SCOR Scientific Statement

Diffuse large B cell lymphoma (DLBCL) is the most common subtype of adult lymphomas, accounting for approximately 25,000 new cases/year in the U.S. Today, the most widely used regimen for the treatment of DLBCL is RCHOP (rituximab, cyclophosphamide, doxorubicin, vincristine, and prednisone). Historically, the CHOP regimen was introduced in the early 1970s. Despite this progress, approximately 50% of patients will have disease progression/relapse after RCHOP, and the majority will die of their disease. Thus, it is clear that chemotherapy-based treatment strategies have reached a plateau, and therefore, new treatment modalities are necessary to improve the cure rate of patients with DLBCL.

Immune based therapies of cancer, including treatment with patient T cells genetically modified to express tumor antigen targeted chimeric antigen receptors (CARs) as well as immune checkpoint (ICP) blockade of T cell inhibitory receptors such as PD-1 and CTLA-4 with monoclonal antibodies, recently demonstrated a promising clinical activity in patients with relapsed lymphoma. Specifically, our group and others have demonstrated that CARs or ICP inhibitors treatment results in approximately 40% response rates in patients with chemotherapy refractory or relapsed DLBCL. The overall goal for this SCOR application is to shift current treatment paradigms by further exploiting T-cell based immune therapy by introducing, developing, and applying novel therapeutic concepts and technologies to improve the cure rate of patients with chemoresistant DLBCL. To ensure success, we have assembled a world-class team-science of immunologists, biologists, oncologists, and pathologists to propose a highly integrated and synergistic projects and supportive core services, each lead by experienced investigator with a track record of scientific excellence and productive collaborations.

In Projects 1 and 2 we will build on our initial experience of developing second generation CARs targeting CD19 expressing DLBCL by addressing 2 complimentary approaches to further enhance the efficacy of our current 19-28z platform. In Project 1 (Brentjens, and Younes, Co-PIs), we will explore the hypothesis that inhibitory tumor microenvironment may include expression of ligands for inhibitory T cell receptors, e.g. Programmed death (PD) -1 ligands, resulting in abrogation of cellular anti-tumor responses. Accordingly, we will explore the potential of armored CAR T cells designed to not only express tumor specific CARs but to secrete biologically active single fragment length antibodies (scFv) targeted to PD-1, designed to modulate the tumor microenvironment and reactivate an endogenous anti-tumor immune response. In Project 2 (Sadelain, PI), we will develop a more potent CD19 CAR therapy for the treatment of DLBCL, by building on our expertise with the 19-28z platform. We
have recently discovered that co-expression of the ligand for 4-1BB (4-1BBL) and 19-28z acquires greater T cell effector functions, proliferative potential and persistence, with diminished acquisition of T cell exhaustion markers. Accordingly, we will establish an all-murine model of DLBCL and evaluate therein the potency and mechanism of action or m1928z+4-1BBL CAR T cells, compared to the currently used 19-28z and 19BBz CARs. Our ultimate goal is to translate this novel approach into a clinical trial in patients with relapsed DLBCL. In Project 3 (Huse, PI), we will leverage the expertise in imaging and cell biological analysis present in the Huse lab and the genetic screening expertise in the Wendel lab to explore determinants of immunotherapy. We will perform systemic and unbiased analyses to define genetic determinants of response to immune therapies including checkpoint blockade or CAR-T cell application. We will explore tumor cell determinants of response, and also investigate the role of TIM3 as a new determinant of T cell action. Finally, in Project 4 (Wendel, PI), we will explore how HVEM mutations contribute to lymphoma biology and to develop tools to restore the HVEM receptor interactions for lymphoma therapy. Furthermore, we will explore the use of HVEM producing 19-28z CAR-T cells that produce and secrete the solHVEM protein to restore its inhibitory interaction with BTLA receptor. Our collaborative SCOR program is expected to improve our strategies to enhance T cell-mediated killing of chemotherapy-refractory lymphoma, which will be translated into novel clinical trials.

