
RECOMBINANT DNA ADVISORY COMMITTEE

Minutes of Meeting

September 9–10, 2014

**U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
Public Health Service
National Institutes of Health**

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[Note: The latest Human Gene Transfer Protocol List can be found on the Office of Biotechnology Activities website at <http://osp.od.nih.gov/office-biotechnology-activities/biomedical-technology-assessment/hgt> .]

**U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
NATIONAL INSTITUTES OF HEALTH
RECOMBINANT DNA ADVISORY COMMITTEE
Minutes of Meeting¹**

September 9–10, 2014

The Recombinant DNA Advisory Committee (RAC) convened for its 139th meeting at 9:15 a.m. on September 9, 2014, at the National Institute of Health (NIH), Rockledge II, 6701 Rockledge Drive, Bethesda, Maryland. Dr. Donald B. Kohn (RAC Chair) presided. In accordance with Public Law 92-463, the meeting was open to the public from 9:15 a.m. until 5:25 p.m. on September 9, 2014, and from 8:30 a.m. until 9:47 a.m. on September 10, 2014. The following individuals were present, either in person or by teleconference, for all or part of the September 2014 RAC meeting.

Committee Members

Scott Antonia, University of South Florida (*incoming*)
Michael Atkins, Georgetown University School of Medicine
Tianxi Cai, Harvard University (*via teleconference*)
Paula Cannon, University of Southern California
Saswati Chatterjee, City of Hope National Medical Center
William Curry, Harvard Medical School
Kevin Donahue, University of Massachusetts Medical School (*incoming*)
Rebecca Dresser, Washington University School of Law
Norman Fost, University of Wisconsin, Madison
Marie-Louise Hammarskjöld, University of Virginia School of Medicine
Angelica Hardison, Georgia Regents University (*via teleconference*)
Patrick Hearing, Stony Brook University (*incoming*)
Howard Kaufman, Robert Wood Johnson Medical School/Rutgers, The State University of New Jersey (*incoming*)
Hans-Peter Kiem, University of Washington School of Medicine/Fred Hutchinson Cancer Research Center
Donald Kohn (RAC Chair), University of California, Los Angeles
David Ornelles, Wake Forest University School of Medicine (*via teleconference*)
Joseph Pilewski, University of Pittsburgh
Lainie Ross, University of Chicago (*incoming*)
Michael Sadelain, Memorial Sloan-Kettering Cancer Center (*incoming*)
Marcella Sarzotti-Kelsoe, Duke University School of Medicine (*via teleconference*)
Richard Whitley, University of Alabama, Birmingham
Dawn Wooley, Wright State University
Laurie Zoloth, Northwestern University

NIH Office of Biotechnology Activities (OBA)

Jacqueline Corrigan-Curay, Office of the Director (OD), National Institutes of Health (NIH)

Nonvoting Agency Representatives

Kristina Borrer, Office for Human Research Protection, NIH
Denise Gavin, U.S. Food and Drug Administration (FDA)

¹ The Recombinant DNA Advisory Committee is advisory to the NIH, and its recommendations should not be considered as final or accepted. The Office of Biotechnology Activities should be consulted for NIH policy on specific issues.

NIH/OD/OBA Staff Members

Linda Gargiulo
Morad Hassani
Robert Jambou
Maureen Montgomery
Chris Nice
Gene Rosenthal

Attendees

There were 56 attendees at this 2-day RAC meeting.

Attachments

Attachment I contains a list of RAC members and nonvoting agency and liaison representatives. Attachment II contains a list of public attendees. Attachment III contains a list of abbreviations and acronyms used in this document. Appendix A includes verbatim public comments from protocol #1406-1320.

I. Call to Order and Opening Remarks

Dr. Kohn, the RAC Chair, called the meeting to order at 9:15 a.m. on September 9, 2014. Notice of this meeting under the *NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules (NIH Guidelines)* was published in the *Federal Register* on August 18, 2014 (79 FR 48749). Issues addressed by the RAC at this meeting included a report from the Gene Transfer Safety Assessment Board (GTSAB, a subcommittee of the RAC), and public review and discussion of five gene transfer protocols.

RAC members introduced themselves by name, affiliation, and research interests. Five incoming members starting their tenure with the RAC with the current meeting were Drs. Antonia, Donahue, Hearing, Kaufman, and Ross.

Dr. Corrigan-Curay reminded RAC members of the rules of conduct that apply to them as Special Government Employees, read into the record the conflict of interest statement, and suggested that related questions be addressed to the OBA committee management officer.

II. Review and Discussion of Human Gene Transfer Protocol #1405-1315: Phase I Trial of Oncolytic Adenovirus-Mediated Cytotoxic and Human Interleukin 12 (IL-12) Gene Transfer for Radio-Recurrent Prostate Cancer

Principal Investigator (PI): Svend Freytag, Ph.D., Henry Ford Health System

RAC Reviewers: Dr. Atkins, Dr. Ornelles, and Professor Dresser

A. Protocol Summary

An estimated 233,000 men will be diagnosed with prostate cancer in the United States in 2014 and approximately one-third will elect radiation therapy (external beam and/or brachytherapy) as their primary treatment. For men with organ-confined, low-risk disease, radiation therapy results in excellent long-term disease control and survival. However, it is less effective against more aggressive forms of the disease, and a significant fraction of men develop disease recurrence within 10 years. The rate of occult distal failure following definitive radiotherapy is less than 10 percent at 10 years. Thus, there is a substantial

window of opportunity after initial recurrence in which the disease may still be largely localized and amenable to local salvage therapies.

The scientific rationale for this Phase 1 study derives from the extensive preclinical and clinical work conducted by Dr. Freytag and colleagues over the past 20 years. The team has developed a multimodal, gene transfer-based approach for the treatment of cancer. The therapeutic platform utilizes an oncolytic adenovirus to deliver a pair of cytotoxic genes to the tumor. The oncolytic adenovirus itself generates an anti-tumor effect by replicating and destroying cancer cells. The therapeutic effect of the adenovirus is enhanced by invoking two cytotoxic gene systems (cytosine deaminase/5-fluorocytosine [5-FC] and herpes simplex virus thymidine kinase/valganciclovir [vGCV]), which render malignant cells sensitive to specific pharmacological agents and sensitize them to ionizing radiation. The combined effects of these three modalities result in significant tumor cell destruction and release of tumor antigens, which, when coupled with the robust immune response elicited by the adenovirus, may generate an environment that fosters tumor antigen processing and cross-presentation culminating in the generation of long-lasting anti-tumor immunity.

The investigators have evaluated the toxicity and efficacy of their multimodal approach in five clinical trials of non-metastatic prostate cancer, including a prospective, randomized, Phase 2 trial. These studies have demonstrated that the multimodal approach is safe and has the potential to improve local tumor control. With respect to safety, there have been no dose-limiting toxicities (DLTs) or treatment-related serious adverse events (SAEs) in 102 subjects treated to date. Only six percent of the adverse events (AEs) have been Grade 3 or higher, with the vast majority being transient and asymptomatic. With respect to efficacy, in the radio-recurrent setting, subjects who received the gene transfer exhibited a significant lengthening of prostate-specific antigen (PSA) doubling time (PSADT), a surrogate endpoint that is highly prognostic for the development of distant metastases and prostate cancer-specific mortality. At 9 and 12 years, there was an improvement in disease-specific survival relative to well-matched historical controls. In the newly diagnosed setting in combination with contemporary dose radiotherapy, there has been a 42 percent reduction in 2-year biopsy positivity in men with intermediate-risk disease and a 60 percent reduction in men with less than 50 percent positive biopsy cores at baseline.

Although local tumor control is important, for most human cancers, new therapies must also target metastatic disease if they are to have an impact on survival. Per this consideration, the investigators have added a fourth modality to their multimodal approach by generating a third-generation adenovirus armed with IL-12 that has the potential to impact both local and metastatic disease. The proposed research will involve an oncolytic adenovirus containing two suicide genes, E. Coli cytosine deaminase (CD) and herpes simplex virus thymidine kinase (HSV-1 TK) and an IL-12 gene (Ad5-yCD/ mutTKSR39rep-hIL12).

Men with radio-recurrent prostate cancer have few therapeutic options that have a high likelihood of eradicating the tumor with a reasonable degree of safety. The primary objective of this Phase 1 study is to determine the dose-dependent toxicity of oncolytic adenovirus-mediated suicide and IL-12 gene transfer in this subject population. Six cohorts of three subjects each will receive a single intraprostatic injection of the adenovirus at one of six dose levels (1×10^{10} viral particles [vp] to 1×10^{13} vp) along with 1 week of 5-FC + vGCV prodrug therapy. The primary endpoint is acute toxicity at 30 days. Secondary endpoints include PSA response, PSADT, 1-year prostate biopsy outcome, and QOL. Exploratory endpoints will examine a possible relationship between primary and secondary outcomes and immunological endpoints.

B. Written Reviews by RAC Members

Eleven RAC members voted for in-depth review and public discussion of the protocol because of the novel use of IL-12 with an oncolytic suicide adenoviral vector. In addition, because there have been some unexpected safety issues arising in ongoing studies using an adenoviral vector with controlled expression of IL-12, the safety of a replication competent adenoviral vector, in which the expression of IL-12 is not controlled, warrants further discussion.

Three RAC members provided written reviews of this proposed Phase I trial.

The reviewers found the study to be well designed and the proposed intervention to be appropriate for the target patient population, for which standard treatment options produce considerable local toxicity and result in less than 50 percent cancer control rates. Drs. Atkins and Ornelles noted that the oncolytic virus has shown to be safe and to possess a suggestion of antitumor effect in men with prostate cancer treated in various clinical settings. Addition of IL-12 has been shown to be safe in animal models and to possibly enhance efficacy. However, all of the reviewers questioned whether the proposed monitoring plan is sufficient to minimize risks of IL-12-associated toxicity.

Dr. Atkins also asked why injection is planned throughout the prostate gland and not directly within the tumor. In addition, he asked whether an activator ligand will be added for the IL-12 vector and what the anticipated blood levels are for IL-12 administered in this setting.

Dr. Ornelles raised concerns about delivery of IL-12 by a replication-competent adenovirus and the potential for unexpected and/or off-target toxicities. Due to AEs associated with systemic IL-12, this proposed study follows the approach of attempting to deliver IL-12 locally, at the tumor site. Preclinical models using the mouse support the notion of restricted, local delivery but are limited by the facts that human adenovirus infects cells of the mouse with low efficiency and that human cells and human tissue in the xenograft model are preferentially infected with human adenovirus. Consequently, the release and dissemination of a limited number of infectious virions in the mouse is unlikely to have significant or measurable consequences in this preclinical model and probably fails to represent what occurs in humans.

Dr. Ornelles noted further that the spectrum of SAEs in three gene transfer protocols intended to achieve local delivery of IL-12 raises the possibility of unexpected or AEs arising from dissemination of the IL-12–expressing virus. The AEs associated with these trials included neutropenia, diarrhea, vomiting, fever, dehydration, hypotension, hyponatremia, renal insufficiency, and delirium or altered mental status. Although many of these symptoms could reflect a systemic toxic effect of IL-12, other events were not so readily explained and may have resulted from dissemination of the virus followed by a localized effect of IL-12. In prior studies of prostate cancer patients (with 1×10^{11} , 1×10^{12} , or 5×10^{12} vp injected into the prostate), non-invasive detection of adenovirus by ^{99m}Tc imaging provided strong evidence against widespread dissemination of the virus through the patient. However, these same experiments demonstrated that expression of the viral reporter gene in the prostate was only detected at the two higher doses. The failure to detect gene expression at the lowest dose may reflect a practical limit of detection. It is possible that dissemination of the virus with subsequent transgene expression at levels lower than that achieved by delivery of 1×10^{11} particles would remain undetected elsewhere in the patient. Dr. Ornelles noted that the product used in one trial (Ad5-CD/TKrep) was detected as viral DNA in the blood as late as Day 76. Given the range of AEs reported in prior trials and the potential limits of detection of the virus outside the target site, the investigators should consider the possibility of the viral vector infecting susceptible cells at other sites in the patient (including micrometastases and gross metastases) where the release of IL-12 triggers a local inflammatory response. The biological consequence of low levels of disseminated virus that may express IL-12 should also be taken into account.

Dr. Ornelles asked whether the definition of clinical failure based on a greater than 25 percent increase in the projected tumor area could be problematic. Because this procedure will likely promote immune cell infiltration and certainly activate immune cells, it seems possible for radiographic measurements to show an increase in tumor size due to cellular infiltration and necrosis rather than tumor cell growth. It is not clear whether the imaging methods used to determine local progression will be able to distinguish between these two possibilities. In addition, the protocol states that a needle biopsy will be used to document the presence of the local tumor, but additional information should be provided to document that this approach is sufficient to identify a mixture of tumor and immune cells.

Exclusion of patients with metastatic disease is reasonable, but the rationale for excluding such patients should be stated.

Professor Dresser made the following comments and suggested modifications regarding the informed consent document (ICD):

- On page 1, sentence 2 says that there are no standard therapies available that have proven to improve survival seems somewhat overstated. Some existing therapies have promising 5-year survival rates, but the research to establish long-term survival has not been done yet. Professor Dresser asked whether it would be better to say that there are treatments available for the patients' type of prostate cancer but that it is not yet known whether those treatments are effective in the long term.
- On page 1, in paragraph 2, line 3 refers to "12 deposits," which sounds like 12 injections, even though there appears to be only one injection. This should be explained better.
- On page 3, in paragraph 3, the term "treatment" should be replaced with "study drug," "experimental gene transfer," or another term that does not imply a proven therapy.
- On page 3, it is not clear whether the term "routine" refers to (1) tests and visits that are part of ordinary care for anyone with this type of cancer or (2) the study eligibility evaluation process. This information might be confusing to patients and should be clarified.
- On page 5, the risks section does not discuss whether there is pain associated with injection and biopsy. Professor Dresser suggested separating the discussion of gene transfer third-party risks (environmental) from risk to the subjects. In addition, before addressing the risk of secondary cancer, it would be helpful to add a sentence or two explaining that vectors can cause secondary cancers if they insert genes in the wrong place, which has happened in trials other vectors.
- On page 7, it is not clear whether the discussion on precautionary measures given the potential for the adenovirus to escape in body fluids is directed at patients, others (e.g., family members), or both. The ICD should state explicitly for whom this information is meant.
- On page 9, the last paragraph under item 6 discusses a subject's decision to stop participating in this study. Some patients may not understand that the vector cannot be removed from the body once it is injected. The investigators should consider adding a statement to explain this.

C. RAC Discussion

During the meeting, the following additional questions, concerns, or issues were raised by RAC members:

- The reviewers found the presentation to be clear and their concerns and questions to be well addressed. The study design of this Phase 1 trial, including a cautious dose escalation plan. However, at the higher doses there could be significant blood levels of IL-12 and interferon-gamma and associated potential IL-12 toxicities due to dissemination of the replication virus to off-target organs. Dr. Atkins noted, however, that the proposed management plan appears to be geared toward less severe toxicities than have been reported in patients who have had complications with IL-12. He suggested adding a low threshold for giving steroids (or other agents, as indicated) in case participants experience more serious adverse events.
- The reviewers and other RAC members recognized that while mouse models provide useful information, replication and infectivity of the human adenovirus in the clinical setting are difficult to predict from the mouse model. Dr. Hammarskjöld noted that mice, unlike humans, do not have preexisting immune responses to adenoviruses and that titers in patients can vary considerably, from negative to very high. She asked whether prior protocols have accounted for preexisting adenovirus responses and, if so, how the studies controlled for these differences. In addition, she asked whether the investigators have considered (or plan to use) a mechanism such as next-generation sequencing of RNA to measure adenovirus gene expression more systematically?
- Dr. Ornelles noted the potentially potent nature of IL-12 in a replication-competent virus and questioned whether a beneficial biology may be missed in preclinical investigations based on some of the unusual effects seen in prior IL-12 studies. The spectrum of AEs in previous IL-12 studies might indicate dissemination of the virus and low-level expression of IL-12 in other organs or tissues. While this is a concern for AEs, it also could be the basis for a positive effect, especially for metastatic disease.
- Dr. Antonia requested additional information about safety concerns, side effects, and "unusual toxicity" associated with IL-12 vectors. Dr. Atkins noted that serious side effects, such as cytokine-related toxicities, interferon gamma (IFN- γ)–related toxicities, transaminitis, neutropenia, and hypotension, have been associated with IL-12–transfected cells for adenoviral or vector treatments. Dr. Corrigan-Curay noted that rates of hypotension, transaminitis, dehydration,

neutropenia, and other complications (often requiring hospitalization) have been higher in trials using an adenovirus that included an activator ligand that could turn on the IL-12 than in trials where IL-12 expression was not controlled in that manner. Some references to local toxicities may be related to a melanoma trial in which the agent is injected directly into the melanoma lesion; one patient on the trial was injected through the chest wall resulting in a pleural effusion. In general, the unusual constellation of SAEs is in studies using an adenovirus with IL-12 and an activator ligand.

- Dr. Donahue inquired about the time course for IL-12 toxicity and whether the proposed monitoring plan is consistent with the development of AEs associated with IL-12. He also asked whether the exclusions for cardiac, renal, and hepatic co-morbidities should be more stringent, given potential toxicities. For example, systemic inflammatory response can lead to fluid accumulation and, in turn, massive vasodilation. Under normal circumstances, Individuals with certain cardiac conditions such as congestive heart failure or systolic dysfunction that is well compensated would not necessarily be in the hospital within 6 months prior to study entry (per the proposed exclusion criteria). However, the stress of this intervention could be catastrophic to such patients, who don't have the reserve to recover. Similar scenarios can be anticipated for patients with moderate renal dysfunction (e.g., when attempts to diurese no longer work) and liver dysfunction. Dr. Atkins noted that the study cohort will include some elderly men who may have subtle cardiac problems and suggested adding cardiac echo with a reasonable ejection fraction.
- Dr. Sadelain asked about the reason for adding IL-12 to this vector, the contribution of the immune response to the current vector versus direct toxicity, and the extent to which an immune response from IL-12 had been documented. In addition, he asked whether there are data (from the toxicology study) that compare the same dose of the vector with and without IL-12 and whether it is possible to determine the contribution of the vector alone versus the added IL-12 on side effects such as neutropenia and transaminitis (seen only at the higher doses).
- Drs. Kohn and Zoloth inquired about the relative sensitivity of humans and mice to IL-12 toxicity. Further, it was noted that some patients in the inducible vector trial developed confusion and depression. Dr. Zoloth asked whether any neurological effects have been observed with IL-12, which could be important in patients facing high levels of stress due to their diagnosis but which could be difficult to detect or assess in animals. Dr. Curry asked what the investigators anticipate the dose-limiting toxicities will be for this product and in this trial. It was suggested that the investigators provide a more detailed response plan to address potential toxicities, including liver toxicity and hypotension.
- Dr. Kiem asked whether the investigators have tested the IL-12 construct in the Syrian hamster model given some of the problems with the mouse models. He also asked about the effect of prodrug administration on IL-12 levels.
- Dr. Whitley asked whether the investigators have compared replication competence of the IL-12 expressing virus versus the non-IL-12 expressing virus. His query is based on studies he and his colleagues have conducted using herpes simplex in which replication competence is greater by two logs with IL-12 than without IL-12. The result is seen in in vitro systems as well as in animal models. Given these findings, Dr. Whitley's team has decided to lower the starting dose in subsequent trials involving genetically modified herpes simplex virus, from 1×10^7 to 1×10^5 . Given concerns about toxicity in the proposed trial, the investigators may want to re-consider the starting dose and build a de-escalating dosage schedule into the protocol to better address potential serious side effects. Dr. Whitley offered to forward dose escalation schemas that will guide dosing (e.g., dropping the dose by half a log) if toxicity is seen at the starting dose.

D. Investigator Response

1. Written Responses to RAC Reviews

The investigators noted that there are three possible strategies that could be used to distribute the adenovirus throughout the prostate: (1) depositing the adenovirus uniformly throughout the prostate regardless of the distribution of cancer, (2) depositing the adenovirus only in those regions (i.e., sextants) known to harbor cancer based on the pre-treatment biopsy, and (3) depositing the adenovirus in all sextants but skewing the adenovirus dose distribution toward those regions known to harbor cancer.

Under the proposed trial, before the adenovirus injection, a prostate biopsy is performed to confirm that the patient has local recurrence of cancer and to map where the cancer resides. A treatment plan (where the adenovirus is deposited throughout the prostate) is then developed based on the results of the pre-treatment biopsy. The investigators plan to use the third strategy (treat the entire prostate) but will skew the adenovirus dose distribution to those regions known to harbor cancer based on the pretreatment biopsy. This strategy was chosen because of the sampling error associated with the needle biopsy and because prostate cancer is typically a multifocal disease. This approach also aligns with definitive treatments such as radiotherapy (and surgery), which typically include the entire prostate in the clinical target volume. In addition, because patients with locally recurrent prostate cancer after definitive radiotherapy have smaller prostates than newly diagnosed patients, complete coverage of the prostate with the adenovirus is likely to be achieved.

The investigators clarified that no activator ligand will be used in this study. In the adenovirus that will be used in the proposed trial, expression of IL-12 is under the transcriptional control of the constitutive cytomegalovirus (CMV) promoter. Gene expression from a similar oncolytic adenovirus has been shown to be short lived, lasting 7 days or less in most patients. The short duration of gene expression is likely limited by the immune response as well as the effects of the 5-FC and vGCV prodrugs on adenovirus replication (i.e., both prodrugs inhibit viral DNA replication). Based on quantification of IL-12 and IFN- γ levels in preclinical toxicology and efficacy studies conducted in the C57BL/6 mouse, the investigators expect serum levels of both compounds to be below toxic levels at adenovirus doses of, at most, 3×10^{12} vp.

The investigators believe that the proposed monitoring plan is sufficient to minimize risks to the subjects. Most of the toxicities associated with the oncolytic adenovirus, prodrug therapy, and IL-12 are acute. Complete blood count (CBC) and blood chemistries will be monitored twice a week during the first 2 weeks after the adenovirus injection. If serious toxicities develop as the adenovirus dose is escalated, the frequency of blood draws will be increased to three times a week or daily, if necessary, in subsequent patients. The research nurse will contact the patient on a daily basis during the first 2 weeks. The treating physician will conduct an on-site toxicity assessment at least once a week during the first 2 weeks. Patients will be instructed to call the PI or research nurse immediately if any serious side effects develop. Post-dosing visits are scheduled at 1 month (i.e., 2 weeks after the initial 2-week period), 3, 6, 12, and 24 and at least annually thereafter. Patients will be monitored and, as needed, treated for AEs associated with administration of IL-12. All SAEs will be reported in writing to the Data and Safety Monitoring Board (DSMB). Following discussion with the PI, the DSMB will determine whether the SAE was treatment related. Those that are deemed to be definitely, probably, or possibly treatment related will be reported in writing to the FDA, OBA, the institutional review board (IRB), and the institutional biosafety committee.

The investigators agree that mouse models fail to fully recapitulate what occurs in humans regarding the cytolytic effects of the oncolytic adenovirus. However, mouse models appear to reflect the human response to a reduced extent. While mouse cells do not generate a viral burst, preclinical studies show that human replication-competent adenoviruses replicate their DNA, yield significant gene expression, and kill mouse prostate epithelial cells with an efficiency approaching that of human prostate adenocarcinoma cells. The investigators note further that the safety of three different oncolytic adenoviral therapies (without IL-12) that are similar to the proposed intervention has already been demonstrated in humans and that these human studies render the mouse studies moot with respect to the toxicity of the oncolytic adenovirus itself. What is unknown and of concern here is the severity (but not the spectrum) of IL-12 toxicity when expressed from a replication-competent adenovirus.

The investigators acknowledged the limitations of their human imaging studies and the possibility of viral dissemination beyond the prostate. In previous trials conducted by the sponsor, about half (42 percent) of patients exhibited transient transaminitis. The vast majority of these events (82 percent) were Grade 1 and returned to within normal limits within 1 week. These events were attributed to dissemination of the adenovirus to liver. The investigators suggest that viral dissemination is a function of three factors: the skill of the urologist doing the transrectal-ultrasonography-guided adenovirus injection, the volume of the adenovirus injection, and the dose of the adenovirus. The protocol includes provisions to address each of these factors.

If a patient fails clinically, it will likely be several years (more than 5) after gene transfer. The interval between administration of the gene transfer and the onset of clinical metastases can be 5 to 10 years, depending on risk level at initial diagnosis. Prior experience indicates that oncolytic adenovirus-mediated cytotoxic gene transfer (without IL-12) delays the need for additional salvage therapy by a mean of 2 years. The addition of IL-12 to the therapeutic platform is expected to delay this even further. If a patient's PSA rises to 10 to 20 ng/mL after the gene transfer, the patient will be offered salvage hormone and remain on hormone therapy until he or she becomes hormone refractory. The investigators do not believe that imaging methods can discern definitively between inflammation and actual tumor progression; however, imaging coupled with PSA kinetics can often discern between inflammation and actual tumor progression. Histopathological analysis of prostate biopsy specimens can easily identify and discern between prostate tumor and immune cells, the latter of which are often seen in early (less than 1 month) post-treatment biopsies.

The investigators noted that men with locally recurrent prostate cancer rarely present with metastatic disease. Patients with metastases will be excluded, given the availability of several FDA-approved treatments for metastatic prostate cancer. The investigators decided that patients with metastases should be offered these standard treatments first because of the seriousness of the patients' disease and the fact that the proposed intervention is unproven in the metastatic setting. If significant clinical activity is observed in the proposed Phase 1 trial, the investigators will reconsider the eligibility criteria in future trials to include men with extraprostatic disease.

With respect to the RAC comments on the ICD, the investigators clarified that the term "deposits" refers to areas within the prostate where the adenovirus is placed; this has been clarified in the revised ICD. The word "routine" is used to refer to tests and visits that are part of ordinary care for anyone with this type of cancer; the term has been defined accordingly in the ICD. The investigators noted that there is some pain and discomfort associated with the prostate biopsy procedure and that 2 percent lidocaine is injected around the prostatic capsule before the needle biopsy to minimize pain/discomfort. This is now stated in the revised ICD. Per the reviewer's recommendation, the discussion of gene transfer risks to the subject has been separated from potential environmental risks to third-parties in the revised ICD. In addition, the ICD now states explicitly that the precautionary measures refer to others including family members.

