RECOMBINANT DNA ADVISORY COMMITTEE

Minutes of Meeting

June 11–12, 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[1]

June 11–12, 2013

The Recombinant DNA Advisory Committee (RAC) convened for its 133rd meeting at 8:00 a.m. on June 11, 2013, at the National Institutes of Health (NIH), Building 31-C, Room 6, in Bethesda, Maryland. Dr. Yuman Fong (RAC Chair) presided. In accordance with Public Law 92-463, the meeting was open to the public from 8:00 a.m. until 5:15 p.m. on June 11, 2013, and from 8:00 a.m. until 3:15 p.m. on June 12, 2013. The following individuals were present for all or part of the June 2013 RAC meeting.

Committee Members

Andrew D. Badley, Mayo Clinic and Foundation
Tianxi Cai, Harvard University
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
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
Marcella Sarzotti-Kelsoe, Duke University School of Medicine
Marshall Strome, St. Luke's–Roosevelt Hospital Center/New York Head and Neck Institute
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/Reviewers/Speakers

Timothy Cripe, Nationwide Children's Hospital *(via teleconference)*
Christoph Klein, University of Munich Medical Center *(via teleconference)*
Paola Leone, University of Medicine and Dentistry of New Jersey
Willis Maddrey, The University of Texas Southwestern Medical Center at Dallas

Nonvoting Agency Representatives

Kristina Borror, Office for Human Research Protections, U.S. Department of Health and Human Services (HHS) *(Day 2 only)*
Denise Gavin, U.S. Food and Drug Administration (FDA)

NIH/OD/OBA Staff Members

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

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

Attendees

There were 94 attendees at this 2-day RAC meeting.

Attachments

Attachment I contains lists of RAC members, ad hoc presenters 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, the RAC Chair, called the meeting to order at 8:00 a.m. on June 11, 2013. Notice of this meeting under the *NIH Guidelines for Research Involving Recombinant DNA Molecules* was published in the *Federal Register* on May 20, 2013 (78 FR 29372). 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 seven gene transfer protocols, updates of two protocols previously reviewed by the RAC, and an update on stem cell gene transfer for the Wiskott Aldrich Syndrome (WAS).

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. Minutes of the March 12, 2013, RAC Meeting

RAC Reviewers: Dr. Hammarskjöld and Ms. Mallino

Ms. Mallino stated that the March 2013 meeting minutes document represented a fair reflection of the meeting.

A. Committee Motion 1

Dr. Fong declared the March 2013 RAC meeting minutes document approved, without objections.

III. Review and Discussion of Human Gene Transfer Protocol #1304-1230 titled: A Phase I Ascending Dose Trial of the Safety and Tolerability of Toca 511, a Retroviral Replicating Vector, Administered Intravenously to Subjects Undergoing Subsequent Resection for Recurrent High Grade Glioma and Followed by Treatment with Toca FC, Extended-Release 5-FC

Principal Investigator:	Steven N. Kalkanis, M.D., Henry Ford Hospital *(via teleconference)*
Additional Presenters:	Doug Jolly, Ph.D., Tocagen, Inc.; Amanda Omlor, M.S., RAC, Tocagen, Inc.; Daniel Pertshcuk, M.D., Tocagen, Inc.
Sponsor:	Tocagen, Inc.

RAC Reviewers: Drs. Cannon, Kiem, and Wooley

A. Protocol Summary

High-grade glioma (HGG) is the most common form of malignant brain tumor in adults, accounting for 50 to 60 percent of primary brain tumors. Despite major improvements in health care during the past decade, the average lifespan for patients diagnosed with glioblastoma multiforme, the most common of the HGGs, is still only 12 to 15 months. Hence, there remains an unmet need to develop effective new approaches against this devastating disease. Gene delivery is one such approach; however, clinical trials of gene-delivery products that involve the use of conventional, replication-defective vectors or cell-killing viral vectors have resulted in disappointingly low and therapeutically inadequate infection and reduction of brain tumor cells.

Tocagen, Inc., is proposing a third Phase I clinical study with its replicating gene delivery viral vector (Toca 511) that will consist of a mouse retrovirus in which the original ecotropic envelope gene has been replaced with an amphotropic envelope gene enabling the virus (referred to as ampho-MLV) to better infect human tumor cells. The vector expresses in the cytosine deaminase (CD) gene. The CD enzyme naturally occurs in yeast, fungus, and some bacteria but not in humans or animals and functions biologically by converting the already approved and widely used antifungal drug flucytosine (5-FC) to the 5-fluorouracil (5-FU), which is an approved and widely used anticancer drug. Based on studies obtained in mice, this viral vector infects dividing tumor cells, which then act as a reservoir for the virus to multiply and spread to additional brain cancer cells. When the prodrug 5-FC is administered orally, most of the drug passes the blood-brain barrier, and the virus-infected brain tumor cells containing the CD enzyme convert 5-FC to the cell-killing drug 5-FU. In this manner, the brain tumor cells are preferentially targeted for destruction.

The viral vector containing the CD gene will be administered only for a short time intravenously at the start of the study; it then will be administered at one time directly into the walls of the remaining cavity after the brain tumor is removed surgically. However, multiple rounds of the oral prodrug 5-FC will be given over time (1) to allow any remaining active virus to infect whatever brain tumor cells remain after the previous prodrug treatment and (2) to maximize killing of all infected brain tumor cells.

Study participants will be patients with recurrent HGG who previously have received surgery, radiation therapy, and chemotherapy. Neither radiation nor chemotherapy will be allowed during this study. Up to five ascending doses of Toca 511 will be evaluated. Participants will wait for approximately 4 weeks while the vector spreads throughout the tumor, after which they will receive daily oral administration of 5-FC for 10 days. This prodrug administration cycle (approximately five weeks off and 1.5 weeks on) will be repeated until the participant's tumor progresses or intolerance to the prodrug develops. Participants will undergo magnetic resonance imaging (MRI) scanning approximately every 10 weeks after dosing with 5-FC.

Safety assessments will include checking 5-FC blood levels; monitoring blood, saliva, and urine for virus; conducting clinical chemistries and hematology at selected time points; and recording adverse events. Efficacy assessments will include tumor response, progression-free survival at six months, and overall survival. All participants will be followed for 32 weeks in this study. Research participants who appear to be benefiting from this experimental treatment may elect to roll into a continuation protocol that would allow them to continue dosing with 5-FC.

B. Written Reviews by RAC Members

Nine RAC members voted for in-depth review and public discussion of this protocol. Key issues included that since intracranial administration had resulted in significant and symptomatic viremia, the risks and benefits of intravenous (IV) administration of a replication-competent retrovirus were deemed to deserve further discussion.

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

Dr. Cannon noted that her major concern following IV injection is the risk of virus replication occurring in noncancer tissues and the possible development of a secondary cancer, such as a lymphoma, even though the risk is mitigated because the treatment with 5-FC is expected to be antiretroviral. The different distribution patterns observed following IV versus intratumoral injection in animal models make it unclear whether such a strategy will result in sufficient antitumor efficacy to justify the risk. Dr. Cannon asked the investigators to discuss what is known about the stability of Toca 511 in humans, especially whether it loses susceptibility to 5-FC during treatment. She also asked, if loss of a functional CD gene were to occur, how this finding would shift the perception of the level of risk from episodes of retroviral replication occurring outside the target tumor tissue. With regard to the informed consent document, Dr. Cannon suggested three changes for clarity and accuracy. In particular, she expressed concern about the accuracy of the statement that Toca 511 had been administered to several animal models without unusually severe side effects, given the history of lymphomas that developed in the BALB/c mouse strains.

Because of the use of a replication-competent retrovirus, Dr. Kiem asked the investigators whether integration could occur in other proliferative cells (e.g., hematopoietic cells) or other tissues and what assays would be used to look for integration in which tissues. He requested that the investigators justify the increased risk of potential side effects from administering the virus intravenously. He also asked whether virus-integration site studies had been conducted in mice. Noting that the research participants will likely have received carmustine or temozolomide thus could have pre-existing blood cell abnormalities, he suggested the importance of conducting careful analysis of retroviral integration sites in blood cells at the study's end (or when participants die). Dr. Kiem asked the investigators to discuss what will happen to participants with viremia and tumor progression, because treatment of the tumor could result in enhanced side effects and the efficacy of the antiretroviral product is unknown in this setting. He requested that the investigators comment on the use of steroids in this study after research participants have been injected with the virus. He also asked whether family members of any participants with viremia would be screened. With regard to the informed consent document, Dr. Kiem suggested one change for increased accuracy. He also asked for clarification on two points: (1) who would pay for costs resulting from side effects of this experimental treatment and (2) what would happen to participants with viremia and progressive disease with regard to their treatment options.

Dr. Wooley expressed her overall concern about this proposed gene transfer study as stemming from detection of virus in peripheral blood following intracranial administration in previous protocols. Proviral DNA was detected in three participants two months after injection in one study, and a serious adverse event (SAE) in a second study involved fever and malaise about four weeks after dosing that was revealed to result from a pulmonary embolism and high viral load in the blood. Dr. Wooley asked the investigators to clarify the highest dose being proposed and to highlight the reasons why they believe IV administration is preferable to intracranial administration. She suggested that the investigators consider a brain shunt for Toca 511 delivery rather than intratumoral or IV injection. She explained that a brain shunt would allow for more widespread delivery of the product to the brain while providing more protection of the peripheral system than would IV administration; a shunt also would allow relief of intracranial pressure if needed. She highlighted several statements in the investigators' Appendix M submission that she believed should be removed or modified for maximal accuracy. Dr. Wooley noted that a plan of action suggested by the RAC in a September 27, 2011, letter in response to Protocol #1107-1120 did not appear to have been considered in this current protocol; she suggested that an alternative antiretroviral therapy should be tested *in vitro* as an effective treatment against Toca 511 if necessary for a research participant or close contact. In addition, Dr. Wooley asked the investigators to provide a brief summary and further explanation about which animal models were studied for IV delivery along with the resulting safety data and biodistribution data for the IV delivery method.

C. RAC Discussion

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

- Dr. Fong asked about long-term reactivation under steroid conditions—whether the investigators have a mechanism for measuring reactivation titers in terminal patients at the end of their lives. He also asked whether the investigators had performed any total body autopsies.
- Agreeing that treating the periphery with IV medication could potentially have a major effect at the core of the tumor, where the blood supply is relatively limited, Dr. Strome asked the investigators whether they have any studies of simultaneous IV and intratumoral injection. He suggested that the investigators add another arm to this study to accommodate this research question.

D. Investigator Response

1. Written Responses to RAC Reviews

In this proposed study, IV administration of Toca 511 will be used as a complementary/supplementary method of vector delivery in conjunction with direct injection of Toca 511 into the tumor bed following resection to maintain the potential benefit of local delivery. The intent of this approach is to evaluate whether Toca 511 given intravenously penetrates the tumor. Immunohistochemical analysis of tumor tissue obtained in mouse glioma studies showed different distribution patterns of Toca 511 in the tumor when given IV versus intracranial-intratumoral (IC-IT). IV delivery results in higher infection of the tumor periphery that may complement the focal and central areas of infection seen with IC-IT delivery. Determining which type of vector coverage of the tumor will be more advantageous in terms of antitumor activity is difficult because each method of delivery alone resulted in increased survival in a mouse syngeneic glioma model.

The architecture of human glioma tumors is complex and heterogeneous. The benefit of IV administration, if effective, could be additive: The different pattern of virus uptake in the tumor by IV administration could be complementary to intratumoral administration. IV delivery might more effectively infect the infiltrating tumor cells in the hypervascular regions of the tumor, leading to more extensive destruction of such infiltrates; and IV administration allows straightforward re-dosing, either on consecutive days to increase the vector uptake or eventually to re-treat patients without subjecting them to further invasive surgery. Combining IV and IC-IT vector delivery strategies should increase the likelihood of vector coverage of multiple areas of the central tumor and tumor periphery.

Toxicity in humans may be unlikely because studies in non-tumor-bearing, virally permissive BALB/c mice showed no difference in the ultimate pattern of tissue distribution of viral vector DNA between IC and IV vector administration. In both modes of administration, vector DNA detection in blood provided an excellent correlative sentinel for the presence of vector elsewhere in the animals. The vector doses evaluated in these mouse models are well above (more than 30-fold) the proposed IV starting dose in the proposed human clinical study. In all the preclinical IV studies, no unexpected or previously unreported toxicities were observed.

The investigators believe that the viral RNA spikes seen in some research participants are unlikely to have come from infected peripheral blood mononuclear cells (PBMCs) as the detection of viral RNA preceded the detection of DNA in the blood. Tumor tissue remains the most likely source for the virus; tissues such as bone marrow or the gastrointestinal (GI) tract are unlikely sources of the viremia because the investigators have not seen any bone marrow or GI toxicity following treatment with 5-FC.

The investigators have proposed considering the use of antiretrovirals that are active against murine leukemia virus (MLV) and approved for use in HIV in the event of persistent viremia. In a prior study, the viral RNA peak in all six research participants with a measurable RT-PCR signal rapidly declined to below the level of quantitation over a period of two to four weeks. In parallel, an antibody response to the virus appeared. This information, in combination with the early published data, suggest that although deletion of the CD gene could occur theoretically, this occurrence would not significantly change the risk assessment for the appearance or clearance of virus in the blood. The investigators reported that through testing, they detected the presence of the CD gene and gene product in resected tumors from research participants after Toca 511 and 5-FC administration. Furthermore, qPCR for DNA in resected tumors from

six research participants after Toca 511 treatment and 5-FC dosing showed that, in the same samples, the copy number for the CD gene and the viral genome are indistinguishable.

Regarding the possible integration of retroviral vector into other proliferative cell types, preclinical studies in MLV-permissive BALB/c mice suggested that detection of DNA in blood was an excellent sentinel tissue assay to monitor the global presence or absence of virus in any other tissues of an animal. MLV has a known distribution pattern to lymphoid-containing tissues, which was verified in the mouse biodistribution studies performed with Toca 511. The investigators agreed to continue attempting to obtain autopsy material from research participants who have received Toca 511; however, thus far, the investigators only have been able to obtain brain and brain tumor tissue. To date, they have seen quantifiable vector RNA in blood from six participants. The investigators have committed to examining integration patterns in DNA samples in blood, but, thus far, no individual's testing has met the criteria requiring such assays. They also have committed to examining any secondary tumors occurring in any research participant after administration of Toca 511, but this too has not occurred. The investigators believe that the most important markers of potential lymphoma in research participants would be lymphocyte counts and overall viral levels in the blood.

The potential clinical advantages for IV administration are (1) the different, potentially complementary tumor distribution pattern of virus uptake seen in mice with intracranial tumors after IV administration; (2) the possibility that IV delivery will more effectively reach the infiltrating peripheral edges of the tumor; and (3) the potential for a relatively noninvasive mode of administration and readministration. Although changing the route of administration could change the risk profile of Toca 511 and mouse studies only accurately predict what will happen in mice, the investigators believe that the risk/benefit ratio is still acceptable, given (1) the grim prognoses of recurrent HGG patients, (2) the fact that all research participants dosed to date have been able to control the virus, (3) the possibility of benefit, and (4) the assessment of risk.

