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STUDY SUMMARIES

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(Listed in alphabetical order by title)

A Modified y-Retrovirus Vector for X-Linked Severe Combined Immunodeficiency		
Study findings in non-technical terms	**Specific biological innovation of study**	**Potential impact on clinical care**
Gene therapy—still experimental but beginning to enter the clinic—attempts to utilize advanced molecular methods to treat and even reverse genetic diseases. The field started in earnest approximately 25 years ago and unfortunately has had several setbacks along the way. However, a renewed international collaborative effort—led by Boston Children's Hospital—is providing hope that gene therapy may be a realistic option for treating some of the most devastating illnesses affecting children. The Transatlantic Gene Therapy Consortium (TAGTC) sought to facilitate a more rapid advancement of gene therapy technology by initiating a new effort shortly after the first trials of gene therapy for X-linked Severe Combined Immunodeficiency (X-SCID) reported leukemia as a serious side effect. TAGTC was formed to address this setback, and in doing so appears to have developed safer gene therapy reagents. TAGTC members shared the costs of their development, and continue to implement new gene therapy trials for additional rare diseases across multiple international sites. TAGTC's initial trial in X-SCID opened in 2011, and accrued patients in London, Paris, Boston, Cincinnati and Los Angeles. In a NEJM paper published in the fall of 2014, TAGTC investigators revealed promising initial results from a clinical trial of gene therapy in boys with a subtype of SCID called X-linked SCID (or SCID-X1). The protocol used a genetically-engineered virus to deliver a working copy of the IL2RG gene (which is broken in boys with SCID-X1) into nine boys with the disorder. One to more than three years post-treatment, eight of the nine boys are alive and well, with functioning immune systems and no sign of SCID-associated infections. One of the eight, however, ended up needing a cord-blood stem cell transplant. The boy who died succumbed to an infection before his genetically corrected cells had enough time to fully restore his immune system.	At the trial's heart was a modified virus vehicle—called a viral vector—designed to more safely shuttle the gene into the patient's cells. In a prior SCID-X1 gene therapy trial in Europe—the first to show that gene therapy could cure a human disease—the vector activated cancer-causing genes. In a clinical trial in the fall of 2002, when it was reported that only one boy had leukemia, it seemed a family history of cancer or recent chickenpox infection might have been at fault. But then a second boy developed leukemia and subsequently 5/20 boys in the trial developed leukemias. By October 2003, the research team identified the cause: the vector had integrated into an oncogene called LMO2. Retroviruses normally home to very active genes, oncogenes among them. But the vector used in the first-attempt SCID-X1 gene therapy included DNA sequences that enhanced the oncogene's activity, revving up cell division resulting in the predisposition to leukemia. The goal with TAGTC's trial's improved vector, called a self-inactivating (SIN) gamma retroviral vector, was to reduce or eliminate the risk of leukemia. Specifically, the team tracked where the vectors inserted into the boys' genomes, and when compared to the trajectories of the vector used in the first trials, the restructured vector appears to avoid activating cancer-associated genes. The working hypothesis amongst the international consortium researchers was that the viral sequences that were replaced with human promoter sequences strong enough to make enough of the errant IL2RG gene, would be less likely—if able at all—to ramp up oncogene action. The vector redesign seems to have paid off. While the TAGTC team will continue to monitor each patient for 15 years for signs of leukemia, genetic and molecular studies show no obvious activation of any cancer-causing genes, including those implicated in the last trial.	The Consortium targeted the critical goal of improving the safety of the vector–the virus that delivers genes into cells to correct the genetic defect. In addition to this very specific biological achievement, two important points are emphasized: Firstly, the study validates that safer viral vectors can be designed which maintain efficacy in treating otherwise fatal diseases. This vector design can be applied to a number of other diseases and an application has been filed with the US Food and Drug Administration for the vector to be approved as an Orphan Disease drug for SCID-X1. Secondly, this study emphasizes the collaborative nature of scientific research. Several previous single institution trials have clearly showed the efficacy of the gene therapy approach. However, in pediatric diseases where the research participant base is limited, inter-institutional—and international—collaboration is essential for progress in treating these diseases. The challenges of mounting this kind of collaborative trial are not insignificant. Although the group adopted essentially the same trial protocol, changing only the vector, it took more than two years for the trial to make its way through the regulatory and funding agencies in the United States alone. This included reviews by the National Institutes of Health (NIH) Recombinant DNA Advisory Committee (which included a full public review), the U.S. Food and Drug Administration, local Institutional Biosafety Committees (IBCs) and Institutional Review Boards (IRBs), the NIH Data and Safety Monitoring Board, the National Institute of Allergy and Infection Diseases (NIAID) and NIAID's Division of Allergy, Immunology and Transplantation Clinical Research Committee. In summary, while daunting in some ways, formalized scientific and clinical trials collaborations are critical to developing new therapies, in a timely and cost-effective manner, for some of the terrible genetic diseases that affect children. Boston Children's is participating in a number of other gene therapy trials using this approach, including trials for Wiskott-Aldrich Disease, Childhood Cerebral Adrenoleukodystrophy, and Chronic Granulomatous Disease and is working to open a trial in Sickle Cell Disease later this year.

