
RECOMBINANT DNA ADVISORY COMMITTEE

Minutes of Meeting

June 11, 2014

**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](http://osp.od.nih.gov/office-biotechnology-activities/biomedical-technology-assessment-recombinant-dna-advisory-committee/human-gene-transfer-protocols-registered-oba) can be found on the Office of Biotechnology Activities website at <http://osp.od.nih.gov/office-biotechnology-activities/biomedical-technology-assessment-recombinant-dna-advisory-committee/human-gene-transfer-protocols-registered-oba>.]

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

June 11, 2014

The Recombinant DNA Advisory Committee (RAC) convened for its 138th meeting at 8:30 a.m. on June 11, 2014, at the Neuroscience Research Center (NIH Building 35), 9000 Rockville Pike, Bethesda, Maryland. Dr. Donald B. Kohn (RAC Chair) presided. In accordance with Public Law 92-463, the meeting was open to the public from 8:30 a.m. until 3:25 p.m. on June 11, 2014. The following individuals were present, either in person or by teleconference, for all or part of the June 2014 RAC meeting.

Committee Members

Michael Atkins, Georgetown University School of Medicine
Paula Cannon, University of Southern California
Saswati Chatterjee, City of Hope National Medical Center
William Curry, Harvard Medical School (*via teleconference*)
Rebecca Dresser, Washington University School of Law
Norman Fost, University of Wisconsin, Madison
Marie-Louise Hammarskjöld, University of Virginia School of Medicine
Angelica Hardison, Georgia Regents University
Donald Kohn (RAC Chair), University of California, Los Angeles
David Ornelles, Wake Forest University School of Medicine
Joseph Pilewski, University of Pittsburgh
Michael Sadelain, Memorial Sloan-Kettering Cancer Center (*incoming*)
Marcella Sarzotti-Kelsoe, Duke University School of Medicine
Marshall Strome, St. Luke's–Roosevelt Hospital Center/New York Head and Neck Institute
Richard Whitley, University of Alabama, Birmingham
Dawn Wooley, Wright State University

NIH Office of Biotechnology Activities (OBA)

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

NIH Office of Science Policy

Amy Patterson, Office of the Director, NIH

Nonvoting Agency Representatives

Kristina Borrer, Office for Human Research Protection, NIH
Denise Gavin, U.S. Food and Drug Administration (FDA)
Daniel Takefman, FDA

NIH/OD/OBA Staff Members

Linda Gargiulo
Morad Hassani
Robert Jambou
Maureen Montgomery
Carolyn Mosby

¹ The Recombinant DNA Advisory Committee is advisory to the 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.

Marina O'Reilly
Gene Rosenthal

Attendees

There were 50 attendees at this 1-day RAC meeting.

Attachments

Attachment I contains a list of RAC members and nonvoting agency and liaison representatives. Attachment II contains a list of public attendees. Attachment III contains a list of abbreviations and acronyms used in this document.

I. Call to Order and Opening Remarks

Dr. Kohn, the RAC Chair, called the meeting to order at 8:30 a.m. on June 11, 2014. Notice of this meeting under the *NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules (NIH Guidelines)* was published in the *Federal Register* on May 19, 2014 (79 FR28740). Issues addressed by the RAC at this meeting included a report from the Gene Transfer Safety Assessment Board (GTSAB, a subcommittee of the RAC), recommendations by an Institute of Medicine (IOM) Committee charged with assessing the role of the RAC in the review of human gene transfer protocols, review of policies and best practices for housing of non-human primates (NHPs) in BL4 laboratories under *NIH Guidelines*, and public review and discussion of two gene transfer protocols.

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

Dr. Corrigan-Curay reminded RAC members of the rules of conduct that apply to them as Special 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 RAC Meeting, March 12, 2014

RAC Reviewers: Drs. Curry and Kiem

Dr. Curry suggested that the RAC approve the minutes of the March 2014 RAC meeting, with no changes; Dr. Corrigan-Curay relayed an email from Dr. Kiem saying that he found the minutes to be acceptable as submitted. No changes to the document were suggested by other RAC members.

A. Committee Motion 1

Dr. Kohn asked the RAC to approve the minutes of the March 12, 2014, RAC meeting. The RAC voted unanimously by voice to do so.

III. Review and Discussion of Human Gene Transfer Protocol #1404-1306: An Open-label Dose Escalation Study of an Adeno-associated Virus Vector (scAAV2-P1ND4v2) for Gene Therapy of Leber's Hereditary Optic Neuropathy (LHON) Caused by the G11778A Mutation in Mitochondrial DNA

Principal Investigator: John Guy, M.D., University of Miami Bascom Palmer Eye Institute
RAC Reviewers: Dr. Chatterjee, Ms. Dresser, and Dr. Sarzotti-Kelsoe

A. Protocol Summary

Leber's hereditary optic neuropathy (LHON) is a maternally inherited form of bilateral optic neuropathy causing severe and permanent visual loss in young adults, usually between the second and fourth decades of life. LHON usually presents as a loss of central vision that typically progresses over weeks without pain, until visual loss ensues. Visual loss may occur in both eyes simultaneously, or in one eye with the fellow eye affected weeks to months later. The deterioration of vision may progress over several months to a maximum loss of function with visual acuities worse than 20/200.

LHON results from a point mutation in the mitochondrial DNA (mtDNA) *ND4* gene, compromising the mitochondrial respiratory chain and causing a severe reduction in adenosine triphosphate (ATP) synthesis. It is generally hypothesized that this dysfunctional respiratory chain impairs ganglion cells in the retina and damages the optic nerve, leading to the visual loss characteristic of LHON. Three primary mutations—at nucleotide pairs 3460, 11778, and 14484—are generally thought to be the main causes of LHON, accounting for 98 percent of all LHON cases. For most individuals with LHON, vision loss is permanent. However, some people spontaneously recover some central vision within a year after the first onset, while others may recover many years later. The likelihood of regaining vision lost by the disease appears to depend on age and the particular mutation involved. Persons whose LHON starts before age 10 tend to have a greater chance of vision recovery. Compared with other forms of LHON, G11778A LHON has the worst prognosis and lowest spontaneous visual improvement, with reported recovery rates around 5 percent. In contrast, LHON with a mutation at 14484 has a reported 37 percent to 71 percent chance of some visual improvement.

To date, an effective treatment for LHON has not been identified. Anecdotal reports of treatment efficacy have been difficult to interpret given the potential for spontaneous recovery in certain genotypes. Treatments including systemic steroids, hydroxycobalamin, cyanide antagonists, and naturally occurring cofactors (e.g., coenzyme Q10, succinate, and idebenone) have not produced definitive evidence of treatment or prevention of visual loss.

Efforts to explore possible gene therapies for LHON have been hampered by the lack of a technology to introduce DNA directly into mitochondria. The investigators have approached this problem by constructing a "nuclear version" of the mitochondrial gene using an adeno-associated virus (AAV) serotype 2 vector expressing the NADH dehydrogenase subunit 4 (*ND4*) gene to produce the study agent, scAAV2(Y444+500+730)-smCBA-P1*ND4v2* (scAAV2-P1*ND4v2*). This vector has been shown to increase cell survival and restore defective ATP synthesis in cybrids cells with 100 percent G11778A mtDNA. In animal models, this construct prevents visual loss and retinal ganglion cell (RGC) and axonal demise that resemble LHON. For the proposed research, intravitreal injection will be used to deliver this construct to RGCs. The cytoplasmically synthesized ND4 protein will include a targeting peptide that should, in turn, allow the corrected ND4 protein to enter the mitochondria.

This study will be an open-label, dose-escalation Phase I clinical trial of a single intravitreal scAAV2-P1*ND4v2* administration to individuals with the most severe form of LHON (i.e., the G11778A mutation). Three different AAV doses will be tested in three different patient groups reflecting the acute, chronic, and presymptomatic stages of LHON disease. Each cohort will include three patients. Enrollment will be limited to adults. The study aims to assess the safety, tolerability, and potential efficacy of the proposed therapy. Ocular and systemic toxicity will be assessed prior to and following vector administration to determine whether there are any adverse changes that may be associated with vector administration.

This protocol represents the first gene therapy trial for a mitochondrial disease and the first clinical trial using a mutated AAV capsid.

B. Written Reviews by RAC Members

Eleven RAC members made a recommendation for in-depth review and public discussion of the protocol. Key issues included that it will be the first human study involving gene transfer for a mitochondrial disease

and because the risks associated with integration and administration of the proposed AAV vector (scAAV2-P1ND4v2) are not precisely known.

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

Several of Dr. Chatterjee's questions and comments focused on the challenges and applicability of the preclinical disease model to the proposed study. She identified several challenges with the mitochondrial disease model used in preclinical studies and pointed out that although the mutant mitochondria exist in all cells, the phenotype appears to be primarily restricted to the eye. For the natural disease, there appears to be a requirement for all mitochondria to carry the mutation, suggesting that even a low level of correction may be therapeutic.

Dr. Chatterjee asked the investigators to provide more information about the disease model generated using an AAV vector encoding a mutant *ND4* gene. The mice normally encode the wild type gene in their mitochondria, and it is not clear why the addition of the mutant *ND4* has such a dramatic effect, particularly when the phenotype is only manifested when 100 percent of the mitochondria are mutated. In this case, AAV delivery of the mutant would result in heteroplasmy, which should not be sufficient for the disease. In the preclinical model, the disease is initiated by AAV delivery of a mutant *ND4* gene to the RGCs of the eye. The therapeutic model, however, involves delivery of the wild-type *ND4* gene to the eye by AAV. Dr. Chatterjee asked the investigators to elaborate on the accuracy of disease modeling for a phenotype that takes years to develop in humans but that is treated within only 3 days of the mutant gene delivery in this model. In addition, she requested further information as to whether it possible that the slow accumulation of the effects of the *ND4* mutation over decades may be more difficult to treat than the acute preclinical model.

Supporting data are needed for the conclusion presented in the protocol that rescue failed when the administration of the therapeutic vector was delayed and that this failure was due to the development of neutralizing antibodies. Dr. Chatterjee inquired as to whether the investigators tested delivery of the wild-type gene with a different AAV or used plasmapheresis to deplete antibodies to determine whether the lack of therapeutic effect was truly due to the development of neutralizing antibodies, rather than a problem with the model.

A recent European clinical trial showed significantly increased integration of AAV sequences in mitochondrial DNA in the human muscle. There was no evidence of insertional mutagenesis, but this finding raises the question of whether AAV can naturally traffic to mitochondria. Dr. Chatterjee asked whether the investigators can provide any data or additional information on this outcome.

Dr. Sarzotti-Kelsoe found the protocol to be carefully designed, but it had some unclear elements. She provided content-oriented comments and questions for clarification and identified some minor corrections. Dr. Sarzotti-Kelsoe asked the investigators to specify how they plan to take into account the issue of spontaneous recovery in the interpretation of the results of the proposed trial. This study will follow patients for 10 years, but rodent and NHP preclinical studies cited in the protocol appear to have been limited to less than 1 year. Information on any long-term animal studies should be provided in addition to the long-term effect of scAAV2-P1ND4v2 that the investigators expect to find in the treated patients. Dr. Sarzotti-Kelsoe also asked that new data on high-dose testing be included in the presentation to the RAC.

Dr. Sarzotti-Kelsoe requested additional information regarding potential toxicities and responses to AAV constructs. She noted that mild to marked increases in creatinine kinase were observed in three rats but that these results were not considered adverse or toxicologically significant. The investigators were asked whether there is any concern of a toxicity trend given these findings. Neutralizing antibody (NAb) titers are listed as one of the planned study evaluations, and an NAb assay is described in Appendix M (p. 124). The value for a positive titer with this assay does not appear to be defined, however. In describing results of an intervention study using the mouse model of LHON, the investigators state that "irrespective of which vector (mutant or normal) is injected first, the introduction of AAV generates a humoral response against the vector that elicits a neutralizing antibody response that prevents expression of the second

vector when this second injection is delayed by 2 weeks or more” (Appendix M, p. 78). Evidence for induction of response should be provided, and the NAb titers should be specified.

Results of a safety study in three NHPs showed NAb titers increasing from less than 5 or 320 at baseline to 5120 within 1 week to 3 months following injection to the eye in the two animals who were given an AAV2 construct, AAV2(Y444,500,703F); the third animal served as a control (Appendix M, Table 37, p. 125). Low-level expression of the vector was detected in the spleen of both animals administered the vector and in the mandibular lymph nodes of one of the two dosed animals. All other major organs were negative for NAb response at the time of sacrifice. The only clinical observation noted was mild local bruising in the injected eye that resolved within 5 to 10 days. Dr. Sarzotti-Kelsoe questioned whether it is correct to conclude that “the test article is safe for human testing,” as stated in Appendix M (p. 128), and whether the investigators are concerned that these findings may be evidence of a systemic immune response induction. She asked further if such a response, if induced, could “break tolerance” of the eye as an immune-privileged site. Dr. Sarzotti-Kelsoe noted that antibodies against human ND4 are not available for detection of transgene expression and asked if the investigators would consider producing a recombinant ND4 for detection of a potential immune response to human ND4.

Dr. Sarzotti-Kelsoe asked whether the operators reading/counting the sections from each eye in the post-mortem and immunohistochemical studies described in Appendix M (pp. 69 and 74) were blinded to the source of treatment.

