RECOMBINANT DNA ADVISORY COMMITTEE

Minutes of Meeting

March 10-11, 2010

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]

March 10-11, 2010

The Recombinant DNA Advisory Committee (RAC) was convened for its 120th meeting at 8:00 a.m. on March 10, 2010, at the Hilton Hotel and Executive Center, Rockville, Maryland. Dr. John Zaia (Acting Chair) presided at the start, and several other RAC members took over the Acting Chair position as needed. In accordance with Public Law 92-463, the meeting was open to the public from 8:00 a.m. until 5:15 p.m. on March 10 and from 8:00 a.m. until 11:50 a.m. on March 11. The following individuals were present for all or part of the March 2010 RAC meeting.

Committee Members

Jeffrey S. Bartlett, Nationwide Children's Hospital/The Ohio State University
Michael J. Buchmeier, University of California, Irvine
Hildegund C.J. Ertl, The Wistar Institute/University of Pennsylvania
Hung Y. Fan, University of California, Irvine
Jane Flint, Princeton University
Yuman Fong, Memorial Sloan-Kettering Cancer Center
Jeffrey P. Kahn, University of Minnesota
Joseph A. Kanabrocki, The University of Chicago
Louis V. Kirchhoff, University of Iowa
Eric D. Kodish, The Cleveland Clinic Foundation
Margaret Mallino, University Park, Maryland
Anna C. Mastroianni, University of Washington School of Law
Bernard Roizman, The University of Chicago
Susan R. Ross, University of Pennsylvania
Scott E. Strome, University of Maryland
Lee-Jen Wei, Harvard University *(Day 1 only)*
David A. Williams, Children's Hospital Boston/Harvard Medical School
James R. Yankaskas, The University of North Carolina at Chapel Hill
John A. Zaia, City of Hope

Office of Biotechnology Activities (OBA)

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

***Ad Hoc* Reviewers, Presenters, and Speakers**

Robert H. Carter, M.D., National Institute of Arthritis and Musculoskeletal and Skin Diseases, NIH
George J. Dover, M.D., Johns Hopkins University School of Medicine and Johns Hopkins Children's Center
James Gulley, M.D., Ph.D., F.A.C.P., National Cancer Institute (NCI), NIH
Rebecca Moritz, M.S., University of Wisconsin-Madison (UW-M)
Gabriele Neumann, Ph.D., UW-M
James Pickel, Ph.D., National Institute of Mental Health (NIMH), NIH
James Turk, M.S., UW-M

[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.

Nonvoting Agency Representatives

Daniel M. Takefman, U.S. Food and Drug Administration (FDA), U.S. Department of Health and Human Services (DHHS)

NIH/OD/OBA Staff Members

Linda Gargiulo
Robert Jambou, Ph.D.
Laurie Lewallen
Maureen Montgomery
Marina O'Reilly, Ph.D.
Gene Rosenthal, Ph.D.
Tom Shih, M.D., Ph.D.
Mona Siddiqui, M.D.

Attendees

There were 8 attendees at this 2-day RAC meeting (the complete count of attendees was not available).

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. Zaia, Acting RAC Chair, called the meeting to order at 8:00 a.m. on March 10, 2010. Notice of this meeting under the *NIH Guidelines for Research Involving Recombinant DNA Molecules* (*NIH Guidelines*) was published in the *Federal Register* on February 24, 2010 (75 FR 8369). 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 five protocols, discussion of a proposal to exempt the mating of certain biosafety level (BL) 1 transgenic rodents from the requirements of Section III-E-3 of the *NIH Guidelines*, and discussion of the University of Wisconsin's application requesting a determination of the appropriate biocontainment for the cloning of Ebola and Marburg complementary deoxyribonucleic acid (cDNA) into *E. coli*.

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 December 1-2, 2009, RAC Meeting

RAC Reviewers: Drs. Kirchhoff and Wei

Dr. Kirchhoff stated that the December 2009 RAC minutes document accurately reflected what happened at the meeting. He and Dr. Wei noted a number of revisions for clarity and enhanced readability that they will convey to the OBA.

A. Committee Motion 1

Approval of the December 2009 RAC meeting minutes was moved by Dr. Kirchhoff and seconded by Dr. Buchmeier. The RAC voted unanimously by voice vote to approve the December 1-2, 2009, RAC meeting minutes.

III. Discussion of Human Gene Transfer Protocol #1001-1024 entitled: Lymphodepletion Plus Adoptive Cell Transfer with CXCR2 and NGFR Transduced T-Cells Followed by High Dose Interleukin-2 in Patients with Metastatic Melanoma

Principal Investigator: Patrick Hwu, M.D., M.D. Anderson Cancer Center
Sponsor: M.D. Anderson Cancer Center
RAC Reviewers: Ms. Mallino, Dr. Yankaskas, Dr. Zaia

A. Protocol Summary

Metastatic melanoma, which starts on the skin, is uniformly fatal when it spreads and metastasizes. The median survival time is less than 1 year despite the currently available therapeutics. While 43 percent of patients respond to current treatment, 57 percent do not. With immunotherapy using T cells, only .01 percent of the injected treatment material gets to the tumors, thus requiring more effective methods to deliver treatment.

T-cell-based immunotherapy is a promising therapeutic modality for cancer. The investigators on this proposal and others have developed therapies to treat human melanomas by isolating and expanding autologous tumor infiltrating lymphocytes (TILs), followed by reinfusion into patients. Although tumor regression can be dramatic in some patients, in other patients no clinical response is seen. The investigators hypothesize that inefficient migration of T cells to tumors is one of the rate-limiting steps in the generation of an effective anti-tumor response. Therefore, the investigators propose to study methods to improve T-cell trafficking to tumors by introducing specific receptor genes that can enhance migration to tumor sites.

Melanomas specifically express the chemokines CXCL1 and CXCL8, which are thought to promote autocrine growth and angiogenesis. However, the investigators have found that tumor antigen-specific T-cells fail to express the chemokine receptors specific for these ligands, including CXCR2. In a strategy designed to direct T cells toward chemokines expressed by tumors, the investigators have developed a system to genetically modify T cells using retroviral vectors encoding CXCR2. They have demonstrated that murine T cells transduced to express CXCR2 exhibited enhanced trafficking to CXCL1-expressing tumors, which led to improved anti-tumor responses and survival. The investigators now propose to translate their findings from mouse models to humans by dosing melanoma patients with autologous tumor-reactive TILs genetically modified to express CXCR2. To compare to control TILs the ability of CXCR2-transduced TILs to migrate to tumor, each research participant will also receive TILs transduced with the truncated nerve growth factor receptor (NGFR) marker gene.

B. Written Reviews by RAC Members

Three RAC members voted for in-depth review and public discussion of the protocol. Key issues included the novelty of this approach for T-cell migration and activation.

Three RAC members provided written reviews of this proposed trial.

Ms. Mallino focused her review on the content and clarity of the informed consent documents from the perspective of a study participant. She requested that the investigators clarify the multi-part screening tests, the paragraph on preparation for cell depletion, and the timeframe during which participants would be allowed to participate in treatment plans. Ms. Mallino asked that the list of side effects of possible antibiotic and antiviral medications include valacyclovir for herpes virus prophylaxis and fluconazole for fungal prophylaxis. In addition, she noted that the timelines needed clarification, even after the current revision by the investigators.

Regarding the study design, Dr. Yankaskas asked the investigators to provide data showing the effectiveness, time course, bone marrow recovery times, and complication rates for the cyclophosphamide and fludarabine lymphodepletion protocol. He also requested an explanation of the criteria for determining the intervals between high dose interleukin-2 (IL-2) infusions, specification of when

the administration of prophylactic Levaquin would begin, reasoning for why the administration of prophylactic Valtrex would start on day 0 rather than on day minus-7 or on another day based on the lymphocyte count, reasoning for why the administration of prophylactic Fluconazole would start on day 0 rather than on day minus-7 or on another day based on the neutrophil count, and discussion of the starting criteria for administering granulocyte colony-stimulating factor. With regard to TIL doses and safety, Dr. Yankaskas requested that the investigators present data demonstrating the safety of infusing the gene-transduced TILs or else consider a dose escalation design to assure research participant safety; he noted that the answer in Appendix M-II-B-2-a does not provide sufficient details to address this concern. Dr. Yankaskas also requested that the investigators change the term “gene therapy” to “gene transfer” throughout the informed consent document.

Dr. Zaia asked the investigators to comment on the potential for this protocol to enhance tumor growth, in the sense that CXCR2 could have effects on the biology of the T cell that could alter their function or have potential negative effects on the tumor microenvironment; in addition, the reduction of IL8 by treatment with CXCR2 TILs could have a deleterious effect on the tumor microenvironment. Noting that the protocol is exceptionally well written, he stated that the major concern is the choice of cell dose and asked for discussion of the appropriate dose. Dr. Zaia asked whether the study was intended to include pediatric-age participants and, if so, whether special consenting procedures for the younger participants would be implemented. He noted that the maximum doses of Cytoxan and fludarabine should be stated consistently throughout the documents. Regarding the informed consent document, Dr. Zaia suggested that the institutional review board (IRB) should determine whether the document is written using comprehensible terminology and that the consent from should include an explanation of the inpatient portion of the protocol. The listing of side effects should indicate which are transient and which permanent, which would require additional treatment to reverse them, and which could lead to hospitalization or might be fatal. He asked the investigators to consider including in the consent form the potential that this experimental treatment could result in the melanoma growing faster.

C. RAC Discussion

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

- Dr. Williams noted that children do get metastatic disease and that no effective therapy exists for them. He stated that he could think of no reason not to enroll participants in this trial who were younger than 16 years old, and he proposed that participants as young as approximately 10 years old could be enrolled in this trial.

- Dr. Kodish suggested the investigators review a paper (Joffe, S., et al., (2006) J. Pediatrics 149:862) regarding assent and the ethics of children participating in cancer research. He also suggested that 9 years old might be an appropriate age cutoff, based on the literature regarding assent.

- Noting that the dose escalation in this protocol spans from 15 billion cells to 75 billion cells, Dr. Ertl requested additional clarity about the dose escalation scheme, noting that she was unsure about the correctness of the proposed dose escalation for a cell that can proliferate within a 6-hour to 8-hour cycle.

- Dr. Takefman asked whether a retroviral vector should be used to mark cells, and whether there is another way to assess whether the transgene is increasing trafficking. He suggested that, at a minimum, the investigators should describe the cell-marking risk clearly in the informed consent document and that they should justify why there is no other way to determine if trafficking is being increased.

- Dr. Kodish reminded the investigators that, for pediatric research participants who have melanoma, weighing the risks and benefits needs to be presented fundamentally differently than weighing the risks and benefits of a melanoma patient who is 18 years old or older. If a component of this study is conducted for any reason other than for the benefit of the child-

participant, the risks of that component need to be analyzed separately, and he reiterated the importance of determining whether the potential benefit of this study could be provided in any other way that would not expose pediatric participants to that risk.

- Dr. Kahn emphasized the importance of disclosing to the parent(s) and to the child, within the informed consent and assent processes, all risks – no matter how theoretical.

- Dr. Zaia queried as to whether the first dose level should be given to adults and not children. He noted that including children for the controlled experiments could only be justified if there exists a different biology in the children. If the biology in children is the same as the biology in adults, it would be difficult to justify including a pediatric population in the controlled part of this trial, i.e., cells marked only for trafficking.

- Dr. Strome asked whether the investigators plan to phenotype their transduced cells to see if those cells are mediating the effector response.

- Dr. Strome argued that the proposed starting dose is reasonable. In this trial, the investigators propose to change the ability of the cell to traffic but will not change the function of the cell once it arrives at its target. More T cells given to research participants is better, as is the plan for this protocol; the same is not true using the chimeric antigen receptor T cell, which is a completely separate therapy.

- Dr. Zaia asked about the ethics of dosing children with a nontherapeutic gene that offers risk but no benefit. Dr. Kodish explained that it is permissible for an IRB to approve a protocol in children if the IRB finds that the risk to child participants is less than a minor increase over minimal risk.

- Dr. Kahn and Dr. Williams suggested that the investigators make an affirmative argument to their IRB about including children in this trial.

D. Investigator Response

1. Written Responses to RAC Reviews

Regarding the waiting period between biopsy and continuation of the next phase of this study, the investigators explained that growing the T cells adequately takes approximately 6 weeks in the laboratory. During those 6 weeks, research participants may receive other treatment if desired. If the participant is planning on T-cell dosing, all other treatments (e.g., systemic chemotherapy) must stop for 28 days prior to lymphodepletion so as to eliminate the medications from the individual's system.

The investigators acknowledged that it would take up to 300 research participants' tumors to obtain 36 evaluable individuals. Reasons for this large number include that not all tumors will grow enough T cells for treatment, the person might decide not to participate in this trial, or the person's disease could progress.

The investigators provided data to show the effectiveness, time course, bone marrow recovery times, and complication rates for the cyclophosphamide and fludarabine lymphodepletion protocol. Data came from 10 representative research participants for recovery of white blood cell and absolute neutrophil counts. No significant complication rates have resulted from the cyclophosphamide and fludarabine protocol, possibly due to the antibiotic prophylaxis and growth colony stimulating factor support used.

