Two is Better Than One: Arming the T-Cells with Dual Cancer-Targeting Antibodies

One way to bring the powerful cellular immunity and antibody-directed targeting together is to construct the chimeric antigen receptor (CAR) T-cells. In this strategy, the patient’s own T-cells are genetically engineered to produce a hybrid molecule (the CAR) on these killing cells. The extracellular portion of the CAR molecule is composed of the antigen-recognizing part of a monoclonal antibody, while the intracellular portion contains the activating motifs of the T-cell receptor. When CAR T-cells are infused into the body, the antigen-recognizing part brings these cells to the targeted cancer cells and drives CAR-T cell proliferation. Upon contact with cancer cells, CAR T-cells will be activated to kill the cancer cells.

Thus far, the most experience was with CAR T-cells targeting CD19 in patients with either acute lymphoblastic leukemia (ALL) or B-cell lymphoma. In fact, the first-in-class CAR T-cells targeting CD19 was approved by FDA to treat patients with B-cell ALL. While the CD19-targeting CAR T-cells can be very effective, not all patients responded to this strategy and the ability of maintaining long term disease control is still to be determined due to concern of emerging resistant cancer cells after CAR T-cell therapy. Recent study has shown that more than half of the disease relapse after CD19-targeting CAR T-cells involved cancer cells lacking the target, CD19 (i.e., the CD19 immune escape). One strategy to overcome this immune escape is to direct the CAR T-cells to additional target(s) on the cancer cells besides

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On November 2, 1987 the first adult patient underwent transplantation at Stanford University. This patient is about to celebrate their 30th anniversary and continues to do well. We are also celebrating this landmark achievement in our Program’s history. Since that day we have treated over 7,000 other patients with life saving autologous and allogeneic transplants to treat a variety of hematological disorders, bone marrow failure states and genetic disorders. Our commitment to you, our colleagues, patients and their families is to provide the most scientifically rigorous, up to date and compassionate care possible to treat our patients during their time of greatest need. Our staff is committed to that goal and we so appreciate the trust you have afforded us.

Hematopoietic cell transplantation was the first place where the concept of using cells for therapeutic purposes was introduced and widely practiced in clinical medicine. In this issue we highlight a new chapter in the field of cell-based therapeutics. With the FDA approval of chimeric antibody receptor (CAR) T cell therapies launches an exciting new opportunity for patients with B cell malignancies such as leukemia and lymphoma that is certain to have a major impact. We are committed to providing these novel therapies to your patients as one of the only sites in Northern California approved for administering these therapies and also through the development of novel approaches to improve outcomes, reduce toxicities and treat other cancers.

As always, we welcome your feedback and referrals. We thank you for your trust and collaboration.

Robert S. Negrin MD
Professor of Medicine and Division Chief
In order to monitor disease after a curative treatment such as allogeneic transplant, pioneer works from Drs. David Miklos and Wen-Kai Weng have established the utility of high-throughput sequencing (HTS) of either B-cell receptor (BCR) or T-cell receptor (TCR) in monitor minimal residual disease (MRD). Once the unique rearranged CDR3 sequences of these receptor (the cancer clonotype) were identified, these patient-specific cancer clonotype(s) is used to measure patient’s disease in a highly-specific, sensitive and quantitative manner using HTS. This new way to monitor MRD clearly outperforms the routine polymerase chain reaction (PCR) or mutliparameter flow cytometry, and has replaced them in daily clinical practice at Stanford Hospital and Clinics.

This new disease monitoring tool using HTS has provided two ways to affect the clinical practice. First, given the utmost patient-specificity and sensitivity, it can be used to predict clinical outcome after allogeneic transplantation. In one such example, HTS was used to measure the patient-specific TCR sequences in patients with cutaneous T-cell lymphoma (mycosis fungoides and Sezary syndrome) after a non-myeloablative allogeneic transplant. Approximately 50% of the patients achieved molecular remission in both skin and blood compartments after allogeneic transplant while the other 50% had persistent MRD detected by HTS. Patients who achieved molecular remission had less chance of disease relapse than those who had detectable MRD (13% chance versus 92% chance of relapse). Even in patients who was in complete clinical remission (no detectable disease by physical examination, flow cytometry, imaging study), persistent MRD detected by HTS translated to a much higher chance of eventual disease relapse, compared to achieving molecular remission (84% chance versus 17% chance of relapse).

