
RECOMBINANT DNA ADVISORY COMMITTEE

Minutes of Meeting

March 3-4, 2009

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' website at www4.od.nih.gov/oba/rac/protocol.pdf.]

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

March 3-4, 2009

The Recombinant DNA Advisory Committee (RAC) was convened for its 116th meeting at 8:30 a.m. on March 3, 2009, at the National Institutes of Health (NIH) Neuroscience Center, 6001 Executive Boulevard, Rockville, Maryland. Dr. Howard Federoff (Chair) presided. In accordance with Public Law 92-463, the meeting was open to the public from 8:30 a.m. until 5:40 p.m. on March 3 and from 8:15 a.m. until 11:30 a.m. on March 4. The following individuals were present for all or part of the March 2009 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 (*present on Day 1; via teleconference on Day 2*)
Hung Y. Fan, University of California, Irvine
Howard J. Federoff, Georgetown University Medical Center
Jane Flint, Princeton University
Kalle Gerritz, The Gramon Family of Schools (*via teleconference*)
Joseph A. Kanabrocki, The University of Chicago
Louis V. Kirchhoff, University of Iowa
Eric D. Kodish, The Cleveland Clinic Foundation
Prediman K. Shah, Cedars-Sinai Medical Center (*via teleconference*)
Robyn S. Shapiro, Drinker, Biddle and Reath (*via teleconference*)
Nikunj V. Somia, University of Minnesota, Twin Cities
Scott E. Strome, University of Maryland
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 (*via teleconference on Day 1*)

Office of Biotechnology Activities (OBA)

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

Ad Hoc Presenters and Speakers

William J. Bowers, University of Rochester Medical Center (*via teleconference*)
John Shively, City of Hope (*via teleconference*)

Nonvoting Agency Representatives

Kevin L. Nellis, Office for Human Research Protections, U.S. Department of Health and Human Services (DHHS)
Daniel M. Takefman, Food and Drug Administration (FDA), DHHS

NIH/OD/OBA Staff Members

Linda Gargiulo
Robert Jambou
Laurie Lewallen

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.

Maureen Montgomery
Marina O'Reilly
Gene Rosenthal
Tom Shih
Mona Siddiqui

Attendees

There were 71 attendees at this 2-day RAC meeting.

Attachments

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

I. Day 1 Call to Order and Opening Remarks

Dr. Federoff, RAC Chair, called the meeting to order at 8:30 a.m. on March 3, 2009. Notice of this meeting under the *NIH Guidelines for Research Involving Recombinant DNA Molecules (NIH Guidelines)* was published in the *Federal Register* on February 12, 2009 (74 FR 7071). Issues addressed by the RAC at this meeting included a report from the Gene Transfer Safety Assessment Board (a subcommittee of the RAC), public review and discussion of five protocols, discussion of appropriate containment for cloning of Ebola virus complementary deoxyribonucleic acid (cDNA), and an update of the consideration of changes to the *NIH Guidelines* regarding non-circulating potentially pandemic human influenza and highly pathogenic avian influenza viruses, as recommended by the Biosafety Working Group (a subcommittee of the RAC).

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 OBA committee management officer.

II. Minutes of the December 3-4, 2008, RAC Meeting

RAC Reviewers: Drs. Bartlett and Kirchhoff

Dr. Bartlett and Dr. Kirchhoff recommended acceptance of the December 2008 minutes document, noting that no substantive changes were needed and that the minutes adequately reflected what transpired at the December 3-4, 2008, meeting.

Committee Motion 1

Approval of the December 3-4, 2008, RAC meeting minutes was moved by Dr. Kirchhoff and seconded by Dr. Bartlett. The RAC voted unanimously by voice vote to approve the December 3-4, 2008, RAC meeting minutes.

III. Discussion of Human Gene Transfer Protocol #0811-955: Herpes Simplex Virus Gene Transfer of Glutamate Acid Decarboxylase for Painful Diabetic Neuropathy

Principal Investigator: David J. Fink, M.D., University of Michigan
Additional Presenter: Darren Wolfe, Ph.D., Diamyd, Inc.
Sponsor: Diamyd, Inc.
RAC Reviewers: Dr. Federoff and Ms. Shapiro

Ad hoc Reviewer: William J. Bowers, Ph.D., University of Rochester Medical Center (*via teleconference*)

A. Protocol Summary

The development of novel effective treatments for chronic pain has been disappointingly slow, in part because side effects limit the use of pain-relieving drugs. To overcome this limitation, the investigators have developed a series of gene-delivery vectors that allow the targeted release of pain-relieving molecules to selectively interrupt pain perception. The first of this class of vectors is currently being tested in patients with pain from cancer.

Pain from nerve damage, which frequently occurs in patients with diabetes, is particularly difficult to treat effectively. The investigators have constructed a series of non-replicating herpes simplex virus (HSV)-based vectors that efficiently target gene delivery to dorsal root ganglia from skin inoculation to effect the release of antinociceptive neurotransmitters in dorsal horn of spinal cord. The first of these vectors – a non-replicating HSV vector expressing preproenkephalin – has been studied in a phase 1 human trial in patients with intractable pain from cancer. But there is a substantial unmet need for effective treatments of neuropathic pain, and preclinical animal studies demonstrate that a glutamic acid decarboxylase (GAD)-expressing HSV vector is particularly effective in rodent models of neuropathic pain including painful diabetic neuropathy. This proposal is for a phase I/II human trial of a GAD-expressing non-replicating HSV vector in patients with painful diabetic neuropathy.

B. Written Reviews by RAC Members

Eight RAC members voted for in-depth review and public discussion of the protocol. Key issues included the use of a novel transgene and indication (diabetic neuropathy), the need for further exploration of the potential impact of preexisting immunity on the feasibility of this approach, the possibility that HSV immunity could attenuate the potential antinociceptive effects induced by the intradermal vector injection, and, if redosing is necessary to sustain pain control, preexisting immunity could limit the effects of additional doses, and ganglia would be resistant to superinfection by HSV.

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

Dr. Federoff asked for a further explanation for the Phase I/II design of this trial, for the selected dose, and why a dose-escalation paradigm was not proposed. He requested comment on the immunological issues regarding tolerance, breaking of tolerance, epitope spreading, safety of the proposed clinical investigation, and the proposed measurements planned for the trial and whether they will address the immunological issues. Dr. Federoff suggested that the investigators should consider, based upon their pre-clinical experience regarding the level of expression from this vector and knowledge of how many neurons are present in the regions where the study agent will be administered, employing a dose in this study that is likely to produce an effect rather than administering the maximum amount of study agent that is physically possible. Because approximately 70 percent of adults are seropositive for HSV type 1, Dr. Federoff asked the investigators to comment on whether potential participants' levels of antiviral antibody titers would be used as an inclusion criterion and whether antiviral titer levels would be part of outcome monitoring. He also asked whether prospective research participants who have frequent cold sores (labial herpesvirus infections) would be excluded from this trial.

Ms. Shapiro offered several examples of therapeutic misconception in the language of the informed consent document. She noted that a number of provisions in the informed consent document seem overly optimistic about trial results and cited two such examples. Other concerns raised by Ms. Shapiro included that the informed consent document's discussion of treatment for research-related injuries in the "Costs" section was vague, the "Privacy and Confidentiality" section did not satisfy Health Insurance Portability and Accountability Act (1996) research authorization requirements, consent for autopsy was not addressed, and a discussion of the possibility of research participants being randomized to saline injection was not included. In addition, she noted that the potential conflict-of-interest issues raised by Dr. Wolfe's involvement in this clinical trial were not addressed in the informed consent document.

Noting the transient nature of the therapeutic effect in animal models, which indicates the potential need for periodic redosing to maintain pain relief, Dr. Bowers asked the investigators to explain the rationale for using the cytomegalovirus (CMV) promoter and noted some uncertainty about the selection of the promoter driving the GAD transgene, especially in future studies in which an immune response to either the vector or transgene may be an issue. He queried whether the investigators had established preexisting HSV exposure or immunity in animal models and whether they then had delivered the HSV-GAD therapeutic to determine the extent to which therapeutic benefit might be compromised and, thus, what the cutoffs might be for anti-HSV antibody titers that would exclude potential research participants. Dr. Bowers asked whether the investigators planned to prescreen potential participants for GAD autoantibodies and to monitor titers after study dosing. He asked whether the investigators had procured data to identify and quantify the transduced cellular targets at the injection site or at proximal lymph nodes. Dr. Bowers also queried whether the investigators had monitored inflammatory gene expression or other measures of inflammation at the site of gene delivery and at distal sites to determine the duration of inflammation in animal models. In the clinical trial, he wondered how neuropathic and inflammatory pain would be differentiated clinically, especially if the inflammatory response is prolonged.

C. RAC Discussion

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

- Dr. Somia asked about evidence of down-regulation of the CMV promoter in human cells, and whether inflammation or secondary infections would turn the CMV promoter back on.
- Dr. Somia asked if remobilization of the vector would be possible if a superinfection by wild type virus occurred.
- Dr. Federoff queried as to whether classes of oral agents prescribed for diabetes—or other classes of agents for comorbid diseases—are known to affect the CMV promoter.

D. Investigator Response

1. Written Responses to RAC Reviews

With regard to the rationale for using the CMV promoter in this study, the investigators explained that, in the context of a genomic HSV vector, the human CMV immediate early promoter (hCMV IEp) produces a high level of transient expression in neurons. This high level is in contrast to the latency associated promoter (LAP2) element that produces biologically meaningful transgene expression that persists for at least 6 months *in vivo*. For this Phase I/II study, the investigators have elected to employ a vector with the hCMV IEp driving transgene expression for reasons of safety; should an unanticipated AE occur as a result of transgene expression, expression from the CMV-driven construct will be self-limited. Once they have firmly established safety and efficacy using this transient expression vector, the investigators will construct and test a long term expression vector using the LAP2 promoter to drive expression of GAD.

In murine studies with HSV vectors expressing several different transgene products that produce analgesic effects (including GAD), the investigators have observed consistently that reinoculation of the vector reestablishes the analgesic effect. These results suggest that any immune response after a single inoculation of the vector in the rodent does not interfere with the therapeutic effect; the investigators have not pursued detailed murine studies of the immune response. In the ongoing Phase I trial of the HSV vector expressing preproenkephalin, the investigators are measuring anti-HSV titers in the serum of research participants prior to and following inoculation of the vector into the skin. For this proposed trial, the investigators do not plan to exclude participants with preexisting anti-HSV antibodies, although post-hoc analysis of the response of participants with and without preexisting immunity might be informative.

The investigators agreed to include measurement of anti-GAD antibodies in the preclinical animal toxicology studies that will be completed prior to initiating the clinical trial. While they had not proposed to

monitor anti-GAD antibodies in the clinical trial, they agreed to incorporate this suggestion in the final study design. Inclusion of these tests will allow a determination of whether the vector stimulates an anti-GAD response.

In the rodent models, the investigators noted that they have not observed any indication of pain caused by injection of the vector or any overt inflammatory responses in the paw. The Phase II aspect of the GAD clinical trial will allow a determination as to whether there are any pain-causing effects of vector injection in research participants.

Regarding the rationale for this Phase I/II design, the investigators explained that they are currently enrolling research participants in a Phase I trial of a similar HSV construct engineered to express preproenkephalin in individuals with intractable focal pain and terminal cancer (NIH OBA 0204-529). The results of that study, which is anticipated to complete enrollment before the end of 2009, will be used to guide the final design of this proposed GAD trial.

The proposed dose is based on the preclinical efficacy and safety assessments and anticipated result of the ongoing Phase I trial (0204-529), and is subject to modification based on the results of that trial. Oncolytic (replicating) HSV has been administered to humans at doses as high as 1×10^{10} plaque-forming units (pfus) without significant toxicity. The proposed trial design reflects the investigators' expectation that the ongoing Phase I trial will support safety of this platform at the highest dose tested in that trial, which is 1×10^9 pfus.

In response to concern about GAD65 being an autoantigen in Type 1 diabetes mellitus and the transgene proposed for this trial, GAD67, sharing considerable homology with GAD65, the investigators explained that there is only a 65 percent protein homology between GAD65 and GAD67, as they are the products of two different genes located on separate chromosomes. While GAD65 is a known autoantigen in the development of Type 1 diabetes and is observed in several rare neurologic syndromes, anti-GAD67 antibodies are not commonly seen in autoimmune disease; several relevant studies provide supporting empiric evidence. To date there has been no evidence of breaking of tolerance to HSV or of epitope spreading.

The investigators stated that they do not plan to exclude participants who have recurrent perioral HSV infections, but they do not intend to dose individuals with active HSV infection in the orofacial region, even though this study proposes delivering the vector to the distal leg.

The investigators agreed to address all of the RAC reviewer comments regarding the informed consent document.

2. Responses to RAC Discussion Questions

Dr. Fink agreed to measure whether GAD antibodies are raised against the transgene when the investigators explore the preclinical toxicology results.

The investigators explained that they chose to test their product using research participants with diabetic neuropathy because that type of pain is relatively constant—it generally does not progress like cancer pain—thus making measurements of effectiveness less complicated.

Regarding the issue of experimenting on larger animals, Dr. Fink stated that the investigators do not intend to test their product in larger animals because no validated pain models exist for painful diabetic neuropathy in larger animals. Specifically, they do not intend to conduct chronic pain studies in primates, as such studies are not usually undertaken by anyone in the research field.

