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**RECOMBINANT DNA ADVISORY COMMITTEE**

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**Minutes of Meeting**

**September 9, 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 <http://oba.od.nih.gov/oba/index.html>]*

**U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES  
NATIONAL INSTITUTES OF HEALTH  
RECOMBINANT DNA ADVISORY COMMITTEE  
Minutes of Meeting<sup>1</sup>**

September 9, 2009

The Recombinant DNA Advisory Committee (RAC) was convened for its 118<sup>th</sup> meeting at 8:15 a.m. on September 9, 2009, at the Hilton Hotel and Conference Center, Rockville, Maryland. Dr. Howard Federoff (Chair) presided. In accordance with Public Law 92-463, the meeting was open to the public from 8:15 a.m. until 4:20 p.m. on September 9. The following individuals were present for all or part of the September 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/University of Pennsylvania  
Howard J. Federoff, Georgetown University Medical Center  
Jane Flint, Princeton University (*via teleconference*)  
Jeffrey P. Kahn, University of Minnesota  
Joseph A. Kanabrocki, The University of Chicago  
Louis V. Kirchhoff, University of Iowa  
Eric D. Kodish, The Cleveland Clinic Foundation  
Bernard Roizman, The University of Chicago  
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

**Office of Biotechnology Activities (OBA)**

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

**Ad Hoc Reviewer**

James M. Church, The Cleveland Clinic Foundation

**Nonvoting Agency Representatives**

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

**NIH/OD/OBA Staff Members**

Linda Gargiulo  
Bob Jambou  
Laurie Lewallen  
Maureen Montgomery  
Marina O'Reilly  
Gene Rosenthal  
Tom Shih  
Mona Siddiqui

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<sup>1</sup> The Recombinant DNA Advisory Committee is advisory to the National Institutes of Health (NIH), and its recommendations should not be considered as final or accepted. The Office of Biotechnology Activities should be consulted for NIH policy on specific issues.

## **Attendees**

There were 34 attendees at this 1-day RAC meeting.

## **Attachments**

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

## **I. Call to Order and Opening Remarks**

Dr. Federoff, RAC Chair, called the meeting to order at 8:15 a.m. on September 9, 2009. Notice of this meeting under the *NIH Guidelines for Research Involving Recombinant DNA Molecules (NIH Guidelines)* was published in the *Federal Register* on August 19, 2009 (74 FR 41914). Issues addressed by the RAC at this meeting included a report from the Gene Transfer Safety Assessment Board (GTSAB, a subcommittee of the RAC), public review and discussion of three protocols, a report from the FDA representative regarding a recent guidance issued by the International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use titled “General Principles To Address Virus and Vector Shedding”, and points to consider regarding applications to lower containment for cloning Risk Group 4 Mononegavirales (Marburg, Nipah, and Hendra viruses) in non-pathogenic *Escherichia coli* (*E. coli*).

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

## **II. Minutes of the June 16-17, 2009, RAC Meeting**

RAC Reviewers: Drs. Buchmeier and Roizman

Dr. Buchmeier provided a summary of the presentations and discussions at the June 2009 RAC meeting. Dr. Buchmeier and Dr. Roizman noted that the minutes document was accurate.

### **A. Committee Motion 1**

Approval of the June 16-17, 2009, RAC meeting minutes was moved by Dr. Buchmeier and seconded by Dr. Roizman. The RAC voted unanimously by a show-of-hands vote to approve the June 16-17, 2009, RAC meeting minutes.

## **III. International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use: General Principles To Address Virus and Vector Shedding**

Presenter: Dr. Takefman

### **A. Presentation**

Dr. Takefman provided background on the International Conference on Harmonisation (ICH) and its Gene Therapy Discussion Group (GTDG). Dr. Takefman explained that the FDA is trying to raise awareness of activities related to international harmonization of gene therapy products and, in particular, is eliciting comments on a recent document on viral/vector shedding. He noted that considerable disharmony remains regarding the conduct of shedding studies, as a number of countries still require patient isolation until multiple negative results have been obtained, even for replication-incompetent viruses.

The ICH was created in 1990 as an agreement between the European Union, Japan, and the United States to harmonize different regional requirements for registering pharmaceutical drug products. These three regions are the core members of ICH; once an ICH guideline is formalized, it becomes FDA guidance. This group is a joint effort by regulators and associated pharmaceutical trade associations; from the United States there are representatives from the FDA and the pharmaceutical industry, and the ICH also includes those U.S. counterparts in the European Union and Japan. In addition to the core voting members of the ICH are various GTDG observers from Health Canada, the World Health Organization, China, and Swissmedic.

The GTDG has been discussing gene therapy topics at the ICH since 1999 and has been an official group since 2001; the goals of this group have been to monitor emerging scientific issues in the field of gene therapy, to proactively set out principles that may have a beneficial impact on harmonization, and to ensure that the outcomes of the GTDG are well understood and widely disseminated. The public ICH Web site includes a GTDG section; after each GTDG meeting, a summary is placed on the Web site in the form of a public communication paper. Recently, the focus of the GTDG has been on writing ICH considerations. Because gene therapy is a rapidly evolving field, it has been difficult to write ICH guidelines on gene therapy topics; therefore, the GTDG believes that consideration papers are a way to proactively set out principles that may have a beneficial impact on harmonization.

Three ICH considerations documents have been published in recent years, including general principles to address the risk of inadvertent germline integration of gene therapy vectors, oncolytic viruses, and the most recent document on viral/vector shedding in June 2009. Currently no FDA guidance addresses these three issues.

Viral or vector shedding is defined as excretion and/or secretion outside the body (e.g., in urine, feces, saliva, etc.). The considerations document provides recommendations on how to design nonclinical and clinical shedding studies, with an emphasis on analytical assays to be used, along with recommendations regarding study interpretation. Shedding studies should be conducted to address potential public health concerns related to the potential risk of transmission to a third party. Although of concern, issues related to the environment have been excluded from the scope of the document. The FDA believes that this document will be helpful in addressing issues regarding the licensure of gene therapy products.

Dr. Takefman reviewed the specifics of this considerations document, including the property of the parental strain from which the virus/vector was derived, replication competence, and any altered tropism or tissue specific replication. Quantitative analytical assays may include PCR to detect nucleic acids and infectivity assays on shed viral samples. Dr. Takefman also reviewed the conduct of nonclinical and clinical shedding studies, sampling frequency and duration, relevance of animal models, route of administration as it relates to sample collection, third party transmission, and study interpretation.

The GTDG intends to formalize this considerations document into an ICH guideline. Noting that formal ICH guidelines eventually become FDA guidance, Dr. Takefman requested that comments from the RAC be provided to Dr. Corrigan-Curay or to Dr. Takefman, or via the ICH Web site ([www.ich.org](http://www.ich.org)); comments on the document as well as additional considerations are welcome, especially from U.S. researchers with significant experience with virus and vector shedding studies.

## **B. RAC Discussion**

The following questions, concerns, and issues were raised:

- Dr. Federoff asked about the timeframe for comments, to which Dr. Takefman responded that the first week in October 2009 would provide enough time to get those comments to the GTDG, which is meeting at the end of October. He noted that quite a few comments have already been received from NIH investigators, who represent that majority of the researchers conducting these studies.

- Dr. Buchmeier asked about the status of screening of stem cell lines in preparation for stem cell trials, noting that currently such research is not reviewed by the RAC. Dr. Takefman responded that the FDA and the NIH are planning a workshop on stem cells specifically in response to the recent *NIH Guidelines for Human Stem Cell Research*. Stem cells would come to the RAC if they are proposed to be transduced with a gene transfer product, even if that product is not intended to be therapeutic.
- In response to Dr. Williams query, Dr. Takefman clarified that the mission of the ICH is to harmonize at the level of pharmaceutical drug products. Harmonization at the clinical trial level would be extremely difficult, especially since there is a considerable amount of disharmony within the European Union.
- Dr. Federoff asked whether the ICH anticipates the need for followup to revisit the nature and stringency of the guidelines as more data is collected that might be representative of a class of viruses or vectors. Dr. Takefman responded that ICH guidelines can be revised; they are intended to be living documents. He noted that this topic (viral/vector shedding) was chosen not necessarily because of the harmony among ICH regions but because the GTDG believed enough data existed in the field such that the provision of useful guidance would not be outdated in one year.

#### **IV. Points To Consider Regarding Applications To Lower Containment for Cloning Risk Group 4 Mononegavirales cDNA into *E. coli***

Presenter: Dr. Corrigan-Curay

##### **A. Presentation**

Dr. Corrigan-Curay reviewed the mononegavirales, which are nonsegmented, negative sense, single-stranded RNA viruses that include Ebola and Marburg viruses of the family *Filoviridae* and Nipah and Hendra viruses of the family *Paramyxoviridae*. The Institutional Biosafety Committee (IBC) of the Rocky Mountain Laboratories, National Institute of Allergy and Infectious Diseases, had contacted OBA to request lowering containment to biosafety level (BL) 2 for cloning of the cDNA from these Risk Group (RG) 4 viruses into *E. coli*.

The RAC's Biosafety Working Group had met several times to consider the related issues, and the full RAC had discussed this request at the March 2009 and June 2009 RAC meetings. The RAC's conclusions were:

- Biosafety: Given the biological properties of these RG4 agents, BL2 is appropriate for cloning of the full-length cDNA of Ebola, Marburg, Hendra, or Nipah viruses into non-pathogenic strains of prokaryotes such as *E. coli*.
- Biosecurity: Research with these RG4 agents at BL2 raises biosecurity concerns and therefore additional biosecurity provisions must be in place for such work.

Based on the RAC's recommendations, OBA advised the Rocky Mountain Lab's IBC and the principal investigator (PI), Dr. Heinz Feldmann, that lowering containment to BL2 for work with the cDNA of Ebola, Marburg, Nipah, and Hendra in non-pathogenic *E. coli* could be considered providing a variety of biosafety and biosecurity conditions were met, many of which had already been proposed by Dr. Feldmann.