A randomized trial of protocol-based care for early septic shock		
Study findings in non-technical terms	**Specific biological innovation of study**	**Potential impact on clinical care**
The Protocolized Care for Early Septic Shock (ProCESS) trial found that protocol-based resuscitation did not improve outcomes in patients with septic shock diagnosed in the emergency department. A single-center prospective randomized trial published more than a decade ago in the New England Journal of Medicine demonstrated that a protocol called early goal directed therapy (EGDT) markedly improved mortality in sepsis. EGDT included multiple elements including fluid resuscitation, mandatory measurement of central venous pressure necessitating placement of a central venous catheter, mandatory measurement of central venous oxygenation, medicine to increase blood pressure if necessary, blood transfusion under certain circumstances (using a different transfusion trigger than is used in other critically ill patients), and medicine to pump the heart harder in certain circumstances. It was impossible from this trial to determine which components of EGDT were helpful. The ProCESS trial randomized patients into three groups. These included a) protocol-based EGDT, b) protocol based standard therapy that did not require that placement of a central venous catheter or blood transfusions or medicine to pump the heart harder, and c) usual care. A total of 1341 patients were enrolled. At 60 days, the number of deaths was similar in the three groups (21.0% vs 18.2% vs 18.9%). There was no difference in the need for organ support. At longer follow-up, there was no difference in mortality at either 90 days or 1 year. Patients randomized to EGDT had significantly more costly and invasive interventions – placement of a central venous catheter, transfusion, and medicine to pump the hard harder – but these interventions did not translate into differences in outcomes.	In order to understand the innovation of the ProCESS trial, it is first necessary to understand best practice prior to its publication. The previously published EGDT was widely accepted in the critical care community. Not only did it improve outcomes, but it also made physiological sense. Clinicians were supposed to a) give fluids to a predetermined endpoint, then b) start medicine to bring up blood pressure, then c) check to see if this was associated with appropriate oxygen delivery and if not d) give blood to increase oxygen delivery and e) if still not effective, start medicine to pump the heart more. However, since these were all bundled into a single protocol, it was unclear whether all of these were beneficial since the critical care literature is replete with interventions that theoretically make physiological sense but are not helpful or are harmful when tested in patients. Within that context, the innovation of ProCESS lies in how much of EGDT is not actually beneficial. First, mandatory placement of a central venous catheter (which is painful and carries potential significant risk) is not necessary. Next, mandatory blood transfusion under certain circumstances is not necessary. Next, mandatory medicine to pump the heart harder under certain circumstances is not necessary. Finally, changes in therapy over time have indicated that even having a mandatory protocol to address all of these – as opposed to "usual care" in high level emergency departments is not associated with improved outcome. So although each of these interventions makes theoretical biological sense, their benefit was not shown in patients, leading to an altered understanding of what actually appears to improve outcome in septic patients (timely antibiotics, rapid fluid administration, blood pressure medicine if needed, good supportive care) and what does not improve outcome (everything else tested in the ProCESS trial).	Prior to the publication of ProCESS, guidelines from professional societies including the Surviving Sepsis Campaign included many of the elements of EGDT in recommendations for treating all patients with severe sepsis. Further, the National Quality Forum included some of these elements (placing a central venous catheter, measuring a central venous oxygen saturation) in recently approved bundles for sepsis. Considering these are the first ever approved bundles for sepsis, this had significant impact. Based upon the publication of the ProCESS trial and a subsequent trial (the ARISE trial), the Surviving Sepsis Campaign is currently altering what is appropriate in severe sepsis. Its website now states "required monitoring of central venous pressure and central venous oxygen saturation via a central venous catheter…does not confer survival benefit in all patients with septic shock who have received timely antibiotics and fluid resuscitation...requiring measurement of CVP and ScvO2 in all patients…after initial challenge and who have received timely antibiotics is not supported by the available evidence." The ProCESS trial is assuredly changing the field rapidly such that patients who present to the emergency department or ICU with severe sepsis will likely have certain interventions (central venous catheter, central venous oxygenation saturation measurement, blood transfusion, initiation of medicine to pump the heart more) initiated on a case by case basis rather than in all patients. This will prevent thousands of patients from receiving mandatory interventions that have not been proven to be beneficial while still allowing for them if clinically indicated.