The interval of 8 hours to 16 hours between doses of high-dose IL-2 is based on long-standing dosing schedules reported in the literature. The FDA-approved schedule for high-dose IL-2 is every 8 hours. However, reversible toxicities such as hypotension or tachycardia often delay dosing, and dosing can continue after resolution of these toxicities. The investigators have found that this proposed schedule is well tolerated in their initial study of TILs in melanoma patients. The administration of prophylactic fluconazole is started on Day 0, rather than on Day -7 or on another day, based on the neutrophil count,

because the investigators have not seen significant fungal infections from this regimen – although fungal infection is a longterm risk. The neutrophil count drops to clinically significant low levels by Day 0, so that is when the fluconazole prophylaxis is scheduled to begin. In addition, the investigators have not seen acute risks of herpes zoster, and therefore believe it is acceptable to begin Valtrex prophylaxis at Day 0 instead of Day -7. Further, the administration of prophylactic Levaquin begins on Day 0, which is when the neutrophil count drops to levels that are clinically significant.

The investigators have suggested a starting dose of 15 billion CXCR2-transduced TILs, a dose that is 10-fold lower than the highest dose of 150 billion TILs in their current trial; this highest dose has been well tolerated. Using a standard 3+3 Phase I design, the current trial proposes three dose levels of CXCR2 transduced TILs, with an equal number of truncated NGFR-transduced control TILs that are not anticipated to have any increased toxicity due to the lack of a cytoplasmic signaling sequence. Cohort 1 will receive 15 billion CXCR2 transduced TILs, Cohort 2 will receive 45 billion CXCR2 transduced TILs, and Cohort 3 will receive 75 billion CXCR2 transduced TILs. In each of these groups, the investigators propose to infuse non-transduced TILs as well, to give up to 150 billion cells per research participant. Thus, participants will be provided with a potential therapeutic benefit from the cells while allowing a Phase I of the transduced TILs. The trial will then continue at the maximum tolerated dose level.

Regarding concern about potential for enhancement of tumor growth by this treatment, the investigators explained that, in studies of CXCR2 transduced peripheral blood lymphocytes and CXCR2 transduced TIL, no indication of increased senescence was encountered. The transduced TILs proliferated well, could migrate toward CXCR2 ligands, and could produce cytokines in response to tumors. Further studies of CXCR2-transduced murine T cells showed that these cells could function well *in vivo* to mediate antitumor immune responses. Moreover, microarray analyses performed in CXCR2 versus control transduced T cells did not show significant upregulation of genes associated with enhanced cell death.

Regarding inclusion of pediatric-age participants, the investigators explained that they see a significant number of pediatric patients with melanoma who have disease progression after standard therapy. Their colleagues in pediatric oncology have urged them to include pediatric patients in these trials due to the limited treatment options available. An assent form and process will be used, and pediatric participants will be required to have an intellectual age of at least 7 years.

The investigators agreed to make the requested changes to and clarifications within the informed consent document.

2. Responses to RAC Discussion Questions

In response to a question about increased immune reaction at the site of the tumor, Dr. Hwu explained that the investigators would be using the natural T-cell receptor rather than an engineered T-cell receptor. The natural T-cell receptor hopefully would become activated at the tumor site and would kill the tumor cell. The goal of this trial is to get more of those natural T-cell receptors to the tumor site and, if all goes well, some additional T-cell receptor antigen reactivity would occur, making possible that some additional cytokine release by the natural T-cell receptor would also occur.

Dr. Hwu explained that the investigators decided to provide prophylactic antibiotics on Day 0 to prevent any infections, although the standard practice is to wait for appearance of a fever to give antibiotics. He offered to consider starting prophylaxis at Day –1, one day earlier than planned.

Regarding the posited problem with trafficking, Dr. Hwu explained that, in mouse models, improved responses resulted when the investigators altered the trafficking; better responses also occurred when more cells were infused. In addition, responses were improved in the few cases in which the investigators threaded catheters into arterial cells compared to the tumor response in the same animals using the intravenous route, which presumably does not result in as robust trafficking to the tumor site. These various lines of evidence indicate that getting more cells to the tumor will result in an improved response.

In response to concern about the number of cells to be infused in the dose escalation scheme, Dr. Hwu stated that, as the methods are perfected and the cells have more proliferative potential, the investigators intend to reduce the number of cells given overall.

Regarding concern about using gene marking, Dr. Hwu stated that this design will answer the question of whether CXCR2 works, so this protocol will be able to tell other researchers whether they should add this receptor to their potential treatments for melanoma. If the informed consent document includes an explanation that the results of this trial will provide an answer to that question by using a marker gene, the majority of potential research participants would likely agree to participate in this trial.

Dr. Hwu agreed with the suggestion that the investigators speak to their IRB to make the case to include children in the full study. If the IRB does not agree with that approach, the investigators can then modify the study; one alternative would be to modify the children's part of the protocol to eliminate the truncated NGFR-transduced cells.

E. Public Comment

No public comments were offered.

F. Synopsis of RAC Discussion and RAC Observations and Recommendations

The following observations and recommendations were made during the RAC's indepth review and public discussion:

Clinical/Trial Design Issues

- Although efficacy is not a primary endpoint, two hypotheses are being tested: 1) does the inclusion of the CXCR2 gene cause the TILs to target the tumor more efficiently and 2) once at the tumor, are the TILs able to attack and eliminate tumor cells. An unaddressed question is whether the TIL cells remain cytotoxic after genetic modification if directly injected into tumor sites. The current study design focuses solely on intravenous delivery, but one way to address this issue is to modify the study design to test whether CXCR2-transduced TILs administered directly into an accessible tumor results in tumor cell elimination and also assess by intravenous administration the ability of the modified TILs to traffic to the tumor.

- One cannot predict the fate of the modified TILs once infused, i.e., there could be significant expansion or rapid elimination. The proposed dose escalation scheme should be informed by what happens to TILs in the prior cohorts. Each participant's peripheral blood should be monitored for 2 weeks post administration to determine the number of gene-marked cells. The dose escalation scheme for the next cohort then should be adjusted according to whether the number of transduced cells is more or less than expected.

- The biopsies that are planned at days 6 and 27, to assess the primary endpoint of gene-modified T-cell migration to the tumor, should examine the phenotype of the T cells. There is new evidence in the literature that the presence of CD4+ cells may be critical to antitumor activity.

Ethical/Social/Legal Issues

- The inclusion of the control TILs complicates the risk-benefit analysis because of continuing questions about retroviral vectors and the risk of insertional mutagenesis. None of the cases of insertional mutagenesis seen to date involved mature T cells (they all involved hematopoietic stem cells) and, in a recent study, investigators were unable to purposely cause insertional mutagenesis in terminally differentiated T cells using a retroviral vector (Newrzela S. *et al.* Resistance of mature T cells to oncogene transformation. *Blood* 2008; 112(6):2278-2286). Nonetheless, because the control TILs may pose a risk but no potential benefit, the IRB should

consider carefully whether the inclusion of the control for pediatric participants is ethically acceptable. In addition, the risk and lack of direct benefit of using the control should be explained more fully in the informed consent document.

- In response to the RAC comments, you stated that participants with an "intellectual age" of at least 7 are being considered for enrollment. If pediatric participants are to be included, the inclusion/exclusion criteria should specify a lower age limit rather than using the less objective "intellectual age." Further, the lower age limit should be set such that the pediatric participants enrolled are able to assent to the protocol.

G. Committee Motion 2

Dr. Zaia summarized the RAC recommendations that will be included in the letter to the investigators and the sponsor expressing the comments and concerns of the RAC. It was moved by Dr. Ertl and seconded by Dr. Williams that the RAC approve these summarized recommendations. The vote was 19 in favor, 0 opposed, 0 abstentions, and 0 recusals.

[At this point, Dr. Strome took over as Acting RAC Chair.]

IV. Discussion of Human Gene Transfer Protocol #1001-1020 entitled: Administration of EBV-Specific Cytotoxic T Cells Expressing HER2 Chimeric Antigen Receptor to Subjects with Advanced Osteosarcoma (ECHO)

Principal Investigators:	Nabil Ahmed, M.D., M.P.H., Texas Children's Hospital
Additional Presenters:	Stephen Gottschalk, M.D., Texas Children's Hospital; Lisa Wang, M.D., Texas Children's Hospital
RAC Reviewers:	Drs. Fong and Kahn

Dr. Williams recused himself from consideration of this protocol due to a conflict of interest.

A. Protocol Summary

Osteosarcoma is the most common primary bone malignancy. Current multimodality therapy consists of radical surgery and systemic chemotherapy. Despite improvements in outcome over the last 30 years for patients with local disease, the 5-year survival rates for patients with metastatic disease at presentation or recurrent disease remains poor. Human epidermal growth factor receptor 2 (HER2) is expressed by more than 60% of primary osteosarcoma samples and correlates with a poor outcome. Although HER2 is a validated target for breast cancer immunotherapy, its expression on osteosarcoma cells is low, rendering HER2 monoclonal antibodies such as trastuzumab ineffective. Genetic modification of T cells with chimeric antigen receptors (CARs) is an attractive approach to overcome this limitation, since the overall avidity of receptors arrayed on a T-cell will be greater than the avidity of a bivalent antibody, and engagement of a limited number of T-cell receptor molecules may be sufficient to trigger a cytotoxic effector response.

CARs can be made by combining the antigen binding domains of the variable regions of a monoclonal antibody (scFv), a transmembrane domain and signaling endodomains derived from CD28 and the T-cell receptor ς-chain. When expressed by T cells, CARs bind the antigen expressed on the target cell surface and activates the lytic pathway of the T cells. We have cloned a CAR targeting the tumor associated antigen HER2 into the retroviral vector SFG. T cells expressing this HER2-specific CAR killed HER2-positive osteosarcoma cells *ex vivo* and in animal models. Incorporation of a single costimulatory endodomain in the CAR cannot readily compensate for the complex temporo-spatial interactions of the multiplicity of costimulatory molecules and ligands involved in the physiological activation of effector T-cell subsets. In previous studies in neuroblastoma, this deficit was overcome by expressing tumor directed CARs in EBV-specific T cells (EBV-CTLs). We now hypothesize that transduced EBV-CTLs will

be activated and persist in response to EBV-positive cells when stimulated through their native TCR in osteosarcoma patients. These bi-specific cells will subsequently be able to kill tumor cells recognized by their HER2-specific CAR.

The Phase I dose escalation clinical trial is designed to evaluate the safety of HER2 CAR EBVCTL in patients with refractory/metastatic osteosarcoma. One SFG vectors will be used to generate autologous HER2 CAR EBV-CTL and seven dose levels will be evaluated namely, $1x10^4$ cells/m^2 (dose level I) , $1x10^5$ cells/m^2 (dose level 2), $1x10^6$ cells/m^2 (dose level 3), $3x10^6$ cells/m^2 (dose level 4), $11x10^7$ cells/m^2 (dose level 5),$3x10^7$ cells/m^2 (dose level 6) and $1X10^8$ cells/m^2(dose level 7). The safety of the infused T cells will be assessed and their survival and antitumor effects measured.

B. Written Reviews by RAC Members

Five RAC members voted for in-depth review and public discussion of the protocol. Key issues included the recent death on another trial using a CAR directed against HER2, which raises questions about potential toxicities and T-cell specificity.

Two RAC members provided written reviews of this proposed trial.

Given the death from acute respiratory distress syndrome of the participant in the previous HER2-specific T-cell trial, Dr. Fong queried as to whether the investigators would consider a prior history of systemic immune disease or high pretreatment cytokine levels in their exclusion criteria, and whether the investigators have a formalized plan or standard procedures for clinical interventions if there is delayed toxicity to HER2-expressing normal tissues, including an algorithm of use for corticosteroids and T-cell antibodies. Regarding participants being classified as “refractory,” he asked whether they would have to show clear progression on chemotherapy or whether they would be so classified if they merely had unresectable disease. Dr. Fong suggested that recovery from the acute toxic effects of prior chemotherapy (an inclusion criterion) should be characterized using hematologic, biochemical, or subjective clinical criteria; alternatively, a minimum time period from last therapy could be utilized. Given that the starting dose is unlikely to produce benefit for the participant, he wondered how long the first participants would be followed before they are allowed to proceed with other therapy and how participants would be noted to have progressive disease between dosing and their first post-dosing scans 6 weeks later. Dr. Fong asked that the investigators be more specific in noting what additional studies would be conducted if a participant shows evidence of cardiac dysfunction, which could be a result of cardiac toxicity from anti-HER2 effectors, and what level of changes in left ventricular ejection fraction would be enough to trigger such evaluation. Noting that only one posttreatment scan – at 6 weeks – is planned for evaluation of tumor response, Dr. Fong asked whether other time points for tumor evaluation should be included in this protocol. He asked whether the cytokines to be assayed include only those listed, and he wondered how and when the investigators would decide to include others. In addition, Dr. Fong suggested separating cytokines and T-cell function, which would allow the investigators to plan more explicitly for assaying for persistence of HER2 T cells and assaying for clonality, depending on the amount of cells available.

Dr. Kahn focused his review on the consent form and process, beginning with the notation that the title “Treatment Consent” that appears on every page should be removed, as this is a Phase I trial that is exploring safety only. In general, he noted that the informed consent document should be written more clearly and should better articulate that the focus of this Phase I trial is safety and toxicity, including rewording terms such as “treatment” and “therapy” that could give potential participants the impression of therapeutic benefit from participation. Dr. Kahn noted that the first paragraph of the informed consent document should be reworded because it currently might give the impression of few, if any, treatment options other than participation in this trial. In addition, he noted that the paragraph describing the modified T cells is unclear and difficult to understand, the paragraph describing side effects of the T cells introduces unexplained names and acronyms, and the section asking participants to notify study personnel if they become pregnant also should include the participant’s partner. While the materials indicate that a substantial portion of the study population will be adolescents and that an appropriate

assent process will be in place, Dr. Kahn noted that the informed consent document does not include any mention of or mechanism for assent by minor participants.

