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**RECOMBINANT DNA ADVISORY COMMITTEE**

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**Minutes of Meeting**

**March 12, 2013**

**U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES  
Public Health Service  
National Institutes of Health**

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*(Note: The latest Human Gene Transfer Protocol List can be found at the Office of Biotechnology Activities' Web site at <http://oba.od.nih.gov/oba/index.html>.)*

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

March 12, 2013

The Recombinant DNA Advisory Committee (RAC) was convened for its 132nd meeting at 8:30 a.m. on March 12, 2013, at the National Institutes of Health (NIH), Rockledge II, Room 9100, in Bethesda, Maryland. Dr. Yuman Fong (RAC Chair) presided. In accordance with Public Law 92-463, the meeting was open to the public from 8:30 a.m. until 3:05 p.m. on March 12. The following individuals were present for all or part of the March 2013 RAC meeting.

**Committee Members**

Tianxi Cai, Harvard University (*via teleconference*)  
Paula M. Cannon, University of Southern California  
Saswati Chatterjee, City of Hope National Medical Center  
E. Antonio Chiocca, Dana-Farber Cancer Institute  
Rebecca Dresser, Washington University School of Law  
Yuman Fong, Memorial Sloan-Kettering Cancer Center (RAC Chair)  
Norman Fost, University of Wisconsin, Madison (*via teleconference*)  
Marie-Louise Hammarskjöld, University of Virginia School of Medicine  
Hans-Peter Kiem, University of Washington School of Medicine/Fred Hutchinson Cancer Research Center  
Walter J. Koch, Temple University School of Medicine  
Donald B. Kohn, University of California, Los Angeles  
Margaret Mallino, Missoula, Montana (*via teleconference*)  
David A. Ornelles, Wake Forest University School of Medicine  
Joseph Pilewski, University of Pittsburgh  
Susan R. Ross, University of Pennsylvania (*via teleconference*)  
Marcella Sarzotti-Kelsoe, Duke University School of Medicine  
Marshall Strome, St. Luke's–Roosevelt Hospital Center/New York Head & Neck Institute (*via teleconference*)  
Dawn P. Wooley, Wright State University  
Laurie Zoloth, Northwestern University

**Office of Biotechnology Activities (OBA)**

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

**Ad Hoc Presenters/Speakers**

Dennis Bente, University of Texas Medical Branch (*via teleconference*)  
Douglas Jolly, Tocagen, Inc. (*via teleconference*)  
Alessandro Lobbia, Tocagen, Inc. (*via teleconference*)  
Stuart Nichol, Centers for Disease Control and Prevention (*via teleconference*)  
Amanda Omlor, Tocagen, Inc. (*via teleconference*)  
Daniel Pertschuk, Tocagen, Inc. (*via teleconference*)

**Nonvoting Agency Representatives**

Denise Gavin, U.S. Food and Drug Administration (FDA)

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

## **NIH/OD/OBA Staff Members**

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

## **Attendees**

There were 53 attendees at this one-day RAC meeting.

## **Attachments**

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

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

Dr. Fong, RAC Chair, called the meeting to order at 8:30 a.m. on March 12, 2013. Notice of this meeting under the *NIH Guidelines for Research Involving Recombinant DNA Molecules (NIH Guidelines)* was published in the *Federal Register* on February 20, 2013 (78 FR 11897). Issues addressed by the RAC at this meeting included a report from the Gene Transfer Safety Assessment Board (GTSAB, a subcommittee of the RAC), public review and discussion of two gene transfer protocols, updates of two protocols previously reviewed by the RAC, and discussion of and recommendations for biosafety considerations for research involving full-length cDNA cloning of single-strand, non-segmented, positive-sense Risk Group 4 RNA viruses.

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

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

## **II. Update and Discussion of Human Gene Transfer Protocol #0510-731: Open-Label, Dose-Escalation Study Evaluating the Safety of a Single Administration of an Adenoviral Vector Encoding Human Aquaporin-1 (AdhAQP1) to One Parotid Salivary Gland in Individuals with Irradiation-Induced Parotid Salivary Hypofunction**

Principal Investigator: Bruce J. Baum, D.M.D., Ph.D., Warren Grant Magnuson Clinical Center, NIH

### **A. Presentation by Dr. Baum**

Dr. Baum provided a review of this protocol and its current results, after noting that he was no longer the principal investigator (PI) for this study as of the completion of the original protocol; the current PI is Dr. Ilias Alevizos (who was present at this RAC meeting). The RAC had reviewed this protocol in December 2005.

Approximately 40,000 new cases of head and neck cancers occur each year in the United States. The oral consequences of radiation therapy to treat these cancers include mucositis, osteoradionecrosis, and salivary hypofunction, the last of which is the focus of this clinical trial. Too little saliva leads to considerable morbidity in these patients, including dry mouth (xerostomia), difficulty swallowing, oral

infections, reduced mucosal healing, oral pain and discomfort, and markedly reduced quality of life. Treatment exists for Grade 1 salivary gland dysfunction, but no effective therapy is currently available for Grade 2, 3, and 4 patients.

AdhAQP1 was delivered by intraductal cannulation and retrograde infusion, first in preclinical trials to rat salivary glands, and then, in the clinical trial, into one parotid gland of each research participant who previously had been irradiated. The first participant was dosed in Summer 2008. The study's objectives were to evaluate the safety of single escalating doses of AdhAQP1 and to evaluate the effectiveness of AdhAQP1 in increasing parotid gland salivary output and reducing complaints of xerostomia. The study was designed as an open-label, single-center, single-dose dose escalation of five cohorts that included three participants each, for a period of 360 days. Dr. Baum displayed the dose escalation scheme and noted that the highest proposed dose had not been connected with any major vector-associated adverse effects and had been well tolerated in previous clinical trials with other tissue targets. Start-up was slower than expected, for a variety of reasons.

Dr. Baum noted that the initial results of the trial were reported in November 2012 (Baum, B., et al (2012) Early Responses to adenoviral-mediated transfer of the aquaporin-1 cDNA for radiation-induced salivary hypofunction. Proc. Natl. Acad. Sci. 109:19403-19407.) Of the 136 patients who were prescreened via telephone for cancer, treatment history, and general health, 17 individuals were deemed suitable for more detailed assessment. Of those 17, 11 were determined to be eligible for this clinical study and were dosed. Dr. Baum presented the baseline characteristics of all dosed participants. Eleven research participants received the vector, all dosed participants tolerated the vector and associated protocol procedures well, and objective and subjective evidence of efficacy was observed in one first-cohort participant, two second-cohort participants, and two third-cohort participants. Adverse events (AEs) were minimal: 91 percent were mild, 9 percent were moderate, and no serious adverse events (SAEs) occurred.

In the second participant in this study, E1-containing adenovirus was detected in saliva after delivery of the vector to his parotid gland. The researchers' conclusion from this event was that this individual most likely had a latent Ad5 infection in the targeted parotid gland that was activated after gene transfer and was without clinical consequence. Discussion of this event was published in *The Journal of Gene Medicine* in November 2009.

In summary, the gene transfer strategy using AdhAQP1 to treat irradiation-damaged salivary glands was developed and tested in preclinical animal models, with results showing the strategy as efficacious and generally safe. Following approval of a clinical protocol, AdhAQP1 was tested in a Phase I study at the NIH Clinical Research Center, the results of which showed that gene transfer to human parotid glands is generally safe, that parotid gland dysfunction can be treated by localized gene transfer, and that positive results in responders did not follow a time course predicted from previous studies in many animal species. In addition, useful measurement tools were tested for future studies: parotid flow rate, visual analogue scale for symptom assessment, and gallium scans to assess inflammation.

When they noticed that all the responders showed a late effect, the investigators requested permission to continue this study past the original 360 days for up to three years; Dr. Alevizos currently is conducting that study. In addition, a Phase I study with the vector AAV2hAQP1 is being conducted, with Dr. Jay Chiorini as the PI, following up on results published in *Gene Therapy* in 2011 that showed this vector to be effective in miniature pigs; all of the research participants who were nonresponders in the original study would be eligible to receive this new vector. The new study is being reviewed within the NIH and has not yet been submitted to the Institutional Review Board.

## **B. RAC Discussion**

Dr. Ornelles asked whether the investigators encountered any indications in the animal studies that might serve as a surrogate to predict research participant outcomes. Dr. Baum responded that none of the animal studies using the Aquaporin vector had been designed for the long term, so no such indications were observed.

Dr. Hammarskjöld asked whether the investigators looked at Aquaporin expression by doing RNA analysis to determine whether expression continued. Dr. Baum answered that the investigators have conducted sialoendoscopic biopsy in one research participant to date and have seen vector past 360 days, which coincides with the investigators' published papers showing that vector remains for at least a year in rats and pigs.

In response to Dr. Fost's query about what the investigators might do differently to minimize start-up delays, Dr. Baum said that he would educate himself further about the NIH protocol approval process. He stated that he had been uncertain whether a quality-of-life protocol, such as this trial, would be approved.

Dr. Pilewski requested data on the ion composition of the salivary secretion before and after vector administration. Dr. Baum explained that the original hypothesis, based on mouse and rat data, was that the fluid would come out of the ducts on a potassium bicarbonate-driven gradient. The pigs showed much more sodium and chloride. The initial analysis of the human data (not yet published) suggests it is more like the pigs', and that sodium chloride, not potassium bicarbonate, plays a role. The responders produced enough saliva that assays could be done; atomic absorption for sodium and potassium was assayed.

### **III. Review and Discussion of Human Gene Transfer Protocol #1301-1200: Phase I/II Gene Transfer Clinical Trial for LGMD2D (Alpha-Sarcoglycan Deficiency) Using SC rAAV8.tMCK.hSGCA**

Principal Investigator: Jerry Mendell, M.D., The Research Institute, Nationwide Children's Hospital  
Additional Presenters: Louis Chicoine, M.D., Nationwide Children's Hospital; K. Reed Clark, Ph.D., Nationwide Children's Hospital; Tom Preston, CCT, CCP, FPP, Nationwide Children's Hospital; Xiomara Rosales, M.D., Nationwide Children's Hospital  
Sponsor: National Institute of Arthritis and Musculoskeletal and Skin Diseases, NIH  
RAC Reviewers: Drs. Chatterjee, Fong, and Fost

#### **A. Protocol Summary**

Limb girdle muscular dystrophy type 2D (LGMD2D) is a devastating form of muscular dystrophy, as severe as Duchenne muscular dystrophy (DMD). As children with this disease get older, they lose significant muscle mass, limiting the amount of muscle potentially available for gene transfer. This protocol represents the first step in vascular delivery for muscular dystrophy.

The proposed clinical trial is a dose-escalation study of self-complementary (SC) adeno-associated virus (AAV) vector, rAAVrh74.tMCK.hSGCA to LGMD2D (alpha sarcoglycan SG-deficient) research participants, delivered via a major lower limb artery of each leg sequentially by modified isolated limb perfusion and isolated limb infusion (ILP/ILI), henceforth designated as isolated limb recirculation for gene transfer (ILR-GT). Two cohorts will undergo gene transfer in a standard three- to six-dose escalation scheme to establish maximum tolerated dose (MTD) using toxicity. A minimum of three research participants will be enrolled in each cohort. The first cohort will receive a total dose of  $2 \times 10^{12}$  vg/kg split between the two extremities ( $1 \times 10^{12}$  vg/kg per limb). The vector will be infused into an indwelling catheter in the femoral artery; this one-time vector infusion will recirculate for 30 minutes in a closed circuit that includes arterial delivery to an isolated limb (proximal tourniquet), return via the femoral vein, and redelivery to the artery via extracorporeal pump. The second cohort will receive  $6 \times 10^{12}$  vg/kg total dose, split between the two extremities ( $3 \times 10^{12}$  vg/kg per limb), delivered to the whole limb according to the same protocol. The total vector genome dose for each participant will be adjusted by rounding down to the closest 10 kg.