In terms of the overall risk, the investigators reported that they have administered the virus intravenously to BALB/c mice (non-tumor bearing), B6C3F1 mice (tumor and non-tumor bearing), and laboratory dogs (non-tumor bearing). The pattern of virus uptake in non-tumor-bearing animals appears to be ultimately the same whether the virus is delivered intracranially or intravenously; the only difference appears to be the speed at which signal appears in the blood. The only animals that had high levels of viral infection in the tissues (and only in some animals) were the permissive BALB/c mouse strain, which was selected as the "worst-case scenario" for toxicology and biodistribution studies. The investigations have not performed integration site analyses in infected mice.

Participants will not be prescreened for potential antibody inactivation of Toca 511. Tumor-bearing B6C3F1 mice with specifically induced pre-existing immune response to Toca 511 had statistically prolonged survival compared to the control group when treated with Toca 511 delivered either IC-IT or IV. Tumor samples obtained during 5-FC treatment from a small number of mice injected with Toca 511, with or without pre-existing immune responses, had similar levels of 5-FU, showing that Toca 511 vector was able to confer prodrug enzymatic activity in the presence of antivector antibodies. The virus should be relatively resistant to complement inactivation because it is made in human cells.

In the clinical studies to date, the investigators have administered 5-FC while participants were viremic and have not observed toxicities that differ in type, severity, or frequency compared to participants who received 5-FC when they were not viremic. The investigators do not consider 5-FC to be the "fail-safe" antiretroviral for these studies. The Toca 511 virus is sensitive to zidovudine (AZT), and three other antiretrovirus agents are expected to have activity against Toca 511. These agents do not rely on CD for their mechanism of action and should retain activity against the vector even if the CD gene is deleted. The antiretroviral therapy (ART) protocol was developed based on the published literature and in consultation with infectious disease experts.

Regarding the use of steroids in this study after participants have been injected with the virus, the investigators explained that the vector is known to possess steroid response elements in the LTR. *In vitro* studies have confirmed that viral replication is augmented in the presence of corticosteroids in some

glioma cell lines. However, the investigators have elected not to use corticosteroids at the time of IV administration of the vector because corticosteroids are known to rapidly decrease vasogenic edema. This effect on blood vessel permeability might reduce the ability of the vector to gain access to the tumor through leaky tumor vessels. Following surgical resection of the tumor and injection of the vector into the walls of the resection cavity, the surgeon will be free to use his or her standard post-operative corticosteroid regimen.

There is no plan to screen family members of viremic study participants. Cohabitation studies with highly viremic BALB/c mice and naïve cage mates have shown that the virus is not transmitted by casual contact. Research participants will be counseled about the need to use condoms and the need to avoid activities that could theoretically transmit the virus, such as sharing razors.

Regarding dosing details, the highest proposed total IV vector dose is much lower than the highest dose studied in the mouse preclinical studies. In the proposed clinical study, participants in the first three cohorts will receive Toca 511 by IV injection on one day only, approximately 8 to 14 days prior to removal of their tumor, followed by injection of Toca 511 into the walls of the resection cavity. Participants in Cohort 4 will receive Toca 511 by IV injection on three consecutive days, with one-third of the total IV dose administered on each of the three days, and participants in Cohort 5 will receive Toca 511 by IV injection on five consecutive days, with one-fifth of the total IV dose administered on each of the five days. In each of the cohorts, the tumor will be resected and Toca 511 will then be administered into the walls of the resection cavity with an intracavitary dose that will not exceed the highest dose safely administered in the surgical resection protocol. Approximately four weeks later, participants will begin treatment with oral 5-FC for ten days. If tolerated, the ten-day courses of 5-FC will be repeated approximately every five weeks until study completion (approximately 32 weeks).

The investigators acknowledged that they have considered use of multiple-day delivery devices for direct intracranial delivery. While not routine, placement of Ommaya or Rickham reservoirs for access to the intracerebral-ventricular (ICV) space has been considered. In mouse glioma models, distinctly different patterns of distribution are observed after ICV, IC-IT, or IV administration of Toca 511. Spread of vector appears to be more limited with ICV than with either IC-IT or IV administration, with only focal areas of infection after ICV. IV delivery takes advantage of the vascularity of the tumor to reach the enhancing areas, typically the periphery of the tumor. Because recurrence after resection can occur at sites not directly in contact with the resection cavity, the investigators believe that IV delivery may be able to target these regions. Combination of different routes of administration may be useful to consider for future studies, but the investigators believe the mechanism of action regarding selectivity of Toca 511 is relevant to IV administration and thus should be considered an advantage in the proposed study.

Toca 511 has been engineered using a replicating retrovirus that spreads via budding from the transfected cell rather than in a lytic manner, potentially reducing the inflammatory immune response compared to that caused by oncolytic viruses. This method allows more time for spreading through the tumor environment.

The investigators took into consideration the RAC's advice in a September 27, 2011, letter from the RAC in response to Protocol #1107-1120. Based on the literature and in consultation with infectious disease experts, the investigators made changes to the protocol language, now included in all current Toca 511 protocols. The new language discusses ART, including a list of drugs likely to be active against Toca 511, and states that the sponsor has devised a standard procedure for post-exposure prophylaxis in the event of inadvertent needle stick exposure to the virus.

The investigators have performed several IV studies in mice and dogs that are not yet published; in these preclinical studies evaluating IV administration of Toca 511, no unexpected or previously unreported toxicities have occurred beyond those observed in the preclinical studies evaluating intracranial administration of Toca 511. No abnormalities were seen in any mouse upon gross necropsy, and there were no signs of leukemic or lymphoma disorders. BALB/c mice were sacrificed at the end of the study (day 189), and no abnormalities were observed. The most striking observation in the mouse biodistribution studies was a strong predictive correlation of the qualitative levels of virus DNA found in

peripheral blood to the detection of vector DNA in other (primarily lymphoid) tissues. This observation underlies why the investigators believe that the presence of higher levels of proviral DNA in whole blood can serve as a sentinel measurement of vector DNA in any other tissue and therefore provide reliance on monitoring peripheral blood as a screening tool in human clinical studies.

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

2. Responses to RAC Discussion Questions

Dr. Pertschuk noted that to date, some research participants have been followed up to 18 months. However, he acknowledged that it is difficult to get people near the end of their lives to participate in data gathering that is more for scientific purposes than for their own benefit, although the investigators continue to try to convince research participants to do so.

Regarding autopsies, Dr. Pertschuk explained that the investigators have performed two or three brain-only autopsies; no total-body autopsies have been conducted. However, the investigators have approximately ten re-resections after administration, and these data have provided valuable information on tumors, such as spread characteristics, location, and the presence of the CD gene. He offered to share those data, which gave the investigators the idea that they needed to administer the vector with real-time MRI guidance to guarantee they were targeting the tumor. The intratumoral protocol has been altered appropriately based on these data.

Drs. Jolly and Pertschuk noted that the investigators have conducted multiple administration studies in mice but not in humans. IV administration in mice results in an even spread throughout the tumor, whereas intratumoral administration in mice is difficult to interpret due to the occurrence of reflux that results in distribution around the outside of the tumor. Dr. Pertschuk pointed out that this clinical trial proposes a two-region administration—IV administration and administration into the tumor bed. He further explained that the investigators believe it is important to demonstrate unequivocally that IV delivery seeds the tumor; a subsequent efficacy study cannot be conducted if that basic premise is not met.

E. Public Comment

No public comments were offered.

F. Synopsis of RAC Discussion and RAC Observations and Recommendations

<u>Preclinical Issue</u>

- The protocol proposes that research participants with evidence of persistent viremia (greater than 30,000 copies/ml by RT-PCR on two occasions separated by at least 1 month) may be considered for ART using the antiviral agents AZT, tenofovir, and raltegravir. Toca 511 replication has been inhibited by AZT *in vitro*; however, the proposal for use of tenofovir and raltegravir is based on published reports of the ability of these agents to inhibit replication of MLV and xenotropic MLV–related virus. The antiviral activity of tenofovir and raltegravir against Toca 511 should be tested directly. If these agents do not inhibit replication of Toca 511, an alternative regimen should be implemented based on the demonstration of antiretroviral activity against Toca 511 by other antiretroviral drugs.

G. Committee Motion 2

Dr. Fong summarized the RAC recommendations expressing the comments and concerns of the RAC. Dr. Fong asked for a vote on this summarized recommendation, which the RAC approved by a vote of 17 in favor, 0 opposed, and 0 abstentions.

IV. Review and Discussion of Human Gene Transfer Protocol #1304-1219 titled: A Phase I/II, Open-Label Dose Escalation Study to Evaluate the Safety and Efficacy of Single Doses of TT-034 in Patients with Chronic Hepatitis C (CHC) Infection

Principal Investigator:	Keyur Patel, M.D., Duke University Medical Center
Additional Presenter:	David Suhy, Ph.D., Tacere Therapeutics, Inc.
Sponsor:	Tacere Therapeutics, Inc.
RAC Reviewers:	Drs. Fong, Kohn, and Zoloth
Ad hoc Reviewers:	Scott Harper, Ph.D., Nationwide Children's Hospital; Willis Maddrey, M.D., The University of Texas Southwestern Medical Center

Dr. Sarzotti-Kelsoe was recused from consideration of this protocol due to a conflict of interest.

A. Protocol Summary

This proposed clinical trial would be the first-time use of an experimental therapy to transfer anti–hepatitis C virus (HCV) genetic sequences into the hepatocytes of patients infected with HCV who have failed the current standard of care for treating their disease. A total of 130 to 150 million people worldwide are infected with chronic hepatitis C (CHC), and CHC is the leading cause of cirrhosis, hepatocellular carcinoma, and liver transplantation in developing nations.

TT-034 is designed as a non-integrating recombinant DNA construct, which is introduced into the nucleus of the cells by a self-complementary, adeno-associated virus serotype 8 (AAV8) vector. The construct provides the template for the cellular synthesis of a small hairpin RNAs (shRNA), which are then processed by the cell into small interfering RNAs. Each shRNA in TT-034 was selected to inhibit a different, well-conserved area of the HCV genome. The simultaneous expression of the three different shRNA provides a pool of therapeutic sequences that target multiple regions of the HCV genome simultaneously. The ability to cleave and degrade the virus genome at three separate sites simultaneously may help prevent the generation of escape mutants. These sequences encoding for the three shRNAs were combined into a single expression construct controlled by three independent Pol III promoters that were modified to produce modest levels of each shRNA.

The primary objective of this research study is to determine whether this vector can be administered safely to humans, and research participants will be followed for side effects of the procedure. Subjects with HCV who agree to participate in this study will be infused with a single dose of the vector through IV administration. A liver biopsy will be taken approximately 28 days following the infusion to assess how well the hepatocytes have taken up TT-034 and to measure the shRNA levels being produced. Blood samples will be taken periodically from the research participants to test for side effects of the procedure and to monitor the levels of HCV in the serum.

The results of this study will determine whether this experimental strategy to treat HCV warrants further evaluation as a method to control this disease.

B. Written Reviews by RAC Members

Seven RAC members voted for in-depth review and public discussion of this protocol. Key issues included that the construct is novel and the protocol is the first to use a vector-delivered shRNA for CHC infection.

Three RAC members and two *ad hoc* reviewers provided written reviews of this proposed Phase I/II trial.

Dr. Fong asked the investigators to explain the expected off-target effects of the three shRNAs. Although only single dosing with TT-034 is planned, success would require 100 percent transduction of liver cells infected with virus; therefore, he noted the importance of knowing whether this experimental vector could be administered a second time and whether preclinical studies indicated if an increased dose of vector could overcome immunity generated against the AAV vectors. Dr. Fong wondered whether the

investigators had considered regional delivery of the vector, which would involve injecting the vector into either the hepatic artery or portal vein using interventional radiology and thus resulting in higher transduction, lower immunogenicity, and lower off-target toxicity. Noting that much of the vector may never reach the liver and therefore the earliest doses are likely not to be effective, Dr. Fong asked whether the research participants would be informed that the goals of the study would not likely be achieved at those earliest doses and whether the participants would know before enrollment to which cohort they would be assigned.

Acknowledging that this clinical protocol has been thoroughly developed, Dr. Kohn summarized the risks of this proposed trial as unknown potential for toxicity to liver or other organs and potential for development of immune responses to AAV, which could impede future gene therapies that use AAV. He noted that the major risk is that this intervention with an AAV8 vector into hepatocytes could be long term, even potentially permanent, in that subtle adverse effects from expression of the shRNA could lead to slow development of liver toxicity. Although no suggestion of this toxicity was encountered in the pharmacology/toxicology studies (which were only a few months long) at clinically comparable doses in nonhuman primates, the shRNA effects could be restricted to human RNA sequence targets or might only manifest over years or decades. Dr. Kohn asked the investigators to describe the assay to be used to measure antibody to AAV8, because it will be a critical enrollment test. He also asked whether any longer-term nonhuman primate studies are planned or in progress.

Dr. Zoloth stated that chronic hepatitis is a difficult, incurable disease and one in which the current therapeutic protocol is often extraordinarily arduous; patients struggle with multiple organ failures, infections, and severe side effects even in the 60 percent of cases in which interferon is effective. Noting that a one-time treatment seems aimed at producing a single strong and permanent effect, she asked what procedures would be followed if the virus changes or escapes and whether administration could be repeated. Dr. Zoloth requested that the investigators discuss the risk that their proposed intervention could permanently shift a research participant's entire immune system. She suggested that this study might be conducted with a wider international sample, because HCV genotypes in other countries differ from the genotype seen in the United States. Although Dr. Zoloth stated that the informed consent document is clear, easy to read, and humble in its claims, she offered several suggestions to increase clarity and accuracy.

Ad hoc reviewer Dr. Harper requested the investigators to clarify in the protocol that AAV8 transduces liver but not exclusively. He also posed several questions related to the data comparing the first-generation product (TT-033), which caused toxicity in animals, to the current TT-034, which appears to be safer and better tolerated while still being effective: (1) whether the investigators have data supporting or ruling out the mechanism of shRNA overexpression–related toxicity; (2) whether the investigators explored whether a differential toxicity exists among the different shRNA sequences at high levels; (3) what might be the possible impacts of modest but chronic changes in natural micro RNA (miRNA) levels that could be affected by TT-034 presence; (4) whether the investigators are concerned about shRNA overexpression inhibiting the production of miR-122 in the liver, alterations in levels of which have been associated with development of liver cancer; and (5) whether the investigators have examined the effects of TT-034 expression on miR-122 maturation and levels (or miR-122 target genes) in animals.

Ad hoc reviewer Dr. Maddrey stated that genotype 1 is the major cause of CHC in the United States but that genotype 3 is more important in other parts of the world; this proposed clinical trial is designed to treat only genotype 1. A one-time treatment for CHC would be a significant advance, because approximately 30 percent of genotype 1 patients do not respond to currently available therapies and many patients are unable to complete a full course of those therapies because of side effects. Dr. Maddey expressed concern about the potential high risk of repeat therapy initiating an immune response. He also noted that the investigators discussed in Appendices A and B how to manage effectively an immune response that might present as an acute allergic reaction or as persistent autoimmune hepatitis; the potential for such reactions would need to be monitored closely. He also expressed concern about how to manage a return of HCV in a dosed research participant if the return is produced by an escaped mutant virus. He asked the investigators whether any cells other than hepatocytes (e.g., Kupffer cells or stellate cells) would be affected. Noting that the data from mouse and monkey models are reassuring, he

wondered whether the follow-up intervals have been long enough. In addition, Dr. Maddrey asked the investigators what protocol or dosing might be offered to the initial participants who will be given what is presumed to be a subeffective dose, and he suggested that the investigators might want to consider using a dose that is more likely to be as effective as the initial dose. In addition, he asked about the possibility that an induced viral mutant would appear and, if so, whether it would be expected to respond to existing drugs.

