March 10, 2012

From: Ellen G. Feigal, M.D., Senior Vice President, Research and Development

To: Independent Citizens Oversight Committee (ICOC)

Re: Pre-read for 2012 Strategic Plan Update at ICOC on March 21, 2012

This pre-read for the ICOC is providing context for the March ICOC discussion on one- and five-year goals, their alignment with the 2012 Strategic Plan and as reference for the discussion on funding priorities and strategies. This document also provides an appendix, starting on page 5, including an executive summary, as well as the more detailed background information on accomplishments to date on CIRM's five-year and ten-year goals as communicated in CIRM's 2006 Strategic Plan.

Goals for CIRM – Accomplishments to Date, and Moving Forward

CIRM's Mission:

"To support and advance stem cell research and regenerative medicine under the highest ethical and medical standards for the discovery and development of cures, therapies, diagnostics and research technologies to relieve human suffering from chronic disease and injury."

Proposed strategic objectives for next five years			
Scientific	**Clinical**	**Economic**	**Community**
Accelerate understanding of stem cell science and its applications towards human diseases and injuries	**Advance stem cell science into clinical trials** to achieve evidence of therapeutic benefit to patients	**Drive economic development** for California from stem cell science and therapies	**Maintain California as the world stem cell leader**

Single most important key outcomes			
Scientific	**Clinical**	**Economic**	**Community**
Achieve transformative research discoveries	**Achieve clinical proof of concept for stem cell therapies**	**Leverage CIRM's investment in California**	**California universally recognized as the "Stem Cell State"**

Strategies for Achieving Success on the 2012 Objectives

CIRM senior leadership, with input from the Independent Citizens Oversight Committee and a wide array of stakeholders, has developed strategies for success for each of the 2012 objectives, summarized in the table below.

Scientific Strategies	Clinical Strategies	Economic Strategies	Community Strategies
▪ Foster an engine of discovery and transformative research ▪ Create a collaborative research community that enhances California's leadership and competitiveness ▪ Realign funding programs, review and decision making with current strategic objectives	▪ Foster disease-specific research toward clinical proofs of concept ▪ Expand multidisciplinary collaborative efforts to enhance clinical outcome ▪ Foster developing a regulatory path for stem cell therapies ▪ Focus, prioritize and evaluate projects to move the most promising forward ▪ Enhance interactions with patients and advocates	▪ Attract co-funding and follow-on financing of CIRM projects ▪ Foster the growth of California's stem cell industry and the creation of stem cell clusters that accelerate investment ▪ Lay the groundwork for development of new therapeutic approaches to treat or cure chronic dis.* ▪ Establish a platform to enable grantees, disease foundations, venture capitalists and others to pursue CIRM's mission upon the expiration of CIRM's bond funding	▪ Communicate value proposition of CIRM and the stem cell field ▪ Engage with stakeholders on why stem cell science matters to them ▪ Create an awareness among stakeholders of CIRM's role in making California the leader in the field

*Recommended at the January 2012 ICOC session, taking into account the intent to take every opportunity to advance therapy candidates that can dramatically reduce the costs of chronic illness and injury.

In striving towards our FY12/13 one year goals, we will continue to be good stewards of public dollars as we seek to achieve the following:

- Ensure CIRM's portfolio includes at least 2 programs with an approved Investigational New Drug (IND) filing with the US Food and Drug Administration (FDA) to enter human clinical trials

- Achieve $50 million in new, outside financial commitment for CIRM

programs (ie., collaborative funding partners, industry, venture capitalists, matching funds from institutions)

- Ensure funding of potentially high-impact projects that could result in transformative research by modifying priorities in CIRM's Request for Applications

- Educate and engage the California community in CIRM's mission and achievements, in part by increasing the number of monthly online engagements from the current 70,000 to 100,000.

- Optimize CIRM's workforce staffing and processes to meet changing priorities within the 6% ceiling

In striving towards our FY17/18 five year goals, we will continue to be good stewards of public dollars as we seek to achieve the following:

These 5 year goals have been modified from the 10 year goals initially set forth in CIRM's 2006 Strategic Plan, which we have included in parentheses, so the ICOC can see the changes:

- Goal I: Through research sponsored by CIRM and others, the factors regulating the self-renewal and tumor-causing potential of stem cells and their derivatives will be identified and characterized.

  (*From CIRM's 2006 Strategic Plan: Goal IX: Through research sponsored by CIRM and others, the mechanisms regulating the self-renewal and oncogenic potential of embryonic stem cells and their derivatives will have been identified and characterized.*)

- Goal II: Through research sponsored by CIRM and others, a thorough description of the steps of differentiation leading to the production of critical cells of the body desired for transplantation will be achieved.

  (*From CIRM's 2006 Strategic Plan: Goal VIII: Through research sponsored by CIRM and others, a thorough description of the steps of differentiation leading to the production of the various cells of the body will have been achieved.*)

- Goal III: CIRM will have funded new approaches for ensuring successful allogeneic cell transplantation that are in clinical development.

  (*From CIRM's 2006 Strategic Plan: Goal IV: CIRM will have funded new approaches for achieving immune tolerance for transplantation that are in pre-clinical development.*)

- Goal IV: Using stem cell research, CIRM-funded investigators will have

established proof-of-principle in preclinical animal models for treatment of >10 diseases.

(*From CIRM's 2006 Strategic Plan: Goal V: Using stem cell research, CIRM-funded investigators will have established proof of principle in preclinical animal models for the treatment of 6-8 diseases.*)

- Goal V: CIRM-funded investigators will have created disease-specific cell lines for 20-30 diseases and used them to gain new information about their underlying pathogenesis, and to identify new drug targets for discovery of new therapeutics.

  (*From CIRM's 2006 Strategic Plan: Goal VI: CIRM-funded investigators will have created disease-specific cell lines for 20-30 diseases and used them to gain new information about pathogenesis, to identify new drug targets and to discover new therapeutics.*)

- Goal VI: CIRM will have enabled development of new procedures for the production of a variety of stem and / or progenitor cells that meet requirements for clinical application.

  (*From CIRM's 2006 Strategic Plan: Goal VII: CIRM will have enabled development of new procedures for the production of a variety of stem and/or progenitor cells that meet GMP requirements.*)

- Goal VII: At least 20 CIRM funded programs will have outside capital commitments for funding development work.

  (*From CIRM's 2006 Strategic Plan: Goal III: CIRM funded projects will have achieved sufficient success to attract private capital for funding further clinical development of stem cell therapies.*)

- Goal VIII: CIRM will have funded 10 therapies in phase I or II clinical trials, in at least 5 different therapeutic areas, based on stem cell research, and have achieved clinical proof-of-concept that transplanted cells derived from pluripotent or progenitor cells can be used to restore function for at least one disease or injury condition.

  (*From CIRM's 2006 Strategic Plan: Goal I: CIRM grantees will have clinical proof of principle that transplanted cells derived from pluripotent cells can be used to restore function for at least one disease; Goal II: CIRM-sponsored research will have generated therapies based on stem cell research in Phase I or Phase II clinical trials for 2-4 additional diseases.*)

- Goal IX: Broaden and reinforce CIRM's efforts to educate and engage the California community in CIRM's mission and achievements, in part by

increasing the number of monthly online engagements to 250,000. (*New, not in CIRM's 2006 Strategic Plan*)

APPENDIX

Executive Summary on Accomplishments

Accomplishments to date on CIRM's five-year goals as communicated in CIRM's 2006 strategic plan serve as milestones to gauge CIRM's progress. CIRM has already met 8 of its 10 goals, is anticipated to meet a 9th goal by the end of 2012, and work is in progress on the goal of CIRM grantees demonstrating methods for transplanted tissues to evade host rejection.

