

Akin to the bone marrow in mammals, the zebrafish kidney marrow is responsible for the production of new blood cells. Research described in this chapter provides evidence that a cluster of cells called melanocytes form a shield to protect blood stem cells in the zebrafish kidney from damaging UV light.

**Top illustration:** The immature zebrafish.

**Bottom left panel:** White arrows point to melanocytes (black spots) that form a cluster that obscures the blood stem cells (red spots) from visualization.

**Bottom right panel:** Close-up (higher magnification) of the boxed area shown in left panel.

*Image courtesy of Leonard Zon, M.D., Department of Stem Cell and Regenerative Biology and Harvard Stem Cell Institute, Harvard University. Kapp FG, Perlin JR, Hagedorn EJ, ...Zon LI. Protection from UV light is an evolutionarily conserved feature of the haematopoietic niche. [Nature](#) 558: 445-448, doi: 10.1038/s41586-018-0213-0, 2018. Reprinted with permission from Springer Nature, copyright 2018.*

# Kidney, Urologic, and Hematologic Diseases

*Diseases of the kidneys, urologic system, and blood are among the most critical health problems in the United States. They affect millions of Americans, and their impact is felt across the lifespan. To improve our understanding of the causes of these diseases, and to identify potential new treatments for them, the NIDDK supports basic and clinical research studies of the kidney and urinary tract and of the blood and blood-forming organs. The overall goal of the NIDDK's research programs is to increase our understanding of kidney, urologic, and hematologic (blood) diseases in order to enhance approaches to prevent and treat these serious conditions.*

Normal, healthy kidneys filter about 200 quarts of blood each day, generating about 2 quarts of excess fluid, salts, and waste products that are excreted as urine. Loss of function of these organs, either for a short period of time or as a consequence of a gradual, long-term decline in kidney function, is a life-threatening condition.

It has been estimated that more than 30 million American adults have impaired kidney function—also called chronic kidney disease (CKD).<sup>1</sup> CKD has two main causes: high blood pressure and diabetes. The increases in obesity and type 2 diabetes in the United States in recent years—especially among children and adolescents—have grave implications for the Nation's health, as young people with these conditions are likely to face serious health complications at an earlier age than people who historically have developed these conditions later in life.

One feature common to kidney diseases arising from varying causes is the deposition of fibrotic scar tissue in the kidney. Research supported by the NIDDK has enhanced our understanding of the origin of this scar tissue, how it can impair kidney function, and how it might be prevented or treated. CKD, especially if undetected, can progress to irreversible kidney failure, a condition known as end-stage renal disease (ESRD). People with ESRD require dialysis or a kidney transplant to live. In 2015, over 701,000 patients received treatment for ESRD: over 493,000 received either hemodialysis or peritoneal dialysis, and

over 207,000 were living with a kidney transplant.<sup>2</sup> Racial and ethnic minority populations in the United States, particularly African Americans, Hispanic and Latino Americans, and American Indians and Alaska Natives, bear a disproportionate burden of CKD and ESRD. Compared to non-Hispanic Whites, ESRD prevalence in 2015 was about 3 times greater in African Americans, 1.3 times greater in Hispanics, 1.2 times greater in American Indians and Alaska Natives, and 1.0 times greater in Asians.<sup>2</sup> In recent years, scientists supported by the NIDDK have uncovered important genetic clues that may play a role in some of the health disparities related to kidney disease susceptibility and progression in minority populations.

The Institute supports a significant body of research aimed at understanding the biology underlying CKD and developing treatment strategies. The NIDDK's chronic renal diseases program supports basic and clinical research on kidney development and disease, including the causes of kidney disease,

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<sup>1</sup> Centers for Disease Control and Prevention. *National Chronic Kidney Disease Fact Sheet, 2017*. Atlanta, Georgia: U.S. Department of Health and Human Services, 2017.

<sup>2</sup> U.S. Renal Data System, *USRDS 2017 Annual Data Report: Epidemiology of Kidney Disease in the United States*, National Institutes of Health, National Institute of Diabetes and Digestive and Kidney Diseases, Bethesda, Maryland, 2017.

the underlying mechanisms leading to progression of kidney disease to ESRD, and the identification and testing of possible strategies to prevent development or halt progression of kidney disease. The NIDDK also supports studies of inherited diseases, such as polycystic kidney disease, congenital kidney disorders, and focal segmental glomerulosclerosis; and immune-related kidney diseases, such as IgA nephropathy and hemolytic uremic syndrome. The CKD Biomarkers Consortium (CKD BioCon) promotes the discovery and validation of novel biomarkers for CKD initiation, progression, and development of complications. A more complete understanding of biomarkers could allow physicians to detect kidney disease earlier and perhaps identify people at greater risk of progression, allowing them to tailor treatments to a specific individual. The Kidney Precision Medicine Project aims to obtain and evaluate human kidney biopsies from participants with acute kidney injury (AKI) or CKD for the purpose of creating a kidney tissue atlas, defining disease subgroups, and identifying critical cells, pathways, and targets for novel therapies.

The NIDDK's National Kidney Disease Education Program (NKDEP) is designed to raise awareness about the problem of kidney disease and steps that should be taken to treat CKD and prevent kidney failure. NKDEP represents a major educational outreach effort to patients, physicians, and the public. NKDEP also promotes the inclusion of estimates of kidney function as a part of routine blood testing and seeks to standardize measurements of protein in the urine, often a sign of underlying kidney disease.

Urologic diseases affect people of all ages, result in significant health care expenditures, and may lead to substantial disability and impaired quality of life. The NIDDK's urology research program supports basic and clinical research on the normal and abnormal development, structure, function, and injury repair of the genitourinary tract. Areas of interest include the causes of and treatments for urologic diseases and disorders such as benign prostatic hyperplasia, urinary incontinence, urinary tract infections, and urinary stone disease. To spur research in urinary stone disease, the Urinary Stone Disease Research Network (USDRN) is: a) conducting a randomized clinical trial to investigate the impact of increased fluid intake and increased urine output on the rate of recurrence of urinary stones in adults and children; b) conducting clinical research to understand and mitigate ureteral stent-related pain and symptoms; and c) providing

data and collecting biological samples from the studies to create a resource for future researchers.

Other disorders of the genitourinary tract, such as interstitial cystitis/bladder pain syndrome (IC/BPS)—also known as IC/painful bladder syndrome (PBS)—in women and men and chronic prostatitis/chronic pelvic pain syndrome (CP/CPPS) in men, are also important research topics of the NIDDK's urology program.

IC/BPS is a debilitating, chronic, and painful urologic disorder. Based on a recent large, national interview survey, it is estimated that among U.S. women 18 years old or older, 3.3 million (2.7 percent) have pelvic pain and other symptoms, such as urinary urgency or frequency, that are associated with IC/BPS.<sup>3</sup> Using a community-based epidemiologic survey, researchers have estimated that among U.S. men ages 30 to 79 years old, 1.6 million (1.3 percent) have persistent urologic symptoms, such as pain with bladder filling and/or pain relieved by bladder emptying, that are associated with BPS.<sup>4</sup>

NIDDK-supported basic and clinical research on IC/BPS and on CP/CPPS is focused on elucidating the causes of these conditions, identifying important subsets of patients to aid diagnostic stratification, and improving treatment and interventions. One example of an ongoing study is the Multidisciplinary Approach to the Study of Chronic Pelvic Pain (MAPP) Research Network, which supports research designed to uncover the underlying causes of IC/BPS and CP/CPPS and to characterize the disease profiles in patients.

Based upon national public health surveys conducted over several years, it is estimated that 1 in 10 U.S. adults (18 years of age and older) suffer from daily urinary incontinence; most of those affected are women.<sup>5</sup> Many suffer in silence due to embarrassment and lack of knowledge about treatment options available. NIDDK-supported studies over the past several years have helped to advance knowledge about the efficacy of surgical treatment of urinary incontinence, as well as to

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<sup>3</sup>Berry SH, et al. *J Urol* 186: 540-544, 2011.

<sup>4</sup>Link CL, et al. *J Urol* 180: 599-606, 2008.

<sup>5</sup>*Urologic Diseases in America*. NIDDK, NIH Publication Number 12-7865, 2012.

provide new insights into non-surgical alternatives. As researchers continue to investigate treatment options, an equally important challenge is to identify and understand the important subgroups of patients with lower urinary tract symptoms (LUTS) through improved measurement of patient experiences of LUTS in men and women. To address this challenge, the NIDDK supports the multi-site Symptoms of Lower Urinary Tract Dysfunction Research Network (LURN). The NIDDK is also leading new efforts to explore whether it may be possible to prevent symptom onset and/or progression, thereby improving health. The NIDDK, in conjunction with the National Institute on Aging and the NIH Office of Research on Women's Health and Office of Behavioral and Social Sciences Research, established the Prevention of Lower Urinary Tract Symptoms (PLUS) Research Consortium to develop the evidence base for normal or healthy bladder function and to identify behavioral and other risk factors for conditions associated with lower urinary tract symptoms in women.