C. RAC Discussion

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

- Dr. Wooley asked whether the shRNAs would act synergistically and, if so, for the investigators to discuss the level of synergism.
- Dr. Maddrey opined that investigators in this field are seeing more fibrosis because they are interacting with older populations. He plans to work with a population of individuals with cirrhosis, because that group has desperate need. He suggested that future-phase trials should include a group that is close to transplantation or development of cancer.

D. Investigator Response

1. Written Responses to RAC Reviews

Extensive animal studies in mice and monkeys have demonstrated that administration of a single dose of TT-034 at and above levels expected to result in close to 100 percent transduction of hepatocytes does not result in pathological or other undesirable consequences. The biodistribution studies support that the vast majority of TT-034 is detected in the liver, as anticipated, and detection in other tissues was not associated with adverse effects when animals were observed for up to 180 days. Exposure to TT-033, the first-generation compound, provides the best insight and predictive indicator into the potential toxicities of shRNA overexpression. In mice administered TT-033, cardiac mineralization was typically seen in one or two of ten animals in the highest dose groups at time points through day 91; however, similar cardiac toxicity was not found in cynomolgus monkeys dosed with TT-033 nor was that toxicity observed in either species when dosed with TT-034.

No current options exist for additional dosing. Initial administration of the vector will trigger a large humoral response that will prevent the ability to transduce the liver efficiently upon readministration of TT-034. Given the potential to elicit a large humoral response and the unknown variable of how vector readministration may exacerbate the potential for additional cell-mediated immune response, vector readministration is not a likely scenario for this product.

The investigators stated that they are not convinced that therapeutic success would require 100 percent transduction of liver cells. Based on the available literature, TT-034 is expected to be effective at doses below those necessary to achieve 100 percent transduction. Adaptive immune responses also may increase with decline in viral burden, and it is possible that virus levels would be suppressed sufficiently well to allow the host immune system to “catch up” and eliminate the remaining infected hepatocytes.

Regarding the choice of IV administration, although hepatic artery or portal vein injection might allow for direct delivery to hepatocytes, cannulation of the hepatic vasculature is an invasive procedure and is associated with risks of complications, such as thrombus, hemoperitoneum, and cholangitis. Other issues, such as the dynamic hepatic circulation, variation in blood flow to the liver, and collateralization with advancing disease, might not prevent off-target toxicity. The redesign of the TT-034 molecule presented favorable toxicology profiles following IV doses of at least $1x10^{13}$ vg/kg in non-GLP (“Good Laboratory Practices”) acute toxicity and GLP toxicity studies, and supported the selection of an IV route of administration.

The informed consent document states that TT-034 is an investigational new drug (IND) that has not been given to humans before. The research participants will be told which cohort they will be placed into before the study begins.

Presence of antibody to AAV8, as an indication of prior exposure, will be determined for each potential study participant using a classic neutralizing antibody biochemical assay. The method planned for use in this clinical trial has been used to screen serum samples from more than 700 cynomolgus monkeys to qualify the animals for the preclinical studies with TT-033 and TT-034. For the human studies, the method will be transferred to a third-party vendor and will be qualified before use.

Regarding studies using nonhuman primates, the investigators explained that a dose range–finding study with TT-033 used 15 cynomolgus monkeys. A bridging study between TT-033 and TT-034 was performed in which six cynomolgus monkeys were dosed with TT-033 and an additional 18 animals were dosed with TT-034. Most recently, 80 cynomolgus monkeys were used in the 180-day IND-enabling GLP studies for the TT-034 clinical product. No additional nonhuman primate studies are currently in progress. If the endpoints are met in the first clinical studies, it is likely that long-term (up to 2 years) carcinogenesis studies may be required in nonhuman primates that have been dosed with TT-034.

If the standard of care has not changed by the conclusion of the 24-week primary trial, then there is nothing else that a physician could offer as a means of follow-up treatment for research participants given subtherapeutic doses or who experience viral breakthrough. If the standard of care has changed to include new options at the time the participants complete the 24-week primary trial, the sponsor may consider offering standard-of-care treatment to research participants who do not have insurance coverage.

The investigators explained that they consider a permanent shift to the entire immune system unlikely. Other researchers have conducted systemic administration of AAV8-based vectors and no global shift in the immune system was noted, although there was seroconversion of research participants. Systemic administration also has been performed with an AAV2 vector. Local administration of AAV-based vectors has been used on hundreds of people to date and the safety profile has been excellent. The most challenging issue associated with systemic administration of AAV is a cell-mediated immune response that has been noted to occur at high levels of systemic vector administration with AAV8 and AAV2. This response typically occurs at seven to ten weeks post–vector administration and principally affects hepatic tissues. The investigators noted that they have accounted for the possibility of a similar event that could occur in research participants administered similarly high doses of TT-034, and the investigators have extended the period of observation to ten weeks before additional dose levels or an increase in dosing can occur for the high-dose levels.

The product profile of TT-034 suggests that this therapeutic candidate has the best chance of achieving meaningful clinical activity when administered at doses capable of transducing nearly all hepatocytes. Within an HCV-infected liver, only 20 to 40 percent of the hepatocytes are infected with the virus at any given time, and the subpopulation of infected cells is constantly changing. When TT-034 is administered and transduces the hepatocytes, the aim is to eliminate HCV in cells that are infected as well as provide prophylaxis to cells that may be subject to infection later. Whether TT-034 truly needs to transduce 100 percent of the cells remains an open question.

As to whether this protocol should be conducted with a wider international sample, the investigators noted that this first-in-human safety study should be conducted in a well-controlled environment. TT-034 has been designed specifically to treat patients with genotype 1 virus, and most of the preclinical testing has been geared toward genotype 1. With a limited sample size (n = 14) and a complex clinical protocol, the investigators believed it best to concentrate efforts on two sites within the United States. In future studies, the investigators intend to include international sites.

All animals were sacrificed at the conclusion of the 180-day study so that tissues could be harvested and examined. Therefore, no long-term carcinogenesis studies are in progress. If the endpoints are met in the

first clinical study, long-term carcinogenesis studies in nonhuman primates that have been dosed with TT-034 will be considered.

The duration of this Phase I/II study is six months for each participant, with a 15-year observational follow-up trial. Like previous trials involving systemic administration of AAV vectors, the restrictions in place for birth control only will apply to the Phase I/II trial, not to the 15-year follow-up trial. The investigators are not advising against any medications in the 15-year follow-up study. However, if the investigators find evidence of persistent vector shedding at the end of the six-month period, they may advise participants to continue using birth control for a longer time; this event is highly unlikely. This process is consistent with the experience from previous trials that used AAV vectors.

Because systemic AAV administration has not occurred on a large scale, limited data exist on the potential side effects associated with these types of compounds. Systemically, AAV2 and AAV8 have been administered to individuals for treatment of hemophilia with no overt clinical signs. Thus, while the investigators expect to be able to reach the highest planned dose level without the kinds of toxicities that patients experience in interferon-based regimens, the protocol must include the potential to stop at a lower dose if unacceptable toxicities are observed. Side effects from systemic administration of siRNA-based therapies have been observed but via a different route of administration.

Efficacy will be assessed by HCV viral load, the presence of HCV target site sequence mutations (i.e., viral escape mutants) in participants with detectable viral load post-dose, as well as viral vector DNA levels and shRNA levels measured within samples taken from the liver biopsy. Typically, drops in HCV serum levels by two logs or greater is considered efficacious.

The use of a liver biopsy taken 21 days post-exposure to TT-034 will provide the material required to assess DNA transduction and shRNA expression levels. The protocol also includes a component in which pharmacodynamics will be assessed by measuring shRNA levels in serum. The investigators predict that long-term persistence of shRNA in serum will be observed as exosomes containing shRNA are shed. The hope is to establish a correlation between serum shRNA levels and DNA transduction and shRNA expression measured in the liver biopsy. This proposed study has been designed to minimize the number of participants who will be administered suboptimal doses of TT-034. Participants will be informed that they could receive a subtherapeutic dose and that the compound cannot be readministered. Although preclinical studies help predict what dose might lead to an efficacious effect, the investigators cannot be certain that lower doses will not be efficacious or that higher doses will be. TT-034 experimental therapy should not limit a research participant's ability to receive other existing and future HCV therapies.

As to whether cells other than hepatocytes could be affected, the investigators cited previous work that demonstrated that Kupffer cells are not transduced by AAV8 in a murine model. The same study implicated that other nonparenchymal cells besides Kupffer cells may be transduced, although the specific subpopulations were not ascertained. More recently, another study showed that a mouse strain using an enhanced yellow fluorescent protein marker as a measure of AAV8 transduction did not show co-localization with liver macrophages, stellate cells, and endothelial cells, indicating that these cell populations are likely not transduced.

The 180-day GLP toxicology studies have been the longest period of time that animals have been followed after dosing with TT-034. These studies consisted of 520 mice and 80 cynomolgus monkeys. The length of the 180-day toxicology study was discussed with the FDA in a formal pre-IND meeting, and the study design appeared to be acceptable to the FDA after the investigators provided rationales to support the study duration and sacrifice time points for each study.

A conscious effort was made during protocol development to minimize the number of individuals who would receive a dose that is anticipated to be subtherapeutic. Considering that this is a first-in-human study, the investigators believe that the dosing schema should provide a balance between safety and the potential for achieving meaningful biologic activity against HCV. Because this is the first time RNA interference will be used systemically and because the nature of the delivery system means it is not possible to withdraw the experimental treatment once administered, the sponsor and the principal

investigators believe that the dosing schema presented in the current clinical protocol takes both factors into account and represents the most prudent approach. Although the participants in the lowest dose levels are not expected to receive benefit, the investigators believe the initial lower doses are necessary to guard against potential unexpected side effects not seen in the animal studies.

Regarding the possibility that induced viral mutants will appear, the investigators explained that neither AAV nor adenovirus vectors integrate into the host cell genome and that, as the transduced cells divide in tissue culture, the vector is rapidly lost from the daughter cells. Therefore, long-term viral escape studies have not been performed. The derivation of point mutants in response to selective pressure against shRNA expression represents only one challenge: Each research participant is likely to harbor dozens or hundreds of quasi species of HCV, some of which will have significant differences in the sequence of the viral genome. Thus, TT-034 will need to be sufficiently active to target already existing changes present within an individual's population of viral sequences. The sequences used in TT-034 were designed with this in mind, and this design, along with the use of three separate shRNAs, should provide good coverage against possible mutants. The clinical trial includes sequencing of the viral genomes in participants at the beginning and end of the trial (if the virus is detected) to monitor such possible changes.

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

2. Responses to RAC Discussion Questions

Dr. Suhy reported that the investigators have not conducted any studies to discern the relative contribution of each shRNA to cleavage; consequently, whether the shRNAs are acting synergistically is unknown.

E. Public Comment

Lloyd B. Glickstein, M.D., of Novartis Institutes, asked if the investigators have tested whether this protocol would work in a fibrotic or anatomically abnormal liver, especially given that a key element of this research proposal is that the investigators would achieve substantial exposure of a majority of hepatocytes to the experimental therapy.

Dr. Suhy responded that the investigators have not directly tested the ability to transduce fibrotic livers. Although unpublished evidence from collaborators indicates it is possible to transduce those organs, it is difficult to maintain long-term transduction because the hepatocytes turn over rapidly. In studies in nonfibrotic animal models, the investigators have been able to achieve near-complete transduction of all the hepatocytes within the liver with clinically relevant doses.

F. Synopsis of RAC Discussion and RAC Observations and Recommendations

<u>Preclinical Issues</u>

- Although the target organ is the liver, this trial proposes an IV route of administration. Further consideration should be given to conducting animal studies to evaluate how direct administration via the hepatic artery or portal vein compares to IV administration. Focused liver delivery should minimize distribution to tissues for which there is no therapeutic need. These data may support an additional cohort using targeted hepatic delivery.

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

- Liver biopsy will be performed at day 21 after dosing to assess for viral vector DNA levels and shRNA levels. The tissue also should be evaluated for changes that would not be expected with CHC infection, such as unusual areas of fibrosis or necrosis or eosinophilia, which are not found in CHC infection.

- The current protocol states that the trial will be stopped if a research participant dies. This statement should be amended to clarify that only a death that is possibly related to the study agent will be a reason to halt the trial, because it is quite possible that participants might die of unrelated causes.

Ethical, Legal, Social Issues

- The informed consent document should discuss more thoroughly the risks of pregnancy during both the active phase and the long-term follow-up portion of the study.

- Hepatitis C is an international problem and, given the costs and length of treatment with traditional therapies, countries with limited resources might not have access to traditional treatments. If this trial is successful, the investigators might want to consider including international sites in the next phase of clinical development.

- A potential advantage of this approach is that virus eradication might occur with one-time administration; however, this is also a potential disadvantage, because (1) if the dose received is not efficacious, readministration likely will not be possible due to the predicted immune response to the AAV vector, and (2) if an adverse event occurs, it may be difficult to stop the action of this agent. The informed consent document should discuss these risks specifically and in more detail.

G. Committee Motion 3

Dr. Fong summarized the RAC recommendations that would be included in the letter to the investigators, expressing the comments and concerns of the RAC. Dr. Fong asked for a vote, and the RAC approved these summarized recommendations by a vote of 16 in favor, 0 opposed, 0 abstentions, and 1 recusal.

V. Gene Transfer Safety Assessment Board Report

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

A. GTSAB Report

Dr. Kiem presented the GTSAB report for the second quarter of 2013. The OBA had received 24 protocol submissions within the past three months, 17 of which were not selected for public review at this RAC meeting. Of the 17 protocols not selected for public review, 13 were oncology protocols, three were for infectious diseases (HIV), and one was for Parkinson's disease. In these 17 protocols, four used adenoviruses, three used plasmids, two used retroviruses, and one each used AAV, HSV, RNA, and transposon. Dr. Kiem stated that information about these trials would be available on the OBA website after this RAC meeting.

The GTSAB reviewed initial and follow-up reports on 13 SAEs from 10 protocols. After analyzing these events, the GTSAB concluded that none warranted public discussion at this RAC meeting, although more information about some of these events will be discussed at the September 2013 RAC meeting.

During this second quarter, the OBA received notification from investigators that 15 protocols were newly open to enrollment.

Dr. Kiem reported on two noteworthy protocol changes that represented responses to RAC public review:

- *OBA Protocol #1169, reviewed in September 2012: Phase I/II Study of Adoptive Immunotherapy After Allogeneic HCT with Virus Specific CD8+ T Cells That Have Been Transduced to Express a WT1-Specific T Cell Receptor for Patients with High Risk or Relapsed AML, MDS, or CML*. Plasma samples will be stored for future cytokine analysis in the event that a systemic inflammatory reaction occurs. The informed consent document has been modified to state

explicitly that approximately one-half of the individuals who participate in this trial are not likely to relapse and thus will not require further treatment.