Dr. Wolfe explained that, in the preclinical data for the Diamyd vaccine, the investigators used as much as 10,000 times the proposed clinical dose in juvenile animals and looked specifically at reproductive toxicity, general toxicity, and long-term toxicity. The doses were meant to break tolerance. Although

massive antibody levels were generated in these animals, no ill effects were observed in any of the myriad of studies.

Although the investigators do not believe that potential participants who frequently suffer from cold sores should be excluded from this protocol, they expressed willingness to do so if discussions with the FDA result in that conclusion. Dr. Wolfe reiterated that individuals with active cold sores would be excluded from this trial.

Dr. Fink reiterated that for the Phase I malignancy trial (0204-529) and for this Phase I/II trial, the investigators propose a single set of injections at one time point only. There is no plan to reinject the research participants, although they will receive usual followup.

In response to a query about reactivating the CMV promoter, Dr. Fink explained that the investigators tried a number of different approaches to determine whether they could reestablish the vector once it was turned off. One such experiment focused on producing a superinfection with another vector that did not have the same transgene; that experiment did not succeed in turning the CMV promoter back on. Since that result, the investigators have not pursued this question in great detail.

With regard to the effect on the CMV promoter's activity of therapeutic drugs being taken by research participants, Dr. Fink offered as evidence of no effect that, in the Phase I trial approved by the FDA, the participants are not restricted as to drugs being taken (other than a restriction on active chemotherapy).

Dr. Fink indicated that he is the clinical investigator responsible for the conduct of the trial at the University of Michigan. Results from the study will be collected by a University of Michigan Data Safety Monitoring Board and transmitted to Diamyd. Diamyd and Dr. Wolfe do not have access to the raw 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 in-depth review and public discussion:

Preclinical Issues

- Given that dendritic cells and other antigen-presenting cells reside within the intradermal space and that HSV-1 vectors transduce such cells with high efficiency, the preclinical toxicology data should include identification and quantification of the transduced cellular targets at the injection site or even proximal lymph nodes. Since transduced dendritic cells could mount an immune reaction to the transgene product, such preclinical data would elucidate the risk of an immune response.
- The short-term expression of the CMV promoter is one of the safety features of the protocol. However, it is possible that standard treatments for diabetes, such as oral hypoglycemic agents, could affect its expression. Preclinical studies to assess this risk should be considered.

Clinical/Trial Design Issues

- It is anticipated that some of the safety data from Protocol #0204-529 will be used to justify proceeding with a Phase I/II design. However, the applicability of those data to this protocol and to this patient population is not clear-cut. The transgene is different, diabetes is a chronic disease, and the patients' lifespan is comparatively long. As such, it would be prudent to plan a deliberate pause at the end of the safety portion of the study, during which the final data are

analyzed, and only then to proceed toward randomization in a Phase II portion of the treatment evaluation.

- The proposed dose in this protocol is not based on a robust, evidence-based analysis. Rather, it is based on preclinical efficacy and safety assessments and will be informed by results from the ongoing trial in cancer patients (Protocol #0204-529). However, the transgene product in Protocol #0204-529 is the substrate preproenkephalin, whereas the transgene in this trial encodes an enzyme (GAD). The dose required to achieve a physiologic response observed when an enzyme is encoded by the transgene may be different than when a substrate is the transgene product. In addition, the dose proposed for this protocol is based on the maximal amount of the vector that can be delivered safely. Further evidence is needed to determine the appropriate dose.

Ethical/Legal/Social Issues

- The local institutional review board should be provided with the suggestions made by the RAC members in their written reviews concerning the informed consent document, including modification of language that may lead to therapeutic misconception, for example, “the HSV vector offers an alternative method of therapy” or “to explore a new therapy to reduce pain, we will inject a recombinant herpes-based vector into the skin.”

G. Committee Motion 2

Dr. Federoff summarized the comments and concerns of the RAC to be included in a letter to the investigators and the sponsor. Dr. Kirchhoff moved (and no second was requested) that the RAC approve these summarized recommendations. The vote was 17 in favor, 0 opposed, 0 abstentions, and 0 recusals.

IV. Ebola Virus cDNA and the NIH Guidelines for Research Involving Recombinant DNA Molecules

Presenters: Michael J. Buchmeier, Ph.D., M.S., University of California, Irvine; Heinz Feldmann, M.D., Ph.D., Rocky Mountain Laboratories (RML), National Institute of Allergy and Infectious Diseases (NIAID), NIH; Nancy P. Hoe, Ph.D., RML and Office of Research Services (ORS), OD, NIH; and Robert C. Jambou, Ph.D., OBA, OD, NIH

Discussants (*via teleconference*): Marshall E. Bloom, M.D., Associate Director, RML; Stephen Dewhurst, Ph.D., University of Rochester Medical Center; Kalle Gerritz, Ed.D., The Gramon Family of Schools; Richard Henkel, Ph.D., CDC; Claudia A. Mickelson, Ph.D., Massachusetts Institute of Technology; Stuart T. Nichol, Ph.D., U.S. Centers for Disease Control and Prevention (CDC); Pierre E. Rollin, M.D., CDC

A. Presentation by Dr. Jambou

The cloning of nucleic acids from a Risk Group 4 (RG4) agent, such as Ebola virus, into nonpathogenic bacteria or lower eukaryotes falls under *Section III-D-2-a* of the *NIH Guidelines* and may be performed under Biosafety Level 2 (BL2) containment if the nucleic acid used is a totally and irreversibly defective fraction of the agent’s genome. In the absence of such a demonstration, work with the full-length genomic nucleic acid must be performed at BL4. Because much has been learned about the biology of Ebola virus in recent years, it may be appropriate to reassess the containment requirements for the cloning of full-length cDNA of this virus.

Section IV-C-1-b-(2)a of the *NIH Guidelines* grants NIH/OBA the authority to lower containment for research falling under *Section III-D*, including research with RG4 agents that do not meet the conditions of *Section III-D-2-a*, in this particular case for Ebola virus, the cloning of a full-length cDNA copy of the viral genome. It is both a responsibility and periodically a necessity for OBA to review occasionally some

of the containment requirements in the *NIH Guidelines* to ensure that these remain appropriate as the science evolves.

B. Presentation by Dr. Feldmann

Dr. Feldmann presented information about the biology of the Ebola virus and the types of experiments that the RML would like to perform. Ebola/Marburg hemorrhagic fever is one of the most severe of the viral hemorrhagic fevers; researchers are now beginning to learn, mostly from animal studies, about mechanisms by which individuals survive infection with these agents. No licensed drug is currently available either for prophylaxis or treatment, although experimental approaches are promising, particularly for vaccines against Ebola and related hemorrhagic fevers.

The virus family to which Ebola and Marburg belong is called Filoviridae; these are nonsegmented, negative-strand ribonucleic acid (RNA) viruses. There is only one species of Marburg virus currently known, whereas there are currently four (and most likely five) species of Ebola virus. The Ebola virus has a filamentous structure resembling a shepherd's hook, it is an enveloped virus with a nonsegmented, single-strand, negative-sense RNA genome that encodes seven structural proteins.

Because filoviruses contain a single-strand, negative-sense RNA genome, the virus cannot rely on cellular machinery for replication. Instead, Ebola virus must package within its virus particle, four essential proteins to ensure its replication and the expression of all other structural and non-structural viral proteins. These essential proteins that are an integral part of the viral particle are the viral RNA-dependent RNA polymerase (or L for "large protein"), and three others called "NP" (for nucleocapsid protein), VP30 (viral protein, 30 kilodaltons -kDa) and VP35 (viral protein, 35kDa). In combination, these essential proteins direct and modulate the activity of the L polymerase to synthesize a full-length sense, or positive strand, of the RNA genome, as well as smaller viral mRNAs that encode all viral components. Throughout its infectious cycle, Ebola never produces any DNA.

A now common laboratory approach, called reverse genetics, used in RNA virus research to study viral pathogenesis, or develop vaccines, antivirals, and diagnostics involves the introduction *in trans* (e.g. by transfection) of six essential Ebola DNA plasmids into a mammalian cell line. In order to initiate synthesis of Ebola virus from these DNA plasmids, it is essential to produce RNA by using a plasmid that expresses bacteriophage T7 RNA polymerase – a DNA-dependent RNA polymerase. Once a properly constituted genomic RNA molecule is formed from the full-length cDNA plasmid of the Ebola genome, the other essential viral proteins expressed from the remaining four plasmids are capable of sustaining viral replication and the formation of infectious Ebola virions. The ability to produce infectious virus – or to rescue virus - from plasmids requires specific experimental conditions and must be performed under BL4 containment. This methodology for viral rescue has been used successfully for Ebola virus since 2001 by several laboratories. There have been no reports to date of any successful rescue by DNA plasmid transfection of mammalian cells using the full-length genomic plasmid alone without the five support plasmids (i.e. T7 RNA polymerase, L, VP30, VP35) and this observation derives from more than 100 failed rescue attempts performed in recent years by U.S. laboratories conducting this kind of work. In addition, there have been no reports of any successful rescue with only one of the essential protein components missing. Because of this experience, researchers working with filoviruses consider that the full-length genomic molecule alone, be it RNA or a cDNA clone, is an irreversibly defective molecule as it relates to its potential for infectivity.

Based on the information presented about the virus structure, virus replication cycle, and the stringent conditions for infectious virus rescue, Dr. Feldmann and colleagues at the RML have proposed that the plasmid carrying the full-length genome of Ebola or any filovirus can be handled safely in a "restricted" BL2 laboratory having limited and controlled access. This proposal is based on the scientific evidence that the genomic cDNA copy contained in a bacterial plasmid is inherently noninfectious and that no infectious virus can be produced in non-pathogenic *Escherichia coli* (*E. coli*). The full-length genomic cDNA plasmids, either wild type or those bearing mutations will be amplified in and purified from *E. coli* only in the restricted BL2 laboratory.

In support of this proposal, Dr. Feldmann described the physical characteristics, entry/exit procedures, and parameters of the “restrictions” within BL2 area as follows:

- The restricted BL2 area is entirely contained within an existing BL2 laboratory (lab within lab design)
- A negative airflow gradient exists between the restricted area, the general BL2 lab, and the common corridor.
- Access to the restricted area is controlled by biometric or keypad devices.
- Dedicated personal protective equipment (PPE) and specific procedures for exit and entry into the restricted area are implemented.
- All waste exiting the restricted area shall be sealed and immediately sterilized with no storage outside the restricted area and no storage allowed at the end of the day.
- Entry procedure requires removal of standard BL2 PPE prior to entry into the restricted area and donning of dedicated PPE to include a disposal lab coat, shoe covers and double gloves.
- Exit procedure requires the ordered removal of outer gloves, then the disposable lab coat, followed by shoe covers and inner pair of gloves. Personnel are required to wash hands prior to exiting the restricted area into the general BL2 laboratory.
- All procedures will be documented in their own specific laboratory manual.
- Inventory of *E. coli* stocks and plasmid DNA preparations, their use and destruction, will be recorded.
- Laboratory staff members will have security clearances and be trained in all laboratory procedures before they will be given access to this restricted area.

Biological materials exiting the restricted area and intended for future experimentation will be placed in a sealed container, separated from any helper plasmids, and transferred to a BL4 lab. All viral rescue experiments to generate infectious Ebola virions will be performed under BL4 conditions.

The specific work that will be performed at the RML in the restricted BL2 laboratory will consist of the religation of mutated subgenomic fragments into full-length clones of Ebola; transformation of appropriate competent *E. coli* in the presence of Zeocin™; selection and culture of positive clones, amplification of full-length clones in *E. coli*, and storage of *E. coli* transformed with full-length genomic plasmids. No helper plasmids will ever be present in that room.

C. Presentation by Dr. Hoe

Dr. Hoe discussed the RML Exposure Response and Management Program, which is the RML’s biosecurity program. She reviewed the components of the RML biosecurity program, enumerated the components of the medical management program for biological exposures, and described the Biological Exposure Assessment Program, which provides for local (RML) and institutional (NIAID/NIH) assessment of incidents, exposures, and illnesses related to the use of biological agents. The crisis management team is activated in response to any large-scale accident or disaster. Because the RML has Select Agents onsite, they also have a Select Agent incident response plan.

The NIH Occupational Medical Service oversees the RML’s general occupational health and safety. Onsite elements of the RML medical management program include:

- An occupational health nurse
- A registered nurse
- An isolation evaluation suite for temporary housing of anyone exposed to a biological agent onsite
- A good relationship with local emergency medical services, with periodic emergency drills
- A consultation contract with a board-certified infectious disease advisor
- A contract with St. Patrick Hospital in Missoula, about 1 hour north, which is the regional referral hospital
- Outreach to the local medical community

The RML's Biological Exposure Assessment Program (BEAP) provides for local, institutional, community, and state assessment of incidence, exposures, and illnesses. Core membership of the BEAP consists of representatives from the NIH Division of Occupational Health and Safety, the NIAID's Division of Intramural Research, and the RML. The RML associate director can activate the BEAP in two situations: either after receipt of notification from the occupational health nurse or designee that an employee has reported sick at work or if an employee becomes ill in the community.

The RML's crisis management team is activated in response to any large-scale accident or disaster, including working with administration, safety, maintenance, public affairs, and law enforcement personnel. The crisis management team utilizes the incident command structure, employs an incident notification system, and provides management of emergency response and recovery. The RML has its own emergency operations center that conducts yearly drills.