The primary objective of this study is to assess the safety of intravascular administration of SC rAAVrh74.tMCK.hSGCA delivered via the femoral artery to the whole limb of both legs of LGMD2D research participants. Safety monitoring during recirculation will include activated clotting times, limb gases, real-time monitoring of arterial and venous access pressures, and perfusate temperature. Safety endpoints will be assessed by changes in hematology, serum chemistry, urinalysis, immunologic response to rAAVrh74 and hSGCA, and reported history and observations of symptoms. Efficacy measures will be used as secondary outcome for these disorders, including a combination of functional (6-Minute Walk Test) and direct muscle testing for strength of lower limb muscles. These quantitative measures will be done at baseline; days 30, 60, 90, and 180; and at the end of the first and second years. Bilateral magnetic resonance imaging (MRI) of leg muscles will be done at day 180 for comparison with pretreatment MRI. Participants will be evaluated at baseline and at the infusion visit (days 0-2) and will return for followup visits on days 7, 14, 30, 60, 90, and 180. On day 180, participants will undergo a muscle biopsy on the injected muscles in one leg to compare with the pretreatment biopsy done at baseline screening in the opposite leg to establish the size of muscle fibers and any potential toxicity from gene transfer. Participants will be seen at the end of the first and second years for a physical exam, functional and strength testing, and immune studies.

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

Twelve RAC members voted for in-depth review and public discussion of this protocol. Key issues included that it is the first trial involving a novel adeno-associated virus (AAV) vector that will be administered via a limb perfusion technique to pediatric subjects, representing the first vascular administration of gene transfer for muscular dystrophy. In addition, the tropism or transduction properties of this novel construct may be different from those previously observed.

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

Dr. Chatterjee asked the investigators to explain the choice of AAVrh74 for alpha-sarcoglycan gene delivery, including whether it transduces skeletal muscle better than other serotypes, what its systemic transduction patterns are, and whether it transduces multiple organs (which might be helpful for better treatment). She wondered why only the lower extremities would be dosed and whether dosing should include the shoulders or should be done systemically, particularly since no toxicity was noted previously, and whether the low-level transduction of back and buttock muscles would be sufficient to allow the research participants to stand and walk. Dr. Chatterjee suggested that it would be more accurate to exclude only those individuals with specific anti-AAVrh74 antibodies, rather than those with anti-AAV8 antibodies, and asked whether participants would be screened for anamnestic responses to AAVrh74 in addition to pre-existing neutralizing antibodies, which might exclude individuals similar to Subject 6 from the first trial. She asked the investigators to explain the reason for peri-transduction immunosuppression, especially since the eligible individuals will be seronegative. Dr. Chatterjee asked for an explanation of the statement found on page 65 of the protocol that no AAV shedding was observed in animal models or clinical trials, since AAV shedding has been described previously in several studies. In addition, if AAV shedding is found, she asked the investigators to discuss the precautions they propose to take to prevent inadvertent transduction of family members and health care workers.

Noting that the investigators have chosen to use a perfusion rather than an infusion and that an infusion would be safer and technically easier, Dr. Fong asked the investigators whether they have preclinical data to show that an infusion would not work or would be more dangerous. He asked whether the proposed perfusion, a 30-minute hypoxic perfusion of vector in mostly Normosol, is optimal for gene transfer, whether other colloids or crystalloids have been tried, and whether shorter perfusion would be just as effective for gene transfer. As most of the safety measures assess toxicity of perfusion and not the vector, Dr. Fong asked whether these measures would lead to trial stoppage or decreasing perfusion time. He asked why the perfusion is being done through the anterior tibial artery when standard practice would be to use the femoral artery. Dr. Fong suggested including a clearer description of the biopsy procedure, including sedation and needles utilized.

Dr. Fost noted that the proposed invasive procedures (arterial cannulation and infusion; muscle biopsies) might make this early-phase study more appropriate among patients capable of providing meaningful consent; he asked about the participants' ages in the earlier placebo-controlled human study involving injection into the digits. He asked the investigators about the lowest age of research participants from whom the investigators will try to obtain sperm, noting that this request could be awkward and embarrassing for young adolescents, providing another reason to include only older participants. Dr. Fost requested clarification as to the kinds of expertise to be represented on the data and safety monitoring board. With regard to the informed consent document, Dr. Fost suggested that it should be made consistent with the protocol by assuring participants that identifiable information will only be disclosed to those entities with a need to know; consistency was also noted as an issue with regard to the statements about whether direct benefit would accrue to the participants. He also suggested that the language in the informed consent document be reworded to conform to the recommended sixth-grade level and that the document clarify whether stored samples would be identifiable and whether identifiers would be released to other investigators.

### **C. RAC Discussion**

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

- Dr. Fost asked whether the investigators would be able to enroll only research participants who would be capable of giving consent, given the moderately invasive nature of the protocol.
- Dr. Fost suggested that the NIH look into the issue of the inclusion in informed consent documents long lists of committees and agencies to which identifiable information could be released. This practice has become common in most protocols.
- Dr. Fong noted that the standard for limb perfusion for cancer is to access the blood vessel from the contralateral side with a needle and then pass a catheter around the iliac and downward, a process that could be repeated many times with minimal morbidity. He asked the investigators why they have not chosen to take this approach in the current protocol.

### **D. Investigator Response**

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

With regard to transduction patterns and possible better choices for treating disease, the investigators explained that their studies using rAAVrh74 for vascular delivery have always used a muscle-specific promoter—tMCK, MCK, or MHCK7—in the transfer of multiple transgenes, including micro-dystrophin, alpha-sarcoglycan, and dysferlin. In all of these preclinical studies, organ-specific expression has been confined to either skeletal or cardiac muscle due to the promoter-driven expression. For muscular dystrophy, skeletal and cardiac muscle are the target tissues.

The current strategy to target the lower limbs was conceptualized more than five years ago. With regard to subjects' functional recovery, the investigators believed it would be overly ambitious to consider correcting all of the muscle groups of the entire body in a single clinical trial with systemic viral delivery. Targeted delivery to the leg muscles would have clear clinical benefit by prolonging ambulation. In the intramuscular gene transfer of AAV.hSGCA, muscle fibers showed high transduction efficiency and muscle fibers increased in size during the six-month studies. This same result to multiple leg muscles responsible for ambulation would represent a successful functional result for this first-ever vascular gene delivery to muscular dystrophy patients, providing a new direction for the field.

The vector doses required for systemic delivery were a rate-limiting consideration. Due to the limits on how much vector could realistically be produced, more research participants could be dosed if delivery was restricted to the legs. In addition, the ILR-GT method developed by the investigators added an element of safety that would be better for subjects and more acceptable for initial trials in LGMD subjects. Once safety and efficacy were demonstrated in the lower limb muscles, testing systemic delivery would be appropriate.

Regarding vector re-administration, the investigators explained that, as part of this study, their first project addressed re-administration of rAAVrh74 in rhesus macaques with high antibody titers to this virus prior to gene transfer. The completed 2-year study has resulted in a manuscript submitted for review, a draft of which the investigators offered to share under a confidentiality agreement. The investigators achieved very high levels of transduction in rAAVrh74-positive macaques following plasmapheresis to remove antibodies. When plasmapheresis was followed immediately by isolated-limb regional gene delivery targeting the gastrocnemius muscle, the investigators were able to achieve high levels of transduction. This result shows the possibility of re-treating research participants following successful therapy.

Natural history data indicates that ambulation in dystrophinopathies and inflammatory muscle diseases can be correlated with strength in either quadriceps or hamstring muscles, predicting that, in clinical trials, improving strength in these muscle groups will have a beneficial effect on the distance walked in the six-Minute Walk Test. This data implies that it could be possible to prolong ambulation by highly targeted gene delivery, and the added muscles that will be transduced in the lower extremities following ILR-GT provide greater assurance that ambulation will improve. Improving back muscles would also help significantly but would require a different delivery approach that is more appropriate for subsequent gene transfer trials, after safety for vascular delivery is established and dose requirements for systemic delivery are reduced.

There is published data on the prevalence of neutralizing antibodies (NAb) to AAV8 compared to AAV2. On average, the prevalence of NAb to AAV8 is 15 percent in infants younger than one year old, 14 percent in toddlers (ages one to less than three years), and 21 percent in older children (ages three to 18 years—the target population for this trial). There is no specific prevalence data available comparing AAV8 to rAAVrh74, so screening for the proposed clinical trial would be targeted to rAAVrh74.

Regarding screening for antibodies following gene transfer, the investigators will prescreen for binding antibodies, an approach that has been shown to be more sensitive and more inclusive in recognizing a potential risk of pre-existing exposure to AAV.

There has been growing concern among clinician scientists who are conducting gene transfer studies that immune responses to AAV can come on rapidly and preclude gene expression. The investigators are in the middle of a trial of AAV1.follistatin and are encountering positive ELISpots in certain participants directed against either transgene or virus. This and other experiences have led the investigators to believe that a course of glucocorticoid treatment for the first six to eight weeks after gene transfer in the alpha-sarcoglycan clinical trial provides the best chance for success with low risk to the research participants. The principal investigator, who has had more than 25 years of experience using prednisone or its equivalent in muscular dystrophy, is confident that it can be administered safely; he was the author of the first randomized, double-blind, controlled trial of prednisone in DMD that has made this drug the standard of care for these patients.

The investigators reported that recombinant adeno-associated virus (rAAV) mediated correction of functional deficits has been documented to persist in their AAV1.follistatin studies in mdx mice for more than two years. In their studies of rAAV.CMV.follistatin gene transfer in nonhuman primates, the investigators documented persistent gene expression in nonhuman primates for 15 months. In recent studies, rAAV.SMN systemic gene transfer delivered within the first five days of life rescued the  $\Delta 7$  SMA mouse beyond 250 days, with no decline over time despite the very young age at which the mice were treated. In studies by other investigators, long-term pharmacologically regulated expression of erythropoietin in primates persisted for six years following AAV-mediated gene transfer.

Although the investigators agree with the RAC reviewers about the importance of monitoring for shedding, they believe it is unlikely to be a major safety issue given that the virus is nonreplicating and noninfectious and causes no human disease. As part of the Institutional Biosafety Committee (IBC) review, the vector is treated as a biosafety level (BL) 1 agent and will be administered in compliance with NIH guidelines for handling such agents, including notice to health care workers during a two-week period postinjection. The informed consent document asks the research participants to refrain from blood

donation and to use contraception for two years postinjection; it also asks the participant and the family to practice good hand hygiene.