Second, HTS can be used to determine the degree of disease progression at the molecular level that requires intervention to prevent the eventual clinical relapse. To realize this aspect of the clinical utility of HTS, Dr. Lori Muffy has started a longitudinal study to monitor MRD status with HTS in patients with acute lymphoblastic leukemia (ALL) who receive either standard chemotherapy or transplant. In this prospective study, blood and bone marrow samples will be collected routinely for multiparameter flow cytometry and MRD determination after therapies. This large scale study will provide a definite data on the advantage of HTS compared to the flow cytometry, and on the clinical significance of MRD determined by HTS after therapies. Most importantly, it will help to shed light on whether intervention(s) based on MRD status will have a positive clinical impact on this patient population.

Bringing cutting-edge technology to daily clinical patient care has been one of the research hallmarks of the Stanford BMT program. In this example, HTS provides an opportunity to care for our patients in a patient-specific manner, that we call Precision Medicine.

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Two is Better Than One

CD19.

The Stanford team lead by Dr. Crystal Mackall, Director of the Stanford Cancer Immunotherapy Program has designed a novel loop CAR molecule that contains two targeting motifs against both CD19 and CD22 (another highly expressed protein on cancer B-cells). The idea is that the additional targeting to CD22 can significantly decrease the chance for immune escape. This novel Stanford-manufactured dual CD19/CD22 CAR T-cells are being tested in an ongoing clinical trial. Another strategy is to apply other immunomodulating agent(s) such as checkpoint blockade along with CAR T-cells. In this case, the addition of immunomodulating agent can enhance the cytotoxic effect of the CAR T-cells.

It is the vision of Stanford team that these novel cellular immunotherapies can be applied to a variety of cancer type, and become standard of care.

The design of the novel dual targeting CD19/CD22 loop CAR molecule

Dr. David Miklos leads the way introducing the clinical use of CAR T-cell therapeutics at Stanford. While the most of initial study was for patients with hematologic malignancies, this new technology can potentially benefit patients with solid tumor. Dr. Miklos will continue to work with Dr. Mackall to develop a robust clinical program, including Stanford-manufactured novel agents.

Blood stem cell activity is arrested by Th1-mediated injury preventing engraftment following nonmyeloablative conditioning.

**JOURNAL OF IMMUNOLOGY**
AM Muller, M Florek, HE Kohrt, NJ Jupper, A Filatenkow, JA Linderman, H Hadeiba, RS Negrin, **JA Shizuru**
2016; 197 (10): 4151-4162

Ibrutinib efficacy and tolerability in patients with relapsed chronic lymphocytic leukemia following allogeneic HCT.

**BLOOD**
2016; 128 (25): 2899-2908

Adoption of pediatric-inspired acute lymphoblastic leukemia regimens by adult oncologists treating adolescents and young adults: A population-based study.

**CANCER**

Coordination of care in survivorship after treatment of hematological malignancies-The journey is not over yet.

**CURRENT HEMATOLOGIC MALIGNANCY REPORT**

Validation of the hematopoietic cell transplantation-specific comorbidity index in nonmyeloablative allogeneic stem cell transplantation.

**BIOLOGY OF BLOOD AND MARROW TRANSPLANTATION**

Increasing use of allogeneic hematopoietic cell transplantation in patients aged 70 years and older in the United States.

**BLOOD**

Foxp3+ regulatory T cells maintain the bone marrow microenvironment for B cell lymphopoiesis.

**NATURE COMMUNICATIONS**
APierini, HNishikii, JBaker, TKimura, HS-KWyon, YPan, YChen, MAlvarez, WStorber, AVelardi, JA Shizuru, JYWu, SChiba, **RS Negrin** 2017; epub

Effect of voriconazole on risk of nonmelanoma skin cancer after hematopoietic cell transplantation.