Dr. Sarzotti-Kelsoe identified the following minor items for clarification or correction:

- The acronyms for WFI and BSS should be defined at first use (clinical protocol, p. 11/63).
- In protocol section 6.19.5.1 (Detailed Study Agent Administration Procedures), three sizes of needles are listed (27, 29, and 30 gauge) for injection. It is not clear whether these are typographical errors or different gauge needles will be used for intravitreal injections. If different size needles will be used, the protocol should specify who will make this decision at the bedside.
- In the Packaging section, the manufacturing date is not included in the labeling. Without manufacturing date, it is impossible to deduce an expiration date (the study agent is stable for at least 12 months at -80 degrees Celsius). This should be corrected.
- Pages 96–117 in Appendix M are repeated twice.

Ms. Dresser considered having a prespecified waiting period after the first subject exposure to be a good approach to reduce risks to study participants. She noted that one of the responses in Appendix M refers to parental permission and assent but that the protocol says the study will be in adults only. This discrepancy needs to be clarified.

Ms. Dresser also suggested the following modifications to the informed consent document (ICD):

- Page 8, paragraph 3: Saying that “there is a high likelihood” that the agent will be safe in humans seems somewhat overstated. The investigators should consider revising the language to use the phrase “it is likely” instead.
- Page 11, paragraph 3: Information about the costs of care for research-related injuries could be stated more clearly. The investigators may want to add a second sentence such as, “Medical care at other hospitals or centers will not be covered by the study sponsors.”
- Page 12, under study withdrawal: It would be helpful to inform participants that the study agent cannot be removed from the body once it is injected.

C. RAC Discussion

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

- Dr. Chatterjee requested additional details about the mutated AAV capsids, including vector genome copy numbers per cell with mutant capsids versus the wild-type gene.
- The reviewers and several other RAC members remained concerned that an NAb response to the vector may prevent future dosing of the second eye. This issue is especially important given

that LHON is a rare condition that typically affects both eyes and that a patient enrolled in the Phase I study may want to enroll in a future study if this research goes forward.

- Dr. Hammarskjöld asked whether any commercially available antibodies can be used for detection of a potential immune response to human ND4.
- Dr. Sadelain inquired about monitoring the early post-injection phase for the emergence of an immune response and what intervention(s) would be used if an immune response occurs.
- Dr. Whitley noted that because the three patient populations proposed for this trial are very heterogeneous, a response may be seen in only one group. The investigators should consider an adaptive clinical trial design, which will allow for expansion of the population in which a significant response is seen to assure that adequate numbers of patients are enrolled to meet the aims of the study, which can be a limitation for rare disease.
- Dr. Atkins asked whether patients who recover from or see improvement in their condition ever subsequently deteriorate.
- Dr. Strome asked whether progression of the disease in the animal model is similar to that in humans, that is, whether both eyes go out simultaneously or one eye loses function before the second eye. Within this context, he questioned whether treatment of the worse eye could adversely affect the eye that is still functional (e.g., because of an immunologic response) or possibly mute the chance of spontaneous recovery, and whether the proposed study allows for sufficient time to make this assessment.
- Dr. Kohn asked about the duration of patient follow-up. He noted that under FDA guidelines, because the AAV is potentially integrating, the requirement is 15 years of follow-up. However, the study appears to propose a 10-year follow-up phase.
- Dr. Kohn advised adding information to the ICD that a potential risk of being treated in one eye is that they may not be able to have the second eye treated in the future if they develop antibodies against the interventional product.

D. Investigator Response

1. Written Responses to RAC Reviews

Summaries of studies in the animal model of LHON and supporting documentation for the proposed study design were submitted with the investigators' written response to the reviewers' comments, were reviewed by Dr. Guy during the RAC meeting, and are described in the protocol and Appendix M.

Development of the mouse model of LHON involved delivery of a mutant *ND4* subunit to the mouse visual system by intravitreal injection. The mutant *ND4* subunit is compatible with the universal genetic code and contains an A-to-H substitution at residue 340. Expression and effects of the mutant *ND4* gene on the optic nerve and cultured RGCs were assessed using MRI, immunohistochemistry, and light and transmission electron microscopy. Results indicated that the targeting sequence directed expression of mutant human R340H and wild-type *ND4*FLAG polypeptides into mitochondria. The investigators report that expression of normal human *ND4* in murine mitochondria posed no ocular toxicity. In contrast, the mutant *ND4* disrupted mitochondrial cytoarchitecture, elevated reactive oxygen species, and induced swelling of the optic nerve head and apoptosis with a progressive demise of ganglion cells in the retina and their axons comprising the optic nerve. Optic nerve swelling in mice with the mutant gene led to the typical optic atrophy characteristic of the later stages of LHON and is consistent with what is observed in patients in the weeks or months preceding, during, and immediately following their acute loss of vision. In contrast, optic nerve heads of mice inoculated with AAV-*GFP* or AAV expressing the allotropic version of wild-type human *ND4* to be used in the proposed Phase I trial appeared histologically normal. Quantification of the degree of swelling of the swollen optic nerve head indicated an almost three-fold increase in mutant *ND4* inoculated eyes relative to controls (AAV-*GFP*; $P < .005$). As seen in patients with LHON, optic edema of mutant *ND4* inoculated mice was transient and resolved within 6 months after inoculation. Thus, delivery of the mutant human *ND4* subunit gene to the mouse visual system functionally mimicked the initial stages of the human LHON phenotype even in the presence of endogenous murine *ND4*.

Although the single-stranded AAV2 vector carrying the wild-type allotopic *ND4* had successfully rescued defective ATP synthesis of LHON cells in animal models, questions were raised regarding heteroplasmy resulting from AAV delivery of the mutant and competition between both human mutant and wild-type *ND4* protein for entry into the mitochondria. The investigators noted that the latter scenario is not applicable to human LHON where the mutated *ND4* sequence exists within the mitochondria. Regarding heteroplasmy, the investigators note that while most LHON patients with the G11778A mutation are homoplasmic with 100 percent mutated mtDNA, 14 percent to 15 percent are heteroplasmic, containing both wild-type and mutant *ND4*, similar to the mouse model. Data indicate that mutation load is an important risk factor for blindness in heteroplasmic men. One study found that approximately one-half of men who harbored more than 80 percent of mutated mtDNA and one-third of men with 60 percent to 80 percent mutated mtDNA were clinically affected and exhibited the phenotypical visual loss. None of those with less than 60 percent mutated mitochondrial DNA had visual loss, however. Women have a much lower penetrance of LHON than men, but the phenotype has been reported in some women with 33 percent, 84 percent, or 97 percent mutated mitochondrial DNA. The investigators note that in both patients and the mouse model, heteroplasmy is sufficient to induce the LHON phenotype.

Questions regarding the possible limitations of the initial mouse model (as described above) led the investigators to develop a second-generation, triple-modified vector with an untagged (no FLAG sequence) transgene, designated as scAAV2-P1*ND4v2*, which is the proposed clinical product. The resulting murine model for LHON is more relevant to the human disease condition. The investigators note that pharmacology studies demonstrate the increased potency of the second-generation test article and provide efficacy data to support the use of human *ND4* gene therapy in G11778A LHON with the intended clinical vector product.

The investigators acknowledge that it is possible that the slow accumulation of the *ND4* mutation over decades, if that is occurring in patients, may be more difficult to treat than the acute animal model. In addition, the animal model and pharmacological effect observed in the preclinical studies are limited by the acute condition manifested by the model. Evidence for slow accumulation of damage in LHON is found in asymptomatic carriers of the mutation who are not yet affected by the visual loss. Some of the carriers have a slightly higher level of an axonal breakdown product called phosphorylated neurofilament heavy chain than normal unaffected controls. In addition, the pattern electroretinogram that identified some asymptomatic carriers shows a progressive decrease in amplitude over time (relative to normal controls). All other parameters (vision, visual field ophthalmoscopy of the optic nerve, optical coherence tomography [OCT] measurements of the retina) appear normal in the carriers. Thus, there is only subtle evidence suggesting an accumulating damage concept.

Clinical findings in G11778A carriers do not indicate a slow accumulation of damage over decades. Rather, damage appears to commence at the onset of visual loss. Preclinical studies in the mouse model induced visual loss and treatment right away, similar to patients with acute visual loss. Patients with chronic visual loss typically have lost approximately half their RGCs; thus, rescue can only be accomplished by gene therapy in the RGCs that are presumably still present but dysfunctional. Some patients in this group have improved spontaneously even with 50 percent loss of their ganglion cells, suggesting that adding in more wild-type *ND4* may allow others who do not recover vision to improve with treatment. In eyes that have not yet lost vision, the last group that will be treated in the planned study (after testing for safety in the blind eyes first) will still have good vision and a normal complement of RGCs. Left untreated, they will go blind within 6 months and half their RGCs will die off. These patients do not have irreversible RGC loss, and results from the LHON animal model suggest that the proposed intervention should be able to maintain good vision in this last group of patients. The proposed small Phase I study is not designed, however, to determine whether the addition of the study agent in patients with no or little normal wild-type *ND4* may reverse the biochemical defect in their remaining RGCs and improve visual function.

As noted by the reviewers, high levels of NAb have been detected in rodents and NHP within 2 months after intravitreal injection of the study agent, with titers going from less than 10 units at baseline to between approximately 5,000 to more than 20,000 post-dosing. A positive NAb titer in the assay described in Appendix M is reported as the highest serum dilution that inhibited self-complementary

AAV2(Y444,500,703F)-smCBA_mCherry transduction of cultured cells by more than 50 percent. Results using the murine model for LHON are consistent with other studies showing the presence of NAb against AAV2 and a lack of transient gene expression in the second eye of animals that have had a previous intravitreal injection into the first eye. The investigators have not done any experiments involving plasmapheresis and could not find any publications of plasmapheresis in small animals such as mice. One report of plasmapheresis in monkeys suggested that removal of NAb improved transgene expression, while a second report in humans showed that plasmapheresis can reduce levels of neutralizing antibody titers against AAV. The investigators stated that the current body of evidence supports their conclusions that intravitreal delivery of a second AAV 3 months after the first dose does not work due to NAb and does not represent a problem with the model but, rather, represents a limitation of the model. Many investigators, including Dr. Guy and his colleagues, are interested in an antibody against human ND4, but producing a recombinant *ND4* for detection of a potential immune response to human ND4 has remained elusive to date.

The investigators note that AAV serotype2 has the highest transgene expression in RGCs following intravitreal injection and that they have not used any different AAV serotypes in their experiments. They agree with the reviewers that using two different AAV serotypes could potentially lessen an NAb response against the mutated capsid and limit the reduction of gene expression of the second vector. They remained concerned, however, that such an approach could confound the study outcome. For example, if the first vector is a different AAV serotype (i.e., not AAV2) carrying the mutant *ND4* to induce disease, it may not provide sufficient transgene expression in a requisite number of RGCs to yield the LHON phenotype. Alternatively, if the second vector carrying the rescue wild-type *ND4* gene is packaged in a different AAV serotype (not AAV2), transgene expression could be insufficient to rescue the phenotype caused by the first injection with the mutant *ND4* packaged in AAV serotype 2.

Neutralizing antibodies are an indication of a systemic immune response. As noted by the reviewers, preclinical studies showed low levels of viral DNA in the spleen of two animals. The investigators commented that expression was not evaluated in these cases, but that histopathology of these tissues was normal, with no evidence of inflammation. The investigators do not anticipate any problems to be caused by the induction of NAb in participants in the proposed study, who will receive a single unilateral injection of the test article. NAb may cause a reduction in expression in the second eye, if in the future an injection into the second eye is done with the same AAV serotype. Second eye injections are not part of the proposed Phase I trial.

Regarding concerns about increased integration of AAV sequences in mtDNA (e.g., as found in muscle of participants in a recent clinical trial), the investigators have examined this possibility in the mouse and found different results depending on the methods used to detect recombination or integration events. Using polymerase chain reaction (PCR), the team found mouse-human chimera following infection with a mitochondrially targeted virus to deliver the wild-type human *ND4* in the mitochondrial genetic code to RGCs of the rodent eye. Using next generation sequencing, however, the investigators failed to find any integration or recombinations of viral DNA with the host mouse mitochondrial genome following intravitreal injection and concluded that PCR resulted in an artifact that suggested recombination.

At most, only a small proportion of patients eligible to enroll in the proposed study are expected to undergo spontaneous recovery. The investigators noted that 95 percent of patients with mutated G11778A mtDNA do not recover and that when recovery does occur, it is often incomplete. For the group with acute visual loss (less than 12 months), some patients showed improved visual acuity (more than 15 letters on the Early Treatment Diabetic Retinopathy Study eye chart, acuity of 20/40 or better). In a natural history study of G11778A LHON patients with chronic visual loss (more than 12 months), however, the team found no spontaneous recovery at all. The power calculations for the study of acute visual loss found that for a therapeutic agent with efficacy ranging from 50 percent to 95 percent, and assuming analysis with McNemar's test (comparing the treated eyes with its untreated contralateral), that 80 percent power could be achieved with sample sizes between 8 and 46 patients. The goal of the planned Phase I study is to test safety at escalating doses of the vector. A protocol for a Phase II trial to test efficacy of the vector in LHON has not been developed yet.

Preclinical studies of two rodent LHON models and disease rescue were carried out for 1 year, almost the entire lifespan of a laboratory mouse. Patients in the proposed trial will be followed for 3 years after a single unilateral intravitreal injection of study drug. At the end of this 3-year follow-up phase, participants will be contacted annually for up to 10 years, presuming continued or renewed funding at the end of the initial 5-year grant.