- *OBA Protocol #1189, reviewed in December 2012: A Phase II, Randomized, Open Label Study of Ad-RTS-hIL-12 Monotherapy or Combination with Palifosfamide-tris in Subjects with Recurrent/Metastatic Breast Cancer and Accessible Lesions.* In the absence of an available tumor lesion, only lymph nodes that are pathologic may be injected. In addition, further studies will be conducted on injected lymph nodes to determine the types of cells that are transduced, the biodistribution of those cells, and the persistence of the vector in those cells. In light of the death of a research participant from infection in a previous trial for melanoma, the investigators received instruction to conduct an analysis to determine whether a potential injectable lesion harbored an infection and, if so, to avoid injection.

Dr. Kiem announced a workshop titled "T-Cell Immunotherapy: Optimizing Trial Design," which will take place on September 10–11, 2013, at the Hyatt Regency Hotel in Bethesda, Maryland. This workshop will examine the state of the science for protocols using modified T cells and chimeric antigen receptors, including a review of the clinical data to date, selection of new targets, the most recent research on preconditioning and T-cell cytokine support, and the management of adverse events. The September 2013 RAC meeting will be held following this workshop.

B. RAC Discussion

No discussion occurred.

C. Public Comment

No public comments were offered.

VI. Update on Human Gene Transfer Protocol #0510-738 titled: A Phase I Dose Escalation Study of Intratumoral Herpes Simplex Virus-1 Mutant HSV1716 in Patients with Refractory Sarcoma or Neuroblastoma

Presenter:	Timothy Cripe, M.D., Ph.D., Nationwide Children's Hospital *(via teleconference)*
Additional presenters: *(all via teleconference)*	Marianne Brunner, R.N., RAC, CCRC, Cincinnati Children's Hospital; Joe Conner, Ph.D., Virttu Biologics, Ltd.; Sue Goldsborough, Virttu Biologics, Ltd.; Robert Spavin, Virttu Biologics, Ltd.; Michele Vaughan, M.D., Nationwide Children's Hospital

A. Presentation by Dr. Cripe

Dr. Cripe explained that successes in childhood cancer therapy mostly have occurred for standard lymphoblastic leukemia, Hodgkin's disease, and a few other tumors, such as the Wilms' tumor; however, for many of the cancers seen in children and young adults, progress has been slow. Even for Ewing's sarcoma, in which patients saw a jump in survival curves with the addition in the 1980s of ifosfamide and etoposide to a three-drug backbone and despite numerous clinical trials since then, little progress has occurred. During the early 2000s, some increase in short-term metrics using a compressed chemotherapy dosing regimen raised hope for more long-term survival. However, despite all this research, progress has been slow, suggesting a need for other kinds of therapies, particularly for individuals who relapse or who have metastatic disease, because their survival rate is essentially zero.

Dr. Cripe and colleagues have been pursuing the use of oncolytic viral therapy as an alternative or adjunct to conventional chemotherapy. Oncolytic viral therapy is the use of live viruses to infect and kill tumor cells, to induce an antitumor immune response, and to express foreign transgenes to induce various therapeutic effects. HSV1716 (Seprehevir®) is an oncolytic virus based on a modified herpes

simplex virus (HSV) that shares certain biological characteristics of the wild-type virus. HSV-1 is a member of the Herpes group of viruses. The usual site for HSV-1 infection is skin or mucous membranes, and it is the causative agent of cold sores. Humans are the only natural host for HSV-1 and, outside the host, the virus is fragile and easily inactivated. Although many other species can be infected experimentally, HSV-1 is not propagated in these species.

Preclinical data for HSV1716 have been generated from a blinded and controlled toxicology and biodistribution study designed with and approved by the FDA. In xenograft models, HSV1716 has demonstrated efficacy in numerous tumor models, including glioma, mesothelioma, melanoma, squamous cell carcinoma, ovarian cancer, and non–small-cell lung cancer. The current clinical trial using HSV1716 has been open for enrollment and features an intratumoral injection. Dr. Cripe and colleagues have proposed an amendment to this trial that would add an IV injection.

Outcomes from seven studies of HSV1716 in HGG, adult glioblastoma multiforme, melanoma, oral squamous cell carcinoma, and mesothelioma include:

- No virus-related toxicity reported at any dose or route of administration
- No virus shedding detected by buccal swab and urine sample
- Administration procedures well tolerated
- Evidence of HSV1716 replication in resection tissue obtained post-administration
- Biopsy tumor tissue permissive for HSV1716 replication
- Notable individual case reports of prolonged survival
- Open study in the United Kingdom administering a higher dose of HSV1716 to the pleural cavity of mesothelioma patients

Based on the accumulated data in 2010, Dr. Cripe and colleagues were able to open their Phase I study, which was originally an intratumoral dosing. This trial was for subjects with non–central nervous system (CNS) solid tumors. The original study objectives were (1) to determine safety and dose-limiting toxicities (DLTs) and (2) to monitor antiviral immune response, look for viremia and virus shedding, and define any antitumor activity.

To date, this study has enrolled five participants, with three enrolled at the first dose level and two at the second dose level. The diagnoses of these research participants are all non-brain solid tumors typically seen in children. No treatment-related SAEs or major toxicities have been observed. All participants in this trial have been assessed for DLTs through the day 28 visit and no DLT has been observed; some adverse events that were possibly related to HSV1716 were mild and transient. All HSV culture results have been negative. Although only one of the five participants was HSV positive at baseline, all participants had become HSV positive at day 28, suggesting they had some exposure to HSV antigens during the prior 28 days.

With regard to efficacy data, the five participants were assessed for initial evidence of efficacy at either day 14 or day 28. The first four participants were determined to have progressive disease; the fifth participant was determined to have stable disease at day 14 but progressive disease at day 28. This trial has generated a total of 284 adverse events, 21 of which warranted SAE reporting but none of which were possibly, probably, or definitely related to the virus but were believed to be due to underlying tumor progression.

Slow enrollment is a significant limitation of the current study, with 23 patients screened since 2010 but only 5 enrolled. Most of the screened patients have opted for other open studies or for off-study salvage regimens that feature systemic treatment because most patients either have extensive disease—and the region being injected in the HSV1716 trial is quite small—or have metastatic disease—and do not want to opt for a localized therapy. This trial has so far shown little efficacy, likely due in part to low doses. The investigators have proposed a higher intratumoral dose as well as an IV dose cohort because of the poor accrual and the need to address these patients' more extensive tumor burden, including metastatic disease.

The investigators conducted an IV toxicology study that included an intraperitoneal administration to stimulate deep tissue injection of virus as well as an IV cohort. In the IV biodistribution study, the tumors appear to be

expressing the viral genes primarily at the site of the tumor; no virus was detected in any organs in this study except in the tumor at various time points, with persistent virus detection beyond three to four weeks. The IV preclinical efficacy study, with IV administration on days one, 14, and 29, indicated that the difference in tumor growth for untreated versus treated was highly significant for two different doses that, per kilo, far exceeded what is proposed to be given in humans. The virus seems to be able to home to, enter, and replicate in tumor when administered intravenously.

Based on these data, the investigators have proposed several amendments to their original HSV1716 trial. The current amendment, called version 12.2, has been approved by the institutional review board (IRB) and the FDA and is open for accrual (although no subjects have yet been enrolled). In addition to the intratumoral dosing, version 12.2 adds a second cohort that will receive an IV dose. Safeguards incorporated into version 12.2 include (1) that the first three research participants to be given IV doses would be adults at least 18 years old, (2) a 28-day observation period between dosing of each of the first three participants, and (3) IRB review of the experience of the first three participants prior to enrolling any younger participants. In addition, no second dose for any participants will be administered until the first three participants have received a single dose and completed the DLT observation period. These safeguards were put in place because this trial would be the first-in-human IV dosing of HSV1716.

Dr. Cripe summarized that numerous studies have demonstrated the safety of various IV oncolytic viruses, and adult studies have shown intratumoral safety of HSV1716, with single and multiple dosing. Adult studies with other HSVs given intravascularly have been published and show safety. The investigators believe that preclinical IV studies of HSV1716 show safety and efficacy, and these studies justify addressing the issues the investigators have encountered with their pediatric/young adult Phase I study—safety at two dose levels but few hints of efficacy, coupled with poor accrual likely due to patients wanting systemic therapy because of their metastatic disease. Version 13 of this trial, which would address these issues, is currently on file with the FDA and is under submission at Nationwide Children's Hospital and Cincinnati Children's Hospital.

B. RAC Discussion

Noting that many musculoskeletal tumors occur in the limb and therefore infusional therapies are sometimes used, Dr. Fong asked whether Dr. Cripe and his colleagues have considered including a study arm with infusion into a specific limb. Dr. Cripe responded that chemotherapy and limb profusion with osteosarcoma has engendered controversy. Although an increased incidence of better tumor necrosis occurs, there is no improvement in survival. The consensus has been that surgery is good for local control, but systemic therapy is needed to address metastatic disease and overall survival. Metastatic disease is most commonly in the lungs, so infusion in the limb would not address that issue.

Dr. Chiocca asked whether the investigators have observed any correlation related to an immune response against these tumors after they are injected with virus or whether the investigators plan to look at that possibility in the future. Dr. Cripe responded that he and his colleagues have not built any biologic correlative studies into the clinical trials, although they have been studying that in the mouse. At this point, they are concentrating on accrual to the clinical trial, as amended, as well as finishing current preclinical studies. Preliminary data in the mouse sarcoma models indicate that virus replication is improved with cyclophosphamide but antitumor immune response is worsened.

C. Public Comment

No public comments were offered.

VII. Discussion of NIH Protocol #0001-381 titled: Long-Term Follow-Up After Gene Therapy for Canavan Disease

Presenter: Paola Leone, Ph.D., University of Medicine and Dentistry of New Jersey

A. Presentation by Dr. Leone

Canavan's disease is a progressive degeneration of the brain, characterized by severe motor and mental impairment, which leads to death during childhood. Considerable evidence indicates that the injury to the brain and the neurological impairments are caused by accumulation of a chemical called N-acetylaspartate (NAA) in the brain, which is present in certain parts of a normal brain but is ordinarily broken down and recycled by the enzyme aspartoacylase (ASPA). When levels of ASPA are too low, the NAA levels in the brain rise to dangerous levels and accumulate. Canavan's is an autosomal-recessive disease characterized by a mutation in the ASPA gene that results in a deficiency of ASPA enzyme in the brain. A total of 98 percent of NAA is synthesized in the brain and by gradient moves to the cerebrospinal fluid (CSF), blood, and urine.

Dr. Leone explained that this protocol represented the first clinical use of AAV in the human brain and the first IND approved for the application of viral vectors for a neurogenetic disorder. The RAC reviewed this proposed Phase I trial in March 2000, and the FDA approved it for accrual in April 2001. The aim of this protocol was to over-express human ASPA enzyme in the brains of patients affected with Canavan's disease. One diagnostic model used to diagnose Canavan's disease is the presence of extremely high NAA in the urine; genetic testing also can be diagnostic if the mutation is known.

Clinical features of Canavan's disease include patients presenting in infancy with hypotonia, lack of head control, macrocephaly, seizures, visual abnormalities, and failure to thrive, followed by severe motor impairment, epilepsy, mental retardation, and blindness. Neuropathologic features include spongy change to the lower parts of the cortex, the subcortical white matter, and the cerebellar cortex. Microscopic features include accumulation of fluid within myelin lamellae, disintegrating of myelin sheaths, swollen astrocytes, and severe vacuolation.

Although the first Canavan's patient examined by the investigators had Ashkenazi Jewish ancestry, within the past seven years the majority of cases do not have this common mutation but instead are rare mutations; 45 novel mutations have been identified during this study. All Canavan's patients are affected by dysmyelination and no normal myelin is being developed. The disease is severe, progressive, and uniformly fatal. Because it is a monogenic disease and the pathology is uniquely represented in the brain, gene transfer seemed the ideal methodology to over-express a human gene and test the safety of a viral vector in the human brain.

Dr. Leone reviewed the design of the original protocol, including clinical phases and enrollment criteria. The investigators have been conducting follow-up with research participants for as long as 14 years since the study began, focusing on the frontal, parietal, and occipital lobes of the brain in which each surgical site received 150 billion genomic particles of AAV2 vector expressing human ASPA. She explained the vector production, vector injection, and surgical procedure in this trial. SAEs were few and included mild post-operative fever, with one exception: participant #13 lost consciousness several days after being discharged from the trial procedure. It was determined that he had no clinical symptoms other than loss of consciousness, but a tiny abscess was discovered at the right occipital burr hole. The abscess was removed surgically, triple antibiotics reversed the pathology, and the life of this research participant was saved; Dr. Leone displayed two photographs of this individual at one year and ten years after gene transfer.

The frontal, periventricular, and occipital areas of the brain were dosed during this trial. Looking at NAA changes in the study, the investigators have noted a significant NAA drop in the frontal and periventricular areas. In the first 4.5 years since gene transfer (for which data were available for all participants), the average concentrations of NAA among all participants decreased to within the normal range in the front subcortical and periventricular regions.

Although results in one two-year-old participant showed that gene transfer has stabilized brain atrophy, the investigators were not able to stabilize atrophy in a participant who was four years old. This age difference suggests that earlier intervention may be more effective. Other results included (1) the presence of neutralizing antibody to AAV did not affect expression of the transgene in the brain and (2) diffusion tensor imaging showed improvement in the bilateral movement of water molecules in one research participant, a movement that occurs only in myelinated fibers. Most of the patients in this trial who underwent the surgery

and the gene transfer procedure showed reduced seizure events at 60 months. In terms of survival, the youngest research participant received gene transfer at 3.7 months of age; she is about to be eight years old. The oldest participant was nearly seven years old and will be 19 years old in August. All of the original participants are alive and well and living with their families.

Dr. Leone presented data on the effect of this gene transfer on neurodevelopment on scales of lying and rolling, alertness, social function, seizure, and survival. Long-term follow-up following AAV2-mediated gene transfer in the CNS indicates that intraparenchymal delivery of recombinant AAV2-ASPA was well tolerated and resulted in a decrease in elevated NAA and in clinical stabilization as well as slowed progression of brain atrophy, especially in the youngest research participant. The low levels of immune response detected in this study, at this dose and with intraparenchymal administration, suggested that rAAV2 is safe. As a result of this trial, the investigators recommend early intervention to maximize benefit.

B. RAC Discussion

Dr. Chiocca asked about the observed subdural and subarachnoid hemorrhages and how long they took to clear out. Dr. Leone explained that these hemorrhages were always attributed to the surgical procedure and cleared out quickly. The participants who experienced the subarachnoid hemorrhages were more likely to experience other events later during the follow-up period.

Dr. Fong asked about the status of the research and plans for the future. Dr. Leone responded that the investigators are continuing to work on Canavan's disease. NAA is the second most abundant metabolite in the brain, although its role remains unknown; the investigators want to unravel the role of NAA because all neurodegenerative diseases feature a drop in NAA. A fully characterized animal model has been developed. The investigators are developing a metabolic therapy that is administered orally and could be administered *in utero* through breast milk. They are working with a company on stem cells because they are seeing an effect of stem cell in graft myelination. The overall goal is to gather the necessary information in the next five years to change Canavan's from a terminal disease to a treatable disease. Dr. Leone and colleagues also are in process of developing a therapy potentially to treat Alzheimer's disease.

Dr. Cai asked whether the investigators scan all areas of the brain for possible therapeutic effect. Dr. Leone explained that, for metabolites, they focused on the six areas of the brain to which the vector had been injected, primarily because this was a Phase I safety study.

