Thus, it is important to study how cells take up and recycle vitamin C.

HT-22 cells, a neuronal cell line derived from mouse hippocampus, were chosen for this study because they contain many SVCT2 and GLUT transporters. Results from a Western Blot showed that the SVCT2 transporter was present at 75 kilodaltons (kD). Results from high-performance liquid chromatography (HPLC) showed that as increasing amounts of ascorbate were added to the cells, there was a progressive uptake of ascorbate up to 16 millimolar (mM) intracellular concentration. When adding increasing amounts of DHA to the cells, there was a linear uptake of DHA and reduction to ascorbate, which only went up to 1 mM ascorbate.

These results illustrated that, since DHA is using the GLUT transporter, glucose could be competing with DHA for entry into the cell. When glucose was removed and DHA was added to the cells, a rapid linear increase in the intracellular levels of ascorbate was observed back up to 16 mM, showing that glucose was competing with DHA for entry into the cell. When increasing concentrations of glucose were added to the cell media, there was a progressive decrease in DHA uptake, and therefore a decrease in recycling DHA to ascorbate.

Clearly, glucose acts as a competitive inhibitor. DHA was shown to be dependent on GSH for reduction to ascorbate because when agents that inhibited GSH synthetase were added, there was a 70-99 percent decrease in intracellular ascorbate, depending on the agent used.

When people without diabetes are exposed to oxidant stress, such as coronary

artery disease, some ascorbate gets oxidized to DHA and goes into the area of ischemia. Once DHA gets into the cells, it is recycled to ascorbate, a form that can act as an antioxidant. DHA uses GLUT transporters for entry into cells. However, people with uncontrolled diabetes lose the ability to uptake DHA due to the competing nature of glucose. Therefore, less DHA is recycled to ascorbate and less ascorbate indicates a decreased ability to deal with oxidant stress. A diabetes patient who develops ischemia will have a problem getting DHA into the cells and recycling it back to ascorbate.[2] This is especially important when considering that the blood-brain barrier lacks SVCT2 transporters but contains DHA transporters. Further studies are being conducted to see how HT-22 cells deal with oxidant stress, such as glutamate, and the effect the stress has on direct reduction with GSH and ascorbate uptake.

The purpose of this project was to better understand the role of vitamin C in the HT-22 cell line. If one understands how these cells use vitamin C, how vitamin C gets recycled and what effect these cells have on oxidative stress, one can then make connections with the importance of certain levels of dietary intake that are required for vitamin C to be beneficial to patients. Researchers can then relate these findings to diseased states so that patients can better understand the importance of vitamin C in the protection from oxidative stress.

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Performance of Statistical Models On a Whole Genome Type 2 Diabetes Scan

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Type 2 diabetes mellitus is one of the most significant health threats in the world. The World Health Organization estimates that approximately 170 million people are afflicted with this disease. Since this number is expected to double within the next two decades,[1] it is important to gain a better understanding of the disease and find better ways to control this growing problem.

The type 2 diabetes mellitus whole genome association study gathers genetic variation[2] by genotyping Single Nucleotide Polymorphisms (SNPs), which are variations of a single nucleotide that occur in at least one percent of the population, from patients with adult onset type 2 diabetes mellitus. This private-public partnership, a collaboration between the Broad Institute, Novartis Institutes for Biomedical Research and Lund University in Sweden, promotes open sharing of information and hopes to show that the benefits of this openness will outweigh the benefits of secrecy. After the quality of the data is assured, the primary analysis results will be posted online for public access.

For this research, case and control subjects from Sweden and Finland were matched using two methods: traditional case control and discordant sibship. For the traditional case control, one or two subjects with adult onset type 2 diabetes mellitus were matched with one or two controls on the basis of sex, body mass index (BMI), age and geographical region. The ages of the controls were within 20 years of the matching cases. The cases and controls were seen at the same clinical site and were from the same geographical location. Cases and controls were determined by the ADA[3] definition for type 2 diabetes and normal glucose tolerance (NGT). For discordant sibship, each sibship group consisted of three or four subjects, including one or two affected siblings and one or two unaffected siblings. This group was chosen using the same selection criteria as the traditional case control.

The study's 2,881 subjects were genotyped using the Affymetrix GeneChip 500k, which contains 500,000 SNPs.

While the Affymetrix GeneChip 500k provides data in terms of SNPs, this

analysis requires the dataset to be organized by genes. One representation was created by matching a compiled list of 32,634 relevant genes to the SNPs within and around the gene. This representation of genes was tested under the stress of multiple statistical methods to determine the statistical method that most accurately embodied the data.[4,5] PLINK, a whole genome association analysis software toolset, was used to analyze this data.

Three gene-based association statistics were compared: 1) Max: a statistical method using the top-performing chi square values for each gene. 2) Sum: a statistical method using the sum and normalization of up to five chi square values for each gene. 3) Hotelling's T-square: a multilocus, genotype-based test. The relationships between the 10 best performing genes of each statistical method were examined. A subset of nine genes, mentioned in literature and thought to be related to diabetes, were chosen as a dataset of more manageable size.

The release of these results is pending the completion of the project and the public release of the genetic and clinical information. In the future, additional statistical methods will be used to consider alternative representations of genes, including a model capturing epistasis (the interaction between multiple genes) and sets of related genes (such as the genes in a particular metabolic pathway).[6,7] Multiple gene representations and statistical tests will hopefully create a clearer picture of the genomic influence on type 2 diabetes and eventually lead to a better understanding of this disease and more effective treatment options.

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Role of Potassium Channels in Brain Plasticity after a Peripheral Vestibular Injury

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A stimulating two-hour lecture on human brain development during my first year of medical school motivated me to seek a summer research position in brain plasticity and function. A Gill Fellowship allowed me to conduct research in the laboratory of my Neurobiology professor, Kenna Peusner, PhD.

My project targeted the changes in vestibular neurons of the brainstem during recovery of function after a peripheral vestibular injury. The vestibular system is primarily responsible for maintaining balance. Balance is maintained by signaling in three neuron reflex pathways composed of vestibular ganglion cells in the periphery, vestibular nuclei neurons in the brain, and motor neurons. After losing the vestibular periphery on one side, people exhibit symptoms of nausea, vomiting, dizziness, nystagmus, and the inability to walk or eat. Without intervention, most of these symptoms disappear naturally over the period of a week, a process known as vestibular compensation. The present understanding is that vestibular compensation is due to brain plasticity, involving a temporary loss of neuronal excitability in vestibular nuclei neurons and their recovery. The process occurs not only in humans, but also in other vertebrates, including birds. I chose the hatchling chicken as my experimental model because the chicken is bipedal like humans, has particularly distinctive vestibular nuclei and young chickens show a higher degree of brain plasticity than adult chickens.[1]

The working hypothesis of Dr. Peusner's laboratory is

that certain developmental events involved in establishing neuronal excitability are re-expressed during recovery of function after injury. Furthermore, the laboratory is focused on investigating specific potassium channels during vestibular compensation. In particular, I studied a potassium channel that can be identified because of its sensitivity to the snake venom dendrotoxin (DTX).