Goal I – CIRM grantees will have six therapies based on stem cell research in pre-clinical development. **CIRM expects to have over 6 therapies in preclinical development before the end of 2012.**
Goal II - CIRM grantees will have developed new methods for making stem cell lines. **This strategic goal has been met.**
Goal III – CIRM grantees will have successfully created disease-specific stem cell lines for four diseases. **This strategic goal has been met**; lines for more than 4 disorders have been derived.
Goal IV – CIRM grantees will have developed methods for growing stem cells in defined media. **This strategic goal has been met.**
Goal V – CIRM will have enabled establishment of a stem cell bank. By supporting development of new lines, encouraging their registration and documentation, and ultimately providing support for self-sustaining banking and distribution efforts**, CIRM has met this strategic goal.**
Goal VI – CIRM funded investigators will have demonstrated methods for inducing immune tolerance in animal models. In June of 2010, CIRM's Stem Cell Transplantation Immunology Awards were issued to 19 investigators whose efforts are specifically devoted to understanding and overcoming immune rejection of stem cell-derived tissues. In addition to probing the immunogenic properties of stem cells, these investigators are exploring a variety of approaches for inducing tolerance or enabling transplanted tissues to evade host immunity. Several of CIRM's Disease Team grantees are also addressing this goal by devising appropriate immunosuppression and/or immunoisolation strategies as part of their preclinical development plan. It is possible that pioneering work by these groups could inform the design of similar approaches in the broader stem cell community.
Goal VII – CIRM will have increased the workforce of stem cell researchers in California. **This strategic goal has been met.**
Goal VIII – CIRM grantees will have established tools for toxicity testing based on stem cell research. **This strategic goal has been met.**
Goal IX – CIRM will have enabled effective partnerships in stem cell research between scientific teams in non-profit and commercial sectors. **This strategic goal has been met.**

Goal X – CIRM will have established national and international collaborations in stem cell research that will allow us to leverage the comparative advantage of California and our collaborators to advance toward therapies. **This strategic goal has been met.**

Accomplishments to date on CIRM's 5 year goals as communicated in CIRM's 2006 Strategic Plan

<u>Goal I:</u> CIRM grantees will have six therapies based on stem cell research in pre-clinical development.

CIRM defines "preclinical development" or "IND-enabling preclinical development" as the stage of translational research that includes those activities required to enable regulatory approval for the initiation and conduct of a clinical trial with a given therapeutic candidate such as process scale-up and production under stage appropriate current Good Manufacturing Practices (cGMP), GLP toxicology and other required safety studies, and pivotal preclinical pharmacology studies.

Progress: As of November 2011, CIRM has invested $225 million dollars and CIRM's Collaborative Funding Partners invested an additional S44.8 million in Disease Team Research Awards I comprising 14 grants to projects in various stages of translation ranging from late discovery research to early preclinical development. CIRM has also committed $240 million to the Disease Team Therapy Development Awards (DTTD), which will fund up to 12 projects seeking to advance a development or therapy candidate through IND-enabling or clinical studies. The first phase of DTTD launched in September 2011, when 19 groups were awarded planning grants to begin assembling teams and putting together competitive proposals for the research phase of the award. Successful applicant projects are expected to receive funding in the summer of 2012.

The goal for each Disease Team I project is an IND submission within four years, whereas the goal for Disease Team Therapy Development projects are to complete IND-enabling studies on existing development candidates and/or advance them to clinical studies within 4 years. Between these two programs alone, **CIRM expects to have over 6 therapies in preclinical development before the end of 2012.**

Outcomes:

- **14 Disease Team I Projects – the ICOC will be provided an update on the Disease Teams at the March 21 session (separate pre-read)**
    - 13 projects continuing on their IND-enabling preclinical development
        - 2 projects anticipated to file IND by end of 2012
        - 1 project revised and continuing on IND-enabling preclinical development
    - 1 project did not meet Go/No-Go milestones and CIRM will end financial disbursements in a wind down of the research by March 31, 2012

- **Disease Team Therapy Development Projects**
    - In August 2011, 19 planning grants (Part 1) were awarded to teams, many of which are in IND-enabling preclinical development, to advance development candidates to IND filing for clinical trials. Those projects that successfully compete for and obtain the research component of these awards (Part 2, to be awarded in July 2012) will allow CIRM to further surpass the milestone set forth in this goal.
    - A few additional projects, through the exceptions process, are bypassing the planning stage and will compete directly for a Disease Team Therapy Development Award, further increasing the number of projects that may impact this goal.

- **Other Projects**
    - A CIRM New Faculty grantee is performing preclinical research and development in the context of an ongoing clinical trial for treating melanoma with genetically modified CD34 cells. Specifically, an improved vector is being developed and will be produced under current Good Manufacturing Practices (cGMP). Following testing in preclinical models, the new vector could be incorporated into a parallel clinical study within the next year or two.
    - One CIRM-funded publication describes the preclinical studies of a small molecule JAK2 inhibitor that has now gone through Phase I/II studies and is the subject of several new clinical trials that are actively recruiting participants., including a phase III trial for myelofibrosis
        - Geron, I., et al. "Selective inhibition of JAK2-driven erythroid differentiation of polycythemia vera progenitors." *Cancer Cell*, 13:321, 2008. **PI: C. Jamieson (SEED, UCSD).** Demonstrated that a JAK2 (signaling kinase) inhibitor could block aberrant erythroid differentiation of polycythemia vera progenitors. Study also provided direct in vivo evidence that a particular mutation in JAK2 (JAK2V617F) is necessary and sufficient to drive aberrant myeloid differentiation characteristic of polycythemia vera. This work provided the basis for clinical trials of the JAK2 inhibitor *TG101348 in polycythemia vera patients*.
    - A publication that was co-authored by a CIRM SEED-funded grantee showed that hedgehog signaling was required for maintenance of cancer stem cells in chronic myelogenous leukemia (CML) (Zhao C., et.al. Nature 458:776, 2009). The researcher, now funded under a CIRM New Faculty II Research award, has subsequently reported in her progress report on preclinical studies on leukemic cancer stem cells with a small molecule inhibitor of the hedgehog pathway. Based in part on these studies, Pfizer has initiated Phase I clinical testing of that inhibitor in CML that is currently recruiting patients.

<u>Goal II</u>: CIRM grantees will have developed new methods for making stem cell lines.