Results of toxicology studies in rats and NHP showed normal histopathology of all tissues and comparable biodistribution of P1ND4v2 at the medium versus high doses with two exceptions. Viral vector was found in the optic nerves of the eyes injected with the high dose, but not in the uninjected eyes or eyes injected with the low dose of the vector. DNA was detected in some of the spleens of the animals given the higher dose. One primate given the higher dose had a haze over the optic nerve that resolved within a month post-injection. OCTs of these animals did not show any cell loss, and electrophysiology did not show any abnormalities. Based on these results, the investigators concluded that the toxicology data show that the vector is safe. Dr. Guy also presented highlights of these studies during the RAC meeting.

Regarding questions about elevated creatinine kinase (CK) levels cited by the reviewers, the investigators noted that they looked at potential toxicity of the study drug in a total of 80 rats, including 40 animals at 30 days and 40 animals at 90 days. On day 30, marked increases in CK were observed in only one male rat (32X fold concurrent control mean). The investigator who performed these studies (Dr. Conlon) concluded that these findings are “most consistent with skeletal muscle injury. Changes in this single male influenced the mean group data, but because they were of an individual nature, they were not considered toxicologically significant. On Day 90, mild increases in CK activity were noted in two females administered scAAV2-P1ND4v2 (up to 1.55X fold concurrent control). Group average analysis including these values was not statistically significant from the control. In addition, the highest dose group did not show these transient findings.

The investigators clarified that enrollment in the proposed study will be limited to adults between ages 18 and 50. Minors will not be eligible to participate in this trial. The acronyms WFI and BSS stand for “water for injection” and “balanced salt solution,” respectively. Some of the language in the ICD reflects suggested modifications from the data and safety monitoring board. The investigators agreed to revise and update the ICD per recommendations by the RAC reviewers, as follows:

Information in the ICD regarding the safety of the study agent has been revised to read: “We have injected scAAV2-P1ND4v2 into the eyes of normal rats as well as normal monkeys and have not caused any visual or general problems. Based on these studies, it is likely that intravitreal administration of scAAV2-P1ND4v2 at the doses proposed in this study will be safe in you. In addition, similar virus vectors are being injected into the eyes of humans in other scientific studies, and these studies also have reported no major problems.”

Information in the ICD regarding the coverage of costs for research-related injuries has been revised to read: “In the event of any physical injury resulting from research procedures, only professional medical care that you receive at the Bascom Palmer Eye Institute will be provided without charge. Medical care at other hospitals or centers will not be covered by the study sponsors. Since this is a research study, your insurance company may or may not pay for any hospitalizations related to study injuries. No other compensation is offered. If you have an illness or injury during this research trial that is determined by the doctors not to be directly related to your participation in this study, you and/or your insurance will be responsible for the cost of the medical care of that illness or injury.”

The ICD has been revised to inform subjects that the study agent remains in the body and cannot be removed after administration. The revised language reads: “You are free to withdraw your consent and stop participation in this research study at any time without penalty or loss of benefits to which you are otherwise entitled at this institution; however, if you wish to do so, regardless of the reason, you should contact the study doctor (Dr. John Guy). Throughout the study, the researchers may notify you of new information that may become available and that might change your decision to be in the study. We ask that you not enroll in the study if you may be considering to withdraw after you have received an injection

of the study drug. It is important to note that the study agent cannot be removed from the body after it has been injected into the eye.”

2. Responses to RAC Discussion Questions

In their search for an agent that would result in significant rescue of a disease that has free radical damage, the investigators tested a variety of products, including single-stranded, wild-type, and single-mutant vectors, none of which yielded the desired outcome. The proposed mutated AAV capsid performed much differently than the other vectors. It appears to preserve the activity of the virus better than unmodified virus in rescuing animals in which LHON has been induced. The capsids that will be used in the proposed study are triple tyrosine to phenylalanine mutants. The tyrosine residues are less susceptible to free radical damage than the other agents tested, which may contribute to the protective mechanism of the mutated AAV capsid. Dr. Guy noted that 80 percent of the vectors with the mitochondria-targeted virus enter the mitochondria. About 40 percent of the non-targeted vectors containing the tyrosine mutant AAV2 capsids reach the mitochondria but are not expressed. Despite the amount of the vector without the targeted virus that gets into the mitochondria, a rescue effect has not been observed with this construct.

The cost of a trial that includes additional monitoring for NAb and other responses, if injection of the second eye is anticipated in the future, would be substantial and would probably require a private or other outside funding source (e.g., pharmaceutical company or drug manufacturer), in part because the additional testing would not constitute high-impact innovative research. Pre-existing immunity would only be a concern for a second eye injection, which is not being planned for the proposed research or in the future. Dr. Guy explained that the neutralizing antibodies are in the capsid, which is rapidly processed and removed from the eye, in contrast with other tissues, which appear to handle the capsid much differently than the eye. The investigators do not consider detection of the vector in the spleen to be a source of concern. Although the presence of the vector beyond the eye suggests some systemic distribution, the levels of DNA in the lymph nodes were very low and are not expected to induce a systemic immune response.

In response to concerns raised regarding the potential effects of generating NABs on future trials that may involve injection of both eyes, Dr. Guy explained that preclinical testing has been done with two different vectors, one produced at the University of North Carolina (UNC) and one produced at the University of Florida (UF). The UNC vector is used for the higher dose and results in a markedly lower NAb response. As noted in the written response, however, even at 1 year post-injection, there was no inflammation or abnormal histology or electrophysiology. The only notable difference in those studies, per the investigators' assessment, was the presence of vector DNA in the optic nerves of the injected eye at the high dose. Ways to mitigate a greater immune response with the UF vector during a second ocular injection include use of plasmapheresis or steroids. Another option would be to give the second injection within 2 weeks of first eye injection.

The monitoring plan for immune response following administration of the vector will include regular blood draws and assaying serum for NAb. Monitoring for T cell-mediated immune response (which was not seen in NHPs) will also be done as an added safeguard. Inflammation in the eye would be treated with antibiotics and steroids, as needed, because it is not clear whether the inflammation is due to an infectious agent or not. Use of plasmapheresis and steroids, as noted above, will be considered. Dr. Guy noted that none of the commercially available antibodies to the human ND4 protein worked and that all four of the previously available antibodies have been taken off the market. Despite these challenges, several labs continue to work on this problem.

Based on data to date from the natural history study, patients who recover do not appear to decline again within 5 years. Whether their condition could deteriorate after 10 or 20 years or longer is not known. Dr. Guy noted that in the animal model, the disease was induced in both eyes and rescue was done (or attempted) in only one eye. In humans, loss of vision in the second eye occurs rapidly, usually within 6 months after the first eye is affected. The proposed study design includes a staggered intervention plan. Patients with chronic bilateral visual loss in both eyes (cohort 1) will be treated first and. The study will

then proceed to treat one eye in a second group of patients with acute bilateral visual loss (cohort 2). Safety data will be collected in the first two groups before proceeding to the third group, patients with acute unilateral LHON for less than 12 months. Using this approach, any adverse effect due to an immune response should be seen in the first two groups and would suggest a potential safety concern.

Dr. Guy recognized the limitations of the proposed study design within the context of the planned patient cohorts and nature of LHON disease and agreed with Dr. Whitley's comments and suggestion regarding the adaptive clinical trial design.

Dr. Guy noted that the plan to follow patients for 10 years was accepted by the FDA. He agreed with the suggestion to revise the ICD to include precautionary language about the possibility that the second eye may not be able to be treated with the test agent in the future.

E. Public Comment

Karen Connelly described her experience as a patient with LHON and provided public comment to the RAC in support of this protocol. Ms. Connelly's testimony is included, verbatim, in Appendix A.

Dr. Kohn thanked Ms. Connelly for attending this RAC meeting and for sharing her story. He noted that it is always helpful for members of the RAC to hear from patients to better understand the patients' perspective and needs.

F. Synopsis of RAC Discussion and RAC Observations and Recommendations

Preclinical Issues

- The protocol proposes to use a novel AAV vector with triple mutant tyrosine to phenylalanine transitions in the VP3 capsid. In the preclinical rodent model, which does not fully reflect the human disease due to differences in the mechanism of disease and the timing of vector administration, rescue of vision was observed only with the mutated vector and not with the wild-type capsid vector. The reasons for this difference are unknown, although it is hypothesized that the tyrosine mutation protects the capsid against the free radicals produced by the disease. If this Phase I trial is successful, you should consider further exploration of the mechanism underlying this difference, as it may have implications for further development of this product.

Clinical and Trial Design Issues

- As many patients with LHON have 100 percent mutated ND4, there could be an immune response to the wild-type protein expressed by the vector. At this time, no antibody to human ND4 is available to conduct immune response studies. As new reagents and methods become available, you should continue to pursue such assays since an immune response to the ND4 protein could have implications for both the safety and efficacy of this product.
- If repeat administration of vector is anticipated in the future, it may be useful at the time of the second administration to collect vitreous fluid from both the previously injected and untreated eyes in order to measure AAV antibody titers and other markers of immune response including lymphocyte proliferation. This would be in addition to any monitoring for systemic immune responses to the vector or transgene.
- The three groups (bilateral, long-standing group; bilateral, recent onset group; and presymptomatic, unilateral group) of research participants proposed for enrollment are clinically heterogeneous. If clinical improvement is observed to be superior in one group in comparison to the others, you may want to continue dosing in that group. Consider an adaptive trial design that will allow the expansion of the cohort with the most favorable response.

Ethical/Legal/Social Issues

modified T cells by administering the drug cetuximab. The investigators have conducted a series of experiments demonstrating that T cells modified with this construct kill ovarian cancer cells *in vitro* and in mice. In addition, this CAR T-cell therapy has been used successfully in patients with B-cell acute lymphoblastic leukemia (B-ALL) using a CAR targeting a different antigen (CD19).

This is a Phase I dose escalation trial to determine the safety of intravenous (IV) and intraperitoneal (IP) infusion (with or without prior cyclophosphamide chemotherapy) of genetically-modified autologous T cells (EGFRt/4H11-28z/IL-12+ T cells) given to women with relapsed fallopian tube, primary peritoneal, or ovarian cancer that expresses the MUC16 protein. The secondary aim is to assess the efficacy of the CAR T cells against the cancers being studied and to determine how long these cells persist in the patient's body. Cohorts of three to six patients will be infused with escalating doses of modified T cells to establish the maximum tolerated dose (MTD) of modified T cells. There are four planned dose levels. Approximately half of the autologous T cells modified with this CAR will be first administered intravenously and, if the research participant remains clinically stable, the T cells will then be administered intraperitoneally. Patients in the first cohort will be treated at the lowest dose level of the modified T cells. Patients enrolled in the second through fifth cohorts will receive cyclophosphamide chemotherapy followed 2 to 4 days later by infusion of modified T cells.

Dose escalation will proceed to the next cohort only if less than 33 percent of patients in a cohort experience unanticipated dose-limiting toxicity (DLT). If unacceptable toxicity is seen in one of three patients in any given cohort, up to six patients will be treated in that cohort using a conventional dose escalation scheme. If two of six patients in any given cohort experience unacceptable toxicity, the MTD of T cells will have been exceeded, and the MTD of T cells will be established at the previous cohort dose level. If the first dose level exceeds the MTD, a subsequent cohort of three to six patients will be treated with a single dose of modified T cells as given in the first cohort (1×10^5 EGFRt/4H11-28z/IL-12+ T cells per kilogram) without the addition of lympho-depleting cyclophosphamide.

B. Written Reviews by RAC Members

Eight RAC members made a recommendation for in-depth review and public discussion of the protocol. This protocol was found to warrant further review because this is the first IP administration of T cells modified by a CAR and because T cells administered into the peritoneal space may have a different safety profile than T cells administered intravenously.

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

Dr. Atkins noted that patients with extensive peritoneal adhesions will not be eligible to enroll in this study; the protocol does not appear to specify how patients will be assessed for such adhesions, however. Dr. Atkins questioned whether IP cetuximab will be administered in cases of IP T cell-associated abdominal-peritoneal toxicity (peritonitis) or severe cytokine release syndrome (CRS), and if so, what provisions are in place to ensure that the cetuximab will reach all sites of inflammation, given the possibility of adhesions from prior surgery, IP chemotherapy, and inflamed CAR T cells. Dr. Atkins also asked the investigators to delineate how they plan to evaluate the contribution of the IP T cells to overall efficacy (e.g., by studying cell persistence or engrafting or by labeling the cells in a way that distinguishes between IP and IV administered T cells). Dr. Atkins expressed concern regarding the safety of the IL-12-containing vector given the vector-associated toxicity reported in the National Cancer Institute (NCI) Surgery Branch IL-12 study. Several melanoma patients in the NCI trial experienced significant liver and kidney toxicity, which was related to high IL-12 secretion in the peripheral blood. Patients who will enroll in the proposed study are likely to have compromised hepatic and renal function and may be at greater risk for these toxicities than the patients with advanced melanoma, who typically have normal kidney and liver function. Dr. Atkins asked how the potential risk will be mitigated or managed for the proposed trial. Dr. Atkins also requested further detail regarding the study design, specifically in relation to the criteria for dose escalation and cohort size expansion based on DLT. He asked whether dose escalation would occur if one-third of patients in a cohort experience a DLT but the fourth patient does not, or if the plan is to administer the same dose in six patients within a cohort before determining whether to proceed to a new dose and cohort.