Surgery to remove the vestibular periphery was performed on four- to five-day-old hatchling chickens, which were sacrificed three days afterward. Five-day-old hatchlings were selected for study because the neurons achieve their mature excitability at this age.[2] During embryonic development, these neurons exhibit high levels of this potassium channel, which is lost after reaching maturity in five-day-old chickens.[3] I performed immunocytochemistry on five-day-old normal chickens and those with surgically removed vestibular periphery to determine whether the level of expression of the protein Kv1.2, an essential component of the DTX-sensitive potassium channel, changed during vestibular compensation.

I studied the principal cells of the chicken tangential vestibular nucleus because these vestibular nucleus neurons participate in the vestibular reflex pathways (Figure 1).[1] My project involved learning immunocytochemistry, behavioral testing of operated hatchlings, and confocal imaging and analysis of these neurons. In performing my tasks, I was fortunate to have the opportunity to work alongside Dr. Peusner as well as two research scientists, Anastas Popratiloff, MD, PhD, and Mei Shao, MD, PhD.

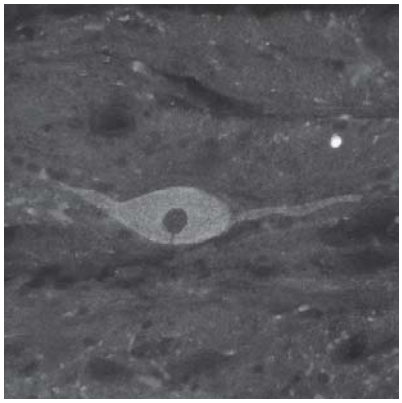

Figure 1. An immunocytochemical image of the principal cell body located in the vestibular system's tangential nucleus. The brighter regions surrounding the soma indicate a greater concentration of the Kv1.2 potassium channel protein subunit.

I began my summer fellowship by watching an experienced investigator perform several vestibular ganglionectomy microsurgeries. While performing the procedure myself, I learned how delicate and demanding microsurgery on the inner ear can be. However, immunolabeling the sections proved to be the biggest challenge. It was critical to determine the appropriate timing to avoid over- or under-labeling the tissue sections with a particular antibody. Two secondary antibodies were used to visualize the neuron cell bodies and their synaptic terminals with Kv1.2. I learned the importance of using fluorescent dyes with excitation and emission curves that do not overlap to prevent false positive labeling.

I mounted the prepared brain sections on glass slides for confocal imaging. This specialized microscope took a fair amount of practice, but after a few days I gained proficiency. Using confocal microscopy and lasers of varying frequencies, I imaged at 60x magnification the Kv1.2 protein expressed in the principal cells and their synaptic terminals.

I captured confocal image stacks containing 50 images for each of 76 principal cells and thousands of synaptic terminals. Using Adobe Photoshop 7.0, I outlined the immunolabeled components and analyzed the images for pixel brightness to quantify the amount of Kv1.2 protein expressed in the synaptic terminals and in the cytoplasm of the principal cells. Mean distribution curves and standard deviation of the data are in progress. I am continuing to work in Dr. Peusner's lab during the school year to finish the project.

The work I performed in Dr. Peusner's lab taught me many lessons, both practical and philosophical. I became proficient at using the confocal microscope and I now have a strong understanding of digital image analysis. As Dr. Popratiloff explained to me, "Image analysis is a highly evolving and useful system in this new age of digital computing." I acquired specific knowledge and skills in behavioral testing of experimental animals, microsurgery and image analysis that have carried over into my classroom studies.

The scientific implications of this work will provide insight into the cellular and molecular mechanisms controlling excitability in vestibular nucleus neurons. As these mechanisms are better understood, molecular targets will be identified and more effective therapies may be devised to treat central vestibular disorders in humans resulting from disease, injury or aging. From this, a more concrete understanding of brain plasticity and function may be achieved. As a Gill Fellow, I witnessed firsthand how ideas and hypotheses about complex systems of body function may be explored in the laboratory, and how the findings may be translated to a better understanding of human health.

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Chemokines: Potentially Efficacious Cancer Vaccine Adjuvants

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Chemokines are a family of low-molecular-weight proteins that appear to be involved in the costimulation of T-cells. For this reason, in addition to their well-established role in chemotaxis, chemokines are being investigated for their use in cancer immunotherapies.

Cancer immunotherapies aim to harness the potential of the body's immune system to combat malignant tumors. When positive therapeutic responses are achieved, they are generally attributed to the induction of cell-mediated immune responses.[1] This has proven challenging, however, tumor cells appear inherently effective at evading immune surveillance. The poor immunogenicity of these transformed, altered self cells can be largely attributed to the suppressive nature of the local tumor microenvironment, owing, in part, to the decreased expression of costimulatory molecules.[2]

Costimulatory signals are critical in the activation of naïve T-cells. Once the T-cell receptor (TCR)/CD3 complex has engaged its cognate peptide-MHC presented by an antigen-presenting cell (APC), naïve T-cells must receive a second signal to drive clonal expansion and differentiation into armed effector cells. In the absence of positive costimulation, T-cells that have encountered antigen become anergic, rendering them nonresponsive. Thus, a costimulatory molecule must be capable of sending and/or receiving information that initiates an intracellular signaling pathway that intersects with antigen-specific signals synergistically to allow for lymphocyte activation.[3]

Most costimulatory molecules belong to the B7/CD28 and TNF/TNFR families, while others belong to the CD2-subset and integrin/cell-adhesion molecule group.[3] Their use in cancer vaccines, often in combination with a known tumor-associated antigen, offers a potentially efficacious means to initiate and maintain cell-mediated antitumor immune responses. Rationally expressing certain costimulatory molecules in the tumor microenvironment, based upon their spatial and temporal significance to the initiation and maintenance of the immune response, immunotherapists can help shift the balance from immunotolerance, tumor growth and survival to T-cell activation and tumor regression.[2]

An exciting finding is that chemokines are emerging as costimulatory molecules.[4] Of particular interest is CCL21, a chemokine that is constitutively expressed by secondary lymphoid tissue and attracts CCR7-expressing cells, which largely include mature antigen-experienced dendritic cells (DCs) and naïve T-cells. The effective communication between these two cell types is crucial to initiate cell-mediated immune responses. For these reasons, CCL21 is being investigated for its role in the coordinated migration of these cell types, as well as its ability to costimulate the proliferation and differentiation of naïve T-cells into armed effectors.

