and to which CD8 T cells that can lyse leukemic cells have been generated. Generating in a timely fashion sufficient numbers of specific T cells with high avidity for leukemia in each patient is a substantive problem, and could be overcome by creating a library of TCR genes from leukemia-reactive T cells that can be introduced into large numbers of T cells from patients whose leukemia expresses the antigen and HLA-restricting allele. However, ultimately the avidity of the transduced T cell is limited by the affinity of the introduced TCR, and high affinity TCRs for tumor antigens that are also normal self antigens are difficult to isolate from the repertoire. Our lab has developed, in collaboration with David Kranz’ lab, methods to mutate/alter the isolated antigen-specific TCR chains prior to introduction into recipient T cells to improve the affinity for the target antigen. These strategies and the results of an ongoing clinical trial of adoptive T cell therapy targeting WT1 in patients with leukemia will be described.

Enhancing the IQ of CAR–modified T Cells

Michael Jensen

The potential therapeutic impact of CAR redirected T cells for cancer immunotherapy is beginning to be realized. However, as the diversity of antigen targeting increases and therapeutic potency is enhanced off target side effects will need to be effectively mitigated. Our program is interested in exploiting the advances in synthetic biology technologies to create new classes of engineered T cells that are programmed to delivery functional outputs based on discreet molecular inputs in near real time. Our progress in this area of research will be presented.

Chimeric Antigen Receptor (CAR) Modified T Cells as a Novel Approach to Immuno–Therapy of B Cell Malignancies

Renier Brentjens

Recently published reports support the novel approach of treating cancer with patient derived T cells genetically modified to express artificial T cell receptors, termed chimeric antigen receptors (CARs), targeted to tumor associated antigens. To this end, initial clinical trial outcomes of patients with B cell malignancies treated with autologous T cells genetically modified to express a CAR specific to the CD19 antigen, expressed on most B cell malignancies, demonstrate that this approach may ultimately prove to be a promising therapeutic intervention which will potentially dramatically alter the standard of care in these malignancies. However, collectively, the currently published clinical trial data regarding CD19 targeted T cells present a multitude of variables which may markedly influence the clinical trial outcomes and influence the optimal design of future clinical trials. Furthermore, promising additional T cell genetic modifications based on pre–clinical studies to enhance the efficacy of this CAR modified T cell therapy in second generation clinical trials will be discussed.

Technical Session Abstracts

Technical Session 1: Imaging Cell Therapy

Chair: Rao Papineni

Molecular Imaging is making rapid strides as a tool in understanding and the cure for several pathologies. Notable are the strategies being developed in the field of stem cell therapy, particularly the ones utilizing multi-modal imaging modules. This session will focus on the recent advances in multimodal imaging modalities and the molecular agents for cell tracking, homing and its function.

Technical Session 2: NK Expansion for Clinical Trials

Chair: Didier Blaise

NK cells are unique lymphocytes that make cytokines and exhibit cytotoxicity without prior sensitization.

1. Allogeneic SCT represent the most reliable form of immunotherapy. Allogeneic immunocompetent cells allows for engraftment and disease control but also for GVH reaction. Among them NK cells represent a peculiar entity: their antitumoral activity as well as the absence of their implication in GVHD reaction is well established. A better understanding in
their function, the supply of efficient and GMP-grade selection tools and new drugs targeting these cells pave the way to new development of NK based immunotherapy in the context of allo-SCT with the aim to provide better antitumor treatment with limited toxicity.

2. Function of NK cells are defined by a cadre of inhibitory receptors capable of recognizing MHC classical and non-classical class I molecules. Engagement of these “self” molecules is capable of protecting a target from lysis as well as a gain in functional capacity by an unknown mechanism. The acquisition of function is referred to as NK cell education or licensing. There is more than one mechanism to activate NK cells. In addition to ligation of inhibitory receptors, NK cells can be activated by cytokines that are present during homeostatic expansion that occurs after lymphodepleting chemotherapy or induced by viral infection such as human CMV. The purpose of this lecture is to evaluate the function of NK cells that circulate in vivo after bone marrow transplant and adoptive transfer. As we gain a better understanding of the NK cell response, newer methods such as Interleukin–15 and choosing donors based on their NK cell receptor genotype hold promise to improve cancer therapy.

Technical Session 3: Cell Engineering

THURSDAY JUNE 7TH | TIME: 7:30AM– 8:30AM

Chair: David DiGiusto

The development of cells as therapeutic agents often requires cell selection, genetic modification, expansion and characterization. This Technical Session on cell engineering will include examples of pre-clinical development and clinical implementation of gene modified central memory T-cells and hematopoietic stem and progenitor cells. The goal of the session is to provide the attendees with examples of how candidate therapeutic cells are isolated, genetically modified and otherwise manipulated in “IND-enabling” studies. Additionally, the speakers will discuss the approach taken to move these cells into Phase I clinical trials.

