



CALIFORNIA INSTITUTE FOR

CIRM

REGENERATIVE MEDICINE

Breaking the Bottleneck: Deriving Definitive Hematopoietic Stem Cells from Human Pluripotent Stem Cells

**A CIRM Mini-Symposium and Focus Section Report
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I. Overview and Summary of Recommendations

The goal of this focus session was to bring together a small group of CIRM grantees and other thought leaders to discuss a longstanding challenge in the stem cell field: the inability to derive fully functional, definitive hematopoietic stem cells (HSCs) from pluripotent stem cells (PSCs). Participants began with brief assessments of the key scientific and technical challenges and provided insights on the methods and tools that are needed to address them. Following additional presentations on how their own work informs derivation of definitive HSCs, participants engaged in a round table discussion with CIRM staff to suggest how CIRM's ongoing and future initiatives might be structured to accelerate advances in the relevant science. Participants also discussed how CIRM and other funding agencies or foundations might indirectly facilitate scientific progress through alliance building and education. The outcomes of these discussions lead to the following recommendations:

- Consider defining HSC research geared towards addressing the bottleneck as a priority within upcoming RFAs such as Tools and Technologies III. Proposed key areas of priority should be 1) studying how to make cells become HSCs (PSCs or reprogrammed); 2) studies on how to expand HSCs; 3) studies on how to test the quality of HSCs.
- Consider allowing funding for priority HSC research of parallel studies of human and mouse systems under initiatives that normally would restrict funding to human cells or limit the use of animal models; strongly encourage investigators using animal models to incorporate human cells into their research programs.
- Consider adjusting review criteria 1) to enable high-risk studies; 2) to enable studies pursuing intermediate objectives towards overcoming the HSC bottleneck (as there are multiple bottlenecks that need to be resolved to ultimately bring PSC-derived HSCs to clinical use); and 3) to put more weight on track record of investigators who have made previous fundamental contributions to the HSC field.
- Consider allowing Co-Principal Investigators to be named on basic research grants to help overcome obstacles to multi-institutional collaborations.
- Consider organizing disease-themed workshops to enable basic researchers, translational scientists and clinicians to interact and synergize their efforts.
- Seek opportunities to collaborate with foundations or other funding agencies towards overcoming the HSC Bottleneck.

II. Introduction

The clinical practice of bone marrow transplantation is one of the earliest examples of success in the field of regenerative medicine. In this procedure, a population of hematopoietic stem cells (HSCs) is transplanted into a patient with a damaged or diseased blood system, whereby the cells subsequently

engraft and differentiate into the entire complement of blood and immune cells that are necessary for survival. These stem cell transplants are curative for a variety of debilitating diseases, but for many individuals, it can be difficult or impossible to find a healthy source of donor tissue that is immunologically compatible. This significant issue could be overcome if it were possible to provide an unlimited and renewable source of functional hematopoietic stem cells from a variety of genetic backgrounds to be used in lieu of those procured directly from primary tissues such as bone marrow and umbilical cord blood. Such a resource would be profoundly transformative for the field of regenerative medicine by greatly expanding the access to these life-changing treatments. Moreover, this technology could be broadly enabling as a platform for gene modification of HSCs, and could lead to the development of unprecedented cures and treatments for a wide range of disorders such as sickle cell disease, beta thalassemia, and diseases caused by blood borne pathogens such as HIV.

HSCs are typically procured from the bone marrow or peripheral blood of a donor (allogeneic source) or for some indications, from the patient him/herself (autologous source). Due to their rarity, a large amount of marrow must be removed from the donor to ensure that sufficient HSCs are available for transplant. In the case of peripheral blood, a donor must be treated with a drug or other agent in order to mobilize HSCs from the marrow to the blood stream for harvest. Umbilical cord blood represents a third source of HSCs for transplant and is especially attractive as an off-the-shelf resource that can be used under conditions of only partial immune matching. Unfortunately, a single cord is typically not sufficient for transplantation into an adult host, and the supply of donated cords is outpaced by the demand.