Dr. Fost noted the poor outcomes and limited treatment options for many patients with recurrent ovarian cancer and the other eligible conditions and asked whether the study should be restricted to patients who have relapsed after second line chemotherapy. Dr. Fost made the following suggestions regarding the ICD:

- Provide information on the outcomes of alternative therapies that would help a patient to decide whether to choose other treatments. A brief summary of the information provided per Appendix M would be appropriate to include in the ICD's "alternatives" section.
- Revise the language regarding potential benefit of participation to reflect the low likelihood of direct benefit in Phase I studies in general and gene therapy trials in particular. Consider adding the recommended NIH language for Phase I gene therapy studies early in the introduction section of the ICD as well as in the "benefits" section. One example of language per the *NIH Informed Consent Guidance for Gene Transfer Trials Subject to the NIH Guidelines* (Informed Consent Guidance) (<http://osp.od.nih.gov/sites/default/files/resources/IC2013.pdf>) document is: "The gene transfer you get in this study is not likely to change the natural course of your disease. This study is not meant to be a treatment for your disease. Instead, the investigators hope that the information learned from this study can benefit patients with [this condition] in the future." Other suggested language can be found in the NIH guidance document.
- Avoid using the word "treatment" and "therapy" in the ICD, per the NIH Informed Consent Guidance document. Consider using "experimental intervention" or "modified T-cells" to better reflect the nature of the intervention.
- Be careful to not overstate the potential outcome of the intervention. The statement that "we were able to cure a cancer similar to ovarian cancer in mice" seems misleading, given the extremely low likelihood of curing the patients who will be enrolled in this study and the results of the animal studies summarized in the protocol. The protocol states that 40 percent of the treated mice, who had a different type of tumor than the patients for the proposed study, were tumor-free at 100 days post-intervention and survived longer than the controls. A more appropriate statement might be, "This kind of treatment showed benefit in mice with a similar cancer. Animal studies do not always predict how humans will respond."
- Add information about the IP infusion being the first in humans for this agent.

Dr. Kohn commented that the clinical setting of recurrent IP carcinomas and their poor prognosis with current therapies merits exploration of novel therapies. The antigen being targeted (MUC16ecto) has not been previously studied in clinical trials of T-cell immunotherapy, and its potential utility as a tumor-associated antigen with minimal normal tissue expression makes it a reasonable and attractive target. Dr. Kohn noted the study team's extensive experience with successful development and performance of CAR T-cell immunotherapy for other malignancies and the capacity to produce the vector and the T cells. Dr. Kohn considered the hypothesis that local IP administration of CAR-transduced T cells could increase efficacy of anti-tumor activity to be reasonable based on available information. However, the rationale for an advantage of the split-route dosing, as planned for this protocol, is not specified and should be clearly delineated.

Dr. Kohn shared Dr. Atkins' concerns regarding the systemic toxicity of the IL-12 vector as was seen in a previous NCI study and questioned whether a different result would be expected in the proposed trial and if so, why. Although the proposed dose escalation schema provides an added safeguard and may detect evidence of toxicities before they are severe, Dr. Kohn questioned whether the expansion potential of T cells could lead to high levels of IL-12 even from low infused cell doses. Dr. Kohn requested additional detail regarding any toxicity and long-term survival in the single cohort of mice receiving T cells with the IL-12-containing CAR, any differences in the sensitivity of mice versus humans on IL-12-mediated systemic toxicity, and how IL-12 increases efficacy. Dr. Kohn also asked the investigators to specify how toxicities from excessive systemic IL-12 would be distinguished from the more typical cytokine storm that may occur with CAR therapies (besides quantifying serum levels of IL-12), including any clinical manifestations or when toxicities typically appear.

Dr. Kohn was concerned about the plan to use the EGFRt “suicide gene” as a “safety switch” to eliminate the CAR T cells in cases of severe patient toxicities. He noted that the presumed efficacy of the EGFRt suicide gene is based on very limited data from a single published report in which *in vivo* data from one mouse given luciferase-labeled anti-CD19 CAR T cells showed a decreased signal from bioluminescent imaging at a single time-point with induction of the EGFRt gene construct. Dr. Kohn pointed out that the higher local dose of CAR T cells given by IP administration may make their eradication by systemically administered monoclonal antibody more difficult. He asked the investigators to provide any additional data demonstrating that cetuximab can be used to eliminate CAR-modified T cells administered by the IP or IV routes.

Dr. Kohn’s suggested changes to the ICD are similar to those offered by Dr. Fost.

C. RAC Discussion

During the meeting, the following additional questions, concerns, or issues were raised by RAC members and by Dr. Steven Rosenberg, an NCI investigator who attended the meeting.

- There was a question about the specificity of the target, MUC16, and provisions for monitoring expression levels and potential toxicities at sites outside the peritoneum, if applicable.
- Dr. Rosenberg noted that IL-12 toxicities occurred in patients in his NCI study at about day 10 to day 12, when the cells began to proliferate dramatically. Problems observed prior to that time (e.g., fever) were related to cytokine release. Dr. Rosenberg advised that the protocol include a sufficient post-infusion monitoring phase as an added patient safeguard.
- Dr. Atkins asked how the investigators would proceed if a patient was found to have significant adhesions (which is exclusionary) after the IP catheter is placed, including whether the study agent would be administered intravenously. Dr. Atkins asked whether there are any data on use of cetuximab in patients who had received an interventional product that included a suicide gene. He noted that this additional issue arose during the recent GTSAB review of a study in which a patient enrolled in another trial died after receiving CAR T cells with the same EGFRt suicide gene planned for the proposed trial (as discussed under Section VII of these minutes). In this case, the patient was not given cetuximab because of the investigators’ concerns about use of cetuximab in the setting of CRS.
- Dr. Kohn cautioned against referring to the EGFRt suicide gene as FDA approved. Use of this truncated gene has been allowed by the FDA under an investigational new drug (IND), but the product has not been approved by the FDA at this point.

D. Investigator Response

1. Written Responses to RAC Reviews

The investigators recognize the safety concerns associated with the administration of an IL-12-containing vector but anticipate that the systemic toxicity encountered with the IL-12 vector to be used with the proposed study will be more favorable than that observed in the NCI IL-12 trials for several reasons. The investigators obtained the vector used in the NCI Surgery Branch studies (MSGIL12) and compared it to two vectors used by in the investigator’s lab in mouse T cells (SFGIL12 and 19mzIRESmIL12). The IL-12 levels for the NCI and SFGIL12 vectors are similar. The levels for these two vectors, however, are two log-fold higher than the IL-12 levels for the 19mzIRESmIL12 vector, which is a construct that has a CAR specific to CD19 followed by mouse IL-12 gene after an internal ribosome entry site (IRES) element. The significantly lower IL-12 levels with the 19mzIRESmIL12 vector are likely due to the fact that the IL-12 is located after an IRES element and that the construct is bicistronic, both of which result in reduced expression of IL-12. The construct that will be used in the proposed clinical trial (EGFRt/4H11-28z/IL-12 T cells) also has the IL-12 gene following an IRES element, and the vector is tricistronic. When these CAR T cells are co-cultured with ovarian tumor cells, the levels of IL-12 are about four log-fold lower than for the MSGIL-12 vector used at the NCI Surgery Branch. The NCI investigators reported similar toxicities in their mouse models as observed in the mouse models studied by the Memorial Sloan -Kettering team.

The IND application for the EGFRt/4H11-28z/IL-12 T cell construct will include formal toxicology studies including complete necropsies and blood work including complete blood count, chemistry, and liver panels (as detailed in Appendix 1 to the protocol) to identify and track toxicities.

The investigators stated further that as an additional safety measure, the vector for the proposed research includes a suicide gene (EGFRt).

The investigators reported that systemic IL-12 levels from their murine tumor model were approximately 40 times lower than those published in humans (100 picograms per milliliter [pg/ml] versus more than 4000 pg/ml) and that IL-12 levels were similar at both 6 and 13 days after injection of 10 million CAR+ T cells in mice. When compared with results of another study, *in vitro* levels of IL-12 by CAR+ T cells were more than five-fold lower than IL-12 levels in CAR expressing flexi-IL12 or nuclear factor of activated T-cells IL-12. The investigators reported further that the amount of IL-12 expressed by EGFR4H1128zIL12 T cells is less than 300 pg/ml per million CAR+ T cells per 24 hours. When these T cells are cocultured with ovarian cancer cells, the levels increase to 300-400 pg/ml per million CAR+ T cells per 24 hours. Use of the suicide gene (EGFRt) is expected to further enhance the safety profile of the study vector by acting as a "safety switch" to eliminate the CAR T cells in cases of severe patient toxicities.

Given the concern that there may be differences in the sensitivity of mice versus humans with respect to IL-12-mediated systemic toxicity, the investigators have included several safety measures in the proposed study design to mitigate the risks to patients. First, the initial starting dose is much lower than that used in the acute lymphoblastic leukemia CAR+ T trial that is currently underway at Memorial Sloan - Kettering Cancer Center. Second, the investigators have incorporated a suicide gene in the vector. Third, the team has developed a comprehensive algorithm that outlines the proposed treatment for patients who encounter CAR+ T-cell toxicity, including steroids and cetuximab. Further experiments have been conducted to assess the efficacy of IL-12 in an orthotopic xenotransplant model (severe combined immunodeficiency disease [SCID]-Beige mice) and an immune competent syngeneic model (C57Bl/6 mice). In both models, mice were injected with 10 million human ovarian tumor cells expressing MUC16ecto, followed 12 to 27 days later with 4H1128zIL-12-expressing CAR+ T cells. Both SCID-Beige mice and C57Bl/6 mice show increased survival with IL-12-secreting CAR+ T cells compared with controls given the 19mzIRESmIL-12 vector.

The investigators acknowledge the difficulties in distinguishing IL-12-related toxicity from the more typical CRS. However, based on current ongoing trials with CAR+ T cells using the 1928z construct, CRS usually manifests with fevers, hypotension, and central nervous system toxicity occurring at day 4 or day 5 post CAR+ T-cell infusion. In contrast, toxicities associated with IL-12 are more typically related to kidney and liver dysfunction and occur 2 or 3 days post-infusion. Patients will be monitored closely, initially in the intensive care unit (ICU), and will also have regular blood work including assessments of hepatic and renal function. The investigators will also monitor cytokine levels (interferon gamma, IL-12, IL-2) and C-reactive protein, which has shown to be useful in CRS in the ongoing clinical trial using CARs to treat patients with leukemia.

The rationale for the split IV/IP route dosing is threefold: (1) IV dosing addresses metastatic disease outside of peritoneum, (2) IP administration has been shown in murine models to have more long-term efficacy compared to IV administration, and (3) the split-dosing route provides an added safeguard. By giving the first CAR+ T-cell dose intravenously, the investigators can monitor patients for any toxicity related to the CAR+ T cells before proceeding to the first-in-human IP administration of CAR+ T cells. Under the proposed study, patients will be monitored for at least 3 days after IV dosing, and assuming very high levels of interferon gamma are not seen during that time, the investigators will proceed with IP dosing. Per the study design, there is a 1-month period between dosing of individual patients and between dose levels. The investigators noted that they are currently in the process of performing toxicology studies where one-half dose will be given IV and one-half dose will be given IP in the same group of mice (per the Acute Toxicity Study Protocol, attached to the clinical protocol as Appendix 1). Complete necropsy and blood work will be done under this toxicology study, and the results will be discussed in detail in the IND.

The investigators will not be able to distinguish the contribution of IP versus IV administration to the overall efficacy. They will, however, study the persistence and engraftment of the T cells. As outlined in the protocol, research blood and peritoneal fluid samples (when feasible) will be collected to serially monitor CAR+ T cells and cytokine levels over time and compare levels in blood versus peritoneal fluid. In addition, because of the delay between the initial IV and subsequent IP administration of CAR+ T cells, the investigators will be able to closely monitor for any toxicities associated with the two routes of administration. T cells will not be labeled to distinguish between IV and the IP dosing.

Regarding the proposed dose escalation plan, the investigators clarified that they will follow a standard 3+3 dose escalation and will expand to six patients should one of three patients in a cohort experience toxicity.

The investigators report that results of *in vitro* testing of the truncated *EGFR* gene show that CAR+ T cells are killed when EGFR-expressing CAR+ T cells (targets) are cocultured with or without cetuximab and NK-92 cells that express CD16 (effector cells) compared to non-EGFR-expressing CAR+ T cells. Additional experiments demonstrated that IP administration of cetuximab after IP administration of CAR T cells in mice eradicated CAR+ T cells (as shown by a decrease in IL-12 levels equivalent to 4H1128z). The FDA has already allowed the use of cetuximab IV to eliminate EGFR+ CAR-modified T cells under IND BB-IND-15829. IP cetuximab will not be administered because of safety concerns. High-dose steroids have been previously given IP to cancer patients, however, and will be included in the toxicity treatment algorithm should toxicity be encountered following IP administration of the CAR+ T cells.

Screening for extensive peritoneal adhesions will include reviewing patients' operative reports for any description of adhesions and screening CT scan (by the study radiologist). Patients with bowel obstruction will be excluded for safety reasons. In addition, the interventional radiologist or surgeon who places the IP catheter will check for flow through the catheter at the time of placement and will inform the study team of any challenges encountered during placement of the catheter. Patients found to have extensive adhesions at the time of the IP catheter placement would be excluded from participation in the study.

