Message from the Dean

Christopher J. Cooper, M.D.
Senior Vice President for Clinical Affairs
Dean of the College of Medicine and Life Sciences

First, let me congratulate Dr. Takashima and his staff on the excellent progress they have made in supporting clinical research at the University of Toledo. As many of you know, clinical research has been a critical part of my own career and is something that I have participated in since my arrival at the Medical College of Ohio in 1994. There was a time when it became increasingly difficult for our clinical researchers. With Dr. Takashima’s leadership and his staff, things have gotten substantially better.

I firmly believe in research as one of the core missions of our University. Certainly, it is the mechanism by which we advance knowledge and have the ability to impact people, not only in Toledo, but across the globe. Equally so, I appreciate the intimate relationship between clinical research and basic research. Simply put, many ideas arise from the bench and many ideas that arise from the patient care can only be tested in translational models.

As a consequence, a focus I have identified with President Naganathan for the Health Science Campus is to expand funded research. It is my belief that this is done best in productive groups working together. I look forward to working with the Department Chairs, faculty and staff on our research enterprise.

My New Grant

Risk stratification for sensitized patients in kidney paired donation program

Stanislaw Stepkowski DVM, PhD, DSc
Professor, Department of Medical Microbiology and Immunology

More than 600,000 patients in the United States suffer from end-stage renal diseases (ESRD), and 66% of these patients have such poor kidney function that they require weekly dialysis procedures in order to remove waste and excess water from their blood. We have long known that the best treatment for ESRD is kidney transplantation. However, to receive a donor kidney there is a growing waiting list of more than 100,000 patients. Kidneys for transplantation can be received either from deceased brain-dead donors (in which both kidneys can be donated) or from a willing, living donor (who can donate one kidney). In the United States, the annual number of kidney transplants resulting from deceased organ donation has remained relatively constant (approximately 11,000 transplants) over the last decade. The annual number of
kidney transplants from willing, living donors is approximately 5,600. Recent research in organ donation suggests this number could be increased by up to 50%, since each year 3,000 patients and their willing, living donors cannot proceed with the transplantation because of a blood group incompatibility (50%) or because the patient is already sensitized to the donor (50%). To accommodate these incompatible pairs, a program called Kidney Paired Donation (KPD) finds matches among the pairs and arranges exchanges of donors. Unfortunately, while the rules for finding matches between blood group incompatible pairs are clear and easy to follow, finding matching donors for sensitized patients is significantly more complicated.

Sensitization occurs when the patient is exposed to cells that have different histocompatibility leukocyte antigens (HLAs) than their own cells. The patient’s immune system is then pre-conditioned to more quickly recognize and attack cells that harbor those particular antigens. Sensitization can occur from previous transplants, blood transfusions, or pregnancies. Therefore, a matching donor for a sensitized patient needs to have cells that either express the same HLAs as the patient’s or express HLAs to which the patient has not previously been exposed. The current methods for the evaluation and matching of HLAs in sensitized patients and donors are outdated and incomplete, requiring significant revisions. My lab was recently awarded a government R21 grant to address this disparity between donors and recipients and, ultimately, increase the number of living donor donations.

My grant primarily addresses the method of identifying HLA types of donors and recipients. The vast majority of transplant centers use methods that identify HLA antigens with less precision, termed low-resolution (2-digit) typing. In addition, many transplant centers follow traditional guidelines in which they analyze the most highly expressed HLA-A, -B, and -DR antigens but do not analyze the HLA-C, -DQ, or –DP antigens. These methods are at odds with the current method of determining patient’s antibody (Ab) sensitivities, which determines reactive HLA antigens with high precision (high resolution, or 4-digits) among all HLA antigens. Having a list of Ab-reactive HLA antigens for each sensitized patient would allow the elimination of potential donors who have these HLAs and, therefore, result in a safer selection of donors to whom the patient is not sensitized. However, the benefits of having precisely defined reactive Abs are lost when donor-typing is imprecise or undefined. The first goal of this project is to develop and validate a method to identify HLA-A, -B, -C, -DR, -DQ and –DP antigens with high resolution, based on the advanced technology of Next Generation Sequencing (NGS). This method is almost 10 times less expensive than the currently used Sanger sequencing method and could thereby reduce or eliminate the financial barrier currently preventing transplant centers from performing these analyses. While NGS for the purposes of HLA typing is being developed and performed by other groups, my lab has collaborated with Dr. James Willey’s lab in the George Isaac Cancer Research Laboratory within the Department of Medicine at the University of Toledo College of Medicine to develop a standardized NGS method which is capable of distinguishing potential failures, a feature unavailable in any other HLA typing facility.