Human PSCs, including embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs) represent an alternative source of HSCs with the theoretical potential to overcome all major obstacles faced by existing clinical sources as they 1) represent a renewable resource with unlimited capacity for self-renewal and expansion; 2) have the potential to differentiate into any cell type of an adult, including adult stem cells and progenitors such as HSCs; 3) in the case of iPSCs, can be generated from easily accessible tissues, such as the skin or blood of patients or donors with matched immune haplotypes; 4) are amenable to genome editing and thus provide a platform for genetically correcting disease mutations; and 5) can be banked to serve as an off-the-shelf- resource. While these advantages are considerable, it has not been possible to differentiate PSCs into HSCs that behave and function like their endogenous counterparts, despite numerous incremental advances over the years.

Given the persistence of this challenge, CIRM sought the collective input from a small group of thought leaders with varying perspectives and experience, including several who have received grants from CIRM to explore the biology of PSC-derived HSCs. These investigators were tasked with defining key scientific and technical gaps that comprise the “HSC Bottleneck” and with providing CIRM with fresh insights on how those gaps could be filled.

III. Defining and Addressing Key Challenges

Transplantation studies using mouse embryos clearly indicate that PSCs have the innate ability to differentiate into fully functional, definitive HSCs in the context of normal development [1]. When differentiated in vitro, however, PSCs give rise to HSC-like cells that are deficient in several ways, including a bias towards myeloid lineages at the expense of lymphoid potential, an inability to self-renew in culture, and a failure to engraft and survive long-term upon transplantation. In vitro expansion and

long-term maintenance defects are also observed for authentic HSCs derived from primary tissues, indicating that an appropriate culture system for ensuring functionality has yet to be defined. In addition, PSC-derived HSCs may acquire unique defects related to an absence of necessary cell context for correct fate specification, as well as the potentially detrimental effects of an incorrect cellular milieu on their endogenous biology. Attempts to improve culture systems are hampered by significant gaps in our understanding of the origin, nature and behavior of adult HSCs, as outlined below.

Understanding the Normal Ontogeny of Human HSC Development

Hematopoietic development during embryogenesis occurs in several distinct temporal/spatial waves, each characterized by its own set of hematopoietic progenitors [2]. Only those produced from the latter, or “definitive” wave give rise to the mature, functional stem cells that are desired for regenerative medicine. During in vitro differentiation, it is believed that many of the HSC-like cells produced are those from the more primitive waves, which are transient and lack potency and capacity for long-term engraftment. The field currently suffers from an incomplete knowledge of the molecular characteristics that distinguish the various human HSCs types from one another. While it is possible to analyze definitive HSCs from adult tissues, there is much less known of the distinct cell populations that emerge during ontogeny, or how they progress at the molecular level to become mature, adult HSCs. Knowledge of these trajectories to date comes largely from studies of animal models and while hematopoietic development is highly conserved across vertebrates, a number of differences are found in human HSCs that have important ramifications for their use in regenerative medicine, such as their cell cycle kinetics, repertoire and functionality of cell surface molecules, and the mechanisms by which HSCs respond to DNA damage [3].