Antidepressant use in pregnancy and the risk of cardiac defects		
Study findings in non-technical terms	**Specific biological innovation of study**	**Potential impact on clinical care**
Clinical depression is diagnosed in 10-15 percent of pregnant women in the United States and increasingly those women are being prescribed antidepressant medications. In 2005, the FDA issued a warning indicating that based on early research results, exposure to commonly prescribed antidepressants may increase the risk of malformations of the heart in the developing fetus. In this new study, we report no substantial increase in the risk of heart malformations attributable to the most commonly prescribed antidepressants in pregnancy. In this large observational study, we analyzed data from 949,504 pregnant women across the United States who were enrolled in Medicaid from three months before conception through one month post delivery, and their live-born infants. We found that 6.8 percent of women used antidepressants during the first trimester, and report that 72.3 per 10,000 infants not exposed to antidepressants were born with a cardiac defect, compared with 90.1 per 10,000 infants who were exposed to the medications. After controlling for depression, depression severity and other potential risk factors, there was no association between use of the most common types of antidepressants and heart malformations in the infants (relative risk = 1.06, 95% confidence interval 0.93-1.22). Moreover, there was no association between paroxetine and right ventricular outflow tract obstruction, and between sertraline and ventricular septal defects; two potential associations that had been of particular concern based on previous studies. In making decisions about whether to continue or discontinue treatment during pregnancy, clinicians and women must balance the potential risks of treatment with the risks of not treating severe depression. Our findings do not provide any evidence that the risk of cardiac malformations should be an important consideration in this decision. The accumulated evidence argues against the existence of important cardiac teratogenic effects for the most commonly used antidepressants.	Our results do not support earlier findings of a spurious association between first trimester antidepressant use and cardiac anomalies in the offspring, specifically paroxetine and sertraline. In contrast to earlier studies, our analysis restricted the cohort to women with a recorded diagnosis of depression, in order to mitigate potential confounding by the underlying psychiatric illness and associated conditions and behaviors, which might increase the risk for structural cardiac malformations through several mechanisms. Smoking, alcohol and drug use, poor maternal diet, obesity, and chronic conditions such as diabetes and hypertension are all more common in depressed patients and are potential risk factors for congenital cardiac anomalies. In addition, depressed, anxious women utilize more health care resources, including ultrasounds, amniocentesis and infant echocardiograms than their healthy counterparts. Hence, there is a higher chance of detecting an infant with a cardiac malformation that might have gone clinically undetected in other women, particularly milder defects such as muscular ventricular septal defect which often close during early childhood. Since treated women are likely to be more complex and more severely depressed, we also adjusted for a large set of predefined and empirical potential confounding variables through conventional and high-dimensional propensity scores, in addition to restricting to women with depression. Although this approach cannot guarantee that all potential confounding is eliminated, it resulted in exposure groups with virtually identical measured characteristics, and moved the risk estimates further toward a null association. This study demonstrates the importance of distinguishing the effects of antidepressants from the effects of the underlying depression when studying the safety of antidepressants during pregnancy, and the feasibility of doing so in the context of large healthcare databases. Moreover, the finding that maternal depression itself appears to confer an increased risk of congenital cardiac malformations suggests that affected women may benefit from antenatal screening tests.	The use of antidepressant medications during pregnancy has increased steadily over time, with reported prevalence of 13% in the US, although pregnant women are systematically excluded from pre-approval randomized trials that help FDA assess the safety of medications. Following the 2005 FDA warning about a potential increased risk of congenital cardiac malformations associated with paroxetine and multiple subsequent studies evaluating the teratogenicity of antidepressants with conflicting findings, pregnant women and their clinicians are left with unclear evidence to help guide their treatment decisions at a most vulnerable time in a woman's live. We designed our study to test the hypothesis of an increased risk of cardiac anomalies associated with first-trimester antidepressant use in a large nationwide cohort, while specifically addressing several biases that may have affected earlier studies. We used the most advanced methods for adjusting confounding by the underlying depression and found that spurious associations with cardiac malformation risk observed in earlier studies disappeared. Decisions by clinicians and women about whether to continue or discontinue treatment with antidepressants during pregnancy must balance potential risks of treatment with the risks of not treating women with severe depression. One of the most concerning adverse effects of medication use during pregnancy is teratogenicity. Our results suggest that concerns about the risk of cardiac defects should not be an important consideration in the treatment decision. The accumulated evidence argues against the existence of important cardiac teratogenic effects for the most commonly used antidepressant medications. This study addresses one important piece of the complex puzzle about the safety of antidepressants during pregnancy. Additional research is needed to evaluate the entire spectrum of risks and benefits of these medications during pregnancy, using sufficiently large cohorts and explicitly accounting for the role of the underlying depression.

Antimicrobial prophylaxis for children with vesicoureteral reflux		
Study findings in non-technical terms	**Specific biological innovation of study**	**Potential impact on clinical care**
Vesicoureteral reflux (VUR, backflow urine with bladder contraction) affects ⅓ of children with a febrile urinary tract infection (UTI) and is associated with an increased risk of recurrent infections and renal scarring. Antimicrobial prophylaxis became standard of care for these children following earlier trials that compared surgery versus antimicrobial prophylaxis and showed no differences in recurrent UTIs and renal scarring. However, lack of placebo or observation groups in these trials precluded determination that either surgery or prophylaxis was effective. Recent randomized trials, mostly unblinded, provide conflicting results regarding the efficacy of antimicrobial prophylaxis in reducing UTI recurrences. The RIVUR study, funded by NIDDK, was designed to avoid limitations of these studies. The study enrolled a representative cohort of 607 children from a variety of clinical settings with VUR (Grade I to IV) diagnosed following UTI, applied stringent diagnostic criteria and used standardized scales to identify predisposing factors. Antimicrobial prophylaxis with trimethoprim- sulfamethoxazole (TMP-SMZ) reduced in half the risk of febrile or symptomatic UTIs (25% vs. 13%). Differences were apparent early on and increased over the 2 years. Differences consistently favored prophylaxis, irrespective of sex, age, degree of VUR and whether the index UTI was caused by a sensitive or resistant pathogen. Children with a febrile UTI and those with bladder and bowel dysfunction (BBD) at baseline derived particular benefit from prophylaxis, with reductions in recurrences of 60% and 80%, respectively. Rates of new renal scarring at 2 years were low and were not reduced by prophylaxis (8%), perhaps because children were enrolled mostly after their first infection and because parents were instructed to be vigilant and seek early medical attention. Not unexpectedly, recurrences in children receiving prophylaxis were more likely to have been caused by resistant pathogens (63% vs. 19%). VUR resolved in 50% of children by 2 years.	