The investigators agreed with the recommendation to restrict the study to patients with limited treatment options. This study is intended to enroll patients who have already progressed on standard chemotherapy and who have a low likelihood of benefiting from further chemotherapy. The protocol has been amended to revise the eligibility criteria to patients who have relapsed after second line chemotherapy (one of which must be platinum-based). Regarding concerns about increased risk for hepatic and renal problems, the investigators noted that to participate in the proposed study, patients must have adequate kidney and liver function, as specified in the eligibility criteria as follows: absolute neutrophil count of at least 1500/mm³, platelet count of at least 100,000/mm³, creatinine at most 1.5 mg/dL or creatinine clearance greater than 60 ml per minute, and alanine aminotransferase, aspartate aminotransferase, and total bilirubin all below 2.5 times the institutional upper limit of normal. The investigators commented that the risks to patients are minimized for the planned study given the lower levels of IL-12 in the proposed construct compared to the construct used at the NCI Surgery Branch and the stringent eligibility criteria that exclude patients with baseline renal/hepatic dysfunction.

Regarding the suggestion to add information on the outcomes of alternative therapies to the ICD, the investigators explained that the proposed ICD includes the standard description of alternatives as required by the local institutional review board (IRB). Also per local institutional guidelines, the consenting physician will discuss the possible alternative treatment options with the patient prior to consenting a patient for the proposed study. The investigators noted that the same language included in the proposed ICD is the same as that used in an approved ongoing trial at Memorial-Sloan Kettering using CD19-targeted CAR-modified T cells for B-ALL. The investigators understand the desire to provide a more comprehensive discussion of alternative therapies in the ICD but do not consider it to be practical, given that information on treatment options would need to be individualized to the patient, taking into account their medical and treatment history, any ongoing toxicities, and other clinical trials for which a patient

might be eligible. The investigators agreed with the other recommendations regarding the ICD and have modified the proposed ICD accordingly.

2. Responses to RAC Discussion Questions

Regarding the specificity of MUC16, the investigators noted that MUC16 is expressed not only in the uterus, fallopian tubes, and ovaries but in low levels on the corneal surface of the eye. Under the proposed protocol, all patients will undergo a comprehensive eye exam at screening, and an ophthalmology assessment and immunohistochemistry are done at 4 to 6 weeks and 3 months after T-cell infusion to monitor for MUC16 expression levels in the eye and any ocular toxicity.

The investigators agree that the timeframe for individual subject monitoring needs to be sufficiently long to monitor and capture any side effects and toxicities following dosing. Patients will be closely monitored for 1 to 3 days following each IV and IP infusion and will undergo comprehensive evaluations and clinical assessments at 4 to 6 weeks and 3 months after T-cell infusion.

Dr. Brentjens noted that no investigational agent would be administered to patients who are found to have extensive adhesions with placement of the IP catheter. The catheter would be removed, and the patient would be withdrawn from the study at that point. The ICD will clearly inform participants of these provisions.

Dr. Brentjens noted that cetuximab is not FDA approved for the specified indication (i.e., CRS in patients given a CAR T-cell construct with a suicide gene) and that there are no human data for the exact case in question. The proposed trial, however, has a comprehensive, time-dependent toxicity treatment algorithm derived from the team's prior experience with CRS. Per this experience, the investigators found that CAR T cells are very sensitive to and rapidly disappear with high-dose steroids. Under the proposed algorithm, patients will be closely monitored post-dosing in the ICU for adverse events and toxicities. Patients with CRS or conditions presumably related to IP T-cell administration would initially be treated with IP cetuximab. Patients who do not show improvement within 12 hours will be given IV and IP high-dose steroids. The protocol includes a neurologist and an ICU-attending physician, who will become an expert on CAR T-cell-related toxicities and who will be referred to each patient coming to the hospital for treatment with T cells. Dr. Brentjens stated that the patient identified in the GTSAB review would have received cetuximab per the algorithm for the proposed study.

Dr. Brentjens noted that although the FDA has allowed for use of the EGFRt suicide gene under an IND, the gene is not FDA approved and the IND does not mean that the FDA has endorsed it as effective.

E. Public Comment

No comments from the public were offered.

F. Synopsis of RAC Discussion and RAC Observations and Recommendations

Clinical and Trial Design Issues In previous trials that employed either a gene-modified T-cell receptor expressing IL-12 (OBA Protocol #1097) or IL-12–modified tumor-infiltrating lymphocytes (OBA Protocol #1037), study participants developed serious toxicities likely related to the IL-12 more than a week after administration of the study agent. This protocol currently proposes to administer the modified T cells intraperitoneally 1 to 3 days after IV administration. A comparison of IL-12 expression from your construct compared to the IL-12 expression from the gene-modified T cells used in the previous trial showed production of IL-12 by your T-cells to be two log-fold lower. However, given the proliferation of T cells *in vivo*, it is difficult to predict with certainty the dose of IL-12 that will be expressed. In light of these data, you should consider whether 1 to 3 days is the optimum interval between the IV and IP administrations, since IL-12 toxicity from the IV administration may not emerge in this time frame.

- As discussed during the June RAC review, you have agreed to amend this trial so it will now only enroll individuals who have failed two prior chemotherapeutic regimens.

Ethical/Legal/Social Issues

- Due to the complications that can arise from placement and use of an IP catheter in patients with extensive adhesions due to previous surgery, such patients will be excluded. This determination may be made by examining radiologic imaging prior to enrollment, but it may also be made at the time of IP catheter placement when the interventional radiologist is assessing for adhesions by looking at flow rates. If there is evidence of adhesions at that time, the subject will not be eligible for the trial. The ICD should include a statement informing patients of this possibility.

G. Committee Motion 3

Dr. Kohn summarized the RAC recommendations to be included in the letter to the investigators, expressing the RAC's comments and concerns. Dr. Kohn asked for a vote on these summarized recommendations, which the RAC approved by a vote of 14 in favor, 0 opposed, 0 abstentions, and 0 recusals.

VI. IOM Report: *Oversight and Review of Clinical Gene Transfer Protocols Assessing the Role of the Recombinant DNA Advisory Committee*

Presenter: Jacqueline Corrigan-Curay, M.D., J.D., OD, NIH

A. Presentation of IOM Report

Dr. Corrigan-Curay presented the key conclusions and recommendations delineated in the IOM report, *Oversight and Review of Clinical Gene Transfer Protocols: Assessing the Role of the Recombinant DNA Advisory Committee* (released in December 2013), and the NIH's response to the IOM report and recommendations. The impact of this assessment, the proposed recommendations, and the NIH response on the RAC review process were discussed.

The impetus for the IOM assessment of the RAC was based in large part on feedback from the American Society of Gene & Cell Therapy (ASGCT) to the NIH Director regarding the role of the RAC in review of individual protocols. ASGCT leadership recognizes the benefits of an open forum to have in-depth discussions of emerging issues in the field and requested that the mission of the RAC be refocused on broader issues relevant to the field that extend across protocols. Other factors taken into consideration in this assessment of the RAC were the NIH's commitment to facilitate translational research and ongoing efforts by the NIH to continue to reevaluate and update its oversight framework for gene transfer and recombinant DNA research.

In response to these issues, the IOM established the Committee on the Independent Review and Assessment of the Activities of the NIH Recombinant DNA Advisory Committee. This Committee was charged with two main tasks: To assess whether the current oversight of individual gene transfer protocols by the RAC continues to be necessary and to offer recommendations concerning the criteria that the NIH should employ to determine whether individual protocols should receive public review. In conducting this review and assessment, the Committee considered the current state of the science; current regulations and policies; and scientific, safety, ethical, and other concerns and objectives that would justify a special level of oversight for this area of research (and potentially others).

As Dr. Corrigan-Curay noted, the IOM Committee's task required understanding the circumstances that led to the creation of the RAC and assessing the current validity of these, and other, concerns. The RAC

plays a central role in gene transfer clinical research. It is a federal scientific advisory committee with up to 20 members who possess broad expertise in clinical and basic research as well as bioethics. The RAC reviews individual gene therapy protocols at quarterly meetings and provides ongoing analysis of safety data across more than 1,300 protocols. Data on individual protocols are made available to the research community and public through the Genetic Modification Clinical Research Information System (GeMCRIS) and via periodic reports and discussions at RAC meetings. In addition, the RAC convenes workshops on emerging issues in the field of gene therapy and is involved in the development and execution of scientific symposia.

Based on its review and within the context of its charge, the IOM Committee concluded that:

- Although gene transfer research continues to raise important scientific, social, and ethical questions and is constantly evolving, not all gene transfer research is novel enough or controversial enough to justify the current forms of additional oversight.
- All individual protocols should continue to be registered with the NIH, but these protocols should not be subject to public review by the RAC “except in exceptional circumstances, such as when novel gene therapy techniques and treatment strategies move into the realm of clinical trials.”
- GeMCRIS is a comprehensive information resource and analytical tool for scientists, research participants, institutional oversight committees, and others and can be used to augment institutional capacity.
- The capacity to enable a public discussion of issues, concerns, and challenges in emerging gene transfer science and other technologies is of great value to the scientific and stakeholder communities and also serves the public’s interest. This capacity must be maintained going forward.

The IOM Committee’s recommendations include:

- Restrict individual gene transfer protocol reviews to exceptional cases that meet specified criteria. Under this provision, the NIH’s Office of the Director should continue to register all gene transfer protocols and, in consultation with appropriate regulatory and oversight authorities, should identify protocols for additional public review only if both items 1 and 2 below are satisfied:
 - (1) Protocol review could not be adequately performed by other regulatory and oversight processes (e.g., IRBs, institutional biosafety committees [IBCs], FDA); and
 - (2) One or more of the criteria below are satisfied:
 - The protocol uses a new vector, genetic material, or delivery methodology that represents a first-in-human experience, thus presenting an unknown risk.
 - The protocol relies on preclinical safety data that were obtained using a new preclinical model system of unknown and unconfirmed value.
 - The proposed vector, gene construct, or method of delivery is associated with possible toxicities that are not widely known and that may render it difficult for local and federal regulatory bodies to evaluate the protocol rigorously.

Even if the protocol does not meet the criteria listed in items 1 and 2, the NIH Director, in consultation with appropriate regulatory and oversight authorities, should have the flexibility to select protocols for review that may present significant societal or ethical concerns.

- All protocols should continue to be registered with the Office of the NIH Director, and information on these protocols would continue to be available through GeMCRIS.
- Review of adverse events across protocols should continue as evaluation of trends may lead to greater awareness of safety concerns.
- The NIH Director may choose to organize workshops to promote greater expertise for reviews of emerging science issues versus review of individual protocols.

The NIH has considered the IOM Committee's review, and the NIH Director, Dr. Francis Collins, announced on May 22, 2014, that the NIH has accepted the IOM's recommendations. Implementation would require revisions to the RAC protocol review process. NIH is considering the a proposal in which:

- All protocols would continue to be registered with the OBA, in the OD. In an effort to streamline the review process, the information included with each protocol document initially submitted for registration and a determination as to whether the study meets the criteria for RAC review would be modified somewhat, to include an abstract, the draft protocol, summary information on the product (e.g., vector, transgene), information on preclinical models, and the draft ICD.
- The NIH would notify the relevant oversight bodies of the registration and share the documents.
- An oversight body would have 3 weeks to review the protocol and make a request to the NIH for RAC review, with the rationale for requesting RAC review.
- If an oversight body requests RAC review, the NIH would then determine whether the protocol meets IOM criterion #2 (i.e., whether the proposed research involves a novel product, preclinical model, or emerging safety data). If the protocol meets this criterion, it will be reviewed by the RAC. If it does not, the NIH will inform the oversight body that requested review why it does not meet the criterion and provide information about previous protocols.
- FDA will continue to work with the NIH to identify issues in the field of human gene transfer research that are in need of further examination through such mechanisms as scientific and safety symposia, as well as discussions with the RAC, as appropriate.
- The NIH Director might request that the RAC publicly review protocols not selected by an oversight body in two exceptional circumstances, that is, that if it fulfills the IOM criterion (as noted above) and public RAC review would provide a clear and obvious benefit to the scientific community or public, or if the protocol otherwise raises significant societal or ethical concerns. This provision is consistent with the IOM recommendation for the NIH Director to have flexibility to select protocols for public RAC review in exceptional cases.
- Reporting of annual reports, amendments, and serious adverse events will continue. Such reporting is already harmonized with FDA reporting requirements.
- The NIH will continue to maintain GeMCRIS as an in-depth source of data on the scope, design, safety, and outcomes of gene transfer clinical protocols (including new and ongoing).
- The NIH will continue to convene workshops on emerging issues of importance to the field.

Prior to implementation of the IOM's recommendations, a *Federal Register* notice announcing the proposed changes with opportunity for public comment will be published. Following consideration of the public comments, the final changes will be published in the *Federal Register*. Implementation of the changes will occur approximately 6 months after publication of the final *Federal Register* notice to provide IBCs and IRBs time to develop new procedures to follow the revised review process.

Some key aspects of how the RAC currently operates will be impacted by the IOM's recommendations. Most notably, in-depth, public RAC review of individual gene transfer protocols would be limited to exceptional cases once the final changes are in place. The number of protocols reviewed each year by the RAC would probably drop by at least 50 percent under the proposed changes. Several fundamental

principles and practices related to the RAC review process will continue even after the IOM's recommendations are fully implemented, however. The proposed changes recognize the evolving expertise of other oversight bodies while providing assistance in review of exceptionally novel protocols. The NIH will continue to provide key information on protocols, including the evolution of their design and details on the products used, to augment institutional review bodies' resources. In addition, the NIH will continue to serve a key role in the analysis of data and the facilitation of public discussion of emerging issues of importance across protocols, thereby enhancing the review and design of new protocols and advancing the field generally. Reporting of adverse events and annual reports will continue under the new process, as will registration of all protocols, even those that are not eligible for review. Dr. Corrigan-Curay pointed out that until the proposed changes are final, there is no change to the process for registration and selection of protocols.