Zinc Finger Nuclease mediated genomic editing of CCR5 in human hematopoietic stem and progenitor cells for clinical application in HIV gene therapy

David DiGiusto

We are currently developing methods to create genetically modified, autologous hematopoietic stem and progenitor cells (HSPC) that will generate HIV-resistant progeny following transplantation in HIV+ individuals. CD34+ HSPC isolated from healthy donors were transduced with an A5/F35 pseudotyped adenoviral vector encoding a pair of CCR5-specific Zinc Finger Nuclease proteins (ZFN). In vitro analysis of hematopoietic potential was used to optimize the genetic modification while ensuring the biological potency of the cells. HSPC were generated with up to 49% of CCR5 alleles disrupted and showed only modest increases in the percentage of monocytes produced during in vitro culture. In vivo analysis was used to confirm the effect of treatment on engraftment of HSPC in immunodeficient mice. The methods used to get high level ZFN activity and results from in vitro and in vivo analysis of hematopoietic potential will be discussed.

Phenotypic and Functional Attributes of Lentivirus Modified CD19-specific Human CD8+ Central Memory T Cells Manufactured at Clinical Scale

Christine Brown

Our group at City of Hope has initiated a first-in-human clinical trial designed to evaluate the safety and immunologic endpoints of CD19-specific chimeric antigen receptor (CAR) engineered central memory T cells (Tcm) for treatment of CD19+ B cell lymphoma. We have developed for clinical application a semi-closed manufacturing process to reproducibly generate genetically modified CD19-specific CAR+ T cells from a defined population of CD8+ Tcm. Clinical scale qualification runs demonstrate that CD8+ Tcm are responsive to anti-CD3/CD28 bead stimulation, can be efficiently transduced with CAR-encoding lentiviral vectors, and undergo sustained expansion in IL-2/IL-15 over 3-6 weeks. Additionally, these cells retain expression of central memory markers such as CD62L/CD28, display in vitro CD19-mediated cytolytic function, and, upon adoptive transfer into immunodeficient NSG mice, exhibit in vivo huIL-15 dependent engraftment fitness and CD19-specific antitumor efficacy. We will discuss progress in our on-going phase I clinical trial designed to evaluate engraftment and persistence of CD19-specific CAR+ Tcm-derived autologous cell products, as well as lymphoma-free survival following high-dose chemotherapy and autologous stem cell transplant.
Technical Session 4: Funding for Early Stage Cell Based Therapies

Chair: Ellen Feigal

Advancing science into therapies for patients is a complex and expensive path to travel, and the uncertainty of success, particularly for innovative technologies, makes the funding of the translational steps towards and into the clinic, particularly challenging. This session, with a focus on NIH and the California Institute for Regenerative Medicine (CIRM), will provide information on the scope of programs, initiatives, funding opportunities and useful contact information, to help investigators make progress in their research.

Technical Session 5: Ex–Vivo and In–Vivo Graft Engineering

Chair: Stephan Mielke

For a variety of patients with lymphomas and leukemias allogeneic blood stem cell transplantation represents the only curative treatment option. The unavailability of an optimal donor requires the use of alternative stem cell sources such as haploidentical family donors or mismatched cord blood units. The introduction of double instead of single cord blood unit transplantation has helped to broaden the applicability of cord blood transplantation by improving engraftment and thereby reducing the rates of transplantation–related mortality (TRM). Nevertheless, the transplantation of a primarily naïve T lymphocyte repertoire with the cord blood is associated with increased rates of infectious complications representing a drawback to its optimal use. Novel ex vivo strategies allow growing memory-type T cells against multiple viruses from the original cord blood source and thereby overcoming viral complications post allografting by adoptive T cell transfer. The feasibility of haploidentical allogeneic stem cell transplantation largely depends on sufficient in vivo and ex vivo T cell depletion strategies to allow engraftment and avoid severe GvHD. As a consequence infectious complications and TRM are increased and limit the overall success of this approach. Therefore novel strategies aim to improve post–transplant immune reconstitution after haploidentical stem cell transplantation to reduce infectious complications. In this context ex vivo and in vivo selective depletion of alloreactive T cells have become the focus of today's translational transplant approaches.