Core A (Administration, Younes) will coordinate all administrative and financial aspects of the SCOR, in addition to coordinating all regulatory and practical aspects of clinical trials that are generated from the SCOR. Core B (Biostatistics and Bioinformatics Core, Seshan) will be responsible for the analysis of all preclinical experiments, in addition will assist in the design of the planned clinical trials.

Integration and synergy. Projects 1 and 2 address 2 complimentary strategies to enhance the cytotoxicity of CAR 19-28z, that are currently being used in clinical trials at MSKCC and elsewhere. Project 1, addresses the potential role of blocking PD-1 signaling, while project 2, addresses the potential benefit of adding a second costimulatory signaling (4-1BBL). Both projects will interact with each others and with projects 3 and 4 which will further investigate mechanisms that contribute to sensitivity and resistance to CAR T cells and to checkpoint inhibitors. We will explore how HVEM mutations contribute to lymphoma biology and to develop tools to restore the HVEM receptor interactions for lymphoma therapy. Furthermore, we will explore the use of HVEM producing 19-28z CAR-T cells that produce and secrete the solHVEM protein to restore its inhibitory interaction with BTLA receptor. Our collaborative SCOR program is expected to improve our strategies to enhance T cell-mediated killing of chemotherapy-refractory lymphoma, which will be translated into novel clinical trials.

Anticipated Results. Our collaborative SCOR program is expected to improve our strategies to enhance T cell-mediated killing of chemotherapy-refractory DLBCL, which will be translated into novel clinical trials. Ultimately, our work should contribute to improving the cure rate of DLBCL.
Scientific Abstract
Diffuse large B cell lymphoma (DLBCL) is the most common subtype of adult lymphoma in the U.S. Approximately 50% of patients will have disease progression/relapse after standard chemotherapy regimens, indicating that new treatment modalities are needed to improve the cure rate of patients with DLBCL. Our group and others have recently demonstrated that T-cell mediated treatment strategies can induce clinical responses even in chemotherapy-refractory DLBCL patients. The central goal of this SCOR is to develop new therapeutic strategies for DLBCL through a collaborative effort involving 4 complementary and highly synergistic research projects. **Project 1**, will investigate the role of PD-1 blockade in enhancing the clinical efficacy of CAR T cells. **Project 2**, will examine whether co-expression of the ligand for 4-1BB (4-1BBL) and 19-28z will enhance the therapeutic efficacy of CAR T cells. **Project 3**, will define genetic determinants of response to immune therapies, including checkpoint blockade or CAR-T cells. We will explore tumor cell determinants of response, and also investigate the role of TIM3 as a new determinant of T cell action. **Project 4**, we will explore how HVEM mutations contribute to lymphoma biology and to develop tools to restore the HVEM receptor interactions for lymphoma therapy. Furthermore, we will explore the use of HVEM producing 19-28z CAR-T cells that produce and secrete the solHVEM protein to restore its inhibitory interaction with BTLA receptor. The projects are supported by 2 core services: Administration and Biostatistics. The ultimate goal of this SCOR is to translate our findings into novel clinical trials to improve the cure rate of DLBCL.
Lay Abstract
Patients with relapsed diffuse large B cell lymphoma (DLBCL) have limited curative options, once their tumor fail to respond to standard chemotherapy regimens. Our group and others have recently demonstrated that activating the patients’ own immune cells can induce clinical responses, even in chemotherapy-refractory DLBCL patients. The central goal of this SCOR is to establish a collaborative team-science aiming at the development of new immune therapeutic strategies for DLBCL. Our ultimate goal is to translate our findings into novel clinical trials to improve the cure rate of patients with DLBCL.
Projects

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**Project 1: scFv secreting CAR T cells that modulate the tumor microenvironment and host anti-tumor response**
Renier Brentjens