The NIDDK's hematology research program uses a broad approach to enhance understanding of the normal and abnormal function of blood cells and the blood-forming system. Research efforts include studies of a number of blood diseases, including sickle cell disease, the thalassemias, aplastic anemia, iron deficiency anemia, hemolytic anemias, thrombocytopenia, and the anemia of inflammation and of chronic diseases. To promote high-impact basic or pre-clinical research, the Institute supports the Stimulating Hematology Investigation: New Endeavors (SHINE) program and includes the following current research topic areas: regulation of blood (hematopoietic) stem cells, factors that play a role in the development of different types of blood cells, and red blood cell maturation. The Institute's SHINE II program seeks to further catalyze research in basic or pre-clinical, proof of principle research projects that are tightly focused and directed at validating novel concepts and approaches that promise to open up new pathways for discovery in benign hematology research. The NIDDK is also keenly interested in the basic biology of adult hematopoietic stem cells, which are used clinically in bone marrow transplants and may have broader application in gene therapy research.

## KIDNEY FORMATION AND FUNCTION IN HEALTH AND DISEASE

**Kidney Protein Could Expand the Window for Developmental Nephron Production:** A study in mice showed that a partial reduction of the protein hamartin in the developing kidney leads to larger numbers of nephrons. Nephrons—the basic functional unit of the kidney—consist of various cells and structures that work together to filter waste products and excess fluid from the blood. The final number of nephrons in the kidney is widely variable, but research has shown that higher numbers of nephrons correlate with improved kidney function, and that nephron loss occurs with aging. While this variability is not completely understood, several factors, such as premature birth, lead to low nephron numbers. In mammals, the production of nephrons in the kidney begins during prenatal development and ends before birth (e.g., in humans) or shortly thereafter (e.g., in mice), at which point the body cannot generate additional nephrons. Therefore, understanding the mechanisms that govern nephron generation during early development has the potential to inform strategies to increase nephron numbers in those at risk, thereby reducing the likelihood of kidney disease later in life.

Previous research demonstrated that a pool of kidney stem cells, called nephron progenitor cells (NPCs), can turn into nephrons when signaled to do so at just the right time during kidney development. A team of scientists discovered that a protein called Mtor seemed to be one of these important signals, and thus used genetic mouse models to investigate the role of Mtor—as well as its functional inhibitor, hamartin—in shaping the number of nephrons in the kidney. Mice engineered to completely lack either Mtor or hamartin in their NPCs did not survive beyond 2 days after birth because they were unable to develop functional kidneys. However, mice engineered to lack just one of the two gene copies encoding Mtor (effectively reducing Mtor levels by half) in NPCs survived but had significantly lower numbers of nephrons and smaller kidneys than their control counterparts. Conversely, genetic deletion of one copy of the gene encoding hamartin led to a greater number of nephrons than in control mice. Further analysis of the mice with a partial reduction in hamartin revealed that the developmental “window” for generating nephrons in the kidney extended by about 1 extra day, resulting

in the significant increase in nephron number that was observed. Surprisingly, by combining these modified genetic backgrounds in mice, the scientists determined that the higher nephron number observed in mice with reduced hamartin was independent of the Mtor pathway. These findings define hamartin as part of an important pathway in mice that determines nephron number by regulating the window of time in which nephrons can form. Additionally, hamartin is coded by the gene *Tsc1*, which is involved in the development of a rare, multi-system genetic disease called tuberous sclerosis complex that causes benign tumors to grow in the kidney and other organs. Thus, future research building on these findings may shed light on cellular pathways in tuberous sclerosis complex, and, if the connection between numbers of nephrons and hamartin is conserved in humans, the hamartin signaling pathway could represent an important therapeutic target for people with or at risk for kidney disease.

*Volovelsky O, Nguyen T, Jarmas AE, ...Kopan R. Hamartin regulates cessation of mouse nephrogenesis independently of Mtor. Proc Natl Acad Sci USA 115: 5998-6003, doi: 10.1073/pnas.1712955115, 2018.*

**Identifying Patients with Chronic Kidney Disease Who Are at Increased Risk of Death:** A recent study has established a correlation between the level of fibroblast growth factor-23 (FGF-23) in the blood of a person with chronic kidney disease (CKD), measured over time, and the risk of death. This research could alert physicians to the need for improved care for these individuals. Previous studies have found that the hormone FGF-23 may play a key role in kidney function and in the initiation and progression of CKD. Premature death from all causes, and from cardiovascular disease in particular, is higher in people with CKD than in healthy adults. Increased FGF-23 levels in the blood have been shown to be associated with increased mortality in CKD. However, it has been unclear how these levels change over time in a person with CKD, and whether repeated testing of FGF-23 levels over long periods of time can better predict clinical outcomes.

In 2001, the NIDDK established the Chronic Renal Insufficiency Cohort (CRIC) study to identify the risk factors for loss of kidney function and the link between kidney and heart diseases, and to apply this acquired knowledge to improving health. In the current study, CRIC investigators examined

circulating FGF-23 levels to see whether they increase over time and whether a rise in FGF-23 levels (*i.e.*, a rising trajectory) is associated with an elevated risk of death among men and women with CKD. FGF-23 levels were measured in 1,135 CRIC participants at enrollment and then again 1, 2, 3, and 4 years later. Compared to levels when the participants first enrolled, FGF-23 levels rose slightly in later years for the group as a whole. However, using a special statistical analysis technique, the investigators identified three FGF-23 trajectory subgroups: stable, slowly rising, and rapidly rising. Unlike the subgroup whose FGF-23 levels remained stable over time, the 37 percent of participants in the slowly rising FGF-23 subgroup were at 4.5-fold higher risk of death, and the 7 percent of participants in the rapidly rising FGF-23 subgroup were at 15.2-fold higher risk of death. Thus, although stable in the majority of CRIC participants, rising FGF-23 levels in some people with CKD place them at exceptionally high risk of death. This finding could help kidney specialists identify patients with CKD at particularly high risk, based on rising FGF-23 levels, and thus provide more personalized care.

*Isakova T, Cai X, Lee J, ...Wolf M on behalf of Chronic Renal Insufficiency Cohort (CRIC) Study Investigators. Longitudinal FGF23 trajectories and mortality in patients with CKD. J Am Soc Nephrol 29: 579-590, doi: 10.1681/ASN.2017070772, 2018.*

**Health Benefits of Intensive Blood Pressure Control Outweigh a Slight Risk of Developing Kidney Disease:** A new study found that in people who do not have chronic kidney disease (CKD), an intensive blood pressure control regimen increases risk of declining kidney function; this risk is generally outweighed by a reduced risk for cardiovascular events and death. Elevated blood pressure is relatively common in the U.S. population and is a risk factor for heart disease, stroke, and kidney disease. The Systolic Blood Pressure Intervention Trial (SPRINT) was designed to test whether using medications to reduce systolic blood pressure to a lower goal than currently recommended will reduce cardiovascular disease risk in people with high blood pressure but not diabetes. ("Systolic" refers to the higher of the two numbers in a blood pressure reading; it measures the pressure in the arteries when the heart beats. "Diastolic" refers to the lower of the two numbers and measures the blood pressure when the heart rests between beats).

SPRINT researchers previously reported that, among the subset of study participants who did not have CKD at the start of the trial, those who received an intensive blood pressure control regimen for more than 3 years were at a slightly higher risk of developing CKD than those who received a standard blood pressure control regimen. Researchers have now followed up on this initial observation by performing a more detailed analysis of SPRINT data to understand the broader health benefits and risks of intense blood pressure control. The scientists analyzed data from more than 6,600 study participants without CKD, about one-third of whom were women, to determine the rates of CKD development, cardiovascular events, and death after about 3 years. The scientists defined new-onset CKD as a reduction in kidney function of at least 30 percent, to a level of function considered less than normal. In the intensive group, 3.7 percent of participants developed CKD as compared to 1.0 percent in the standard group. The researchers also found that 4.9 percent of the intensive group experienced cardiovascular events or death, compared to 7.1 percent in the standard group. Further computational analysis of these data revealed that when compared with standard care, intensive blood pressure control led to a 2.6 percent increase in risk of developing CKD, but the risk of death or of cardiovascular events decreased by 2.2 percent. Through their calculations, the scientists predicted that statistically, for each death or cardiovascular event prevented by the intensive therapy regimen, there would be 1.2 cases of new-onset CKD.

In the intensive group, the reasons for and long-term consequences of the observed reduction in kidney function remain unclear. The researchers point out that because kidney filtration rates are dependent on blood pressure, reduced kidney function to some degree would be expected. Because cardiovascular events and, of course, death are far more serious outcomes, the health benefits of intensive blood pressure therapy outweigh the risk of developing CKD. However, the scientists note that additional follow-up time will help in understanding the longer-term risks and benefits of the two blood pressure control regimens described in the study. They also caution that in some cases, intensive therapy might not be the best option for blood pressure control because the risk of CKD development could be a higher priority than other health considerations. These findings, which add to those from other SPRINT studies, could help provide

valuable insights that inform decisions made by patients and health care providers.