The investigators propose combining components of ILP and ILI. There is general consensus that ILI is safer and can be performed as a minimally invasive alternative to ILP. In this experimental paradigm, the investigators have taken the template for ILI and optimized it by adding a servo-regulated pump to achieve broad distribution of vector and transgene expression in muscles of the lower extremities of nonhuman primates. The investigators are confident that ILR-GT will be appropriate for the research participants, given the similarity in the anatomy of the primate circulation. Another major modification from ILP is that the investigators plan to forego using an oxygenator, which typically accompanies ILP, for vascular delivery. Infusion without an oxygenator leads to a tissue environment with relative hypoxemia, resulting in vasodilation of the vascular beds throughout the limb and thus enhancing vector tissue exposure. Unlike a single-infusion approach, these modifications provide a recirculating delivery system; recirculation has been shown to distribute gene expression more evenly because the tissue is exposed to vector during its multiple passes through the muscle. The use of Normosol in the current recirculation protocol adds some additional buffering capacity over that provided by the crystalloid, normal saline.

Normoxia would not likely improve gene transfer or decrease complications. The addition of the oxygenator medium combined with the inherent increase in circuit volume to prevent hypoxia would result in vector sequestration and dilution, leading to reduced levels of circulating vector. In contrast, hypoxia and acidemia are both potent vasodilators that improve vector delivery across the lower limb, since more vascular beds are opened with reduced oxygen levels. The investigators have not observed deleterious changes in preclinical outcomes in nonhuman primates, and there have been no physiologic or histologic changes in targeted muscle groups with the degrees of hypoxia generated in the preclinical protocols, including 30 minutes of recirculation in six nonhuman primates (ten limbs).

All safety endpoints will be measured and assessed for decisions regarding protocol modification and/or trial stoppage. Real-time monitoring of arterial and venous pressures avoids concerns regarding compartment syndrome. Maintenance of normal body temperature will reduce the risk of muscle breakdown or muscle damage, which also will be monitored with creatine kinase (CK) testing and urinalysis for myoglobinuria. Serum electrolytes will provide insight into muscle breakdown. Hematology studies will help monitor blood loss or sequestration in muscle from rupture of vessels of the vascular bed or possible increases in white blood cells related to infection. The magnitude of the changes in any of these parameters will dictate the response. Any evidence of compartment syndrome that leads to surgical decompression would be an indication for stopping the protocol; such problems are unlikely, given the investigators' experience in nonhuman primates and with both ILI and ILP in human studies. A CK elevation of 10-fold or greater would require a modification of the perfusion time as the initial step. Any functional change in the participant that would prevent ambulation or pose a risk to the extremity for loss of function would be an indication to stop the study.

The investigators agreed that cannulating the femoral artery and vein should be the initial approach, and the protocol will be amended to reflect this change. The vessels will be visualized and cannulated via direct cutdown at the inguinal ligament. Any collateral vessels visualized will be snared and temporarily occluded for the recirculation procedure. Alternative sites of cannulation will be reserved for participants presenting with access difficulty.

Muscle biopsies are a routine part of the investigators' laboratory and clinical studies. In patients more than ten years old, the investigators perform muscle biopsies with anesthesia standby depending on the child's maturity. These children receive light sedation with lorazepam, and the procedure is done under local anesthesia. For children younger than ten years, the identical procedure is done in the operating room with an anesthesiologist administering agents that impair consciousness. A designated surgeon who has worked with the investigators' team for many years conducts the muscle biopsy. In either case the muscle biopsy procedure is the same: An incision is made over the quadriceps muscle and carried down through fascia to expose the muscle. Two small pieces of muscle, about the size of a pencil eraser, are removed, and the technical team immediately freezes the muscle in isopentane cooled in liquid nitrogen. The fascia, subcutaneous tissue, and skin are closed in layers and the skin sutures are

reinforced with sterile strips. The histopathology laboratory, which is part of the Center for Gene Therapy, performs all the sectioning, staining, and cutting tissue for Western blots, immune cell infiltration, and quantitative polymerase chain reaction (PCR) to validate gene transfer.

The investigators agreed that it would be preferable to include only research participants who can provide meaningful consent, and they will enroll as many individuals as possible who are at least 14 years old.

Regarding sperm collection, the investigators agreed to clarify that they only will request semen samples from sexually active males who are at least 18 years old.

The investigators agreed to change and clarify language in the informed consent document as requested by the RAC reviewers.

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

Dr. Mendell noted that the investigators would most likely be able to limit protocol enrollment to participants who were capable of giving consent (i.e., age 14 or older).

Regarding whether to use percutaneous perfusion, Dr. Chicoine explained that the proposed process of doing a cut-down on the femoral artery and vein is the process that was used with the nonhuman primate model, and that this clinical trial is attempting to reconstruct the nonhuman primate experience as closely as possible. In addition, the catheters needed to use the contralateral side and go up over the bifurcation are much longer than the catheters currently used by the investigators, and the increased length could decrease the flow needed to achieve success for these subjects. Dr. Preston added that that the difference between the cancer treatment process and this proposed process is that the investigators anticipate using both limbs, whereas the cancer treatment focuses primarily on one limb. Dr. Mendell also explained that a sample large enough for researchers to conduct all the analyses for this trial cannot be obtained from a needle.

### **E. Public Comment**

No public comments were offered.

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

#### Preclinical Issues

- Administration of the vector by isolated limb recirculation should optimize uptake of the vector by the lower extremity muscles. After application of a tourniquet to prevent systemic circulation, the vector will be delivered into the femoral artery and recirculated through the limb vasculature via the femoral vein with return to the femoral artery through an external pump. As this is a novel method of delivery for gene transfer, it is not known how long the vector should optimally recirculate. Based on clinical data from other indications, for example limb perfusion for chemotherapy delivery, a 30-minute recirculation time is expected to be safe; however, it is important to obtain data on the time needed for vector uptake. This can be done in a preclinical model by sampling the venous effluent at various time points and determining the concentration of vector still circulating. It may take less than 30 minutes for the vector concentration to drop below the level expected to be required for additional muscle transduction.

#### Clinical and Trial Design Issues

- For the reasons outlined above, during the clinical trial the venous effluent should also be sampled to determine the remaining circulating vector levels in research participants. If the data indicate that almost no vector remains to recirculate after a shorter period of time, this may help guide the design of future trials that use this procedure.

- In order to avoid systemic vector circulation, the limb perfusion procedure ends with a washout of any remaining vector before removal of the tourniquet and resumption of normal circulation. However, these research participants have other muscles that are affected by the disease, for example those of the upper extremities. Given that the preclinical data indicate that this vector and transgene are expected to be relatively safe for systemic administration, the investigators should reconsider whether the potential benefit of permitting a relatively low dose of remaining vector to be delivered systemically outweighs the risk.
- As was done in the preclinical nonhuman primate studies, the femoral artery and vein will be accessed by a surgical procedure that allows for direct visualization of these vessels. However, this procedure is more invasive than percutaneous cannulation of the vessels, which is often performed when isolated limb perfusion is used for other clinical indications. While it is important that the clinical trial closely follow the procedures previously tested in the preclinical studies, the investigators should consider whether percutaneous access is a feasible alternative that may minimize risk and could be more easily replicated in future trials.
- While some data in neonatal mice indicate an association between AAV vector administration and development of hepatocellular carcinoma (HCC) (e.g., Donsante, A., *et al.*, 2007, *Science* 317:477), the investigators should reconsider whether long-term testing of alpha-fetoprotein levels to screen for HCC is supported by the totality of data regarding risk of hepatic tumors from administration of an AAV vector or whether alternative monitoring strategies may be appropriate.

#### Ethical, Legal, Social Issues

- This research presents more than minimal risk. Children may enroll in research that involves more than minimal risk provided that there is the prospect of direct benefit; otherwise, the research protocol will require special review by the Department of Health and Human Services. Therefore, the informed consent document should include a discussion of the potential for direct benefit.
- Given the invasive procedures (e.g., arterial cannulation and infusion, muscle biopsies), enrollment in this early-phase study should be limited to research participants who are at least 14 years of age, as they are more likely to be capable of providing meaningful assent.
- The informed consent document includes a long list of “people or companies authorized to use, disclose, and receive protected health information collected or created by this research study.” As it is unlikely that even analysis of a severe adverse event would require release of identifiable information to most of the entities listed, the informed consent document should be modified so that participants are not required to provide such broad authorization for release of protected health information.

#### **G. Committee Motion 1**

Dr. Fong summarized the RAC recommendations that would be included in the letter to the investigators, expressing the comments and concerns of the RAC. A motion to approve these recommendations was made but not seconded, and the RAC approved these summarized recommendations by a vote of 17 in favor, 0 opposed, and 0 abstentions.

#### **IV. Review and Discussion of Human Gene Transfer Protocol #1301-1202: Phase I Study of Safety and Immunogenicity of ADU-623, a Live Attenuated *Listeria monocytogenes* Vaccine (*LmΔactA/ΔinlB*) Expressing EGFRvIII-NY-ESO-1, in Patients with Treated and Recurrent WHO Grade III/IV Astrocytomas**

Principal Investigator: Marka Crittenden, M.D., Ph.D., Providence Cancer Center

Additional Presenters: Keith Bahjat, Ph.D., Providence Cancer Center; Dirk G. Brockstedt, Ph.D., Aduro BioTech, Inc.; Tom W. Dubensky, Jr., Ph.D., Aduro BioTech, Inc.; Dung Le, M.D., Johns Hopkins University  
Sponsors: Earle A. Chiles Research Institute, Providence Cancer Center; Aduro BioTech, Inc.  
RAC Reviewers: Dr. Chiocca, Ms. Dresser, Dr. Pilewski, and Dr. Strome

## A. Protocol Summary

In 2012, there were projected to be 22,910 newly diagnosed individuals with primary malignant brain tumors in the United States. The most common brain tumor subtype in adults is glioblastoma multiforme (GBM), which has the worst prognosis, with a five-year survival rate of only 3.3 percent. Standard-of-care therapy for GBM, including surgical resection, adjuvant radiation therapy, and temozolomide chemotherapy, has resulted in little improvement in survival. While ongoing studies using bevacizumab either alone or in combination with standard-of-care chemotherapy/radiotherapy as candidate first-line or second-line therapies are promising, the results may represent only an incremental advance of existing approved therapies. The continuing poor prognosis with the available treatment options for patients with GBM underscores the urgent need for significantly improved therapies. This proposed clinical trial will test a novel immunotherapy regimen.

Dysregulated signaling via the epidermal growth factor receptor (EGFR) family is believed to contribute to the progression of a diverse array of cancers, including GBM, by promoting proliferation and survival via the RTK/Ras/PI3K pathway. The most common variant of the EGFR, EGFRvIII, results from a consistent and tumor-specific in-frame deletion of exons 2-7 of the EGFR gene. The deletion of exons 2-7 generates a novel glycine at the junction and leads to constitutive, ligand-independent signaling through this receptor. Significant to the ADU-623 investigational agent, this junction forms a novel tumor antigen with demonstrated immunogenicity in both mice and humans. The EGFRvIII receptor variant has been found in 40% of glioblastomas while being virtually absent from normal tissues. In addition to the tumorigenic effects of constitutive signaling, EGFRvIII-expressing cells are less sensitive to radiation therapy, tyrosine kinase inhibitors and anti-EGFR antibodies that have been effective against tumors overexpressing native EGFR

The restricted expression pattern of EGFRvIII and its demonstrated immunogenicity make EGFRvIII an ideal target for immunotherapy. Elicitation of a potent and long-lived EGFRvIII-specific T cell and antibody response would both eliminate EGFRvIII-expressing cells and exert immunoselective pressure in favor of those cells not expressing EGFRvIII, potentially facilitating treatment of residual tumor with existing targeted therapeutics.