Knowledge of Intrinsic HSC Programs

Elucidating the genetic networks that govern the transition of embryonic progenitors to mature HSC fate will be important for pinpointing the origin and nature of defects that emerge during HSC derivation in vitro, and for identifying gene programs that could be manipulated to restore or circumvent defective processes during in vitro differentiation, or enhance the ability of derived cells to either self-renew or differentiate appropriately into the desired lineages. A recent development in this area was presented by Dr. Dong-Er Zhang, who explored the roles of different Runx1 isoforms in the differentiation of human (h) PSCs to blood cells [4]. Runx1 is a major transcriptional regulator of the hematopoietic program during embryogenesis and is essential for establishment of the definitive hematopoietic system. In humans, there are at least 12 different Runx1 mRNA isoforms that can be expressed in a cell-specific manner due to alternative splicing. Dr. Zhang found that forced expression of the Runx1a isoform in hPSCs promotes formation of definitive HSC-like cells that can expand ex vivo, engraft and survive for at least 9 weeks upon transplantation, giving rise to myeloid, lymphoid and erythroid lineages. While notable, these capabilities are not as robust as those observed for cord blood HSCs expressing Runx1a, indicating that expression of this single isoform is not sufficient to reverse all the deficiencies of PSC-derived HSCs. Nonetheless, these findings mirror reports of additional key factors whose manipulation enhances different aspects of HSC biology, such as HOXB4, SCL, and as recently reported, retinoic acid signaling [5-8]. There is growing enthusiasm that continued efforts to define the spectrum of regulatory mechanisms that govern HSC specification and behavior will lead to a “critical mass” of knowledge, enabling rational molecular bioengineering approaches to be developed for overcoming deficiencies inherent to in vitro hematopoiesis.

In addition to understanding the genetic underpinnings of hematopoietic development, it is fundamentally important to understand the epigenetic context in which these intrinsic programs are functional, and to understand how culture conditions and in vitro procedures can perturb genetic programs through alterations of the epigenetic landscape. To illustrate this point, Dr. Hanna Mikkola described her recent efforts to compare gene expression profiles of PSC-derived hematopoietic stem/progenitor cells (HSPCs) with those from their fetal liver-derived, primary counterparts (manuscript in preparation). Dr. Mikkola noted that despite the lack of self-renewal ability in PSC-derived HSPCs, there was very good correlation in terms of overall gene expression, indicating that HSPCs from both sources possess similar cellular identity in terms of the general HSPC transcriptional program. However, PSC-derived HSPCs were unable to induce the expression of the HOXA gene cluster, consistent with progenitors from earlier stage of human development obtained from first trimester placentas, and implying that the developmental maturation of PSC derived HSPC is incomplete. The importance of this difference was confirmed by a demonstration that forced reduction of HOXA gene expression in fetal liver-HSCs disrupts their ability to self-renew. Dr. Mikkola's work further suggests that the presence of an inhospitable chromatin conformation, or epigenetic barrier, prevents the activation of the HOXA cluster genes by their upstream regulators. Dr. Mikkola has also identified additional transcriptional regulators, the expression of which correlates highly with self-renewal capacity, that may be required together with HOXA genes to convey self-renewal capacity to PSC-derived HSCs.

In assessing functionality, it is important to consider that definitive HSCs persist for the lifetime of an adult organism in a largely quiescent state, becoming activated (proliferative) only when necessary to replace blood cells lost to normal wear and tear, or in response to injury or illness. Any disruption in this delicate balance can lead to serious consequences such as leukemia, anemia or autoimmune disorders. To date, studies directed towards diagnosing and overcoming deficiencies in cultured HSCs are largely focused on achieving multipotent, self-renewing cells that can engraft and survive long-term in a living organism. While these properties are fundamental to the utility of HSCs in regenerative medicine, it is not clear that all cells with those desired properties would be free from deficiencies revealed later in life, or revealed only under certain circumstances such as stress or injury. Dr. Ellen Rothenberg offered an example from her own research to highlight this concern. She pointed out that several genes that are normally expressed in the initial stages of T-cell lineage commitment are associated with T-cell lymphomas if reactivated, or not fully repressed in the later stages. She provided examples of hidden defects due to violations of developmental checkpoints, such as those observed in NOD genotype mice prone to autoimmunity and diabetes [9]. These mice can be protected from autoimmunity by mutations that block T-cell receptor gene rearrangement, but they go on to develop T-cell lymphoma with very high penetrance. While their T-cell precursors appear normal at birth, a defect is revealed after 6-8 weeks as the regulation of developmental genes in these cells becomes defective. The cells fail to turn off the immaturity-specific regulatory genes and fail to respect a second developmental checkpoint, which normally controls access to later differentiation programs. The collapse of these two regulatory points causes the T-cells to become pre-leukemic, with broad upregulation of early T-cell commitment genes within the context of a more mature gene program, and these cells then go on to become malignant. This tendency for regulatory breakdown would not be discerned simply from features of the stem cells unless their developmental fates were also tested. As another example of a latent quality control feature, Dr. Rothenberg noted that Bcl11b, a key developmental checkpoint controller with recurrent roles in later immune function, must be activated in the appropriate stages of T-cell development by removal of methylation marks [10]. As PSC-derived HSCs in culture should have a methylated Bcl11b locus, this