*Beddhu S, Rocco MV, Toto R, ...Whelton PK; for the SPRINT Research Group. Effects of intensive systolic blood pressure control on kidney and cardiovascular outcomes in persons without kidney disease: a secondary analysis of a randomized trial. Ann Intern Med 167: 375-383, doi: 10.7326/M16-2966, 2017.*

## KIDNEY STONE TREATMENT

**Treatment for Common Urinary Ailment No Longer Carved in Stone:** Newly published results from a large clinical trial indicate that a drug frequently used in the emergency room (ER) to treat people with urinary stone disease has no benefit if the stones are below a certain size. Urinary stones, also called kidney stones, are pebble-like masses that can form in the kidneys if there is an excess of certain minerals in the urine. Stones vary in size from a grain of sand to (rarely) a golf ball. Those that get lodged in the ureters—the tubes that carry urine from the kidneys to the bladder—cause extremely painful symptoms. Although many symptom-causing stones subsequently pass out of the body on their own, appropriate medical evaluation is important because, if they do not, they can lead to infection and loss of kidney function. Men are much more prone to stones than women, although recent research suggests that rates may be rising in women. People with symptomatic urinary stones are often first seen in emergency departments. Under current guidelines, if a special imaging technique reveals a stone up to a certain size trapped in a person's ureter, a recommended first line of treatment is to prescribe a type of drug thought to promote stone expulsion, called an alpha adrenergic receptor blocker, or alpha blocker. One such drug is tamsulosin. The drug is taken daily by mouth for about a month, along with medication to alleviate pain until the stone passes.

However, because recent studies have called into question whether alpha blockers actually promote passage of stones that are below a certain size (including stones commonly considered large), NIDDK initiated the Study of Tamsulosin for Urolithiasis in the Emergency Department (STONE) clinical trial. The goal of the trial was to determine whether symptomatic patients given tamsulosin actually passed a symptomatic stone at

a significantly higher rate over the course of 28 days than patients given placebo (no medication) pills. Conducted at six different emergency departments, the STONE trial enrolled 512 participants 18 years of age or older with a symptom-causing stone of less than 9 millimeters (*i.e.*, up to the size of a large green pea); the average size was close to 4 millimeters, and over half of the symptom-causing stones fell within the 3 to 4 millimeter range. About three-quarters of participants were men. Participants were randomly assigned to either tamsulosin or an identical looking placebo pill to be taken once a day, and then contacted by phone multiple times during the treatment phase to find out whether the stone had passed—*i.e.*, whether he or she had seen or even collected it after urination. The STONE researchers found that, among the 497 persons for whom they had data, 49.6 percent of those in the tamsulosin group and 47.3 percent in the placebo group reported stone passage at 28 days—this small difference was not statistically significant (*i.e.*, it likely happened by chance). Further, while the researchers also did not find significant differences between the groups in factors such as pain medication usage, time away from work, or return ER visits due to a stone, they did find that men on tamsulosin were more likely than men on placebo to report problems with ejaculation (a side effect of tamsulosin that has been noted in the past). These findings, in combination with similar findings from two other trials conducted in the United Kingdom and Australia, indicate no benefit of tamsulosin for promoting passage of stones of the sizes tested and suggest that guidelines for ER treatment of urinary stone disease may need to be revised.

Meltzer AC, Burrows PK, Wolfson AB, ...Brown J. Effect of tamsulosin on passage of symptomatic ureteral stones: A randomized clinical trial. *JAMA Intern Med* 178: 1051-1057, doi: 10.1001/jamainternmed.2018.2259, 2018.

## URINARY TRACT INFECTIONS AND THE MICROBIOME

**Microbial “Iron”—Bacteria Provide Keys to Battling Urinary Tract Infections:** Researchers have identified two different molecules that can limit growth of microbes that cause urinary tract infections (UTIs), adding to approaches being pursued to develop new strategies for clinical treatment of UTIs. UTIs are very

common and primarily affect women, who often go on to suffer long-lasting or repeated infections. The majority of UTIs are caused by bacteria. Although still treatable with available antibiotics, a steady and alarming increase in antibiotic-resistant bacteria has led researchers to seek out other ways to prevent or treat UTIs. Exploiting the knowledge that bacteria need host-supplied iron in order to survive—and that human hosts limit iron access during infection—is one very promising avenue of research.

UTIs are most commonly caused by uropathogenic *Escherichia coli* (*E. coli*), or UPEC. Whereas *E. coli* normally live in the human intestinal tract and benefit health, some strains possess factors that help them, with varying degrees of success, to colonize the urinary tract and cause disease. For example, UPEC that possess a genetic element called the *Yersinia* high pathogenicity island (HPI) can make a special iron-scavenging molecule, called a siderophore, that helps them obtain iron even as the host tries to fight infection by limiting its availability. Scientists have now found evidence that the HPI also enables UPEC to produce a second molecule, called escherichelin, that inhibits a siderophore produced by a totally different species of bacteria that can also cause UTIs. This finding—together with some initial findings in humans—not only provides insight into how some UPEC may eliminate microbial competitors, but also suggests that *E. coli* strains that can colonize the urinary tract and produce escherichelin but not cause disease (asymptomatic strains) might be useful as “probiotics” to prevent opportunistic UTIs caused by other bacteria.

In a second study, a different team of researchers sought out molecules that could inhibit UPEC growth itself. One of the first antibiotics ever identified, penicillin, is a natural product of a certain mold, and kills susceptible bacteria by inhibiting a key step in how they create their unique cellular “wall.” Similarly, the research team looked to an extensive library of naturally produced molecules, rather than synthesized chemicals, to screen for any that could limit UPEC growth under low iron conditions similar to those found during UTI. They hoped to find molecules that didn’t bind up (chelate) iron and further reduce its availability to have this effect, but instead could target UPEC cellular processes active during “normal” infection. From their screening process, they isolated a novel molecule produced by a bacterial species called *Streptomyces nicotiae* (*S. nicotiae*), which they

termed nicoyamycin A, or NicA. Testing revealed that purified NicA and two similar molecules subsequently isolated from *S. nicoyae* are potent inhibitors of UPEC growth in low iron conditions. However, despite efforts during the initial screens to exclude iron-chelating molecules, purified NicA (as well as the other two molecules) was subsequently found to be a potent iron chelator. Still, it is known that some antimicrobial molecules, such as the antibiotic tetracycline, have iron-chelating properties, even though that is not their primary mode of action. Thus, further studies of NicA and related molecules from *S. nicoyae* could reveal if this is the case, and, if so, the relative contributions of different molecular properties to their inhibition of UPEC growth. Encouragingly, a second set of molecules similar to NicA that were also tested inhibited UPEC growth but did not potently chelate iron, suggesting a greater likelihood that NicA and the other molecules act through another mechanism. In the meantime, the identification of multiple bacterial-produced molecules, such as escherichelin and NicA, that hamstring UTI-causing organisms under conditions of low iron is an encouraging step forward in finding new therapeutic strategies to combat UTIs.

Mike LA, Tripathi A, Blankenship CM,...Mobley HLT. Discovery of nicoyamycin A, an inhibitor of uropathogenic *Escherichia coli* growth in low iron environments. *Chem Commun (Camb)* 53: 12778-12781, doi: 10.1039/c7cc07732g, 2017.

Ohlemacher SI, Giblin DE, d'Avignon DA, Stapleton AE, Trautner BW, and Henderson JP. Enterobacteria secrete an inhibitor of *Pseudomonas virulence* during clinical bacteriuria. *J Clin Invest* 127: 4018-4030, doi: 10.1172/JCI92464, 2017.

## INTERSTITIAL CYSTITIS DISEASE MEDIATORS

**A Molecule in the Urine May Hold the Key to Improved Bladder Health for People with Interstitial Cystitis:** New research has revealed that reduced levels of menthol in the urine are associated with interstitial cystitis, a finding with implications for improving both diagnosis and treatment. Interstitial cystitis (IC), also called IC/bladder pain syndrome (BPS), is a urologic chronic pelvic pain syndrome with symptoms that include urinary urgency and frequency, and pelvic pain. The causes and risk

factors for IC are not well understood, and there is no fully effective treatment. A better understanding of IC could lead to improvements in diagnosis and the development of new therapeutic approaches. Because people with IC often report foul odors in their urine, the researchers, in a recent study, examined a class of odorant molecules called volatile organic chemical compounds (VOCs) that may be altered with disease progression.

The scientists sought to identify differences in VOCs by comparing urine samples from women with IC to those from women with normal bladder health. Using sophisticated techniques, the researchers separated the various urinary VOCs from one another, determined their identities, and compared the results. These analyses yielded twelve distinct VOCs that were different between urine samples from the two groups. One of these compounds, menthol, was reduced in urine from women with IC. Menthol is widely used for a range of medicinal purposes, in part due to its anti-inflammation properties. Because inflammation of the bladder is a hallmark of IC, the researchers tested whether menthol plays a role in disease progression. They exposed mouse macrophages—immune cells that initiate inflammation—to menthol under conditions that instruct the macrophages to begin the inflammatory response. These cells did not turn on the genes or secrete the proteins necessary for inflammation, in contrast to the robust inflammation program initiated in cells that were not treated with menthol. Further analysis identified the specific cellular pathways normally triggering inflammation that were disrupted by menthol. These findings suggest that urinary menthol could serve as a useful diagnostic indicator of IC. Additional research will be needed to determine whether menthol, or the cellular pathways targeted by the VOC, could be utilized as a treatment to quell bladder inflammation in people with IC.

Shahid M, Lee MY, Yeon A,...Kim J. Menthol, a unique urinary volatile compound, is associated with chronic inflammation in interstitial cystitis. *Sci Rep* 8: 10859, doi: 10.1038/s41598-018-29085-3, 2018.

