Exploring the State of the Science in the Field of Regenerative Medicine

CHALLENGES OF AND OPPORTUNITIES FOR CELLULAR THERAPIES

Proceedings of a Workshop

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Forum on Regenerative Medicine

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BOXES

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Acronyms and Abbreviations

$A_{2A}R$	adenosine A2A receptor
AAV	adeno-associated viruses
ADPKD	autosomal dominant polycystic kidney disease
ALS	amyotrophic lateral sclerosis
AMD	age-related macular degeneration
CADUCEUS	Cardiosphere-Derived Autologous Stem Cells to Reverse Ventricular Dysfunction (trial)
CAR	chimeric antigen receptor
CDC	cardiosphere-derived cell
CF	cystic fibrosis
CNS	central nervous system
CRISPR	clustered regularly interspaced short palindromic repeat
DEB	dystrophic epidermolysis bullosa
DMD	Duchenne muscular dystrophy
DNA	deoxyribonucleic acid
ESRD	end-stage renal disease
FDA	Food and Drug Administration
GFR	glomerular filtration rate
GMP	good manufacturing practice

GMT	Gata-4, Mef2c, and Tbx5
GVHD	graft-versus-host disease
hESC	human embryonic stem cell
HIV	human immunodeficiency virus
HLA	human leukocyte antigen
hPS	human pluripotent stem (cell)
HSCT	hematopoietic stem cell transplantation
HSPC	hematopoietic stem/progenitor cell
HuCNS-SC	human neural stem cell
huKO	humanized Kusabira–Orange
IMPD	investigational medicinal product dossier
IND	investigational new drug
IPF	idiopathic pulmonary fibrosis
iPS	induced pluripotent stem (cell)
ISSCR	International Society for Stem Cell Research
LEAES	LZRSE-Col7A1 engineered autologous epidermal sheets
MLV	murine leukemia virus
MRI	magnetic resonance imaging
PERV	porcine endogenous retrovirus
PKD	polycystic kidney disease
PPMD	Parent Project Muscular Dystrophy
RCS	Royal College of Surgeons
REGROW	Reliable and Effective Growth for Regenerative Health Options to Improve Wellness
RNA	ribonucleic acid
ROI	return on investment
RPE	retinal pigment epithelium
RPESC	retinal pigment epithelial stem cell
SNP	single nucleotide polymorphism

UK	United Kingdom
VST	virus-specific T cell
X-linked SCID	X-linked severe combined immune deficiency
ZFN	zinc finger nuclease

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Introduction[1]

Regenerative medicine holds the potential to create living, functional cells and tissues that can be used to repair or replace those that have suffered potentially irreparable damage due to disease, age, traumatic injury, or genetic and congenital defects. The field of regenerative medicine is broad and includes research and development components of gene and cell therapies, tissue engineering, and non-biologic constructs. Although regenerative medicine has the potential to improve health and deliver economic benefits, this relatively new field faces challenges to developing policies and procedures to support the development of novel therapies are both safe and effective. Additionally, there is hope that in light of increasing health care costs, regenerative medicine therapies may help reduce the total costs of patients care, even if the treatments are expensive at the outset.

The potential applications of cellular therapies are broad, ranging from the use of islet cell transplantation and regeneration to cure type 1 diabetes, to regenerative neurobiology approaches to treat injuries and degenerative diseases like spinal cord injury and amyotrophic lateral sclerosis (Feldman et al., 2014; Shapiro et al., 2000). Other areas of ongoing cellular therapy research include restoring vision through cell regeneration in the retina, repairing or restoring function in the musculoskeletal system, and improving or restoring function in various organs, including the heart, liver, lungs, and kidney (Trounson and

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McDonald, 2015; Tsukamoto et al., 2016). Although novel research findings in regenerative cellular therapies are promising, it is possible that they might benefit from a deeper understanding of basic biological concepts underlying the differentiation, engraftment, behavior, and survival of implanted cells in vivo. Such knowledge may help researchers address the scientific and technical hurdles related to assessing and ensuring successful long-term outcomes of cell therapies, controlling cell differentiation, and refining processes for production on a scale that is commercially sustainable and yields quantities of product that have the potential to be clinically effective. Some areas of regenerative medicine, such as hematopoietic stem cell transplants for the treatment of blood cancers, have experienced success in treating patients, and lessons and best practices could potentially be applied to other areas of research (Chhabra et al., 2016). It is also possible that increased communication and collaboration across fields may facilitate the sharing of these lessons to inform and advance ongoing research.

Current cellular therapies in regenerative medicine use several delivery approaches, including the introduction of cells that have been modified, expanded, or genetically manipulated into diseased tissue, with or without supporting biologic or nonbiologic materials such as key signaling molecules or scaffolding to facilitate the delivery and success of these therapies. (Researchers use the term "expand" to mean increase the number of cells through cell division.) Much of the research on these approaches is carried out using in vitro or rodent models, neither of which closely mimics the complex environment of the human body. Translating promising research from these models into clinical studies is a challenging process, and, as the field matures, there may be opportunities to develop guidelines for the safe and proper use of regenerative medicine advances, to highlight translational barriers, and to explore the regulatory environment. Potential challenges surrounding the process of characterizing cells and defining the critical quality attributes that cells and cell products must meet throughout the regulatory and manufacturing processes and maintain over time, also exist. Additionally, the study and use of regenerative medicine therapies are complicated by the ethical and social debate surrounding the use of adult, embryonic, and induced pluripotent stem (iPS) cells as well as cell products such as exosomes and hybrid devices.

BOX 1-1
Objectives of the Workshop

- To examine the state of the science for therapies that generate, repair, or replace tissues by convening scientists, clinicians, industry, patient experts, and other stakeholders.
- To highlight the challenges, successes, and lessons learned with respect to the translation of regenerative therapies from early discovery into clinical practice with the goal of reaching patients.
- To illuminate the next steps for the field and ways that the Forum on Regenerative Medicine could be a facilitator of progress.

During the first half of 2016, the National Academies of Sciences, Engineering, and Medicine's Health and Medicine Division launched the Forum on Regenerative Medicine to highlight and discuss important scientific and policy issues that are emerging in this relatively nascent field. The forum members spent time considering many of the challenges facing the field.

On October 13, 2016, the Forum on Regenerative Medicine hosted its first public workshop with the goal of developing a broad understanding of the opportunities and challenges associated with regenerative medicine cellular therapies and related technologies. Stakeholder groups, including research scientists, clinicians, and representatives from patient groups and industry, presented their perspectives and participated in discussions during the workshop, which focused on an exploration of the state of the science of cell-based regenerative therapies within the larger context of patient care and policy. The specific workshop objectives are listed in Box 1-1. The statement of task for the workshop may be found in Appendix C.

OVERVIEW OF THE WORKSHOP

In order to demonstrate the breadth of the field and highlight advances in areas that are further along than others in terms of developing therapies that are safely available for patients, each session of the workshop focused on a different tissue or organ system: skin and musculoskeletal tissues, hematology and immunity, neurological and ophthalmological tissues, cardiovascular and lung tissues, and renal tissues. In each session, experts discussed the state of the science of regenerative medicine

in that particular area of research and identified challenges and successes. Several sessions also included a patient perspective. Following the presentations in each session, the speakers convened as a panel, highlighting common themes that had emerged during the day and reflecting on ways to address challenges and move the field forward in order to bring new therapies to patients. Although the presentations were divided by tissue area, the workshop was designed to encourage cross-fertilization and to highlight shared challenges. Cynthia Dunbar, the workshop co-chair and president of the American Society of Gene and Cell Therapy, asked presenters and participants to focus on describing the gaps in basic scientific knowledge, identifying resources that did or would help move regenerative medicine forward, and discussing common challenges such as reproducibility and standardization.

There are a number of factors that are coming together to increase the power and success of regenerative medicine, said Lorenz Studer, the keynote speaker and director of the Center for Stem Cell Biology at the Memorial Sloan Kettering Cancer Center. First, he said, while existing therapies such as bone marrow transplantation and skin grafts have shown what is possible for cell-based therapies, a number of therapies are on the horizon, such as iPS cells, which are adult cells that are reprogrammed to have the capacity to give rise to any type of cell in the body. Developing the properties of the right cell to use, whether in vitro or in vivo, has been an important factor contributing to success, with promising therapies emerging in the areas of macular degeneration, spinal cord injury, and type 1 diabetes. Second, Studer said, access and scalability are likely to be keys to the field's eventual success. Recent advances in cell culture and manufacturing have increased the ability to scale up cell production, allowing millions or billions of cells to be generated, he said, but more research is needed. Third, he said, it will be important to use proof-of-concept studies to address unmet clinical needs. As industry recognizes the unmet needs of patients and the enormous potential of cell-based therapies, commercial investment in the area of regenerative medicine will rapidly increase, creating more opportunities for research to advance. Finally, Studer emphasized the importance of using robust animal models to move the field forward.

Studer listed some of the common shared problems in the field of regenerative medicine, some of which related to his ideas about factors that contribute to success. One major challenge, he said, is getting the right cell to the right target with the right function. This issue has been a bottleneck in the process of turning hypotheses into clinical therapies,

Studer said, but he added that this challenge is on the brink of being overcome in many diseases. Another challenge is understanding how to control cell maturation. In many cases, introduced pluripotent cells mature at a slower rate, which can not only delay the point at which they become clinically effective, but potentially create safety issues as well. Developing accurate models for testing therapies is critical; however, Studer said, current animal models often do not match the necessary physiology well enough to predict the effects of a therapy in the human body. For example, a small animal whose heart beats at 500 beats per minute does not serve as a good model for cardiac therapies for humans. The difficulty of finding an appropriate animal model, Studer suggested, may be eased in the future by the development of functional organoid-like structures, which could mimic organs and microenvironments in the human body. Also, cell-based therapies must overcome immunological barriers. Studer suggested several future possibilities for creating immunological compatibility, including using allogeneic off-the-shelf products, using patient-derived cells, building human leukocyte antigen (HLA)-matched cell banks, and developing universal donor cells. In addition to the scientific issues, Studer said, other logistical challenges remain, including the regulatory uncertainties surrounding cell-based therapies due to their complexity and inability to rely on established pathways, the lack of manufacturing experience in academia, and the lack of incentives for academics to pursue translational medicine.

Studer discussed some of these issues within the context of his own work on Parkinson's disease. Motor symptoms of Parkinson's are caused by the loss of dopaminergic neurons. The current therapeutic approach is to provide the drug L-dopa, which is taken up by cells and converted into dopamine; however, this approach becomes less effective over time. Studer's team has attempted instead to replace the affected nerve cells, using pluripotent cells to make dopaminergic neurons. The team has had success using this approach in a mouse model of Parkinson's. They grafted pluripotent cells into the mouse brain and demonstrated that the transplanted cells not only produced dopamine, but also resulted in an improvement in Parkinson's symptoms.

Studer said that he and his team have faced a number of challenges in this line of work. First, it has taken an enormous amount of time; he has worked on this approach for 22 years, and it is still not yet a commercially available clinical therapy. Studer's work on Parkinson's began in 1995 and focused on fetal dopamine grafting in patients with the disease. Over the course of his research, further testing on other types of

cells indicated that pluripotent cells were more effective at making dopaminergic neurons, but it was not until 2011 that Studer's team finally had a proof of concept for human cell-derived dopaminergic neurons. As of 2016, Studer's lab had produced approximately 1,000 doses of human pluripotent stem cells using good manufacturing practice (GMP) standards, keeping them in frozen storage for later use in humans. In addition to this long period of research, other challenges that the team faced included

- developing an understanding of the mechanism of action of the therapy;
- adapting the cells to a GMP protocol, including creating a protocol that allows for the freezing and thawing of neurons while retaining viability and ensuring the shelf-stability of the cells;
- defining the cellular product by establishing functional cellular markers; and
- obtaining necessary expertise, resources, and regulatory approvals to manufacture cells.

Looking toward the future of regenerative cellular therapies, Studer pointed to several areas where he predicted that advances in science and technology will accelerate the progress of regenerative medicine. He suggested, for instance, that technologies that allow the better characterization of cell products will help researchers more clearly define their cells and improve the production of many different cell types. Another example of an emerging technology is the organoid, an organ-like structure made up of multiple cells and resembling whole organs; in the near term, it can be used to model disease, but it could also be used as a therapy for tissue replacement in the future (Lancaster and Knoblich, 2014). Studer also predicted that enhanced technologies to assess therapeutic effects in vivo will be critical to approaches that depend on manipulating and controlling cells in vivo. Understanding and controlling the maturation of cells, he said, is another issue that must be addressed, and it will require a thorough understanding of the mechanisms of cells.

Studer offered a suggestion of where the field may be in 5 years. Given the current state of the field and new scientific developments, he predicted that there will be a few products at the level of market approval, with many products in early-stage trials. While regenerative medicine holds promise, he said, scientists and other stakeholders should be careful to convey realistic expectations to patients and the public and to

make sure that new therapies are supported by strong scientific evidence. Studer said that he is concerned that poorly designed studies could move forward and negatively affect the entire field of regenerative medicine. Conducting high-quality research and moving the field forward will require collaboration and investment in the translational research pathway, he said. Translational research career paths should be created and included within academia, and grants and investments should be made to fund the translation of basic research into experimental clinical therapies, he suggested. In addition, he said, collaboration with regulators will be important since there is not an established pathway for the development of regenerative therapies. Patient and provider communication and involvement are critical, both to advancing the field and to preventing unrealistic expectations, Studer said.

ORGANIZATION OF THE PROCEEDINGS OF A WORKSHOP

Following this introductory chapter, Chapters 2 through 6 examine the state of the science in research and novel applications of regenerative medicine, discuss the obstacles that hinder progress as well as the elements that may contribute to success, and identify opportunities to move the field forward for various tissues and organ systems.

Chapter 2 explores the state of the field in regenerative medicine for skin and musculoskeletal tissues. Speakers with expertise in these tissue areas highlighted how successes in their fields may inform other areas of research and discussed the emerging challenges associated with manufacturing and scaling up treatments to be effective at a clinical level.

Chapter 3 describes how the field of hematology has used cellular therapies to treat immunological and hematological conditions and how lessons learned from those advances have informed the field of regenerative medicine as it continues to develop. Speakers on the hematology and immunity panel shared their views on the state of the science in hematopoietic stem cell transplantation, gene editing, and T cell therapies.

Chapter 4 delves into the scientific and clinical advances in regenerative medicine for neurological and ophthalmological tissues. Speakers on the neurological and ophthalmological panel explored the state of the science for regenerative therapies designed to treat a range of conditions including age-related macular degeneration and spinal cord injury.

Chapter 5 summarizes the presentations and discussions during the panel on cardiovascular and lung tissues. Speakers in the session shared

their insights on recent advances in the field, discussing in vivo cellular reprogramming, the therapeutic potential of exosomes, and the development of and uses for organoid-like structures.

Chapter 6 explores the state of the science and the potential applications of regenerative medicine for renal tissues. The panelists discussed the prevalence of renal failure, polycystic kidney disease, and emerging technologies related to organoids.

Chapter 7 offers some reflections by a panel of stakeholders on the common themes that emerged during the workshop along with a look toward the future of cell-based regenerative medicine approaches.

2

Skin and Musculoskeletal Tissues

Important Points Highlighted by Individual Speakers

- Consortia are critical to the success of new cell-based therapies; collaboration and the sharing of data can make the development of new therapies more efficient and help therapies reach patients faster. (Oro)
- The costs of product development, manufacturing, and obtaining regulatory approval for cell-based therapies are considerable; in order to be successful, a product must be profitable. Innovations in science, technology, manufacturing, and data sharing may reduce costs and increase profitability. (Ratcliffe)
- There are significant challenges related to identifying the right cells to use, delivering them to the right target, and ensuring that the cells have a positive effect on the target tissue. (Furlong)
- While animal models can be helpful to a certain degree in judging safety and efficacy, it is important for researchers to understand the questions that these models are not capable of answering and to move into human trials when appropriate. (Oro, Ratcliffe)
- Researchers and physicians should be educated on how to best communicate with patients and how to avoid overestimating the potential for therapies on the horizon. (Furlong)

Autologous and allogeneic skin therapies have a long history, from skin grafts that were performed more than 4,000 years ago to modern-day hair transplants, said Anthony Oro, a professor of dermatology at Stanford University. Remarkable advances have been made in the ability to grow healthy skin and musculoskeletal tissues for potential use in the

treatment of chronic wounds; neuromuscular diseases; trauma to bone, cartilage, tendons, or ligaments; and bone tumors. While promising, many of these approaches have faced roadblocks throughout the course of their research and development, and they still have not become routine in clinical care. Speakers in this panel discussed the significant clinical needs for patients with musculoskeletal and skin diseases, outlined the challenges facing research and patients, and described potential ways forward for the field.

CELL THERAPIES IN SKIN

Skin is composed of many types of cells, including keratinocytes, hair follicle cells, melanocytes, and fat, among numerous others, which together act to provide a barrier to the outside world. Because skin is externally accessible, it is fairly straightforward to perform autologous transplants from one part of the body to another, Oro said. However, if there is not sufficient tissue from the patient's body, autologous tissue must be scaled, presenting a challenge.

Patients with recessive dystrophic epidermolysis bullosa (DEB) have a defect in one of the keratin proteins that adhere the epidermis to the underlying dermis, resulting in impaired adherence, and patients suffer from severe blistering, scarring, deformity, squamous cell carcinoma, and often early death, Oro said. He and his team have been working on the regeneration of autologous skin tissue in order to treat DEB. In 2014 Oro's team conducted Phase I clinical trials on an autologous retroviral-corrected keratinocyte sheet product called LEAES (for LZRSE-Col7A1 engineered autologous epidermal sheet). The sheet product is made from affected keratinocytes taken from a patient's unscarred skin, and, using gene therapy, the genetic mutation is corrected in the keratinocytes, and the edited cells are cultured in sheets, which are then transplanted back to a patient's wound sites. In a recent study of the application of this technique to treat DEB, researchers created these sheets by taking a patient's unscarred skin tissue, using a murine leukemia virus (MLV) to correct the mutated *COL7A1* gene, and culturing the tissue to make six skin grafts. Although Phase I trials are not meant to test the effectiveness of a treatment, the trials did successfully demonstrate safety and the presence of collagen in the four patients who participated, Oro said (Siprashvili et al., 2016).

A number of challenges have arisen during the process of scaling up autologous cells for regenerative therapies for DEB, Oro said. First, DEB patients have low keratinocyte stem cell numbers because of chronic wounding, which makes it difficult to produce sufficient skin grafts for the whole body. Second, gene transfer is ineffective for forms of the disease caused by dominant negative mutations. Finally, many patients' skin cells have preclinical premalignant lesions, which will eventually result in squamous cell carcinoma, Oro said.

In order to address these issues, Oro and his collaborators used a new process called therapeutic reprogramming, in which iPS cells are created from the patient's skin cells and genome editing is used to correct the mutation that causes DEB. The edited cells are screened to select for those that do not have mutations for squamous cell carcinoma, and then downstream differentiation techniques are used to make human skin cells for transplantation (Sebastiano et al., 2014). A schematic of this process is found in Figure 2-1. Oro noted a number of benefits of using therapeutic reprogramming to treat DEB. First, Oro's patients are highly susceptible to squamous cell carcinoma, and the technique allows for the screening and selection of cells that do not have mutations that predispose them to squamous cell carcinoma. Second, the genome editing techniques such as clustered regularly interspaced short palindromic repeat (CRISPR)/Cas9 allow researchers to correct the mutation that is responsible for DEB. However, Oro noted, there remains the questions of whether CRISPR/Cas9 should be used to make changes to other genes, and if so, which ones. There are also issues associated with therapeutic reprogramming with regard to safety and efficacy, Oro said. Individual iPS cell lines vary in their ability to make the same keratinocytes, which can affect therapeutic efficacy. A detailed map of the differentiation pathway is needed to understand how to manufacture a defined product from different clones, he said.

Consortia are critical to the success of these new therapies, Oro said. He noted that the development of LEAES took 18 years, partially because of a lack of collaboration and sharing within and across groups. To further develop the use of iPS cell–derived keratinocytes, Oro and others have formed a DEB consortium to establish a coordinated process.[1] In addition, the Stanford University Center for Definitive and Curative Medicine brings together groups working on different cell therapies to share best practices in order to improve and accelerate the practice.

[1]For more information about the Epidermolysis Bullosa Research Consortium Study, see http://med.stanford.edu/dermatology/resources/gsdc/eb_clinic/trials/eb-ebcrc.html (accessed December 12, 2016).