C. RAC Discussion

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

- Dr. Strome asked how the vector proposed for this trial differs from others being used at the investigators' institution.

- Dr. Wei queried as to whether the dose escalation scheme could be more aggressive between cohorts.

- Dr. Strome asked how the investigators justify not using lymphodepletion in this population. He noted that, although the death in the other trial occurred in the context of lymphodepletion, it is also clear that lymphodepletion is the reason for the potential of therapeutic benefit. Without lymphodepletion and using adoptive immunotherapy, efficacy is reduced significantly.

- Dr. Strome asked the investigators to justify administering 10,000 cells – a dose that will not treat even a mouse – in a participant cohort for which they already have an ongoing trial.

- Dr. Kodish discussed the potential for great therapeutic misconception in single-disease Phase I trials. The clustering of a lung cancer trial with an osteosarcoma trial and others helps participants, through the architecture of the trial, to understand that the goal for Phase I trials is safety and determining the appropriate dose for a Phase II trial.

- Dr. Ertl suggested that the investigators either add lymphodepletion so there is a better chance of seeing efficacy or start at a much higher dose and increase that dose even higher – and convince the FDA that this scheme is prudent.

- Dr. Strome asked the investigators to consider how to parse their three ongoing osteosarcoma trials, all of which have low doses and overlapping populations. He suggested that they consider proposing to combine the three trials so that, once enough evidence is gathered that the low dose is safe, the investigators could progress more rapidly to a dose that might be therapeutic. At the least, he suggested that the investigators provide a rationale written into each trial so that participants in all three trials do not get dosed at the $1x10^4$ level, which everyone agrees will not be of benefit, particularly in the absence of lymphodepletion.

- Dr. Kahn noted that the discussion about this protocol had centered on the issue of Phase I research with no potential therapeutic benefit being conducted in a pediatric population. He suggested the investigators collect some early-phase data from adults that could then be applied to a pediatric participant population at a higher dose, at which dose some potential for therapeutic benefit might be possible.

- If the current trial were to start at the current proposed dose but data become available from similar trials that indicates safety at higher doses, Dr. Fong suggested that the investigators should approach the FDA for either rapid acceleration of dose or a different scheme for dose escalation.

D. Investigator Response

1. Written Responses to RAC Reviews

The investigators explained that the eligibility criteria were developed to prevent serious complications. This trial does not propose to use lymphodepletion and all participants must have recovered from the

acute toxic effects of prior chemotherapy, so participants are not expected to have elevated cytokine levels. The investigators agreed that it would be possible to modify the exclusion criteria to exclude individuals with systemic autoimmune disease.

Delayed toxicities to HER2 expressing normal tissues would be mediated by T-cell effector function and would be analogous to acute graft-versus-host disease (GvHD) observed after hematopoietic stem cell transplantation. If such T-cell-mediated toxicities occur, participants will be treated according to the investigators' standard procedure, which uses high-dose steroids and, if necessary, T-cell and T-cell cytokine antibodies.

To clarify the term "refractory, the inclusion criteria were modified to state diagnosis of refractory HER2-positive osteosarcoma or metastatic HER2-positive osteosarcoma, not treatable by surgical resection and with disease progression after receiving at least one prior systemic therapy.

Life expectancy should be ≥6 weeks at the time of infusion of the cell product.

The investigators explained the starting dose for this trial, which was a consistent concern of RAC reviewers. The primary objective of this Phase I dose-escalation clinical study is to evaluate the safety of escalating doses of autologous HER2-CAR EBV-CTL. The starting dose level of $1x10^4$ cells per square meter was requested by the FDA for the investigators' other two clinical studies with HER2-specific T cells, due to the adverse event observed with HER2-specific T cells in another trial (discussed at the December 2009 RAC meeting). After completion of the initial toxicity evaluation (6 weeks), participants will come off study if they have progressive disease. However, participants can come off study earlier if there is evidence of progressive disease and they will be eligible for alternate treatments, although none are of known efficacy for the patient group that will be enrolled in this trial.

Regarding the mechanism for assent, the investigators stated that the procedures and treatments involved and their attendant risks and discomforts will be explained carefully to the potential participant and/or the parent(s) or guardian(s) prior to enrollment and dosing. Assent will be obtained for each child who is capable of providing assent based on age, maturity, and psychological state. Assent will be documented using the "child clause" within the informed consent document.

The investigators agreed to make the requested changes to and clarifications within the informed consent document.

2. Responses to RAC Discussion Questions

Dr. Gottschalk explained that the investigators have used gene-marked EBV-specific T cells for reconstitution after hematopoietic stem cell transplantation. Longterm followup data, published in 2009 in *Blood*, showed no tremendous expansion of gene-marked T cells. The majority of the research participants in that longterm followup cohort received $2x10^7$ EBV-specific T cells per square meter.

Noting that the investigators are in active discussion with the FDA, Dr. Gottschalk agreed to go back to the FDA and try to modify the regimens related to the three similar trials. However, he explained that the current situation is that there is little data to indicate that the low dose (proposed in this trial) is safe, so it is not possible to propose starting this trial at a significantly higher dose.

When the investigators discussed the starting dose with the FDA, they took advantage of the data that are known about donor lymphocyte infusion. Donor lymphocyte infusions are safe at very low doses but they are inefficient, and at very high doses they are lethal because they cause GvHD; however, there is a middle ground at which antitumor activity occurs and the dose is considered safe. Dr. Gottschalk explained that the investigators wanted to start at the lower end, knowing that donor lymphocyte infusions have not caused GvHD at that level.

Dr. Ahmed explained the differences among the three trials (either approved or to-be approved) that will be undertaken by these investigators. Although the three trials might look the same because HER2 is

involved in all of them, one trial is in adult participants, mostly towards the older age, who are getting HER2 and TGF-β grafted on a T cell; the second trial is the first protocol using stem-cell blasts that have HER2; and the third trial (currently under consideration at this RAC meeting) uses CTLs that are specific for EBV and have a different reactivity if grafted with HER2.

E. Public Comment

No public comments were offered.

F. Synopsis of RAC Discussion and RAC Observations and Recommendations

The following observations and recommendations were made during the RAC's indepth review and public discussion:

Clinical/Trial Design Issues

- Because of concerns about the safety of CTLs expressing a HER-2 specific CAR, the proposed starting dose is well below that used in previous cancer trials using gene-modified T cells. Enrollment in this study should be deferred until additional data are gathered from the initial dose cohorts from similar studies already underway or about to start. Protocol #0903-971, which uses EBV-specific lymphocytes expressing a HER-2 CAR and a TGF-∃ dominant negative receptor in participants with advanced HER-2 positive lung cancer and Protocol #0903-969, which uses non-EBV-specific T lymphocytes expressing a HER-2 CAR for advanced osteosarcoma, both use the same or similar starting doses and a similar dose escalation scheme. Rather than having three different trials establish the safety of the new initial dose, it would be prudent to use data from the two existing protocols to ascertain an optimum starting dose and dose escalation plan.

- Although the protocol lists corticosteroids and T-cell antibodies as treatments that are available in the event of an unexpected toxicity resulting from the T cells targeting normal tissues that express HER-2, the protocol should include a formal plan for the use of these interventions that describes the clinical indication for each intervention and an algorithm for its use.

- It has been postulated that the participant who died in a trial using T lymphocytes with a CAR directed at HER-2 may have been uniquely susceptible to some component of the cells and the history of multiple drug allergies may have been indicative of increased susceptibility. As such, it would be prudent to exclude patients who may have increased susceptibility to allergic reactions, e.g., patients with autoimmune disease.

- The current protocol employs a continual reassessment statistical model for dose escalation. Adjustments in dose are to be made based on toxicity. To further optimize the dose escalation schema, biological activity parameters also should be factored into the analysis.

Ethical/Social/Legal Issues

- The IRB will need to assess carefully the risk-benefit ratio, particularly if the protocol goes forward with the proposed dose, in order to approve the enrollment of pediatric participants.

- The following changes should be made to the informed consent document:

    - Revise the informed consent document so it is clear that this is a Phase I safety study with no prospect of benefit. For example, since not all of the T cells infused are cytotoxic T cells, the consent should refer to T-cell infusion. The statement "it is possible that your immune system may begin to kill the cancer cells" suggests therapeutic benefit and should be revised.

- Revise the confusing statements about alternatives to participation. In one place, the consent states that there are "no standard treatments for your disease" but later discusses alternatives. If the point is that there are no alternative treatments with established efficacy, it is important to make this distinction clear.

G. Committee Motion 3

Dr. Strome summarized the RAC recommendations to be included in the letter to the investigators and the sponsor expressing the comments and concerns of the RAC. It was moved by Dr. Yankaskas and seconded that the RAC approve these summarized recommendations. The vote was 18 in favor, 0 opposed, 0 abstentions, and 1 recusal.

[At this point, Dr. Fan took over as Acting RAC Chair.]

V. Discussion of Human Gene Transfer Protocol #1001-1023 entitled: Gene Transfer for Patients with Sickle Cell Disease Using a Gamma Globin Lentivirus Vector: An Open Label Phase I/II Pilot Study

Principal Investigator:	Punam Malik, M.D., Cincinnati Children's Hospital Medical Center (CCHMC)
Additional Presenters:	Stella M. Davies, MBBS, Ph.D., MRCP, CCHMC; Karen Kalinyak, M.D., CCHMC; Lilith Reeves, M.S., Indiana University School of Medicine/CCHMC
RAC Reviewers:	Drs. Fan, Kodish, and Ross
Ad hoc Reviewer:	George Dover, M.D., Johns Hopkins Children's Center

Dr. Bartlett and Dr. Williams recused themselves from consideration of this protocol due to conflicts of interest.

A. Protocol Summary

Sickle cell disease (SCD) is a hereditary blood disease caused by an A to T transversion in the 6th codon of the β-globin gene. The resulting sickle hemoglobin (Hb S) tetramer polymerizes upon deoxygenation to form long fibers, changing the shape of the normally discoid red blood cells (RBCs) to abnormal sickle shapes. Repeated cycles of sickling damage RBC membrane resulting in dense, poorly deformable RBCs that have difficulty traversing the micro-vasculature and are hemolyzed or removed by the reticuloendothelial system. These chronic periodic occlusions of blood vessels cause cumulative damage to organs and tissues, have an enormous impact on a patient's quality of life, and shorten the lifespan of individuals with SCD, despite the significant cost to the healthcare system. More than 80,000 Americans have SCD and 2,000 infants with SCD are born in the United States each year; greater than $1.1 billion is spent annually in the United States for healthcare related to SCD.

The treatment of SCD is primarily focused on symptomatic management of acute events and prevention of infections. Patients with severe disease, are placed on chronic transfusions to reduce the sickle hemoglobin (Hb S). Bone marrow transplant is curative but is limited by the lack of available donors and high toxicity. Hydroxyurea is the only drug approved for use in SCD that reactivates fetal hemoglobin (Hb F) which results in reducing hemolysis, decreasing the frequency of vaso-occlusion and acute chest syndrome, and prolonging survival. However, hydroxyurea is not tolerated by a proportion of patients as it is either ineffective or associated with significant side effects related to marrow suppression. It may have an unknown long-term oncogenic potential and has not been widely embraced by patients. However, hydroxyurea and hematopoietic stem cell transplant remains the only targeted therapeutic strategies that are in active clinical use or trials.

The lentivirus vector, sGbG, incorporates the γ-globin exons and β-globin non-coding regions and regulatory elements, and showed complete correction of the murine sickle cell phenotype following gene transfer into HSC and myeloablative transplants. Additionally, the investigators determined critical

parameters necessary to correct disease pathophysiology using a reduced intensity transplant. Complete correction of the hematological and functional RBC parameters, inflammation and organ pathology was seen in SCD mice with following myeloablative conditioning and transplant. Correction was sustained long-term in primary and secondary transplant recipients. There was 100% 6-month survival of genetically-corrected mice, compared to 20% survival of mock-transplanted mice. Subsequently, using reduced-intensity conditioning, and simulating conditions of autologous transplant, different proportions of gene modified sickle HSC were transplanted into sub-lethally irradiated sickle mice to define the minimal proportions of genetically-corrected HSC, Hb F, Hb F-containing RBC (F-cells) and Hb F/F-cell required for correction of SCD

The Phase I/II pilot gene transfer study proposes the transfer of sGbG lentivirus vector into human bone marrow CD34+ cells of subjects with SCD utilizing a reduced intensity melphalan conditioning and autologous transplant. Study aims include the safety, feasibility and efficacy of a) obtaining sufficient CD34+ cells via a bone marrow harvest in patients with SCD, b) ex-vivo gene transfer of the sGbG lentivirus vector into the sickle CD34+ cells, c) a reduced intensity chemotherapy conditioning and d) transplant of autologous gene modified CD34+ cells into SCD subjects. The eventual goal of gene transfer is to offer a patient the potential of a one time cure of SCD without the immunological side effects associated with allogeneic transplant, such as graft rejection and graft versus host disease.

B. Written Reviews by RAC Members

Eight RAC members voted for in-depth review and public discussion of the protocol. Key issues included the novel use of a lentiviral vector for SCD and the inclusion of pediatric research participants. In two prior X-SCID gene transfer studies using gamma-retroviral vectors, five research participants developed T-cell proliferative disorder due to insertional mutagenesis at or near oncogenes; one of those research participants subsequently died. While there is some evidence to suggest a self-inactivating (SIN) lentiviral vector may pose less risk of insertional mutagenesis compared to the gamma-retroviral vectors used in the X-SCID trials, there has been a recent report from a French β-thalassemia trial of a clonal population of cells due to insertional mutagenesis by a mechanism other than enhancer-mediated gene activation.