The investigators did not agree with the suggestion to add information about secondary cancers caused by other viruses. Although secondary cancers owing to insertional mutagenesis have been documented in humans with retroviral vectors, adenoviruses do not insert their DNA into the host cell genome and there is no evidence that they cause cancer in humans.

The investigators agreed with the other recommendations regarding the ICD and have modified the proposed ICD accordingly.

2. Responses to RAC Discussion Questions

IL-12 is being added to the previous generation of the vector to boost immune response. Dr. Freytag noted that in the absence IL-12, antitumor immunity may develop. He referred to a case in which a patient had a rapid decline in PSA followed by a slowing of PSADT long after the virus was no longer present. He noted that the virus typically remains in the patient for less than 7 days based on gene expression and about 60 days based on viral DNA. The basis for this long-term effect after the therapeutic agent has cleared is not known, but in the absence of IL-12, there may be development of antitumor immunity. The addition of IL-12 may enhance that effect. Another hypothesis is that the virus could kill more biologically active tumor cells, leaving less biologically active cells behind. There are no hard data at this point to document any added effect of IL-12 with this platform, however.

The toxicology study described during the presentation did not include a virus lacking IL-12, but the investigators have conducted six other toxicology studies with adenoviruses lacking IL-12. The experiments in the different studies are not exactly the same, but the data could be compared for outcomes with and without IL-12.

Neutralizing antibodies to adenovirus were measured in the first three trials in which preexisting adenovirus responses were assessed. Two of the trials were in the radio-recurrent setting and with radiation. Responses were measured before and about 30 days after the adenoviral injection. All patients had low levels of preexisting antibodies, and their levels markedly increased in all patients in both trials. The change in antibody levels failed to show any correlation with toxicity or efficacy, however. The investigators discussed the findings with the FDA and concluded that these assessments do not add anything to the research. Upon further consideration, the decision was made to drop this testing from future studies.

Dr. Freytag agreed with the reviewers and committee members that the mouse does not perfectly recapitulate what happens in humans and will take into consideration the suggestion to look at adenovirus gene expression more systematically (e.g., with next-generation sequencing of RNA). He noted further that while the wild-type adenovirus replicates relatively well in Syrian hamsters, E1B- attenuated viruses do not. The investigators discussed this issue with the FDA and concluded that the Syrian hamster is not better than the mouse for studying the team's viruses. The best approach to assess the toxicity of the viral construct at this stage is in a carefully designed Phase 1 trial, starting with a safe dose and very carefully escalating the dose and closely monitoring patients.

Dr. Corrigan-Curay noted that the time course for IL-12 toxicity can vary, with some events occurring within a day after dosing, while other side effects develop over several days. In studies where subjects receive the vector and an activator ligand, there is increased expression of IL-12 due to the ligand, but side effects usually occur within the first week of dosing. Dr. Kohn added that hypotension and neurotoxicities may be seen within days after the viral product is given, with IL-12 possibly contributing to these events. Dr. Corrigan-Curay pointed out, however, that more severe problems (e.g., severe liver toxicity) have been seen in different settings (e.g., immunotherapy protocols in which T cells containing a gene for IL-12 expression are administered IV). Dr. Freytag noted that the Grade 3 transaminitis appears to be due to viral leak out of the prostate and infection of hepatocytes. In the imaging trial, 18 patients were treated, and the last six patients were given a dose of 5×10^{12} viral particles. The last two patients developed transient Grade 3 transaminitis, which resolved within about 2 weeks. Several participants developed Grade 3 transaminitis. The reason for the higher-grade transaminitis may have been due to the volume injected rather than the dose per se, while the Grade 2 events may have been related more to infection of hepatocytes. These results raise concerns about possible hepatocyte damage from infection with adenovirus and how this would be treated, since use of steroids to manage IL-12 toxicity would be contraindicated. Given the multiple mechanisms that may be acting on the liver, the investigators will consider the suggestion to integrate liver biopsies to guide management of potential hepatic injury. The study team will discuss the range of comments regarding exclusion of co-morbidities and will consider revising the eligibility criteria as suggested.

Regarding possible neurological effects of the study intervention, Dr. Freytag noted that most patients who would be eligible for the proposed study are not expected to be depressed, because their disease is relatively benign. Having a diagnosis of prostate cancer, particularly recurrent disease could cause subjects to become anxious or distressed, potentially confounding assessment of any mood disturbances.

Dose-limiting toxicities for the proposed study are expected to be hypotension, edema, ascites, dehydration, and related conditions. In contrast, lymphopenia and hematologic toxicities are not expected to be of great concern for patients, and flu-like symptoms are manageable. Most cases of transaminitis in prior trials were acute, and patients were asymptomatic.

Dr. Freytag noted that prodrugs inhibit viral replication, which is one of the reasons why the investigators wait 2 days after the adenoviral injection is given before starting prodrug therapy. The 2-day window is to allow replication to occur. Both of the prodrugs that will be used in the proposed trial, 5-FC and GCV, block replication, thereby also reducing expression. Results of imaging studies show that virus expression peaked on day 1-2 and then gradually declined. While this decay presumably is due in large part to the immune response to the adenovirus-infected cells, prodrug therapy may also play a role in containing the virus.

The investigators have compared two replication competent viruses, one with and one without IL-12, at peak viral load, but they have not compared the quantity of viral replication in culture versus in mouse model. Dr. Freytag noted that he is not familiar with how the dose de-escalation strategy would work and will look into this concept and review information from Dr. Whitley (and others) in considering this suggestion.

E. Public Comment

Dr. Borror noted that the draft consent still includes references to the experimental intervention as “therapy” and “treatment” and says that the study agent will help destroy cancer cells, even though the efficacy of the intervention has not been demonstrated yet. Statements in the consent that participants will be required to make scheduled visits to the clinic for the rest of their life should be revised (e.g., replace with “you will be asked” or “you will be expected”) to take into account that subjects can choose to no longer be followed and withdraw from participating in the study. In addition, Dr. Borror noted that the way the screening and routine tests are described in the consent is confusing. Participants need to understand that specific tests and procedures will be performed to determine whether or not they can receive the injections and that these tests are part of the research. Similarly, the consent should clearly explain that screening is part of the research study and tests done as part of the screening process will be performed only if the subject is eligible and decides to continue in the research.

F. Synopsis of RAC Discussion and RAC Observations and Recommendations

- In the mouse preclinical studies using the same adenoviral vector with murine IL-12, you observed toxicities such as elevated serum liver enzymes and low white blood cell counts. It would be helpful to understand whether these toxicities are due to IL-12 or the vector. A comparison of results of current toxicology studies to previous toxicology studies that you have performed with the same adenoviral construct that does not express IL-12 may be informative.
- IL-12 has the ability to enhance viral replication. Consider performing experiments that compare the peak viral load and viral replication of this vector to the same construct that does not express IL-12. A significant increase in viral replication, due to the expression of IL-12, may inform your planned dose escalation.
- Since the mouse model being used in the preclinical studies is unlikely to be completely predictive of IL-12 toxicity in humans, consider adding a dose de-escalation to your proposed dosing cohorts so that you can reduce the dose below your starting dose in the event of a dose-limiting toxicity in the initial cohort.
- In previous clinical trials using an adenoviral vector expressing IL-12, there have been several serious adverse events that involved hypotension, metabolic abnormalities, liver enzyme elevations, “cytokine release,” and mental status changes. While this is a different construct and the IL-12 expression is not under the control of an activator ligand, consider more conservative inclusion criteria in this initial study by excluding research participants with other co-morbidities that might complicate the analysis, management, and outcome of an adverse event. For example, the current exclusion criteria limit participation to those who have a severe co-morbidity, such as a myocardial infarction within six months, unstable angina, or heart failure requiring hospitalization within six months. It may be prudent to be more conservative regarding such underlying vascular conditions. In addition, since participants are likely to be elderly, a screening echocardiogram to document relatively preserved cardiac function should be considered.
- In your previous trial with the same vector that does not express IL12, there was some evidence of transaminitis (indicating that the vector reached the liver). IL-12 expressed from this construct could lead to liver toxicity as well. You should consider developing a protocol to address any unexpected serious toxicity, including liver toxicity and hypotension.

- The informed consent document contains a number of references to “treatment” and a statement that the IL-12 will stimulate your immune system to attack your prostate cancer. Such statements should be changed because they may create an impression that the intervention has been shown to be efficacious.
- There is a section in the consent document called “Routine Tests and Procedures to be Performed Whether or Not You Decide to Participate in This Research Study.” This section should be clarified to make it clear that screening tests are part of the research study, but only those who are eligible for the study will be subject to the remaining tests.
- In a previous trial that used an adenoviral vector to express IL-12, some of the adverse events observed included neurological toxicities. This should be included as a potential risk in the informed consent document.

G. Committee Motion 1

Dr. Kohn summarized the RAC recommendations to be included in the letter to the investigators, expressing the comments and concerns of the RAC. Dr. Kohn requested a vote, and the RAC approved these summarized recommendations by a vote of 16 in favor, 0 opposed, 0 abstentions, and 0 recusals.

III. Review and Discussion of Human Gene Transfer Protocol #1404-1304: A Phase I, Multicenter, Open-Label, Single-Dose Study to Assess the Safety and Tolerability of Autologous Hematopoietic Stem Progenitor Cells Modified at the BCL11A Gene by Zinc Finger Nucleases (SB-BCLmR-HSPC) to Increase Fetal Hemoglobin Production in Subjects with Transfusion-Dependent β -Thalassemia

PI: Mark Walters, M.D., University of California, San Francisco, Benioff Children’s Hospital

RAC Reviewers: Drs. Hammarskjöld and Zoloth

Ad Hoc Reviewers: Drs. Segal and Tisdale

Drs. Cannon, Chatterjee, Kiem, and Kohn were recused from consideration of this protocol due to conflicts of interest. As a result of Dr. Kohn’s recusal, Dr. Hammarskjöld chaired this section of the September 2014 RAC meeting.

A. Protocol Summary

Patients with β -thalassemia are born with a defect in hemoglobin, a protein in red blood cells that carries oxygen to vital organs such as the brain, heart, lungs, and kidneys. Normally, human fetal globin (γ -globin) gene expression is high at birth, but levels decline progressively and are replaced by adult hemoglobin during the first year of life. In adults, the BCL11A gene ordinarily turns off fetal hemoglobin and allows the adult type of hemoglobin to be made. Individuals with thalassemia cannot make adult hemoglobin and develop severe anemia after the fetal hemoglobin is no longer made. Patients must receive monthly blood transfusions to survive and must have chelation treatment for the potentially fatal iron overload that develops due to transfusion. It is possible to replace the defective red blood cells with normal blood cells in persons with thalassemia by a bone marrow transplant. However, a bone marrow transplant is an intensive medical procedure with serious short- and long-term risks. In addition, not all persons with thalassemia have a suitable donor for this procedure.

Given these factors and complications, new treatments that have the potential to cure thalassemia are being sought and tested. The proposed research builds on the knowledge that for patients with β -thalassemia, production of fetal hemoglobin (HbF) is associated with milder disease and improved outcomes. Genome-wide association studies show that specific alleles of BCL11A (i.e., those associated

with reduced BCL11A levels) lead to elevated HbF levels and protection from symptoms of β -thalassemia and sickle cell disease. Knockout of BCL11A eliminates symptoms of sickle cell anemia in animal models by elevating γ -globin levels, with no other observable effect on erythropoiesis, while partial knockdown of BCL11A expression via RNA interference robustly elevates γ -globin levels.

This multicenter Phase I study will investigate the safety and feasibility of transplanting a research participant's own blood stem cells (i.e., autologous hematopoietic stem and progenitor cells [HSPC]) after they have been genetically modified at the BCL11A gene by a zinc finger nuclease (ZFN). Use of the BCL11A-specific ZFN is expected to disrupt the programmed repression of γ -globin, allowing for the production of HbF. To achieve long-term repression of BCL11A levels, mRNA encoding the BCL11A-specific ZFN will be delivered to the HSPC by the process of electroporation. These modified HSPC will then be reinfused (in a single IV administration) into adult subjects with transfusion-dependent β -thalassemia following myeloablative busulfan preconditioning. The protocol calls for sequential enrollment of 10 patients between ages 18 and 40 who currently require at least eight blood transfusions each year.

The ultimate goal of this research is to elevate total hemoglobin in the patient's circulation via reactivation (or derepression) of the previously silent fetal hemoglobin gene to attain lifetime relief from the need for transfusion. Because each subject on the trial will serve as her or his own donor, the investigators hope to avoid the risks of both acute and chronic graft-versus-host disease associated with a traditional bone marrow transplant.

B. Written Reviews by RAC Members

Five RAC members voted for in-depth review and public discussion of the protocol. This protocol was found to warrant further review because of the novel BCL11A target for the zinc finger nuclease that will be used in HSPC. The risks and benefits of this approach for this disease, particularly the data supporting the proposed in vivo function of the transplanted cells, deserve further discussion.

Two RAC members and two ad hoc reviewers provided written reviews of this proposed Phase I trial.

The reviewers identified several potential on- and off-target safety and scientific concerns with use of the modified BCL11A gene, which will be tested for the first time in humans in the proposed trial. Expression of the BCL11A gene is essential for normal B-cell development, as discussed in the protocol. It is surmised that sufficient numbers of normal, unmodified HSPC will persist in the subjects to allow normal immune function reconstitution. Since B-cell dysfunction is possible, however, the investigators should consider assaying for this at the follow-up visits. It is not clear whether the "CBC with differential" assay will test specifically for the concentration of B-cells. The gene to be modified has also been implicated in development of leukemia in both mice and humans. It is therefore important to assess "oncogenic" potential of the SB-BCLmR-HSPC cells. Since BCL11A is a transcription factor that appears to be capable of expressing multiple protein isoforms, it may have other functions yet to be elucidated. Since the protocol will use SB-BCLmR, an mRNA designed to transiently express ZFN specifically targeting BCL11A, there are likely no major "genotoxic" risks or concerns with the vector per se. However, the permanent deletion of BCL11A in the modified cells may be associated with unknown "on-target" and potential "off-target" effects.

Unintended off-target mutations are always a concern with the therapeutic application of a targetable nuclease. The investigators have examined predicted off-target sites and state in their submission that they are performing soft agar transformation assays and an animal preclinical safety assessment study in which a full human cell dose is tested across 150 mice, where each animal receives more than 10 times the human dose. The protocol states that in the planned mouse experiments, if a tumor is found, then the investigators will look for disruption of BCL11A and attribute the tumor to this intervention if such disruption is found. The protocol does not appear to account for tumors that may be found without disruption at BCL11A or whether an off-target disruption could lead to the development of a tumor. In addition, the protocol does not specify whether the modified cells from each patient will be assessed in any way for "oncogenic" potential before transfusion and, if so, which assays will be used for this testing. The description of these experiments seems oriented toward the discovery of solid tumors. Dr. Segal

asked whether the NSG mouse model for evaluation of tumorigenicity would be able to detect non-solid cancers such as leukemia, which would be more likely. The reviewers requested clarification as to whether any of these experiments have been performed and, if so, what results were generated. The investigators should also clarify whether the clinical trial would begin before these results are obtained and whether the clinical trial would be stopped if a particular result were observed.

The investigators stated that the safety and biodistribution of ex vivo modified autologous HSPC using an integrating lentivirus has been documented in a previous study in AIDS-related lymphoma. A reference for this study does not appear to be provided, however. In addition, language describing the prior trial implies that it was a study targeting BCL11A, which does not appear to be correct and needs to be clarified. The investigators explain that SB-BCLmR mRNA is an ancillary product that provides only transient expression of the BCL11A ZFNs and undergoes significant degradation and dilution as the cells divide; therefore, toxicology and biodistribution studies to assess SB-BCLmR or human HSPC safety is not proposed. Dr. Hammarskjöld commented that while the “transient” strategy using mRNA is unlikely to lead to any integration of the vector, there is still the potential for “on-target” toxicity leading to adverse effects, including cancer (as discussed in the protocol). Dr. Tisdale pointed out that the prior trial in humans infected with HIV targeted a terminally differentiated population, not stem cells (as in the proposed trial), which have a higher risk of a leukemic event. In addition, the prior study used a different platform to deliver the ZFN. Thus, the extent to which the prior study provides safety data for the proposed study is limited. Dr. Tisdale also asked whether there are any large animal data regarding this approach targeting CD34+ cells. Dr. Hammarskjöld requested that the investigators address whether these concerns would warrant similar toxicology and biodistribution studies for this trial, as such studies may help to address potential unknown on- and off-target effects.

Dr. Segal asked the investigators to clarify the following statements regarding the predicted off-target site studies reported on page 32 of the clinical protocol:

- “Very low levels of modification (at a frequency below one in 500)” seems inaccurate. In contrast with this statement, data in Table 8 on page 32 of the research protocol show that some modifications occur at up to 8 percent, which is about 10 percent of the on-target frequency, while modifications at two of the three loci were in range above the 0.2 percent suggested by the sentence in the clinical protocol.
- The implication that off-target mutations are so rare that they would not matter also seems inaccurate and misleading. For example, an 80-kg subject would receive 240 million modified cells. Off-targets occurring at even 0.2 percent at three different loci would produce 1.5 million cells with off-target events. If the frequency were as high as 8 percent at one locus, there would be 19 million cells with off-target events, including about 1.5 million with bi-allelic events. If these mutations had a functional effect, they would be of concern even at very low frequencies.
- The locus for which modification was as high as 8 percent is in a DNase I hypersensitivity site, which, according to the UCSC Genome Browser, appears to also be a region of H3K4Me1 histone methylation in some cells, suggesting that this locus, while intergenic, might have regulatory function.

The investigators report that deletion of BCL11A has been shown to increase p53 levels and apoptosis in lymphoid cells. The protocol does not specify, however, whether any assays have been performed or are planned to look at effects on p53 and/or increased apoptosis in SB-BCLmR–modified cells. Other reports indicate that BCL11A is subject to significant alternative splicing, with the potential expression of multiple isoforms. Dr. Hammarskjöld asked whether the investigators have verified that SB-BCLmR will result in the absence of all potential BCL11A protein expression in the modified cells that will be used in this trial.

Per the proposed study design, for the first three subjects, hematopoietic stem cell transplantation (HSCT) for the next subject in the queue will not occur until the previous subject has engrafted with SB-BCLmR-HSPC as determined by hematopoietic reconstitution. The protocol needs to clearly define hematopoietic reconstitution and specify whether evidence of “immune reconstitution” will be required before the subsequent subject can be transfused.

A statement in Appendix M (page 7) regarding the impact of knockout of BCL11A needs to be revised. The statement reads, “Knockout of BCL11A eliminates symptoms of sickle cell anemia in animal models via elevating γ -globin levels, with no other observable effect on erythropoiesis (Xu et al., 2011).” In these studies, a complete knockout of BCL11A was shown to be embryonic lethal. Thus, conditional knockouts using EpoR CRE were generated, and this is the model in which the referred studies were done. The statement should thus be clarified as it is misleading as written. In addition, Appendix M (page 40) includes the statement, “No SB-BCL specific effects on erythroid cell growth have been observed...,” even though deletion of BCL11A prevents B-cell development.

The reviewers found the consent to be uneven and technical in many places. Part of the document appeared to be beyond the recommended sixth- to eighth-grade reading level. Dr. Hammarskjöld found the ICD to be overly long and redundant. The investigators should try to rewrite the document to be simpler, less redundant, and more comprehensible.

Dr. Hammarskjöld made the following specific comments and suggestions regarding the ICD:

- Under “Possible Side Effects of SB-BCLmR-HSPC,” the reference to “the most common side effects so far” implies that SB-BCLmR-HSPC has been used previously in humans. This is potentially very misleading, since this is the first-in-human trial of this agent.
- .
- The statement, “Because of the unknown possible effects of the SB-BCLmR-HSPC, there could be serious harm to unborn children or children who are breastfeeding,” could be confusing, because there are also the “known” potential risks of the busulfan conditioning that is part of this protocol. This statement should be revised for clarification.

Dr. Zoloth made the following additional comments and suggestions regarding the ICD:

- In the description of the protocol, the risks associated with interfering with B-cell production are described, but this is not explained to the patient in the ICD.
- The consent must state and explain very clearly that the proposed study is an experimental, Phase 1 trial. Dr. Zoloth commented that the start of the consent form includes one of the best statements about this issue seen to date and commended the team for this language. However, the document subsequently speaks about the possibility of this approach being able to cure the disease. While it is reasonable to be optimistic, the goal of clinical equipoise needs to be consistently stated.
- Certain technical terms were not clearly defined, for example, the term “zinc finger”.
- The ICD should explain that the drugs used in the protocol are chemotherapy drugs and that taking these drugs will make them sick, just like in cancer treatments.
- It must be made very clear to patients that the cells being called stem cells are not the human embryonic stem cells that they may have heard about. The difference between these types of stem cells needs to be clearly explained.
- It is not clear what is meant by the statement that participants can “leave the study at any time.” The fate and impact of the modified stem cells given to participants are not known, except in mice. Additional details such as how long these cells will survive in the body need to be provided, and the phrase “leaving the study” needs to be clearly explained.
- The section on what happens if a subject is injured or “sick” is vague. Specific details are needed to explain what is meant by the statement that medical care “will be available,” including whether this care will be paid for under this study and whether—and, if so, when—the patient could incur costs. The entrance to the care seems to be determined by the study doctor, which is not adequate and presents a clear conflict of interest for a private company.

Dr. Tisdale noted that the consent could better describe the experimental part of the trial (ZFN modification) versus the routine aspects of the study (autologous transplantation). The risks of ZFN treatment of cells should be more clearly articulated and should be moved to the risks sections of the consent document. In addition, the risk section should indicate the likelihood of potential or anticipated adverse events or side effects rather than simply listing these events.

Dr. Segal noted that “dose” is defined as the number of modified cells used to treat the patient ($\geq 3.0 \times 10^6$ cells/kg).

Gamete cryopreservation will be offered for the myeloablative treatment, but it is not clear who will pay for this treatment if the offer is accepted.

Appendix M, Section IIA-2-b, should identify any alternative methodologies for the delivery of ZFNs to CD34+ HSPC other than via recombinant nucleic acids. The authors should discuss protein-based delivery of ZFNs, which might have similar benefits, such as transient expression and no viral vector or viral DNA. Dr. Segal cited two references by Gaj and colleagues to support this suggestion.

Dr. Segal identified a number of additional minor concerns and typos in the clinical and research protocols and the ICD.

Dr. Tisdale noted that work done to date in vitro and in the xenograft supports the safety of the proposed approach. However, the protocol does not describe any secondary transplant studies during which the stressed hematopoiesis may unveil abnormalities not noted during the brief follow-up of the xenografted animals. While animal testing does not always offer the best way to determine the safety of an intervention, the results are not sufficiently detailed in the protocol to have confidence in the data. To address these issues, Dr. Tisdale requested information on the number of animals followed in the preclinical studies conducted thus far, whether all transplanted animals were alive during follow-up, and the procedure used to evaluate animals that died during the studies. The investigators should also consider adding information on secondary transplants and specify whether secondary transplants will be performed and whether (or which of) these studies will be performed before applying for an IND.

Dr. Tisdale noted that much of the preliminary data on HbF is expressed as a ratio or is normalized to another parameter, making it difficult to determine the likelihood of therapeutic efficacy. Furthermore, there are stringent methods for growing erythroid cells that do not promote endogenous HbF expression. If 80 percent of progenitors are disrupted, it seems that erythroid cells derived from these progenitors should be expressing HbF at levels detectable by HPLC or other quantitative methods. Dr. Tisdale commented that the overall levels achieved are more important than the percentage of cells that have disruption or make some HbF.

The protocol states that CD34+ cells will be expanded after electroporation, but clarification is needed as to how this will be accomplished and whether there are any data to support that this expansion will have no detrimental effect on engraftment.

In the follow-up of patients post-transplant, HbF levels will be monitored. It is known that HbF levels can increase after transplantation without disruption of BCL11a. The protocol should delineate how HbF levels will be correlated with BCL11A disruption during follow-up.

Dr. Tisdale noted that the inclusion criterion of transfusion dependence is defined as more than eight transfusions per year, which will likely include thalassemia minor. However, the long-term risks attributed to the disease that justify entry into the protocol derive from thalassemia major patients. The investigators need to clarify whether thalassemia minor patients will be eligible for this protocol.

C. RAC Discussion

During the meeting, the following additional questions, concerns, or issues were raised by RAC members:

- The reviewers noted that many of their comments and suggestions were similar. During the discussion, they focused on issues for which further clarification was needed.
- The rationale for the proposed research is supported by both preclinical and clinical data presented by the investigators. In addition, given that there is no curative treatment for thalassemia except for bone marrow transplant, which is associated with serious risks, studying an alternative intervention with therapeutic potential is warranted. The investigators have

prepared for potential expected and unexpected complications that could develop in this first-in-human trial involving perturbation of BCL11A using a gene transfer approach.