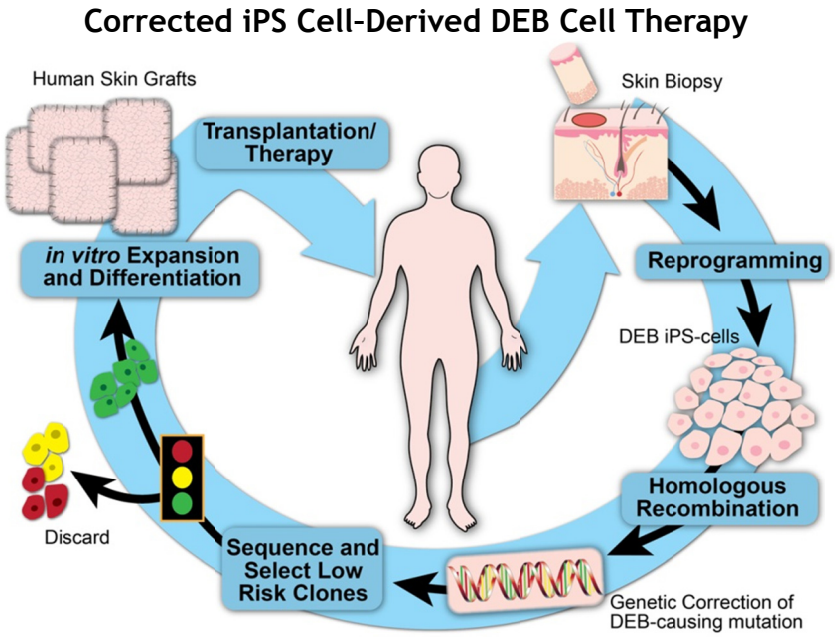

FIGURE 2-1 Therapeutic programming to treat dystrophic epidermolysis bullosa (DEB) using induced pluripotent stem (iPS) cells.
SOURCES: Anthony Oro, National Academies of Sciences, Engineering, and Medicine workshop presentation, October 13, 2016. From an internal report based on Sebastiano et al. (2014).

TRANSLATION OF RESEARCH DISCOVERIES INTO CLINICAL CARE

There are numerous opportunities for the use of cell-based therapies in the treatment of skin and musculoskeletal tissues. Despite the wealth of opportunities, there are several roadblocks that prevent advances from reaching the market, said Anthony Ratcliffe, the president and chief executive officer of Synthasome, Inc. There are challenges to the translation of research discoveries into products, Ratcliffe said, noting that in order to be successful, a product must not only be safe and effective, but must also be profitable.

The regulatory pathway from discovery to approval for musculoskeletal products takes years and millions of dollars, Ratcliffe said. A 510(k)

Food and Drug Administration (FDA) submission, which is for medical devices that are "substantially equivalent" to devices legally already on the market, can cost between $5 and $20 million and can take more than 3 years. The approval of a new biologic or drug can take more than 8 years and cost up to $300 million (Ratcliffe, 2004). There must be a positive return on investment (ROI) to make the development of a product worth the time and money, Ratcliffe said. In other words, the income generated by a product, taking into consideration the cost of manufacturing and selling it, must be greater than the costs of development (Ratcliffe, 2004).

There are challenges involved in making a product profitable, including technical difficulties such as sourcing and developing the product, manufacturing, and predicting market opportunity. Ratcliffe noted that scientific and technical advances may improve profitability. Innovative manufacturing processes may make safe, efficient, and consistent production possible; for example, Aesculap Biologics has developed a system for manufacturing cartilage that takes cartilage biopsy tissue, isolates and expands the cells, and then seeds them onto a scaffold to grow.

There are various roadblocks in the research and development process, including challenges with cell sourcing that could be mitigated by the development and implementation of clear standards and quality measures, Ratcliffe said. He also addressed the challenges faced when carrying out clinical trials, including the uncertainty about what data already exist and how a lack of data sharing can lead to inefficiencies and make it difficult to complete safety and efficacy studies. Ratcliffe mentioned several other challenges and proposed potential ways to mitigate them (see Table 2-1).

TABLE 2-1 Challenges and Opportunities to Profitability of Cell-Based Therapies

Challenge	Opportunity
Cost	Increase efficiencies, standards
Time	Streamline studies required
Technical difficulty	Identify standards for cell sourcing
Clinical uncertainty	Improve clinical databases
Regulatory uncertainty	Develop standards
Manufacturing	Improve scalability, standards
Predicted market opportunity	Increase availability of data
International opportunities	Improve harmonization of standards

SOURCE: Anthony Ratcliffe, National Academies of Sciences, Engineering, and Medicine workshop presentation, October 13, 2016.

PATIENT PERSPECTIVE: DUCHENNE MUSCULAR DYSTROPHY

Duchenne muscular dystrophy (DMD) is a rare disorder that affects 1 in 4,600 live male births, said Patricia Furlong, the chief executive officer of Parent Project Muscular Dystrophy (PPMD). Patients are usually diagnosed between the ages of 3 and 5, and the disorder causes weakness and wasting of muscles, beginning with trunk muscles and eventually affecting the heart and gut. The life expectancy of patients is around 25 years.[2] Furlong's two sons were diagnosed with DMD in 1984, and she immediately became involved in the search for a treatment or cure. After working independently for several years, Furlong founded PPMD in 1994. The organization's mission is to accelerate DMD research, advocate for DMD causes, demand optimal care for all young men with DMD, educate the global community, and, ultimately, end DMD. PPMD was instrumental in passing the Muscular Dystrophy Care Act in 2001,[3] which has delivered $700 million into muscular dystrophy research and development.

One of the first potential therapies for DMD was myoblast transfer, in which allogeneic nondystrophic muscle cells are injected into the patient's muscles (Karpati et al., 1993). Furlong's sons underwent this experimental therapy, but they experienced issues with the techniques used for cell delivery and migration, and the cells were rejected. Similar problems were encountered in the 2000s, when an Italian clinical trial using human mesenchymal cells was unsuccessful and even resulted in severe negative side effects for one child (Maffioletti et al., 2014). Noting the challenges that remain with cell delivery and engraftment, Furlong said that the following questions should be asked with regard to work using myloblast transfer as a therapy for patients:

1. What kinds of cells are being used, and how do you know they will have the intended effect?
2. How will the cells be delivered?
3. Will the cells migrate?

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[2]See Parent Project Muscular Dystrophy at http://www.parentprojectmd.org/site/PageServer?pagename=Understand_about (accessed December 12, 2016).

[3]The Muscular Dystrophy Community Assistance, Research and Education Amendments of 2001 is available at https://www.congress.gov/bill/107th-congress/house-bill/717 (accessed May 15, 2017).

The cause of death for most DMD patients is cardiac failure, although little is known about the mechanism behind it, Furlong said. Currently, a company called Capricor[4] is pursuing a promising approach in which allogeneic cardiospheres are delivered into the patient's myocardium using a cardiac catheter; the hope is that these cells will coax cardiac stem cells to regenerate normal cardiac cells. Phase I studies have been completed, with 24 boys over 12 years of age participating.[5] The study is not without its challenges, Furlong said; for instance, the fact that so little is known about DMD-related heart failure has made it difficult to develop outcome measurements to indicate success. In addition, if the therapy proves beneficial, patients who receive it may need repeated doses of cardiospheres delivered on a regular basis, which will present a risk for a fatal arrhythmia. Early studies in mice by Capricor have demonstrated that the delivery of cardiosphere exosomes also results in the growth of new heart cells. However, mouse models may be of minimal value, Furlong said, commenting that "the mouse has been treated and cured many, many times; the boys not very many times." Furlong described several other avenues that are being pursued, including the use of iPS cells to create more effective disease models to study cardiomyopathy and skeletal muscle pathologies.

While there has been success in the muscular dystrophy field over the past 30 years, much remains to be learned about how cell-based therapies may or may not be useful in treating the disease and creating better models to study DMD, Furlong said. She reiterated that there are significant challenges in identifying the right cells to use, delivering them to the right target, and ensuring that the cells have a positive effect on the targeted muscle tissue.

PANEL DISCUSSION

Commercialization

Workshop speakers and participants discussed the commercialization of these cellular therapies and whether and how regulatory requirements should or could be relaxed for potential treatments of rare diseases. The recent approval of Sarepta Therapeutics' drug for DMD demonstrates the

[4]For more information on Capricor, see Chapter 5.

[5]For more information on the HOPE–Duchenne Study, see http://capricor.com/hope (accessed December 15, 2016).

difficulty of approving and paying for these types of therapies, Furlong said. FDA debated the approval of the drug, with some officials opposed to approval because the increase in dystrophin production was quite small, while those who wanted to approve the drug argued that while the production was small, it was statistically significant. Ultimately, FDA approved the drug under the accelerated approval pathway for rare diseases. While the increase in dystrophin in skeletal muscle was small, the fact that there was any effect indicated that the drug hit its target, Furlong said. She argued that in a "rare disease with a high unmet medical need and no options," therapies that are safe and potentially beneficial need to be "out there on the market" so that patients can use them and researchers can learn more about how and whether the therapies work.

One insurance company has already announced that it will not pay for the Sarepta therapy, indicating that even therapies with regulatory approval may encounter challenges in entering the market.[6] This potential roadblock could prevent companies or investors from initially investing in therapies that are not guaranteed commercial success. The traditional model of funding is one in which investors are motivated by the desire to make money, rather than to cure disease, Ratcliffe observed. He added, however, that there is "no reason why that has to be the only option." If a product is developed for an application with a high unmet medical need and few existing therapeutic options, the product may require an alternative type of funding mechanism that is driven by a desire to get the product into the hands of those who need it most, he added. Dunbar noted that there may be similarities between the commercial structure for these skin or musculoskeletal therapies and the structure for vaccine development, because the potentially far-reaching benefits of these products may change the equation.

One workshop participant commented that part of the appeal of regenerative medicine is the "hope of a cure for diseases that are currently either untreatable or managed with chronic therapy," but current U.S. payment systems are geared toward chronic therapy rather than one-time therapies, which may compromise commercial viability for some therapies. While one-time therapies may be cost-effective, the cost savings may not be manifest for years. Ratcliffe agreed, noting that payers are reluctant to foot the bill for an expensive treatment when the

[6]The Anthem Medical Policy Statement on Eteplirsen can be found here: https://www.anthem.com/medicalpolicies/policies/mp_pw_c192386.htm (accessed December 15, 2016).

patient may switch insurance companies at any time, taking any future cost-savings with him or her. When there is the potential that a one-time therapy will not be paid for, the incentive to initially invest in a therapy is lessened, the participant suggested.

State of the Science

When considering the state of the science for skin and musculoskeletal cell–based therapies, Dunbar said that there are two important questions to ask: What types of models are used to study potential therapies? Is there enough information to understand whether the models are effective at representing the disease and how the treatment might work? For DMD, the mouse model has traditionally been used because researchers were able to produce a mouse with the mutation for DMD, Furlong said, but the more expensive models of dogs and pigs are used once the mouse model shows promise. Unfortunately, she said, even these models cannot represent the complexity of the multi-system disease as seen in humans with DMD. The lack of a perfect animal model means that tough decisions will have to be made about how much evidence is needed before moving a potential therapy into humans, Furlong said. Oro concurred, noting that mouse and human skin are quite different, so clinical trials must move forward with only partial results from the animal model. While animal models can be helpful in determining some level of safety and efficacy, it is important for researchers to understand the questions that these models are *not* capable of answering and to move into human trials when appropriate, Ratcliffe added.

Meeting Quality and Production Standards

One workshop participant asked how, during the manufacturing process, one can define and measure the critical quality attributes for a cell therapy product in order to ensure that it is safe, effective, and the same from batch to batch. This is an important issue because manufacturing and regulatory standards for biologics, such as cell therapies, are unclear, Oro said. Developing quality attributes begins with characterizing and understanding the cell that will be manufactured, he said. It is then possible to develop a GMP pathway and assays to measure the quality and safety of those cells. Ratcliffe said that he starts by applying a large number of assays and measurements and gradually

determines which assays are critical for identifying his unique cell population. Once those assays are identified, he said, he uses them to assess the quality of subsequent cells.

The topic of scaling up and expanding cell populations for use in cellular therapies was brought up by a workshop participant. How can scientists and manufacturers make sure that their expanded cell populations retain purity and potency? For example, fibroblasts are easily expanded, but may not retain the desired characteristics. This issue remains a significant challenge in the field of regenerative medicine, Ratcliffe said. In many cases, scientists can easily identify a defined cell type, but they do not know how to expand that cell population. Ratcliffe said that there is an opportunity for the field to study potential solutions to this problem.

Communication

How, asked one workshop participant, can research findings be best translated and communicated so that patients and other stakeholders can understand the science and manage expectations about product development timelines and what clinical outcomes are possible? Families approach researchers with money in hand to ask for a cure, and researchers have told the families, "Within 2 years, I will be giving you a prescription for this therapy," Furlong said. These types of promises and highly publicized experimental therapies make it challenging to effectively communicate with families and patients who are desperate for help. Scientists need to be realistic about the time that it takes to move from the laboratory to helping a patient, Oro added. It is critical not only to educate patients on how to engage with researchers, but also to educate investigators about how to speak with patients about the research process and the possibilities, Furlong said.

3

Hematologic and Immunologic Applications

Important Points Highlighted by Individual Speakers

- Scientists who unraveled the intricacies of gene editing were often focused on fundamental basic science projects, a fact that underscores the importance of basic science research in advancing clinical medicine. (Urnov)
- Gene editing of patients' cells (e.g., the knockout of *CCR5* to treat HIV) has great potential in regenerative medicine; pharmaceutical companies have recognized the genome as a legitimate drug target and are making investments in the field. (Urnov)
- Hematopoietic stem cell transplant is a well-established treatment for a number of conditions. Recent and emerging advances in the therapy include the use of haploidentical related donors, conditioning regimens that do not use chemotherapy or radiation, and the early detection and treatment of infections. (Malech)
- It takes a long time to move cell-based therapies from discovery into the clinic. The efficient development of therapies can be facilitated through collaboration between researchers, network building, and education of patients and providers. (Sadelain)
- Patients should be viewed as "active participants of humanity-based research" rather than as study subjects; an active and involved patient base is critical to research funding and support. Early engagement with patients is necessary to avoid pushback when and if negative effects of new therapies emerge. (Fields)
- While some of the risks of gene editing may be foreseeable, there will be others that are unexpected and will require researchers, regulators, and physicians to work together to identify and address them. (Malech)

- Sharing data is critical to learning more and moving the field forward, but cost is a significant obstacle to the collection of sufficient data. (Sadelain)

Several exciting advances in cell-based regenerative medicine have taken place in the fields of hematology and immunology over the past two decades. These include harnessing gene-editing technology to treat human immunodeficiency virus (HIV), using modified cells and gene therapy to improve clinical outcomes for patients who undergo hematopoietic stem cell transplantation (HSCT), and the use of engineered T cells for the effective treatment of cancer. The speakers in this session provided a high-level overview of the state of their respective fields, described the challenges and successes they encountered along the way, and discussed how those lessons can be applied moving forward.

CLINICAL APPLICATIONS OF GENOME EDITING

Gene editing has become a clinical reality, said Fyodor Urnov, the associate director of the Altius Institute for Biomedical Sciences. It is being used in multiple clinical trials and multiple open investigational new drugs, both ex vivo and in vivo, and there is great potential for its use with regenerative medicine, he said. The basic concept underlying gene editing, Urnov said, is that when a DNA double-strand break is created, the cell repairs the break either through non-homologous end-joining or homology-directed repair. Scientists can leverage the repair pathways, using gene editing to drive specific outcomes at targeted loci within the cell or tissue of interest. Urnov spoke about three approaches to targeted gene editing: an insertion/deletion "indel," which can create a "knockout" by inactivating a gene; a single nucleotide polymorphism (SNP), which can correct a mutation; or a transgene, which can produce a targeted integration. The first gene editing tool used to make targeted breaks in a cell's DNA was the zinc finger nuclease (ZFN) (Klug, 2010). Since that original use of ZFN, researchers have developed various other nucleases to perform gene editing, and current approaches now include TALE (transcription activator–like effector nuclease) nucleases, meganucleases, and RNA-programmable nucleases such as the clustered regularly interspaced short palindromic repeat (CRISPR)/Cas9 (Doudna and Charpentier, 2014). Each genome editing tool has advantages and disadvantages, Urnov said, and the technologies will continue to evolve

and be refined in years to come. Pharmaceutical companies have come to recognize the potential of gene editing, he said, and they have created partnerships in order to pursue the genome as a "legitimate drug target" despite the inherent challenges of a long and costly research and development process. This investment, he said, "has provided the infrastructure, the funding . . . and the courage to go after larger disease indications."

As examples, Urnov went on to describe two gene editing approaches taken by researchers at Sangamo BioSciences that have reached the clinic for the treatment of HIV-1 infection. Both examples involve targeting the *CCR5* gene for inactivation, with the first approach being undertaken in T cells and the second in CD34+ hematopoietic stem and progenitor cells (HSPCs). The ability of HIV-1 to enter cells depends on the binding of viral gp120 Env protein to the host's CD4 receptor and a chemokine co-receptor, most commonly *CCR5* (Holt et al., 2010; Wu et al., 1996). Individuals who are homozygous for a frameshift-causing 32-base-pair deletion in *CCR5* (CCR5Δ32) are profoundly resistant to HIV-1 infection (Liu et al., 1996). This finding, in combination with evidence from the "Berlin patient," an individual cured of HIV-1 infection after receiving an allogeneic transplant with stem cells carrying nonfunctional *CCR5*, spurred researchers at Sangamo BioSciences to develop *CCR5*-targeted HIV treatments (Tebas et al., 2014; Zou et al., 2013). The first approach, Urnov said, involved harvesting CD4+ T cells from patients with HIV, using ZFN to disrupt *CCR5* in those cells, and then transplanting the edited cells back into the patient. Preclinical efficacy tests were rather straightforward and involved showing that the edited T cells functioned in vitro and were efficacious in a mouse model. So far, he said, this approach has been well tolerated by patients and has produced an antiviral effect. There has also been evidence that the edited cells home to the gut-associated lymphoid tissues and persist in the body for at least 4 years (Tebas et al., 2014). In the most recent cohort, 60 percent of patients were able to control their viral load in the absence of antiretroviral therapy, Urnov said. The trial protocol will continue to be refined as ZFN technology improves, he said, and there is now an established good manufacturing practice (GMP) pathway that meets criteria for efficiency and specificity. The company has worked closely with the Food and Drug Administration (FDA) to develop and meet regulatory requirements.

The second approach also targets *CCR5* for HIV, but uses less mature CD34+ HSPCs that can differentiate into a variety of blood cell

types. This approach has met standards for safety and efficacy, Urnov said. A clinical trial using this therapeutic approach is currently open, but information from the trial was not available at the time of the workshop.

Milestones and Avenues for Improvement

The approaches to targeting *CCR5* for the treatment of HIV have paved a path for the use of gene editing in the clinic, Urnov said. Both approaches have established clinical scale efficiency and specificity, he said, as evidenced by the development of an FDA pharmaceutical quality/chemistry, manufacturing, and controls path, a GMP path, and with toxicology evidence. In addition, the researchers from Sangamo BioSciences have worked with FDA and the Recombinant DNA Advisory Committee of the National Institutes of Health[1] to develop a regulatory framework from which other gene-editing therapies could benefit. Avenues to improve the quality and reduce the cost of these potential therapies in the future would include better, more efficient cell processing; allogeneic off-the-shelf products that reduce the need to expand and edit autologous cells; and in vivo delivery of gene therapy, Urnov said. Low yields of CD34+ HSPCs are a current challenge, so finding a way to create greater numbers of targeted HSPCs would drastically change the landscape of therapies for that cell type, he said.

ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION

Allogeneic HSCT, or bone marrow transplantation, has been well established for nearly 30 years as a standard-of-care treatment for many monogenic diseases and cancers, said Harry Malech, chief of the Laboratory of Host Defenses and chief of the Genetic Immunotherapy Section at the National Institute of Allergy and Infectious Diseases. It has become the standard of care for hemoglobinopathies, inherited bone marrow failure syndromes, primary immune deficiencies, lysosomal storage diseases, and some metabolic enzyme deficiencies and leukodystrophies. According to Malech, successful HSCT requires that the following four conditions be met:

[1]The charter of the Recombinant DNA Advisory Committee is available at http://osp.od.nih.gov/sites/default/files/resources/RAC_2015-2017_Charter_Updated.pdf (accessed December 4, 2016).

1. Access to a suitably HLA-matched donor
2. An adequate and safe conditioning regimen to attain permanent engraftment
3. A preventative regimen to reduce or prevent graft-versus-host disease
4. Prevention, detection, and effective treatment of viral, bacterial, and fungal infections that may occur due to the immunodeficient status of transplant patients

Increasing the Pool of Suitable Donors

Often, the best donor is an HLA-matched sibling, Malech said; however, the availability of matches through unrelated donors has vastly increased in recent years due to the National Marrow Donor Program, and the best donor is increasingly likely to be found through that program.[2] One challenge that still remains is that individuals of mixed ethnic heritage may have trouble finding a donor within the National Marrow Donor Program, Malech said. To expand the potential donor pool, researchers have also been exploring the possibility of performing haploidentical HSCT using a relative who is a partial HLA match (Locatelli et al., 2013). Successful haploidentical HSCT was recently demonstrated in a patient with chronic granulomatous disease, a genetic disorder that causes recurrent infections and autoinflammation, and in another patient with *DOCK8* deficiency, which results in combined immunodeficiency (Freeman et al., 2016; Parta et al., 2015). The HCST conditioning regimen for both of the patients involved administering high-dose cyclophosphamide administration post-transplant to help with immunosuppression.