NY-ESO-1 is a cancer/testis (C/T) antigens expressed in a variety of human cancers but not in normal tissue, except for testis. Although NY-ESO-1 is not widely found in cancers of the brain, NY-ESO-1 is one of the best characterized antigens to date and NY-ESO-1 specific humoral and cellular immunity has been detected frequently in cancer patients

Aduro BioTech is developing a therapeutic vaccine that targets EGFRvIII and NY-ESO-1 concurrently in hopes of improving survival in patients with brain cancer. Wild-type *Listeria monocytogenes* (*Lm*), a type of bacteria commonly found in the environment, may occasionally cause a severe illness when it is passed to humans through contaminated food. ADU-623 is a form of *Lm* that has been modified in the laboratory to be 1,000 times less toxic than wild-type *Lm* when administered to mice. ADU-623 has also been modified by the insertion of genes that cause the bacteria to express EGFRvIII and NY-ESO-1. *Lm* is a powerful activator of nonspecific immune responses (innate immunity), which helps in the development of adaptive immunity that targets specific diseases. *Lm* activates primarily the cellular arm of the immune response, comprising cytotoxic T lymphocytes (CTLs) and helper T lymphocytes. The goal of therapeutic cancer vaccines is to induce CTLs to recognize cancer cells as foreign and then target them for destruction. In addition, CTLs stimulated by *Lm* recognize *Lm*-infected cells in the infected host as foreign and kill them. If *Lm* cells are engineered to express antigens that are present in high numbers within tumor cells (such as EGFRvIII and NY-ESO-1), CTLs induced by administering these types of

modified *Lm* vaccines will recognize and selectively destroy both *Lm*-infected cells (thus clearing the bacteria from the patient's body) and tumor cells that overexpress the target antigen(s).

Studies have shown that giving ADU-623 to mice can induce EGFRvIII- and NY-ESO-1-specific T-cell responses. Clinical studies with therapeutic vaccine strains on which ADU-623 is based or with a similar strain that encodes a different tumor-associated antigen have demonstrated that these *Lm*-based vaccines can be administered safely to cancer patients. The most commonly reported side effects after infusion of the vaccines were fever, chills, and nausea. Subjects also experienced drops in lymphocyte counts and phosphate levels after infusion, but all side effects resolved on their own within days. Although a Phase I study with CRS-207 (a similar strain that encodes human mesothelin antigen) was not designed to assess survival, six of the 17 end-stage subjects (with a typical life expectancy of three to six months) lived for 15 or more months after receiving CRS-207, and five of these six subjects developed a mesothelin-specific immune response induced by the CRS-207 vaccine. CRS-207 is currently being evaluated in a randomized, controlled Phase II study in patients with metastatic pancreatic cancer. In addition to the ongoing Phase II trial, CRS-207 is being evaluated in combination with chemotherapy in a Phase IB study in patients newly diagnosed with pleural mesothelioma.

This proposed Phase I study will enroll subjects with a pathologic diagnosis of WHO Grade III/IV astrocytomas and who (1) have completed standard-of-care external beam radiation therapy and concurrent temozolomide followed by adjuvant temozolomide or (2) show radiographic evidence of progression following standard-of-care radiation and chemotherapy treatment, including those who had a second surgical resection. Research participants will receive four doses of ADU-623 given four weeks apart. For safety, research participants will be followed with physical exams, adverse event reporting, and lab work, and they will receive MRI scans every three months for two years or until disease progression. The primary purpose of this study is to identify the MTD (up to a dose of  $1 \times 10^9$  CFU IV) and to characterize the safety profile of the ADU-623 vaccine. In addition, the study will determine progression-free survival, time to progression, and overall survival rates in participants vaccinated with ADU-623. Evaluation of the EGFRvIII-, NY-ESO-1-, and vector-specific and innate immune responses will be performed.

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

Eleven RAC members voted for in-depth review and public discussion of the protocol. Key issues included the novelty of combining these two tumor antigens and a *Listeria* vector platform, for which there is a limited safety profile. In the first trial using this attenuated *Listeria* vector,  $1 \times 10^{10}$  CFU was determined to be the MTD, after the first participant to receive this dose intravenously developed significant hypotension and required admission to the intensive care unit. Several modifications were made to the protocol as a result, including specific premedication and infusion protocols. OBA protocol #932 proposed using an attenuated *Listeria* vector expressing two hepatitis C viral proteins in participants who had hepatitis C; this protocol was closed prematurely due to the development of an anaphylactoid reaction in one participant. An ongoing Phase II trial (OBA protocol #1075) enrolls two participant cohorts: One cohort receives the GVAX pancreatic cancer vaccine (allogeneic pancreatic tumor cells transduced with the gene for human granulocyte monocyte colony-stimulating factor) with cyclophosphamide, and the other cohort receives the GVAX vaccine, cyclophosphamide, and intravenous administration of a *Listeria* vector expressing human mesothelin. At least 41 participants have been accrued, and four SAEs have occurred that were assessed as being probably or definitely related to the gene transfer agent, including pyrexia, elevated liver function test results, chills, and hypophosphatemia. While a number of participants have tolerated the vector, this protocol was chosen for public review in order to examine the safety data and to discuss how that data will inform the design of the trial, which will also use a novel combination of tumor antigens.

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

Dr. Chiocca asked the investigators to provide experimental evidence that NY-ESO-1, an antigen that is not expressed in most malignant gliomas, is an appropriate target for these tumors. He also asked for rationales for dose escalation first by a full log and thereafter by half a log, the wait period of 45 days from

craniotomy to treatment (which he thought was excessively long), and the selected EGFRvIII immunogen peptide, which is different from the one in current clinical trials. Because only some malignant glioma patients express EGFRvIII, a plan to prescreen patients for EGFRvIII (and/or NY-ESO-1) expression before accrual is needed. He noted that steroid treatment is an exclusion criterion but that the majority of these patients are likely to be taking steroids to control edema, thus posing a risk that accrual would be poor or that accrued research participants would have to be excluded from analyses after dosing. Dr. Chiocca requested clarification as to when MRI measurements would be obtained, whether an additional MRI between day 0 and day 90 would be beneficial to assess responses, whether the investigators plan to look for testicular toxicity due to the NY-ESO-1 antigen, and what the effects of bevacizumab on this experimental treatment might be, since participants will be allowed to take this drug.

With regard to safety issues, Ms. Dresser noted that the only nonhuman data come from mice and asked the investigators to explain why testing in an additional nonhuman species is unnecessary to assess human safety. Because other trials using *Listeria*-based vectors have produced hypotension and other systemic symptoms soon after infusion, she suggested that the investigators consider increasing the frequency of vital signs monitoring, consider whether four hours is a sufficient time period for post-infusion monitoring, and consider developing a specific response plan to address hypotension and other serious immune reactions, including developing predetermined criteria for stopping (and resuming) an infusion. Regarding the informed consent document, Ms. Dresser noted that “treatment” is an inappropriate term for a Phase I trial, that the information on possible benefits should be more precise, that the statement on liability seems inconsistent with the statement on costs in Appendix M, and that it is important to alert potential participants that health insurance coverage is not always available for injuries that occur in early-phase trials.

Noting that this protocol is well written and is a first step toward developing a new therapy for a devastating malignancy, Dr. Pilewski asked the investigators to discuss the choice of antigens expressed by the *Listeria* strain, because of the question of relevance given the limited expression of EGFRvIII and NY-ESO-1 in brain cancer. He asked whether the investigators knew of any preclinical model systems relevant to astrocytomas that provide evidence for antitumor effects. Dr. Pilewski stated that *Listeria* is a well-described cause of meningitis and, as a result, he wondered whether the presence of an altered blood-brain barrier in patients with recurrent astrocytomas might significantly predispose those individuals to SAEs, even though no vaccine-related SAEs have been reported in the Phase IB or Phase II studies with CRS-207 administered at  $1 \times 10^9$  CFU, the maximum dose proposed in this trial. Given the high frequency with which fever, rigors, and hypotension occurred, he suggested that the investigators consider a longer observation period for the first dose in each research participant; in addition, he suggested the investigators consider a more conservative definition of “clinically significant heart disease” and broader exclusion criteria for cardiac disease. Dr. Pilewski asked the investigators to clarify the proposed dose escalation description and discrepant statements in the informed consent document and Appendix M with regard to payment of costs if a research participant is injured during this study.

Dr. Strome asked the investigators to explain why they chose not to limit enrollment to GBM patients who express EGFRvIII, especially given that the primary objective of this trial is to identify the maximally tolerated dose and safety. Because NY-ESO-1 is not widely expressed in brain malignancy, he questioned why it is being included in the construct for this pathology, noting that the two reasons given by the investigators for inclusion are questionable; he suggested instead that researchers consider limiting enrollment to tumors expressing both EGFRvIII and NY-ESO-1 (although he acknowledged that such a restriction could affect the rate of enrollment). Dr. Strome asked the investigators to share detailed information from animal trials using this novel construct to provide reassurance of its safety profile, especially given that *Listeria* vaccination has been associated with significant hypotension, anaphylactoid airway events, and immune reactions. He asked what dose levels, if any, have shown significant elevation in liver laboratory values. With regard to the informed consent document, Dr. Strome asked for inclusion of more information about standard therapy so that potential participants are made aware of standard survival rates and markers that may affect survival; participants also should be made aware of the percentages of tumors that express EGFRvIII and NY-ESO-1. He suggested that the investigators include more emphasis on potential respiratory events such as severe short-term breathing difficulty that

could require hospitalization. Dr. Strome commended the sponsor's willingness to cover the cost of untoward events that might result from this trial.

### C. RAC Discussion

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

- Dr. Hammarskjöld asked whether the proposed vector was originally made for other cancers or whether it was produced specifically for GBM.
- Dr. Kiem asked about the amount of time between each participant's cessation of temozolomide and the start of experimental treatment.
- Dr. Fong asked about the standard procedure for when research participants are allowed to go home after dosing, particularly in reference to concerns about tachycardia and orthostatic changes.
- Dr. Wooley asked about the investigators' plan for dealing with an unanticipated autoimmune reaction.
- Dr. Hammarskjöld reiterated her concerns about using two antigens rather than one.

### D. Investigator Response

#### 1. Written Responses to RAC Reviews

Although EGFRvIII and NY-ESO-1 are not detected broadly among individuals with GBM or in all tumor cells from a given positive individual, it is well recognized that, as a mutant form of an endogenous gene (EGFRvIII) and a cancer testis antigen (NY-ESO-1), there is not profound peripheral tolerance for these antigens, making them desirable targets to elicit a functional cytolytic tumor-specific immune response. It is well known that tumor destruction resulting from antigen-specific CTLs leads to the presentation of additional tumor antigens to the immune response and to priming of CTLs with specificities beyond the cognate vaccine antigens, a process known as antigen spreading. The investigators have shown previously that treatment of tumor-bearing syngeneic animals with the attenuated *Lm* vector itself induces pro-inflammatory cytokines, activating natural killer (NK) cells and resulting in NKG2D-mediated tumor cell recognition and lysis, leading to priming of tumor-specific immunity and a survival benefit. Additionally, researchers showed several years ago that treatment of brain tumor-bearing Fischer rats with attenuated recombinant *Lm* provided therapeutic efficacy and a survival benefit due to both tumor-specific cellular immunity induced by vaccine-encoded antigens and through tumor-antigen spreading mechanisms. Thus, the investigators believe that the ADU-623 investigational agent has the capacity to induce broad GBM-specific cellular immunity specific for vaccine-encoded EGFRvIII and NY-ESO-1 antigens, and against additional GBM-specific antigens through cross presentation and antigen-spreading mechanisms. Recent reports have shown that NY-ESO-1 is expressed in a population of GBM cancer stem cells, implying that NY-ESO-1 may represent a strongly desirable tumor antigen for targeting the self-renewing GBM stem cell population. NY-ESO-1 is currently being targeted in an ongoing clinical study to evaluate the safety and immunogenicity of an investigational therapeutic vaccine in individuals with NY-ESO-1-expressing solid tumors, including GBM.