**Prevention of recurrent UTIs** - Uncertainty regarding the efficacy of antimicrobial prophylaxis was resolved by the RIVUR study. This study demonstrated that antimicrobial prophylaxis is a safe and effective measure to prevent recurrent UTIs in children. Prophylaxis reduced recurrences irrespective of sex, age, degree of reflux, number of UTIs, or index infection pathogen. Although children with grade III-IV VUR had more recurrences, children with grade I-II derived the most benefit from prophylaxis. **Subgroup analyses** – Children with bladder and BBD and children whose index UTI was febrile had high levels of reduction in UTI recurrences when receiving antimicrobial prophylaxis (80% and 60%, respectively). The RIVUR study highlighted the importance of screening for BBD (>50% of toilet-trained children at entry). **Antimicrobial resistance as secondary outcome** – UTI with resistant E. coli occurred more frequently among children receiving prophylaxis (63% vs. 19%). Breakthrough UTIs while on prophylaxis are usually caused by bacteria resistant to the prophylactic antimicrobial, and are treated with a different drug. This needs to be addressed with families in the context of reduced recurrences. **Prevention of UTI recurrences regardless of VUR resolution** - VUR resolved in 50% of children by 2 years. The reduction in UTI recurrences favored prophylaxis whether VUR had resolved, improved, not changed, or worsened. **Accurate, standardized and central interpretation of kidney scans for renal scarring** - Although not a primary measure of outcome for the RIVUR study, no difference in overall, severe and new renal scarring between groups was noted. **Ancillary studies** - The RIVUR study enabled 3 ancillary studies evaluating (a) risk factors for renal scarring, (b) an intervention to enhance recruitment in multicenter trials, and (c) reasons for parents to agree/decline participation, which will inform patient recruitment in future large scale pediatric clinical trials.	**Prophylaxis** - Experts have long disagreed about the importance of VUR, its management, and its causal effect on the occurrence of UTIs in young children. The RIVUR study findings allow clinicians to confidently prescribe antimicrobial prophylaxis with TMP-SMZ to decrease the risk of recurrent febrile UTI in children with VUR, particularly for those with a history of febrile UTI or those with BBD. **BBD** - Careful evaluation and aggressive management of BBD, including treating constipation, timed voiding, double voiding, and adequate hydration should be integrated into mainstream primary care of young children, even before a diagnosis of febrile or symptomatic UTI. **Resistance** - Because of concerns about the emergence of bacterial resistance, UTIs that lead to the diagnosis of VUR and institution of prophylactic antimicrobial therapy need to diagnosed using stringent criteria as in the RIVUR study, including fever or symptoms related to the urinary tract, the presence of inflammatory response (pyuria, or white cells in the urine), and single primary pathogens at appropriate colony counts in properly collected urine specimens. **VUR resolution** - Although the standard of care for management of VUR has been to repeat the VCUG annually, the RIVUR study found approximately a 50% resolution of VUR at 2 years. Accordingly, it would be reasonable to consider repeating the VCUG every 2 years to decrease children's exposure to an uncomfortable procedure and unnecessary radiation, and reduce parental anxiety. **Renal scarring** - The RIVUR study showed no differences between groups in overall, severe or new renal scarring. While reassuring, this finding also highlights the importance of increased awareness about early diagnosis and treatment of young children with febrile or symptomatic UTIs in reducing the likelihood of renal scarring.

Detection of a Genetic Abnormality (DNAJB1-PRKACA Chimeric Transcript) in Fibrolamellar Hepatocellular Carcinoma		
Study findings in non-technical terms	**Specific biological innovation of study**	**Potential impact on clinical care**
Fibrolamellar hepatocellular carcinoma (FLHCC) is a pediatric cancer of the liver. One year ago, little was known about it: Was it one disease or many? Was it a variant of hepatocellular carcinoma or its own disease? Was it genetically inherited, induced by virus, or other origin? The symptoms for fibrolamellar are non-specific, such as weight loss and abdominal pain, and there are no useful biomarkers (1). Even with a biopsy, the diagnosis of FLHCC is not unambiguous (2). As a result, FLHCC is often diagnosed fairly late, after the tumor has metastasized. Before metastasis, FLHCC can be completely resected, but otherwise effective therapeutic options are limited; FLHCC does not respond to chemotherapy (3-5). This study, which is the first to characterize the basis for the disease, found the chromosomes in the tumorous tissue were unremarkable. There were none of the expected deletions (where part of a gene is missing), amplifications (where part or all of a gene is repeated), or inversions. The one exception was a deletion in one copy of one of the chromosomes which joined the front end of one protein involved with cell maintenance onto the bulk of the body of a protein that regulates many important cellular function. This chimera was fully active, losing much of the regulation of its activity. In the initial report which is the basis for this nomination (6) this chimera was found in 15 out of 15 patients. Subsequently the results were repeated 119 out of 119 patients and reproduced in labs from Stanford (7), University of Washington, Washington University, and others. The results have helped answer many of the unknown questions about FLHCC, the results are the basis for two clinical trials under consideration at the FDA, and they have informed the design of the first blood tests for FLHCC.	There are many levels of innovation in this study which depended upon a few critical decisions. The first was choosing a pediatric cancer. This was done in the belief, subsequently substantiated by this group's work, that there should be very few mutations in the somatic cells and few mutations in the tumor, given the age of the patients. The extraordinarily low level of mutations (8) made it relatively easy to pick out the one genomic alteration of consequence: A deletion in one copy of chromosome 19 that formed a fusion between the heat shock protein (DNAJB1) and the catalytic subunit of protein kinase A (PRKACA) (6). The second decision was the choice of a rare disease. Studying a rare disease is often problematic: Any one institution sees few patients and it is difficult to get samples and difficult to get a critical mass of data about the disease. The advantage of a rare disease is that it is often distinguishable enough from other diseases, thus there is a better chance of getting a more homogeneous population. This choice was validated by the demonstration that every patient tested has the same genetic alteration in the tumor. To overcome the limitation of a rare disease, the investigators made their third critical decision to partner with patients. This partnership resulted in patients recruiting other patients to donate tissue, to donate funds and even to work in the lab. The group is now receiving over 50% of all patient's samples in North America. A fourth critical decision was the choice to do a whole genome analysis, rather than just exome, and RNA-seq on both coding and non-coding RNA. This has allowed them to identify a novel chimera that otherwise would not be detectable over a background of normal copies of the genes.	