D. Presentation by Dr. Buchmeier

Dr. Buchmeier presented the RAC Biosafety Working Group (BWG) assessment of the RML request to clone full-length Ebola cDNA in *E. coli* at BL-2. The BWG put forth recommendations regarding biosafety containment and biosecurity procedures, both of which were intended solely for Dr. Feldmann's research at the RML. The conclusions of the BWG were that cloning of the full-length Ebola cDNA in *E. coli* K12, a nonpathogenic prokaryote, can be performed safely at BL2 due to the inherent properties of the cDNA in this biological system.

The BWG recommended that the biosecurity procedures for research with full-length Ebola cDNA in *E. coli* K12 at BL2 should parallel the security policies and procedures described in the *Biosafety in Microbiological and Biomedical Laboratories (BMBL)* manual's principles of biosecurity and that the RML proposal should encompass the BMBL recommendations and include any additional elements identified by the BWG. Those additional elements would include the appointment of an appropriate official to oversee the security plan on behalf of the institution and the establishment of a training program for security aspects of the research in addition to normal biosafety training. Such a program should be reevaluated periodically and updated as needed. Dr. Feldmann and the RML are responsible for developing and implementing an appropriate biosecurity plan that addresses the principles of biosecurity outlined in the *BMBL* and is commensurate with the risk assumed by this activity.

Biosecurity is defined as the protection of microbial agents from loss, theft, diversion, or intentional misuse. However, biosafety and biosecurity programs share some common components, such as risk assessment; personnel expertise and responsibility, including training and control of and accountability for research materials; access to critical elements of a process; material transfer documentation and training; emergency planning; and program management. The RML has considered all of these issues in appropriate detail.

The BWG recommendations also included a biosafety risk assessment, which concluded that cloning full-length cDNA into *E. coli* can be done safely at BL2.

The BWG recommendation to work with the cDNA of Ebola at BL2 with the nonpathogenic prokaryote *E. coli* K12 is specific to Dr. Feldmann and the RML. OBA should consider any future requests for research involving RG4 viral agents in the order Mononegavirales on a case-by-case basis. In consultation with the RAC as needed, OBA will conduct a biosafety risk assessment to determine whether such requests for research can be conducted safely at BL2 in other research facilities.

In keeping with the approach outlined in the “Principles of Biosecurity” section of the *BMBL*, it is the responsibility of the research institution to perform a biosecurity risk assessment and develop an appropriate plan, both of which should be included in the institution’s application to OBA.

E. RAC Discussion of the Presentations

RAC members discussed various aspects of the presentations. Concerns about the elements of a security clearance were not definitively resolved. Dr. Ertl asked about treatment and security of the waste products at the RML laboratory and was assured that the biohazard waste would be autoclaved and then incinerated onsite.

General discussion resulted in the conclusion that the chance of a random mixture of plasmids coming together through cross-contamination in a laboratory, and coming together in the correct ratios and in the presence of transfection solution to effect the transfection of mammalian cells, is extremely unlikely. The various plasmids must be combined in specific ratios to be able to generate infectious virus. In general, a plasmid concentration matrix must be used in order to obtain a successful rescue event.

Dr. Nichol noted that additional safety would not be gained by insisting that the plasmids be used in separate laboratory areas. Laboratories at his institution have operated for several years manipulating all of the Ebola plasmids in a BL3 area within a Select Agent restricted lab; there has not been one accidental generation of infectious Ebola virus in that area. It is possible to handle each of the different plasmids individually in the same lab and do so in a safe and responsible way. The most important element is to have restricted access to the lab where the full-length clone exists and thus address the critical biosecurity concern, as opposed to a biosafety concern, which is minimal.

Dr. Henkel asked about the possibility of unsupervised graduate students or postdoctoral fellows performing experiments that the PI was not aware of and noted such an instance in which drug-resistant mutants of a Select Agent were generated. To address this issue of potential nonmalicious experimentation, Dr. Feldmann reminded the Committee that the purpose of this work ultimately is to generate infectious virus. Thus, any individual conducting this type of research would need access to a BL4 laboratory in order to complete this type of experiment. Because there are few BL4 laboratories, few academic centers would have access to such facilities and therefore could not justify the need to work with a full-length cDNA genomic molecule of any filovirus.

F. RAC Discussion of the BWG Recommendations

Dr. Kodish asked about the distinction between biosafety and biosecurity. Dr. Buchmeier noted that it is not specifically within the jurisdiction of the BWG or the RAC to rule on biosecurity issues, but that biosecurity must be considered as part of the overall recommendations. Dr. Buchmeier reiterated that *biosecurity* is protection against loss or theft of an agent, whereas *biosafety* is protection of the individual working with the agent, through training and physical and procedural precautions, as well as protection of the public health. Noting that she had worked on the biosecurity chapter in the *BMBL*, Dr. Mickelson defined biosafety as looking at the management aspects of working with the biological material and biosecurity as looking at people management in terms of prevention of access and control of unauthorized use. All agreed that biosafety and biosecurity programs share common components, including risk assessment, personnel expertise and responsibility, control and accountability for research

materials, access to controlling elements, material transfer documentation, training, emergency planning, and program management.

Dr. Ertl asked whether the research material is ever in the hands of nonsecured personnel. Dr. Feldmann responded that waste from research involving Select Agents in BL4 facilities is autoclaved and then, under a chain of custody, is incinerated. The waste material is always under the documented control of a responsible individual until its destruction. The autoclaving process itself is validated periodically, and although the incinerator is not located in the restricted area of the laboratory building, it is located on the secured RML campus.

General discussion ensued about the use of incineration for material that has already been autoclaved and is therefore considered nonviable. Several RAC members stated that it would be very unlikely to recover any useful biological material from autoclaved DNA samples, since these are physically and chemically denatured as a result of high heat and pressure during the autoclaving process. It was noted that incineration is not widely available at many research facilities and will most likely be less available in the future due to environmental concerns. However, the RML does have that capability at present.

Dr. Kanabrocki suggested that the RAC write and publish performance-based recommendations that other institutions could follow to petition the RAC more easily for subsequent action. Dr. Federoff agreed that the RAC should develop a “points-to-consider” document that could be useful for other laboratories requesting authorization for work with the full-length cDNA genome of Ebola virus. It was agreed generally that the RAC should develop a list of questions that would guide investigators and inform them of the kind of information needed by the RAC to make case-by-case decisions about containment and other issues surrounding this area of research. A points-to-consider document should include details of the actual benefits of studying these high-risk agents.

Dr. Kanabrocki underscored the importance that those who are responsible for this area of research be well informed about the science behind it. He noted that a body such as the RAC would be an appropriate venue to oversee this research because the RAC could evaluate the science appropriately.

Dr. Feldmann explained that the research being conducted by the RML and discussed at this RAC meeting is justified by public health and biodefense rationales.

G. Public Comment

No public comments were offered.

H. Committee Motion 3

It was moved by Dr. Kanabrocki and seconded by Dr. Buchmeier that the RAC accept the recommendations of the BWG. The RAC voted unanimously by voice vote in favor of the BWG recommendations, with a strong recommendation that the BWG develop a points-to-consider document for use by subsequent requesters; this document would be reviewed by the RAC prior to public dissemination.

V. Gene Transfer Safety Assessment Board Report

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

Dr. Zaia reported that of the 14 protocol submissions received by OBA in the past 3 months, 9 were not selected for public review at this RAC meeting. A total of 188 amendments, annual reports, and PI or site changes were submitted to OBA in this 3-month period, and 14 trials began enrolling research participants. Three M-I-C-1 responses were discussed:

Protocol #0204-529, “Gene Transfer for Intractable Pain: A Phase I Clinical Trial to Determine the Maximum Tolerable Dose of a Replication Defective Human HSV Vector Expressing Human

Proenkephalin,” employs an HSV vector expressing human proenkephalin for pain for individuals with cancer; this protocol was reviewed at the June 2002 RAC meeting. All of the RAC recommendations concerned the informed consent document and process. The IRB and the University of Michigan conflicts of interest committee were consulted regarding the RAC recommendations, and the consent document has been amended in accordance with the recommendations of the University of Michigan committee.

Protocol #0710-877, “Phase 2 Safety and Efficacy Study Evaluating Glutamic Acid Decarboxylase Gene Transfer to the Subthalamic Nuclei in Subjects with Advanced Parkinson’s Disease,” employs an adenoassociated viral (AAV) vector expressing two isoforms of glutamic acid decarboxylase; this protocol was reviewed at the December 3-5, 2007, RAC meeting. The RAC noted that there were several differences between that protocol and the initial Phase I study, including the composition of the infusion, the dose, bilateral infusions, and the delivery catheter. Therefore, the RAC recommendation was that a small Phase I study be conducted to look at safety prior to proceeding to Phase II. The sponsor noted that the dose had been reduced so that the total dose delivered per standard of care should not be greater than that delivered in the Phase I trial. With respect to the composition of the product, bilateral delivery, and a new catheter, the sponsor submitted data from preclinical studies and positron emission tomography imaging from a Phase I study to support her decision to move directly to a Phase II study. However, the investigators will institute a 10-day waiting period between surgeries for the first three participants. The blind will be broken for all participants after 6 months, and the investigators chose not to follow the recommendation to lengthen the data-gathering portion to 12 months; they believe they will be able to collect statistically significant data since the vector reaches peak expression at 4 weeks and data from the Phase I trial indicate that expression remains stable for 12 months. Once the blind is broken, participants who received sham surgery will be able to participate in an open-label study or pursue other options.

Protocol #0807-930, “A Double-Blind, Placebo-Controlled (Sham Surgery), Randomized, Multicenter Study Evaluating CER-110 Gene Delivery in Subjects with Mild to Moderate Alzheimer’s Disease,” employs an AAV vector expressing beta nerve growth factor; this protocol was reviewed at the September 2008 RAC meeting. In response to the recommendations that an interim analysis using an adaptive study design be considered so that meaningful cognitive changes of less than 50 percent could be detected, the sponsor indicated that change would be considered depending on the availability of resources to carry out a larger study. In response to the RAC request to discuss further the decision to require general anesthesia in the control arm rather than conscious sedation, the sponsor noted that general anesthesia was necessary to help maintain the blind and maintain unbiased information.

Dr. Zaia discussed the AEs that were reported to OBA during this reporting period. A total of 157 AEs were reported from 27 trials, of which the majority were unrelated to the gene transfer products. There were 43 initial and followup reports in which the AE was possibly related to the gene transfer products, one of which was sufficiently significant to discuss publicly at this RAC meeting. Protocol #0807-932, “A Randomized, Placebo-Controlled, Double-Blind, Dose-Escalation Study to Evaluate the Safety, Tolerability, and Pharmacodynamics of Multiple Intravenous Doses of ANZ-521 in Treatment-Naive Hepatitis C Patients,” was discussed at the September 2008 RAC meeting. This protocol has been closed after two AEs indicated that the vector might be immunogenic—two participants developed a cough after dosing. There were no signs or other symptoms to indicate that an anaphylactic reaction had occurred, but given the two reactions, a decision was made to close the trial.

VI. Discussion of Human Gene Transfer Protocol #0901-966: A Prospective, Randomized, Controlled, Multicenter, Unblinded, Safety, and Early Efficacy Trial of ExpressGraft™_{Enhance} Skin Tissue versus Wet-to-Dry Dressings in the Treatment of Recently Occurring, Noninfected Foot Ulcers in Diabetic Patients

Principal Investigator: Michael J. Schurr, M.D., University of Wisconsin-Madison
Additional Presenters: B. Lynn Allen-Hoffman, Ph.D., University of Wisconsin-Madison and Stratatech Corporation; Allen R. Comer, Ph.D., Stratatech Corporation; John M. Centanni, M.S., Stratatech Corporation
Sponsor: Stratatech Corporation

RAC Reviewers: Drs. Shah, Strome, and Zaia
Ad hoc Reviewer: John Shively, Ph.D., M.S., City of Hope (*via teleconference*)

A. Protocol Summary

Diabetes affects more than 20 million people in the United States, and the number of people diagnosed with diabetes is expected to double in the next 30 years. Diabetes can increase the risk of numerous complications, including diabetic foot ulcers. Of patients who have been diagnosed with diabetes, 15 percent to 20 percent will develop a diabetic foot ulcer, and many of these ulcers will be resistant to healing, in part due to infection of the ulcer. As a result, these patients will be at increased risk for serious complications such as amputation and death. As of 2006 infection of foot ulcers had resulted in 82,000 amputations each year.

Current treatments for diabetic foot ulcers fall short because at least 50 percent of all diabetic foot wounds are infected when the patient seeks treatment, and there is no currently approved therapeutic for infected ulcers. These infected ulcers must be treated to eliminate the infection before any attempt to close the ulcer is made. The goal of this proposed Phase I/II clinical trial is to test a novel skin substitute (ExpressGraft™_{Enhance}) that has antimicrobial activity to determine whether diabetic foot ulcers can be healed sooner through the use of this novel antimicrobial application compared with conventional therapy (cadaver allograft). ExpressGraft™_{Enhance} tissue is prepared from a consistent and uniform source of human allogeneic epidermal keratinocytes that have been stably-transfected with a plasmid-based linear expression fragment encoding the cathelicidin (hCAP-18/LL-37) host defense peptide. Because the transgene is stably integrated into the genome of the stably-transfected cells, no transfer of vector DNA to autologous (patient) cells is anticipated in this protocol. The copy number and integration site have been characterized and found to be stable for several passages beyond the point of anticipated clinical use. The keratinocytes from which the genetically-modified cells were derived are a consistent and uniform source of pathogen-free and clinically-tested human epidermal keratinocytes (NIKS® cells). ExpressGraft™_{Enhance} tissue secretes elevated levels of bioactive cathelicidin protein, and exhibits enhanced antimicrobial activity *in vitro* and *in vivo* compared to tissue prepared from unmodified keratinocytes.