- The investigators have provided evidence indicating that there is no particular risk with the proposed modification of BCL11A. However, the research is potentially complicated by BCL11A levels and function and any unknown deleterious effects of the deletion (e.g., if modified by other genes). Several pre-IND studies are planned and/or are in progress to address many of these issues, including increased risk of leukemia and increased p53 and apoptosis with BCL11A perturbation. As the investigators stated, results of these studies will be available before the clinical trial is initiated.
- Dr. Hammarskjöld noted that information in Appendix M regarding the impact of knockout of BCL11A was revised as recommended to clarify that elimination of symptoms in animal models, in association with elevated fetal globin levels, occurred only with an EPO-specific deletion.
- In light of data suggesting an interplay between BCL11A and p53-induced program cell death, which would require overexpression of BCL11A, Dr. Hammarskjöld cautioned that making a small deletion and targeting an exon could result in splicing differences that generate unintended products of BCL11A. She asked whether the investigators will look at (or have considered looking at) what happens to mRNA expression from BCL11A either in healthy volunteers or patients. She suggested that the team assess whether there is a potential for protein expression with the RNAs obtained.
- For the first three subjects, the investigators will not dose a subject until the previous subject has engrafted, as determined by hematopoietic reconstitution; if the first three subjects engraft, then the subsequent seven subjects in the study will be treated without waiting. Dr. Hammarskjöld inquired further about how hematopoietic reconstitution will be defined for this trial.
- Dr. Tisdale asked whether the tumorigenicity study includes a positive control (e.g., disruption of a known tumor suppressor gene in the mouse model) to ensure that the planned assays are able to detect off-target effects of the investigational product.
- Dr. Tisdale noted that while the consent is technically accurate, the details regarding the experimental versus standard interventions are somewhat overwhelming and may not be clear to participants. For example, mobilization and conditioning with busulfan and related side effects, including potential for infections, are all routine with any autologous transplantation and could be presented to explain that these types of transplants have been done for clinical purposes for many years and that the side effects are well known (and then list the side effects and risks). The consent could then describe the experimental aspect of the study, including the disruption of BCL11A in the hematopoietic stem cells and the inherent risks of this procedure. Participants should be informed as to what is different with respect to the planned experimental intervention in comparison with the routine clinical aspects of the trial. The other reviewers agreed that participants need to understand that not all of the risks of the research intervention are known and that this needs to be clearly spelled out in the consent.
- Dr. Segal requested that the investigators further clarify how results of the pre-IND and the IND-enabling studies will be used. For example, it isn't clear who will evaluate the results and make a decision as to whether to proceed or not (i.e., the investigators, the RAC, or FDA).
- Dr. Zoloth asked why umbilical cord blood transplantation, which was mentioned during the presentation but not elaborated on further, is not a possible treatment for this patient population.
- Dr. Sadelain inquired about the efficacy of the study intervention in balance with the risk-benefit analysis in this patient population given the concerns about off-target effects. Among those concerns is the potential of administering too few stem cells that have been disrupted at both alleles, in which case there would be no chance for success.
- Dr. Sadelain also asked about the expected rise in the expression of fetal globin, specifically the amount expected to be induced with proper disruption at both alleles and in relation to patients selected for this trial. Although the transfusion-dependence criterion is more than eight transfusions per year, it is not clear whether the planned intervention would be sufficient for individuals with β^0 -thalassemia or whether testing should be done in a subset of patients with β^+ -thalassemia, in which the anticipated gain in fetal globin expression might be therapeutic.
- Additional information was requested regarding how the level of gene disruption (61 to 76 percent) translates in a bi-allelic knockout, how many of these disruptions are truly functional

knockouts, and whether some have in-frame open reading frames and thus do not disrupt the gene functionality. In addition, further clarification was requested regarding the fraction of hematopoietic stem cells or CD34+ cells with long-term reconstitution potential in which double copy disruption can be achieved.

- Given the potential adverse outcomes with the BCL11A knockout, the investigators were asked about disrupting the binding site for BCL11A at the globin gene as an alternative strategy. A potential complication of this approach, however, is that by knocking out the binding site, the extension of the genes might also be knocked out.
- The investigators were asked to explain why myeloablative conditioning is required instead of using reduced intensity conditioning, and to specify the impact of this process on reproductive capacity and the risk of death with the transplant procedure.
- Dr. Zoloth advised that all reproductive implications, including provisions for in vitro fertilization (IVF) and why modifying enrollment in the protocol has implications for pregnancy and nursing, be fully explained in the consent. This discussion needs to be written at the target sixth- to eighth-grade reading comprehension level. The investigators should consider having an actual 6th grader read the revised consent for comprehensibility and clarity. Dr. Zoloth noted further that many people may not know that there are different types of stem cells or understand the difference between human embryonic stem (ES) cells and hematopoietic stem cells. As a result, they may mistakenly presume that all stem cells are human ES cells. These distinctions need to be clearly explained in the consent to assure that participants are properly informed, particularly given that individuals may have different views regarding the use of embryonic stem cells.

D. Investigator Response

1. Written Responses to RAC Reviews

The investigators clarified that individuals with thalassemia minor and β -thalassemia intermedia will not be eligible to participate in this trial. In determining eligibility criteria for this study, the investigators chose to focus on patient phenotype rather than genotype. The requirement for more than eight transfusions per year will make patients with β -thalassemia major solely eligible for the trial. Patients with thalassemia minor exhibit symptoms equivalent to the trait condition and can have minor anemia but typically do not require multiple transfusions per year. Similarly, patients with β -thalassemia intermedia will be excluded as these patients do not require more than eight transfusions per year.

Hematopoietic reconstitution or engraftment is defined as having an absolute neutrophil count (ANC) of more than 500/ μ L for 3 consecutive days and a platelet count of more than 50,000/ μ L for 3 consecutive days and at least 1 week after the most recent platelet transfusion. Subjects who do not engraft by day 42 will be transplanted with the backup unmodified CD34+ cells. Treatment is staggered for the first three subjects so that the previous subject must be engrafted with SB-BCLmr-HSPC before HSCT for the subsequent subject. Immune reconstitution after autologous transplant may take 6 months to 1 year (independent of BCL11A modification) and will be tracked quantitatively and qualitatively at specified time points in the study.

The investigators agree with the reviewers that a clinical dose of the cell product ($>3 \times 10^6$ cells/kg) will contain cells harboring both on- and off-target modifications and that unintended mutations are a key concern of the proposed research. Low-frequency off-target events will be present in a considerable number of cells (per patient dose), and understanding the functional consequences of off-target activity is important, even if present at a relatively low frequency. Direct high-throughput sequencing of potential off-target sites is done through a procedure called SELEX and begins with *in vitro* recognition of the specificity of the zinc finger. A benefit of site-specific gene modification is that the off-target sites are not random (as might be the case for a traditional viral vector approach) but occur at a limited number of sites that share substantial homology to the intended target (e.g., the three known off-target sites identified by SELEX for the ZFN targeting BCL11A). Assessment of the functional consequences of the known off-target sites as well as any potential additional off-target sites that were not identified by using the SELEX-based approach will be done under the tumorigenicity study, which is evaluating a clinical-scale lot of ZFN-modified cells from multiple individual donors. This scale of safety assessment ensures that a

representative number of both on- and off-target genome modification events are placed on study. All studies that aim to evaluate the potential risk of off-target mutations (including the tumorigenicity study) will be fully reported in the IND filing. Neither IND filing nor clinical trial initiation will occur before completion of these studies. Secondary transplants will not be performed. The poor chimerism (less than 1 percent) in secondary transplant recipients when using the clinically relevant mobilized adult human HSPC as the cell source for the primary transplant (independent of whether they have been edited with ZFNs) markedly reduces the number of cells that could be evaluated and thus limits the utility of secondary transplants for the assessment of tumorigenicity.

The goal of the tumorigenicity study is to determine whether the cell product, SB-BCLmR-HSPC, harbors cells with tumorigenic potential. This study evaluates a clinical-scale lot of modified cells from multiple individual donors and thus places a large number of modified CD34 HSPC on study in primary-recipient NSG mice. Ensuring that sufficient numbers of cells are studied was the primary goal of the team's safety assessment strategy. The IND-enabling tumorigenicity study, which is currently in progress, is following 114 animals in the control group and 163 animals in the ZFN-treated group. All of the animals on study have been transplanted, with a total of 114 million cells infused in the control group and 163 million infused in the ZFN-treated group. Unscheduled deaths are treated per an IACUC-approved protocol. All animals that die during the study undergo a complete macroscopic postmortem examination, after which bone marrow is analyzed by Giemsa staining and genotyping, and a comprehensive histopathology assessment on all major organs (brain, heart, lung, liver, lymph nodes, ovary, adrenal gland, and skin) is performed. For tumors assessed to be of human origin, the target locus, BCL11A, will be genotyped to determine whether the origin of the tumor can be traced to the intervention. The investigators note that while it is theoretically possible that an off-target disruption could lead to the development of a tumor without a concomitant disruption of BCL11A, it is mechanistically highly unlikely. Therefore, if a tumor of human cell origin is identified but is unmodified at BCL11A, an analysis of a panel of off-target loci to determine whether the event was related to the treatment will be run.

The investigators agree with the reviewers that risk of a leukemic event is higher with a cell product that uses stem cells versus a terminally differentiated cell and that the delivery platform may also alter the risk profile. For this reason, the preclinical safety assessment program for SB-BCLmR-HSPC is modeled on a more advanced program that uses targeted disruption of the *CCR5* gene in human HSPC following mRNA electroporation. This program has been reviewed both by the FDA Office of Cellular Tissue and Gene Therapies (in a pre-IND meeting) and by public review at the NIH RAC (September 13, 2013). A CBC with measurement of absolute numbers of neutrophils, lymphocytes, and monocytes will be performed at sacrifice. This will detect total numbers of cells and will indicate whether leukemia is present. Histopathology will detect the source of the leukemia or lymphoma in the bone marrow and lymphoid organs and provide histologic features of cellular phenotype and differentiation. Further characterization can be done on frozen sections and extracted DNA from these hematopoietic tissues. Overexpression of BCL11A has been observed in both human leukemias and mouse models of cancer. The planned approach (targeted BCL11A knockout) is engineered to downregulate rather than upregulate BCL11A function. Whether an inadvertent upregulation of BCL11A nonetheless occurs will be assessed in the tumorigenicity study in NSG mice.

The preclinical efficacy study described in Appendix M followed five animals in the control group and nine animals in the group engrafted with cells bearing a disruption of BCL11A at exon 2. All transplanted animals were alive until their scheduled termination (up to 28 weeks post-administration).

The team has completed three additional preclinical studies at Jackson Labs using human HSPC bearing more than 50 percent targeted disruption at exon 2 of BCL11A. In total, these studies followed 34 control animals and 54 animals engrafted with ZFN-modified cells. No difference in unscheduled deaths was observed between the two groups.

Studies in a nonhuman primate (NHP) model could, in principle, be a useful way to assess the consequences of BCL11A disruption. To date, however, no such studies in large animals have been conducted. To address this question, the investigators have an active research program with the goal of determining conditions for gene modification and robust engraftment of NHP-derived CD34+ HSPC in the

NHP model. Such a study would not assess the human cell product itself. Rather, the xenogeneic NSG human CD34 engraftment model is used because it provides time in the correct hematopoietic niche to evaluate oncogenesis, engraftment, and differentiation of the human BCL11A modified CD34 cells manufactured exactly per the methods supporting the clinical trial. In addition, this model also allows for evaluation of cell products from multiple donors and for testing of a large number of modified human cells (reaching the size of a clinical lot). Finally, it is noteworthy that in humans, hemizyosity for a naturally occurring allele of BCL11A that lowers its expression by 40 percent is not associated with any effect in erythropoiesis other than a marked elevation of fetal globin, and hemizyosity for a BCL11A knockout in humans is not associated with any known hematopoietic abnormality.

The target gene product is required for B-cell-poiesis, as noted by the reviewers. The investigators anticipate that following myeloablation and transplant in the planned trial, B cells will be provided by both unmodified and heterozygous cells in the infused cell product and the subject's own cells. In support of the former product, in preclinical NSG mouse studies, comparable B-cell human chimerism was observed in animals engrafted with control cells and animals engrafted with cells bearing a BCL11A disruption; however, lower marking at BCL11A was observed in the B-cell compartment. B-cell functional reconstitution will be monitored during follow-up.

In response to the paper cited by Dr. Hammarskjöld (Yu et al., 2012), which reports an effect of BCL11A on p53 signaling in lymphoid cells, the investigators note that in the proposed trial, following transplant, the unmodified cells as well as the monoallelically disrupted CD34+ HSPC are expected to sustain normal lymphopoiesis. This anticipated outcome is supported by data showing similar growth kinetics during in vitro erythropoiesis of control and SB-BCLmR-modified cells without loss of edited alleles during an 18-day culture and expansion period. Comparable chimerism is seen in NSG mice engrafted with control and SB-BCLmR-modified cells, including absolute numbers of human CD19+ cells; in these experiments, modified cells have persisted in NSG mice for up to 28 weeks (the longest time assessed). These data suggest that no significant activation of programmed cell death pathways occurs in ZFN-modified cells (outside the context of lymphocytes, where, as the reviewer points out, BCL11A is required).

The investigators recognize that the BCL11A gene locus encodes multiple isoforms and may have as-yet-unknown functions. In the context of hematopoiesis, a full knockout of BCL11A has well-studied effects, which are addressed in the preclinical assessment and proposed clinical follow-up plan. In the context of erythropoiesis, a BCL11A knockout has no known effects beyond a dramatic increase in fetal globin. The naturally occurring human variation in BCL11A that controls fetal globin levels that the reviewer alludes to is the one that the investigational product is engineered to mimic. The investigators note further that exon 2, which is included in all known BCL11A isoforms, is targeted in the BCL11A disruption. Thus, out-of-frame deletions induced by the ZFNs at this position are expected to generate a knockout of all isoforms.

The investigators have assayed HbF levels following in vitro erythroid differentiation by using two distinct HPLC-based fractionation strategies. Results with reverse phase HPLC (which assays individual globin chains) indicate that γ -globin represents 34.9 percent of all β -like globins. Results using the cation exchange HPLC (which measures hemoglobin tetramers) indicate that HbF represents 43.1 percent of all hemoglobin tetramers. In the in vitro differentiation system, higher than normal levels of HbF are observed in control cells, but these levels never exceed 5 to 10 percent by either assay. These data are summarized in table form in the investigators' written response.

The investigators note that with very rare exceptions (four subjects reported out of several thousand transplanted), HbF induction after myeloablative therapy in clinical allogeneic HCT is not sustained. Thus, a transient induction of HbF post-transplant should be distinguishable from a sustained level of expression following BCL11A modification during a 2-year follow-up period. The levels of BCL11A disruption will be determined by deep sequencing the ZFN target sites in BCL11A across distinct cell lineages obtained from peripheral blood and bone marrow of trial participants.

Regarding quantification of transcript levels after ZFN treatment, the investigators explained that their approach interferes with BCL11A function at the DNA level by inducing small insertions and deletions (indels) in the BCL11A gene. These indels at the DNA level are copied into the BCL11A mRNA, where

they result in frame shifts that encode truncated peptides of about 50 amino acids (the wild-type BCL11A protein has 835 amino acids). Because the promoter of BCL11A is not affected by this approach, nascent transcript levels are not expected to change. For a subset of human genes (about 25 percent in a recent comprehensive assessment), spliced mRNA bearing a premature stop codon is degraded via a process called nonsense-mediated decay (NMD). The investigators have not detected any statistically significant decrease in BCL11A mRNA levels following ZFN treatment, suggesting that the BCL11A gene belongs to the approximately 75 percent of human genes that escape NMD.

With respect to the levels and frequency of modification, the investigators note that it is important to place the off-target effects of a site-specific technology (such as ZFNs) in context of the state of the art in the field. Classical gene therapy approaches to β -thalassemia involve the transduction of autologous HSPC with a transgene-bearing lentivirus. Lentiviral vectors must integrate into the genome to achieve any therapeutic effect but do so in a semi-random fashion in the genome, exhibiting a bias for transcribed regions. Per a recent report on this approach presented at the June 2014 European Hematology Association Meeting, the vector copy numbers (i.e., numbers of transgenes per cell) in two treated subjects were 1.5 and 2.1, respectively. Thus, every single cell genetically modified to provide therapeutic benefit bears, on average, approximately two randomly integrated transgenic constructs. By definition, since lentiviral integration cannot be controlled, every transgenic cell in the product bears two off-target events—typically integrations into a transcribed gene. Analysis of the peripheral blood in subjects reveals more than 2,000 unique integration events. By comparison, when ZFNs has been used to disrupt BCL11A, 60 percent to 80 percent on-target disruption is attained, and a small number of off-target cleavage events are observed at a limited number of sites that share substantial homology to the intended target (e.g., at the three known off-target sites identified by SELEX). When placed in the context of current approaches, the investigators propose that the ZFN strategy possesses very low levels of “off-target” modification.

The investigators acknowledge that the site-specific gene modification approach offers an important advance over classical viral vector approaches, and they recognize the importance of understanding the functional consequences of off-target activity (even if present at a relatively low frequency). Assessment of the functional consequences of the known off-target sites (as well as any potential additional off-target sites that were not identified using our SELEX-based approach) will be done under the tumorigenicity study, which, as described above, is evaluating a clinical-scale lot of ZFN-modified cells from multiple individual donors. This scale of safety assessment ensures that a representative number of both on- and off-target genome modification events are placed on study.

All engineering runs used to support the safety of SB-BCLmR-HSPC will be performed per the GMP manufacturing process. Characterization will include molecular assessment of on- and off-target activity, in vitro soft agar transformation assay, in vitro cytokine-independent growth assays, and a clinical-lot-scale in vivo tumorigenicity study in immunocompromised NSG mice with full histopathology examination as described above. Each clinical lot will be released according to the Certificate of Analysis for SB-BCLmR-HSPC (including sterility, purity, viability, etc.). This testing will not include a patient-specific assessment of the “oncogenic potential,” because this type of assay would consume the majority of the cells and thus preclude dosing the subject. The clinical cell product that will be used to treat study participants can, at the present state of the field, be assessed in an immunodeficient mouse model. The model has well-established limitations (e.g., no erythroid progeny of human HSPC are found in the peripheral circulation of the animals). With respect to assessing both potential on- and off-target toxicity, however, the team has engrafted a clinical lot of ZFN-treated cells into NSG mice with the goal of determining whether ZFN action has any unwanted consequences on the cells.

The investigators anticipate that the proposed study will adequately determine whether off-target modification of the specified sites (as identified in Appendix M) has any functional consequences affecting the safety of the product.

The investigators consider protein-based delivery to be a promising (albeit early) nuclease delivery modality. The discussion in Appendix M is limited to methodologies that, in the investigators’ view, are ready for clinical application; thus, protein-based delivery is not included at present.

Regarding the rate of efficiency of the ZFN knockout of human BCL11A, the maximum level of modification observed (as stated in the protocol) uses the “up to” qualifier to define the upper limit for considerations of on-target toxicity.

No minimal acceptable amount of modification has been set in the proposed clinical protocol. The investigators note that the relationship between level of marking in the cell product, long-term marking post-engraftment observed in the patient, and levels of HbF represent a key endpoint from the Phase 1 trial. Variations in marking levels are expected and represent an important component of early-stage clinical efforts. As a draft guideline, the investigators will not condition or transplant a patient if the product has fewer than 1×10^6 CD34+ cells after modification per kilogram of body weight. More than 50 percent modification has been reproducibly observed in manufacturing runs at clinical scale, which would be sufficient to exceed this threshold.

The study sponsor, Sangamo BioSciences, Inc., will generate a clinical trial agreement with the clinical study sites that will include resources to conduct gamete cryopreservation. This option will be offered at no cost to study subjects. At the Oakland site, subjects who pursue this option will be referred to the UCSF Fertility Center, where this service has been established for other clinical studies.

The reference for the HIV study using a ZFN to disrupt CCR5 in T cells was provided. The language will be reworded to ensure that the description of this study does not refer to a ZFN-driven modification of BCL11A.

The investigators agreed with the other minor recommendations regarding the protocols and ICD and have modified the proposed documents accordingly.

2. Responses to RAC Discussion Questions

The investigators have assessed mRNA expression from the modified BCL11A, which fails to show any downregulation of BCL11A mRNA following treatment with the ZFNs. To generate the product used in the preclinical experiments (and for the planned trial), the investigators take CD34 cells from G-CSF-mobilized volunteers, purify them, electroporate them at the clinical scale with the ZFN that cut BCL11A, and then perform in vitro erythropoiesis, which is an 18-day process. At the end of this process, testing is done to determine whether or not fetal globin has been elevated using a range of analytical techniques. Results of these analyses show, for example, that with this BCL11A knockout, fetal globin mRNA is increased 8.1-fold after 18 days of erythropoiesis and fetal globin (upon disruption of BCL11A) is markedly increased, accounting for between 35 and 43 percent of all hemoglobin. Dr. Urnov, the project leader on this study, noted that this result is very reproducible. Researchers who work on NMD were consulted, and the consensus from those investigators is that between half and three-quarters of the mRNAs produced by the human genome resist being degraded by that pathway. Thus, the simplest interpretation of these findings is that the BCL11A locus encodes a messenger RNA that is resistant to NMD. Dr. Urnov commented that the study investigators have not looked to see whether any alternative splicing pattern could generate a protein, nor have they looked for any such proteins. The team will consider this analysis as suggested. Dr. Urnov added that the team has not directly looked at the effects on p53 and increased apoptosis in the modified cells. He noted, however, that in in vitro erythroid cultures in which cells expand by several logs, there is no statistically significant difference in the growth rate between control cells and cells bearing the BCL11A knockout, suggesting that at least in this setting, there is not a pronounced activation of p53. In addition, marked cells have been observed in the periphery of immunodeficient mice to the longest time studied thus far, 28 weeks.

Dr. Walters explained that the study will use a standard definition of hematopoietic reconstitution and stagger the first three subjects, pending results in the prior subject. Specifically, for this determination, the ANC must not be greater than 500 by 42 days after the infusion, at which time the unmodified cells will be thawed and reinfused, and the subject will be scored as having delayed or inadequate engraftment.

Regarding allelic function and globin levels in affected patients, Dr. Urnov described two pediatric patients who are heterozygous for a full knockout of BCL11A. These patients have had neurological abnormalities since birth due to a requirement of BCL11A in the central nervous system, but neither has any detectable hematological or immunological abnormality. The only blood-related symptom for these two patients is a dramatic and sustained elevation of fetal globin (16 and 24 percent, respectively), which is about 25 percent of the total hemoglobin level in a non-affected individual. In addition, there is a natural and somewhat prevalent allele that knocks down BCL11A by 40 percent and that appears to have no effect other than a sustained elevation of fetal globin levels, which is known to be disease-protective for individuals with thalassemia.

Results of in vitro testing with the investigational product (using two HPLC approaches as described above) consistently show fetal globin comprising one-third to 43 percent of all hemoglobin. Baseline levels of fetal globin are typically higher in these in vitro systems, however. For example, in most unaffected adults, fetal globin constitutes about 0.5 percent to 1 percent of total hemoglobin. In in vitro erythropoietic systems, the level is usually around 5 percent but can be as high as 10 percent. Dr. Walters added that with a small population of erythroid progenitors that can be properly immunoglobulinized (i.e., make a normal level of β -like globin), those cells will be enriched and confer a benefit beyond what would be seen in an individual who has normal hemoglobin genes. Thus, with enrichment of the corrected cells, the globin levels should be higher than what is observed in healthy volunteers.

The investigators have studied the percentage of a functional knockout and the fraction of cells that have one versus two alleles of BCL11A edited. In brief, of all the alleles that the ZFN generate in CD34 cells, 80 percent are small insertions and deletions that disrupt the reading frame. Thus, about 20 percent are in frame. The investigators posit that that even with the loss or gain of a few amino acids at that specific position of BCL11A, functionality is retained. Per these findings, 80 percent of the alleles of BCL11A in the investigational product are functional knockouts. Using a methylcellulose followed by direct genotyping they determined that about three-quarters of the colonies are marked on both alleles of BCL. About 20 percent of the colonies are heterozygotes and the remaining are wild-type. The range of modification in the investigational product is between 60 and 75 percent. These experiments were done with cell samples on the higher end of that marking spectrum. In experiments looking at mRNA-based delivery of ZFN to presorted populations of stem cells versus the full population, there was no significant difference in the level of cleavage in the two populations. However, with the introduction of more elaborate modifications to the genome in human hematopoietic stem cells, the cleavage activity was found to be robust in both the general cell population and the selected long-term hematopoietic stem cells.

Dr. Urnov commented that the elimination of the target site of BCL11A from the fetal globin loci is an elegant and attractive proposition because that approach would disrupt the specific molecular circuit that is being targeted, that is, the repression of fetal globin genes by BCL11A. However, the specific position within the beta-globin region that is necessary for the silencing effect that BCL11A exerts on its target has not been mapped yet. Without that information, the target region is too big to be eliminated, given the existing methodology.

The tumorigenicity study does not include a positive control. However, the duration of the study is based on transplants into the NSG mouse model, which showed evidence of tumors in other studies, and is expected to provide sufficient time for tumor development. One of the reasons for the extended time in preclinical studies was to allow for presentation of B cells to determine which cells or products gave rise to both solid and liquid tumors within that time frame. These studies do not test the type of mechanism of gene disruption used in the proposed trial, however.

The study investigators and sponsor will initially evaluate results of the preclinical studies and then file an IND application with the FDA. The FDA will review the submission over a 30-day period and contact the investigators if there are any questions. Two pre-IND meetings have already been held with the FDA team to discuss the proposed research, with a focus on the toxicology and safety of the experimental

intervention. Thus, while review of the data will be a joint effort, the final decision as to whether the research will proceed will be made by the FDA.

Dr. Walters explained that umbilical cord blood collection contains an order of magnitude fewer cells than is contained in a typical marrow harvest and that most of these umbilical cord blood units, in turn, were HLA mismatched to the recipient. Because of the HLA disparity and the small number of stem cells, there is a high probability that patients would reject the grafts, making this approach likely to fail.

Myeloablation was chosen based on how subjects will be selected for this protocol and how the study is structured. Most patients will be relatively healthy young adults, and those who are at high risk for untoward toxicity from the myeloablative dose of busulfan will be excluded. The incremental toxicity at a half dose versus a full dose of busulfan is not substantial and needed to be taken into consideration with the proposed plan. In addition, the investigators wanted to avoid exposing the endogenous hematopoietic stem cells to an alkylating agent. Ablating the cells reduces the possibility of long-term tumorigenicity related to the busulfan exposure and pushes the balance in favor of engrafting the modified cells. Increasing the dose of busulfan is not expected to impact reproductive capacity, but the sponsor is providing gamete storage before commencing therapy at no expense to the patients to accommodate any concerns.

The risk of death from the transplant procedure is estimated to be less than 5 percent, and most likely between 1 and 3 percent based on autologous transplantation in patients in the similar age range as the planned cohort.

Regarding the eligibility criteria, it might appear that those who have thalassemia intermedia or a higher baseline hemoglobin level might make better enrollees, because a smaller increment in hemoglobin might still yield a clinical effect. However, the investigators have not been able to identify a biological difference between the planned cohort and other thalassemia patient groups in which any increment in the hemoglobin would be beneficial to both.