Noting that the vector and technology are about 12 years old, Dr. Kohn asked about the possibility of using more efficient vector. Dr. Leone responded that the localized NAA drop did not deliver the expected myelin development. She acknowledged that the field is moving forward quickly and it may be possible to use a vector that would express enough human ASPA to make a difference in myelin development. The investigators are focusing on the possibility of oral administration at present. The investigators have not seen any T1 or T2 abnormalities within their trial.

Dr. Chatterjee asked why ASPA gene transfer does not result in more myelination. Dr. Leone speculated that timing and/or where the gene is expressed could be the reason. Potency of the vector could also be involved.

Dr. Chatterjee asked whether the investigators would consider neonatal gene transfer with a serotype like AAV9 that can go everywhere in the CNS. Dr. Leone noted that brain surgery can be associated with significant risk, as was seen with the child who developed an abscess. If the same benefit could be delivered with an oral medication, it also could be administered to a pregnant woman. Dr. Leone expressed confidence that she would be able to reach her goal, which is to turn Canavan's into a curable disease.

C. Public Comment

No public comments were offered.

VIII. Review and Discussion of Human Gene Transfer Protocol #1304-1226 titled: A Phase I Study of Guanylyl Cyclase C (GCC)-Encoding Replication-Deficient Human Type 5 Recombinant Adenovirus Vaccine (Ad5-hGCC-PADRE) in Stage I and II Colon Cancer Patients

Principal Investigator:	Takami Sato, M.D., Ph.D., Thomas Jefferson University Hospital
Additional Presenters:	Adam E. Snook, Ph.D., Thomas Jefferson University; Scott Waldman, M.D., Ph.D., Thomas Jefferson University
RAC Reviewers:	Drs. Fong, Ornelles, and Pilewski

A. Protocol Summary

Colorectal cancer is the fourth leading cause of cancer and the second leading cause of cancer-related death in the United States and the world. Despite surgical, chemotherapeutic, and radiotherapeutic interventions, approximately 50 percent of patients with colorectal cancer die of metastatic disease. An established racial disparity in survival manifests as African-American patients with early-stage disease (Stages I and II) having a 40 percent greater mortality from metastatic disease compared with Caucasian patients. These observations illustrate the unmet clinical need for novel approaches to prevent metastatic colorectal cancer.

This is a feasibility study of the guanylyl cyclase C (GCC)-encoding replication-deficient human type 5 recombinant adenovirus vaccine Ad5-hGCC-PADRE) in stage I and II colon cancer patients. GCC is selectively expressed by intestinal cells and universally over- expressed by metastatic colorectal cancer cells. It exhibits a unique “split” tolerance comprising GCC-specific “killer” CD8+ T cells and antibody-producing B cells, but not “helper” CD4+ T cells. These unique characteristics have been leveraged by incorporating CD4+ T cell epitopes, like PADRE, into an Ad-based vaccine. Such vaccines generate robust T cell and antibody immune responses to GCC, providing effective life-long protection from colon cancer metastases in mice. Moreover, protection is achieved in the absence of intestinal autoimmunity, reflecting the well-established separation of mucosal and systemic immune compartments.

Ad5-hGCC-PADRE is composed of a recombinant, replication-deficient E1/E3-deleted Ad5 virus possessing an hGCC-PADRE expression cassette comprising the human cytomegalovirus (CMV) immediate early promoter driving expression of a fusion product of the extracellular domain of hGCC residues 1-430) and the CD4+ T cell epitope PADRE. *In vivo* expression produces the hGCC-PADRE immunogen that induces an immune response against hGCC.

In this first-in-human study, 22 African American and 22 Caucasian early-stage colon cancer subjects will receive a single dose of Ad5-hGCC-PADRE in the upper arm. Primary objectives include defining the safety of a single injection of Ad5-hGCC-PADRE and whether an antibody response to GCC is produced at one month following vaccination. Exploratory objectives will determine whether Ad5-hGCC-PADRE induces killer T-cell immune responses to GCC and whether and to what extent African American subjects and Caucasian subjects respond immunologically to the vaccine.

It is expected that risks associated with Ad5-hGCC-PADRE are low. No vaccine-related toxicities were observed in animal studies, and numerous clinical trials have demonstrated that Ad5-based delivery systems are safe. The investigators believe that risks associated with the vaccine are lower than the expected benefit from this clinical trial. In that context, this trial will make an important first assessment of the Ad5-hGCC-PADRE vaccine, which could reduce metastatic colorectal cancer. Thus, research participants in this trial could benefit directly from the vaccine, and the scientific community could benefit from the knowledge gained.

B. Written Reviews by RAC Members

Nine RAC members voted for in-depth review and public discussion of this protocol. Key issues included that it is a first-in-human trial.

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

Dr. Fong requested that the investigators provide their rationale for using GCC as an immuno-target, given that this protein is detected in normal intestines in addition to being detected in almost every colon cancer specimen. One of the endpoints of this study is to assay the lymph nodes collected at the time of colectomy; if that result is suggestive of micrometastases, Dr. Fong asked whether that information would be shared with the research participant. If that information will be shared, he queried as to how soon after vaccination these participants would be allowed to enter other trials without being removed from the current one. Dr. Fong asked how recent adjuvant chemotherapy might affect action of the current study agent vaccine and how such action would be taken into account for analysis. He suggested that a third cohort of other ethnicities, in addition to African American and Caucasian, might be useful. Because one of the side effects from resection of the large intestine is frequent bowel movements or liquid stools and the greatest off-target toxicity concern of GCC vaccination is on the intestines, Dr. Fong asked whether the investigators plan to use the number and consistency of stools as inclusion or exclusion criteria.

Dr. Ornelles noted that the safety of adenovirus vectors in immune-competent individuals has been well established and little concern exists regarding the inherent risks associated with a single intramuscular (IM) delivery of the agent in this study. He stated that the principle concerns associated with this study focus on the novel use of hGCC as an antigen expressed by adenovirus. Immunity derived from vaccination by an adenovirus platform appears to be particularly durable, and antibody responses to adenovirus-delivered antigens seem to persist. However, he wondered whether the persistence of this viral vector and prolonged expression of the transgene could have consequences beyond stimulating the intended immune response. He cautioned that the outcome of the STEP trial (an adenoviral vectored HIV vaccine efficacy trial) highlights the need to be vigilant in evaluating adenovirus-elicited immunological responses among individuals of varying immunological competence because of the correlation noted in that trial between immunity toward adenovirus and acquisition of HIV, which may have been due to qualitative differences in the individual's immune system, rather than the action of the adenovirus vector. Dr. Ornelles asked the investigators to discuss the possibility that the E1 deleted vector could replicate in tumor cells and in a participant with a high occult tumor burden could result in a disproportionately greater amount of hGCC antigen through the tumor cells, which could either enhance the immune response or, by recruiting tumor-associated immunosuppressive cells, diminish the effective immune response. Dr. Ornelles suggested that the investigators consider evaluating samples obtained for immunological studies for the presence of the adenoviral vector or gene products. Noting that preclinical studies in the mouse model suggest that self-immunity to murine GCC has no impact in the gut and because hGCC is expressed in dopaminergic neurons, Dr. Ornelles asked whether the mouse model provides an adequate test of consequences in the CNS or the enteric nervous system. Regarding the exclusion criteria, he also asked if many potential participants are expected to have been given a previous adenovirus vaccine.

Dr. Pilewski opined that the protocol is well written, and he noted that the hypothesis is based on mouse studies that suggest immune responses and reduction of tumor burden with a similar vaccine targeting murine GCC. He asked the investigators to discuss the rationale for enrolling participants during the period of two months to two years after surgical resection, especially given that many cancers recur early. Dr. Pilewski asked how the vaccine dose was chosen for this single-dose study: although the proposed vector dose is within that reported in prior human studies, it appears the murine dose necessary to elicit an immune response and tumor effect is much higher, and therefore the proposed dose in humans might be insufficient. Given that the major toxicity concern appears to be autoimmune inflammatory bowel disease, he asked the investigators how they will be certain of consistent surveillance for GI toxicity, and he suggested they consider using a GI symptom diary or a set of symptom questions at routine visits to ensure consistency. With regard to the informed consent document and process, Dr. Pilewski noted three statements that needed clarification, and he asked about coverage of medical expenses that are a direct result of this study and about the potential for investigators' financial conflicts of interest.

C. RAC Discussion

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

- Dr. Badley asked the investigators to discuss the evidence that an immune response against GCC helps thwart disease progression.

- Dr. Zoloth expressed concern that the African American and Caucasian research participants are self-selected for those groups. She asked whether the investigators planned to validate those groupings.

D. Investigator Response

1. Written Responses to RAC Reviews

Although GCC is expressed throughout the normal GI tract from duodenum to rectum, the investigators hypothesized that it could act as an ideal target for metastatic colorectal cancer, producing antitumor immunity in the absence of autoimmunity. In a patient with metastatic colorectal cancer, GCC is found in essentially two locations: in the intestine and in the metastases that typically occur in lung and liver. Thus, an ideal vaccine would induce GCC-specific immune responses in lung, liver, and other systemic tissues but not in intestines, producing immunity against metastases but not normal intestine. The immune system is compartmentalized to produce this exact scenario, in which immune responses can be produced in the systemic immune compartment but not in the intestinal compartment. This hypothesis is supported by approximately ten years of preclinical experimentation with GCC-based vaccines (adenovirus, poxvirus, and rhabdovirus vectors) that produced no GI toxicity and did not exacerbate experimentally induced inflammatory bowel disease.

One of the endpoints of this study is to assay the lymph nodes collected at the time of colectomy; if that result is suggestive of micrometastases, the results of the lymph node testing will not be shared with research participants because of the experimental nature of the technique.

Recent adjuvant chemotherapy would be expected to reduce the immunogenicity of the vaccine by compromising the patients' immune response. In contrast, growing literature suggests that some forms of chemotherapy can augment endogenous or vaccine-induced immune responses to cancer antigens. Prior chemotherapy is an exclusion criterion for this trial; future studies are required to define the impact of chemotherapy on Ad5-hGCC-PADRE efficacy.

The investigators explained that this trial was designed to include specific cohorts of African American and Caucasian research participants, reflecting the disparate outcomes in colon cancer and the need to define mechanisms underlying that disparity and identify novel treatments that can be effective in African American and Caucasian patients. Ad5-hGCC-PADRE will be tested in other ethnicities in the future, but inclusion of other ethnicities is beyond the scope of this first-in-human trial.

The number and consistency of stools will not be an inclusion/exclusion criterion. Although GI toxicity was not observed in preclinical animal studies, GI toxicity is the most likely form of toxicity that may be observed in study participants. Therefore, the investigators have expanded GI safety monitoring, employing weekly participant self-assessment questionnaires along with guidelines from the Rome III Diagnostic Criteria for Functional Gastrointestinal Disorders. In the questionnaire, symptoms of GI toxicity, including nausea, vomiting, retching, diarrhea, stool color, constipation, abdominal discomfort, and bloating, will be monitored.

Intramuscular administration of adenoviral vectors transduces muscle cells (myoblasts and myotubes) and stromal cells (e.g., fibroblasts, endothelial cells). Expression of transgenes can persist for months or years in muscle tissue; the mechanism for persistence is not defined. Although adenoviral DNA remains epichromosomal and the virus theoretically cannot be replicated by the cellular machinery alone in the absence of E1, elimination of vector DNA requires dilution by repeated cellular division or death/clearance of the transduced cells. Wild-type adenoviruses acquired by natural infections also persist for years in lymph nodes and tonsils, suggesting that a similar mechanism may operate for natural and recombinant vectors. Beyond sustained immune responses to vector transgenes, the implications of adenoviral persistence remain unknown.

With regard to the STEP trial and similar trials, the investigators explained that the apparent increase in HIV susceptibility following Ad5-based vaccines appears to be restricted to Ad5 vaccines for HIV. Ad5-

based vaccines for other infectious diseases and for cancer have not resulted in increased susceptibility to their respective indications. As a precaution based on the STEP results, the investigators plan to exclude individuals with HIV from this study. The investigators will monitor Ad5-specific antibody and T-cell responses pre- and post-vaccination to determine whether Ad5 immune status correlates with GCC-specific response rates.

Biodistribution studies in mice revealed negligible persistence in blood. However, the investigators said they will consider quantifying Ad5-hGCC-PADRE DNA levels in blood samples obtained for immunological evaluation prior to vaccination and at the 30-, 90-, and 180-day time points following administration.

The extracellular domain of GCC shares less than 20 percent homology with other guanylyl cyclases, and heterodimer formation among any of the guanylyl cyclases is not known to occur. Thus, it is unlikely that the GCC vaccine construct could act in a dominant negative fashion with other guanylyl cyclases.

GCC is selectively expressed only in GI and some CNS tissues, but not in muscle or immune cells that would be exposed to and/or express the truncated GCC vaccine construct. Thus, the expressed GCC protein within the vaccine is unlikely to affect endogenous GCC or other guanylyl cyclase function. The investigators have not observed any effect of GCC-specific immune responses on endogenous GCC function. In addition, they have not observed any effects of GCC-specific antibodies in serum or monoclonal antibodies produced by GCC vaccination on GCC activity *in vitro* or *in vivo*.

The investigators have shown previously that GCC is expressed in hypothalamic neurons, mediating a gut-brain endocrine axis regulating appetite in mice and humans, indicating that the mouse models employed in the preclinical safety and efficacy studies are representative of humans. CNS toxicity is not a concern; the investigators have shown that GCC immunization produces GCC-specific immune responses and antitumor immunity in mice, yet no gross or histologic toxicity is observed in any tissue, including GCC-expressing intestines and CNS tissues. Transgenic mice lacking GCC expression possess disrupted satiation after eating, consume more food daily, and gain weight over their lives, yet GCC vaccines caused no change in food intake or body weight in various studies. Therefore, the investigators conclude that GCC vaccination does not cause histopathologic or behavioral toxicity targeting CNS-expressed GCC. The phenomenon known as immune privilege protects the CNS from GCC-specific immune responses; the blood-brain barrier effectively excludes antibodies from penetrating CNS tissue.

Investigators do not expect many research participants to have been exposed previously to an adenovirus vaccine, and serious reactions to adenovirus vaccines are rare. However, the use of adenoviral vectors is rising, and large trials (primarily international trials) of HIV and tuberculosis vaccines employing adenovirus have been conducted. While the likelihood of a previous serious reaction to adenovirus vaccine in a potentially eligible patient is unlikely, the risks associated with exposing a patient to Ad5-hGCC-PADRE after experiencing a serious reaction to another adenovirus vaccine justifies excluding those individuals. The prior experimental medications exclusion criteria apply only to experimental colon cancer treatments; individuals who received an experimental adenovirus vaccine for any indication except colon cancer would be eligible for this trial.

The investigators acknowledged that enrolling research participants between two months and two years after surgical resection would affect the exploratory aims. However, those aims are exploratory only. The investigators explained that reducing the window of eligibility would significantly reduce their ability to enroll a sufficient number of participants to answer the primary study objectives of safety and immunogenicity.

The investigators explained why a single dose of $1x10^{11}$ vp of Ad5-hGCC-PADRE was selected for this Phase I trial. The safety of Ad5 vectors has been confirmed during the past ten years, and $1x10^{11}$ vp of Ad5 can be given IM to healthy volunteers with short-lived and self-limited symptoms of headache, myalgia, malaise, and fever. While immunity to Ad5, present in 32 percent of the population, can limit the effectiveness of Ad5 vaccines, doses of $1x10^{11}$ vp induce full cell–mediated and humoral responses to the antigens of interest. Doses of $1x10^{11}$ vp were immunogenic and well tolerated in mice, confirming the

safety of this vaccine dose in mice. In the context of the established safety of adenovirus vectors in humans and Ad5-mGCC-PADRE in mice, the investigators concluded that a dose escalation study that would lead up to $1x10^{11}$ vp was not warranted.