Optimizing Conditioning Regimens

Conditioning regimens, which prepare a patient's body to accept the graft by depleting lymphocytes and HSPCs, have traditionally relied on chemotherapy or radiation. A variety of other serotherapies, such as anti-thymocyte globulin or anti-CD52 monoclonal antibody (alemtuzumab), are available to use instead of radiation and chemotherapeutic agents to deplete lymphocytes, Malech said, but it is still a challenge to completely

[2]More information about the National Marrow Donor Program can be found by visiting https://bethematch.org (accessed December 4, 2016).

eliminate host HSPCs without using radiation or chemotherapy. One approach to overcoming this challenge is to use a monoclonal antibody therapy that targets c-Kit, a protein found on HSPCs and downstream hematopoietic progenitors. A recent study in mice found that a combination approach using anti-c-Kit monoclonal antibody plus anti-CD47 antibody resulted in the depletion of host HSPCs in immunocompetent recipients with efficient engraftment of donor cells (Chhabra et al., 2016). Another recent study in mice used immunotoxin (saporin)-conjugated anti-CD45 antibody to achieve similarly efficient HSPC engraftment in immunocompetent mice (Palchaudhuri et al., 2016). Targeted HSPC conditioning regimens that use only biologic agents represent a disruptive innovation that could transform HSPC transplantation, Malech said. Looking toward the future, studies may focus on improvements in approaches to toxin-conjugated antibodies that more specifically target HSPCs or even possibly the development of chimeric antigen receptor T cells that specifically target HSPCs, he said.

New Targets to Prevent Graft-Versus-Host Disease

Patients who undergo HSCT are at high risk for graft-versus-host disease (GVHD), a condition that occurs when donor cells recognize the recipient's normal cells as foreign and mount an immune response, resulting in an array of symptoms that can include inflammation, gastrointestinal distress, jaundice, and dryness of mucus membranes (Leukemia & Lymphoma Society, 2016). Currently, the overall success rates of HSCT are hampered by morbidity and mortality associated with GVHD (Lappas et al., 2010). According to Malech, preventing GVHD is an extremely important concern for patients who undergo HSCT for indications other than the treatment of hematologic malignancies (e.g., for the treatment of monogenic diseases such as immunodeficiencies and hemoglobinopathies). Although immunosuppressive agents such as rapamycin, cyclosporin A, tacrolimus, mycophenolate mofetil, and methotrexate are used clinically to induce tolerance, they have only been partially successful, and there is a pressing need to develop new methods to prevent GVHD, Malech said. One new potential approach to prevent GVHD is the use of highly specific adenosine A_{2A} receptor ($A_{2A}R$) agonists (Lappas et al., 2010). The $A_{2A}R$ is involved in the termination of inflammatory signals, and the selective activation of the $A_{2A}R$ has been shown to limit inflammation and tissue damage in several models of inflammatory disease (Awad et al., 2006; Lappas et al., 2006; Naganuma

et al., 2006; Zarek et al., 2008). Although $A_{2A}R$ agonists have not yet entered the clinic for use against GVHD, Malech said, they are a very exciting prospect.

Combating Infections in Transplant Recipients

HSCT recipients are at increased risk of serious bacterial, viral, and fungal infections because of their lowered immunity. A majority of patients who receive HSCT acquire an infection and 17–20 percent of those infections result in death (Leen et al., 2014). HSCT recipients are especially vulnerable to infections for reasons that include the immunosuppressive drugs they receive pre-transplant, the cytotoxic chemicals used to prevent GVHD, and the symptoms of GVHD itself (Leen et al., 2014). In recent years, Malech said, great strides have been made in the ability to rapidly detect and control post-transplant viral infections such as cytomegalovirus and Epstein-Barr virus. One such advance has been with the use of virus-specific T cells (VSTs) that can be used "off-the-shelf" because they are derived from individuals with common HLA polymorphisms (Leen et al., 2013). Despite the fact that off-the-shelf VSTs have reached the clinic and shown efficacy in reducing mortality and bridging the gap to reacquisition of post-transplant immunity, many open questions remain in this rapidly emerging area, Malech said.

Advances in Gene Therapy

The first evidence of clinically beneficial gene therapy for monogenic immune deficiencies was observed using infusions of murine gamma retrovirus vector-transduced autologous HSCs to treat X-linked severe combined immune deficiency (X-linked SCID) or adenosine deaminase-deficient severe combined immune deficiency (Aiuti et al., 2009; Gaspar et al., 2011; Hacein-Bey-Abina et al., 2010, 2014). However, gamma retrovirus vector gene therapy used to treat X-linked SCID, chronic granulomatous disease, or Wiskott-Aldrich syndrome has been associated with vector-insertion-related genotoxic effects leading to development of leukemia or myelodysplasia (Braun et al., 2014; Hacein-Bey-Abina et al., 2008; Howe et al., 2008; Stein et al., 2010). Self-inactivating lentivectors derived from HIV-1 appear to show enhanced transduction of long-term engrafting human HSPCs while at the same time appear to be less capable of activating nearby oncogenes. Gene

therapy–based approaches that involve an infusion of lentivector-transduced autologous HSPCs show clinical promise for the treatment of several monogenic disorders without evidence of genotoxicity (Malech and Ochs, 2015). At the conclusion of his presentation, Malech briefly reported that several recent trials of lentivector gene therapy have demonstrated promise for significant long-lasting clinical benefits for patients with monogenic illnesses including thalassemia (Cavazzana-Calvo et al., 2010), X-linked SCID (De Ravin et al., 2016), Wiskott-Aldrich syndrome (Hacein-Bey-Abina et al., 2015), metachromatic leukodystrophy (Sessa et al., 2016), and X-linked adrenoleukodystrophy (Cartier et al., 2009). "In our ongoing clinical trial of lentivector with busulfan conditioning for older children and young adults with X-linked SCID, we have been able to restore immunoglobulin production in our patients," Malech said (De Ravin et al., 2016). Although self-inactivating HIV-1-based lentivector has become the current vector of choice for ex vivo transduction of human HSPC, related integrating lentivectors such as those derived from foamy virus should be explored, he said. An important goal for the future, Malech said, would be to discover methods to create "druggable" versions of these integrating vectors that are delivered and targeted in vivo.

THE PROMISE OF T CELL ENGINEERING

Although several emerging oncology treatments can induce impressive responses, there is still a serious lack of cancer therapies that are both specific and curative, said Michel Sadelain, the director of the Center for Cell Engineering Immunology Program at the Sloan Kettering Institute's departments of medicine and pediatrics and the Memorial Sloan Kettering Cancer Center. A drug that is specific to a given target should be both safe and efficacious, Sadelain said, and T cells, a component of the adaptive immune system, have evolved to target molecules in an exquisitely specific fashion that provides long-lasting immune support. "T cells do not always have the potency required to fight cancer," he said, "and that's where T cell engineering comes into play." T cell engineering is a technique used to reprogram T cells to harness and improve on their natural immunological abilities to increase their potency and achieve a superior immune response.

Chimeric antigen receptors (CARs) are artificial receptors that are designed to target T cells to respond to specific antigens of choice. They

mediate T cell antigen recognition and activation, and they augment T cell functionality and persistence (Sadelain, 2015). In order to get CARs expressed on the surface of T cells, CAR cDNA must be introduced into T cells, Sadelain explained, and this is often accomplished via retroviral or lentiviral vectors. Once the T cells express the CAR molecule, they become known as CAR T cells, and they have the ability to recognize target antigens on the surface of tumor cells and attack those cells. The reprogrammed CAR T cells are expanded ex vivo and infused back into the patient.

Sadelain and his lab began researching CAR T cells over a decade ago, focusing their work on exploring the therapeutic potential of primary human T cells that were genetically modified to recognize and kill tumors that express the B cell lineage-specific antigen, CD19 (Brentjens et al., 2003). CD19 is a transmembrane protein that is expressed on normal B cells, follicular dendritic cells, and the cells in many types of blood malignancies, including acute lymphoblastic leukemia and chronic lymphocytic leukemia (Wang et al., 2012). Over the years, Sadelain and his colleagues created a manufacturing platform within their academic setting. CAR T cell development within the manufacturing platform begins with collection of a patient's T cells by apheresis, Sadelain said. The T cells are then activated by incubation with antibodies to CD3 and CD28, a viral vector is applied, and the cells are cold cultured and expanded to allow for gene transfer to occur and to increase the number of genetically edited T cells. Within 8–10 days, and following a few additional biosafety tests, the cells are ready for infusion back into the patient, or they can be frozen for deferred use, Sadelain said.

Recent studies of CAR T cells produced in this way have shown great promise, Sadelain said, describing the results of one of his lab's studies that was presented at the 2015 American Society of Hematology conference. Forty-five adults with refractory (treatment-resistant) acute lymphoblastic leukemia received the treatment, and 82 percent went into complete remission. Complete remission is considered a molecular remission, he said, meaning that the tumor is undetectable by deep sequencing. Even though it will take several years to determine if the patients are cured, the early results are extraordinary, he said. These early findings resulted in *Science* naming cancer immunotherapy 2013's Breakthrough of the Year, highlighting the work on CAR therapy and another approach called checkpoint blockade, which is aimed at blocking inhibitors of the immune response.

When asked about the potential of CAR T cell therapy to treat solid tumors, Sadelain said that a number of different cancer cells carry the CD19 surface marker, but CAR T cell therapy works better on some than on others. This difference in effectiveness, he said, is likely due to the fact that different tumor types have different microenvironments, each unique to the particular tumor type, and solid tumors tend to have more inhibitory mechanisms present than cancers like acute lymphoblastic leukemia. Research is being conducted on CAR T cells to improve their effectiveness in the tumor microenvironment, but designing and using cells as an "ultra-targeted" chemotherapy is a complex undertaking, Sadelain said.

Previous T cell–based therapeutic approaches have relied on finding and expanding the right cell in the patient or in the donor. The new paradigm, Sadelain said, is likely to use gene transfer, gene editing, and synthetic biology to manufacture T cells that have the optimal properties for the intended use. Sadelain listed what he sees as four priority research areas needed to move the field forward:

1. Optimization of CAR design (e.g., second generation CARs, armored CARs)
2. Additional basic research on T cell differentiation in order to identify which subpopulation is the most well-suited for this type of therapy
3. Innovations in cell-manufacturing sciences to get the product to patients more efficiently
4. Integration of gene transfer and gene editing technology with CAR therapy

The Future of CAR T Cell Therapies

T cells have been used as medicines for a number of years; however, progress in adoptive T cell therapy has been slow because of a lack of antigen-specific human T cells (Themeli et al., 2013). A new paradigm is emerging in which researchers use pluripotent stem cells as the source material to manufacture reservoirs of engineered cells devoid of alloreactivity, Sadelain said. The ultimate goal, he said, would be to have a "cell pharmacy" with prepared cells that can be administered to patients for multiple conditions.

Sadelain offered three important lessons from his experience with CAR T cells. First, these types of breakthroughs take time: 21 years

lapsed between the beginning of T cell engineering and FDA designation of CAR T cells as a breakthrough therapy (a breakthrough therapy designation is intended to allow expedited development and review of drugs for serious or life-threatening conditions[3]). Second, while the involvement of industry is critical to moving a therapy into manufacturing and commercialization, the role that academia plays cannot be understated. Academic teams have the benefit of cross-fertilization between researchers who are working in different but related fields. Third, education and network building are crucial components of developing these therapies. Both patients and providers were reluctant to participate in early CAR T cell therapies, Sadelain said, and moving forward would not have been possible without addressing concerns and educating people about how the therapy worked.

PATIENTS AS ACTIVE PARTICIPANTS IN HUMANITY-BASED RESEARCH

Research participants are often viewed simply as "study subjects," but Jennifer Fields, a patient advocate, argued that they should be considered "active participants of humanity-based research." Having an informed patient population is critical to supporting continuing research in the field, she said, but there is a significant communications gap in the current scientific research process. Researchers and patients move in separate spheres and are only connected by physicians or organizational liaisons who facilitate communication between the groups. If researchers engaged patients earlier in the scientific process, they would have a more trusting patient base that would actively participate in clinical trials and would advocate for research funding and support, Fields stated.

Patients want to be involved in the research process, and they want to understand and trust research, Fields said, suggesting that in order to involve patients, researchers must shift some resources into patient engagement and should use knowledgeable patients as liaisons between the research and patient communities. She also emphasized the importance of continuous communication and feedback between the communities as a way of creating a true relationship that would benefit all involved.

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[3]For more information on breakthrough therapies see http://www.fda.gov/RegulatoryInformation/Legislation/SignificantAmendmentstotheFDCAct/FDASIA/ucm341027.htm (accessed December 1, 2016).

PANEL DISCUSSION

Potential Risks of Gene Editing and Cellular Therapies

What are some of the potential "off-target effects" from gene editing or cell therapies, Dunbar asked, and how we can predict and prevent some of the most likely effects? When gene therapy was nascent, there were a variety of concerns about negative effects from the therapy, Malech said. Cancer was one potential side effect, but studies of early retroviral vectors in mice did not show cancer as an outcome, he said, so it only became clear that it was a real danger of the therapy once trials began and a number of patients developed cancer. Gene editing may be similar in that it may be possible to foresee and prepare for some of the effects of the therapy, but there will likely be some effects that were not predicted before implementation in patients. Controlling the risks of these therapies is an iterative process that requires investigators, regulators, and clinical physicians to work together closely to quickly address effects as they are identified, Malech said. Sadelain noted that, in addition to the failure of mouse models to predict cancer as an effect of gene therapy, mouse models also failed to predict heart failure as a result of CAR T cell therapy. Mouse models can be very valuable, but it is clear that it will not be possible to predict all potential risks using these types of models.

When transplanting stem cells, it is possible to sequence the genome and identify a mutation within a cell, but it is difficult to know if the mutation will pose a clinical risk or not, Urnov added. The gene editing and gene therapy community is "acutely aware" of the challenges associated with identifying and addressing risk in these therapies, he said, and it is working to improve existing systems and building new ones to continue to lower the risk of these therapies.

The potential of using the genomic profile of patients to reduce the risk of toxicity of a therapy was also discussed. This area is still in its infancy. For example, fully sequencing a patient's genome results in only about 60 to 70 actionable pieces of information concerning, for instance, the patient's susceptibility to anesthesia, Malech said. However, the area is growing rapidly, and personalized medicine has the potential to influence the provision of these therapies in the future, he said.

The Use of Data and Data Registries

The collection and sharing of outcomes data has been instrumental in making advances in the transplant field, as can be seen in developments in immunosuppression and in the prevention of GVHD, Dunbar said. She said that because many trials are quite small, sharing data may be the only way that investigators can solve manufacturing issues or learn how cells behave in a patient. Several workshop participants added that data likely need to be collected across a broad number of endpoints such as genetics, age, gender, ethnicity, and immune profile, among others. Currently, funding is an obstacle to collecting truly comprehensive data, Sadelain replied, noting that trials are already expensive and that adding more data collection points will increase the cost.

Patient Involvement

A workshop participant asked speakers about the best ways for researchers to engage patients and inform them about the risks of new therapies in order to avoid stymieing progress in the field in the case of unintended side effects. Patients need to be engaged early on in the process, Fields responded, and they need to be made to feel as if they are partners in the research. Patients should feel that researchers are working for them and with them, she said. Dunbar added that researchers need to strike a balance—they need to convey their enthusiasm and optimism about the possibilities of the research in order to get patients interested in participating in trials, while at the same time avoiding overpromising and leading patients to believe that a cure is right around the corner. Dunbar suggested that liaisons who are informed about the science and about the realities of clinical trials could act as brokers between researchers and patients to present a more balanced view.

4

Neurological and Ophthalmological Tissues

Important Points Highlighted by Individual Speakers

- Implantation of cells into the macula in order to replace the retinal pigment epithelium (RPE) is under investigation for the treatment of age-related macular degeneration. Potential cells for the therapy include adult retinal pigment epithelial stem cells (RPESCs), human neural stem cells, and RPE derived from human embryonic stem cells. (Coffey, Temple, Tsukamoto)
- There are a number of challenges involved in the transplantation of neural cells, including immunosuppression; the delivery, integration, and survival of the cells; identifying and producing the authentic cell type; and the potential for adverse effects. (Temple)
- Developing ways to activate RPESCs in vivo would be beneficial because it could potentially avoid the complications associated with cell removal, replication, and transplantation. (Temple)
- Unproven therapies currently being administered in clinics are deeply concerning because without proper safety and efficacy data, patient health is at risk along with patients' trust in the medical system. (Temple)
- The regulatory approval pathway can be lengthy and costly; however, engaging with regulators early on can make the process more efficient. (Coffey)
- Arranging an interdisciplinary team of engineers, regulators, manufacturing experts, patients, and clinicians can accelerate and streamline the therapeutic pathway from the lab to the clinic. (Coffey)
- A mechanism for sharing data among researchers working on age-related macular degeneration could be beneficial because it would mean that mistakes are not repeated; however, there need to be incentives to create such consortia. (Temple)

- It is important for patient advocates and clinicians to share patient perspectives with regulators to convey unmet clinical needs. (Coffey)

Currently there is a huge unmet medical need for effective therapies to treat neurological disorders, including Alzheimer's disease, Parkinson's disease, multiple sclerosis, spinal cord injury, psychiatric disease, amyotrophic lateral sclerosis, stroke, and age-related macular degeneration, said Sally Temple, a principal investigator and the scientific director of the Neural Stem Cell Institute. All of these diseases have a common mechanism of cell loss, which provides a rationale for contemplating the replacement of these cells with stem cell–based products, she said. The neurological system is extraordinarily complex, containing multiple cell types and involving complex interactions within and between each neural region; therefore, it is challenging to produce the specific neural cell types that are authentic and appropriate for each specific region. Despite the complexity, there has been progress made in several areas, including retinal pigment epithelium transplantation, oligodendrocyte replacement, and the transplantation of human neural stem cells. During this session, speakers discussed opportunities in these areas, along with challenges encountered and lessons learned.

STEM CELL THERAPY FOR AGE-RELATED MACULAR DEGENERATION

Adult Retinal Pigment Epithelial Stem Cells

Retinal pigment epithelium (RPE) transplantation is under investigation as a potential method for treating age-related macular degeneration (AMD). AMD, the major cause of vision loss in adults over 65 is caused by the degeneration of RPE cells (AOA, 1994), which are highly specialized cells that support the health and integrity of photoreceptors and the choriocapillaris, the network of capillaries that supplies nutrients to the retina (Sonoda et al., 2009). Patients with AMD experience vision distortion that leads to central vision loss, which can significantly impair their ability to read or recognize faces. Temple and her colleagues are attempting to replace the damaged RPE with new cells from donor eyes. Their protocol involves the harvesting of adult retinal

pigment epithelial stem cells (RPESCs) from donor eyes; these cells are normally dormant but can be encouraged to self-renew in vitro. Because the starting material is specific to the eye and is the natural precursor to RPE, donor RPESCs are especially well suited to make RPE compared to other types of stem cells, Temple said. Once the donor cells are purified and expanded, 50,000 to 150,000 cells are transplanted into the patient's eye beneath the retina. In the future, Temple said, it would be ideal if RPESCs could be activated in vivo, so that the process of cell removal, replication, and transplantation could be avoided.

Several studies are under way to investigate different sources of RPE for transplantation, and each has its own benefits and drawbacks, Temple said. For example, using embryonic stem cells or induced pluripotent stem (iPS) cells has the benefit of unlimited expansion, but it also has the potential drawback of tumorigenicity. In contrast, RPESCs will likely not increase the risk of tumors in the recipient, but the cells cannot be perpetually replicated.

The effects of advancing studies that use poorly defined cell types or cells that are not normally present in the nervous system are a major concern, Temple said. Unproven therapies springing up in clinics are deeply concerning because they are of unproven safety and efficacy and risk patient health as well as patient trust in the medical system.

Human Embryonic Stem Cell–Derived RPE

An alternative stem cell–based approach to treat AMD was described by Peter Coffey, a professor at the Neuroscience Research Institute of the University of California, Santa Barbara. He told participants about a United Kingdom–based partnership between the University College London Institute of Ophthalmology, the Moorfields Eye Hospital, and Pfizer Neusentis which investigated the possibility of using human embryonic stem cells (hESCs) for the treatment of AMD. As discussed above, RPE cells are critical for retinal function and maintenance of vision. Coffey and his team aimed to develop and deliver a small artificial membrane, or "patch" of RPE (derived from hESCs), to replace damaged or lost RPE in patients' eyes. The success of the project relied on four main steps according to Coffey: proper cell characterization, high-quality manufacturing, the development and validation of quality control assays, and a demonstration of the safety of hESC-derived RPE.