These findings have totally changed our understanding of FLHCC and are contributing to the first ever change in patient care. The work has altered our understanding by answering the key unknowns of FLHCC. First, as a result of the work of this group it is now believed that FLHCC is single disease. The ubiquity of the DNAJB1-PRKACA chimera (6) in the background of no other changes in the genome (8), and the consistency of the changes of gene expression in tumor tissue (Simon, submitted) suggest that FLHCC is a single disease rather than a collection of diseases that happen to affect the same organ. Second, the work demonstrates that the molecular pathology of FLHCC, as well as the changes of transcription, are distinct from HCC. Third, the results show that the disease is not genetically inherited: The alteration in the DNA was found only in the tumor and not in the adjacent liver tissue. This suggests that if the tumor could be completely resected, it should not recur. The work is also impacting patient care. First, many clinicians are no longer trying drugs that are appropriate for other cancers (e.g. hepatocellular carcinoma) that were thought to be similar to FLHCC. Second, the work has led to the development of blood tests for the disease. Different tests are currently being tested for their ability to detect the chimera in the blood and the urine. An effective test can be used diagnostically, to hopefully catch the disease at earlier stages before metastasis. The text can also be used prognostically to follow the efficacy of the treatments that have been developed based on this work.

Door-to-Needle Times for Tissue Plasminogen Activator Administration and Clinical Outcomes in Acute Ischemic Stroke Before and After a Quality Improvement Initiative

Study findings in non-technical terms	Specific biological innovation of study	Potential impact on clinical care
The only proven drug treatment for acute ischemic stroke is the clot-dissolving medication tissue plasminogen activator (tPA). As the benefits of tPA are time-dependent, national guidelines recommend that treatment be started within 60 minutes of patient arrival, i.e. that the "door-to-needle" (DTN) time be 60 minutes or less. However, fewer than 30% of US patients were being treated within this time window. Target: Stroke was therefore launched as a national quality improvement initiative to improve DTN times for tPA administration, targeting DTN times of under 60 minutes in 50% or more of patients. The Target: Stroke initiative disseminated 10 care strategies to achieve faster DTN times for tPA administration, provided clinical decision support tools, facilitated hospital participation, and encouraged sharing of best practices. The study population included 71,169 patients with acute ischemic stroke treated with tPA at 1030 US hospitals prior (2003-2009) and after (2010-2013) launch of the Target: Stroke initiative. Measures of DTN time for tPA administration improved significantly during the postintervention period compared with the preintervention period. The annual rate of increase in the proportion of patients with DTN times of 60 minutes or less heightened from 1.4% to 6.2% per year. As a result, during the intervention period, the overall proportion of patients treated within 60 minutes increased from 27% to 53%. The improvement in guideline-recommended door-to-needle times were observed among clinically relevant subgroups of patients, including men and women; patients older and younger than the median age of 72 years; white, black, and Hispanic patients; and patients with greater and lesser stroke severity. Clinical outcomes improved concurrently with the accelerated treatment. In hospital death rates declined from 9.9% to 8.3%. Symptomatic brain bleeding declined from 5.7 to 4.7%. Greater functional independence, permitting discharge directly to home, increased from 37.6% to 42.7%.	This study applied to reengineering of clinical practice insights from biological studies in preclinical stroke models and in human imaging studies and randomized trials. In acute ischemic stroke, a clot blocks a blood vessel, cutting off the flow of oxygen and nutrients to the region of the brain the vessel supplies. The longer the artery remains occluded, the more brain tissue that will be lost. In a typical ischemic stroke, every minute that goes by without reopening of the artery, 2 million nerve cells are lost and 14 billion synapse (connections between nerve cells) are lost. Because time lost is brain lost, it is crucial to accelerate the start of thrombolytic tPA therapy. The study disseminated multiple methods to accomplish the neurologic evaluation and brain imaging of patients more quickly, including having paramedics provide pre-arrival notification to the hospital that they were on their way with a stroke patient, having single group page to activate all stroke team members, and pre-mixing lytic drug even before brain imaging was completed. The study also provided several new biological insights. The finding that outcomes improved more for ischemic stroke patients treated with tPA than for other types of stroke patients provided evidence that faster reperfusion was the direct cause of the improved clinical outcomes. The study's large size enabled it to demonstrate that improvements in tPA treatment speed not only reduced patient disability, as suggested by clinical trials, but also lowered bleeding and mortality rates, effects which clinical trials were underpowered to detect.	Stroke is the second leading cause of death and a leading cause of disability worldwide. The study demonstrated a direct and substantial impact on patient care of the national quality improvement campaign. At the study hospitals, during the intervention period, there was a 1.87% absolute mortality reduction, which equals 977 lives saved. There was 5.70% absolute increase in patients able to be discharged home (rather than less independent and more costly locations like skilled nursing care facilities), which equals 2977 extra patients being able to be discharged home. And there was a 2.4% increase in the proportion of patients treated with tPA at all, which translates into 5,883 patients with reduced long term disability. Prior to performance of this study, there were concerns that attempting to achieve shorter DTN times might lead to rushed assessments, inappropriate patient selection, dosing errors, and greater likelihood of complications; instead, the study found that more rapid reperfusion therapy in acute ischemic stroke is feasible and could be achieved not only without increasing rates of symptomatic intracranial hemorrhage, but with actual reductions in complications. Lastly, the success of this study in stroke suggests the potential of quality improvement campaigns to beneficially affect care for many conditions nationally, when the initiatives are integrated into an environment that includes explicit goals; collaborative, interdisciplinary teams; a patient focused organizational culture; engaged clinical leaders and senior management; and detailed data feedback.