Three RAC members and one *ad hoc* reviewer provided written reviews of this proposed Phase I/II pilot study.

Dr. Fan stated that this protocol is well designed and based on substantial preliminary data, and he raised concerns in two areas. Because SCD leads to progressive dysfunction of hematopoiesis, he asked the investigators to discuss the feasibility of obtaining sufficient CD34+ cells for the protocol, a concern that is compounded by the (justifiable) approach of enrolling participants 18 years old and older. While the sGbG vector has characteristics that would appear to reduce the potential for insertional oncogenesis, Dr. Fan noted that recent experience with a SIN lentiviral vector in a β-thalassemia trial raises the possibility that this class of vectors could cause malignancies by a different insertional mechanism. He asked about the concentration of CD34+ cells in the SCD bone marrow compared to the bone marrow from normal individuals in the previous gene-marking studies with lentiviral vectors expressing GFP on SCD CD34+ cells. Regarding the stopping rules for the clinical protocol, Dr. Fan requested that the investigators provide additional details on the criteria or procedures that would be employed if participants were to show a monoclonal outgrowth of transduced cells.

Noting that the protocol is well written and clear, Dr. Kodish asked the investigators to provide additional information on the comparative merits of hydroxyurea, particularly regarding the risk:benefit ratio for children, and whether children would be eligible for this study based on hydroxyurea being ineffective, on noncompliance, or on parent choice. Regarding management of potential conflict of interest, more detail is needed beyond simple signoff on eligibility by a nonconflicted investigator, in part because the principal investigator (PI) has applied for a patent for the lentiviral vector. With regard to the consenting process, Dr. Kodish commended the investigators for preparing three well-written and accessible documents to reflect the important distinctions among informed consent, parental permission, and participant assent. He suggested several wording changes in the informed consent documents, including adding

reassurance that using the modified HIV vector will not cause HIV disease or AIDS, and suggested changing “gene therapy” wording to “gene transfer” to avoid the therapeutic misconception. On the assent form, Dr. Kodish noted that younger individuals might be confused about the use of the phrase “to follow you,” taking it literally, and he suggested rephrasing.

Because of the recent report of clonality in a β-thalassemia trial in humans using a similar vector, Dr. Ross asked whether the investigators have looked at the clonality of the engrafted cells in the SCD mice at the 6-month timepoint, which is the first timepoint for which integration site analyses will be conducted in humans. This vector is new; therefore she queried the investigators as to whether they have looked at expression of the transduced vector in any cell lineages besides erythroid in the engrafted SCD mice, and whether they can transduce human hematopoietic stem cells and, *in vitro*, differentiate them to show that the vector is erythroid-lineage specific. Dr. Ross asked whether the investigators plan to use the same criteria for younger research participants as are being proposed for the first three participants (who will be at least 18 years old), particularly those under the age of 5 who may not yet have experienced the symptoms of severe SCD. Given the recent results in the β-thalassemia trial in France, she wondered whether integration site clonal predominance in any of the original three participants would preclude further enrollment and whether other criteria, such as evidence of hematologic cell proliferation, would be used. Dr. Ross suggested that the investigators wait 6 months (or longer) to carry out integration site analysis prior to proceeding with additional research participants.

Ad hoc reviewer Dr. Dover noted that a concern of similar protocols is the failure of investigators to recruit sufficient participants, which leads to a lack of advancement in the field as well as unnecessary risks to the few participants entered into the incomplete studies. Given the number of exclusion and inclusion criteria, he asked what assurance the PI has that it will be possible to recruit the required number of participants. Dr. Dover stated that the rationale for extending this study to children as young as 3 years old is not justified by the PI, and suggested that, in a Phase I study, it might be more prudent to confine the study to participants at least 18 years old. Although the evidence from the animal studies is impressive and well documented, he noted that the statements that this intervention reverses the sickle phenotype and reverses the sickling process are not substantiated by the data, primarily because the transgenic models do not completely reproduce the human SCD phenotype. For this reason alone, Dr. Dover stated that initial participation in this trial should be limited to individuals with proven organ damage and severe disease. While the protocol clearly outlines its objectives, all the objectives except the last secondary one will likely be assessed in the first 2 years of the study while the participants are on chronic transfusion therapy. Therefore, Dr. Dover suggested that all the primary objectives could be tested on research participants who are presently on chronic transfusions, and eligibility and exclusion criteria could be based on a well-defined group of patients on chronic transfusions. He discussed several minor concerns, including that the inclusion/exclusion criteria should be more clearly defined, the rationale for excluding patients with sleep apnea should be explained, and individuals with poor venous access or prior complications to central lines should be excluded. Additionally, Dr. Dover suggested that the transfected gamma globin gene be marked by a different coding sequence in order to distinguish recruitment of Hemoglobin F cells from normal stem cells from those of the transfected stem cells.

C. RAC Discussion

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

- Dr. Fan recommended conducting additional analyses on the SCD mouse model, including sequencing the integration sites, because one of the important considerations for this trial is the overall propensity of the proposed vector to activate oncogenes. He acknowledged that an oncogene that is activated in humans might be different from an oncogene that is activated in mice.

- Dr. Dover recommended that adults with alpha-thalassemia should be excluded from participation in this study.

- Dr. Dover stated that this study should be conducted in adults and the investigators should wait an appropriate period of time after the adult trial for the possibility of clonal expansion. Only after that time should the investigators decide whether it would be safe to expand this study to include children.

- Dr. Zaia asked whether peripheral blood progenitor cells could be used as the source of cells in this population, rather than from bone marrow.

- Dr. Strome asked the investigators to clarify the term "clonal dominance" because such a result could be considered desirable; however, such a result could also lead to malignant transformation and myelodysplasia. He noted that the desirable situation results in a clonal dominance among red blood cell producing cells and the undesirable situation could result in a premalignant condition or leukemia.

- Dr. Strome asked how clonality would be assessed.

- Dr. Strome asked about the difference between repopulating bone marrow in adults compared with children. He asked whether it would be possible that children might benefit from this approach when adults do not and, if this approach is deemed safe in adults but the levels of expression are low, whether it is possible to conclude that this approach will not work in children.

D. Investigator Response

1. Written Responses to RAC Reviews

The investigators believe they will be able to collect adequate numbers of CD34+ cells from SCD patients for this study. Based on their experience of collecting CD34+ cells from children and adults with SCD, they expect to get a much higher yield of CD34+ cells from younger patients. They have also observed a high proportion of erythroid lineage-committed progenitors in the CD34+ population in patients with SCD.

The investigators agreed to add two additional stopping rules: (1) If monoclonal dominance is detected in one participant, enrollment into the trial will be placed on hold. The RAC, the FDA, the data and safety monitoring board (DSMB), and the IRB will be informed and the investigators will seek their guidance to continue. (2) If two participants develop monoclonal dominance, enrollment into the trial will be suspended and participants will be followed until the DSMB, the FDA, and the RAC can determine the clinical importance of the event.

Hydroxyurea is currently the preferred approved therapy for SCD. Hydroxyurea is effective at increasing fetal hemoglobin in patients and reduces mortality, but small numbers of patients do not tolerate the drug due to side effects. Some adults cannot respond to or tolerate the drug due to a limited bone marrow reserve, while others refuse the drug due to potential longterm effects and the need for contraception. The adverse short-term side effects of taking hydroxyurea include mild gastrointestinal discomfort and lowering of blood counts requiring periodic dose de-escalation or stoppage with monthly monitoring to achieve a stable maximum tolerated dose. The longterm risks for hydroxyurea therapy are largely unknown, although current experience is that use of hydroxyurea for longer than 15 years in adults and longer than 12 years in children is relatively safe. The potential of hydroxyurea as a teratogen in animals provides the rationale for contraception, but no teratogenicity has been observed to date in humans. The investigators do not plan to enroll those individuals who are currently tolerating hydroxyurea and have stable disease. Gene transfer would be offered only to those who have been treated with hydroxyurea and have not tolerated it, have not responded to hydroxyurea, or have chosen not to take hydroxyurea.

In this protocol, adults are being studied first to test the safety and feasibility of performing a harvest, obtaining sufficient cells, administering chemotherapy conditioning, and engrafting gene-modified cells. The investigators will wait for a 6-month post gene transfer assessment of the first three adults enrolled prior to offering enrollment to a younger age group. If clonal dominance is observed in any adult, it will be reviewed by the DSMB and reported to the RAC and the FDA, during which time enrollment will be

suspended. The investigators recognize that clonal dominance and insertional mutagenesis are risks; however, the magnitude of risk is unknown and may not be apparent for years post gene transfer. The clonal dominance seen in one participant in the French thalassemia trial was observed has been beneficial for that patient so far, and has resulted in transfusion independence with no perturbation in hematopoiesis and stable bone marrow chimerism of the dominant clone. It is unknown whether that clonal dominance will remain beneficial in the long term or will result in a serious adverse event. If the safety profile of the first three participants does not provide sufficient evidence for safety and feasibility, the investigators would expect to continue to enroll only adult participants until more compelling evidence allows a reduction in the age for inclusion.

Eligible SCD patients in the Cincinnati area include 58 who are 3 years to 18 years of age and 41 who are 18 years to 30 years of age; all of these SCD patients are currently followed at CCH and the University Hospital, University of Cincinnati. The investigators have begun discussions with the sickle cell community; initial results from those discussions and a survey indicate that the majority of the community has an interest in such a trial.

The rationale for excluding subjects with sleep apnea is that they have a high incidence of developing acute chest syndrome with general anesthesia.

Regarding chronic transfusion therapy, it is anticipated that during the period of neutropenia following melphalan, transfusions of platelets and RBC will be needed. For subjects not previously on transfusions, a gradual wean will be planned beginning at 3 months until 6 months post transplant to allow a selective advantage to Hb F producing RBC and to assess the secondary objectives. For subjects previously on transfusions, the wean will continue until 2 years post gene transfer.

The investigators agreed to make the requested changes to and clarifications within the informed consent document.

2. Responses to RAC Discussion Questions

Dr. Malik explained that the investigators have proposed to wait 6 months for the first three to six adult participants in order to evaluate the safety and feasibility of the procedure and to provide an indication of potential benefit, using the gene-marked cells; 6 months is expected to provide sufficient time to evaluate benefit. If significant numbers of gene-marked cells have engrafted and no indication of clonal dominance is observed in the first three adult participants for 6 months post dosing, the investigators plan to approach the regulatory advisors regarding expanding the participant population to minors. In addition, the protocol will not be opened to children unless a potential benefit is seen in the adult research participants. If no fetal hemoglobin is observed or poor gene transfer occurs, this protocol will not be offered to children. If the adults in this trial experience benefit, the investigators would expect even more benefit to accrue to child participants.

Dr. Kalinyak explained how the investigators ensure compliance in hydroxyurea patients, and therefore how the inclusion criterion of having failed – versus being noncompliant with – hydroxyurea would be determined.

Regarding the possibility of using peripheral blood to study erythroblasts, Dr. Malik stated that, while the literature indicates that peripheral blood progenitors can be present in sickle patients, it is unknown whether it represents the gene transfer; therefore, the investigators have decided to compare peripheral blood and bone marrow side by side. Colonies will be made from both types of cells, and data will be compared to see if they are concordant.

Dr. Malik explained that the investigators are erring on the side of safety – if they see a particular clone dominating among all the integrations in one research participant, the trial will be stopped at least temporarily until the investigators uncover what is occurring in that participant. They plan to conduct a high-throughput integration site analysis that will produce a map of integration sites to determine whether all individual colonies are from the same integration.

Regarding the differences between adults and children, Dr. Malik noted that the limitation in adults is that they have many fewer stem cells. Preliminary data on the sickle bone marrow aspirates clearly indicate a decline in the number of CD34+ cells in older sickle patients.

E. Public Comment

No public comments were offered.

F. Synopsis of RAC Discussion and RAC Observations and Recommendations

The following observations and recommendations were made during the RAC's indepth review and public discussion:

Preclinical Issues

- The preclinical data suggests that the vector may have a tendency to integrate into the genome at particular sites. Dominant bands appeared in the linear amplification-mediated polymerase chain reaction (LAM-PCR) analysis of the bone marrow from Berkeley sickle cell mice. As an additional safety measure, those insertion sites should be sequenced to determine whether the vector has a propensity to activate proto-oncogenes.

Clinical/Trial Design Issues

- Based on preclinical data, which showed that the vector might cause hematological changes that are often associated with alpha-thalassemia, patients with alpha-thalassemia should be excluded from the trial.

- Sickle cell studies that involve bone marrow transplantation often experience challenges in recruiting a sufficient number of participants. While outreach efforts have been made to assess the patient community's level of interest in participating in the study, it appears that the bone-marrow harvesting component of the study may not have been included in the study description. Before initiating the research, further efforts should be made to assess whether there is sufficient interest in a study involving bone marrow harvesting.

Ethical/Social/Legal Issues

- For ethical reasons, additional data on the safety and efficacy of the intervention should be gathered before considering the enrollment of pediatric patients. The additional data also will be needed to help establish that the prospective benefits of the intervention outweigh the risks. The assessment of the DSMB should be based on data from at least six adult participants.

- Hydroxyurea is an established therapy for sickle cell disease. For ethical reasons, only pediatric patients who cannot tolerate the treatment should be eligible for the trial. Non-adherence in pediatric patients should not be a reason for inclusion in the study if/when the research moves to the stage of enrolling children.