B. RAC Discussion

Dr. Fost questioned the IOM's conclusion regarding the evolving expertise of other oversight bodies. He noted the very complex and cutting edge nature of gene transfer protocols and that substantive issues have been raised for nearly all studies reviewed by the RAC. The changes resulting from these reviews have improved the design and the consent process for those protocols. Dr. Fost was concerned that IRBs at individual institutions, including preeminent academic clinical centers that oversee large numbers of studies each year, do not have the appropriate expertise to properly review gene transfer studies. Without the appropriate technical knowledge, these local oversight bodies, in turn, will not be able to fully assess the risks and benefits of these clinical trials. Dr. Fost suggested converting the RAC into a central IRB as an alternative to the proposed changes and identified several organizations and committees that have been successful using this approach, such as the NCI IRB, the Western IRB, the Veterans Administration, and the Cystic Fibrosis Foundation.

Dr. Hammarskjöld noted further that while an IBC may have the knowledge about the biosafety and the molecular biology of the vector of interest, and the IRB has the ethical and other medical expertise to assess subject risk and benefit, these two committees are usually not coordinated at the local level. Dr. Sarzotti-Kelsoe raised the issue of a potential conflict of interest of the local IRB or IBC regarding review of protocols submitted by investigators at the same institution, even with recusals. The current process avoids this issue because the RAC is separated from the local institution. Dr. Corrigan-Curay recognized the importance of an independent review and noted that per the NIH's oversight of IBCs, for example, formal policies need to be in place to avoid such conflicts. Most IRBs have similar provisions. Evaluating whether the local IRB and IBC are capable of properly reviewing a protocol is not one of the NIH's or IOM's criteria. Dr. Hammarskjöld asked what remedies and resources are available, or could be made available, to local oversight bodies to address a lack of expertise to appropriately review a protocol. Dr. Corrigan-Curay noted that several options could be considered, depending on a number of factors. For example, if a protocol meets either of the two exceptional circumstances described above, the NIH Director could request a RAC review. If a protocol involves an agent that has already been reviewed, or is very similar to a product in a previously reviewed study, the NIH could provide the local IRB with the complement of materials used with the prior review, including the relevant RAC meeting minutes, podcasts, and other in-depth reviews. Another option would be to work with the NIH to identify appropriate ad hoc reviewers who could contribute to the local board's reviews.

Dr. Sadelain asked whether the two protocols reviewed during the current meeting would meet the criteria for "exceptional circumstances" as proposed under the new plan to limit RAC reviews to studies that reach this threshold. Dr. Corrigan-Curay replied that both studies would be expected to meet these criteria because they involve new genetic material and first testing in humans. The process for making this determination, however, would be different once the final changes to the review process are in place. The local IRB and IBC would need to request a RAC review in writing, including the rationale for this request, for the NIH to determine whether a registered study meets the IOM criteria for RAC review.

Dr. Sadelain also inquired as to the status of emerging knowledge gained from genome editing under the proposed changes to the RAC review process. For example, it is not clear whether transient expression of nuclease or other mechanisms that lead to a permanent change in the genome but reflect an

endogenous repair rather than an actual gene transfer would meet the new review criteria. Dr. Corrigan-Curay noted that this question has been previously raised and that the outcome could vary depending on the mechanism of the genome modification. The NIH would refer to the guidelines for gene transfer clinical research, which specify the deliberate administration of recombinant genomes. Dr. Sadelain questioned whether the guidelines need to be clarified to encompass permanent genome changes resulting not only from adding a gene but also by modifying the genome.

Dr. Sarzotti-Kelsoe noted that one important value of the RAC is the committee members' opportunity to review a large number of diverse protocols each quarter and over their tenure with the RAC. This experience, in turn, enables the members to compare and contrast prior versus current research through the lens of a multi-disciplinary group and to build on the institutional history and knowledge gained since the RAC's inception. It is unlikely that the same level of interaction and review would be possible under the new paradigm, and it is not clear how members would be able to optimize the review process to judge how, or whether, individual protocols should proceed. Dr. Corrigan-Curay commented that while RAC members could continue to see the protocols that go through the local IRBs and IBCs, they would no longer select protocols for review. She noted further that the RAC currently reviews only about 20 percent of all registered protocols. The new process will provide for more formal direct interaction with and feedback from local IRBs, in contrast with the current process, which is more unidirectional. There are some distinct differences as well as some overlap between the current guidelines and the proposed IOM criteria for RAC reviews. Questions related to social and ethical issues and to products that have already undergone scientific review would not fall under the new IOM guidelines. However, questions relating to the criteria of novelty (e.g., use of a new vector or new gene delivery system, or a new clinical application or unique application of gene transfer) and for determining whether sufficient preliminary *in vitro* and *in vivo* safety data have been obtained in appropriate models are consistent with the proposed criteria. This latter set of issues comprises a sort of "swing clause" for the NIH and the RAC, which serves in an advisory capacity to the NIH Director. If a protocol fulfills the IOM criteria and public RAC review will provide a clear and obvious benefit to the scientific community or the public, then that study could be flagged, and the NIH Director could decide whether to request a RAC review.

Under the current system, public RAC review of a protocol is triggered if at least five RAC reviewers make that request. The proposed system, in contrast, largely depends on the local oversight body requesting a RAC review. Dr. Strome expressed concern that local oversight bodies will not seek a RAC review, even if the local IRB does not have the appropriate expertise to conduct the review, and questioned whether the criteria for determining whether a RAC review is done could be modified to take this issue into account. Dr. Wooley shared Dr. Strome's concerns and asked how, for example, a novel feature that is missed by an IRB or IBC would be flagged. Dr. Corrigan-Curay explained that the NIH will continue to look at all registered protocols. Studies are entered into and tracked via GeMCRIS, and adverse event data are monitored for all products and trials. The NIH will use available information and existing guidelines to determine whether an incoming protocol meets the IOM criteria and whether it should be flagged for the NIH Director. Thus, the process recommended by the IOM incorporates many of the steps and critical elements and criteria currently used by the RAC (i.e., per Appendix M). The NIH is also considering changing the RAC meeting schedule once the proposed changes are finalized. Regular quarterly meetings probably will not be necessary with the smaller number of protocols expected to undergo public RAC review. Options being considered include convening bimonthly meetings and holding virtual instead of in-person meetings. A benefit of revising the meeting schedule is that investigators will not have to wait a full quarter for the RAC to review their protocol.

Dr. Kohn asked whether a comprehensive protocol package needs to be submitted to the IRB for consideration of a RAC review (funding terms, potential conflicts of interest, etc.) or whether a partial submission with key information is acceptable, given the short timeframe in which the IRB needs to act. Dr. Corrigan-Curay explained that the 3-week timeframe for the IRB review and assessment was identified to keep within the current 8-week cycle. Local IRBs may want to set up a process to accommodate gene therapy protocols, including whether a full protocol package is needed to determine whether a RAC review request is warranted.

Ms. Dresser asked whether one of the recommendations should be to have a standing or new NIH committee to review clinical applications of novel technologies, neurobiology, synthetic biology, nanotechnology, and gene transfer. Ms. Dresser also asked whether the RAC members will receive a summary of the protocols sent to the local IRB and IBC to provide additional expertise regarding the science of the proposed studies. Dr. Corrigan-Curay replied that establishment of a new committee has not been proposed at this time. Regarding the second question, the local institutions will not receive a summary of the protocol from OBA, as the RAC currently does. The goal is to have the local institution make a decision regarding requesting a RAC review without introducing any bias (or perceived bias) from the NIH or the RAC. The IRB should make a decision based on the protocol itself, along with any specific information from the investigators regarding the primary objectives of the proposed research and how the study fits into the field of gene therapy. Part of the NIH's role in facilitating the new review process will be increased outreach to educate IRBs and IBCs, with a focus on using GeMCRIS for internal evaluations.

RAC members will be kept informed as to the upcoming *Federal Register* notice and how to submit comments in response to the notice.

C. Public Comment

No public comments were offered.

VII. Gene Transfer Safety Assessment Board Report

RAC Reviewers: Drs. Atkins, Curry, Kiem, Kohn, Pilewski, Sadelain, Strome, and Whitley

A. GTSAB Report

Dr. Atkins presented the GTSAB report for the second quarter of 2014. Within the past 3 months, the OBA received a total of 18 protocol submissions, 15 of which were not selected for public review at this RAC meeting. Review of one of the three protocols selected for the current RAC meeting was postponed at the request of the sponsor. Of the 15 protocols not selected for public review, 13 were oncology protocols and two were infectious disease protocols, both for HIV. Among these 15 protocols, seven used lentiviruses, three used retroviruses, three used plasmids, one used herpes simplex virus, and one used vaccinia. (Information about these trials will be made available on the OBA website after this RAC meeting.)

The GTSAB reviewed initial and follow-up reports on 18 serious adverse events from 13 protocols. After analyzing these events, the GTSAB concluded that none warranted public discussion at this RAC meeting. Dr. Atkins did, however, refer to a death in one patient in a study with suicide gene therapy in which participants were not given cetuximab. Upon further review, the GTSAB found that no patients have been given cetuximab in any of the protocols involving a suicide gene intervention. As a result, it cannot be determined whether or not use of this agent constitutes a salvage approach for toxicity.

During this quarter, the OBA received notification from investigators that 16 protocols were newly open to enrollment, six of which had been reviewed publicly by the RAC. One protocol had previously submitted responses to the issues raised. Dr. Atkins reviewed the responses of the other five protocols:

- OBA Protocol #1145, reviewed by the RAC in March 2012: Phase I Study of Direct Administration of AdVEGF-All6A+, a Replication Deficient Adenovirus Vector Expressing a cDNA/Genomic Hybrid of Human Vascular Endothelial Growth Factor to the Ischemic Myocardium of Individuals with Diffuse Coronary Artery Disease via Minimally Invasive Surgery
- OBA Protocol #1192, reviewed by the RAC in December 2012: A Study to Infuse ROR1-Specific Autologous T Cells for Patients with CLL
- OBA Protocol #1201, reviewed by the RAC in March 2013: Phase I Study of Safety and Immunogenicity of ADU-623, a Live Attenuated *Listeria monocytogenes* Vaccine (Lm)

ΔactA/ΔinlB) Expressing EGFRvIII-NY-ESO-1, in Patients with Treated and Recurrent WHO Grade III/IV Astrocytomas

- OBA Protocol #1230, reviewed by the RAC in June 2013: A Phase 1 Ascending Dose Trial of the Safety and Tolerability of Toca 511, a Retroviral Replicating Vector, Administered Intravenously to Subjects Undergoing Subsequent Resection for Recurrent High Grade Glioma and Followed by Treatment with Toca FC, Extended-Release 5-FC
- OBA Protocol #1266, reviewed by the RAC in December 2013: A Phase 1, Open-Label Clinical Trial Evaluating the Safety, Tolerability and Immunogenicity of Intradermally Administered ID-LV305 in Patients with Locally Advanced or Metastatic Cancer Expressing NY-ESO-1

B. RAC Discussion

No discussion occurred.

C. Public Comment

No public comments were offered.

VIII. Housing of Non-human Primates in BL4 Laboratories under the *NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules*

Presenters: Jacqueline Corrigan-Curay, M.D., J.D., OD, NIH
Dee Zimmerman, University of Texas Medical Branch

A. Presentation

Dr. Corrigan-Curay provided an overview of practices and principles of biosafety containment, including a summary of current biosafety guidelines regarding housing of Non-Human Primates (NHPs) in biosafety level (BL) 4 laboratories, animal welfare considerations for NHPs, the types of primary containment caging available, and the challenges associated with BL4 containment.

The fundamental principles and practices of biosafety containment follow a hazard control hierarchy that includes four main components. 1. Elimination involves the removal of the hazards from the workplace. 2. Engineering controls include designs or modifications to facilities, equipment, ventilation systems, and processes that reduce the source of exposure. 3. Procedural controls alter the way the work is done, including timing of work, policies and other rules and work practices such as standard operating procedures (i.e., training, housekeeping, equipment maintenance, and personal hygiene practices). 4. Personal protective equipment includes the complement of equipment worn by individuals to reduce exposure.

In the late 1990s, the Centers for Disease Control and Prevention (CDC)/NIH Biosafety for Microbiological and Biomedical Laboratories (BMBL) guidance was revised to allow flexibility in the use of primary containment caging for animals at BL4, including housing of NHPs in open caging in dedicated animal rooms. If these labs perform recombinant research, they are required to implement primary containment caging as required under the present *NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules (NIH Guidelines)*.

The current biosafety requirements for animal containment under the *NIH Guidelines* are specified in Appendix G, which applies to common “laboratory animals” generally of small size and easily contained (e.g., rodents, rabbits, ferrets, NHPs), and Appendix Q, which applies to “research animals [that] are of a size or have growth requirements that preclude the use of containment for laboratory animals” (e.g., cattle, swine, horses, poultry). In brief, for small animals involved in BL4 experiments, primary containment caging is required and is frequently in the form of individually ventilated cages with solid sides and bottom and bonnets (typically for rodents) or partial containment cages enclosed in isolators (e.g., for ferrets). In contrast, for large animals, where primary containment caging is not feasible, animals

are held in pens and the room becomes the primary containment vessel; the facility must be designed so that the holding area is completely isolated from adjacent laboratory areas and thus cannot function as a “multi-use” (or “integrated”) research facility.

