RECOMBINANT DNA ADVISORY COMMITTEE

Minutes of Meeting

September 11–12, 2013

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 at the Office of Biotechnology Activities' Web site at http://oba.od.nih.gov/oba/index.html.)

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
NATIONAL INSTITUTES OF HEALTH
RECOMBINANT DNA ADVISORY COMMITTEE
Minutes of Meeting[1]

September 11–12, 2013

The Recombinant DNA Advisory Committee (RAC) convened for its 134th meeting at 1:20 p.m. on September 11, 2013, at the Hyatt Regency Hotel in Bethesda, Maryland. Dr. Donald B. Kohn (RAC Chair) presided. In accordance with Public Law 92-463, the meeting was open to the public from 1:20 p.m. until 5:25 p.m. on September 11, 2013, and from 8:30 a.m. until 1:25 p.m. on September 12, 2013. The following individuals were present for all or part of the September 2013 RAC meeting.

Committee Members

Tianxi Cai, Harvard University
Saswati Chatterjee, City of Hope National Medical Center
William Curry, Harvard Medical School (Pending)
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
Hans-Peter Kiem, University of Washington School of Medicine/Fred Hutchinson Cancer Research Center
Walter J. Koch, Temple University School of Medicine
Donald B. Kohn (RAC Chair), University of California, Los Angeles
David A. Ornelles, Wake Forest University School of Medicine
Joseph Pilewski, University of Pittsburgh
Michael Sadelain, Memorial Sloan-Kettering Cancer Center (Pending)
Marshall Strome, St. Luke's–Roosevelt Hospital Center/New York Head and Neck Institute
Dawn P. 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 C. Borror, Office for Human Research Protections, U.S. Department of Health and Human Services (HHS)
Denise K. Gavin, U.S. Food and Drug Administration (FDA) *(Day 2 only)*
Daniel M. Takefman, FDA *(Day 1 only)*

NIH/OD/OBA Staff Members

Linda Gargiulo
Robert Jambou
Maureen Montgomery
Marina O'Reilly
Gene Rosenthal
Carolyn Mosby

[1] The Recombinant DNA Advisory Committee is advisory to the National Institutes of Health (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.

Attendees

There were 67 attendees at this two-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.

I. Call to Order and Opening Remarks

Dr. Kohn, the RAC Chair, called the meeting to order at 1:20 p.m. on September 11, 2013. Notice of this meeting under the *NIH Guidelines for Research Involving Recombinant DNA Molecules (NIH Guidelines)* was published in the *Federal Register* on August 20, 2013 (78 FR 51196). Issues addressed by the RAC at this meeting included a report from the Gene Transfer Safety Assessment Board (GTSAB), a subcommittee of the RAC; public review and discussion of four gene transfer protocols; an update of one protocol previously reviewed by the RAC; an update on the use of lentiviral vectors for long-term gene correction for Wiskott Aldrich Syndrome (WAS) and metachromic leukodystrophy (MLD); guidance for industry regarding consideration for the design of early-phase clinical trials of cellular and gene transfer (CGT) products; an update on a proposal to exempt certain gene transfer trials from institutional biosafety committee (IBC) review; and an update to Appendix B of the *NIH Guidelines*.

RAC members introduced themselves by name, affiliation, and research interests.

Dr. Corrigan-Curay reminded RAC members of the rules of conduct that apply to them as Special Federal 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 #1307-1236, Titled "Infusion of Allogeneic, Third-Party CD19-Specific T cells (CD19RCD137 T Cells) in Patients with Refractory CD19+ B-Lineage Malignancies"

Principal Investigators:	Laurence J.N. Cooper, M.D., Ph.D., M.D. Anderson Cancer Center (MDACC); Partow Kebriaei, M.D., University of Texas, MDACC
Additional Presenters:	Philip D. Gregory, D.Phil., Sangamo BioSciences, Inc.
Sponsor:	Sangamo BioSciences, Inc.
RAC Reviewers:	Drs. Kiem, Pilewski, and Wooley

Drs. Cannon, and Kohn were recused from consideration and discussion of this protocol due to conflicts of interest.

A. Protocol Summary

This single-center, proof-of-concept human gene trial evaluates the safety, feasibility, and persistence of adoptively transferred "off-the-shelf" CD19-specific allogeneic T cells in research participants with advanced B-lineage cell malignancies. The goal is to manufacture the T cells *a priori* of need and infuse them on demand as a "drug". The T cells, derived from third party umbilical cord blood, are rendered specific for CD19 using a scFv derived from a mouse monoclonal antibody (clone FMC63). This is achieved using a Nucleofector device to co-electro-transfer two *Sleeping Beauty* (SB) DNA plasmids expressing (i) SB11 hyperactive transposase and (ii) transposon coding for chimeric antigen receptor (CAR), designated CD19RCD137, that can activate T cells through chimeric CD137 and CD3-zeta endodomain upon binding cell surface CD19 independent of human leukocyte antigen (HLA). T cells are further genetically edited using zinc finger nucleases (ZFNs) targeting T-cell receptor (TCR) β constant

regions to disrupt the expression of endogenous TCR αβ receptor to avoid an un-intended deleterious allogeneic immune response. In compliance with current good manufacturing practice (cGMP) for phase I/II trials, the genetically modified and edited T cells are recursively expanded to clinically-meaningful doses on gamma-irradiated artificial antigen presenting cells (aAPC) derived from a master cell bank (or a derived working cell bank) of K562 that were genetically modified by lentiviral transduction to co-express (i) CD19 (ii) 4-1BBL (CD137L) (iii) CD86 (iv) CD64 and (v) a membrane-bound IL-15 (mIL-15). These donor-derived TCRnegCD19RCD137CAR+ T cells will be cryopreserved as a bank until use for infusion into multiple research participants. Prior to adoptive immunotherapy research, participants will receive intravenous lympho-depleting chemotherapy in the form of cyclophosphamide at 60 mg/Kg IV x 2 consecutive doses, fludarabine 25 mg/m^2 IV x 5 doses, followed by at least a day of rest. Research participants will be eligible to receive one intravenous infusion of thawed donor-derived (allogeneic) genetically modified ex vivo-propagated T cells. The primary objectives of this study are to assess the safety and feasibility of allogeneic, 3rd party, genetically modified, CD19-specific CAR+TCRneg T cells administered into research participants with refractory CD19+ B-lineage malignancies. The secondary objectives are to assess the (i) screen for the development of host immune responses against the CD19-specific CAR, (ii) describe the homing ability of the infused T cells, and (iii) assess disease response. This study will employ the 3+3 design to find the maximum tolerated dose (MTD) of these CD19-specific T cells. The first research participant will be enrolled in the first cohort at Dose level A (not to exceed $10^6/m^2$) and following the completion of three T-cell infusions, the successive cohorts of research participants at the subsequent dose levels will be enrolled. Beginning with the second research participant receiving the CD19-specific T cells, a research participant will not be treated with CD19-specific T cells until a minimum of 15 days have elapsed between the period in which that research participant and the prior research participant was infused with the CD19-specific T cells. The T-cell infusion dosing for subsequent cohorts are as follows: Dose level A>$10^6/m^2$, but less than or equal to $10^7/m^2$, Dose level B>$10^7/m^2$ but less than or equal to 5 x $10^7/m^2$, Dose level C >5 x $10^7/m^2$ but less than or equal to $10^8/m^2$, Dose level D >$10^8/m^2$ but less than or equal to 5 x $10^8/m^2$, Dose level E >5 x $10^8/m^2$ less than or equal to $10^9/m^2$, and Dose level F >$10^9/m^2$ but less than or equal to 5 x $10^9/m^2$. A maximum of 42 research participants at a rate of approximately one research participant per month will be infused.

B. Written Reviews by RAC Members

Eleven RAC members voted for in-depth review and public discussion of this protocol. Key issues included that although the use of second-generation anti-CD19 CAR T cells in research participants with hematologic malignancies is not novel, this protocol is the first to use a zinc-finger endonuclease to target the TCR alpha-beta receptor with the goal of developing an off-the-shelf T-cell product.

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

Dr. Kiem questioned the investigators' requirement of a minimum of 10 percent CAR+ T cells, citing the potential for significant toxicity variance among research participants and cohorts. He asked about the experience with cord blood cells, especially with regard to the ability to isolate and expand cord blood mononuclear cells, and whether the phenotype after expansion is similar to peripheral blood mononuclear cell expansion and modification. Dr. Kiem asked why the release criteria do not specify a certain CD8+ cell percentage. Noting that graft rejection remains possible even though these cells are not supposed to cause graft-versus-host disease (GVHD), he asked how graft rejection would be assessed and how it would affect the protocol design and analysis. Regarding the informed consent document, Dr. Kiem stated the importance of clarifying that any costs related to the care and treatment of injuries or side effects from the experimental treatment would not be covered by the study and would need to be covered by the participant's insurance or the individual participant.

Noting that the protocol, consent, and responses to Appendix M were thorough and clearly written, Dr. Pilewski asked the investigators to clarify the methods for evaluating the secondary objective to assess the homing ability of the infused T cells and the safety assay to be used to detect the presence of anti-human leukocyte antigen (anti-HLA) antibodies in peripheral blood. He suggested that the investigators consider monitoring pulmonary function with spirometry at regular intervals after dosing, given the concerns for pulmonary toxicity akin to GVHD, and he asked whether four hours of observation after

infusion would be sufficient based on prior adverse reactions during donor lymphocyte infusion. Dr. Kiem suggested that the informed consent document should clarify which costs would not be billed to insurance and, based on experience with prior T-cell protocols, should provide an assessment of potential expenses for participation in this protocol. He asked the investigators to discuss methods other than sequence similarity/homology analysis that could be used to assess the risk of insertional mutagenesis.

Dr. Wooley noted that little information is provided in the submitted documentation regarding off-target effects, a major concern. She suggested that *in vitro* or cell-model studies should be conducted to test the actual activity of the proposed zinc-finger nucleases (ZFNs) before this experimental treatment is used in humans. She asked the investigators to explain the proportions expected for CD56+ and CD4+ lymphocytes in the culturing process and whether range limits are set for these cells and, if not, how the investigators plan to compare results from different batches of cells if the proportions are dramatically different. Dr. Wooley requested that the investigators delineate the steps being taken to ensure that the aAPCs are not contaminating the T-cell population and that they discuss planned testing for potential contamination and proposed removal steps. She also asked about the specific ablation method to be used in response to a serious adverse event (SAE). Dr. Wooley noted that the informed consent document is not clear about why the participants or their insurance companies should pay for the chemotherapy or the infusion; she opined that these costs and any complications or injuries from these experimental treatments should be covered by the study or by MDACC health providers. If MDACC will not cover these costs, she suggested that a strongly worded statement be included in the informed consent document to indicate that many insurance companies do not cover experimental treatments or their complications and that, therefore, the participant might be responsible for these costs.

C. RAC Discussion

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

- Dr. Fost asked Dr. Corrigan-Curay why this protocol had not been given a specific ethics review. Dr. Corrigan-Curay responded that the three RAC reviewers of this protocol provided ethics-related comments, so the OBA determined that a focused ethics review was not necessary. The ethicist members of the RAC always have the opportunity to provide any additional comments during the meeting discussion.
- Dr. Hammarskjöld pointed out that there are myriad examples of diseases caused by retrotransposition.
- Dr. Hammarskjöld asked the investigators to explain how potential participants in this trial would be informed about their possible eligibility for other, concurrent clinical trials in which they could participate in lieu of participating in this trial.
- Dr. Zoloth suggested several variations and changes to the informed consent document.
- Dr. Sadelain requested further discussion about inducing $CD3^+$ negativity by T-cell activation.
- Dr. Sadelain noted a recent report of anaphylactic response upon multiple infusions of RNA-transfected cells that was thought to be mediated by an antibody to the CAR; he asked the investigators how that information has changed their perspective.
- Dr. Sadelain asked the investigators whether they would be able to tell the difference between the function of the CAR and allorejection if the cells disappear quickly, a result that is possible although not expected with this CAR.
- Ms. Hardison suggested that the informed consent process include clear information for potential participants about standard treatment versus experimental treatment related to this study.
- Dr. Kiem asked whether it is possible to screen potential participants to determine whether they could be sensitized to a second infusion.

D. Investigator Response

1. Written Responses to RAC Reviews

The investigators explained that they will use a 10 percent cutoff (of CAR+ T cells) based on their prior four trials infusing CD19-specific CAR+ T cells. However, the percentage of CAR expression on clinical-grade T cells electroporated and propagated typically falls within a known range. They assume that the T-cell product from a third-party donor with CAR expression will be above 10 percent. The therapeutic activity will be reported, based in part, on expression of the CAR on the off-the-shelf T cells.

The investigators are currently infusing donor-derived CD19-specific CAR+ T cells after allogeneic umbilical cord blood (UCB) transplantation. They have since transitioned the program to develop off-the-shelf T cells from UCB as well as from peripheral blood (PB). Their approach to manufacturing, based on electrotransfer of DNA and mRNA species and propagation on aAPCs, can be applied readily to T cells from both UCB and PB. The percentage of genetically modified and propagated CD4+ and CD8+ T cells varies from donor to donor. The investigators have profiled selected mRNA species on CAR+ T cells and have not seen major differences between PB and UCB donors.

The release criteria do not predefine a percentage of CD8+ T cells in the genetically modified product, because there is donor-to-donor variation, CD4+CAR+ T cells appear to be cytotoxic, and the investigators do not know the “ideal” percentage of CD8+ T cells.

The infused, genetically modified T cells from a third party will be HLA mismatched with the recipient and thus will be subject to immune-mediated clearance due to the recipient “recognizing” major and minor histocompatibility antigens (as well as the CAR). This situation may be mitigated through the addition of lymphodepleting chemotherapy prior to adoptive transfer of the T cells. Loss of the infused T cells is expected, and one of the proposed trial’s priorities is to evaluate the T cells’ persistence using quantitative polymerase chain reaction (Q-PCR) using CAR-specific primers and, if applicable, flow cytometry using CAR-specific monoclonal antibodies. Future trials may infuse CAR+ TCRneg T cells that also have been engineered using ZFNs to eliminate HLA.

This trial is not limited to research participants who have undergone prior hematopoietic stem cell transplantation (HSCT). In patients who have had a prior HSCT, a minimum of three months from transplantation to infusion of CAR+ T cells is required. Secondary graft failure is possible in participants with prior HSCT and, because of its relationship to T-cell infusion, it would be counted as a dose-limiting toxicity (DLT).

The potential for homing of infused T cells is estimated based on researchers’ ability to measure infused T cells in bone marrow, lymph nodes, and other tissues when these biologic sites are sampled, as clinically indicated.

The investigators plan to determine the presence of HLA-specific antibodies based on testing already undertaken at MDACC in support of the engraftment of hematopoietic stem cells. The presence of anti-HLA antibodies to third-party T cells will be determined by testing the research participants’ sera with a panel of fluorescent beads coated with single HLA antigen preparations, and the anti-HLA antibody reactivity will be detected in a Luminex platform. The reactivity of HLA antibodies will be defined by testing two panels of HLA molecules that include a set of single-antigen preparations for assignment of specificity, to be confirmed by comparison with the reactivity against a panel of HLA class I or II antigen preparations extracted from lymphocytes of individual research participants. The final HLA antibody will be assessed by comparing the high-resolution types of the patient and antigen panels. Antibody levels will be interpreted as normalized fluorescence intensity (FI) as defined by the kit’s manufacturer against HLA antibody mismatch.