G. Committee Motion 4

Dr. Fan summarized the RAC recommendations that will be included in the letter to the investigators and the sponsor expressing the comments and concerns of the RAC. It was moved by Dr. Kodish and seconded that the RAC approve these summarized recommendations. The vote was 16 in favor, 0 opposed, 0 abstentions, and 2 recusals.

VI. Gene Transfer Safety Assessment Board Report

RAC Reviewers: Drs. Federoff, Strome, Williams, Yankaskas, and Zaia

Dr. Yankaskas reported that, of the 18 protocol submissions received by the OBA in the past 3 months, 12 protocols were not selected for public review at this RAC meeting and 1 selected for review was deferred to a subsequent RAC meeting. Of the 13 protocols not selected for public review at this March RAC meeting, 8 are oncology protocols, 3 are for infectious diseases, 1 is for peripheral vascular disease, and 1 is for GvHD. The vectors used in these 13 protocols are six plasmids, two adenoviruses, two retroviruses, one saccharomyces, one lentivirus, and one measles virus.

Eight protocols indicated enrollment had begun, and one protocol was reviewed at a public RAC meeting – Protocol #0704-849, a Phase I Study Evaluating the Use of Allo-Depleted T Cells Transduced With an Inducible Caspase-9 Suicide Gene After Haploidentical Stem Cell Transplantation. For participants who develop GvHD, the RAC recommended identifying whether the reactive cells were transduced by the transgene and could be eliminated by the dimerizer drug, AP1903, or whether GvHD was caused by nontransduced cells. Blood samples will be obtained from participants who develop GvHD to monitor for the effects of AP1903 on persistence of the transgene. Two participants have developed mild GvHD to date and responded within 24 hours to the administered drug. The potential immunogenicity of the construct was a concern due to the slightly mutated tacrolimus binding domain. In participants dosed to date, cells have perished beyond 6 weeks without an apparent immune response but transduced T cells disappear rapidly from the circulation. Evidence of an immune response will be sought but may be technically challenging due to the fact that participants remain lymphopenic for 1 year after transplant.

Dr. Yankaskas noted that 18 serious adverse events were reviewed from 12 protocols, including both initial and followup results. In followup to the December 2009 RAC meeting discussion of a death on a trial regarding chimeric T-cell receptor against HER2/neu, a safety symposium entitled “Gene-Modified T Cells: Challenges for Clinical Trial Design” is being planned for June 15, 2010, in conjunction with the RAC’s June 2010 meeting.

Four publications of note were discussed briefly by Drs. Williams and Yankaskas:

- A publication concerning the development of myelodysplastic syndrome in two research participants in the Frankfurt, Germany, trial for chronic granulomatous disease; this serious adverse event had been reported previously in the literature.

- Retrovirus gene therapy for X-linked granulomatous disease can achieve stable longterm correction of oxidase activity in peripheral blood neutrophils, a report on three participants with treatment refractory infections who enrolled in a trial using a murine onco-retrovirus vector expressing $gp91^{phox}$ after preconditioning with busulfan.

- Virus-specific T cells engineered to co-express tumor-specific receptor persistence in antitumor activity in individuals with neuroblastoma – infusion of these cells was associated with regression or necrosis in four of the eight participants with evaluable tumors, and one participant achieved complete remission that was sustained for 12 months without additional therapy.

- Overall survival analysis of a Phase II randomized controlled trial of pox-virus-based, PSA-targeted immunotherapy in metastatic castration-resistant prostate cancer – the treated group showed a 44 percent reduction in the death rate and an 8.5-month improvement in median survival when compared to the control group.

VII. Updates from the Office of Biotechnology Activities/Dr. Corrigan-Curay

A. Presentation

Dr. Corrigan-Curay presented the conclusions for the Major Action on Chlamydia that was discussed at the December 2009 RAC meeting. On the basis of the data about fluoroquinolones, the OBA has decided to amend the RAC recommendation regarding allergies to azithromycin. The OBA talked to the investigators and an agreement was reached that excluding individuals with a documented allergy to azithromycin would be prudent because only one antibiotic would be available to treat a potential chlamydia infection.

Dr. Corrigan-Curay noted that the OBA is cohosting a conference on June 30 and July 1 at the Hyatt Regency Hotel in Bethesda, Maryland, on the use of sham neurosurgical procedures. The RAC has reviewed a number of protocols that have used sham surgical procedures, in particular for Parkinson's disease and Alzheimer's disease.

Regarding the changes in the *NIH Guidelines* to cover synthetic biology, Dr. Corrigan-Curay reported that some substantive changes have been made to Section III; the draft of those changes will be circulated to those who were involved in helping craft that language. The recommendations are being reconsidered in light of public comments that have been received. She suggested the possibility of revisiting some of those comments at the June 2010 RAC meeting and engaging in further discussion.

B. RAC Discussion

Dr. Kirchhoff noted that some of the revised language from the Major Action regarding allergy and sensitivity to azithromycin differed from the wording in the December 2009 RAC minutes document. Dr. Corrigan-Curay stated that a footnote would be inserted into the minutes to explain the subsequent change to the language.

VIII. Discussion of Biocontainment for University of Wisconsin's Proposed Research Involving the Cloning of Ebola and Marburg cDNAs into *E. coli*

Presenters: Joseph Kanabrocki, Ph.D., RAC Member, and Gabriele Neumann, Ph.D., UW-M
Other Discussants: Rebecca Moritz, M.S., UW-M, and James Turk, M.S., UW-M
Discussants via teleconference: Naomi Rosenberg, Ph.D., Tufts University, and Jan Parker-Thornburg, Ph.D., MD Anderson Cancer Center

A. Presentation by Dr. Kanabrocki

Dr. Kanabrocki reviewed the recommended BL containment under the *NIH Guidelines*; provided a summary of previous biosafety and biosecurity recommendations from the RAC for similar work with Ebola, Marburg, Nipah, and Hendra Risk Group 4 (RG4) Mononegavirales at Rocky Mountain Laboratories (RML); and enumerated the differences between the prior RML proposal and the current proposal by the UW-M. The current proposal is being offered by the laboratory of Yoshiro Kawaoka, D.V.M., Ph.D., at the UW-M. The key differences between the current proposal and prior proposals are that the cDNA will be transferred from the UW-M to the RML for generating infectious virus under BL4 containment and that liquid waste will be decontaminated using a phenolic disinfectant and discarded in the public waste stream.

B. Presentation by Dr. Neumann

Dr. Neumann presented background on filoviruses and the reverse genetics of the Ebola virus. Ebola virus and the closely related Marburg virus cause hemorrhagic fevers with mortality rates as high as 90 percent. Currently, neither vaccines nor antiviral treatments are available and, for these reasons, filoviruses are classified as biosafety level 4 agents. She described the rationale for the proposal that will be conducted at the Influenza Research Institute (IRI), who will have access to the IRI and the laboratory, the workflow and the proposed flow of material in to and out of the laboratory, communications plans,

proposals to deal with visitors and maintenance staff, training requirements, and incident reporting and response.

No Ebola virus or Marburg virus will be generated in this work; the UW-M proposes to work only with the full-length cDNA clones. Transfers of full-length cDNA constructs in liquid suspension will conform to the IATA/DOT requirements specified for shipping Dangerous Goods classified in Division 6.2 except that the nucleic acid material cannot be labeled as such by regulation since it is not infectious. Additional procedures (other than package labeling) will conform to those required for infectious agents, in particular that sender and recipient be aware of any shipment in transit and that in the event of non-delivery within a pre-determined interval (*e.g.* 24-48 hours), a written follow-up plan will be initiated for shipment tracking and recovery. The plan includes notification of institutional local law enforcement officials as well as the designated responsible officials at both RML and UWI.

Dr. Neumann compared the safety and security of Dr. Hans Feldmann's already-approved program at the RML to the program being proposed for the UW-M. Both programs will work or are proposing to work with full-length cDNA clones; there will be no work with protein expression plasmids, no cell culture work, and no virus rescue; and both programs have strict rules and requirements for recordkeeping.

C. RAC Discussion

RAC members questioned where the fragments of Ebola come from, whether the 70 percent ethanol solution proposed as a disinfectant would kill what needs to be killed, and why this laboratory wants to change its procedures. A lengthy discussion about the details of shipping these materials ensued.

D. Public Comment

No public comments were requested.

E. RAC Recommendations

Dr. Kanabrocki read the proposed recommendations of the RAC, which would allow the UW-M to operate similarly to the RML, with amendments that included decontamination with bleach and shipping comparable to Select Agents but without notification to the USDA Animal and Plant Health Inspection Service or to the Centers for Disease Control and Prevention. These recommendations would apply only to this laboratory at the UW-M.

F. Committee Motion 5

It was moved by Dr. Buchmeier and seconded by Dr. Ertl that the RAC approve these summarized recommendations. The vote was 16 in favor, 0 opposed, 0 abstentions, and 0 recusals.

IX. Discussion of Proposal To Exempt the Mating of Certain Biosafety Level 1 Transgenic Rodents from the Requirements of Section III-E-3 of the *NIH Guidelines*

Facilitator: Dr. Flint
Presenter: James Pickel, Ph.D., NIH Animal Research Advisory Subcommittee on Genetically Modified Animals, NIMH, NIH

A. Presentation

Dr. Flint summarized the current *NIH Guidelines* requirements and the impetus and rationale for the proposed exemption. Transgenic rodents that may be contained under BL1 conditions do not pose an appreciable biosafety risk to humans. The *NIH Guidelines*, in Appendix C-VI, currently exempts the purchase or transfer of transgenic rodents that require BL1 containment. The overwhelming majority of matings of transgenic rodents that require BL1 containment will result in a rodent that can be housed at

BL1 and would therefore not pose an appreciable risk to human health. While each registration is not a significant burden, the total number of registrations required leads to an administrative burden on the institutional biosafety committee and on researchers that does not appear to be commensurate with the biosafety risk.

As a member of the NIH Animal Research Advisory Subcommittee on Genetically Modified Animals, Dr. Pickel discussed the history of using transgenic rodents and stated that transgenic rodents that may be housed under BL1 conditions do not pose an appreciable health risk. He noted a long history of safety with these model systems. Mice that are assigned to be housed under BL1 conditions are assigned to that level based on their registration with recombinant DNA evaluations and they are all covered by human pathogen registration controls. As an example of the administrative burden of registration, he noted that the building in which he works houses about 80,000 rodents, more than half of which are transgenic and all of whom are mating. The proposed regulations are proportional for the posed risk; not only would they relieve a burden on investigators and committees charged with oversight but they would educate the public about real dangers as opposed to model systems that are dealt with daily that are not hazardous.

Dr. Flint presented language for a proposed exemption regarding the mating of transgenic rodents:

> The mating of two different transgenic rodents or the mating of a transgenic rodent with a non-transgenic rodent with the intent of creating another transgenic rodent that requires BL1 containment will be exempt from the *NIH Guidelines* if:
>
> 1. Both parental rodents require BL1 containment
>
> <u>AND</u>
>
> 2. Each parental transgenic rodent does not contain any one of the following genetic modifications:
>
> a) A transgene that codes for amyloid or a prion; or
> b) More than 50 percent of the genome of an exogenous virus from a single family; or
> c) Expression of the transgene is under the control of a retroviral long terminal repeat;
>
> <u>AND</u>
>
> 3. It is anticipated that the transgenic rodent that results from this mating will not contain more than 50 percent of an exogenous viral genome from a single family or contain a transgene that codes for amyloid or a prion.

B. RAC Discussion

Questions raised by RAC members included whether risk is increased by mating and why mated rodents should be treated differently than a *de novo* creation of a transgenic rodent. Dr. Ross noted that there may not be the same risk of recombination between endogenous murine retroviruses and certain types of retroviral long terminal repeats such as those from mouse mammary tumor virus. Removing #2c of the proposed exemption was proposed and agreed to be discussed further.

C. Public Comment

No public comments were requested.

D. Committee Motion 6

It was moved by Dr. Flint that the RAC approve seeking public comment on the proposed exemption; if no substantive comments are received, the OBA will implement this exemption. The vote was 15 in favor, 0 opposed, 0 abstentions, and 0 recusals.

X. Day 1 Adjournment/Dr. Fan

Dr. Fan, Acting RAC Chair, adjourned Day 1 of the March 2010 RAC meeting at 5:15 p.m. on March 10, 2010.

XI. Day 2 Call to Order and Opening Remarks/Dr. Bartlett

Dr. Bartlett, Acting RAC Chair, called Day 2 of the March 2010 RAC meeting to order at 8:00 a.m. on March 11, 2010.

XII. Discussion of Human Gene Transfer Protocol #0912-1016 entitled: A Phase II Study to Determine the Efficacy and Safety of Allogeneic Human Chondrocytes Expressing TGF- β1 in Patients with Grade 3 Degenerative Joint Disease of the Knee

Principal Investigator:	Michael A. Mont, M.D., Sinai Hospital of Baltimore *(not present)*
Presenter:	Kwan Hee Lee, M.D., Sinai Hospital of Baltimore
Sponsor:	TissueGene, Inc.
RAC Reviewers:	Dr. Bartlett, Dr. Ertl, and Ms. Mastroianni
Ad hoc Reviewer*:*	Robert Carter, M.D., National Institute of Arthritis and Musculoskeletal and Skin Diseases, NIH

Dr. Strome recused himself from consideration of this protocol due to a conflict of interest.