Per Appendix G (section II-D-4-a), a BL4 facility for housing small laboratory animals consists of either a separate building or a clearly demarcated and isolated zone within a building. Outer and inner change rooms separated by a shower are provided for personnel entering and exiting the facility. A double-doored autoclave, fumigation chamber, or ventilated airlock is provided for passage of materials, supplies, or equipment that is not brought into the facility through the change room. The guidelines identify two options for housing small laboratory animals involved in experiments requiring BL4 physical containment (Appendix G-II-D-2-l): either in cages contained in Class III cabinets, or when personnel are required to wear one-piece positive pressure suits, in partial containment caging systems (e.g., Horsfall units), open cages placed in ventilated enclosures, or solid-wall and -bottom cages placed on holding racks equipped with ultraviolet irradiation lamps and reflectors located in a specially designed area. For large animals involved in experiments requiring BL4 physical containment, the laboratory must provide a double barrier to prevent the release of recombinant or synthetic nucleic acid molecules containing microorganisms into the environment, and the design of the animal facility shall be such that if the barrier of the inner facility is breached, the outer barrier will prevent release into the environment (Appendix Q-II-D-2-g). Passage through two sets of doors is the basic requirement for entry into the animal area from access corridors or other contiguous areas. In addition, the animal containment area must be physically separated from access corridors or other laboratories or activities by a double-door clothes change room equipped with integral showers and airlock. Supplies and materials needed in the animal facility must be brought in by way of the double-door autoclave, fumigation chamber, or airlock, which is to be appropriately decontaminated between each use. Passage through two sets of doors for entry into the animal area from access corridors or other contiguous areas is not among the facility design features specifically addressed in Appendix G of the current *NIH Guidelines*.

BMBL guidance states that infected animals should be housed in a primary containment system, such as those listed in Appendix G-II-D-2-l of the *NIH Guidelines*. BMBL guidance requires all procedures to be conducted by personnel wearing a one-piece positive pressure suit ventilated with a life support system. All manipulations of potentially infectious agents must be performed within a Class II biosafety cabinet (BSC) or other primary barrier system. Infected animals must be housed in ventilated enclosures with inward directional air flow and high-efficiency particulate air (HEPA) filtered exhaust, and should be handled within a primary barrier system, such as a Class II BSC or other equivalent containment system.

The containment guidelines for NHP housing are being revisited at this point in time to respond to concerns from investigators who -argue that the use of primary containment caging has created new risks that outweigh any potential biosafety benefit compared to open cages in a dedicated animal holding room. Therefore, because each laboratory has unique design and operational characteristics, most investigators contend that a risk assessment is required to best determine the appropriate selection of microbiological practices, safety equipment, and facility standards that are essential in preventing laboratory acquired infections.

As part of the process of reviewing the current guidelines and assessing the risks of primary containment caging, the OBA and CDC’s Division of Select Agent and Toxins, which regulates much of this research, hosted a workshop on March 28, 2014, to explore the challenges of using primary containment caging systems for NHPs, evaluate best practices for research with NHPs at BL4, and consider alternative strategies to achieve primary containment. Nine U.S. BL4 laboratories and five international laboratories were represented at the meeting.

The main considerations for biosafety at biological laboratories include the protection of personnel working in the laboratory, the community and environment outside of the laboratory, animals involved in the research, and protecting specimens in the laboratory from contamination. Biocontainment at BL4 labs is achieved through a two-part strategy involving primary and secondary containment. Primary containment equipment and devices are used to protect laboratory workers when conducting animal studies, animal necropsy, agent production activities, and activities relating to maintenance, service, or

support of the laboratory facility. Secondary barriers protect laboratory staff, persons outside the laboratory, and persons and animals in the community from infectious agents that may be released within the laboratory in the event of an accident. The physical design of a facility and its systems constitute the secondary barrier and include features such as ventilation systems with monitoring, HEPA filtration, decontamination equipment (e.g., autoclaves, dunk tanks, disinfectant showers), controlled access zones, airlocks at laboratory entrances and exits, alarms and security measures, and separate buildings or modules to isolate the laboratory from surrounding areas. Dr. Corrigan-Curay noted that airlocks are designed to serve a double purpose. The airlock may be a room used for clothing changes, animal food storage and preparation, other storage, a shower, or a laboratory of lower biosafety level where less hazardous work can be conducted.

Many factors affect animal welfare requirements for NHP housing, including caging systems and habitable minimum space, environmental variables (temperature and humidity, ventilation, directional air flow, lighting), husbandry practices, sanitation schedule, veterinary care, social housing and enrichment, and adequate opportunity for interaction between animals. To accommodate these factors, investigators need to consider the type of caging and the room layout (e.g., self-contained isolator caging versus open caging in a ventilated enclosure versus open caging in a dedicated animal room). Advantages of primary containment caging include minimization of the spread of animal infectious waste throughout the room, minimizing exposure to contaminated aerosols, and the release of infectious material outside of the animal holding area. Isolator or ventilated enclosure caging also presents challenges to animals as well as investigators and handlers. Animals' limbs may become trapped in the barriers around the cages, and animals may be adversely affected by the increased noise and the reduced visual stimuli due to opaque enclosures. Animals may also destroy the soft wall barriers, thus compromising containment. Personnel may have to work in cramped conditions under poor lighting and reduced visibility caused by the soft barriers.

Dr. Corrigan-Curay noted the following discussion points and conclusions from the March 28 OBA/CDC workshop as a basis for reviewing the current guidelines for containment of NHP at BL4 labs:

- NHPs have welfare requirements and behavioral characteristics that present unique challenges in the use of standard containment strategies as compared to most common laboratory animals.
- Most investigators and biosafety officials from the laboratories represented concluded that primary containment caging for NHPs in BL4 offers minimal benefit compared to open containment caging in a dedicated animal room, and in certain cases, creates new risks.
- Most containment facilities for NHPs do not or cannot meet the full design requirements set forth in Appendix Q of the *NIH Guidelines*, where the room serves as the primary containment vessel.
- Containment proposed by a number of labs would be a hybrid approach between the requirements of Appendix G and Appendix Q, regarding the use of open caging within a dedicated animal holding room.

Ms. Zimmerman continued the presentation with additional information and details regarding Level 4 labs and facilities. She noted that BL4 labs and buildings have been in use for decades and are unique in their construction. They are designed to allow for work with pathogens that have the ability to cause serious, life-threatening illness. Preventing microorganisms from escaping is critical to the engineering and design features of a BL4 lab. Over time, lab designs have been refined to allow for increased flexibility and systems that do not compromise the containment envelope, thereby reducing risk of transmission and maximizing safety for animals and staff. Ms. Zimmerman focused on what is known as a "suit lab," in which personnel accessing the lab wear a positive pressure suit (sometimes referred to as a space suit).

A key component to successful operation of a BL4 lab is having highly trained personnel who understand the structure and design of the facility and are proficient in BL4 practices and techniques, including emergency response procedures. The physical design and layout of a basic BL4 facility includes a "buffer" (service) corridor, double-door autoclaves, dunk tanks, clean entry, and suit room. The facility entry requires a complete change of clothing. Personnel pass through a personal shower, at which point the BL4 containment envelope usually begins. The chemical shower for most U.S. facilities lasts about 8 to 10 minutes, half of which is chemical (MicroChem), and half of which is plain water. The layout of

most newer labs includes separate *in vitro* and *in vivo* areas. All effluent is removed through an effluent decontamination system, where the material is autoclaved, and then exits through the sanitary sewer system. Any material that enters the lab, whether it is an animal carcass or other research material, is autoclaved out. The only items that come out of a Level 4 lab are samples that are potentially going to an irradiator, or have been chemically disinfected. Viruses may also be shipped between Level 4 labs worldwide.

Current NHP housing options include isolator caging, which is a cage (or cages) protected by Plexiglas; the bioBubble unit, in which caging is enclosed in plastic "housing"; and DuoFlo units, which are attached to self-contained filtration system and include cages inside a plastic curtain. These types of caging may be referred to or defined as partial containment because to get to the animal for any purpose (e.g., feeding, husbandry, cleaning), personnel have to open the cage door. Once the door is open, the barrier is not maintained, and the room at that point becomes the primary barrier. Daily sanitation is difficult, and the electrical cords and plastic in some units can present a hazard to the animals. In addition, this type of containment may impair animal welfare because the social interaction cues that are olfactory, visual, and auditory stimuli, all important to NHPs, are compromised or absent. Another disadvantage is that this type of structure limits the use of closed circuit TVs to monitor or observe the animals (e.g., post-inoculation monitoring) because of the opacity of the Plexiglas barrier and the caging. The additional visual checks are important to understanding the clinical aspects of the research but also require an increased level of contact with the animals, which can be difficult because of the puffy and bulky nature of the pressurized suits. The available partial containment systems decrease the amount of space personnel have for movement in and around the unit, thereby increasing the chance for animals to grab suits or air supply hoses while personnel are performing routine procedures and places the suit in contact with potentially contaminated inner cage walls.

Given the concerns and potential risks associated with partial containment, alternative designs and policies are being considered. Among the questions being posed is whether open cages in a dedicated animal holding room can provide appropriate biosafety containment for infected NHPs in an integrated BL4 research facility where both *in vitro* and *in vivo* research activities occur concurrently. Any final configuration must meet the minimum design requirements for all BL4 facilities with NHP containment using primary barriers, specifically, a double-doored autoclave (with appropriately interlocked doors), fumigation chamber, or ventilated airlock for passage of materials, equipment, or supplies that are not brought into the facility through the change room, and pressure differentials with an air flow directed inwards toward the areas of highest potential risk. In addition, lighting should be diffused throughout an animal holding area and provide sufficient illumination for the animal's well-being while permitting good housekeeping practices, adequate animal inspection including the bottom-most cages in racks, and safe working conditions for personnel. Part of this assessment includes identifying which barriers should be required between animal holding rooms and non-containment areas, and the conditions under which these barriers would provide adequate containment. For example, is a single air pressure-resistant (APR) door that is locked and sealed sufficient to ensure appropriate containment when the laboratory is actively working with Risk Group (RG) 4 agents (i.e. the laboratory is "hot"), if that door is only opened after the room has been decontaminated? Is a closed or padlocked APR door functionally equivalent to a wall? Should there be a second door in an access corridor to create a secondary barrier?

In considering these questions, the Biosafety Working Group made the following recommendations:

- If primary containment caging is not used and the room becomes the primary containment for the animals, there should be two physical barriers between the animal holding room and the outside laboratory space.
- When the animal room is in use, any access doors to non-containment spaces should be closed and not accessible (e.g., fitted with appropriate and redundant lockout mechanisms).
- The ability of the APR door to act as a "wall" when the animal holding room is being used should be verified.
- Access to the non-contained space immediately outside of the animal holding rooms should be restricted.
- There should be a second solid door (designed to not corrode, split, or warp) that controls access into a service ("buffer") corridor bordering the animal holding rooms. This second door does not

need to be an APR door, but it should be interlocked with the animal holding room APR doors so that it cannot be opened if an animal holding room door is open.

- The animal holding room should maintain a negative air pressure differential compared to the “buffer corridor.”
- To minimize the risk of failure of decontamination of the animal holding room, which could lead to a potential release of contaminated material into uncontained space, cages should be removed for autoclaving prior to decontamination of the room.
- To mitigate the risk of infectious material in the animal room, caging should be chosen with the intent to reduce the amount of animal detritus that can be thrown out of the cage and into the room, and clean/dirty flow procedures should be implemented to minimize the spread of contamination from within the animal room to other parts of the lab.

When considering options for NHP containment in BL4 labs, it is important to remember that “one size does not fit all.” Facility design, practices, and staff expertise are imperative when implementing a containment plan and system. Decisions are best made by risk assessment at the local level, which, in turn, will dictate procedural activities when conventional caging is in use. Opening a decontaminated animal room into a buffer corridor is no different than opening a recently decontaminated airlock. In the end, a well-engineered and operated BL4 facility is far superior to less sophisticated containment systems.

B. RAC Discussion

Dr. Fost expressed concern about the stress of containment on NHPs in particular and the documented negative impact on animals’ behavioral, psychosocial, and physical well-being when held under such conditions. These conditions, in turn, make it difficult to draw any conclusions about the interventions used with these animals. He recognized the dedication and efforts of the investigators and others involved in developing guidelines and conducting this research but is not convinced that the potential benefit is justified by holding animals under the conditions being discussed, whether in large or small cages or large open rooms.

Dr. Cannon noted that open caging in dedicated containment rooms might allow for more social interaction among animals. She asked about the purpose of the central buffer corridor in the BL4 setting, which is outside the containment area, given that the dedicated holding rooms are completely contained behind not one but two sealed doors. For example, it is not clear if the corridor is in place for convenience, such as for restocking the lab or transporting new animals into the room after decontamination. Ms. Zimmerman explained that the corridor allows personnel to use that area as a general acclimation room for the animals without having to bring the animals into the lab room before the study goes “hot”. Current facilities are designed and engineered so that once the APR door seals are pressurized, a solid wall is created. The APR doors are then fully sealed and locked and cannot be overridden or accidentally opened by, for example, entering a PIN or other code number into the magnetic locking mechanism. The doors are also padlocked from the outside as an added safeguard. Dr. Corrigan-Curay suggested specifying how the doors are physically secured and stating clearly that they are not to be used as emergency egresses.