The characterization of the cells was fairly straightforward, Coffey said. The team performed a series of analyses of phenotype, cellular

ultra-structure, immune activity, and in vitro and in vivo function to demonstrate that the hESC-derived RPE was indistinguishable from native fetal or adult RPE. The group went on to perform whole genome transcript analysis on approximately 40 cell lines of RPE to demonstrate that the hESC-derived RPE was identical to other sources of RPE.

Manufacturing the RPE patches in the UK required submission of an investigational medicinal product dossier (IMPD), which is a document that details the steps that will be taken to manufacture the product and how safety and quality will be ensured.[1] Preparing the IMPD took Coffey's team 9 months and required the development of an extremely detailed manufacturing process. During the process of therapeutic development, researchers often acquire new information and may wish to make modifications; however, the regulatory pathway requires that the process be "locked down" at some point so that the regulators can perform their evaluation. Any non-minor changes to the process are challenging, time-consuming, and costly, Coffey said.

Quality control assays were developed in order to test various aspects of the hESC-derived RPE cells, including their sterility, viability, identity, purity, potency, sensitivity, accuracy, and robustness. Tests for the presence of contaminating hESCs were critical because of concerns about tumorigenicity and teratoma formation. Stringent regulatory requirements stated there must be zero pluripotent cells on the RPE patch, Coffey said. Therefore the team employed specific RPE culture conditions that do not support hESC survival, and they confirmed total hESC depletion using image analysis and flow cytometry, he said. Regulators also required that the hESC-derived cells be transplanted into an animal model to evaluate for tumorigenicity. A 6-month-long tumorigenicity study in immunodeficient mice demonstrated that the cells did not form teratomas, Coffey said.

After a lengthy and complex regulatory process that involved seven different regulatory bodies and cost approximately £10 million, the group received regulatory approval to conduct the first round of clinical trials. In August 2015 the first human patient received the small patch of hESC-derived RPE, which was placed behind the retina, and Coffey's team expects the 12-month clinical outcomes data in late 2016. The team is now working with a chip manufacturer to build a process for scaling up

[1]For more information on IMPDs, see http://www.impd.eu (accessed November 29, 2016).

production of the patches, with the hope of eventually getting the patch licensed as a therapeutic.

Despite the fact that the product has taken 8 years to develop to this point, the process was streamlined and accelerated because the project's interdisciplinary team, which included engineers, regulators, good manufacturing practice facilities, and clinicians, had all the necessary skill sets to take the therapy from development to clinic, Coffey said. Rather than approaching the process serially, beginning with laboratory development and moving onto the other steps as appropriate, the team tried to tackle the project in a more holistic manner.

HUMAN NEURAL STEM CELLS

Ann Tsukamoto, the former executive vice president of Scientific and Strategic Alliances at StemCells, Inc., shared her company's experience with developing human neural stem cells (HuCNS-SCs®). StemCells, Inc., worked with HuCNS-SCs for more than 18 years before shutting down operations in May 2016. With a fluorescence-activated cell sorting protocol, HuCNS-SCs are isolated and purified from human fetal brain tissue, then expanded, banked, and cryopreserved under conditions suitable for clinical applications, Tsukamoto said. The banked cells are tested for safety and biological properties including genetic modifications, normal karyotype, and potency. HuCNS-SCs do not require pre-differentiation before transplant and do not form tumors in vivo, Tsukamoto said.

In animal studies using immunosuppressed or immunodeficient mice, HuCNS-SC cells were shown to migrate throughout the central nervous system (CNS) and differentiate in a site-specific manner depending on where the cells took up residence. For example, HuCNS-SCs differentiated into myelin-producing oligodendrocytes in white matter areas of the CNS, Tsukamoto said. The HuCNS-SCs self-renew in vivo, which is an important feature for cells that are being used to correct a lifelong disorder, she said. Furthermore, HuCNS-SCs exhibit several neuroprotective properties that could be beneficial in trying to regenerate disease target areas within the CNS, such as the production of neurotropic factors, phagocytic activity, and an anti-inflammatory effect, she said.

HuCNS-SCs have been tested in humans for a variety of disorders of the brain, retina, and spinal cord. In total, HuCNS-SCs have been

transplanted into 55 patients, and there have been no safety concerns observed, Tsukamoto said. Evidence from a mouse model of infantile neuronal ceroid lipofuscinosis (also known as Batten disease, which is a fatal, inherited disorder of the nervous system) demonstrated that, upon transplantation, HuCNS-SCs engrafted and produced therapeutic benefit through the protection of endogenous neurons (Tamaki et al., 2009). Subsequent human clinical studies were performed in patients with Batten disease, and the transplanted HuCNS-SCs migrated, self-renewed, and engrafted, and in port-mortem exams researchers found that the donor cells had survived long term. One of the patients with Batten disease who received HuCNS-SCs is still alive, 8 years post transplant, Tsukamoto said.

HuCNS-SCs were also tested in patients with Pelizaeus Merzbacher disease, a leukodystrophy characterized by the inability to form myelin, the insulating sheath that is wrapped around nerve axons to facilitate the conduction of electrical impulses. In a mouse model of the disease, injection with HuCNS-SCs resulted in mature, compact myelin formation (Uchida et al., 2012). Following cell transplant, diffusion tensor imaging of the children's brains showed de novo myelination, Tsukamoto said. Unfortunately, Phase II trials for Batten disease and Pelizaeus Merzbacher disease were not carried out because of a lack of eligible patients and a poor understanding of the natural history of the disease.

HuCNS-SCs have also been investigated as a potential treatment for the dry form of AMD, with the cells being injected into the sub-retinal space. Testing in the Royal College of Surgeons (RCS) rat, a model of inherited retinal degeneration, demonstrated that the photoreceptor layer was protected long-term by an injection of neural stem cells. The protective effect was the result of multiple mechanisms, including phagocytosis of the outer segments, stabilization of the synapses of the cells, neuroprotection through secretion of neurotrophic factors, and proliferation of endogenous RPE layers, Tsukamoto said. In a Phase I clinical study of 15 human patients with AMD, there was a decelerated progression of the rate of geographic atrophy and improved visual acuity.

Researchers went on to test HuCNS-SCs as a possible treatment for thoracic and cervical spinal cord injuries. In animal models of thoracic spinal cord injury, injected cells migrated, engrafted, and resulted in restored motor function (Cummings et al., 2005). These findings led to a clinical study in which 12 patients received injections of 20 million cells each at four injection sites. There was overall sensory improvement in 7

of the 12 patients, with sustained effects beginning at 3 months post transplant, Tsukamoto said. In a clinical trial conducted in patients with cervical spinal cord injury, and most patients experienced a significant increase in upper extremity motor strength. Immunosuppression was a key factor in getting the HuCNS-SCs to survive, Tsukamoto said. Clinical trials for thoracic and cervical spinal cord injuries showed that most patients experienced restoration of sensory function, but the restoration was not permanent for all patients. Thoracic patients received immunosuppression for 9 months, and there was no loss of function at 12 months. Cervical patients, on the other hand, received only 6 months of immunosuppression and experienced a decline in restored motor function by 12 months. The mixed results of these studies unfortunately resulted in the company closing its doors, Tsukamoto said.

Looking Ahead and Learning from Past Challenges

One major hurdle for researchers in the AMD field, Temple said, is to determine how to replace other damaged cell types such as photoreceptors, the cells that lie adjacent to the RPE and rely on it for normal function and survival. Another goal for the field will be to produce a functional retina of full thickness from three-dimensional organoids. Finally, it will be important to overcome the challenge of getting regenerated or transplanted retinal ganglion cells to project inside the optic nerve in order to connect back to the brain, Temple said.

At the conclusion of their presentations, Temple, Coffey, and Tsukamoto discussed specific challenges and lessons learned during the process of developing a cell replacement strategy, and their individual ideas are listed in Box 4-1 below.

BOX 4-1
Challenges and Lessons Learned During the Development of Stem Cell–Based Therapies for Neurological Diseases (as presented by Coffey, Temple, and Tsukamoto)

Challenges

- Producing authentic neuronal cell types at sufficient purity and quantity. (Temple, Tsukamoto)
- Identifying the optimal cell stage for transplantation. (Temple, Tsukamoto)
- Managing the lack of an appropriate animal model of AMD. (Coffey, Temple)

- Determining the optimal method for delivery, cell integration, and survival. (Temple)
- Measuring cellular potency and the mechanism of action. (Temple)
- Handling lengthy experimental time-lines and difficulty defining appropriate endpoints. (Temple)
- Determining the required duration of immunosuppression. (Temple, Tsukamoto)
- Avoiding adverse events, including the disruption of neural function and pain. (Temple)
- Securing clinicians, clinical sites, or institutional review board approval in the face of the political and ethical controversies surrounding the use of fetal-derived cells. (Tsukamoto)
- Navigating a confusing and lengthy regulatory pathway. (Coffey, Temple).
- Managing the high costs of translation. (Coffey, Temple)

Lessons Learned

- Communicate with patients and clinicians early in the translational process to determine their needs and find out what is compatible with clinical workflow. (Temple)
- Engage regulators in an ongoing and structured dialogue early on to accelerate and clarify the approval process. (Coffey)
- Carefully select an appropriate patient population. (Coffey)
- Do not underestimate the costs and time associated with safety studies. (Coffey)

PANEL DISCUSSION

Cell Dosing and Source

In the discussion following the presentations, a workshop participant inquired about the rationale underlying the selection of the cell dose for these therapies. Temple, whose approach to AMD therapy uses 50,000 to 150,000 cells per injection, said that their decision was based on the relatively small size of the diseased area and the number of cells that needed to be replaced, in addition to data from a previous study. The selection of number of cells for the patch for AMD was limited by the size of the patch (3 × 6 millimeters) and by how many cells could actually grow on it, Coffey said. In using HuCNS-SCs to treat AMD,

Tsukamoto said, she and her colleagues chose to dose with 20 million cells because that type of cell migrates and they wanted to cover a larger area. In the case of using HuCNS-SCs to treat spinal cord injuries, she said, researchers at StemCells, Inc., used allometric scaling based on the volume of the patient's spinal cord compared to the cord size used in animal model tests.

Brian Fiske asked about the rationale for choosing the source of cells for the therapies described by the speakers. Tsukamoto said that research on fetal-derived neural stem cells began well, before important discoveries were made using other cells such as hESCs or iPS cells. The easiest cell population to identify, purify, and expand was the brain stem cells, so her team pursued that, she said, and ultimately the rationale behind the source of the cells they chose was the timing of the science. Previous work that demonstrated that RPE cells from organisms such as salamanders could regenerate the RPE and the neural retina in about 4 weeks was the inspiration for her team, Temple said. This evidence spurred Temple's team to look for adult cells that were multipotent and could self-renew, to see if a similar regenerative process could be activated in human retinas, she said.

Tolerance and Immunosuppression

The challenge of achieving permanent tolerance in a setting with ongoing inflammation was an issue raised by Cynthia Dunbar, a workshop co-chair and the president of the American Society of Gene and Cell Therapy, and she asked the speakers to comment on why and when they decided to stop immunosuppression regimens after transplantation. There are currently no good models with which to examine graft-versus-host disease and tolerance induction, Tsukamoto said, and for their experiments they looked to data that had been published on Parkinson's disease patients who received fetal tissue grafts. Although they cannot rule out differences in the patient population (thoracic versus cervical spinal cord injury) that may have impacted the clinical outcome of the trial, shortening the length of the immunosuppression may also have contributed to the loss of the transplanted cells and subsequent decrease in motor function, she said.

Data Sharing

The speakers in this session were all involved in developing cell-based therapies for AMD, and a workshop participant asked if they had any mechanism for sharing data between them. "I think that would be wonderful," Temple said, adding that it is critical to share the failures and negative results from trials so that mistakes are not repeated. However, she said, there need to be incentives to create consortia. There has been a good amount of information sharing among those involved in the RPE trials, Coffey said, noting that knowledge sharing is critically important for the entire cell-based therapy community, not just those working on RPE.

Regulatory Oversight

Panelists were asked if they believed that the regulatory burden imposed on the therapies is appropriate for the seriousness of the disease, particularly from a patient perspective. The impact of disease on patients' lives may be something that regulatory agencies do not fully understand, the workshop participant noted, and perhaps risk–benefit analysis may be disease specific. It is important for charities, patient advocates, and hospitals to inform the regulators about how large the unmet clinical need is, and to share the perspective of the patients, Coffey said. Though they had an overall positive experience with FDA, it would have been helpful to have more interaction and a two-way conversation between the researchers and the regulators, Temple said. In the United Kingdom, the relationship between researchers and regulators is one of continual back and forth, Coffey said, which is markedly different than the relationship in the United States. At The Michael J. Fox Foundation, Fiske said, researchers have found ways to engage with FDA and have more open conversations by hosting workshops on specific topics.

5

Cardiovascular and Lung Tissues

Important Points Highlighted by Individual Speakers

- Converting resident cardiac fibroblasts into cardiomyocytes has potential as a regenerative therapy for heart disease, but challenges remain in delivery, safety, and regulation. (Srivastava)
- Testing cell-based therapies in large-animal models (e.g., pigs) may accelerate translation since the porcine heart is more akin to the size of a human heart. (Srivastava)
- Exosomes—bioactive nanoparticles that are secreted by all eukaryotic cells—mimic the regenerative benefits of cardiosphere-derived cells in treating heart conditions such as heart failure or cardiomyopathy. (Marban)
- Important information may be acquired from the study of newt cells and their exosomes, because newts have the exceptional capacity to regenerate limbs and parts of their visual system. (Marban)
- There is a great unmet clinical need for lung regenerative therapies, but the field lags behind other organ systems, perhaps because of the extraordinary structural and functional complexity of the lung. (Gomperts)
- Recently developed cells and organoids that mimic the three-dimensional architecture of lung tissue may be very useful for complex lung disease modeling and drug screening. (Gomperts)

Chronic cardiac and lung disease are among the leading causes of morbidity and mortality worldwide, and currently there are very few, if any, treatments available to alleviate the damage from heart and lung conditions and prevent mortality. Experimental approaches involving in

vivo cellular reprogramming, the therapeutic administration of exosomes, and bioengineered three-dimensional lung models have emerged as exciting new ways for understanding and treating these conditions. However, several challenges need to be overcome before these technologies can move forward in the research and development process or be or applied clinically. This chapter explores new advances in cell and exosome-based approaches to treating heart and lung disease and includes a discussion of remaining questions and challenges that need to be addressed in order for clinical translation to take place.

REPROGRAMMING APPROACHES TO CARDIOVASCULAR DISEASE

In the United States, heart disease is the number one cause of death in men and women and is the leading noninfectious cause of death in children, according to Deepak Srivastava, the Younger Family Director of the Gladstone Institute of Cardiovascular Disease, the director of the Rodenberry Center for Stem Cell Biology and Medicine, and a professor at the University of California, San Francisco. There are 6 million people in the United States who are living with heart failure, and about half of heart failure patients die within 5 years of diagnosis (CDC, 2016). Currently, there is no disease-modifying therapy available for heart failure, so there is tremendous excitement for the potential of cell-based approaches, Srivastava said.

There are several cell replacement strategies to treat heart failure that are currently under investigation. One approach involves the injection of cardiomyocytes or multipotent cardiac progenitor cells directly into the heart. A recent study demonstrated that fibroblast-derived induced pluripotent stem (iPS) cells that were differentiated into cardiomyocytes were able to improve cardiac contractile function in the damaged hearts of nonhuman primates, although there were concerns about post-transplant arrhythmias (Shiba et al., 2016). Furthermore, there is still uncertainty about the mechanism underlying the functional benefits observed in the hearts of the nonhuman primates. Although the approach generated a lot of excitement and has a great deal of potential, it will be important to address the issue of cell maturity in cardiomyocytes that are derived from iPS cells, Srivastava said. Another approach to treating heart failure involves introducing inductive signals into the heart to stimulate resident progenitor cells to regenerate the damaged tissue. A third

strategy involves stimulating resident cardiomyocytes to re-enter the cell cycle and divide in order to regenerate the heart. A fourth strategy, the focus of Srivastava's presentation, involves reprogramming endogenous and resident fibroblasts into new cardiomyocytes.

Cardiac fibroblasts are in abundance in the adult human heart, comprising approximately half of the heart's cells, Srivastava said. The cell-based approach that Srivastava described supposes that converting the resident fibroblasts in situ directly into cardiomyocyte-like cells may induce a regenerative effect. The first step for Srivastava's team involved determining how fibroblasts could be converted into cardiomyocyte-like cells by cellular reprogramming. To do so, they leveraged the vast knowledge amassed over the past two decades about cardiac cell fate decisions during embryogenesis, and they found that a cocktail of three transcription factors (Gata4, Mef2c, and Tbx5, referred to as GMT) was sufficient for generating functional cardiomyocytes from mouse postnatal cardiac or dermal fibroblasts (Ieda et al., 2010). Interestingly, the cell fate conversion did not involve transition through a progenitor or stem cell stage, but rather the cells converted directly from one adult somatic cell type to another, he said.

Srivastava and his team went on to demonstrate that it was possible to do in vivo reprogramming of murine cardiac fibroblasts into cardimyocyte-like cells by delivering GMT via myocardial injection (Qian et al., 2012). The reprogrammed cells exhibited a binucleate structure, assembled sarcomeres, ventricular action potentials, beating upon electrical stimulation, and evidence of electrical coupling (Qian et al., 2012). The cardiomyocytes derived from this conversion are electrically most similar to adult ventricular cardiomyocytes, whereas stem cell–derived cardiomyocytes are less mature, he said.

Even in vivo the cell reprogramming process was somewhat inefficient, Srivastava said, so his team used a high-throughput screening process to identify chemical modulators that could improve the process. It was discovered that Wnt signaling and TGF-β signaling inhibitors could independently improve the efficiency, quality, and speed of direct cardiac reprogramming in vitro, and when used together they had the remarkable effect of producing beating cardiomyocytes in a cell culture dish within 1 week. When researchers used an approach that introduced the genes for the three transcription factors (GMT) in combination with Wnt and TGF-β signaling inhibitors, they observed a greater rate of cardiac reprogramming than achieved when using the gene therapy approach alone (Mohamed et al., 2017).

Srivastava and his team hit a roadblock when they discovered that the cell reprogramming protocol that had worked well in mouse cells and models did not translate well into human cells. The researchers re-screened for other factors and found that the addition of MESP1 and ESRRG to the three original factors was sufficient to convert human cardiac fibroblasts to human induced cardiomyocytes (Fu et al., 2013). To further investigate this treatment in a larger heart more akin to the size of a human heart, they decided to use pigs as a model system, Srivastava said. The experimental protocol in porcine models involved inducing myocardial infarction at day 0, performing magnetic resonance imaging (MRI) to measure baseline damage at 3 days post infarction, delivering reprogramming factors via retrovirus at 5 days post infarction, and measuring cardiac function at 56 days post damage. Compared to the control group, the pigs that received the cardiac reprogramming genes had improved cardiac function at day 56 as measured by magnetic resonance imaging. The team is in the process of repeating the experiment using the chemical factors in addition to the gene therapy, Srivastava said.

Further refinement of the technique has reduced the number of factors necessary to reprogram human fibroblasts to three: MEF2C, TBX5, and Myocardin. One important remaining challenge is the issue of delivery, Srivastava said. The use of a retrovirus for gene delivery is not optimal because it may integrate in vivo, while adeno-associated viruses (AAVs) work well for targeting cardiomyocytes but generally have low rates of infectivity in cardiac fibroblasts. A positive-negative screen of AAV variants resulted in the identification of one AAV variant (A2) that has greater tropism for human cardiac fibroblasts and lower efficiency of infection in cardiomyocytes. Although approaches to direct cardiac reprogramming have drastically improved over the last several years, there are important challenges that remain, Srivastava said, including delivery, safety, and regulatory issues.

During the panel discussion a workshop participant asked Srivastava if converting fibroblasts into cardiomyocytes results in a depletion of the fibroblast pool. “Fortunately, [fibroblasts] can reenter the cell cycle and proliferate,” Srivastava said, and his team has not to date observed an issue with depleted fibroblast pools.

EXOSOMES AS NEXT-GENERATION THERAPEUTIC CANDIDATES

The process of culturing cardiosphere-derived cells (CDCs) was first described in 2007 and has since been reproduced by at least 26 labs worldwide, said Eduardo Marban, the director of the heart institute and a professor of medicine at Cedars-Sinai. To create CDCs, small amounts of biopsied cardiac tissue are placed in dishes coated with fibronectin. Stromal-like cells arise from adherent cardiac explants, and they are then re-plated onto non-adherent plastic, poly-lysine-coated dishes where they self-assemble into three-dimensional organoids called cardiospheres. The cardiospheres are then transferred to fresh growth medium and their numbers divided multiple times to yield the therapeutic candidate CDCs. CDCs are uniformly positive for the TGF-β receptor accessory subunit endoglin (also known as CD105) and negative for CD45 and all other hematogenous markers, Marban said. CDCs secrete stromal cell-derived factor (SDF-1), he said, and can induce the secondary secretion of SDF-1 via exosomes that contain a distinctive panel of microRNAs and other non-coding RNAs.