## RED BLOOD CELL MATURATION AND RETENTION

**New Tool Reveals Insights into Maturation of Red Blood Cells:** Using detailed genetic data along with a recently developed analytical tool called “population

balance analysis” (PBA), investigators have developed models capable of predicting the mature blood cell type of early stage (progenitor) blood cells. Scientists have grappled with how to design studies that will reveal what controls or drives progenitor cells to undergo a process that ultimately leads to becoming more specialized mature cells (*i.e.*, red blood cells). With previous significant technological advances, scientists are now able to use an approach called single-cell expression profiling to begin to understand what cell and tissue types will arise from an individual progenitor cell. In single-cell expression profiling, the “transcriptome” is characterized to provide a measure of a single cell’s gene activity. Active genes produce transcripts, which may serve as instructions for making proteins. A transcriptome is a collection of all the transcripts present in a given cell and gives an internal snapshot of what the cell is doing or how it is changing.

Using the transcriptome data of 4,763 individual cells, researchers showed that progenitor cells from female mice mature along distinct branches leading to one of seven blood cell fates. To understand pathways that progenitor cells might follow as they mature, the investigators had previously developed the analytical tool, PBA, to predict cell fate likelihood using snapshots of single-cell transcriptomes. PBA identified the commitment likelihood for each blood progenitor cell to each of the seven blood cell fates, and these predictions were confirmed by tracking the cell fate process. Unlike the classical models of blood cell maturation, the details provided by PBA revealed that the process is a continuous progression (rather than discrete stages of cell development). For example, the researchers were able to map the continuity of the red blood cell maturation process, called erythropoiesis, from the earliest progenitor cell to the final maturation stage. During these experiments, the researchers uncovered new insights in red blood cell development, including the first identification of IL-17A, a growth factor, as a strong stimulator of red blood cell maturation. The new knowledge obtained from single-cell data combined with PBA has allowed an in-depth examination of early stage blood cell maturation. This approach may provide similar insights into other cell maturation processes, and provides tools to further the fields of stem cell biology and regenerative medicine.

*Tusi BK, Wolock SL, Weinreb C, ...Socolovsky M. Population snapshots predict early haematopoietic and erythroid hierarchies. Nature 555: 54-60, doi: 10.1038/nature25741, 2018.*

**Zebrafish Employ Cellular Shield To Protect Blood Stem Cells from Damaging Ultraviolet Light:** While studying the blood stem cells that reside in the kidney of the zebrafish embryo, researchers observed that cells called melanocytes were positioned above the stem cells in an umbrella pattern, and subsequently showed this umbrella served as a shield protecting stem cells from damaging ultraviolet (UV) light. When stem cells divide, they can form more stem cells or other cells that perform specialized functions. The blood stem cell niche is crucial for regulating the process of new blood cell formation, and its location varies among species—for example, it is found in the bone marrow of adult mammals and in the kidney of the majority of fish species. Little is known about the factors (*e.g.*, environmental) that have influenced the placement of stem cell niches in mammals and fish.

A recent report described an opportune observation that a cluster of melanocytes formed an umbrella pattern directly above the kidney stem cell niche in zebrafish. Zebrafish are an important animal model system that scientists use to better understand human physiology and disease. One distinct advantage that zebrafish embryos have is that they are “transparent”—allowing scientists to watch the fertilized eggs grow into fully formed baby fish under a microscope. Previous research has shown that the melanocyte is a cell in human skin that produces and contains the pigment melanin; melanin protects skin cells from DNA damage caused by UV light.

This led the research team to hypothesize that melanocytes may serve as a shield to protect the blood stem cells in the zebrafish kidney from damaging UV light. They tested this hypothesis by studying a mutant form of zebrafish embryo that lacks melanocytes. After UV-light exposure, stem cells from mutant zebrafish embryos contained significantly more DNA damage compared to stem cells from normal zebrafish embryos with melanocytes. Furthermore, mutant zebrafish embryos showed a significant decrease in stem cell numbers compared to those in normal embryos. These findings suggest that the melanocytes were protecting the stem cell niche from damaging UV light. The researchers then sought to determine whether it was the “orientation” of the melanocyte umbrella that played a protective role. When anesthetized, zebrafish embryos flip upside-down—this change in orientation no longer positions the melanocyte umbrella between the UV light and

kidney stem cell niche. Under these conditions, stem cell numbers in normal zebrafish were reduced to the same level as those in mutant embryos after UV treatment. These findings confirm that the orientation of the melanocyte umbrella is critical to protect stem cells from UV damage and suggests that melanocytes form an optical shield.

The research team also showed that other types of fish contain a melanocyte shield and postulated that land-based animals such as mammals employ a “bone” shield to protect the blood stem cell niche in the bone marrow from damaging UV light. These new findings suggest that protection against damage from UV light plays an important role in influencing the placement of animals’ stem cell niche.

*Kapp FG, Perlin JR, Hagedorn EJ,...Zon LI. Protection from UV light is an evolutionarily conserved feature of the haematopoietic niche. Nature 558: 445-448, doi: 10.1038/s41586-018-0213-0, 2018.*

## TREATING BLOOD DISORDERS

### Identification of Potential Therapeutic Target for Sickle Cell Disease and Other Hemoglobinopathies:

Researchers recently identified a protein governing production of a type of hemoglobin, called fetal hemoglobin (HbF), in adult human red blood cells, a finding that may have important implications for treating red blood cell diseases. People with sickle cell disease suffer from chronic anemia and episodes of bone, joint, and muscle pain, as well as other complications because their red blood cells form rigid, “sickle” shapes, leading to impaired blood flow in small blood vessels and reduced oxygen delivery to tissues. The disease results from genetic mutations that affect the form of hemoglobin often called “adult” hemoglobin, even though its production begins soon after birth. Such diseases, affecting hemoglobin structure and/or production, are referred to as hemoglobinopathies. Individuals with another hemoglobinopathy, called  $\beta$ -thalassemia, also suffer from chronic anemia. In this case, impaired adult hemoglobin production results in reduced numbers and viability of red blood cells. Although HbF is mostly undetectable in adults and children (after about 6 months of age) in the general population, increased levels safely persist to varying degrees in some people. Researchers previously observed that

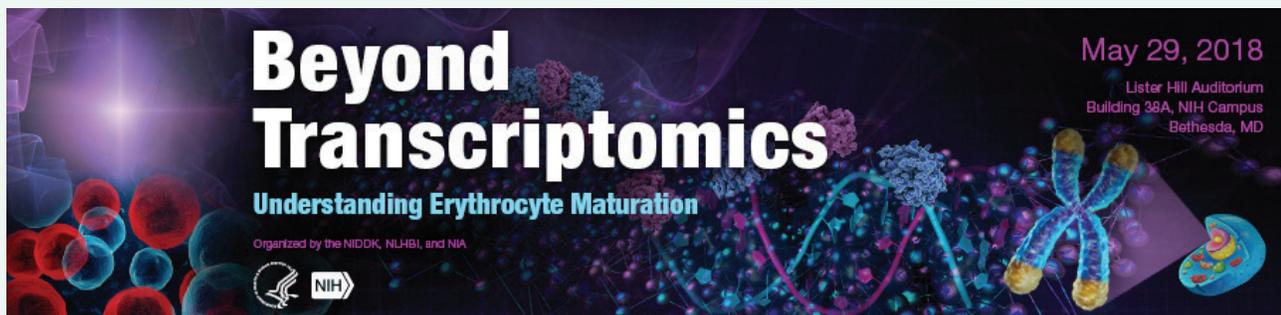
people with sickle cell disease who retain higher HbF levels have less severe disease. Thus, one potential treatment approach is to reactivate HbF production to sufficient levels in adult red blood cells such that it compensates for the defects in hemoglobinopathies.

One way to achieve such reactivation of HbF would be to use a drug to target proteins that regulate its production. Thus, for the current study, the researchers aimed to identify regulators of HbF production that would potentially be “druggable.” To do this, they designed a screen for proteins called kinases in adult red blood cells, to see if any of the numerous kinases in human cells might affect HbF production. They focused on this type of protein because many protein kinases are druggable with small molecules that inhibit their activity. For their screen, the scientists used a genome editing tool called the CRISPR/Cas9 system to target genes encoding protein kinases. The CRISPR/Cas9 system allows for a gene’s DNA to be edited with unprecedented precision, and the resulting changes can be screened for the desired outcome—in this case, a change in a sought-after regulator would result in a change in HbF levels. This approach led to the identification of a protein kinase called heme-regulated inhibitor (HRI) as a repressor of HbF production. Specifically, HRI depletion in normal adult red blood cells resulted in significantly increased HbF levels. Furthermore, in subsequent experiments the scientists found that HRI depletion in red blood cells from patients with sickle cell disease led to increases in HbF levels, which suggests that this strategy may be beneficial to people with sickle cell disease.

The discovery of HRI as a regulator of HbF opens the possibility for future research to identify a small molecule drug that would selectively inhibit it; such a drug may have profound clinical benefit to people with sickle cell disease or  $\beta$ -thalassemia by increasing HbF to sufficient levels to overcome the pathology associated with these red blood cell diseases.