Multiplatform analysis of 12 cancer types reveals molecular classification within and across tissues of origin		
Study findings in non-technical terms	**Specific biological innovation of study**	**Potential impact on clinical care**
Tumor classification is an essential first step in determining treatment options; many different features are used to characterize tumors, including tumor genetics and the tissue site where the tumor arises, e.g., breast, lung, or ovary. In fact, tissue of origin is one of the most important clinical determinants for treatment options. Recent genomic studies have revealed that very different tumors, with different genetics and behavior, can arise within a single tissue site. In the Hoadley et al. paper submitted for this award, the authors developed an entirely new method for classifying cancers based upon several new cutting edge technologies combined together. This new approach placed the tumors into groups, or "subtypes," based on similar features. The resulting subtypes included many known tumors that were similar to their tissue of origin, but many other tumors were new and showed both differences within a tissue site and similarities across different tissue sites. For example, tumors from the colon and rectum were more similar and grouped together, while breast cancer separated into two distinct tumor subtypes. The authors also found that some tumors from the head and neck, lung, and bladder were more similar and grouped together across these different tissue of origin sites. These new subtypes provided valuable new clinical information that can be used to improve patient prognosis. The new tumor classification suggests that the tumors of 10%-20% of patients may be erroneously classified, and thus these patients may be receiving sub-optimal therapies. If validated with new groups of patients, reclassifying the tumors of 10% to 20% of patients could have a profound effect upon personalized medicine and should improve patient outcomes.	The innovations of this study were two-fold. First, this was the first "pan-cancer" study, which used >3500 samples and 6 different genomic technologies (gene expression, DNA mutations, DNA copy number changes, DNA methylation, microRNA expression, and proteomics) to arrive at a novel classification of twelve cancer types at once. The authors used newly developed statistical and bioinformatics methods to develop a robust and objective classification schema that builds upon the genome-wide strengths of the 6 powerful genomic platforms used. Second, the new classification system, cluster-of-cluster assignments (COCA), identified 11 broad tumor subtypes where some previously distinct tumor types merged into a single group (colon and rectum converged), while other single tissue of origin groups diverged (bladder split into three main groups and breast split into two groups). Each of the 11 COCA Super Groups were defined by distinctive genetic events (both DNA mutations and Copy Number Changes), and each showed a predictable prognosis. For example, the Colon and Rectum COCA Group was defined by APC mutation/loss, and the Squamous COCA Group composed of squamous cancers of the lung, head and neck, and bladder was defined by TP53 loss and TP63 gain/amplification. Furthermore, the bladder cancers that clustered with the squamous samples had a worse survival outcome than bladder cancers that were classified into other COCA groups. Even across COCA groups we found similarities. For example, TP53 mutation and genome-wide copy number aberrations were seen in the squamous, basal-like breast, and ovarian groups. Each of our 6 genomic and epigenomic data platforms helped characterize the COCA subtypes and promoted the idea that an integrative approach is required to fully understand cancer.	Cancer patient care is based upon many features, including tumor histology, stage (node and tumor size), molecular markers, and the body site of tumor origin. Recent genomic studies have revealed that in some single tissue sites, different cancers can arise, suggesting that reliance on an anatomic tissue of origin classification may be flawed. Hoadley et al. confirmed this suspicion and suggested that as many as 10%-20% of the tumors studied might have been erroneously classified. This simple potential mis-classification could have a profound effect upon patient care since many treatments are still offered based solely upon tissue of origin. For example, the majority of brain tumors do not originate in the brain, but instead are metastases derived from the breast or lung. Therefore, the development of a multi-platform, biologically based tumor classification system for cancer patients that is based upon tens of thousands of data points, rather than a few biomarkers such as IHC, is an advancement that could profoundly impact the cancer clinic. The novel "COCA subtype predictor" described in the submitted publication is being further validated on additional data sets, and the set of features reduced to the minimal number needed for classification, which would make it easier to implement the system in CLIA compliant pathology laboratories. We are also increasing the number of tumor types to over 30 in the next phase of analysis. By understanding the similarities and differences of tumors within and across tissue sites, we may be able to leverage effective treatment information to tumors with similar characteristics and try alternative treatments for tumors that diverge from tumors of the same anatomical site. If validated, this classification could be used on all solid tumor type cancer patients, and it might change the standard of care treatments for tens of thousands of patients each year.