CDCs have been tested in the clinic several times, and the results of the first prospective, randomized trial, titled Cardiosphere-Derived Autologous Stem Cells to Reverse Ventricular Dysfunction (referred to as CADUCEUS), were published in 2012 (Makkar et al., 2012). In the CADUCEUS trial, autologous CDCs were administered to 17 eligible patients, with 8 patients receiving the standard of care. In this Phase I, proof-of-concept clinical trial, patients who received CDCs had an increase in viable myocardial tissue and regional contractility, along with a reduction in scar mass and regional systolic wall thickening (Makkar et al., 2012). The CADUCEUS trial demonstrated that CDCs are safe, and researchers have gone on to create an allogeneic version that is viable even in non-immunosuppressed patients, Marban said. Enrollment was just completed for a Phase II multi-center, randomized, placebo-controlled, double-blinded study of allogeneic CDCs in patients with mild heart failure after myocardial infarction. Other trials are under way to study allogeneic CDCs for advanced heart failure and for Duchenne muscular dystrophy–related cardiomyopathy.

Even though CDCs are cardiac progenitor cells, their mechanism of action is paracrine in nature, Marban said. CDCs have regenerative effects on the heart, including the promotion of cardiomyogenesis, the prevention of cardiomyocyte apoptosis, and increased anti-fibrotic and

anti-inflammatory effects. Transplanted CDCs do not proliferate, differentiate, or produce new tissue of donor origin, Marban said, but rather they survive for several weeks while secreting factors that lead to new healthy tissue of host origin. Discovering this led researchers to wonder if there was a single entity that could mimic all the salient benefits of CDCs which could be developed into a cell-free therapeutic approach. One possible solution was exosomes, bioactive nanoparticles that are secreted by all eukaryotic cells and present in all body fluids. Exosomes are 30 to 150 nanometers in diameter and contain a unique complement of microRNAs and other bioactive contents that vary depending on the cell type and culture conditions. A recent study demonstrated that CDC-secreted exosomes reproduce the therapeutic regeneration associated with the administration of CDCs and that inhibiting the production of exosomes in CDCs negates their positive therapeutic effects (Ibrahim et al., 2014). In response to a workshop participant's question about reproducibility, Marban said that his team has sent exosomes to various other labs to see if their results can be replicated and if there is bioactivity in other model systems. Their results have been verified in a few other models, he said, noting that CDC-derived exosomes inhibited human T cell degranulation in antibody-dependent cell-mediated cytotoxicity, and induced regenerative effects on skeletal muscle in the mdx mouse model of Duchenne muscular dystrophy. Although CDCs are initially derived from heart tissue, their bioactivity may be applied elsewhere in the body, Marban said.

Several studies have indicated that CDC-derived exosomes have the same regenerative, anti-inflammatory, anti-fibrotic, anti-apoptotic, and immunomodulatory effects as CDCs themselves (Aminzadeh et al., 2015; Chimenti et al., 2010; Ibrahim et al., 2014; Li et al., 2012; Makkar et al., 2012; Smith et al., 2007; Tseliou et al., 2014a,b). In an attempt to understand if one specific component of exosomes was responsible for the regenerative effects, researchers compared the microRNA profiles of exosomes from CDCs to those from normal human dermal fibroblasts. One particular microRNA, miR146a, was heavily enriched in CDC exosomes, but no single RNA species can account for all the benefits of CDC-derived exosomes, Marban said. Instead, "it is the totality of the contents that are required for full manifestation of bioactivity," he said.

Recently, Marban's team has turned their attention to newts, amphibians with an exceptional capacity for regeneration. Although newts separated from the mammalian lineage approximately 300 million years ago, there may be important lessons to learn from newt cells, Marban said.

Early experimental results indicate that A1 cells, a naturally immortal type of cell found in newts, have the ability to generate exosomes that contain eight times as much RNA per particle as mammalian exosomes and are bioactive in mammalian injury, Marban said.

Bringing therapeutic exosomes to the clinic required that the technology be transferred to a company that could take it to the next step, Marban said. Therefore, in 2005 he co-founded Capricor, Inc., to further the research on CDCs and exosomes, among other technologies. Marban emphasized that the company is managed exclusively by scientists and clinicians and is supported by grants from the California Institute for Regenerative Medicine, the Department of Defense, and the National Institutes of Health. Capricor's business model includes a wide range of activities including discovery through development, manufacturing, regulation, and clinical trials design and management, he said.

REGENERATIVE THERAPIES FOR LUNG DISEASE

There is a great unmet clinical need for lung regenerative therapies, but unfortunately the field lags behind many other tissues and organ systems, said Brigitte Gomperts, an associate professor of pulmonary and pediatric medicine at the University of California, Los Angeles. This may be due, in part, to the structural and functional complexities of the lung, she said. In the upper airways, the proximal cartilaginous airways are in direct contact with the environment, and they produce mucus to trap bacteria and viruses and other particles from, for example, pollution. There are also ciliated cells which beat unidirectionally to move the mucus up and out of the body. In the lower airways, gas exchange occurs at the level of the alveolar sacs, which provide a very large surface area where the epithelial and endothelial cells come together to allow the diffusion of oxygen into the capillaries and of carbon dioxide back into the alveoli spaces to be breathed out. The lung contains more than 42 different cell types, Gomperts said, and there does not seem to be one specific stem cell that makes all lung cell types. Lung diseases are complex because all of the anatomical areas of the lung and multiple cell types are affected.

The field of lung disease research tends to be divided into two main areas, Gomperts said. The first area is focused on monogenic lung diseases, such as cystic fibrosis (CF), and researchers in this area have made great therapeutic advances, she said. The second focus is on

complex lung diseases, where unfortunately progress has been much slower for a number of reasons, she said (see Table 5-1).

CF results from an inherited mutation in the cystic fibrosis transmembrane conductance regulator (*CFTR*) gene. CFTR functions as an ion channel, and although CF is a systemic disease, it has major implications in the lungs, Gomperts said. Currently there are two Food and Drug Administration–approved drugs available for CF; however, there are subsets of patients with specific mutations who will not respond to these drugs, Gomperts said, and stem cell therapies may hold a great deal of therapeutic potential for those individuals. The strategy underlying potential cell-based therapies for CF involves generating iPS cells from patients, correcting the genetic defect in the stem cells, differentiating them into lung stem or progenitor cells, and transplanting them back into patients. A few of those steps have been completed, including the creation of iPS cells from CF patients and the ex vivo gene correction of the most common CF variant, Gomperts said. However, there are many challenges remaining, including determining the correct stem or progenitor cell to use, finding novel ways to expand the cells, and identifying the optimal delivery and engraftment approaches.

TABLE 5-1 Successes and Challenges in Regenerative Therapies for Lung Diseases

	Monogenic Lung Diseases	Complex Lung Diseases
Pathogenesis understood	√	×
Representative mouse models	√	×
Therapeutic strategies identified	√	×
Disease examples	Cystic fibrosis and pulmonary alveolar proteinosis	Idiopathic pulmonary fibrosis and chronic obstructive pulmonary disease

NOTE: √ indicates that the specific challenge or issue in the left-hand column has been overcome and × indicates that further research is required.
SOURCE: Brigitte Gomperts, National Academies of Sciences, Engineering, and Medicine workshop presentation, October 13, 2016.

The progress made toward CF therapies is in stark contrast to research on complex lung diseases, such as complex obstructive pulmonary disease or idiopathic pulmonary fibrosis (IPF), for which there are few therapies currently available. This may be due in part to a lack of understanding about the interactions between the genetic and environmental factors that contribute to these diseases, Gomperts said. To address the therapeutic gap for complex lung diseases, Gomperts and her team investigated the possibility of using iPS cells as a model to study IPF. Their protocol involved removing fibroblasts from the damaged lungs of IPF patients when they were undergoing transplantation. Although the IPF lung fibroblasts came from extensively damaged lungs, the cells were phenotypically and genotypically almost identical to normal fibroblasts, Gomperts said. Next her team generated iPS cells from the IPF fibroblasts, allowed them to spontaneously differentiate, and placed them onto 12-kilopascal hydrogels, which mimic the stiffness of the IPF lung. After about 2 weeks on the hydrogels, the cells exhibited a progressive phenotype of aggregation. Immunostaining was used to test the cell aggregates for markers of fibrotic foci, which are the hallmark of IPF. The researchers found alpha smooth muscle actin staining, a marker of activated fibroblasts, as well as collagen production and evidence of proliferation. Further examination revealed that the patient-derived iPS cells had elevated levels of cytokines and chemokines, increased levels of TGF-β activity, damaged associated molecular patterns, and increased cellular stiffness—all of which are similar to features of the lung tissue of IPF patients. These cells may be a very useful model of IPF in vitro and could be used for disease modeling as well as drug screening, Gomperts said.

Recently researchers have made exciting advances in bioengineering which will greatly aid lung disease research, Gomperts said. One such advance is the development of a human lung "small airway-on-a-chip," which consists of primary cells seeded in a two-chambered microfluidics device, allowing for the analysis of organ-level lung pathophysiology (Benam et al., 2016). In her lab, Gomperts and other researchers are attempting to mimic the lung's cellular architecture in a three-dimensional model system. To do this, researchers allow cells to adhere to alginate beads coated with collagen and dopamine. A rotating bioreactor encourages the cell-coated beads to come together to form organoid-like structures. Sectioning through the organoids reveals that the tissue very closely mimics the three-dimensional structure of normal

human alveolar lung tissue, Gomperts said. This bioengineered "lung" could be very useful for disease modeling and drug discovery.

Looking toward the future of lung disease research, Gomperts commented on possible next steps. One goal should be to generate three-dimensional lung models complete with respiratory membranes that mimic gas exchange, she said. Ideally, regenerative approaches for lung diseases in the future would involve introducing a scaffold or a large amount of functional tissue back into patients, she said.

PANEL DISCUSSION

Partial Reprogramming

A workshop participant queried the panelists about the issue of cells that only get partially reprogrammed during in vivo reprogramming. The participant hypothesized that partially reprogrammed cells may have some deleterious effects. Srivastava responded that he is concerned about partially reprogrammed heart cells acting as a nidus for rhythm disorders because they are not as electrically mature. While they have seen evidence that some of the cells are indeed partially reprogrammed, they have not yet seen evidence of arrhythmias in animal models. There is evidence of a percentage of fibroblasts expressing the reprogramming genes, but not of them undergoing conversion to cardiomyocytes, Srivastava said. These cells remain in a fibroblast-like state, he said, but they do not function like normal fibroblasts and may not lay down as much collagen. These partially reprogrammed, altered fibroblasts may contribute to the improvements observed after attempted cell reprogramming.

Unique Challenges

"What are the unique challenges for this area of medicine besides the fact that this is a new field?" asked Jiwen Zhang, the senior director of regulatory affairs in the Cell Therapy and Regenerative Medicine Division at GE Healthcare and the session moderator. Unlike the case for a single drug or single therapy, these new therapies are extremely complicated, Srivastava said, and likewise, the development and regulatory processes are also complicated. However, he noted, there is a huge unmet medical need that has no other readily available solution, so researchers should not be deterred by the complexities. Marban added

that one of the challenges is having a thorough understanding of the complexities in order to be comfortable with safety and mechanism of action of a new therapy. "In the future it may be possible to come up with defined cocktails of the active factors that may reproduce many of the desired effects of cell therapy as next-generation products," he said. Cell-based therapies for lung diseases are likely not going to be solutions that use just one type of cell, Gomperts said, but instead they may be organoids or scaffolds. She said that these types of therapies are likely still very far away, but that cell-based disease modeling and drug screening are "low hanging fruit" that may be achievable in the near future.

Physician Involvement

Next, Zhang queried the panelists about how clinicians can be engaged in the research process, specifically in terms of managing patients and helping them navigate these new technologies. All three panelists noted that in addition to their research roles, they are physicians who still work in the clinic and see patients. The training that physician scientists receive is very important, Marban said, because it introduces a certain level of "healthy skepticism," whereas clinical training alone may not provide that. It was extremely challenging to get physicians to administer new cholesterol-lowering medications, Srivastava said, indicating that the adoption of cell-based therapies by physicians might also be a huge challenge. Even though she works closely with a pulmonologist, Gomperts said, it has been very difficult to collect patient samples, and she added that it "is going to take a lot of work and a lot of interactions and collaborations to really get the physicians on board."

Scaling Up

Organs such as the lungs and heart are much bigger than the macula, noted a workshop participant, who went on to ask the panelists whether it will be a challenge to scale up cell-based therapies for larger organs. The challenges of such a scale-up will be significant but not impossible, Marban said, noting that bioreactors and other amplifying mechanisms may be useful. Exosomes may be another part of the answer, he continued, because a single eukaryotic cell can make several thousand exosomes per day, which explains why "a few cells seem to make a difference." The scale-up of production to generate a billion or more

pluripotent stem cell–derived cardiomyocytes is less of a problem today, Srivastava said. The major challenge is getting those cells to engraft and survive upon transplantation, he said. That is one reason why his research is increasingly focusing on harnessing the regenerative power of the resident cells.

6

Renal Tissue

Important Points Highlighted by Individual Speakers

- Researchers have made strides in using human pluripotent stem cells to build kidney-like organoids that could be used for disease modeling, drug discovery, and toxicity testing for drugs. (Humphreys)
- The current treatments for renal disease—dialysis and kidney transplant—are expensive and difficult; advances in regenerative therapies for renal disease have the potential to make a big difference in patients' lives and in the cost of treatment. (Baron)
- Therapy for polycystic kidney disease should begin much earlier in the course of the disease, meaning that disease detection must improve and that the therapy will need to be safe and tolerable, potentially for decades. (Baron)
- Blastocyst complementation and xenotransplantation are promising concepts, but they are still very early in the discovery phase. Understanding the scientific basis and complex ethical issues related to both concepts will require years of additional research before they reach the clinic. (Humphreys)

There has not been a new treatment for end-stage renal disease (ESRD) developed in nearly 40 years, said Ben Humphreys, the chief of the Division of Nephrology in the Department of Medicine at Washington University School of Medicine in St. Louis. For many patients with kidney disease, the only treatment is dialysis and, potentially, a kidney transplant, but research advances in recent years have generated hope that new therapies based on gene editing, organoids, and even xenotransplantation may one day be available.

END-STAGE RENAL DISEASE

Current approaches to addressing ESRD are not optimal, Humphreys said. According to the U.S. Renal Data System annual data report, more than 660,000 Americans are being treated for ESRD, with 468,000 of those patients being treated with dialysis, a procedure that filters a patient's blood through an "artificial kidney" to remove waste, salt, and extra water from the body; maintain safe levels of potassium, sodium, and bicarbonate in the blood; and control blood pressure (National Kidney Foundation, 2015, 2016a). Dialysis is a life-saving procedure, and without it people with ESRD would die within 2 weeks, Humphreys said. However, it is a costly therapy. ESRD patients typically receive a 4-hour dialysis procedure three times a week, resulting in costs of about $82,000 per year to treat a single patient (U.S. Renal Data System, 2013). While dialysis is a widely accessible treatment and does extend life, patients on dialysis have a much lower life expectancy than their healthy peers. The only alternative treatment to dialysis is kidney transplantation, which costs less in the long term and improves life expectancy, but is less feasible because there are not enough donor kidneys available to meet the needs of a growing number of patients. More than 100,000 ESRD patients are on the transplant list (National Kidney Foundation, 2016), and most will die before they receive a kidney, Humphreys said. The relative success of dialysis, combined with its high costs, has hampered further development in the field by shunting money away from basic research, he said.

The development of stem cell and regenerative medicine approaches to treat kidney disease lags behind the development of some other fields, but there has been remarkable progress in recent years, Humphreys said. Researchers have started using human pluripotent stem (hPS) cells and induced pluripotent stem (iPS) cells to grow kidney organoids (Takasato et al., 2015). The approach involves culturing hPS cells (or iPS cells) and exposing them to a variety of signaling molecules at specific concentrations and times to mimic the conditions of normal in vivo embryonic development. By observing normal embryonic development and analyzing the signaling found in the pluripotent cells that eventually give rise to the kidney, researchers were able to mimic the migration and signaling pathways to culture two unique progenitor cell populations: ureteric bud cells and metanephric mesenchyme cells. These cells are collected, disaggregated, and then recombined via centrifuge to form a pellet composed of both cell types. The cell pellet is cultured over a

period of 2 to 3 weeks, during which time the progenitor cells continue to differentiate and self-organize into small, heterogeneous clumps of cells that resemble the basic structure of a kidney. These structures, called organoids, contain roughly 15 different cell types (of a possible 26 cell types) typically found in the mature kidney (Al-Awqati and Oliver, 2002). They also contain nephron-like structures that consist of the proximal tubule, the glomerulus, the distal tubule, and the collecting duct. In the immediate future, these organoids hold promise for several applications, including disease modeling, toxicity testing for drugs, and drug discovery, Humphreys said. While organoids hold the potential to improve the quality and accuracy of research models in the near future, there is also hope that scientists will eventually be able to grow functional kidney tissue intended for clinical applications in vitro using the technique established to develop organoids. There remain significant challenges that must be overcome before this approach may be used in a clinical setting to treat patients; specifically, the technical challenge of scaling up the size and improving the morphology of organoids to more closely match those of a healthy, mature kidney remains a significant hurdle. Currently, the organoids developed in Humphreys's lab are very expensive to produce and measure only about 5 millimeters, whereas the human kidney is 10–12 centimeters. Organoids developed in vitro also tend to have a "fried egg" morphology because of the effects of gravity, although researchers have addressed this through the use of a miniature bioreactor that can produce a sphere-shaped organoid.

XENOTRANSPLANTATION AND BLASTOCYST COMPLEMENTATION

Alternatives to traditional human kidney transplant, such as the xenotransplantation of pig kidneys into humans and blastocyst complementation, have been explored and continue to remain an attractive opportunity to develop more accessible and functional organs for transplant in ESRD patients, Humphreys said. Pfizer first investigated xenotransplantation of pig kidneys into humans in the mid-1990s, but the research was stopped because of concern over porcine endogenous retroviruses (PERVs), he said. PERV genomes are integrated into the larger genome of a pig and, depending on the class of PERV, can undergo replication in normal pig cells and infect human cells when exposed in culture or via transplant. Unlike other zoonotic pathogens,

PERVs cannot be eliminated through traditional approaches such as biosecure breeding.

Numerous studies have proven the infectivity of subclasses PERV-A and PERV-B when primary pig cells are co-cultured with primary human cells in vitro (Le Tissier et al., 1997; Patience et al., 1997), but it is unclear whether the rate of infectivity of an in vivo transplant would result in widespread infection or the development of clinical symptoms (Wilson, 2008). A study conducted in mice with severe combined immunodeficiency found that the transplantation of pig pancreatic islet cells into the mice resulted in limited PERV infections with no related symptoms. Scientists at The Scripps Research Institute have suggested that immunodecificient mice may provide a good model for the study of xenotransplantation in humans, but they cautioned that further research is required (van der Laan et al., 2000). There are few studies of porcine xenotransplantation into humans because of the potential risks associated with PERVs; however, the limited clinical examples of human exposure to pig organs or xenotransplant products have not demonstrated any clinical evidence of PERV infection.

With the advent of clustered regularly interspaced short palindromic repeat (CRISPR)/Cas9 technology, there has been renewed interest in the potential of porcine xenotransplantation, because CRISPR/Cas9 may be used to inactivate PERVs in the porcine genome and remove the risk of PERV infection, which previously could not be eliminated. Initial research into this approach was conducted by researchers at Harvard University, who recently reported the successful inactivation of all PERVs found in a porcine kidney epithelial cell line (PK15) using CRISPR/Cas9 technology. Genomic analysis of the PK15 cell line showed 62 copies of PERVs in the cell genome. To accurately target and inactivate all 62 copies, the researchers used polymerase chain reaction to identify distinct, highly conserved DNA sequences unique to PERVs. These sequences, identified as *pol* genes, code for a reverse transcriptase that is vital for PERV replication and infection. By designing a Cas9 guide RNA to specifically target the *pol* gene, the researchers were able to achieve a 1,000-fold reduction in in vitro PERV transmission to human cells as compared with non-edited PK15 cells. This approach has only been applied in vitro to the PK15 cell line, but it does demonstrate the possibility for clinical application in the future (Yang et al., 2015). However, even with the use of CRISPR/Cas9 to inactivate PERVs within the porcine genome, xenotransplantation of pig kidneys into humans remains a substantial challenge, Humphreys said, noting that organ

rejection remains an issue and that life-long immunosuppression would still be required to maintain tolerance of the transplanted kidney.