Following allogeneic HSCT, pulmonary function tests will be performed on any research participants who develop pulmonary GVHD.

The investigators explained that four hours of observation after infusion is the timeline they follow when infusing allogeneic donor lymphocytes and for their current protocols infusing CAR+ T cells. To date, they have not observed any immediate acute toxicity following infusion of CAR+ T cells.

As they do in all their clinical trials, the investigators pledged to seek financial clearance from each participant's insurance company to reimburse for lymphodepleting chemotherapy and all required tests prior to dosing (such as echocardiograms) to ensure that the intended recipient can safely undergo this experimental treatment and can receive routine follow-up tests afterward. Costs associated with the T-cell product manufacturing process will be charged as research.

Regarding the risk of insertional mutagenesis and subsequent malignancy related to third-party T cells, the investigators explained that, in collaboration with the University of Minnesota, they have undertaken high-throughput sequencing of the *Sleeping Beauty* (SB) integration events, evaluating the location of CAR insertions in 33 separate populations of genetically modified and propagated T cells and resulting in 7.4 million reads. The vast majority (96.5 percent) of intragenic insertions are intronic. The integration profiles for SB-modified T cells compare favorably to those of T cells transduced with retrovirus.

Regarding the concern about off-target effects, the investigators explained that, prior to the initiation of human studies, the specificity of the ZFNs targeting tagged red blood cells (TRBCs) will be assessed experimentally. They have performed a preliminary assessment of the top 15 putative off-target sites found in the genome. They have determined experimentally the subset of DNA binding sites that each individual ZFN is capable of binding *in vitro* and have used this information to guide a genome-wide bioinformatic prediction of the most similar putative off-target sites in the human genome. Analysis of the top 15 such sites failed to detect evidence of ZFN-induced mutations (although on-target TCR β constant region cleavage was measured at up to 48 percent of alleles). As the clinical trial moves forward, the investigators propose to undertake in-process testing that will be extended to include the top 30 such sites, a ZFN mRNA dose in excess of that used in the clinical manufacturing process, and a direct DNA sequencing assay run at a minimum of 10,000 sequence reads per target site. In addition to these molecular analyses, the modified T-cell product will be characterized via in-process testing and will be shown to be unable to sustain autonomous growth in the absence of activation signaling and exogenous cytokines (specifically, interleukin-2 [IL-2]).

The ratio of CD4+ to CD8+ T cells is donor-dependent, and the ideal composition is not known. The investigators reported that their current practice is to manufacture CAR+ T cells containing a ratio of CD4/CD8 cells that emerges upon co-culture with aAPCs in the presence of IL-2/IL-21. They prefer to retain flexibility to derive a T-cell product with a desired amount of these two T-cell products, in the eventuality that one subset is demonstrated to be therapeutically superior. The removal of CD56+ (CD3neg) natural killer (NK) cells is necessary to prevent their overgrowth and competition for the aAPC (feeder) cells. The investigators have established criteria for the removal of NK cells.

Regarding contamination of the T-cell population by aAPCs, the investigators explained that the aAPCs are excluded from the final product based on (a) the use of lethal gamma irradiation of the aAPCs prior to use, (b) evaluation of the T-cell product for contaminating presence of aAPCs based on flow cytometry for CD32+ and CD19+ (endogenous gene and introduced transgene), and (c) testing of the final product for absence of autonomous growth.

Regarding the specific ablation method to be used in response to an SAE, the investigators explained that corticosteroids would be administered initially, followed by other immunosuppressive reagents. Systemic administration of corticosteroids has been used successfully to treat adverse events attributed to CAR+ T cells.

The investigators pledged to work with their institutional review board (IRB) to modify statements in the informed consent document that the RAC reviewers had noted were not clear.

2. Responses to RAC Discussion Questions

Dr. Kebriaei explained that potential participants in this trial would be told about other trials going on at MDACC at the time they consider enrolling in this trial. Dr. Cooper added that a potential participant's condition could mean that it would be difficult or impossible to make enough T cells in the needed timeframe for that individual. Such patients would be informed of this challenge and would likely be advised to enroll in other approved clinical trials.

Explaining how and when to use T-cell activation to induce CD3 negativity, Dr. Cooper said that the final product would be propagated *ex vivo* for several weeks to ensure that neither replication-competent retrovirus nor CD3 re-emerged on the surface.

Regarding the recent report of anaphylactic response upon multiple infusions of RNA-transfected cells that was thought to be mediated by an antibody to the CAR, Dr. Cooper explained that information about this response will be included in the informed consent document, along with a statement about the unknown risk of anaphylaxis with a subsequent infusion. The investigators' standard of care for all biologic infusions is to have an anaphylaxis kit next to the bedside. He further explained that the investigators will adjust their practice based on any additional data obtained about multiple infusions.

Dr. Cooper explained that the investigators will look for allorejection vectors in each research participant, using two different assays.

Dr. Cooper also said that that no easy method exists to screen potential participants' susceptibility to anaphylaxis when given a second infusion.

E. Public Comment

No public comments were offered.

F. Synopsis of RAC Discussion and RAC Observations and Recommendations

Clinical Issues

- While the goal is to eliminate the endogenous T-cell receptor, one risk of this protocol is GVHD from the infused cells if all of the T-cell receptors on the allogeneic cells are not deactivated. Because the infused cells will track to the pulmonary circulation, pulmonary GVHD would be one risk. As pulmonary GVHD may be detected prior to the onset of clinical symptoms by routine testing, either by spirometry or a scheduled pulmonary function test, the investigators should consider adding scheduled testing of pulmonary function.

- One step in the production of the zinc-finger mRNA involves a DNase treatment. To ensure that DNA is sufficiently degraded prior to product infusion, the investigators should consider adding an assessment to the release testing that can detect residual DNA, such as a PCR test.

Ethical/Legal/Social Issues

- The costs of chemotherapy and certain other aspects of the protocol procedures will be billed to the research participants' insurance. The current informed consent document describes this generally and states, "Before taking part in this study, you may ask which parts of the research-related care may be provided without charge, which costs your insurance provider may pay for, and which costs may be your responsibility." It would be more informative and to the point to state that the insurance company may not cover the costs for all care associated with this study and that the participant would then be responsible for those costs. To ensure that participants are adequately informed about the potential costs of this trial, it may be prudent to give all participants information on which procedures would be considered standard of care and billed to their insurance and to encourage participants to speak with their health insurance providers about coverage for trial procedures not covered by MDACC.

- The informed consent document mentions the option to withdraw from the study at any time, but this needs to be clarified, because the infusion of the T cells is not necessarily reversible and these types of products have the potential for long-term survival. It should be made clear that withdrawal from the protocol will only include withdrawal from any subsequent protocol-specified procedures and tests and long-term follow-up. In addition to clarifying the terms of withdrawal in the context of this protocol, it is important to inform the participant of the potential disadvantages of declining to participate in long-term follow-up.

- The informed consent document does tell participants that this procedure is experimental and is a first-in-human protocol that may have unexpected risks and may not provide benefit to the individual. However, the consent also says that "receiving the T-cell infusion may help to control the disease," a statement that makes this experimental intervention sound like therapy and that should be revised to be consistent with previous statements that enrollment may not provide individual benefit.

- There is a case report of a CAR that was transduced with an mRNA in which the subject received a second infusion and had an anaphylactic reaction to the CAR (Maus, M. *et al*., 2013. T Cells Expressing Chimeric Antigen Receptors Can Cause Anaphylaxis in Humans. *Cancer Immunol. Res.* Epub April 7, 2013. Doi:10.1158/2326-6066). Since this protocol will include an option for reinfusion, the informed consent document should reference the possibility of such a reaction as a potential risk of reinfusion.

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 asked for a vote on these summarized recommendations, which the RAC approved by a vote of 12 in favor, 0 opposed, 0 abstentions, and 2 recusals.

III. Update on the Use of Lentiviral Vectors for Long-Term Gene Correction for Wiskott Aldrich Syndrome (WAS) and Metachromic Leukodystrophy (MLD)

Presenter: Luigi Naldini, M.D., Ph.D., San Raffaele Telethon Institute for Gene Therapy (HSR-TIGET), University of Milan, Italy *(via teleconference)*

A. Presentation by Dr. Naldini

Dr. Naldini reviewed the early trials of hematopoietic stem cell-based gene transfer that used gamma-retroviral vectors in primary immunodeficiencies. With at least 13 years of follow-up data, these early trials show stable correction of the disease in a significant number of subjects. However, there are two important issues related to these early studies: the requirement for a selective advantage of the corrected cell, and the occurrence of vector-related leukemia in some patients, which raised concern about the safety of the procedure.

The use of lentiviral vectors has been proposed to address the current issues in this field: to increase the efficiency of gene transfer in hematopoietic stem cells, to better regulate transgene expression to avoid toxicity or abnormal growth by constitutive or ectopic expression of the transgene, and to alleviate the risk of insertional mutagenesis by random vector integration.

Metachromatic Leukodystrophy (MLD) studies. Dr. Naldini discussed several new trials that have resulted in efficient gene transfer compared with the early trials, starting with data from trials targeting MLD, a disease for which there is currently no effective treatment. MLD results from mutations in the gene encoding arylsulfatase A, an enzyme involved in myelin catabolism, and affects oligodendrocytes, neurons, and microglia. Physicians at HSR-TIGET have been treating the most severe form of MLD, which features early onset in the first 2 years of life. In preclinical studies from which Dr. Naldini presented

data, mice with the disease were transplanted with lentiviral transduced progenitors that corrected the disease phenotype; the correction was based and dependent on achieving overexpression of the enzyme in the hematopoietic cell. Based on this data, the clinical protocol was started three years ago.

Dr. Naldini showed the published data from the first three research participants. Vector copies have been high in the blood monocytes, myeloid cells, granulocytes, and bone marrow, and with polyclonal reconstitution. These three individuals are experiencing normal development of motor skills and are conducting essentially normal lives for their ages, with IQs in the normal range.

In total, the investigators have completed experimental treatment of nine MLD subjects. The experimental therapy has been well tolerated and appears safe. Transduced cell engraftment has been sustained at unprecedented levels, and polyclonal reconstitution with a safe vector integration profile has occurred. Enzyme activity in hematopoietic cells and the central nervous system has been restored, and therapeutic benefit has resulted.

WAS trial. WAS is a primary immunodeficiency that affects platelets and myeloid cells and is accompanied by autoimmunity and predisposition to tumor. Wiskott-Aldrich protein is a protein involving cytoskeletal regulation and signal transduction. Allogeneic stem cell transfer is curative, but also has high morbidity and mortality and is not available to all WAS patients. Gene transfer was shown previously to be efficacious in this disease using a gamma-retroviral vector, but more than 50 percent of the research participants have developed leukemia at different time points post-infusion. The investigators seek to determine whether benefit can be achieved while improving safety.

Dr. Naldini presented data from the WAS trial that showed that research participants' phenotypes improved, their immune function was fully reconstituted, and bleeding platelet number rose, although not to normal levels. Although they have not experienced full myeloid reconstitution, these research participants no longer suffer from spontaneous bleeding. The investigators have completed dosing of six research participants, and the experimental therapy has been well tolerated and appears safe. Stable hematopoietic stem cell (HSC) gene marking has been shown at 30 to 50 percent, the vector integration profile is clearly distinct from retroviral-based gene transfer at the same follow-up, and therapeutic benefit has been shown to be comparable to that of allogeneic HSC transplant.

Summary. Dr. Naldini said that data from both the MLD and WAS trials show that HSC-based gene transfer can be an effective treatment that is available to all patients and that might lower transplant morbidity, because GVHD is not present and milder conditioning can be used. When HSCT is not effective, gene-modified HSCs may have the advantages of being able to increase the therapeutic gene dosage, even surpassing levels expressed from normal cells, as shown in the MLD study, and allowing delivery of functional enzymes into the brain.

B. RAC Discussion

Dr. Kohn asked whether a single factor is responsible for the high level of marking in the MLD studies. Dr. Naldini responded that the result likely occurs due to a combination of factors. Having a well-optimized manufacturing process results in high vector infectivity and therefore high levels of transduction. High-quality vectors, a relatively short *ex vivo* protocol, and fresh cells are also factors.

Dr. Kohn noted the apparent absence of genotoxicity in the MLD and WAS trials. He asked whether Dr. Naldini had suggestions about what to look for with regard to preclinical safety data. Dr. Naldini responded that the self-inactivating design of lentiviruses could be a major contributing factor. He added that, if the safety data are maintained going forward, the different paths of viral integration will be a predictive indicator.

In response to a query from Dr. Hammarskjöld, Dr. Naldini reported that the MLD trial has dosed nine of its planned ten participants and the WAS trial has dosed all six of its planned participants. All participants have shown a high marking level, with some variation among them. The experimental treatment was well

tolerated in all participants. In terms of clinical benefit, the first five participants (from both trials) show benefits; data from the other dosed participants has not yet been published.

IV. Review and Discussion on Human Gene Transfer Protocol #1307-1241, Titled "A Phase I Study of Intracranially Administered Carboxylesterase-Expressing Neural Stem Cells in Combination with Intravenous Irinotecan for the Treatment of Recurrent High-Grade Gliomas"

Principal Investigators: Karen Aboody, M.D., City of Hope; and Jana Portnow, M.D., City of Hope
RAC Reviewers: Drs. Koch, Kohn, and Zoloth

Dr. Chatterjee was recused from consideration and discussion of this protocol due to a conflict of interest.

A. Protocol Summary

Despite advances in imaging, surgery, radiation, and chemotherapy, high-grade gliomas are virtually incurable, with survival typically measured in terms of months for glioblastoma, the most common glioma in adults. Treatment failure is largely attributable to the diffuse, invasive nature of glioma cells and ineffective delivery of chemotherapy to tumors in the brain.

Neural stem cells (NSCs) may provide a safe, effective way to deliver cancer-killing drugs to brain tumors, because of NSCs' natural ability to home to tumor cells throughout the brain. This cancer-targeting property can be harnessed to localize high concentrations of powerful chemotherapeutic agents to invasive tumor sites while reducing toxicity to healthy tissues and associated side effects. The research team has modified an established human NSC line (HB1.F3.CD) to secrete a highly active modified human form of the enzyme carboxylesterase (CE; hCE1m6). When the NSCs are administered into the brain, they migrate to tumor sites, after which the prodrug irinotecan is given intravenously. When it encounters the CE-producing NSCs, the irinotecan is converted to a more powerful chemotherapy agent, SN-38, selectively killing surrounding brain tumor cells while sparing normal brain tissue.

In effect, the NSCs produce tumor-localized chemotherapy, decreasing the side effects associated with systemic chemotherapy. The investigators' preclinical data support this hypothesis, demonstrating increased SN-38 levels at brain tumor sites and smaller tumors in mice that received CE-NSCs and irinotecan than in controls. No serious side effects were detected.

Based on these compelling preclinical data, the investigators propose to perform a multicenter Phase I clinical trial, in partnership with the NIH-funded Adult Brain Tumor Consortium, that will define the maximum tolerated doses (MTDs) and toxicities of the combination of CE-NSCs and irinotecan in patients with recurrent high-grade gliomas who are undergoing tumor resection or biopsy. The investigators hypothesize that, upon administration into the brain, the CE-NSCs will distribute themselves throughout the primary tumor site as well as targeting infiltrating tumor cells.