The PI noted that the draft consent describes the collection and long-term storage of gametes (sperm and eggs) for future use but may not include all reproductive implications. Information on possible coverage of IVF for future pregnancies will need to be added to the consent.

E. Public Comment

No comments from the public were offered.

F. Synopsis of RAC Discussion and RAC Observations and Recommendations

- You have examined the impact of disruption of BCL11A gene on mRNA levels and have found the mRNA level to be unchanged even after disruption of the BCL11A gene. Given this finding, it would be important to look at both the specific mRNAs expressed, and splice isoforms, as well as potential protein(s) translated from those mRNAs.
- Deletion of BCL11A has recently been shown to increase p53 levels and apoptosis in lymphoid cells [see Yu et al., J. Exp. Med. 209:2476 (2012)]. Consider examining whether there is any change in p53 levels in the cells that have been modified by the BCL11A specific ZFNs.
- The informed consent reviewed by the RAC was quite lengthy, and the terminology was complex. You have stated that you intend to revise the consent so that it is at a Grade 6–8 reading level. In addition to simplifying the language, the following changes would improve the document.
 - Research participants will undergo hematopoietic stem cell transplant using myeloablative conditioning followed by administration of the ZFN modified autologous hematopoietic stem cells. The section on risks in the informed consent document should be reorganized to separate the risks that are due to the stem cell transplant protocol, which are known risks based on past experience, and the risks that are due to the experimental approach, i.e., the

gene modified stem cells. Such a re-organization should improve the subject's understanding of the experimental risks.

- There have been several previous gene transfer trials for β -thalassemia using integrating vectors. Some research participants may be familiar with these previous trials, for example, through information provided on websites for patients and families. Since your protocol is using an mRNA approach without an integrating vector, it will be important to not only describe the approach but perhaps include some information on the differences between this gene transfer approach and others that patients may be familiar with.
- The consent uses the term "blood stem cells" in some places and in others, "stem cells." In order to avoid any confusion as to the source of stem cells, consider consistently using the term blood stem cells. In the media, stem cell research is sometimes used interchangeably with embryonic stem cell research.
- The informed consent document speaks about leaving the trial, but as this is a gene transfer approach and the modified cells are designed to persist, this concept of leaving the trial needs to be clarified. Consider adding language such as the following to the informed consent: "If you decide to withdraw from the study, the gene modified cells cannot be removed from your body and can potentially persist indefinitely. By withdrawing from the study, you are withdrawing from further monitoring."
- The busulfan conditioning that will be given prior to infusion of the gene modified cells may affect fertility and therefore there is a plan to offer collection and storage of gametes in this protocol. This needs to be explained in the informed consent, together with some information about the potential risks and costs of future artificial reproductive services.
- The section titled "What if I Get Hurt or Sick While I am in this Study?" could be further clarified. The first sentence does state that the sponsor will pay the costs of reasonable medical treatments as long as the participant followed directions and the participant's health plan will not pay those costs. The next paragraph states that study-related injuries or illnesses might not be covered by a health plan and then goes on to state that "medical treatment will be available." It is not clear whether "available" as used in that context means payment is also available.

G. Committee Motion 2

Dr. Hammarskjöld summarized the RAC recommendations to be included in the letter to the investigators, expressing the RAC's comments and concerns. Dr. Kohn asked for a vote on these summarized recommendations, which the RAC approved by a vote of 10 in favor, 0 opposed, 0 abstentions, and 4 recusals.

IV. Review and Discussion of Human Gene Transfer Protocol #1405-1313: A Phase I Trial of the Safety and Immunogenicity of a Multiple-Antigen Vaccine (STEMVAC) in Human Epidermal Growth Factor Receptor 2 (HER2)-Negative, Advanced-Stage Breast Cancer Patients

PI: Mary (Nora) Disis, M.D., University of Washington School of Medicine

RAC Reviewers: Dr. Chatterjee, Dr. Pilewski, and Ms. Hardison

A. Protocol Summary

It is estimated that one in eight women will be diagnosed with breast cancer at some point in her lifetime. For women with metastatic breast cancer, new approaches are especially needed for the delay or prevention of relapse. Vaccines that target pathogens have been one of the most successful interventions used in humans to prevent disease. Infectious disease vaccines have been effective because the proteins in the pathogen can elicit a destructive immune response. Studies in breast cancer have identified specific proteins in the tumor that can induce immune responses in breast cancer patients. For example, biologically relevant epithelial-to-mesenchymal transformation (EMT)-associated proteins that are involved in breast cancer initiation and cancer stem cell-like morphology have been identified. The

investigators hypothesize that a tumor vaccine targeting these proteins could delay or prevent disease progression in women with limited treatment options and who have a high likelihood of recurrence.

The five antigens selected for this multi- vaccine (STEMVAC) trial, CD105, YB-1, SOX2, CDH3, and MDM2 are involved in diverse biological pathways required for tumor initiation, growth, recurrence, and drug resistance. In addition, all five antigens are overexpressed in breast tumors compared to normal breast tissue. A construct expressing these antigens has not been used before. The unique fusion protein expressed by this plasmid vector encodes extended epitope regions that were selected for the ability to induce Type 1 T helper (Th1) cell responses in samples from cancer patients and healthy volunteers. The human protein sequences in STEMVAC are 79 to 100 percent homologous to the mouse sequences. A cell line derived from a mouse mammary gland tumor demonstrates expression of the STEMVAC antigens. Vaccination of a murine model used to evaluate the efficacy and potential toxicities of the STEMVAC antigens with STEMVAC followed by implantation of this syngeneic cell line resulted in a significant decrease in the mean tumor volume compared to control animals.

The proposed Phase 1 trial will assess the safety and immunogenicity of the multiple antigen STEMVAC vaccine in advanced stage breast cancer patients who are HER2 negative and currently show no evidence of disease or stable bone disease only. Patients must have completed primary or salvage therapy to be eligible for this study. Subjects will be assigned sequentially to one of three intradermal doses of the vaccine: arm 1 (150 mcg), arm 2 (300 mcg), and arm 3 (600 mcg). Up to 10 patients will be enrolled in each arm.

B. Written Reviews by RAC Members

Six RAC members voted for in-depth review and public discussion of the protocol. This protocol was found to warrant further review because it is the first in human testing of a novel product and because the safety of inducing immunity against the antigens in the STEMVAC vaccine is unclear.

Three RAC members provided written reviews of this proposed Phase I trial.

Dr. Chatterjee stated that the design and study of immune-based therapies for advanced-stage, HER2-negative breast cancer patients are worth pursuing. The major concerns regarding this protocol deal with the potential off-target effects of immune responses to cellular proteins targeted in STEMVAC, most of which are expressed on normal tissues. Induction of cytotoxic T cells targeting these proteins may thus result in immunotoxicity. Similarly, induction of high levels of cytokines in response to these proteins in normal tissues may result in a cytokine storm. Dr. Chatterjee asked that the investigators comment on these possibilities or rule out these concerns. The investigators should specify whether they have tested human T-cell clones and lines against a panel of normal human tissues to ensure that toxicity due to off-target effects will not occur in humans.

The mouse model used to validate the immunization strategy utilizes a tumor target similar to human breast cancer. Additional information was requested to clarify how closely expression of the target antigens in a mouse resembles that in humans both in terms of expression and epitopes. In a related question, Dr. Chatterjee asked how homologous are the antigenic targets derived from epitope spreading and cross-presentation between mice and humans, especially in light of the differences between the human and murine immune systems.

Dr. Pilewski found the submitted protocol materials to be thorough and clearly written. The target patient population is one of high need, as there are currently no effective therapies for patients with Stage III or IV breast cancer, many of whom will relapse with metastatic disease.

Dr. Pilewski noted that the peptide sequences in the vaccine were modeled to preferentially elicit Type I but not Type II cytokine responses. While this approach has been safe thus far in a vaccine targeting HER2, the protocol does not discuss expression and function of the target antigens in normal tissues to help assess the risk of autoimmunity and other off-target effects. Of specific concern is that some of the target antigens are not expressed on epithelial or cancer cells but rather are upregulated on tumor

microvessels or activated endothelium. The potentially deleterious effect on wound healing of blocking EMT and the effect of disrupting adhesion molecules on normal epithelia are not considered. In addition, the potential that interfering with signal transduction may impair stem cell renewal in normal tissues is not discussed. Lastly, the likelihood of success for targeting intracellular proteins is not provided from a mechanistic or risk-benefit perspective.

Safety monitoring consists of observation for an hour after vaccine administration and follow-up clinical evaluation and laboratory studies monthly. Research subjects are encouraged in the ICD to report “any side effects,” but monitoring for specific symptoms of cardiopulmonary or other dysfunction is not provided. The investigators should consider adding contact (by phone) between visits to follow-up with a symptom survey to more systematically and reliably elicit potential toxicities.

The study cohort includes only patients with HER2-negative breast cancer. The rationale for excluding patients with HER2-positive breast cancer needs to be specified.

Per the responses to Appendix M, Dr. Pilewski noted that the preclinical data in a murine model are described in limited detail, limiting assessment of the likelihood of success in human studies. Animal toxicity data are also limited because the longer-term necropsy studies are still in progress.

Dr. Pilewski offered the following comments and suggestions regarding the ICD:

- The ICD is reportedly written at an eighth-grade comprehension level, but it contains numerous scientific words that are not defined and are unlikely to be understood by most patients. Examples include “immunogenic proteins,” “metastasize,” “autoimmune disorders,” and “post-vaccine monitoring.”
- The ICD does not clearly describe the risk of inducing an immune response to normal cells and the development of an autoimmune disease.
- The description of indemnification is clear, but the lack of compensation for damages related to the study is disappointing. The ICD should explicitly state that the insurer may refuse payment, leaving the subject with uncovered medical care costs.

Ms. Hardison reviewed the protocol with emphasis on the following areas: the treatment plan, patient selection, plan of treatment, withdrawal from study, and an in-depth review of the ICD. Ms. Hardison’s comments and questions focused primarily on the ICD, as follows:

- She suggested clarification of the following statement: “The vaccine being used in this study, STEMVAC, targets immunogenic proteins expressed in breast cancer stem cells which are the components of the breast cancer that is resistant to chemotherapy and has the ability to metastasize”. Alternative suggested language: “The vaccine being used in this study, STEMVAC, targets the proteins expressed in breast cancer stem cells which cause or are capable of causing an immune response and are the components of breast cancer stem cells which are resistant chemotherapy and have the ability to spread to other tissues.”
- Regarding the statement, “The DNA we are using in this study contains instructions for your body to produce parts of the 5 proteins we identified,” she suggested the following: “The DNA that we are using in this study contains instructions for your body to produce parts of the five immunogenic proteins that we identified.”
- In the section titled “What are the benefits?” there is a statement, “We do not know if this study will benefit patients with breast cancer. However, future patients with breast cancer may benefit from your participation in this study.” Ms. Hardison commented that because the proposed trial is a Phase I study, this statement improperly suggests possible benefit to the participant and should be rephrased to indicate that it is unlikely that the participant will receive benefit from this investigational vaccination. While it is acceptable to mention the altruistic benefits derived from participation, there should not be any implication that the individual will receive benefit.
- Referring to the sharing of data from this study, the ICD states, “These people are interested in the study data, not your personal information. Personal information is information that can identify you. Data may include your name, date of birth, social security number, phone number, or other information.” She suggested rewording this paragraph to indicate that personal identifiable data

will be protected with sufficient safeguards to protect the identity of the individuals involved with the research. Suggested language to convey this information: “We will do our best to keep your personal information confidential, but we cannot guarantee total confidentiality. Your study chart will be kept in a locked filing cabinet in a secured building. Study charts are kept for 30 years after the close of the study per University of Washington policy.” Provisions to ensure adequate provisions for sufficient long-term storage of data need to be specified.

- The section titled “Your rights” discusses the participants’ right to withdraw their consent to participate in the research protocol. The ICD should clearly state that although consent may be withdrawn at any time and investigators may withdraw a participant at any point during the study, the intervention is permanent. As a result, effects of the drug may be permanent.

C. RAC Discussion

During the meeting, the following additional questions, concerns, or issues were raised by RAC members:

- The reviewers found the presentation to be clear and most of the responses to their questions to be satisfactorily addressed. They noted that the consent has been revised for simplicity and clarity. Dr. Chatterjee commented that with vigilant monitoring for, and when needed treatment of, untoward immune responses, the responses are satisfactory.
- Dr. Pilewski found the animal and efficacy data to be helpful because the protocol does not seem to clearly delineate the likelihood of benefit versus the major risks, which seem to be autoimmunity. He noted that in Appendix M, the length of the chronic toxicity studies is not clear and that potential concerns regarding any long-term AEs in humans based on preclinical studies need to be delineated.
- Dr. Zoloth strongly advised the investigators to drop language about future childbearing, because it is unrealistic and does not help women in this study, who will be facing the reality of a fatal diagnosis. She found the entire discussion to be inappropriate for this cohort and suggested restricting the patient population to postmenopausal women or doing a screening test for hormone levels to see whether potential participants are menopausal (if not otherwise documented). She suggested adding language to the consent to recognize the altruism and bravery of patients because they are volunteering to participate in this research near the end of their life.
- Dr. Antonia did not agree with the suggestion to exclude women of childbearing potential and questioned the ethics of restricting enrollment because of the ability to bear children. Such an approach would unfairly deny those with advanced-stage disease the opportunity to participate. Dr. Antonia commented that, based on his experience, it is very important to end-stage cancer patients to be able to participate in clinical research when therapeutic options are no longer available to them, and many become distraught when they are excluded. He suggested using language such as, “Many of you may already have [reached] menopause because of your age or because of your treatment. You will be screened [for menopausal status], and if you are not [menopausal], you will be required [or you will be counseled] to take birth control for the rest of your life.”
- Dr. Wooley noted that this is a Phase 1 study with no direct benefit to participants and that exclusions based on menopausal status vary by study but are not unusual. The efficacy and risks of the study intervention are unknown, and participating in this experimental research is distinct from being treated for cancer. As long as participants understand these differences and the risks of the study and are given the support for their choice, their decision to participate is a dignified and courageous choice. Further, because the vaccine is experimental, excluding women capable of bearing a child would not be denying them treatment. In such cases, as noted by the PI, only a small fraction of potential participants (3 percent) would be affected.
- A question was raised as to whether this is any potential medical benefit from participating in clinical trials even if the intervention does nothing for the patient’s disease. Dr. Kaufman noted one paper published out of CTEC indicating that more than 50 percent of cancer patients in Phase 1 trials experience some sort of meaningful clinical benefit in terms of response rates, progression-free survival, or overall survival. This finding appears to question the expectation of absolutely no benefit from participating in a Phase I study. Dr. Donahue inquired as to whether

this more general finding about participation of cancer patients in Phase 1 studies applies to the proposed trial. He noted that independent of this study, patients may clinically benefit with medical care and prognosis of their disease. However, it would be unfair to have the study investigators be responsible for that care or any potential outcomes from such treatment or evaluations.

- Some members commented that a more compelling scientific or safety rationale for exclusion of women of childbearing potential would be needed to restrict enrollment (e.g., vertical transmission and propagation through the germline with plasmids, teratogenicity, cross-reaction of the immune response against the fetus).
- Dr. Kaufman described experience with trying to generate an immune response in melanoma both through vaccines and more recently with T cell checkpoint inhibitors. Autoimmune reactions with checkpoint inhibitors are seen against a range of off-target sites, including endocrine organs, peripheral nerves, the optic nerve, and other tissues. The key point, however, is that autoimmunity can be well controlled with low doses of corticosteroids and other measures. He noted that when autoimmunity can be generated, a very favorable response for tumor rejection is often seen, although whether this reflects a direct relationship isn't certain at this time. Thus, seeing some signs of autoimmunity in this study could be encouraging.
- Dr. Antonia asked about the number of mice treated with the construct that had a neurologic condition and died and how the investigators know that these outcomes are not related to the vaccine.

D. Investigator Response

1. Written Responses to RAC Reviews

The investigators provided a detailed response to the questions and concerns regarding off-target toxicity. They explained that the proteins targeted by STEMVAC are expressed widely at basal levels in normal tissues and that this basal-level of expression is key to maintaining normal immune recognition of these proteins. In contrast, in cancer, the STEMVAC proteins become markedly overexpressed. Studies in autoimmune disease have demonstrated that overexpression of self-proteins results in a unique major histocompatibility complex (MHC)–peptide repertoire display associated with unmasking of subdominant epitopes that have not been previously available for immune recognition. Thus, overexpression of cancer-associated proteins will confer selectively of the T-cell response to cancer tissues due to a higher expression of subdominant epitopes. The immune system will recognize either intracellular or extracellular proteins. Mechanistically, T cells do not recognize antigens as intact proteins but rather as peptides presented in MHC molecules. Immunization to foreign proteins normally elicits immunity to only a subset of potential epitopes, operationally defined as dominant epitopes, whereas other potentially immunogenic epitopes, operationally defined as subdominant epitopes, are ignored. Immunization to self-proteins usually fails to elicit immunity. It has been shown that autologous T cells recognize and become tolerant to the dominant epitopes of self-proteins but “ignore” the subdominant epitopes that are not available in great enough concentrations in the MHC for recognition. Overexpression of self-proteins can result in display of subdominant epitopes at high-enough concentrations to trigger T-cell recognition of an MHC-peptide repertoire display unique to cancer and not found in tissues where the protein is expressed at basal levels.

A systemic review of 239 Phase I vaccine studies published between 1990 and 2011 that enrolled an aggregate of 4,952 patients was published recently (Khleif et al., *Clinical Cancer Research*, 2014). Trials using synthetic vaccines accounted for the greatest number and more than half the subjects enrolled. Approximately one third of the trials employed autologous vaccines and enrolled approximately one-third of the total number of subjects. A small percentage employed an allogeneic vaccine. Self-tumor antigens studied in these trials included but were not limited to carcinoembryonic antigen, melanoma antigen recognized by T cells 1, Ras, p53, HER2, cytochrome P450 1B1, cyclophilin B, survivin, and telomerase. Cell-based vaccines included allogeneic and autologous tumor cells, which express the self-repertoire of proteins, as well as vaccines immunizing with fixed endothelium cells and constructs that have been shown to secrete cytokines such as granulocyte-macrophage colony-stimulating factor (GM-CSF), interferon alpha, and CD40L over extended periods. Among the 4,952 subjects assessed, there were 162

Grade 3 and five Grade 4 treatment-related toxicities reported. Of these toxicities, 60 were local reactions, 40 were constitutional symptoms, and five were related to the adjuvants used in the vaccines. The remaining 62 systemic AEs were reported by the investigators to be at least “possibly related” to the vaccines. The overall rate constitutes 1.25 AEs per 100 patients. The highest vaccine-related toxicity rate was reported in trials that used bacterial vectors for antigen delivery (3.97 events per 100 patients).

The investigators report a similar experience in two recently completed clinical trials in which patients were immunized against antigens reported to be associated with cancer stem cells or cells undergoing EMT. The first trial enrolled 66 Stage III and IV breast cancer patients vaccinated against the HER2 intracellular domain encoded by the same plasmid proposed for use in this trial. Data suggest that that HER2 expression drives a stem cell-like phenotype in breast cancer. Vaccination with the HER2 intracellular domain was found to be safe and immunogenic. A total of 990 AEs were collected in the 66 patients. Of these, 517 AEs across all arms were considered possibly, probably, or definitely vaccine related. The most common vaccine related AEs ($n = 188$), defined as comprising at least 5 percent of vaccine-related AEs (all grades) across all arms, included injection site reactions (23 percent), flu-like syndrome (7 percent), and fatigue (7 percent). The majority of all vaccine-related toxicities were Grades 1 (89 percent) and 2 (11 percent) and were not significantly different across the three dose arms. In arm 3, one Grade 3 toxicity (less than 1 percent), an elevated serum glutamic-pyruvic transaminase, was observed; this event was deemed to be possibly related to vaccination and normalized during the study. There were no Grade 4 toxicities. A total of 16 abnormal autoimmune serologic events potentially attributed to vaccination across all arms; these events included elevated antinuclear antibody (ANA) and transient decrease in complement levels. All autoimmune toxicities observed were Grade 1, and no patients developed detectable anti-double-stranded DNA (dsDNA) antibodies or clinical signs of autoimmune disease.

In another trial, the investigators immunized 25 Stage III and IV ovarian cancer patients with a plasmid DNA vaccine encoding the N-terminus of insulin-like growth factor binding protein 2 (IGFBP-2), which is associated with stem cells and EMT. A total of 177 AEs were reported, with the majority classified as Grades 1 (81 percent) and 2 (15 percent). Like the HER2 intracellular domain vaccine, the most common related AEs were reaction at the injection site, fatigue, myalgia, headache, pruritus, nausea, lymphopenia, and leukopenia. One patient was withdrawn from the study after experiencing a possible allergic reaction to the first vaccine. There was one death from myelodysplastic syndrome (MDS), which was not considered related to the study. There were three abnormal autoimmune serologic events with low-complement C3 potentially attributed to vaccination. All autoimmune toxicities observed were Grade 1 or 2, and no patients developed detectable anti-dsDNA antibodies or elevated ANA or symptoms of autoimmune disease.

Both vaccines were immunogenic, as demonstrated with IFN- γ enzyme-linked immunoSpot assays. More than 75 percent of patients immunized with either vaccine developed antigen-specific immunity. Booster immunizations were administered in the ovarian cancer study. No patient demonstrated evidence of a cytokine storm even after subsequent booster immunizations with the IGFBP-2 plasmid based vaccine.

In addition, there appears to be minimal risk associated with the use of plasmid-based vaccines. In one study of a plasmid based HIV vaccine, nearly 300 patients were followed for a median of 5.2 years. Infectious diseases and injuries accounted for almost 50 percent of the 175 reported clinical events, of which more than 95 percent were mild or moderate in severity. There were 36 normal pregnancies and births in the cohort. No potentially vaccine-related medical conditions, immune mediated diseases, or malignancies were reported. Ninety-three percent of volunteers reported good health at their last visit.

Collection of long-term follow-up (LTFU) safety data for the HER2 intracellular domain vaccine began in September 2005 and consisted of a questionnaire sent to the study subject’s primary physician. The investigators have collected and reviewed 176 questionnaires, and 96 percent reported no new findings. Of the 28 new findings, 82 percent were deemed unrelated to vaccination, since events were either present at baseline or due to disease metastasis. Only five AEs could not be attributed to existing disease: development of carpal tunnel syndrome, new diagnosis of Parkinson’s disease, colitis, anemia, and dizziness. Collection of LTFU safety data for the IGFBP-2 vaccine started in November 2012. To

date, the investigators have collected 22 physician notes from 13 patients. No long-term toxicity has been observed that could potentially be a result of vaccination. These data, in combination with the extensive negative toxicities studies performed in mice using STEMVAC, would indicate that the vaccine has a reasonable chance of a favorable safety profile.

The investigators have designed this study to address the concerns for the development of autoimmunity. They have an established long-term monitoring program for all vaccine studies and will be collecting data on the patients for the next several years. The monitoring plan includes complete history and physical exams each month that will focus on toxicity assessment and monthly toxicity studies consisting of comprehensive blood and chemistry analysis and autoimmune evaluation. The study has rigorous stopping rules based on limited Grade 3 and 4 toxicity. If autoimmune toxicity were to occur, the same treatment strategies that have been employed successfully with the use of ipilimumab would be used (as described in Protocol Section 9).

The investigators provided additional information regarding the proposed safety monitoring plan and problems encountered with prior plans that included other provisions, such as phone contact for toxicity evaluations. In the vaccine trial involving the HER2 intracellular domain plasmid-based vaccine, study staff contacted patients by phone 48 hours and 7 days after vaccination to evaluate for both local- and systemic-type reactions. A large percentage of patients did not answer the phone or call back when a message was left. In addition, several patients declined phone contact, as they did not want messages left on their phone. The IRB had concerns about continuing to call a patient to document contact per protocol when the patient did not reply. For these reasons, at each visit, the investigators have begun to counsel patients to call or email with any complaint. This approach has resulted in extensive collection of AEs between visits. The AEs reported in this time frame were local injection site reaction (e.g., tenderness, erythema, and induration), fatigue, and flu-like syndrome. All these AEs were Grades 1 and 2 and resolved by the following monthly vaccine visit. There were no reported signs or symptoms of other systemic dysfunction, such as cardiopulmonary AEs.

Regarding the exclusion of individuals with HER2+ tumors, the investigators noted that use of trastuzumab elicits HER2-specific immunity as well as epitope spreading to other antigens expressed in the patient's tumors. In addition, a potential future goal for use of STEMVAC is in the preventive setting (e.g., with high-grade ductal carcinoma in situ [DCIS]). In the diagnosis and treatment of DCIS, evaluation of estrogen receptor (ER) status but not HER2 status is the current standard. Thus, the planned study cohort, which includes ER-positive, ER-negative, and HER2-negative patients, is more representative of both triple-negative breast cancer and the targeted future DCIS patient population.

Homology of antigenic targets derived from epitope spreading and cross-presentation between mice and humans is unknown, primarily because there are no model systems that allow ready study of epitope spreading after cancer vaccination.

Testing human T-cell clones and lines against a panel of normal human tissues for toxicity assessment would require not only an autologous, antigen-specific T-cell line but also autologous tissues (maintained in cell culture) derived from the same individual. The investigators noted that if the line were tested against cells with unmatched MHC molecules, there would be T-cell recognition directed against foreign MHC.

Information on long-term storage of research documents has been added to the ICD as follows: "Study charts are kept for 30 years after the close of the study per University of Washington policy. In addition, the University of Washington has a secure facility where research documents can be stored." Technical terms have been simplified or more clearly described, and the ICD now states, "If your insurance refuses to pay [for research-related injuries], you may have to cover the costs out of pocket." The investigators agreed with the other recommendations regarding the ICD and have modified the ICD accordingly.

2. Responses to RAC Discussion Questions

Dr. Disis explained that the animal studies are designed to reflect the ultimate goal of this research: to be able to use vaccines to prevent breast cancer. Some of the animals in these prevention studies are still alive almost a year after vaccination without untoward effects in either immunizing cohorts of the new transgenic mice or immunizing cohorts of the C3T/tag mice from 6 weeks of age forward.