Because OBA might receive similar requests in the future, Dr. Corrigan-Curay noted that OBA would review future requests in consultation with the RAC as needed, that OBA approval to allow an IBC to lower containment for such research to BL2 would be specific to a PI at a specific institution, and that an IBC is not required to lower containment based on OBA's assessment. Dr. Corrigan-Curay stated that information required for submitting requests would be posted on the OBA Web site.

## B. RAC Discussion

The following question was raised:

Dr. Buchmeier asked whether the Select Agent status of these DNAs would change as a result of the lowering of biosafety requirements, also noting that the Select Agent rules are the province of the Centers for Disease Control and Prevention. Dr. Corrigan-Curay responded that the DNA of this family of viruses is not a select agent, only the viruses.

## V. Discussion of Human Gene Transfer Protocol #0907-991: A Phase I Study of an IL-2 Expressing, Attenuated *Salmonella enterica typhimurium* in Patients with Unresectable Hepatic Spread from Any Non-Hematologic Primary Cancer

Principal Investigator:	Edward W. Greeno, M.D., University of Minnesota
Additional Presenter:	Lance Augustin, Ph.D., M.S., University of Minnesota
Sponsor:	Daniel Saltzman, M.D., Ph.D., University of Minnesota
RAC Reviewers:	Drs. Kirchhoff, Williams, and Yankaskas

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

### A. Protocol Summary

It is estimated that 130,000 new cases of colorectal carcinoma occur in North America each year. Of these patients, 40 percent to 50 percent will experience a recurrence within 5 years. Furthermore, it is known that 75 percent to 80 percent of patients with a recurrence will have the liver as one of the involved sites for metastasis, with 15 percent to 20 percent having the liver as the only site of failure. Surgical excision of the hepatic metastases is the only potential for cure in these patients. Unfortunately, when a diagnosis of hepatic metastases is established, the majority of these patients have unresectable disease. Unresectable metastatic carcinoma of the liver – regardless of the primary site – continues to have a very poor prognosis despite recent advances with chemotherapeutic and radiotherapeutic strategies, chemoembolization, radiofrequency ablation, and cryotherapy.

This Phase I clinical trial is proposed to determine the highest nonharmful oral dose of an investigative new drug (IND) for treatment of cancer that has metastasized to the liver. The agent is a strain of attenuated *Salmonella enterica typhimurium* (*S. enterica*) that expresses truncated human interleukin-2 (IL-2) (–SalpIL2). The bacteria have been genetically engineered to diminish the ability to cause illness and still survive inside the body long enough to colonize tumors. Significant efficacy was demonstrated in extensive preclinical studies involving oral administration of SalpIL2 in mouse models of human cancer, without significant toxic side effects.

This dose escalation study has a primary endpoint of safety of human administration. Up to 18 research participants will be enrolled – 3 at each of five dose levels, plus an additional 3 at the maximum tolerated dose. Enrollment is expected to occur at a rate of 1 to 2 participants per month. The ultimate goal of studying this IND in humans is to induce or enhance an antitumor immune response due to SalpIL2 localization and expression of IL-2 protein in hypoxic regions of established tumors.

### B. Written Reviews by RAC Members

Five RAC members voted for in-depth review and public discussion of this protocol. Key issues included the use of a novel attenuated strain of *S. enterica* (clinical experience with attenuated strains of *S. enterica* to treat malignancies has been limited) and the need for more indepth discussion of the ability of this orally delivered agent to primarily target the tumor cells and the potential for off-target expression of IL-2.

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

Dr. Kirchhoff asked the investigators to explain why they chose to use a clone with a truncated IL-2 coding sequence rather than continuing the molecular work until a recombinant plasmid encoding a full-length IL-2 was obtained. He stated that the use of the truncated IL-2 raises two issues: (1) in all work done with the truncated IL-2, comparisons with the research results of other investigators who use full-length IL-2 will be difficult because the two molecules are so different; and (2) if no significant antitumor effect is observed, the investigators will be left with the question of whether the result would have been different had full-length IL-2 been encoded by the plasmid. In addition, Dr. Kirchhoff suggested that the general preventive goal of this protocol should be that the study bacteria not be transmitted from the participants to anyone else, and therefore the protocol should be revised to indicate that participants would be instructed not to prepare food for anyone. In view of the fact that persons with advanced cancer may not be immunologically normal, Dr. Kirchhoff asked the investigators to explain what convinces them that the study participants will not become infected with SalpIL2 and how they will be monitored for this potentially serious outcome. Noting that the basic criterion for entry into this study will be the presence of a solid tumor at any site that has metastasized to the liver and that tumors arising in different organs differ greatly, Dr. Kirchhoff suggested that the investigators might want to focus on participants with a single tumor type.

Dr. Williams asked about the risks of disseminated and uncontrolled disease if a participant has an attenuated immune system since SalpIL2 appears to be able to multiply intracellularly, how the investigators will ensure that research participants do not spread the attenuated bacteria to other individuals, how participants who become chronically infected and shed bacteria would be treated, and what effect development of antibodies to IL-2 might have on a participant's immune system. As the C-terminal truncation appears to have been derived unintentionally during construction of the rescue plasmid, Dr. Williams requested that the investigators provide data showing that this truncated protein encodes an IL-2 activity, since the assays in the protocol are indirect. He asked that the protocol focus more precisely on the tumor types anticipated to be encountered in the potential participants. Dr. Williams requested additional information about the mechanism of the large effect on neuroblastoma of SalpNG (bacteria not expressing IL-2) as well as a prediction of whether the gastroenteritis seen in test mice might also be encountered in humans. In addition, he asked for clarification of why the adverse events seen in mice (e.g., a twofold increase in platelet counts and leukocytes, and two mice that developed meningitis and cerebritis) were not considered by the investigators when they declared that no adverse events were encountered.

Dr. Yankaskas asked how the investigators propose to evaluate the benefits derived from expression of truncated IL-2 and its effects on lymphocytes and other cells, how the effects of the truncation on clinical benefits and adverse events would be determined, and how the replication capability and safety of this vector *in vivo* would be assessed. Because the good-hand-washing technique and disposal-of-potentially-infectious-feces protocol will require meticulous application several times per day for several weeks to be effective, Dr. Yankaskas wondered how adherence to these practices would be monitored and what actions would be taken if inadvertent transmission were suspected or detected. Antibiotic courses may be prescribed for persistent infections, but he asked for more specifics about how the antibiotics would be selected and how participants would be monitored, and suggested that the informed consent document be modified to indicate these plans and the possible side effects of the antibiotic courses. In addition, Dr. Yankaskas urged that autopsy studies be performed that would detect SalpIL2 in the gastrointestinal tract, Peyer's patches, lymph nodes, tumor, normal liver, or other organs to assess the mechanisms of action and/or potential limitations of the vector.

### **C. RAC Discussion**

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

- Dr. Federoff asked whether there is direct evidence of biological activity that can be compared with full-length IL-2.

- Dr. Ertl suggested that the investigators plan to store peripheral blood mononuclear cells.
- Dr. Federoff requested further explication regarding the pathological results seen in some of the experimental mice.
- Dr. Strome asked the investigators whether they had any evidence of their drug binding the human IL-2 receptor. He suggested performing straightforward biocore assays to compare directly the binding of IL-2 to the experimental truncated protein; doing so would allow a solid understanding of the kinetics of the drug. He also suggested conducting these assays against the receptors that share the common gamma chain with IL-2 (IL-4 and IL-7) so as to understand any potential off-target effects of the drug.
- Dr. Buchmeier asked about the possibility with large tumor burden of an endotoxin shock outcome due to a nearly synchronous release of large amounts of dead tumor tissue along with Salmonella.

## **D. Investigator Response**

### **1. Written Responses to RAC Reviews**

While it has been reported that Salmonella targets tumors, multiplies within them, and invades tumor cells, the mechanism by which Salmonella targets tumors is unclear. Because Salmonella is a facultative intracellular parasite that can thrive in hypoxic conditions, it is hypothesized that there is a direct antitumor effect of these organisms due to their survival in the relatively hypoxic areas of tumors and their ability to invade tumor cells. SalpIL2 possesses these properties. Although the investigators have seen an antitumor effect with Salmonella lacking the IL-2 gene, they note that Salmonella containing the IL-2 gene appears to have a more substantial antitumor effect. Animals administered this organism develop a significant elevation in cellular populations (NK and CD8 T-cells) primarily responsible for tumor cell killing. Despite the fact that this engineered bacterium contains a truncated gene for IL-2, the investigators reported their observation of a significant antitumor response in more than 100 experiments during the last 15 years of study.

The investigators have determined that their experimental organism is susceptible to a multitude of antibiotics, including ciprofloxacin, trimethoprim/sulfamethoxazole, and gentamicin. Antibiotics will be selected based on each study participant's allergy profile and the susceptibility pattern of the organism. Participants will be monitored as any individual with a suspected infection.

Truncation of the IL-2 polypeptide in SalpIL2 was not apparent until DNA sequencing of the pIL-2 plasmid was undertaken in response to a request from the FDA following submission of the investigators' original IND application in 2005. Upon discovering the frame shift, the investigators sequenced samples of pIL-2 from the laboratory freezer and found that all of their experiments had been conducted with the truncated IL-2. Given the repeatedly observed antitumor effect of SalpIL2 that prompted the proposal of a clinical trial, the investigators concluded that proceeding to determine the safety of SalpIL2 in a Phase I trial was warranted. The truncation may provide important insight into the vasopermeability activity of IL-2 that is responsible for the toxicity that precludes increased dosing in systemic IL-2 anticancer therapy. The 36 amino acid sequence responsible for the vasopermeability activity of IL-2 (2) is contained within the truncated IL-2 polypeptide expressed in SalpIL2 and this may be the activity responsible for the enhanced anti-tumor effect observed with SalpIL2 vs. SalpNG.

To maximize safety, study participants will be instructed not to prepare food for anyone until their stool cultures indicate that the organism has cleared their system. Study participants will meet with a study nurse, one on one, to review the necessary guidelines for hygiene and handling of excrement. Universal precautions will be taught to these participants. Furthermore, subjects will be asked to keep a diary of these daily activities. If an inadvertent transmission is suspected or detected, in addition to looking for

clinical signs of infection, the investigators will communicate with those medical providers primarily responsible for those affected and antibiotics will be offered.