Following irinotecan administration, the CE-NSCs will convert irinotecan locally to SN-38, killing surrounding tumor cells. A brain catheter will be placed in research participants to allow for repeat rounds of this experimental treatment on an outpatient basis. The dose of NSCs and number of treatment rounds will be increased in a step-wise fashion. The CE-NSCs' ability to convert irinotecan to SN-38 in the brain will be evaluated using microdialysis catheters to measure the amounts of SN-38 in the brain over time. The investigators also plan to use serial magnetic resonance imaging (MRI) to follow tumor response and track the migration of iron-labeled NSCs in a subgroup of participants. The clinical and regulatory teams have the expertise and experience to successfully conduct this Phase I study, having recently completed the first-in-human pilot feasibility study with the parental NSC line.

B. Written Reviews by RAC Members

Eleven RAC members voted for in-depth review and public discussion of this protocol. Key issues included the use of a novel transgene/prodrug combination to generate a chemotherapeutic agent. In addition, the use of an adenoviral vector rather than a retroviral vector means that the carboxylesterase may only be expressed transiently in the neural stem cell line.

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

Noting that this protocol is generally well written and straightforward, Dr. Koch asked the investigators to discuss, from a preclinical and potentially clinical perspective, what they believe the advantages for potential benefit of this proposed trial are, compared with the previous trial conducted using CD/5-FU. CE adenovirus is the gene transfer agent, but virtually no information about it appeared in the protocol; the production and infection of the NSCs should be detailed in the protocol. Dr. Koch asked whether the investigators plan to look specifically at an antigenic response to the CE and whether the CE will be measured in brain or plasma for persistence of expression. Because these cells produce high amounts of secreted CE and the chemotherapeutic agent is not administered for two days, he asked the investigators to explain whether significant amounts of CE could get into peripheral circulation and if so, what the expected outcome would be.

Dr. Kohn asked why the investigators changed from the CD to the CE gene and whether there is data comparing the antitumor activity of the two different prodrug-converting systems. He wondered whether the presence of the CE gene in the NSCs increases the sensitivity to irinotecan (acting like a “suicide gene” for the NSCs) or whether the enzyme was mostly secreted with conversion to SN-38 occurring extracellularly. With regard to the change from a retroviral vector to an adenoviral vector, Dr. Kohn asked the investigators to explain the expected duration of expression by the implanted NSCs, the reason for using an adenoviral vector that would be present only transiently and that would lead to the absence of a potential suicide-gene mechanism for late-arising tumors, and whether the adenoviral vector-transduced cells express adenoviral protein from the E1/E3-deleted vector, which tends to be leaky. Dr. Kohn also suggested that participants be monitored for immune responses to adenoviral proteins with repeat administration. He asked the investigators to discuss the parameters for acceptable toxicity in terms of persistence of NSCs and formation of tumors. Noting that this protocol represents these investigators’ first clinical use of the Rickham reservoir to deliver NSCs, Dr. Kohn asked how the site of deposition of the NSCs changes, compared with the intra-operative approach in which NSCs are distributed along needle tracts in the brain/tumor parenchyma, and he requested that the investigators discuss their experience in using these devices to deliver cells. Dr. Kohn said that the informed consent document does a good job explaining the complicated study design and multiple variations of the interventions.

Dr. Zoloth focused her review on the ethical issues in the proposed study. She summarized the informed consent document as thoughtful, well defined, and thorough, listing in detail the many possible severe side effects of experimental treatment and being realistic about the Phase I nature of such studies. However, three important issues are not yet adequately described in the informed consent document: The term “fetal tissue” is not explained, the Rickham catheter needs to be more fully described and a picture of the device provided, and the promise to participants that they can “withdraw” from the study if they choose “at any point” should be revised to indicate that withdrawal from the study is not really possible. Dr. Zoloth discussed two ethical issues in this trial: whether such seriously ill and vulnerable patients, understandably desperate as they face a terminal illness, can be considered as research participants and, if so, whether the protective mechanisms proposed for this study are sufficient to protect the research participants from unreasonable or unfair harm. She said that a one-time informed consent interview may not be adequate for long-term, highly invasive protocols such as this one, and she suggested that the investigators might want to assign a patient advocate or social worker to the study for ongoing, real-time support.

C. RAC Discussion

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

- Dr. Hammarskjöld asked whether the investigators plan to test for previous adenovirus-5 (Ad-5) exposure.
- Dr. Ornelles asked if the investigators have conducted additional studies on the neural-derived cell line after those cells were infected with adenovirus.
- Dr. Curry suggested that the investigators specify the interpretation of the pathology readout regarding recurrent tumor and when to proceed, to ensure consistency among the participating institutions.
- Dr. Curry asked the investigators why they have chosen to exclude patients for whom gross total resection is a possibility.
- Dr. Sadelain asked whether the investigators plan to measure migration of cells and correlate that finding with outcomes.

D. Investigator Response

1. Written Responses to RAC Reviews

The investigators explained that the clinical development plan will continue to assess the safety and efficacy of both the first-generation (HB1.F3.CD) and second-generation (HB1.F3.CD.hCE1m6) NSC products in parallel, to determine individual safety/toxicity profiles. This plan will be modified depending on the safety and efficacy results. It is still unclear whether the second-generation NSC product will be superior to the first-generation product. It is also unclear whether adenoviral transduction of a modified human CE in the second-generation NSC product will elicit immune responses that might limit its clinical use. Therefore, for now, the investigators will continue to develop both NSC products. A Phase I study of repeat intracranial doses of HB1.F3.CD NSCs in combination with oral 5-FC is planned to begin next year.

If participants develop anti-NSC antibody and/or T-cell responses, the investigators plan to look into whether the antigenic response is specifically due to the hCE1m6 or the adenovirus. They also will look for the presence of hCE1m6 in any subsequent brain tissue samples obtained from participants who undergo an additional debulking craniotomy or brain biopsy in the future, including autopsy specimens if available.

In addition to monitoring the peripheral persistence of the NSCs, the investigators will look for the presence of hCE1m6 in the systemic circulation of study participants. They explained that they do not anticipate that detectable amounts of hCE1m6 will pass from the brain into the peripheral circulation; however, even if the entire amount of hCE1m6 expressed by the NSCs were to be released into the systemic circulation, the amount of enzyme would be negligible compared with the total quantity of CE present in the liver and gut. Therefore, no significant impact on systemic CE activity or increased systemic toxicity is anticipated if the hCE1m6 from the NSCs gets into the systemic circulation.

Regarding the change from the CD gene to the CE gene, the investigators explained that the CE-secreting NSC line starts with the original HB1.F3.CD NSC line, which has already demonstrated safety and feasibility in an initial clinical trial. The use of hCE1m6, a secreted enzyme, provides the opportunity to exploit an application of the NSC platform with a highly potent therapeutic agent that, in theory, would have a more extended radius of action than the intracellular CD in combination with 5-FC. Irinotecan currently is being administered to patients with glioblastoma as a second-line treatment. The recombinant CE enzyme can increase the tumor-localized concentration of the activated drug SN-38, potentially increasing efficacy. The investigators are currently gathering preclinical data to assess the safety and efficacy of using HB1.F3.CD.hCE1m6 NSCs in combination with both 5-FC and irinotecan in order to exploit the full potential of these second-generation NSCs. The CE gene can act as a suicide gene *in vitro*, while the NSCs are dividing; however, the NSCs are resistant to the action of SN-38 when they are not dividing, which is what is expected *in vivo*.

For the experimental treatment regimen to be effective, CE expression needs to last only as long as irinotecan is present. The investigators plan to treat with irinotecan two days after NSC administration, when the CE activity is expected to be greatest. Irinotecan has a terminal half-life of six hours in plasma;

therefore, the prodrug levels are expected to fall by approximately 95 percent within 24 hours (four half-lives) following the irinotecan dose. Each subsequent weekly dose of irinotecan would be preceded by another administration of NSCs, so it is not necessary for the cells to continue to produce CE beyond the first 72 hours after administration.

The investigators explained that the final product contains both the retrovirally integrated CD gene as well as the adenovirally transduced CE gene (HB1.F3.CD.hCE1m6). While it is expected that the adenoviral expression of hCE1m6 will wane over time, the NSCs will still express the CD gene. Therefore, even after loss of CE expression, CD expression can still be exploited for conversion of 5-FC to 5-FU, which is a potential suicide gene mechanism if the NSCs were to proliferate at a later time.

The two main reasons for using adenovirus for CE transduction are that expression levels are reportedly higher using adenovirus than retrovirus and that adenovirus transduction circumvents the risk of insertional mutagenesis, because the genes are transcribed without chromosomal integration. The investigators have already sequenced the HB1.F3.CD NSCs, which showed no proto-oncogenes within a 2000-bp stretch on either side of the single CD gene or single *v-myc* gene.

Although they have not determined whether the transduced cells express adenoviral proteins from the E1/E3-deleted vector, the investigators anticipate that they do. Therefore, the group will monitor all study participants for development of anti-NSC antibody and T-cell responses. If participants develop increasing anti-NSC antibody and/or T-cell responses, the immunogenic component will be investigated further.

To date, regardless of whether hCE1m6 NSCs are implanted into normal brain tissue or glioma-bearing tissue, no tumorigenicity of the NSCs has been observed. One week after implantation into normal brain tissue, NSCs are Ki67 negative and appear to be undergoing apoptosis. One week after implantation into orthotopic brain tumor, after one bolus dose of irinotecan, remaining NSCs appear to be Ki67 negative, with single cells scattered at the tumor site. In a long-term safety/toxicity study that is currently under way, mice are being monitored for clinical symptoms on a twice-daily basis. In similar studies with the HB1.F3.CD NSC line, no tumorigenicity has been observed, with no detectable viable NSCs at 1 to 3 months.

The parameters for the long-term safety toxicity study are as follows:

1. No NSC tumorigenicity based on histological evaluation and PCR for v-*myc* in all harvested tissues.
2. Complete blood counts and blood chemistries not significantly different from the control group consisting of tumor and irinotecan only.
3. No statistically significant difference in clinical symptoms, monitored twice daily, between dosed and control groups.
4. No statistically significant difference in gross necropsy observations between dosed and control groups.

Although this study will be the investigators' first use of a Rickham reservoir/catheter system to deliver NSCs, the City of Hope Brain Tumor Program has significant experience with using Rickham systems for intracranial administration of cellular therapy. In a recently completed immunotherapy study of genetically modified T cells, the investigators successfully infused repeated doses of the T cells during a two-week period via a Rickham placed within tumor tissue.

In this Phase I NSC study, a Rickham will be placed in tumor tissue at the time of surgery. The site of deposition of the NSCs when delivered through a Rickham is the same as when the NSCs are initially administered by direct injection intra-operatively—that is, into tumor/peritumoral tissue. In order to determine whether it would be feasible to use a Rickham to deliver repeat doses of NSCs intracranially, the investigators performed an experiment to assess the viability and binding of NSCs administered through a Rickham. To simulate as much as possible the way the NSCs would be delivered repeatedly to study research participants, researchers administered HB1.F3.CD NSCs (first-generation NSCs) by convection-enhanced delivery into the reservoir of a Rickham. Results indicated no loss of NSCs due to binding to tubing, and the viability of the NSCs remained constant during six hours of convection-

enhanced delivery, which is the maximum amount of time needed to infuse the largest dose of NSCs that will be tested in this Phase I study. This experiment demonstrated that the planned system for delivering repeat doses of NSCs intracranially works well, resulting in 100 percent recovery of the NSCs through a Rickham after flushing, and that NSC viability does not decrease for at least six hours after the cells are thawed and administered through a Rickham.

Research participants will be monitored closely throughout the experimental treatment. They will be allowed to continue on study as long as they are tolerating the treatment well and follow-up brain MRIs show no evidence of progressive disease. “Tolerating the treatment well” is defined as experiencing or developing side effects that are no more severe than CTCAE v.4 grade II. Up to two dose reductions are allowed before a participant would be taken off study, if he or she were to develop a grade III nonhematologic toxicity that is at least possibly related to the combination of NSCs and irinotecan, and one dose reduction is allowed if a participant were to experience a grade IV toxicity related to the NSCs and irinotecan.

The investigators agreed to include a description of the gene transfer agent, how it was produced, and how it transduced the NSCs with hCE1m6 in the background section of the protocol.

2. Responses to RAC Discussion Questions

Dr. Portnow said that, after consultation with several virologists, the investigators have decided not to screen out potential participants based on antibodies to Ad-5.

Dr. Aboody elaborated that the investigators always look at Ki67 for division and for tumor formation. In mice, they have checked the tumorigenicity of the transduced cell line and the karyotype to make sure no chromosomal changes have occurred.

Regarding exclusion of patients for whom gross total resection is possible, Dr. Portnow explained that the blood-brain barrier is more disrupted in patients with gross total resections and that the investigators want to ensure that as much of the prodrug gets to the stem cells as possible. However, for this first-in-human study, it would be acceptable to include patients with gross total resection, because the investigators were not concerned about 5-FC getting into the brain. This criterion might change for a Phase II trial.

Dr. Portnow agreed that pretesting for participants’ exposure to Ad-5 would provide a useful baseline, and he agreed with Dr. Hammarskjöld that the investigators should include this pretesting.

Regarding measurement of cell migration and correlation with outcomes, Dr. Aboody responded that samples will be taken from autopsied brains to pinpoint the location of viable cells and conduct histopathology on adjacent areas. For tracking not at autopsy, iron labeling is being developed so that MRI can be used to indicate where and how far the stem cells have migrated.

Dr. Portnow agreed to reword the “withdrawal” language to indicate that the investigators do not recommend withdrawing from long-term follow-up in this study, although participants are allowed to do so. Participants cannot truly stop being affected by this study once the experimental treatment has been administered, but they can choose not to be contacted by the investigators during the follow-up period.

E. Public Comment

Dr. Borror expressed concern about the possibility of therapeutic misconception being caused by the informed consent document, especially in the use of “treatment,” and she suggested replacing such words with other language, such as “infusion of stem cells” or “experimental treatment.” She also reminded the investigators that they cannot follow participants who choose to withdraw.

F. Synopsis of RAC Discussion and RAC Observations and Recommendations

<u>Clinical and Trial Design Issues</u>

- The neural cell line will be transduced at a multiplicity of infection of 20 (e.g., for 1 million cells, 20 million adenoviral vector particles will be used). This level is higher than that in many other studies employing adenoviral vectors and likely will result in significant adenoviral protein expression that could induce an adenoviral-specific immune response to the transduced cells. In order to understand whether anti-adenoviral immunity affects this approach, it is recommended that antibody titers against the adenoviral vector (Ad-5) be measured before administration to establish a baseline. For research participants who will receive a second dose of the cells, it will be helpful to look at adenoviral immunity to evaluate whether adenoviral-specific immunity to the transduced cells alters the response to readministration. An immune response against an early indicator of adenoviral gene expression, such as expression of the 72Kd DNA binding protein, may provide a more sensitive way to answer this question.

- At the time of craniotomy, a pathologist will determine whether there is tumor recurrence or treatment-induced effects from prior radiation and/or chemotherapy. Only one pathologist or group will make this determination at each institution. Since this new trial will be conducted at several sites in addition to the City of Hope, assessment of tumor recurrence should be standardized among participating institutions.