*Grevet JD, Lan X, Hamagami N,...Blobe GA. Domain-focused CRISPR screen identifies HRI as a fetal hemoglobin regulator in human erythroid cells. Science 361: 285-290, doi: 10.1126/science.aao0932, 2018.*

# Scientific Workshop: Exploring Approaches To Bolster Understanding of Red Blood Cells



To identify gaps in our knowledge of red blood cell (also known as the erythrocyte) maturation, the NIDDK, in collaboration with the National Heart, Lung, and Blood Institute and the National Institute on Aging, held a workshop titled “Beyond Transcriptomics: Understanding Erythrocyte Maturation” in May 2018.

In a healthy person, the production and destruction of erythrocytes is maintained at a certain level. With respect to production, approximately two million newly formed erythrocytes leave the bone marrow and enter the blood stream every second in the adult human. Unlike most cells of the body, the mature erythrocyte no longer contains a nucleus—the cellular compartment that serves as the cell’s command center by sending directions to the cell to grow and mature. Pathways critical to understanding how the erythrocyte matures and how the cell maintains its metabolism after the nucleus is jettisoned are not completely understood. Knowledge gained from new research into pathways and metabolism may shed light on disorders that affect erythrocyte levels such as certain types of anemia (decreased erythrocyte level) or polycythemia (increased erythrocyte level).

The main purpose of the workshop was to stimulate research interest beyond the recent progress that has been made using an approach called “transcriptomics” – the study of messengers, or “transcripts,” between genes and proteins that eventually carry out the instructions encoded in the genome. To explore a range of other research avenues, workshop attendees, who were drawn from across the United States, Canada, and Europe, had expertise in several different scientific disciplines including cellular biology, molecular biology, molecular genetics, physiology, bioinformatics, developmental biology, pathology, and hematology. By the conclusion of the meeting, the participants identified several research questions that, if answered, could move the field forward, including the: characterization of the structures of the erythrocyte cell membrane that ensure integrity and survival in the blood; identification of external and internal factors that modify erythrocyte structure and function in normal and diseased states; determination of the mechanisms in the bone marrow that allow for and regulate the differences found in normal erythrocytes; and identification of the signals for destruction and production of proteins in developing early stage erythrocytes or fully mature erythrocytes.

# Workshop Charts a Path Forward to Imaging the Kidney and Its Function

On July 12–13, 2018, the NIDDK held a workshop on the NIH campus in Bethesda, Maryland, to discuss current approaches to renal (kidney) imaging and to improve the characterization of kidney function, structure, and damage.

Kidney disease remains an enormous public health burden, but discovery of novel treatment and prevention strategies has been challenging. Research to develop new therapies for kidney disease would greatly benefit from an improved ability to visualize the structure of the kidney in finer detail and to evaluate its functional status. In this workshop, NIDDK brought together experts from different fields to explore opportunities to advance the development of new technologies and approaches to kidney imaging. A range of topics was covered in the presentations, including current cutting-edge science in renal imaging, examples of clinical areas in which imaging approaches have been successfully applied, and technological hurdles that impede translation of laboratory discoveries to clinical settings. The workshop featured a poster session that focused on additional

technologies and recent studies, and provided an opportunity for junior investigators and trainees in this area to present their research.

The workshop also convened several breakout group sessions to explore topics in greater depth. Through these sessions and subsequent discussion, ideas emerged to tackle several important challenges in renal imaging development and implementation, such as identifying research areas essential for scientific advancement; defining critical measurements of kidney structure and function; improving the drug development process; and validating clinical imaging methodologies. The workshop revealed that a robust scientific community is currently working toward each of these goals. However, workshop participants agreed that the development of multidisciplinary teams that include nephrologists (specialists in kidney disease), radiologists, engineers, and the biomedical industry will likely accelerate progress. Scientific opportunities discussed during the meeting are expected to inform new approaches to renal imaging, thereby paving the road for the next generation of kidney disease therapeutic strategies.

# Workshop Forges Collaborative Approach to Understanding Chronic Kidney Diseases in Agricultural Communities

On June 25–26, 2018, the NIDDK co-sponsored— together with the National Institute of Environmental Health Sciences (NIEHS) at NIH—a workshop in Bethesda, Maryland focusing on research to understand the causes of and potential treatments for chronic kidney diseases in agricultural communities. This workshop brought together clinicians, basic scientists, epidemiologists, and public health officials to discuss current gaps in knowledge and to develop a coordinated scientific research agenda on this topic. In addition to NIDDK and NIEHS staff, other organizers included representatives from the National Institute for Occupational Safety and Health at the Centers for Disease Control and Prevention (CDC), the Fogarty International Center at the NIH, and the Instituto Nacional de Ciencias Médicas y Nutrición Salvador Zubirán in Mexico. The workshop was part of ongoing efforts to advance research in this area, including NIEHS’s formation of a working group on this topic in December 2017 and related work through their collaborating center with the World Health Organization.

In recent years, chronic kidney diseases of unknown or unexpected origins have been on the rise, particularly in agricultural communities around the world, including in the United States. These diseases go by many names depending on where they occur, including chronic kidney disease of unknown or non-traditional etiology, chronic interstitial nephritis in agricultural communities, and Mesoamerican nephropathy. Possible contributing factors identified to date include heat stress, dehydration, drugs or herbal supplements, environmental chemicals, and a combination of exposure with genetic susceptibility. Many questions remain about them, such as: 1) whether these are one common disease or a syndrome of related conditions that are unique to a given region, and 2) which factors are most important to their development and progression.

Workshop participants reflected the international and interdisciplinary expertise needed to tackle this problem through building consensus around the scientific path forward. The speakers presented on available information, challenges, and research opportunities related to these chronic kidney diseases in agricultural communities occurring in such regions as the Americas (mainly the United States and Central America), Sri Lanka, the Balkans, and other parts of the globe. Session themes focused on defining these diseases, using geography to provide new insights, investigating causes, studying the agricultural environment, gaining trust in the community, and studying the entire population for genetic and epidemiologic clues. Breakout working groups were tasked with addressing challenges in areas such as defining cases, identifying common data elements, understanding ethical barriers to studying agricultural workers, and creating a research agenda to advance understanding of this mysterious and far-reaching disease.

The meeting concluded with a summary of the wide range of inter-related topics touched on by the speakers that will inform future research on chronic kidney diseases in agricultural communities, including key questions in defining the disease, its causes, and its susceptibilities; developing necessary research tools; designing animal and clinical studies; and identifying opportunities for prevention. Meeting organizers plan to develop a summary for publication in the scientific literature describing the workshop proceedings. Scientific opportunities discussed during the meeting will inform future efforts to advance research addressing the many unanswered questions surrounding chronic kidney diseases affecting agricultural communities.

# STORY OF DISCOVERY

## Getting a Notch Up on Cord Blood Cell Transplantation

Blood stem cell transplants can be life-saving for people with a number of conditions. However, it can be challenging to find blood stem cells in needed quantities from a donor whose cells are similar enough to a patient's cells to be a sufficient “match” for transplantation. Cells from bone marrow and from circulating blood have been used in treatments for many years, and blood from the umbilical cord, collected after a baby is born, has been another source of blood stem cells used more recently. Cord blood from donors is available in public cord blood “banks,” and the match between donor and recipient may not need to be quite as close as is needed for transplants of other types of blood cells. However, cord blood from each donor exists in only limited quantities. Researchers supported by NIDDK have discovered key factors that promote blood stem cell growth and maturation, and, with these insights, have developed ways to expand the numbers of cells from cord blood for use in transplants. This research may increase the availability of such treatments to benefit many more people.

### **CORD BLOOD CELL TRANSPLANTATION**

Cord blood is found in blood vessels of the placenta and the umbilical cord—tissue that is normally discarded. It is collected after a baby is born and after the umbilical cord is cut between the mother and the baby. Approved by the U.S. Food and Drug Administration only for use in blood (hematopoietic) stem cell transplantation procedures, cord blood has been transplanted into thousands of patients who have serious medical conditions such as bone marrow failure syndromes, blood disorders, immunodeficiencies, certain metabolic disorders, and cancers. Cord blood stem cells (which are

different from embryonic stem cells) facilitate healing and repair of damaged cells and tissue—that is, they are early stage blood cells that, like other blood stem cells, provide a source for the regrowth of all the types of specialized blood cells. Cord blood transplantation, therefore, is an example of regenerative medicine.

A critical advantage of cord blood is that cord blood stem cells can be easily transplanted; the transplanted cells do not have to be an exact match with the recipient's tissues. However, the main disadvantage is that a single umbilical cord is insufficient; a single umbilical cord contains a limited number of stem cells that do not mature into a sufficient number of specialized types of blood cells needed for adults. Cord blood cell transplants (from a single umbilical cord) could thus place an adult patient at increased risk of life-threatening infections due to the inadequate number of infection-fighting types of blood cells. Such patients may need two or more units of cord blood. Yet, they may still be at risk because, compared with conventional bone marrow transplantation, it may take longer for transplanted cord blood stem cells to engraft—that is, to start working properly in a patient's body—and give rise to other types of blood cells. For this reason, cord blood cell treatment is used more often in children, who have a small body size and thus require fewer cells. Basic research on blood cells has sparked ideas for expanding the numbers of cells in a unit of cord blood.