Murine CCL21 has already been shown to costimulate naïve T-cell expansion and Th1 polarization of non-regulatory $CD4^+$ T-cells, and to a lesser extent, the increased proliferation of $CD8^+$ T-cells.[5] However, it cannot be assumed that human CCL21 is capable of the same, as significant discrepancies exist between the innate and adaptive immune systems of mice and humans.[6]

Researchers have previously demonstrated the costimulatory expansion of peripheral blood mononuclear cells (PBMCs) with the structurally related, but functionally dissimilar chemokines CCL3, CCL4 and CCL5.[7] These three chemokines are members of the inflammatory chemokine subfamily, whereas CCL21 belongs to that of the homeostatic or lymphoid chemokines. While both subfamilies have chemotactic properties, their differential expression profiles may carry

implications toward their intrinsic ability to function in costimulation.[8]

As our interests are inclined toward translational research, it is of great importance to provide supportive evidence that the observations made in murine models may also hold true in the clinical setting. We have begun to conduct numerous *in vitro* cell proliferation assays to test the ability of human CCL21 to costimulate cell division in PBMCs, as well as sorted $CD4^+$ and $CD8^+$ T-cells, all of which have been harvested from healthy human donors. Positive findings here will help provide a supportive rationale for integrating human CCL21 into cancer vaccines as an adjuvant to therapy. As the immune system is elegantly regulated by a cascade of complex interactions that resemble a rheostat, rather than an on-off switch,[9] immunotherapists must tip the balance of positive and negative costimulatory signals in a way that achieves appropriate levels of T-cell activation that can lead to the eradication of tumors, devoid of significant, undesired autoimmunity.[10] Targeting specific costimulatory pathways, particularly those of the chemokine family, can allow for the fine tuning of various spatial and temporal aspects of cell-mediated immune responses, helping to increase the safety and efficacy of cancer vaccines.

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Second Messenger Activity with Immune Cell Activation

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Cancer is a leading cause of death in the United States each year. With current standard therapy options, the long-term survival rate over a five-year period hovers around 65 percent.[1]

With a growing understanding of the immune system, researchers have determined that the immune system fights off foreign pathogens and is utilized against malignancy.[2] These discoveries have opened the door for a new cancer treatment method—cancer immunotherapy.

Cancer immunotherapy uses the body's own natural immune defenses to fight cancer. It has two main benefits over current treatment options. With the use of antibodies, therapeutic treatment can be directly targeted to specific tumor sites. This allows for more concentrated drug delivery and effective results. Targeted delivery also localizes toxicity while decreasing harmful systemic side effects.

The Tumor Necrosis Treatment (TNT) antibody was engineered to bind to intracellular antigens in degenerating cells. The antibody binds to necrotic regions of solid tumors all over the body.[3-5] Specifically, the liver expression chemokine (LEC)/chTNT-3 fusion protein, which binds to the necrotic region and chemoattracts T-cells, B-cells, and NK-cells to the tumor site, has proven to greatly reduce tumor load and metastatic spread of the tumor.[6] However, the intracellular consequences of LEC binding to and activating immune cells are unknown.

In this study, second messenger and receptor tyrosine

kinase pathway protein expression levels were analyzed by Western blot in THP-1-0 cells, a monocytic leukemia cell line. Protein levels were measured before and after LEC stimulation to determine the intracellular signaling molecules responsible for immune cell activation.

Protein kinase C (PKC) delta is a specific protein kinase in the Phospholipase C pathway, a very common signal transduction pathway. In previous LEC studies, blockers of this pathway have led to decreased chemotactic activity in response to LEC by immune cells.[7] A Western blot was performed to determine phosphorylated (phospho) and unphosphorylated PKC delta protein levels in monocytic leukemia cell lines exposed to LEC. A significant change in PKC delta protein levels was not seen, but there was a significant increase in phospho PKC delta protein concentration 18 hours after LEC stimulation (Figure 1). These results indicate that PKC delta plays a role in the LEC stimulation pathway.

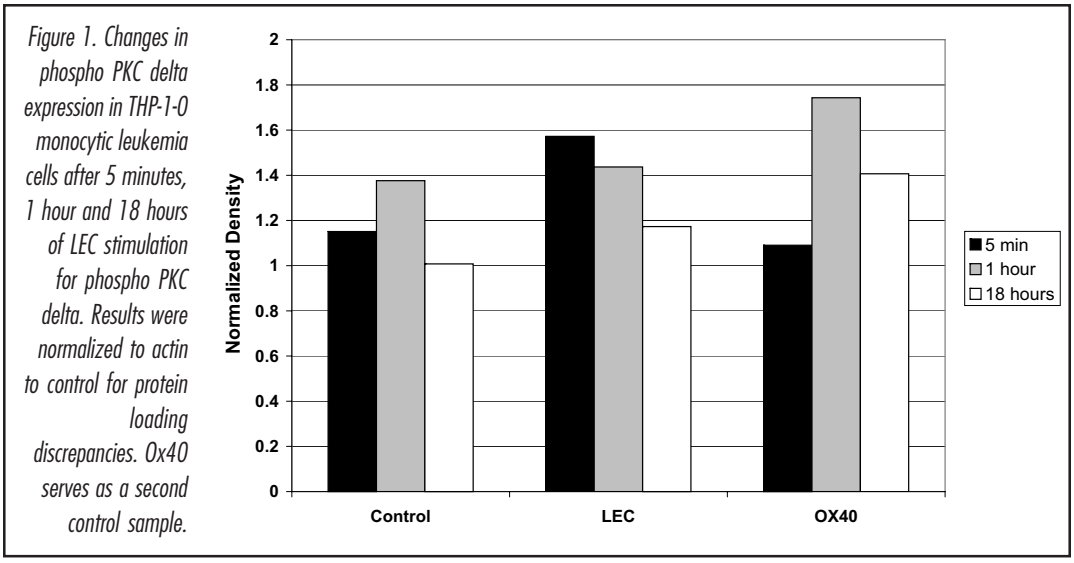

Figure 1. Changes in phospho PKC delta expression in THP-1-0 monocytic leukemia cells after 5 minutes, 1 hour and 18 hours of LEC stimulation for phospho PKC delta. Results were normalized to actin to control for protein loading discrepancies. Ox40 serves as a second control sample.