Progress: CIRM has funded numerous projects seeking to develop or optimize methods for generating new stem cell lines. In addition to deriving new human embryonic stem cell lines from blastocysts, CIRM grantees have explored the use of transcription factors, chemicals, proteins, cell fusion, nuclear transfer, and small RNAs for generating induced pluripotent stem cells (iPSC) or other reprogrammed cell types. Investigators are creating and using new methods for producing stem cell lines with desired properties such as disease- or patient-specific phenotypes, ethnic and genetic diversity, expression of reporter constructs, correction of genetic defects, or production of therapeutic agents. In total, CIRM has funded 118 projects with the goal of a) deriving, engineering or refining a human stem cell line for research and/or development purposes; or b) developing tools or techniques for modifying or deriving stem cell line*s or derivatives. While this goal was specifically targeted by the New Cell Lines Awards, several* grants from CIRM's other initiatives have had impact, including projects from the SEED, Comprehensive, New Faculty, Tools and Technology, Early Translational, Basic Biology and Disease Team Initiatives.

Outcomes: Many CIRM grants addressing this goal are ongoing, but a number have already led to significant discoveries and insights.

- Data from progress reports indicate that 36 projects have generated novel insights and/or methods. To date, 32 publications have emerged from these studies, documenting work using small molecules and microRNAs to induce pluripotency and make significant refinements to stem cell line derivations. Some notable recent findings include:
    - Identification of a family of microRNAs whose targets promote somatic cell reprogramming in both human and mouse fibroblasts. Subramanyam et al, *Nature Biotechnology,* May 2011 and Judson et al, *Nature Biotechnology*, April 2009. **PI: R. Blelloch (SEED, New Faculty, UCSF),** with contribution from **Bridges Intern, SFSU).**
    - Discovery of a rapid and efficient approach for deriving and expanding primitive neural progenitor cells from hESC, a population of keen interest to the regenerative medicine community. Li et al, *PNAS*, May 2011. **PI: S. Ding (New Faculty, Scripps**).
    - New protocols for hESC derivation and xeno-free culture conditions revealed through mechanistic analyses and comparisons of hESC derived from various embryonic subpopulations. Krtolica, A., et al, *Differentiation*, April 2011. Genbacev, O., et al. *Stem Cells*, July 2011. Ilic. D, et al. *Stem Cells Dev*. November 2009. **PI: S. Fisher (Comprehensive, New Cell Lines, UCSF)**

This strategic goal has been met.

<u>Goal III</u>: CIRM grantees will have successfully created disease-specific stem cell lines for four diseases.

Progress: CIRM has funded over 40 grants with a goal of developing disease- or patient- specific stem cells lines targeting over 20 disorders. Data from progress reports indicates that many such lines have been successfully created and are being used to generate novel findings (see below).

Outcomes: Disease- or patient-specific stem cell lines (embryonic, induced pluripotent or cancer stem cell) have been created for the following disorders:

Ataxia Telangiectasia*
Alzheimer's Disease (sporadic and genetic forms)
apoE (Alzheimer's Disease predisposition)
Chronic Pulmonary Obstruction Disease
Duchenne Muscular Dystrophy
Ewing's Sarcoma*

Fanconi Anemia
Frontotemporal Dementia (multiple genetic forms)
Huntington's Disease
ICF Syndrome
Lesch-Nyhan Syndrome
Leukemia (CML)

Long QT Syndrome
Monosomy X*
Marfan Syndrome
Parkinson's Disease (genetic forms)*
$p53^{-/-}$ (cancer predisposition)*
Rett Syndrome*

trisomy (various)*
schizophrenia*

* published

Twelve publications have resulted from this work thus far. A few noteworthy examples from 2011 include:

- Byers, B. et al. *PLoS One,* November, 2011. "SNCA Triplication Parkinson's Patient's iPSC-derived DA Neurons Accumulate α-Synuclein and Are Susceptible to Oxidative Stress". **PI: R. Reijo Pera, New Cell Lines and Shared Labs, Stanford).** This study demonstrated that the relevant Parkinson's disease (PD) mutation is intrinsically capable of perturbing normal cell function in culture, conferring a cell autonomous disease manifestation that is independent of exposure to the entire complexity of a diseased brain.
- Brennand, K. et al. *Nature,* April 2011. "Modeling schizophrenia using human induced pluripotent stem cells." **PI: F. Gage (New Cell Lines, Salk Institute) and Training Grant.** This study reports the development of hiPSC neuronal phenotypes and gene expression changes associated with schizophrenia (SCZD). SCZD hiPSC neurons showed diminished neuronal connectivity in conjunction with decreased neurite number, PSD95-protein levels, glutamate receptor expression and impairment of key signaling pathways.
- Liu, et al. *Cell Stem Cell*, May 2011. "Targeted Gene Correction of Laminopathy-Associated LMNA Mutations in Patient-Specific iPSCs". **PI:**

J.F. Loring (Early Translation I, Scripps Institute) and Training Grant. The study shows that helper-dependent adenoviral vectors (HDAdVs) provide a highly efficient and safe method for correcting mutations in large genomic regions in human induced pluripotent stem cells and can also be effective in adult human mesenchymal stem cells. This type of approach could be used to generate genotype-matched cell lines for disease modeling and drug discovery and potentially also in therapeutics.

- Marchetto, M. et al. *Cell*, November 2010. "A model for neural development and treatment of Rett syndrome using human induced pluripotent stem cells." **PI: F. Gage (Comprehensive, New Cell Lines, Salk Institute).** The model generated from this data recapitulates early stages of a human neuro-developmental disease and represents a promising cellular tool for drug screening, diagnosis and personalized treatment.

This strategic goal has been met; lines for more than 4 disorders have been derived.

<u>Goal IV</u>: CIRM grantees will have developed methods for growing stem cells in defined media.

Progress: CIRM has funded 17 grants that are focused on developing methods or identifying molecules or tools that enable stem cells to grow effectively in defined, xeno-free media. In addition, efforts to develop GMP-grade cell lines or therapy candidates amongst CIRM's Development Portfolio projects could lead to insights that could further impact this goal.

Outcomes:

- Data from progress reports indicate that about 30 grants have generated new insights in this area. Some of the highlights include:
  - Use of defined, xeno-free conditions for more efficient derivation of patient-specific stem cell lines
  - Use of screening platforms and microfluidic technologies to rapidly identify ligands, chemicals and matrix formulations that promote stem cell expansion and pluripotency or replace non- defined components of culture media
  - Identification of specific molecules or compounds that promote differentiation to specific lineages including neural, cardiac and hematopoietic cell fates

- Thus far, 8 publications addressing this strategic goal have resulted from CIRM funding. Some notable findings include:
  - Hasagewa, K. et al, *Stem Cells Translational Medicine*, December 2011, "Wnt signaling orchestration with a small molecule DYRK inhibitor provides long-term xeno-free human pluripotent cell expansion" **PI: M. Pera**

- Krtolica, A., et al, *Differentiation*, April 2011. "GROalpha regulates human embryonic stem cell self-renewal or adoption of a neuronal fate." and Ilic. D., et al. *Stem Cells Dev*. November, 2009 "Derivation of human embryonic stem cell lines from biopsied blastomeres on human feeders with minimal exposure to xenomaterials." **PI: S. Fisher (Comprehensive, New Cell Lines, UCSF)**
- Brafman, D., et al. "Long-term human pluripotent stem cell self-renewal on synthetic polymer surfaces." *Biomaterials*, December 2010 and "Defining long-term maintenance conditions of human embryonic stem cells with arrayed microenvironment technology." *Stem Cells Dev*, March 2009. **PIs: S. Chien (SEED, UCSD), S. Varghese (New Faculty, UCSD) and K. Willert (Shared Labs, UCSD).** These publications describe the use of array technology to identify fully defined and optimized conditions for the culture and proliferation of hESCs. The authors screened extracellular matrix proteins, signaling molecules and synthetic polymers in order to develop and characterize a defined culture conditions for the long-term self-renewal of hESC lines.
- Swistowski, A., et al. "Xeno-free defined conditions for culture of human embryonic stem cells, neural stem cells and dopaminergic neurons derived from them." *PLoS ONE*, July 2009. **PI: X. Zeng (Shared Labs, Buck Institute).** This paper describes the use of chemically defined, xeno-free media to propagate hESCs, differentiate them into human neural stem cells, induce dopaminergic neuron precursors and mature these precursors into neurons expressing midbrain and A9 dopaminergic markers (the cells lost in Parkinson's disease). The grantee writes that this "four-step scalable process is readily transferable to a Good Manufacture Practice (GMP) facility for the production of functional dopaminergic neurons from hESCs for potential clinical uses."