The investigators will assess EGFRvIII and NY-ESO-1 expression in the tumors of participants in this study and will determine whether expression of these antigens alters either the toxicity of the vaccine or immune outcomes. These results could inform whether this restriction should be in place in subsequent studies and what method of EGFRvIII detection is optimal.

Regarding steroid treatment as an exclusion criterion for this trial, the investigators explained that such a criterion will limit enrollment for those patients who are having significant and rapid disease progression and who require systemic steroids for symptom management. Some potential research participants will have completed adjuvant temozolomide and will not have progressed and will not be on steroids, and some potential participants with recurrent disease who are on bevacizumab will not require systemic steroids; all of these individuals will be eligible for this protocol.

The rationale for dose escalation follows the design of the Phase I safety, tolerability, and immunogenicity study of ANZ-521, a strain that is based on the platform strain ANZ-100 and engineered to express two nonstructural proteins of the hepatitis C virus. The starting dose of  $3 \times 10^7$  CFU is 30-fold lower than the dose currently being administered to individuals with metastatic pancreatic cancer. More than 150 IV infusions of  $1 \times 10^9$  CFU have been administered so far in the Phase II study, and the two-hour infusion at this dose level has been well tolerated. The investigators anticipate that the dose levels of  $3 \times 10^7$  and  $3 \times 10^8$  CFU will be well tolerated with minimal infusion-related toxicities. The lower dose level of  $3 \times 10^7$  CFU also was chosen based on the results from the ANZ-100 Phase I study, demonstrating biological activity with minimal toxicity. Full-log escalations are proposed for this trial at the lower dose levels in order to more rapidly identify a potentially beneficial dose while carefully monitoring safety. The half-log increase from  $3 \times 10^8$  CFU is proposed in order to reach the expected MTD, which is anticipated to be  $1 \times 10^9$  CFU.

A major obstacle to cancer immunotherapy has been the availability of tumor-specific antigens that are not subject to profound tolerance mechanisms that largely diminish the ability to generate an effective tumor-specific immune response. Cancer-testis antigens such as NY-ESO-1 are largely encoded by the X chromosome, and expression in healthy individuals is restricted to immune-privileged testicular germ cells due to the lack of expression of major histocompatibility complex class I molecules on these cells. Because other NY-ESO-1-targeted vaccines in clinical trials have not shown it, testicular toxicity is not anticipated in participants dosed with the ADU-623 investigational agent.

The post-craniotomy period of 45 days will be in the setting of recurrent disease, and patients will be eligible to initiate bevacizumab as a standard option in recurrent GBM. The concern about initiating vaccine too close to surgery—the increased incidence of meningitis in the perioperative period—needs to be balanced with the rapid recurrence that can be seen in this disease, and a 45-day healing period was believed to be appropriate. The investigators noted that they would be amenable to shortening this period if the RAC consensus was that a shorter time frame would be safe.

Bevacizumab use in GBM is associated with low-grade bleeding, hypertension, impaired wound healing, and proteinuria. Rates of SAEs such as gastrointestinal perforation, reversible posterior leukoencephalopathy syndrome, cardiac failure, and wound-healing complications in GBM studies are low, at a 2 percent incidence for each. Deep vein thrombosis and pulmonary embolism have been reported in studies evaluating bevacizumab-containing therapy in recurrent GBM. Overlapping toxicity between bevacizumab and ADU-623 is not expected. The preclinical toxicities observed in mice and nonhuman primates treated with anti-VEGF antibodies did not fully predict the toxicities that were observed in the clinical setting. Therefore, the investigators believe that careful clinical evaluation of the combination starting with a very low dose of ADU-623 and careful dose escalation will be more appropriate. The investigators agreed to perform a post hoc exploratory analysis to assess the effect of bevacizumab on immune responses in this study.

ADU-623 has the same genetic background as ANZ-100 and two other investigational agents based on ANZ-100 that encode different antigens (CRS-207 and ANZ-521). ANZ-100 and CRS-207 at the proposed route of administration and dose for this study have been shown to be safe and well tolerated in subjects with advanced cancer. The preclinical safety package for ADU-623 was designed to demonstrate that safety in mice is comparable to CRS-207 and ANZ-100, which have been used safely in clinical studies. This comparability was demonstrated in both acute toxicity and biodistribution studies and by monitoring standard serum chemistry and peripheral blood hematology parameters following a single IV administration. The data confirmed that the safety and toxicology of the ADU-623 investigational agent is consistent with the preclinical studies conducted with CRS-207 and ANZ-100, and there appear to be no added toxicities due to the encoded antigens. A Pre-Pre-IND meeting with the FDA was conducted on January 9, 2013, to discuss the ADU-623 pharmacology and toxicology studies in support of the proposed Phase I multidose, dose-escalation study of ADU-623 in patients with Grade III/IV astrocytomas. Based on that discussion, the FDA agreed that the presented preclinical studies are appropriate and sufficient to support the careful Phase I testing of ADU-623 in humans with fatal brain tumors.

The recommendations regarding infusion of the study drug are based on the results from the Phase I and Phase II studies that are ongoing or have been completed using the same vector platform strain *Lm*  $\Delta actA/\Delta inlB$  as used in the ADU-623 construct. The recommendation for four hours of observation is based on the onset of symptoms related to hypotension initiation and peak in completed and ongoing Phase I and Phase II studies. However, the investigators agreed to modify this protocol by extending the observational window to six hours for the first administration of ADU-623 for each participant. Observation after subsequent administrations in the same individual may be reduced to four hours if no significant reaction is observed with the first administration.

A financial counselor who has experience in counseling patients and helping them navigate insurance coverage questions is available to all patients treated at Providence Cancer Center, and therefore will be available to the research participants in this study.

Mouse models using syngeneic tumors show that cellular and humoral immunity specific for EGFRvIII can have antitumor effects. These responses have been elicited using peptide vaccines, dendritic-cell vaccines, and whole-cell vaccines. In other preclinical brain tumor models, it has been demonstrated that tumor-specific T cells can readily move into the brain and exert antitumor effects. These data are reflected in the recommendations of the National Cancer Institute's cancer antigen prioritization task force, which placed EGFRvIII fifth among a collection of more than 50 tumor-associated antigens.

The investigators believe that limiting enrollment to individuals with tumors expressing both EGFRvIII and NY-ESO-1 would not take into account the well-known property of attenuated *Lm*-based vaccines to broaden the tumor-specific cellular response beyond vaccine-encoded antigens.

The ADU-623 construct was developed for inducing immune response against two targets—EGFRvIII and NY-ESO-1—and has applicability in multiple malignancies. Some of these malignancies will express more of one target than the other. Neither target is expressed in normal tissues (except testis, an immune-privileged site), and the antigenic construct itself has no biological activity. Therefore, toxicity related to these tumor antigens is expected to be minimal with respect to cross-reaction to normal tissue. In the case of GBM, EGFRvIII has a higher expression rate than NY-ESO-1 does. However, recent researchers have reported NY-ESO-1 expression in populations of GBM cancer stem cells. Because the investigators believe that the ADU-623 investigational agent has the capacity to induce broad GBM-specific cellular immunity specific for vaccine-encoded EGFRvIII and NY-ESO-1 antigens, immunizing patients to this antigen and targeting this cell population may be of benefit. Many studies of immunotherapy have included additional antigens or antigen fragments, such as tetanus, hepatitis B virus antigens, and others, with the intent to track immune responses to well-known and -characterized T-cell epitopes. In addition to its antigenicity, NY-ESO-1 has well-characterized epitopes that can fulfill this role without the need to introduce an additional antigen into the vaccine vector.

No vaccine-related SAEs or unexpected grade 3 or higher adverse events, including hypotension or increase in liver function tests, have been reported. The airway events reported in the ANZ-521 Phase I study in subjects with chronic hepatitis C have not been observed in the studies with ANZ-100 or CRS-207 in subjects with advanced cancer. Based on a report from an independent allergist/immunologist, the airway events (i.e., cough) observed with ANZ-521 are not believed to be an anaphylactoid response as initially reported by the original study sponsor. Hypotension and airway events that were noted in the Phase I studies were not observed in preclinical mouse or nonhuman primate studies with the same vaccine strains. Therefore, the investigators believe that the existing clinical experience with vaccine strains that are based on the same platform strain as ADU-623, preclinical mouse toxicology performed with ADU-623, and a carefully performed Phase I study provide the most rational approach to determining the safety of ADU-623 in subjects with Grade III/IV astrocytomas who received standard care.

Participants in this protocol will have received the standard therapy for GBM and would be enrolled in this protocol either after completion of the adjuvant temozolomide during the observation period or at recurrence. At recurrence, subjects would first be treated surgically and/or initiated on salvage bevacizumab, if deemed appropriate by the treating physician and surgeon, and then offered enrollment in the study. Since patients will be at different stages in their cancer treatment, standard therapy will vary

for research participants and is best addressed by the treating oncologist. To direct the potential participants to discuss what standard treatment(s) would be appropriate based on their tumor characteristics, the investigators modified the informed consent document to report the percentages of tumors that express EGFRvIII and NY-ESO-1.

The investigators agreed to change and clarify language in the informed consent document as requested by the RAC reviewers.

## 2. Responses to RAC Discussion Questions

Dr. Crittenden explained that the investigators believe the vector will activate innate immune cells with peripheral trafficking properties. The NK cells are expected to traffic peripherally and can have antitumor immune responses. In the case of research participants who have neither EGFRvIII expression nor NY-ESO-1 expression, the *Listeria*'s activation of innate immunity could have a positive effect by activating the immune system and secreting cytokines. Type 1 cytokines increase following *Listeria* administration; either a pre-existing T-cell or NK-based response may be reactivated by *Listeria* administration, which revs up the immune response. The only published preclinical model that involved brain tumors and tested this hypothesis is a study in a rat model of glioblastoma; the study showed that immune responses to the encoded antigen were induced with antitumor activity as well as antigen spreading.

Dr. Dubensky summarized a study by Dr. Bahjat that demonstrated that mice that had tumors that were treated with *Listeria* (that did not encode any antigens) generated a strong NK-cell response. Those NK cells were able to traffic to and kill the tumor. Upon rechallenging those mice, Dr. Bahjat and colleagues showed that the mice had a tumor-specific immune response that was initiated by the *Listeria*. As a result, the investigators believe that administering this ADU-623 experimental agent to research participants in the proposed study, regardless of whether the participants express either the NY-ESO-1 or EGFRvIII antigens, will result in tumor-specific T-cell responses via the NK-cell-mediated, cross-presentation mechanism.

Dr. Crittenden explained that the proposed vector was made specifically for GBM but could apply to other EGFRvIII- or NY-ESO-1-expressing cancers. From the beginning, the investigators proposed this vector for GBM, although initially they considered it only for EGFRvIII. Subsequently, the investigators discussed adding other antigens that could potentially be beneficial, and NY-ESO-1 was determined to be an appropriate target because it is not widely expressed elsewhere.

