RECOMBINANT DNA ADVISORY COMMITTEE

Minutes of Meeting

June 19, 2012

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]

June 19, 2012

The Recombinant DNA Advisory Committee (RAC) was convened for its 129th meeting at 3:30 p.m. on June 19, 2012, at the National Institutes of Health (NIH), Building 31-C, Conference Room 6, in Bethesda, Maryland. Dr. Yuman Fong (RAC Chair) presided. In accordance with Public Law 92-463, the meeting was open to the public from 9:15 a.m. until 3:20 p.m. on June 19. The following individuals were present for all or part of the June 2012 RAC meeting.

Committee Members

Andrew D. Badley, Mayo Clinic and Foundation
Michael J. Buchmeier, University of California, Irvine (via teleconference)
Saswati Chatterjee, City of Hope National Medical Center
E. Antonio Chiocca, Ohio State University Medical Center
Rebecca Dresser, Washington University School of Law
Yuman Fong, Memorial Sloan-Kettering Cancer Center (RAC Chair)
Norman Fost, University of Wisconsin–Madison
Joseph A. Kanabrocki, University of Chicago
Hans-Peter Kiem, University of Washington School of Medicine
Walter J. Koch, Thomas Jefferson University
Donald B. Kohn, University of California, Los Angeles (via teleconference)
Margaret Mallino, Missoula, Montana (via teleconference)
Anna C. Mastroianni, University of Washington School of Law
David A. Ornelles, Wake Forest University School of Medicine
Susan R. Ross, University of Pennsylvania
Marshall Strome, St. Luke's–Roosevelt Hospital Center/New York Head & Neck Institute
James R. Yankaskas, University of North Carolina at Chapel Hill

Office of Biotechnology Activities (OBA)

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

Ad Hoc Presenters and Speakers

Carl June, University of Pennsylvania
Martin Lotz, The Scripps Research Institute and Sanford-Burnham Medical Research Institute
Ronald Mitsuyasu, UCLA AIDS Institute and University of California, Los Angeles
Michael A. Mont, Sinai Hospital of Baltimore

Non-Voting Agency Representatives

Kristina C. Borror, Office for Human Research Protections, U.S. Department of Health and Human Services
Denise Gavin, U.S. Food and Drug Administration (FDA)

[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.

NIH/OSP/OBA Staff Members

Linda Gargiulo
Chezelle George
Robert Jambou
Maureen Montgomery
Marina O'Reilly
Gene Rosenthal

Attendees

There were 52 attendees at this one-day RAC meeting.

Attachments

Attachment I contains lists of RAC members, ad hoc reviewers and speakers, and nonvoting agency and liaison representatives. Attachment II contains a list of public attendees. Attachment III is a list of abbreviations and acronyms used in this document.

I. Call to Order and Opening Remarks

Dr. Fong, RAC Chair, called the meeting to order at 9:15 a.m. on June 19, 2012. Notice of this meeting under the *NIH Guidelines for Research Involving Recombinant DNA Molecules* (*NIH Guidelines*) was published in the *Federal Register* on February 14, 2012 (77 FR 31624). 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 two gene transfer protocols, update and discussion of one clinical trial previously reviewed by the RAC, update on immunotherapy trials for HIV and cancer, and discussion of first-in-human use of gene transfer agents in "compassionate use" protocols.

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. Minutes of the March 7–8, 2012, RAC Meeting

RAC Reviewers: Dr. Kanabrocki and Ms. Mallino

Dr. Kanabrocki stated that the minutes document was well written and accurately reflected the proceedings of the March 2012 RAC meeting. He moved to approve this document; no one seconded the motion.

A. Committee Motion 1

The RAC members voted verbally and unanimously to approve the March 2012 RAC meeting minutes document.

III. Update on Immunotherapy Trials for HIV and Cancer

Presenter: Carl June, M.D., Perelman School of Medicine, University of Pennsylvania

A. Presentation

Dr. June presented background and data regarding long-term follow up on one HIV trial and an update on two cancer trials that were presented to the RAC in September 2010.

Protocol #9912-365, entitled A Phase I/II Study of the Safety, Survival, and Trafficking of Autologous CD4-ζ Gene-Modified T Cells With and Without Exogenous Interleukin-2 in HIV-Infected Patients

The first clinical experience with chimeric antigen receptors (CARs) and cancer was in ovarian cancer and renal cell carcinoma, resulting in disappointing clinical activity because the CARs did not proliferate in the research participants and failed to reach the appropriate effector-to-target ratio. However, the first CAR trials were in HIV, sponsored by Cell Genesys. As a result of the requirement for 15-year follow-up, Dr. June is now able to report persistence and safety of the transgene in follow-up studies out to eleven years. He shared analysis of data from three of these trials, which infused similar doses with different schedules and without chemotherapy conditioning. The investigators expected that the CARs would decay and disappear from the research participants' bodies, but instead they are currently detectable at high levels, with the average half-life of these CARs being projected as between 16 years and 24 years in the three trials. The clonal persistence in humans of natural T cells is unknown but has been reported at 14 years to 16 years in B cells and T cells. The results from these three trials are informative for toxicity because of the long-term survival of the CD4-ζ CAR cells and because analysis indicates no genotoxicity or development of clonal dominance suggesting that integrating murine gammaretroviruses are safe in human T cells.

Dr. June summarized that infusions of the CD4-ζ CARs have been well tolerated with no clinical safety signals. With more than 540 years of participant follow-up, 41 out of the 43 participants indicate CAR persistence in peripheral blood mononuclear cells. All research participants who had rectal biopsies as long as 1 year after dosing show CARs in the rectal biopsy specimens, indicating CAR persistence in the mucosal lymphoid system. This persistence is indistinguishable from normal T cells. It is unknown whether CARs persist in secondary lymphoid tissue such as lymph nodes and spleen.

Protocol #1007-1056, entitled A Phase I, Dual, Cohort, Two Site, Clinical Trial Evaluating the Safety and Activity of Redirected Autologous T-cell Expressing a High Affinity TCR Specific for MAGE-A 3/6 or NYESO-1 Administered Post ASCT in Patients with Advanced Myeloma

and

Protocol #1007-1057, entitled Phase I Study To Assess the Safety and Activity of Enhanced TCR Transduced Autologous T Cells Against Cancer-Testis Antigens in Metastatic Melanoma

The clinical investigators for these two trials are Edward A. Stadtmauer, M.D., Abramson Cancer Center, University of Pennsylvania; Aaron P. Rapoport, M.D., Greenebaum Cancer Center, University of Maryland; and Gerald P. Linette, M.D., Ph.D., Siteman Cancer Center, Division of Oncology, Washington University School of Medicine. These trials both used affinity-enhanced T-cell receptors (TCRs). Dr. June presented information about experience with TCRs, backgrounds of both trials, data about the two serious adverse events (SAEs) that occurred following administration of the affinity enhanced MAGE-A3 T-cell receptors, and interim results. Both trials are sponsored by AdaptImmune, headquartered in Great Britain, and conducted at various institutions in the United States.

Cancer/testis (CT) antigen expression is upregulated in a variety of cancers. Normal expression is restricted to male germ cells in the testes and not usually expressed in adult somatic tissues. The NY-ESO-1 family of CT antigens is a small family of X-linked genes that includes NY-ESO-1 and LAGE1. The MAGE family of CT antigens is a large family of more than 45 X-linked genes. Spontaneous humoral and cellular responses have been demonstrated against the NY-ESO-1 and MAGE-A families, and Phase III cancer trials targeting immune responses against these tumor antigens are in progress.

Dr. June presented the rationale for using affinity-enhanced TCRs, including some data on TCR affinities for viral versus self antigens, how including affinity-optimized TCRs were generated, an overview of the preclinical safety studies for MAGE-A3 TCRs, and how the T-cell product was manufactured for the NY-ESO-1 and MAGE-A3 trials.

Safety data from the NY-ESO-1 TCR myeloma trial show no reportable SAEs related to study treatment. Eleven research participants have been dosed to date with NY-ESO-1 T cells. Manageable gastrointestinal toxicity has been observed. Four reversible SAEs were reported, with three being unrelated to the experimental treatment, and one (colitis) possibly related to T cells but not to the NY-ESO-1 TCR. Overall, this NY-ESO-1 study shows that the experimental treatment is basically nontoxic, with some clinical activity.

Regarding safety issues following infusion with cells expressing the MAGE-A3 affinity-enhanced TCR, two participants have been dosed to date—one on the melanoma study and one on the myeloma study. Both participants died within five days following T-cell infusion, as a result of cardiac-related events. Investigators discovered that the melanoma patient had previously undetected heart disease that was found at autopsy. The myeloma patient was screened more extensively after the first SAE occurred, but initial investigation implicates direct T-cell-mediated cardiac myonecrosis. Both protocols have been suspended pending further investigation.

Dr. June provided specifics of both SAEs. The melanoma patient, Case 1, was a 63-year-old man with metastatic melanoma who died in the summer 2011. Autopsy revealed extensive acute myocardial infarction (MI) and prior ischemic damage to the left ventricle with two prior “silent” transmural MIs, despite normal screening electrocardiogram and echocardiogram. This individual had 95 percent blockage of the proximal left anterior descending artery, minimal occlusion in other coronary arteries, and neutrophils in tissue. Investigators concluded that this research participant had prior undetected and significant heart disease and the anemia and metabolic demands from fever of unknown origin, along with the potential contribution of cyclophosphamide, which is known to induce transient cardiac toxicity, contributed to this event. No evidence was found that the MAGE-A3 specificity of the cells contributed to the event. This SAE underscored the importance of screening out patients with underlying ischemic cardiac disease, and the investigators implemented nuclear cardiac stress tests in all studies as a screening procedure and added explicit language regarding the death and the additional cardiac screen to the informed consent documents on all trials containing the MAGE arm. In addition, MAGE arms in all trials were placed on hold immediately following the event; after FDA review of the final report and protocol modification, the MAGE arms were reopened in Spring 2012.