Dr. Disis recognized the concerns regarding issues of childbearing for this patient population. She noted that it is rare even to screen women of childbearing potential in this group. Many have experienced menopause due to chemotherapy. About 3 percent of the advanced-stage breast cancer patients enrolled in prior studies have not had documentation of menopause by hormones and underwent a surgical procedure to allow them to qualify for the study. A test to screen for menopausal status could be added to the protocol. As for a rationale to exclude women of childbearing potential, the vaccine is not expected to be teratogenic, but rejection of the fetus might be possible. The PI explained that this is less of a problem for selective T-cell vaccines, because trafficking across the placental barrier is poor compared to, for example, vaccines that generate antibodies. One of the anticipated advantages of this protocol is the lack of toxicity. Furthermore, Dr. Disis noted that the 5-year survival for those with Stage IV is about 20 percent and the 5-year survival for Stage III disease is less than 50 percent. Dr. Disis explained that at these stages, breast cancer patients repeatedly relapse and that while their overall survival might be long with respect to living with cancer, their chance of a cure at later stages of the disease is nil. Study investigator Dr. Salazar concurred, noting that the progression-free survival is typically short and depends whether the patient is hormone receptor positive or not. Dr. Salazar noted further that checking follicle stimulating hormone (FSH) and estradiol levels to determine menopausal status isn't practical because many patients will be on long-term endocrine therapy, which would confound test results, and because it would not be ethical to ask patients to go off endocrine therapy just to determine menopausal state.

Dr. Disis noted that 6 out of 30 mice (20 percent) given the vaccine construct developed neurological disease and died, which is consistent with what is reported in the literature. The same frequency was seen in the control mice. The veterinary histopathologist determined that these events were classic epileptic syndrome, which has been defined in the FVB mouse.

E. Public Comment

Dr. Borrer commented that the nontechnical abstract does a good job of describing the study in lay terms and suggested using some of this language in the ICD (in lieu of the current description). In addition, because most people have a certain idea about what vaccines are, it would be helpful to use a term such as "experimental vaccine" or "study vaccine" when referring to the intervention.

F. Synopsis of RAC Discussion and RAC Observations and Recommendations

- The target population for this trial is likely to be infertile, either due to the side effects of previous chemotherapy, current hormonal therapy, or the stage of their disease. Thus, the section of the informed consent regarding reproductive risks for women who can become pregnant, as currently written, could be misinterpreted as an assessment of the potential for success of this approach (i.e., their long-term survival is likely and/or the potential for future pregnancies). This section should be rewritten to state that pregnancy is an unlikely event but nonetheless for those women who are still fertile, contraception is required.
- The section on the right to withdraw from the study should be clarified to make it clear that once the gene transfer agent is administered, it cannot be withdrawn. Withdrawal from the study is withdrawal from future monitoring and not from any potential effects of the gene transfer agent.

- The use of the term “vaccine” may lead research participants to think that this is an established approach, like other vaccines. This term should be modified using an adjective to make it clear that this is an experimental approach, e.g., “experimental or study vaccine.”
- The background information on breast cancer and the rationale for a gene transfer vaccine approach that was provided in the non-scientific abstract was clearly written and understandable. You should consider incorporating some of that language into the informed consent document.

G. Committee Motion 3

Dr. Kohn summarized the RAC recommendations to be included in the letter to the investigators, expressing the RAC’s comments and concerns. Dr. Kohn asked for a vote on these summarized recommendations, which the RAC approved by a vote of 15 in favor, 0 opposed, 0 abstentions, and 1 recusal Dr. Kiem).

V. Review and Discussion of Human Gene Transfer Protocol #1406-1320: Phase I Study of T Cells Expressing an Anti-Cluster of Differentiation-22 (CD22) Chimeric Receptor in Children and Young Adults with B-Cell Malignancies

PI: Terry Fry, M.D., National Cancer Institute, NIH

RAC Reviewers: Drs. Sarzotti-Kelsoe, Kohn, and Fost

A. Protocol Summary

Acute lymphoblastic leukemia (ALL) represents the most common pediatric malignancy in the United States, accounting for approximately 25 percent of all childhood cancers. Although disease-free survival rates are high for children with ALL, approximately 20 percent will die of their underlying disease, making ALL the leading cause of cancer death in childhood. For those who relapse, long-term prognosis is poor. The expected complete remission rate to salvage therapy depends on relapse number. Disease-free survival rates of less than 50 percent are expected with a second remission and fall to about 27 percent with a third remission. Given these outcomes, new treatment approaches are needed to address the incidence of relapse in pediatric subjects.

Adoptive transfer of donor T cells following bone marrow transplantation has shown activity in recurrent leukemia. One approach to adoptive T cell therapy is to engineer T cells to express chimeric antigen receptors (CARs). CAR-expressing T cells can mediate antitumor activity, as demonstrated through tumor shrinkage in neuroblastoma, lymphoma, and leukemia. Therapy with anti-B-lymphocyte antigen CD19 CAR T cells (CARTs) has demonstrated marked clinical responses in patients with ALL, including pediatric patients. Not all patients respond to CD19 CAR therapy, however, and CD19-negative leukemias have been observed following this treatment.

The current trial proposes anti-CD22 CART therapy as an alternative to the CD19 CAR construct. Encouraging responses targeting CD22 with an antibody-based immunoconjugate have been seen in patients with recurrent and refractory ALL, including children. CD22 represents a promising target for patients with B cell malignancies. CD22 is restricted to cells of B-lineage and is expressed on the surface of mature B cells. It is widely expressed on B cell malignancies, including 96 to 100 percent of pediatric B-precursor ALL. The proposed trial will use an anti-CD22 CAR that was produced by the study investigators. The construct contains a human single-chain variable fragment (ScFv) to CD22 and the 41BB co-stimulatory domain and is delivered by a lentiviral vector.

The primary purpose of this Phase I dose escalation trial is to assess the feasibility and safety of administering the anti-CD22 CAR-engineered T cells in children and young adults with B-cell malignancies after lymphodepleting chemotherapy. Secondary objectives are to determine whether this therapy can cause regression of B-cell cancers and to measure the persistence of the transduced T cells

in patients' blood. Cognitive function tests will be correlated with serum cytokine levels. To be eligible for this study, patients must be between 1 and 30 years of age, have CD22-expressing B-cell ALL or lymphoma, have persistent or relapsed disease, and be deemed incurable by standard therapy.

The study will follow a 3+3 cell dose escalation schema to assess safety and feasibility as primary endpoints and evidence for persistence of the CAR+ T cells and clinical efficacy as secondary endpoints. The study will test four dose levels of anti-CD22 T cells to determine the maximum tolerated dose (MTD). The first patient at each dose level must be at least 18 years old. Up to 24 patients will receive the highest safe dose tested to further evaluate the effects of this therapy; these patients will be divided into two groups of 12 patients (i.e., those who have been given CAR T cells before and those who are CAR naïve). Efforts will be made to balance enrollment between patients less than 12 years of age and those who are 12 or more years of age, with a total of 12 patients in each stratum (including those treated to determine the MTD). Up to 57 patients may participate to meet the study objectives.

Patients will be screened for eligibility and apheresis within 28 days before beginning a preparative regimen. They will undergo leukapheresis for collection of the blood cells that will be used to make the autologous anti-CD22 CAR T cells. The cells will be cryopreserved at that point or put into culture immediately following harvest. All patients are expected to receive a fresh infusion, but the protocol includes an option to cryopreserve the CAR-transduced cells if clinical condition warrants. All patients will receive identical preparative regimen chemotherapy to allow for an intent-to-treat analysis in this cohort. Two chemotherapy drugs (fludarabine and cyclophosphamide) will be given starting 4 days before the cell infusion, which will be done on an inpatient basis. They will receive CAR T cells on day zero and will be assessed for toxicity response 28 days after infusion of the CAR T cells. Patients will be evaluated frequently following infusion to promptly manage any signs of cytokine release syndrome and for several weeks and months after this treatment. Subjects will have blood taken intermittently for research tests and safety tests. Patients will be monitored closely via physical exam, vital signs, laboratory evaluation, cytokine levels, and clinical response measures. Patients will be discharged from the hospital between 5 and 7 days after infusion of the modified anti-CD22 CAR cells, depending upon recovery. Patients will return to NIH every month for 2 to 3 months, then every 3 to 6 months for evaluation of their cancer disease status. Blood samples and physical evaluations will be done annually for 5 years; thereafter, patients will be contacted to evaluate their status for at least 15 years.

B. Written Reviews by RAC Members

Nine RAC members voted for in-depth review and public discussion of the protocol. This protocol was found to warrant further review because this will be the first-in-human testing of anti-CD22 CAR adoptive cell therapy and will involve use of a novel CAR target in pediatric patients.

Three RAC members provided written reviews of this proposed Phase I trial.

Dr. Sarzotti-Kelsoe found the protocol to be well written but to include a few unclear elements. For example, there was some confusion as to whether documentation of CD22 expression on tumor cells would be an inclusion criterion.

Dr. Sarzotti-Kelsoe requested additional information regarding CAR gene transfer in relation to the statement in the protocol, "Successful CAR gene transfer for each transduced PBL population will be defined as >15% CAR positive cells." If known, the percentage range of anti-CD22 CAR-positive cells in PBMCs from different patients (transduction efficiency) should be specified. In addition, the protocol should include information on how the percentage of CAR-positive cells corresponds to the number of transduced T cells/kg given to each patient cohort. Furthermore, the investigators need to clarify whether the "+20%" specified in the Dose Escalation Table refers to the variability in transduction efficiency or the variability in the total number of transduced T cells given at each administration.

The protocol states that, should an illness or infection before CART administration occur, "cells" will be maintained in culture for an additional 72 hours. It was not clear whether "cells" in this context refer to already transfected CARTs or whether this extended culture period has been tested and validated. Dr.

Sarzotti-Kelsoe also asked about the viability of CAR surface expression and RCL levels in cells that have been cultured for a longer period. The protocol states, “If more than 72 hours from planned cell harvest elapses, the cells will be cryopreserved.” The protocol does not specify whether and, if so, how CAR surface expression and RCL levels of cryopreserved CARTs have been compared to those from freshly cultured cells or whether the use of fresh versus cryopreserved cells has been validated.

Dr. Kohn noted the experience of the study investigators with similar trials, including one with anti-CD19 CARTs for pediatric ALL. He found the eligibility criteria to be well chosen and appropriate and the design and starting doses to be reasonable. The two ICDs that will be used, one for initial enrollment for screening for eligibility and a second for the CAR treatment protocol, are clear and include essential information.

Dr. Kohn pointed out that a critical issue in this first-in-human targeting of CD22 is the evidence for the specificity of expression of the antigen to the target cell types (i.e., B lineage malignant cells and normal B lineage cells) and not to other organs and tissues. The protocol states that CD22 is not expressed on pluripotent hematopoietic stem cells (HSCs), which would be important to prevent aplasia. However, data supporting the lineage-restricted expression of CD22 are not cited in the protocol. Furthermore, although monoclonal antibodies (mAb) to CD22 have been administered without off-target toxicities, there have been other examples where CARs show potency and toxicities not predicted by mAb (e.g., Her2neu). Because this trial represents first-in-human testing of anti-CD22 CAR adoptive cell therapy and has the unknown risks for off-target toxicity, the investigators may wish to consider limiting enrollment of the initial subjects to the older subjects (e.g., ages 18 to 30) of the proposed cohort.

Dr. Kohn inquired as to the basis for the cutoff value chosen for the percentage of leukemic cells expressing CD22 (more than 15 percent by immunohistochemistry or more than 30 percent by flow cytometry) required for inclusion. He noted that these percentages seem low and that, if only a minority of the cells express detectable CD22, it is hard to expect a significant tumor response.

Dr. Kohn requested clarification regarding the two planned cohorts, CAR-naïve subjects and subjects who have previously received CAR T cells, presumably anti-CD19, and are now in relapse or have a resistant disease. Subjects treated primarily with the anti-CD22 CAR in this trial may receive subsequent treatments with the anti-CD22 CAR, but it is not clear whether these patients would then be considered part of the first cohort or the second for the outcome analysis.

Dr. Foster requested additional information and clarification regarding the PI’s background and prior gene transfer research.

Dr. Foster noted the low likelihood of direct medical benefit to the research participants and the substantial discomforts, particularly for children (seven or more lumbar punctures and seven or more bone marrow biopsies), and asked whether the study should be conducted only on consenting adults and/or older adolescents, given the risks and potential for benefit. The table on page 8 of the ICD states that five or more visits “may” involve lumbar puncture and/or bone marrow biopsy in the first year, every 6 months for the next year, and then annually. The scientific rationale and necessity for this schedule should be delineated, including (1) how the investigators will determine whether these procedures will be done at each visit and (2) whether any of these procedures are optional.

Dr. Foster asked that the nature and composition of the Safety Monitoring Committee (SMC) be more clearly defined, including whether this committee and its members will be independent of the study and the research team. The schedule and timeframe for monitoring by the SMC need to be clarified. The protocol states that review by the committee will occur annually, but recruitment is expected to be completed within 1.5 to 2 years. Dr. Foster suggested that the SMC review data more frequently or after a defined number of subjects have been studied.

Dr. Foster had some additional comments regarding the ICD and the consent process:

- The benefit section of the ICD states, “We hope that you will get personal medical benefit, but we cannot be certain. The potential benefits could include reduced amount of cancer and related symptoms.” These statements imply that the investigators think that there probably will be benefit from participating in this trial study, but the statements may overstate the potential benefit of the study intervention. Dr. Fost noted the low likelihood of benefit in Phase I studies in general (generally less than 5 percent) and gene therapy trials in particular (less than other Phase I studies) and suggested using the following recommended NIH language for Phase I gene therapy studies, early in the introduction section and in the “benefits” section: “The gene transfer you get in this study is not likely to change the natural course of your disease. This study is not meant to be a treatment for your disease. The main purpose of the study is to find if the experimental intervention is safe. The investigators hope that the information learned from this study can benefit patients with [this condition] in the future.” The source of this statement and other suggested formulations can be found in the *NIH Informed Consent Guidance for Human Gene Transfer Trials* (<http://osp.od.nih.gov/office-biotechnology-activities/biomedical-technology-assessment/hgt/guidance/web-based-informed-consent-guidance>).
- The investigators should avoid using the words “treatment” and “therapy” throughout the ICD and consider using terms such as “experimental intervention” or “modified T cells” instead.
- The protocol states that cognitive function will be assessed before and after administration of the anti-CD22 CARTs. However, it is not clear how this testing will be done in infants and young children or how often. Information on cognitive testing and concerns about the effects of the anti-CD22 infusion on cognitive function should be added to the ICD, to be consistent with the protocol.
- The protocol states that consent will be obtained from children aged 12 years or older. The more common cutoff is 7 years. Given the low likelihood of benefit and the substantial discomforts for young children, the importance of obtaining meaningful consent (i.e., clarifying that the child is under no obligation to participate) is heightened. Furthermore, given parents’ understandable desire for anything that might help a child with a fatal disorder, it would be helpful to have another way of ensuring that a child’s preference not to participate in the study is respected. The investigators should consider developing written draft language for an ICD or a process that states clearly the facts that would be of most importance to a young child (e.g., that this study is not part of his or her “treatment”; that it involves many uncomfortable needle sticks, including in the bone and back; that the child does not have to do it).
- The investigators should consider adding consent monitoring—that is, assessment by someone other than the investigators of the child’s understanding (at an age-appropriate level) of the key elements of the study.

C. RAC Discussion

During the meeting, the following additional questions, concerns, or issues were raised by RAC members:

- The reviewers summarized the responses to the written reviews during the discussion session of the meeting. Dr. Sarzotti-Kelsoe found the responses to be satisfactory and did not have any additional questions.
- Dr. Kohn requested clarification as to the age of the first patient tested for each dose cohort. He noted that pediatric patients should not be excluded from potentially therapeutic trials and that the plan to have the first patient in each dose cohort be an adult is reasonable. Dr. Kohn also requested further clarification regarding the inclusion criteria (cut-offs) for CD22-positive leukemic cells and eligibility based on prior exposure to CD19 CAR. Dr. Kohn found having a screening consent and a consent for the treatment/intervention phase of the study to be an acceptable approach.

- Dr. Fost expressed concern about including very young children in the proposed research. While the primary purpose of a Phase 1 study is to assess safety and dosage, the potential for clinical benefit is low. An older report in *NEJM* (from 2005) indicated that the response rate for novel agents in Phase 1 gene therapy trials at that point in time was less than 3 percent. Dr. Fost noted that in the NCI's Pediatric Oncology Branch's (POB's) CD19 trials, a majority of patients had a favorable effect, but three of the 16 patients had a severe adverse event, one of which was life-threatening. Although these results are for a different construct, they are not trivial and may be similar for the CD22 product. Given the aims, risk: benefit assessment, and number of invasive/uncomfortable procedures for the planned study (including seven LPs and seven bone marrow biopsies), Dr. Fost stated that it would be preferable for the new agent to be tested in subjects who are capable of consenting. The proposed trial, as currently written, anticipates enrollment of infants and children as young as 1 year old (at least 15 kg). Children around age 10 or 12 and older, especially those who have had multiple hospitalizations and have undergone numerous procedures, can understand why the proposed testing is needed and decide whether or not to proceed. Very young children (e.g., age 5 and under) are unlikely to have that level of understanding, however, or be able to make the same kind of decision. Dr. Fost considers adolescence as starting at age 12 and commented that age 14 is generally the age at which children are capable of thinking more like adults in weighing risks and benefits.
- Dr. Zoloth shared Dr. Fost's concerns about obtaining assent from younger children but noted that some portion of younger children can give meaningful assent. Because this is research, however, there needs to be clinical equipoise. Dr. Zoloth underscored the importance of parents (and children, depending on developmental level) understanding that the proposed intervention is experimental and could fail. The patients may have a life expectancy of only weeks to months and no or few other treatment options, and they and their families are likely anxious or even desperate to find something that works. Drs. Zoloth and Kiem also inquired as to other interventions or treatments for study participants, specifically, whether patients only have the choice of palliative care or if other options are available.
- Dr. Zoloth agreed with Dr. Fost's suggestion to avoid use of the words "therapy" and "treatment" in the ICD and when discussing the study with participants and their parents, since the intervention is experimental.
- Additional information was requested regarding cognitive testing in very young children, including why a comprehensive neurologic exam is not sufficient for this purpose. Dr. Ross noted that understanding the long-term neurological and cognitive impact of the study intervention would be especially important when more than one therapy is available for patients (or parents) to choose from. Dr. Ross added that the percentage of children with leukemia who are enrolled in cooperative group studies has been decreasing over the past decade and that many of the patients seen in follow-up clinics are unaware that they were treated for cancer when they were young (e.g., under age 5).
- Dr. Antonia expressed concern about the ability of the transduced cells to produce severe toxicity, including unexpected off-target related deaths. Given this possibility, and as noted by Dr. Kohn, the distribution of CD22 expression is important. The immunohistochemistry assay being used for this assessment has low sensitivity (based on the "Human Protein Atlas"), however, and thus may not be able to determine whether there is off-target expression. Other testing to detect non-target or systemic expression of CD22 should be provided. Dr. Antonia also noted that the toxicities appear to vary based on the type of toxin conjugate and questioned whether the conjugate, not the target, is being utilized.
- Dr. Sadelain asked whether, based on what is known about CD19 and CD22, the investigators expect more CD19-positive ALL tumors resulting in the emergence of a CD19-negative variant or more CD22-positive tumors leading to the emergence of a CD22-negative variant.

- Dr. Sadelain noted that the response in the CD19 CAR trials has been very encouraging, with a 50 to 70 percent complete remission rate observed in multiple trials, including the trial being conducted at the NCI POB. The response rate with the CD22 CAR construct is unknown, however. He asked how the proposed study will proceed given that potential participants may be eligible for both the CD19 and the CD22 CAR trials and that treatment (at the expansion phase) involves CD22-positive patients who have relapsed after CD19 CAR treatment.

D. Investigator Response

1. Written Responses to RAC Reviews

The investigators clarified that CD22 expression testing will be required as an element of screening for eligibility on this study and will remain as an inclusion criterion.

The investigators confirmed that, per FDA guidelines, results of RCL testing will not be available before cells are infused into the patient. However, results from a quantitative real-time polymerase chain reaction (qPCR) assay for the Vesicular Stomatitis Virus G envelope protein, which is under control of the CMV promoter, will be available before cell infusion and required to be negative to meet release criteria.

The investigators noted that transduction efficiency evaluated during the optimization and validation of the manufacture process for the production of anti CD22 CARTs (using GLP and GMP vector) has always been greater than 50 percent. This assessment includes two anti-CD22 CART products generated from two leukemia patients previously enrolled in the CD19 CAR trial. Transduction efficiency is evaluated on each end product and is used as release criteria (i.e., minimum cutoff sets at 15 percent by immunohistochemistry or 30 percent by flow cytometry). The final cell dose to be administered will be corrected for the transduction efficiency to ensure that the infused CART number per kilogram is the same for each patient per dose level.

The extended time in culture has been studied and validated for this trial and for the ongoing CD19 CAR trial. Viability and transduction efficiency for the extended culture is acceptable and comparable to shorter culture periods. An RCL assay is performed in process testing at least 24 hours before fresh infusion or on final product before cryopreservation. The investigators do not expect changes in RCL levels with the extended culture. Furthermore, as noted above, results of the qPCR assay for viral envelope will be available before release of all products. Stability studies have been performed on multiple products at time of thawing. The investigators stated that cell recovery, viability, and transduction efficiency has always been acceptable; in all cases, viability has been greater than 80 percent and transduction efficiency similar to the product before cryopreservation. Five cryopreserved products manufactured by the NIH Clinical Center's Department of Transfusion Medicine Cell Processing Section have been tested in xenograft models with comparable activity to non-cryopreserved cells confirmed in all cases.

The investigators clarified that there are actually four potential dose cohorts (not three) during the dose escalation portion of the study (as corrected in the protocol). Once the MTD has been determined, the MTD dose will be expanded into two groups of subjects (two strata) to evaluate any differences in safety, feasibility, and clinical response between the two groups. The two strata include the CART-naïve subjects (first stratum) and the subjects who have previously received CARTs (i.e., anti-CD19 CART therapy; second stratum). These two strata have been taken into account in the power calculations.

The investigators agreed with the reviewers that the data for expression of CD22 on HSCs and in normal tissues are limited, but a limited number of reports indicate that CD22 expression is restricted to the B-cell lineage. To further explore this question, the investigators performed flow cytometric evaluation of CD22 expression on CD34+ and CD133+ cells in two pediatric bone marrow samples and analyzed expression of CD22 on purified human CD34+ cells collected during four GM-CSF–mobilized peripheral blood stem cell collections. Results of these experiments showed that CD22 was not found on any of the HSCs. To confirm prior reports that CD22 had restricted expression on normal tissues, the investigators have asked a collaborator (Dr. Paul Sorensen) to run a human tissue microarray for CD22 expression.

The investigators pointed to additional provisions to reduce any potential risk of off-target expression and any possible AEs. The starting dose in the dose escalation schema is low (1×10^5 CARTs/kg), and the protocol includes a very detailed algorithm for management of cytokine release syndrome (CRS) with tocilizumab (anti-human interleukin 6 [IL-6]) and steroids, both of which have been shown to be effective in CD19 CAR trials. The algorithm was developed by a multi-institutional group of investigators with experience in CAR therapy and as part of a cooperative pediatric immunotherapy grant.

In addition to safety testing for CD22 expression in nonmalignant human tissues (as described above), the investigators have incorporated specific strategies and procedures into the trial to mitigate the risks to the pediatric patients who will be eligible for this study. For example, the protocol includes staggered enrollment, predefined post-infusion safety assessment periods after each patient completes the dosing regimen, and close safety monitoring of individual subjects. The dose escalation plan requires that the first patient at each dose level be an adult, and 4 weeks must elapse after completion of cell infusion in the final patient in a cohort before accrual to the next dose level can begin. The starting dose is a full log lower than that being used as the effective dose in the majority of CD19 CAR trials. The review schedule for the SMC review has been revised from annually to have the first review after 6 months of patient enrollment, and the IRB will review this study every 6 months.

Patients will be enrolled into two cohorts (CAR naïve, and previously CAR-treated subjects) during the expansion phase but not during the dose escalation phase, because no differences in feasibility of generating the cell product or in safety are expected between the two groups. The rationale for two cohorts during the expansion phase is the poor response rate to retreatment with CD19 CARTs, which may be due to the murine derivation (and immunogenicity) of the ScFv used in that construct. In contrast, the CD22 ScFv used for the construct in the proposed trial was derived from a human B cell phage display. Evaluation for efficacy in the expansion phase would be restricted to the first cell infusion and the patients would, thus, be placed into cohorts based on whether they had received CAR cells before enrollment. Retreatments with CD22 CARTs under this protocol would be restricted to those patients responding to the initial infusion and for whom there is the highest likelihood of benefit from retreatment.

The investigators explained that the CD22 cutoff value chosen was the same value used in the team's prior CD22 immunotoxin trial. As discussed in the background section (with additional graphic data included in the written response), almost all ALL samples express CD22, and when they do, the entire population is generally CD22 positive. Because it is not known what expression level is required for activity, the investigators would like to maintain the current eligibility guidelines to be consistent with the CD22-targeted immunotoxin trial.

There has been extensive preclinical testing of the CD22 CAR construct containing the 4-1BB co-stimulatory domain in Dr. Fry's laboratory. This work has been presented at the American Society of Hematology Meetings in 2013 and is being prepared for submission as a manuscript.

The investigators acknowledged that the likelihood of objective response on early phase clinical trials has historically been low (in the 5 to 10 percent range) but point out that the prospect of direct benefit is a critical part of any clinical protocol involving children. Recent data suggest that response rates to early-phase clinical trials in children with cancer may be increasing and that these response rates do not capture patients with stable disease who may still derive benefit from the treatment. The response rate in the CD19 CAR trials has been high, and a 50 to 70 percent complete remission rate has been observed in multiple trials. As described in the protocol, in the Phase I study using anti-CD19 CART therapy, 10 of 16 subjects experienced complete response, and two subjects had stable disease. The purpose of the expansion cohort in the CD22 CAR trial is to determine whether comparable response rates are seen with the CD22 CAR construct.