Ethical/Legal/Social Issues

- The neural cell line used in this protocol was derived from fetal tissue. The informed consent document has been amended to state that these cells were originally derived from a 15-week fetus. The exact source of these cells is not known, but some participants may be concerned about whether these cells were originally derived from an aborted fetus. The lack of certainty regarding the origin of these cells should be disclosed (e.g., by stating that the cells came from a cell line derived more than a decade ago and that it is not possible to determine the original source of these cells).

- Cells will be administered via a Rickham catheter. Including a clear diagram of this device in the informed consent document will be critical to informing the participants about this intervention.

- The informed consent document must make it clear that, once administered, neither the transduced cells nor the catheter can be removed. The revised informed consent document states that participants will be followed indefinitely, but participants can decline to be followed if they withdraw from the study. It is important that each participant understand the risks and benefits of withdrawing completely from the study, including from long-term follow-up.

- The use of the term “treatment” in the title and in the informed consent document could lead to a misapprehension that this is a therapeutic approach, not an experimental protocol. While irinotecan is a treatment for glioblastoma, it is important that participants understand that administration of the neural stem cells is experimental and has not been shown to provide benefit.

G. Committee Motion 2

Dr. Kohn summarized the RAC recommendations to be included in the letter to the investigators, expressing the RAC’s comments and concerns. Dr. Ornelles moved and Dr. Zoloth seconded that the RAC approve these summarized recommendations, which the RAC approved by a vote of 13 in favor, 0 opposed, 0 abstentions, and 1 recusal.

V. Gene Transfer Safety Assessment Board Report

RAC Reviewers: Drs. Kiem, Kohn, Pilewski, and Strome

A. GTSAB Report

Dr. Kohn presented the GTSAB report for the third quarter of 2013. Within the past three months, the OBA had received 15 protocol submissions, 11 of which were not selected for public review at this RAC meeting. Of the 11 protocols not selected for public review, four were oncology protocols and one each was for HIV, peripheral artery disease, brain injury, eliminating GVHD, wound healing, arthritis, and muscular dystrophy. In these 11 protocols, four used plasmids, two each used retrovirus and lentivirus, and one each used adeno-associated virus (AAV), modified listeria monocytogenes, and Venezuelan equine encephalomyelitis replicon. Dr. Kohn said that information about these trials would be available on the OBA website after this RAC meeting.

The GTSAB reviewed initial and follow-up reports on 23 SAEs from 13 protocols. After analyzing these events, the GTSAB concluded that none warranted public discussion at this RAC meeting.

During this quarter, the OBA received notification from investigators that six protocols were newly open to enrollment. None of these six protocols had been reviewed publicly by the RAC.

B. Report on AAV Integration in a Clinical Trial

Dr. Chatterjee reported on an unexpected finding from an AAV vector integration site study of clinical samples from a gene transfer trial for lipoprotein lipase deficiency (LPLD) that was recently published (Kaeppel, C., et al., (2013) A Largely Random AAV Integration Profile after LPLD Gene Therapy. *Nature Medicine* 19:890-892.). In this study involving the first approved gene therapy drug, Glybera, muscle biopsies from five subjects were analyzed for vector integrations by linear amplification mediated-polymerase chain reaction (LAM-PCR) and deep sequencing.

Previous integration studies were conducted primarily on transformed cell lines, mouse tissues, nonhuman primate tissues, and human fibroblasts. This study was the first to look at relevant human tissue samples. The study consisted of an intramuscular injection of the AAV1 vector encoding the LPLS447X gene.

The investigators concluded that AAV integration was random, and no preferential integration was encountered into any genes, CpG islands, palindromes, or ribosomal DNA, contrary to previous reports. The AAV vector frequencies seen were relatively high. The most surprising result reported was that the most common integration sites mapped to human mitochondrial DNA, a result that had not been described before. Chromosome-AAV junctions were found throughout the length of the vector and not limited for the inverted terminal repeats (ITR).The overall integration frequencies were tenfold higher than previous estimates but still well below that of retroviral and lentiviral vectors. No integrations into hepatocellular carcinoma or any other oncogene or tumor suppressor gene sites were observed.

C. RAC Discussion

Dr. Kohn asked whether this result is AAV-specific—whether the same result might be encountered with an adenovirus or a plasmid—or whether the AAV was merely efficient. Dr. Chatterjee responded that the investigators did report a high copy number per cell and that she was unaware of other investigators conducting similar integration studies after adenovirus or plasmid injection.

In response to a question from Dr. Hammarskjöld, Dr. Chatterjee explained that the investigators went to great lengths to distinguish the nuclear mitochondrial DNA from mitochondrial DNA. As a result, they saw some integration into the translocated chromosomal mitochondrial DNA but, even excluding those results, the frequency of integration into mitochondria was much higher than expected.

Dr. Dresser asked whether this result means that there is a potential risk that has not been recognized. Dr. Chatterjee responded that the information in this published paper is primarily descriptive. There is significant controversy in the field about AAV integrating into the hepatocellular carcinoma locus and giving rise to liver cancers ;however, this research did not detect such integration.

D. Public Comment

No public comments were offered.

VI. Day 1 Adjournment

Dr. Kohn adjourned Day 1 of the September 2013 RAC meeting at 5:25 p.m. on September 11.

VII. Day 2 Opening

Dr. Kohn opened Day 2 of the September 2013 RAC meeting at 8:30 a.m. on September 12.

VIII. Minutes of the June 11–12, 2013, RAC Meeting

RAC Reviewers: Drs. Pilewski and Strome

Dr. Pilewski reported that he had reviewed the June 2013 RAC meeting minutes document and requested that the minutes be approved by the RAC.

A. Committee Motion 3

Dr. Pilewski moved and Dr. Ornelles seconded that the June 2013 RAC meeting minutes document be approved. By a show of hands and with no opposition, the RAC approved the June 2013 RAC meeting minutes.

IX. Unexpected False-Positive PCR Test for HIV Following a Lentiviral Gene Therapy for X-Linked Severe Combined Immunodeficiency (X-SCID; OBA Protocol #0901-964)

Presenters: Suk See De Ravin, M.D., National Institute of Allergy and Infectious Diseases (NIAID), NIH; and Harry L. Malech, M.D., NIAID

A. Presentation by Dr. De Ravin

Dr. De Ravin explained that the main objective of this protocol is to study the efficacy and safety of lentiviral gene transfer for older subjects with X-SCID. X-SCID is caused by IL-2 receptor-gamma (gamma C) gene mutations that result in profound cellular and humoral immune deficiency. The severe form is fatal in infancy unless the patient receives a stem-cell transplant or gene therapy. Transplantation from HLA-matched siblings results in the best outcome; however, this option often is not available. Haploidentical transplantation from parents is another option that is commonly performed without conditioning; the procedure saves lives but most often results in incomplete immune reconstitution.

The target population in this trial is X-SCID patients who have received earlier stem-cell transplants but have achieved only partial correction. These patients usually have limited or waning donor T-cell function, generally without any B- or NK-cell function. The vector used in this trial is a third-generation self-inactivating HIV-1 derived vector. It is pseudotyped with vesicular stomatitis virus glycoprotein (VSV-G) and has an EF1 promoter that drives the codon-optimized human gamma-c cDNA. The vector is produced by a stable lentivector producer cell line made at St. Jude Children's Research Hospital Vector Facility.

Subject 1 in this protocol received a false-positive test for HIV. Dr. De Ravin described Subject 1 as a 24-year-old male who received a stem-cell transplant from his haploidentical mother when he was five months old and subsequently received a boost when he was nine years old. He had a history of recurrent

infections and lymphopenia with waning T-cell function, and he is dependent on intravenous immunoglobulin. As part of the entry criteria, he was screened for a panel of viruses, including HIV, and the viral screen was repeated at regular intervals after dosing. Because the X-SCID research participants do not produce antibodies, HIV RNA PCR assays were used for screening. At six months post-treatment, the viral screen with Roche COBAS® Ampliprep/COBAS® Taqman HIV-1 v2.0 was positive for HIV RNA.

Three months ago, Dr. De Ravin and colleagues dosed the second research participant, a 24-year-old male who received previous haploidentical transplants and has indications for treatment similar to Subject 1. Given the data from the first subject, the investigators looked for HIV RNA earlier in the second participant; at two months post-infusion, HIV RNA was detected at approximately 1,000 copies/mL. The gene marking in multiple lineages in this individual, as in Subject 1, is showing a stable myeloid cell lineage and increasing lineage marking in the B cells. Dr. De Ravin noted that it is too early to see a rise in T cells.

Some of the potential explanations for the positive HIV RNA assay include a carryover from the initial *in vitro* transduction of the CD34 cells, a wild-type HIV infection, replication-competent virus generated by recombination, and the possibility that vector sequences could be detected from the gene-modified cells. Dr. De Ravin reviewed how each of these options was investigated. Serial measurements of HIV RNA did not decline over time ruling out carryover. Assays involving multiple primers were negative for natural HIV infection. No replication-competent lentivirus (RCL) was detected, as indicated by absence of detectable quantities of p24 protein and reverse transcriptase. The investigators collaborated with other HIV colleagues from the Laboratory of Immune Regulation at NIAID, who performed a similar quantitative co-culture assay on the sample. Collaborators at St. Jude also performed an extensive series of studies in which RCL was not detected. However, HIV psi packaging sequences present in the vector were detected, but not amplified by reverse transcriptase. The investigators concluded that the HIV PCR assay detects genomic proviral vector sequence in the gene modified cells.

Dr. De Ravin expressed concern about the effect of a false-positive HIV test on social and economic issues, as well as potential impact on research participants' health insurance coverage. Steps taken to address this false-positive result include amending the protocol and consent/assent documents to inform the participants and providing a letter to advise the participant in case a positive test result occurs (e.g., a health care or blood donation situation) and contact numbers for further information from the investigators.

B. RAC Discussion

In response to Dr. Wooley's query, Dr. De Ravin clarified that the assay was not detecting RNA but proviral DNA sequence in the cell lineages. The positive results in the plasma tests were due to DNA contamination from cells.

Dr. Kohn noted that this newly reported risk—the potential for a false-positive HIV assay—is not a medical risk but a social risk. He suggested sending a letter to investigators about adding a reference to this potential risk to informed consent documents for HIV lentiviral vector trials. Dr. Corrigan-Curay stated that the OBA sent a letter to all lentiviral investigators registered with the OBA about today's presentation by Dr. De Ravin, and the OBA will send a follow-up letter, along with Dr. De Ravin's slides, so that all relevant investigators have this information. Dr. De Ravin agreed that this course of action is appropriate. She added that the investigators disclosed the results to Subject 1 and explained the different possibilities, noting that he has a very low risk for infectious HIV, but in spite of that information he was worried about the result.

Dr. Zoloth expressed concern about the length of time it takes to differentiate a false-positive result from a real-positive result and the possibility that this may delay commencement of therapy. Dr. De Ravin explained that the results took a few months for this initial event. Dr. Kohn suggested that one conclusion from this false-positive result is that the ultra-sensitive Roche RNA assay should not be used for HIV monitoring of research participants who have had lentiviral gene transfer. Dr. De Ravin explained that it is possible that such an individual could be given that test when coming into an emergency department for

example and a misleading result would occur. Dr. Wooley pointed out that the Roche test is one of the most prominent tests currently available and it is used typically to test for viral load.

Dr. Malech said that an HIV test was performed on these research participants because these young adults are capable of contracting HIV. Therefore, the investigators have not changed their periodic testing for HIV but now are using a test that will not provide a false-positive result. People of sexually active age or who might get infusions should continue to be tested periodically for HIV—with the appropriate test.

Dr. Wooley pointed out that there may not be other commercially available tests that do not involve the long terminal repeat or packaging sequence primers that may detect vector sequence. She added that these individuals might need to be tested by a special laboratory because other commercial tests would continue to provide false-positive results. Dr. De Ravin responded that there was a commercial test that detected HIV DNA but did not detect vector sequence.

Regarding disseminating this information, Drs. Curry and Kohn asked whether Dr. De Ravin and colleagues were planning to publish their report of this finding. Dr. De Ravin responded that they were, with help from the vector facility. She anticipates that this issue will be significant, given the application of lentivector trials in HIV-infected patients.

Dr. Kohn congratulated the investigators on the early results that appear to indicate that research participants are showing signs of making new lymphocytes: Participants' B-cell counts are rising, and immune improvement may follow.

X. Review and Discussion of Human Gene Transfer Protocol #1307-1239, Titled "Phase Ib Study Using Patient-Derived Tumor Cells Expressing a Streptococcal Antigen in Patients with Indolent Non-Hodgkin Lymphoma"

Principal Investigator:	Philip Bierman, M.D., University of Nebraska Medical Center
Additional Presenters:	Kelly Creighton, Ph.D., Clinipace, Inc.; Brenda Fielding, Clinipace, Inc.; Michael Lawman, Ph.D., Morphogenesis, Inc.; Patricia Lawman, Ph.D., Morphogenesis, Inc.; and Curtis Scribner, M.D., RRD International
Sponsor:	Morphogenesis, Inc.
RAC Reviewers:	Dr. Chatterjee, Ms. Dresser, and Dr. Strome

A. Protocol Summary

Non-Hodgkin lymphoma (NHL) is a form of blood cancer that can start in the lymphatic system, possibly a lymph node, skin, or the gastrointestinal tract, and is characterized by abnormal proliferation and progressive accumulation of malignant immune cells called lymphocytes. While a number of factors have been connected with increased risk for NHL, the causes have not been discovered yet. Occurrence of NHL is higher in developed countries and increases with age (the average age at diagnosis is 66) and is slightly higher in men than women. In 2012, an estimated 70,130 new cases of NHL were diagnosed in the United States.

More than 60 subtypes of NHL have been characterized, and they range from fast-growing to slow-growing. The diversity of lymphoma subtypes presents many challenges in researching and treating this complex disease. Diagnosis of NHL is confirmed by identifying specific proteins on the surface of lymphocytes derived from analysis of a lymph node biopsy specimen and by analyzing the genetic makeup of these cells. Patients with NHL may experience fever, excessive sweating, unexplained fatigue, loss of appetite, weight loss, itching, cough, and/or abdominal pain.

Current therapies for NHL include chemotherapy and radiation therapy. The side effects of chemotherapy vary with the drugs and doses administered and can include hair loss, poor appetite, nausea and vomiting, mouth and lip sores, dizziness, infertility, and increased risk of developing a second cancer. Side effects from radiation therapy are also dose dependent and can include hair loss, skin disorders,

sore throat, numbness in limbs and lower back, nausea and vomiting, diarrhea, urinary discomfort, infertility, increased risk of developing a second cancer, and increased susceptibility to infections. The treatment regimen a patient receives, as well as the intensity of the treatment, is determined by the disease subtype, the stage of the disease, and prognosis, as well as the overall physiological condition of the individual.

The purpose of this study is to test the safety and tolerability of an investigational therapeutic vaccine for NHL, called IFx-hu1.0, in patients with slow-growing NHL who either were previously untreated or who had received standard therapy. Usually, vaccines are made to help the body's immune system recognize and kill foreign invading organisms. IFx-hu1.0 alerts the immune system to the presence of cancer cells and "teaches" the immune system to target and kill those cancer cells effectively.