Predictive correlates of response to the anti-PDL1 antibody MPDL3280A in cancer patients		
Study findings in non-technical terms	**Specific biological innovation of study**	**Potential impact on clinical care**
In this study, treatment with an investigational immune checkpoint inhibitor led to consistent responses across multiple types of cancer (including melanoma and cancers of the lung, kidney, colon, GI tract, and head and neck), particularly in patients with suppressed immune systems that appeared to be reinvigorated by the drug. Tumors were evaluated for expression of PD-L1, a protein expressed by many tumor types that acts to halt the immune system from attacking cancer cells. Of the 175 patients enrolled, 21% showed partial or complete response to the therapy, with some being rapid and durable. Across all tumor types, 46% of patients with high PD-L1 expression on non-tumor immune cells showed a partial or complete response.	In recent years it has become clear that modulating the immune system can be an effective cancer therapy, however the understanding of human cancer immunology is incomplete. This study focused on understanding the biomarkers related to the PD-L1 pathway that protects tumors from the immune response utilizing an investigational monoclonal antibody that specifically binds to PD-L1. It identified markers to predict which tumors will respond to treatment with this agent and which will not. The study differed from other immune therapy trials in that it incorporated serial biopsies before and during treatment to identify a tumor profile that predicts response to treatment. Although it was known that expression of PD-L1 in tumor cells is critical in blocking the immune system, it was not known that expression of PD-L1 in non-tumor cells such as macrophages also predicted drug response.	This study shed light on enhancing the immune response in those responding to immune therapy and overcoming resistance in those who don't respond. The identification of markers to predict which patients may or may not respond to treatment is a compelling finding. Furthermore, these findings have broad applicability across many types of tumors, as illustrated by this study and additional findings published simultaneously on the response of advanced bladder cancer patients who did not respond to other treatments. The findings of this work can be applied to both advanced cancers and for use in frontline therapy for primary and metastatic disease.

Upper-Airway Stimulation as a Novel Treatment for Obstructive Sleep Apnea		
Study findings in non-technical terms	**Specific biological innovation of study**	**Potential impact on clinical care**
Obstructive sleep apnea (OSA) is a chronic condition characterized by frequent upper airway collapse at the base of the tongue and soft palate during sleep, followed by arousal to resume breathing. OSA affects 18 million adult Americans. OSA decreases quality of life and productivity, and increases risks for drowsy driving, high blood pressure, heart failure, stroke, diabetes, and all-cause death. Treatment with positive airway pressure by a facemask (PAP) can be transformative, restoring alertness and quality of life, and reducing rates of hypertension, stroke, and all-cause death. Unfortunately only 50% of those with moderate to severe sleep apnea will use PAP therapy. For these persons, there are limited and less predictable options. This nominated paper describes the Stimulation for Apnea Reduction (STAR) trial, designed to test safety and effectiveness of a novel pacemaker-like device. This trial recruited participants with moderate to severe OSA who had failed CPAP treatment. The device consists of a pacing wire attached to one nerve to the tongue (the hypoglossal nerve), a breathing sensor placed in the chest, and a pulse generator, all placed under the skin and turned on and off at night by a hand-held remote. Triggered by breathing, a mild electrical stimulation to the nerve activates the tongue muscle to open the airway. Twelve months after implant, all participants were using the device over 85% of the time. There were no device related major adverse events. Sleep apnea was reduced approximately 70%. Participants (and spouses) reported improved sleep quality, and improved daytime sleepiness into the normal range. Stopping stimulation for one week showed return towards baseline levels of OSA severity, and bothersome snorts and snoring. Follow-up studies continue to show benefit and safety. The findings were a crucial part of FDA assessment of the device, and its approval for clinical use in 2014.	Like diabetes or asthma, adult sleep apnea is a complex disease. There are no unique genetic causes, and variable endotypes and consequences. One can acquire sleep apnea alone or in combination through four general pathways- a collapsible upper airway, an unstable breathing pattern (high loop gain), a low arousal threshold (awakening before stable sleep is achieved), and inadequate muscle activation or recruitment- with each pathway having genetic, developmental, and physiologic controllers. This study was based on a fundamental observation that the beginning of an apnea (cessation of breathing) or hypopnea (partial collapse) occurs during sleep with a reduction in neural drive. Reduced activation leads to multi-site collapse of the upper airway at the back of the tongue (oropharynx) and/or behind the soft palate (velopharynx). This study shows that direct stimulation of the hypoglossal nerve which activates the protrussor muscle of the tongue (the genioglossus) can be effective treatment not only by enlarging the oropharynx but the velopharynx as well. Therefore, sleep apnea can be prevented by neural stimulation alone, largely independent of changes in weight, head form, or neck size. This therapy prevents multi-site airway collapse during sleep. These findings shift the field of discovery. First, one is a new focus on the soft palate and velopharynx (rather than the tongue) as key feature in how the upper airway collapses during sleep and how this site opens in response to neuromuscular stimulation of a hypoglossal nerve. The neuromechanical linkages that predisposes to airway collapse during sleep probably came about secondary to human expressions in speech and song. Second, nerve activation alone can be a transformative treatment in a subset of sleep apnea patients and provides an avenue for drug discovery- pharmacologic manipulations which could keep the airway awake while letting the brain sleep.	Phasic stimulation of the genioglossal nerve gated with inspiratory efforts during sleep represents a new class of therapy for patients with moderate to severe OSA who cannot accept or adhere to positive airway pressure therapy. This is a patient population particularly vulnerable to cardiovascular events and car crashes. The other secondary tier treatments- anatomic surgery or oral appliances- either are cumbersome, expensive, or less predictable. As a result, patients frequently go untreated and are at continued risk for adverse medical outcomes. By augmenting neural output to the genioglossal muscle, airway collapse is prevented because caliber is increased directly at the level of the retrolingual airway (oropharynx) and via mechanical coupling at the level of the soft palate (velopharynx). Economic modeling of benefit has demonstrated a favorable lifetime discounted Cost/QALY at approximately $40,000 (US), largely because of reduced mortality and morbidity (hypertension, heart failure, stroke) from treated apnea. This incremental cost effectiveness associated with the neurostimulation system compares favorably with lifetime discounted Cost/QALY associated treatment of other common chronic conditions such as medications for hypertension, and insulin pumps and monitoring for type II diabetes. Alternatives to standard positive airway pressure therapy have not been uniformly effective and can be associated with increased morbidity and cost. These include oral appliance therapy and upper airway bypass or reconstruction. As opposed to neurostimulation, upper airway reconstruction results in irreversible changes of the upper airway structures. Attempts at drug therapy have been inconsistent; however, the success reported in isolated patients should now be reexamined knowing what this shows about the effects of increasing neural drive. In summary, gated phasic neurostimulation of the upper airway provides a clinically and cost effective option to at risk patients who are unable to accept or adhere to positive pressure therapy for OSA.