This proposed clinical study will test the safety and effectiveness of ExpressGraft™_{Enhance} skin tissue in the closing of diabetic foot ulcers. The study will enroll 24 research participants with recently occurring, uninfected, full-thickness diabetic foot ulcers. Of the 24 participants, 16 will be randomly assigned to receive ExpressGraft™_{Enhance} skin tissue, and 8 will be randomly assigned to a control group that will receive standard care (wet-to-dry saline dressings). This study aims to determine whether diabetic foot ulcers close more rapidly and remain closed when treated with ExpressGraft™_{Enhance} skin tissue compared with wet-to-dry dressings. The incidence of infection will be monitored for each participant, and safety will be assessed throughout the trial.

B. Written Reviews by RAC Members

Nine RAC members voted for in-depth review and public discussion of the protocol. Three RAC members and one *ad hoc* member provided written reviews of this proposed trial.

Dr. Shah asked the investigators to enumerate the stopping rules for this trial and to discuss what is known about the duration of viability of an allograft. He asked the investigators whether they would be measuring circulating transgene product. If systemic spillage of the transgene product would be harmful to the research participant, Dr. Shah asked how the investigators planned to counter that effect.

Dr. Strome asked whether the StrataGraft™ tissue (not wet-to-dry dressings) would be a more appropriate control for the ExpressGraft™_{Enhance} skin tissue, requested more specifics about how the ExpressGraft™_{Enhance} skin tissue would be applied, and asked whether the graft survives or is replaced by the recipient's skin. He noted that more information is needed in the protocol regarding the rationale for the choice of the gene (hCAP-18/LL-37) for this study. Dr. Strome asked why the ExpressGraft™_{Enhance} skin tissue is not expected to be antigenic and how the investigators will evaluate the possibility that the

graft is merely inciting a local inflammatory response that enhances wound healing. He noted that the proposed antibody-testing strategy is not specific for the ExpressGraft™_{Enhance} product and that it might be helpful to consider conducting immunohistochemistry on the sloughed grafts to obtain more information on immunologic response.

Dr. Zaia asked the investigators to clarify the elements derived from humans, rats, and mice within the “dermal equivalent” and to explain whether the preclinical studies have evaluated the effects of treatment on the overall resolution of ulcerating wounds in an animal model. He asked for a description of the controls that would be used in the mixed lymphocyte response and chromium release (51-Cr) assays. He suggested adding functional status as an eligibility criterion, and recommended that the investigators revise the stopping rules so that they are more objective. Dr. Zaia asked for clarification of the language concerning the sponsor’s payment for research costs. Regarding the informed consent document, Dr. Zaia recommended that potential participants who are incapable of giving informed consent should be considered ineligible for this study and that the investigators should more clearly describe the storage of blood and tissue and the potential for additional tests. Because the risk of acquiring a disease from the mouse cells is so remote, its inclusion in the consent document might raise unwarranted anxiety; however, the risks associated with this first use of human cells should be presented more clearly, including noting the potential risks of allergic reactions and enhanced inflammation.

Dr. Shively noted that LL-37 stimulates the migration of neutrophils into wounds, yet the investigators did not report the neutrophil counts in the burn model. (LL-37 is the processed protein product from the hCAP18 gene; it is a natural antimicrobial produced by keratinocytes and infiltrating leukocytes.) In addition, LL-37 causes necrosis of annexin V-positive cells, and cellular necrosis in the model was not reported. Dr. Shively explained that lack of this information is not a major flaw in the proposed study, but addressing these issues might be pertinent if complications arise or if the potential therapy is not effective.

C. RAC Discussion

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

- Dr. Flint asked about the need for additional preclinical trials to elucidate the benefit of the antimicrobial activity,
- Dr. Bartlett questioned whether the investigators had gathered any preclinical data showing that the ExpressGraft™_{Enhance} skin tissue product is superior to the StrataGraft® tissue.
- Dr. Strome noted that the NIH has a service for diabetic animal models that will help investigators acquire the appropriate mice; he offered to provide the investigators with that information.
- Dr. Federoff asked whether the large amounts of proteins being produced undergo organized assembly to form amyloid.

D. Investigator Response

1. Written Responses to RAC Reviews

The investigators rewrote the stopping rules for this protocol to be more clearly defined. AEs will be determined using the toxicity grading scale for healthy adult and adolescent volunteers enrolled in preventative vaccine clinical trials. Based on discussions with the FDA and to maximize participant safety, the investigators propose to limit participant exposure to a single ExpressGraft™_{Enhance} skin tissue application in the first participant cohort prior to implementing multiple tissue applications in subsequent cohorts. A stopping point includes generating an interim safety report that will be submitted to the data and safety monitoring board after the first cohort and prior to enrollment of additional research participants.

The investigators plan to store participant serum samples to examine circulating levels of transgene product in participants receiving the ExpressGraft™_{Enhance} skin tissue. A preclinical mouse safety study will use a polymerase chain reaction (PCR)-based method to evaluate the presence of transgene DNA sequences in blood and selected distant organs. The results of this preclinical safety study will be used to evaluate the ability to detect circulating levels of hCAP18/LL-37. As a result of this effort and pending input and discussions with the FDA, a safety-monitoring program will be established that may include the evaluation of transgene product in circulation. Specific assessment of ExpressGraft™_{Enhance} skin tissue-derived hCAP18/LL-37 protein levels in the circulation is not possible since the transgene and endogenous products are both wild type and therefore indistinguishable. With regard to countering effects of systemic h-CAP-18/LL-37 spillage, physiological levels of apolipoprotein A-I protein in human plasma should act to scavenge and inactivate hCAP-18/LL-37 protein activity in the circulation.

The investigators explained that ExpressGraft™_{Enhance} skin tissue is similar to StrataGraft® tissue in that it consists of a fully stratified epidermal layer containing viable keratinocytes and a dermal equivalent containing viable human fibroblasts. Following engraftment of StrataGraft® tissue onto nude mice, the tissue maintains this histological architecture for several months. The viability of StrataGraft® tissue in a human clinical setting was assessed 7 days after application to full-thickness skin wounds. In general, the StrataGraft® tissue remained tightly adherent to the wound bed and did not exhibit signs of necrosis or acute rejection. In contrast, cadaver skin (the control treatment) frequently appeared brown and necrotic after 1 week and became detached from the wound bed. The production process for the ExpressGraft™_{Enhance} skin tissue is comparable to that of the StrataGraft® tissue and includes the same sources of raw materials such as collagen, culture medium, and normal human dermal fibroblasts. Although the investigators anticipate that the ExpressGraft™_{Enhance} skin tissue, like the StrataGraft® tissue, will remain viable for at least 1 week, the viability of the ExpressGraft™_{Enhance} skin tissue in the environment of diabetic foot ulcer remains to be determined.

Regarding using the StrataGraft® tissue as the control for ExpressGraft™_{Enhance} skin tissue in this proposed trial, the investigators explained that the StrataGraft® tissue cannot be used because it is untested in diabetic ulcers. Wet-to-dry dressings are the standard of care for diabetic foot ulcers; therefore, represent the most appropriate control for the ExpressGraft™_{Enhance} skin tissue.

The ExpressGraft™_{Enhance} skin tissue product is approximately 250 μm thick and forms a multilayered, living skin equivalent with hallmarks of a normal differentiating epidermis. It is supplied as a sterile, circular, suturable sheet of skin tissue with a diameter of 7.5 cm and a surface area of 44 cm². The dermal equivalent and epidermal layers of this skin substitute are uniform in thickness throughout the entire tissue. A consistent manufacturing process in conjunction with required quality control testing specifications for release of each tissue lot will ensure that all tissues used in this clinical study will be of comparable thickness, quality, purity, and potency. ExpressGraft™_{Enhance} skin tissue will be applied to the wound and cut to fit, allowing for limited but essential overlap with the tissue in the ulcer periphery.

The ExpressGraft™_{Enhance} skin tissue is anticipated to be replaced eventually by the research participant's cells during the later stages of wound healing and remodeling. Although the ExpressGraft™_{Enhance} skin tissue is not anticipated to function as a permanent graft, the investigators stated that they anticipate it will survive in the wound environment long enough to provide a continuous fresh source of hCAP18/LL-37 and other wound-healing factors. Multifunctional antimicrobial peptides, such as the human cathelicidin hCAP18/LL-37, are expected to enhance innate host defense mechanisms against invading microorganisms and promote wound closure through neovascularization and reepithelialization. The biological activities of human cathelicidin directly address the underlying pathophysiology of diabetic ulcers, which include infection, poor circulation, and impaired wound healing.

With regard to concern that the graft enhances wound healing only by inciting local inflammation, the investigators stated that results from preclinical and clinical evaluations of the StrataGraft® tissue indicate no acute inflammatory response to the NIKS® cells, to normal human dermal fibroblasts, or to other components of the ExpressGraft™_{Enhance} skin tissue. It is possible that expression of hCAP18/LL-37 might elicit an enhanced inflammatory response to the ExpressGraft™_{Enhance} skin tissue; however, there is

no evidence of an overt cellular immune response to ExpressGraft™_{Enhance} skin tissue in the preclinical animal studies conducted to date. In addition, elements of the wound-healing response that are inflammatory in nature may be desirable as long as safety is not impacted and the ultimate outcome is improved wound healing. The investigators emphasized that, at this point in clinical development, identification of the precise mechanism of action is not as important as demonstration of safety.

Regarding neutrophil counts in the *in vivo* infected burn wound model, the investigators explained that they did not perform these counts because the focus of these studies was assessment of bacterial growth. However, the tissues were removed from the mice and sectioned, and the hematoxylin- and eosin-stained sections were examined. No significant inflammatory infiltrate was seen in the dermis or epidermis of the ExpressGraft™_{Enhance} skin tissue when evaluated in this model.

Although replication-inactivated murine feeder cells are used during the monolayer culture expansion of keratinocytes in the initial stages of the tissue manufacturing process, no murine components remain in the dermal equivalent. Stratatech Corporation has developed and qualified procedures to demonstrate that the replicative capacity of each lot of feeder cells has been completely eliminated. Stratatech also tests each lot of final tissue product for the presence of residual murine feeder cells using a proprietary, species-specific PCR-based assay. These procedures ensure that no viable feeder cells remain within the final ExpressGraft™_{Enhance} skin tissue product.

In response to a request for direct evidence that the LL-37-expressing cells will inhibit the usual flora in diabetic foot ulcers, the investigators stated that analyses of the microbial flora associated with these ulcers and other chronic wounds have demonstrated that most wounds are colonized with multiple bacterial species, with an average of 1.6 to 4.4 different organisms identified per wound. Gram-positive aerobic bacteria account for the majority of organisms identified in wound cultures, with *Staphylococcus aureus* the most commonly encountered; other organisms commonly identified in diabetic foot ulcers include *Staphylococcus epidermidis*, *Pseudomonas aeruginosa*, *Enterococcus faecalis*, and *Acinetobacter baumannii* (*A. baumannii*). The investigators explained that they focused on *A. baumannii* because of its significant ability to rapidly develop antibiotic resistance. *In vivo* preclinical studies have demonstrated directly that the ExpressGraft™_{Enhance} skin tissue exhibits potent antimicrobial activity against *A. baumannii*.

Although the investigators do not anticipate that expression of LL-37 will exacerbate wound infection, the clinical evaluations and stopping rules implemented by the investigators and sponsor are designed to put participant safety first by detecting and responding to any adverse reaction that might result from participant exposure to the ExpressGraft™_{Enhance} skin tissue.

The investigators explained that Stratatech Corporation is in the process of finalizing an investigator's brochure, which will be available after refinements are made to the preclinical and clinical programs for the ExpressGraft™_{Enhance} skin tissue product. The brochure will be made available to all appropriate regulatory authorities and safety monitoring committees for this clinical trial, including the RAC.

The investigators and sponsor agreed to address all of the RAC reviewer comments regarding the informed consent document.

2. Responses to RAC Discussion Questions

Dr. Schurr explained that the investigators have limited enrollment based on the pulse volume recordings, in which blood pressures in the arm and the leg are assessed; this is an accurate test for vascular insufficiency to the lower extremities in diabetic patients. If pulse volume recordings dip below 0.9, then the investigators will obtain a vascular surgery consult. If the research participant's arm or leg can be revascularized, then that individual may be able to enroll in the study. This procedure will be an important part of the safety analysis.

Regarding the comparator group for this Phase I trial, Dr. Schurr stated that the investigators would like to compare the effectiveness of the ExpressGraft™_{Enhance} skin tissue with both the StrataGraft® tissue and

the wet-to-dry dressings (the current standard of care). However, as this proposed trial is a Phase I study to determine safety, such a three-way trial would not be appropriate.

Dr. Schurr stated that the diabetic foot ulcer model is problematic in terms of healing because it has bacterial contamination, problems with blood flow to the wound, and elevated proteinases in the wound that inhibit wound healing. The investigators' intention is to debride the wounds and then apply an initial dose of ExpressGraft™_{Enhance} skin tissue, although there is a likelihood that that initial treatment will not engraft. However, the initial treatment is expected to improve the wound environment to a point where the second or third application might engraft and stay in the wound until the body heals the rest of the wound and the cells are displaced by native keratinocytes for final wound closure.