The myeloma patient, Case 2, was a 53-year-old man initially diagnosed with multiple myeloma who died in the spring of 2012, within five days of receiving the MAGE-A3 Cells. This individual had a past medical history of hypertrophic obstructive cardiomyopathy and atrial fibrillation, but nuclear cardiac stress test prior to enrollment revealed no evidence of ischemia, no arrhythmias, and normal heart wall motion, and he was enrolled in the study following cardiologist evaluation that his past history posed no additional risk. A provisional autopsy revealed 500 ml of serous pericardial fluid, no evidence of acute ischemic MI, extensive myonecrosis similar to allograft cardiac rejection, and a heart that contained significant hematopoietic infiltrate composed of CD3+ cells. Additional investigation revealed high levels of interferon gamma (IFN-γ) in the pericardial effusion, but no tumor necrosis factor alpha. Other serum cytokines were present at levels similar to the CD19 CAR or NY-ESO-1 arms. There was a T-cell infiltrate in cardiac tissue sections in this subject. However, to date we cannot detect reactivity to cardiomyocyte cell lines from normal donors.

In Case 1, the T cells did not expand out of proportion to expansion observed in other research participants; T cells in other studies expanded by about 60 percent but in case 1 the T cells were approximately 30% of lymphocytes. In case 2, the participant’s T cells expanded up to 90 percent. Engraftment and expansion of gene-modified cells in both cases were similar to what was seen in other studies. Cytokine profiles showed that various cytokines increased in Case 1 and Case 2, by 10-fold to greater than 100-fold above baseline, but Dr. June stated that this increase was not the result of a cytokine storm occurring immediately after infusion because the increase was observed on days 2

through 5. In Case 1, on autopsy, the most significant T cells infiltration was seen in spleen and heart cells. In Case 2, the T cells were highest in pericardial fluid, were low in heart, and were not seen in skeletal muscle. In neither case were T cells found in testes.

What is known to date is that the TCR-transduced T cells expanded, the T cells expressed MAGE-A3 TCR and trafficked to tissues including heart. Cytokine storm was not the cause of these two deaths. MAGE-A3 is not known to be expressed on normal heart. Issues to be investigated include whether this is an on-target effect, whether the target is upregulated in response to IFN-γ or chemotherapy, whether the etiology of each event is similar, and whether these SAEs are a class effect with MAGE-A3 TCRs. (Dr. June noted that a human leukocyte antigen (HLA) A2 restricted MAGE-A3 TCR trial at the National Cancer Institute (NCI) was stopped due to unexpected central nervous system toxicity.) Planned studies following the MAGE-A3 SAE include evaluation of the effect of IFN-γ and chemotherapy on MAGE-A3 expression in heart and other tissues, investigating a screening panel of similar epitopes that potentially may be cross reactive, investigating the possibility of alloreactivity, and investigating the possibility of mispairing.

Dr. June concluded that NY-ESO-1 restricted T-cell receptors have shown a good safety profile and encouraging antitumor effects. Revising the protocol exclusion criteria in trials that have potent T-cell therapeutic effects should be considered. The current *in vitro* tests are not predictive of the *in vivo* outcomes. Off-target effects and toxicities clinically are established only in well-designed Phase I trials. Dose reduction may not ameliorate risk and may only delay onset of toxicity. In other trials with CARs, investigators have found that low doses of CARs merely delay the effects of the infused T cells and, for intravascular infusion, dose reduction will not manage this toxicity. Corticosteroids did not ablate the toxicity in Case 2, and it is unknown whether suicide systems or another form of immunosuppression might have avoided that toxicity.

B. RAC Discussion

Dr. Fong requested clarification about the cytokine and immune cell infiltrate levels, which Dr. June provided. He also asked about future of the MAGE programs at the University of Pennsylvania and the NCI.

Dr. Kiem asked about the chemotherapy for both research participants. Dr. June stated that the melanoma research participant received Cytoxan at 60 mg/kg on two days and the myeloma participant received melphalan at 200 mgs/m^2.

Dr. Badley asked whether the investigators are considering adding a suicide transgene to the vector. Dr. June stated that, if the toxicity occurred in the kidney, rather than the heart, there might be more time to utilize a suicide vector.

C. Public Comment

Dr. Steven A. Rosenberg, M.D., from the NCI, discussed the future of their MAGE program. NY-ESO has no off-target toxicities. He has treated 23 research participants with the NY-ESO TCR targeting the same epitope and has seen no toxicities. Of nine research participants treated with an HLA-A2 restricted MAGE-A3 directed TCR, two had complete regressions that are ongoing; however, two participants developed an unexpected severe neurologic toxicity that led to necrosis of the white matter. Although MAGE-A3 is not expressed in the brain, MAGE-A12 is expressed in the brain at low levels. MAGE-A12 has the same epitope but with a methionine in the second position, which leads to an increase in binding to HLA-A2; therefore, the MAGE-A12 epitope is highly recognized as the MAGE-A3 epitope. Dr. Rosenberg and his colleagues believe that situation is what caused the toxicity in these two research participants.

He suggested testing all the members of the MAGE family to see if any epitopes are similar to the epitope being targeted or if any epitopes might react because of amino acid substitutions. If those searches of peptides in the MAGE family are conducted carefully, it would be safe to proceed, although at a slower dose escalation. Because this is such an extraordinarily good target due to its widespread

expression, and because these research participants are endstage patients, Dr. Rosenberg opined that MAGE as a target still has promise. However, MAGE-A12 and MAGE-A9 are expressed in the brain, a fact that had not been recognized previously.

IV. First-in-Human Use of Gene Transfer Agents in "Compassionate Use" Protocols

A. Presentation by Dr. Fong

Dr. Fong's presentation and the ensuing RAC discussion focused on first use of a novel gene transfer agent in single-subject protocols and the role of the RAC in reviewing such protocols. He discussed the type of single-subject gene transfer protocols registered with the OBA to date, how often a novel gene transfer agent is used first in a single-subject protocol, and the challenges these protocols raise regarding informed consent and for the field generally, and facilitated a discussion about the optimal role for the RAC in reviewing these protocols.

Single-subject protocols are exemptions to the reviewed protocol design and are usually time sensitive, and they involve judgments about the suitability of gene transfer for an individual patient based on that person's unique clinical circumstances. Such protocols usually are reviewed by the Gene Transfer Safety Assessment Board (GTSAB). These protocols do not necessarily come to the full RAC for review, the rationale being that the goal of such protocols is to provide to an individual, who is usually very sick and likely has a very short life expectancy, an experimental therapy. The individual nature of these protocols with the focus on a single patent's clinical condition, as well as time constraints for review, make public discussions of such protocols difficult. In many cases, the agent has been used in humans and such protocols do not raise as much concern. However, protocols that propose using an agent that has not been given to humans do raise some concerns.

Exempting single subject protocols when the agent has been used in the clinic from full RAC review is appropriate in some cases. Some examples include redosing a research participant already enrolled on a protocol, dosing a research participant who has the same disease but who missed participating in a closed protocol for a promising agent, or dosing a research participant who fails to meet inclusion criteria on an open protocol but who has the same or similar disease. For immune therapies, the disease may be different but the target antigen is the same.

However, other examples from past single subject protocols are not as clear-cut, including single-subject protocols that propose to dose subjects with a different disease that features the same mechanism, protocols using a new gene transfer product never given to humans, and protocols attempting to treat rare diseases. In one example in leiomyosarcoma, the proposed vector brought together two transgenes previously used separately; the subject passed away due to disease before receiving the transgene, and this vector has not been proposed in an OBA-registered clinical trial since that time. Another example in lung cancer featured a new target receptor for Epstein-Barr virus specific cytotoxic T cells; the subject received the gene-modified cells but passed away shortly thereafter due to complications from the disease, and this vector has not been proposed in an OBA-registered clinical trial since that time. A question for discussion that arises from these two examples is whether the field learns anything when subjects die soon after dosing or never get to dosing and a more extensive clinical trial program using the experimental product is not pursued.

In another example in hereditary inclusion body myositis, a new product was proposed and the RAC recommended public review of the proposed protocol. Subsequently, the principal investigator requested permission to proceed prior to the RAC meeting due to the patient's clinical condition. The subject was dosed in August 2008, and the RAC reviewed the protocol at the September 2008 RAC meeting. Data was published, and the conclusion of the article was that this single-subject protocol served as a proof of principle for manufacturing of clinical grade product and clinical safety and activity were demonstrated. In another new-product protocol in pancreatic cancer, the subject with Stage IV disease has been dosed, but no data is yet available. A question for discussion that arises from these two examples is why develop

an agent for a single use protocol and can that initial use in a very ill patient potentially derail a promising avenue of research.

Dr. Fong focused on the question of whether single-subject protocols are research or treatment. He pointed out that such protocols often involve the use of an unapproved experimental agent with limited or no efficacy record to support its characterization as treatment, the protocols have as their primary goal to ameliorate disease in patients with no other treatment options, and the patient's interest in experimental treatment often takes precedence over the ability to produce generalizable knowledge. Limited data is likely to be generated from a study with a single subject. The *NIH Guidelines,* however, define human gene transfer research as the transfer of recombinant DNA, or DNA or RNA derived by recombinant DNA, into one or more individuals. Therefore, such protocols require registration with the OBA and RAC review.

Dr. Fong framed the RAC discussion. He noted that various circumstances might lead an investigator to pursue compassionate use in the clinic, including that final approvals for a full protocol are pending but are expected in a short time and that the disease is extremely rare. However, significant key questions arise, including:

- What data can be expected from such protocols?
- Is there a risk that an SAE could derail development of this agent? What are the ethical implications of taking that risk for a single patient if doing so could set back a promising approach for multiple patients?
- How is selection of the research participants made for an individual protocol?
- How can issues surrounding informed consent and therapeutic misconception be addressed?
- How can the RAC contribute to this process?

B. Invited Comment from Dr. Nemunaitis

Dr. Fong introduced John J. Nemunaitis, M.D., a practicing oncologist and executive medical director at the Mary Crowley Medical Research Center and director of the U.S. Oncology Network Phase I Research Program. He was invited to address this RAC meeting (via telephone) because he has submitted several single subject protocols.