Once high resolution HLA typing of donors and recipients has been established, a more detailed matching algorithm called a Virtual Crossmatch (VXM) is used to predict the best transplant matches. Although the algorithm may indicate the patient would not react against a particular donor kidney, the transplant is not done unless there are negative results with a final flow crossmatch (FXM). Correct negative VXM results should correlate with negative FXM results. However, our recent analysis suggested as many as 25% of VXM may fail to detect dangerous donor-specific Abs (DSA) in sensitized patients. Therefore, the second portion of the awarded grant is designed to better evaluate sensitization of patients, thereby reducing false negative VXM results.

Current VXM algorithms rely primarily on HLA typing. The best choices are donors with matching HLA types followed by donors with HLA types to which the patient is not sensitized. However, this paradigm treats all mismatched HLA types equally. Our grant aims to improve the HLA matching algorithm by performing each analysis at the molecular level. Because all mismatched HLA antigens are not created equal, the modified matching algorithm takes into account epitope mismatches and provides a stratified list of potential donors. Epitope matching is performed by the HLAMatchmaker program (http://www.HLAMatchmaker.net) which is designed to account for Ab reactivities by listing dangerous epitopes (with documented reactive Abs) and excluding self-epitopes or non-immunogenic epitopes (called “acceptable mismatches”). Following epitope analysis, the HLAMatchmaker program provides a list of specifically determined unacceptable HLA antigens that contain dangerous epitopes. In fact, careful analysis of epitope reactivities can occasionally prevent donors from being erroneously excluded based on low resolution serological typing, in which donors are assigned 2-digit HLA types based on a single shared epitope. The resulting increased selection of donors for sensitized patients is predicted to reduce the waiting time for a transplant. Although the infrastructure for these analyses is already present in the HLAMatchmaker program, it can only be fully exploited in combination with a cost-effective 4-digit HLA typing method.

Overall, our R21 grant will provide a fully validated NGS method for 4-digit HLA typing at a fraction of the current cost. We will revise the subsequent matching algorithms to better account for the Abs causing transplant failure, with a goal of improving the VXM accuracy and reliability. Most importantly, patient and donor 4-digit HLA typing and epitope analysis is expected to allow for the most effective selection of donors for sensitized patients both in KPD programs and through the traditional deceased donor system. In collaboration with Dr. Michael Rees from the Department of Urology at the University of Toledo College of
Medicine, the new NGS method and advanced analysis of epitope-based matching will be introduced into the KPD computer program to improve donor/recipient selection. We predict that these changes will revolutionize the selection of donors for sensitized patients and therefore significantly increase the number of performed kidney transplants in kidney paired donation program.

Investigators involved in the project (from left to right): Dr. Michael Rees, Dr. James Willey, Dr. Stanislaw Stepkowski, Caitlin Baum (MD/PhD student), Erin Crawford (senior research assistant) and Dr. Thomas Blomquist.