Blastocyst complementation, a technique in which a recipient blastocyst is induced to generate exogenic organs resulting in a chimeric organism, has also emerged as a potential approach to growing human kidneys in pigs, Humphreys said. He cited a study published by Nakauchi et al. in 2013, in which the researchers successfully developed apancreatic pigs by introducing transgenes that inhibit pancreatic development into mature oocytes. The procedure resulted in male pancreatogenesis-disabled fetuses that were capable only of developing a vestigial pancreas. The apancreatic pigs were cloned using somatic nuclear cell transfer. Concurrently, the team has induced donor pig embryos to express the protein humanized Kusabira-Orange (huKO), which fluoresces orange. The apancreatic pig embryos were allowed to mature to the morula stage, at which point they were injected with blastomeres from the morula stage donor embryos that expressed huKO. The chimeric host morulae were cultured in vitro and then transferred to the uterus of a recipient sow and allowed to mature until the late-term fetus stage, when they were analyzed for pancreas development. Fetuses from the host blastocysts (non-chimeras) did not develop a pancreas, while those from the chimeric blastocysts and donor blastocysts did develop pancreata. Notably, the pancreata in the chimeric fetuses fluoresced orange, indicating that they were derived from the donor blastomeres. The success of this approach in this study and others provides the basis for research into the production of human organs in pigs. With current CRISPR technology, the potential to create pig embryos that lack kidneys and other target organs is increasingly feasible, Humphreys said, and the injection of human iPS cells into the CRISPR-edited blastocysts could result in the development of pig chimeras that produce human organs for transplant into patients in need. The immunorejection of the xenogenic organ in the host animal and the potential for organs derived of a mix of host animal and xenogenic tissues remain technical challenges (Kemter and Wolf, 2015). While promising, Humphreys said, the approach is still early in the discovery phase, and the scientific technique and complex ethical issues related to the concept will require years of additional research before the technique reaches the clinic (Nagashima and Matsunari, 2016).

There remains tremendous clinical need for new therapies to treat chronic kidney disease, Humphreys concluded. We have not had a new drug in the chronic kidney disease space in decades, he said, noting that

the costs of treatment are remarkably high. Kidney organoids are changing the way researchers in the field approach their pursuits, and with continued investment and collaboration, there is reason for cautious optimism, he said.

POLYCYSTIC KIDNEY DISEASE

Polycystic kidney disease (PKD) is a fatal, monogenic disease and is the fourth leading cause of renal replacement therapy, said David Baron, the chief scientific officer of the PKD Foundation. Autosomal dominant PKD (ADPKD), the most common monogenetic kidney disease, is caused by mutations in one of two genes: *PKD1*, which accounts for about 85 percent of patients with autosomal dominant PKD, and *PKD2*, which generally results in a more mild phenotype. Spontaneous mutations are responsible for up to 10 percent of patients with ADPKD, with most individual mutations occurring at a low frequency. The correlation between phenotype and genotype is variable, and some mutations are quite rare. There are about 600,000 people in the United States who have been diagnosed with ADPKD, Baron reported (PKD Foundation, 2016). Autosomal recessive polycystic kidney disease is a similar disease that is caused by a mutation in a different gene that is very rare and that affects the kidneys, livers, and lungs of children (National Kidney Foundation, 2016c).

PKD results in the very rapid growth of the kidneys, and the issues that confront patients include hypertension, infection, hematuria, kidney stones, electrolyte imbalance, pain, fatigue, and, ultimately, ESRD and the need for dialysis or transplant. An increased risk of retroperitoneal bleeds is another effect of PKD, Baron said. The long-term impact of retroperitoneal bleeds can be severe since blood transfusions, which can complicate the ability to accept a kidney transplant, are often used to treat them. Because PKD is a systemic ciliopathy, and because most cells of the body contain a primary cilium, the disease affects far more than just the kidneys, Baron said. For example, other manifestations of PKD include mitral valve prolapse, abdominal wall hernias, diverticulosis, and diverticulitis (National Kidney Foundation, 2016c).

ADPKD is a progressive disease, with cysts most likely developing in utero, but patients are frequently not diagnosed until the third or fourth decade of life, Baron said. The kidney grows rapidly over time, and the disease is usually diagnosed when cysts are found by ultrasound,

although MRIs and other imaging techniques can show cysts as well. Glomerular filtration rate (GFR) is a surrogate biomarker for PKD, Baron said, but by the time that GFR begins to decline, the number of parenchyma-destroying cysts has grown so substantially that it is unlikely that renal function can be maintained at that point (Grantham et al., 2006). Another way to detect PKD is through genetic testing, which may be appropriate if there is a family history of PKD or if magnetic resonance imaging or ultrasound imaging shows an uncertain diagnosis of PKD (National Kidney Foundation, 2016c). Ideally, therapy for PKD should occur much earlier in the course of the disease, meaning that disease detection must improve and that any therapy will need to be safe and tolerable, potentially for decades, Baron said.

The current treatment approaches for PKD include symptom management through diet and lifestyle and medication, dialysis, and transplantation, although there is a shortage of available kidneys (NIDDK, 2015). There have been advances in transplantation immunology that have improved how people can live and work with transplants. For example, Baron is on a steroid-free regimen. Advances in cardiovascular therapies have resulted in better control of hypertension and of the effects that declining renal function has on the heart. Although there is still controversy over how the mutation actually causes cysts, researchers are continually improving the knowledge base regarding the cellular and molecular mechanisms of the disease, Baron said. However, he noted that there have been no recent therapeutic advances in the United States. A new therapeutic called tolvaptan, a vasopressin V2 receptor antagonist, has been approved in Canada, the European Union, and Japan, but not yet in the United States (Business Wire, 2013; PKD Foundation, 2013). It decreases the growth of cysts over time, but it also causes extreme thirst, polyuria, and an increase in liver enzymes in some patients (ASHSP, 2016). There are a number of ongoing trials for tolvaptan as well as repurposed drugs such as metformin, pioglitazone, niacinamide, tesavatinib, and lanreotide (PKD Foundation, 2013). However, because of the complexity of the disease, it seems unlikely that any one drug will be able to address the multiple pathways of this disease, Baron said. The regulatory path for the approval for new and novel PKD therapeutics is still quite ill-defined, he said.

Investments in regenerative medicine research for PKD have the potential to be hugely cost-effective, Baron said, because the current standard of renal replacement therapy is so expensive. About $4 billion is spent each year on renal replacement therapy for patients with ADPKD,

Baron estimated. Funding for renal research needs to be increased, he said, because the "savings are obvious"—the fewer people on dialysis, the greater the benefit to Medicare.

The applications of regenerative medicine to treat PKD are being explored, although potential therapies are still many years away from clinical application. According to Baron, possible regenerative therapies for PKD include

- embryo selection at the 32-cell stage to avoid the occurrence of PKD;
- directed drug delivery into the cyst, such as folate receptor targeted delivery of folate-conjugated rapamycin to the cells that line renal cysts in PKD or the use of dimeric immunoglobulin A antibodies to introduce antibodies against growth factors implicated in the development of renal cysts, such as epidermal growth factor, ouabain, TGF-α, TGF-β, TNF-α, and IL-1β (Olsan et al., 2015; Shillingford et al., 2012);
- autologous genetically "corrected" stem cell infusion;
- the infusion of exosomes containing corrected forms of the polycystin-1 protein or mRNA; and
- implants of autologous genetically corrected kidney organoids or non-immunologic hybrid kidneys made from autologous corrected kidney cells seeded onto a non-immunologic bioengineered scaffold.

Most of these potential therapies are years away from the clinic, Baron emphasized, though he noted that because treating PKD involves nephron regeneration or cell repair, advances in this area may be generalizable to other renal diseases or other ciliopathies. Moving forward, well-informed PKD patients will be needed to inform assessments of the risks versus benefits of potential therapies and to assist the Food and Drug Administration and other regulators in deciding what therapies to move forward with, he said.

PANEL DISCUSSION

Patient Awareness

Some participants asked if there was any value in promoting awareness and the early diagnosis of PKD, perhaps through kidney volume imaging of children at ages 10 and 20 to see if the size of the kidney has increased. That is possible, Baron said, although there is an ethical dilemma involved with telling a young person that he or she has a disease that has no treatment.

Bioscaffolding and Organoids

The panel was asked about the possibility of using acellular structures or three-dimensional printing with bio-material to create a functioning organ. This is an area of great interest, Humphreys responded, noting that early research indicates that using progenitor cells to "build" a kidney on a scaffold has resulted in some cells differentiating as they should, while others do not. However, this approach is "forcing something that is a little unnatural," he said. While we should not rule out new ideas, the most promise lies in direct differentiation, when cells "simply do what they want to do" and create nephrons. Right now, he said, it is possible to create about 200 nephrons in a small organoid, but in the future it will be possible to scale up production to produce the million nephrons that are present in an adult kidney. Hoshizaki added that investment in creating kidney organoids may be fruitful not only as a potential therapeutic organ, but as a research and assay tool.

Gene Editing and PKD

Several workshop participants observed that standard genetic editing may not be possible for PKD, because the affected gene is too large for traditional delivery vehicles. Genetic researchers have had success in treating other diseases by targeting second-site suppressors, that is, genes in a second site that prevent or alleviate a disease that would otherwise be present due to a mutation. Because some PKD patients have milder forms of the disease, there was a discussion about whether this could be due to a second-site suppressor and if these genes could be targets for PKD treatment. This idea is worth investigation, Baron said, but many

factors that explain the disease, including environmental factors, are still unknown.

A workshop participant asked about identifying and using targets in the kidney to grow new nephrons in vivo. To date, this approach has not shown success in humans, Humphreys responded. However, the fact that other species—particularly fish—can grow new nephrons indicates that perhaps the developmental signaling pathways could be reactivated in mammals and result in new nephrons. The activation of this pathway would need to be balanced against the potential for carcinogenesis, Humphreys said.

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Looking Toward the Future: Concluding Thoughts

Over the course of the workshop, several themes emerged, highlighting common challenges, areas of opportunity, and prospects for future innovation. In the final session of the workshop, George Daley, the director of the Stem Cell Transplantation Program at the Boston Children's Hospital and the Dana-Farber Cancer Institute and the dean of Harvard Medical School, provided his thoughts on the state and direction of research in regenerative medicine. Daley and a panel of stakeholders then summarized their individual views of the key themes that emerged throughout the workshop and added their perspectives about the prospects for regenerative medicine and the roadblocks that must be addressed in order to move forward.

HYPE AND THE PROMISE FOR CHANGE

Daley began by proposing a paradox, prompted by a *Boston Globe* opinion piece by Eric Lander titled "Hype vs. Hope in Medical Research."[1] In the article, Lander explored whether the promise of genomic medicine was overhyped, arguing that the hype surrounding genomic medicine is contradictory, because there is great potential for genomics to change the state of medicine, but it will be many years before its full potential is realized. The field of regenerative medicine faces the same issue, Daley said. In Lander's piece he invokes Amara's Law, which states that we tend to overestimate the effect of a technology in the short run and

[1]To read the full article from the *Boston Globe,* see https://www.bostonglobe.com/opinion/2016/10/12/hype-hope-medical-research/nY3hXS67HT0mQ78BGmQQfJ/story.html (accessed December 15, 2016).

underestimate the effect in the long run. Amara's Law applies to regenerative medicine as well, Daley said, because while these exciting cell-based approaches are unlikely to drastically change medical care in the near term, the field holds great promise for transformation in the long term.

THE PATHWAY TO DEVELOPING NEW TECHNOLOGIES

Emerging medical technologies usually take 20 to 30 years to mature, Daley said, reflecting on the advent of recombinant DNA technology by Stanley Cohen and Herbert Boyer in 1973, which enables targeted, individual fragments of DNA from a donor genome to be inserted into vector DNA molecules such as plasmids, which can then be amplified in bacteria (Griffiths et al., 1999). Recombinant DNA technology allowed researchers to target DNA sequences that code for specific proteins, which could then be inserted into plasmid vectors and used to produce the desired protein in bacteria. Remarkably, the first recombinant protein product, Humulin, was brought to market by Eli Lilly in 1982, Daley said, but the broader impact of the technology was not realized until the release of Epogen in 1989, which was followed by several other protein therapeutics brought to market in the early 1990s.

Monoclonal antibodies, pioneered by Kohler and Milstein (1975), followed a similar trajectory. Orthoclone OKT3, an immunosuppressive drug used to prevent rejection in solid organ transplants and the first monoclonal antibody approved for use in humans, was approved by the Food and Drug Administration (FDA) in 1985, but again, it took more than 20 years before monoclonal antibody drugs became commonplace with the development of Rituxan, Herceptin, and others in the late 1990s, said Daley. Fire et al. (1998) successfully used RNA interference (RNAi) to manipulate gene expression in C. elegans in 1998, shared Daley, but there is still no product clinically available that uses that technology.

Stem cells and cellular therapies have followed this pattern, too, said Daley. Society is already reaping the benefits of years of research and investment in some cell-based therapies, such as hematopoietic stem cell therapy, he stated, highlighting the recent progress in applying gene editing and recombinant DNA approaches to T cell modification and other hematopoietic stem cell therapies. However, therapies that rely on other kinds of stem cells are still years away from success in the clinic. Research on embryonic stem cells, which were isolated and characterized in mice in 1997, has resulted in clinical data with indications of efficacy in

treating macular degeneration, spinal cord injury, and Parkinson's, but has yet to result in a commercially available Food and Drug Administration (FDA)-approved product, Daley said. It has been also challenging to develop clinical therapies using induced pluripotent stem (iPS) cells, which were pioneered in 2007. They have proven invaluable as a tool for modeling disease and screening for potential drugs, but there have been few cases where patients have received iPS cell therapies in the clinic (Scullideri, 2016). The hope that accompanied the emergence of both embryonic stem cells and iPS cells as potential new regenerative approaches to treat disease has been tempered by the decades-long research and development process that has yet to yield an FDA-approved product, Daley said, but the field is on the cusp of success, with promising therapies to treat neurologic diseases currently in clinical trials and with remarkable progress having been seen in the field of in vitro gametogenesis to treat infertility.

CHALLENGES FACING THE FIELD

Deriving the medically relevant cell type is a significant challenge in the field of regenerative medicine, Daley said. Finding, characterizing, and growing the right cell is a decades-long investment, he said, as demonstrated by Lorenz Studer's work with pluripotent stem cells and his success in "pharmaceuticalizing" a cell by understanding its identifying characteristics, potency, and developmental pathway. There is no substitute for a deep mechanistic understanding of the way cells work, Daley said. Progress is hampered by a lack of clear definitions of cell identity and cell function, which are necessary to assign sufficient confidence in a given cell type and predict its therapeutic efficacy.

Related to the challenge of defining the right cell is the challenge of identifying the right time for the clinical translation of new research. Because the research and development pathway is still unclear for the field, there is a risk of premature clinical translation, Daley said. Without clear definitions or a strong mechanistic understanding of a proposed therapy, there is an increased chance that weak clinical hypotheses will be pushed through the regulatory process, only to result in expensive failures. The field is "drowning in failures and in the expense," he said, advocating that the solution should not be to reduce regulatory burden, but to hold the scientific community to a higher standard of understanding at the preclinical level and to bring stronger hypotheses to the clinic for testing.

INTERNATIONAL SOCIETY FOR STEM CELL RESEARCH GUIDELINES FOR STEM CELL RESEARCH AND CLINICAL TRANSLATION

Holding research to a higher standard begins with the scientific community. The International Society for Stem Cell Research (ISSCR) acts as a steward of the field of regenerative medicine by bringing together scientists and clinicians involved in stem cell research and promoting high scientific standards through communication and the development of guidelines for the responsible conduct of research, Daley said. In May 2016 the ISSCR released an updated set of guidelines for stem cell research and clinical translation.[2] The initial set of guidelines was meant to assist researchers in the controversial field of embryonic stem cells, but it has evolved into a broader effort to guide the clinical translation of stem cell therapies. The updated ISSCR guidelines set very high standards and aspirational goals for the scientific community rather than concrete criteria because there is so much variability between cell types and potential therapeutic applications, Daley said. The new guidelines are intended as a roadmap for the field moving forward to support the conduct of high-quality research and safe and effective clinical trials. The ISSCR's principles, as outlined by Daley, are below:

- Clinical protocols should undergo independent, expert peer review that is free from conflicts of interest in order to set a high standard for any clinical hypotheses brought into clinical testing.
- Clinical trials should be held to a high standard of safety and efficacy, and the potential benefit of protocols should be easily and clearly weighed against well-defined risks.
- Standards for manufacturing and processing must be high to support the development of products that are consistent, safe, and effective.
- There should be high standards for efficacy and a mechanistic understanding of a therapy as a precondition for entering clinical trials and the eventual marketing of the therapy. Efforts to reduce the regulatory hurdles should be met with "healthy skepticism"

[2]To read the complete *Guidelines for Stem Cell Research and Clinical Translation* from ISSCR, please see http://www.isscr.org/docs/default-source/guidelines/isscr-guidelines-for-stem-cell-research-and-clinical-translation.pdf?sfvrsn=2 (accessed December 15, 2016).

because lower standards will risk patients' safety and increase the risk of failure.

- In a fledgling field, such as regenerative medicine, there is the potential for financial conflicts of interest that can corrupt the process of product development. The scientific community should consider carefully whether efforts in the regulatory or product development pathways are driven by commercial interests or patient need.

These principles must be considered in the review and implementation of new regulatory laws, Daley said. Congressional efforts such as the Reliable and Effective Growth for Regenerative Health Options that Improve Wellness (REGROW) Act pose a risk to the field by reducing the regulatory burden on cellular therapies through conditional approval on the basis of preliminary evidence of safety and efficacy, he said. The ISSCR is opposed to the REGROW Act and to any efforts that seek bring therapies to patients faster at the cost of failing to conduct sufficient research into the mechanism and safety of a new product, Daley said, emphasizing that the majority of new drugs fail, even after collecting early Phase II clinical trial data.

MOVING FORWARD

"In the 20th century, scientists learned how to turn chemistry into medicine," Daley said. "Regenerative medicine is going to be the medicine of the 21st century." He added that it will take decades but that scientists will learn how to transform cells into medicines. In short, he said, the path will be long and difficult, but regenerative medicine is going to transform medicine. The integrity of the research enterprise should be foremost in planning for the future. Patient welfare, respect for research subjects, transparency around the research process, and access to new regenerative therapies will be essential, he continued. The Forum on Regenerative Medicine can support this effort by continuing to convene stakeholders in the field and by illuminating opportunities and challenges of regenerative medicine in a responsible way, he suggested. By investing in the deepest scientific understanding of regenerative therapies, the field will continue to sustain support from the National Institutes of Health and from the investors who will carry promising research forward into the clinic.

PANEL DISCUSSION

Daley and a panel of presenters reflected on the workshop and shared their insights about issues that emerged over the course of the day. Panel participants identified and discussed several common themes that had emerged throughout the workshop. These themes are described below.

Understanding and Characterizing Cells

One major challenge facing research in regenerative medicine, Daley said, is deriving the medically relevant cell types and defining them in such a way that they can be assigned an identity and produced with reliable potency that can enable the development of a dose–response relationship in clinical trials. In addition to assembling a deep understanding of how cells work, Srivastava said, more research is needed on the development of cell identity in order for researchers to be able to harness the ability of endogenous cells to regenerate or convert to other cell types. It is not enough to have the right cell and understand the biology of the cell, Tsukamoto said; in order to see the biological activity that is hypothesized, "you have to have the right disease target [and] the right kind of patients for your first clinical trial."

Another challenge with using cells in clinical therapies is understanding and manipulating the maturation process, Srivastava said. Most human cells that are generated follow the maturation timeline of human development, he said, citing Studer's keynote talk. For example, when cardiomyocytes are made from human pluripotent cells, they take months to fully and functionally integrate. During this time, they are not providing benefit to the patient, and they may also have negative consequences such as triggering arrhythmia. In his experimental therapies for Parkinson's, Studer said, pluripotent stem cells take between 6 and 12 months to mature, which is not optimal for patients and also makes the conduct of clinical trials more challenging. The maturation of cells is a universal problem across cell types and disease areas, Srivastava said, adding that he hopes that if the issue can be solved for one type of cell, the solution may apply to all cells. There is pressure to do in vitro cell characterization because in vivo characterizations are more challenging and take longer, Tsukamoto said, noting that with certain cells, such as human neural stem cells (HuCNS-SCs®), in vitro characterization does not seem to be predictive of activity in vivo. The in vitro properties of the cells that ended up engrafted and survived long term were identical to the in

vitro properties of the cells that did not engraft, she said. As cells are scaled up in vitro, it will be important to ensure that bioactivity is not lost during the manufacturing process, Tsukamoto said.

Improved Model Systems

Several participants spoke about the lack of good model systems for testing regenerative cell-based therapies. While animal models are not perfect, they may be useful for some purposes. For example, as a workshop participant noted, the Royal College of Surgeons (RCS) rat is not a good model for age-related macular degeneration (AMD), but because the RCS rat has inherited retinal degeneration, it can be used to study how cellular therapies to replace retinal pigment epithelium (RPE) will operate in a host whose RPE is not functioning. Animal models have also been used with success to study therapies for other diseases, such as Parkinson's, as evidenced the by success of Studer's research using a mouse model. The mdx mouse model for Duchenne muscular dystrophy has not been as successful, however. The mouse has seemingly been cured many times, while the afflicted boys have not been cured, Furlong said. Moving away from animal models may be appropriate for some types of research. For example, a workshop participant said, iPS cells have the potential to be more useful as a model for AMD. Over the course of the day, many speakers emphasized that animal models are useful for answering basic questions about safety and efficacy but that ultimately the effectiveness of a therapy can only be determined by using it in humans.