The JAK-STAT signaling pathway takes part in the regulation of cellular responses to cytokines and growth factors. The pathway transduces the signal carried by extracellular polypeptides to the cell nucleus, where activated STAT proteins modify gene expression. The pathway plays a central role in principal cell fate decisions, regulating the processes of cell proliferation, differentiation and apoptosis. It is particularly important in hematopoiesis. Because of its involvement in blood cell gene expression, we chose to look into its possible role in immune cell activation. There were no significant changes in STAT3, a protein-coding gene involved in the JAK-STAT pathway. There were also no significant changes in the catalytic subunit of protein kinase A (PKAc) involved in another G protein coupled receptor pathway.

Protein kinase G (PKG) is another protein kinase downstream to a G protein coupled receptor pathway. One hour after LEC stimulation, THP-1-0 cells showed a significant decrease in PKG protein levels compared to unstimulated cells (Figure 2). These results elucidate the intracellular signaling pathways involved in LEC-mediated cellular activation. In the future, these findings can be used to understand the mechanism of LEC-induced immune cell recruitment to fight cancer.

Another research project aims to selectively knock out T-regulatory cells from the rest of the T-cell population since T-regulatory cells limit the immune response against malignancy.[8,9] This Western blot technique could be used to investigate intracellular signaling pathways targeted to specifically destroy T-regulatory cells while keeping other T-cell populations intact.

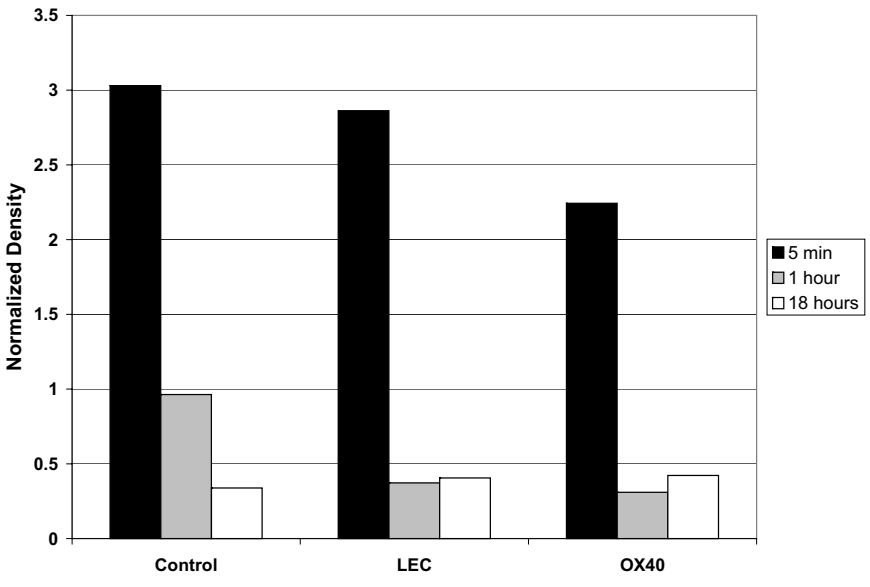

Figure 2. Changes in PKG expression in THP-1-0 monocytic leukemia cells after 5 minutes, 1 hour and 18 hours of LEC stimulation for PKG. Results were normalized to actin to control for protein loading discrepancies. Ox40 serves as a second control sample.

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Central Mechanisms for Auditory Fear-Conditioned Learning

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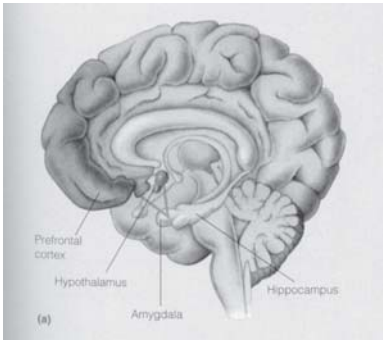
Georgetown University (for PhD work)
and the National Institutes of Health
Washington, DC

The physiological stress response in animals, including humans, is an adaptive mechanism to fight or flee from a threat. Observing this response to stress in the laboratory allows one to understand the sensory information and mechanisms associated with stress.



(Courtesy of Marieb E. Human Anatomy and Physiology. California: Benjamin/ Cummings; 1995.)

Long-term stress disturbs homeostasis and leads to a host of physiological and mental disorders and reduces the ability of the organism to cope with its environment. One of the major stress-activated neurotransmitters is neuropeptide Y (NPY), which appears to inhibit the stress response and improve memory in the brain.[1] The three goals of this project were to elucidate the neural mechanisms that enhance learning and memory under stress, identify the physiological limits at which these mechanisms fail and determine the role of NPY and its receptors in these processes.

Auditory fear conditioning provides a quantifiable paradigm for dissecting out the neural circuits and synaptic mechanisms that regulate memory and learning and can be modified by stress. The resultant physiological stress response can be quantified by measuring associated changes in activation of the following systems: sympathetic nervous (catecholamines), adrenal cortical (corticosterone) and cardiovascular (heart rate and blood pressure). To determine if these changes are related to activation of the NPY system in the brain, expression of the peptide and its receptors (Y1-Y5) were studied in different parts of the brain, including the cortex, amygdala, hippocampus and hypothalamus. It was predicted that NPY would play a significant part in modulating stress in neurons.

In order to understand NPY's role in modulating stress, mice were conditioned by pairing an unconditioned stimulus/stressor (mild electric foot shock) with a battery of complex auditory stimuli (harmonic

stacks of frequency modulated sweeps). This established a learning curve for categorical perception of the conditioned stimulus. Next, NPY or specific NPY receptor agonists were pressure injected into the lateral ventricles prior to conditioning to see direct behavioral changes to a stressor with increased NPY.

Although NPY's role in the physiological stress response has not yet been established, there is strong evidence suggesting a direct mechanism in regulating the cardiovascular system. Intracerebro-ventricular injection of NPY has been shown to lower an increase in heart rate associated with auditory fear conditioning.[2] It was also predicted that this effect would reduce the neuro-endocrine stress response, thereby extending the ability of the animal to recognize fine distinctions between categories of auditory stimuli. Further insight into NPY's role will be gathered by monitoring levels of stress by measuring plasma corticosterone levels and heart rate prior to testing and before and after fear conditioning and changes in NPY receptors in the relevant brain areas.