**JOURNAL OF THE AMERICAN ACADEMY OF DERMATOLOGY**
LFKuklinski, SLi, MR Karagas, **W-K Weng, BY Kwong** 2017; 77 (4): 706-712
Teaching a young dog new tricks: Story of a kinase and its inhibitor

The development of normal lymphocytes is a well-orchestrated process, that begins in the bone marrow. This process involves a functional antigen receptor (B-cell receptor or T-cell receptor) and a dozen of intermediators, including adaptor proteins and kinases that form a network of signaling pathway inside the cells. One such intermediary in the B-cells is Burton’s tyrosine kinase (BTK). While BTK was identified decades earlier, its role in the development of B-cells was first elucidated by the association of its mutation with a severe immunodeficiency, called X-linked agammaglobulemia (XLA) by two back-to-back landmark papers in 1993. It turns out that BTK is not only critical for the development of normal B-cells, it also plays an important role in maintaining the survival of malignant B-cells. Therefore, blocking the activity of BTK can potentially be a way to treat B-cell malignancies. Indeed, ibrutinib, a small molecule that binds permanently to BTK, inhibits the function of BTK and drives the chronic lymphocytic leukemia (CLL, a B-cell leukemia) cells into self destruction. In a pivotal clinical trial, ibrutinib alone resulted in clinical response in ~70% of the patients with mantle cell lymphoma. This result led to the FDA approval of ibrutinib to treat patients with relapsed/refractory mantle cell lymphoma in 2013. This is quite remarkable on several points. First, ibrutinib is another proof that targeted therapy can be very effective in treating cancer. In addition, this successful story occurred because of the tremendous basic research works on the biology and pathobiology of diseases. Second, ibrutinib is a oral drug, which makes it very convenient for the patients. Its low toxicities also make it possible for long term use. Subsequent clinical trials further demonstrated the efficacy of ibrutinib in treating CLL and Waldenstrom’s macroglobulinemia, both now are FDA approved indication for ibrutinib treatment.

After allogeneic transplant, both donor B-cells and T-cells can play critical roles in the process of chronic graft-versus-host disease (cGVHD) by generating immunity against the recipient. While the standard immuno-suppressive agents such as steroid, calcineurin inhibitor, mTOR inhibitor can help to manage cGVHD, prolonged use of these agents carry significant toxicities and they do not work all the time. Therefore, other less toxic way to treat cGVHD is needed. One candidate is ibrutinib. In the pre-clinical study, ibrutinib inhibits both the BTK in B-cells, and the Interleukin-2-inducible T-cell kinase (ITK) in the T-cells. Therefore, ibrutinib can potentially block the deteriorating effects of GVHD-triggering donor B-cells and T-cells at the same time. In a clinical trial lead by Dr. David Miklos, 42 patients with steroid-dependent or -refractory cGVHD were treated with ibrutinib. Nearly 70% of the patients had clinical benefit from the ibrutinib, by either improvement of the GVHD symptoms or ability to taper the dose of systemic steroid. The study also showed that patients tolerated ibrutinib quite well. Supported by this study, the FDA has approved the use of ibrutinib for the additional indication of chronic GVHD in August 2017. Additional trials are being conducted to test whether ibrutinib can be used in the GVHD prophylactic setting. With our ongoing work, we anticipate this young dog will learn more new tricks that can help our patients.