While the mechanism of tumor cell destruction is probably multifactorial, the investigators believe that an immunological basis for tumor cell destruction remains most likely because an antitumor effect was more pronounced in preclinical studies in animals administered Salmonella with the IL-2 gene. Thus, study participants with known human immunodeficiency virus (HIV), the need for chronic steroids or other immunosuppressant drugs, or with any other condition in which there is immunosuppression to a significant degree will be excluded from this study. Individuals with an overwhelming cancer burden and who are deemed immunosuppressed by the values of their complete blood count will also be excluded.

Regarding the suggestions to focus on a single tumor type, the investigators explained that the protocol calls for those individuals with any gastrointestinal malignancy (adenocarcinoma) with metastases to the liver. The bulk of the in vivo studies were conducted with adenocarcinoma. In addition, anti-tumor activity with this system was observed with primary neuroblastoma, pancreatic adenocarcinoma and metastatic osteogenic sarcoma.

Mice treated with the attenuated bacterium showed twofold increases in platelet counts, and leukocytes (including eosinophils, monocytes, and neutrophils) were all increased. However, other than the platelet count, which is a typical response seen in humans with infections, the other blood values that increased were not statistically significant nor were those values much different than controls. To monitor those values in study participants, complete blood counts will be drawn prior to the start of the study; at weeks 5, 9, and 13; and every four weeks up to week 24.

The meningeal inflammation observed in the SalpIL2-treated mice was seen in one mouse at weeks 6, 48, and 50. Vacuolization of the white matter in the cerebrum and cerebellum of control mice was also observed. Despite culturing Salmonella in several of the experimental mice, at no time did these mice behave clinically different than the control animals. In conjunction with the fact that this antitumor system is effective in multiple tumor types, this finding may imply a response to brain tumors.

In the preclinical necropsy studies, the investigators observed pyogranulomas in the liver tissue of control mice (saline fed) as well as animals fed SalpIL2. Pyogranulomas were observed in the pulmonary tissue of only one Salmonella-treated mouse at week 20. A pyogranuloma is a focal concentration of neutrophils that could be expected with bacterial invasion of a tissue. The investigators believe that this finding is not the result of gene transfer. In tumor-laden mice, the investigators have found a large concentration of Salmonella within the tumor tissue, suggesting a mechanism by which the bacteria facilitate immune-cell-mediated destruction of tumor cells.

Regarding the possible immune response to the truncated IL-2, the investigators noted that while most of the proteome produced by SalpIL2 will be immunogenic, the truncated IL-2 polypeptide produced in SalpIL2 is identical in amino acid sequence to the corresponding sequence of native human IL-2 except for the N-terminal methionine necessary to define the start of translation. Therefore the IL-2 peptides in major histocompatibility complexes on any antigen presenting cells that sample the truncated IL-2 protein will be identical to peptides presented during elimination of self-recognizing lymphocytes in the thymus and bone marrow. It is extremely unlikely that antibodies to IL-2 will be produced as a result of SalpIL2 administration.

## **2. Responses to RAC Discussion Questions**

Dr. Saltzman explained that universal precautions will be taught to all the study participants. If there is a suspected or actual inadvertent transmission, the investigators will communicate with the primary care physicians and will offer antibiotics. (This organism is susceptible to the conventional antibiotics that are known to eradicate Salmonella.) In addition, the investigators have determined in the laboratory how much bleach is needed to kill the Salmonella in the excrement – allowing a little bit of bleach to sit in the toilet will eradicate all the Salmonella. This organism cannot survive on many carbon sources in the environment; it can survive only on glucose because it is a hyper-deletion mutant.

Dr. Saltzman acknowledged that an autopsy will be offered to all study participants and the investigators will communicate with the pathologist to obtain all relevant data.

Regarding the appearance of bacteremia in the study of 12 patients using *Salmonella typhi* with a similar method of attenuation, four participants developed bacteremia that was detected only in blood cultures and not via clinical signs. Dr. Saltzman pledged that the investigators would monitor participants closely in this proposed trial for any signs of infection, determining blood counts as well as looking for systemic disease. In the laboratory, the experimental organism only lasts 4 to 6 weeks in the natural host; the investigators are hoping for a similar result in this proposed trial.

Initially *in vitro* studies looking at proliferation assays of YAK1 cells (which only tend to multiply in the presence of IL-2) showed no activity in the spleens of mice fed Salmonella without the IL-2 gene and significant activity in the spleens of mice fed Salmonella with the IL-2 gene. Dr. Saltzman explained further that, in mice, the gene for IL-2 is remarkably similar to the gene for human IL-2, so the investigators hypothesize that the same process will occur in humans.

Dr. Saltzman noted some studies conducted by other investigators that suggest that Salmonella has antiangiogenic properties, which could suggest a possible mechanism of the antitumor effect that was observed in the preclinical studies.

Regarding the experimental animals, Dr. Saltzman reiterated that there was no evidence of any clinical abnormalities. The mice continue to act normally – feeding and drinking and not acting moribund or sick in any way.

Dr. Saltzman noted that increasing the amount of tumor burden results in a less-effective therapy. The investigators are considering this potential therapy as a way to decrease tumor burden in the liver such that a patient would benefit by the change of unresectable disease into resectable disease.

#### **E. Public Comment**

No public comments were offered.

#### **F. Synopsis of RAC Discussion and RAC Observations and Recommendations**

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

##### Preclinical Issues

- The IL-2 protein that is to be produced in this trial will be lacking one-third of the amino acids found in endogenous IL-2. As a result, the protein's conformation may be altered, which could lead to the exposure of new immunogenic epitopes and potentially induce a B-cell response. Understanding the potential immunogenicity of this protein is critical and should be evaluated in a mouse model. Moreover, since the molecule is not identical to endogenous IL-2, the reagents chosen for the analysis must be specific to the truncated version. In other words, commercial reagents used in assays for IL-2 may not be appropriate.
- While antitumor activity was demonstrated in the murine model, it has not been established that the effects were due to the truncated IL-2 nor is it clear that the protein has the same biological activity as endogenous IL-2. Studies should be undertaken to determine whether the truncated IL-2 binds to the mouse IL-2 receptor. In addition, assays should be done to document that the truncated IL-2 binds to the human receptor for IL-2 as well as receptors that share the common gamma chain with IL-2.

##### Clinical/Trial Design Issues

- As noted above, the truncated IL-2 is potentially immunogenic. Assays for antibodies to the truncated IL-2 protein should be developed and used to monitor for adverse effects. In addition, peripheral blood mononuclear cells, pre- and post-administration, should be collected and cryopreserved to enable additional immunological studies if needed.
- While it is important to demonstrate that the truncated IL-2 produced by the plasmid can bind the IL-2 receptor, such a finding is not sufficient to establish that the biological activity of the truncated IL-2 is equivalent to that of the endogenous molecule. Assays to measure the truncated IL-2's biological activity will be needed in order to show that it has antitumor effects.
- Since many of the subjects in the study may have compromised immune systems from being treated with chemotherapeutic agents, they may be more susceptible to systemic infection with Salmonella. Using white blood cell counts as a screen for compromised immune status will be helpful in this regard, but additional criteria should be developed to identify participants who may be at greater risk of disseminated infection with Salmonella.
- Longitudinal shedding studies should be undertaken to document whether the attenuated bacteria persist.

#### Ethical/Legal/Social Issues

The following changes should be made in the informed consent document and addressed during the consent process:

- As one of the dose limiting toxicities is “sepsis,” the risk of sepsis and proposed steps for its management should be discussed.
- The fact that the IL-2 protein being used in the study is not the same as endogenous IL-2 and that, as such, it may not have the same immune system and antitumor effects as endogenous IL-2 should be made clear.
- To avoid misleading participants, the experimental agent should not be referred to as a “treatment.” A term such as “study agent” should be used instead.

#### **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. It was moved by Dr. Williams and seconded by Dr. Kodish that the RAC approve these summarized recommendations. The vote was 13 in favor, 0 opposed, 0 abstentions, and 1 recusal.

#### **VI. Gene Transfer Safety Assessment Board**

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

##### **A. GTSAB Report**

Dr. Yankaskas reported that, of the 11 protocol submissions received by OBA in the past 3 months, 8 protocols were not selected for public review at this RAC meeting; all 8 protocols were for cancer. Three adenoviruses, two plasmids, one retrovirus, one RNA transfer, and one vaccinia virus vectors were proposed to be used in these protocols. A total of 16 serious adverse events (SAEs) were reviewed by the GTSAB from 10 protocols, including initial and followup reports. Analysis of these events was completed and the GTSAB concluded that no events raised issues that needed public discussion.

Of trials that have initiated enrollment in the past 3 months, 11 protocols submitted M-I-C-1 responses to OBA, of which four had been reviewed by the RAC at a public meeting. Dr. Yankaskas provided highlights of responses to the RAC recommendations. Those protocols were:

- #0704-843, A Phase I Study of Autologous T-Cells Genetically Modified at the CCR5 Gene by Zinc Finger Nuclease SB-728 in HIV-Infected Patients (*reviewed June 2007*)
- #0707-868, A Phase 1 Safety Study of Heat/Phenol-Killed, E. coli-Encapsulated, Recombinant Modified Peanut Proteins Ara h 1, Ara h 2, and Ara h 3 (EMP-123) in Normal Volunteers Followed by Subjects Allergic to Peanuts (*reviewed September 2007*)
- #0710-881, Phase 1b, Open Label Trial to Define the Safety, Tolerance, Transgene Function and Immunological Effects of Intratumoral Injection(s) of Adenoviral Transduced Autologous Dendritic Cells Engineered to Express hIL-12 Under Control of the RheoSwitch® Therapeutic System in Subjects with Stage III and IV Melanoma (*reviewed December 2007*)
- #0901-967, Phase I/IIa, Dose Escalation, Safety, Pharmacokinetic, and Preliminary Efficacy Study of Intraperitoneal Administration of DTA-H19 in Subjects with Advanced Ovarian Cancer (*reviewed March 2009*)

Dr. Yankaskas reviewed an amendment to protocol #0807-923, A Phase I Compassionate Trial of Nanocomplex Mediated GNE Gene Replacement in Hereditary Inclusion Body Myopathy-2, which had been reviewed by the RAC in September 2008. In this single subject protocol, the subject received intramuscular injection of a liposome encapsulated plasmid encoding GNE. The GNE gene encodes a rate limiting enzyme that catalyzes the first two steps of sialic acid biosynthesis. Decreased sialic acid production consequently leads to decreased sialylation of a variety of glycoproteins including the critical muscle protein alpha-dystroglycan ( $\alpha$ -DG). Dr. Yankaskas reviewed the RAC's recommendations and the modifications that had been made to the protocol since those recommendations were promulgated. John J. Nemunaitis, M.D., Mary Crowley Cancer Research Centers, PI of this protocol, provided an update of results and the intramuscular (IM) and initial intravenous (IV) dosing experience in the single research participant as well as a plan toward development of a full Phase I trial.