Ongoing research will test for any augmentation in the stimulus-evoked local field potentials (LFPs) in response to the conditioned stimuli within the amygdala and hippocampus to localize the sites of conditioned learning. Mapping experiments will determine stability (memory) of the representation of novel frequency modulation stimuli with repeated conditioning. The effects of iontophoretic administration of NPY or receptor agonists at the recording site on the spontaneous and stimulus-evoked responses will be studied as well as LFP responses at sites where augmentation is observed. These results will delineate the neural substrates and synaptic mechanisms involved in conditioned learning and the role of stress in either augmenting or reducing this effect. This knowledge will shed light on how to prevent the detrimental effects of stress by either pharmacological or behavioral interventions during a stressful experience rather than treating the physiological damage from stress afterward at a much greater cost.

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Erythropoietin's Dual Role May Be More Harmful Than Helpful in Cancer Patients

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Erythropoietin (EPO) is a hormone that regulates red blood cell production and is a powerful drug used to manage anemic patients. However, recent evidence suggests that EPO's use in treating anemic cancer patients may be more harmful than helpful.

EPO, also known by its trade names Procrit and Epogen, is manufactured by pharmaceutical giants Johnson & Johnson (J&J) and Amgen. It is one of the 10 most prescribed drugs in the United States, with nearly six billion dollars of combined sales in the past year. Clinical oncologists had high hopes of using EPO to treat cancer patients suffering from anemia, a common side effect resulting from the toxicity of radiation and chemotherapy on the vascular system.

> *Administration of pharmaceutical erythropoietin promoted cancer cell proliferation and invasion.*

Two clinical trials designed by J&J and Hoffman-La Roche aimed to test the safety and efficacy of EPO in cancer patients. These trials were prematurely terminated when researchers discovered high mortality rates and poor disease-free survival in the patient treatment groups that were receiving EPO.

Until now, few researchers understood the mechanism behind such findings. The laboratory of Ajay Verma, MD, PhD, at the Uniformed Services University of the Health Sciences, has focused its efforts on elucidating the mechanisms behind EPO's

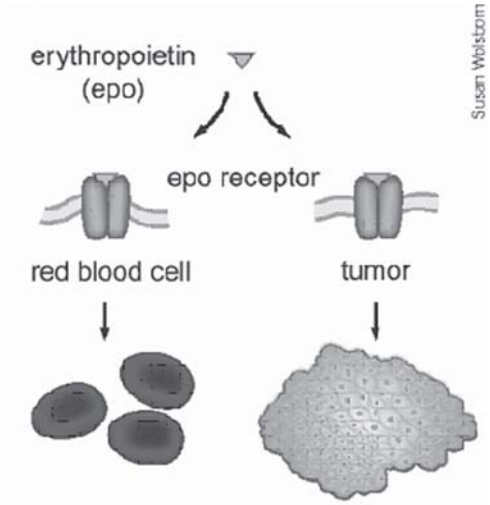

Figure 1. EPOR expression on red blood cell precursors and tumor cells. (Courtesy of Brower V. Erythropoietin may impair, not improve, cancer survival. Nature Medicine. *2003; 9(12): 1439.)*

role in cancer. Recent studies by Dr. Verma that will be published in the *Journal of Neurosurgery*[1] provide insight on the underlying mechanisms behind these clinical observations. By studying the roles of EPO and its receptor (EPOR) in brain cancer progression and invasion, this research may have determined how EPO mediates these deleterious consequences.

The hypothesis was built on several lines of research that demonstrated that EPO was a pleiotropic hormone with a broad spectrum of functions outside of the hematopoetic system. Several groups have demonstrated that the EPOR is found on tissues in addition to red blood cell precursors, including the brain, uterus and breast (Figure 1). Surprisingly, it is highly expressed on the surface of cancer cells derived from these tissues.[1-3]

Using several cancer cell lines derived from tissues utilizing EPO signaling, this research showed that the EPOR was highly upregulated in these cells compared to normal tissue. In fact, administration of pharmaceutical EPO promoted cancer cell proliferation and invasion. It was postulated that ectopic EPO and EPOR expression in tumors is used as an autocrine/paracrine mechanism for tumor proliferation and metastasis to escape several tumor stressors, such as local tumor hypoxia, that develops from an imbalance of tumor vascular supply to tumor volume ratio.

To empirically approach this hypothesis, a dominant negative EPOR that was missing the cytoplasmic domain for cell signaling was developed. Inhibition of EPO signaling in cancer cells reduced proliferation, invasion and *in vivo* tumor growth in mice. Tumor volumes of brain cancer cells with truncated-EPOR transplanted in nude mice were almost three to four times smaller than control cells (Figure 2).

This work provides insight into how EPO promotes cancer malignancy. Human cancers express EPO and functional EPORs. High expression of these proteins in several human cancers makes EPO signaling an attractive therapeutic target and underscores the importance of reevaluating the indiscriminate use of EPO in the treatment of anemia associated with cancer therapy.