Dr. Nemunaitis summarized his opinion that single subject protocols should result in knowledge to advance the field. When deciding to initiate a single subject IND, he focuses on the rationale and the scientific milestones that can be achieved with minimal risk to the research participant in order to move the field forward into a position to help other research participants more efficiently through traditional trial design/IND database formulation. In his view, if the rationale is well thought out regarding the patient target and targeted therapy within the single patient IND, even with no prior human experience, the target population could still derive benefit. Given the advancing ability to define molecular targets, relationship of these targets to disease, and proof of principle for molecular target control in vitro and in animal testing, the investigative/medical community can provide with greater confidence the justification of a single subject IND involving molecular directed technologies against relevant targets, which do not have molecular evidence of toxicity.

He discussed the results provided by the four single subject protocols he and his colleagues filed and that the RAC reviewed. Because the data prior to a Phase I trial involves expression of a particular vector or use of a technology in animals only, studies with a single research participant could still provide some information regarding the mechanistic activity seen in animals to date and could be helpful in designing subsequent preclinical studies to justify a Phase I trial. For example in the hereditary inclusion body myositis (HIBM) study, the disease is felt to be caused by a mutated GNE gene, so the justification of treatment is based on providing the functioning GNE gene. Thus, based on the relevance of molecular targeting and molecular relationship to disease, the first scientific milestone was to determine whether GNE nanoplex injection of a single local muscle (extensor carpi radialis longus muscle specifically) could induce local wildtype GNE expression as had already demonstrated in animal models. The results of the intramuscular administration protocol supported proceeding to intravenous injections. The single subject

studies provided not only some clinical benefit to the subject but data regarding toxicity and multi-dosing useful for the design of the planned Phase I protocol.

Sometimes the lessons learned from single subject protocols may not necessarily be used to move forward in the exact patient population with that specific rare disease, but the knowledge becomes part of the information database that helps investigators move to other Phase I and Phase II trials. Dr. Nemunaitis noted that he and his colleagues used data regarding the vector, nanoparticle delivery system, or other technology from the four single subject studies to move forward in other research. The same patient population was not involved, but the knowledge of the technology allowed completion of Phase I testing of 52 subjects receiving a single vector; that vector is being used currently in three Phase II studies. In addition, the knowledge of the nanoparticle also helped move that experience into Phase I trials.

C. RAC Discussion

Dr. Corrigan-Curay explained the current process for reviewing single subject protocols. When the protocols are submitted to OBA, they are either considered an amendment to an existing protocol or a new protocol if the agent has never been used in humans. The GTSAB provides a more rapid process for reviewing amendments, protocols and any relevant clinical safety data. Single subject protocols are not likely to be brought before the RAC for public review due to time sensitivities. RAC member comments should be contributed in the pre-review process, recognizing that those comments do not represent RAC consensus. Because they are gene-transfer protocols, single subject protocols always are also reviewed by the local institutional review board (IRB) and the institutional biosafety committee.

Dr. Fost enumerated several purposes for IRB and RAC review: (1) to check the credentials of the investigator(s) proposing to conduct the protocol; (2) to confirm a plausible scientific purpose for the proposed protocol, which an IRB may not be qualified to do but which the RAC is; and (3) to obtain assistance from the IRB, with help from the RAC, in constructing the informed consent document and the consent process. The data should be reported in some way and a limit on the number of subjects who can be recruited into such a trial should be established, so such trials do not become a mechanism for conducting an uncontrolled or poorly designed clinical trial. For first-of-its-kind agents proposed for compassionate-use gene transfer trials, Dr. Fost stated his belief that the threshold for approving such trials should be high. He also stated that the ethical dilemma for the patients of such trials is no different than in any other clinical trial in which many desperate, terminally ill patients with hopeless conditions are willing to try anything.

Dr. Strome expressed concern about the ability to obtain truly informed consent in such patients, who are likely to be thinking that they are participating in such a trial for therapeutic reasons when, realistically, the compassionate-use trial is being conducted for investigational reasons. He noted that the majority of these patients might turn down participation in these compassionate-use trials if they had a reasonable life expectancy (which most of these patients do not have), if they have received truly informed consent, and if they truly understand the small percentages of these trials that have meaningful results.

Ms. Dresser noted that harm could result from these protocols—to the subject and also to science, if an event happens that sets back the research field or if data are not collected in a systematic way. The quality of the subject's consent decision often is not good. Some cases could justify conducting these protocols so some kind of review is necessary, but the timing makes it impossible to conduct the regular RAC review. She noted that, in her experience, IRBs do not provide a rigorous review for these protocols.

Dr. Fong summarized that it appears that compassionate-use protocols are acceptable if they propose an agent already being used for another disease, the same disease, or a similar mechanism of disease. In the case of compassionate use in which it must be acknowledged that the patient is unlikely to benefit, if the informed consent document and process are done correctly, compassionate use also would be acceptable. The primary concern is in the case of a new agent that has not been used in humans. The threshold for moving forward with such protocols should be high. A good reason must be put forth to

administer that new agent to someone who has low likelihood of benefit and from which the field is unlikely to learn anything.

Dr. Chiocca added that disservice to the patient might also be of concern. Because of the focus on the compassionate-use protocol, that individual might not be entered into a clinical trial that could be helpful. All of these factors must be considered carefully before offering a novel agent. That has not yet been tested in humans, to a single subject under compassionate use.

D. Public Comment

Dr. Bryan, FDA, provided the FDA's perspective on single-patient protocols. These protocols come under the FDA's expanded-access program, which is intended to make investigational agents available for the treatment of patients; the FDA considers these protocols as primarily for treatment and not as having a primary research objective. The potential benefit to the patient must justify the risks to the patient; risks to the patient should not be balanced by acquisition of generalizable knowledge or in service to a primary research objective. The FDA encounters these protocols at all stages of protocol development.

Some research data is gathered as a result of these protocols, which the FDA considers primarily safety data. Dr. Bryan cautioned that the RAC may encounter what the FDA has already seen in other fields—sponsored investigators who may be using this mechanism to try to bypass the need for preclinical studies or as a mechanism to gather initial safety data rather than conducting a Phase I safety study. For example, with small molecules, expanded-access protocols have recently enrolled more participants, followed by the sponsor using that experience as evidence of efficacy to support approval. Particularly in rare disorders, the FDA is concerned that using the investigational agent for this purpose could interfere with ultimately developing the product and gathering the safety and efficacy data necessary for marketing approval.

V. Review and Discussion of Human Gene Transfer Protocol #1202-1164 titled: A Phase I/II, Open Label Study Evaluating the Safety and Efficacy of Gene Therapy in Subjects with ß-Thalassemia Major by Transplantation of Autologous CD34+ Stem Cells Transduced Ex Vivo with a Lentiviral $ß^{A\text{-}T87Q}$-Globin Vector

Principal Investigator:	Donald B. Kohn, M.D., University of California, Los Angeles
Additional Presenters:	David Davidson, M.D., bluebird bio, Inc.; Mitchell H. Finer, Ph.D., bluebird bio, Inc.; Alan R. Cohen, M.D., Children's Hospital of Pennsylvania
Sponsor:	bluebird bio, Inc.
RAC Reviewers:	Ms. Dresser, Dr. Ornelles, and Dr. Ross

Drs. Kiem and Kohn were recused from consideration of this protocol due to conflicts of interest.

A. Protocol Summary

bluebird bio, Inc., is developing lentiviral based gene transfer product for treating β-thalassemia, a rare and potentially life-threatening disease caused by the absence or insufficient production of the β chain of hemoglobin. The gene transfer agent consists of autologous CD34+ hematopoietic stem cells transduced with lentiviral vector encoding the human $β^{A\text{-}T87Q}$-globin gene suspended in cryopreservative solution.

Mutations in the genes for the hemoglobin protein cause a group of hereditary diseases called the hemoglobinopathies. One of the best known hemoglobinopathy is β-thalassemia. In adult humans, the most common hemoglobin type is a tetramer, which contains four subunit proteins called hemoglobin A, consisting of two α and two β subunits.

β-thalassemia results from the impaired production of β-globin and leads to a relative excess of α-globin. Unable to form hemoglobin tetramers, the excess α-globin chains become insoluble and form a precipitate that damages the developing red blood cell precursors (erythroblasts). The clinical implications

of the α-globin/β-globin imbalance are twofold: 1) patients lack sufficient red blood cells and hemoglobin to effectively transport oxygen throughout the body, and 2) abnormal red blood cells suffer an elevated rate of hemolysis that can lead to morbidities through chronic damage to the vasculature and iron overload.

In the most severe cases, called β-thalassemia Major, patients require more than eight red blood cell transfusions per year to compensate for the anemia caused by the disease, since they produce as little as 1 to 7 g/dL of total hemoglobin. Transfusions are aimed at maintaining steady-state hemoglobin levels at about 9–10 g/dL. However, regular transfusions introduce an excessive amount of iron in the body (iron overload) that over time can lead to mortality through iron-associated heart and liver toxicity. To prevent iron-overload-associated risks, patients must take iron chelators orally or subcutaneously. However, patients do not always take their iron chelation treatment and, even with current therapies, overall survival until the age of 30 is only 55 percent for β-thalassemia-major patients.

The only cure for β-thalassemia is allogeneic hematopoietic stem cell transplant (HSCT). Because of the significant risk of transplant-related mortality, graft versus host disease (GVHD), and graft rejection with allogeneic HSCT, transplants are offered primarily to patients with available HLA-matched sibling donors (less than 25 percent of cases). The experience with unrelated donor umbilical cord blood (UCB) HSCT has been less promising, with reported overall and disease-free survival rates of 77 percent and 65 percent, respectively.

To provide an effective and safer alternative to allogeneic HSCT, bluebird bio is proposing a clinical protocol to evaluate the use of this gene transfer agent in research participants with β-thalassemia who are transfusion dependent, defined as requiring greater than 100 ml/kg/year of packed red blood cells, or at least eight transfusions, per year.