This strategic goal has been met.

Goal V: CIRM will have enabled establishment of a stem cell bank.

Progress: Multiple grants have been awarded to groups developing new stem cell lines (see Five Year Goals 2 and 3). Although CIRM has developed a system for registering and documenting these lines, recent policy changes at the National Institute of Health has led to the NIH Registry becoming the repository of choice for the research community. More recently, CIRM has developed a comprehensive initiative to support the establishment of a physical infrastructure to bank and distribute stem cells and human induced pluripotent stem cell lines of appropriate quality that have been developed, or will be developed by CIRM grantees.

Outcomes:

- As of September 2011, CIRM Grantees have reported derivation of nearly 200 human pluripotent stem cell lines, including embryonic and induced pluripotent cells, representing a diversity of disease, gender, ethnicity, and derivation methods.

- In June 2011, the ICOC approved CIRM becoming a member of the public private partnership initiative sponsored by the National Institute of Neurological Disorders and Stroke (NINDS) at the NIH to develop and bank well characterized hiPSC lines for neurodegenerative diseases, and to make them publicly available. CIRM is contributing funds to a consortium that develops lines from patients with Huntington's Disease, Parkinson's Disease, and Amyotrophic Lateral Sclerosis (ALS).
- A series of RFAs are planned for 2012 to facilitate the procurement, derivation and banking of iPSC lines from patients with complex genetic disorders, and to enable banking of existing human pluripotent cell lines that have been derived by CIRM grantees and meet appropriate inclusion criteria. A cell bank will be established in California to manage and distribute these lines as a resource to the scientific community.

By supporting development of new lines, encouraging their registration and documentation, and ultimately providing support for self-sustaining banking and distribution effort, CIRM has met this strategic goal.

<u>Goal VI</u>: CIRM-funded investigators will have demonstrated methods for inducing immune tolerance in animal models.

Progress: In June of 2010**,** CIRM's Stem Cell Transplantation Immunology Awards were issued to 19 investigators whose efforts are specifically devoted to understanding and overcoming immune rejection of stem cell-derived tissues. In addition to probing the immunogenic properties of stem cells, these investigators are exploring a variety of approaches for inducing tolerance or enabling transplanted tissues to evade host immunity. Several of CIRM's Disease Team Grantees are also addressing this goal by devising appropriate immunosuppression strategies as part of their preclinical development plan. It is possible that pioneering work by these groups could inform the design of similar approaches in the broader stem cell research community.

Outcomes:

- October, 2011: CIRM organized and participated in a round table discussion with the FDA to evaluate the current challenges facing cell therapy development with respect to the immune system, and the current technologies and approaches that are being used to address them.
- 19 three-year grants were awarded in the area of Stem Cell Transplantation Immunology and have been active for approximately 1 year. Approaches being explored include use of tolerogenic dendritic cells; induction of central tolerance; mixed chimerism; regeneration of thymic epithelium; manipulation of regulatory T cells or NK cells; engineering the adaptive immune system; reducing the immunogenicity of stem cells; use of *in utero* methods; various specialized biologic strategies.
- In addition to the above, CIRM has funded 10 awards across various initiatives that address this strategic goal. Data from progress reports indicate that CIRM researchers have successfully developed a tool for modulating HLA expression

on hESC-derived hematopoietic stem cells; have optimized and refined protocols for differentiating pluripotent stem cells into defined populations of T cells and dendritic cells; and developed a SCID model that is capable of mounting a T cell-mediated allorejection response.

- Thus far, 6 publications addressing this strategic goal have resulted from CIRM funding. Some notable findings from 2011 include:
    - Stem cell allografts can survive when transplanted into the hippocampus. However, Chen et al (*PLoS One*, March 2011) found that MHC mismatch decreases surviving cell numbers and strongly inhibits the differentiation and retention of both graft-derived and endogenously produced new neurons. These effects were ameliorated by nonsteroidal anti-inflammatory drugs but not cyclosporine A, revealing an unexpected role for innate immunity in the survival and function of mismatched cellular grafts. **PI: T. Palmer (Comprehensive, Stanford).**
    - Cells derived from murine iPSCs elicited an immune response when transplanted into a genetically matched host, possibly due to abnormal expression of immunogenic proteins in the reprogrammed cells. This result cast doubt on the premise that autologous iPSC-derived transplants would necessarily be tolerated, and future studies to understand and mitigate rejection of autologous tissues will be warranted. Zhao, T. et al. *Nature*, May, 2011. **PI: Y. Xu (Early Translational, UCSD**).
    - Survival of human spinal stem cells after intraspinal transplantation into an SOD1 model for ALS was significantly improved by use of a combined, systemic immunosuppression regimen as opposed to monotherapy. Hefferan M., et al. *Cell Transplant*, June 2011. **PI: M. Marsala (Comprehensive)**.

<u>Goal VII</u>: CIRM will have increased the workforce of stem cell researchers in California.

Progress: CIRM continues to invest in several programs to support the training and career development of the next generation of stem cell scientists, including:

- 17 Training Grants to support graduate students, postdoctoral and clinical fellows at universities and institutes across California. These programs have recently been renewed for a third round of funding, beginning in 2012.
- 16 Bridges to Stem Cell Research Grants to provide stem cell training and education to undergraduate and Master's level students at a variety of universities and colleges across California. These programs have been extended for another three years, beginning in 2012.
- A new training program, the Creativity Awards, will be formally implemented in 2012. These grants will support summer internships for high school students in stem cell laboratories.
- $80 million has been allocated for New Faculty Physician Scientist Translational Research RFA, which will be launched in 2012.

- The Research Leadership Awards program, which enables top California institutions to recruit the most productive and rapidly rising stem cell scientists from out of state, was extended.

Outcomes:

- CIRM has supported 914 undergraduate and graduate students, postdoctoral fellows and clinical fellows through its various training grants. A pilot version of Creativity Awards supported summer internships for 22 high schools students.
- The careers of 45 investigators have been jump started through New Faculty Awards.
- Two Research Leadership Awards have enabled the successful recruitment of Dr. Robert Wechsler-Reya, from Duke University to the Sanford-Burnham Institute, and Dr. Peter Coffey from the Royal College of London to University of California, Santa Barbara. A third award to recruit Dr. Zhigang He from Children's Hospital and Harvard Medical School to University of California, Berkeley has been approved by the ICOC.
- When last assessed, more than 130 faculty-level researchers had moved to California's non-profit institutions from around the world since CIRM began operations.