Ongoing Trials on Ibrutinib’s Effect on GVHD

BMT 302: A Phase 2 Study of Ibrutinib Maintenance After Reduced-Intensity Conditioning and Allogeneic Hematopoietic Cell Transplantation for Acute Leukemia (Andrew Rezvani, MD)

BMT 316: Optimizing Post-Allogeneic Hematopoietic Cell Transplant Outcomes for Lymphoma Using Ibrutinib (David Miklos, MD, PhD)
The many challenges to success

Since the establishment of Stanford BMT program in 1987, many aspects of the clinical practice have changed. First, the number of transplant has increased dramatically in recent years. For the 2016-2017 academic year, 446 patients have received transplantation at Stanford with autologous and allogeneic about equally. Second, the upper age limit for transplant has also increased significantly. While most myeloablative regimen is limited to patients younger than 60 years, the reduced intensity regimens have been offered to patients up to 75 years old. In addition, our unique non-myeloablative regimen using total lymphoid irradiation (TLI)/antithymocyte globulin (ATG) has no upper age limit due to its low toxicity and excellent safety record. Third, due to the smaller family size, it becomes harder to find HLA-matched sibling donor. Therefore, the number of transplant with unrelated donor or alternative graft source including haploidentical graft and double cord blood stem cells have increased.

While modern-day supportive measures have provided better ways to manage side effects/complications associated with transplant, there are still numerous challenges. One challenge is to assess the risk of a patient regarding potential toxicity/mortality prior to transplant. With the complexity of older patient population and different transplant regimens, it becomes a difficult task. To this end, Dr. Laura Johnston has analyzed the value of Hematopoietic Cell Transplantation-Specific Comorbidity Index (HCT-CI) in predicting the outcome of patients who underwent TLI/ATG non-myeloablative regimen at Stanford. In this cohort, about 1/3 of the patients each fell into the HCT-CI low, intermediate and high categories. While the higher HCT-CI correlated with lower overall survival, contrast to previous studies, higher HCT-CI did not predict higher transplant-related Mortality (TRM) or risk for graft-versus-host disease (GVHD) in this specific cohort. This can be due to the overall low TRM and low incidence of GVHD using TLI/ATG regimen. This result suggests that the adverse effect of pre-transplant comorbidities can be alleviated with safer or less toxic regimen. However, it also showed that we need more sophisticated tool in assess patient’s risk especially for the elderly population.

With safer regimen and better supportive measures, more patients achieve long term survival after transplant. Different challenges arise in these patients, mostly related to late complications that might be associated with transplantation procedure. These include cardio-pulmonary compromise, renal insufficiency, hormonal disturbance such as hypothyroidism and early menopause, and secondary malignancy. One of the most common secondary malignancy is skin cancer especially the squamous cell carcinoma. To understand the incidence and risk for skin cancer, Drs. Wen-Kai Weng and Bernice Kwong (Dermatology) have conducted a retrospective cohort study with the entire Stanford BMT clinical database. With 2600+ patients included in this analysis, they found an increased risk for developing skin squamous cell carcinoma (6 times risk) and basal cell carcinoma (3 times risk) in patients with allogeneic transplant compared to the ones with autologous transplant. The median time to diagnosis ranged from 1.6 to 2.9 years after transplant. In addition, they found an 80% increase in risk for squamous cell carcinoma for allogeneic transplant patients who received voriconazole.

These are the two examples of different challenges to success, which will require team work and the dedication of the BMT team to overcome.
Ridhi Gupta, MD

 grew up in India where she completed her medical degree at Maulana Azad Medical College at New Delhi. She then came to the United States and did a surgical oncology research fellowship in the Melanoma division at Harvard Medical School. After that, she found her passion in medicine and finished her Medicine residency at New York Medical College, followed by a Hematology/Oncology fellowship at University of South Carolina. She has been very active academically and has presented her works in several local and national meeting including Annual BMT Tandem Meeting by American Society of Blood and Marrow Transplantation (ASBMT). She aspired to be a clinical investigator focusing on hematological malignancy in an academic medical center.

Francisco Socola, MD

 received his medical degree from Cayetano Heredia University at Peru. He finished his Medical residency at University of Miami and a Hematology/Oncology fellowship at University of Arkansas. He has been very active in clinical research and involved in many projects related to different solid organ malignancies and leukemia. He has more than half a dozen publications on a variety of different topics. He has special interest in the role of natural killer (NK) cells in allogeneic transplant.