Dr. Allen-Hoffman explained that the process of producing StrataGraft® tissue removes the feeder cells prior to completion and includes thorough testing to confirm that no feeder cells with either replicative capacity or species-specific DNA remain. The investigators are making headway in not using feeder cells at all, which is the goal.

The total number of patients who have received the StrataGraft® tissue is 15.

Dr. Schurr acknowledged that the investigators have struggled with developing a chronic wound model in animals. The animal model of infection differs significantly from a chronic ulcer in humans with a significant bioburden; an animal model for chronic human ulcers does not exist. The burn model is an infection model that generates data but is not exactly what the investigators propose to study clinically. Because of the ongoing difficulty of modeling the exact infected chronic ulcer in animals, the investigators have excluded individuals with overtly infected ulcers from participation in this clinical trial.

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 in-depth review and public discussion:

Preclinical Issue

- The protein product cathelicidin undergoes posttranslational processing resulting in the release of LL-37. It is possible that the assembly of the final protein products could lead to aberrant protein aggregation and the formation of amyloid-like deposits. Since amyloid-like deposits could promote local inflammation, it would be prudent to conduct preclinical studies to look for aggregation of the protein.

Clinical/Trial Design Issues

- The proposed immunologic assay, a modified mixed leukocyte response, may not be the optimal assay to detect a CD8+ T-cell response to major histocompatibility complex class I (MHC I) antigens. Data presented to support the assertion that ExpressGraft™_{Enhance} skin tissue will not be immunogenic comes from short-term preclinical studies (i.e., up to 7 days postengraftment). Since an inflammatory infiltrate could develop later, additional immunological studies may be needed to determine whether there is a CD8+ T-cell infiltrate. If there is evidence of an infiltrate, specific assays also will be needed to examine the target of the cellular response.

- In light of recently published data that subclinical concentrations of LL-37 can increase the virulence of Group A streptococcus², the study should be designed to explore the effect of LL-37 on other normal skin flora.
- A recent paper found that in patients with psoriasis, whose plaques contain very high LL-37 levels, LL-37 binds extracellular self-DNA fragments into aggregated particles that enter plasma dendritic cells and trigger robust interferon responses. Although the levels of LL-37 in this trial are expected to be much lower, serologic tests should be conducted to monitor for the development of double-stranded DNA antibodies in the trial participants.

G. Committee Motion 4

Dr. Federoff summarized the comments and concerns of the RAC to be included in a letter to the investigators and the sponsor. Dr. Somia moved and Dr. Zaia seconded that the RAC approve these summarized recommendations. The vote was 17 in favor, 0 opposed, 0 abstentions, and 0 recusals.

VII. Discussion of Human Gene Transfer Protocol #0901-963: A Pilot Feasibility Study of Gene Transfer for X-Linked Severe Combined Immunodeficiency (X-SCID) in Newly Diagnosed Infants Using a Self-Inactivating Lentiviral Vector to Transduce Autologous CD34+ Hematopoietic Cells

Principal Investigators: Brian P. Sorrentino, M.D., St. Jude Children's Research Hospital, and Mary Ellen Conley, M.D., St. Jude Children's Research Hospital
Other Presenters: John T. Gray, Ph.D., St. Jude Children's Research Hospital
RAC Reviewers: Drs. Ertl, Fan, Kodish, and Somia (*for this protocol and the one immediately below*)

AND

Discussion of Human Gene Transfer Protocol #0901-964: Lentiviral Gene Transfer for Treatment of Children Older Than 1 Year of Age with X-SCID

Principal Investigators: Suk See De Ravin, M.D., Ph.D., NIAID, NIH; Elizabeth M. Kang, M.D., NIAID, NIH; and Harry L. Malech, M.D., Ph.D., NIAID, NIH
Sponsor: Brian P. Sorrentino, M.D., St. Jude Children's Research Hospital
RAC Reviewers: Drs. Ertl, Fan, Kodish, and Somia (*for both protocols*)

Dr. Federoff explained that the RAC would review both of these proposals together because they involve similar sets of research participants and because both groups of investigators are collaborating.

A. Summary of Protocol #0901-963

Drs. Conley and Sorrentino presented a summary of this protocol. The RAC reviewers discussed their concerns, with time allotted for responses, and the RAC members posed additional questions.

X-linked severe combined immunodeficiency (SCID-X1) is a catastrophic disease of childhood caused by mutations in the common gamma chain (γ c) gene, necessary for the growth and function of T- and B-lymphocytes. Affected patients typically die of infections in the first year of life if immune reconstitution is not achieved. Patients can be successfully treated by hematopoietic stem cell transplantation, particularly if they have an HLA matched sibling donor. In most cases, a matched sibling donor is not available and the majority of patients receive transplants from parental donors. These patients have significantly poorer

² Gryllos I, Tran-Winkler HJ, Cheng MF, Chung H, Bolcome R 3rd, Lu W, Lehrer RI, Wessels MR. Induction of group A Streptococcus virulence by a human antimicrobial peptide. Proc Natl Acad Sci U S A, 2008 Oct 28;105(43):16755-60. Epub 2008 Oct 20.

outcomes with 10 year survival rates ranging between 55 and 75%. Gene therapy using retroviral vectors to transduce autologous hematopoietic stem cells can be used for patients that lack a matched sibling donor and results in significant immune reconstitution in most patients. However, 5 out of 20 patients have developed leukemia due to insertional mutagenesis in these initial gene therapy trials. The investigators propose to use a novel self-inactivating lentiviral vector (CL20-4i-EF1 α -hyc-OPT) that has been designed to reduce the potential for insertional mutagenesis. In contrast to the Moloney Leukemia Virus (MLV) vectors that have been used in past trials, the lentiviral vector is self-inactivating (SIN) and contains no viral transcriptional elements such as the LTR enhancer that has been strongly implicated in the transformation events seen in the previous trials. A novel stable packaging cell line has also been developed that produces high quality, clinical vector preparations. These vector supernatants will be used to treat newly diagnosed SCID-X1 patients who lack a matched sibling donor, who are less than 12 months of age but are older than 3 months of age. Bone marrow CD34+ cells will be obtained in the operating room, transduced with the lentiviral vector, and reinfused without any myeloablative conditioning. Patients who do not show evidence for early T-cell reconstitution 16 (\pm 1) week after cell infusion will be considered an early failure and offered an allogeneic transplant. For all other patients, the primary endpoint assessing the efficacy of this approach will be T-cell immune reconstitution 52 weeks (\pm 2) weeks after transplantation. Continued and detailed evaluation of all aspects of immune reconstitution, protocol-related toxicity, and retroviral integration sites will also be performed. This study will evaluate the first use of a SIN lentiviral vector for the treatment of SCID-X1 and may lead to a new form of therapy that could be applied to the majority of newly diagnosed patients.

B. Summary of Protocol #0901-964

Drs. De Ravin and Sorrentino presented a summary of this protocol. The RAC reviewers discussed their concerns, with time allotted for responses, and the RAC posed additional questions.

XSCID results from defects in the *IL2RG* gene encoding the common gamma chain (γ_c) shared by receptors for Interleukin 2 (IL-2), IL-4, IL-7, IL-9, IL-15 and IL-21. At birth XSCID patients generally lack or have a severe deficiency of T-lymphocytes and NK cells, while their B-lymphocytes are normal in number but are severely deficient in function, failing to make essential antibodies. The severe deficiency form of XSCID is fatal in infancy without intervention to restore some level of immune function. The best current therapy is a T-lymphocyte-depleted bone marrow transplant from an HLA tissue typing matched sibling, and with this type of donor it is not required to administer chemotherapy or radiation conditioning of the patient's marrow to achieve excellent engraftment and immune correction of an XSCID patient. However, the great majority of patients with XSCID lack a matched sibling donor, and in these patients the standard of care is to perform a transplant of T-lymphocyte depleted bone marrow from a parent. This type of transplant is called haploidentical because in general a parent will be only half-matched by HLA tissue typing to the affected child. Whether or not any conditioning is used, haploidentical transplant for XSCID has a significantly poorer prognosis than a matched sibling donor transplant. Following haploidentical transplant, XSCID patients are observed to achieve a wide range of partial immune reconstitution and that reconstitution can wane over time in some patients. That subset of XSCID patients who either fail to engraft, fail to achieve adequate immune reconstitution, or lose immune function over time suffer from recurrent viral, bacterial and fungal infections, problems with autoimmunity, impaired pulmonary function and/or significant growth failure. Some patients with XSCID have mutations in the *IL2RG* gene that allow some γ_c function, where these hypomorphic mutations are associated with sufficient immune function to allow a child to survive early infancy, often leading to a late diagnosis of XSCID. XSCID patients with hypomorphic mutations associated with residual immune function (variant XSCID) often have sufficient immune function to prevent establishment of a clinically beneficial graft, but not enough immune function to prevent the problems of recurrent infection, autoimmunity, pulmonary dysfunction and growth failure.

This is a Phase I/II non-randomized clinical trial of *ex vivo* hematopoietic stem cell (HSC) gene transfer treatment for X-linked severe combined immunodeficiency (XSCID, also known as SCID-X1) using a self-inactivating lentiviral vector incorporating additional features to improve safety and performance. The study will enroll 12 research participants with XSCID who are between 1 and 20 years of age and who have clinically significant impairment of immunity. Research participants in Group A have persistent clinically significant defects in immunity despite prior haploidentical hematopoietic stem cell transplant.

Research participants in Group B have hypomorphic mutations of the *IL2RG* gene associated with clinically atypical XSCID characterized by delayed diagnosis of XSCID with survival past one year of age without having received a haploidentical transplant. Group A participants have failed the standard of care haploidentical transplant option available to XSCID patients who lack a matched sibling donor, and our current gene transfer treatment protocol from this perspective can be regarded as a salvage/rescue protocol for Group A. Although research participants in Group B have not received the haploidentical transplant treatment option, their hypomorphic mutation and age make it much more likely that they either will not engraft or will not achieve an adequate immune reconstitution from haploidentical transplant. For this reason, gene transfer treatment may have the potential to be superior to a first haploidentical transplant provided at greater than 1 year of age to Group B patients. Patients will receive busulfan 5 mg/kg/body weight to condition their bone marrow, and this will be followed by a single infusion of autologous transduced CD34⁺ HSC. Patients will then be followed to evaluate engraftment, expansion and function of gene corrected lymphocytes that arise from the transplant; to evaluate improvement in laboratory measures of immune function; to evaluate any clinical benefit that accrues from the treatment; and to evaluate the safety of this treatment. The primary endpoint of the study with respect to these outcomes will be at two years, though data relevant to these measures will be collected at intervals throughout the study and during the much longer term follow up required by regulation for patients participating in gene transfer clinical trials.

C. Background for Protocols #0901-963 and #0901-964

Eight RAC members voted for in-depth review and public discussion of Protocol #0901-963, and five RAC members voted for in-depth review and public discussion of Protocol #0901-964. Four RAC members provided written reviews of both of these two proposed trials.

For both protocols, Dr. Ertl explained her primary concerns as whether gene transfer would result in improved immune reconstitution compared with traditional stem-cell transplants for individuals who do not have a human leukocyte antigen (HLA)-matched sibling and whether the risk of the gene transfer is less than that of transplantation. Although the investigators had conducted and described an impressive array of preclinical studies, the data from these studies were not shown, and she requested that the investigators provide several specific sets of data from the mouse and dog studies. Dr. Ertl indicated that her primary safety-related concern was insertional mutagenesis and therefore requested additional information about assays that would show the reduced likelihood of the lentiviral vector causing insertional mutagenesis compared with the retroviral vectors used in prior trials. Regarding Protocol #0901-964, she asked whether the investigators would view the lack of circulating immunoglobulin or poor natural killer (NK) cell functions as an indicator of unsuccessful stem-cell transplant for these older children.

For both protocols, Dr. Fan noted that no literature citations or primary data were shown in the response to *Appendix M* of the *NIH Guidelines*; therefore, he recommended that the protocols should be revised to include appropriate literature references and presentation of critical data. He stated that the protocols were a logical and appropriate extension of previous gene transfer experiments for X-SCID; the data presented support testing the hypothesis that the new lentiviral vector CL20-4i-EF1 α -h γ _c-OPT will provide sufficient levels of γ _c expression and may have enhanced safety with respect to oncogenesis, and the inclusion of myeloreductive conditioning is reasonable. However, Dr. Fan requested that the investigators provide experimental data showing efficacy of the CL20-4i-EF1 α -h γ _c-OPT vector in γ _c -/- mice, data on testing the CL20-4i-EF1 α -h γ _c-OPT vector in the myeloid immortalization assay, and references to previous experiments on transducing macaque CD34⁺ cells. If only a few corrected stem cells will be sufficient for corrective benefit, he asked how this would affect the use of clonality measurements in monitoring the development of neoplasms. For Protocol #0901-963, he asked the justification for not using myeloreductive conditioning and for the choice of 16 weeks as the criterion for early failure of engraftment.