The lentiviral vector encodes a single codon variant of the β-globin gene, $\beta^{A\text{-}T87Q}$, which conserves the protein's function while allowing for quantification relative to other globin species. The individual's own (autologous) hematopoietic stem cells are transduced by the lentiviral vector thereby significantly reducing or eliminating the risk of GVHD and graft rejection. The lentiviral vector is genetically engineered so that it is not infectious and cannot reproduce.

bluebird bio has reported preliminary clinical safety and efficacy data from three research participants dosed with autologous CD34+ stem cells transduced with a different lentiviral vector in an ongoing Phase I/II trial in France. The first dosed participant failed to engraft and received rescue cells. The second dosed participant became transfusion independent 12 months following dosing with with sustained expression of $\beta^{A\text{-}T87Q}$-globin protein for nearly four years. The last participant in this trial was dosed in November 2011; neutrophil engraftment occurred on Day 20 post transplant, and delayed platelet engraftment occurred on Day 129 post transplant. No gene transfer related adverse events have been attributed to the genetically modified cells (i.e., no GVHD-like adverse events), and no leukemias or lymphomas have been related to insertional mutagenesis. Based on these preliminary clinical results, bluebird bio proposes to initiate a Phase I/II clinical study in the United States to treat β-thalassemia major with a modified lentiviral vector.

B. Written Reviews by RAC Members

Five RAC members voted for in-depth review and public discussion of this protocol. Key issues included the modification of the vector delete the insulators, which were included in the prior French trial of this vector. RAC members believed that the effect of deleting the chromatin insulator sequences on the risk of insertional mutagenesis deserved further discussion with respect to the optimum balance among improving lentiviral vector yield, transduction efficiency, and overall safety.

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

Regarding risk-benefit concerns, Ms. Dresser noted that conventional treatment is effective for many patients and asked whether methods exist to limit participant selection to those most likely to be at risk

with conventional therapy. Given that adherence to chelation therapy is a major reason for early death and that it is also an exclusion criterion for this study, she asked for clarification as to whether this means that participants will be selected from among those who might do well with conventional treatments. Since unrelated umbilical cord blood has had some success, Ms. Dresser asked why the proposed intervention is a potentially better treatment than cord blood and whether it would be possible to recruit participants who are unlikely to benefit from cord blood transplants. She suggested that people with sickle-cell disease be excluded from this study because filgrastim carries special risk for those individuals. With regard to the informed consent document, Ms. Dresser noted that the form frequently uses therapeutic terminology to describe the investigational intervention and the β-globin gene; she requested that the investigators change that wording. Ms. Dresser also recommended several wording changes to enhance clarity and precision.

Dr. Ornelles noted that this protocol is well described in both the clinical protocol and responses to Appendix M, with an excellent plan of experimental benchmarks to maximize safety and efficacy in the course of the trial and over the long term. Key elements of concern that could lead to suspension of this study include the detection of leukemia due to vector-mediated insertional mutagenesis, the appearance of vector-derived replication-competent lentiviruses, and clonal dominance of a population of cells that might presage leukemia. His single major concern stemmed from recent observations that the lentiviral vectors introduce numerous cryptic signals that increase the likelihood of aberrant splicing events in adjacent (or integrated) genes. A similar lentiviral vector used in France was discovered to lead to aberrant processing of the mRNA for the HMGA2 gene in one research participant; the clinical consequences of this event continue to be monitored. Since lentiviral vectors have been shown to have the potential to induce aberrant transcripts that might contribute to genotoxicity, Dr. Ornelles requested information about how the investigators would remain vigilant for such aberrant events. Because of the novel nature of these vectors, he suggested determining the relative abundance of aberrant transcripts in patient samples that show mild skewing of transduced cells, possibly using linear amplification polymerase chain reaction (PCR) on reverse-transcribed RNA. Although relatively long-term studies in animals with the related vector HPV569 support the safety of this class of vector in the mouse, human trials suggest that species-specific differences may be significant.

Regarding concerns about insertional mutagenesis, Dr. Ornelles requested that the investigators review the latest findings, which speak to the tendency of this vector to elicit aberrant effects, on the ongoing studies performed *in vitro*, in animal models, and in the French study.

Additional questions raised by Dr. Ornelles included (1) how detection of clonal skewing or clonal dominance would affect continuation of this trial; (2) whether an insertional event in the HMGA2 gene, as occurred in the French trial, would stop this proposed study; (3) whether inclusion in this study would preclude a participant from receiving subsequent allogeneic HSCT from an appropriately matched bone marrow donor; and (4) whether mobilized hematopoietic stem cells (HSCs) or bone marrow HSCs from a person with β-thalassemia major are significantly more difficult to harvest or to transplant and, if so, whether this information should be included in the informed consent document.

Dr. Ross commented that the protocol is clear, that extensive preclinical work has been done with the new vector as well as the previous vectors containing the cHS4 insulators, and that the French study indicates that this approach has significant potential. With regard to enrollment suspension criteria, the protocol states that detection of leukemia/lymphoma due to vector-mediated insertional oncogenesis will be one criterion for temporary suspension of the protocol; however, Dr. Ross suggested the investigators consider clonality in any research participant as a criterion for suspension/evaluation. Regarding transduction efficiency, she asked the investigators to provide information about the minimum and maximum copy numbers per cell for determining whether sufficient numbers of cells have been transduced prior to engraftment, and whether the investigators will attempt to correlate initial transduction efficiency with final hemoglobin levels as a means of optimizing the therapy as the trial proceeds. She also asked whether the investigators can measure the actual transduction frequency (number of cells transduced) between the different participant samples to determine whether variation exists in the number of integrated vectors per cell, since copy number per cell could have an impact on integration site effects.

Additional questions from Dr. Ross included (1) whether the HSC vector transductions and analyses would be performed at the three different sites or at a single site, and whether a standard procedure would be in place for use at the different sites; (2) whether integration site or transcription

analysis has been performed on the HPV-569 transduced cells found to have increased replating frequency in the *in vitro* immortalization (IVIM) assay; and (3) whether additional information is available regarding the third research participant in the French study.

C. RAC Discussion

During the meeting, no additional questions, concerns, or issues were raised by RAC members.

D. Investigator Response

1. Written Responses to RAC Reviews

This study will enroll only adults with β-thalassemia major, which is a population requiring frequent red blood cell transfusions, defined in the protocol as at least eight per year. Current therapy with red blood cell transfusions and iron chelation is life saving, but achieving a normal quality of life and normal life expectancy is not yet possible for most of these patients. While some patients are able to normalize iron levels with strict compliance to chelation therapy, long-term success remains a significant challenge for many patients due to problems of poor tolerability, lack of efficacy, and non-compliance, especially in individuals with cardiac iron overload who require combination chelator therapy. Despite transfusion therapy, many patients are regularly fatigued as their hemoglobin levels drop prior to their next transfusion. Furthermore, the requirement for chronic red blood cell transfusions may lead to red blood cell allo-immunization, complicating the identification of a suitable donor for red blood cells; achieving adequate venous access can also be problematic. Patients with poor compliance are excluded from this clinical trial because they are not likely to be compliant with the intensive monitoring required by this protocol and because advanced iron overload leads to diffuse tissue damage that would predict a poor outcome from transplantation. The potential to achieve transfusion independence offers benefit with regard to reducing the morbidity and mortality from iron overload, as well as other transfusion-related complications.

The primary factor that limits the application of unrelated umbilical cord blood (UCB) transplant in adults is the low number of progenitor cells present in umbilical cord blood. As a consequence of the relatively low number of HSCs in UCB transplant, patients may experience an increased risk of early fatal complications due to the lower engraftment rate of donor hematopoiesis, delayed kinetics of neutrophil recovery, and lack of adoptive transfer of pathogen-specific memory T cells because UCB lymphocytes are naïve cells. Increasing cell dose with dual UCB transplant may improve engraftment, especially in adults, though at a cost of increased GVHD. However, little published evidence is currently available on the long-term immune reconstitution and clinical benefit of dual UCB transplant in non-malignant diseases in patients without an HLA-matched sibling donor. Consequently, at this time it is not possible to define a population of adult patients with β-thalassemia major who should be excluded from the trial to receive UCB transplant.

Two recent studies describe evaluation of potential alternative splice sites following gene transfer with lentiviral vectors; however, it is not clear how these contribute, if at all, to genotoxicity. Genotoxicity due to integration can arise from (1) over-expression of a gene at the integration target site by interaction of the viral transcriptional controls with local cellular transcription units, (2) knockout of gene expression by gene disruption at the integration site, (3) truncation of the transcript due to insertion of a poly-A site by integration within an intron of a cellular gene, or (4) through alternative splicing by cryptic splice sites present in the vector.

With regard to the concern about aberrant splicing events, the investigators explained that they propose to use a self-inactivated lentiviral vector, which has a significantly reduced propensity to engage in aberrant splicing compared with that of vectors carrying active LTRs. The investigators have mapped the cryptic splice site in the truncated insulator region in the HMGA2 integration-containing clone. The insulator and the cryptic splice site have been deleted in their new lentiviral vector to be used in the proposed clinical study. In the article published by Ronen et al. in 2011, no integrations into genes controlling proliferation were identified when murine cells were transduced with the previous vector and

used to reconstitute β-thalassemic mice. HMGA2 integration was not identified with the French clinical lentiviral vector in this preclinical study. The investigators have carried out in-vitro immortalization (IVIM) assays to assess the functional implication of genotoxic events. While these RNA-based assays can identify all potential combinations of aberrant transcripts, functional assays such as the IVIM assay are designed to assess functional consequences of genotoxicity. In two independent IVIM assays carried out by Dr. Christopher Baum's group in Hannover, Germany, the mutagenic potential of the current vector and the one used in the French trial versus the published positive control vectors, with the conclusion that both vectors are believed to be significantly less genotoxic compared to positive controls.