Technical Session 6: TPP Workshop

Chair: Ellen Feigal

This session will provide participants with an understanding of the components of the target product profile (TPP), and the different usages of the TPP in the product discovery effort and development plan. The TPP is a useful planning tool for preclinical, IND-enabling studies and clinical trials, and for effective communications with the FDA, and is a component of the California Institute for Regenerative Medicine's (CIRM’s) translational and clinical grant applications. This session should enable interested participants to more fully employ this tool and more effectively prepare a TPP.

Technical Session 7: Freezing of Living Cells for Cellular Therapy: A Challenge for Biomedical Scientists

Chair: Dayong Gao

Life is a biochemical process that has evolved in relation to the thermal conditions on our planet. Rate of the biochemical process is temperature-dependent. Low temperature can be used to slow down the life process, cryopreserving living cells, tissues and organs for transplantation, gene/cellular therapy, bio-banking, and conservation of endangered or transgenic animal species (by gamete preservation). However, there is an apparent contradiction between the concept of the cryopreservation and experimental findings that the living cells can also be damaged by the cryopreservation process itself. To optimally employ low temperatures in these biomedical applications, one must develop a fundamental understanding of the freezing process and its effects on the biological systems at low temperatures.

Contrary to popular belief, the challenge to cells during freezing is not the cells' ability to endure storage at very low temperatures (below -190°C); rather it is the lethality of an intermediate zone of temperature (-15 to -60°C) that the cells must traverse twice ¾ once during cooling and once during warming. The central theme of this presentation is to report our research work on: (1)
the mechanism of the cell-tissue cryoinjury and cryopreservation; (2) optimization of cryopreservation conditions and methods to prevent the cryoinjury; and (3) development of novel technology to (A) achieve the optimal cryopreservation conditions to ensure the survival of living cells/tissues during cryopreservation, (B) to remove potentially-toxic cryoprotective agents from the cells after cryopreservation, and (C) to precisely control the cell concentration as well as the cell-suspension volume of either cryopreserved cells or ex vivo expanded stem cells/progenitor cells before various applications.

Workshop Abstracts

Workshop 1: ISCT/USP Joint Workshop on Ancillary Materials

Co-Chairs: Elizabeth J. Read and Fouad Atouf

This workshop will focus on the importance of ancillary materials, and the practical challenges for their selection, qualification, and use in cell therapy development and manufacturing in academic and industry settings.

Ancillary Materials: Definitions, Regulatory Framework, and USP’s Risk-Tiered Approach

Elizabeth Read

Ancillary materials are those materials that come into contact with a cell or tissue therapy product during manufacturing, but are not intended to be part of the final product formulation. Although not regulated as medical products, ancillary materials are subject to regulatory oversight in the context of CMC review because of their impact on product quality, safety, and efficacy. USP chapter <1043> presents a risk-tiered approach to qualification of ancillary materials for cell, tissue, and gene therapies. Additional chapters defining standards for materials such as cytokines and fetal bovine serum have been published, and physical reference standards are in development. Examples of ancillary materials and qualification activities will be discussed.

Ancillary Materials in Cell Therapy Product Development

Nicole Provost

Ancillary materials are commonly used in the development and production of biotherapeutic products. This talk will briefly summarize the unique potential advantages and disadvantages presented by ancillary materials in the development of cell and tissue therapy products. Risk-based approaches will be presented, so that potential hazards can be predicted, assessed, mitigated, and eliminated. Ancillary material issues of special concern to cell product developers will also be discussed, along with relevant real-world examples.

Cytokines and Growth Factors for Ex Vivo Cell Culture

Felicia M. Rosenthal

Cytokines, growth factors, and media are commonly used in the processing of cells for therapeutic applications. The quality of these ancillary materials is crucial for the quality of the finished therapeutic product. In parallel with the evolving clinical field of cell therapy and regenerative medicine, regulatory and standard-setting agencies are developing guidelines that outline general risk-mitigation strategies and qualification programs which can be used to select appropriate reagents or which outline specific quality attributes for cytokines and growth factors (like USP Chapter <92> “Growth Factors and Cytokines Used in Cell Therapy Manufacturing”). Risk classification, qualification, and performance testing are of major importance. To minimize concerns over safety, BSE and other contaminations, animal-derived component-free (ADCF) raw materials are increasingly employed also in the production of ancillary reagents. The use of GMP grade and ADCF ancillary materials will significantly reduce qualification and validation efforts of cell therapy manufacturers and help to ensure consistency, safety, and purity of the final cell therapy products.

Workshop 2: Induced Cells for Neurological Disorders

Chair: Josef Priller

Induced pluripotent stem (iPS) cells provide an unlimited source of patient-specific cells to study and treat diseases of the central nervous system. Improved methods for the differentiation of iPS cells into neuronal and glial cell types have allowed to generate stem cell models of neurological diseases. These