Regarding the IM results, after multiple doses were injected, the investigators did not observe any significant toxic effects. The initial injection on the left side, which involved both left biceps, was a total dose of 400 micrograms ( $\mu$ g); three subsequent injections on the right side involved 200 $\mu$ g per injection. The subject developed transient fever but no significant toxic effects with dosing at those levels. Additionally, the investigators demonstrated brief transient muscle function improvement on the left and the right extensor carpi radialis longus (ECRL), which were the specific muscles injected.

Dr. Nemunaitis discussed the rationale for IV administration. The participant's muscle function is deteriorating over time, the IM injection of 400 $\mu$ g of pUMVC3-GNE DNA lipoplex is safe and well tolerated in this individual, and treatment has demonstrated transient direct and local regional muscle benefit. The proposed IV dosing schema begins with a 400 $\mu$ g IV dose, which is 1/400<sup>th</sup> of the IV no-observed-adverse-effect level in mice. The investigators and others hypothesize that sialic acid may be produced outside of muscle and utilized by muscle, thereby alleviating symptoms of hereditary inclusion body myopathy (HIBM). Given all of this information, the IV amendment was moved forward and approved by the FDA on June 25, 2009.

Results of IV administration in the single subject showed no significant toxic effect except transient Grade 1 fever and Grade 2 headache, and muscle function improvement in the previously injected left ECRL was noted.

Future plans include collection of serum samples for cumulative analysis of sialic acid at the end of the study. A concurrent multi-injection IV safety, expression, and limited toxicity study in animals is ongoing, to include multiple organ analysis; results are critical for continuation of the dose escalation.

Biodistribution and toxicology studies have been approved by the local animal use committee; these results are critical for Phase I trial submission. Once the database is complete, a Phase I intravenous protocol will be submitted to the RAC.

## **B. RAC Discussion**

The following additional questions, concerns, and issues were raised:

- Dr. Williams noted that the biochemical readouts of sialic acid levels showed an increase in muscle content from 22 nanomoles per milligram of protein to 28 nanomoles; he wondered whether that increase is biologically relevant. Dr. Nemunaitis responded that the level in normal muscle is “much higher” but he was uncertain as to the exact number. Because this response has not been studied well, the investigators are correlating what they are learning in this trial with dynamometer testing so that it may be possible in the future to validate changes as significant.
- Dr. Ertl asked how to explain the differing results between the left and right arms of the study participant. Dr. Nemunaitis explained that this observation is typical with HIBM patients. The muscle function is a significant reflection of the muscle remaining in the tissue, and the participant’s right side, over time, has deteriorated to the point of having less muscle tissue and more fatty and scar tissue; thus, compared to the left arm, there exists less muscle to respond to the treatment. The left side still has some muscle present and appears to have responded to some degree.
- Dr. Strome expressed concern about administering a drug based on mouse data that does not show that the drug is distributed at appropriate levels to the tissues of interest. Human data from this single subject trial indicates that, even if the drug were distributed appropriately, those tissues likely could not respond because they already have been replaced by fatty infiltrate. Therefore, he opined that the benefit of giving this drug IV seemed questionable at best. Dr. Nemunaitis responded that the investigators focused on the capability that could be preserved in the muscles that had not deteriorated to zero function; in those muscles, the investigators have demonstrated a positive and significant change. That change has allowed the participant to accomplish small but significant tasks like holding a purse or moving a wheelchair, which help preserve her independence. The investigators understand that this drug cannot yet be considered a “treatment,” and they will move this study forward as carefully as possible, including further animal testing, a formal Phase I trial, and a formal Phase II trial.
- Dr. Ertl queried as to whether the improvement experienced by the participant was a random response. Dr. Nemunaitis explained that the investigators examined all the participant’s other muscles and saw a steady decrease during the time period of the study, which was not the case in the muscles that purportedly responded to the experimental drug. Therefore, the investigators concluded that the improvement was not a random response.
- Dr. Federoff opined that the data were inadequate to determine whether IV administration could be expected to lead to evidence of biochemical and functional restoration of muscle function.

Dr. Corrigan-Curay clarified why the change of dosing in this single subject trial did not go through a new protocol process. She noted that OBA receives a number of such requests for changes, which are usually treated as an amendment to an existing protocol and are shared with the GTSAB. The majority of these requests are new dosing of participants who have finished on the trial or newly do not meet the inclusion or exclusion criteria. The RAC has a primary role in promoting the safety of gene transfer research, including single-participant protocols. Therefore, amendments such as these have been shared with the GTSAB. The GTSAB will continue to provide feedback to OBA that can be shared with the investigator and the appropriate regulatory bodies, and the GTSAB will bring these amendments to the RAC for further comment and discussion as needed.

**VII. Discussion of Human Gene Transfer Protocol #0907-989: A Phase 1 Open-Label, Escalating-Dose Study, of the Safety and Tolerability of Single Daily Doses of CEQ 508 an RNAi-Based Therapy for Familial Adenomatous Polyposis**

Principal Investigator: Gideon Steinbach, M.D., Ph.D., Fred Hutchinson Cancer Research Center  
Additional presenters: Patrice Courvalin, M.D., Institut Pasteur; Johannes Fruehauf, M.D., Cequent Pharmaceuticals Inc.; Alison Silva, Cequent Pharmaceuticals Inc.  
Sponsor: Cequent Pharmaceuticals Inc.  
RAC Reviewers: Drs. Federoff, Kodish, and Zaia  
*Ad hoc* Reviewer: James M. Church, MB, Ch.B., F.A.C.S., F.R.A.C.S., The Cleveland Clinic

**A. Protocol Summary**

Familial Adenomatous Polyposis (FAP), an autosomal dominant disorder with an estimated prevalence of approximately 1:10,000 persons, is one of the well described forms of hereditary colorectal cancer. FAP is caused by mutations in the adenomatous polyposis coli (APC) gene located on chromosome 5, which results in low levels of functional APC protein required to regulate intracellular levels of CTNNB1 ( $\beta$ -catenin). This dysregulation and accumulation of  $\beta$ -catenin initiates an activation of downstream target genes, resulting in uncontrolled cellular proliferation, hyperplasia, adenoma formation, and an increased risk of colon cancer development. In addition, the APC gene also has a role in chromosome segregation through microtubule binding and cell polarity. Almost all of the cancer-causing mutations result in a truncated APC devoid of its C-terminal region that functions in chromosomal segregation, leading to chromosome instability, a hallmark of cancer. Typically, FAP results in the formation of multiple (hundreds to thousands) polyps in the large and small intestine. While these polyps start out benign, malignant transformation into colon cancer occurs 100% of the time when left untreated. By age 35, 95% of individuals with FAP have developed polyps. Without surgical intervention, the mean age of colon cancer onset in these individuals is 39 years of age (range of 34-43 years). There is no relationship between the disease and any particular gender or race. Left untreated, without surgical intervention, the leading cause of death is colon cancer.

FAP patients are also at increased risk of developing other cancers, including brain tumors and cancer of the liver, pancreas, thyroid, and biliary tree. The only available treatment for FAP involves a complete colectomy (surgical removal of the entire large intestine) to forego the risk of colon cancer, which is usually performed in the late teenage years or early twenties. However, the risk remains of cancer forming in the remaining stump of the rectum and in the small intestine. Although pharmaceutical interventions have been evaluated, none have been proven to be safe and effective. Thus, there are no currently available, approved, nonsurgical therapies for treating FAP.

RNA interference (RNAi) can be initiated by direct delivery of short interfering RNAs (siRNAs) into the target cell or by short hairpin RNAs (shRNAs), which are either transcribed from DNA-based plasmids or viruses (i.e., lentivirus or adenovirus), or in the case of *tk*RNAi are generated and delivered by live bacteria to the cytoplasm of eukaryotic target cells. shRNAs are processed into siRNAs in the cytoplasm by Dicer RNase III, a protein required for RNAi function. The generated siRNAs work in concert with a naturally occurring cellular protein complex called the RNA-induced silencing complex (RISC). The RISC complex binds to the siRNA duplex, unwinds it and generates a stable complex between itself and the single-stranded antisense component of the siRNA. The single-stranded form of the siRNA, termed the guide strand, will pair with its complementary sequences within the targeted mRNA and facilitate cleavage of the mRNA by RISC, thus making it susceptible to complete degradation by cellular RNases. In the case of CEQ508, that complementary sequence is contained within the mRNA of  $\beta$ -catenin (CTNNB1), and this results in reduction of  $\beta$ -catenin levels in the treated cell.