Because the investigators understand the possibility of species-specific differences that might not be accounted for in the functional assays that assess the genotoxicity of their vector in preclinical models, they agreed to carry out integration site analysis by linear amplification-mediated PCR (LAM-PCR) and 454 pyrosequencing of DNA derived from participants' peripheral blood to assess integration site preference. If genes controlling cell cycle or proliferation are affected, they planned to analyze whether transcription of the relevant gene is altered. If the target gene is known to control differentiation or proliferation, or if it is identified within the Retrovirus Tagged Cancer Gene Database, the investigators will initiate an analysis of whether insertion leads to aberrant transcripts of these targeted genes.

A review of the preclinical and clinical data from the French study, alongside the recently generated preclinical data with their new vector, suggests that the vector to be used in the proposed clinical study should have an improved safety profile compared to the one used in the French trial. The HMGA2 insertion site was not identified in the investigators' published preclinical animal studies of gene transfer with vector used in the French trial into mouse bone marrow followed by reconstitution of thalassemic and normal recipients. In Subject 1003 in the French study LG001, the presence of the HMGA2 clone did not affect homeostasis of red blood cells or nucleated cells in peripheral blood, and that research participant remains clinically stable and transfusion independent 5 years after dosing. In the currently proposed clinical study, the insulator sequences have been deleted from new lentivrial vector add comment covered earlier.

Detection of clonal skewing or clonal dominance will affect continuation of this trial on a case-by-case basis, with decisions on suspending or stopping enrollment for clonal skewing in the absence of overt malignancy made in collaboration with the principal investigators at the clinical sites, the study Data Monitoring Committee (DMC), the regulatory authorities, and the sponsor. The clonal skewing associated with the HMGA2 integration event stabilized at a relatively low percentage of peripheral blood nucleated cells (2–3 percent) and has not been associated with any adverse events in five years of follow-up. Therefore, this clonal skewing has not been considered a stopping event, and a similar perspective would be applied to HGB-204.

While it would be possible for a patient to receive a subsequent allogeneic HSCT following participation in this trial, the repeated exposure to myeloablative chemotherapy could increase the risk of adverse events. This procedure would fall outside of the this study, and the treating physician will be responsible for the decision regarding whether to proceed with allogeneic transplant.

The key criteria used to release the lentiviral vector are infectious titer, expression of the desired protein, and the absence of replication-competent lentiviral vector. The key criteria for release of the drug product are transduction efficiency in CD34+ cells, $\beta^{A\text{-}T87Q}$-globin protein expression in subject bulk erythroid cell progeny, and cell count and viability.

Studies in allogeneic transplant suggest that as little as 10–15 percent mixed donor chimerism results in transfusion independence. As the cells in an allogeneic transplant contain two gene copies, a vector copy number of 0.3 should be sufficient for transfusion independence; however, as the vector level of expression is only 70 percent of the endogenous gene per copy, the investigators have increased the minimum vector copy number for release of the transduced cells (drug product) to 0.5. The maximum vector copy number for release of the drug product has been set at a conventionally adopted upper target of 3, which is thought to minimize the risks of insertional oncogenesis. In the French study, transduced cells from Subject 1003 had a mean vector copy number of 0.6 at release, and most recently (month 55

post transplant), the mean vector copy number from his peripheral blood was 0.105. This individual has shown clinical benefit five years after transplant with no gene transfer associated serious adverse events.

Correlates of biomarkers and clinical outcome, including correlation of initial transduction efficiency with hemoglobin levels, will be assessed. OBA Protocol 1164 is an open-label study, and ongoing analysis of the data will occur and could influence the conduct of the trial. The investigators will measure integrated vector copy number in CD34+ cells pre-infusion for drug product release by assessing mean vector copy number. During the clinical study, the investigators intend to determine vector copy number in peripheral blood nucleated cells by qPCR. Through the use of appropriate reference standards and assay controls, the investigators will assess the relationship between vector copy number at infusion and long-term hemoglobin expression levels. They also can compare mean vector copy number in peripheral blood among different participants who will be enrolled in the clinical trial.

The manufacturing process (transduction of autologous hematopoietic stem cells with lentiviral vector) will be conducted at a single manufacturing site. The process will be conducted under cGMP conditions at the contract manufacturing organization, Progenitor Cell Therapies in Mountain View, California. All analysis associated with in-process testing for the drug product manufacturing process also will be conducted at Progenitor Cell Therapies. The clinical sites will ship the cells to the manufacturing site, and the cells will be transduced there and sent back to the clinical sites for infusion into the research participants.

The slight increase in replating frequency with French trial vector observed in the IVIM assay was well within the assay variance and was not statistically significant. However the integration sites of the expanded clones for both vectors are being analyzed and will be compared and evaluated.

The third research participant in the French study is clinically stable and has returned to work. She achieved neutrophil and platelet engraftment, and at the 6-month post-transplant time point she has levels of cell marking and corrected globin, detected by high-performance liquid chromatograph, that are comparable to what was seen with Subject 1003 at similar time points.

In response Ms. Dresser's question regarding the risk of filgrastim to patients with sickle cell disease, the investigators clarified that subjects with sickle cell disease will not be enrolled in this trial. The investigators agreed to remove references to sickle cell disease from the informed consent document.

The protocol will exclude enrollment of patients with an available 10/10 HLA-matched HSC donor.

The investigators agreed to make the various requested modifications to the informed consent document.

E. Public Comment

No public comments were offered.

F. Synopsis of RAC Discussion and RAC Observations and Recommendations

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

- The protocol states that if there is "detection of leukemia due to vector-mediated insertional mutagenesis … enrollment <u>will be</u> temporarily suspended and the event assessed by the DMC. After evaluation, the DMC will make a recommendation regarding whether enrollment may recommence (underline added)." It is reasonable not to include an absolute stopping rule for detection of a clonal population of cells. Nonetheless, it should be noted that if clonality is detected, the protocol may be suspended pending consultation with the regulatory bodies and the DMC. In addition, the protocol should state that, depending on the nature of the event, clonality alone may lead to suspension of the trial.

G. Committee Motion 2

Dr. Fong summarized the RAC recommendations that would be included in the letter to the investigators, expressing the comments and concerns of the RAC. Although no motion was made or seconded to approve this recommendation, the RAC approved this summarized recommendation by a vote of 15 in favor, 0 opposed, 0 abstentions, and 2 recusals.

VI. Gene Transfer Safety Assessment Board Report

RAC Reviewers: Drs. Badley, Chiocca, Fong, Kiem, Kohn, Strome, and Yankaskas

A. GTSAB Report

Dr. Yankaskas reported that OBA had received 19 protocol submissions in the past 3 months, 17 of which were not selected for public review at this RAC meeting. Of the 17 protocols not selected for public review, 13 were oncology protocols, and one each were for peripheral artery disease, HIV and infectious disease, oral mucositis, and monogenic congenital retinal disease (achromatopsia). In these 17 protocols, five used adenovirus vectors, three each used plasmid and retrovirus vectors, two each used lentiviruses and modified bacteria, and one each used AAV and vaccinia vectors.

Twenty SAEs from 12 protocols were reviewed by the GTSAB, including initial and follow-up reports. Dr. Yankaskas stated that summaries of these SAEs will be available on the OBA Web site by the next quarter. After analysis of these events, the GTSAB concluded that none warranted public discussion at this RAC meeting.

The OBA received notification from investigators that 13 protocols were newly open to enrollment. Five of those 13 had previously been reviewed at a RAC public meeting, and three of those five presented responses to the issues raised following public review.

Dr. Yankaskas summarized the particulars of a three-session gene transfer and rare diseases workshop scheduled for September 13, 2012, cosponsored by the OBA and the NIH Office of Rare Diseases Research. Session I will focus on clinical experience with hemophilia, Leber congenital amaurosis and other eye diseases, and blood cell disorders. Session II will work on defining opportunities for data sharing across protocols, using common pharmacology and toxicology studies and vector platforms. Session III will review the current resources, including the Rare Diseases Clinical Research Network, the National Gene Vector Biorepository, bridging interventional development gaps, and the Gene Therapy Resource Program. Dr. Yankaskas expressed the GTSAB's hope that RAC members will participate in this meeting.

B. RAC Discussion

No discussion occurred.

C. Public Comment

No public comments were offered.

VII. Review and Discussion of Human Gene Transfer Protocol #1202-1153 titled: Stem Cell Gene Therapy for HIV in AIDS Lymphoma Patients

Principal Investigator: Mehrdad Abedi, M.D., University of California, Davis, Cancer Center
Additional Presenters: Joseph Anderson, Ph.D., University of California, Davis

RAC Reviewers:	Drs. Badley, Chatterjee, and Fost
Ad hoc Reviewer:	Ronald Mitsuyasu, M.D., University of California, Los Angeles

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

A. Protocol Summary

The human immunodeficiency virus (HIV) remains a major health problem, with millions of people infected worldwide. HIV infects cells of the immune system, becomes part of the cells' genetic information, stays there for the rest of the life of these cells, and uses these cells as a factory to make more HIV. In this process, the immune cells get destroyed; thereafter, a condition called Acquired Immunodeficiency Syndrome (AIDS) sets in, leaving the immune system unable to fight common infections. If left untreated, death from severe infections occurs within eight to ten years. Although advances in treatment using small molecule drugs have extended the lifespan of HIV-infected individuals, neither a cure for HIV infection nor a well-working vaccine has been developed. Drug treatment is currently the only option to keep HIV-infected individuals alive. HIV patients must take a combination of drugs daily and reliably for the rest of their lives, and some patients cannot take these drugs due to severe side effects. If not taken regularly, HIV becomes resistant to the drugs and continues to destroy immune cells.

Stem cell gene transfer for HIV may offer an alternative treatment. Blood-forming stem cells, also called bone marrow stem cells, make all blood cells of the body, including immune-system cells such as the T cells and macrophages that HIV destroys. If "anti-HIV genes" were inserted into the genetic information of bone marrow stem cells, these genes would be passed on to all new immune cells and would make them resistant to HIV. Anti-HIV-gene-containing immune cells can multiply in the presence of HIV and fight the virus.