Clinical Translation

Additional basic research and higher standards of evidence are needed, Temple said, noting that high-quality and rigorous science tends to propel the most promising things into the clinic. Advancing only high-quality hypotheses would use time and resources in the most effective way to bring new and effective therapies to patients. Workshop participants discussed the challenge of determining what endpoints to measure in order to assess quality, potency, and function for regenerative cellular therapies, asking the panel how the need for a deep scientific understanding of the therapy can be balanced with innovation and the role of commercial investment in clinical translation. "We need to sustain a culture of innovation," Daley replied. Public funding in the field does not have the resources to move research beyond the discovery phase, and invest-

ment by the biotechnology field is needed for clinical translation, he said. Increasing public funding of basic science may support the development of strong clinical hypotheses.

Overcoming Challenges Associated with Immunomodulation

The issue of immunomodulation was mentioned throughout the day as a major barrier to cell-based therapies. Studer listed five avenues that regenerative medicine could pursue in the future to address this issue: autologous cells, patient matched cells, human leukocyte antigen (HLA)-matched cell banks, allogeneic off-the-shelf products, and universal donor cells. Each source of cells has its benefits and drawbacks. For example, Srivastava commented, while autologous cell transplants do not risk immune rejection, they may be "too expensive and face too many regulatory hurdles to be a realistic approach." There are efforts under way to develop iPS cell banks with multiple HLA qualities that could be used for off-the-shelf products, he noted. Participants pointed out other methods on the horizon for avoiding immune rejection, including advances in conditioning therapies and the reprogramming of endogenous cells.

Another consideration, Tsukamoto said, is whether a single stem cell treatment will be sufficient to last for a patient's lifetime. Transplanting cells into a patient has an immunological impact, and researchers should consider what the effect would be if patients must undergo multiple transplants. If there is an immune response the first time, Tsukamoto asked, what will happen with subsequent treatments?

Navigating Regulatory Pathways

There is a great deal of regulatory uncertainty with cell-based therapies because the regulatory path is still quite undefined, Studer said in his opening remarks, noting that FDA is grappling with how to regulate novel therapies and where to draw the line on the level of safety and efficacy evidence that is required before a treatment can enter clinical trials.

Participants asked whether the current regulatory framework is sufficient for these complex areas, including cell-based therapies and gene editing. FDA has multiple accelerated approval pathways, and these pathways are malleable and responsive to new science (e.g., the use of surrogate endpoints and biomarkers), Daley said. There is no need to change or relax the regulatory framework, he said, because if the research is based on a "deep mechanistic understanding of disease," the

efficacy of the therapy should be evident. Regenerative medicine will transform what we do in the future, but it is hard and it is going to take time, Furlong said. Strong regulatory standards are important, but it will also be important to keep the patient in mind, she said, adding that advocacy groups, researchers, and regulators should consider partnering in their efforts to move the field forward.

Rethinking Funding Models

The decision to invest in the development of cell-based therapies is fraught with potential complications: the costs of research, development, and manufacturing may be untenably high; there may be uncertainty about whether a product will be approved by regulatory bodies; insurance companies may not pay for the therapy; and there is the possibility that patient or provider demand for a therapy will be lower than expected. In order to get these therapies to the patients who need them most, a workshop participant said, it may be necessary to develop alternative funding mechanisms. For example, reducing the cost of upfront investment through government incentives or other means may encourage companies to invest in cell-based therapies, commented a workshop participant, mentioning a UK program called the Regenerative Medicine Platform in which the government funds research in several key areas and makes the resulting data available for the entire research community. This solution increases the amount of research being done and facilitates the collection and analysis of data that may support future research. A workshop participant also discussed another UK-based program that provides government funding for clinical translation and conversion to good manufacturing practices. If a biotechnology company finds a new therapy promising, it is freely given to the company to continue the development process. It is unfortunate that there are not more public funds directed toward basic science, Daley said, noting that the return on public investment in fundamental research is "amplified and multiplied many fold."

Several participants expressed concern that once cell-based therapies do reach the clinic, they will likely be one-time therapies and likely quite expensive. Our health care system is better geared toward paying for chronic therapy over many years, rather than for one-time procedures that will not recoup costs for many years, a participant said. Insurance companies are hesitant to "take the hit" on a high payment for such a therapy because there is no guarantee that the patient will stay with the

insurance company in the future, Ratcliffe said. Advocacy from patient groups could help overcome this hurdle, Furlong suggested.

Potential Future Approaches for Cell-Based Regenerative Therapies

There will be at least two major areas of growth for regenerative medicine, Srivastava said. One will be cell-based, and therapies in that area will be applied to treat diseases such as Parkinson's, diabetes, and spinal cord injury. For cell-based therapies, the issues of maturation and cell differentiation will be significant hurdles to overcome, Srivastava predicted. The other area of growth will be in the development of treatments for diseases that are not responsive to cellular therapies. Regenerative approaches for these conditions will focus on harnessing the regenerative capacity of endogenous cells through reprogramming, stimulating cell division, or introducing external materials such as exosomes, he said.

Refocusing on Patients

While the workshop focused primarily on the state of the science of cell-based therapies, many individual speakers urged participants to keep the needs of patients in focus. There is an inherent tension, Furlong said, between giving patients the opportunity to access potentially life-saving therapies and upholding scientific and regulatory standards in order to ensure that products entering the market are safe and effective. Furlong added that for patients who may "only have one shot" at a potentially curative therapy, the risk–benefit analysis may be different than for other patients. "We have to rigorously address and adhere to standards," she said. "But we also must keep the patient at the center of this and recognize desperate times call for desperate [measures]. We need to really address the patient first and always." There are unproven therapies being offered by clinics across the country, catering to patients who are desperate for new therapies, Temple said, suggesting that researchers should increase their efforts to educate the patient community. Patient organizations should speak with a unified voice and call for higher standards, so that patients are not taken advantage of by these clinics, Tsukamoto said. Expectations need to be managed for patients and families as well as for providers, Furlong added, so that everyone involved understands that the path from discovery to clinic can be a long and winding one.

CONCLUDING THOUGHTS

In her concluding comments, forum co-chair Alta Charo noted that the need for scientific rigor in the research and development process as a precursor to strong clinical hypotheses had been a common area of emphasis across all of the panels, and she said that further study on standards and quality control, as well as on finding ways to reduce the up-front costs of developing new therapies, will support efficient and useful research. Although the workshop presentations covered different tissue areas, different cell types, and different health conditions, the challenges and opportunities that each presenter discussed were quite interrelated, Charo said. The Forum on Regenerative Medicine was designed for this very type of cross-fertilization, and its continued work will encourage stakeholders in the field to look for commonalities and overlapping interests "that will fire the imagination" and inspire collaboration, she concluded.

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A

Workshop Agenda

The State of the Science in the Field of Regenerative Medicine: Challenges of and Opportunities for Cellular Therapies: A Workshop

October 13, 2016

National Academy of Sciences Building
Room 125
2101 Constitution Avenue, NW
Washington, DC 20418

MEETING OBJECTIVES

- To examine the state of the science for therapies that generate, repair, or replace tissues by convening scientists, clinicians, industry, patient experts, and other stakeholders.
- To highlight the challenges, successes, and lessons learned with respect to translation of regenerative therapies from early discovery into clinical practice with the goal of reaching patients.
- To illuminate next steps for the field and ways that the forum could be a facilitator of progress.

AGENDA

8:30 a.m. **Welcoming Remarks**

R. Alta Charo, *Forum Co-Chair*
Warren P. Knowles Professor of Law and Bioethics
School of Law and School of Medicine and Public Health
University of Wisconsin–Madison

Jay Siegel, *Forum Co-Chair*
Chief Biotechnology Officer
Head, Scientific Strategy and Policy
Johnson & Johnson

INTRODUCTION: OVERVIEW OF THE FIELD OF REGENERATIVE MEDICINE AND FOCUS OF THE WORKSHOP

Objectives: To describe and explore the broad field of regenerative therapies at a high level and to highlight successful research and clinical applications as well as barriers to scientific and therapeutic advances as the field moves forward.

8:35 a.m. **Charge to Workshop Speakers and Participants: Considering the State of the Science in Regenerative Therapies**

Cynthia Dunbar, *Workshop Co-Chair*
President
American Society of Gene and Cell Therapy
Senior Investigator, Molecular Hematopoiesis Section
National Heart, Lung, and Blood Institute

8:45 a.m. **Areas of Challenge and Success in Regenerative Therapies**

Lorenz Studer
Director, Center for Stem Cell Biology
Memorial Sloan Kettering Cancer Center

9:05 a.m. Clarifying Questions

SESSION I: SKIN AND MUSCULOSKELETAL TISSUES

Objectives: To examine the state of the science in research and novel applications of new technology to repair, regenerate, or renew skin or musculoskeletal tissues; to consider the obstacles that hinder progress in research; and to

highlight scientific successes, lessons learned, and opportunities to move the field forward with the goal of bringing new therapies to patients.

Moderator: **Audrey Kusiak,** Scientific Program Manager, Rehabilitation Research and Development Service, Office of Research and Development, Department of Veterans Affairs

9:15 a.m. Anthony Oro
Professor of Dermatology
Member of Program in Epithelial Biology, Institute for Stem Cell Biology and Regenerative Medicine
Stanford University

Anthony Ratcliffe
President and Chief Executive Officer
Synthasome, Inc.

Patricia Furlong
Chief Executive Officer
Parent Project Muscular Dystrophy

10:00 a.m. **Discussion with Speakers and Attendees**

10:30 a.m. **Break**

SESSION II: HEMATOLOGY AND IMMUNITY

Objectives: To examine the state of the science in research and novel applications of new technology to repair, regenerate, or renew tissue and function in the blood and the immune system; to consider the obstacles that hinder progress in research; and to highlight scientific successes, lessons learned, and opportunities to move the field forward with the goal of bringing new therapies to patients.

Moderator: **Cynthia Dunbar,** President, American Society of Gene and Cell Therapy; Senior Investigator, Molecular Hematopoiesis Section, National Heart, Lung, and Blood Institute

10:45 a.m. Fyodor Urnov
Associate Director
Altius Institute for Biomedical Sciences

Harry Malech
Chief, Laboratory of Host Defenses
Chief, Genetic Immunotherapy Section
National Institute of Allergy and Infectious Diseases

Michel Sadelain
Director, Center for Cell Engineering and Gene Transfer and Gene Expression Laboratory
Memorial Sloan Kettering Cancer Center

11:30 a.m. **Patient Perspective**

Jennifer Fields
Patient Advocate

11:35 a.m. **Discussion with Speakers and Attendees**

12:00 p.m. **Working Lunch**

SESSION III: NEUROLOGICAL AND OPHTHALMOLOGICAL TISSUES

Objectives: To examine the state of the science in research and novel applications of new technology to repair, regenerate, or renew neurological and ophthalmological tissues; to consider the obstacles that hinder progress in research; and to highlight scientific successes, lessons learned, and opportunities to move the field forward with the goal of bringing new therapies to patients.

Moderator: **Brian Fiske,** Senior Vice President, Research Programs, The Michael J. Fox Foundation for Parkinson's Research

1:00 p.m. Sally Temple
Principal Investigator and Scientific Director
Neural Stem Cell Institute

Ann Tsukamoto
Former Executive Vice President, Scientific and Strategic Alliances
StemCells, Inc.

Peter Coffey
Professor, Neuroscience Research Institute
University of California, Santa Barbara

1:45 p.m. **Discussion with Speakers and Attendees**

SESSION IV: CARDIOVASCULAR AND LUNG TISSUES

Objectives: To examine the state of the science in research and novel applications of new technology to repair, regenerate, or renew cardiovascular and lung tissues; to consider the obstacles that hinder progress in research; and to highlight scientific successes, lessons learned, and opportunities to move the field forward with the goal of bringing new therapies to patients.

Moderator: **Jiwen Zhang,** Senior Director, Regulatory Affairs, Cell Therapy and Regenerative Medicine, GE Healthcare

2:15 p.m. Deepak Srivastava
The Younger Family Director, Gladstone Institute of Cardiovascular Disease; Director, Rodenberry Center for Stem Cell Biology and Medicine; Professor, University of California, San Francisco

Eduardo Marbán
Director, Heart Institute
Professor of Medicine
Cedars-Sinai

Brigitte Gomperts
Associate Professor, Pulmonary and Pediatric Medicine
University of California, Los Angeles

3:00 p.m. **Discussion with Speakers and Attendees**

3:30 p.m. **Break**

SESSION V: RENAL TISSUES

Objectives: To examine the state of the science in research and novel applications of new technology in repairing or regenerating tissue in the kidney, to consider the obstacles that hinder progress in research; and to highlight scientific successes, lessons learned, and opportunities to move the field forward with the goal of bringing new therapies to patients.

Moderator: **Deborah Hoshizaki,** Program Director, Division of Kidney, Urologic, and Hematologic Diseases, National Institute of Diabetes and Digestive and Kidney Diseases

3:45 p.m. Ben Humphreys
Chief, Renal Diseases Division, Department of Medicine
Washington University School of Medicine

David Baron
Chief Scientific Officer
PKD Foundation

4:15 p.m. **Discussion with Speakers and Attendees**

SESSION VI: FINAL DISCUSSION AND NEXT STEPS

Objectives: To reflect on the current state of the science of regenerative therapies, explore the existing and potential scientific barriers to advancing the field of regenerative medicine, and to discuss strategies and lessons learned for facilitating efficient, effective, and translatable research.

Moderator: **Alta Charo,** Warren P. Knowles Professor of Law and Bioethics School of Law and School of Medicine and Public Health, University of Wisconsin–Madison

4:35 p.m. **Looking Toward the Future: The Promise and Challenges of Regenerative Therapies**

George Daley
Director, Stem Cell Transplantation Program
Boston Children's Hospital and Dana–Farber Cancer Institute
Dean, Harvard Medical School

4:55 p.m. **Reflections on the Day/Next Steps**

Patricia Furlong
Chief Executive Officer
Parent Project Muscular Dystrophy

Deepak Srivastava
The Younger Family Director, Gladstone Institute of Cardiovascular Disease; Director, Rodenberry Center for Stem Cell Biology and Medicine; Professor, University of California, San Francisco

Sally Temple
Principal Investigator and Scientific Director
Neural Stem Cell Institute

Ann Tsukamoto
Former Executive Vice President, Scientific and Strategic Alliances
StemCells, Inc.

5:15 p.m. **Discussion with Speakers and Attendees**

5:35 p.m. **Concluding Remarks**

R. Alta Charo, *Forum Co-Chair*
Warren P. Knowles Professor of Law and Bioethics
School of Law and School of Medicine and Public Health
University of Wisconsin–Madison

Cynthia Dunbar, *Workshop Co-Chair*
President
American Society of Gene and Cell Therapy
Senior Investigator, Molecular Hematopoiesis Section
National Heart, Lung, and Blood Institute

5:45 p.m. **ADJOURN**

B

Speaker Biographical Sketches

David Baron, Ph.D., received his B.A. (Biology) and Ph.D. (Anatomy) from The University of Chicago. Following his postdoctoral fellowship in pathology and pharmacology at the Medical University of South Carolina (MUSC), with the support of a National Institutes of Health Program Project grant, Dr. Baron founded the Core Structure-Function Laboratory in the Department of Pharmacology and joined the MUSC faculty with a joint appointment in the Departments of Pharmacology, and Anatomy and Cell Biology. He later joined Searle Pharmaceuticals as a research scientist, later becoming a Monsanto, then Pharmacia senior science fellow. Dr. Baron became the first Director of Toxicology at Takeda Pharmaceuticals, U.S. (Deerfield, Illinois) rising to Vice President, Nonclinical Safety Evaluation, for the United States and Europe. He has been a grant reviewer for the National Cancer Institute, given numerous invited seminars and has served on several national scientific boards (International Consortium for Innovation and Quality in Pharmaceutical Development, International Serious Adverse Event Consortium). Dr. Baron, who has polycystic kidney disease, became the Chief Scientific Officer for the Polycystic Kidney Disease (PKD) Foundation in 2015. During his career he has focused on the structural correlates of electrolyte and water transport across epithelia, and the safety and pharmacology of pioglitazone while at Takeda, a type 2 diabetes drug now in a pilot clinical trial for the treatment of PKD through the support of the PKD Foundation and the Food and Drug Administration (FDA). He will begin a 3-year term as a member of the board of the Kidney Health Initiative January 2017.

Peter Coffey, D.Phil., is the director of the London Project to Cure Blindness and a professor of cellular therapy and visual sciences at the Institute of Ophthamology, University College London (UCL). His achievements include the launch of the London Project to Cure Blindness, which aims to develop a stem cell therapy for the majority of all types of age-related macular degeneration; seminal work on retinal transplantation; and the development of a cell-based therapy for a currently untreatable form of macular degeneration, age-related macular degeneration (also called dry AMD). He is the principal author and co-author of two landmark papers demonstrating the use of human cells to halt visual deterioration in models of dry AMD. Also, Dr. Coffey has a laboratory at the University of California, Santa Barbara (UCSB), and is co-director of UCSB's Center for the Study of Macular Degeneration. He is the director of translation at UCSB's Center for Stem Cell Biology and Engineering and is a member of the Neuroscience Research Institute.

Dr. Coffey has received many honors and awards, including the prestigious Estelle Doheny Living Tribute Award in 2009, Retinitis Pigmentosa International's Vision Award in 2009, the California Institute for Regenerative Medicine (CIRM) Leadership Award in 2010, and recently the New York Stem Cell Foundation's Robertson Award for translation stem cell work. CIRM reviewers characterized Dr. Coffey's work as "truly innovative, novel, ambitious and important . . . highly significant, with a potential to revolutionize the field." He is engaged in public service endeavors to explain stem cell research to the lay public, including talks to the British Parliament and the Vatican. Dr. Coffey received his D.Phil. degree at Oxford University and was a member of the faculty at Oxford and later the University of Sheffield, as lecturer and senior lecturer, before joining the faculty at UCL as head of the Ocular Biology and Therapeutics Research Department.

George Q. Daley, M.D., Ph.D., is the dean designate of Harvard Medical School and the Robert A. Stranahan Professor of Pediatrics and a professor of biological chemistry and molecular pharmacology at Harvard Medical School. He is also the director of the Stem Cell Transplantation Program at the Dana-Farber/Boston Children's Cancer and Blood Disorders Center and a Howard Hughes Medical Institute investigator.

Dr. Daley is a world-renowned expert on stem cells, cancer, and blood disorders. He received his bachelor's degree, magna cum laude, from Harvard University (1982), a doctorate in biology from the Massachusetts Institute of Technology (1989), where he worked with Nobel

laureate David Baltimore, and his medical degree from Harvard Medical School (1991), where he was only the 12th individual in the school's history to receive the degree summa cum laude.

Dr. Daley pursued clinical training in internal medicine at Massachusetts General Hospital, where he served as chief resident (1994–1995), and a clinical fellowship in hematology/oncology at Brigham and Women's Hospital and Boston Children's.

He was a founding member of the executive committee of the Harvard Stem Cell Institute and served as president of the International Society for Stem Cell Research from 2007 to 2008 and as its clerk from 2012 to 2015. He anchored the special task forces that produced the society's guidelines for stem cell research (2006) and clinical translation (2008) and their subsequent revisions and updates (2016).

Dr. Daley's research uses mouse and human disease models to unravel the mechanisms that underlie various cancers and blood disorders. Important contributions from the Daley laboratory include the creation of customized stem cells to treat a genetic immune deficiency in mice, the differentiation of germ cells from embryonic stem cells, the generation of disease-specific pluripotent stem cells by direct reprogramming of human skin and blood cells, and demonstration of the role of the LIN28/let-7 signaling pathway in the development of cancer.

Previously, Dr. Daley's work demonstrated the central role of the BCR/ABL protein in the development of human chronic myeloid leukemia (CML), a finding that provided the critical target validation for the development of Gleevec, a highly successful treatment for this disease.

Dr. Daley has been elected to the National Academy of Medicine, the American Society for Clinical Investigation, the American Association of Physicians, the American Pediatric Societies, the American Academy of Arts and Sciences, and the American Association for the Advancement of Science.

Dr. Daley was an inaugural winner of the National Institutes of Health Director's Pioneer Award for highly innovative research and has received the Judson Daland Prize from the American Philosophical Society for achievement in patient-oriented research, the E. Mead Johnson Award from the American Pediatric Society for contributions to stem cell research, and the E. Donnall Thomas Prize of the American Society of Hematology for advances in human-induced pluripotent stem cells.