This strategic goal has been met.

<u>Goal VIII</u>: CIRM grantees will have established tools for toxicity testing based on stem cell research.

Progress: CIRM has funded two projects that explicitly target the development of assays for predicting or evaluating toxicity. In addition, a third project sought to identify agents toxic to hESCs, the insights from which could inform our understanding of developmental/reproductive toxins and their mechanisms of action. CIRM funds about 25 other projects that are seeking insights towards developing more authentic, mature heart or liver tissues, the basic tools that are needed for toxicity studies. CIRM will continue to address this goal by encouraging additional grant submissions through future Basic Biology, Early Translational and Tools and Technology initiatives.

Outcomes:

- Data from progress reports suggests that thus far, 8 projects have yielded specific tools (reporter lines, patient-specific stem cell derivatives) or insights that could be useful for predicting or evaluating developmental or cardiotoxicity.
- CIRM grantees have made excellent progress in elucidating the molecular basis of lineage specification towards the cardiac or hepatic fate, including the notable recent publication:
    - Willems, E., et al. "Small-Molecule Inhibitors of the Wnt Pathway Potently Promote Cardiomyocytes From Human Embryonic Stem Cell-Derived Mesoderm," Circ Res, July 2011. **PI: M. Mercola (Comprehensive, Sanford-Burnham)**. This study employed pharmacological inhibition of Wnt signaling via small molecules to drive human mesoderm cells to form

cardiomyocytes. This method could yield novel tools for the benefit of pharmaceutical and clinical applications, including predictive toxicology.

- Espejel, S, et al., "Induced pluripotent stem cell-derived hepatocytes have the functional and proliferative capabilities needed for liver regeneration in mice." *Journal of Clinical Investigation*. September, 2010. **PI: H. Willenbring (New Faculty, UCSF).** This study examined whether iPS cell-derived hepatocytes have both the functional and proliferative capabilities needed for liver repair in a model of liver damage and established the feasibility of using iPS cells generated in a clinically acceptable fashion for rapid and stable liver regeneration.
- Duan, Y., et al., "Differentiation and characterization of metabolically functioning hepatocytes from human embryonic stem cells." *Stem Cells*, February 2010. **PI: M. Zern (Comprehensive, UC Davis).** This paper describes the multi-step differentiation of hESCs into cells with many of the markers and metabolic activities characteristic of primary human liver cells. While these hESC-derived hepatocytes may not be fully equivalent to mature hepatocytes, they represent an important step towards that goal and a potentially valuable tool for toxicity testing.

This strategic goal has been met.

<u>Goal IX</u>: CIRM will have enabled effective partnerships in stem cell research between scientific teams in non-profit and commercial sectors.

CIRM has funded multiple industry/nonprofit collaborations encompassing a variety of relationships and will continue to do so, particularly as more of its programs enter the translational and clinical landscapes. These partnerships are best illustrated by the Disease Team I Awards, in which teams are effectively leveraging the disparate resources and skills that will be necessary to bring such complex and ambitious projects to fruition. Examples include:

- 2 projects with principal investigators or co-principal investigators at industry and non-profit organizations
- 8 projects with academic principal investigators that include CIRM-funded, industry-based subcontracts for critical activities including GMP manufacturing, vector development, preclinical safety studies, sample and data analysis, project management, and access to specific reagents, supplies or technologies

This strategic goal has been met.

<u>Goal X</u>: CIRM will have established national and international collaborations in stem cell research that will allow us to leverage the comparative advantage of California and our collaborators to advance toward therapies.

CIRM has established 16 such partnerships and is actively pursuing additional agreements (Argentina and Brazil the end of March). From these programs, a total of 20 collaborative projects have emerged.

- **<u>Funding Agreements</u>**

Andalucian Initiative for Advanced Therapies (IATA)
State of Victoria, Australia
Canadian Cancer Stem Cell Consortium (CSCC)
Chinese Ministry of Science and Technology (MOST)
Medical Research Council, UK (MRC)
Juvenile Diabetes Research Foundation (JDRF)
Japanese Science and Technology Agency (JST)
Scottish Enterprise, Scotland
Spanish Ministry of Science and Innovation (MICINN)
Federal Ministry of Education and Research, Germany (BMBF)
Maryland Technology Development Corporation (TEDCO)
National Institutes of Health (NIH)
National Research Agency, France (ANR)
Indian Institute of Stem Cell Science and Medicine (inSTEM)
New York Stem Cell Foundation (NYSTEM)
Australia (NH&MRC)

- **Awarded Projects (as of November, 2011)**
  6 Disease Team Awards (with MRC, CSCC, BMBF, JDRF)
  2 Basic Biology Awards (with JST, BMBF)
  2 Transplantation Immunology Awards (with State of Victoria)
  10 Early Translational Awards (with State of Victoria, BMBF, TEDCO)
  1 iPSC cell line award (with NIH)

This strategic goal has been met.

Accomplishments to date on CIRM's 10 year goals as communicated in CIRM's 2006 Strategic Plan

Goal I: CIRM grantees will have clinical proof-of-principle that transplanted cells derived from pluripotent cells can be used to restore function for at least one disease.

In summer of 2011, CIRM issued a $25 million loan as part of its Targeted Clinical Development program to Geron Corporation, who sought to demonstrate safety and preliminary evidence of efficacy for a human embryonic stem cell-derived therapy for acute spinal cord injury. Although the study was discontinued for business reasons and funds were returned to CIRM, 5 patients have already been treated. Continued monitoring of these individuals will ensure that useful knowledge is obtained from these groundbreaking studies and inform future endeavors towards achieving this goal. In the mean time, CIRM continues to build a pipeline of potential pluripotent-derived cell therapies through the Early Translational and Disease Team Research Initiatives, which currently support 19 active projects to develop pluripotent stem cell-based therapies for 16 different indications.

<u>Goal II</u>: CIRM-sponsored research will have generated therapies based on stem cell research in Phase I or Phase II clinical trials for 2-4 additional diseases.

Progress: CIRM currently funds a "Development Portfolio" of 43 potential therapeutic candidates for approximately 26 different indications, a number that will increase in 2012 with funding of the Early Translational III and Disease Team Therapy Development Awards. Thirteen projects from Disease Team I are continuing in IND-enabling preclinical development, and a subset are likely to have advanced to Phase I or Phase II studies within the next few years (see Five Year Goal, 1).

Outcomes: CIRM has, in part, sponsored research leading to a Phase I/II clinical trial for a small molecule inhibitor of the JAK2 pathway for treating polycythemia vera and a Phase I clinical trial for a small molecule inhibitor of the hedgehog pathway for treating CML. If only a few additional IND applications emerge from the 43 potential therapeutics in CIRM's current pipeline, this goal will be achieved. Moreover, CIRM will fund several Disease Team Therapy Development Awards in 2012, some of which are expected to initiate Phase I and/or Phase II clinical studies within the next few years. Based on these estimates, CIRM is on track to reach this goal.

<u>Goal III</u>: CIRM funded projects will have achieved sufficient success to attract private capital for funding further clinical development of stem cell therapies.