Dr. Crittenden stated that the amount of time between stopping temozolomide and starting the experimental treatment will be based on each research participant's functional blood cells. Because of temozolomide's impact on platelets and white blood cell counts, participants need to have enough time to recover an appropriate immune response in order to participate in this clinical trial.

The investigators agreed to incorporate additional guidelines, as recommended by the RAC, for when participants can be released to go home after the four-hour observation period that follows dosing.

With regard to an unanticipated autoimmune reaction, Dr. Crittenden explained that such a reaction could result in treatment with antihistamines or systemic, topical, or inhaled steroids. Dr. Urba clarified that the first line of treatment would be antibiotics to rule out infection; secondarily, such a reaction would be treated with steroids.

Dr. Crittenden stated that the investigators believe it is the ethical obligation of the treating physician to discuss with the patient/potential research participant the full range of standard-of-care therapy, along with vaccine or other clinical protocols. He offered to include a reference to cancertrials.gov within the informed consent document, along with language to encourage potential participants to speak with their treating physicians and check that website. He noted that guidance from the treating physician is important because reading about cancer trials can be intimidating.

Although agreeing that the use of two antigens increases the complexity of this clinical trial, Dr. Crittenden reiterated the primary endpoint of this Phase I trial—finding a safe dose—and stated that the use of two antigens does not compromise the safety of the research participants. Dr. Brockstedt added that the chosen antigens for this trial are not expressed in healthy tissue or in testes, thus precluding the possibility of an autoimmune response.

## **E. Public Comment**

No public comments were offered.

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

### Clinical and Trial Design Issues

- Neither of the two antigens included in this attenuated vector is widely expressed on the tumor being targeted in this study. EGFRvIII is expressed in less than 40 percent of glioblastomas and NY-ESO-1 is expressed very rarely. The rationale for enrolling participants in a vaccine trial when the majority of tumors will not likely express either of the tumor antigens appears to be based in part on preclinical data that suggests that *Listeria* can induce innate immunity and lead to an immune response to antigens that are not included in the vaccine. While there was considerable discussion during the RAC meeting regarding whether EGFRvIII tumor expression should be a criterion for enrollment, this was not recommended, provided that (1) antigen expression on participants' tumors are analyzed and correlated with the safety and efficacy data and (2) the informed consent process explains the rationale for using a vaccine that employs antigens expressed in a minority of tumors.
- For those participants who will have resection of their tumor prior to administration, the proposed waiting period of 45 days from craniotomy to administration of the vector may be too long, as a number of tumors may recur in this time period. The investigators should consider whether a 30-day waiting period is sufficient to address the increased risk of meningitis in the perioperative period.
- Because some participants have experienced systemic reactions to the *Listeria* infusion (including alterations in blood pressure, heart rate, or fevers), participants will be observed for 6 hours after the infusion. It is important that the decision to discharge participants at the end of this observation period be based on uniform criteria and be documented. Therefore, it is recommended that a standard operating procedure be developed for this discharge assessment.

### Ethical, Legal, Social Issues

- The two antigens in this cancer vaccine are likely to be expressed in a minority of the participants' brain tumors. As the potential for benefit may be dependent on whether the participant's tumor expresses at least one of these antigens, participants should be told in the informed consent process that a minority of glioblastomas express these antigens and lack of expression could impact the potential for benefit and possibly safety. As the informed consent process includes a discussion of alternative clinical trials, the investigators should consider including information on cancer trial websites in the informed consent document for those participants who would like to research alternative trials.

## **G. Committee Motion 2**

Dr. Fong summarized the RAC recommendations to be included in the letter to the investigators, expressing the comments and concerns of the RAC. Dr. Chiocca moved to approve these recommendations; the motion was not seconded, and the RAC approved the summarized recommendations by a vote of 18 in favor, 0 opposed, and 1 abstention (Dr. Strome).

## V. Minutes of the December 4–5, 2012, RAC Meeting

RAC Reviewers: Drs. Badley and Kiem

Dr. Kiem stated that the December 2012 meeting minutes document was accurate and complete, with one minor change needed.

### A. Committee Motion 3

Dr. Fong declared the December 2012 RAC meeting minutes document approved.

## VI. Updates from the Office of Biotechnology Activities

Presenter: Dr. Corrigan-Curay

Dr. Corrigan-Curay noted several upcoming events of interest. A workshop titled “Gene Therapy: Charting a Future Course” will be presented on April 12, 2013, on the NIH campus in Building 31C, Room 10. A safety symposium regarding T-cell immunotherapy is scheduled for September 10–11, 2013, in conjunction with the September 2013 RAC meeting.

She also reported that the NIH will be commissioning an Institute of Medicine (IOM) study on the RAC’s role in reviewing gene transfer protocols. This review may include opportunities to participate in IOM meetings.

## VII. Gene Transfer Safety Assessment Board Report

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

### A. GTSAB Report

Dr. Kohn presented the GTSAB report for the first quarter of 2013; the Board met in March 2013. The OBA had received 13 protocol submissions in the past three months, 11 of which were not selected for public review at this RAC meeting. Of the 11 protocols not selected for public review, nine were oncology protocols, one was for an eye disorder, and one was for control of graft-versus-host disease after stem-cell transplant. In these 11 protocols, five used retroviruses, two used vaccinia viruses, and one each used lentivirus, adeno-associated virus, *Saccharomyces cerevisiae*, and transposon. Dr. Kohn stated that information about these trials would be available on the OBA website after this RAC meeting.

Reports on 12 SAEs from ten protocols were reviewed by the GTSAB, including initial and follow-up reports. After analyzing these events, the GTSAB concluded that none warranted public discussion at this RAC meeting.

The OBA received notification from investigators that 14 protocols, three of which had been reviewed previously at a RAC public meeting, were newly open to enrollment.

Dr. Kohn reported on three noteworthy protocol changes that represented responses to RAC review:

- *OBA Protocol #964, reviewed in March 2009: Lentiviral Gene Transfer for Treatment of Children Older than 1 Year of Age with X-Linked Severe Combined Immunodeficiency.* Preclinical data was submitted comparing the risk of insertional oncogenesis between the lentiviral vector being used in this protocol and the retroviral vector used in the clinical studies for X-SCID in which five research participants developed leukemia.

- *OBA Protocol #1005, reviewed in December 2009: A Safety and Efficacy Study in Subjects with Leber Congenital Amaurosis (LCA) Using Adeno-Associated Virus Vector to Deliver the Gene for Human RPE65 to the Retinal Pigment Epithelium (RPE) [AAV2-hRPE65v2-301].* The primary endpoint has been changed from pupillary light reflex to mobility testing. In addition, the Phase III eligibility criteria now are restricted, with respect to the primary endpoint, to exclude individuals with more moderate retinal degeneration and those with impairment so severe that improvements cannot be detected.
- *OBA Protocol #1117, reviewed in September 2011: A Phase I/II Safety, Pharmacokinetic, and Pharmacodynamic Study of APS001F with Flucytosine and Maltose for the Treatment of Advanced and/or Metastatic Solid Tumors.* APS001F is a recombinant *Bifidobacterium longum* strain expressing cytosine deaminase. Additional preclinical data was submitted regarding biodistribution of vector over time; cytokine levels for IL-6, INF- $\gamma$ , IL-1 $\beta$ , and TNF- $\alpha$  will be measured, as elevations in TNF- $\alpha$  were seen in animal studies at the higher doses and in some of the animals who died in these studies. Because a necrotic tumor could serve as a nidus for abscess formation, the protocol includes a guideline on management of abscesses.

Recent publications of interest included an article in *Science Translational Medicine* reporting on results from OBA Protocol #0001-381, *Gene Therapy of Canavan Disease Using AAV for Brain Gene Transfer*, a 10-year look back at research participants. This protocol investigated whether early detection and treatment with gene transfer-mediated enzyme replacement in the neonatal period might offer the best opportunity for a reduction in symptoms and long-term stabilization in subjects with Canavan disease. The principal investigator will present her results at the June 2013 RAC meeting. Also of interest was a February 2013 article in *Human Gene Therapy* reporting on results from OBA Protocol #9711-221, *Long-Term Follow-Up Assessment of a Phase I Trial of Angiogenic Gene Therapy Using Direct Intramyocardial Administration of an Adenoviral Vector Expressing the VEGF121 cDNA for the Treatment of Diffuse Coronary Artery Disease*. From 1997 to 1999, AdVEGF121 was administered by direct myocardial injection to an area of reversible ischemia in 31 research participants with severe coronary disease, either as an adjunct to conventional coronary artery bypass grafting or as minimally invasive standalone therapy, using a minithoracotomy. This study provides long-term (median, 11.8 years) follow-up on the research participants. Both publications reported hints of efficacy, and both approaches were shown to be safe.

## **B. RAC Discussion**

No discussion occurred.

## **C. Public Comment**

No public comments were offered.

## **VIII. Update and Discussion of Human Gene Transfer Protocol #1107-1120: A Phase I Ascending-Dose Trial of the Safety and Tolerability of Toca 511, a Retroviral Replicating Vector, Administered to Subjects at the Time of Resection for Recurrent High Grade Glioma and Followed by Treatment with Toca FC, Extended-Release 5-FC**

Presenter: Daniel Pertschuk, M.D., Tocagen, Inc. (*via teleconference*)  
Additional Presenters: Douglas Jolly, Ph.D., Tocagen, Inc. (*via teleconference*); Alessandro Lobbia, Ph.D., Tocagen, Inc. (*via teleconference*); Amanda Omlor, M.S., RAC, Tocagen, Inc. (*via teleconference*)

### **A. Presentation by Dr. Pertschuk**

Dr. Pertschuk presented a brief overview of Toca 511; a review of the two studies currently in progress including a summary of enrollment and safety data from those two studies; a brief review of the study in

which an adverse event occurred, a review of that event, and biodistribution and shedding data for this vector; and a summary of protocol changes going forward.

Toca 511 is a retroviral replicating vector that is based on a murine leukemia virus. The ecotropic envelope has been changed to an amphotropic envelope to allow infection of human cells and the cytosine deaminated gene has been inserted between the envelope and the 3' Long Terminal Repeat (LTR). This gene catalyzes the conversion of the antifungal drug 5-FC to the cytotoxic drug 5-FU, and an internal ribosome entry sequence facilitates transgene expression.

The two studies currently in progress are "the Intratumoral Study" (protocol 0904-976) and "the Surgical Resection Study" (1107-1120). The indication for both studies is recurrent high-grade glioma, and both studies use the vector Toca 511. In the Intratumoral Study, the vector is injected stereotactically transcranially through a small burr hole into the tumor; in the Surgical Resection Study, participants who wanted to have a repeat craniotomy have the vector injected into the walls of the resection cavity at the time of tumor removal. Doses studied are essentially the same, and both studies use the same protocol design. Both studies use the same Prodrug (5-FC); there are some subtle differences in the cycle times for 5-FC administration, but both studies have the same main objective, which is to study safety and tolerability and to identify the MTD. Participants in both studies are referred into the long-term follow-up study to assess safety.

The Intratumoral Study has enrolled 24 research participants to date and is currently enrolling in the top dosing cohort. The Surgical Resection Study has enrolled 19 participants to date, with dosing in the top cohort expected to begin in early April 2013. The adverse event that defined the dose-limiting toxicity (DLT) occurred in the  $1 \times 10^5$  dose cohort in the Surgical Resection Study, and the investigators studied three additional participants in that cohort without identifying a subsequent DLT. They also have studied six participants in the next cohort without encountering a DLT. To date, no DLTs have occurred in the Intratumoral Study, and the Surgical Resection Study has seen only this one event.