### **NOTCH: THE STORY BEGINS**

In 1994, while investigating the cellular and molecular mechanisms underlying the production

# STORY OF DISCOVERY

and function of blood cells, NIDDK-supported researchers discovered for the first time that in human early stage blood cells, a gene encoding a protein called Notch is “turned on.” NIDDK-supported researchers then set out to determine whether Notch influences the function of blood stem cells. Notch, which sits in the cell membrane, transmits signals originating from the outside of the cell to direct the turning on or turning off of genes. Using laboratory mice, they demonstrated, in 2002, that activated Notch had two distinct activities— 1) the inhibition of stem cell maturation into different blood cell types and 2) the stimulation of stem cell self-renewal (*i.e.*, an increase in stem cell number). In 2003, with support from NIDDK and other sources, researchers reported that they could induce early stage mouse blood cells to proliferate by exposing the cells to an engineered protein that binds to Notch; this protein was a modified version of a protein called Delta1. Interestingly, the researchers did not see this effect if they simply mixed the modified Delta1 protein into the liquid in which the cells were growing; through their experiments, they realized they had to immobilize this protein on the dish in which the cells were growing for it to have the desired effect of increasing the numbers of cells.

## NOTCH-DRIVEN ENGRAFTMENT OF CORD BLOOD CELLS IN HUMAN PILOT STUDY

Taking advantage of their knowledge of the Notch signaling pathway and blood stem cells and the modified Delta1 protein, investigators supported by NIDDK and others were able to significantly increase, by greater than 100-fold, the number of early stage blood cells from a unit of cord blood. The researchers then conducted, and reported in 2010, a pilot clinical study of 10 participants with leukemia to begin to assess the safety of infusing patients with cord blood stem cells that had been expanded to greater numbers using the modified Delta1 protein/Notch procedure, and to begin evaluating the engraftment properties of the expanded stem cells. In the course of their

treatment for leukemia, the participants were given radiation therapy and chemotherapy to destroy the blood cancer cells; these treatments also destroyed the stem cells in their bone marrow. Cord blood cell transplantation was subsequently used to repopulate the bone marrow. Each participant received two units of cord blood—one unit of non-expanded blood and one containing expanded numbers of blood cells, or two units of non-expanded blood. In this small group of participants, no safety issues were encountered. In addition, the time for engraftment, measured in terms of white blood cell recovery, was significantly shorter for participants who received Delta1/Notch-expanded cells than for those who received only non-expanded cord blood.

## MANIPULATING BLOOD STEM CELLS INTO MATURE BLOOD CELLS

Blood stem cells mature along two tracks: 1) the myeloid lineage, which produces white blood cells (*e.g.*, macrophages and neutrophils), red blood cells, and other cells, and 2) the lymphoid lineage, which produces T cells, B cells, and other cells. The immune system consists of two major pillars: the innate (general defense) and the adaptive (specialized defense). Both systems work closely together and take on different tasks. Researchers have sought to identify a minimum cocktail of factors necessary to enable the reconstitution of both myeloid and lymphoid lineages from blood stem cells. Previous NIH-supported research found that experimentally increasing production of a factor called HoxB4 in mouse early stage blood stem cells could reconstitute the myeloid lineage but only minimally reconstituted the lymphoid lineage. In further experiments to try to reconstitute both lineages, in 2016, investigators supported by NIDDK and others discovered that Delta1/Notch activation of mouse early stage blood stem cells, in combination with increased HoxB4 in these cells, enabled the cells to fully reconstitute the myeloid and lymphoid lineages when transplanted into mice lacking their own blood cells.

# STORY OF DISCOVERY

## **CLINICAL TRIAL ASSESSMENTS OF NOTCH-ACTIVATED CORD BLOOD CELL TRANSPLANTATION**

The NIDDK-supported research to increase numbers of blood stem cells led one of the investigators to start a clinical cell therapy company. The company is investigating (in two Phase 2 clinical trials) whether Delta1/Notch-expanded cord blood cells could reduce the time to white blood cell recovery and reduce the rate of infections in individuals with blood cell cancers.

## **NIDDK-SUPPORTED RESEARCH**

The translation of scientific knowledge and technology into improvements in the practice of medicine is central to the missions of the NIH and the NIDDK. As this story illustrates, the initial investment in basic science research has led to the development of a laboratory-based methodology to expand the stem cell population in cord blood for potential beneficial transplantation treatment of people with a myriad of blood disorders and diseases.

# SCIENTIFIC PRESENTATION

## Dr. Iain Drummond—Kidney Regenerative Medicine and the (Re)Building a Kidney Consortium

*Dr. Iain Drummond is an Associate Professor at Massachusetts General Hospital and Harvard Medical School, and the Director of the Kidney Group of the Harvard Stem Cell Institute. An expert in kidney developmental biology and kidney disease, Dr. Drummond is a member of the NIDDK-supported (Re)Building a Kidney Consortium.*

*At the September 2018 meeting of the National Diabetes and Digestive and Kidney Diseases Advisory Council, Dr. Drummond presented an overview of regenerative medicine challenges and approaches as applied to the kidney and kidney disease, with a special focus on the work of the (Re)Building a Kidney (RBK) Consortium, including work from his own laboratory.*

Regenerative medicine is a multidisciplinary field focused on developing ways of replacing, engineering, or regenerating human cells, tissues, or organs to restore, establish, or enhance normal biological function that has been lost to disease or trauma. Its progress relies on concerted and collaborative efforts in the life and physical sciences and engineering. Currently, the scientific knowledge and approaches to regeneration vary greatly by organ and tissue. A common thread is that directing regeneration in any tissue is a complicated process that will likely require multiple strategies.

Many of the diseases and conditions within the NIDDK research portfolio, from diabetes to liver and digestive disorders to problems of the bladder and kidney, are amenable to regenerative medicine approaches. Regarding kidney disease in particular, the last few years have seen fundamental advances

that have helped contribute to different strategies toward rebuilding a kidney or regenerating kidney tissue. Human kidneys are bean-shaped organs about the size of a fist that perform many critical tasks, including cleansing metabolic waste products from the blood and maintaining proper salt and mineral balance and fluid volume in the body; each of the two kidneys normally found in a person is comprised of approximately 1 million basic functional units, called nephrons, that carry out the majority of these tasks. Loss of kidney function can thus lead to build up of toxins in the blood and other problems, and total kidney failure is deadly without dialysis or a kidney transplant. In his presentation, Dr. Drummond described recent advances, pressing questions, and the development and testing of various strategies by which researchers may be able to accomplish restoration of kidney function via regenerative medicine approaches.

### **KIDNEY REGENERATIVE MEDICINE AND HUMAN DISEASE: MULTIPLE CHALLENGES, TWO OVERARCHING GOALS**

Dr. Drummond reminded the group of the prevalence of chronic kidney disease, which has a number of causes, including diabetes, hypertension, and inherited diseases such as polycystic kidney disease. There has been little advance in the last 50 years in treating chronic kidney disease or end-stage renal disease (ESRD, or kidney failure) since dialysis treatment became available—a treatment, he noted, that is really a “holding pattern” as it does not solve the underlying problem. Kidney transplantation

# SCIENTIFIC PRESENTATION

to replace the function of at least one damaged or diseased organ can be life-saving for people with ESRD, but limited organ availability means that people die every day waiting for a kidney. Regenerative medicine could thus be an answer if it provides functional kidney tissue or entire organs—but how to go about it?

Dr. Drummond broke down the regenerative medicine challenge of building a kidney, or making new kidney tissue, into steps, using the analogy: An iPhone is composed of many component parts—phone, camera, touch screen, microphone, *etc.*—that together enable the device to act as a single complex unit capable of carrying out numerous tasks. At the same time, each contributing component can be developed independently of the others and as such provide milestones of success for development of the final, complex device. Similarly, while much more complicated than a smartphone, the kidney has many component parts with specific functions that contribute to overall organ function, but which can potentially be isolated and worked on individually.

Looking at the kidney in this way, according to Dr. Drummond, the challenges of generating and using a kidney/kidney tissue *de novo* become somewhat more tractable as specific questions:

- How do we make kidney cells?
- How do we know we have made the right kidney cells?
- How do we organize them into useful structures?
- How can we show they are useful for functional replacement?
- How do we integrate them into patient tissue to augment or replace renal function?

At the same time, he noted, it is equally important to consider how it might be possible to keep people from progressing to ESRD through studies to better understand deterioration and damage in key parts of the kidney. This leads to a sixth question:

- How do we promote regeneration of damaged kidney tissue?

Approaches for addressing the burden of kidney disease and kidney failure—for organ/tissue replacement and prevention of disease progression—are being pursued in the (Re)Building a Kidney (RBK) Consortium. RBK was started in 2015 to optimize approaches for isolation and expansion of different kidney cell types, and their integration into complex structures that replicate human kidney function.

Dr. Drummond broke down the RBK mission in terms of the two overarching goals: (1) engineer and engraft new, functional kidney tissue and (2) identify repair mechanisms in the injured kidney that can be exploited therapeutically.

Multiple research groups in the United States, Australia, and New Zealand are part of RBK, tackling the different challenges. Using a published schematic diagram that captures the six challenges and how they fit together as a reference map, Dr. Drummond proceeded to lead the audience on a journey highlighting scientific advances from the RBK Consortium and other research groups that are occurring along the way to the ultimate goals of functional kidney tissue and reparative processes.