Tamna Wangjam, MD

 was born and raised in Northeast India. She received her medical degree from University of Delhi/Lady Hardinge Medical College. While she was interested in obstetric during medical school, she decided to pursue a career in medicine after she came to the United States. She did her Medicine residency at Johns Hopkins University Program at Sinai Hospital, and a Hematology/Oncology fellowship at University of Texas at San Antonio. Her clinical interest is in immunotherapy and novel targeted therapy of lymphoma and myeloma. She is interested in becoming a practicing hematologist focusing on transplantation.

Alejandro Sica, MD

 received his medical degree from University of Buenos Aires, Argentina before he came to the United States. He then spent several years at University of Pittsburgh and University of California-Davis for basic research focusing on DNA repair mechanism. He subsequently finished Medicine residency at Tufts University, and a Hematology/Oncology fellowship at University of Illinois at Chicago. His research works have been presented in a variety of national meetings. He want to pursue a career in cellular therapeutics including CAR T-cells.

Abdulwahab Albabtain, MD

 is a native of Saudi Arabia. He received his medical degree from King Saud University, Saudi Arabia prior to coming to the United States. He started his clinical training at St John Hospital in Detroit for Medicine residency, followed by a Hematology/Oncology fellowship at Wayne State University Karmanos Cancer Institute. Dr. Albabtain’s goal is to become a transplant specialist at a major medical center in his home country after this one year of dedicated fellowship.
OUR VISION

With the newly expanded Cellular Therapeutics Facility (CTF) and Laboratory for Cell and Gene Medicine (LCGM), we have started a new chapter of cellular therapeutics. The Stanford BMT program is now in the prime position to develop novel life-saving treatments and implement state-of-art cellular therapeutics such as chimeric antigen receptor (CAR) T-cells to daily clinical practice. To this end, the first Stanford-made CAR T-cells were successfully manufactured at the LCGM, and have been infused back to the patient under a clinical trial on September 22, 2017. With the leadership of Drs. Crystal Mackall and David Miklos, we plan to build a robust, world-class cellular therapeutics program that focus on different hematological and solid organ malignancies.

The Year of Celebration
This is the 30th anniversary of the BMT program with more than 7,000 patients received transplantation at Stanford. The program is supported by grants from the National Institutes of Health including a Program Project Grant now in the 26th year of funding and a T32 Training Grant for next generation of transplantors. As a part of celebration, a Stanford Cancer Immunotherapy and Blood and Marrow Transplantation Symposium will be held at Arrillaga Alumni Center on April 20-21, 2018. This symposium will focus on the specific topics related to transplant on the first day and cellular therapeutics on the second day. Many experts in the field from all over the country will speak at this symposium. For additional information, please visit https://med.stanford.edu/cme/courses/2018/bmt.html or contact program director, Drs. Laura Johnston and Wen-Kai Weng.

How You Can Help
As a referring physician, encouraging your patients to work with our team will be a tremendous benefit. In addition, if you or someone you know would like to make a philanthropic donation to the BMT program, please contact Michele Thompson at 650-725-1109 or visit our website: http://bmt.stanford.edu

CLINICAL TRIAL HIGHLIGHTS

Phase I dose escalation study of CD19/CD22 chimeric antigen receptor (CAR) T cells in adults with recurrent or refractory B cell malignancies (David Miklos, MD, PhD)

A phase I study to evaluate the safety and tolerability of tandemly-purified allogeneic CD34+ CD90+ stem cells administrated following conditioning with AMG 191 to achieve engraftment and immune reconstitution in patients with severe combined immunodeficiency (SCID) (Judith Shizuru, PhD, MD)

Phase I/II trial for patients undergoing myeloablative allogeneic HCT with a T cell depleted graft with simultaneous infusion of conventional T cells and regulatory T cells (Everett Meyer, MD, PhD)

A phase I/II multi-center study evaluating the safety and efficacy of KTE-C19 in combination with Atezolizumab in subjects with refractory diffuse large B-cell lymphoma (DLBCL) (David Miklos, MD, PhD)