The investigators provided several reasons to enroll children with refractory ALL in the proposed study. ALL is the most common malignancy in children, and the patients eligible for this trial (those for whom a standard upfront regimen and one salvage therapy has failed) will have no curative options. The pediatric patients in this trial will have a life expectancy of weeks to months, and many will have very limited treatment options other than palliative care. In addition to the CD19 CAR trial, the investigators have

experience in safely conducting phase I trials using genetically engineered adoptive cell therapy in the pediatric patient population. Furthermore, as detailed above, substantial efforts have been made to reduce the risks to children enrolled on this trial.

The investigators explained that the required bone marrow biopsies and lumbar punctures (and the frequency of these procedures on this trial) are considered standard practice evaluations for patients with the targeted hematologic malignancies who are undergoing treatment for their disease. These procedures are also standard on all NCI leukemia trials and those conducted through the pediatric oncology cooperative. Disease evaluation procedures are required regardless of whether the child is receiving an investigational agent or a standard of care therapy. The ability to identify early recurrence in the bone marrow or central nervous system is critical to define when these patients should be offered other therapy.

As per the NIH Center for Cancer Research's Standard Operating Procedure for Data and Safety Monitoring Plans, all protocols using gene transfer or gene therapy methodology require oversight by an SMC. The NCI SMC is made up of a Chair, four reviewers who serve in the role of PI on NIH clinical trials, a statistician, and a patient advocate. No investigator from the NIH NCI branch from which the protocol originates can be assigned as a reviewer. Although the NIH SMC typically evaluates a protocol after the first year and then decides whether it should be reevaluated again in 6 months, the protocol has been revised to incorporate an evaluation after 6 months.

The investigators used the document titled "NIH Informed Consent Guidance for Gene Transfer Research," to make modifications to the ICD to meet the recommendations for statements regarding potential benefit of the proposed research.

The assessment tools for cognitive function and the frequency of their examination will be performed as outlined in Appendix G, with the associated age range for the tests. The youngest testing age for these tests is 2.5 years. The investigators noted that although NIH rarely sees patients younger than this age and the apheresis guidelines on this protocol make it likely that all patients will be more than 5 years old, any children younger than age 2.5 will be evaluated using the neurologic exam as part of the daily physical examination performed on days 1 through 14 and then twice weekly. Any changes in the level of consciousness, speech, or motor movement will be captured for subjects of all ages during this daily and then biweekly evaluation.

The investigators appreciate Dr. Fost's comments and share his view on the importance of obtaining consent from children enrolled on clinical trials. The investigators stated, however, that a written ICD is not mandated for clinical trials conducted by the Children's Oncology Group (COG), nor is it mandated in the Office for Human Research Protections guidelines. Rather, the details of the consent process are left to local IRBs. The investigators noted that although the NCI IRB does not require a written ICD signed by the child, the investigators must obtain consent and document it in the subject's medical record. The requirement for an advocate is not viewed as standard, nor is it required in any of the COG trials except HSC transplantation trials involving minor donors, where the parent signs consent for both the donor and recipient and may be potentially conflicted. The investigators believe that parents can advocate for their children in clinical research where they consent for the affected child only. They reiterated that consent is a critical component for all children enrolled in their clinical trials and have changed the language in the protocol to read, "Pediatric patients will be included in age appropriate discussion. Verbal assent will be obtained for those >7 years of age when deemed appropriate by the clinician and the child's parents or guardian. In such cases, the parent or guardian will sign the designated line on the ICD attesting to the fact that the child has given assent." The investigators have asked, however, that they not be required to prepare a written ICD for the reasons given.

The proposed study will be the fourth genetically modified T cell trial opened in NCI's Pediatric Oncology Branch (POB). The investigators have addressed the other minor corrections and clarifications suggested by the reviewers.

2. Responses to RAC Discussion Questions

Dr. Fry clarified that the first patient at each dose level will be an adult, that is, age 18 or older.

Regarding screening for CD22-positive cells, Dr. Fry noted that based on experience with leukemia patients and the team's CD22 immunotoxin trials, distribution of CD22 expression across the entire blast population tends to be fairly consistent. Some MLL rearranged patients tend to have a wider or lower distribution of CD22 levels (for unknown reasons). The number of cells needed to express CD22 or the site density needed for a response isn't clear at this point, however. Dr. Fry commented that the criterion for the CD22 expression level could be increased without losing many patients based on screening done to date for other trials, but the goal is to try to be as consistent as possible across all studies. Study analyses will include comparing the percent of CD22-positive cells and response rate. CD22 expression and CD19-negative leukemias appear to be comparable, but these outcomes have not been studied systematically and are based on a small number of patients. Thus, the investigators also hope to learn whether CD19 is a unique antigen that is not replicated with other targets from the proposed trial.

The response to the CD22 CAR in ALL patients is not known at this time. In contrast, the response rate in the multiple CD19 CAR trials conducted to date has been excellent. In the NCI POB Phase I study using anti-CD19 CART therapy, 10 of 16 subjects experienced complete response, and two had a stable disease. The purpose of the expansion cohort on the CD22 CAR trial is to determine whether comparable response rates are seen with the CD22 CAR construct. Dr. Fry noted that the team discussed whether to require patients to have received CD19 CAR therapy before enrolling on the proposed trial but decided to limit this criterion to the expansion arm of this study. Because the anti-CD19 data are from Phase I trials, the efficacy seen in the CD19 trials may not be seen with the CD22 CAR.

Subjects treated previously with CD19 CAR will be eligible for the expansion phase of the proposed study; outcomes for this group will be analyzed in a separate stratum based on the first infusion of CAR T cells. Dr. Fry noted that both CD19 CAR trials are currently open and that CD19-negative leukemia patients from those trials who either did not respond or relapsed will likely be among the first potential participants to be enrolled on the CD22 targeted trial. Similarly, other options may be available to patients in the planned trial; depending on the eligibility criteria, they may be able to enroll in one of the other open POB trials, including the CD19 CAR trial and the Phase 2 CD22 immunotoxin trial. Decisions about a second transplant for patients who respond to the CD22 CAR would be made by the referring institution.

Dr. Fry understood Dr. Fost's and others' concerns about enrolling very young children in this trial. He pointed out, however, that all of the patients enrolled in the proposed trial will have received the same types of procedures as part of their prior treatment. The tests and procedures are also standard across the Children's Oncology Group protocols and the POB's CD19 CAR trial for a leukemia patient. Participants will not necessarily undergo a total of 14 LPs and bone marrow biopsies under the proposed study. A bone marrow biopsy or an LP is required for eligibility and needs to be done within 2 weeks before initiation of preparative regimen chemotherapy. In prior trials, almost all patients were able to use the same bone marrow for eligibility in the pre-treatment phase. The procedure sometimes needs to be repeated closer to the infusion date to assess a possible response. Another bone marrow biopsy is done on day 28. Patients who stay on protocol will continue to undergo these procedures. Dr. Fry noted that most of the patients on the CD19 CAR trial moved on to consolidated hematopoietic stem cell transplant at day 28, at which time they were taken off the CAR trial; these participants therefore had three bone marrow biopsies (for eligibility, on day 28, and maybe one more). Participants have the same number of LPs unless the patient has central nervous system (CNS) leukemia, in which case giving intrathecal chemotherapy and radiation are considered standard for management of this condition. Thus, a study participant could have as few as a total of six LP and bone marrow procedures. If the patient remains on trial, has durable responses, and does not go on to transplant, he or she would continue to be followed and potentially could have the higher number of procedures (e.g., up to 14 in total).

Dr. Fry did not agree that the potential for therapeutic benefit in the proposed trial is remote. Based on more recent data than that presented in the 2005 *NEJM* article, the response rate in Phase 1 gene therapy trials involving novel products appears to be improving. He noted the high complete remission

rate (70 percent) in the CD19 CAR trials and response rates of 25 to 50 percent in large numbers of patients including children with ALL in other CAR trials using the same construct (with the exception of the scFv, which binds CD22) and toxin-conjugated antibodies. The exploratory expansion cohort is built into the proposed trial and will proceed if a CR rate of at least 30 percent is seen in the earlier phases of the study.

Dr. Fry noted that he and his team and other pediatric oncologists in the POB take the issue of assent very seriously and routinely obtain assent from children at different ages and stages of development. The investigators did not agree, however, that signed consent is necessary for children younger than age 12. The PI noted that per discussions with leadership at the COG, consent has not been required for younger children enrolled in COG protocols and that provisions for obtaining consent (e.g., providing information sheets for minor participants) have been left to local IRBs. Dr. Fost was surprised with this reply based on his experience with the NCI IRB and the inclusion of consent language on all NIH-funded cancer trials. One study investigator, Dr. Mackall, noted that one of her roles as a member of the National Heart, Lung, and Blood Institute (NHLBI) IRB is to evaluate pediatric transplant protocols. While older children and adolescents may, as a group, be more developmentally mature and better able to understand the risk and benefits of a research study than younger children, setting an arbitrary age cut-off and singling out the proposed trial would likely exclude younger children from a potentially curative therapy. Dr. Mackall commented that pediatric transplant protocols have written assent documents that are developed in consultation with school teachers to assure that the assent forms provide age-appropriate language for children as young as 5 years old. Assent language for 5- to 7-year-olds, for example, includes statements such as "This is your choice. You can say no if you don't want to do this," and for slightly older children, "This is not part of your treatment and you don't have to do this if you don't want to."

Drs. Fry and Mackall agreed that there probably was a misunderstanding on the investigators' part as to what was being requested (i.e., a signed ICD for young children versus an information sheet or written script for those under age 12). The investigators consider a written script with age-appropriate language and explanations of the study and risks to be reasonable. They agreed to draft consent language that includes age-appropriate information about the study. The investigators also agreed to use terms other than therapy and treatment when referring to the study intervention in the ICD and the consent process. The investigators noted that when describing the proposed intervention to parents and participants, they explain that the anti-CD22 CART "treatment" is experimental and that the intervention may succeed or fail. Dr. Mackall offered to email Dr. Fost sample language from other pediatric protocols.

Dr. Fry explained that one of the secondary endpoints in the proposed trial is to assess cognitive function after CAR therapy. These assessments were incorporated because CNS toxicity was observed during serious adverse events involving cytokine release syndrome in the CD19 CAR trials. The CNS effects in these patients were reversible, but the investigators want to monitor and evaluate these effects systematically. Neuropsychologists on the trial will assist in these assessments and screenings. Cognitive function will be assessed as long as participants are enrolled in the study, but this testing is expected to primarily capture any acute CNS effects in the majority of patients within the 28-day evaluation period. Evaluating long-term cognitive toxicity in this patient population would be valuable but also presumes an increased life expectancy and a "curative" therapy. The design of the proposed study builds on experience from the CD19 CAR trials. To add long-term cognitive assessment as a systematic secondary endpoint would be very difficult, however, because of the uncertainty regarding the number of patients who will remain in the study over time and be available for those evaluations.

Dr. Fry added that gene transfer is a relatively new treatment in the field of oncology and that the Childhood Cancer Survivors Group has been performing very systematic evaluations of long-term survivors of childhood cancer treatments. As a whole, investigators in the field are learning that in spite of some overall positive outcomes for pediatric cancer patients, there are long-term sequelae associated with therapies that are just being realized now that the first group of survivors is, for example, 30 years out from treatment and includes large enough numbers to generate meaningful data. Thus, the field is active and will continue to gather information on efficacy and toxicities since the vast majority of children treated for leukemia (80-plus percent) in the United States are enrolled in COG trials and are followed at COG institutions. Individual trials with small numbers of patients focus on toxicities specifically associated

with the intervention being studied. Thus, studying this cohort in isolation wouldn't be very informative, but comparing outcomes with other children should contribute to the existing body of knowledge.

Dr. Fry acknowledged that the assay used to detect off-target/systemic expression could be better. He noted that the team is collaborating with investigators (e.g., with Dr. Sorensen, as noted above) on a large multi-institutional pediatric immunotherapy to develop and validate more sensitive assays for systemic evaluation. A comprehensive confirmatory screen of CD22 expression is in process; preliminary results indicate that CD22 expression is restricted to lymphoid tissues.

The Lead Associate Investigator on the study, Dr. Niroli Shah, noted that the response (toxicities) to different conjugates has been variable. For example, in a Phase II study of children and adults with relapsed or refractory ALL given a CD22 monoclonal antibody conjugated to the toxin calicheamicin, increased risk of veno-occlusive disease post-transplant is thought to be partially related to the immune conjugate. In another study, ALL patients given CD22 monoclonal antibody conjugated to pseudomonas exotoxin had an increased risk of hemolytic uremic syndrome and capillary leak caused by platelet and hemoglobin dysfunction, which seem to be a result of a cumulative dose effect of the toxin. Dr. Shah noted that to date, no cardiac problems have been seen in these studies and the more common toxicities are mild (e.g., lab abnormalities of no clinical significance). Results from the CD19 CAR dose escalation trial suggest a correlation between disease burden and toxicity. One of the questions for the ongoing expansion cohort in the CD19 CAR study is to try to establish whether debulking with antibody before CAR T cell infusion to limit the cytokine would reduce the likelihood of at least Grade 3 CRS in patients with high disease burdens using chemotherapy. Because assessment of toxicity in a Phase I trial is challenging, the proposed study does not require this additional provision. However, patients will receive cyclophosphamide and fludarabine before infusion of the CARTs.

Dr. Fry noted that the biology of CD22 in ALL is really not well understood, but his lab and other researchers are pursuing this area of research as well as looking at differences between CD19 and CD22. Dr. Fry explained that biologically CD22 in non-malignant B cells appears to involve an inhibitory signal and may act as a receptor, because it is part of the pre-B-cell receptor. Loss of CD22 in mice, for example, does not have a specific phenotype because of redundancy within the system due to another molecule that can substitute in some of the double knockout mice, which have a loss of regular B cell populations. In co-culture experiments using CD19-targeted therapy, CD19-negative leukemia can be more easily generated than CD22-negative leukemia, suggesting that it might be more important functionally for blasts. The roles and differences between the two agents have not been systematically evaluated, however, and warrant further investigation.

E. Public Comment

The following individuals provided public comment to the RAC in support of this protocol (listed below in order of appearance):

- Carlos Sandi
- Suzy Matter
- Lisa Wilkins
- Nancy and Greg Sanders (via letter, read by Dr. Kohn)
- Sang Nguyen
- Waleed Haso

The parents described the experiences of their children, their advocacy efforts, and their support for this trial. Testimony of the above individuals is included verbatim in Appendix A.

Dr. Kohn thanked the patients and family members for attending this RAC meeting and for sharing their stories. He noted that it is always helpful for members of the RAC to hear from patients and family members to better understand the patients' perspective and needs.

F. Synopsis of RAC Discussion and RAC Observations and Recommendations

Before proceeding to the recommendations, Dr. Kohn congratulated the investigators for developing the vector and the CAR that will be used in this study.

- Potential research participants in this trial may also be eligible for your anti-CD19 CAR trial. In your Phase 1 trial with the anti-CD19 CAR, you had an impressive 70 percent response rate, and other groups have had similar success using anti-CD19 CAR for patients with ALL. Because there is an open trial using anti-CD19, it is important that research participants who are eligible for both the CD19 trial or this C D22 trial receive information about both trials.

Given the attention that the CD19 trials have received, both in the scientific literature and media, research participants may not appreciate that the efficacy seen in CD19 CAR trials may not necessarily be seen with this trial. Consider including a statement that clearly communicates that this is the first study with this agent and that it is unknown whether it will be safe or provide any benefit. The NIH Informed Consent Guidance for Gene Transfer (<http://osp.od.nih.gov/sites/default/files/resources/IC2013.pdf>) contains sample language such as: *The investigators' goal is to find out whether this approach is safe. This is the first step in studying whether it can be used in humans with your disease.* In addition, the consent document should avoid using the word “therapy” or “treatment” and instead consider “experimental or study intervention” or “gene modified T-cells.”

- The intent is to obtain assent for children over age seven years. While a signed assent form is not necessary, consider developing a written document that provides a consistent age appropriate message regarding the uncertain benefit that this protocol offers and the risks and procedures that will be required. Such a document should facilitate consistency in the assent process and assist these children in making an informed decision, taking into account their age and decision-making capacity.

Before proceeding with the motion and vote on the summarized recommendations, the RAC re-visited the issues associated with enrollment of younger versus older pediatric participants.

Dr. Fost noted that he would be more comfortable if the lower age for entry were increased so that the study population includes older children who are capable of participating more actively in the choice to enroll and participate in this trial. Dr. Whitley, who is a pediatrician, did not agree with this suggestion. He commented that the target population for this research is children in the younger age groups, who should be included in the study to define the safety of the intervention and for future considerations. He gave the example of a soon-to-be-launched study of an experimental intervention for respiratory syncytial virus (RSV), which causes disease primarily in children less than 2 years of age. To date, the intervention has not been tested in patients, but the FDA has agreed that this study can proceed in children under age 2 based on the risk-benefit assessment and what is known about the safety of the approach both in animal models and in Phase 1 pre-clinical human toxicity studies. For RSV infections, mortality is highest in very young children and infants and immunocompromised individuals who have the most significant disease. Further, the animal models of RSV are not ideal.

With respect to the proposed trial, Dr. Whitley commented that, per his understanding of the disease, the patients who theoretically will teach investigators and clinicians the most will not be older children (e.g., those who are 14 years old and older) but those who are under 8 or 9 years old. Although all patients must have relapsed to be eligible for the proposed trial, the safety and other outcomes seen in younger children are more likely to be applicable in the future for this group, compared with older children and young adults. Further, if this intervention is therapeutically efficacious, younger children would derive the greatest benefit from it. By limiting enrollment to only older children and young adults, a large number of patients who could benefit from participating in the study and who would at least provide safety data would be excluded. In addition, recruitment of the population that could benefit the most (i.e., younger children) would be slowed if the study immediately jumps into the older patients. Dr. Mackall pointed out that ALL is most common between the ages of 2 and 9. Thus, as Dr. Whitley noted, if the group that is most likely to get the disease is excluded from the early trials, testing in this group will have to be done later. Dr. Kohn similarly did not favor restricting participation to older children and young adults because of the track record of CARs. Although the safety and efficacy of the specific product that will be tested in

the proposed trial are unknown, the preclinical data suggest it may have similar activity to other well-studied CARs. In addition, it is important to take compassionate considerations into account, which seem to be reasonable for this patient population. Dr. Kaufman commented on the importance of obtaining safety data in the Phase 1 study and supported proceeding with younger children to assure that this information is available going forward. Dr. Hammarskjöld agreed that more will be learned with the inclusion of younger children. She echoed Dr. Kohn's comments by pointing out that options for these pediatric patients are very limited and that these children are unlikely to live long, productive lives without availability of other treatments.

Dr. Wooley asked whether the safety profile is expected to differ by age group and if so, if it's important to test the age group that will be targeted in the future. Dr. Whitley stated that trials should enroll the populations that will need to be ultimately treated and that for these conditions, that includes children in the 4- and 9-year-old age range. He commented that in this case, starting in adults and working down to children is an archaic approach. Some additional complicating factors were noted, however. For example, Dr. Kiem pointed out that the first patient in each dose cohort will be an adult. It was noted further that adult ALL is different from pediatric ALL. Dr. Fry commented that if enrollment were restricted to older patients, the design of the proposed Phase 1 study would need to be discussed at length and would likely need to be redone. In addition, a separate dose escalation trial would need to be conducted in a younger age group to generate the toxicity data.

Dr. Kohn called for a straw poll (not a vote) to see how many committee members favor adding a minimum age restriction of 12 or 14 compared with those who favor keeping the lower age for eligibility as currently proposed. By a show of hands, the balance of the committee favored keeping the criteria as planned.

G. Committee Motion 4

Dr. Kohn summarized the RAC recommendations to be included in the letter to the investigators, expressing the RAC's comments and concerns. Dr. Kohn asked for a vote on these summarized recommendations, which the RAC approved by a vote of 14 in favor, 0 opposed, 1 abstention, and 0 recusals.

VI. Gene Transfer Safety Assessment Board Report

RAC Reviewers: Drs. Atkins, Curry, Kiem, Kohn, Pilewski, Sadelain, and Whitley

A. GTSAB Report

Dr. Kohn presented the GTSAB report for the third quarter of 2014. Within the past 3 months, the OBA received a total of 31 protocol submissions, 27 of which were not selected for public review at this RAC meeting. Of the 27 protocols not selected for public review, 19 were oncology protocols, four were monogenic disease protocols, two were infectious disease protocols, one was a heart failure protocol, and one was a renal disease protocol. Among these 27 protocols, six used AAVs, four used retroviruses, four used plasmids, three used RNA, three used lentiviruses, three used adenovirus, two used modified organisms, one used herpes simplex virus, and one used measles. (Information about these trials was made available on the OBA website after this RAC meeting.)

The GTSAB reviewed initial and follow-up reports on 16 serious AEs from 13 protocols. About half of the events were from protocols using T cells that express CARs, mainly CD19 CARTs. Seven of these events involved CRSs of varying severity. The interval from dosing to the event ranged from 24 hours to 11 days. Three of these events were mild (e.g., fever, mild drop in blood pressure), while four required intensive care unit care due to combinations of severe hypotension, hypoxemia, and other symptoms. In one CD19 CAR recipient, there were neurologic symptoms, seizures, and encephalopathy. Two case outcomes were complicated by the development of concomitant infections or sepsis. Elevated IL-6 levels were reported for four events. Elevated ferritin C-reactive protein consistent with a hemophagocytic

complication was reported for three events. In some cases, anti-IL-6 drugs (tocilizumab and siltuxumab) and steroids were used to reverse the CRS. Dr. Corrigan-Curay noted that at least 25 patients were treated in the protocols reporting these latter events. Thus, about one-third of patients in those trials had these symptoms, which is consistent with published case reports reporting that 25 to 75 percent of patients have these kinds of complications with the CARs.

Dr. Kohn summarized published results from a CAR protocol reviewed by the RAC several years ago, “Chemotherapy-Refractory Diffuse Large B-Cell Lymphoma and Indolent B-Cell Malignancies Can Be Effectively Treated with Autologous T Cells Expressing an Anti-CD19 Chimeric Antigen Receptor” (OBA Protocol #940). Fifteen patients with advanced B-cell malignancies were treated per this protocol with autologous T cells that express anti-CD19 CAR 3 after lymphodepleting chemotherapy. Nine of these patients had diffuse large B-cell lymphoma (DLBCL), two had indolent lymphoma, and four had chronic lymphocytic leukemia (CLL). Eight of the 15 patients achieved complete remissions, four achieved partial remissions, one had stable lymphoma, and two were not available for response. Four of the seven available patients with chemotherapy-refractory DLBCL achieved a complete response; three of these complete responses are ongoing with durations of 9 to 22 months. The study authors note that this is a first report, to their knowledge, of successful treatment of DLBCL with anti-CD19 CARTs (Kochenderfer et al., *Journal of Clinical Oncology*, 2014).

During this quarter, OBA received notification that 10 new protocols opened, two of which were publicly reviewed. One protocol had previously submitted responses to the issues raised. Dr. Kohn reported on the following noteworthy changes that represented responses to RAC public review of the second protocol:

- *OBA Protocol #1101-1023, reviewed in March 2010: Gene Transfer for Patients with Sickle Cell Disease Using a Gamma Globin Lentiviral Vector: An Open Label Phase I/II Pilot Study.* In response to concerns regarding insertional oncogenesis following a report of a patient in France with β -thalassemia with a clonal expansion, additional preclinical studies in mice and nonhuman primates were conducted and demonstrated no evidence of vector-derived malignancy in any animal model. To gather further safety data before enrolling pediatric subjects, the first six subjects enrolled in this pilot study will be adults. In addition, based on discussion during the RAC review about hydroxyurea and its established therapy for sickle cell disease, only children who cannot tolerate hydroxyurea will be eligible to enroll, and adults may participate only if they refuse this therapy.

B. RAC Discussion

Dr. Zoloth asked whether it is typical to have 4 years pass between public review and enrollment of the first participant into a protocol, as occurred with OBA Protocol #1101-1023. Dr. Corrigan-Curay noted that such statistics have not been analyzed but that the timeframe from RAC review to study initiation can vary considerably based on numerous factors. Dr. Kohn replied that a 4-year lag time is probably not unusual, because the RAC is often the first group that investigators approach for feedback before submitting a study for subsequent reviews by several other bodies, including regulatory agencies, and obtaining approvals and assurances of funding and other resources. Dr. Corrigan-Curay added that while all protocols report when enrollment starts whether they undergo public review, only those reviewed by RAC are highlighted during meetings, because the investigators are required to provide information on the response to the RAC’s recommendations.

C. Public Comment

No public comments were offered.

VII. Day 1 Adjournment

Dr. Kohn adjourned Day 1 of the September 2014 RAC meeting at 5:25 p.m. on September 9.

VIII. Day 2 Call to Order and Opening Remarks

Dr. Kohn opened Day 2 of the September 2104 RAC meeting at 8:30 a.m. on September 10. Following the discussion of the minutes of the June 2014 meeting, RAC members introduced themselves by name, affiliation, and research interests.

IX. Minutes of RAC Meeting, June 11, 2014

RAC Reviewers: Drs. Cannon and Wooley

Dr. Cannon found the minutes of the June 2014 RAC meeting to be acceptable as submitted. Dr. Wooley found the summary of the review of the protocols to represent the Committee's discussions. However, she felt that the section of the minutes on housing of nonhuman primates in BL-4 laboratories did not fully capture the extensive discussion on issues pertaining to the bioBUBBLE units and use of individual self-contained pressurized suits, which are key reasons for the recommendations to move to open caging. In addition, Dr. Wooley commented that some of this discussion was listed as public when it was a continuation of the RAC discussion. No changes to the document were suggested by other RAC members.

Regarding practices and principles of biosafety containment for housing of animals, and non-human primates in particular, in BL-4 labs under the *NIH Guidelines*, Dr. Corrigan-Curay noted that implementation of the recommendations will likely be phased in after further consultation.