The investigators have not considered higher doses for two reasons. First, doses of Ad5-mGCC-PADRE reliably produced mGCC-specific antibody responses at doses of $1x10^{11}$ vp and $1x10^{10}$ vp. While the magnitude of response increased with dose, the frequency of positive responses was unaffected by dose. In the absence of pre-existing Ad5 immunity, doses of $1x10^{10}$ vp and $1x10^{11}$ vp are expected to be comparable with regard to antibody response rate in humans, which is a primary objective. However, the presence of pre-existing Ad5 immunity in humans would likely reduce the effectiveness of a dose of $1x10^{10}$ vp. T-cell responses to GCC were less robust than antibody responses, reflecting the established limitations in cryopreserving mouse splenocytes for ELISpot analysis. Human PBMC samples, in contrast, are unaffected by cryopreservation, suggesting that T-cell responses might be better in humans than those observed in mice, reflecting the likely underestimation of T-cell responses in mice. Second, doses above $1x10^{11}$ vp have been used safely in human research participants but are less common. Because the primary objective of this study is determining Ad5-hGCC-PADRE safety and doses of greater than $1x10^{11}$ vp have not been used routinely, the investigators have chosen a more cautious approach. If $1x10^{11}$ vp of Ad5-hGCC-PADRE is safe and well tolerated in this study, future studies will examine dose escalation and repeated dosing. The FDA has agreed with the investigators' rationale for employing $1x10^{11}$ vp.

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

2. Responses to RAC Discussion Questions

Regarding the evidence that an immune response against GCC will help thwart disease progression, Dr. Snook explained that little is known about GCC immunology. The investigators have shown that, in mice, the vaccine generates immune responses that are protective. However, in humans, no data are available and therefore this Phase I study is needed.

Regarding the subjectivity of research participants self-identifying as African American or Caucasian, Dr. Waldman indicated his familiarity with the data that state that people who describe themselves as African American could be 90 percent genetically Caucasian and vice versa. Much of the data that demonstrate a disparity in colon cancer outcomes between the African American population and the Caucasian population are based on self-descriptions provided by African American or Caucasian individuals; the investigators are specifically planning to study the disparity that encompasses self-description. Another study conducted by the investigators looked at occult tumor cells in lymph nodes in a self-described African American population and a self-described Caucasian population; the investigators found a disparity in outcomes—the self-described African-American population had worse outcomes than the self-described Caucasian population.

E. Public Comment

No public comments were offered.

F. Synopsis of RAC Discussion and RAC Observations and Recommendations

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

- Although this is a replication-defective adenovirus vector, it is known that E1-deleted adenoviruses can replicate under certain conditions and in particular in transformed cells. Therefore, the investigators should consider analyzing tissue and blood samples that are collected for immunologic studies for the presence of replicating adenoviral vector or its gene products.

- GCC is expressed on normal intestinal cells and therefore targeting of GCC may have the potential to cause GI toxicity. This toxicity may manifest as recurrent loose stools or frequent bowel movements. However, participants in this trial will have undergone colectomies for their cancer, and colectomy is also a risk for loose stools or frequent bowel movements. Therefore, the investigators should consider adding an exclusion criterion based on the frequency of stools so as not to confound the analysis of adverse effects.

Ethical, Legal, Social Issues

- The investigators have conducted a study of 257 patients with colorectal cancer and with no known nodal involvement. In these research participants, nearly 2,600 lymph nodes were assessed for GCC mRNA expression. The appearance of GCC mRNA expression was a significant predictor of cancer recurrence. This study will examine lymph nodes collected at the time of colectomy for the presence of GCC mRNA using quantitative RT-PCR. The collected data suggest that if mRNA levels are determined to be elevated, the individual has micrometastases. Research participants may be interested in knowing this information. The current plan is not to disclose these data, because the test is still considered experimental and is not approved by the FDA. Despite the fact that this is an experimental marker and such information could be used by participants to make health or treatment decisions, given the strength of the association found in the study, further discussion with the IRB is recommended about whether and how this information should be shared.

G. Committee Motion 4

Dr. Fong summarized the RAC recommendations to be included in the letter to the investigators, expressing the comments and concerns of the RAC. Dr. Fong asked for a vote, and the RAC approved these summarized recommendations by a vote of 19 in favor, 0 opposed, and 0 abstentions.

IX. Review and Discussion of Human Gene Transfer Protocol #1304-1224 titled: E10A (Endostatin Adenovirus) for the Treatment of Recurrent/Metastatic Squamous Cell Carcinoma of the Head and Neck

Principal Investigator:	George Yoo, M.D., Karmanos Cancer Center, Wayne State University
Additional Presenters:	Bob Choy, Ph.D., Marsala Biotech, Inc.; Nancy Stewart, Ph.D., Marsala Biotech, Inc.; George R. Thomas, Ph.D., Marsala Biotech, Inc.
Sponsor:	Marsala Biotech, Inc.
RAC Reviewers:	Ms. Dresser, Dr. Koch, and Dr. Strome

A. Protocol Summary

The creation of new blood vessels, known as angiogenesis, is a critical element in tumor formation and growth. Endostatin, a naturally occurring protein, inhibits angiogenesis. Antitumor activity of endostatin protein has been demonstrated in various mouse and human studies without detectable toxicity. Phase I trials of endostatin administered by daily IV infusion or continuous infusion have confirmed the safety of endostatin.

E10A is replication deficient E1 and E3 deleted adenovirus containing the human endostatin gene. Endostatin has been shown to block endothelial cell proliferation, survival, and migration. Antitumor activity of endostatin protein has been demonstrated in various murine and human tumors in animal studies without any detectable toxicity. Endostatin gene therapy can directly express a highly bioactive protein *in vivo* allowing for post-translational modification and proper protein folding, as well as overcoming the challenge of long-term storage and cumbersome daily administration of endostatin protein. After transfecting tumor cells, E10A expresses human endostatin, which inhibits vascular endothelial cell proliferation and tumor angiogenesis, and blocks tumor blood supply, thereby specifically inhibiting tumor growth and inducing apoptosis of tumor cells. Pre-clinical models have demonstrated the

anti-tumor activities of E10A. The safety and efficacy of E10A in treating head and neck cancer has also been demonstrated to be favorable in Phase I and II studies. Preclinical studies demonstrated that intratumoral injection of E10A provided significant tumor growth inhibition and sustained elevation of endostatin in blood and tumor tissue in hepatocellular carcinoma, nasopharyngeal carcinoma, and tongue cancer animal models. E10A treatment provides an additional benefit in combination with chemotherapy. In one example, E10A, in combination with a weekly low-dose of cisplatin in a xenograft mouse model for head and neck cancer, enhanced the tumor growth inhibitory effects of cisplatin, inhibited angiogenesis, and induced high levels of apoptosis. In addition E10A increased the platinum concentrations in tumors to five times higher than that of which occurred with cisplatin treatment alone

The purpose of this study is to determine whether E10A in combination with chemotherapy is more effective than chemotherapy alone. The chemotherapy given is not a research drug; it is approved for the treatment of this type of cancer. To date, 100 research participants have been dosed in previous studies with E10A.

B. Written Reviews by RAC Members

Ten RAC members voted for in-depth review and public discussion of this protocol. Key issues included the first use of this agent in the United States. In addition, several aspects of the design of this Phase III study deserve further discussion, including the rationale for additional chemotherapy agents not included in the Phase II trial, the exclusion of a chemoradiation arm for comparison, and whether tumor location will be factored into the analysis.

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

With regard to the risk/benefit ratio, Ms. Dresser noted that participants in this trial will receive different chemotherapy agents than those received by participants in the earlier-phase trials. Therefore, she asked the investigators to discuss the evidence that exposure to different chemotherapy agents would not produce a different (possibly toxic) response in the Phase III participants and whether a smaller trial of participants receiving the same chemotherapies is needed before this Phase III trial is conducted. In addition, if this study goes forward, the informed consent document should indicate this difference as well as state that participants in this study could experience additional side effects that were not detected in the earlier human trials. Ms. Dresser observed that the informed consent document contained numerous references to the investigational intervention as “therapy” or “treatment,” implying that the intervention has been shown to be safe and effective; she suggested that the investigators consider using such terms as “investigational drug,” “study drug,” or “gene transfer agent.” She requested clarification of the statement in the informed consent document that the investigators expect no benefit to participants in this trial, which raises the question of why a phase III trial is being pursued.

Dr. Koch summarized this protocol as well written and straightforward, and noted that the vector has proven to be safe with multiple injections and has shown some trends toward a positive benefit. He suggested clarifying some inconsistency in injection numbers and requested data in animals or humans that show whether the peak endostatin expression remains the same or wanes due to neutralizing antibodies after this multitude of injections. He asked whether there has been human experience with up to 12 repeated administrations of this vector and the resultant transgene expression, and he wondered about the expected liver effects of this multiple dosing, including the effect on liver enzymes. With regard to exclusion criteria, Dr. Koch suggested that angina status or other indices of underlying coronary artery or ischemic heart disease should be addressed or clarified. In addition, he asked the investigators to discuss how the vector would be stored at the multiple sites, how much would be on hand, and whether stocks over time would be tested *in vitro* or in animal models for sustained effectiveness.

Dr. Strome offered several suggestions related to the inclusion criteria:

- The investigators should consider adding to this trial a radiation ± chemotherapy arm. The likelihood of chemotherapy alone curing metastatic or recurrent head and neck squamous cell

carcinoma is nil, and adding E10A has not had a significant impact relative to cure. Therefore, to show any meaningful data, this additional arm should be included.

- Only one chemotherapy regimen was used in the Phase I and II trials, but this trial proposes to include additional chemotherapeutic regimes; the response in combination with the construct might differ. These additions make the proposed trial unnecessarily complicated and could prove to be detrimental.
- The inclusion of patients refusing standard therapy who could be curable in favor of palliative care raises questions relative to a properly executed informed consent document and process.
- Throughout the protocol, “advanced” versus “non-resectable” are used interchangeably, which is incorrect. Untreated advanced disease is often resectable and, if necessary, treatable with adjuvant post-operative chemoradiation. Therefore, the only non-treated group appropriate for inclusion in this trial would be those in Stage 4C (distant metastatic disease at presentation).
- It is important to know the locations of the primary tumors in the reported data (the China studies) and whether they are relatively evenly distributed between arms. Significant differences in responsivity to chemotherapy regimens relate to site.
- It is necessary to know the human papillomavirus (HPV) status in all research participants. HPV status is significant in that positivity portends a significantly better prognosis for all stages with standard therapy. Sixty-three percent of oropharyngeal cancer in the United States is HPV associated (mostly tonsil and tongue base).
- Given that most of the participants in the researchers’ prior studies were Asian, he asked how relevant this data would be to African Americans.

With regard to the use of Avastin, Dr. Strome asked the investigators why they believe this trial is better than considering Avastin in place of E10A; Avastin inhibits angiogenesis and sensitizes tumor cells to cisplatinum. He asked why the investigators plan to end this study at 12 months following the last dose, and he suggested a follow-up interval of at least five years or as long as there are survivors. He suggested that an inclusion criterion of four weeks following the last treatment for entry may be too short, and he suggested waiting eight weeks to be certain that all malignant cells with the potential to respond to radiation or chemotherapy have done so. Dr. Strome also noted that endostatin is primarily cleared by the liver and that some of the newly included chemotherapy regimens have the potential to cause liver cellular injury; adding endostatin could lead to severely compromised function and/or associated severe myelosuppression. Dr. Strome offered several suggestions for changes to specifics in the protocol. In addition, he noted several points in the informed consent document that needed clarification or elaboration.

C. RAC Discussion

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

- Dr. Fong stated that the investigators should ensure that the data supports a Phase III trial. If repeat intensity-modulated radiation therapy (IMRT) is a standard of care, that fact needs to be stressed somewhere in the protocol, especially if it is possibly curative.
- Dr. Fong expressed concern that the agents to be used in this Phase III trial have not been tested in prior-phase trials.
- Dr. Fong was concerned that the investigators would be conducting a Phase III trial without Phase I or Phase II data on the combination drugs. In addition, because they are using combination therapies with new drugs, the investigators do not fully know the toxicities or whether the regimen will silence the vector and deliver no viable vector or expressible gene. This situation could sabotage this Phase III trial.

D. Investigator Response

1. Written Responses to RAC Reviews

The investigators explained that no additional adverse effects are anticipated from E10A given in conjunction with the different chemotherapeutic agents, because most effects from E10A are seen as local toxicity. This study will closely monitor for toxicity and has dose modification protocols in place if Grade 3 or Grade 4 toxicities are observed. The study design of this trial is adequate to detect additive toxicities.

In animal models, only a single injection of E10A has been examined. Dynamic levels of E10A DNA *in vivo* last 11 days. In BALB/c mice, endostatin protein levels rose to peak values day 5 post-injection, with the highest levels in tumor tissue followed by liver and blood. Endostatin levels in tumors remained elevated after nine days post-injection, whereas levels in other tissues returned to normal.

In the Phase II trial, serum levels of adenoviral E10A DNA peaked at three days after the first dose (day 1), and four days after the second dose (day 8). After 21 days, E10A DNA was not detected in the serum in almost all of the research participants in the Phase II trial, and low levels of DNA were detected in only two individuals. This result was consistent over repeated treatment cycles (up to 6 cycles or 12 repeated administrations). Serum levels of endostatin peaked at three to five days after the first dose and 3 to 5 days after the second dose. Endostatin levels returned to baseline after 26 days for each cycle, which was consistent over repeated treatment cycles. Twelve out of 20 participants had pre-existing immunity to Ad5 prior to the study; all developed anti-Ad5 immunoglobulin G and immunoglobulin M by the end of the study.

Regarding the expected liver effects of this proposed multiple dosing, the investigators explained that intratumoral administration maximizes initial delivery of vector into the tumor; it is possible that virus particles could be quickly lost from the tumor, either by drainage into the circulation lymphatic or blood vessels or by fluid seepage back through the site of injection. Adenoviral DNA can be found in the liver after 30 minutes, presumably trafficking there via the bloodstream, which suggests rapid clearance into the liver from the blood. Rapid hepatic accumulation of systemically delivered adenovirus is mainly a result of capture by phagocytic Kupffer cells, which causes a nonlinear dose response for adenovirus vector–mediated transgene delivery into hepatocytes. Adenoviral interactions with Kupffer cells induce activation of a variety of proinflammatory responses.

Each clinical batch of E10A will be stored at -70ºC and distributed from the manufacturing facility. A certain amount of each lot is retained for stability testing. Each clinical batch is tested for stability testing upon production and every three months subsequently, which includes integrity of the viral particles, endostatin production, and activity *in vitro*. Onsite, viral stocks will be stored at -70ºC in a secured, limited-access location. Each clinical trial site will be given enough stock to dose five research participants. In the event that any clinical lot reaches the end of its lifespan or fails stability testing, any stock present at the clinical sites will be withdrawn and replaced with a new lot.