A. Protocol Summary

Degenerative arthritis is the rnost frequently encountered orthopedic disease associated with cartilage damage and impacts one in seven people. The pathogenesis of degenerative arthritis is the degeneration of the hyaline articular cartilage that becomes deformed, fibrillated, and eventually excavated during the course of the disease. Most methods for treating degenerative arthritis focus on the reduction of the symptomatic pain. If the degenerated cartilage could be regenerated, most patients would be able to function without debilitating pain. Treatment using autologous human chondrocytes to replace damaged cartilage entails two operations with an excision of the soft tissues and requires a lengthy recovery time. Further, these autologous chondrocytes have a limited capacity to regenerate hyaline cartilage.

One of the most important factors in the production of type II collagen by cartilage cells is the presence of transforming growth factor-β1 (TGF-β1). TGF-β1 is a multifunctional cytokine, playing a regulatory role in cell growth, differentiation, and extracellular matrix protein synthesis and has been reported to induce osteogenesis and chondrogenesis. Studies have also suggested that TGF-β1 stimulates chondrocyte proteoglycan synthesis and the growth of articular chondrocyte cells. In addition to its stimulatory action on chondrocytes, TGF-β1 has been shown to possess anti-inflammatory and immune suppressive properties. However, widespread clinical applications of this protein have been limited due to its short-term effects as a result of a short half-life.

TissueGene-C represents a cell-mediated cytokine gene therapy approach for cartilage regeneration. TissueGene-C is a 3: I mixture of non-transduced allogeneic human chondrocytes (hChonJ) and irradiated allogeneic human chondrocytes transfected with a retroviral vector encoding TGF-β1 (hChonJb#7). Cellular production of TGF-β1 can provide longer-term effects of TGF-β1 in stimulating hyaline cartilage generation from only a single product injection without requiring an operation or a lengthy rehabilitation time. The non-transduced chondrocytes are included as cells for filling the defect site and as target cells for TGF-β1 expressed from transduced cells. When injected into the damaged knee joints of rabbits, dogs, and goats transduced human chondrocytes have exhibited sustained TGF-β1 release and proliferation of regenerative cartilage with no overgrowth or adverse histological findings. TissueGene-C has been administered to a total of 21 patients from two Phase I clinical trials conducted in the U.S. (OBA Protocol #0307-594; 9 subjects) and Korea (12 subjects). Subjects were intramuscularly

injected with doses of either 3 x 10^6, 1 X 10^7, and 3 x 10^7 cells per patient. No serious adverse events were observed that were related to TG-C administration. TissueGene herein is proposing a Phase II study to investigate the safety and efficacy of TissueGene-C in patients with Grade III chronic, degenerative joint disease (DJD) of the knee joint refractory to existing therapies. This will be a 2-year multicenter, randomized, comparator-controlled, elective crossover study. In these patients data will be collected on symptoms, joint pain, knee functionality, incidence and dose of analgesic and anti-inflammatory medication, as well as patient initiated delays in knee arthroplasty post-dose administration. Further, this study will evaluate the regeneration of hyaline cartilage as determined by 3T MRI.

B. Written Reviews by RAC Members

Six RAC members voted for in-depth review and public discussion of the protocol. Key issues included concerns about the safety of administering potentially immunogenic allogeneic cells transduced by a retroviral vector that expresses a growth factor. While initial dose-finding studies found administration to be relatively well tolerated, it was believed that these data should be reviewed carefully to evaluate the risks and benefits of proceeding in a larger population with potentially less severe (Grade III or Grade IV) osteoarthritis (OA) that is disabling but not life-threatening.

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

Dr. Bartlett noted that preclinical studies are described using surgical cartilage defect models in several species but, because the etiology of OA is so poorly understood, an animal model that more accurately mimics the human disease is desirable. He asked the investigators to comment on their failure to carry out either safety or efficacy studies in an animal model that might mimic human disease more accurately, with specific emphasis on the influences of mechanical load, posture, and body weight on cartilage regeneration. He asked whether it would be possible to discriminate TG-C encoded TGF-β1 from endogenous TGF-β1, whether the ELISA assay that would be used to detect TGF-β1 in human blood has been validated, what were the systemic levels of TGF-β1 pre-dosing, and whether an increase in systemic TGF-β1 would be seen even if the TG-C product were delivered systemically. Dr. Bartlett asked the investigators to explain the logistics of TG-C administration, specifically with regard to differences that might arise between sites and how these might impact performance, and suggested that assessment of viability. He noted that the design and interpretation of this trial would be strengthened significantly if the effect of the experimental agent were shown on the same endpoint in a valid animal model. Noting that the use of nonsteroidal anti-inflammatory drugs (NSAIDs) would be restricted during the initial 72 hours following TG-C administration, he asked the investigators to discuss how NSAIDs might influence outcome determinations. In addition, he suggested that the investigators add protocols for blood and/or tissue sample collection in the event of an SAE that results in the participant being under the care of a physician not involved in the trial.

Noting that dosing is poorly rationalized, Dr. Bartlett asked the investigators to clarify the following issues: (1) whether dose is predicated solely on the Phase I safety data and whether data exist to support efficacy at this dose; (2) whether the dose ratio study in an injury model was conducted only once; (3) whether irradiated cells were used in this preclinical determination; (4) whether similar timepoints were analyzed to assess efficacy; and (5) whether any preclinical studies assessed replication of transplanted cells.

Additional discussion requested by Dr. Bartlett included: (1) whether any evidence suggests that the non-transduced cells contribute to the regenerated cartilage; (2) the cross-species activity of TGF-β1; (3) the rationale for not developing an assay to detect cellular immune responses, and for not banking cells that might be used to exclude such an immune response in the event primary or secondary measures of efficacy are not realized; and (4) histological analyses that would be performed on resected tissue when total knee arthroplasty is performed and the benefit of these analyses. He suggested that therapeutic misconception be addressed more explicitly in the consent documentation especially since the PIs would be responsible for participant recruitment and consent.

Noting that cell-mediated immune responses were not measured in the Phase I trials nor does the current trial plan to assess them, Dr. Ertl stated that testing for such responses using quantitative assays is essential. Regarding the cell lines proposed, she asked about the karyotype of both, she wondered why

only the TGF-producing cell line would be irradiated, and she asked the investigators to discuss the stability of these cell lines. Dr. Ertl requested that the investigators provide a brief summary of the animal results, indicating how many animals died and the cause of those deaths as well as which group(s) showed indication of joint repair. The drug used in the preclinical studies produced human TGF-β, but she asked whether studies were conducted with a TGF-β corresponding to the species of the transplant recipients. In addition, Dr. Ertl suggested two clarifications within the informed consent document.

Ms. Mastroianni stated that the informed consent document is missing some important information and also requires some clarifications:

- The investigators should describe the meaning of efficacy more accurately for participants.
- To prevent confusion by potential participants, the purpose of this protocol should be stated clearly and concisely in one place.
- The gene transfer aspect of the trial is minimized and not adequately explained in the Purpose section.
- Participants might be confused by the words “treatment,” “treated knee,” and “patient,” which imply direct benefit to the research participant.
- The informed consent document should have a full discussion of the planned 15-year longterm followup, including its purpose, when and where the participants would have study visits, how contacts for that length of time would be arranged, and whether compensation and expenses would be paid. It should be made clear that this requirement will not apply to participants injected with the control substance.
- Women and men should be advised more clearly in the consent document regarding the use of contraception, and the reason for birth control measures should be explained.
- The Risks section should be reviewed for appropriate terminology for lay understanding. This section should also include potential risks associated with the control.
- The option of elective crossover is not reflected in the consent document, and should include how research participants might make this judgment, what the followup procedures would be, and what information should be disclosed to them.
- Because individuals with mental handicaps might be considered for participation, the investigators should provide an assent document for review.

Ad hoc reviewer Dr. Carter noted the lack of either a DSMB or an independent data monitoring committee, and he asked that the statement in the informed consent document that “your blood levels of TGF-β1 could increase” be clarified for improved participant understanding. In addition, he requested that the investigators discuss the following questions:

- Several participants in the study in Korea reported pain, warmth, itching, or redness in the knee after the procedure, which likely represents an immune response to the study agent; was there any reason related to formulation or delivery that could result in this occurring more frequently in the Korean study than in the U.S. study? What studies will be conducted in the proposed trial to screen for an immune response?
- One participant who received active agent had newly detected cystic changes; what was the nature and time course of these cysts?
- What steps have been taken to standardize and validate the TGF-β1 assays?
- In the preclinical studies in animals, there appear to have been many deaths in the experimental groups; can the investigators be sure that these deaths do not indicate a risk from the intervention?
- What will be done to examine joints for new bone growth, and what is the sensitivity of this technique? What technique will be used to examine cartilage quality and what is the sensitivity of this technique?
- Might the proper control be only allogenic cells without the cells expressing TGF-β1?

C. RAC Discussion

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

- Dr. Bartlett expressed concern about the lack of efficacy seen in the preclinical studies. Especially as the investigators now move into Phase II, not to have those validated endpoints assessed first in a preclinical model results in a lower level of confidence that efficacy could result in the Phase II trial.

- Noting that the investigators' hypothesis is that increasing the level of TGF-β1 in the joint will have clinical benefit, Dr. Bartlett emphasized that the investigators need to know the starting level of TGF-β1 in the knee joint of each participant in order to determine efficacy in this trial.

- Dr. Bartlett asked how the investigators plan to separate out the research participants who take few or no NSAIDs, as requested in this study, from those participants who do not adhere so carefully to the request to refrain from NSAID use. The results of this trial could be different depending on participants' use of NSAIDs, with regard to outcome measures of pain and range of motion.

- Noting that the assays proposed by the investigators (enzyme-linked immunospot [ELISpot] and intracellular cytokine staining) would be easy to conduct, Dr. Ertl strongly encouraged the investigators to find someone who is familiar with conducting those assays so that, at the time of the trial, the assays are in place. She suggested that the investigators use banked cells left from the investigators' Phase I trial to determine if an allergic response occurs. She suggested that a local allergic reaction could do more harm than good, because the immune response could destroy the knee further. Dr. Carter reiterated the critical importance of understanding any evidence of a local allergic reaction.

- Dr. Takefman explained that the FDA requires 15-year longterm followup for gene transfer products and specifically for all products using retroviral vectors. The investigators for this trial propose to conduct their safety and efficacy analysis up to 2 years; after that time, the research participants will be enrolled into the longterm followup protocol, which involves specific testing and monitoring.

- Agreeing that the Phase I study was designed for safety purposes, Dr. Carter stated that Phase I data should be used to generate outcome variability so that analyses in subsequent studies can be conducted with sufficient power. Phase I data should be used to determine the "noise" in the proposed assay for a Phase II trial; this information is needed to inform the statistics proposed in the design of a Phase II efficacy study.

- Dr. Zaia noted that a key flaw in this study is that it will be conducted as a single blind, which will result in dependency on the subjective assessment of the participants. He explained that a study like this has a high likelihood of a placebo effect.

- Considering the lack of data, both preclinically and clinically, and considering that a major safety concern – the potential for an allogenic reaction – was not addressed in the Phase I trial, Dr. Ertl expressed her belief that this trial is not ready for Phase II. She suggested the need for an additional Phase I trial, possibly a bridging study, to assess immune responses. If in 10 participants there are no immune responses, the Phase II trial could proceed; if immune responses are observed, a Phase II trial should not be conducted.

- Dr. Barlett expressed his concern that the failure to take into account the recommendations set forth by RAC members could severely set back the gene transfer field. In addition, the investigators would not have tools with which to assess the efficacy or safety of this Phase II trial.

D. Investigator Response

1. Written Responses to RAC Reviews

The data available in the field indicate a low probability that TG-C will induce an immune response. However, to address the concerns of the reviewers and in order to better assess the potential for immunogenicity of TG-C during this Phase II trial, the investigators agreed to evaluate the participants for erythrocyte sedimentation rate, C-reactive protein, white blood cell count, and interferon-γ. Additionally, to better measure potential cell-mediated immune response, the investigators propose to test peripheral blood mononuclear cells from participant blood samples in an IFN-γ ELISpot assay. The severity of any potential immune reaction will be assessed through an evaluation of the levels of these markers, how long any increases persist, temporal correlation to injection, and whether there are any corresponding local or systemic symptoms.

The karyotype of the hChonJ (untransduced chondrocytes) is normal. A cytogenetic analysis of karyotyping results for the hChonJb#7 suggests that the cell lines are stable in spite of being aneuploid. Additionally, the growth rate and TGF-β expression levels of the hChonJb#7 cells are stable in vitro during long-term passaging under standard culture conditions.

The TGF-producing cell line will be irradiated because these cells were shown to be immortalized *in vitro*. As a safety measure, therefore, and in discussion with and recommendation of the FDA, these cells will be irradiated to render them replication incompetent and they are expected to die off within 2 weeks after irradiation.

Several Good Laboratory Practice (GLP) safety and toxicity studies were performed on the various experimental animals. TG-C was found to be well tolerated following intra-articular administration, with no apparent evidence of overgrowth or overt effects outside the site of administration, nor were there any apparent systemic effects in SCID mice at systemic doses up to $1x10^6$ cells, localized effects in rabbits at intraarticular doses as high as $9x10^6$ cells/cm^2, or in female goats at doses as high as $2x10^7$ cells/knee.