Progress: While CIRM funded research is only just starting to move toward the clinic, CIRM is engaging in a number of actions to define pathways forward, shorten timelines and remove obstacles for those projects that demonstrate potential for clinical success. Ongoing initiatives range from promoting CIRM programs in one-on-one meetings with pharmaceutical companies, to spearheading, along with the Alliance for Regenerative Medicine, the first-ever regenerative medicine partnering and investor conference in November 2011. In addition, CIRM's board recently approved the concept for a $30 million Strategic Partnership Funding Program, which will foster collaborations of CIRM-funded researchers with partners from industry or investments from venture capital.

Outcomes: Progress towards this goal appears to be on target considering the long timeline. CIRM has learned that companies have attributed their ability to attract funding, in part, to the prospect of obtaining CIRM funding. Also, CIRM has funded, in part, research relating to the use of a small molecule inhibitor of the JAK2 pathway (owned by TargeGen), which resulted in a high impact publication prompting further research in this area. TargeGen was recently acquired by Sanofi-Aventis, who continues to explore the therapeutic potential for this drug. Viacyte very recently attracted additional funding from JDRF to their project for beta cell replacement also funded by CIRM through a Disease team I award.

<u>Goal IV</u>: CIRM will have funded new approaches for achieving immune tolerance for transplantation that are in pre-clinical development.

Progress: See Five Year Goal VI.

Outcomes: CIRM's Disease Team Projects are currently in IND-enabling preclinical development, each with a different strategy or consideration for addressing immune issues. One project is pursuing a novel encapsulation strategy to protect transplanted cells from host immune attack. Other projects are exploiting immune privileged sites and/or autologous cell populations to thwart or otherwise evade immune rejection. Knowledge gained from these efforts may elicit broader insights that could be applicable to other stem cell transplantation paradigms. Finally, mechanistic insights from CIRM's Stem Cell Transplantation Immunology and other research programs may lead to novel findings that will overcome existing scientific and/or regulator bottlenecks on the path to the clinic.

Goal V: Using stem cell research, CIRM-funded investigators will have established proof of principle in preclinical animal models for the treatment of 6-8 diseases.

Progress: As described previously, CIRM's Development Portfolio, which will continue to grow over the next few years, presently comprises 43 projects that are seeking to demonstrate, or already have demonstrated, proof of principle in preclinical models of disease or injury. Furthermore, several additional grants from CIRM's other programs have also led to insights and methods that impact this goal.

Outcomes:

- Diseases represented in CIRM's current Translational/Development Portfolio include type 1 diabetes, glioblastoma, cancer (hematologic and solid tumor), macular degeneration, corneal injury, epidermolysis bullosa, stroke, ALS, HIV, anemia, arthritis, Parkinson's Disease, cardiovascular damage, Alzheimer's Disease, epilepsy, muscular dystrophy, spinal cord injury, traumatic brain injury, Canavan's Disease, spinal muscular atrophy, autism, diabetic foot ulcers, osteoporotic bone fractures, liver failure and Huntington's disease.
- Analysis of recent progress reports from CIRM's ongoing grants indicate that several projects have made headway towards this goal. Examples include:
    - Demonstration of potentially beneficial effects from hESC-based cell populations in models of retinal degeneration, Parkinson Disease, radiation damage, and melanoma
    - Progress towards establishing proof of principle for bone repair, cardiovascular disease, intestinal disorder, myeloproliferative disorders, muscular dystrophy, multiple sclerosis, and HIV
- Notable recent publications include:
    - Fierro, F. A., *Stem Cells*, November 2011. "Effects on Proliferation and Differentiation of Multipotent Bone Marrow Stromal Cells Engineered to Express Growth Factors for Combined Cell and Gene Therapy." **J.A. Nolta (Early Translation, UC Davis).** This study provided scientific evidence to bolster the rationale that the therapeutic properties of mesenchymal stem cells/bone marrow stromal cells (MSCs) could be improved by

genetically modifying them to express higher levels of specific growth factors. **PI: J. Nolta (Early Translational, UCD).**

- Cho, E. et al. "MEF2C Enhances Dopaminergic Neuron Differentiation of Human Embryonic Stem Cells in a Parkinsonian Rat Model." *PLoS One* August, 2011. This publication describes functional and anatomical benefit of transplanted hESC-derived neural progenitors that are programmed to express constitutively active MEF2C in a rat model of Parkinson's disease. **PI: S. Lipton (Comprehensive, Sanford Burnham Institute).**
- Minear, S., *Sci Trans Med*, April 2010. "Wnt proteins promote bone regeneration" **Co-Investigator: J. Helms (Early Translational, Stanford).** This study demonstrated that bone healing after injury is accelerated when Wnt signaling is increased, either by genetic mutation or upon delivery of purified Wnt3a protein to skeletal defects, which stimulates the proliferation of progenitor cells and accelerates their differentiation into osteoblasts, the cells responsible for bone growth. As Wnt signaling is conserved across mammals in tissue repair, these findings may find widespread application in regenerative medicine.
- Rossi, S. L., et al. "Histological and functional benefit following transplantation of motor neuron progenitors to the injured rat spinal cord." *PLoS ONE*, July 2010. **PI: H. Keirstead (Comprehensive, UC Irvine).** This publication describes the transplantation of hESC-derived motor neuron progenitors (MNPs) to treat a rat model of spinal cord injury. While these MNPs didn't integrate at the site of injury, they improved endogenous neuronal survival, neurite branching and performance on a balance beam task, presumably through trophic effects.
- Acharya, M., et al. "Rescue of radiation-induced cognitive impairment through cranial transplantation of human embryonic stem cells." *Proc. Natl. Acad. Sci. USA,* November 2009. **PI: C. Limoli (SEED, UCI).** This paper demonstrated the potential for hESCs to ameliorate radiation-induced tissue injury (such as that which occurs during treatment of certain cancers), and that such strategies may provide useful interventions for reducing the adverse effects of irradiation on cognition.
- Blurton-Jones, M., et al. "Neural stem cells improve cognition via BDNF in a transgenic model of Alzheimer disease." *Proc. Natl. Acad. Sci. USA*, July 2009. **PI: F. LaFerla (SEED, UC Irvine), Postdoctoral trainee: M. Blurton-Jones.** This paper reported memory improvement following mouse NSC transplant in a mouse model of Alzheimer's disease. Dr. LaFerla is the recipient of an Early Translational award to expand upon these findings using hESC-derived NSCs.
- Sun, L. et al. "Mesenchymal stem cell transplantation reverses multiorgan dysfunction in systemic lupus erythematosus mice and humans." *Stem Cells*, June 2009. **PI: S. Shi (New Faculty, USC).** This paper reported that allogeneic mouse mesenchymal stem cell (MSC) transplant improved multiple organ function and measures of immune function in a mouse model of systemic lupus erythematosus (SLE). This paper further

demonstrated the safety and efficacy of allogeneic hMSC transplant in 4 human SLE patients, but that part of the study was performed in China and not funded by CIRM.

<u>Goal VI</u>: CIRM-funded investigators will have created disease-specific cell lines for 20-30 diseases and used them to gain new information about pathogenesis, to identify new drug targets and to discover new therapeutics.

Progress and Outcomes: See progress for Five Year Goal III. CIRM researchers have already developed at least 20 different disease/patient lines and have used them to explore disease pathology. Such lines are also being used to identify drug targets and novel therapeutic approaches.