The George Isaac Cancer Research Laboratory

James C. Willey, M.D.
Professor of Medicine and Pathology
George Isaac Professor for Cancer Research

It is likely that everyone in Northwest Ohio remembers a friend or loved one that died from lung cancer. This is expected because lung cancer is the leading cancer cause of death in the United States, annually killing more than breast, colon, and prostate combined. Moreover, Northwest Ohio has one of the highest lung cancer incidence rates in the nation. The reason lung cancer is so deadly is that it tends not to cause symptoms until it has become too advanced to be cured through surgery. Fortunately, recent positive trends in public health and advances in lung cancer diagnosis and treatment are likely to significantly reduce the incidence and increase the survivability of lung cancer in the coming years. The research group that I supervise is playing a significant role in implementing effective lung cancer screening methods and smoking cessation programs, and in developing more accurate diagnostic tests and molecular diagnostic testing methods. These efforts currently are funded by the National Heart Lung and Blood Institute (NHLBI, HL108016) and the George Isaac Cancer Research Fund and have been funded in recent years by the National Cancer Institute (e.g. CA148572).

Results from the 10 year National Lung Screening Trial were reported in 2011 with the conclusion that annual chest CT screening detects lung cancer at a significantly earlier stage, and reduces mortality by at least 20%. Following this report, our laboratory, working with the Department of Radiology, started a University of Toledo Lung Cancer Screening Program in 2012 as one of the International Early Lung Cancer Action Program (I-ELCAP) sites. In 2014, the United States Preventive Services Task Force (USPSTF) determined on the basis of results from the 10 year National Lung Screening Trial that annual chest CT screening detects lung cancer at a significantly earlier stage, and reduces mortality by at least 20%. Following the USPSTF decision, the University of Toledo screening program has been available to the community as standard of care. This program enrolls subjects at high risk for lung cancer based on age (55-80 years) and heavy smoking history. Interested individuals may inquire regarding their eligibility by calling 419 383-3927, and may visit the website at [http://utmc.utoledo.edu/news/lung-cancer-screening.html](http://utmc.utoledo.edu/news/lung-cancer-screening.html). Subjects who participate in the screening program receive a low dose chest CT annually. A Radiologist on the screening team reads the CT scan immediately and reviews it with a Pulmonologist on the team, who then discusses the findings with the patient, including recommendations for follow-up of any abnormal findings. If there were no abnormal findings, which is the case for most subjects, they are told to return in one year for the next screening CT. Another important feature of the screening program is counseling of each patient who continues to smoke by a smoking cessation counselor. Typically, the CT scan, Radiologist review, interview with Pulmonologist, and counseling from smoking cessation expert takes less than one hour.

While annual screening with chest CT is effective and now standard of care, there is definitely room for improvement. Key challenges include high cost for screening across the nation (estimated to be $10 billion/year) and a large number of false positive findings. Thus, there is great interest in the efforts of this laboratory to develop an accurate test for lung cancer risk so that those at highest risk can be prioritized for screening and those at lowest risk may be advised with confidence not to undergo annual CT screening.
Toward this goal, this laboratory developed a 14 gene test for lung cancer risk (http://cancerres.aacrjournals.org/content/69/22/8629.full.pdf+html) and recently completed enrollment of 385 subjects into a prospective, multi-site trial of this test. Erin Crawford, M.S. our laboratory manager has played a key part in the development of this test and in organizing the clinical trial. It is expected that results from the study will be available in early 2016. In the meantime, this laboratory is engaged in understanding the inherited genetic variation responsible for inter-individual variation in regulation of the key genes comprised by the risk test. Xiaolu Zhang, a graduate student working toward her Ph.D, is doing key work in this area. Ms. Zhang has generated evidence supporting the hypothesis that DNA variation altering expression of key DNA repair genes contributes lung cancer risk. She is building on studies by Dr. Blomquist and Ms. Crawford (http://carcin.oxfordjournals.org/content/28/12/2552.full.pdf+html, http://carcin.oxfordjournals.org/content/31/7/1242.full.pdf+html, http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3712557/pdf/qrsb-7-2013-125.pdf).