In most of the previous stem cell gene transfer clinical trials for HIV, only one anti-HIV gene was used. However, these investigators propose to use a combination of three anti-HIV genes to have a potentially more potent effect. To demonstrate safety and effectiveness of this experimental treatment, the investigators propose to perform a clinical trial in HIV lymphoma patients. In such patients, destruction of the immune system by HIV led to the development of a type of leukemia called B-cell lymphoma.

The proposed study is a two-arm clinical study evaluating the safety and toxicity of a novel triple combination of anti-HIV genes transferred by a single lentiviral vector into isolated, mobilized peripheral blood CD34+ cell from HIV infected individuals with relapsed lymphoma. The three anti HIV genes are: shRNA against the CCR5 receptor, a secondary receptor of HIV, inhibiting entry of HIV into the target cell, a humanized Trim5alpha molecule inhibiting uncoating of the viral capsid and a TAR decoy, preventing upregulation of HIV expression post integration. This triple combination of anti-HIV genes is hypothesized to strongly inhibit HIV entry into the target cells, and should be active against macrophage and T cell tropic HIV strains. Use of three targets should help prevent resistant mutants of HIV from developing, just as combination drug therapy does.

High-dose chemotherapy together with the transplantation of the patient's own bone marrow stem cells cures B-cell lymphoma. The investigators propose to insert anti-HIV genes in the patient's bone marrow stem cells and then transplant these gene-containing cells into the HIV-infected lymphoma patient. The gene-containing bone marrow stem cells will produce a new immune system, and the newly arising immune cells will be resistant to HIV. Assuming this plan is successful, the patient's leukemia will be cured and the patient will have been given an HIV-resistant immune system that will be able to fight HIV.

B. Written Reviews by RAC Members

Eleven RAC members voted in-depth review and public discussion of this protocol. Key issues included the novelty of this vector containing three anti-HIV genes, including the chimeric Trim5α. Because autologous stem cell transplant for HIV-related lymphoma leads to three-year survival of greater than 50 percent, RAC members believed that the risks and benefits of using these transduced cells in the transplant setting deserved further discussion.

Three RAC members and one *ad hoc* reviewer provided written reviews of this proposed trial.

Dr. Badley noted that the application is accompanied by a significant body of preclinical data demonstrating efficient expression and function of the anti-HIV genes *in vitro* and *in vivo* in a humanized mouse model. A critical component of this application is the assumption that the HIV-resistant CD4 T cells that result will need to be functional in order for immune competency to be restored in treated subjects; therefore, Dr. Badley requested that the investigators supply the data for cytokine secretion profiles of transduced cells versus non-transduced cells that have engrafted the RAG1 mice, in conditions of mitogen stimulation or resting. Because a secondary objective of this protocol is to evaluate immune reconstitution, he encouraged the investigators to incorporate an immunization step with a replication-incompetent antigen, such as tetanus toxoid or rabies, so that acquisition of immunity against a neoantigen could be assessed. Dr. Badley requested clarification about (1) how the investigators plan to recruit research participants from outside their catchment area, (2) why the first three participants will be infused with transduced and unmanipulated product, (3) how often and for how long after gene transfer the investigators will monitor participants for insertional oncogenesis and clonality, and (4) the composition of the data and safety monitoring board. He suggested the investigators include more explicit stopping rules for the antiretroviral therapy interruption phase.

Dr. Chatterjee noted that the preclinical data generally support the ability of the transduced cells to engraft and show HIV resistance against laboratory strains of HIV as well as evidence of transgene function. Because the proposed lentivirus construct does not include insulator sequences to prevent activation of neighboring genes, particularly oncogenes, she asked whether the addition of a “corrected” insulator sequence would increase the safety of the vector. Dr. Chatterjee queried as to whether immortalization assays had been performed with this vector and, if so, she asked the investigators to share the results of those experiments. She also requested data on integration site analysis of this vector on the *in vivo* engrafting of human CD34 cells. Dr. Chatterjee asked why no cell death was observed in the enhanced green fluorescent protein (EGFP) only transduced cultures at 21 days post infection, since these cells are not protected against HIV infection. She asked the investigators to comment on (1) the cause of the reduced levels of CD14/CD45 in xenografted mice as compared with untransduced controls, (2) whether monocyte/macrophage toxicity is associated with vector transduction, and (3) the plans to monitor research participants for clonal proliferation of transduced cells (an event that has been observed in several lentivirus trials to date).

Noting that a secondary objective is to examine the role of antiretroviral therapy (ART) interruption on peripheral blood cells and gut mucosa for presence, quantity, and duration of gene-modified HIV-resistant immune cells, Dr. Fost asked the investigators to clarify why this approach is being tried first on individuals with HIV infection plus lymphoma, rather than on those with just HIV infection. He requested clarification of the evidence that discontinuing ART for eight weeks is safe, discussion of why the first 15 participants (as stated in the study summary) would only receive unmanipulated product, and additional information on past experience with bone marrow transplantation for refractory or relapsed non-Hodgkin’s lymphoma in general, the BEAM (carmustine, etoposide, cytarabine, and melphalan) regimen in particular, or gene-modified stem cells. Dr. Fost offered several suggestions for clarification and correction within the informed consent document, including information about previous failures of gene transfer using HSCs, compensation and billing, changes to wording that might foster therapeutic misconception, and explanation of whether a research participant could decline the sub-study while continuing to participate in the primary stem-cell study.

Ad hoc reviewer Dr. Mitsuyasu noted that the protocol is clear and provides information about the extensive preclinical work *in vitro* and in an HIV-infected humanized mouse model. He asked how much expression and inhibition each anti-HIV gene provides individually and whether additive or synergistic inhibition occurs with the combined anti-HIV gene approach. Dr. Mitsuyasu requested that the investigators discuss whether the HSC-derived T cells *in vitro* similarly express the transduced genes, function normally, and are protected against HIV infection, compared with the macrophages. Noting that the exploratory endpoint is a comparison with pre-ART HIV viral set point, thus requiring documentation of pre-ART viral load, he suggested a record of this viral load be available as part of entry criteria, or that

only potential participants with this available information should be included in the exploratory analysis of this endpoint. He also suggested specification of the viral load criterion that will be used as a termination criterion for dosing interruption after month six. Because many immune reconstitution assays and DNA monitoring tests will be conducted post transplant, Dr. Mitsuyasu suggested that the investigators should ensure that adequate blood samples are obtained and that blood volume guidelines are not exceeded at any time point. He also requested two clarifications within the informed consent document.

C. RAC Discussion

During the meeting, the following additional questions, concerns, and issues were raised:

- Dr. Chatterjee noted that the investigators plan to monitor the participants for viral load and CD4 after ART has been stopped and the transduced cells have had a chance to grow out. If the viral load increases in the presence of transduced cells, she asked whether that would signify escape mutants, although she acknowledged that the chance of generating escape mutants is low.

- Dr. Fost suggested that the investigators add to the appropriate section in the informed consent document about the risk associated with stopping ART and that the investigators will be monitoring for this risk to mitigate it.

- Dr. Kiem asked how the investigators plan to proceed if engraftment does not occur with the gene-modified cells for the first three research participants.

- Dr. Fong asked how the investigators will define “success” in this trial.

- Dr. Fost commented on the issue of reimbursement of research-related injury. He noted that few institutions are indemnified for open-ended commitment for any research-related injury, and few make that promise. Most institutions do offer generally to provide medical care, and they sometimes offer to pay for emergency medical care.

D. Investigator Response

1. Written Responses to RAC Reviews

Data for the individual anti-HIV genes were not included in this application since their actions, individually, have been described in peer-reviewed publications by other researchers. All three genes are strong inhibitors of HIV. The main reason for adding them together was to inhibit the formation of escape mutants.

In regard to the question about studies in CD4 T cells, the investigators responded that an *in vivo* model that can generate functional T cells from transduced human CD34+ cells, as was shown in the investigators’ preclinical model, is a more relevant model for this stem cell gene transfer approach and can provide more meaningful data for a planned Investigational New Drug submission. In the *in vivo* HIV challenge experiments, for example, *in vivo* generated T cells expressed typical T cell markers, i.e., CD3, CD4, and CD8.

No primary isolate challenges were used for preclinical challenge experiments with the current vector. In preclinical experiments that these investigators performed for previous HIV stem cell gene transfer clinical trials, several primary isolate challenges were done *in vitro*. Looking at the inhibition of HIV output after challenge, the investigators saw no significant differences in primary isolate challenges versus laboratory virus challenges, even with single anti-HIV genes. Although the investigators have considered the relevance of primary isolate challenges as a part of correlative studies for this clinical trial, they decided that meaningful primary isolate challenges would only come from isolates obtained from research participants, as these challenges would provide relevant data of potential resistance versus selective survival advantage of HIV target cells for the individual patient.

More than 90 percent of HIV-positive adults demonstrate recall reactivity to SEB and CEF antigens, so the investigators have used these antigens as standard positive controls. However, based on Dr. Badley's suggestion, the investigators agreed to include tetanus toxoid vaccination as a part of their evaluation for immune reconstitution post transplant.

Regarding accrual of research participants, the investigators noted that the CARES (Center for AIDS Research, Education, and Services) clinic was originally created as a consortium of local hospitals, including UC Davis, Sutter Memorial, the Kaiser Hospitals, and Mercy Hospital. Close collaborations with the HIV providers at these institutions continue, including regular personal and email correspondence regarding open clinical trials. In addition, the investigators have received verbal and/or written commitments from many of the outside bone marrow transplant programs to refer these patients to the investigators' trial. Collaboration with Dr. Kaplan at the University of California, San Francisco (UCSF) is important because UCSF has one of the largest populations of individuals with HIV-related lymphomas and has a good track record of enrolling these patients on transplant clinical trials.

The dosing of the first three research participants with transduced and unmanipulated cells is a feasibility study, an initial step that will test the feasibility of the proposed approach and will troubleshoot unforeseen complications without compromising participant safety. The FDA has suggested a similar approach in previous stem cell gene transfer clinical trials for treatment of HIV-related lymphomas. If the approach appears feasible, the investigators will continue with the remainder of the clinical trial.