<u>Goal VII</u>: CIRM will have enabled development of new procedures for the production of a variety of stem and/or progenitor cells that meet GMP requirements.

Progress: CIRM is currently funding 60 grants that either directly or indirectly impact this goal. Included among these are:

- 5 grants developing methods or cell lines specifically for GMP production
- 33 translational cell therapy projects (in CIRM's current Development Portfolio) which will, if successful, develop GMP and GMP-compatible methods, cell lines and banks over the course of their progression towards an IND application
- 10 projects addressing quality control of cell preparations, assays for detecting teratomas, assurance of cell integrity and functionality
- Also see Five Year Goal IV: 12 additional grants seeking to develop defined media conditions could lead to insights that may indirectly impact this goal

Outcomes: While still in the early stages, several projects have generated preliminary data by comparing and evaluating growth and behavior parameters for multiple pluripotent cell lines or cell therapy candidates using different conditions and media formulations for expansion. Most recently, CIRM investigators published a significant report describing GMP-compatible procedures for deriving tissues from somatic cells via a pluripotent (hiPSC) intermediate (see below).

- Karumbayaram, S. et al, "From Skin Biopsy to Neurons through a Pluripotent Intermediate Under Good Manufacturing Practice Protocols" *Stem Cells Trans Med,* December 2011*.* The authors describe a successful framework for producing GMP-grade derivatives of hiPSCs that are entirely free of xenobiotic exposure, from collection of patient samples through reprogramming, cell maintenance, identification of reprogramming vector integration sites, and terminal differentiation of clinically relevant cells. A primary set of Standard Operating Procedures for these practices were provided to facilitate their widespread adoption. **CIRM PIs: W. Lowry** (**SEED, Basic Biology**), **K. Plath** (**New Faculty, Basic Biology), J. Zack (New Cell Lines**), **A. Clark** (**New Cell Lines**), **UCLA.**

<u>Goal VIII</u>: Through research sponsored by CIRM and others, a thorough description of the steps of differentiation leading to the production of the various cells of the body will have been achieved.

Progress: CIRM has funded about 175 projects that could inform our understanding of the mechanisms by which cell identity is established. CIRM will continue to target additional studies in this area, particularly through the ongoing Basic Biology Initiative. Currently funded grants include:

- About 70 grants studying specification of neural fate
- About 20 grants investigating the cardiac lineage
- About 30 grants focused on hematopoietic and/or immune differentiation
- Multiple grants focused differentiation towards skeletal muscle, liver, pancreas, retinal epithelium, trophoblast and other early lineages
- One or two grants each exploring specification of lung, kidney, bladder, vascular, skin, hair cells cells, bone/cartilage, germ cells, intestine, and/or dental fates

Outcomes: Major strides have been made in understanding differentiation into many cell lineages. Most of CIRM's strategic impacts, thus far, have been towards this goal and derive largely from the earliest rounds of research funding, the SEED, Comprehensive and New Faculty Awards.

- Analysis of progress reports from CIRM's active and recently concluded grants suggest that 118 grants thus have had measurable impacts on this strategic goal, many of which have yet to be published
- To date, CIRM grantees have produced about 90 publications detailing aspects of the differentiation process of stem/progenitor cells into various phenotypes. Some notable recent examples include the following:
    - Ritner, C. et al. "An engineered cardiac reporter cell line identifies human embryonic stem cell-derived myocardial precursors." *PLoS One,* January 2011. **PI: H. Bernstein (Comprehensive, UCSF).** The investigators identified heart stem cells derived from hESCs and showed that they could give rise to all of the different types of heart muscle found in the patients with heart disease.
    - Pozniak, C.D., et al. "Sox10 directs neural stem cells toward the oligodendrocyte lineage by decreasing Suppressor of Fused expression" *PNAS*, Nov 2010. **PI: S.J. Pleasure (Comprehensive, UCSF)**. Oligodendrocyte precursor cells (OPCs) are lineage-restricted progenitors generally limited *in vivo* to producing oligodendrocytes. This study shows that the certain transcription factors can induce multipotent neural precursor cells (NPCs) from the early postnatal subventricular zone (SVZ) to become OPCs in an autonomous manner. Mechanisms controlling genesis of OPCs are of interest because of their importance in myelin development and their potential for regenerative therapies in multiple sclerosis and dysmyelinating syndromes.
    - Oshima, K., et al. "Mechanosensitive hair cell-like cells from embryonic and induced pluripotent stem cells." *Cell*, May 2010. **PI: S. Heller**

(Comprehensive, Stanford). In this study, the authors describe a stepwise protocol for directing mouse embryonic stem and induced pluripotent stem cells towards a hair cell-like fate. Hair cells are specialized mechanosensory cells that play a central role in hearing and balance. Cells produced from this methodology possessed sterociliary bundles and responded to mechanical stimulation. This study lays the foundation for future therapeutic advances for treating hearing loss due to hair cell damage.

- Cordes, K.R., et al. "miR-145 and miR-143 regulate smooth muscle cell fate and plasticity." *Nature,* 2009. **PI: D. Srivastava (Comprehensive, Gladstone Institute)** MicroRNAs are regulators of myriad cellular events, but evidence for a single microRNA that can efficiently differentiate multipotent stem cells into a specific lineage or regulate direct reprogramming of cells into an alternative cell fate has been elusive. These findings demonstrate that a specific microRNA can direct the smooth muscle fate and that a combination of microRNAs functions to regulate the quiescent versus proliferative phenotype of smooth muscle cells.
- Karumbayaram, S., et al. "Directed differentiation of human-induced pluripotent stem cells generates active motor neurons." *Stem Cells*, April 2009. **PI: W.E. Lowry (SEED, UCLA).** The authors found that human induced pluripotent stem (iPS) cells could be differentiated to form motor neurons with a similar efficiency as hESCs. This represents the first demonstration that human iPS-derived cells are able to generate electrically active motor neurons and demonstrates the feasibility of using iPS-derived motor neuron progenitors and motor neurons in regenerative medicine applications and *in vitro* modeling of motor neuron diseases.
- Oh, S., et al. "Stem cell fate dictated solely by altered nanotube dimension." *Proc. Natl. Acad. Sci. USA*, January 2009. **PI: S. Chien (Comprehensive, UCSD); Trainee: S. Oh.** This paper demonstrated that engineered microenvironments could be used to direct the fate of stem cells. In this case, the dimensions of nanotubular-shaped surface structure (geometric cues) could be manipulated to either augment human mesenchymal stem cell (hMSC) adhesion, or specify differentiation into osteoblasts.

<u>Goal IX</u>: Through research sponsored by CIRM and others, the mechanisms regulating the self-renewal and oncogenic potential of embryonic stem cells and their derivatives will have been identified and characterized.