We can target T cells to B cell malignancies by utilizing a chimeric antigen receptor (CAR) specific for CD19, termed 1928z. While this therapy has shown remarkable promise in patients with B cell acute lymphoblastic leukemia (B-ALL), the results in patients with DLBCL have been more modest. Our hypothesis is that inhibitory tumor microenvironment may include expression of ligands for inhibitory T cell receptors, e.g. Programmed death (PD) -1 ligands, contribute to abrogation of cellular anti-tumor responses. The overall goal in this proposal is to explore the potential of armored CAR T cells designed to secrete biologically active single fragment length antibodies (scFv) targeted to PD-1, to modulate the tumor microenvironment and reactivate an endogenous anti-tumor immune response. We predict that, when translated to the clinical setting, this armored CAR T cell approach will exhibit enhanced anti-tumor efficacy when compared to current CD19 targeted CAR T cells. **Aim 1:** To utilize a previously established immune-competent syngeneic murine model of CD19+ tumor to assess the ability and mechanism of CD19 targeted CAR T cells further modified to secrete an anti-murine PD-1 blocking scFv to eradicate advanced tumors. **Aim 2:** To generate human CD19-targeted CAR T cells designed to secrete anti-human PD-1 blocking scFv, as well as express a truncated EGFR (EGFRt) as a suicide target, and to further assess anti-tumor efficacy in previously established xenotransplant murine models of human CD19 malignancies. **Aim 3:** To translate scFv secreting CD19 targeted armored CAR T cell technology to the clinical setting treating DLBCL patients in a phase I clinical trial.

**Project 2: Optimizing Second generation CAR therapy for DLBCL**
Michel Sadelain

This proposal builds on our expertise with CD19 CAR therapy (Sadelain, JCI, 2015) and our recent discovery that co-expression of the ligand for 4-1BB (4-1BBL) together with the 19-28z (the CAR that is presently in use at MSK in phase I trials for ALL, CLL and NHL, and is the platform used in Project 1) vastly increases T cell potency (Zhao, Cancer Cell, 2015). Specifically, we have found that CAR T cells co-expressing 19-28z and 4-1BBL acquire greater T cell effector functions, proliferative potential and persistence, with diminished acquisition of T cell exhaustion markers. More intriguingly yet, we found that these T cells engineered induce
IRF7 expression and activate the interferon-β pathway in vitro and in vivo. These findings open exciting new prospects for developing a more potent CD19 CAR therapy for the treatment of DLCL. Aim 1: To establish an all-murine model of DLCL and evaluate therein the potency and mechanism of action or m1928z+4-1BBL T cells, compared to the conventional 19-28z and 19BBz CARs. Aim 2: To examine the role of NK cells and Tregs in regulating CAR T cell cytotoxicity. Aim 3: To examine whether immune checkpoint blockade can further enhance the cytotoxicity of 1928z+4-1BBL CARs. Our ultimate goal is to conduct a clinical trial in patients with relapsed DLBCL, consisting in a single infusion of 19-28z+4-1BBL CAR T cells following conditioning with CY/Flu. Clinical grade vector (not paid for through this award) will be available in May 2016.

Project 3: Novel Molecular Strategies for Improving T Cell Cytotoxicity

Morgan Huse

The overarching goal of this project is to develop novel strategies for boosting tumor cell destruction by therapeutic cytotoxic T lymphocytes (CTLs). Our preliminary studies have implicated the cell surface receptor TIM3 in the potentiation of target cell killing. TIM3 is a receptor for phosphatidylserine (PS), a lipid that becomes exposed on the cell surface during apoptotic cell death. Our preliminary studies have implicated the cell surface receptor TIM3 as a critical positive regulator of target cell killing. We aim to establish the molecular and cellular bases of TIM3 function in this context and to explore the potential of using TIM3 expression to enhance CAR-mediated immunotherapy. We have also identified the orphan nuclear hormone receptor Nr4a3 as a candidate transcriptional regulator of a cytotoxic gene expression program that includes TIM3. Our specific Aims will investigate how Nr4a3 regulates TIM3 and how TIM3 regulates CTL-mediated killing. We will also assess the importance of these molecules for anti-tumor responses in a B cell leukemia model. Aim 1) Determine the mechanism by which TIM3 boosts cytotoxicity. Based on our preliminary data, we propose that TIM3 enhances cytotoxicity by promoting efficient dissociation from dying target cells. To investigate this hypothesis, we will employ quantitative single cell imaging methods in combination with gain- and loss-of-function perturbations. Aim 2) Characterize the transcriptional regulation of TIM3 in CTLs. We have found that TIM3 expression is associated with the upregulation of the transcription factor Nr4a3. In this Aim, we will investigate the molecular mechanism by which Nr4a3 regulates the expression of TIM3 and other effector molecules that are important for CTL-mediated killing. Aim 3) Determine if TIM3 modulates CTL-mediated responses against B-cell lymphoid malignancies. This Aim will examine whether TIM3 and Nr4a3 potentiate anti-tumor responses during CAR immunotherapy. We will make use of the murine CD19+ B cell ALL model recently developed by our collaborator Michel Sadelain (Project 2).