Dr. Kodish noted that both protocols and the informed consent documents were written clearly, detailing rationally and persuasively the approach to mitigating risk. He offered several suggestions for additional clarification of the informed consent document and a suggestion to decrease the risk of therapeutic

misconception by changing “gene therapy” to “gene transfer” and not using “treatment.” Dr. Kodish also suggested wording improvements in the informed consent document that would delineate the anticipated length of hospitalization after dosing and clarify when the search for a matched unrelated donor (MUD) would be initiated. For Protocol #0901-964, he offered three suggestions for improving the wording in the assent document and five suggestions for improving the wording in the consent document.

For both protocols, Dr. Somia asked whether the investigators had data resulting from dosing X-SCID-deficient mice with and without clinically relevant doses of busulfan pretreatment, particularly given that the investigators have argued that the X-SCID deficiency itself may be a genetic factor for tumors. He asked the investigators to provide additional preclinical data about the nature of the transduced vector in the safety studies. Regarding Protocol #0901-964, Dr. Somia requested comment about potential consequences if some donor marrow cells are still resident and could subsequently be transduced with CL20-4i-EF1 α -h γ c-OPT, resulting in cells overexpressing the γ c chain. He requested additional data and other details regarding the data from several models to test the safety of CL20-4i-EF1 α -h γ c-OPT and regarding the vector packaging cell line.

D. RAC Discussion of Protocols #0901-963 and #0901-964

During the meeting, the following additional concern was raised:

- Dr. Williams noted that research participants may appear to fail the gene transfer if evaluated at 12 weeks but do not fail if evaluated later at 16 weeks.

E. Investigator Responses

1. Written Responses to RAC Reviews of Protocols #0901-963 and #0901-964

The investigators presented data from myeloid transformation assays, Jurkat LMO2 activation assays, XSCID/Arf-null mice, XSCID dog transplant studies, and from CD34+ cell transduction studies from both normal subjects and XSCID patients. In preclinical models, the vector does result in immune reconstitution and correction of the X-SCID phenotype. However, with regard to tumor formation, the SCID/Arf mice model is not responsive to insertional mutagenesis and therefore not suitable for testing vector safety design elements.

A limitation of the myeloid immortalization assay is that it is associated only with a relatively modest degree of sensitivity for transformation. Transformation rates with the control SFFV vector are the highest in this system, but less so for other LTR vectors such as MSCV. Nonetheless, the complete absence of transformed clones seen with the lentiviral vector argues for its relative safety when compared to the transformation rates seen with either the MSCV or the SFFV vector. As Dr. Ertl suggested, attempts are being made to increase the sensitivity of this assay.

The overall definition of success for this gene transfer trial will be reconstitution of T cells, because research participants who have improved T-cell function are likely to experience direct clinical benefit; therefore, the main objective of this trial is to improve T-cell function with minimal risk. In the first group of participants, those who have improved T-cell function but whose B-cell and/or NK-cell functions are not improved would not be considered failures. Data on B-cell and NK-cell functions will be collected to determine the rate at which these immune parameters can be corrected.

The goal in designing this trial was to test the investigators' safety-modified lentiviral vector in nearly the same context as that used in the prior French and United Kingdom (U.K.) trials. Because both of these prior trials were conducted without any form of myeloablation and because most participants achieved significant degrees of immune reconstitution, the investigators retained lack of conditioning as a design feature. In addition, not using myeloreductive conditioning reduces the overall risk of this experimental protocol and reduces the chances of adverse interference with a subsequent allogeneic transplant, should one be necessary.

The investigators chose a 16-week time point as the criterion for early failure of engraftment based on an analysis of the rates of T-cell reconstitution seen in the prior French and United Kingdom trials. In both of these trials, the published data show that a substantial proportion of the participants began to show clinical signs of T-cell reconstitution in the interval between 12 and 16 weeks after cell infusion. Some participants took longer than 16 weeks to display signs of immune reconstitution. Therefore, the investigators considered the risk of performing an allogeneic transplant in an individual previously exposed to the risks of gene transfer, who otherwise could display significant immune reconstitution if the individual were allowed to wait 2 to 3 more weeks. The investigators stated that they believed this risk would be unacceptably high using a 12-week evaluation of T-cell reconstitution and that it would expose too many participants to the risk of allogeneic transplant at a time of imminent immune reconstitution due to gene transfer.

Because Protocol #0901-963 does not propose to include treating participants with any form of myeloreductive therapy, participation in this trial should not negatively affect a subsequent attempt at allogeneic transplant. The investigators will initiate a search for a MUD donor at the time of each participant's enrollment, so little to no time will be lost in identifying a donor for allogeneic transplant because identification of MUD donors can take 12 weeks or longer.

It is not possible to predetermine the length of time each research participant will remain hospitalized, because the length of time in the hospital will be determined by the attending transplant physician and the physician who follows all transplanted X-SCID patients. Participants who are well enough after the gene transfer could be followed closely on an outpatient basis, although many of them will require continuous IV therapy for infections and nutritional support. The investigators will alter the informed consent document to convey this information more clearly.

Based on available data from mouse studies and the human X-SCID gene transfer studies performed to date, there has been no evidence that overexpression of the γ chain will result in increased phosphorylation of its downstream associated tyrosine kinase JAK3. Although the γ chain is an essential subunit, it requires the complementary subunit for each respective cytokine receptor for signal transduction to occur. Therefore, because differential expression of the other subunits is required, most investigators have concluded that common γ chain expression is not rate limiting.

Using a variety of assays, the investigators have concluded that there is no detectable transmission of tTA genomes above the background level of their negative control and that there is no detectable stable human immunodeficiency virus *gagpol* transfer. They have not tested for transmission of *rev* or vesicular stomatitis virus glycoprotein sequences.

The investigators and sponsor agreed to address RAC reviewer comments regarding the informed consent document.

2. Responses to RAC Discussion Questions of Protocols #0901-963 and #0901-964

Dr. Malech explained that busulfan would be used in this protocol because of its potent effects on primitive hematopoietic progenitors and its lack of organ toxicity at reduced doses in nonmyeloablative regimens for hematopoietic stem-cell transplant. Studies in nonhuman primates show that low-dose busulfan provides a strong competitive advantage for the survival and proliferation of transplanted stem cells during suppression of endogenous hematopoiesis. The investigators propose using busulfan at 6 mg/kg total, which they believe is the highest level that can be used and probably not compromise the fertility of the research participant. In addition to busulfan, the investigators propose to add palifermin, a keratinocyte growth factor that is an FDA-approved agent to reduce mucositis.

For those potential participants who have a MUD donor available, Dr. De Ravin stated that the investigators plan to present the pros and cons of the gene transfer and the MUD transplant to the individual and the family in a nonbiased fashion, including a reminder that, for the MUD transplant, the patient would be exposed to subablative conditioning as well as the risk of graft-versus-host disease. The investigators will advise the individual and family to seek consultation from other transplant and

immunodeficiency centers; armed with available information, the potential participant and the family then can decide which course to pursue. The MUD transplant will be offered at the NIH or at another transplant center.

Regarding the level of gene transfer necessary for a therapeutic effect to take place, the investigators stated that animal model experiments in dogs showed that at least 1 million CD34 corrected cells per kilogram of body weight were needed to get reliable full immune correction. In humans, many published studies from the French and U.K. groups concluded that the few research participants who failed to achieve any benefit from the gene transfer received less than 1 million corrected cells per kilogram. Yet another study in humans found that even more cells produce superior results. Given these various findings, the investigators are targeting a minimal number of 1 million corrected cells per kilogram.

The investigators have chosen to use busulfan because there is much experience with this drug in children, including many long-term data about the risk of later malignancy. Using busulfan adds a malignancy risk; however, that risk is described in the literature and is considered acceptable in the transplant community. As to whether use of busulfan in the background of X-SCID would increase the malignancy possibility, Dr. Malech responded that the data to answer that question are probably available from SCID patients but have not been gathered from the various U.S. centers.

Dr. Malech stated that it would be possible to treat mice with lower doses of busulfan so that the mice survive and then follow them for several months to look for tumors. It would be an easy experiment to conduct that would provide informative data.

Dr. Conley reported that more than 100 patients with X-SCID have been dosed with busulfan at 16 mg/kg and that over the long term there have been no reports of T-cell malignancies in those patients.

F. Public Comment on Protocols #0901-963 and #0901-964

No public comments were offered.

G. Synopsis of RAC Discussion and RAC Observations and Recommendations

1. Protocol #0901-963

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

Preclinical Issues

- Of foremost importance in supporting the decision to move forward in a clinical trial using a SIN lentiviral vector for X-SCID is evidence that such vector is not expected to lead to insertional mutagenesis that could result in the development of leukemia as seen in two previous X-SCID trials using a MFG retroviral vector. Data already have been developed to support the safety of the SIN lentiviral vector. Nonetheless, additional studies should be considered that compare the safety of the SIN lentiviral vector to the MFG retroviral vector.
 - If possible, an animal model of insertional mutagenesis should be developed to allow for comparison of the leukemogenic potential of the SIN lentiviral vector to the MFG retroviral vectors.
 - The myeloid immortalization assay should be repeated and scaled up to provide additional comparison of the risk of insertional mutagenesis between the SIN lentiviral vector and the MFG retroviral vector. In addition, it would be of interest to compare the SIN lentiviral vector to the SIN version of the MFG retroviral vector.
- Data were presented on the immune reconstitution studies in the X-SCID dog model. The X-SCID

dog's response to phytohemagglutinin appeared to differ from the normal dog's response. An explanation of the difference and the SIN lentiviral vector's role in causing it should be submitted to OBA.

- The vector packaging cell line was developed in 293 T cells, which express the SV40 large T antigen. Some of the plasmids used to construct the packaging cell line contain the SV40 polyA sequence. Because homology exists between the SV40 large T antigen and sequences neighboring the vector, target cells should be tested for transfer of SV40 large T-antigen sequences.

Clinical/Trial Design Issues

- If there is no evidence of immune reconstitution and/or gene expression by 16 weeks after vector administration, the protocol states that an allogeneic stem-cell transplant will be offered. Preparations during the 16 weeks should be undertaken to minimize the delay in providing an allogeneic transplant.

2. Protocol #0901-964

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

Preclinical Issues

- Of foremost importance in supporting the decision to move forward in a clinical trial using a SIN lentiviral vector for X-SCID is evidence that such a vector is not expected to lead to insertional mutagenesis that could result in the development of leukemia as seen in two previous X-SCID trials using a MFG retroviral vector. Data already have been developed to support the safety of the SIN lentiviral vector. Nonetheless, additional studies should be considered that compare the safety of the SIN lentiviral vector to the MFG retroviral vector.
 - If possible, an animal model of insertional mutagenesis should be developed to allow for comparison of the leukemogenic potential of the SIN lentiviral vector to the MFG retroviral vector used in the clinical studies in which five children developed leukemia due to insertional mutagenesis.
 - The myeloid immortalization assay should be repeated and scaled up to provide additional comparison of the risk of insertional mutagenesis between the SIN lentiviral vector and the MFG retroviral vector. In addition, it would be of interest to compare the SIN lentiviral vector to the SIN version of the MFG retroviral vector.
- Data were presented on the immune reconstitution studies in the X-SCID dog model. The X-SCID dog's response to phytohemagglutinin appeared to differ from the normal dog's response. An explanation of the difference and the SIN lentiviral vector's role in causing it should be submitted to OBA.
- The vector packaging cell line was developed in 293 T cells, which express the SV40 large T antigen. Some of the plasmids used to construct the packaging cell line contain SV40 polyA sequence. Because homology exists between the SV40 large T-antigen sequences and sequences neighboring the vector, target cells should be tested for transfer of SV40 large T-antigen sequences.
- Although participants who have received busulfan preconditioning in gene transfer studies for adenosine deaminase-deficient (ADA)-SCID and chronic granulomatous disease have not developed cancer, there is a very small risk that busulfan can induce malignancy. Given the possibility that the common γ chain deficiency may be a genetic factor that contributes to tumor

development, the use of busulfan in the X-SCID population could elevate the risk of malignancy. Recognizing that it may be difficult to find an appropriate X-SCID animal model, preclinical studies of the potential oncogenic effect of busulfan should be undertaken.

Ethical/Legal/Social Issues

- The risk of infertility should be included in the assent document using age-appropriate language.

H. Committee Motion 4

Dr. Federoff summarized the comments and concerns of the RAC to be included in a letter to the investigators and the sponsor for each of the two protocols. Dr. Ertl moved (and no second was requested) that the RAC approve these summarized recommendations. The vote was 17 in favor, 0 opposed, 0 abstentions, and 0 recusals.

VIII. RAC Discussion of the X-SCID Recommendations/Dr. Corrigan-Curay

Dr. Corrigan-Curay led a brief discussion reviewing the RAC's X-SCID gene transfer recommendations in 2003 and 2005. She highlighted some proposed revisions, which the RAC members discussed. The conclusion was that having an HLA identical related match is an exclusion criterion for gene transfer, as is being younger than 3½ months. However, MUD transplants vs. gene transfer should be reviewed on a case-by-case basis in each protocol, because RAC members were less comfortable making categorical statements because of the complexities of MUD transplants. Those complexities included the use of ablative therapy and the length of time needed to identify a suitable MUD donor. It was generally agreed that the 3½-month threshold would be used; that time point was based on the data and the clinical experience provided by Rebecca H. Buckley, M.D., Duke University Medical Center, on Day 2 of the December 3-4, 2008, RAC meeting.

It was decided that RAC members should deliberate informally overnight about these proposed changes and discuss them further and vote on them during Day 2 of this RAC meeting.