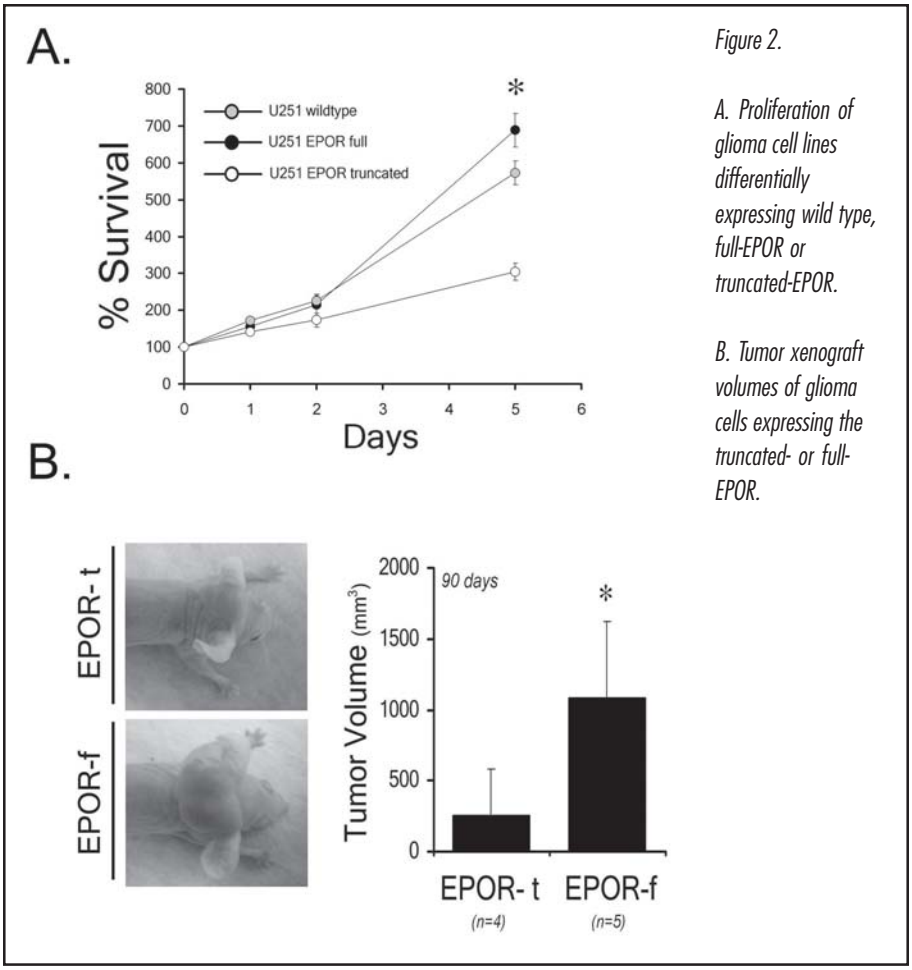

Figure 2.

A. Proliferation of glioma cell lines differentially expressing wild type, full-EPOR or truncated-EPOR.

B. Tumor xenograft volumes of glioma cells expressing the truncated- or full-EPOR.

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Effects of TGF-ß1 on Cell Differentiation and IGFBP-3 Expression in C2C12 Myocytes

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Children's National Medical Center
Washington, DC

Duchenne muscular dystrophy (DMD) is a degenerative muscle wasting disease caused by mutations in the dystrophin gene. DMD has a high incidence in all world populations, due to the high rate of new mutations in the dystrophin gene. DMD patients show myofiber membrane instability from birth. Muscle weakness usually begins around age four followed by a gradual replacement of skeletal muscle with fibrous and fatty tissue.[1]

Transforming growth factor beta (TGF-ß) is a family of proteins secreted by many cell types. These proteins regulate a variety of cellular processes and have been implicated in many disease processes, including DMD.[2] For example, with some vascular conditions, such as pulmonary hypertension, TGF-ß1 has been shown to stimulate excess synthesis of extracellular matrix proteins and inhibit matrix degradation.[3] Additionally, TGF-ß1 inhibits *in vitro* myoblast proliferation in some cultured muscle cell lines and primary myogenic cells.[4] It also suppresses proliferation and delays muscle regeneration *in vivo*.[4] It has been demonstrated that TGF-ß1 secretion by C2C12 myoblasts leads to downregulation of myogenic proteins such as MyoD and increased expression of fibrotic proteins.[5]

Insulin-like growth factor binding protein 3 (IGFBP3) has recently been shown to interact with the TGF-ß pathways. IGFBP3 has been shown to suppress proliferation of various cell types by blocking cell cycle and inducing apoptosis.[6,7] Besides their IGF-dependent mechanism of action, IGFBP3 may affect cells by IGF-independent mechanisms including binding to cell surface receptors, including the TGF-ß1 receptors.[7-9] Recent studies show that IGFBP3 is upregulated in mid-to-late stages of DMD. Currently, research is being performed to test the hypothesis that TGF-ß1 pathways lead to fibrosis and defects in muscle regeneration, which, in turn, leads to muscle wasting in later stages of DMD. Also, there is ongoing research to identify downstream targets of TGF-ß1.

In this research, the effects of TGF-ß1 on C2C12 myoblast phenotype and IGFBP3 expression were studied. This preliminary study stemmed from the hypothesis that the inhibition of myogenesis/regeneration of dystrophin-deficient muscle in later stages of DMD is mediated, at least in part, by IGFBP3.
This research used the mouse myoblast cell line C2C12. These cells have the ability to become skeletal muscle cells and are often used in experiments looking at myogenesis (skeletal muscle development) and cell differentiation. Bbecause they are cells that are not terminally differentiated, one can see how external factors affect the process of these less mature cells becoming more mature skeletal muscle cells.

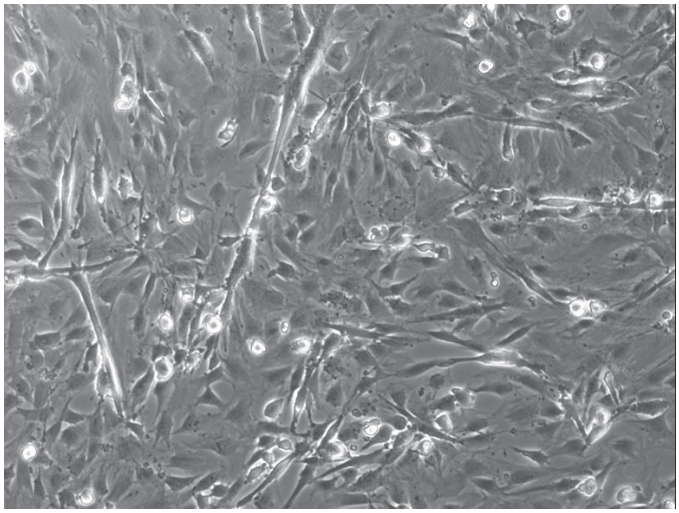
C2C12 cells were incubated in differentiation medium containing Dulbecco's Modified Eagle's Medium (DMEM), two percent fetal bovine serum and penicillin/streptomycin (1:100) to facilitate cell maturation with and without TGF-ß1 for 72 hours. Cell differentiation was inhibited in cells treated with higher TGF-ß1 concentrations compared to cells treated with lower TGF-ß1 concentrations or cells not treated with TGF-ß1 at all. It is likely that the exposure to high concentrations of TGF-ß1 led to the downregulation of myogenic proteins, such as MyoD, and the upregulation of fibrosis-related proteins, such as fibronectin. As a result, cultures with a high concentration of TGF-ß1 showed fewer myotubes (Figure 1).

In cultures with no TGF-ß1 and low concentrations of TGF-ß1, the transition from expression of myogenic proteins to fibrotic proteins does not likely occur to the extent as in cultures with high concentrations of TGF-ß1. Thus, in cultures where the frequency of myoblasts was high, expression of the

MyoD family of transcription factors was likely high, leading to myogenic differentiation by expression of various muscle specific genes.[10]

An increase in IGFBP3 expression due to TGF-ß1 was previously shown in various cells.[11-13] Levels of IGFBP3 and TGF-ß1 have been shown to be higher in patients in later stages of DMD, and it was hypothesized that the muscle wasting and increased fibrosis seen in later stages of DMD was the result of TGF-ß1 pathways upregulating IGFBP3. Our results, however, do not show a correlation between TGF-ß1 exposure and IGFBP3 expression in C2C12 myoblasts. These studies may need to be repeated with greater sample size to confirm the results.