The investigators explained that this protocol is intended to study those patients who are not suitable for surgery or radiotherapy; therefore, patients who are suitable for either treatment regimen are excluded. Comparison against a radiation ± chemotherapy arm would be inappropriate because it represents a different patient population.

For patients with distant metastatic disease, a local therapy (radiotherapy) would not be added (National Comprehensive Cancer Network [NCCN] Guidelines Version 1.2013, ADV-3 Very Advanced Head and Neck Cancer). For those with unresectable recurrent or persistent disease, most will have failed radiotherapy. Both ADV-2 and ADV-3 guidelines recommend chemotherapy for those patients who have failed radiotherapy. Reirradiation ± chemotherapy is an allowed option in the NCCN guidelines for recurrent/unresectable disease. It will be up to each site to determine whether reirradiation is an option and thus would constitute an exclusion criterion from this study.

The investigators' overall survival data are encouraging, since 18.8-month survival in this patient population is better than the historical survival data of eight to ten months. This study will be looking at patients with Stage IV-B (recurrent) and IV-C cancer. Standard therapy for these stages is chemotherapy, according to NCCN guidelines. Enrollment in this trial will be limited to those individuals for whom no other local therapy is available and to patients with metastatic disease who need systemic chemotherapy.

The Phase II trial looked at the standard-of-care chemotherapeutic regimens in China; the proposed Phase III trial is intended to look at the current standard of care for chemotherapy in North America in recurrent, unresectable, or metastatic (incurable) head and neck cancer. The investigators will be testing the synergistic and additive effects of adding E10A to standard cytotoxic chemotherapy. The regimens for this trial will be restricted to one of three most common regimens to minimize complication in randomization and analysis. Stratification of the different chemotherapy regimens will be included within the randomization programming for the trial, which also will be monitored during enrollment to ensure a proper balance is maintained between the combination (E10A + chemotherapy) and chemotherapy-alone arms.

HPV status was not measured in the Phase I and Phase II trials, but HPV status will be collected in the proposed trial if that information is available. HPV status has been correlated with favorable oropharyngeal carcinoma prognosis, although its correlation with clinical outcome in other head and neck cancers is unclear. As patients with HPV-positive oropharyngeal tumors are generally newly diagnosed and likely would be eligible for surgical and/or radiation/radiochemotherapy, they would not be eligible for this trial. According to NCCN guidelines, HPV testing is not indicated as a routine consideration in treatment selection.

Research participants were not randomized based on primary site or N Stage in the Phase II study; however, that distribution appears to be random between the E10A + chemotherapy and chemotherapy-alone groups. There is no significant difference in survival for the primary site and N stage when dealing with recurrent/unresectable and distant metastatic disease. Randomization stratification will be based on the chemotherapy and whether a patient is refractory to chemotherapy or has a first-time recurrence to chemotherapy.

The sponsor recognizes the higher incidence of nasopharyngeal carcinoma in patients enrolled at the sites of the previous studies compared to that seen in the North American population, but the majority of the primary Asian sites enrolled non-nasopharyngeal research participants. The investigators do not anticipate any other differences between Asians with head and neck cancer and non-Asians with head and neck cancer, except for the proportion of nasopharyngeal cancers. No study shows differences in survival between Asian and non-Asian populations when nasopharyngeal cancer is excluded.

The sponsor is currently evaluating E10A in conjunction with standard-of-care treatments as outlined in the NCCN guidelines. However, the sponsor is not currently considering comparison to other off-label use of medications (such as Avastin) for head and neck cancer.

All human hypopharyngeal cancer xenografts in BALB/c mice showed increased sensitization to cisplatin in combination with E10A versus cisplatin alone. Tumor tissue samples after treatment were analyzed for cisplatin levels; treatment with E10A significantly increased the intratumoral cisplatin concentration. Lewis lung cancer tumors in C57BL/6 mice also exhibited sensitization to cisplatin when combined with an adenovirus expressing endostatin (Ad-Endo); treatment with a combination of cisplatin and Ad-Endo resulted in marked inhibition of tumor growth.

Longer follow-up is not part of the trial design because the median endpoint—survival—is expected to be reached within six months of enrollment. No long-term toxicities have been observed with previous Ad5 vectors.

No statistically significant increase in liver enzymes AST and ALT or in liver adverse events was observed in the Phase I and Phase II studies. The sponsor is aware of the historical data for adenoviral vectors and will include stringent monitoring for liver toxicity in this study. In addition, dose modification

recommendations are included if Grade 3 or Grade 4 toxicities are observed. Clinical trials using adenoviral vectors similar to E10A have been shown to have low toxicity, with either no liver toxicity or mild, transient increases in AST and ALT. Liver enzymes recovered to pretreatment values within 10 days.

Based on RAC reviewer suggestion, the investigators will add further stringent vascular disease exclusions into the exclusion criteria.

The investigators agreed to add the following two statements to the informed consent document to reflect specific suggested clarifications:

> 1) "This study uses different chemotherapy drugs than those used in the previous human studies. There may be other risks and side effects associated with the use of E10A and the chemotherapeutics being used that are not known at this time."
>
> 2) "There may not be any benefit to you, but your participation in this research study may help to develop knowledge for others in the future."

In addition, the investigators agreed to change and clarify language in the informed consent document as suggested by the RAC reviewers.

2. Responses to RAC Discussion Questions

Dr. Yoo explained that this trial offers a different design than most targeted therapy trials. The investigators for this trial will enroll a group of subjects with poor prognosis and will use acceptable chemotherapy regimens on them. This clinical trial is testing the anti-angiogenesis property of E10A with standard cytotoxic therapy in what is considered to be standard chemotherapy. Reradiation may be curative in a single-institution setting but not in a randomized, controlled multi-institution setting. For this trial, the heterogeneous population of patients with different chemotherapy exposures will receive the standard of care plus anti-angiogenesis.

E. Public Comment

No public comments were offered.

F. Synopsis of RAC Discussion and RAC Observations and Recommendations

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

- This trial proposes to use multiple chemotherapy agents, but the available animal and previous clinical data are from studies that have only tested E10A with cisplatin. While a goal of this trial is to allow participants to receive standard-of-care therapy, which includes chemotherapeutic agents that have not been tested in either animal or clinical trials in combination with E10A, the addition of these agents that have not been tested with E10A may lead to unexpected toxicities and could confound the analysis. The investigators should consider limiting this Phase III trial to those who have had previous non–cisplatin-based chemotherapy and for which cisplatin would therefore be standard of care, thus limiting the number of chemotherapy agents that would be studied with E10A. Alternatively, the investigators could gather additional data—for example, using a Phase II/III trial design to test E10A in combination with the other chemotherapeutic agents—prior to embarking on this Phase III trial.

- Because the investigators did not collect HPV status in the Phase I or Phase II trials, no data exist on how E10A performs in HPV+ cancers compared with HPV- cancers. Given the more favorable prognosis of head and neck cancer with positive HPV status, this trial should gather data on HPV status of participants because this will help to inform the results.

- The trial screening procedures include a CAT scan of the lungs or abdomen if distant metastases are suspected. However, a chest x-ray, CAT scan, or PET scan are adequate to evaluate for lung metastasis in individuals in whom metastases are not suspected. New data indicate that a low-dose CAT scan has greater sensitivity albeit with some increase in radiation. Having some individuals have a chest x-ray and others have the more sensitive CAT scan might lead to inconsistent results in detecting lung metastases and staging patients. The investigators should consider whether a low radiation dose CAT scan should be required to screen for lung metastases in all potential research participants to ensure consistent screening results.

Ethical, Legal, Social Issues

- The patient population eligible for this trial consists of individuals who have recurrent or metastatic disease and are not eligible for surgery or radiation. The standard of care has evolved and reradiation with IMRT is an option for a number of patients who recur after radiation/chemotherapy. However, some sites for this trial will not have IMRT and, therefore, there is a concern that a patient could be referred to this trial because he or she is seeking treatment at a site that does not have IMRT as an option. The informed consent document should inform potential participants who enroll at sites that do not offer IMRT that this is a potentially curative therapy and provide information on clinical sites that may be able to offer IMRT. In addition, the informed consent document should clarify that chemotherapy is not curative for this population.

- Avastin has been used in head and neck cancer as an anti-angiogenic agent and has been shown to sensitize tumor cells to cisplatin. This alternative approach should be discussed in the informed consent document.

G. Committee Motion 5

Dr. Fong summarized the RAC recommendations to be included in the letter to the investigators, expressing the comments and concerns of the RAC. Dr. Fong asked for a vote, and the RAC approved these summarized recommendations by a vote of 17 in favor, 0 opposed, and 2 abstentions (Drs. Strome and Zoloft).

X. Certificates of Appreciation to Retiring RAC Members

Presenter: Amy Patterson, M.D., OD, NIH

Dr. Patterson presented the following departing RAC members with certificates of appreciation for their service on the RAC: Dr. Badley (served since 2010), Dr. Chiocca (served since 2010), Ms. Mallino (served since 2009), Dr. Ross (served since 2009), and RAC Chair Dr. Fong (served since 2009 and chaired the RAC since 2011).

Dr. Patterson announced that Dr. Kohn will take over as RAC Chair beginning with the September 2013 RAC meeting.

XI. Day 1 Adjournment

Dr. Fong adjourned Day 1 of the June 2013 RAC meeting at 5:15 p.m. on June 11.

XII. Day 2 Call to Order and Opening Remarks

Dr. Fong opened Day 2 of the June 2013 RAC meeting at 8:00 a.m. on June 12.

XIII. Update on Stem Cell Gene Transfer for the Wiskott Aldrich Syndrome

Presenter: Christoph Klein, M.D., Ph.D., Dr. von Haunersches Kinderspital, University of Munich Medical Center *(via teleconference)*

A. Presentation by Dr. Klein

Dr. Klein discussed his and his colleagues' current experience in retrovirus stem cell gene transfer for individuals with WAS, which is a rare disorder of the immune system. Clinically, affected boys present with recurrent, life-threatening infections, autoimmunity, eczema, autoimmune hemolytic anemia, nephritis, and vasculitis. WAS protein (WASp) deficiency causes low counts of platelets and a bleeding disorder, and patients have an intrinsic susceptibility to develop lymphoma or leukemia. Most patients with lymphoma have lymphoma associated with Epstein-Barr virus, and life expectancy is shortened. Patients with serious WAS die within the first two decades of life unless they are treated with allogeneic hematopoietic stem cells (HSCs) using classic bone marrow transplant procedures. Patients who do not have access to a source of human leukocyte antigen (HLA)-–identical allogeneic stem cells have a dismal outlook. Only about 50 percent of patients survive a non–HLA-identical transplantation. The incidence of WAS is between 1 in 500,000 and 1 in 1 million.

Because a variety of ethical aspects and issues needed to be resolved, the investigators teamed up with bioethicists at the University of London. Their collective basic insight was that extramural therapy should only be conducted in children if there is solid preclinical evidence that the individual children could benefit directly from this experimental procedure. WAS patients were recruited from several countries, which was logistically difficult because all the patients came back for follow-up every three months initially, then every six months, and now once a year.

Based on multiple preclinical studies conducted in mice and in human cells, the investigators proposed a scheme that would purify HSCs from children. At the time the investigators designed this protocol, only conventional retroviral vectors were available, so this protocol was based on a classical first-generation gamma retroviral construct. WAS patients were conditioned and prepared with busulfan at a dose of 8 mg/kg of body weight, which causes myelosuppression and facilitates engraftment of genetically modified cells.

Dr. Klein showed data on the reconstitution of WASp expression in the peripheral blood cells of the ten research participants, including CD4, CD8, CD19, CD3-/CD56+, monocytes, and platelets. A CXCR4 antagonist was used in eight of the ten research participants to help engraftment. T cells, B cells, and natural killer (NK) cells show rapid increase in the percentage of corrected cells. Myeloid cells are slower to reconstitute, which likely is related to the fact that lymphoid cells (in contrast to myeloid cells) have a stronger advantage when they express WASp in comparison to their WASp non-expressing counterparts. Gene marking has shown that 10 to 20 percent of the cells are reconstituted functionally. In most participants, this procedure has resulted in complete normalization of the platelet count, although a few participants have subnormal counts; however, none of these participants had any significant bleeding episodes after the procedure.

Clinically, the ten research participants in this trial initially showed all the striking clinical improvements. They stopped bleeding, none had any severe infections (with the exception of one individual who had a sepsis episode), and many participants had substantial improvement with respect to autoimmunity. One participant had colitis and, after gene transfer, evinced intact mucosal aspects. Another participant had severe and refractory eczema prior to gene transfer that resolved over time and has not recurred. Overall, the investigators saw clear evidence of good gene marking and functional reconstitution of platelets, T cells, B cells, and NK cells.

Dr. Klein acknowledged that the investigators knew at the start of this study that retroviral gene transfer can be associated with severe side effects, including activation of oncogenes. Classical gamma-retroviral vectors have a tendency to insert themselves at transcriptional sites, so the investigators planned long-

term monitoring of the retroviral insertion sites. In total, the investigators have characterized more than 70,000 integration sites in these ten research participants during the years after the clinical trial. Initially, the results looked promising; with the exception of participant 3, who did not have any sustained engraftment and had to drop out of the trial, the other participants had large numbers of insertion sites.

The first sign of concern was participant 6. As a four-year-old boy, he had gene transfer in June 2009. Initially, like the other participants, he recovered well and showed clinical improvement and unremarkable hematopoiesis with no evidence of cytopenia, chromosomal translocation, or cytogenetic aberration of the bone marrow. He had a normal blood count on day 458 followed by a few days of fever and bronchitis, and he came back on day 488 with full-blown acute lymphoid leukemia (ALL). Dr. Klein showed a summary of the six of ten study participants who developed malignancies. With a total of ten participants, one dropped out early, five developed T-cell ALL, one developed myelodysplastic syndrome (MDS) and acute myeloid leukemia (AML), and three are currently healthy and remain in follow-up without signs of any problems.

Within the past two weeks, two study participants who initially presented with T-cell ALL, and who were treated for 1½ to 2 years without chemotherapy, returned with secondary leukemias. The individuals with T-cell ALL have LMO2, a few have TAL1, and the one individual who presented with AML has MDS1 insertions. Dr. Klein noted that LMO2 has been the most critical gene in T-cell ALL development in the X-SCID trial in France, and MDS1 was affected in the German trial for chronic granulomatous disease.

The investigators now are working on other vector systems. Dr. Klein expressed the hope that novel configurations of inactivated viruses might add safety features while maintaining the striking efficacy already observed. Their current studies focus on preclinical investigations in cells and mice.

Dr. Klein summarized the investigators' experience in this clinical trial: ten research participants showed stable expression of the WAS protein in relevant hematopoietic lineages upon transplantation of autologous gene-modified cells. Leukocytes and platelets were functionally corrected. Immunodeficiency, bleeding, and diathesis resolved completely, and autoimmunity resolved partially. Polyclonal hematopoiesis occurred with insertions in multiple loci. However, unacceptably high toxicity occurred—six of the ten study participants developed acute leukemia. As a result, it is clear that improved systems, better vectors, and novel genetic engineering tools are needed to treat these devastating diseases.