A. Committee Motion

There was no motion or vote on the June 2014 RAC minutes at the September 2014 meeting. A motion to approve the minutes of the June 11, 2014, RAC meeting will be considered pending revision of the minutes per the comments regarding the discussion on BL-4 containment.

X. Review and Discussion of Human Gene Transfer Protocol #1406-1319: A Phase 1 Study of CAR-Modified T Cells Targeting Natural Killer Group 2D (NKG2D) Ligands in Patients with Acute Myeloid Leukemia (AML) or Advanced Myelodysplastic Syndrome (MDS) and Multiple Myeloma (MM)

PI: Susanne Baumeister, M.D., Dana-Farber Cancer Institute (DFCI)

Co-PI: Glenn Dranoff, M.D., DFCI

Sponsor: Celdara Medical LLC

RAC Reviewers: Drs. Kiem, Zoloth, and Whitley

Dr. Curry was recused from consideration of this protocol due to a conflict of interest.

A. Protocol Summary

Despite significant advances in supportive care, post-remission chemotherapy, HSC transplantation, and improvements in the overall survival of patients with AML, treatment failure due to either primary refractory or relapsed disease still occurs in approximately 60 percent of patients due to the persistence of minimal residual disease. Similarly, while alkylating agents and autologous stem cell transplantation result in median survival times of 3 to 5 years, and despite promising novel drugs such as proteasome inhibitors and immunomodulatory agents, most patients with MM, the second most common hematological malignancy, will experience disease relapse or will die of this disease.

Novel therapies are clearly needed for these diseases, and immunotherapy holds promise to fulfill this need. Of interest for the current research is the NKG2D receptor, which plays an important role in protecting the host from infections and cancer. Approximately 70 percent of the targeted AML and MM tumors express NKG2D proteins that can become a target for T cells. By recognizing ligands induced on infected or tumor cells, NKG2D modulates lymphocyte activation and promotes immunity to eliminate ligand-expressing cells. Because these ligands are not widely expressed on healthy tissue, NKG2D ligands may present a useful target for immunotherapeutic approaches in cancer.

The proposed Phase 1 dose escalation trial will test the safety and feasibility of single-dose intravenous (IV) administration of CM-CS1 T cells without prior lymphodepleting conditioning. The CM-CS1 T-cells have been permanently modified to recognize human NKG2D ligands. CAR-T cells have been utilized experimentally for several other diseases, but this construct represents the first-in-human use to address NKG2D ligands. The study population will include patients with specific NKG2D ligand-expressing hematologic malignancies: (1) AML or advanced MDS that is not in remission and for which standard therapy options are not available and (2) relapsed or refractory progressive MM. The dose escalation will follow a 3+3 design. Subjects will be enrolled serially, and enrollment will be balanced between disease states. At least 30 percent of patients on each dose level must be subjects with AML, and at least 30 percent must be patients with MM. Safety assessments will be initiated after infusion of CM-CS1 cells (Day 0). The MTD by the intravenous route of administration will be defined as the dose level at which no or one patient among six enrolled experiences DLT. There will be a dose expansion cohort of six patients per disease at the MTD level.

B. Written Reviews by RAC Members

Eleven RAC members voted for in-depth review and public discussion of the protocol. This protocol was found to warrant further review because this is the first-in-human use of CAR T cells targeted to NKG2D ligands, which are expressed on cancers including AML and MM.

Three RAC members provided written reviews of this proposed Phase I trial.

Dr. Kiem found this research to be important and the proposed study to be well written. The inclusion criteria and the planned dose escalation strategy are reasonable for a Phase I study. The patient population is appropriate, as NKG2D ligands are expressed on cancers including AML and MM. The staggered enrollment/dosing and safety assessment periods after infusion of the first subject in each cohort will minimize risks to participants. The definition for DLT of the T-cell therapy (i.e., any new Grade 3 non-hematologic or greater AE) is acceptable.

The vector backbone and packaging cell line (PG13) to produce the proposed gamma-retroviral vector with the MFG promoter have been used before. Dr. Kiem noted that while there have been no side effects reported with this gamma-retroviral promoter, a self-inactivating (SIN) configuration would probably be beneficial for future studies.

The main issue and concern with the proposed research is the potential for off-target effects. Dr. Kiem noted that the amount of information on expression of NKG2D ligands on other cells (i.e., non-malignant cells) to assess potential off-target toxicity appears to be limited. NKG2D ligands are part of the immune system's intrinsic stress and DNA damage pathway. The investigators report that upregulation of these ligands can be expected in tissues that are actively infected, underwent DNA damage due to recent radiation therapy, or experienced other types cellular stress. These conditions, in turn, could result in serious AEs. Of relevance to this research is inflammation in gastrointestinal epithelium due to normal low-level NKG2D ligand expression on these epithelial surfaces, and the potential for the CAR T cells to induce NKG2D ligand upregulation and amplify inflammatory response. The investigators should provide any additional data about the upregulation of NKG2D ligands in particular disease settings (e.g., chemotherapy in patients) and identify any other ways to evaluate potential off-target effects (e.g., in an animal model) to address this issue.

Dr. Kiem found the ICD to be well written but noted that the document does not include risk of death from CRS.

Dr. Whitley also found the protocol to be well written and to address an important unmet medical need. The proposed study includes careful assessments of host response, both immune generated and CRS. Dr. Whitley noted that the investigators have carefully weighed the potential for toxicity, especially as predicated upon prior experience with other CAR T cells. He commented that the scientific approach is sound and justifies the implementation of the study.

Dr. Whitley noted the following issues and items in the protocol to be clarified or addressed:

- The flow diagram (protocol page 2) implies that all cohorts will be recruited simultaneously, which conflicts with statements indicating sequential cohort recruitment indicating that subjects will be enrolled serially. This aspect of the recruitment and enrollment plan needs to be clarified, and the diagram and information in the protocol needs to reflect how subjects will be recruited.
- The number of patients intended for each cohort should be specified early in the protocol. A discrepancy in the number of study participants specified in the protocol and the ICD needs to be resolved.
- The investigators need to ensure that the DSMB is an independent body. Information on the planned members of the DSMB, including their backgrounds and affiliations, should be provided. Any employees of Celdara Medical should be identified. In addition, the protocol should specify who makes judgments about AEs.
- Patients will be tested for a variety of chronic infections that could be affected by therapy. Because these patients may be immunocompromised, it might be prudent to screen for tuberculosis using contemporary serologic tests (e.g., QuantiFERON instead of a routine skin test).
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- Given the potential for cytokine release syndrome and macrophage activation syndrome, a period for post-infusion AE observation/monitoring in the clinic should be specified.
- The composition of the overall patient cohort needs to be clarified. The protocol appears to account for only 60 percent of the cohort (i.e., at least 30 percent of patients will have AML and at least 30 percent will have MM). Dr. Whitley asked whether the remaining 40 percent could be either of these two diseases. If so, the investigators should specify the potential impact of leaving this portion of the cohort “open” to either condition on the study and whether the cohort should be divided equally between patients with AML and MM.

Dr. Whitley stated that the ICD accurately reflects the studies that will be performed and suggested minor wording changes.

Dr. Zoloth raised concerns regarding the accuracy of some information and statements in the ICD:

- She felt that the statements regarding the risks and discomforts of participation in this study do not sufficiently differentiate the unknown risks of a Phase I clinical trial from the risks associated with standard cancer treatments. In addition, the current consent language implies that the very speculative intervention used in the proposed trial is a treatment. Dr. Zoloth recommended removing these statements from the ICD. She noted that another section of the ICD provides a much better and clearer explanation of these issues.
- The ICD does not indicate the serious consequences facing this patient cohort, with or without the proposed intervention. References to pregnancies and follow-up periods of 15 years seem out of place and inconsistent with these patients’ prognosis. Sections of the document with this information should be reworked, and the ICD should include some acknowledgement that the researchers understand and appreciate the gravity of the situation.
- The potential for SAEs is high. Patients participating in this trial face risk of CRS, or macrophage activation syndrome, included the potential for severe and potentially permanent neurological effects, and the risk of insertions leading to other cancers. The ICD needs to stress the reasons that such risks are defensible. Dr. Zoloth noted that researchers have done a commendable job in explaining that patients participating in this study may help other patients in the future.

- Statements that subjects can stop being in the research study at any time are not correct. Participants cannot withdraw once the cells are injected and can choose only to no longer be observed. The ICD needs to make these distinctions clearly to ensure that participants understand the nature of this research and their options.
- Patients should be informed that because they are using their own cells for development of the engineered T cells, the DNA signature is a part of the research records. Thus, in a sense, even if the study and data are theoretically anonymous, there will be a link to the participant in ways that the subject may not fully understand.

C. RAC Discussion

During the meeting, the following additional questions, concerns, or issues were raised by RAC members:

- Drs. Kiem and Whitley reviewed their written comments and the investigators' responses and noted that most the issues raised in their written reviews have been addressed. Off-target toxicity is addressed with the proposed monitoring plan and through the exclusion criteria, including avoiding patients with obvious inflammation or infection and no prior chemotherapy or radiation. In addition, the investigators have clarified that there is no animal model to test these products and that concerns regarding insertional oncogenesis have not been a problem thus far with the current vector. The ICD should include the risk of death from CRS, which can occur in this setting.
- Dr. Kiem inquired what the meeting schedule is for the DSMB (e.g., after the first patient) and how "reasonable" will be defined for the purposes of this protocol if there is no "reasonable" standard treatment available for patients with AML or MDS.
- Dr. Zoloth noted that many of the ethical issues have been addressed, but some of the consent language needs to be revised. The ICD includes language that places the study intervention among other cancer treatments instead of clearly informing participants that the intervention is still experimental. The terms "treatment" or "therapy" to describe the study intervention are not accurate and should not be used in the ICD. The potential of this intervention should not be overstated, and the boundary between research and treatment needs to be maintained. In addition, by saying that this therapy may not have any benefit, the investigators imply that the therapy may or may not have benefit, and therefore they should be clearer. The statement that subjects can stop being in the research study at any time should be removed, with the clarification that subjects can only stop being a part of the observation aspects of the research.
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- Dr. Kohn relayed questions submitted by Dr. Sadelain, who could not attend the second day of the meeting. Dr. Sadelain asked in which tissues NKG2D ligands are expressed other than certain tumors, the tumor neovasculature, infected tissues, and gut epithelium. In addition, Dr. Sadelain also asked whether activated T cells express these ligands.
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- The investigators were asked about uniformity of expression on the target leukemia or myeloma cells and the potential to turn off expression and escape and why a suicide gene has not been incorporated into the investigational product.
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- Dr. Hammarskjöld expressed concern about unanticipated off-target effects in tissues such as the brain that may not be fully addressed with the proposed management plan, especially without a suicide gene. She referred to a review article that points to evidence for NKG2D ligand expression in healthy cells (beyond being stress-induced) and further complications given both post-transcriptional and post-translational modifications. She asked whether the investigators have considered looking more comprehensively at mRNA and protein/ligand expression using immunohistochemistry or a similar method.
- Dr. Kohn inquired about the mechanism for inducing a host immune response with a secondary effect (i.e., as a result of the first round of T cells). Dr. Atkins noted the response regarding off-target effects on normal cells, specifically, regulatory T cells (Tregs) and myeloid derived suppressor cells (MDSCs), and the potential not only for immunity against tumors but also for autoimmunity since those cells may be preventing colitis or other autoimmune reactions. The

absence of a suicide gene further underscores the importance of being prepared to manage toxicities via selection criteria and clinical treatment when needed.

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- Dr. Antonia asked about clinical experience with adoptive transfer of CAR T cells in the absence of lymphodepleting chemotherapy and whether lymphodepleting chemotherapy is necessary or not. Dr. Kohn found the rationale for starting the proposed trial without lymphodepleting chemotherapy to be reasonable. It isn't clear, however, if patients' lymphocyte status will be monitored to assess whether they are lympho-deficient as a result of their prior therapy and if so, whether those patients have a better response than patients who have normal T cell numbers.
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- Given the potential for gut toxicity with the study intervention, the eligibility criteria might need to be more stringent and/or more specific as to the types of gut diseases that are exclusionary. The investigators should consider adding diverticulitis and possibly other conditions to the list of excluded conditions. In addition, the GI toxicity and CRS management algorithms need to be more specific as to, for example, dosage and schedule of treatment.

D. Investigator Response

1. Written Responses to RAC Reviews

The investigators explained that the retroviral promoter (MFG) and packaging cell line (PG13) proposed for this study were chosen because they have been used safely in several CAR T cell trials. For potential subsequent studies, the team will take the use of a SIN vector into consideration, as suggested by Dr. Kiem.

As noted in the protocol and by the reviewers, upregulation of stress-induced NKG2D ligands on healthy cells could result in on-target, off-tumor effects, and the resultant inflammatory response could further augment the off-tumor CAR T cell response. Limited information is available regarding NKG2D ligand upregulation in particular disease settings such as chemotherapy. Histone deacetylase (HDAC) inhibitors upregulate NKG2D ligands in various cancer cell lines and in primary human hepatocellular carcinoma cell samples, whereas in primary human hepatocytes, treatment did not induce ligand expression. One study has reported upregulation of NKG2D ligands on AML blasts in patients receiving all-trans retinoic acid or valproic acid in vivo. Upregulation of NKG2D ligands on multiple myeloma cell lines and primary patient samples in response to melphalan, doxorubicin, and bortezomib has also been reported, as has been hydroxyurea-enhanced NKG2D ligand expression in myeloid leukemia cell lines alone and in synergism with HDAC inhibition. These findings may reflect future opportunities to enhance killing of malignant cells. In light of insufficient characterization of the effect on healthy tissues and the potential for toxicity, however, the proposed study will exclude patients who have received chemotherapy or radiation therapy within 3 weeks before entering the study.

The investigators explained that while the NKG2D or NKG2D ligand axis exists in mice, the ligands differ significantly from those in humans. Furthermore, there is no functional homologue of MHC class I chain-related protein (MIC) A (MICA) or B (MICB) in mice. Use of a non-obese diabetic/severe combined immunodeficiency disease (NOD/SCID) xenograft model would therefore unlikely reveal potential off-tumor effects. MIC sequences have been identified in rhesus monkeys, but phylogenetic analysis of these genes indicates that these are not orthologs of human MICA or MICB genes and appear to have evolved after the separation of humans and rhesus monkeys from a common ancestor. Studies evaluating a murine NKG2D CAR targeting NKG2D ligands in several murine models by Dr. Sentman and colleagues (as provided to the FDA in the toxicity report and found to be acceptable) did not reveal autoimmune effects at the pharmacologically effective dose. At very high dose levels, elevations in systemic cytokines were detected, as might be expected based on the experience with CRS in humans. However, even when CRS was present in the mice, no histologic autoimmune or inflammatory pathology was observed.

The investigators fully recognize the potential for off-tumor toxicity in humans and have incorporated the following provisions into the protocol as safeguards to address these concerns and mitigate the risk:

- The starting dose is considerably lower than in other CART trials. Participants will receive a single flat cell dose where other CART trials employ similar doses per kilogram of patient weight and/or administer multiple doses. The starting dose in this clinical trial will be well below the estimated values of the no observed AE level (NOAEL) and the pharmacologically effective dose for CM-CS1 T cells in humans (at 1×10^{10} and 2 to 10×10^9 cells, respectively). The dose escalation regimen was further adjusted to half-log increments per FDA recommendation.
- Several patient populations will be excluded to minimize the risk for off-tumor toxicity.
- The protocol includes specific criteria and guidelines for management of CRS to limit the potential for an escalating inflammatory response.
- Participants will be evaluated on the day of NKG2D CAR T cell infusion to ensure that they meet the criteria for NKG2D CAR T cell infusion, which include the absence of fever or clinically significant acute infection (e.g., positive blood cultures for bacteria or fungus or evidence of acute viral infection within 48 hours of scheduled T-cell infusion).
- Per FDA recommendations, the protocol will stagger enrollment between the initial two subjects in each cohort (rather than in the first cohort only) by 30 days and allow for assessment of potential toxicities before enrolling subsequent patients.

The investigators clarified that the study is designed to dose escalate sequentially over four cohorts and agreed that the flow diagram in the protocol is not clear and may imply that cohorts are being recruited simultaneously. This issue will be addressed during the IRB review process at the DFCI.

As specified in the protocol, in accord with the proposed study design, up to 24 patients will be enrolled in the dose escalation part of the study, depending on the number of cohorts required to estimate the MTD and on the number of dropouts who do not complete dosing. An additional 12 patients (six AML patients plus six MM patients) will be enrolled in the expansion cohort at the MTD. If the study proceeds through all dose levels, 12 to 24 patients (depending on DLTs) will be enrolled in the dose escalation part of the study with an additional 12 patients in the expansion cohort. Based on these numbers, an estimated 24 to 36 subjects will participate in the study; this is the same range cited in the ICD. The investigators will consider including this information in Section 1.1 of the protocol for ease of identification.

The investigators noted that due to the requirements of the funding mechanism for the proposed research, the NHLBI DSMB will be monitoring this study. The NHLBI DSMB is an independent DSMB. The investigators planned to introduce this protocol to the NHLBI DSMB on September 12, 2014. The investigators' written response includes contact names for the NHLBI DSMB.

Regarding infectious disease testing, including testing for latent tuberculosis in study participants, the investigators explained that in the absence of additional risk factors or clinical concerns, such screening is not standard practice in the oncology population receiving chemotherapy. In addition, NKG2D CAR T cells are not expected to exert an additional immunosuppressive effect. The study proposes to screen for several viral infections, both to ensure the subjects' safety and for cell manufacturing purposes. While it would be challenging to screen for every possible latent infection, the protocol contains several safeguards to protect against administration of CAR T cells to individuals with active infection or fever.

Cell processing for this study will be performed at the Connell O'Reilly Cell Manufacturing Core Facility (CMCF) at DFCI. All procedures are performed in accordance with current GMPs for cell and tissue processing. The CMCF has been accredited by the Foundation for the Accreditation of Cellular Therapy and is licensed by Clinical Laboratory Improvement Amendments to perform high-complexity testing. The laboratory is also FDA registered to package, process, store, label, and distribute 351 and 361 cell therapy products such as hematopoietic progenitor and therapeutic cells and somatic cellular products. The FDA has reviewed the cell manufacturing section of the investigational new drug (IND) application and approved the IND.

Per the reported literature, CRS, when it occurs, generally evolves not in the first hours after infusion but after several days. As part of the safety plan, patients will be monitored closely during and for 2 hours after infusion of CM-CS1 T cells and three times a week (via clinic visits) for the first 21 days following

CAR T cell administration, which is considered the most vulnerable time frame for the evolution of CRS. In addition, as specified in the protocol, any patient who exhibits signs of evolving CRS will be admitted to the hospital for further observation and management. The team will also focus on providing thorough patient education with respect to recognition of symptoms that would prompt emergent medical evaluation. This will allow expeditious recognition of toxicities and CRS, in particular, even if such toxicities were to occur outside of the typical time frame observed in CD19-CART trials. Patients will be cared for by clinicians with significant experience in managing high-risk patients who may require urgent admissions for febrile neutropenia or other oncologic or stem cell transplant emergencies.

Each disease category (AML or MDS and MM) will be represented at each dose level. In the absence of DLTs, each dose level will enroll three patients. Thus, a 1:1 randomization is not feasible. Instead, the protocol ensures that per group of three patients, each disease is represented at least once (more than 30 percent). This design should ensure that disease-specific toxicity differences are captured. Once the MTD is declared, the study provides for a disease-specific dose expansion cohort with six patients from each disease category to ensure that the safety profile for each disease can be clearly defined.

The investigators noted that the ICD was developed based on the standard Dana-Farber/Harvard Cancer Center ICD template and that any institution-specific requirements will be addressed during the ongoing IRB review process. Issues and questions raised about the ICD or its language were addressed as follows:

- The investigators agreed that the ICD could make clearer the potential risk of death from CRS.
- The current version of the ICD (page 11) discusses the possible risk of autoimmune disease and the beneficial, potentially beneficial, and adverse effects of the CM-CS1 T-cells.
- The investigators commented that, taken in isolation, the statements regarding the risks of a Phase I clinical trial and those of standard cancer treatments might mislead a participant to believe that the cancer treatment under investigation in the research study has a side effect profile comparable to established cancer treatments. However, when taken in context with the preceding and subsequent paragraphs, which discuss suspected side effects and state that the side effects associated with this research study are not fully known and are potentially life threatening, the investigational nature and unknown risks of a Phase I trial are put into perspective for participants. The investigators note further that no cancer treatment is free of potential side effects, and even standard chemotherapy can lead to life-threatening complications. In addition, to be eligible for this trial, patients must have exhausted all standard treatment options.
- The lack of statements in the ICD that specifically point out the poor prognosis of the disease status to the participant is consistent with institutional practice at the DFCI. The ICD does, however, specify the disease and disease state required for inclusion. Discussions surrounding the individual patient's prognosis will have preceded a consent discussion for this trial and are best conducted by the patient's respective primary oncologist. The investigators noted that while pregnancy may not be a frequent occurrence in the eligible patient population, it is nevertheless a possibility, and the study aims to protect unborn life from the unknown risks of the investigational treatment by incorporating pregnancy as an exclusion criterion. The 15-year follow-up period is mandated by the FDA for all gene transfer studies and is included in the protocol and ICD for this reason.
- The investigators agree with Dr. Zoloth that, once administered, the infusion itself is not reversible and that subjects who have already received an infusion cannot stop being in the research study. These subjects could, however, choose not to participate in study-specific procedures anymore. The ICD language will be revised to ensure that these points are clear to participants. This issue will also be addressed during the IRB review process.

The investigators clarified that this study does not include DNA banking. Research studies will strictly focus on elucidating the biologic properties of CM-CS1 T-cells and serve to analyze and monitor the immune response to the therapeutic product as described in the protocol.

The investigators will ensure that abbreviations are sufficiently clear in the protocol. (Per Dr. Whitley's query, "HBS" is the acronym for hepatitis B surface antigen.)

2. Responses to RAC Discussion Questions

Dr. Baumeister noted that the initial DSMB meeting will be held after the first patient and the meeting interval is every 6 months. The investigators will discuss this plan with the DSMB.

The disease-specific eligibility criteria for patients with AML or MDS are histologically confirmed AML and MDS-RAEB (MDS-refractory anemia with excess blasts) that is not in remission (defined as more than 5 percent blasts in bone marrow or peripheral blood) and for which there are no reasonable standard treatment options. The term “reasonable” in this case refers to patients who have already received induction chemotherapy, have either persistent or relapsed disease, and are not eligible for HSC transplantation.

The PI noted that the goal of describing this research to participants is to state very clearly in the ICD that the intervention is an experimental therapy being studied in a Phase 1 trial, that the risks of the intervention are unknown, and that there is no anticipated therapeutic benefit to the patient. In addition, the ICD notes that any other treatment options are associated with risks as well.

The PI explained that small areas of the thymus have been reported to express NKG2D ligands but that in this tissue, the ligands appear to function to help CD8+ T cells complete their development before they travel to the periphery. Given this role, expression in the thymus is not expected to cause problems in a clinical trial. The focus on potential GI toxicities is based on an initial report of expression in the gut epithelium. Subsequent studies have shown that the location of expression is actually intercellular. In addition, the ligands are expressed and may play a role in some autoimmune diseases, such as inflammatory bowel disease, celiac disease, and rheumatoid arthritis. Further, the ligands can be induced under certain conditions of extreme cellular stress or damage. Given these factors, the investigators recognize that they will need to remain vigilant, particularly regarding low-level expression in the gut, via close monitoring, plans to intervene when needed, and excluding patients with certain medical conditions (e.g., those with active autoimmune disease).

The PI described a paper involving about 200 patients with leukemia that showed more than 75 percent express NKG2D ligands (often multiple ligands) and that 100 percent of these patients actually have detectable soluble ligands. Thus, one feature of tumor cells is that they are able to shed soluble ligands, which in turn is associated with poor prognosis in many of these diseases. The PI added that some of Dr. Sentman’s studies show that even in the face of soluble ligands, the CM-CS1 CAR T cells can effectuate the killing of tumor cells.

Reports vary regarding expression of NKG2D ligands on activated T-cells in vitro. Some studies report HIV-infected T cells expressing some ligands. In one study that looked at NKG2D CAR expressing T cells in vitro, there was a peak expression in about 20 percent of the T cells that rapidly declined by about Day 10, indicating about 1 percent expression. Enrichment of NKG2D CAR T cells was observed during that process, suggesting that ligand expression in vitro leads to a sort of fratricide in the T-cell culture. Where T cells proliferated very well, expression might also be dependent on how the T cells are activated and stimulated. In addition, there is a small shift to CD8+ NKG2D T cells, which has not been formally explored but is not expected to occur in this trial. The PI noted that when the T cells are cultured, they do not make IFN- γ , which would suggest that this is not a risk once the patients receive the T-cell construct.

Several other CAR T cell trials incorporate either a suicide gene or a gene marker such as a truncated growth factor receptor (GFR) that could be targeted with an antibody. Dr. Baumeister noted that the safety and efficacy of suicide genes is not yet validated in the CAR T cell field, however, and this mechanism has not been employed in clinical CAR T cell trials of significantly ill patients. On the other hand, emerging evidence suggests that intervening in the inflammatory cascade with an agent such as tocilizumab can alleviate symptoms and improve the patient’s condition while retaining the therapeutic benefit of the CAR T cell therapy. This strategy is included in the management algorithm in the proposed protocol. Corticosteroids will also be available if significant toxicity is seen. Details for management of gut

toxicity and CRS (e.g., dose, treatment schedule) are delineated in the protocol but not in the management algorithms.

Dr. Baumeister explained that the doses will be increased in half-log increments. A previous version of the protocol specified escalating the dose in log steps, but this plan was changed based on feedback from the FDA during the IND approval process.

Dr. Sentman explained that instead of killing targets one by one, the proposed cell therapy generates cytokines that can change the microenvironment around the tumor. Specifically, they appear to deplete and modify the function of local immunosuppressive cells that support tumor growth and survival. The team's experiments suggest that interferon-gamma and GM-CSF from the modified cells impacts how myeloid cells act. Some tumors bring in these immunosuppressive cells as a protective mechanism and to prevent local immune responses. Some immunosuppressive cells, such as Tregs and myeloid-derived suppressor cells (MDSCs), also express NKG2D ligands. The experimental evidence indicates that the combination of attacking the tumor, promoting tumor antigen presentation, and changing the microenvironment acts in place of the host immune system, which appears to explain the effectiveness of the intervention.