CEQ508 is an experimental bacterial agent that uses a concept called *transkingdom* RNA interference (*tkRNAi*). The *tkRNAi* system consists of live non-pathogenic *E.coli* bacteria (CEQ221) genetically modified to possess two important novel properties: (1) Production of high levels of intracellular shRNA under the control of a plasmid-based *E.coli* promoter system, and (2) An ability to enter host cells and release the expressed shRNA through the use of two unrelated proteins, invasin and listeriolysin O, that have been engineered into the CEQ508 strain. Invasin is encoded by the *inv* gene derived from *Yersinia pseudotuberculosis*. It is expressed on the bacterial surface and results in the uptake of invasin-expressing bacteria into non-phagocytic cells, (i.e., epithelial cells), through an interaction with  $\beta$ 1-integrins expressed on the surface of these cells, where the bacteria subsequently enter in an endosomal vesicle and lyse due to an engineered nutrient auxotrophy. CEQ508 also produces listeriolysin O (LLO), which is encoded by the *hly* gene derived from *Listeria monocytogenes*. LLO is a pore forming protein that selectively ruptures the endosomal membrane which, in conjunction with bacterial lysis, results in the release of shRNA into the host cell cytoplasm where it interacts with the RNAi machinery (the RISC complex) to induce degradation of the  $\beta$ -catenin mRNA. Suppression of  $\beta$ -catenin may arrest or slow the growth of colon cancer cells and has the potential to prevent polyp formation in the context of the APC mutation.

The proposed Phase I clinical trial is a dose-escalation trial to evaluate the safety and tolerability of CEQ508 in individuals diagnosed with FAP. Trial participants will receive an oral suspension of CEQ508 once daily at the prescribed dose for a total of 28 consecutive days. Four dose groups are planned, and each group will consist of three individuals. In each group, only one individual (of three) will initially receive the specified daily dose of CEQ508. After 1 week, the second individual in that group will begin receiving CEQ508. After the second individual in that dosing group has received CEQ508 for 1 week, the third and final research participant in the group will begin receiving CEQ508. After all individuals in a dose group have received CEQ508 for at least 2 full weeks, the first individual at the next highest dose level will begin receiving CEQ508, and dosing of the next two participants in that group will also be staggered by 1 week as with the previous dose group.

Trial participants will be followed to evaluate safety and potential biological effects related to activity of the investigational product. At the highest dose found to be safe, an additional six participants will be enrolled and dosed for 28 days. These participants may be re-enrolled from a previous dosing cohort (provided certain criteria are met) or they may be newly enrolled participants. All research participants will undergo upper endoscopy (esophago-gastro-duodenoscopy) as well as lower endoscopy (sigmoidoscopy or pouch endoscopy, where appropriate) with tissue samples taken at baseline, before the start of dosing, and on the day of endpoint examination (between Day 26 and Day 28).

The primary objective of this trial is to evaluate the safety and tolerability of CEQ508 following daily dosing after 28 days in research participants diagnosed with FAP. Secondary objectives include evaluation of CEQ508 shedding in stool samples, as well as the evaluation of gene expression and polyp histological changes following the 28-day dosing cycle. All participants will be followed for an additional 28 days after the conclusion of the 28-day dosing study.

## **B. Written Reviews by RAC Members**

Nine RAC members voted for in-depth review and public discussion of this protocol. Key issues included the novel aspects of this study, including use and safety of a modified bacterial strain and two proteins – invasin and listeriolysin – that will be expressed from the bacterial construct to assist in the bacteria's ability to invade the host cell and release the RNAi into the cytoplasm of the target cell.

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

Dr. Federoff asked whether invasin and listeriolysin are immunogenic. He asked the investigators to elaborate on the justification of including post-colectomy patients in this Phase I trial to assess safety and tolerability. In selecting participants for the stable dosing level, Dr. Federoff noted that the investigators would consider previously dosed individuals; he expressed concern that data interpretation might be difficult if previously dosed individuals are re-enrolled for the stable dosing phase. He asked about the

relationship between the PI and the study participants, particularly whether the PI is likely to be the treating physician. Dr. Federoff requested that the investigators comment on the apparent differences in cytokine responses in wildtype versus antigen presenting cell minimal (APCmin) mice, and whether these differences might be relevant to the clinical status of potential investigational participants. He queried as to why the sponsor of this protocol would not support the medical costs of a participant if an SAE occurred after administration of CEQ508.

Dr. Kodish asked the investigators to explain the reasons for including both FAP patients who are status post-colectomy and those who have not yet had surgery, including a discussion of any concern that patients might defer definitive therapy (i.e., surgery) in hopes that the study drug would delay or avoid the need for colectomy. Dr. Kodish asked the investigators to explain why a participant's insurance company should be expected to cover costs that are a "direct result of study procedures." He noted that, in general, the informed consent document is well written and explains the study clearly, and explicitly dispels potential therapeutic misconception.

Dr. Zaia noted that the mouse toxicology studies suggest the possibility of an on-target effect of "detachment of the epithelial layer from the stroma and changes in the cellular composition of the mucosa" and that this AE was not seen in the nonhuman primate (NHP) studies (although CEQ508 was not used in the NHP studies). He asked whether the data support this AE as a rare but possible event, whether it is possible to rely on the NHP studies in this regard, and whether this possible AE should be mentioned in the informed consent document. Dr. Zaia asked whether the calculations of the possibility of transfer of the pMBV43-H3 plasmid to other bacteria by conjugation, transduction, or transformation are theoretical and whether the investigators have tested this scenario *in vitro*; the possibility of transfer should be mentioned in the informed consent document. He requested that the data and safety monitoring board (DSMB) be defined more clearly in the protocol and that the DSMB review any SAE that halts the trial. Dr. Zaia stated that the protocol should include a plan for determining how the decision will be made for continuing dosing versus removing a participant from the study for intercurrent illness plus how to determine who will treat that sick individual. He suggested that either specific criteria be developed to assist the visiting nurse or PI in this decision or that a non-study physician be involved in assessing and treating the participant for all intercurrent illnesses. In addition, Dr. Zaia suggested two rewordings to clarify the informed consent document.

*Ad hoc* reviewer Dr. Church confirmed the appropriateness of using FAP as a model for studying a therapy aimed at the primary molecular pathway of colorectal carcinogenesis, although he noted that the authors overstate the dangers of cancer developing after routine surgical treatment of FAP. He stated that a preferable study rationale would be for patients for whom the sequelae of surgery are more significant. Regarding selection of study participants, Dr. Church asked about the wisdom of including individuals taking nonsteroidal anti-inflammatory drugs, in particular sulindac, as these drugs might suppress polyp formation in the large intestine or pouch and produce ulcers in the small bowel and large bowel, in the pouch, and at the ileorectal anastomosis, all of which could complicate assessment of the secondary endpoint (mucosal disruption). He suggested that persons with a detectable polyp burden in their remaining lower gastrointestinal tract would be the best participants, and that individuals within 1 or 2 years of an ileorectal anastomosis or ileal pouch-anal anastomosis and individuals with ulcerations of the pouch or rectum could be excluded. Regarding likely effectiveness, Dr. Church noted that the results from a preclinical experiment look impressive regarding the ability of  $\beta$ -catenin abrogation to suppress adenoma development; these results bode well for Phase II studies of RNAi.

### **C. RAC Discussion**

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

- Dr. Ertl noted that the preclinical data indicated that this experimental agent could prevent formation of polyps but that it could not result in resolution of polyps. The current clinical trial is designed for participants who already have polyps, so the results of the animal studies are not relevant regarding potential benefit. She asked, therefore, whether the investigators' eventual target population is a pediatric population, in whom polyps will not have developed.

- As a surgeon who treats patients with FAP, *ad hoc* reviewer Dr. Church commented on the evolving strategy to postpone colectomy because of the resulting significant negative effects on quality of life. During the last 10 years, it has been popular to offer colectomy at the first sign of polyps, usually in the patient's late teens; however, because of the risk of complications and the risk of altered lifestyle in these teenagers, the strategy now is to defer surgery if possible. .
- Dr. Ertl asked whether this trial should be conducted in participants at least 16 years old, who would be old enough to give consent.

## **D. Investigator Response**

### **1. Written Responses to RAC Reviews**

Current surgical options have resulted in a lower rate of cancer development in FAP patients. The investigators believe that, given the overall rarity of the disease and the resultant paucity of available research participants as well as the fact that there is effectively no available pharmaceutical alternative to radical surgery in any case, it is appropriate to evaluate the safety and tolerability of CEQ508 in the proposed participant population. Based on the results of the proposed Phase I trial, it may be appropriate in the future to conduct trials to evaluate efficacy in defined subsets of individuals with FAP, such as in subjects with upper gastrointestinal (GI) manifestations. Upper endoscopy, including upper GI tissue biopsy, that will be performed in the proposed Phase I trial will aid in determining if CEQ508 has the expected activity on upper GI tissues.

The investigators agreed to revise the protocol to specify that patients with ulcerations or active ongoing inflammation at baseline examination will be excluded. For this Phase I trial, all FAP patients should be eligible whether or not they have had a surgical procedure, provided that they do not fall under any of the other exclusion criteria. The investigators do not anticipate that the presence or absence of the colon, or of a pouch in the case of ileal pouch anal anastomosis-operated patients, will significantly change the risk of treatment with CEQ508, and the secondary endpoints of biomarker determination will help in decisionmaking about the selective enrollment of subclasses of patients for a future Phase II trial. Patients post-colectomy remain at risk for duodenal adenomas and cancer, and for ileal pouch adenomas; duodenal polyps and ampullary cancer are more difficult to treat surgically compared to the colonic manifestations. If found safe and efficacious, CEQ508 ultimately might become a treatment for both pre- and post-colectomy patients.

The investigators pledged to revise the protocol to reflect the requirement that, where medically appropriate, NSAIDs would be discontinued during the course of the trial, with the exception of low-dose aspirin if given for cardiovascular indications. In any case, aspirin should be discontinued within 5 days of an anticipated endoscopic procedure. The study drug will be recommended to be administered with a sodium bicarbonate buffer.

The toxicity data is expected to be valid in naïve participants as well as in those who were previously treated in this trial with a lower dose. Including previously treated participants enables the investigators to limit the total number of individuals exposed to the agent until safety is established, and it will facilitate the conduct of this study in this rare study population. Previously treated participants will undergo a washout period between the end of their dose escalation phase and enrollment in the stable dose phase. Any individuals considered for reenrollment will be treated according to the protocol and as if they were naïve participants, meaning they will undergo a new baseline and endpoint endoscopy that will help avoid confounding of results.