property would not reveal whether epigenetic awakening of this gene would occur normally at a later stage of development. While it would be challenging to elucidate stem-cell defects that may not reveal themselves in terms of immediate phenotype and proliferation assays, Dr. Rothenberg's points illustrate the importance of incorporating controlled differentiation assays to look beyond the immediate phenotype of HSCs to identify other prognosticators of HSC quality and function.

Knowledge of Extrinsic HSC Programs

Human HSCs become specified not only through their intrinsic genetic programs, but also via interactions with neighboring cells and tissues, or their niche. Importantly, the niche changes as cells progress from the embryonic stage, where expansion and proliferation are favored, to the more mature stages, where HSCs take up residence in the bone marrow and become quiescent. This natural progression can be at odds with the *in vitro* culture environment, where PSCs are pushed to differentiate into definitive HSCs through inductive signals, and lack the context of dynamic feedback from their microenvironment (timing and dosage of signals), including maintenance cues or "breaks" that may be necessary for them to develop normally. There is a significant gap in our understanding of what niche signals are required for controlling the behavior and potential of human HSCs, whether derived from PSCs or primary sources, and how those could be manipulated in a culture system to favor the desired properties and behaviors.

While the timing and location of HSC emergence during hematopoietic development has provided important clues about the instructive role of the niche, there is much to learn about the actual molecular mechanisms that underpin these interactions. Dr. David Traver has made progress in this area using zebrafish models to dissect the function of various conserved genes that are upregulated during HSC emergence (personal communication). He found that depletion of *jam1a*, a junctional adhesion molecule expressed on the surface of posterior lateral mesoderm (PLM) cells, causes loss of HSCs without affecting more primitive waves of hematopoiesis or vascular development. A subset of migrating PLM cells with depleted *jam1a* appeared to arrest at the physical boundary of the somite, suggesting that the defects caused by *jam1a* loss may be related to an improper interaction with the somite. A similar loss of HSCs and PLM migration defect was observed upon depletion of *jam2a*, which is expressed on the surface of somitic cells. Dr. Traver provided biochemical evidence that *jam1a* and *jam2a* interact with one another, supporting the idea that *in vivo* interactions between migrating PLM and the somite transmit important cues for HSC specification. Further analysis using these approaches is helping to elucidate the temporal and spatial requirements for key signaling inputs that are known to be important for HSC specification including the Notch, Hedgehog and FGF signaling pathways.

While many investigators focus on the embryonic aorta-gonad-mesonephros (AGM) region as the site of origin for definitive hematopoiesis, Dr. Irv Weissman presented historical data supporting a model whereby the yolk sac acts to prime an early set of progenitors, which ultimately give rise to blood forming progenitors in the AGM [11, 12]. Elucidating the various mechanisms by which niche factors affect HSC fate and behavior, including those HSCs and progenitors that emerge from more primitive embryonic waves, should prove invaluable for understanding how to better manipulate the properties of a culture system for expanding and maintaining HSCs *in vitro*. Dr. Juan Carlos Zúñiga-Pflücker provided an important reminder of how useful such knowledge can be when he discussed the use of OP9 cells in co-culture with embryonic stem cells to promote their differentiation to HSCs [13]. OP9 is a stromal cell line generated from bone marrow, which acts as a surrogate niche *in vitro* by providing key growth factors to developing HSCs. ESCs differentiated in co-culture with OP9 produce HSC-like cells with