IFx-hu1.0 consists of the subject's own cancer cells, made to look "foreign" to the subject's immune system by making the cancer cells express a bacterial antigen called *Emm55*. Emm55 is a highly antigenic protein, one of very few bacterial antigens to have been successfully expressed on the surface of mammalian cells, and which exhibited no toxic effect in nonclinical studies and 88% long-term survival in a murine tumor model shown to be relevant to human disease. The tumor cells will be inactivated so that they cannot divide and then will be injected into the skin. A single course of eight doses will be administered once a week for four weeks and then once a month for four months. Research participants will be monitored for adverse reactions and their blood tested for immune response activity.

The primary objective of this study is to assess the safety of IFx-hu1.0 vaccination. Safety will be assessed by the evaluation and comparison of hematology, serum chemistry, and physical examinations. The secondary objective is to evaluate the antitumor responses induced by IFx-hu1.0 in research participants dosed with this vaccine, as determined by specific tests that follow the immune response and tumor burden assessments.

B. Written Reviews by RAC Members

Nine RAC members voted for in-depth review and public discussion of this protocol. Key issues included the administration of a novel antigen in a population of patients who are asymptomatic and may have a favorable clinical prognosis.

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

Because all cells from the biopsied lymph node will be transfected with the *Emm55* plasmid (including tumor cells and potentially normal lymphocytes) and therefore autoimmune responses might be induced, Dr. Chatterjee asked the investigators whether it is possible to estimate the probability of autoimmunity induction. She also asked the investigators whether it is possible to estimate the frequency of tumor cells to normal cells in the biopsied lymph nodes and whether the investigators could determine the relative transfection efficiencies of primary lymphoma cells as compared with normal cells. Dr. Chatterjee asked what criteria would be used to determine potential induction of autoimmunity besides antinuclear antibodies, why the transfected cells are proposed to be cultured for 48 hours prior to administration, whether the criterion of 10 percent transfection efficiency relates to all cells or just tumor cells, and the backup plans if the 10 percent level of efficiency is not attained. Noting that they report an incidence of 3 percent tumor formation at the injection site in mice, Dr. Chatterjee requested that the investigators explain why they see any tumor formation at all from the irradiated cells and whether this result in mice poses a risk for the human participants in this trial. She further asked the investigators to explain the observation that chemotherapy did not interfere with the development of an antitumor response after administration of *Emm55*-transfected tumor cells in mice. In the canine model, Dr. Chatterjee noted, there was a wide variability in responses, with some dogs responding well and living for years after dosing while others succumbed within weeks; she asked the investigators to discuss whether they believe there is any correlation of success of the experimental treatment with specific factors.

With regard to the risk-benefit ratio, Ms. Dresser asked about the opportunity costs of trial participation for individuals with previously untreated NHL such as, if the vaccine is unsafe or shows no antitumor

response, whether participants would have a reduced likelihood of benefiting from standard therapies. She provided six specific suggestions for improving the informed consent document.

Dr. Strome questioned the investigators' selection of research participants with asymptomatic, early-stage disease, given that the preclinical canine study featured dogs with advanced disease and that first-in-human gene transfer trials usually have limited success; he noted that the patients to be enrolled might survive for years without medication. He suggested making this a "clean" study by selecting for inclusion only those patients with follicular lymphoma, the most common of the groups suggested for inclusion. Dr. Strome asked the investigators to specify the clinical and laboratory data that will be used to stop patient participation in favor of current applicable therapeutic alternatives, to delineate the panel of tests that will be used to determine immune status prior to dosing and what the exclusion immune criteria would be, and to state the response data for given percentages of cells expressing *Emm55*. He noted that insurance plans generally will not cover complications from experimental trials and suggested that such complications be covered by the sponsor. Dr. Strome suggested five changes and enhancements to the informed consent document. In addition, he suggested that the informed consent document state clearly that life-threatening events are not precluded in humans, even though the animal data did not include any such events.

C. RAC Discussion

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

- Dr. Curry said that he was comfortable with the chosen patient population for participation in this Phase I study. His primary reasons were the difficulty of generating a vigorous immune response quickly enough to handle advanced and rapidly progressive disease and because of previously demonstrated safe application of cellular immunotherapy.
- Dr. Zoloth expressed concern that the investigators were presenting an overly optimistic potential for benefit, based on the canine study.
- Dr. Fost opined that standards for consent should be especially high in a study like this one. Potential participants should completely understand the risks and benefits, as well as the treatment alternatives. To ensure this understanding, he suggested that the investigators include some form of written consent monitoring.
- Dr. Strome recommended that the investigators limit enrollment to the population that was used for the idiotype vaccines—people who received either rituximab or chemotherapy and who, following their response, were treated in an adjuvant setting. In addition, their immune system would need to have recovered.
- Dr. Chatterjee explained that participants' lymphocyte population will be different early in the disease compared with late in the disease. When the investigators enroll older individuals who are in remission, they will be testing different cells than they would be if they were testing patients earlier in the disease. Therefore, for the questions they are asking in this study, the proposed participant population is reasonable.
- Ms. Hardison requested clarification of the description of "no known risks" associated with the vaccine, as presented in the informed consent document. She also suggested that the rare side effects associated with the use of genetic material, which were described elsewhere in the protocol, be enumerated within the informed consent document.

D. Investigator Response

1. Written Responses to RAC Reviews

Biopsied lymph nodes from NHL patients will contain normal lymphocytes as well as tumor cells. However, the vast majority of cells in the affected lymph nodes will be lymphoma cells, transfection efficiency is low and not all cells from the lymph node will be transfected, there are no established methods for differentiating between normal lymphocytes and lymphoma cells. Standard-of-care therapies such as rituximab are specifically directed against CD20 (a cluster differentiation antigen present on most normal B cells, not just lymphoma cells). Normal T lymphocytes are usually refractory to most kinds of

nonviral DNA delivery methods, and while it is not currently possible to predict the induction of autoimmunity, the incidental transfection of normal lymphocytes does not automatically result in autoimmunity to normal lymphocytes. Delivery of nucleic acids as antigenic payload to normal dendritic cells and B lymphocytes is a recognized means to generate cytoxic T lymphocytes that kill tumor cells associated with the antigen payload. As indicated in the study protocol, participants will be monitored for autoimmune response during the course of this Phase 1b trial, and the data obtained in the study may provide ways to estimate autoimmune induction, if any, in the future. Currently, the criteria for assessing autoimmunity are under consideration.

The 48-hour culture of the cells following transfection is designed to allow the cells to recover from the electroporation process and to allow time for them to express the *Emm55* antigen.

The 10 percent transfection efficiency criterion was established based on prior electroporation experience and takes into account that there will most likely be one opportunity to obtain a tissue specimen from each research participant and thus only one opportunity to produce vaccine for that individual. If 10 percent transfection efficiency is not obtained or if insufficient numbers of tumor cells are available for transfection and another biopsy cannot be performed, vaccine cannot be produced for that participant and he/she will not be enrolled in the study.

The text in Section 2.6 of the protocol describes a preliminary study conducted to determine the tumorigenicity of Neuro-2a tumor cells that had been transfected with the *Emm55* gene but that had not been irradiated. The three percent tumor formation (and subsequent regression) noted in this experiment indicates that *Emm55*-expressing tumor cells that were not irradiated were detected by the immune system and were unable to form tumors. In the proposed trial, therapeutic vaccine cells will be irradiated and therefore will pose no risk to trial participants in this regard.

In preclinical studies, it was noted that chemotherapy did not interfere with the development of an antitumor response after administration of *Emm55*-transfected tumor cells. Since cell proliferation is required for an effective immune response, several issues could explain this result:

- The effect of chemotherapy on the immune system may be more complex than previously assumed.
- Some studies suggest that chemotherapy can augment antitumor immune responses, perhaps by the release of tumor antigens, the reduction of a suppressive environment, and/or the augmentation of antitumor lymphocyte expansion.
- In most cases, the use of chemotherapy in veterinary applications is not curative, due to the adverse reactions of pets; that is, high therapeutic doses are not routinely administered because pet owners are not tolerant of extremely adverse reactions in their “family members” and are not prepared to administer the necessary supportive care.
- Based on anecdotal evidence, dogs seem to have hardy immune systems that can withstand the effects of chemotherapy to some degree.

The variability of response seen in the dog study is most likely due to multiple factors: the aggressiveness of the tumor and the tumor burden; the time from diagnosis to vaccine; the time between the pet owner’s observation of swollen lymph nodes and veterinary diagnosis; the animal’s age, sex, and breed; the concurrent standard-of-care therapies; the route of vaccine administration; the number of vaccine doses received; and the animals’ general health conditions. These factors help explain why dogs with naturally occurring cancer make good models for human disease.

The investigators stated that, when considering the appropriate population for this study, they seriously considered whether participants would have a reduced likelihood of benefiting from standard therapies if the vaccine proves to be unsafe or shows no antitumor response. They concluded that this experimental treatment would not confer either a positive or negative advantage for further treatment modalities. The investigators believe there would be no increase or decrease in chemotherapy drug sensitivity, no immunologic effect should prevent the use of biological therapies (e.g., antidrug antibodies), and they can find nothing that would preclude previously untreated patients from receiving subsequent therapies, either conventional or experimental, since this vaccine confers no resistance to future therapies. Given the

natural history of NHL, by selecting a population that is asymptomatic (and therefore not in immediate need of therapy) and not significantly immunocompromised from multiple rounds of chemotherapy, the investigators believe that their use of these research participants will provide the best opportunity to test the safety and activity of the experimental agent while not compromising the utility of any future therapeutic interventions. Participants will receive the experimental therapy and no other concurrent therapy without the potential for a positive clinical effect; therefore, the opportunity costs of trial participation for previously untreated individuals will be negligible.

The investigators explained that the asymptomatic subject population was chosen specifically because it would not be practical or ethical to include symptomatic subjects who required immediate therapy. Because one of the purposes of this study is to obtain information about the efficiency of producing the vaccine in humans and it will take time to conduct the biopsy and manufacture the vaccine, symptomatic patients would be exposed to the risk of further progression during the biopsy and production interval or to the possibility that the vaccine could not be manufactured for them. This situation is of particular concern because of the many treatment options with known efficacy that are available for symptomatic patients. This reasoning is why other vaccine trials first treated subjects with conventional therapy and then use vaccines as adjuvant treatment. The use of asymptomatic subject was also chosen to increase the ability to reach the primary and secondary endpoints of safety and development of an immune response. The investigators decided not to risk subjects having symptomatic progression and then requiring treatment before the endpoints could be evaluated. The protocol plan was modified to this effect based on comments received from the FDA.

Regarding the choice of subject population, the investigators explained further that asymptomatic patients do not necessarily have “early-stage disease.” It is anticipated that many patients will have advanced-stage disease or bulky disease, even though they are asymptomatic. These “asymptomatic” subjects have been included in recent Phase III trials for follicular lymphoma and are considered candidates for treatment in guidelines published by the National Comprehensive Cancer Network and the European Society of Medical Oncology.

Symptomatic patients require effective therapy, and enrolling such patients in a Phase I study could be considered impractical or unethical. Therefore, the investigators believe that asymptomatic untreated patients are an ideal population for a Phase I vaccine trial specifically because they may be likely not to need therapy for some time. Thus, the primary and secondary aims of the Phase I study could be completed without concern that the subjects might progress during the trial period. Use of this experimental therapy will not preclude other forms of therapy, should a subject progress and become symptomatic.

Recent data from several sources have demonstrated remarkable improvements in overall survival for patients with follicular lymphoma, and a watch-and-wait policy is appropriate for many patients with follicular or other indolent histologic subtypes of lymphoma. However, the investigators stated that they do not agree that these patients should not be candidates for therapeutic trials. In fact, there are many examples of asymptomatic patients with follicular lymphoma being included in trials, often with more aggressive therapies.

With regard to the suggestion that this study be confined to follicular lymphoma patients, the investigators agreed that this idea is reasonable. However, since the primary aim of this study is to examine safety and the development of an immune response, the investigators wanted to expand subject eligibility so that the study could be completed in a timely manner.

Subjects will be eligible for treatment with conventional agents at their request or at the request of their physician and at any time. Subjects will be taken off the study if they require treatment with conventional agents or should they have any of the other recognized indications for treatment, such as bulky or symptomatic lymphadenopathy or splenomegaly, systemic symptoms, cytopenias, or histologic transformation. Further, general stopping criteria are included within the consent form.

Because the primary endpoints of this study are to evaluate safety and the development of an immune response, the investigators believe that inclusion of selected subjects with mantle cell lymphoma is appropriate.

Between 50 percent and 80 percent of the cells in the vaccine administered to the first 18 dogs in the lymphoma study expressed *Emm55*. These cells had been expanded and selected in culture. The final two dogs in the lymphoma study and all subsequent animals treated with autologous vaccine had expression rates ranging from 11 percent to 30 percent. The investigators reported that they have observed no difference in humoral, cellular, or clinical response among animals receiving vaccines with various levels of *Emm55* expression.

It is standard operating convention for a pharmaceutical company to self-insure or to buy clinical trial insurance specifically to cover harm to the research participants, especially in the face of SAEs. Morphogenesis, Inc., believes it is prudent and important to purchase clinical trial insurance for this study. However, the duration of such a policy is usually limited. The sponsor reported that it is their understanding that none of the long-term trials for gene transfer, where a 15-year follow-up is recommended by the FDA, will indemnify or pay for the treatment of future events that may or may not be caused by the initial experimental treatment.

The investigators reported that the informed consent document has been revised to address the RAC reviewers' comments.

2. Responses to RAC Discussion Questions

Dr. Scribner provided additional insight into the investigators' evaluation of the appropriate population for this clinical trial. Regarding the autoimmune question, the investigators are already ensuring they are not enrolling participants with a potential for autoimmune dysfunction.

Dr. Bierman explained that the investigators envision using this vaccine, if it proves to be effective, in several ways: in the population proposed for this study by administering eight doses of vaccine after the initial diagnosis so that those individuals may never need treatment, as initial upfront therapy, for patients who had relapsed, or as an adjuvant.

Dr. Lawman clarified that the investigators know of no adverse side effects related to the use of this vaccine. Possible side effects have been shown in other vaccine trials but are not associated with this trial.

E. Public Comment

Dr. Borror suggested that, to avoid therapeutic misconception, "experimental" be inserted alongside "therapeutic vaccine" in the informed consent document. She added that "we hope" should be appended to the description about what is expected to occur when the antigen is expressed in participants' immune systems, and that the statement "there are no known risks or side effects associated with this use" be removed or altered, because that statement is misleading.

F. Synopsis of RAC Discussion and RAC Observations and Recommendations

<u>Clinical Issues</u>

- Patients who will be eligible for this protocol include those with indolent NHL who do not require immediate treatment. This population is diverse in terms of their long-term prognosis but a recent publication found that for patients with extranodal disease who were diagnosed before they reached 75 years of age, 10-year survival is as high as 70 percent with current standard of care (Pulte, D., Gondos, A, and Brenner, H., *Arch. Int. Med.* 2008. 168 (5): 469-476).

  The rationale for enrolling a population with asymptomatic disease is to maximize the potential to evaluate whether the vaccine is safe and can elicit an antitumor immune response.