Use of gene editing in humans to block HIV without drugs		
Study findings in non-technical terms	**Specific biological innovation of study**	**Potential impact on clinical care**
The current standard of care for HIV infection relies on a maintenance strategy of daily antiretroviral drugs designed to reduce viral replication and keep the infection in check. There are approximately 30 antiretroviral drugs approved by the FDA and almost all are designed to inhibit some stage of the pathway of viral replication. Currently available drugs do not cure HIV infection or AIDS. They can suppress the virus, even to undetectable levels, but they cannot eliminate HIV from the body. Hence, people with HIV need to take antiretroviral drugs on a daily basis which is costly and can have significant undesirable side effects. There is no therapeutic approach available which protects CD4+ T-cells, reduces viral load and does not require continuous daily dosing. Thus, the unmet medical need is to develop a functionally curative approach for HIV/AIDS, so that the patient does not have to take daily medications and is unable to transmit the infection. The study "Gene Editing of CCR5 in Autologous CD4 T Cells of Persons Infected with HIV", which appeared in the New England Journal of Medicine, along with an accompanying editorial, is the first published report of any gene editing approach in humans, a long sought holy grail of genetic engineering. Carl June and a team of University of Pennsylvania researchers reported the successful genetic engineering of the immune cells of 12 HIV positive patients to resist infection. The approach used zinc finger nuclease technology, which function as a "molecular scissors," to mimic the CCR5-delta-32 mutation, which is naturally occurring variation that provides a natural resistance to the virus, but is only found in 1 percent of the general population. By inducing the mutations in the ccr5 gene, there was reduced the expression of CCR5 surface proteins, rendering the patients' cells resistant to infection.	In preclinical studies, June's laboratory along with scientists at Sangamo Biosciences designed Zinc Finger Nuclease (ZFN) pairs consisting of two 4-finger proteins that bind to a target site within the human chemokine receptor 5 gene (CCR5). ZFNs are artificial restriction enzymes that can be designed to cleave DNA at specific sites. In preclinical tests, CCR5-modified CD4 T cells were protected from human immunodeficiency virus (HIV) infection, and reduced HIV RNA levels in a humanized mouse model involving xenotransplantation of HIV infection (Perez EE,...June CH. Establishment of HIV-1 resistance in CD4+ T cells by genome editing using zinc-finger nucleases. Nat Biotechnol. 26:808-16, 2008). Therapies based on the CCR5 have gained interest after a man known as the "Berlin Patient" was "functionally" cured after a stem cell transplant from a donor who had CCR5 mutation in both alleles. June's team is attempting to replicate this phenomenon using autologous cells because a bone marrow transplant is not a practical solution for HIV patients who do not have blood cancers. In the current study, to knock-out CCR5 in autologous CD4 T cells, June's team used an adenoviral vector constructed to express ZFNs. Since mutations are commonly induced during the natural repair of these breaks, it becomes possible to disrupt the ability of the targeted allele to make a functional protein, in this case CCR5. 12 patients were infused and CD4 T cell counts increased in all participants. After 36 weeks of follow up, the median increase was 615 cells. The genetically modified cells persist in vivo with a half-life of nearly a year; they also appear to be protected from HIV infection, because when antiretroviral therapy is stopped, they are depleted at a slower rate than are unmodified cells. In summary, this is the first example in humans that targeted gene modification can be used to knock-in a disease resistance gene.	According to UNAIDS/WHO, over 2.5 million people were newly infected with HIV in 2011 with an estimated 1.7 million people dying of AIDS in the same year. There are now over 34 million people living with HIV and AIDS worldwide. The CDC estimates that, in the United States alone, there were 1.2 million people living with HIV/AIDS, approximately 50,000 new infections and 21,000 deaths in 2009. Therefore the development of functionally curative approaches for HIV/AIDS would be a substantial advance. An accompanying editorial in the New England Journal of Medicine (PMID 24597871) had the following concluding paragraph: "This proof-of-principle study is an important first step, not just in the treatment of those infected with HIV but also for genome editing in a broader sense…The potential future of gene knockout by ZFNs and other techniques is not restricted to HIV infection. There are now methods that can be used not only to inactivate a gene but also to make specific nucleotide changes in a specific site in the genome and gene addition. These methods will be useful in fixing genes that contain harmful mutations and in supplying therapeutic proteins. Through repeated trips from bedside to bench and back again, it is likely that these approaches represent a basis for effective future therapeutic interventions." Thus, targeted gene editing has the potential to cure a number of monogenic congenital diseases such as the hemoglobinopathies and hemophilia. The data from the study using CCR5 specific ZFNs to edit CD4 cells from patients with HIV infection showed that genome editing of human cells was safe and associated with an acceptable adverse-event profile and that the cells persisted in vivo. This study paves the way for future studies to generate genetically edited cells for hemoglobinopathies and hemophilia.