## MAKING (ENOUGH) KIDNEY CELLS WITH THE POTENTIAL TO WORK TOGETHER

Dr. Drummond began with the fundamental question: how do we make cells? It turns out that the initial objective is to begin with human-derived pluripotent stem cells—*i.e.*, cells that retain the potential to be coaxed into becoming many different types of cells found in the human body, find a way to drive them specifically into a nephron progenitor cell state (nephrogenic stem cells), and optimize the expansion of those cells to produce the billions of cells that will be needed to create replacement tissues for patients.

Dr. Drummond summarized a number of advances in the field that have yielded a group of signals and growth factors that, when sequentially applied to pluripotent cells, produce cells that resemble early nephrogenic cells found during kidney development.

# SCIENTIFIC PRESENTATION

He noted that successful generation of these cells in the laboratory is usually measured using the level of expression of certain marker genes (e.g., *OSR1*, *Six2*, and *PAX2*). Further, he noted that the field is at a stage now where people have optimized these procedures, citing in particular the work of RBK member Dr. Melissa Little and researchers at the Harvard Stem Cell Institute (Drs. Ryuji Morizane and Joseph Bonventre), in which almost 90 percent of pluripotent cells driven through their protocol emerge with markers denoting them as potential kidney progenitor cells.

RBK has adopted these successful and highly reproducible strategies to create new nephron progenitor cells—and to expand their numbers. Dr. Drummond described how RBK scientist Dr. Leif Oxburgh has generated growth conditions that support the development and expansion of mouse kidney cells and adapted that to nephrogenic cells derived from human-derived pluripotent stem cells—meaning that researchers can now reliably create mouse and human kidney stem cells and expand them.

Moreover, these cells apparently have the potential to work, as suggested by experiments in which Dr. Oxburgh has taken some of these nephrogenic cells, allowed them to differentiate, and implanted them in mice under the outer covering (capsule) of the kidney, a procedure referred to as engraftment. The result was the growth of a ball of cells that contained many elements of a fully functional kidney (segmented tubules, podocytes, and vascular components), a very encouraging finding.

However, according to Dr. Drummond, the current challenge is whether researchers can actually integrate this type of engrafted tissue into the body such that it has basic kidney functions—e.g., blood filtration (cleansing) and drainage into a host kidney collecting system. So, stepping back, kidney regenerative medicine scientists are working on generating “organoids”—miniature, multicellular, three-dimensional structures that resemble and/or mimic (but usually do not yet fully replicate) corresponding organs, such as was seen in the mouse

kidney capsule experiment—in the laboratory, outside of a living organism, for subsequent engraftment and testing. This means first taking the experimentally created nephrogenic progenitor cells and trying to drive them into organs and tissue structures, and there are multiple approaches to doing that.

For example, Dr. Little and her research group have basically concentrated nephrogenic progenitor cells into small pellets, finding that this approach enhances their differentiation (maturing into more specialized cells) into organoids in which different nephron segments and kidney cell types can be detected visually through special cellular staining techniques. Dr. Drummond pointed out that this experiment and others underscore how, intriguingly, most of the approaches to creating kidney organoids do not involve use of growth factors at the final differentiation stage, suggesting that there is a lot of self-differentiation and self-organization of tissue on the part of these cells, once they get started—and also a great deal of mystery about how these nephrons are forming. Hence, there is also a lot to learn about whether it might be possible to develop directed and organized approaches to drive the cells into specific/desired structures and shapes. This is another highly complex research problem being taken on by RBK scientists (led by Drs. Oliver Wessely and Jan Jensen), who are using specialized computational approaches to help reduce the number of variables and experiments that need to be done in order to answer some of the questions about what factors are driving differentiation into what types of cells in the organoids. The caveat remains, however, that the kidney organoids are still limited—in their structure, level of maturity, and functional capacity versus what exists in a normal, human adult kidney.

## “HAVE WE MADE THE RIGHT CELLS?”

Dr. Drummond turned to another of the fundamental challenges for kidney regenerative medicine: what are all of the cells in the kidney? That is, do scientists even know what they all are and their key functional features?

# SCIENTIFIC PRESENTATION

For example, he noted that the kidney stroma—the mixture of supportive cells and molecular matrix surrounding more discrete structures—was originally thought to be homogeneous, but studies from RBK member Dr. Thomas Carroll's group are showing that it has at least six different zones defined by different gene activity patterns in the kidney. This is important because kidneys without stroma do not make nephrons; thus, Dr. Drummond observed that in the organoids there are no doubt stromal cells that are understudied, and we need to know how they support growth of the kidneys. Similarly, work from RBK member Dr. Ondine Cleaver's research group is showing that blood vessels in the kidney are highly heterogeneous; a greater understanding of these vessels is critical to future strategies for conveying blood to the filtration unit of newly generated/regenerated kidney tissue.

Another consideration, Dr. Drummond observed, is that most of the work on kidney development has been performed in animal models—specifically, mice and zebrafish—meaning that it is critical to figure out how the data derived from those studies applies to human biology. For example, the human and mouse kidney differ in shape and size, and that is reflected in differences in gene expression (which genes are turned on or off), as revealed in part by research performed by RBK member Dr. Andrew McMahon and his group; they continue research toward understanding the differences in factors governing gene expression and also differences in cell types among species.

Dr. Drummond also remarked on how, in addition to a static description of cell types and the genes they express, developmental studies inherently need to integrate a time factor and account for cellular changes, similarities, and interactions that occur over the course of and during transitions in development of an organ. This perspective can also be applied to models of the progression of injury in the kidney, and to understanding where cells have productive responses and where cells do not.

## STRUCTURAL CONSIDERATIONS AND PLUMBING

For externally generated kidney cells, tissues, and organoids to be tested and ultimately useful, Dr. Drummond noted they will need to be placed in structures that can be used by the body, so the essential question is: how to build these and make them do what they need to do? Dr. Drummond described a number of approaches being pursued in RBK. For example, RBK member Dr. Jennifer Lewis and her group are focused on “printing” materials—*i.e.*, cells and supports—and showing what can be done with intentional organizations of tissue. Her group's approach allows the researcher to start with simple structures and build up iteratively. Dr. Drummond noted the hope that this research will, in the future, enable use of organoids or simple kidney tissues to build a complex three-dimensional structure; currently, Dr. Lewis and her group are working on printing tubular structures and testing them for function.

Dr. Drummond then described some of his own group's research on zebrafish, which are of interest because, unlike humans, they can regenerate entirely new kidney tissue as adults if injured. The relevance of this model to structural considerations for human kidney tissue replacement is that all nephrons ultimately must fuse with the collecting system that carries away fluid containing filtered waste, *etc.* So, similarly, new nephrons that grow out in the course of regeneration in the zebrafish must establish proper connections (plumbing joints) to enable flow and filtering of blood and excretion of wastes.

Dr. Drummond's group has found that a signaling factor highly conserved among animals, called Wnt, is essential to the interconnection of regenerating nephrons in zebrafish. This finding means that kidney regenerative medicine researchers have a new guidepost for understanding how to build a complex structure if they can provide signals that promote tubule interconnection at key parts of an assembly.

# SCIENTIFIC PRESENTATION

Another facet of his research is building on the finding that zebrafish that regenerate their kidneys activate growth factors that humans and other mammals use during early development (embryogenesis). With those observations in mind, Dr. Drummond's group is pursuing the idea of "turning a mouse into a fish." The concept is to use a genetic approach designed to introduce and produce growth factors in a kidney-specific fashion in mice—creating discrete sites of growth factor or morphogen expression—and then introduce laboratory-grown nephrogenic progenitor cells and ask whether it is possible to promote development and connection of organoids, and also whether it is possible to promote blood vessel growth in the new tissues. This is a prime example of combining technologies to achieve a regenerative medicine end: derivation of human pluripotent stem cells and subsequent nephrogenic progenitor cells, promoting differentiation to kidney cells types, and providing the right environment for them spatially for assembly into essential interconnected structures.

## TOWARD FUNCTIONAL REPLACEMENT

While pursuing the generation of kidney cells and organoids, dissecting and facilitating proper organization, and developing methods and structures, key issues that needs to be considered simultaneously are how to determine and demonstrate that they will actually work as kidney replacements. Approaches under consideration include attempting to restore function to mutant mouse kidneys with implanted organoid tissue, and generating transgenic organoids that secrete molecules that can be measured in the urine to show there is functional interconnection.

Currently, some other research is being pursued in RBK to get a better handle on the function of various types of engineered cells and tissues. For example, Dr. Drummond noted that Dr. Lewis and her group are testing their three-dimensional "printed" kidney structures for functions such as active transport of larger molecules between physically distinct but adjacent tubular structures, mimicking what would

be observed in a normal nephron, and thus far is seeing positive results. Another RBK member, Dr. Lisa Satlin, is studying the Lewis group's printed tubules to assess how fluid flow rate affects the flux of essential salt ions, potassium and sodium, in the engineered structures—to see how well these generated tubules mimic the native kidney. Dr. Drummond also noted the work of a recent addition to the RBK consortium, Dr. Thomas Kleyman, who is examining organoid cells and is finding that they possess physiologically relevant and active potassium transport channels.