Many tissues might have NKG2D ligands; data suggest that these ligands are regulated by micro RNAs. The PI pointed out that the original paper described expression not only in the gut epithelium but in many different organs and that ligand expression was not found in the brain. With respect to any toxicities in the brain, the option to use dexamethasone instead of methylprednisolone to facilitate better penetration of the brain barrier has been incorporated into the management plan.

Several studies involving adoptive T cell therapy have given lymphodepleting chemotherapy, and recent trials have shown improved efficacy with lymphodepleting chemotherapy. Dr. Baumeister commented that it might be helpful to give lymphodepleting chemotherapy in a future study to perhaps create a niche for the incoming CAR T cells. Additional information is needed regarding the impact on healthy tissue, however, and the current focus needs to be on safety. Dr. Kohn noted that a few adoptive T cell therapy trials that haven't used lymphodepleting chemotherapy have shown some efficacy (e.g., studies targeting GD2 in neuroblastoma). In these studies, the endogenous viral antigens might be driving the T cells to expand.

E. Public Comment

No comments from the public were offered.

F. Synopsis of RAC Discussion and RAC Observations and Recommendations

- NKG2D ligands are usually not expressed on normal tissues, except if there is cellular stress, for example, during infection or in the face of certain chemotherapy. However, certain NKG2D ligands are expressed on the gastrointestinal epithelial cells. To reduce risk of a serious toxicity due to activation of the T cells against the gut epithelium, consider adding an explicit exclusion criterion for patients with a history of inflammatory bowel disorders (e.g., diverticulitis) in addition to the current exclusion for autoimmune disease, which would capture certain inflammatory bowel diseases, such as Crohn's disease or ulcerative colitis. It is also important to develop a specific protocol for management of potential gastrointestinal toxicities, including which agents and doses of such agents would be used.
- This study will not use chemotherapy to deplete lymphocytes prior to infusion of the gene modified T cells, as is done on most other protocols for hematologic malignancies that use CAR expressing T cells. Lymphodepletion is thought to contribute to the efficacy seen in these CAR T cell trials. The decision to forgo chemotherapy is due to concerns about upregulation of NKG2D ligands on normal cells in response to chemotherapy. To determine whether lymphodepletion could impact the efficacy of this approach, consider assessing the immune status of the participants and in particular whether the CAR T cells are more potent in research participants who are lymphopenic due to previous therapies or their underlying disease.
- Although the consent provides detailed information on the possibility of a severe adverse reaction due to cytokine release syndrome (CRS), it should be clearly stated that there is a risk of death from CRS.
- The informed consent document contains the following statements: "All cancer treatments can have side effects, which can range from mild and reversible to severe, long lasting and possibly life-threatening. There is a great deal of variability among side effects of different cancer treatments and between individuals." These statements can be misinterpreted to mean that this approach is equivalent to other established cancer treatments and should be revised. In general, the word "treatment" should be avoided in a consent document for a Phase 1 trial.
- The prospect of direct benefit is unknown in this Phase 1 study. While investigators would not bring an investigational agent into a clinical trial if they did not have preclinical data supporting potential efficacy, the primary goal is to test safety, and many Phase 1 trials do not result in clinically significant benefit for the participants. Therefore, research participants need to understand that enrollment in the study is not likely to lead to a direct clinical benefit. The informed consent contains statements about a 15-year follow-up and pregnancy, which could be seen as a possible indication that the investigators expect long-term survival for research participants. There is nothing in the consent document regarding the fact that these research participants have very limited therapeutic options and a terminal disease, making the decision to enroll very altruistic. While these are sensitive issues to put in writing, additional language such as "there is no known or established treatment that can cure your disease" would communicate this clearly and balance other statements about 15-year follow-up.
- The language on withdrawal should indicate that once you receive the modified cells, they can persist indefinitely, and therefore withdrawal from the study would mean withdrawal from monitoring for potential long-term effects of the experimental intervention.

G. Committee Motion 5

Dr. Kohn summarized the RAC recommendations to be included in the letter to the investigators, expressing the RAC's comments and concerns. Before the motion and vote on these recommendations, the language in the last recommendation was re-read to ensure that it does not include the word "therapy." Following clarification of that point, Dr. Kohn asked for a vote on these summarized recommendations, which the RAC approved by a vote of 14 in favor, 0 opposed, 0 abstentions, and 1 recusal.

IX. Closing Remarks and Adjournment

Dr. Kohn thanked the RAC members and the OBA staff and adjourned the September 2014 RAC meeting at 9:47 a.m. on September 10, 2014.

(Note: Actions approved by the RAC are considered recommendations to the NIH Director; therefore, they are not considered final until approved by the NIH Director.)

Jacqueline Corrigan-Curay, J.D., M.D.
RAC Executive Secretary

I hereby acknowledge that, to the best of my knowledge, the foregoing Minutes and the following Attachments are accurate and complete.

This Minutes document will be considered formally by the RAC at a subsequent meeting; any corrections or notations will be incorporated into the Minutes after that meeting.

Date: _____

Donald B. Kohn, M.D.
Chair, Recombinant DNA Advisory Committee

**Attachment I:
Recombinant DNA Advisory Committee Roster**

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**Attachment II:
Public Attendees**

(This list includes only individuals who are not identified elsewhere in this document. It does not include one individual whose name is illegible on the sign-in sheets.)

Dale Ando, Sangamo BioSciences, Inc.
David Berg
Glenn Dranoff, DFCI/Harvard Medical School (*via teleconference*)
Philip Gregory, Sangamo BioSciences, Inc.
Hunter Gunn, LLS
David Lee, NCI
Crystal Mackall, NCI
Suzy Matter
Sang Nguyen, NCI and ALL cancer survivor
Jake Reder, Celdara Medical LLC
Lupe Salazar, University of Washington
Carlos Sandi
Charles Sentman, Dartmouth College
Niroli Shah, NCI
Fyodor Urnov, Sangamo BioSciences, Inc.
Waleed Haso, NCI
Lisa Wilkins

Attachment III: Abbreviations and Acronyms

AAV	adeno-associated virus
AE	adverse event
ALL	acute lymphoblastic leukemia
AML	acute myeloid leukemia
ANA	antinuclear antibody
ANC	absolute neutrophil count
BL-4	biosafety level 4
CAR	chimeric antigen receptor
CART	chimeric antigen receptor T cell
CBC	complete blood count
CDC	Centers for Disease Control and Prevention
CLL	chronic lymphocytic leukemia
CMCF	Connell O'Reilly Cell Manufacturing Core Facility
CMV	cytomegalovirus
CNS	central nervous system
COG	Children's Oncology Group
CRS	cytokine release syndrome
CV	curriculum vitae
DCIS	ductal carcinoma in situ
DFCI	Dana-Farber Cancer Institute
DLBCL	diffuse large B-cell lymphoma
DLT	dose-limiting toxicity
dsDNA	double-stranded DNA
DSMB	Data and Safety Monitoring Board
EMT	epithelial-to-mesenchymal transformation
EPO	erythropoietin
ER	estrogen receptor
ES cells	embryonic stem cells
5-FC	5-fluorocytosine
FDA	Food and Drug Administration
FSH	follicle stimulating hormone
FVB	Friend virus B-type
GFR	growth factor receptor
GLP	good laboratory practice
GM-CSF	granulocyte-macrophage colony-stimulating factor
GMP	good manufacturing practice
GTSAB	Gene Transfer Safety Assessment Board
HER2	human epidermal growth factor receptor 2
HDAC	histone deacetylase
HLA	human leukocyte antigen
HPA	Human Protein Atlas
HPLC	high performance liquid chromatography
HSC	hematopoietic stem cell
HSPC	hematopoietic stem and progenitor cells
IACUC	Institutional Animal Care and Use Committee
ICD	informed consent document
IFN- γ	interferon gamma
IGFBP-2	insulin-like growth factor binding protein 2
IL-6	human interleukin 6
IL-12	human interleukin 12
IND	investigational new drug
IRB	institutional review board
IV	intravenous
IVF	in vitro fertilization
LTFU	long-term follow-up

mAB	monoclonal antibodies
MDS	myelodysplastic syndrome
MDSCs	myeloid derived suppressor cells
MDS–RAEB	myelodysplastic syndrome–refractory anemia with excess blasts
MFG	milk fat globule (promoter)
MHC	major histocompatibility complex
MIC	MHC class I chain-related protein
MICA	MHC class I chain-related protein A
MICB	MHC class I chain-related protein B
MM	multiple myeloma
MTD	maximum tolerated dose
mRNA	messenger RNA
mtDNA	mitochondrial DNA
NCI	National Cancer Institute
NEJM	New England Journal of Medicine
NHLBI	National Heart, Lung, and Blood Institute
NHP	non-human primate
NIH	National Institutes of Health
<i>NIH Guidelines</i>	<i>NIH Guidelines for Research Involving Recombinant DNA Molecules</i>
NKG2D	natural killer group 2D
NMD	nonsense-mediated decay
NOAEL	no observed adverse effect level
NOD/SCID	non-obese diabetes/severe combined immunodeficiency
NSG	NOD SCID gamma
OBA	Office of Biotechnology Activities, NIH
OD	Office of the Director, NIH
PBMC	peripheral blood mononuclear cell
PI	principal investigator
POB	Pediatric Oncology Branch, NCI
PSA	prostate-specific antigen
PSADT	PSA doubling time
QOL	quality of life
qPCR	quantitative real-time polymerase chain reaction
RAC	Recombinant DNA Advisory Committee
RCL	replication-competent lentivirus
RSV	respiratory syncytial virus
SAE	serious adverse event
ScFv	single-chain variable fragment
SELEX	Systematic Evolution of Ligands by Exponential Enrichment
SIN	self-inactivating
SMC	Safety Monitoring Committee
Th1	Type 1 T helper
Tregs	regulatory T cells
UCSF	University of California at San Francisco
vGCV	valganciclovir
vp	viral particles

Appendix A: Public Comments on Human Gene Transfer Protocol #1406-1320

[This testimony is provided verbatim, as reported in the transcript of the September 2014 RAC meeting.]

Testimony of Carlos Sandi

May I please comment? I'll monopolize the 10 minutes and then some. Definitely much livelier discussion than I was anticipating based on some of the past videos [laughter].

Rather than add to that dynamic, I'm just going to stick with my written comments. So here we go. It's a little long winded, but there are no PowerPoints, so sorry.

My emergent study in pediatric oncology began on a February afternoon in 2006, when I received a panic phone call from my wife asking me to leave work and come home immediately. She had taken our daughter, Althea [ph], to the doctor for an ear infection, and from there our day unfolded into a typical diagnosis story of shock and despair, common to every parent of a childhood cancer patient.

In Althea's case, the diagnosis was leukemia. The word was terrifying enough on its own, but as the first few days went by, the news grew steadily worse. We quickly learned the difference between lymphoblastic and myeloid, and we were crushed to find out that hers was myeloid. We then learned about the FAB classification system and the various subtypes of ALL. Hers was called "status M7 megakaryoblastic."

We read everything we could find on the subject, and we were repeatedly confronted with the nauseating phrase "dismal prognosis." Our oncologist put it to us gently when he told us, "She has a chance; it's just not a very good one." From the age of 16 months to just over 2 years, our daughter simply endured. She went through round after round of intensive chemotherapy, bone marrow aspirations, surgeries, infections, radiation, and an ill-fated cord blood transplant. As a parent, it's incredibly painful to submit your child to that level of suffering, but what carried us through was an unshakeable oath that with the right therapy and a lot of luck, our daughter might have a real shot at survival. Unfortunately, that wasn't to be. On December 1, 2006, she died at home surrounded by her family in her mother's arms.

I vividly remember the conversation on Unit 5200 at Duke when Althea's transplant doctors gave "the talk" that we hoped against all odds to never have. I didn't have the presence of mind to write down exactly what was said, but I think anybody who has spent enough time in pediatric oncology knows the script: "Things are not going as well as we had hoped, and at this point, there are no other treatment options and we need to focus on keeping her comfortable now."

Our primary thought that one of these days, leukemia will be something you cure with a single injection, but that's just not where we are right now.

I tried my best to get on with the business of living but couldn't resist the occasional urge to search the Web for any signs of real progress against AML. The news was either absent or consistently depressing, until I read the first press releases coming out touting Emily Whitehead's incredible against-all-odds recovery from otherwise incurable leukemia. Did they really use HIV [inaudible] to fight cancer? Like many people, I was absolutely blown away by the novelty of the concept and pleasantly shocked that finally we were getting close to a real breakthrough. It wasn't a cure for AML, but at least it was going to help somebody else in that hopeless situation.

As it turns out, after Althea's death, my wife and I decided to go ahead and try for another child. Some people might think that after losing a child to a rare cancer, we would be more skeptical about probabilities, but we felt pretty certain that a disease as rare as hers wasn't anything hereditary. The data indicated only a very minor increase in the chances that a sibling might have similar issues, and besides, a lot of other cancer kid families went on to have children who were doing just fine.

So we did what parents do and on June 7, 2008, our son Phineas joined our [inaudible]. Phineas [inaudible], and for 4½ years, life was good and almost normal again, until March 2013. A month or so after I first heard about the CAR T cells, my wife took Phineas to the doctor. He had had a persistent low grade fever and sore throat that she wanted a quick check to rule out an infection.

On short notice, she set up an appointment for a pediatrician at our local practice. His eardrums looked fine, but then the doctor found swollen lymph nodes and pointed a thin rash on his torso. Trying to swallow her rising panic, my wife asked him, "Does that look like a TK [ph] to you?" She told him about Althea, and then he agreed to run a CBC.

After a while, he returned and said there was something wrong with the first sample. The numbers were way off, probably a clot, and he needed to repeat the test. At that point, she knew before the poor pediatrician did, and I got the second worst phone call of my life.

As we went through the intake process at UNC, we were convinced we were about to repeat that awful year. But to our surprise, the first pathology reports indicated lymphoblastic leukemia, the good kind. His presenting leukocyte count was somewhere in the neighborhood of 150,000, and he was initially classified as high-risk ALL; but at that point, all we focused on was “Thank God it’s not myeloid.” Baffled but hopeful, we went through the high-risk induction protocol and celebrated his good morphological response.

All of that was turned completely upside down when his first MRD came back at 2 percent positive. Over the next 3 months, for several rounds of chemo, an anaphylactic reaction to PEG-asparaginase, ridiculous liver enzyme issues, and pretty much every other side effect in the book, his MRD levels dipped ever so slightly and then began creeping up again, until it became blazingly obvious that we’re dealing with primary refractory disease.

We began scrambling to find some way to save our boy. Chemotherapy was not going to do the job, and bone marrow transplant was a long shot at best unless we could get him into a solid MRD-negative remission.

At the time, CAR 19 trials were actively recruiting at three facilities: CHOP, Sloane, and NIH. We begged the team at UNC to reach out to all three, and fortunately for us they did. Unlike others who [inaudible], Dr. Lee at NIH enthusiastically returned the call, and after a few false starts, Phineas was able to get into their trial. He had a very robust response and, by the end of September, was declared MRD negative.

He underwent a BMT for unmatched, unrelated donor October 23, 2013; and long story short, thanks to an amazing combination of luck, persistence, excellent health care providers, and good old-fashioned science, our now genetically modified, fully irradiated, chemotological transgendered 6-year-old chimera started kindergarten last month. Yes, he had a pretty scary run of cytokine release syndrome; and, no, the inpatient room service menu descriptions at NIH are not terribly representative of the food that gets delivered [laughter]; but calories are calories, and the low toxicity of the CAR approach gave him time to heal and grow stronger before beginning transplant prep. I’m not going to say this transplant was an easy process for him, but he went in healthier than most and overall the experience was a far cry from the medieval torture our daughter had endured years before.

Clearly, our family is not very good at picking the future; but for now, our son is off all his meds, and he is thriving. We are cautiously optimistic and incredibly grateful for all of the amazing people we had the privilege of depending on throughout this ordeal.

The team at NIH is one of the most competent, innovative, and compassionate groups that I have ever met. In triumph and tragedy, they consistently excelled, and I am deeply hopeful that they can continue their work to advance science and save children’s lives with the new anti-CD22 variant CAR as soon as humanly possible. I personally know of several families of children with B-cell malignancies who have also had “the talk” with their treatment teams. They have very little time and no good options left. Moving this trial forward now will not save all of them, but for many, it’s their best shot at a real future together. It might not be the single injection cure we’re looking for, but it’s infinitely better than a dismal prognosis. Thank you.

Testimony of Suzy Matter

Hello and thank you for the opportunity to be here today. I want to personally thank you for the work that you do. My name is Suzy Matter, but most people know me as Colton’s mom. Five years ago today, we heard the words for the first time, “Your son has cancer.” Those words are scary enough, but believe it or not, Colton has been called to battle leukemia five times in five years—five times too many. With each battle cry, there have been blessings of remission in between. During those blessed remission months, I know doctors like you are working tirelessly in their labs, researching, testing, experimenting, and losing sleep at night while taking baby steps and thankfully giant steps towards finding a cure.

With each battle call, the conversations with doctors get increasingly more terrifying. The list of options to treat and cure Colton’s cancer gets shorter and shorter. When Colton relapsed last year, we were told by our doctor that there was nothing else he could do. To hear those words as a parent, it changes you. It can shrivel you up on your bedroom floor or make you fight like you’ve never fought before.

That is why I traveled here today from Seattle. Colton isn't done fighting. He isn't done living. He is my 14-year-old son who just started high school, who loves to laugh, who loves to play a mean game of baseball, loves life, and just wants to be normal.

Thankfully, our doctor was wrong last fall, and after additional research, there was something else for Colton. And after many trips to Philadelphia, he was blessed to be Pediatric Patient #22 on the clinical trial targeting CAR 19 cells. After this treatment, he went into immediate and complete remission and stayed that way for 9 beautifully blessed months, until we learned his cancer was back last month. To say Colton is a warrior is an understatement. He has battled through three bone marrow transplants and one revolutionary T-cell immunology treatment, and he's not done yet. And if you saw him walking down the street, you would never know the battles he has faced and won.

Colton is a prime candidate for this clinical trial targeting the CD22 cells, because that is how his cancer has come back, expressing CD22 and no longer CD19. In many ways, Colton is helping the world find a cure to this horrible disease by helping doctors figure out what cancer can and can't do and also what patients are willing to do to live.

Our T-cell doctors in Philadelphia have now treated more than 35 kids, with 90 percent of them going into a complete remission. I know in my heart that this clinical trial and discussion here today will do the same and save so many like Colton who have run out of options and that this is their only hope left.

Colton's bone marrow was 40 percent leukemic cells a month ago and, after his first round of chemo, is now less than 1 percent. Amen.

When he should be making plans for that Friday night football game, Colton is now starting his second round of chemo to keep this horrible disease at bay the best we can until there is an option. This trial is the option he needs to save his life or give a shot at it.

I don't say these words lightly. I say them with every fiber of my being. Through his actions, through his words, Colton isn't done living. He's got plans to attend football games, he's got plans to go to college, and he's making new plans every day, because he's a fighter and he has hope. Please don't delay in approving this clinical trial. Please don't let him die waiting.

This is Colton [showing picture]. I feel like there is sometimes no face behind the name.

So thank you very much for your time.

Testimony of Lisa Wilkins

I don't have a script, so I'm just kind of going off the cuff, but I just thought it was important to talk. My name is Lisa Wilkins. My son is Nicholas. He is Patient #15 at Children's Hospital Philadelphia. When he was 4, he was diagnosed with acute lymphoblastic leukemia, underwent 2½ years of chemotherapy, and came off treatment when he was done. Three years short of being off treatment for 5 years, we found that he relapsed. He went through 15 weeks of intensive chemotherapy and was lucky enough to go through a bone marrow transplant with an exact-match sibling—from his older sister. At his 3-year follow-up appointment at Duke, we found out that he relapsed again. He's now been battling cancer for over 12 years. When we found out he relapsed, we went and immediately started chemotherapy again. A month after treatment, we found out he was now resistant to—he became resistant to chemotherapy.

His docs fortunately had referred us to Children's Hospital Philadelphia, and at the time that we went to go visit CHOP, there were only results of two patients at the time: Emily Whitehead and a second patient. So at the time, as a parent, you know, we did as much research as we could, which quite honestly was not a lot at the time; there was not a lot of data. CHOP provided no guarantees of this being a cure, but of course, as a parent, this is hope for us; this is something that we had to do because it would give him a chance for something. Fortunately, he underwent his—his T cells were reengineered, and he had them given to him in May of 2013; and a month later, we found out that he was now MRD negative. He is now 15 months post-reengineering for seeing his T cells and is just basically like any normal kid—wants to play baseball, is a junior in high school, and just living the life. We were lucky enough last Friday to be at Stand Up to Cancer, where his video was shown with two other patients to really kind of show the effects of immunology. So I truly believe that this is where we are headed and feel strongly that this is where we need to go. Thank you.

Testimony of Nancy and Greg Sanders (via letter, read by Dr. Kohn)

Thank you for allowing us to make comments on this critical issue of why Protocol 1406-1320 should continue to enroll pediatric patients and why there should not be any kind of delay in continuing this protocol with those pediatric patients.

As a parent, you do everything to cure, heal, and comfort your child when he or she is diagnosed with ALL. You have very difficult discussions with the doctors and hear words you never thought you would have to hear in connection with your child, but the tough discussions are held with that child. You are faced with explaining to your child what is making him so sick. You are faced with telling your child that the cancer that he fought so bravely for 3½ years that everyone thought was gone has returned. You are also the one who has to tell him that there is really no choice in the way of options in order to beat this cancer again. It's heartbreaking to have to sit down and go through the list of possible complications and have death be a major side effect mentioned over and over.

Since 2005, when nelarabine was approved ALL, the only other drug approved in the treatment in ALL has been Erwinaze in 2011. There are many drugs approved for the treatment leukemia—chronic forms of leukemia. Our son was one of those who received Erwinaze in 2008. He received it again this past December, when he relapsed after being off treatment for 2 years and 4 months. The Erwinaze did not work the second time around. He received nelarabine to get him into remission after relapsing. So our son did benefit from both of the only two drugs that have been discovered and developed in the last 9+ years.

There should be more than just two new drugs that have shown effectiveness against children's cancer that have been discovered in the past 10 years. There should be more to choose from to beat this beast called ALL. Children suffer mightily from all of the side effects of the standard-of-care protocols that are offered to cure leukemia. They suffer in so many ways, but you all know that. You are the researchers who are trying to spare our kids and offer them a gentler approach, but one that will still offer a cure.

Our son Dale relapsed with T-cell ALL in November 2013, and while we know and understand the protocol that is being discussed today would not have benefited our son, we still believe that we have much to say and that our comments are very pertinent.

When our 17-year-old son relapsed, we were told our only choice was to have a bone marrow transplant. There was nothing else to try. We could have tried just chemo, but we were told there was a very high chance that the leukemia would return and be resistant and we might never get Dale into remission. We chose to pursue a bone marrow transplant, and even then we almost didn't get him into remission, as the leukemia was resistant to the first round of chemo that we tried, which—many of the same chemos that were used in 2008 at first diagnosis.

Dale passed away on July 26, 2014, at Day +135, after suffering numerous complications due to the bone marrow transplant and how harsh it was. While there is no evidence for our son having any leukemic cells left in his body when he died, he died due to infections that were able to proliferate due to severe graft-versus-host disease issues. If you had other options, options that were not available to him, he might still be alive. He might not have had so many things go wrong and suffer so in the end.

We are here to beg and plead on behalf of other pediatric patients. We pray that these children currently hearing that their cancer has returned are given the chance of conquering their leukemia without having to go through what our son endured. If our son had had any other options, we would have pursued them. We did not have this option. Please make this option available to other children by keeping the protocol open to pediatric patients now, not by making children wait until a protocol is vetted first by adults. Children can't wait, and a wait of even a few months might be a death sentence. None of us wanted to hear the word "cancer," none of us wanted to hear "relapse," and none of us want to hear that there are very few good options to try. Please keep this promising option available by keeping Protocol 1406-[1]320 open and enrolling new pediatric patients.

Sincerely, Nancy and Greg Sanders.

Testimony of Sang Nguyen

My name is Sang. I go to, like—this meeting is, like, coincidence, because one of the RAC member[s] brought me to here. So I'm actually [inaudible] member, but Dr. Fry informed, like, all the communities from, like, parents of, like, children.

So I'm actually a cancer survivor for over 15 years, and I actually got ALL, like, back when I was really young. So, like, the thing I want to say to you—because, like, the work that you are doing, like, helping for the patients in the past, like, helping for us, like, getting help for us—and also right here that we try again and like to keep that help increasing for all the people and also for the future patients.

So I hope, like—because I see every day people in my lab and also, like, Dr. Fry and all the people in, like, the Pediatric Oncology Branch try really hard, like, to pursue and also that you get help for the patient. So I hope, like, you guys, like, push this one forward, because I know, like, the reason you

want to become a doctor: because you want to save the life for the kids. So that's it—like, the thing. Keep that thing.

And even though, like, back in the day—like, I was really young; I was just a kid, but I can tell you the children are real strong, really strong, and we know that, like, with [inaudible] around and [inaudible] believe, like, all of you, like, do that for us.

Testimony of Waleed Haso

So my name is Waleed Haso. I was a scientist doing most of the preclinical work, and I have to comment on how difficult it was to, like, work in the lab, and I was, like, very motivated to see all the patients and all the trials open. It took me more than 4 years working on making the CD22 CAR. I was very happy to see successful. It took me so many sleepless nights in the lab. I worked so hard, and the only motivation was seeing the patients. People like Sang working with me in the lab made me more motivated to work harder and harder to find more therapies, making novel therapies through the immunotherapies proven to be difficult. And we are very lucky to have the CD22 CAR active, just as active as a CD19 CAR. It took me 2 years. I was 22 years old when I first joined the lab doing research, and I'm only motivated by seeing all the patients, all the families, striving to find cures. Thank you very much. And I really support this trial, and I hope it doesn't get delayed. Thank you.