In many cases, the study PI will not be the primary treating physician of the study participants, who will remain under the treatment of their primary treating physician during the study period. The PI will discuss any changes to medication or management required during the trial with the participant's treating physician. The study nurse and PI will evaluate participants for potential toxicities during the study period.

In the NHP toxicology studies, CEQ501 showed strong reduction (approximately 50 percent) of monkey  $\beta$ -catenin levels in tissues; this reduction did not lead to any ontarget toxicity. *In vitro* CEQ501 is more potent and efficacious on monkey epithelial cells than is CEQ508, thus all target-related toxicities are more likely to be seen when using CEQ501. To address any nontarget-related potential toxicities, the investigators conducted a bridging study in mice, which resulted in no therapeutic agent toxicities observed with either CEQ501 or CEQ508. Because CEQ508 has better potency on human cells compared to CEQ501, the dose escalation schedule in the proposed Phase I trial was designed to start at very low doses, to maximize safety.

IgA and IgG anti-invasin antibodies have been detected by ELISA in the sera of mice after oral administration of CEQ508. There are no data for listeriolysin with CEQ508; however the presence of antibodies against LLO should not be a cause of concern, since it is contained intracellularly within the therapeutic bacteria. This anti-invasin antibody response is secondary to the well-documented interaction of the invasin-expressing bacterial vector with the intestinal Peyer's patches. It may contribute to keeping the bacterial vector at the luminal site of the intestinal mucosa. Indeed, CEQ508 was never found in the mesenteric lymph nodes or internal organs in mice. At this point, it is unclear to which extent the secreted IgA is inhibitory and might result in a reduction of efficacy with long term treatment, but waning of efficacy has not been observed thus far in animal studies conducted to date. Additional experiments are being initiated to address this question *in vitro* as well as in a longer-term *in vivo* experiment.

The levels of TNF- $\alpha$  observed in the serum of the experimental animals were very low, almost to the point of being below the detection threshold, and therefore do not represent a significant cause for concern. A significant elevation in TNF- $\alpha$  would manifest in the individual being febrile, which was not observed in mice or in NHPs.

The sponsor will cover all medical costs for related SAEs if they occur during the study period; the informed consent document will be changed to reflect this amendment.

The investigators have conducted experiments to evaluate the risk of horizontal transfer. In mouse samples analyzed to date from CEQ508 and precursor studies, no evidence was found of horizontal transfer of the therapeutic plasmids to other bacteria. However, mice are of limited value in predicting the situation in the proposed clinical trial, since *E. coli* are not usually part of their flora. The investigators are currently preparing to conduct an experiment in Streptomycin-pretreated animals that are reconstituted with a more human-like flora in order to mimic this situation. In humans as well as in NHP samples, there is a high amount of background Kanamycin resistance, which makes it virtually impossible to use clinical samples or the NHP stool samples to screen for potential horizontal transfer because of the lack of a useful selection marker.

The DSMB will be established in compliance with the appropriate requirements, even though National Cancer Institute guidelines do not require a DSMB for Phase I studies. The DSMB will be composed of three experts, in the fields of medicine and science that are applicable to the study, who are not investigators or collaborators on the study. The investigators will revise the protocol to include a full DSMB review of any SAE that halts the trial.

The mention of epithelial layer detachment comes from a paper published by Ireland *et. al.*, (Gastroenterology 2004), in which the authors created a conditional  $\beta$ -catenin knockout mouse and observed that villus epithelium was detached in sheets from crypt to villus tip. The ablation of  $\beta$ -catenin expression was uniform from the stem cells residing in the crypts to the mature enterocytes at the villus tip. Such a complete and uniform reduction in  $\beta$ -catenin expression is an extreme occurrence that can only be achieved through the use of genetic deletion strategies and the likelihood of inducing a similar effect using *t*kRNAi delivery strains is remote for the following reasons. First, CEQ508 and bacteria in general, are able to access only the upper areas of the villus and are unable to access the intestinal crypts due to production of the alpha and beta defensins by Paneth cells residing at the base of the crypts. This ensures that the crypt is sterile and that bacteria (i.e. CEQ508) cannot contact the epithelial stem cells and, in the case of CEQ508, cannot influence expression of  $\beta$ -catenin in these cells. Secondly,

*tkRNAi* mediated silencing of  $\beta$ -catenin is not absolute, and it may not need to be in order to be efficacious in FAP patients as the dysplastic polyp epithelial cells have a significantly elevated expression compared to normal enterocytes. Reduction of  $\beta$ -catenin in these cells to levels comparable to normal enterocytes may be sufficient to delay adenoma formation. Lastly, studies conducted in both rodents and non-human primates, using CEQ508 and CEQ501, have failed to demonstrate the dramatic epithelial detachment observed by Ireland and colleagues in their knockout mouse model.

## **2. Responses to RAC Discussion Questions**

Dr. Fruehauf acknowledged that the taste of the drug might be problematic. The investigators have not had the time or the ability to reengineer the bacteria to change the taste or to add flavoring; however, the bacteria will be administered in a sodium bicarbonate buffer, which is required to allow the bacteria to transit the acidic milieu of the stomach, and the investigators hope that doing so will make the taste more palatable.

Regarding participants' thoughts of delaying needed surgery because they believe this trial would be curative, Dr. Fruehauf reiterated that the investigators are making it very clear to potential participants that, within this 1-month Phase I study, there is no expectation of any effect on the polyps. Although the investigators will be delighted if they see any changes in gene expression in that 4-week period, they will make it clear to all potential participants that they cannot expect to be cured by this clinical trial. In addition, an endoscopy will allow for discovery of significant change from the previous endoscopic result at the beginning of the study; if multiple new suspicious-looking polyps were to appear, that research participant would be excluded from the study and referred immediately to a surgeon.

Dr. Fruehauf explained that this potential therapy is anticipated to be a chronic treatment, taken every 3 days, for as long as patients tolerate it. The current formulation must be given daily because of the transient effects of the agent.

With regard to the results of the preclinical experiments as they relate to humans with polyps, Dr. Fruehauf stated that there is a hypothetical possibility that longer dosing periods might lead to benefit on existing polyps, although that hypothesis was not tested in the animals. He explained further that what might come out of this product is a preventive reagent for patients with a diagnosis of FAP, probably a pediatric population that would benefit by offering a delay for colectomy. If new polyp formation could be slowed down in these individuals, surgeons would be more comfortable in allowing these people to wait longer – possibly until 25 or 30 years of age – before undergoing colectomy to prevent development of colon cancer. In adults, the hope is the agent may have a preventive effect on duodenal polyposis and cancer. Dr. Fruehauf reiterated that the investigators consider this product a preventive agent rather than a therapeutic agent.

In response to a query about conducting this proposed trial in participants at least 16 years old, Dr. Steinbach explained reasons for studying this disease in adults. The majority of FAP patients have attenuated polyposis and present with a small number of polyps; those patients would benefit from the preventive strategy being proposed for testing in this clinical trial. Also duodenal disease occurs late in adult life, so intervention in adults could be of benefit in delaying or preventing duodenal disease.

### **E. Public Comment**

No public comments were offered.

### **F. Synopsis of RAC Discussion and RAC Observations and Recommendations**

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

#### Preclinical Issues

- Additional data should be gathered on the immunogenicity of the *listeriolysin O*, and a serologic assay developed to enable monitoring for such a reaction during the trial.
- The invasin protein will interact with  $\beta$ 1-integrin on the surface of the gastrointestinal cells to allow the modified *E. coli* to enter the cell. Further studies should be carried out to determine whether other cellular machinery in addition to  $\beta$ 1-integrin is necessary for invasin to facilitate entry of the *E. coli* into epithelial cells and whether these cellular components may be altered in the dysplastic cells of FAP.

#### Clinical/Trial Design Issues

- Given the molecular basis of the desired therapeutic mechanism, i.e., downregulation of the  $\beta$ -catenin mRNA, it is critical to enroll only participants with documented family history of FAP, an autosomal dominant disease, or those with documentation of the APC mutation by genotype testing.
- Biopsies will be taken during endoscopy prior to dosing and at the conclusion of the dosing period to evaluate for histological changes, proliferation rates, and target gene expression levels. Although the protocol specifies that both an upper endoscopy as well as a flexible sigmoidoscopy will be performed, it does not specify the number of biopsies planned during endoscopy, how many polyps will optimally be removed, and the criteria for removal of additional polyps. These details should be added to the protocol and the informed consent document.
- The dose escalation plan is to enroll three research participants per cohort and an additional six participants at the maximum tolerated doses. Participants in the initial cohorts may be included in the last cohort. For completeness, please include the statistical basis for this design.
- The APC mutation results in increased  $\beta$ -catenin, which in turn leads to upregulation of a series of genes involved in cell proliferation, including oncogenes such as *c-myc*. In order to determine whether there is a correlation between the effect of RNAi on  $\beta$ -catenin levels and any clinical outcome, the protocol should investigate the downstream suppression of oncogenes such as *c-myc*.

#### Ethical/Legal/Social Issue

- The rationale for enrolling FAP patients rather than healthy individuals is justifiable given the possible risks associated with any novel treatment, the specific risks associated with this investigational agent, and the fact that this population is most likely to benefit from the knowledge gained. However, given the lack of nonsurgical therapies and that even surgery does not prevent the development of cancer in all patients with FAP, there is considerable potential for therapeutic misconception. The current consent document is carefully written to reduce the chances of therapeutic misconception; attention to this issue during the consent process is equally important.

#### **G. Committee Motion 3**

Dr. Federoff summarized the comments and concerns of the RAC to be included in a letter to the investigators and the sponsor. It was moved by Dr. Zaia and seconded by Dr. Ertl that the RAC approve these summarized recommendations. The vote was 14 in favor, 0 opposed, 0 abstentions, and 0 recusals.