Animal models of OA include mouse and guinea pig spontaneous OA, meniscectomy and ligament transection in guinea pigs, meniscectomy in rabbits, and meniscectomy and cruciate transection in dogs. None of these models has a proven track record of predictability in human disease because no agents have been shown to provide anything other than symptomatic relief in human OA. Additionally, the onset of OA is not uniform in spontaneous OA animal models. In order to generate uniform, reproducible defect in cartilage, the investigators employed a surgical method to introduce a cartilage defect. They used two different animal models (rabbit and goat) for safety and efficacy preclinical studies. A large animal (goat) model was added to assess more adequately the effects of mechanical load and body weight on cartilage regeneration compared to humans.

The restriction on the use of NSAIDs during the initial 72 hours following TG-C administration is intended to allow evaluation of potential inflammatory reactions at the time of dosing without interference from anti-inflammatory medications. Additionally, this will allow assessment of any potential initial effects of TG-C, considering that TGF-β1 has anti-inflammatory properties, without the potentially confounding effects of concomitant NSAIDs.

The dose and ratio of cells to be used in the proposed Phase II study is based on a combination of preclinical and clinical data. These data indicate that the dose level selected for Phase II is at a level where both safety and efficacy were observed.

In preclinical studies, testing was conducted to determine whether normal (nontransduced) human chondrocytes alone, transduced TGF-β1-expressing (hChon-TGF-β1) chondrocytes alone, or varying ratios of mixed cells (nontransduced to transduced chondrocytes) were able to stimulate the repair of a hyaline cartilage defect; cells were implanted into an artificially created partial defect of a rabbit knee joint. This study found that mixed cells are capable of inducing hyaline-like articular cartilage more efficiently than are TGF-β1-expressing chondrocytes or untransduced chondrocytes alone *in vivo*. Further testing was performed in rabbits to confirm the optimal cell ratio of normal chondrocytes to TGF-β1-expressing chondrocytes for inducing cartilage formation; that optimal ratio was determined to be 3:1.

It is not possible to discriminate TG-C encoded TGF-β1 from endogenous TGF-β1. The systemic levels of TGF-β1 can vary widely from patient to patient. Based on the assay package insert, the normal range for TGF-β1 levels was set at between 18,289 to 63,416 pg/mL. The half-life of TGF-β1 in blood is short so any additional TGF-β1 produced by cells administered systemically would not persist. Further, if delivered systemically, it is expected that the small amount of TGF-β1 would be sufficiently diluted in the blood volume to not cause an increase in TGF-β1 levels.

The investigators agreed that the design and interpretation of the trial would have been strengthened if the effect of the agent were shown on the same end-point in a valid animal model. However, MRI assessments were not performed in preclinical studies. At the FDA Cell, Tissue and Gene Therapy Advisory Committee meeting held on May 15, 2009, the committee expressed their opinion that, for now, MRI is has not been validated as a primary endpoint.

When total knee arthroplasty is performed after this Phase II trial, the resected knee tissue will undergo histological analysis, which will detect potential positive response to TG-C therapy and will evaluate any potential adverse effects of TG-C dosing such as abnormal tissue formation.

Several participants in the Phase I study in Korea reported pain, warmth, itching, or redness in the knee after the procedure. The precise cause of the reactions seen in the Korean study is unknown. Similar reactions were not seen in the Phase I U.S. study in participants treated with either vehicle control or TG-C. The formulation and delivery method of TG-C in the Korean study was identical to that used in the U.S. study.

In the original adverse event report, cystic lesions were reported as newly detected in one participant who received active agent. However, upon further review of this adverse event, the investigator noted that these cystic lesions were present in pre-dose (screening) X-rays of the individual's knee. Therefore, this adverse event was redesignated as not related to TG-C administration.

The participants' knees will undergo 3.0 Tesla (3T) MRI evaluation. This 3T MRI is a higher resolution than the proton density MRI used in the Phase I study and will evaluate any potential abnormal bone formation as well as cartilage formation and/or repair.

The investigators' testing in animals has indicated that treatment with non-transduced cells alone is not efficacious. If there is the possibility of an immune reaction to the allogeneic cells, then treatment with allogeneic cells alone would be unethical as it would present an unnecessary risk to the research participants while offering no possible efficacy. The control included in this protocol was selected specifically as standard treatment that might offer some measure of efficacious treatment for individuals with significant disease, thereby optimizing the risk/benefit ratio.

TGF-β1 is highly conserved across species. There is sequence homology of 100% with bovine, canine, porcine, sheep and primate TGF-β1, and 99% homology with rat and mouse TGF-β1. The cross-species activity has been seen with a number of species including human, canine, porcine, rat and mouse TFG-β1. The distribution and modulation of the cellular receptor is similar across species and in a wide variety of cell types.

The investigators agreed to utilize an independent data monitoring committee for this study as we did for the Phase I study.

The longterm follow up will be performed under a separate protocol, which is designed to accept "rollover" individuals from any study with TG-C. As a separate study, it accordingly has its own informed consent document. The longterm followup study has been intentionally kept as a separate, discrete study so that the results of individual shortterm studies can be evaluated and reported in the near term. The investigators believe that including all the information for both studies in one consent form would have made it unduly long and cumbersome, and might have been confusing enough to hinder potential research participants from reading the informed consent document carefully.

The investigators agreed to make the requested changes to and clarifications within the informed consent document, and a revised form was submitted to the RAC.

2. Responses to RAC Discussion Questions

Regarding an animal model, Dr. Lee indicated that the investigators have been unable to find any controlled degenerative arthritis model; the goat is as close as possible to human disease. The investigators have seen new cartilage-like formations in the area of spontaneous osteoarthritis in the goat, which has provided evidence to move to human trials with this product. In addition, in Korea some research participants showed satisfactory evidence of regeneration.

Dr. Lee explained that NSAID use will be allowed in this study, but the investigators plan to check how long NSAIDs are used by each participant and will use that information as a factor in evaluating the trial data.

E. Public Comment

No public comments were offered.

F. Synopsis of RAC Discussion and RAC Observations and Recommendations

The following observations and recommendations were made during the RAC's indepth review and public discussion

Preclinical Issues

- Insufficient evidence has been gathered thus far to support the hypothesis that the cells remain in the joint, adhere to the cartilage, and secrete TGF-β1 in the joint. As such, and because it may not be possible to confirm these effects in human studies, it will be important to continue preclinical studies.

- There have been a number of deaths of animals in the preclinical studies. In each case, a cause-and-effect relationship could not be established to link the death to the study agent. Nonetheless, before advancing to a Phase II study, further analyses of the animal data should be carried out to determine whether or not there was a mortality trend in the animals that received the agent.

- Participants in the Phase I studies experienced unexpected variations in serum TGF-∃1 levels. These variations were attributed to platelet shearing and release of TGF-∃1 caused by the use of a small-gauge needle. Simply specifying that a large gauge needle be used for blood draws is insufficient. Data should be gathered to validate the TGF-∃1 assay and to determine whether the use of a larger-gauge needle eliminates the variations in TGF-∃1 levels observed.

Clinical/Trial Design Issues

- Since the primary endpoint depends in part on subjective measurements of pain, the double-blind study design is important because it will help prevent potential bias. However, differences in the injection volumes of the study agent and the control may undermine the blind. This problem needs to be addressed.

- Since it has not yet been established from the preclinical data that the transduced cells adhere to the joint and produce TGF-β1, synovial fluid should be obtained to try to confirm that TGF-β1 levels are produced by the cells in the joint and to detect inflammation, for example by measuring interferon gamma.

- A total of 500 MRIs (five in each participant) are planned to measure cartilage regeneration, a secondary endpoint of the study. To optimize the information gathered through these images, a standard quantitative and statistical analysis should be developed. In addition, the MRIs from the Phase I study should be used to inform the analysis of the MRIs in Phase II.

- While the protocol precludes NSAID use within 14 days of the baseline visit and during the initial 72 hours following administration of the study agent, pain medication use may vary considerably in the 2-year followup period after administration of the agent. Since pain is a primary outcome measure, the use of NSAIDS or other pain medication potentially will confound this assessment. The statistical analysis will need to control for this variable.

- The two allogeneic cells populations that will be administered have the potential to induce an immune response, an effect that has not been studied in the preclinical models that used xenogeneic rather than allogeneic cells. The Phase I clinical trials also failed to include assays to detect an immune response. Since the donor cells are human leukocyte antigen typed and the potential immunogenic antigens are known, participants' baseline serum/plasma and peripheral blood mononuclear cells should be evaluated for preexisting antibodies, and T cells and corresponding serial blood samples should be evaluated for an immune reaction against these antigens. These assays should be developed prior to initiating the trial.

- Currently, the transgene product and endogenous TGF-β1 cannot be distinguished. This is a significant drawback because, in the event of a serious adverse event, it will confound any analysis about the role of transgene production. Unless a change can be made in the transgene to allow an assay to distinguish TGF-β1 produced by the cells from endogenous TGF-β1, at the least an assay should be developed to differentiate the messenger ribonucleic acid (mRNA) from the transgene and the mRNA expressed endogenously.

- The study provides for an elective crossover at 6 months. The rationale for choosing a 6-month time point when the safety and efficacy endpoints are to be carried out to 2 years should be further explained in the protocol and the informed consent document.

<u>Ethical/Social/Legal Issues</u>

- Additional language earlier in the informed consent document is needed regarding the fact that participants who receive TG-C are expected to agree to 15 years of followup in accordance with current regulatory requirements. As currently written, clear distinctions have not been made between the study followup period of 2 years and the longterm followup period of 15 years.

- The informed consent document should be revised as follows:

    - Include the rationale for requiring contraception during the trial. The data on which this requirement is based, including any limitations in the data, should be explained.
    - Clarify that participants will receive the results of clinically significant abnormal screening tests, e.g., pregnancy test results.
    - Revise wording that perpetuates the therapeutic misconception.

G. Committee Motion 7

Dr. Bartlett summarized the RAC recommendations to be included in the letter to the investigators and the sponsor expressing the comments and concerns of the RAC. It was moved and seconded that the RAC approve these summarized recommendations. The vote was 13 in favor, 0 opposed, 0 abstentions, and 1 recusal.

[At this point, Dr. Yankaskas took over as Acting RAC Chair.]

XIII. Discussion of Human Gene Transfer Protocol #1001-1026 entitled: A Phase I Neoadjuvant Study of In-situ REIC/Dkk-3 Therapy Followed by Prostatectomy in Patients with High Risk Localized Prostate Cancer

Principal Investigator:	Simon J. Hall, M.D., Mount Sinai Medical Center
Additional Presenter:	Yasutomo Nasu, M.D., Ph.D., Okayama Medical School (Japan)
Sponsor:	Momotaro-Gene, Inc.
RAC Reviewers:	Drs. Buchmeier, Flint, and Kahn
Ad hoc Reviewer:	James Gulley, M.D., Ph.D., F.A.C.P., NCI, NIH

A. Protocol Summary

Prostate cancer is the ninth most common cancer in the world, but in the United States it is the second most common cancer in men; in 2005, prostate cancer affected 18 percent of American men and caused death in 3 percent of those affected. Symptoms, physical examination, prostate-specific antigen (PSA) testing, or biopsy can signal the presence of prostate cancer. Treatment options are limited for patients at high risk of metastasis or who are refractory to hormone treatment. Because all treatments can have significant side effects, such as erectile dysfunction and urinary incontinence, treatment discussions often focus on balancing the goals of therapy with the risks of lifestyle alterations.

The Kattan nomogram can predict recurrence after surgery and/or radiation therapy, based on data available either at time of diagnosis or after surgery; the Kattan score represents the likelihood of remaining free of disease at a given time interval following surgical prostatectomy. Patients classified as high risk using the Kattan nomogram are considered good candidates for adjuvant therapy to prevent the spread and potential reoccurrence of the cancer.

A normal protein called reduced expression in immortalized cells (REIC) exists in all human cells but is missing or greatly reduced in some cancer cells. In the absence of REIC/Dkk-3 (Dkk is the Dickkopf family of genes), cancerous cells fail to initiate apoptosis and thus become immortalized or cancerous. Expression of REIC/Dkk-3 in abnormal cells has been identified as a potential mechanism to induce tumor suppression and trigger apoptosis of cancer cells, even in the presence of normal cells that are already expressing physiologic levels of REIC. The forced expression of REIC/Dkk-3 using a plasmid vector was shown to inhibit cell growth in cervical and liver cancer cell lines. An adenovirus delivery system that efficiently carries REIC/Dkk-3 into cells has also been demonstrated to induce cancer-specific apoptosis in prostate cancer, testicular cancer, and malignant mesothelioma cells, while producing no changes in normal cells. Advanced prostate cancer represents a suitable model to test the effectiveness of Ad-REIC/Dkk-3 in a clinical setting prior to advancing to other cancer that are REIC/Dkk-3 deficient. Prostate cancer cells produce PSA that can be measured in human plasma and is an excellent biomarker for cancer cell activity.

The primary purpose of this first-in-humans Phase I trial is to evaluate safety, but the prostate cancer model also allows for the opportunity to detect some efficacy of the Ad-REIC/Dkk-3. Momotaro-Gene, Inc., has conducted preclinical pharmacology and efficacy studies to demonstrate the potential benefit of Ad-REIC/Dkk-3 in human cancer models. The clinical trial will be conducted as a classic dose escalation with three research participants per cohort until a maximum tolerated dose or maximum feasible dose is reached. Based on these results, additional clinical trials will be designed to further explore the utility of Ad-REIC/Dkk-3 in human cancer types that are REIC-deficient leading to immortalization. To date, Ad-REIC/Dkk-3 has not been evaluated in any formal human clinical trial.