multilineage potential, but which lack the ability to form T-cells. Dr. Zúñiga-Pflücker reasoned that these stromal lines could be engineered to provide additional niche factors that could influence the lineage potential of PSCs or HSCs. He went on to demonstrate that OP9 cells expressing the Delta-1-like notch ligand promoted formation of functional T-cell progenitors from murine ESCs, which engrafted and mounted an effective immune response upon transplantation and challenge with virus [14]. Assessing competence to generate T-cells is a valuable guide to identify subsets of cells with definitive stem cell function. In collaboration with Dr. Gordon Keller, Dr. Zúñiga-Pflücker has now optimized a protocol for generating stem cells with apparent T-cell generating potential from human PSCs, demonstrating that it is possible to generate useful blood lineage cells in vitro, even if the definitive HSC stage is transient or premature, and providing hope that PSC-derived blood cells could prove therapeutically useful even if the bottlenecks to their derivation are not readily overcome [15]. He is currently collaborating with the Notarangelo group to translate these findings to hiPSCs cells for modeling diseases of T-cell development [16].

Beyond Development: Alternative Contexts for Fate Specification

There is little doubt that an in depth understanding of hematopoietic development will shed much light on the limitations and deficiencies of our current methodologies for deriving and maintaining HSC. However, it is entirely possible that there are alternative mechanisms that can be exploited to produce HSCs with the desired properties, as evidenced by several recent and exciting developments in the field. First, two independent studies demonstrated that when injected into immunocompromised adult mice, human iPSCs could give rise to engraftable, multipotent HSCs within the resulting teratomas, providing the first proof of principle that human HSCs can be appropriately specified from hPSCs [17, 18]. More recently, Pereira et al. identified 4 key transcription factors that when co-expressed, are sufficient to induce an HSC/progenitor fate in mouse fibroblasts [19]. These developments are compelling because they demonstrate that something as biologically disorganized as a teratoma can provide a suitable niche for HSC development, lending hope that a more sophisticated three dimensional/co-culture system could be feasible for reconstructing the niche. Indeed, teratomas themselves could be used as a basic research tool to dissect the molecular requirements of such a system. Moreover, the ability to reprogram fibroblasts to HSC with only 4 factors, in a two dimensional culture system, suggests that it may be possible to circumvent the requirements for niche signaling and epigenetic remodeling in the derivation of functional HSCs, allowing them to become “reprogrammed” to the definitive HSC state rather than converted through a process meant to recreate embryogenesis. Indeed, the use of small molecules, microRNAs and other manipulations to redefine somatic cell fate continues to be an active and productive area of investigation, with growing numbers of reports describing conversion of fibroblasts or other cell types into a variety of specialized lineages and even multipotent progenitor-like cells [20, 21]. In a variation on this theme, Doulatov and Daley recently defined a set of five factors that convert human PSC-derived lineage restricted myeloid precursors to more immature, HSC-like cells with short term engraftment potential and the ability to be expanded in vitro [22]. They suggest that the use of stem cell transcription factors to revert or produce somatic tissue stem cells from more differentiated, but closely related progenitor types may be particularly important for achieving prolonged tissue reconstitution in blood and other short-lived tissues such as skin, mesenchyme and intestinal epithelium [23]. While it remains a challenge to derive cells that exhibit all functional capabilities of definitive HSCs, there is growing optimism that a combination of rational cell engineering techniques, along with fundamental advances in our understanding of HSC biology, will eventually lead to the achievement of this long elusive goal.