The FDA and the RAC approved this protocol in September 2011. The IND Safety Report occurred in August 2012; the FDA placed this clinical trial on hold on September 28, 2012, and removed it from hold on November 20, 2012.

Dr. Pertschuk provided a timeline of the SAE that occurred in the Surgical Resection Study. On July 16, 2012, the affected participant received Toca 511; PCR and RT-PCR (reverse transcription PCR) were both negative at that time. Approximately 18 days later, he developed cough and fever, followed by atelectasis, at which time he had 193,000 copies of virus/mL in plasma. Later, right lung opacity developed and he was hospitalized for weakness. Pulmonary embolism was diagnosed, at which time the virus had fallen to below the level of quantitation without specific therapy, and the participant was discharged on hospital day 4.

Dr. Pertschuk offered the investigators' current interpretation of the transient viremias. Following injection, the tests do not detect virus, so the RNA lag with a subsequent peak suggests the virus has infected a population of cells, is being actively produced, and is entering the bloodstream, where it is detected. Infected tumor cells are the likely source of the viremia, as the investigators have not seen any toxicities, including hematologic or gastrointestinal toxicities. If this interpretation is accurate, then the viremia could be a marker for productive tumor infection and the viremia is controlled by the immune system without specific therapy.

In summary, Toca 511 5-FC has been administered to more than 40 participants in two studies. It has been shown to be safe and well tolerated, especially for an anticancer drug. There has been one DLT, which was transient Grade 3 weakness in a research participant who had intercurrent medical problems of pulmonary embolism and possible upper respiratory infection. The viremia was self-limiting and in this case was associated with flu-like symptoms, but other participants who have been viremia have been asymptomatic. Viremia appears to be associated with the use of higher postoperative doses of corticosteroids. This vector appears to have limited shedding potential, with only one participant having RNA detected in the saliva or urine, and the shedding does not appear independently from viremia.

In agreement with the FDA, the investigators have added ad hoc viral testing in participants who are symptomatic or who have high-grade viremia. The testing is intended to help better define the time courses of viremia, the relationship to symptoms, and the relationship to shedding. The investigators have agreed to study the effect of corticosteroids in a systematic fashion by studying three research participants with and three without higher-dose steroids at each remaining vector dose.

## **B. RAC Discussion**

Dr. Cannon asked whether the investigators have looked at the integrity of the virus in the participants who have exhibited viremia, and in particular at whether the CD transgene is being retained. Dr. Jolly responded that experiments are in process but data is not yet available. Dr. Pertschuk elaborated that the investigators were able to obtain virus from a number of brain tumor specimens during reoperation or autopsy and after participants had received one or more courses of 5-FC; those specimens contained the CD gene.

Dr. Hammar skjöld asked the investigators whether they had conducted any studies of the immune status or T-cell responses in the research participant who had the high level of viremia, and she wondered whether all of the participants had been treated with high levels of corticosteroids. Dr. Jolly explained that these research participants have received temozolomide, and all temozolomide-treated individuals have reduced white blood cell counts. Dr. Pertschuk added that the participants with high levels of viremia all had some degree of immunosuppression from previous radiotherapy and temozolomide and they had all failed the original standard treatment (an inclusion criterion). There is a minimum absolute lymphocyte count to get into the study, but the high-viremia research participants do not appear to be any different than the other participants—immunologically, by their absolute lymphocyte counts, or by their CD4 or CD8 counts—apart from the fact that they all received higher-dose postoperative steroids. It is difficult to conclude that the high level of viremia is an immune-mediated event. Even in the participant who had a very high level of viremia, the DNA copy number was relatively low. The RNA peak always precedes the DNA peak, so it is unlikely that what is seen in the blood is a consequence of white-cell replication.

Dr. Jolly explained that, as the animal studies showed, the investigators expected the tumor cells to be infected once and that viral manufacture would occur in those cells and spread to further tumor cells, potentially also spreading to passing blood cells.

Dr. Kohn pointed out a recent paper reporting that the transient inflammatory response induced by lentiviral vector delivery could be blocked by dexamethasone resulting in increased liver transduction in animals. Dr. Jolly said that he and his colleagues were intrigued by this paper and wondered whether it was reporting a direct effect on the innate immune system.

Dr. Fong asked how many research participants who were viremic had received 5-FC after the viremia and whether the circulating level of 5-FU or toxicities change according to level of viremia. Dr. Pertschuk responded that all of these individuals had received at least one cycle of 5-FC; there have not been any toxicities, the participants are generally asymptomatic, and the 5-FC has been well tolerated. The investigators in this trial do not measure circulating 5-FU, because of its short half-life and because it is difficult to measure in the clinic. The investigators have measured 5-FU in brain tumors from animals, and they are trying to devise experiments that would provide neuro-pharmacokinetic data on 5-FC and 5-FU in those brain tumors.

In response to Dr. Zoloth's query, Dr. Pertschuk clarified that the only adverse event experience has been this one viremic episode in the one research participant in the Surgical Resection Study; he had an unusually high level of viremia and developed asthenia that required hospitalization. No other adverse events have occurred, and most of the participants with viremia have been asymptomatic. This one research participant had flu-like symptoms with low-grade fever, but the doctors taking care of him were prescribing antibiotics because they thought he had a concurrent respiratory infection as well as a pulmonary embolism.

### C. Public Comment

No public comments were offered.

## IX. Biosafety Considerations for Research Involving the Full-Length cDNA Cloning of Single-Strand, Non-Segmented, Positive Sense Risk Group 4 RNA Viruses (e.g., Flaviviruses)

### A. Reverse Genetics of Tick-Borne Flaviviruses: Glycoprotein Contributions to Pathogenesis

Presenter: Bevan Sawatsky, Ph.D., University of Texas Medical Branch

Dr. Sawatsky presented an overview of tick-borne flaviviruses and the contributions of the glycoproteins to pathogenesis. Flaviviruses can be categorized as either mosquito-borne viruses, including yellow fever, dengue virus, and Japanese encephalitis, or tick-borne viruses, tick-borne encephalitis, Omsk hemorrhagic fever, Kyasanur forest disease virus, and Alkhurma hemorrhagic fever virus. The four tick-borne viruses are classified as Risk Group (RG) 4 agents in the United States.

Dr. Sawatsky reviewed the structure of flaviviruses, which are non-segmented positive-sense single-stranded RNA viruses. The genome consists of one large open reading frame that encodes a polyprotein of between 3,000 and 3,500 amino acids, depending on the virus. This genome is recognized by ribosomes and initiation factors when it enters the cytoplasm. Because of this, the RNA genome can be translated without further processing by viral proteins. The purified flavivirus RNA genome needs to be handled with care as it is considered to be potentially infectious.

The life cycle of the virus begins when the virus attaches to a host cell receptor. Once inside the cell, the virus is released from the capsid and translated by ribosomes in the cytoplasm. The polyprotein that is produced is cleaved by various proteases. The genome generates a full-length negative-sense anti-genome intermediate that is used as a template for synthesis of new positive-strand genomes; the new genomes are encapsidated by the C protein and meet up with the glycoproteins at the endoplasmic reticulum membrane during assembly. Ultimately, mature virions are released from the plasma membrane.

Dr. Sawatsky discussed some of the practical considerations for working with flavivirus genetic material, particularly genomic RNA. The approved method for removing genomic RNA from BL4 containment is to use Trizol; his protocol uses a fourfold excess of Trizol plus surface decontamination by Micro-Chem. After removal to the BL2 laboratory, the extraction is performed essentially by phenol/chloroform separation and then precipitated by isopropanol and washed in 70 percent ethanol, and then this extracted genomic RNA is resuspended in water. To generate materials that can be used in a BL2 laboratory, Dr. Sawatsky and colleagues do reverse transcription to generate cDNA. After the cDNA reaction is run, the genomic RNA is destroyed and the remaining reaction mixture contains only cDNA. To clone flavivirus genomes, full lengths are broken into four pieces, and all assembly or re-assembly is performed in the full-length cloning room. Any genomic fragments can be used in the BL2 laboratory.

Reverse genetics for flaviviruses requires generation of genomic RNA, using plasmids with the T7 promoter. No other supporting viral plasmids are necessary. Providing T7 polymerase results in the generation of viral genomic RNA, which can also be translated by ribosomes to generate the viral proteins. He explained the specifics of his transfection protocol, noting that the procedure he and his colleagues will follow will maintain complete separation of the cells and the transfection mix in the BL2 lab.

Dr. Sawatsky and colleagues are interested in using this system because they want to look at the encephalitic disease manifestations typically caused by flaviviruses. Viruses like tick-borne encephalitis, Japanese encephalitis, West Nile, and St. Louis encephalitis typically cause neurological disease, whereas viruses like dengue, Omsk hemorrhagic fever, and Kyasanur forest disease tend to cause mostly hemorrhagic manifestations. Dr. Sawatsky and colleagues want to construct recombinant viruses and then investigate the effect of these proteins on disease outcome as well as virus replication and other

*in vitro* characteristics. In addition, the investigators want to determine why flavivirus glycoproteins have so few N-glycans compared with other viruses. These N-glycans are at the same location in mosquito- and tick-borne viruses and do not appear to be important for structure or stability; therefore, the investigators want to construct recombinant viruses that express glycoproteins that are hyperglycosylated to see how these glycoproteins affect virus replication and disease.

Regarding safety considerations, Dr. Sawatsky explained that the only materials used in cloning and recovery of recombinant genomes are pipette tips and other plasticware, so no sharps will be involved in this research. Biological safety cabinets will be decontaminated with a multipurpose disinfectant such as CaviCide in a manner similar to what is done in the BL4 laboratory.

Personnel for this research will be Dr. Sawatsky as the primary researcher, along with Dennis A. Bente, D.V.M., Ph.D., University of Texas Medical Branch. Any other personnel will be trained appropriately and approved for this type of work.

### **B. Ad Hoc Comments**

Commenter: Stuart Nichol, Ph.D., Centers for Disease Control and Prevention

From the working group discussion, Dr. Nichol noted that the recommendation would be that the RNA itself be considered potentially infectious. Because these are RG4 agents, the full-length RNA should be handled only in the BL4 laboratory. Second, Dr. Nichol asked whether the full-length clone room is separated from the main BL2 laboratory by restricted access; Dr. Sawatsky responded that a card reader is on the door to restrict access to approved personnel only.

### **C. Recommendations from the Biosafety Working Group**

Presenter: Dr. Cannon

Dr. Cannon stated that the Biosafety Working Group (BWG) met via teleconference on February 25, 2013, to address the biosafety and biosecurity issues regarding cloning cDNAs from Risk Group 4 flaviviruses. She presented a description of the current guidelines on biosafety conditions for cloning Risk Group 4 viruses in nonpathogenic bacteria under the *NIH Guidelines* and an explanation of the OBA guidance for cloning cDNAs from Risk Group 4 negative-strand viruses. Risk Group 4 flaviviruses are positive-strand RNA viruses, which means the RNA inside the virus—the genomic RNA—is similar to messenger RNA. The RNA itself is potentially infectious, and if it is introduced into a cell, it can generate replication-competent viruses.