While it is unusual to enroll such an early-stage population in Phase I gene transfer trials for lymphoma, the investigators note that the risks of this plasmid-based vaccine are likely to be low, given the preclinical data and previous experience with plasmid vaccines. Furthermore, enrolling only alternative populations might not enable the investigators to answer the key scientific questions. For example, if only people with bulky disease who are at greater risk of progression were enrolled, the patients might progress during this trial, which will take about 8 months to complete. Once a participant's disease progresses, other therapies would be indicated, likely confounding any analysis of the safety and immune response to the vector. Also eligible for the trial are those who have already completed treatment and recovered from any treatment-related toxicity. However, many treated patients may have long-term immunosuppression and potentially a different immune response to the vaccine due to the effects of treatment. Therefore, enrolling only previously treated patients may not provide data on the population that might be enrolled in future trials. Nonetheless, while there may be a reason for enrolling patients with indolent, untreated disease, the investigators should consider whether there are other factors—for example, age, biomarkers such as LDH, or cytogenetic, molecular, or tumor histology—that could be used to identify patients within this population who are at higher risk for disease progression and may have a less favorable long-term prognosis.

Ethical/Legal/Social Issues

- As stated above, some individuals who enroll in this trial may be asymptomatic and not require therapy for years. Therefore, it is important that patients clearly understand the potential risks of enrolling in a clinical trial that is unlikely to provide clinical benefit. To achieve this goal, the following revisions to the informed consent document and process are recommended:
    - Include a discussion of the patient's long-term prognosis, clarifying that the patient may not need any therapy for their disease for many years, if at all. In addition, include a robust discussion of alternatives to enrollment.
    - Include a statement that this is a safety study and that no clinical benefit is expected; the goal is to test for toxicity of this approach and evaluate any potential immune response to this agent.
    - Revise the phrase "you may not get benefit from being in this research study" to a statement that more accurately reflects that individual clinical benefit is unlikely, and remove references to "therapeutic vaccine," which can be misleading. While this is an innovative approach and the preclinical data is encouraging, the overwhelming majority of new therapeutic agents that enter Phase I studies fail to advance into standard medical care.
    - Revise the statement "there are no known risks or side effects" to reflect the fact that, while side effects were not detected in the canine studies, one of this study's primary goals is to establish whether there are any risks.
    - Clarify the statement "with the use of genetic material other rare side effects could occur which are not described in this consent form" by providing information on what those risks might be.

- It is important that the informed consent process include a mechanism to evaluate whether a participant has a good understanding of the risks and benefits, such as a written document that tests comprehension and/or use of a consent monitor.

G. Committee Motion 4

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 asked for a vote on these summarized recommendations, which the RAC approved by a vote of 13 in favor, 0 opposed, 1 abstention, and 0 recusals. (Dr. Strome abstained due to concerns about the selection of patients with indolent lymphoma, who have a long-term favorable clinical prognosis, for a Phase I safety trial.)

XI. Guidance for Industry: Consideration for the Design of Early-Phase Clinical Trials of Cellular and Gene Therapy Products

Presenter: John Hyde, M.D., Ph.D., Food and Drug Administration

A. Presentation by Dr. Hyde

Dr. Hyde presented an overview of this draft guidance document. He said that the motivation for providing the guidance is that, since cellular and gene transfer products have distinctive features compared with small molecules or other biologic products, such as monoclonal antibodies and therapeutic proteins, the design of early-phase trials is often different from that of trials for other pharmaceuticals. This document was intended to provide perspectives to improve the early development of cell and gene therapies. The document is primarily educational and is intended to make investigational new drug (IND) sponsors aware of issues when the sponsors propose their early-phase trials and to provide recommendations for addressing those issues. The hope is that this will help IND sponsors design their early-phase trials and enhance their interactions with the FDA. The guidance document does not set forth any new requirements. The intended scope covers biologic products for which the Office of Cell Tissue and Gene Therapy has regulatory authority; it is not for tissue-based products, devices, or biologics regulated by the Center for Drugs.

This guidance document was issued on July 2, 2013; a Federal Register Notice was published with a call for public comment, and Dr. Hyde reported that on October 23 the draft document will be presented for discussion at an FDA advisory committee meeting. The comment period closes on November 22, 2013, and the plan is to finalize the guidance document as soon as feasible after the comment period closes.

Dr. Hyde noted that the core of the guidance document is Sections III and IV, which enumerate special features of CGT products and how those features affect clinical trial design. Section III provides more specifics about features of CGT products that would affect the design of early-phase clinical trials, including the relative novelty of these products. Some products can have effects that persist for a long time, and it is not unusual for invasive administration to be needed; common examples are cardiac catheterization, injection into the central nervous system, and procedures that might require surgery to get the product to its intended location. Cellular products may require donors, either the patient or another person. Other special features of CGT products result from the complexity of the manufacturing process for certain products, and in some cases there are limits on the range of doses or concentrations that can be manufactured. There are special considerations regarding the role of preclinical data.

Section IV of the document discusses how the specific issues might affect the way early-phase trials are designed and makes general recommendations about approaches to addressing those issues. The primary objective of early-phase trials should be safety, but there are often additional objectives including dose exploration, preliminary assessment of feasibility relating to logistic issues of manufacturing and delivery, initial assessment of bioactivity, and use of a biomarker to see whether the product is present and eliciting at least some of the expected effects. Regarding choosing a study population, the draft guidance document describes the considerations that should go into making the choice and provides general advice. The need for potential benefit often means that healthy volunteers are unsuitable; the specific regulations regarding research and pediatric participants are reviewed in the document. Control groups can be valuable in early-phase studies for making preliminary safety and activity assessments; however, some CGT products require invasive administration. For small molecules, there are widely used methods for scaling up to human dosing from animal studies, but for CGT, the conventional allometric scaling may be less precise, which can make it difficult to establish a safe initial starting dose. Therefore, the guidance document suggests that initial exploration of those issues could be objectives of early-phase trials. The guidance document also explains staggering schemes and considerations for choosing the observation time between participants. Special considerations for CGT products regarding monitoring and follow-up include immunogenicity, persistence, migration, shedding, and growth and development; for most CGT products, follow-up up to 1 year is appropriate, although special considerations for pediatric participants are necessary. The guidance document includes a subsection discussing considerations for stopping rules and explains the reason behind them and how they operate.

Section V encourages sponsors to interact with the FDA, including before sponsors submit an IND, and lists a number of clinical topics as suggestions for issues that might be appropriate to discuss with the FDA. Section VI points to additional information about submitting an IND. It lists other relevant FDA guidance documents about what to provide in a submission, includes brief general advice, and suggests that sponsors consider the overall development plan early in a product's development.

The draft guidance is available in the guidance section of the FDA/CBER website at http://www.fda.gov/downloads/BiologicsBloodVaccines/GuidanceComplianceRegulatoryInformation/Guidances/CellularandGeneTherapy/UCM359073.pdf. Anyone can provide comments on the draft guidance document by referring to docket number FDA-2013-D-0576. Questions about the document and the comment process can be directed to the FDA's Office of Communications, Outreach, and Development at ocod@fda.hhs.gov or 1-800-835-4709.

B. RAC Discussion

Dr. Kohn commented that the guidance document is written clearly and would be useful for investigators entering the field. He said that the slides for Dr. Hyde's presentation would be posted on the OBA website.

Dr. Hammarskjöld noted that this guidance is timely.

C. Public Comments

No public comments were offered.

XII. Review and Discussion of Human Gene Transfer Protocol #1307-1240, titled "A Phase I, Open-Label Study to Assess the Safety, Feasibility, and Engraftment of Zinc Finger Nucleases CCR5 Modified Autologous CD34+ Hematopoietic Stem/Progenitor Cells (Sb-728mR-HSPCs) with Escalating Doses of Busulfan in HIV-1 (R5)–Infected Subjects with Suboptimal CD4 Levels on Antiretroviral Therapy"

Principal Investigator:	Amrita Krishnan, M.D., City of Hope; and John Zaia, M.D, City of Hope
Additional Presenters:	Dale Ando, M.D., Sangamo BioSciences, Inc.; Paula Cannon, Ph.D., City of Hope *(via teleconference)*; Dave DiGusto, Ph.D., City of Hope *(via teleconference)*; Philip Gregory, Ph.D., Sangamo BioSciences, Inc.; Michael Holmes, Ph.D., Sangamo BioSciences, Inc.; and Rodica Stan, Ph.D., City of Hope
Sponsor:	Sangamo BioSciences, Inc.
RAC Reviewers:	Drs. Fost, Hammarskjöld, and Ornelles

Drs. Cannon, Chatterjee, Kiem, and Kohn were recused from consideration and discussion of this protocol due to conflicts of interest. As a result of Dr. Kohn's recusal, Dr. Hammarskjöld chaired this section of the September 2013 RAC meeting.

A. Protocol Summary

This is a Phase I study to determine the safety and feasibility of transplantation of autologous hematopoietic stem and progenitor cells (HSPC) that have been genetically disrupted at the CCR5 locus using a Zinc Finger Nuclease (ZFN). CCR5 is a co-receptor of HIV-1 in human T cells. It is expected that once the CCR5 gene is disrupted in the HSPC, the progeny of these cells, including CD4 cells, is also protected from HIV-1 entry.

Transfer of the gene-modified cells will be carried out in HIV-1 (R5) infected subjects who are on combined antiretroviral therapy (cART) and have undetectable HIV-1 RNA, but suboptimal CD4 levels.

Due to the suboptimal CD4 levels (≥200 and <500 cells/ L), these subjects are at increased risk of HIV-1 rebound and possibly other infections, as well as non-AIDS mortality.

The proposed study also aims to identify optimal conditions for engraftment of the gene-modified cells. Thus, six research participants will be enrolled into two cohorts based on the reduced-intensity busulfan regimen used. Patients in Cohort one will receive 1.6 mg/kg/day intravenous (IV) busulfan for two days before research product infusion, and research participants enrolled in Cohort two will receive 3.2 mg/kg/day IV for two days before the infusion.

Peripheral blood progenitor cells will be mobilized with G-CSF/plerixafor and collected in one or two aphereses. The CD34+ stem cells will be selected using the Miltenyi CliniMACSTM system from the cells collected by apheresis. The ZFN mRNA vector targeting CCR5 (SB-728mR) will be electroporated into HSPC, and the resulting investigational agent, SB-728mR-HSPC, will undergo release testing and cryopreservation prior to infusion. At the time of infusion, the SB-728mR-HSPC (2.0×10^6/kg) will be thawed and administered intravenously. The research participants will be followed for safety and for evidence of CCR5 gene modification in the peripheral blood and gut mucosa.

Following HSPC infusion and engraftment, research participants demonstrating >1% gene disruption of CCR5 in peripheral blood mononuclear cells (PBMC) in at least two consecutive time points two weeks apart, and for whom the CD4+ T cell counts have reached 500 cells/ L post-infusion will start an analytical treatment interruption (ATI). The ATI, which is planned for a maximum of 16 weeks, will be governed by protocol-defined stopping rules linked to HIV levels or clinical events aims to study the selection of CCR5-disrupted cells post SB-728mR-HSPC infusion.

B. Written Reviews by RAC Members

Eight RAC members voted for in-depth review and public discussion of this protocol. Key issues included the novelty of using the ZFN technology in stem cells and because the response to DNA editing in stem cells may be different than what has been seen in mature T cells. In addition, unexpected outcomes are possible in a trial that uses a more efficient strategy to knock down CCR5 in hematopoietic stem cells.

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

Dr. Fost suggested that the investigators summarize in the informed consent document all studies that have evaluated the safety and tolerability of ZFN-treated stem cells in humans and that all alternative therapies and trials available to the participant population be enumerated in that document. He asked the investigators to clarify the wording regarding affirmation that the research participant is willing to consent to the various blood, bone marrow, and biopsy tests; if research participants are allowed to participate in the gene modification study without participating in any of these components, the informed consent document should explain that option more explicitly. Dr. Fost also suggested that the investigators clarify closer to the beginning of the informed consent document that the research has two components: an essential component, which all participants are expected to undergo, and several optional components, including some of the blood and bone marrow sampling, rectal biopsy, and the antiviral discontinuation study.

Although this protocol will not use a retrovirus or other virus, where vector integration itself could cause genetic changes that might result in oncogenic transformation, Dr. Hammarskjöld noted that the potential exists for genotoxicity because of ZFN expression designed to cause double-stranded DNA breaks. The protocol mentions that off-target effects have been studied and that some disruption of CCR2 was observed, but she asked whether other off-target DNA disruptions have been observed with SB-728 and to what extent these disruptions have been analyzed. She wondered whether any studies or oncogenicity tests had been conducted in HSPCs and whether karyotyping will be performed on modified HSPCs. Dr. Hammarskjöld asked whether the investigators plan to monitor the possibility of a potential shift to CXCR4 using viruses (if and when virus rebounds after treatment interruption), especially if the engraftment is efficient as previous protocols that used short-hairpin RNA and other strategies were designed to knock down CCR5, not disrupt the gene. She suggested that the investigators discuss the

rationale for choosing the stated endpoints and that they clarify whether treatment would be reinitiated if only one of the two criteria for reinitiation were met. In light of the increased spread of West Nile virus (a flavivirus) in the United States, Dr. Hammarskjöld said that both the protocol and the informed consent document should include discussion of reports of an association between CCR5 deficiency and increased severity of disease with certain flaviviruses observed in studies using CCR5-deficient mice. She asked the investigators to clarify whether six or 12 research participants would be enrolled in this protocol. Dr. Hammarskjöld requested clarification or rephrasing of wording in three locations in the informed consent document.

Dr. Ornelles noted that the protocol and supporting documents are well constructed and well written and that the target subject population, clinical outcomes, and approaches—including analytical interruption of antiretroviral treatment—appear well considered and well justified, but he described two major concerns. First, he noted that the underlying method of introducing a double-stranded DNA break in an exon of CCR5 gene followed by imprecise repair of the lesion by nonhomologous end-joining seems to be the principal risk in the proposed approach. Although ZFNs have shown remarkable specificity, off-target cleavage as high as 6.2 percent has been reported, with a contribution arising from sites that did not share obvious homology with the cognate site. Although a number of studies have shown an absence of deleterious effects in altered T cells, extensive studies appear not to have been performed on CD34+ HSPCs. Weighed against the existing evidence for ZFN specificity and the proposed studies to explore the impact in CD34+ cells, he said that there is not sufficient concern to merit altering the proposed plan of action beyond encouraging that dosed individuals be evaluated for possible hematologic abnormalities that could reflect unexpected mutations in the stem cell compartment.

Second, Dr. Ornelles expressed concern regarding the uncertain nature of the cellular response to DNA damage. Since dividing cells typically experience as many as a dozen double-stranded DNA breaks during cell division, the single break (or pair of breaks) introduced by the ZFN is not likely to constitute an overwhelming genotoxic stress. However, unlike breaks associated with DNA replication, the break introduced in a cell maintained at low temperature and probably not engaged in DNA synthesis might elicit a sharply different DNA-damage response. The responses of human stem cells to genotoxic stresses are not well understood, and human hematopoietic stem cells and embryonic stem cells have been shown to exhibit a more robust response to DNA damage than do fully differentiated cells. Differences in response to DNA damage raise the possibility that lessons learned in other cells may not directly transfer to HSPCs. Dr. Ornelles said that he was not suggesting further experimentation or changes to the existing protocol based on these speculative concerns; however, he wanted to reiterate the need to be vigilant in screening for changes to the hematopoietic compartment of dosed individuals. Dr. Ornelles also listed several minor concerns, including whether the CD4/CD8 ratio is expected to change because of disruption to the CCR5 locus or the busulfan conditioning, whether genomic or epigenetic changes have been postulated to affect the differentiation and development of hematopoietic cell compartment, the rationale for accepting subjects with viruses with a mixed tropism, and the importance of including descriptions in the informed consent document regarding how the cellular DNA will be cleaved and allowed to repair in an “imprecise” manner to introduce a mutation.