Although acute retroviral syndrome is difficult to define, the criteria for restarting ART, described as "at the discretion of the provider and patient," would fall under this category. In the investigators' experience with multiple treatment interruption protocols enrolling dozens of research participants, this has happened in just one case where malaise, low-grade fevers, and lethargy prompted the participant to request restarting ART. Because a participant could have a very low initial CD4 count followed by a confirmed CD4 count below 200 soon after discontinuing ART, the investigators have revised the protocol by modifying the CD4 count prior to ART interruption to 300, thus providing a larger interval.

LAM-PCR will be performed every three months for the first two years of follow-up for the duration of the study. Thereafter, it will be performed only if clinical and/or laboratory evidence of a clonal process is observed.

For this vector, the investigators do not believe that insulators would add significantly to the safety profile. The safety measures already built into the vector include (1) the vector is self-inactivating, (2) a poly-A signal in the 3'UTR is present to stop transcription from the MNDU3 promoter, and (3) the U6 promoters have their own transcription termination signals (a string of six thymidines) at the end of the expression cassettes.

Although the investigators did not perform immortalization assays with the vector proposed for this trial, they have performed many *in vivo* experiments in immunodeficient mice, which are good indicators for tumor formation. They did not detect any tumor formation in the *in vivo* experiments.

The investigators do not believe that toxicity is associated with vector transduction for macrophages or monocytes. They have not observed toxicity in their *in vitro* experiments with anti-HIV vector transduced hematopoietic progenitor cell-derived macrophages. On average, for their *in vivo* experiments, the numbers are significantly lower; however, not all mice displayed lower levels of CD14/CD45+ cells.

The prerequisite to show efficacy of this experimental treatment is engraftment of anti-HIV transduced stem cells in the recipient. The best engraftment levels usually occur in the setting of a myeloablative preparative regimen. The fully ablative transplant is a relatively toxic regimen with long-term hospital admission, high short-term morbidity, and a small but real chance of mortality. Patients with relapsed/refractory HIV-related lymphomas require stem cell transplantation as a part of their standard of care management. This platform will provide the opportunity to test the gene-transduced stem cells in research participants who need a myeloablative stem cell transplant anyway. Once the safety and

engraftability of anti-HIV gene-transduced stem cells are defined, the investigators plan to enroll subjects with HIV without any malignancy in their subsequent clinical trial.

Most or all of the patients with HIV-related lymphoma who undergo stem cell transplant will be covered by private insurance companies or would be eligible for coverage by Medicare/Medicaid. The investigators agreed to clarify this issue by stating that the research participant will not be billed for the experimental part of this research.

The investigators explained that they did not derive T cells *in vitro*. T cells generated *in vivo* from their anti-HIV gene-transduced CD34+ cells are more relevant for answering the protocol questions. The T cells derived *in vivo* were shown to be functional and were protected from infection.

Upon suggestion, the investigators added further clarification throughout the protocol, stating that the efficacy of the candidate product will be measured at three months post transplant.

Because plerixafor (Mozobil) has some effect on transduction of stem cells, and may also have some effects on CXCR4 (C-X-C chemokine receptor type 4), the investigators have discouraged the use of Mozobil in their stem-cell mobilization setting. However, the investigators are concerned that the heavily pretreated HIV-related lymphoma patients may not mobilize adequate numbers of stem cells in all cases using conventional stem cell mobilization approaches. Therefore, they prefer not to eliminate completely the use of Mozobil for exceptional cases when, despite all efforts, only a limited number of stem cells have been collected. In such cases, the use of Mozobil will be recorded to avoid confusion in data interpretation.

The investigators stated their belief that viral load does not consistently correlate with disease progression. However, they suggested a cutoff of confirmed 500,000 copies of HIV per ml if the RAC deemed it necessary.

The investigators will comply with the American Red Cross Guidelines to assure that not more than 450 ml of blood is drawn per six weeks, with less than 5ml per kg of body weight per blood draw.

The investigators agreed to make suggested changes in the informed consent document to increase clarity and comprehension.

2. Responses to RAC Discussion Questions

Dr. Abedi explained that the investigators are expecting to see an increase in the viral load with a plateau after that initial increase. If the viral load gets to the level of 300,000 copies, the research participant will be put back on ART. The investigators plan to conduct extensive studies, including blood work and gastrointestinal biopsies before and after dosing, to look at immunological reaction and to see whether or not anti-HIV immune T cells and macrophages expand.

Dr. Abedi agreed to add to the informed consent document a few comments about the risk of stopping ART. The investigators believe the risk to be minor because of the short duration compared with other trials and the rigorous monitoring.

Regarding engraftment with the gene-modified cells in the initial three research participants, Dr. Abedi explained that the investigators plan to continue with the remainder of the study even if engraftment does not reach the target level of 0.5 percent. The first three participants will receive transduced and untransduced cells to ensure safety of the procedure and product.

Dr. Abedi stated that the investigators define “success” for this trial as safety, but eventually they want to produce clinically relevant numbers of immune cells that have anti-HIV genes.

Dr. Abedi reiterated that, for the two-year follow-up period of this trial, the investigators’ institution will cover any expenses directly related to participation in the trial.

E. Public Comment

Dr. Borror asked about the gastrointestinal biopsy informed consent document. She requested explanation of why a lower endoscopy was being done, for whom it would be done, and whether or not biopsies would be taken from the lower gastrointestinal tract.

Robert J. Reinhard, a global health consultant and patient advocate for HIV Cure Research, asked two questions via email:

1. Preclinical safety study for the protocol considered EGFP expression and also used an MTT assay to measure immune cell activation. Please confirm the adequacy or known limitations of that report or the use of other tools to determine the safety of the products and to alert participants for informed consent.
2. Adverse events (off-target effects) or other expenses for experimental procedures like this are mostly not covered by insurance. In these novel first-in-human studies requiring a high degree of voluntarism, please direct that the study sponsors cover and reimburse causally related research injury (only physical injury, not economic) and other unreimbursed expenses of research participants. These protections mitigate public consequences that could derail later research program advances.

F. Synopsis of RAC Discussion and RAC Observations and Recommendations

Preclinical Issue

- In an immunodeficient mouse model, transplanted transduced CD34+ cells were allowed to mature into macrophages, and analyses demonstrated a normal surface phenotype. Because the target cell of this study is CD4+ T cells, the investigators should consider using this mouse model to evaluate the phenotype of mature, transduced CD4+ T cells.

Clinical and Trial Design Issue

- A secondary objective of this trial is to evaluate immune reconstitution. T-cell responses to recall antigens will be tested using cytomegalovirus, Epstein Barr virus, and influenza virus antigens. However, in the absence of re-exposure to, or reactivation of, these viruses post transplant, there may not be a positive response. Adding a recall antigen such as tetanus toxoid is recommended.

Ethical, Social, and Legal Issues

- The changes to language in the informed consent document made in response to the written reviews reduce the likelihood that a potential participant would confuse this experimental approach with therapy. In addition to those changes, the informed consent document should be amended to include:
  - A discussion of the potential health risks due to an interruption in ART, recognizing that the monitoring program for this trial is designed to mitigate the risk
  - A brief discussion of the risks of HSCT, recognizing that there will be a separate, more-detailed consent document for that procedure
  - A simple statement that consent for the intestinal mucosa biopsies is not required for enrollment in this trial

- The section titled “What Happens If I Am Injured Because I Took Part in This Study?” was amended to state that the research participant will not be billed for any treatment necessitated by complications associated with the experimental part of this research. As the intent of this protocol is to create a population of gene-modified cells that can persist for many years, it should also be acknowledged that long-term adverse effects of this approach may not be recognized immediately, and therefore expenses due to those long-term effects possibly will not be compensated.

G. Committee Motion 3

Dr. Fong orally summarized the RAC recommendations to be included in the letter to the investigators, expressing the comments and concerns of the RAC. A motion was made, and no second was called for, that these comments be approved by the RAC. The RAC approved these summarized recommendations by a vote of 15 in favor, 0 opposed, 0 abstentions, and 1 recusal.

VIII. Presentation of Certificates of Appreciation for Departing RAC Members

Dr. Corrigan-Curay presented certificates of appreciation on behalf of the NIH to the RAC members whose terms of service were completed at this RAC meeting; she offered brief remarks about the contributions of each to the RAC. Departing members were Dr. Buchmeier, Dr. Kanabrocki, Professor Mastroianni, Dr. Roizman, and Dr. Yankaskas.

IX. Update on Protocol #0912-1016 titled: A Phase II Study To Determine the Efficacy and Safety of Allogeneic Human Chondrocytes Expression TGF-β1 in Patients with Grade 3 Chronic Degenerative Joint Disease of the Knee

Principal Investigator:	Michael A. Mont, M.D., Sinai Hospital of Baltimore
Additional Presenter:	Ogden Copeland, TissueGene, Inc.
Sponsor:	Kwan Hee Lee, M.D., Ph.D., TissueGene, Inc.
Ad hoc Commenter:	Martin Lotz, M.D., Research Institute, La Jolla, California

A. Presentation

Dr. Mont discussed the background and purpose of the study, some of the nonclinical data since he last presented to the RAC, the study design, a status update of the study, and some adjustments made to the study. He provided a quick overview of osteoarthritis of the knee, and noted that the lifetime risk of developing osteoarthritis is under-exaggerated at 45 percent and, with increase body mass index, increases to 60 percent. Few adults are *not* affected by knee osteoarthritis.

This new technology was developed by Dr. Lee in Korea and then brought to TissueGene, Inc., in the United States. The protocol is designed to assess the effects of a single intra-articular injection of TissueGene-C (TG-C) in patients with knee osteoarthritis. TG-C is a 3:1 mixture of normal human chondrocytes and irradiated human chondrocytes that have been transduced to express TGF-β1 to stimulate cartilage regeneration. Irradiation renders the cells replication incompetent, and the irradiated cells die off within 2 weeks after injection. In preclinical studies, TG-C showed a paracrine mode of action with the TGF-β1 from the transduced cells, acting on the injected normal chondrocytes to stimulate cartilage formation.