B. Written Reviews by RAC Members

Seven RAC members voted for in-depth review and public discussion of the protocol. Key issues included the use of the novel REIC/Dkk-3 transgene that encodes an apoptotic protein that has not been

used previously in human gene transfer research; the risks and benefits of this approach in this population of patients was deemed to deserve further discussion.

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

Dr. Buchmeier expressed concern about the selection of research participants – enrolling and dosing potentially curable patients at a stage of disease in which the tumors might be operable could delay and possibly compromise any opportunities for a conventional cure that exist at the time of enrollment. Dr. Buchmeier suggested that the investigators add to the exclusion criteria individuals with ornithine transcarbamylase deficiency, an X-linked genetic disorder that renders the individual sensitive to adenovirus-induced pathology. Additionally, he noted that the safety of the proposed studies is in doubt because of the paucity of background information available on the use of the proposed vector in prostate cancer.

Dr. Flint requested that the investigators summarize the data supporting the conclusion that REIC/Dkk-3 expression induces apoptotic cell death and antiproliferative status selectively in human immortalized and transformed cell lines, in particular the effects of REC/Dkk-3 on human prostate cancer cells and normal prostate cells. She also asked for discussion of the proposition that exogenous or secreted REIC/Dkk-3 at a tumor site might activate immune cells and enhance antitumor immunity, discussion of the data demonstrating that overproduction of REIC/Dkk-3 does not harm normal cells including normal prostate cells, and discussion as to whether REIC/Dkk-3 produced from the vector is likely to persist for longer than 7 days and whether the amount produced would be sufficient to be therapeutically effective. Dr. Flint asked for evidence that Ad-REIC/Dkk-3 does not replicate in normal human prostate cells. Additionally, she requested further information as to whether the assays to be used to measure secondary endpoints would allow assessment of the hypothesis that REIC/Dkk-3 might activate immune cells.

Dr. Kahn stated that the informed consent document is generally well written, in language that is appropriate for the participant population and with sufficient detail and explanation. However, he offered several suggestions for additions and clarifications. Wording regarding this protocol being a Phase I, first-in-human trial should be added, along with an explanation of the meaning of those terms. Dr. Kahn suggested that a statement on page 4 is overly optimistic, given that this is a first-in-human trial, and should be reworded. He also suggested that the directions regarding the period of time before it is advisable to engage in unprotected sex should be consistent throughout the protocol documents.

Regarding the potential participant population, *ad hoc* reviewer Dr. Gulley suggested that the inclusion criteria state which Kattan nomogram is to be used and the quartile information should be provided. He noted that the potentially relevant data about the clinical safety of adenoviral vectors in the neoadjuvant setting is not addressed in either the protocol or responses to Appendix M. He suggested that an alternative participant population for this first-in-human safety study could be patients with biopsy-proven local recurrence following local therapy such as radiation therapy. Noting that the investigators offer no background information on other clinical trials with this vector or similar vectors in prostate cancer, Dr. Gulley stated that several clinical trials of adenoviral vectors injected intratumorally in the neoadjuvant setting and in the locally recurrent setting might provide additional insight into safety with this vector.

C. RAC Discussion

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

- Dr. Flint asked whether the investigators have proof that the proposed replication-competent vector does not replicate in normal human cells. She noted that, in general, the replication of replication-competent vectors is both cell-type dependent and multiplicity of infection dependent. Because of the greater likelihood of escape if this vector does replicate, she recommended that preclinical studies be conducted to determine that this vector does not replicate in the relevant cell types.

- Dr. Gulley suggested defining the potential participant population as individuals with a primary Gleason pattern of 4 or greater.

- Dr. Ertl suggested that the investigators collect and store blood samples before and after dosing to check for T cells related to adenovirus.

- Dr. Zaia asked why lymphopenia greater than 7 days was excluded for determining dose-limiting toxicity. (Dr. Gulley explained that such an event is not a clinically relevant decrease in lymphocytes, and no clinical sequelae occur.)

- Dr. Buchmeier asked whether the investigators plan to test for high levels of pre-existing anti-adenovirus antibodies that could trigger unexpected inflammatory responses, which could complicate surgery and other downstream events.

D. Investigator Response

1. Written Responses to RAC Reviews

The intention of the investigators is to enroll research participants at high risk of recurrence of cancer after prostatectomy. The initial protocol was envisioned to use the Kattan nomogram as the primary entry criteria. However, the sponsor and the investigators agreed to modify the entry criteria, eliminating the Kattan nomogram; the main entry criteria will be altered to state, "Patients with histologically confirmed prostate cancer and Gleason Score of 7-10, or PSA of 10 ng/mL or greater." This entry criterion defines a high-risk population that is appropriate for experimental dosing with an adenovirus-mediated *in situ* protein therapy in the prostate cancer setting.

The safety of REIC and Ad-hREIC/Dkk-3 has been demonstrated in numerous animal studies that include both healthy and tumor-bearing animals, two of which were GLP toxicology studies in relevant species. These studies include animal efficacy and pharmacology studies as well as both tumor-bearing toxicology studies in the mouse and GLP toxicology studies in the rat and dog. Protein homology, *in vitro*, and animal experiments demonstrate that the mouse, rat, and dog are relevant species and predictive of human toxicology for the REIC protein. No significant toxicity was observed in GLP and non-GLP studies.

The potential for REIC/Dkk-3 to induce an immunological response has been studied in several published experiments. The REIC/Dkk-3 gene has been reported to be a tumor suppressor and the expression is significantly downregulated in a broad range of cancer cell types. The protein is secretory and studies have been conducted that demonstrate that recombinant REIC/Dkk-3 protein induces differentiation of human CD14+ monocytes into a novel cell type (called REIC/Dkk-3Mo) which resemble immature dendritic cells. Data suggest a cytokine-like role of REIC/Dkk-3 protein in monocyte differentiation.

Normal prostate cell lines have been tested and demonstrated to be unaffected by Ad-hREIC/Dkk-3. Several preclinical studies have demonstrated low potential for induction of apoptosis in normal cell lines, including endothelial cells and fibroblast cells that make up the majority of the prostate.

The *in situ* production of REIC/Dkk-3 protein from the Ad-hREIC/Dkk-3 is anticipated to persist only for the period of time during which the virus is present within cells; thus, clearance is expected within 7 days. The effect of REIC is primarily an acute induction of apoptosis as demonstrated by *in vitro* and *in vivo* methods and, therefore, the short-term infusion of REIC protein is considered to be adequate to initiate apoptosis and the short-term (7-day) stability of Ad-hREIC/Dkk-3 is considered acceptable.

Measurements for cytokines and white blood cell counts were considered adequate and appropriate to measure immune response to the REIC/Dkk-3 in the Phase I trial. The investigators have not planned to assess immune system activation in this Phase I safety trial, but will consider such evaluations in Phase II.

The 4- to 6-week period between the biopsies and scheduling of the prostatectomy is the standard of care; it is the typical time to allow the prostate to heal after biopsy and the practical time to schedule prostatectomy. Therefore, this timing does not represent a delay in the standard-of-care treatment for these research participants nor does it present any abnormal risk to them.

Ornithine transcarbamylase (OTC) is a urea cycle disorder that results from a genetic deficiency leading to reduced ammonia incorporation and symptomatic hyperammonemia. The frequency of this disorder is between 1:80,000 and 1:20,000 live births, and individuals with severe cases have low survival past childhood. While the sponsor and the investigators are agreeable to testing for this disorder, the data regarding OTC indicate that this disorder is a very low risk in elderly male prostate cancer patients.

The investigators agreed to make the requested changes to and clarifications within the informed consent document.

2. Responses to RAC Discussion Questions

Dr. Nasu explained that the REIC protein remains in the prostate for a few days, but in this study the investigators will check for the presence or absence of the transduced protein in the prostate by using the immunohistochemical stain after the prostate has been removed. The investigators have not measured its half-life in cells in culture.

Dr. Hall agreed to expand the section in the protocol that discusses the clinical experience with adenovector, to include instructive additional data.

If this Phase I trial proves safety of this approach, Dr. Hall stated that this experimental therapy would be tried on similar prostate cancer patients using more than one injection. Experience with adenovirus herpes simplex virus thymidine kinase (Ad-HSV-TK) in radio-recurrent disease has shown that repetitive injections result in enhanced benefit.

E. Public Comment

No public comments were offered.

F. Synopsis of RAC Discussion and RAC Observations and Recommendations

The following observations and recommendations were made during the RAC's indepth review and public discussion:

<u>Preclinical Issues</u>

- To enhance understanding of the mechanism of action, studies should be carried out to determine the half-life of the REIC protein in cultured cells.

- The adenoviral vector to be used in this study is described as replication defective (deletion of E1A, E1B, and E3 genes). However, the lack of E1A or E1B proteins in infected cells is cell-type specific and depends on multiplicity of infection. Studies should be done to determine whether vector replication occurs in prostate cells, whether normal or cancerous. If the vector can replicate in prostate cells, there is a higher risk of vector dissemination outside of the prostate. If such studies already have been conducted using the same adenoviral vector, the data should be described in the protocol.

- The premise of the protocol is that adenoviral vectors will infect prostate tumor cells. Preclinical studies have not established the types of cells that the vector infects in the prostate. These studies should be performed.

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

- The inclusion criteria are designed to limit enrollment to research participants at high risk of reoccurrence after prostatectomy. The protocol requires a minimum Gleason score of 7, which is one predictive factor of the risk of reoccurrence. A Gleason score is based on a composite of two readings by the pathologist. To ensure appropriate participant selection, the application of the Gleason score should be refined by putting more weight on the primary score, i.e., only those with a primary Gleason score of 4 or greater and total Gleason score of at least 7 should be enrolled (as opposed to those with a primary Gleason score of 3 and a total Gleason score of at least 7).

- According to data presented at the RAC meeting, a number of gene transfer protocols have used intraprostatic injection of adenoviral vectors containing different transgenes prior to prostatectomy. Since these trials are being used as a rationale for the conduct of this trial and to guide selection of participants, data from these trials and their results to date should be included in the protocol.

- In the event of an adverse event or any unexplained finding in the trial, it would be important to be able to differentiate between an immune response against the transgene product and one against the adenoviral vector. Distinguishing between the two is important because many research participants may have preexisting cellular and humoral immunity against adenovirus. To facilitate such studies, peripheral blood mononuclear cells and serum should be collected and stored before and after vector administration.

Ethical/Social/Legal Issues

- The protocol and the informed consent document should be revised to clarify that barrier contraception is required only during the period between vector administration and the total prostatectomy.

G. Committee Motion 8

Dr. Yankaskas summarized the RAC recommendations that will be included in the letter to the investigators and the sponsor expressing the comments and concerns of the RAC. It was moved by Dr. Flint and seconded by Dr. Buchmeier that the RAC approve these summarized recommendations. The vote was 14 in favor, 0 opposed, 0 abstentions, and 0 recusals.

XIV. Closing Remarks and Adjournment/Dr. Yankaskas

Dr. Yankaskas thanked the RAC members and the OBA staff and adjourned Day 2 of the March 2010 RAC meeting at 11:50 a.m. on March 11, 2010.

[Note: Actions approved by the RAC are considered recommendations to the NIH Director; therefore, actions 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: _____

_____
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Chair
Recombinant DNA Advisory Committee

Attachment I: RAC Roster
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Attachment II
Public Attendees

A complete list of attendees for this meeting is unavailable.

Adriana Byrnes, NCI, NIH
Tim Cripe, Cincinnati Children's Hospital
Syed Husain, Center for Biologics Evaluation and Research, FDA
Scott Kennedy, Solving Kids' Cancer
Rick Morgan, NCI, NIH
Maryann Redford, National Eye Institute
Joyce Reyes, Jennerex
Karl Seldane, FDA

Attachment III
Abbreviations and Acronyms

3T	3.0 Tesla
BL	biosafety level
CAR	coxsackievirus and adenovirus receptor
CCHMC	Cincinnati Children's Hospital Medical Center
cDNA	complementary DNA
DHHS	U.S. Department of Health and Human Services
DNA	deoxyribonucleic acid
DSMB	data and safety monitoring board
EBV	Epstein-Barr virus
ELISpot	enzyme-linked immunospot
FDA	Food and Drug Administration, DHHS
GLP	Good Laboratory Practice
GTSAB	Gene Transfer Safety Assessment Board
GvHD	graft-versus-host disease
Hemoglobin F	fetal hemoglobin
Hemoglobin S	sickle hemoglobin
HER2	human epidermal growth factor receptor 2
HIV	human immunodeficiency virus
IL-2	interleukin-2
IRB	institutional review board
IRI	Influenza Research Institute
LAM-PCR	linear amplification-mediated polymerase chain reaction
mRNA	messenger ribonucleic acid
NCI	National Cancer Institute, NIH
NGFR	nerve growth factor receptor
NIH	National Institutes of Health
NIH Guidelines	*NIH Guidelines for Research Involving Recombinant DNA Molecules*
NIMH	National Institute of Mental Health
NSAIDs	nonsteroidal anti-inflammatory drugs
OA	osteoarthritis
OBA	Office of Biotechnology Activities, NIH
OD	Office of the Director, NIH
OTC	ornithine transcarbamylase
PI	principal investigator
PSA	prostate-specific antigen
RAC	Recombinant DNA Advisory Committee
REIC	reduced expression in immortalized cells
RG	risk group
RML	Rocky Mountain Laboratory, NIH
SCD	sickle cell disease
SIN	self-inactivating
TG-C	TissueGene-C
TGF-ß1	transforming growth factor beta 1
TILs	tumor-infiltrating lymphocytes
UW-M	University of Wisconsin-Madison