C. RAC Discussion

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

- Dr. Wooley asked about the CD4 cutoff points for reinitiating antiretroviral therapy. She wondered whether participants whose CD4 counts were extremely low could be harmed by restarting therapy at that point.
- Dr. Zoloth noted that treatment interruption was once considered a form of therapy and was built into nearly every AIDS trial; this fact made her more comfortable with the limited use of ATI in this trial.
- Dr. Sadelain expressed uncertainty as to whether 20 weeks is enough time to detect transformation in the transplant assay.
- Dr. Sadelain asked whether the proposed starting dose of three milligrams per kilogram of busulfan is likely to be effective.

- Dr. Hammarskjöld reminded the investigators that disrupting a gene in a subject who normally has that gene is different than deleting the gene.

D. Investigator Response

1. Written Responses to RAC Reviews

Previous studies have been performed with ZFN-modified CD4 T cells, but this proposal represents the first use of ZFN-modified HSPCs (called SB-728mR-HSPCs). The SB-728-T studies used an adenovirus to deliver the ZFNs. This SB-728mR-HSPC trial will deliver the ZFNs to stem cells using ex vivo processing with electroporation. The investigators did not include a reference to the T-cell study in the original submission because they thought it might mislead the participants. In response to the RAC reviewer's comment, they agreed to add text to the informed consent document to be clear on this point.

The investigators explained that they left Appendix M-II-A-1d ("What alternative therapies exist?") unmodified because no approved alternative therapies are available. However, two experimental studies are currently listed on www.clinicaltrials.gov as research alternatives, so the consent form has been modified appropriately.

The City of Hope IRB requires a separate list of procedures performed solely for research purposes, as opposed to therapy-related procedures, when the research procedures represent more than minimal risk. The investigators agreed that the inclusion of the "yes/no" response results in confusion, and the City of Hope IRB has agreed to review a request to list these procedures only as an acknowledgement. If granted, the change would state that the study involves the procedures listed, which are for research and unrelated to any routine treatment of the participant's illness.

The two components of the study are the stem cell transplantation procedure and, when engraftment is well documented, the analytical treatment interruption (ATI) for observation of the effect on HIV-1 biology and on cell selection. Participants must agree to the first portion of the study in order to participate, but will be re-consented for the ATI. Some of the listed procedures, such as rectal biopsy, extend across both portions of the study. Thus, once a participant has consented using the main consent (for the first part of the study) or re-consented for the ATI, individual research procedures are not an option. For clarity, a sentence has been added to the informed consent document.

The investigators have performed a preliminary analysis (via deep sequencing) of the top 23 potential ZFN off-target sites of SB-728mR mRNA electroporated CD34+ HSPCs, as determined from published empirical observations. Modification at the noncoding sites by SB-728mR would not be expected to have an impact on gene expression or function, and the remaining 19 sites analyzed by deep sequencing show no modification above background levels. CCR2 can also serve as an HIV-1 coreceptor, thus adding to potential anti–HIV-1 effects of this ZFN treatment.

CCR2 is involved in inflammation and monocyte trafficking. In animals, the CCR2-knockout mice develop normally with only an impaired ability to recruit monocytes and macrophages to sites of induced inflammation. No defects in monocyte trafficking were found in heterozygous (CCR2 +/−) mice, so the investigators explained that they would expect biallelic modification of CCR2 to be required in order to see a phenotype. Given that a CCR2 polymorphism has been associated with protection from AIDS progression, it is unlikely that this off-target effect will have any adverse effect on the research participant. Prior to initiating human studies, the investigators will perform additional deep sequencing studies on CD34+ HSPCs treated with SB-728mR mRNA at clinical scale to confirm the specificity of the CCR5 ZFNs. They also will evaluate the frequency and duration of double-strand break formation in SB-728mR–treated HSPCs by immunohistochemistry using antibodies against 53BP1 (a known factor recruited to the sites of DNA repair). In addition, they will karyotype a sample of SB-728mR electroporated HSPCs to look for any chromosomal abnormalities caused by the ZFNs.

With respect to oncogenicity tests on modified HSPCs, prior to beginning human studies the investigators will complete a tumorigenicity study to examine an approximate human clinical dose of SB728mRNA–modified HSPCs in immunodeficient mice, using cells manufactured from three different human donors.

To increase the stringency of this assay, the researchers will electroporate HSPCs with a twofold higher dose of SB-728mR to maximize ZFN activity. The investigators do not plan to perform exome sequencing on ZFN-treated HSPCs. Exome sequencing would provide comprehensive data on a limited number of genomes (from single cells). Instead, the investigators are focused on methods that can identify rare off-target events within a large population of modified cells (i.e., studies that can interrogate a large number of genomes simultaneously).

The investigators are aware of the possibility of a shift toward CXCR4-tropic virus, although this has not been seen in the studies of gene-modified CD4 T cells or in the study of maraviroc. They plan to monitor the research participants for CXCR4-tropic HIV-1 before and during the ATI, at the points indicated in the clinical protocol. Subjects accrued on this trial must be receiving combination antiretroviral therapy (cART) and must have undetectable HIV-1 (< 50 GC/mL) for at least 12 months prior to baseline evaluations; subjects will be prescreened using an HIV proviral DNA-based assay to evaluate HIV tropism. Patients with detectable X4-tropic virus will be excluded from the clinical trial. Although the investigators have not observed a selection toward X4-tropic or dual-tropic virus in the preclinical or clinical studies using CCR5 ZFN-modified CD4 T cells, they plan to monitor viral loads and HIV tropism in participants during the ATI. Once viral loads are above 1000 GC/mL, samples will be submitted for analysis to assess viral tropism. Any participant with detectable X4-tropic virus during the ATI will be placed back on cART and will continue to be monitored until viral loads return to baseline.

The rationale for choosing the study endpoints is participant safety. A viremia above 100,000 GC/mL has been used in other ATI trials and is well tolerated, with return to aviremia levels after reinstitution of cART. CD4 count below 200/μL is recognized as a cutoff for risk of opportunistic infection. When either of these events occurs, the investigators will reinstitute cART.

The investigators have modified the clinical protocol to explain some of the risks associated with CCR5 deficiency. The maraviroc trial was not associated with increased symptomatic West Nile virus (WNV) infection. Nevertheless, the investigators agree that an increased risk of WNV exists in CCR5 homozygous deficient patients, and this information will be disclosed in the informed consent document, with the recommendation that participants follow mosquito-protection guidelines when WNV is active locally.

This study uses a standard 3 + 3 design, in which dose cohort 1 will have three participants and escalate to dose cohort 2 if there are no DLTs. The dose cohorts refer strictly to the busulfan dose, as the dose of SB-728mR-HSPCs does not change between cohorts. If there is one DLT at dose 1, then three additional participants will be added and dose escalation will occur if no additional DLT is observed. The MTD will be the dose at which no more than one of six treated participants has a DLT. At the MTD, three additional participants will be dosed to obtain added safety and efficacy data if needed. Based on this design, the study will include either nine participants (if there are no DLTs) or 12 participants (if one DLT occurs at both dose levels).

To thoroughly examine ZFN specificity and any detrimental impact ZFN-induced modification might have on treated HSPCs, the investigators will perform the following preclinical safety studies:

- Deep sequencing of the top 23 off-target sites identified either by bioinformatics analysis of the human genome or by mapping the locations of integrase-defective lentiviral integration in CCR5 ZFN-treated cells by LAM-PCR
- Quantification of double-strand break formation in electroporated HSPCs over time by immunohistochemistry using antibodies against factors involved in DNA repair
- Karyotyping of ZFN-treated HSPCs to look for chromosomal rearrangements
- Evaluation of the tumorigenic potential of an approximate human clinical dose of HSPCs electroporated with SB-728mR (at a twofold higher dose of RNA than used in clinical manufacture, to maximize the potential for ZFN-induced genotoxicity)

This battery of preclinical safety studies should provide a thorough assessment of any safety issues caused by treating HSPCs with SB-728mR mRNA. However, to evaluate safety during the clinical trial, the investigators plan to monitor participants for any hematological defects, changes in CD4 cell counts and the CD4/CD8 ratio, and changes in the level of modification at CCR5 over time.

The investigators are aware of the studies on the impact of ionizing radiation on stem cells, and they agree that the limited number of induced DNA breaks expected from SB-728mR treatment should have little to no impact on the survival or development of HSPCs. This conclusion is supported by the *in vitro* and *in vivo* studies they have performed so far to determine the impact SB-728mR treatment might have on the hematopoietic potential of HSPCs. Their data demonstrate the successful electroporation of HSPCs at large scale with SB-728mR mRNA, without any deleterious impact on the subsequent engraftment or differentiation potential of the cells *in vitro* or *in vivo*.

Similarly, while the inclusion of a transient 30°C incubation period (16 to 18 hours) significantly increased the level of CCR5 disruption achieved, the investigators observed no impact on HSPC engraftment or differentiation potential. Together, the panel of characterization and release assays they have performed and will perform provide support for the application of these cells in individuals with HIV. Even though they do not expect to observe hematological defects, the researchers will monitor participants to ensure that the procedure has not resulted in hematopoietic changes over time. If participants experience hematological failure, the investigators have a backup HSPC product with which to treat the participants.

While the investigators do not know if there will be changes in the CD4/CD8 ratio in humans, it is possible that an increase in the CD4/CD8 ratio could occur in participants with suboptimal CD4 counts, due to protection of CD4s via ZFN-induced modification. The investigators plan to monitor participants for changes in the CD4/CD8 ratio. Animal studies in mice and in macaque monkeys suggest that the ratio of CD4/CD8 cells will change with HIV-1 challenge.

The investigators have not observed any issue with the transient incubation of HSPCs at 30°C. No changes in lineage differentiation were observed between the adenoviral-transduced HSPCs and electroporation/hypothermia–treated HSPCs. No further work has been performed on characterizing how transient hypothermia can enhance ZFN activity in cells. The investigators stated that they do not know of any genomic or epigenetic changes caused by temporary incubation (16 to 18 hours) of HSPCs at 30°C that could affect HSPC function. The researchers have not observed any difference in the efficiency of HSPC engraftment or in the potential to support multi-lineage development compared to untreated cells, based on their mouse engraftment studies.

The protocol has been updated to clarify that participants infected with dual-tropic virus will be excluded from this study.

The investigators agreed to change and clarify language in the informed consent document as suggested by the RAC reviewers.

2. Responses to RAC Discussion Questions

Dr. Zaia explained that the CD4 cutoff point for reinitiating cART is 200μL. He acknowledged that immune reconstitution syndrome could occur as an individual's CD4 count returned to higher levels.

With regard to waiting only 20 weeks to detect transformation, Dr. Holmes explained that the investigators looked at their own and other investigators' experiences with genotoxic events and the transformation rate in immunodeficient mice and found that 20 weeks was the correct balance between seeing some type of event and missing an event due to increased mortality.

Regarding the starting dose of 3 milligrams busulfan per kilogram of body weight, Dr. Zaia said that the investigators decided to determine the extent of potential toxicity by starting at a low dose of busulfan.

E. Public Comment

Dr. Hammarskjöld read into the record the written letter from Dr. Robert Reinhard, addressed to Dr. Corrigan-Curay. The letter is included as Appendix A.

In response to this public comment, Dr. Zaia thanked Dr. Reinhard and said that the investigators are in the process of collecting advice from the AIDS community. This protocol will be presented for input from an AIDS Clinical Trial Group Subcommittee in late September 2013. The questions related to ATI are particularly important. One question for discussion among AIDS experts will be whether the investigators could design the eligibility for ATI around certain other biological markers.

F. Synopsis of RAC Discussion and RAC Observations and Recommendations

Preclinical Issues

- The SB-728mR encodes a bicistronic mRNA expression cassette in which two ZFNs are linked by a 2A "self-preprocessing" peptide sequence derived from the *Thosea asigna* virus of insects. The investigators should consider conducting studies to determine whether dimerization occurs between the 2A regions in the product. The dimerized ZNFs may compete with monomer binding or form a pre-assembled complex with increased off-target activity.

Clinical Issues

- In participants who undergo a 16-week ATI, cART will be reinstituted before the end of the 16 weeks if a participant's CD4+ T-cell counts drop to below 200/μL or HIV-1 RNA rises above 100,000 GC/mL on two consecutive measurements (with tests every 2 weeks). However, many participants who enroll in this study may never have had a CD4 count as low as 200/μL, a level at which the risk of opportunistic infection significantly increases. Because this is primarily a safety study, the investigators should consider whether the CD4+ count threshold for reinstituting cART could be raised without compromising the information to be gained from the ATI.

- The goal of this protocol is to down-regulate CCR5. Some HIV patients are already on maraviroc, a CCR5 antagonist. Since this drug acts on the same target as the gene transfer, the investigators should consider excluding these patients from enrollment, because use of this drug could complicate analysis of the effect of the gene-modified cells. In addition, participants taking maraviroc may need to change their antiviral drugs should the therapy be successful and completely down-regulate CCR5 and/or lead to a phenotypic shift to a CXCR4-tropic virus. Because this is an initial safety study, it might be prudent to enroll participants who would not need to change their antiviral therapy due to the intervention.

- The preparation of the mRNA product includes a DNAase treatment to remove the linearized plasmid DNA template. To ensure the purity of the product, a PCR-based assay should be used to detect whether any residual DNA may be present in the final product.

Ethical/Legal/Social Issues

- The informed consent documents for both the gene transfer and the ATI portions of the study should include a clear explanation of the risks of the ATI.

- The investigators should consult their IRB regarding the use of the term "treatment" in the informed consent document when describing the administration of the gene-modified cells. It may be preferable to use another term that would not imply that this experimental approach will lead to clinical benefit. While the investigators hope, based on the preclinical research, that this approach may ultimately have clinical benefit, the very low success rate of experimental therapies moving from Phase I through to licensing means it is important to provide research participants with a realistic assessment of the relatively remote potential for clinical benefit.

- The rationale for targeting CCR5 in stem cells is based in part upon the results experienced by the "Berlin patient," whose levels of circulating HIV-1 were undetectable after he received an allogeneic bone marrow transplant for acute myelogenous leukemia from a donor who was

homozygous for CCR5 deficiency. However, the informed consent document should clearly state that the ZFN-mediated disruption of the CCR5 gene will be different from the CCR5-Δ32 mutation present in the Berlin patient's donor cells and, therefore, this approach may not result in the "functional cure" experienced by the "Berlin patient."

G. Committee Motion 5

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 asked for a vote on these summarized recommendations, which the RAC approved by a vote of 11 in favor, 0 opposed, 0 abstentions, and 4 recusals.