While TGF-ß1 and IGFBP3 expression may not be correlated in mouse C2C12 myoblasts, it is possible that this relationship does exist in other species, including humans. Other cell lines, particularly primary cells, should be tested. Studying the effects of molecular pathways, such as the TGF-ß1 pathway, is important because it may eventually lead to a model for DMD pathogenesis and uncover potential targets of therapy for DMD and other muscle wasting disorders.



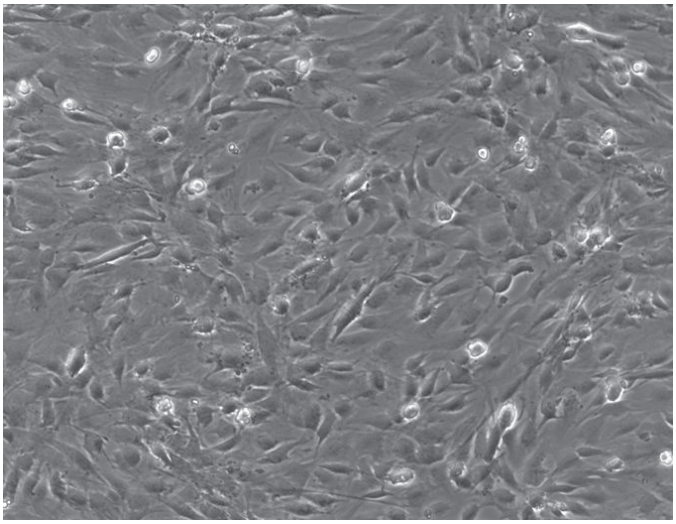


Figure 1. C2C12 cells in culture after 72 hours without TGF-ß1 (above) and with a high concentration of TGF-ß1 (below). Cells without TGF-ß1 show a high degree of myotube formation, whereas formation seems to be inhibited with a high concentration of TGF-ß1.

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X-ray Crystallographic Studies on the Binding Mechanism of a Potential Antimetastatic Molecule

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Advisors: Pradman K. Qasba, PhD, and Boopathy Ramakrishnan, PhD
National Cancer Institute
Frederick, MD

The difference could be in an atom or two. When reading the structural maps of proteins, a crystallographer tries to accurately determine the location of an amino acid building block, side chain or substrate molecule. Within the limits of technology, the crystallographer must carefully identify molecules, attempt to discover the order of nature and perhaps learn why a potential drug may be able to fight cancer.

Glycosylation is the "ornamenting" process of all cells. Tumor cells that display specific sugar "ornaments" are directly involved in the metastasis of certain lung and colon cancers. Preliminary results from a mapping study at the National Cancer Institute suggest that a unique disaccharide compound is capable of inhibiting glycosylation activity of 1,4-galactosyl-transferase.[1] Experimental studies performed by Brown et al have shown positive signs of the compound's antimetastatic potential.[2]

To see how the molecule fits into the substrate binding "pocket" of 1,4-galactosyltransferase, forming the crystal structure of the enzyme-substrate complex was vital. High-quality crystals were obtained with the human mutant enzyme, Met344His-Gal-T1,[3] bound to the acceptor substrate, GlcNAcß1,3Galß-O-naphthalenemethanol (the disaccharide moeity), after purifying the enzyme to about 99 percent homogeneity. Figure 1 shows crystals of the mutant galactosyltransferase in complex with the disaccharide obtained after 48 hours.

> *A crystallographer must carefully identify molecules, attempt to discover the order of nature and perhaps learn why a potential drug may be able to fight cancer.*

I began to learn the steps involved in protein crystallography as part of my summer internship in Dr. Pradman Qasba's lab at the National Cancer Institute in Frederick, MD, under Dr. Boopathy Ramakrishnan's mentorship.

I learned how to crystal-

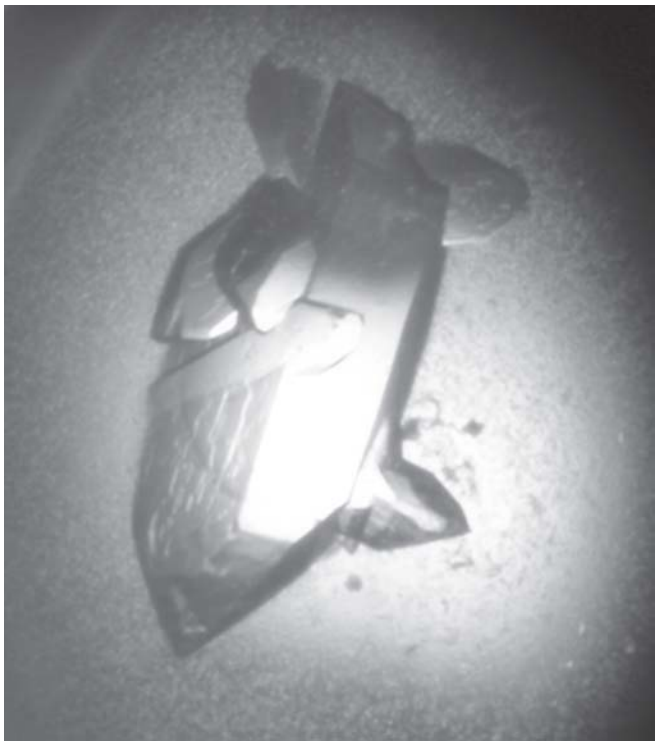

Figure 1. Crystals of the mutant galactosyltransferase in complex with the disaccharide obtained after 48 hours. (Courtesy of Najma Khorrami)

lize the enzyme, given a relatively complex recipe from Dr. Ramakrishnan. In one of my first attempts to crystallize the disaccharide substrate with the enzyme, I plated a set of 18 wells with microliter-sized protein droplets and waited for crystals to appear.

After waiting 48 hours, using the hanging drop vapor diffusion method (in which the basis for crystallization is a concentration gradient between the droplet and solvent within the same well), I noticed a large diamond-shaped crystal in a droplet under the microscope. While some parts of the crystallization process were tedious, others made the wait and the work seem near trivial.

Preliminary x-ray results showed that the disaccharide compound bound to the substrate site with equal or higher affinity than a natural trisaccharide molecule analyzed previously.[4] The results support the hypothesis that the disaccharide "decoy" is able to bind tightly to the molecule and divert glycosylation of natural substrates and ultimately inhibit tumor cells from binding to blood platelets, which enables the cancer to metastasize.