IV. Tools and Techniques

Addressing the knowledge gaps required to overcome the HSC bottleneck will necessitate the utilization and development of a wide variety of tools and approaches. As technology for high throughput and single cell profiling become less expensive and more robust, it will be feasible to expand the depth of molecular profiling that be achieved in different populations of HSC and related cells, enabling scrutiny of their intrinsic genetic programs and their epigenetic states. These studies should lead to the identification of new biomarkers that can be used to purify specific populations for further study, or whose expression can be manipulated to control cell fate. To probe the biology of HSC and identify deficiencies in their intrinsic or extrinsic programs, it will be necessary to develop better model systems as readouts that can be used to assess long term potential and viability of PSC-derived HSC. There must be continued and rigorous efforts to understand the biology of human HSC and how it is similar and different to that of other vertebrates and how these cells can be most effectively exploited for practical applications. Continued evolution of new techniques for enabling complex and dynamic culture environments are likely to be very helpful if becomes necessary to recapitulate aspects of forward development in order to feasibly obtain definitive HSCs.

A list of approaches and techniques that workshop participants deemed critical include the following:

- Tools (biomarkers, antibodies, reporter cell lines) that enable purification of cells at various stages of differentiation and development, particularly those for the human system
- Molecular profiling of HSC intrinsic programs (transcriptome, epigenome, proteome, alternative splicing, microRNA, lncRNA, etc.)
- Molecular profiling of extrinsic factors (signals from endogenous niche during development) and signals from artificial or surrogate niches (stromal cells, teratoma)
- Comparative studies between human HSC developmental stages and those of animal models; use the latter to test hypotheses in vivo
- Improved humanized mouse models for studying long term engraftment and integrity of human HSCs (example: knock out innate immunity genes/knock in human cytokine genes)
- Surrogate in vitro assays to distinguish functional HSC from deficient variants
- Tools for expressing and manipulating combinations of factors within differentiating HSCs
- Culture systems that enable dynamic control of niche signaling (timing, dosage), ability to modulate cytokine environment and biophysical properties of growth matrix; and the use of engineered human stromal lines for co-culture
- Immunodeficient large animal models and/or effective immunosuppression regimens for testing HSCs
- Methods for facilitating epigenetic changes and/or stabilizing epigenetic states
- Molecular indicators that predict quality of HSC in terms of developmental checkpoint integrity and epigenetic integrity

V. Opportunities for CIRM

CIRM's Mission is to support and advance stem cell research and regenerative medicine towards development of cures, diagnostics, and research technologies. As of August 2013, approximately 14% of the ~430 project-oriented grants awarded by CIRM have focused specifically on HSC biology, ranging

from basic research studies to early translation and the development of HSC-based therapeutics. ~30 of these, representing a \$52 million dollar investment, address fundamental issues directly relevant to understanding and overcoming the HSC bottleneck, including multiple efforts to derive HSCs from hESCs or hiPSCs using variety of approaches. While primarily focused on the human system, CIRM funds several studies of hematopoietic development using model organisms, including zebrafish and mice. Given this background, workshop participants provided insights as to how CIRM might go above and beyond what has been done previously to advance progress towards overcoming the HSC bottleneck, within the constraints of ongoing initiatives and competing priorities. One of the most relevant suggestions included the designation of a special funding priority for HSC research within ongoing and future initiatives, particularly those studies that seek to understand 1) how to make cells become functional HSCs (starting from PSCs, somatic cells, or lineage intermediates); 2) how to expand HSCs; 3) how to test or predict the quality of HSCs. To help such projects compete more favorably, participants recommended adjustment of review criteria to ascribe merit to high risk, potentially high gain types of approaches and to place added value on achieving intermediate goals as opposed to transformative outcomes. A second major suggestion was to increase the ability of CIRM-funded investigators to work together by 1) removing institutional barriers to collaboration by enabling appointment of co-principal investigators to research grants; and 2) by seeking new opportunities to fund, or leverage funding from collaborators outside of California. Participants felt it would be extremely useful for CIRM to organize disease-themed workshops in which basic and translational scientists as well as clinicians have opportunities interact and learn from one another, and to synergize their efforts towards developing HSCs for regenerative medicine applications. Finally, participants suggested CIRM use its position of influence to work with other funding agencies towards common goals, and to forge alliances, disseminate information, and foster communication between the many scientists and stakeholders whose collective efforts could break the HSC bottleneck.