The RAC publicly reviewed this protocol at its March 2010 meeting. Preliminary immune response testing was performed, additional animal testing was initiated, and the protocol was revised and resubmitted as a result of that meeting. After post-Phase I discussion with the FDA and further revisions based on those discussions, enrollment in this Phase II trial was initiated on May 17, 2011. Dr. Mont showed data on the original 77 research participants enrolled in this study.

Nonclinical studies completed since March 2010 were conducted to answer some of the questions raised by the RAC concerning TG-C; the findings included:

- Greater than 98 percent of the loaded cells adhered to knee cartilage in the rabbit after 30 minutes.
- In humans, an *ex vivo* cell adhesion study showed that 40–60 percent of loaded cells adhered to the cartilage surface after 60–120 minutes; exposed bony surfaces showed lower adhesion rates.

- No tumorigenicity was seen except at 3,000 times the dose used in any clinical or preclinical protocol.
- A test of cell fate in a rabbit surgical defect model showed that human cells were detected in regenerated cartilage 8 and 12 weeks after transplantation of TG-C.
- Histopathological examination revealed inflammation including synovial cell hyperplasia, synovitis, vascularization in the synovium and pannus formation consistent with known TGF-β1 effects that were reversible.

Dr. Mont provided a brief synopsis of the clinical protocol and the results so far. Because this study is blinded, he has access only to general data. There is a 2:1 ratio of 100 participants getting TG-C versus control injections. Participants are between the ages of 18 and 70, with Grade 3 chronic knee degenerative joint disease. Participants in this study will receive a single intra-articular injection of TG-C or hyaluronan control. Because no treatment-related adverse events were observed in the U.S. Phase I trial, the investigators have decided to continue with the maximum dose level of $3x10^7$ cells used in that study. Hyaluronan was chosen as a control so the effects of TG-C could be compared to a standard treatment method and so research participants would not have the chance of getting no treatment if randomly assigned to placebo.

Safety assessment of TG-C administration in the U.S. Phase II study will be based on observations of the injection site for irritation or other abnormalities, incidence and severity of adverse events, and changes in physical examination findings and laboratory tests through 24 months. Dr. Mont reviewed the primary pharmacokinetic endpoints, which will include evaluation of TGF-β1 expression levels and for the presence of vector DNA and replication-competent retrovirus. The investigators will analyze patient blood samples to assess the potential for immunogenicity of TG-C. Immunosuppressants will not be used prior to product injection (and were not used in Phase I or either of the Korean studies) because they would confound immune response analysis. Dr. Mont reviewed the primary and secondary efficacy criteria and the methods used to evaluate those endpoints.

A total of 77 research participants have been dosed as of June 18, 2012. A total of 138 AEs were reported from 55 participants, of which 67 (48.5 percent) of the adverse events are considered possibly or definitely related to treatment (active or placebo). Enrollment was suspended on March 5, 2012, pending regulatory review of the more severe adverse events related to inflammation of the knee joint; the study was re-initiated on May 7. All five sites have re-received IRB approval and are enrolling. No “serious” adverse events have occurred related to TG-C administration; some were moderate but all were self-limited. Inflammation of the knee joint is not unexpected with TG-C, based on the results from the acute rabbit toxicity study that showed inflammation due to TGF-β1. Interim analysis at six months after enrollment of the last participant is likely to occur in August 2012. Dr. Mont planned to return to the RAC to show results after analysis.

Dr. Mont shared the data on his 23 blinded patients at Sinai. They do not appear different than the normal profile of patients with knee pain. Visual analog scale pain scores at 24-hour, 4-week, and 3-month follow-up points after injections show a slight initial pain increase and subsequent pain intensity decrease. (Dr. Mont said that two of his research participants were so happy with the results of their injections—with visual analog scales at zero—that each wanted to go to Korea to have their other knee injected.) A few swelling episodes occurred, and some participants had to use a cane and take extra pain medications.

Moderate and severe side effects, but not serious ones, caused some concern by the institutional data monitoring committee, which thought that participants should be cautioned about the possibility of swelling and pain; therefore, the investigators revised the risk section of the informed consent document to include the side effects seen in this study. The protocol also has been revised to redefine the stopping criteria to include a localized cutaneous immune response; the study would no longer be stopped just for swelling of the knee. In addition, a handout will be provided to participants, subject to approval by each site’s IRB, to describe post-injection instructions such as icing the injected knee, avoiding strenuous exercise for 2–3 weeks, and taking Advil or Aleve for pain, swelling, or discomfort. Further guidance for investigators in the Investigator Brochure now includes the adverse events seen in this study to date, the procedure described in the protocol for guided injections has been reviewed with the sites’ investigators,

and a standardized regimen for addressing pain and inflammation has been provided to the investigators so research participants who experience these types of adverse events in the future will receive uniform treatment. Dr. Mont stated that, in hindsight, he would recommend giving the guidance information to research participants and investigators earlier.

B. RAC Discussion

In response to a query from Dr. Lotz, Dr. Lee stated that, after injection, no TG-C was discovered in the synovial membrane. After two to three weeks, an inflammatory change due to TGF-β could no longer be found. The investigators checked all other tissues for TGF-β and concluded that TGF-β does not penetrate into the serum and is confined to the knee joint region. Preclinical studies performed with labeled cells showed no remnant cells. Previously conducted biodistribution studies showed that even if TGF-β cells penetrate into the circulation via technical error during injection, they are eliminated. Dr. Copeland further responded that the investigators have conducted several preclinical studies using doses equivalent to and greater than the planned clinical dose, subcutaneously and via injection. When mice were tested with subcutaneous injections, a small bolus formed initially that dissipated after a few days; the cells died and no toxicities or adverse events, including fibrosis and persistent swelling, were seen from those subcutaneous injections.

Dr. Lotz requested a summary of the two research participants who had the most profound inflammatory response, including how soon that response was seen after injection and how soon it resolved. Dr. Copeland explained that nearly all participants experienced a noticeable inflammation at 24 hours after receiving the injection. The inflammation resolved for most participants within a few days; for the few participants who had a more significant response, complete resolution took a little more than 30 days. Dr. Mont added that the two individuals with the most significant response were treated initially with anti-inflammatory medications followed by a narcotic or a corticosteroid dose pack. In addition, both were put on crutches for one to two weeks. The synovial fluid was not analyzed in these participants. Dr. Lotz suggested that aspirating and analyzing the effusions in the future would assist in clarifying the underlying mechanism. Dr. Mont agreed that analysis for TGF-β and inflammatory mediator levels would be appropriate.

At the suggestion of Drs. Fong and Strome, Dr. Mont agreed to compare the initial magnetic resonance imaging (MRI) scans to the scans at three-month follow-up to determine which research participants have persistent fluid in the knee. This comparison could provide information about whom to dose and whom not to dose. Dr. Lotz noted that identifying an injection site cutaneous reaction versus a knee effusion should be straightforward for someone who is familiar with examining knees; he suggested that this characterization of synovial versus cutaneous reaction should be made clearer to avoid a similar discussion in the future.

Dr. Fong summarized the ensuing discussion: If significant swelling around the knee joint occurs and if investigators see, via MRI, fluid in the joint, fluid should be extracted from the knee joint to determine the reason for the swelling. Characterization of these fluid samples will assist in the current protocol as well as in planning future studies.

C. Public Comment

No public comments were offered.

X. Closing Remarks and Adjournment

Dr. Fong thanked the RAC members and the OBA staff and adjourned the June 2012 RAC meeting at 3:20 p.m. on June 19, 2012.

(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: _____

_____
Yuman Fong, M.D.
Chair
Recombinant DNA Advisory Committee

Attachment I: RAC Roster
Recombinant DNA Advisory Committee

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Attachment II
Public Attendees

(This list includes individuals who are not identified elsewhere.)

Gerhard Bauer, University of California, Davis
Wilson Bryan, FDA
Anne-Virginie Eggiman, bluebird bio, Inc.
Sandra Gurgo, National Institute of Allergy and Infectious Diseases (NIAID)
Nancy Jones, NIAID
Kathleen Kirby, bluebird bio, Inc.
Carolyn Larment/Tarment, NCI
Mark Lee, FDA
Harrison Noh, TissueGene, Inc.
Moon Jong Noh, TissueGene, Inc.
John J. Nemunaitis, Mary Crowley Medical Research Center
Steve Rosenberg, NCI
Gabor Veves, bluebird bio, Inc.
Youngsuk Yi, TissueGene, Inc.
Yao-Yao Zhu, FDA

Attachment III
Abbreviations and Acronyms

AIDS	acquired immunodeficiency syndrome
ART	antiretroviral therapy
CARs	chimeric antigen receptors
CT	cancer/testis
DMC	data monitoring committee
EGFP	enhanced green fluorescent protein
FDA	Food and Drug Administration, U.S. Department of Health and Human Services
GTSAB	Gene Transfer Safety Assessment Board
GVHD	graft-versus-host disease
HIV	human immunodeficiency virus
HLA	human leukocyte antigen
HSCs	hematopoietic stem cells
HSCT	hematopoietic stem cell transplant
IFN-γ	interferon gamma
IRB	institutional review board
IVIM	in vitro immortalization
LAM-PCR	linear amplification-mediated PCR
MI	myocardial infarction
MRI	magnetic resonance imaging
NCI	National Cancer Institute, NIH
NIAID	National Institute of Allergy and Infectious Diseases, NIH
NIH	National Institutes of Health
NIH Guidelines	*NIH Guidelines for Research Involving Recombinant DNA Molecules*
OBA	Office of Biotechnology Activities, NIH
OD	Office of the Director, NIH
PCR	polymerase chain reaction
RAC	Recombinant DNA Advisory Committee
SAEs	serious adverse events
TCRs	T-cell receptors
TG-C	TissueGene-C
UCB	umbilical cord blood
UCSF	University of California, San Francisco