In addition to diagnosing lung cancer at early stage through screening, it is important to develop lung cancer diagnostic tests that accurately predict to which chemotherapeutic a particular lung cancer is most likely to respond. A post-doctoral fellow in the laboratory, Ji Yeo, Ph.D. is making important advances on this problem. Dr. Yeo, while a graduate student in our laboratory, developed a more accurate method for diagnosis by real-time PCR, a commonly used instrument in clinical diagnostic laboratories (http://www.plosone.org/article/info%3Adoi%2F10.1371%2Fjournal.pone.0089395, http://www.plosone.org/article/info%3Adoi%2F10.1371%2Fjournal.pone.0071851). The advances in PCR diagnostics by this laboratory were noted in both Nature Biotechnology (http://www.nature.com/nmeth/journal/v10/n5/full/nmeth.2443.html) and PCR News (http://www.genomeweb.com/pcrsample-prep/toledo-group-publishes-method-multiplex-rt-qpcr-lung-cancer-dx-ffpe). Dr. Yeo also contributed to development of a reliable method for diagnostic testing based on next generation sequencing. The key to this method is generation of targeted multiplex competitive PCR amplicon libraries which were developed by Tom Blomquist, M.D/Ph.D, who was a Post-Doctoral fellow in the laboratory, and is now a second year Pathology Resident. This method was published in 2013 (http://www.plosone.org/article/info%3Adoi%2F10.1371%2Fjournal.pone.0079120) and now is used by this laboratory as well as other laboratories at UTMC. The technology is patent pending and licensed to Accugenomics, Inc.

A new MD/Ph.D graduate student in the laboratory, Ben Chaney is implementing advanced methods for growing normal human bronchial epithelial cells in the incubator. The Conditionally Re-programmed Cell culture method enables long term culture of normal cells for mechanistic studies, while maintaining normal genetic code. Mr. Chaney and Ms. Zhang will use these methods to investigate specific mechanisms by which DNA variants alter regulation of key DNA repair and antioxidant genes in airway epithelium. Such knowledge is key to understanding why some individuals are more susceptible to development of lung cancer or chronic obstructive pulmonary disease (COPD). These studies are supported by grant HL108016 from NHLBI.

From left to right: Ben Chaney, M.D./Ph.D graduate student, Xiaolu Zhang, Ph.D. graduate student, Ji Yeo, Post-doctoral fellow, Diego Morales, M.S. in Bioinformatics graduate student, Dan Craig, M.S. in Medical Sciences graduate student, James C. Willey, Director of George Isaac Cancer Research Laboratory, Erin Crawford, M.S., Laboratory Manager

RSP Corner

NIH Resubmission Policy and Practice
Rick Francis, Ph.D., C.R.A.

Many investigators are well aware that earlier this year the NIH changed its policy on resubmission applications. NOT-OD-14-074, released April 2014, was fairly clear:

...for application due dates after April 16, 2014, following an unsuccessful resubmission (A1) application, applicants may submit the same idea as a new (A0) application for the next appropriate due date. The NIH and AHRQ will not assess the similarity of the science in the new (A0) application to any previously reviewed submission when accepting an application for review. Although a new (A0) application does not allow an introduction or responses to the previous reviews, the NIH and AHRQ encourage applicants to refine and strengthen all application submissions.

Additional information may be found here: http://grants.nih.gov/grants/guide/notice-files/NOT-OD-14-074.html

Several months later, the research community has learned more about how NIH is implementing this policy, occasionally through unpleasant surprises. Some of these may be avoided by paying close attention to the FAQs, updated this month (http://grants.nih.gov/grants/policy/resubmission_q&a.htm), but here is a key point:

1. **What distinguishes a new application from a resubmission application?**

   A resubmission application must contain an Introduction, which addresses the comments from the previous review and often changes [sic] marked in the text; *a new application makes no reference to a previous submission.* (emphasis added)

   “New” applications do not have an introduction to address reviewer concerns, but that last point is being enforced by NIH such that proposals that merely mention changes based on comments have been withdrawn (!). Applicants should avoid mentioning scores or even enthusiasm expressed by previous reviewers. Be extra vigilant! The proposal you save may be your own.

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