The normal glycosylation process is close to, but not exactly, what happens when the "decoy" is in place. Here, a dangling naphthalene molecule, which is bound to the disaccharide, conforms to the substrate binding site similarly to the third sugar molecule in the natural trisaccharide. Eventually, "cloaking" occurs with cells missing the natural trisaccharide, triggering hematogenous metastasis.

Jillian R. Brown, PhD, of the University of California will continue experimental studies using the "decoy" disaccharide compound. Her clinical-focused research on the molecule's ability to target colon and lung cancer cell metastasis, both *in vitro* and *in vivo*, has lasted close to five years. She understands the important benefits of a potential antimetastatic drug if it entered the market.

"Nothing clinically useful is available for the treatment of metastasis," says Dr. Brown. "At the molecular level, there are lots of hypotheses but little is known (mechanistically)." The studies conducted in the laboratory of Jeffrey Esko, PhD at the University of California, have examined the disaccharide's ability to inhibit metastasis in rats by limiting the ability of tumor cells to proliferate in the body by binding to blood platelets via signature sugar chains on cell membranes. "The idea of compounds acting as 'primers' by decoying the synthesis of endogenous glycoconjugates has been ongoing in our lab for many years. The first identification was in an experiment with U937, a human lymphoma cell line," says Dr. Brown. "Now, the disaccharide is being developed further and will enter preclinical trials as an antimetastatic agent."

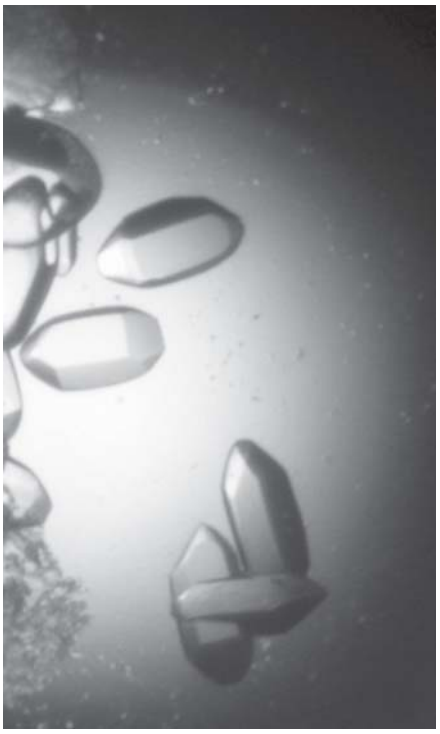

Figure 2. Crystals of the mutant galactosyltransferase in complex with the disaccharide were obtained after 48 hours (Courtesy of Najma Khorrami)

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Planning Ahead ...

Fusion **is the annual publication of the GW chapter of The Beaumont Society to showcase student achievements in the fields of biomedical research, public health research, clinical research and international health related travel experiences.**

Submissions for next year's issue of the journal will be accepted beginning September 2007 from the Class of 2010 as well as the incoming Class of 2011. More information about the submission process will be provided during the summer of 2007. If students have any questions or comments, contact the Beaumont Society at gwbeaumont@gmail.com.

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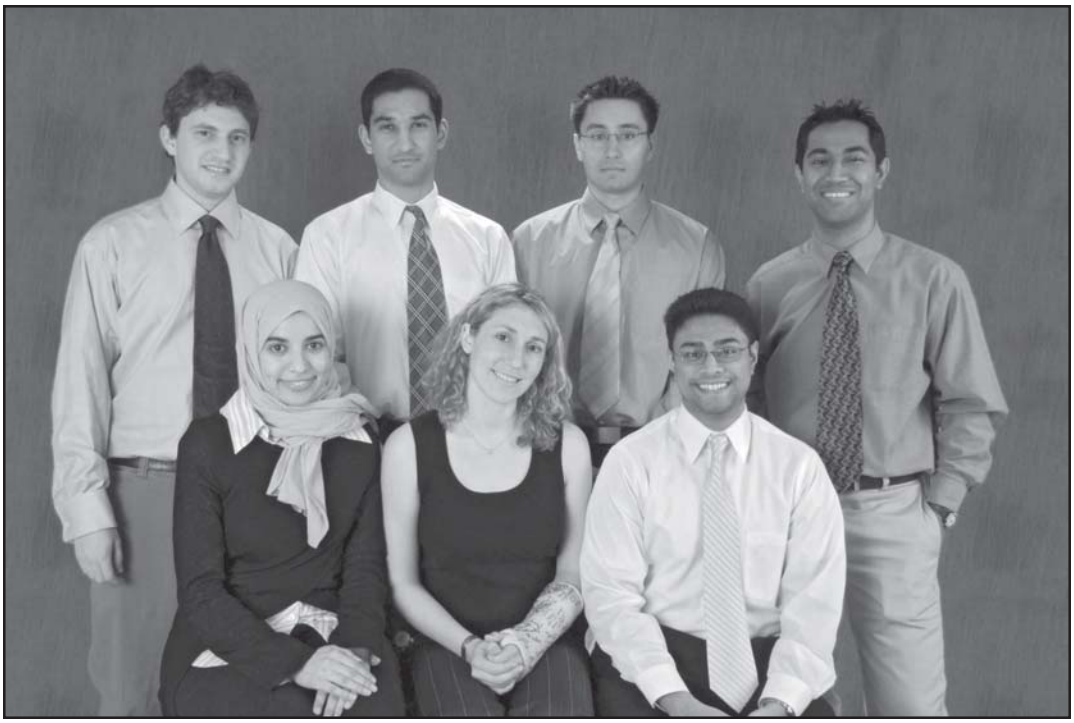

Beaumont Leaders

2006-2007 officers of the William H. Beaumont Society at The George Washington University include, top row, from left: Andrew Lerner, president; Vivek Patil, co-vice president; Steven Kardos, secretary; and Rahul Arya, research journal chair; seated, from left: Rasha Al-Bawardi, co-vice president; Jennifer Goldstein, research day coordinator; and Ajay Wadgaonkar, MSI student representative.

The William H. Beaumont Society is an honorary research society of medical students that was established in 1935 in honor of Dr. William Beaumont, a pioneer in physiology research. The organization seeks to foster a continuing interest in biomedical research and to promote its value in the practice of medicine. As a part of this mission, the Society integrates current research topics with the curriculum, develops a research journal showcasing GW student research, makes available information on research opportunities and seminars throughout the area, including the William T. Gill Summer Fellowship for GW medical students, and highlights student and faculty research accomplishments at the annual GW Research Day.

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