VI. Conclusion

CIRM has acted on recommendations from this focus session by defining the HSC Bottleneck as an area of priority in the ongoing the Tools and Technologies III RFA, and altering its eligibility requirements so that co-principal investigators could be appointed to this program. CIRM has recently experimented with new funding models in the Basic Biology V initiative by creating a new award track for potentially transformative or high risk, high gain studies, including those using animal models, alongside its ongoing commitment to prioritize investigations of key human stem cell mechanisms. CIRM will continue to refine and refocus future Basic Biology Initiatives to better capture the most timely and relevant research for achieving its mission. Finally, CIRM will strive to create new opportunities for scientific and financial collaboration by organizing workshops, communicating insights, adding to its network of collaborative funding partners, and by judicious use of the External Innovation program.

VI. Acknowledgements

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VII. References

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Breaking the Bottleneck: Deriving Definitive Hematopoietic Stem Cells from Human Pluripotent Stem cells

August 29, 2013	
10:00 am	<p>Welcome and Introduction <i>Sohel Talib, CIRM Scientific Officer</i></p>
10:15 - 11:00 am	<p>Deriving Functional, Definitive HSC from Human Pluripotent stem Cells <i>Chair: Ellen Feigal, CIRM Senior Vice President of Research and Development</i></p> <p>1) What are the bottlenecks? 2) What approaches are needed to address them?</p> <p>Presenters (5-10 minutes each):</p> <ul style="list-style-type: none"> • Juan Carlos Zúñiga-Pflücker , Sunnybrook Research Institute • David Traver, UCSD • Dong-Er Zhang, UCSD • Juan Carlos Izpisua Belmonte, Salk Institute • Hanna Mikkola, UCLA • Irv Weissman, Stanford University • Ellen Rothenberg, California Institute of Technology
11:00 - 12:00 am	<p>Panel Discussion of the Bottleneck</p>
12:00-1:00 pm	<p>Lunch – CIRM Lobby</p>
1:00-3:00 pm	<p>Addressing the Bottleneck- Ongoing Research Contributions <i>Chair: Gil Sambrano, CIRM Associate Director, Review</i></p>
1:00-1:15 pm	<p>Kelly A. Shepard, CIRM Scientific Officer <i>CIRM's Hematopoietic Portfolio</i></p>
1:15-1:30 pm	<p>Ellen Rothenberg, California Institute of Technology <i>Developmental checkpoint regulation and long-term quality control in stem-cell derived lineages</i></p>
1:30-1:45 pm	<p>Hanna Mikkola, UCLA <i>Defining the transcriptional barriers for generating self-renewing HSCs</i></p>

1:45- 2:00 pm	Dong-Er Zhang, UCSD <i>Targeting transcriptional regulation of hematopoiesis in regenerative medicine</i>
2:00-2:15 pm	David Traver, UCSD <i>Environmental instruction of HSC fate by the somite</i>
2:15-2:30 pm	Juan Carlos Zúñiga-Pflücker, U. Toronto/Sunnybrook Research Institute <i>If you go from PSCs to T lymphocytes, do you pass by an HSC?</i>
2:30-2:45 pm	Coffee Break – CIRM Lobby
2:45-3:00 pm	Irv Weissman, Stanford (via Skype) <i>The phenotype of mES-HSC is the same as embryonic yolk sac HSCs</i>
3:00-3:30 pm	How CIRM Could Impact the Bottleneck Chair: <i>Michael Yaffe, CIRM Associate Director of Scientific Activities, Research</i> Group Discussion
3:30-4:00 pm	Closing Remarks and Next Steps <i>Pat Olson, CIRM Executive Director of Scientific Activities</i>
5:00 pm	Dinner