XIII. OBA Updates: Comments on Proposal to Exempt Certain Human Gene Transfer Trials from Institutional Biosafety Committee Review and Updates to Appendix B of the *NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules*

Presenter: Dr. Corrigan-Curay
CDC representative: Dean Erdman, Dr.P.H., CDC

A. Proposal to Exempt HGT Trials from IBC Review

In June 2013, the OBA published in the Federal Register a proposal to amend the *NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules* to exempt certain HGT trials from IBC review, a proposal that had been discussed with the RAC. The impetus for this proposal was that a number of gene transfer clinical trials are conducted using vectors for which considerable clinical experience exists and the biosafety risks are well characterized. The OBA was asked whether a mechanism could be created to streamline the review of trials of certain low biosafety risk without compromising the safety of trial conduct, with the goal of facilitating research, particularly for multisite or Phase III trials.

With input from the RAC, the OBA decided that the vectors eligible for exemption would be non-integrating viral vectors derived from Risk Group 2 or lower agents: adenovirus serotypes 2 or 5, poxviruses except for vaccinia, HSV-1, and all serotypes of AAV. Viral vectors eligible for exemption must be attenuated, as demonstrated preclinically and by experience in clinical trials. To be eligible for the exemption, investigators must have conducted an initial trial that was comparable in design, with the same product and delivery method, performed in the same country.

This proposal is intended to give IBCs more flexibility by removing the requirement that they review all such trials and allowing each institution to decide what works for them regarding the process for exemption, including the possibility of relying on another institution's decision that a trial is exempt. This flexibility would not affect whether a trial must be registered with the OBA and undergo RAC review; those requirements would remain the same.

The OBA received nine public comments from institutions and individuals, including the American Biological Safety Association, biosafety officers or IBC representatives from several institutions, a representative from a commercial IBC, an investigator from the U.S. military's HIV research program, a director from a rare disease foundation, and a global health consultant. A few comments advocated moving forward and recommended that the OBA consider going further by exempting from IBC review all trials that are exempt under Appendix M-VI, the so-called "vaccine exemption." The majority of the comments supported the general goal of making the review of multisite trials more efficient but mentioned significant concerns about safety, site-specific protocols, uniformity of quality and depth of IBC review, vector choice, determination of attenuation, trial design, and general implementation issues.

In light of these expressed concerns, Dr. Corrigan-Curay said that the OBA wishes to revisit the proposal with the RAC to consider ways to address two outstanding issues raised in the comments: first, the

potential for this exemption to reduce oversight and the resulting implications for safety, and, second, the ability to implement this change uniformly across IBCs.

B. Updates to Appendix B

Dr. Corrigan-Curay reported that the OBA will soon release a Federal Register Notice to update Appendix B of the *NIH Guidelines*, which designates the Risk Group classification of microorganisms based on their ability to cause disease in healthy adults and the ability to treat or prevent such disease. An organism's risk group does not determine the organism's containment level for research, but generally the risk group and the level of containment are correlated, e.g., a Risk Group 3 agent would generally be worked on at a BL3 level of containment. Requests to work with a Risk Group 3 agent at a lower level of containment must be addressed to the OBA for review.

Using consultation with experts from the NIAID, the NIH, and the CDC, and with current and former RAC members of the Biosafety Working Group, the OBA would like to update Appendix B to add Middle East respiratory syndrome (MERS) coronavirus as a Risk Group 3 coronavirus and to add *Pseudomonas aeruginosa* as a Risk Group 2 bacterium.

MERS is an emerging pathogen identified in Saudi Arabia in 2012. It causes a severe pulmonary syndrome similar to severe acute respiratory syndrome (SARS) coronavirus. SARS is currently listed as Risk Group 3 under the *NIH Guidelines*. The mortality rate for MERS is high, at approximately 50 percent, and no vaccines or therapeutics exist. At this time, MERS is characterized as posing a high individual risk but a low community risk, as sustained person-to-person transmission has not been seen. The *NIH Guidelines* currently classify all coronaviruses except for SARS as Risk Group 2, so MERS coronavirus, under the *NIH Guidelines*, is a Risk Group 2 virus. The OBA believes it is important to raise MERS coronavirus to at least Risk Group 3, the same level as SARS.

Pseudomonas aeruginosa primarily affects serious diseases in individuals who are immunocompromised or hospitalized. It can also be contracted from inadequately decontaminated water, ear infections, or contact lens infections. Antibiotics are available to treat this infection, although resistance is becoming a concern. Therefore, the OBA believes it is appropriate to classify *Pseudomonas aeruginosa* as a Risk Group 2 bacterium.

NIH Guidelines Appendix B changes go into effect immediately and, although not required, opportunity for public comment will be available.

C. RAC Discussion

Dr. Kohn agreed that the IBC issues of oversight and centralizing should be considered further by the RAC in light of the public comments.

Dr. Chatterjee asked about further information on MERS infectivity and the potential for person-to-person transmission. Dr. Erdman explained that MERS coronavirus can be transmitted person-to-person but that transmission does not appear to be sustained. He noted reports of MERS cases that appear to be asymptomatic or to result in mild respiratory illness. The definition of this illness is broadening as more data are gathered. Dr. Corrigan-Curay added that the *New England Journal of Medicine* recently published an article about MERS hospital cases, looking at an index case and possible spread in the health care setting.

D. Public Comment

No public comments were offered.

XIV. Closing Remarks and Adjournment

Dr. Kohn thanked the RAC members and the OBA staff and adjourned the September 2013 RAC meeting at 1:25 p.m. on September 12, 2013.

(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.)

Alex Annala, City of Hope
Jeffrey Bartlett, Calimmune
Ray Ebert, NHLBI
Anne-Virginie Eggimann, bluebird bio
Lucy Ghoda, City of Hope
Marty Giedlin, Sangamo BioSciences, Inc.
Allison HakeAya Jakobovitz, Kite Pharma
Manel Juan, Hospital Clinic
William Lee, Cato Research
Margaret Marshall, Kite Pharma
Marcella Maus, University of Pennsylvania
Allison Nance, Celgene
Philip Potter, St. Jude Children's Hospital
Antoni Ribas, University of California, Los Angeles
Angela Shen
Minkyung Song, National Cancer Institute (NCI)
Timothy Synold, City of Hope
William Trimmer, NCI
Gabor Veres, bluebird bio
John Zaia, City of Hope

(plus one individual from the M.D. Anderson Cancer Center whose name was illegible on the sign-in sheet)

Attachment III: Abbreviations and Acronyms

aAPC	artificial antigen-presenting cell
AAV	adeno-associated virus
ATI	analytic treatment interruption
CAR	chimeric antigen receptor
cART	combination antiretroviral therapy
CE	carboxylesterase
CGT	cellular and gene transfer
DLT	dose-limiting toxicity
FDA	Food and Drug Administration, HHS
FI	fluorescence intensity
GTSAB	Gene Transfer Safety Assessment Board
GVHD	graft-versus-host disease
HHS	U.S. Department Health and Human Services
HLA	human leukocyte antigen
HSC	hematopoietic stem cell
HSCT	hematopoietic stem cell transplantation
HSPC	hematopoietic stem and progenitor cell
HSR-TIGET	San Raffaele Telethon Institute for Gene Therapy
IBC	institutional biosafety committee
IL-2	interleukin-2
IND	investigational new drug
IRB	institutional review board
LPL	lipoprotein lipase
MDACC	M.D. Anderson Cancer Center
MERS	Middle East Respiratory Syndrome
mRNA	messenger RNA
MLD	metachromic leukodystrophy
MRI	magnetic resonance imaging
MTDs	maximum tolerated doses
NCI	National Cancer Institute
NHL	non-Hodgkin's lymphoma
NIAID	National Institute of Allergy and Infectious Diseases, NIH
NIH	National Institutes of Health
NIH Guidelines	*NIH Guidelines for Research Involving Recombinant DNA Molecules*
NK	natural killer
NSCs	neural stem cells
OBA	Office of Biotechnology Activities, NIH
OD	Office of the Director, NIH
PB	peripheral blood
RAC	Recombinant DNA Advisory Committee
RCL	replication-competent lentivirus
Q-PCR	quantitative polymerase chain reaction
SAE	serious adverse event
SARS	severe acute respiratory syndrome
SB	*Sleeping Beauty*
TCR	T-cell receptor
TRBC	tagged red blood cells
UCB	umbilical cord blood
VSV-G	vesicular stomatitis virus glycoprotein
WAS	Wiskott-Aldrich Syndrome
WNV	West Nile virus
X-SCID	X-linked severe combined immunodeficiency
ZFNs	zinc-finger nucleases

Appendix A:
Public Comment on Human Gene Transfer Protocol #1307-1240, titled "A Phase I, Open-Label Study to Assess the Safety, Feasibility, and Engraftment of Zinc Finger Nucleases CCR5 Modified Autologous CD34+ Hematopoietic Stem/Progenitor Cells (Sb-728mr-HSPCs) with Escalating Doses of Busulfan in HIV-1 (R5)–Infected Subjects with Suboptimal CD4 Levels on Antiretroviral Therapy"

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September 9, 2013

Jacqueline Corrigan-Curay J.D., M.D.
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RE: RAC Meeting - September 12, 2013 Discussion on Human Gene Transfer Protocol #1307-1240 titled: *A Phase I, Open-Label Study to Assess the Safety, Feasibility and Engraftment of Zinc Finger Nucleases CCR5 Modified Autologous CD34+ Hematopoietic Stem/Progenitor Cells (Sb728mr-Hspc) With Escalating Doses Of Busulfan in HIV-1 (R5) Infected Subjects with Suboptimal CD4 Levels on Antiretroviral Therapy ("ZFN Trial")*

Dear Dr. Corrigan-Curay:

Thank you for the opportunity to submit public comments regarding the ZFN Trial. I serve as a community representative on the International AIDS Society HIV Cure Industry Collaboration Group and the Community Advisory Board of the defeatHIV Cure Collaboratory. I work with investigators and stakeholders as a long time advocate for HIV prevention and treatment programs.

This phase I trial will add to the developing knowledge base for this important technology, extend possible application to progenitor cells and validate unique manufacturing processes. The study targets a set of trial participants

that are underserved in other attempts to advance permanent therapeutic options for HIV patients. I agree with much enthusiasm for this effort in principle. This is an important study. However, significant weaknesses in the scope of informed consent and issues of trial design affecting protection of research participants should be corrected. Please use the following to craft Committee recommendations:

[Note: I will refer to the relevant document or appendix or page number of the 222 page protocol materials sent to me describing the study]

1. **The Analytical Treatment Interruption (ATI)and Restart Scheme Is Not Justified.**

Although identification of altered cells and progeny is desirable, the automatically triggered interruption plan could be changed. The study proposes to measure other biological markers that should be verified prior to ATI, including measuring a positive effect on viral reservoir reduction. Even then, it is reasonable to question whether ATI is necessary at this early phase I stage in so few patients and whether it should be reserved for later trials based first on durable CD4 increases and other progeny evaluation.

The cART restart criteria may be harmful to patients and do not sufficiently account for patient risks, standard of care or consulting with physicians. Under the current proposal, cART resumption would occur at values ***below*** the CD4 baseline for study inclusion and significantly ***above*** VL criteria (p. 41 of 222, Protocol). Considering the underlying immune dysfunction of the patients, this design element is not explained or justified. The potential for recovery to baseline and pre-study function under possibly altered nonindividualized regimens is not characterized. The scheme is not protective.

The choice of cART either during the study or in ATI or during restart is insufficiently characterized. The drug substitution plans described in the proposal (p. 40 of 222, Protocol) do not account for the individualized regimens that may have been necessary for these patients before, during or after the study. Use of other newer drugs such as dolutegravir or combinations should be included in the study description and not left by chance to external care procedures.

The Protocol does not describe considerations for possible ATI during early HSPC mobilization prior to study treatment (p. 32 of 222, Protocol). These

should not be left solely to the investigator's decision as proposed without consultation with patients or their caregivers.

2. Treatment for Research Related Injury

On January 1, 2014, new statutory protections under the Affordable Care Act take effect to grant new rights for clinical trial participants. (A copy of the new provisions and recent related HHS guidance is attached.) The scope of these rights to secure treatment for research related injury in HIV cure trials has not been entirely clarified by government agencies or insurors . Operating definitions by some private insurors as to requirements to cover "routine costs" in clinical trials for life threatening conditions suggest that many trial related injury costs could possibly be covered. Important guarantees for continued coverage during trial participation are also part of the new law. "Grandfathered" insurance plans under the ACA are excluded from these new provisions. On such an important issue, it is vitally important that the sponsors exercise greater care to understand and secure the new optimal protections for these at risk participants and supplement them.

The IAS HIV Cure Ethics Working Group recommends that participants in HIV cure research should receive appropriate medical care for study related physical injuries at no cost to them**(Lo, Grady et al. 2013)**. This protection is particularly important for the ZFN Trial given the considerable unknowns, very burdensome procedures, frailty of the study population and potential for unanticipated events. The ZFN Trial authors cite to recent FDA hearings on values important to patients in HIV cure trials and purport to agree with patient stakeholders (p. 80 of 222, Appendix M). Patient stakeholders in those hearings unequivocally seek treatment for research related injury at no cost to participants.

The RAC previously supported use of treatment for research related injury as an important design feature for the Calimmune proposal, a study which has much in common with the ZFN Trial and which these ZFN authors cite to as precedent (p. 32 of 222, Protocol).

3. Deficiencies in Informed Consent

The draft informed consent document is insufficient in several respects. Among the gaps, it does not inform participants sufficiently of:

- The new ACA protections of rights for participants in clinical trials, application of routine care coverage to trial related injuries and possible limitations on those rights. (Previously used boilerplate language on these issues does not serve the new rules).
- Risks from off target effects, that off target editing is possible or possible effect on progeny other than CD4 cells such as myeloid cells. (The protocol and consent are unclear about the depth of study of myeloid cells or macrophages and that should be added.)
- Risks from cellular contaminants described in the appendix, Section M;
- The potential use of ATI during HSPC mobilization and its consequences;
- The fact that participation in the trial may preclude future enrollment in other HIV Cure trials or that the potential for accruing benefit from other cure strategies may be adversely affected as a result of undergoing these procedures.
- Although full consent information for ATI was not provided, it is unclear if participants will be apprised of all risks, the potential for full recovery and function to baseline and the uncertainty that restart medications would be effective. Participants should also be reminded of their rights to discontinue ATI at any time and return to cART.
- That alternatives to participation may include a number of regimen alteration possibilities that a patient may not be aware of from their otherwise delivered care.

As a trial practice element, the lack of any payment other than transportation costs for these excessively burdensome procedures is not appropriate. Payments could be provided without exerting undue influence.

Finally, this is a very complex study and poses many challenges to explain in a consent form. The readability and reading level of this draft does not match other models and would benefit from major edits for style and comprehen-

sion. The language can be misleading. (e.g. "*when* [not "if"] your CD4 count reaches 500" or "The study doctor will be able to treat most side effects" [is that without regard to severity? payment by participant?]

The FDA forum referred to earlier focused on exactly these issues of clarity, fullness and scope. This draft consent form would not meet the standards articulated by patient and agency stakeholders at the FDA's workshop. see Docket **FDA-2013-N-0473 at http://www.regulations.gov**

Thank you for considering these requests. As a person strongly supportive of novel HIV therapeutic interventions for full or functional cure, I am very glad these proposals are coming forward. However, responsible GCP, complete protection of participants and attention to setting ethical precedents mean we should all work together to improve the way these trials are conducted.

Sincerely,

Robert Reinhard

Robert Reinhard, Public/Global Health Consultant

w/attachments