## PROMOTING REGENERATION OF DAMAGED KIDNEY TISSUE TO PREVENT PROGRESSION

Alluding to the other overarching goal of RBK, Dr. Drummond remarked that as the consortium's research leads to discoveries of new growth and differentiation factors, some of these might be therapeutic targets for regeneration or regrowth of existing kidney structures when they are damaged.

Thus, in addition to their work on the relationships between gene expression and kidney differences in the human and mouse, he noted, as one example, that Dr. McMahon's group is using gene expression profiling to discover how to distinguish states of cells that can repair certain damaged kidney structures versus those that result in scarring (fibrosis) or cell death. Already, the group has identified genes, such as *SOX9*, that seem to be activated in injury and recapitulate certain developmental events in repair. These approaches, along with work from RBK member Dr. Benjamin Humphreys' lab to develop an "atlas" of cells active during repair, are enabling researchers to examine how well current mouse models replicate human data sets.

## FINAL REMARKS

In the time remaining, Dr. Drummond acknowledged the work of all the individual research groups involved in RBK and provided

# SCIENTIFIC PRESENTATION

some perspectives on what has made the RBK Consortium work well thus far both scientifically and logistically. These include a highly effective coordinating center that maintains an informative website for the consortium; effective communication across the RBK, including discussion of failures that sometimes leads to solutions; development of new collaborations within the Consortium over time; addition of new groups who bring new technologies and other expertise to the Consortium; and public data-sharing that benefits the entire NIDDK investigator community. In addition, the RBK has a partnership program that actively recruits new labs with grant funding, which, Dr. Drummond noted, recently brought in expertise to advance RBK's research; collaborating projects are brought in as well.

Looking forward, Dr. Drummond noted activities and efforts that could ultimately emerge from RBK's research even though they are not part of its near-term aims, such as drug screens that could yield small molecule therapeutics to help heal damaged tissues, and studies of disease using the organoids. He noted the importance of communication with other organizations who have shared goals in reducing kidney disease. Finally, he expressed his appreciation for the NIDDK program staff and leadership most closely involved in the initiation and continued work of the RBK Consortium to pave the way to regenerative medicine approaches for treating people with kidney disease and damage.

# PATIENT PROFILE

## Charles: Participation in a Pragmatic Clinical Trial To Bring Hope to People Coping with Multiple Chronic Diseases



**Charles**

People who have multiple chronic diseases often lead difficult lives, struggling with effects of these conditions on their health and well-being. “Well, everything with me has slowed down ... I’m not so active anymore,” says Charles, who has type 2 diabetes, hypertension (high blood pressure), and chronic kidney disease (CKD). “I used to be active all day.” In his mid-thirties, Charles was initially told by his doctor that his blood glucose (sugar) levels were elevated. Although he has struggled with his weight, he was

somewhat surprised to learn of this diagnosis because he had always led an active life, playing sports such as football. “I had never been the person who went to the doctor because I was always athletic ... always feeling good,” he explains, “all my life, I always felt good.” As his condition worsened to type 2 diabetes, he began taking insulin to manage his blood glucose levels. In the years that followed, he was also diagnosed with CKD and hypertension. For Charles, coping with these three chronic diseases would prove to be a major focal point in his life. By choosing to participate in the NIH-supported Improving Chronic Disease Management with Pieces (ICD-Pieces) clinical trial, Charles is helping researchers find new pathways to better health for people with these life-altering diseases.

### THE CONTINUAL CHALLENGES OF MANAGING MULTIPLE CHRONIC DISEASES

Charles, now in his late-fifties, has adjusted many aspects of his life to manage these diseases. His health was a factor in his decision to work as a driver for a day care facility, noting that he was limited in what he could physically do. “Standing—I can’t do that ... [lifting] heavy things—I can’t do that either,” he notes, “I’m just able to get by by driving ... so I do that.” However, as is often the case, these three chronic diseases may have been the causes of other health issues that have come up to further complicate Charles’s life. He also has other

# PATIENT PROFILE

conditions, including bone disease and eye-related health issues. A few years ago, Charles faced another serious health scare—he suffered a stroke, which left an enduring mark, still affecting the right side of his body. “My body is breaking down a little bit,” Charles reflects. In order to manage these diseases, he takes different medications that require specific timing during the day. After his stroke, Charles’s long-term memory is not quite what it used to be, so he has come up with a plan to make sure he takes all of his medicines in a timely manner. “I make things simple for myself,” he explains, “everything is laid out for me in a simple location, in a simple bag ... I try to keep it plain and simple for me because I will, I will forget.” Adherence to the proper regimen for each of his medications is just one of the many daily challenges faced by Charles and others with type 2 diabetes, hypertension, and CKD, as well as the spectrum of health complications that these diseases can cause.

*“Well, everything with me has slowed down ... I’m not so active anymore,” says Charles, who has type 2 diabetes, hypertension (high blood pressure), and chronic kidney disease (CKD). “I used to be active all day.”*

## THE ICD-PIECES TRIAL—A PRAGMATIC APPROACH TO CLINICAL RESEARCH

Charles’s struggles illustrate how multiple chronic conditions can exact a serious toll on people’s health and their daily lives. Although research studies over the years have identified potential treatments for these diseases, the application of scientific advances to usual clinical settings has proven difficult. Earlier studies have focused on each disease individually, but not in combination—a complex, clinically important health scenario for many people in the United States. In 2014 the NIH Common Fund and NIDDK began supporting the Improving Chronic Disease Management with Pieces (ICD-Pieces) study—a clinical trial designed to improve health

outcomes in people with coexisting type 2 diabetes, hypertension, and CKD. Four health-care systems, three based in Texas and one in Connecticut, are participating in the study. The study implemented a new technological tool, called “PIECES,” that uses electronic health records (EHRs) in the four participating health care systems. The PIECES platform, developed by Pieces Technologies, utilizes data from EHRs in real time to help researchers and clinicians identify patients managing the triad of chronic diseases, improve data collection, discover complications of the three diseases in patients at an early stage, and coordinate care for study participants. Patients enrolled in the ICD-Pieces trial are assigned to one of two groups. In one group, the participants’ health care does not change, but their outcomes are closely monitored—this will serve as the “control” group. In the second group, the patients’ primary care physicians collaborate with subspecialists, for example, kidney disease specialists (nephrologists); these collaborations are supported by “practice facilitators” who work with physicians to help implement best practices and tailor health care plans based on available resources at each site. Frequency of hospitalization for any reason, over the course of 1 year, is the main, or primary, outcome measured in the study. However, researchers are also evaluating a number of secondary outcomes, including disease-specific hospitalizations, emergency room visits, cardiovascular events (e.g., stroke or heart attack), and death.

*“I make things simple for myself,” he explains, “everything is laid out for me in a simple location, in a simple bag ... I try to keep it plain and simple for me because I will, I will forget.” Adherence to the proper regimen for each of his medications is just one of the many daily challenges faced by Charles and others with type 2 diabetes, hypertension, and CKD, as well as the spectrum of health complications that these diseases can cause.*

# PATIENT PROFILE

ICD-Pieces falls under the “pragmatic clinical trial” category of research studies, which uses real-world, large-scale, often multiple-center health care settings to evaluate different approaches to disease management, leading to findings that are likely to have broader translatability to clinical care. However, in order to conduct a pragmatic clinical trial, several practical challenges unique to these settings must be overcome, such as implementation of appropriate and standardized rules and regulations across the different health care systems. By contrast, more traditional “randomized controlled trials” often occur at a smaller scale and in settings where most variables can be controlled, but findings may not necessarily be as applicable beyond the clinical settings used in the trial. ICD-Pieces plans to enroll more than ten thousand study participants who are already patients across the four participating health care systems. This engagement of diverse health care systems and a broad range of study participants will help expand translation of the research findings.

***Charles remains positive about his circumstances, noting that he didn't choose to have these conditions. “And I can only adapt to it,” he says, “and keep going with my life, and don't give up.”***

Charles, a patient within one of the Texas-based health care systems participating in ICD-Pieces, was identified as a potential candidate for the study. He agreed to enroll and in February of 2018 began his 12-month participation in the trial, in which he continued to receive care from his primary care physician, but also began seeing new subspecialists for his chronic conditions. Other health care

professionals, including a nutritionist and physical therapist, provided additional support and guidance as well.

***Findings from ICD-Pieces and other clinical trials will continue building the foundation for better health in people living with complex multiple chronic diseases. “I keep moving,” Charles reflects, “I keep moving every day, and not let this disease ... get me down.”***

## HOPE THROUGH RESEARCH

Charles struggles daily with the burden of managing the many health conditions he has—the primary effects of which can change over time. “[My back pain] has made it difficult for me to function like a normal person would, all day,” he explains; “now my back is really my biggest problem.” However, Charles remains positive about his circumstances, noting that he didn't choose to have these conditions. “And I can only adapt to it,” he says, “and keep going with my life, and don't give up.” With the invaluable participation of Charles and thousands of others, ICD-Pieces is paving the way to improved treatments for people with type 2 diabetes, hypertension, and CKD, identifying therapeutic approaches that have the highest likelihood of improving health in the real world, not just in an experimental setting. Findings from ICD-Pieces and other clinical trials will continue building the foundation for better health in people living with complex multiple chronic diseases. “I keep moving,” Charles reflects, “I keep moving every day, and not let this disease ... get me down.”