Progress: CIRM has funded 133 grants with the potential to impact this goal. Included amongst these projects are:

- Those that elucidate oncogenic mechanisms (genetic instability, tumor suppressor function) in stem cells

- Mechanisms of self-renewal in pluripotent, adult and cancer stem cells
- Mechanisms by which pluripotency can be established or maintained
- Non-viral methods for induction of pluripotency
- Evaluation and mitigation of teratoma risk in stem cells and their derivatives
- Consequences of reprogramming and culturing methods on genetic and epigenetic integrity of stem cells

Outcomes: Analyses of progress reports indicate that more than 70 projects have had substantial and/or measurable impacts on this goal, many of which have yet to be published. In addition, CIRM funding has contributed to more than 44 publications describing the mechanisms regulating the self-renewal and oncogenic potential of embryonic stem cells and their derivatives. These publications include:

- Gore, A., et al. "Somatic coding mutations in human induced pluripotent stem cells." *Nature*, Mar 2011. **PI: K. Zhang; L.S. Goldstein (Comprehensive and Training, UCSD)**. This study compared 22 human induced pluripotent stem cell lines (hiPSC) reprogrammed using five different methods and showed that each line contained an average of five protein-coding point mutations in the regions sampled. The majority of these mutations were non-synonymous, nonsense or splice variants, and were enriched in genes mutated or having causative effects in cancers. At least half of these mutations pre-existed in the fibroblast progenitors at low frequencies, whereas the remainder occurred during or after reprogramming. These data suggest that extensive genetic screening may be necessary to ensure hiPSC safety before clinical use.
- Hawkins, R. D., et al. "Distinct epigenomic landscapes of pluripotent and lineage-committed human cells." *Cell Stem Cell*, May 2010. **PI: B. Ren (SEED, New Faculty II, Ludwig Institute).** This paper reported that hESCs differ vastly from their lineage-committed progeny in their DNA modification profile, or epigenome. The group analyzed different types of DNA modifications in different cell types using high-throughput, genome-wide approaches. The differences they discovered between hESCs and their differentiated progeny may comprise novel epigenetic mechanisms underlying pluripotency and lineage commitment in human cells.
- Lee, A. S., et al., "Effects of cell number on teratoma formation by human embryonic stem cells." *Cell Cycle*, August 2009. **PI: J. Wu (SEED, Comprehensive, Stanford).** In this paper Dr. Wu's group utilized fluorescent reporter genes and long-term, non-invasive imaging techniques to determine the minimum number of hESCs required for teratoma formation in immunodeficient mice. They found that a minimum of 100,000 hESCs transplanted into the heart and 10,000 hESCs into skeletal muscle were required, demonstrating that both cell number and transplant site play important roles in teratoma formation.

- Gaspar-Maia, A., et al. "Chd1 regulates open chromatin and pluripotency of embryonic stem cells." *Nature*, July 2009. **PI: Miguel Ramalho-Santos (SEED & New Cell Lines, UCSF).** This paper reports the identification of a protein, Chd1, required for hESC self-renewal and pluripotency as well as the epigenetic mechanism responsible for this regulation. This discovery will impact work on stem cell differentiation, reprogramming and oncogenicity.
- Xu, N., et al. "MicroRNA-145 regulates OCT4, SOX2, and KLF4 and represses pluripotency in human embryonic stem cells." *Cell*, May 2009. **Trainee: Na Xu (UC Santa Barbara).** This paper reports the identification of a novel microRNA regulator of hESC self-renewal and pluripotency. The authors demonstrated that this microRNA directly regulates known transcription factors responsible for pluripotency, and its expression inhibits hESC self-renewal. This is an important discovery with implications for controlling the differentiation and potential oncogenicity of hESCs.
- Zhu S, st al. "A small molecule primes embryonic stem cells for differentiation." *Cell Stem Cell*. May, 2009. **PI Schultz, SEED, Scripps)**. The authors utilized a high-content screen to identify stauprimide, a small molecule that interacts with NME2 and inhibits its nuclear localization, thereby leading to downregulation of c-Myc, a key regulator of the pluripotent state. These findings identified a chemical tool that primes ESCs for efficient differentiation and reveals an important role for NME2 in ESC self-renewal.

<u>Goal X</u>: CIRM will have enabled development of new methods for tissue replacement based on stem cell research.

Progress: CIRM is funding a significant number of grants that address this goal:

- 27 grants exploring the use of matrices, biomaterials, co-culture techniques or scaffolding to control cell fate/ improve cell authenticity or function
- An additional 22 grants exploring the effects of cellular microenvironment or niche on cell behavior

Recently, CIRM has designated tissue engineering as one of several priority areas to be targeted by the Basic Biology IV Awards, which was released in November of 2011. Moreover, CIRM has organized a workshop on Tissue Engineering that convened in January of 2012. Here, leading experts in the field discussed the potential opportunities and challenges, including immunological issues, scaffold choice, translation/scale-up, and funding, in tissue engineering whereby CIRM might make a contribution.

Outcomes: While most grants in this area were funded only recently, CIRM investigators have already generated novel insights with the potential to impact our understanding of tissue architecture, particularly in the areas of cardiac biology but also in such organs as the eye, the brain, intestine and liver. CIRM has contributed funding towards 30 publications that focus on tissue engineering, tissue

regeneration/replacement, and/or microenvironment interactions of stem cells. Notable examples include:

- Zhou, P., *Liver Transpl*, 2011. "Decellularized liver matrix as a carrier for the transplantation of human fetal and primary hepatocytes in mice." **PI: M. Zern (Comprehensive, UCD).** Efforts improve the level of engraftment of primary hepatocytes upon transplantation led to the discovery that decellularized liver matrix provides an excellent environment for long-term survival and maintenance of the hepatic phenotype.
- Gilbert, P. M., et al. "Substrate Elasticity Regulates Skeletal Muscle Stem Cell Self-Renewal in Culture." *Science*, July 2010. **PI: H. Blau (Tools & Technologies I, Stanford).** In this groundbreaking study, the authors report that freshly isolated muscle stem cells (MuSCs) could be maintained on a bioengineered substrate that recapitulates key biophysical and biochemical niche features. Furthermore, these MuSCs contributed extensively to muscle regeneration when transplanted into mice. This study provided novel evidence that by recapitulating physiological tissue rigidity, propagation of adult muscle stem cells was possible, renewing the promise of cell-based therapies for treating muscle wasting diseases.
- Yu, J., et al. "The use of human mesenchymal stem cells encapsulated in RGD modified alginate microspheres in the repair of myocardial infarction in the rat." *Biomaterial*, June 2010. **PI: R. Lee (Comprehensive, UCSF).** The combination of scaffold material and cell transplantation therapy has been extensively investigated in cardiac tissue engineering. However, many polymers are difficult to administer or lack the structural integrity to restore left ventricle function. This study developed a technique using human mesenchymal stem cells (hMSCs) encapsulated in RGD modified alginate microspheres that were capable of facilitating myocardial repair. The surface modification and microencapsulation techniques were successfully combined with cell transplantation, which led to the maintenance of left ventricle geometry, preservation of left ventricle function, increase of angiogenesis and improvement of cell survival.
- Nakayama, K. H., et al. "Decellularized rhesus monkey kidney as a three-dimensional scaffold for renal tissue engineering." *Tissue Eng Part A*, February 2010. **PI: A. Tarantal (Comprehensive, UC Davis). Trainee: K. H. Nakayama.** This paper describes the optimization of kidney decellularization techniques and the characterization of the resulting structures. The authors demonstrate that decellularized kidney sections retain critical properties necessary for use as a three-dimensional scaffold. This study represents an important first step toward new strategies for renal tissue engineering and repair.