#### **VIII. Discussion of Human Gene Transfer Protocol #0907-988: A First-in-Human Safety and Dose-Finding Study of a New Type-16 Human Rhinovirus (RG-HRV16) Inoculum in Healthy Volunteers**

Principal Investigator: James Gern, M.D., University of Wisconsin School of Medicine and Public Health  
RAC Reviewers: Drs. Ertl, Flint, and Kahn

## **A. Protocol Summary**

Human rhinoviruses (HRVs) are the most frequent cause of the common cold. For most people, HRV causes a self-limited upper airway illness; however, HRV can cause more serious illnesses in certain populations. HRV contributes to most exacerbations of asthma – it is detected in 85 percent of exacerbations in children and about half of exacerbations in adults. HRV infections can cause wheezing illnesses and pneumonia in young children and the elderly. There are no antivirals for preventing or treating HRV infections, which represents a major unmet medical need, and understanding HRV pathogenesis is an important first step toward identifying therapeutic targets. HRV pathogenesis can be investigated by observational studies of naturally acquired infection or through the use of experimental inoculation. The experimental inoculation model is ideal for studying host factors, since the strain of virus is held constant, in contrast to natural infection, which is comprised of more than 100 strains. In addition, experimental inoculation allows the time of infection to be planned, thus enabling kinetic studies, and the inoculating dose can be a constant.

To improve the safety and stability of the inoculum virus, a cDNA clone using reverse genetics was used to generate source virus. The manufacturing procedure had the following steps: 1) development of a cDNA clone derived from a previous inoculum, 2) transcription of viral RNA, 3) transfection of human lung fibroblasts with the viral RNA to produce RG-HRV16 virions, and 4) purification of the viral particles by ultracentrifugation. Recombinant technology is used in the production of the inoculum; the RGHRV16 is in fact synthesized within the fibroblast cell line. The main difference is that the source of the viral RNA is the plasmid rather than virions from another person's nasal secretions.

Eligible participants will be inoculated with RG-HRV16 on day 1 after baseline assessments including physical examination, vital signs, cold symptom diaries, collection of blood for HRV16 antibody titer and nasal lavage. The first 5 participants will return to the research unit 24h after RG-HRV16 inoculation for review of cold symptoms and collection of adverse events. Nasal lavage, cold symptom diaries and adverse events will be collected on all participants 48h, 72h, 96h, 7-10 days and 21-28 days after inoculation. A physical exam will be repeated at 7 days for the first 5 participants exposed to RG-HRV16 and for all participants at study discharge. Blood will be drawn for HRV16 antibody conversion and final safety labs at study discharge.

## **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 recombinant virus in healthy volunteers with no anticipated benefit; although the recombinant virus is predicted to behave similarly to non-recombinant viruses isolated and administered in other studies, it was deemed important to discuss this protocol further and to analyze the risk-benefit ratio.

Three RAC members provided written reviews of this proposed first-in-human trial.

Dr. Ertl focused her review on questions regarding the virus and the study design. She noted that that the investigators repeatedly state that the genetically engineered virus does not differ from the virus that was used previously by isolation from nasal swabs of an acutely infected individual. Dr. Ertl asked the investigators to discuss whether this newly derived virus reflects a rare variant or a virus that was not present in the viral stock; in either case, she expressed concern that previous safety results obtained with the original viral stock might be meaningless for this new virus. Questions directly about the nature of the virus included the purity of the virus preparation, the stability of the genetically engineered virus, whether any of the mutations affect antibody-binding sites, and whether any of the mutations affect other viral domains pertinent to viral pathogenicity. Dr. Ertl asked for clarification of what "close monitoring" of participants would entail, as that process was described unevenly throughout the protocol, and she stated that it did not seem appropriate for study participants to be responsible for the cost of emergency care if

they are injured as a result of participating in this study. She asked for a clearer definition of the first dosing cohort and suggested adding asthma or allergic reactions to the individual stopping rules. In addition, Dr. Ertl strongly encouraged the investigators to conduct a more in-depth analysis than what was proposed in the response to Appendix M-II-B-4, in case a study participant dies under circumstances that could possibly, probably, or definitively be related to the study.

Dr. Flint suggested that assessment of the impact and broader benefit of the protocol would be enhanced by a more precise description of the questions about pathogenesis that the investigator wishes to address as well as which antivirals might be tested. She noted that lay descriptions of this information included in the informed consent document would be of value to study participants, who will receive no direct benefit from their participation. One rationale given for creating and testing RG-HRV16 is that the cDNA clone should provide a stable source of viral genome sequences reducing mutation and the variation observed among different inocula prepared from natural isolates; however, Dr. Flint noted the likelihood of that sequence variation would still occur among different preparations of the RG-HRV16 inocula when the RNA genomes were amplified by the error prone viral RNA polymerase. To allow assessment of this putative advantage of RG-HRV16, she asked the investigators to address questions about the error rates of the polymerases, and cycles of amplifications and whether the investigators anticipate that a single preparation of RG-HRV16 would be sufficient to complete all subsequent studies of interest. Dr. Flint requested that the investigators indicate which human pathogens – particularly respiratory pathogens – are proposed to be tested for in the viral preparations and how those tests would be conducted. She also requested that the investigators provide data regarding the ability of the quantitative polymerase chain reaction (PCR) assay used to confirm infection and assess viral load to distinguish RG-HRV-16 from other circulating rhinoviruses.

Dr. Kahn focused his review on the protection of research participants in this study and some aspects of the informed consent document and process. While the protocol makes mention of close monitoring of the health of participants, the consent form is noncommittal about treatment of symptoms or side effects; therefore, Dr. Kahn asked the investigators to discuss their plan for addressing potential (though unlikely) side effects. He noted that this discussion is especially important because of the protocol's enrollment of healthy participants for whom there is no potential for medical benefit through their participation and in whom the research goal is to induce a viral infection. Dr. Kahn noted that the informed consent document is generally well written and provides clear explanations of what will happen to participants. However, some significant potential side effects are possible from administering the recombinant virus in healthy participants. While effects of a head cold will be well recognized and therefore easily understood by most participants, Dr. Kahn stated that the risks of nasal infection or pneumonia are not trivial and, therefore, the informed consent document and process should better describe how participants will be monitored for these and other side effects, as well as how they will be treated.

### **C. RAC Discussion**

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

- Dr. Ertl expressed her agreement with the approach of waiting one week between each of the first several participants, to make sure the dose is safe. However, she suggested that the plan to dose several participants together at the higher doses was not prudent.
- Dr. Buchmeier expressed concern about the inadequacy of merely taken nasal swab samples if a participant dies. A full autopsy is necessary, sampling all the organs so as to understand the distribution and pathogenesis of the virus that may have caused the death.
- Dr. Strome asked how the investigators plan to deal with the illness of someone who is close to a research participant who may be exposed to this virus but who did not consent to be exposed. He wondered what would happen if that person died, which does occur with the common cold. Further, he expressed concern that this protocol would potentially expose a population of people – who never consented to be in this study and never consented to get sick – to a new virus that may or may not be wide spread in the community.

- Dr. Buchmeier questioned the procedures in place for dealing with college-student participants, who come into contact with others transiently during class, at mealtimes, in a dormitory, and while sharing materials.
- Dr. Federoff asked about the timeframe for recruiting participants and whether there is a financial incentive for participating in this clinical trial.
- Dr. Federoff asked whether enough is known about the implications of a particular viral variant entering a population vis-à-vis the extent to which that could be conveyed to other individuals who are not part of the study. He wondered if it were possible that the spread of this HRV16 would be more likely given that the investigators plan to introduce it in late summer or early fall.
- Dr. Ertl suggested that it might be safer to conduct this study in the “off season” for HRV; the “high season” is purported to be September-October and April.
- Because college students are likely to be recruited as research participants, Dr. Kirchhoff queried whether student health officials would be notified about this clinical trial in relation to the possible appearance in their clinics of students with RG-HRV16.

Dr. Strome and Dr. Ertl stated that they were both struggling with the concept of releasing a new virus, with the sole objective of determining if it can be used to test for antivirals and for scientific studies on exacerbation of asthma. This concern is amplified by the fact that the investigators will be exposing people to this new virus who have not consented to be exposed – family members and close contacts.

Noting that a virus generated in this way will not be significantly different than wild type virus, Dr. Williams suggested that a survey be completed in the first cohort of research participants along with additional assays for close contacts to characterize the lack of transmission. Dr. Zaia noted that a previous clinical trial of a recombinant virus required that participants’ contacts sign the informed consent document; while recruitment was difficult, that approach might be appropriate for this trial. Dr. Flint suggested that a recommendation be added to quarantine at least the first cohort of participants, to give the investigators time to study symptoms and find out if there is any reason to expect this recombinant virus to be more pathogenic than other HRVs.

Dr. Williams suggested a middle ground in which the first group of participants would not be students but would be individuals in “stable social situations”; doing so would mean not having to quarantine anyone while also recognizing that, for the first group of five participants, a little more assurance is needed that there is nothing “different” about this recombinant virus. With the addition that it might be prudent to consider consenting some of the close contacts, the RAC members agreed to this middle-ground suggestion. Dr. Kahn suggested the addition of the concept of “social distancing” so that research participants are not quarantined but that they do refrain from attending large public gatherings.

## **D. Investigator Response**

### **1. Written Responses to RAC Reviews**

Although RNA viruses, including rhinoviruses, exist as quasispecies due to the high error rate of the viral RNA polymerase, RG-HRV16 is derived from the consensus sequence of the dominant nucleotide sequences in the original viral stock. It is not a rare mutant. So far, the investigators’ results have showed that RG-HRV16 is as stable as the original stock with no loss of infectivity after months of storage. Its growth characteristics are not different from that of the clinical isolate, and the HRV16-specific antibody can neutralize it efficiently.

The RG-HRV16 inoculum will have one less possible source for introducing contaminants compared with inocula using standard technology. Viral inocula produced using standard technology could be contaminated by pathogens in the seed virus (nasal or respiratory secretions), the cell line, or the

process. With the current RG-HRV16, the seed virus has been taken out of this equation, which the investigators believe is a protocol improvement that lessens the risk of introducing other respiratory contaminants. The Master Cell bank has proven to be free of contaminating viruses and other human pathogens. Because the reagents are all certified as pathogen free and the source of viral seed is produced by reverse genetics instead of human respiratory secretions, the investigators have not proposed additional testing for pathogens other than the sterility, endotoxin, and mycoplasma tests described in Appendix M-II-B-3-e.