Project 4: Restoring The HVEM - BTLA Receptor Interaction In Lymphoma

H.Guido Wendel

The HVEM (TNFRSF14) immune receptor is mutated and /or deleted in >50% of germinal center (GC) lymphomas. Mutations have been variably linked to poor outcomes. The goal of this study is to explore how HVEM mutations contribute to lymphoma biology and to develop tools to restore the HVEM receptor interactions for therapy. The HVEM receptor engages in cell-cell interactions with stimulating receptors (LIGHT, CD160) and the inhibitor receptor (BTLA: B- and T- lymphocyte attenuator). How HVEM loss contributes to lymphoma is not known. We will use a murine model of FL that recapitulates the genetics and pathology of the human disease to explore the consequences of HVEM loss in vivo. Moreover, tumor suppressive interactions between surface receptors such as HVEM and BTLA may be amendable to repair and we will explore this concept using a purified soluble ectodomain fragment of HVEM (solHVEMP37-V202)
that binds to LIGHT and BTLA receptors. The following tasks will be accomplished in the Wendel lab at MSKCC and in collaboration with Renier Brentjens and Michel Sadelain. First, we will characterize HVEM’s tumor suppressor function in germinal center lymphomas. We will explore how loss of HVEM contributes to GC malignancy using a mouse model of FL, primary human FL cells, and cell lines. We will examine the cell autonomous effects of HVEM loss and effects on the lymphoma microenvironment. Secondly, we will generate tools to restore the inhibitory HVEM interactions in GC lymphomas. Our preliminary results indicate that the purified ectodomain fragment of the HVEM receptor (solHVEM\textsuperscript{P37-V202}) can engage the inhibitory BTLA receptor and block mitogenic signals, proliferation, and induce lymphoma regressions in vivo. These findings indicate a potential therapeutic opportunity. We propose to develop and test reagents for the systemic delivery of solHVEM\textsuperscript{P37-V202} in order to restore its inhibitory interaction with the BTLA receptor in vivo. Third, we are exploring the use of HVEM producing and CD19-targeted CAR-T cells that produce and secrete the solHVEM protein. We speculate that these cells may be especially effective against HVEM mutant B cell lymphomas.
Cores

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**Core A: Administration**
Anas Younes
The administrative core will coordinate all administrative, financial, and regulatory aspects of the SCOR. The core will maintain records pertaining to all IACUC protocols, IRB protocols, publications, and meeting reports. The Core will coordinate all aspects of clinical trials supporting the proposed projects, including the development of IRB-approved clinical trials, and data management of these trials. The Core will coordinate a monthly SCOR meeting for all SCOR investigators. The administrative core will organize annual meetings of the internal and external advisory boards. The Core will coordinate all communications with the LLS leadership, and will be responsible for providing an annual progress reports.

**Core B: Biostatistics and Bioinformatics Core**
Venkatraman Seshan
The role of the Biostatistics and Bioinformatics Core is to support the investigators of the SCOR in their research efforts, including laboratory and mouse experiments, molecular studies and the design and analysis of clinical trials. The Core will provide informatics infrastructure to enable collaboration and data sharing among the various projects.