Jennifer Fields, M.P.H., a North Carolina native, was diagnosed at the age of 2 months with sickle cell anemia (HgbSS) disease. Ms. Fields possesses a broad background in health and human services and nonprofit operations, including strategic development and grant management, totaling more than 14 years of experience. She obtained a master of public health degree from East Carolina University and is presently working as a consultant with the Sickle Cell Treatment Demonstration Program at Sickle Cell Disease Foundation California and the Sickle Cell Disease Newborn Screening Program at the Sickle Cell Disease Association of America in Baltimore, Maryland. Ms. Fields's work has included managing several federal awards, including awards from the National Institutes of Health, Health Resources and Services Administration, Centers for Disease Control and Prevention, and the Federal Emergency Management Agency at the Department of Homeland Security, totally several millions of dollars. Ms. Fields now resides in Raleigh, North Carolina, with her 4-year-old son, Roman.

Patricia Furlong is the founding president and chief executive officer of Parent Project Muscular Dystrophy (PPMD), the largest nonprofit organization in the United States solely focused on Duchenne muscular dystrophy (Duchenne). Its mission is to end Duchenne. It accelerates research, raises its voice in Washington, demands optimal care for all young men, and educates the global community.

Duchenne is the most common fatal, genetic childhood disorder, and it affects approximately 1 out of every 4,600 boys each year worldwide. It currently has no cure.

When doctors diagnosed her two sons, Christopher and Patrick, with Duchenne in 1984, Ms. Furlong did not accept "there's no hope and little help" as an answer. She immersed herself in Duchenne, working to understand the pathology of the disorder, the extent of research investment, and the mechanisms for optimal care. Her sons lost their battle with Duchenne in their teenage years, but she continues to fight—in their honor and for all families affected by Duchenne.

In 1994, Ms. Furlong, together with other parents of young men with Duchenne, founded PPMD to change the course of Duchenne and, ultimately, to find a cure. Today, she continues to lead the organization and is considered one of the foremost authorities on Duchenne in the world.

Brigitte Gomperts, M.D., is a physician-scientist at the University of California, Los Angeles (UCLA), in the Departments of Pediatrics and Pulmonary Medicine. She earned her medical degree from the University of Witwatersrand in Johannesburg, South Africa, and trained in general pediatrics and pediatric hematology/oncology at Washington University in St. Louis, Missouri. She is an associate vice chief of education in the Department of Pediatrics, the vice chief of research for the Pediatric Hematology-Oncology Division, and a co-director of the Jonsson Comprehensive Cancer Center Cancer Stem Cell and Biology Program at UCLA. She is also affiliated with the Eli and Edythe Broad Center of Regenerative Medicine and Stem Cell Research at UCLA and the Molecular Biology Institute.

Her research focuses on the repair and regeneration of the lungs and how the normal repair mechanisms go awry in lung diseases. Her lab is using novel human three-dimensional models to understand repair and regeneration in the proximal and distal lung and has expertise in adult stem cell models of lung diseases and patient-specific induced pluripotent stem cell lung disease modeling and high-throughput drug screening. The ultimate goal is to use this knowledge to develop novel targeted therapies and prevention strategies for lung diseases. Major areas of interest include lung fibrosis, mucociliary clearance, chronic obstructive pulmonary disease, and premalignant lesions with stepwise progression to lung cancer. Because she is a physician-scientist, her lab is particularly interested in translational research that will result in new therapies for lung diseases.

Benjamin D. Humphreys, M.D., Ph.D., is the Joseph Friedman Associate Professor in Renal Diseases and the chief of the Division of Nephrology at Washington University School of Medicine in St. Louis. Prior to joining Washington University in St. Louis, Dr. Humphreys was the director of the Harvard Stem Cell Institute Kidney Program and an associate professor of medicine at Harvard Medical School and Brigham and Women's Hospital. Dr. Humphreys is a member of the American Society of Clinical Investigation and an established investigator of the American Heart Association. He is the recipient of the National Kidney Foundation Young Investigator Award and the American Society of Nephrology Gottschalk Research Scholar Award. His National Institutes of Health–funded laboratory focuses on adult kidney injury and repair. The laboratory has special expertise in genetic mouse models of kidney disease, stem cell biology, and the generation of kidney organoids from human

pluripotent stem cells, and it employs these approaches to identify new treatments for patients suffering from kidney disease. Dr. Humphreys earned his bachelor of arts degree from Harvard College and his medical and doctor of philosophy degrees from Case Western Reserve University. He completed a residency in internal medicine at Massachusetts General Hospital and a fellowship in nephrology at Brigham and Women's Hospital in Boston. He has authored more than 100 publications and multiple book chapters, and he holds 5 patents.

Harry L. Malech, M.D., is the chief of the Laboratory of Host Defenses and the Genetic Immunotherapy Section in the National Institute of Allergy and Infectious Diseases of the National Institutes of Health (NIH). At NIH, Dr. Malech cares for and studies patients who have a variety of inherited immune deficiencies, with a long-term focus on children and young adults with chronic granulomatous disease (CGD) or X-linked severe combined immune deficiency (XSCID). His clinical service has research programs of study of allogeneic hematopoietic stem cell transplant and autologous stem cell ex vivo gene therapy for CGD, SCID, and other immune deficiencies. Laboratory research is focused on achieving efficient genetic correction of patient hematopoietic stem cells. Related work includes studies of the generation of induced pluripotent stem cells from patients with CGD and XSCID and the use of gene targeting methods to genetically correct induced pluripotent stem cells or hematopoietic stem cells. Dr. Malech is an elected member of the Association of American Physicians and the American Society for Clinical Investigation. He is a recent past president for 2014–2015 of the American Society of Gene & Cell Therapy.

Eduardo Marbán, M.D., Ph.D., is an international leader in cardiology and a pioneering heart researcher. His 30-plus years of experience in patient care and research have led to key discoveries in gene and stem cell therapies for heart disease. Those discoveries have formed the basis for multiple startup companies.

Dr. Marbán attended public schools through high school and later Wilkes College, where he earned a B.S. in mathematics. Thereafter, Dr. Marbán completed a combined M.D./Ph.D. program at Yale University. Postgraduate training took him to the Osler Medical Service at the Johns Hopkins University, where he eventually spent 25 productive years. During his tenure there, he served in a variety of academic and research positions, including chief of cardiology.

In his research career, Dr. Marbán, a cellular electrophysiologist by training, has pursued questions of relevance to heart disease (ischemia, heart failure, and arrhythmias). The Marbán laboratory elucidated the fundamental pathogenesis of myocardial stunning, pioneered the concept of gene therapy to alter electrical excitability, and created the first de novo biological pacemaker as an alternative to electronic pacemakers. He first became interested in stem cells in 2002, building on his work on biological pacemakers. Since 2004 the lab has been intensively studying cardiac progenitor cells, in particular, their origins and their therapeutic potential. The basic work has come full circle in that Dr. Marbán's cardiac-derived cell products form the basis for four grant-funded clinical trials, one of which has been completed (CADUCEUS) and the other three ongoing (ALLSTAR, DYNAMIC, and HOPE-Duchenne). The CADUCEUS trial was the first to show that cell therapy can repair "irreversible" tissue damage caused by heart attacks, ushering in the concept of therapeutic regeneration in humans.

In 2007 Dr. Marbán became the founding director of the Cedars-Sinai Heart Institute, a multidisciplinary entity that brings together adult and pediatric cardiologists, cardiac surgeons, imaging specialists, and researchers to foster discovery and enhance patient care. The institute is built on a long tradition of excellence and innovation at Cedars-Sinai, including the invention of the Swan–Ganz catheter. The Cedars-Sinai Heart Institute, ranked as the top heart program in the western United States, performs more heart transplants annually than any other institution worldwide.

Among the many honors Dr. Marbán has received are the Basic Research Prize of the American Heart Association (AHA), the Research Achievement Award of the International Society for Heart Research, the Gill Heart Institute Award, and the distinguished scientist awards of the AHA and the American College of Cardiology.

Anthony Oro, M.D., Ph.D., is a professor of dermatology, associate director of the Center for Definitive and Curative Medicine, a member of the Institute for Stem Cell Biology and Regenerative Medicine and the Stanford Cancer Institute at Stanford University, and the Cancer Biology and Stem Cell graduate student programs. He trained in the medical scientist program at the Salk Institute under Ron Evans lab, working on developmental functions of novel orphan nuclear receptors in model systems. During dermatology residency/fellowship in Matthew Scott's lab at Stanford, he helped solidify the link between the hedgehog path-

way and human cancer. In his own lab in the Program in Epithelial Biology at Stanford, Dr. Oro has extended the original studies focusing on the role of skin stem cells to understand in tissue regeneration and carcinogenesis. He has a long-standing interest in the mechanisms of hedgehog signaling in hair follicle regeneration, and in the pathogenesis of the most common human tumor, basal cell carcinoma of the skin. As a practicing physician, he led the clinical trials developing the first human hedgehog pathway inhibitor in skin cancer. His recent focus is on tumor evolution and novel resistance-associated signaling pathways. His interest in the mechanisms of human skin development and early ectodermal differentiation has led to the development of Therapeutic Reprogramming, the use of in vitro human skin differentiation protocols and genome editing tools to produce clinical grade, corrected, autologous human skin from patient-specific induced pluripotent (iPS) cells. He is focusing his efforts to treat the blistering disease Epidermolysis bullosa. Dr. Oro has received numerous awards including the Marion Sulzberger Memorial and Montagna Awards, and is a member of the American Society for Clinical Investigation. Dr. Oro has 18 patents and published more than 70 peer-reviewed articles, commentaries and chapters.

Anthony Ratcliffe, Ph.D., is president and CEO of Synthasome, Inc. Dr. Ratcliffe obtained his B.Sc. in biochemistry in 1977, and Ph.D. in immunology in 1980, from the University of Birmingham, United Kingdom. He then joined The Kennedy Institute for Rheumatology, London as a research scientist, and in 1987 he moved to Columbia University, New York, as associate professor in the Department of Orthopaedic Surgery. In 1996 he joined Advanced Tissue Sciences in La Jolla, where he served as vice president for Research until 2002. Dr. Ratcliffe has focused his work on musculoskeletal research, tissue engineering, and product development. He has had a number of external leadership positions, including serving as a member of the Board of Directors of the Orthopaedic Research Society, Study Sections for the National Institutes of Health, co-chairman of the Grant Review Committee for the Orthopaedic Research and Education Foundation, co-chairman of the Tissue Engineering Committee for the American Society for Testing and Materials, and has published more than 100 papers.

Michel Sadelain, M.D., has made major contributions to the generation and optimization of CAR T cells to treat cancer as well as to the devel-

opment of stem cell therapies for blood disorders. Dr. Sadelain's work has focused on developing novel strategies to extend the survival of CAR T cells in the body and to enable T cells with increased potency to overcome the resistance imposed by tumor and other cells in the tumor microenvironment. In 2002 his group was the first to report the design of "second-generation" CARs that, in addition to a binding domain outside of the T cell and a signaling domain inside, included a costimulatory domain designed to promote cell proliferation and survival. These advances provided a broad platform to enhance CAR T cell therapy, leading directly to the development of new CAR T cell therapies that are showing increasing efficacy in patients. Building on proof-of-principle experimentation in mice bearing CD19+ malignancies, the Memorial Sloan Kettering Cancer Center (MSKCC) team led by Dr. Sadelain has recently obtained dramatic clinical responses in adult patients with acute lymphoblastic leukemia.

Dr. Sadelain is the founding director of the Center for Cell Engineering and the head of the Gene Transfer and Gene Expression Laboratory at MSKCC, where he holds the Stephen and Barbara Friedman Chair. Dr. Sadelain is also a member of the departments of medicine and pediatrics at Memorial Hospital and the molecular pharmacology and chemistry program of the Sloan Kettering Institute.

Dr. Sadelain received his M.D. from the University of Paris, France, in 1984 and his Ph.D. from the University of Alberta, Canada, in 1989. After completing a clinical residency at the Centre Hospitalier Universitaire Saint-Antoine in Paris, Dr. Sadelain carried out a postdoctoral fellowship with Richard Mulligan, Ph.D., at the Whitehead Institute for Biomedical Research at the Massachusetts Institute of Technology, before joining MSKCC in 1994 as an assistant member. Dr. Sadelain is a member of the American Society of Hematology, the American Society of Human Genetics, and the American Society of Cell and Gene Therapy, where he served on the board of directors from 2004 to 2007, and he is an elected member of the American Society for Clinical Investigation. He has authored more than 150 scientific papers and book chapters.

Deepak Srivastava, M.D., is the director of the Gladstone Institute of Cardiovascular Disease and the Roddenberry Stem Cell Center at Gladstone and is also a professor at the University of California, San Francisco (UCSF), Medical Center. Dr. Srivastava received his B.S. from Rice University and his M.D. from The University of Texas, and he

trained in pediatrics at UCSF and in pediatric cardiology at Harvard Medical School.

Dr. Srivastava's laboratory discovered genetic bases for cardiac septal and valve defects and revealed complex signaling, transcriptional, and translational networks that regulate progenitor cells to adopt a cardiac cell fate and subsequently fashion a functioning heart. He has leveraged this knowledge to reprogram fibroblasts directly into cardiomyocyte-like cells for regenerative purposes. Dr. Srivastava is a member of the American Academy of Arts and Sciences and the National Academy of Medicine.

Lorenz Studer, M.D., is the director of the Center for Stem Cell Biology and a member of the Developmental Biology Program at Memorial Sloan Kettering. A native of Switzerland, he received his M.D. and doctorate degree from the University of Bern where he co-developed the first cell-based therapy for Parkinson's disease in the country. He subsequently trained as postdoctoral fellow with Ron McKay at the National Institutes of Health pioneering the therapeutic application of neural stem cell–derived neurons in models of neurodegeneration. In his laboratory, he has established techniques that can turn human pluripotent stem cells into many of the diverse cell types of the nervous system. He was also among the first to realize the potential of patient-specific stem cell in modeling human disease and in drug discovery. Furthermore, he is currently leading a multidisciplinary consortium to pursue the first clinical application of human stem cell–derived dopamine neurons for the treatment of Parkinson's disease. Dr. Studer's work has been recognized by numerous awards including the Boyer Young Investigator Award and the Annemarie Opprecht Award.

Sally Temple, Ph.D., is the co-founder and scientific director of the Neural Stem Cell Institute, located in Rensselaer, New York. Dr. Temple's group is focused on studies of neural stem cells and on using this knowledge to develop therapies for central nervous system disorders.

Dr. Temple trained at Cambridge University and University College London with Dr. Martin Raff, FRS. In 1989, Dr. Temple discovered that the embryonic mammalian brain contained a rare, multipotent stem cell that could be grown in tissue culture, producing both neurons and glia. Since then, her group has continued to make pioneering contributions to the field of neural stem cell research, identifying cell-intrinsic and extracellular niche factors that participate in their self-renewal and differentia-

tion into diverse cell types. Using patient-derived neural, retinal pigment epithelial, and induced pluripotent stem cells, her research group is building novel models to study disease mechanisms of age-related neurodegeneration, with the aim of identifying new targets to slow or stop the disease process. In recognition of her work, Dr. Temple has received the Royal Society Stothert Research Fellowship, the Javits National Institutes of Health merit award, the MacArthur award and the Ellison investigator award. Dr. Temple is currently the president of the International Society for Stem Cell Research.

Ann Tsukamoto, Ph.D., has been working in the stem cell field for almost 27 years. Her most recent position was executive vice president for Scientific and Strategic Alliances at StemCells, Inc. During her 18 year tenure at StemCells, Dr. Tsukamoto led the scientific team that discovered the human central nervous system stem cell (HuCNS-SC®) and a second candidate stem cell for the liver and that transitioned the human neural stem cell into early clinical development in all three components of the CNS: brain, spinal cord, and eye. The biological potential and activity of these HuCNS-SC® cells was demonstrated in some patients and reflected results seen in preclinical rodents studies. The many challenges of developing a cell therapy in a small biotech firm led to the closure of StemCells, Inc., in August 2016.

Prior to her time at StemCells, Inc., Dr. Tsukamoto worked at the first stem cell company, SyStemix, Inc., where she co-discovered the human hematopoietic stem cell (hHSC) and played a leading role in the launch of the clinical research program for this cell. The purified hHSC was shown to be cancer-free when isolated from the cancer-contaminated hematopoietic mobilized blood of patients with disseminated cancer, and it successfully regenerated the patients' blood-forming system after myeloablative chemotherapy. Dr. Tsukamoto is an inventor on seven issued U.S. patents, of which six are related to the human hematopoietic stem cell. She received her Ph.D. in microbiology and immunology at the University of California, Los Angeles, and did her postdoctoral work with Dr. Harold Varmus at the University of California, San Francisco, where she worked on the wnt-1 gene and developed a transgenic model for breast cancer. Wnt-1 was later discovered to be a key player in the stem cell self-renewal pathway.

Fyodor Urnov, Ph.D., is an associate director at the Altius Institute for Biomedical Sciences. Prior to joining Altius in August 2016, Dr Urnov

was the vice president of discovery and translational research at Sangamo BioSciences, where he most recently led an effort to expand genome editing and targeted gene regulation technologies to new disease indications, including beta-thalassemia and sickle cell anemia. As a co-developer of genome editing, Dr. Urnov led the company's research and development efforts to deploy genome editing for crop trait engineering in partnership with Dow AgroSciences, and he co-managed Sangamo's partnership with Sigma-Aldrich for the generation of engineered cell lines for manufacturing, transgenic animals, and research reagents.

He is an author of more than 70 scientific publications and an inventor on more than 100 issued and pending U.S. patents related to genome editing and targeted gene regulation technology. Dr. Urnov is also an adjunct professor in the department of Molecular and Cell Biology at the University of California, Berkeley, where he teaches upper-division undergraduate and graduate classes in the life sciences as well as Biology for Voters, a class for students who are not majoring in the sciences. Prior to joining Sangamo, Dr. Urnov was a postdoctoral fellow at the National Institutes of Health in the laboratory of Alan P. Wolffe, where he trained in the study of chromatin-based genome regulatory processes in metazoa. Dr. Urnov received a B.Sc. in biology from Moscow State University and his Ph.D. in biology from Brown University, where he studied chromatin-based integration of genome control in the laboratory of Susan A. Gerbi.

C

Statement of Task

An ad hoc committee will plan and conduct a 1-day public workshop to examine and discuss the state of the science in the field of regenerative medicine. The goal of the workshop will be to begin with an overview to set the foundation for the field, then to focus in on opportunities and challenges for future work in regenerative medicine, concentrating on understanding the underlying biology of potentially promising cellular therapies. Gathering this information will also help facilitate future forum discussions around the issues of implementing regenerative medicine therapies and technologies, such as developing standards, examining regulatory pathways, addressing reimbursement issues, and considering bioethical matters. Discussions during this workshop will be held with a broad array of invited stakeholders, which may include research scientists, clinicians, patients, payers, regulators, and representatives from pharmaceutical and biotech companies. The planning committee will develop the workshop agenda, select and invite speakers, and moderate the discussions. Proceedings from the workshop will be prepared by a designated rapporteur in accordance with institutional policies and procedures.

D

Registered Attendees

Salvatore Alesci
Takeda Pharmaceuticals

Rasha Alhazzaa
Howard University

David Baron
PKD Foundation

James Beck
Parkinson's Foundation

Steven Becker
National Eye Institute

Kapil Bharti
National Eye Institute

Lucie Bruijn
ALS Association

Denis Buxton
National Heart, Lung, and Blood Institute

Rosa Canet-Aviles
Foundation for the National Institutes of Health

Preethi Chander
National Institute of Dental and Craniofacial Research

Alta Charo
University of Wisconsin–Madison

Peter Coffey
University of California, Santa Barbara

George Daley
Boston Children's Hospital, Harvard Medical School

Ray DeBert
National Heart, Lung, and Blood Institute

Nancy DiFronzo
National Heart, Lung, and Blood Institute

Robert Drape
WiCell

Cynthia Dunbar
American Society of Gene & Cell Therapy

Debra Egan
National Heart, Lung, and Blood Institute

John Elliott
National Institute of Standards and Technology

Luis Espinoza
National Institutes of Health

Jennifer Fields
JRE Enterprises

Donald Fink
Food and Drug Administration

Brian Fiske
The Michael J. Fox Foundation for Parkinson's Research

Erin Hammers Forstag
Consultant

Christopher Fox
American Association for Dental Research

Stephanie Fox-Rawlings
National Center for Health Research

Mark Frohlich
Juno Therapeutics

Patricia Furlong
Parent Project Muscular Dystrophy

Ellen Gadbois
National Institutes of Health

Allyson Gage
Cohen Veterans Bioscience

Lawrence Goldstein
University of California, San Diego

Brigitte Gomperts
University of California, Los Angeles

Thomas Greenwell
National Eye Institute

Rachel Haddock
GlaxoSmithKline

Michael Halter
National Institute of Standards and Technology

Andrea Harabin
National Heart, Lung, and Blood Institute

Deborah Hoshizaki
National Institute of Diabetes and Digestive and Kidney Diseases

Susan Howley
Christopher & Dana Reeve Foundation

Benjamin Humphreys
Washington University

Seigo Izumo
Takeda Pharmaceutical Company Ltd.

Shekhar Jha
National Eye Institute

Elizabeth Jungman
The Pew Charitable Trusts

Christine Kelley
National Institute of Biomedical Imaging and Bioengineering

Sage Kim
National Institutes of Health

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