The error rates of the viral polymerase and the T7 polymerase used for reverse genetics are similar. Using reverse genetics, sufficient genome sequence will be provided to allow enough RG-HRV16 to be produced for the entire study from only one *in vitro* transcription and one cycle of viral growth. Therefore, the number of mutations should be less than that of inoculum from natural isolates which require multiple amplifications and lengthy passages of the virus.

Regarding the specificity of the PCR detection assay for HRV16, a baseline nasal lavage will be conducted on the day of inoculation prior to RG-HRV16 administration and will be tested for known respiratory viruses. If a rhinovirus is detected, sequencing of the 5' non-coding region can identify the strain. A sample of nasal lavage taken at peak of the cold will be analyzed using a TCID50 neutralization test using serum specific for RV16 and sequencing analysis will be performed to confirm infection by RG-HRV16.

The informed consent document and protocol were amended to provide additional information about possible future studies to better understand how rhinoviruses can contribute to worsening of asthma.

Sinus infection as a result of a cold may be experienced in fewer than 5 percent of cases and pneumonia even less frequently. The informed consent document has been revised to indicate how adverse outcomes will be monitored – study visits at days 1 (in a subset of individuals), 2, 3, 4, and 7 with access to a study physician, if warranted, at any of those visits. If a research participant presents with a sinus infection, the study physician can write a prescription for an antibiotic that may be filled by the research participant at the pharmacy of their choice, at their own cost.

The State of Wisconsin is prevented by policy from paying for research related non-study procedures. The study group will be provided with acetaminophen for symptom relief.

## **2. Responses to RAC Discussion Questions**

Dr. Gern explained that the investigators have conducted extensive sampling of rhinoviruses in the community in Wisconsin, monitoring every peak common cold season since September 2006. In general, they have found 20 or 30 strains that circulate in the community at any one time. Comparing fall to spring, which are the two main rhinovirus seasons, 90 percent of the fall strains are no longer present in the spring and new strains appear. Since monitoring studies began in September 2006, HRV16 has not been detected. Therefore, the chances of finding another HRV16 in the community would be remote, although methods will be in place to monitor for that possibility.

Regarding safeguards for the close contacts of research participants, Dr. Gern explained that potential participants in high-risk groups as well as potential participants who have a family member with a high-risk condition would be excluded from this clinical trial. High-risk groups are defined as the very young, elderly individuals, and people with chronic lung disease.

The investigators will provide research participants with specific instructions on how not to spread common colds, which have been shown in many settings to be effective procedures.

The investigators made this new virus through recombinant techniques that have provided the opportunity to study its growth in tissue culture, where it behaves like the parental and progeny viruses. Therefore, the investigators are confident that its replication, infectious profile, growth curve, and other aspects are similar to viruses with which they have worked for 30 years.

Dr. Gern offered to add to the protocol a rule that the study will be stopped and a safety review conducted if any spread of the virus is documented.

With regard to where the participants for this trial are likely to originate, Dr. Gern stated that their experience has been that 75 percent of participants during the school year are students and, during the summer months, many more community members participate compared to the number of students.

Dr. Gern briefly described a birth cohort study being conducted since 1999 in which the investigators monitor for HRV year-round; results show that HRV never goes away. The peak prevalence for HRV is September-October and April, but HRV is responsible for at least 50 percent of colds at all times of the year. The only exception is during the peak of influenza season, when HRV is overshadowed by influenza, but HRV infections are still present.

Dr. Gern explained that colds are spread most efficiently in the first few days of the illness, even before symptoms appear. The reason that handwashing and other precautions are expected to be so effective is that participants will know they are infectious and they can take the appropriate precautions to minimize the chance that this virus will be spread. That knowledge is not normally available for the usual “wild-caught” colds. Although exposure to colds is ubiquitous and a significant-enough exposure is likely to result in catching a cold, these research participants know they have colds, they know the precautions to take to avoid spreading those colds, and therefore the investigators are confident that they can send these people into their usual environments with the expectation that the colds will not spread. Dr. Gern offered to monitor participants’ household contacts to document whether spreading occurs.

#### **E. Public Comment**

No public comments were offered.

#### **F. Synopsis of RAC Discussion and RAC Observations and Recommendations**

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

##### Preclinical Issue

- Because the viral RNA polymerase lacks an error-correcting function, mutations occur frequently during virus replication resulting in the generation of quasi-species. Efforts should be made to analyze (e.g., through deep sequencing) the quasi-species of RG-HRV16 that may have arisen during propagation of the virus in the human embryonic lung fibroblast cell line as well as those isolated from nasal secretions from infected research participants.

##### Clinical/Trial Design Issues

- Given the unknown risks associated with administration of a possibly novel virus, the initial research participants should be closely monitored for unexpected reactions. In addition, if there are novel attributes to the virus, limiting spread in the community is of utmost importance. Research participants who are initially enrolled should have stable, limited contacts (i.e., should not live in a communal setting). In order to enhance surveillance of close contacts, it may be prudent to consider requiring close contacts of each research participant to consent to the study. In addition to providing instructions to research participants about how to prevent the spread of the virus to close contacts, information should also be provided about how to prevent wider transmission through social distancing.
- Even a few amino acid changes could potentially modify the pathogenicity of the RG-HRV16 viruses in unexpected ways. While replication of the recombinant virus in cell culture was shown to be comparable to naturally occurring virus, the immune response to RG-HRV16 can only be

evaluated clinically. For safety reasons, the current plan for the initial low dose cohort is to widely space each participant in order to monitor for any adverse events. The low dose is 10-fold lower than the dose that produced either mild colds or asymptomatic infections in previous studies with a nonrecombinant virus. Since adverse events may not be seen at the low dose, the same precautions should be taken with the higher-dose cohorts.

- Given that research participants could potentially expose relatives and close contacts to a novel virus, the investigators should consider including in the protocol another stopping rule in the event that contacts develop respiratory infections in the time period one would expect infection to develop from exposure to the research participant. In addition, if the contact is willing to be tested, every effort should be made to determine whether the RG-HRV16 virus was the source of the infection in the contact.
- The quantitative polymerase chain reaction assay used to detect respiratory viruses in research participants' nasal secretions should be specific for HRV16 and, ideally, for RG-HRV16.
- If the risk of spreading the virus through the community is greater in certain seasons, the study should not be conducted during those periods.

#### Ethical/Legal/Social Issues

- Given that development of a cold of at least moderate intensity is a primary endpoint of the study, provisions should be made for caring for any illness arising from infection with the virus, including reimbursement for care. Policies addressing research-related injury are not applicable to this type of research. In the case of the usual research-related injury, the injury is not the goal of the study treatment. However, in this case the intention of the intervention is to create illness; therefore, being prepared to provide care for the full spectrum of related illness, regardless of the seriousness, should be part of the protocol.
- The informed consent document should indicate that in the event of the death of a research participant, no matter the cause, a request for an autopsy will be made of the family in order to obtain vital information about the safety and efficacy of the research. Research participants should be asked to inform their families that such a request will be made and why it is scientifically and medically important. See Appendix M-III-B-2-c of the *NIH Guidelines* and NIH Guidance on Informed Consent for Gene Transfer at the OBA web site: Request for Autopsy ([http://oba.od.nih.gov/oba/rac/ic/appendix\\_m\\_iii\\_b\\_2\\_c.html](http://oba.od.nih.gov/oba/rac/ic/appendix_m_iii_b_2_c.html)).

#### **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. It was moved by Dr. Roizman and seconded by Dr. Williams that the RAC approve these summarized recommendations. The vote was 14 in favor, 0 opposed, 0 abstentions, and 0 recusals.

#### **IX. Closing Remarks and Adjournment**

Dr. Federoff thanked the RAC members and the OBA staff and adjourned the meeting at 4:20 p.m. on September 9, 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.

Date: \_\_\_\_\_

\_\_\_\_\_  
Howard J. Federoff, M.D., Ph.D.  
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## Attachment I Recombinant DNA Advisory Committee Roster

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## **Attachment II Public Attendees**

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Parai Au, FDA  
Lance B. Augustin, University of Minnesota  
Lajos Baranyi  
Charles Bonds, Auciq  
Connie Edmondson  
Steven Fleischer, FDA  
Joydeep Gosh, FDA  
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Daniel A. Saltzman, University of Minnesota  
Mercedes Serabian, FDA  
Ramtay Vatsan, FDA  
Allen Wensky, FDA  
Hatchem Zayed, Lentigen Corporation

## Attachment III Abbreviations and Acronyms

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APC	adenomatous polyposis coli
BL	biosafety level
cDNA	complementary deoxyribonucleic acid
DHHS	U.S. Department of Health and Human Services
DNA	deoxyribonucleic acid
DSMB	data and safety monitoring board
<i>E. coli</i>	<i>Escherichia coli</i>
ECRL	extensor carpi radialis longus
FAP	familial adenomatous polyposis
FDA	Food and Drug Administration, DHHS
GI	gastrointestinal
GTDG	Gene Therapy Discussion Group
GTSAB	Gene Transfer Safety Assessment Board
HIBM	hereditary inclusion body myopathy
HIV	human immunodeficiency virus
HRV	human rhinovirus
IBC	institutional biosafety committee
ICH	International Conference on Harmonisation
IL-2	interleukin-2
IM	intramuscular
IND	investigative new drug
IV	intravenous
NHP	nonhuman primate
NIH	National Institutes of Health
<i>NIH Guidelines</i>	<i>NIH Guidelines for Research Involving Recombinant DNA Molecules</i>
NSABB	National Science Advisory Board for Biosecurity
OBA	Office of Biotechnology Activities, NIH
OD	Office of the Director, NIH
PI	principal investigator
RAC	Recombinant DNA Advisory Committee
RG	risk group
RG-HRV16	type-16 human rhinovirus
RNA	ribonucleic acid
SAEs	serious adverse events
<i>S. enterica</i>	<i>Salmonella enterica typhimurium</i>
shRNA	short-hairpin RNA
siRNA	short-interfering RNA
<i>tkRNAi</i>	<i>transkingdom</i> RNA interference
ug	micrograms