C. Public Comment

Ms. Nancy Hoe, who is the biosafety officer and responsible official at Rocky Mountain Labs, which houses one of the BL4s, noted that failure testing has been done on mechanisms used to seal the containment room (e.g., magnetic lock plus gasket system), including testing of air flow. One of the strategies used at Rocky Mountain Labs is to put a pin through the lock on the inside of the door to simulate a catastrophic APR door failure. Under those test conditions the door moves only a fraction of an inch. After 10 to 15 minutes of testing, adequate negative pressure was maintained, as was inward air flow. Thus, with this set-up, both the magnetic lock and the gasket would need to fail to compromise the seal and risk contamination of the corridor. Ms. Hoe did question, however, whether interlocking doors to the dedicated animal room are necessary if access to the corridor and the main room is restricted and other systems are in place. Although the purpose of the interlocking doors is to prevent concurrent

opening of doors between a hot/contaminated side and a non-contaminated side of an enclosure, the only time the service door to that lab is going to be opened is after decontamination of the holding room, at which point it is no longer a contaminated room.

Dr. Peter Jahrling, from the National Institute of Allergy and Infectious Diseases Integrated Research Facility at Fort Detrick, noted that the introduction of the buffer corridor was an obvious solution to mitigate the inconvenience of having to transport animals through the shower and fumigation room and to facilitate decontamination testing and restocking of naïve animals. He agreed that inadvertently opening of the pressurized doors is not feasible because of multiple security devices and safety redundancies. At the Fort Detrick facility, the doors are locked and padlocked, and there is a steel-framed door at the end of the corridor. As at other sites, access is highly restricted and there is no remotely controlled access to the labs and facilities. Dr. Jahrling also noted that the Centers for Disease Control and Prevention (CDC) had approved a study to put Ebola virus into pigs that were in an open pen within an animal room. This statement was corrected by CDC officials after the meeting. This experiment was not approved by the CDC and was done outside of the US.

Lt. Col. Neal Woollen, U.S. Army Medical Research Institute of Infectious Diseases, commented on hazards resulting from exposure to, for example, contaminated animal fecal matter and urine. Changing caging systems does not change animals' behaviors, it only affects where the contaminated material may end up (e.g., the floor, the walls of the draping surrounding the cages, footwear, headgear, protective clothing). Contamination resulting from a breach of the positive pressure encapsulating suit or seam separation of shoes or gloves is of particular concern. Personnel should be acutely aware of these risks, especially if they are in contact with any urine- and fecal-contaminated equipment. Lt. Col. Woollen noted that the systems described during the current meeting increase risks to employees entering these environments and degrade the quality of life of NHPs inside those caging systems. He questioned the value of these systems, which appears to be limited, given that when the cages are opened, the contaminants are released into the room. The preferred alternative should be working in a room with fewer obstacles, less potential for employee hazards, and better welfare for the animals. As noted by Dr. Wooley, that type of environment is more similar to the BL3 agricultural (ag)-type system, which uses open cages. Ms. Hoe pointed out, however, that under BL3 ag conditions, personnel usually wear tieback protective outerwear and do not go through a chemical shower. In contrast, the BL4 containment barrier for personnel is the chemical shower.

Dr. Wooley further inquired about the amount of organic material that could end up on a positive pressure suit and if the use of open cages would increase the likelihood of soiling the suit, thereby affecting the ability to effectively decontaminate it. Lt. Col. Woollen indicated that it would be more likely to contaminate the outside of a pressure suit by coming into contact with the plastic sheeting of a containment module. Animal rooms are cleaned on a daily basis to prevent the build-up of contaminated organic matter on floors, walls and cages. When cages that are holding NHPs are placed inside a soft-wall containment module, it is difficult to remove the cages and decontaminate the inside of the module effectively. Animals may remain in the containment modules for a week, thus the organic load on the curtain is often greater than what would be on a floor given a daily cleaning regimen of an animal room. Dr. Wooley agreed that it would be less likely to contaminate the exterior of a pressure suit in an animal room using open caging as opposed to caging that is maintained in primary containment modules.

Ms. Zimmerman explained that even if the suit or other gear becomes contaminated as a result of opening cages or coming into contact with animals or the inside of cages or plastic coverings, personnel go through a double decontamination process: The first, when they leave the main room/lab and the second, before they leave the facility. She said that at the University of Texas BL4 site, there has not been a problem with decontamination of suits, boots, and other gear with this system. Lt. Col. Woollen noted a Canadian study that showed that the physical process of washing the positive pressure encapsulating suits is key to killing the pathogen and decontaminating positive pressure encapsulating suits, perhaps more than the chemical agent itself. Ms. Zimmerman added that the rooms and cages need to be cleaned daily, as is done in a partial containment setting. Facility-specific provisions to reduce risk of specimen contamination also need to be in place. Factors such as dirty and clean materials /

personnel flows and use of completely separate *in vitro* and *in vivo* labs and changing rooms with an APR door between labs need to be taken into account in designing a facility.

Ms. Hoe provided some historical background on the development of the *NIH Guidelines* for containment of NHPs. The original *NIH Guidelines* developed in 1976 did not contain language that stipulated a requirement for primary containment of animal caging. Revisions to these *Guidelines* 2 years later included supplementary information on physical containment (Appendix D), which stated that “animals exposed to biohazardous aerosols should be housed in ventilated cages in gas-type cabinet systems or in rooms designed for protection by personnel by use of ventilated suits. Animals inoculated by means other than aerosols should be housed in equipment suitable for the level of risk involved.” Dr. Corrigan-Curay also discussed the evolution of the *NIH Guidelines*. She noted that the original 1976 *NIH Guidelines* language that included the term “should” was sent out for public comment, investigators and agencies from regions and countries around the world stated that NHPs must be housed in primary containment caging. This led the wording of the *NIH Guidelines* to be changed from “should” to “shall” in the 1978 edition such that primary containment caging became mandatory. Through 2000, the BMBL used language that NHP “are to be housed,” not “should” or “shall be housed.”

D. Synopsis of RAC Discussion and RAC Observations and Recommendations

Dr. Corrigan-Curay clarified that if the *NIH Guidelines* are amended to allow open caging of NHPs in a dedicated animal room, such caging would not be a requirement under the *NIH Guidelines*. Investigators and facilities may choose to use soft-wall barriers or containment caging in lieu of a dedicated animal holding room. The following recommendations and subsequent motion and vote relate to laboratories that propose to implement open caging of NHPs in a dedicated animal holding room in BL-4 laboratories. Dr. Corrigan-Curay noted further that these recommendations will apply to primate-specific housing guidance in Appendix G of the *NIH Guidelines*.

- To assure proper and secure containment of all animals and any test agents and contaminants and to restrict access to authorized personnel only, two physical barriers need to be in place: An APR door providing direct access to an animal holding room from a non-containment service corridor, and 2) a solid second door that restricts access to the non-containment service corridor when an animal holding room door is opened. The second door does not need to be an APR/high-containment door. Each lab needs to develop procedures that will prevent concurrent opening of both the APR door and the second barrier door.
- A negative pressure differential should be maintained between the decontaminated animal holding room and the service corridor such that air will flow into the animal holding area when the APR door is opened.
- Clean and dirty personnel traffic flows should be implemented to minimize or prevent the spread of contaminants originating from the animal holding room to other locations within the containment laboratory.
- To further minimize risk of contaminating the non-containment service corridor, cages should be removed from the animal room prior to surface decontamination of the holding area.

E. Committee Motion 4

Dr. Kohn summarized the RAC recommendations, expressing the RAC’s comments and concerns. Dr. Kohn asked for a vote on these summarized recommendations, which the RAC approved by a vote of 12 in favor, 1 opposed, 0 abstentions, and 0 recusals.

IX. Closing Remarks and Adjournment

Dr. Kohn thanked the RAC members and the OBA staff and adjourned the June 2014 RAC meeting at 3:25 p.m. on June 11, 2014.

(Note: Actions approved by the RAC are considered recommendations to the NIH Director; therefore, they are not considered final until approved by the NIH Director.)

Jacqueline Corrigan-Curay, J.D., M.D.
RAC Executive Secretary

I hereby acknowledge that, to the best of my knowledge, the foregoing Minutes and the following Attachments are accurate and complete.

This Minutes document will be considered formally by the RAC at a subsequent meeting; any corrections or notations will be incorporated into the Minutes after that meeting.

Date: _____

Donald B. Kohn, M.D.
Chair, Recombinant DNA Advisory Committee

**Attachment I:
Recombinant DNA Advisory Committee Roster**

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**Attachment II:
Public Attendees**

(This list includes only individuals who are not identified elsewhere in this document. It does not include one individual whose name is illegible on the sign-in sheets.)

Althea Capul, Division of Occupational Health and Safety/National Biosafety and Biocontainment Training Program, NIH
Gene Connelly
Karen Connelly
Marcus Corat, National Heart, Lung, and Blood Institute, NIH
Amy Cuevas
Philip Gregory, Sangamo
Nancy Hoe, NIH
Peter Jahrling, National Institute of Allergy and Infectious Diseases, NIH
Mythlili Koneru, Memorial Sloan-Kettering Cancer Center
Gerry Pierson, National Institute of Allergy and Infectious Diseases, NIH
Richard Ruzicka
Rizwana Sproule, Kite Pharma
Fyodor Urnov, Sangamo
Louise Wideroff, National Eye Institute, NIH
Neal Woollen, U.S. Army Medical Research Institute of Infectious Diseases

Attachment III: Abbreviations and Acronyms

AAV	adeno-associated virus
ALL	acute lymphoblastic leukemia
APR	air pressure-resistant
ASGCT	American Society of Gene & Cell Therapy
ATP	adenosine triphosphate
B-ALL	B-cell acute lymphoblastic leukemia
BL	biosafety level
BMBL	Biosafety for Microbiological and Biomedical Laboratories
BSC	biosafety cabinet
CAR	chimeric antigen receptor
CDC	Centers for Disease Control and Prevention
CK	creatinine kinase
CRS	cytokine release syndrome
DLT	dose-limiting toxicity
EGFRt	truncated human epidermal growth factor
FDA	Food and Drug Administration
GeMCRIS	Genetic Modification Clinical Research Information System
GTSAB	Gene Transfer Safety Assessment Board
IBC	institutional biosafety committee
ICD	informed consent document
ICU	intensive care unit
IL	interleukin
IND	investigational new drug
IP	intraperitoneal
IRB	institutional review board
IRES	internal ribosome entry site
IV	intravenous
HEPA	high-efficiency particulate air
LHON	Leber's hereditary optic neuropathy
MTD	maximum tolerated dose
mtDNA	mitochondrial DNA
NAb	neutralizing antibody
NCI	National Cancer Institute
<i>ND4</i>	NADH dehydrogenase subunit 4
NHPs	non-human primates
NIH	National Institutes of Health
<i>NIH Guidelines</i>	<i>NIH Guidelines for Research Involving Recombinant DNA Molecules</i>
OBA	Office of Biotechnology Activities, NIH
OD	Office of the Director, NIH
OCT	optical coherence tomography
PCR	polymerase chain reaction
RAC	Recombinant DNA Advisory Committee
RGC(s)	retinal ganglion cell(s)
scAAV2-P1 <i>ND4v2</i>	scAAV2(Y444+500+730)-smCBA-P1 <i>ND4v2</i>
SCID	severe combined immunodeficiency disease
UF	University of Florida
UNC	University of North Carolina

**Appendix A:
Public Comments on Human Gene Transfer Protocol #1401-1307**

[This testimony is provided verbatim, as reported in the transcript of the June 2014 RAC meeting.]

Testimony of Karen Connelly

Good morning. My name is Karen Connelly, and I've had this disease for about five years, and I'm so lucky to have found Dr. Guy to give me some hope. It did start first in the left eye, and I was still seeing. As a matter of fact, my husband has a glass eye and he lives life perfectly. Another odd thing is that my brother had this disease when he was maybe 5 or 6 years old. He's older than me. They had no clue what it was then, and he was one of the 12 percent or 11 percent that got better. The doctors wanted to test him and, you know, my mother had a nervous breakdown.

She swore it as Padre Peo's oil, but 7 years later, when he went to doctors, when they knew what the disease was, they told him. So when this started happening to me, I had no clue because it was supposed to be something that generally is passed on to males.

All the doctors I was going to, sending me for spinal taps, brain tumors and, you know, because they said I didn't fit the mold and blah blah blah, and actually Dr. Guy was recommended. Surprisingly, they even grew up in the same neighborhood.

But I don't know. If there's any questions—I mean I would give anything for one eye to have somewhat of a normal life right now. I cannot—all I can see are shadows. I can't cross the street, I can't see, you know, what's on the screen, and my life was—I was a special education teacher. I want to get back there, and Dr. Guy is the hope.

Gene checked everything, everywhere and every place for years on the computer when this first happened. So I don't know. Does anyone have any questions? I can't see.

Chairman Kohn: I don't think there's any questions, but I want to thank you very much for coming and speaking. It's always helpful to us to hear from patients their perspective and their need. You know, we tend to look at it as scientists and as doctors.

Ms. Connelly: I understand. That's why—right. I want to, you know. (Simultaneous speaking.) The hope of one eye right now to give me a life back. I'm trapped in my house, you know. I can't drive, I can't, you know. I can't teach, I can't see—I have to sit like this off from a TV, you know, to see somewhat. So it's—I mean I'm not complaining, I'm just explaining what it does to you, and what that doctor was saying about one eye is to start on the one eye, I'd be thrilled. Thank you very much.

Chairman Kohn: Thank you very much to you and your husband for coming here and helping inform us about this.

Ms. Connelly: Thanks, doc.