IX. Day 1 Adjournment

Dr. Federoff adjourned Day 1 of the December 2008 RAC meeting at 5:40 p.m. on March 3, 2009.

X. Day 2 Call to Order and Opening Remarks

Dr. Federoff, RAC Chair, opened Day 2 of the March 3-4, 2009, RAC meeting at 8:15 a.m. on March 4, 2009.

XI. Biosafety Working Group Recommendations for the *NIH Guidelines: Human H2N2, Reconstructed 1918 H1N1, and Highly Pathogenic Avian Influenza (HPAI) H5N1 Viruses*

Presenter: Dr. Kanabrocki

A. Presentation

Dr. Kanabrocki reviewed the current status of biosafety guidance for research with the human H2N2, reconstructed 1918 H1N1, and HPAI H5N1 viruses provided in the *NIH Guidelines* and in the *BMBL* (fifth edition), discussed the charge and questions for the BWG, and presented the BWG's recommendations.

Currently, all influenza viruses are classified as RG2 agents under the *NIH Guidelines*. No distinction is made between potentially pandemic strains and potentially less dangerous influenza viruses.

There are four risk groups:

- RG1 agents are not associated with disease in the healthy adult human.
- RG2 agents are associated with human disease that is rarely serious and for which preventive and therapeutic interventions are often available.
- RG3 agents are associated with serious or lethal human disease for which preventive or therapeutic measures may be available; these agents are thought to represent high individual risk but no community risk.
- RG4 agents cause serious or lethal human disease for which preventive or therapeutic interventions are not usually available; these agents represent high individual risk as well as high community risk.

Containment levels are set approximately analogous to the RG levels, with containment levels 1 through 4 corresponding to RG1 through RG4, but the containment level can be raised or lowered depending on the comprehensive risk assessment conducted by the institutional biosafety committee (IBC).

Dr. Kanabrocki discussed the *BMBL* recommendations for research with these viruses. For human H2N2, increased caution is recommended due to the pandemic potential—BL3 containment plus several enhancements—although research with cold-adapted, live-attenuated H2N2 vaccine strains could continue to be managed at BL2. The *BMBL* urges extreme caution in managing fully reconstructed 1918 H1N1 due to the pandemic potential and recommends BL3 with several enhancements; research with large animals such as nonhuman primates should be housed in primary barrier systems in animal BL 3 (ABL3) facilities. In addition, fully reconstructed 1918 H1N1 is a Select Agent, and registration with the CDC Select Agent program is required for work with that virus. For HPAI H5N1 with the potential to infect humans, BL3 or ABL3 practices, facilities, and procedures should be followed, with respiratory protection enhancements; loose-housed animals should be kept in a BL3 facility. The U.S. Department of Agriculture (USDA) Animal and Plant Health Inspection Service (APHIS) regulates viruses that contain the hemagglutinin (HA) gene from HPAI H5N1 as Select Agents, and additional measures may be required according to the APHIS.

The recommendations of the BWG were as follows:

- *RG classifications.* Human H2N2, reconstructed 1918 H1N1, and HPAI H5N1 strains in the Goose/Guogdong/96-like H5 lineage (HPAI H5N1) should be classified as RG3.
- *General containment considerations.* Human H2N2, reconstructed 1918 H1N1, and HPAI H5N1 viruses should be contained using BL3 facilities, practices, and procedures, with specific enhancements for facilities, personal protective equipment, and practices and procedures. These enhancements include the use of powered air-purifying respirators and protective clothing.
- *Animal research.* Research on animals using human H2N2, reconstructed 1918 H1N1, and HPAI H5N1 viruses should be contained at BL3, with additional recommendations for work with HPAI H5N1.
- *Training of laboratory workers.* Proper training, periodic retraining and assessments, and reporting of all incidents are key elements. Recommendations include implementing methods to avoid inadvertent cross-contamination and maintain antiviral susceptibility.

- *Occupational health.* Key elements include developing a detailed occupational health plan, implementing an incident reporting system, distributing medical cards for laboratory workers, and mandatory reporting of symptoms.
- *Community risk.* Based on past history, human H2N2 and reconstructed 1918 H1N1 viruses represent a risk to the community. In case of a known laboratory exposure, treatment with appropriate antiviral agents should be implemented, the possibly infected person(s) should be isolated until infection is confirmed or refuted, and the local health department should be notified. HPAI H5N1 viruses represent less of a community risk because the virus is not efficiently transmitted from human to human; self-isolation should be required, antiviral prophylaxis should be offered, and the local health department should be notified.
- *Antiviral prophylaxis.* Preexposure prophylaxis with antiviral agents should not be mandated for work with 1918 H1N1. Antiviral agents for preexposure prophylaxis should be discussed with laboratory workers, including a discussion of the data on the safety of taking these drugs for 6- to 12-weeks, their ability to reduce the risk of clinical disease, and the limited data regarding protection of close contacts in the community. Antiviral agents for postexposure prophylaxis should be provided only after medical evaluation. The BWG does not recommend provision of home supplies in advance.
- *Lowering of containment.* Work with recombinant influenza viruses containing the H2N2 specific HA shall be conducted at BL3 with enhanced containment. Work with H2 HA gene in cold-adapted live-attenuated vaccine strains may be conducted at BL2 provided the segments with mutations controlling this cold-adapted attenuation are retained in the recombinant virus; the maintenance of this cold-adapted attenuation must be confirmed when a recombinant virus is generated. For work with HPAI H5N1 for nonselect agent research, if the influenza recombinant virus contains a majority of gene segments from the RG3 influenza virus, this recombinant should be managed with BL-3 enhanced unless in consultation with OBA staff a decision is made to lower containment. If a recombinant influenza virus contains a majority of gene segments from the RG2 influenza virus, the *NIH Guidelines* permit this research to be conducted at BL2; however, the IBC may raise containment based on their risk assessment. In deciding to lower containment, an IBC should consider whether in two animal models, there is evidence that a resulting influenza virus shows reduced replication and virulence at relevant doses compared with the RG3 parent virus.
- *Containment considerations for recombinants.* Work with recombinant viruses containing any genes or segments from 1918 H1N1 should be conducted at BL3 enhanced until there is an agreement on a set of experiments that can be used to demonstrate, prior to lowering containment, that such viruses are safe to work on at BL2. The identification of this set of experiments will be developed with input from the scientific community through a *Federal Register* comment period.

After approval by the RAC, the next steps for these recommendations are immediate implementation under the *NIH Guidelines* and publication in the *Federal Register* to obtain additional expert consultation and comment. OBA will revisit these recommendations as new information emerges.

B. RAC Discussion

Questions, concerns, and suggestions posed by RAC members included the following:

- Dr. Corrigan-Curay explained that OBA wants to implement these recommendations immediately to provide guidance. Although it is permissible to implement them immediately without *Federal Register* comments, OBA decided to ask for comments, particularly those that may help define the experiments that could be used to lower containment for certain recombinants with genes from the 1918 H1N1 virus. OBA also wants to provide additional opportunity for comment and input regarding experiments on the HPAI H5N1 viruses.

- Dr. Buchmeier noted the importance of maintaining perspective and flexibility, because it has become apparent that experts on influenza do not agree precisely on what conveys virulence or avirulence. There is likely to be additional input in the future on this issue, and to maintain objectivity, decisions should be evaluated and reached on a case-by-case basis.
- Dr. Kanabrocki explained that the intent of the BWG is to provide the opportunity for ongoing evolution of these guidelines and to seek continuing input from experts and the scientific community.

C. Public Comment

Dr. Swain, Agricultural Research Services, USDA asked whether protective suits and double shoe coverings are absolute requirements. After some discussion of the purpose of these recommended protections (including the use of tieback suits and complete removal of street clothes), Dr. Swain agreed to assist in drafting the appropriate language for this part of the recommendations.

D. Committee Motion 5

It was moved by Dr. Kanabrocki (and a second was not requested) to accept the recommendations of the BWG. The vote was 17 in favor, 0 opposed, 0 abstentions, and 0 recusals.

It was decided that additional details regarding the occupational health recommendations would be clarified through the BWG and that any such clarifications would not represent a substantive change in the approved recommendations.

XII. RAC Discussion of the X-SCID Recommendations/Dr. Corrigan-Curay

A. RAC Discussion

RAC members discussed proposed revisions to RAC recommendations, approved between 2005 and 2008, regarding retroviral gene transfer studies for X-SCID. Discussion centered on the lower age limit (3½ months) for including participants in gene transfer studies and various wording clarifications. Dr. Corrigan-Curay explained that the details on exclusion and inclusion regarding patients who might have a MUD donor would continue to be reviewed on a case-by-case basis. The RAC's original recommendations include a requirement that an individual would have failed haploidentical stem-cell transplantation to participate in gene transfer; however, that requirement may be less than optimal for older children because of lower efficacy of haploidentical transplantation compared to gene transfer.

Dr. Williams reminded the RAC that the 3½-month time point was based on the presentation by Dr. Buckley at the December 3-4, 2008, RAC meeting, in which she presented data based on chronological (not developmental) age after birth in relation to the success of haploidentical transplantation. Dr. Buchmeier suggested language such as "experience has proven that children older than 3½ months have a less favorable outcome from a haploidentical transplantation." Dr. Kodish added that the RAC does not want to completely exclude all children younger than 3½ months because it is important to retain the potential for clinical judgment by the physicians caring for these patients.

B. Final Recommendations

After discussion, the RAC crafted this final recommendation:

Gene transfer studies for X-linked SCID that propose to use integrating vectors that have been shown in preclinical studies to reduce the risk of insertional mutagenesis compared to the retroviral vectors used in the original X-SCID trials that led to leukemias should be reviewed, on a case-by-case basis, and should ordinarily exclude patients who:

- Have an HLA identical related donor available for stem-cell transplantation, as this remains first line therapy, or
- Are younger than 3½ months, who often have clinical improvement with haploidentical transplant.

Case-by-case review of the other inclusion and exclusion criteria would include an appropriate risk-benefit analysis accompanied by implementation of appropriate informed consent and monitoring plans. Further revision of this recommendation will be developed once more data on the leukemogenic potential of these vectors are developed in clinical investigations.

C. Committee Motion 6

It was moved by Dr. Yankaskas and seconded by Dr. Ertl to accept these revised recommendations. The vote was 17 in favor, 0 opposed, 0 abstentions, and 0 recusals.

XIII. Discussion of Human Gene Transfer Protocol #0901-967: A Phase I/IIa, Dose-Escalation, Safety, Pharmacokinetic, and Preliminary Efficacy Study of Intraperitoneal (IP) Administration of DTA-H19 in Subjects with Advanced-Stage Ovarian Cancer

Principal Investigator:	George Coukos, M.D., Ph.D., University of Pennsylvania
Additional Presenters:	Lana Kandalaft, Pharm.D., Ph.D., University of Pennsylvania; Janet H. Ransom, Ph.D., Fast-Track Drugs & Biologics, LLC
Sponsor:	BioCancell Therapeutics, Inc.
RAC Reviewers:	Ms. Shapiro, Dr. Williams, and Dr. Yankaskas

Drs. Ertl and Kodish recused themselves from consideration of this protocol due to conflicts of interest.

A. Protocol Summary

Ovarian cancer is the fifth most common cancer in women, with about 1 in 55 women developing an ovarian malignancy during her lifetime. There are approximately 21,650 new cases diagnosed annually in the United States, with 15,520 deaths estimated in 2008, making ovarian cancer the most common cause of death from gynecologic malignancy. Epithelial ovarian cancer comprises a majority of the ovarian malignancies and typically occurs in women between the ages of 40 and 65 years. Unfortunately, 60 percent to 70 percent of patients present with stage III or stage IV disease, with an associated 5-year survival rate of less than 25 percent. Improvements have been made in recent years in the treatment of advanced epithelial ovarian cancer, but the single most common outcome is tumor reduction following chemotherapy and surgery, followed by relapse and eventual death from progressive disease. New approaches are needed to improve the outcome of these women.

The investigators hypothesize that incorporation of DTA-H19, a plasmid that carries the gene for the diphtheria toxin A chain (DT-A) under the regulation of the H19 promoter sequence will delay ovarian cancer disease progression by selectively destructing tumor cells. The investigational agent, BC-819, (DTA-H19) is a plasmid that carries the gene for the DT-A chain under the regulation of the H19 promoter sequence. DT-A chain expression is triggered by the presence of H19 transcription factors upregulated in tumor cells. The selective initiation of toxin expression results in selective tumor cell destruction via inhibition of protein synthesis in the tumor cell, enabling highly targeted cancer treatment. DTA-H19 is being developed for the treatment of cancers that have upregulated levels of H19 expression. One of the first indications under development is ovarian cancer. In all clinical trials, patient's tumors must test positive for H19 ribonucleic acid (RNA) in order to be eligible for treatment. H19 is an oncofetal gene that encodes only RNA and no protein. It appears to play a role in promoting cancer progression, angiogenesis, and metastases. Expression of H19 has been shown in many cancers including ovarian, but not in surrounding normal tissues. *In vitro* studies support the mechanism of action of DTA-H19 where

protein inhibition by DTA chain under the regulation of the H19 promoter occurs only in tumor cells that express H19 RNA regulatory sequences. This is because tumor cells upregulate transcription factors that drive H19 expression, and these also enable expression of DTA-H19.

Intraperitoneal (IP) administration of DTA-H19 has the potential to reach ascites tumor cells and deliver its intracellular toxin without targeting normal tissues, which would help reduce tumor burden and fluid accumulation, improve the individual's quality of life, and prolong the individual's life.