In the *NIH Guidelines*, cloning full-length cDNAs of Risk Group 4 RNA viruses falls under Section III-D-2-a, which states, in part, “Experiments in which DNA from Risk Group 4 agents is transferred into nonpathogenic prokaryotes or lower eukaryotes may be performed under BL2 containment after demonstration that only a totally and irreversibly defective fraction of the agent’s genome is present in a given recombinant.” In the absence of such a demonstration, BL4 containment is the default; anything considered infectious must be worked with under BL4.

In September 2009, after consultation with the RAC, the OBA issued a set of recommendations allowing for cloning of full-length cDNA genomes from the negative-strand viruses—such as Ebola—and the biosafety level was reduced from BL4 to BL2. In light of this guidance, a request was made to lower the biosafety containment level for cloning of full-length cDNAs of the risk group for positive-strand viruses such as flaviviruses. The OBA was asked to make a determination as to whether the same practices that have been described for working with the negative-strand viruses could be applied to the risk group for flaviviruses.

Dr. Cannon summarized the considerations: Unlike the negative-sense viruses, the positive-sense, Risk Group 4, RNA genomes alone can produce infectious virus; they hijack cellular machinery and do not need any other viral proteins to produce infectious virus. Introduction of the RNA genome into tissue culture cells, directly into animals via inoculation, or into laboratory workers by accidental self-inoculation could result in viral replication. One of the discussion points in the BWG’s deliberation was that a full-

length cDNA in a bacterial plasmid, even without an intended mammalian cell promoter, could be considered to have a risk of generating an appropriate infectious RNA genome.

The BWG first considered whether the "Points to Consider" document that had been generated for cloning with negative-strand RNA viruses had appropriate biosafety recommendations that would also apply to the risk group for flaviviruses. The BWG concluded that the same security concerns applied in both cases but that the biosafety concerns were considered of a higher level because of the infectious nature of the RNA from flaviviruses.

The "Points to Consider" document states that research should be conducted in a dedicated BL2 laboratory with physical and procedural measures to limit access; inventory flow of materials and waste should be controlled; separation of full-length cDNAs and any rescue plasmids, which are needed for the negative-strand viruses, should be maintained; and all personnel should have adequate training. These guidelines were deemed necessary for flavivirus cDNA, but the BWG also recommended that a written biosecurity plan be developed using the approach outlined in the *Biosafety in Microbiological and Biomedical Laboratories (BMBL) 5th Edition*, that all personnel with access to the full-length cDNAs have at least a Public Trust Level 5 security clearance, that an official at the institution be appointed for oversight, that there be periodic re-evaluation of procedures, and that an annual report to the IBC be copied to the OBA. In addition, the BWG deemed it prudent to recommend enhanced BL2 practices specifically for flaviviruses, with the main point being that work should be performed in a dedicated, strictly prokaryotic cloning laboratory, defined as having no mammalian cell culture or tissue culture work conducted in that laboratory. The typical BL2 lab, where a variety of research would be going on, would not be appropriate. The intent of this recommendation is to prevent expression plasmids from being introduced into a mammalian cell where they could generate infectious virus. For the same reason, no *in vitro* transcription work should occur in that lab, including T7 polymerase-based transcription.

In addition, the full-length cDNA clones should be secured appropriately, and no supporting expression plasmids, including a T7 polymerase expression plasmid, should be stored in the dedicated cloning laboratory. Regarding the enhanced BL2 practices, the use of sharps and glassware should be avoided, with plastic alternatives recommended. Personal protective equipment must include adequate mucosal membrane protection, the lab must have suite-dedicated or disposable lab coats, and the gloves should be chosen to resist chemicals commonly used in the cloning procedure.

#### **D. RAC Discussion**

Dr. Cannon summarized the process as the investigators working initially in a BL2 lab, mixing together the transfection reagents with the T7 polymerase expression plasmid, taking that mixture into the full-length cloning lab where the genomic plasmid is added, and then taking that mixture into the BL4 lab. She noted that this procedure seemed like an unnecessary detour through the full-length cloning lab. Dr. Cannon recommended instead that the investigators take the transfection T7 polymerase mixture directly into the BL4 lab and then independently bring a full-length clone into the BL4 lab, conducting the mixing in the BL4 lab. Her concern was introducing T7 polymerase expression plasmids into the full-length cloning lab, which she deemed an unnecessary extra step and a risk. Dr. Sawatsky agreed that the investigators could use Dr. Cannon's suggested procedure.

Dr. Sarzotti-Kelsoe asked the investigators how they can be certain that all of the RNA is destroyed at the end of the cDNA reaction. Dr. Sawatsky admitted that the investigators do not currently have a test to demonstrate that all the RNA is destroyed, but they would be willing to develop such a test if deemed necessary.

Dr. Zoloth asked for justification as to why these two investigators are asking to work in an enhanced BL2 lab with agents that have previously been designated as appropriate for BL4 containment. Dr. Cannon summarized the BWG discussions: requiring lab staff to work in a BL4 lab, an environment with biohazards, is onerous and a risk, especially if working in such an environment is not necessary for doing relatively routine cloning. Unless they are inserted into a mammalian cell, these cDNAs are not infectious. Dr. Corrigan-Curay explained that the research with cDNA may be conducted at BL2 containment.

However, the RNA must remain at BL4.

Dr. Wooley added several suggestions to the BWG recommendations already presented by Dr. Cannon. She recommended that full-length, wild-type RNA not be brought in at the beginning and that no cells be housed in the special BL2 room. Minimum personal protective equipment should be specified as ANSI Z87-approved eyewear, lab coat, and gloves, and face shields could be worn depending on the procedure. In addition, a laboratory-specific biosafety manual should be prepared, adopted, and available and accessible. Dr. Sawatsky said that he has dedicated procedures for those rooms but not necessarily a dedicated manual. Dr. Wooley reiterated the importance of having a manual specifically for the suite in which the proposed cloning would be conducted, and the manual, which should be readily accessible in the room, should include standard operating procedures for how to handle the materials and protocols to be carried out in that suite. Dr. Jambou explained that the OBA received a large binder that had specifics of what research is planned for that BL2 lab, plus a manual of BL2 procedures at the investigators' institution that detailed the specifics for the suite. Dr. Hammar skjöld added that the requirement for a specific manual is similar to what is currently done with HIV in BL2 enhanced labs, in that a specific manual is created and everyone entering that space needs to sign off as having read it.

Dr. Sawatsky reiterated that the research he is proposing would only manipulate subgenomic segments, not whole genomes; therefore, infectious virus cannot be reconstituted. In light of the need to keep RNA in purified form at BL4, he asked the RAC what procedures would be necessary to bring cDNA into the BL2 lab in order to do the cloning manipulations, which is not typically done inside BL4 labs. Dr. Cannon responded by restating that the RNA alone is infectious. cDNAs that represent partial genomes may be worked with in BL2 containment. When they are recombined into a full-length genomic cDNA, the guidelines suggest that that work would have to be conducted in a prokaryotic lab with no capability of *in vitro* or *in vivo* transcription and no possibility of an RNA being generated. Under the current guidelines, the RNA cannot be taken out of BL4 containment. Generating cDNAs and reagents from that RNA is allowed, but taking the RNA out of the BL4 is not allowed because it is infectious. Dr. Sawatsky agreed that the recommendations are clear that full-length RNA cannot be brought out of BL4 containment but that pieces of the RNA would be allowed; cDNA would be generated in BL4 conditions, and then pieces would be brought out to the BL2 enhanced lab.

Dr. Corrigan-Curay summarized the BWG recommendations to the RAC. The BWG recommended that all of the requirements that were put in place for Ebola virus, including the standard guidance of a dedicated BL2 laboratory, security, inventory, disposal of waste, a biosecurity plan, an officer who oversees this work, and an annual report. For flavivirus, Dr. Cannon summarized the additional recommendations that recognize the infectious nature of a full genomic RNA, which could be produced inadvertently if a cDNA got into a mammalian host. All work involving the full-length cDNA is to occur in a room that has only prokaryotic capabilities, and no mammalian tissue culture may be used or stored in that room. In addition, no reagents or systems could be used to produce an RNA either *in vitro* or *in vivo*, including, but not limited to, the T7 polymerase system. Researchers must develop a dedicated laboratory standard operating procedure that discusses how the material that comes into the lab is generated and what material can be worked with in that lab, and the procedure must recognize that the RNA version of these Risk Group 4 flaviviruses is a Select Agent and is only appropriately handled at BL4 containment. The minimum personal protective equipment should be specified, and no glass or sharps of any kind should be used. Demonstration of the absence of remaining RNA must also be performed.

Dr. Nichol noted that these recommendations are valuable for researchers in general and will assist researchers in getting work that is very low risk out of the high-risk environment of a BL4 laboratory.

#### **E. Committee Motion 4**

Dr. Fong requested a vote on these recommendations. The RAC approved the BWG recommendations, with the discussed additions, by a vote of 13 in favor, 0 opposed, and 0 abstentions.

**X. Closing Remarks and Adjournment**

Dr. Fong thanked the RAC members and the OBA staff and adjourned the March 2013 RAC meeting at 3:05 p.m. on March 12, 2013.

*(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.)*

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

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

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

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

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

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

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**Chair**

**FONG**, Yuman, M.D.  
Murray F. Brennan Chair in Surgery  
Memorial Sloan-Kettering Cancer Center  
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**FOST**, Norman, M.D., M.P.H.  
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**Members**

**BADLEY**, Andrew D., M.D., FRCP(C), FACP, FIDSA  
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Associate Director of Research Resources,  
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Professor of Medicine, Division of Infectious  
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**HAMMARSKJÖLD**, Marie-Louise, M.D., Ph.D.  
Professor of Microbiology  
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**Attachment II:  
Public Attendees**

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Kelly R. Clark, Nationwide Children's Hospital  
Ana Cotrim, NIH  
Aimee Murphy, Aduro BioTech, Inc.  
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Joachim Trobaugh, Aduro BioTech, Inc.  
Walter Urba, Providence Cancer Center

### Attachment III: Abbreviations and Acronyms

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AAV	adeno-associated virus
AE	adverse event
BSL	biosafety level
BWG	Biosafety Working Group
CFU	colony-forming units
CK	creatine kinase
CTLs	cytotoxic T lymphocytes
DLT	dose-limiting toxicity
DMD	Duchenne muscular dystrophy
EGFR	epidermal growth factor receptor
FDA	Food and Drug Administration, U.S. Department of Health and Human Services
GBM	glioblastoma multiforme
GTSAB	Gene Transfer Safety Assessment Board
HCC	hepatocellular carcinoma
IBC	Institutional Biosafety Committee
ILP/ILI	isolated limb perfusion and isolated limb infusion
ILR-GT	isolated limb recirculation for gene transfer
IOM	Institute of Medicine
IV	intravenous
LGMD2D	limb girdle muscular dystrophy type 2D
<i>Lm</i>	<i>Listeria monocytogenes</i>
MRI	magnetic resonance imaging
MTD	maximum tolerated dose
Nab	neutralizing antibodies
NIH	National Institutes of Health
<i>NIH Guidelines</i>	<i>NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules</i>
NK	natural killer
OBA	Office of Biotechnology Activities, NIH
OD	Office of the Director, NIH
PI	principal investigator
PCR	quantitative polymerase chain reaction
rAAV	recombinant adeno-associated virus
RAC	Recombinant DNA Advisory Committee
SAE	serious adverse event
SC	self-complementary