B. Written Reviews by RAC Members

Eleven RAC members voted for in-depth review and public discussion of this protocol. Key issues included the potential safety issues raised by expressing a highly lethal toxin molecule as an antitumor agent and selective expression being dependent on H19 being present only in the target tumor cells and not in normal cells. Three RAC members provided written reviews of this proposed trial.

Ms. Shapiro asked the investigators to provide additional information about the person proposed as medical monitor and the timing of the AE reviews to be conducted by that individual. She noted that the informed consent document included many words such as "treatment" that raise concerns about therapeutic misconception.

Given that thrombocytopenia and anemia were seen as toxicities in the compassionate-use setting of DTA-H19, Dr. Williams requested a more detailed analysis to assess whether low-grade disseminated intravascular coagulation or marrow suppression are potential toxicities. He asked whether the investigators had performed toxicology studies in rats or mice regarding antibody development and requested that the number of animals used in these preclinical studies be specified. Dr. Williams asked about the analysis of expression of the transgene construct in normal tissues and whether any studies had directly examined apoptosis and/or growth of cells after transfection and expression of DTA-H19. Given that plasmid has been detected in the blood after injection in test animals and with compassionate use in humans, Dr. Williams asked whether the investigators were concerned about rapidly dividing nonmalignant cells, particularly blood vessels during wound healing.

Dr. Yankaskas asked whether the investigators had assessed H19 expression in blood leukocytes or any other normal tissues and whether the pharmacokinetic (PK) studies of ascites fluid and blood would be evaluated for plasmid expression in tumor as well as nontumor cells. He asked about the fate of the DTA-H19 plasmid that is not incorporated into tumor cells and whether its presence and effect should be assessed in blood leukocytes or other cells at additional intervals after the PK studies have been completed. Dr. Yankaskas requested clarification of the platelet count and hemoglobin exclusion criteria in the informed consent document and elsewhere in the protocol.

C. RAC Discussion

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

- Dr. Fan asked about the percentage of cells expressing the experimental molecule.
- Dr. Bartlett asked about ways to develop *in vitro* assays that would allow testing of the ascites cells to correlate with the clinical response.

D. Investigator Response

1. Written Responses to RAC Reviews

Regarding development of antibodies to the plasmid, the investigators explained that DNA plasmids administered by the planned route of administration are not expected to be immunogenic. The repeat-dose toxicokinetics studies performed in mice and rats suggest no evidence for antibody formation. In these studies, the plasmid was administered either IV or IP, and PK samples were collected after the first

and sixth doses of plasmid. If antibodies had formed, it would be expected that plasmid clearance would have been faster. In addition, there is no concern for antibodies to DT-A chain, as it is expressed intracellularly at its site of action, and if antibodies had developed, these would only accelerate extracellular removal of the toxin from the blood stream. Although there is no plan to test for antibody development to the plasmid in the proposed clinical trial, the investigators plan to evaluate PK after the first and fourth IP infusions of plasmid. Therefore, it will be possible to determine whether the kinetics of plasmid in the blood has changed, indicating altered clearance, which would indicate the presence of antibodies that could interfere with plasmid delivery.

In the rat and mouse toxicology studies, standard hematology parameters were evaluated. At a dose tenfold higher than the starting dose planned for the clinical trial, there were no clinically significant changes in any of those parameters. These data suggest that neither low-grade disseminated intravascular coagulation (DIC) nor marrow suppression is a potential toxicity. One of the 18 participants with superficial bladder cancer who were administered DTA-H19 intravesically in the Phase I/IIa clinical trial had a single and transient incident of mild-grade thrombocytopenia and mild-grade anemia. There was no evidence of deep vein thrombosis, DIC, or bone marrow suppression in any of these participants, nor have there been reports of any of these adverse effects in the ongoing Phase IIb bladder cancer trial. The only case of thrombocytopenia and anemia in the compassionate-use participants was in one individual with a liver metastasis—with low platelet counts and hemoglobin fluctuation once in 20 evaluations. The investigators did not consider the mild and transient changes in clinical laboratory measures in the absence of any symptomatic evidence of coagulation problems to suggest a need for clinical laboratory monitoring other than that already proposed in the study protocol.

To study DTA-H19 toxicity in H19 expressing cells versus H19 non-expressing cells, an *in vitro* model using luciferase RNA and protein expression was evaluated. In this model, the cell-specific expression driven by the H19 promoter region was demonstrated in human bladder carcinoma cells, T24P, but not in human fibroblast cells, IMR-90. The investigators also concluded that the collective data from rodent models and humans indicate that DTA-H19 causes minimal toxicity in normal tissues, including in organs with highly proliferative cell compartments such as bone marrow or intestine.

The starting dose in the proposed clinical study is 60 mg, which translates to a 1.0-mg/kg dose in a 60-kg human. As the no-observable-adverse-effect levels in mice and rats were greater than 10 mg/kg, this is a safety margin of at least tenfold. Given the lack of toxicity at a 10-mg/kg dose in mice and rats and the safety in the compassionate-use clinical trials, the proposed starting dose is considered reasonably safe for IP administration in research participants with ovarian cancer.

Regarding concerns about rapidly dividing nonmalignant cells in blood vessels during wound healing, the investigators explained that the intracompartamental administration approaches to deliver the maximal concentration of plasmid to the tumor sites are expected to minimize exposure of normal cells to the plasmid and minimize possible uptake. No transfection enhancer will be added to the plasmid, which also should minimize exposure of other cells via transient circulating levels of plasmid. Given that the study population consists of ovarian cancer patients who are refractory to other standard therapies, have ascites fluid accumulation and associated painful side effects, have a poor quality of life, and expect minimal long-term survival, the risk-benefit ratio that was observed in the compassionate-use ovarian cancer patient is considered by the investigators to be acceptable.

In response to a question about the fate of any DTA-H19 plasmid not incorporated into tumor cells, the investigators explained that tissue samples from the rat and mouse toxicology studies were collected and are being stored frozen for possible future biodistribution studies. Collection and storage of tissues were recommended specifically by the FDA as part of the toxicology studies. In the event that unexpected toxicities are observed in the clinical trials, evaluation of these stored tissues is an option and will be discussed with the FDA. In the compassionate-use trial, the PK of the plasmid in blood and urine was evaluated via PCR, and detectable levels of plasmid were found in the blood for the first 48 hours; after that initial period, levels of plasmid were undetectable, and plasmid was excreted in the urine.

Regarding tests for apoptosis, Dr. Ransom explained that *in vitro* studies of typical apoptotic cell assay outcomes do not seem to be sensitive in the cell culture conditions used. Therefore, the best evidence for apoptosis is for *in vivo* intratumoral administrations and focal necrosis at the injection sites, both of which demonstrate the toxicity of DTA-H19.

Dr. Coukos explained that the purpose for which this drug is proposed is palliation of ascites. The main reason for morbidity in these patients is because of the ascites. Ascites creates overwhelming pressure in the abdomen, reduces the ability to breathe properly, and paralyzes the entire gastrointestinal tract so that patients stop eating and become emaciated. By reducing the burden of ascites, patients are given a chance for prolonged survival and improved quality of life. It is not the purpose of the IP injection to treat the disease but to focus on the palliative aspects of reducing the ascites. However, many doctors who treat ovarian cancer believe that taking care of the ascites problem provides a new opportunity for therapeutic interventions in these patients, including more time to try more drugs or to try more innovative approaches and surgery.

Dr. Ransom stated that the ovarian cancer compassionate-use patient had high levels of H19 expression and that H19 appeared to be ubiquitously expressed over all the tumor cells that were examined. BioCancell has developed a grading system that is still being perfected to look at expression patterns and clinical outcomes in these studies to understand the type of staining patterns that might be predictive of clinical response.

The investigators and sponsor agreed to address all of the RAC reviewer comments regarding the informed consent document.

2. Responses to RAC Discussion Questions

Regarding expression of H19 in normal tissue, Dr. Coukos explained that, in the ovarian study, H19 was detected by *in situ* hybridization only in the tumor cells and not in the associated leukocytes in the tumor and the ascites. In the liver, H19 was expressed by the tumor and at very low levels by desmoplastic reactions surrounding the tumor, but not in normal hepatocytes distant from the tumor. Following intrahepatic or intratumoral injection of the plasmid, liver function tests were mildly and transiently elevated, indicating no substantial hepatic toxicity. In bladder biopsies where tumor and normal tissues were present, H19 was documented in the tumor but not in adjacent normal tissue. Direct tissue-related studies in which tumor and normal tissues were next to each other in humans clearly demonstrate a sharp contrast between the expression of H19 in normal tissue vs. tumor tissue.

The investigators believe that the IP approach to treatment will protect sites that are distant from the injection site, because the amount of H19 that would reach distant sites should be low.

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 in-depth review and public discussion:

Preclinical Issues

- The safety of this approach is based on selective expression of DTA in cells that express H19. Data presented to document the lack of H19 expression in normal cells consisted largely of the expression of luciferase under transcriptional control of an H19 promoter. To verify further the lack of H19 expression, the development of a more specific assay for H19, for example, by reverse transcriptase PCR, should be considered.

- It would be prudent to gather additional data on the selective expression of the H19 promoter in the plasmid, as is predicted by endogenous expression, to document further the specificity of expression of the H19 promoter.
- The vector will be delivered by an IP catheter that may be in place for several months or more, and there is a risk of infection at the site of the catheter or from other sources. Since inflammation in tissues is known to upregulate H19, additional studies should be considered to assess the potential impact of the vector on wound healing.

Clinical/Trial Design Issues

- The preclinical data do not show a uniform correlation between H19 expression in cells and DTA expression by the plasmid. Therefore, the clinical response seen in the Phase I bladder cancer trial and, more to the point, in the compassionate-use patient with ovarian cancer, cannot necessarily be attributed to DTA expression. As the protocol moves forward, specific assays should be developed to assess whether clinical response correlates with expression of DTA in the target cells. This would provide additional evidence of transgene expression.

G. Committee Motion 7

Dr. Federoff summarized the comments and concerns of the RAC to be included in a letter to the investigators and the sponsor for each of the protocols. No formal motion was made, and no second was requested, but Dr. Federoff asked that the RAC approve these summarized recommendations. The vote was 14 in favor, 0 opposed, 0 abstentions, and 2 recusals.

XIV. Closing Remarks and Adjournment

Dr. Federoff thanked the RAC members and OBA staff and adjourned the meeting at 11:30 a.m. on March 4, 2009.

[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.

These Minutes will be formally considered 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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Attachment I Recombinant DNA Advisory Committee Roster

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Marshall E. Bloom, RML/NIAID, NIH (*via teleconference*)
John M. Centanni, Stratatech Corporation
Allen R. Comer, Stratatech Corporation
Mary Ellen Conley, St. Jude Children's Research Hospital
George Coukos, University of Pennsylvania
Suk See De Ravin, NIAID, NIH
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Nicholas Muzyczka, University of Florida (*via teleconference*)
Stuart T. Nichol, CDC (*via teleconference*)
Janet H. Ransom, Fast-Track Drugs & Biologics, LLC
Pierre Rollin, CDC (*via teleconference*)
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Carolyn Wilson, FDA, DHHS
Darren Wolfe, Diamyd, Inc.
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Attachment III Abbreviations and Acronyms

<i>A. baumannii</i>	<i>Acinetobacter baumannii</i>
AAV	adenoassociated viral, adenoassociated virus
ABSL	animal biosafety level
AE	adverse event
APHIS	Animal and Plant Health Inspection Service, USDA
BEAP	Biological Exposure Assessment Program, RML
<i>BMBL</i>	<i>Biosafety in Microbiological and Biomedical Laboratories</i> manual (fifth edition)
BMT	bone marrow transplant
BSL	biosafety level
BL2	biosafety level 2
BWG	Biosafety Working Group (a subcommittee of the RAC)
CDC	Centers for Disease Control and Prevention, DHHS
cDNA	complementary deoxyribonucleic acid
CMV	cytomegalovirus
DHHS	U.S. Department of Health and Human Services
DIC	disseminated intravascular coagulation
DNA	deoxyribonucleic acid
<i>E. coli</i>	<i>Escherichia coli</i>
FDA	Food and Drug Administration, DHHS
GAD	glutamic acid decarboxylase
HA	hemagglutinin
hCMV IEp	human CMV immediate early promoter
HLA	human leukocyte antigen
H5N1	highly pathogenic avian influenza
HSV	herpes simplex virus
IBC	institutional biosafety committee
IP	intraperitoneal
IRB	institutional review board
IV	intravenous, intravenously
LAP2	latency associated promoter
MUD	matched unrelated donor
NIAID	National Institute of Allergy and Infectious Diseases, NIH
NIH	National Institutes of Health
<i>NIH Guidelines</i>	<i>NIH Guidelines for Research Involving Recombinant DNA Molecules</i>
NK	natural killer
OBA	Office of Biotechnology Activities, NIH
OD	Office of the Director, NIH
PCR	polymerase chain reaction
pfu	plaque-forming unit
PI	principal investigator
PK	pharmacokinetic
RAC	Recombinant DNA Advisory Committee
RG	Risk Group
RML	Rocky Mountain Laboratories
RNA	ribonucleic acid
SIN	self-inactivating
U.K.	United Kingdom
USDA	U.S. Department of Agriculture
X-SCID	X-linked severe combined immunodeficiency