B. RAC Discussion

Dr. Kiem wondered whether the other four research participants have shown clone abnormalities or worrisome integration sites. Dr. Klein responded that one individual was treated with haplo-identical transplantation and is no longer participating in the study. The remaining three participants return regularly every six months for evaluation. One individual is completely fine. Another individual initially proved worrisome because of an MDS1 clone ranging up to 15 percent in a fluctuating pattern; however, at his latest visit last week, his bone marrow was fine, and cytogenetic and molecular data are pending. The third individual will come back next week. Although he is the oldest study participant, had the slowest engraftment, and has the worst level of chimerism of WASp positivity T cells, ranging only to about 30 to 40 percent, he did not have any discernible clones.

In response to Dr. Kiem's query about using haplo-identical transplantation for these children, Dr. Klein explained that the investigators opted to transplant two of the T-cell ALL participants immediately, strictly following the ALL protocol. For those who did not meet the risk criteria for allotransplant, the investigators treated them using chemotherapy. Dr. Klein noted that if the investigators had known that those individuals would come back with AML, they probably would have received transplantation up front. So far, the transplant procedure has been successful in every child except one, participant #5. He had T-cell ALL and a transplant was done, with a relapse early after transplantation. Approximately five weeks after the transplant, this study participant came back with leukemia cells. This early relapse could not be controlled and he died two weeks later.

Dr. Sarzotti-Kelsoe asked whether there was a correlation between age at treatment and the leukemic complications. Dr. Klein explained that the investigators have not been able to establish any risk factors associated with the onset of leukemia. They tried a variety of parameters, including number of cells, reconstitution time, and age. Looking at the molecular pattern reinforces the idea that a number of stochastic events cannot be followed, at least in T-cell ALL. The investigators have not seen an increasing percentage of a premalignant clone. Everything looks quiet and suddenly, within a month, full-blown leukemia occurs. This course of development suggests that early T-progenitor cells are acquiring an unknown number of additional mutations that then causes leukemia.

With regard to the two study participants who developed secondary AML, Dr. Klein stated that the data are not yet available as to whether the clones contain a vector. He promised to share this information once the investigators receive it.

C. Public Comment

No public comments were offered.

XIV. Review and Discussion of Human Gene Transfer Protocol #1304-1223 titled: A Phase I/II, Non-Randomized, Multicenter, Open-Label Study of G1XCGD (Lentiviral Vector Transduced CD34+ Cells) in Patients with X-Linked Chronic Granulomatous Disease

Principal Investigator:	Donald Kohn, M.D., University of California, Los Angeles
Additional Presenters:	Peter E. Newburger, M.D., University of Massachusetts Medical School; Adrian Thrasher, Ph.D., University College London Institute of Child Health *(via teleconference)*; Leo Wang, M.D., Ph.D., Harvard Medical School and Boston Children's Hospital; David A. Williams, M.D., Harvard School of Medicine and Dana Farber Cancer Institute
RAC Reviewers:	Ms. Dresser, Dr. Kiem, and Dr. Ross

Drs. Cai, Cannon, Chiocca, Fost, Koch, Kohn, and Zoloth were recused from consideration of this protocol due to conflicts of interest.

A. Protocol Summary

X-linked chronic granulomatous disease (XCGD) is an inherited disorder with severe recurrent infections in which white blood cells are unable to kill bacteria and fungus. XCGD patients can be managed with medical measures, such as prophylactic and therapeutic antibiotics, but some patients have severe or persistent infections and other complications. Transplantation of bone marrow or other sources of blood-forming HSCs, such as umbilical cord blood or mobilized peripheral blood stem cells (PBSCs) from a healthy, well-matched donor can lead to production of normal white blood cells, which could improve infections. However, transplants from less-well-matched donors have higher risks for complications, and suitable matched donors may not be found for some patients who could benefit from transplantation.

X-linked Chronic Granulomatous Disease (XCGD) results from mutations in the *CYBB* gene encoding the gp91phox protein, a component of the anti-microbial oxidase activity in phagocytic leukocytes. Allogeneic hematopoietic stem cell transplantation (HSCT) can be clinically beneficial for XGCD, by allowing production of normal leukocytes from the donor hematopoietic stem cells (HSC) which may provide anti-microbial immunity. Graft versus host disease risks and lack of well-matched donors may limit the availability of HSCT for some XCGD patients. Transplantation of autologous HSC that are genetically corrected may provide similar benefits as allogeneic HSCT, although prior clinical trials for CGD had severe complications from the gene transfer vector causing leukoproliferation, myelodysplasia and leukemia. A new gene transfer vector has been developed for XGCD that may have reduced potential to cause this complication, although this has only been shown using murine cells in culture and transplants. This is a prospective non-controlled, non-randomized Phase I/II clinical trial to assess the safety and efficacy of cellular gene therapy in subjects with XCGD. Transplantation will be performed using

autologous G-CSF mobilized CD34-selected peripheral blood stem cells (PBSC) transduced *ex vivo* by the G1XCGD lentiviral vector, carrying a codon-optimized human *CYBB* cDNA under transcriptional control of a chimeric promoter derived from cellular genes expressed in myeloid cells. It is packaged by transient transfection in a VSV-G pseudotype, concentrated by down-stream processing, and certified to meet release criteria for potency, identify, sterility and absence of replication competent lentivirus. Subjects will be ten males with XCGD and refractory infections or inflammatory complications lacking an HLA-identical sibling or unrelated donor, meeting defined eligibility criteria. Subjects will undergo mobilization of PBSC with Granulocyte-colony stimulating factor (G-SCF) for up to 5 days followed by leukopheresis. A portion of the leukopheresis product will be cryopreserved without processing to serve as a “back-up”. CD34+ PBSC cells will be isolated from the remainder of the leukopheresis product and transduced by *ex vivo* culture with the G1XCGD lentiviral vector in the presence of recombinant hematopoietic growth factors. During this time, the subjects will receive non-myeloablative conditioning using busulfan (with dose adjustment based on measurement of busulfan pharmacokinetics) over three days. Final cell products (G1XGCD-transduced autologous CD34+ PBSC) that meet release criteria will be transplanted by intravenous infusion. Standard in-patient clinical care for hematopoietic stem cell transplantation will be provided. The follow-up period will include an initial two years of out-patient post-transplantation visits at regular intervals, for monitoring safety and efficacy end-points, followed by offer of enrollment into a long-term follow-up study during years 3-15. The objectives of this study are to assess safety and efficacy, in terms of reconstitution of biochemical and functional leukocyte oxidase activity, improvement of immunity to bacterial and fungal infections, as well as evaluation of post-transplant HSC engraftment kinetics and stability.

B. Written Reviews by RAC Members

Nine RAC members voted for in-depth review and public discussion of this protocol. Key issues included the use of a novel lentiviral vector with a chimeric promoter in a pediatric population. In addition, the data supporting the safety of this vector, compared to a previous retroviral vector used for chronic granulomatous disease (CGD) that led to clonal expansion due to insertional mutagenesis, deserve further discussion.

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

With regard to selection of research participants, Ms. Dresser asked the investigators whether it would be possible to limit the study population to older children and adults, noting that, in high-risk studies similar to this study, the most ethically defensible approach has been to rely on participants who are able to understand the risks and trade-offs and to decide for themselves whether to enroll. She also asked whether it is possible to establish in as little as two years that children will do poorly on conventional therapy and whether one persistent infection is sufficient to demonstrate this conclusion. Ms. Dresser noted that the informed consent document includes a statement about finding a suitable donor for participants whose immune systems fail to recover after participation in this trial; that statement prompted her to question whether the eligibility criteria should be changed to require a longer search for a suitable donor before enrollment or, if not, to clarify that transplant is an unlikely option. Ms. Dresser stated that the informed consent document should clarify that this research study is the first human trial of this experimental intervention, and she offered ten additional suggestions for clarifications and changes within that document.

Noting that this protocol is well written and will address some of the major problems with previous CGD studies, Dr. Kiem inquired as to the longest follow-up of *in vivo* expression in human CGD-affected and non-affected cells. He asked the investigators to detail the number of NOD SCID gamma (NSG) mice with persistent expression and whether any secondary mouse studies were conducted with human cells. For bone marrow or peripheral blood graft harvest in advance of the experimental treatment, he asked about the criteria of harvesting marrow versus peripheral blood and the timing before harvest for the CD34 cells for transduction. He wondered whether granulocyte colony stimulating factor (G-CSF) or Plerixafor, if used for the backup, could influence the composition of marrow or peripheral blood collection used for the transduction and possibly also the effect of the conditioning. Dr. Kiem asked the investigators whether they plan to test for the possibility that an immune response to the vector/transgene could limit

engraftment based on results from other CGD studies in which nonmyeloablative conditioning regimens did not result in significant engraftment beyond 3 months in the absence of insertional mutagenesis. He also asked whether they have considered including fludarabine in the conditioning regimen. Dr. Kiem requested that the investigators discuss the basis for setting an endpoint of 5 percent of cells expressing granulocytes at 12 months, whether a research participant who has reached 5 percent would continue to have infectious complications, and whether such individuals would be candidates for HSC transplantation (HSCT) if a donor could be identified. With regard to the informed consent document, Dr. Kiem suggested that the investigators mention the existence of a risk for prolonged cytopenia (likely for at least eight weeks) in case of graft failure, because backup marrow would not be infused until six weeks after transplant and would likely take another two weeks to engraft.

Dr. Ross noted that this protocol is exceptionally well written and the rationale is clear for moving to lentiviral vectors with myeloid-specific promoter/enhancer regions to correct the gp19phox deficiencies. She stated her appreciation for the care with which the investigators qualify their statements regarding whether lentivirus vectors are more efficacious in transducing cells or are safer than gamma-retroviral vectors. She expressed concern about testing a potentially oncogenic vector, despite its possible greater safety, in such a young population; all previous CGD gene transfer trials involved research participants older than age 21 years. Dr. Ross asked the investigators to consider whether a three-month safety review of the first three participants is a sufficient amount of time before proceeding with additional dosing, particularly because a previous study of gamma-retrovirus gene transfer with CGD resulted in the appearance of myelodysplasia five months after dosing. She asked which safety issues would be measured at three months and how those results would be used to determine whether additional research participants should be enrolled. She also asked whether the investigators have considered conducting integration site analysis at the first monitoring at three months to determine if clonality is occurring. In addition, Dr. Ross asked, if poor engraftment were to be seen in any of the research participants, would additional participants be enrolled so that a total of ten individuals with engrafted, vector-transduced HSCs could be followed.

C. RAC Discussion

During the meeting, RAC members did not raise any additional questions, concerns, or issues.

D. Investigator Response

1. Written Responses to RAC Reviews

This protocol is a direct adoption of the European protocol, only changing the investigators and sites but leaving protocol elements, such as inclusion/exclusion criteria, identical to allow best comparison of data for trials conducted under separate regulatory authorities. Participant ages will range from 23 months and older, including adults, and is not limited to participants younger than 18 years old. Several previous clinical trials of gene transfer for CGD have included pediatric participants, and the investigators explained that inclusion of some pediatric participants is ethically and scientifically justified. In addition to the absence of a matched sibling or unrelated donor, the inclusion criteria limit enrollment to individuals with ongoing or resistant severe infections or auto-inflammatory complications, all of which represent a life-threatening situation for patients with CGD. Gene transfer has been demonstrated to be efficacious in resolving such infections in several trials, even trials in which gene-modified white blood cells were present only transiently. The investigators expect that the relatively intensive myelo-reductive conditioning regimen and the use of effective HSC transduction by a lentiviral vector will lead to long-lasting engraftment of gene-corrected stem cells making functional phagocytic cells. This result could significantly improve antimicrobial immunity with as low as between 1 and 5 percent gene-corrected neutrophils.

This specific vector will be used for the first time in humans in this trial; however, previous trials with gamma-retroviral vectors have shown production of sufficient amounts of the gp91phox protein to restore phagocyte antimicrobial activity. Therefore, the prospect of benefit exists for pediatric participants who do not have a fully matched donor for allogeneic HSCT. Pediatric participants might have better gene

transduction or engraftment of their stem cells compared with adults; this greater potential benefit for pediatric participants would not be realized if they are excluded from this trial.

The XCGD patients eligible for this study presenting with “ongoing or resistant severe infections” are deemed highly likely to continue to have a bad disease course and therefore would be referred for an allogeneic HSCT if a matched donor were available.

The initial three-month observation is related to the peri-transplant issues of conditioning regimen–related toxicity and recovery, engraftment, and gene transfer and expression. Endpoints include clinical toxicities, engraftment/prolonged aplasia, and absence of replication-competent lentivirus. Based on preclinical and clinical studies, many factors give rise to prevalent integration sites, including technical biases, HIV integration preferences, oligoclonal engraftment of stem/progenitor cells, and physiological stem-cell or T-cell expansion as a consequence of hematopoietic stress or infection. There is no known predictive value of integration site analyses in the short term; longitudinal studies and validation tests are needed to correlate over-representation of sequence reads and cell expansion.

For the statistical calculations, the level of 5 percent expression of granulocytes at 12 months is based on the target set in the primary endpoint. This value is based on multiple observations of clinical benefit in antimicrobial activity from as low as 5 percent granulocyte expression in (a) affected females with skewed X-inactivation, (b) recipients of allotransplants with only low levels of donor chimerism, and (c) clinical gene transfer studies. It is unknown whether this level will be fully or partially effective; the relative strength of the novel chimeric promoter of this vector could be a factor, which might be learned from this study. Having undergone autologous transplant with gene-corrected HSC should not preclude an allogeneic HSCT if a suitable donor is identified, but increased risks of toxicity could exist from a second round of conditioning.

Participants will undergo either marrow harvests or PBSC collections but not both, so effects of prior G-CSF on subsequent bone marrow harvest is not germane. Effects of G-CSF mobilization on second mobilization and effects on transduction of those PBSCs have not been studied, but the investigators stated that there is no reason to postulate adverse effect.

The choice of bone marrow versus PBSC will be made by the physicians and participants on a case-by-case basis. For participants ten to 13 years old or who weigh less than 20 kg, bone marrow harvest is often more practical than G-CSF administration and leukophoresis. The dosages of CD34+ cells needed for autologous recovery in the case of marrow aplasia are not known, but a lower limit of $1x10^6$ CD34+ cells/kg will be set. Because bone marrow is likely to yield lower numbers of CD34+ cells than PBSC will, participants who will have bone marrow obtained as the HSCT source will undergo two bone marrow collections, at least one month apart.

The investigators explained that they expect a lentiviral vector to lead to higher rates of sustained engraftment than has been the case in previous trials, a conclusion based on prior work in large animals and observations from recent trials of HSC gene transfer for metabolic diseases. Although the potential for immune responses against the normal gp91phox protein leading to graft rejection is possible, the risks of this response are unknown and have not been documented to date. If the frequencies of gene-corrected neutrophils recede, the investigators might consider modifying the conditioning regimen.

The “suitable donor,” referenced in the informed consent document, who might be used for a rescue transplant—in the dire situation of marrow aplasia after the autologous gene transfer/transplant and despite administration of the backup—would likely be an incompletely matched donor, a situation that has increased risks for graft-versus-host disease (GVHD) but might be lifesaving. A new, fully matched donor becoming available in the months after transplant is unlikely, given that none was found during the initial search of more than 20 million individuals in the donor registry. The investigators agreed to clarify this reality in the informed consent document.

Although cord blood transplants may be done with less than 10/10 HLA matching, these transplants carry increased risks for non-engraftment and GVHD. It is unlikely that a transplant using two incompletely

matched cord blood units would be done for CGD as primary therapy, and doing so would be considered experimental.

Human G-CSF–mobilized PBSCs from an XCGD research participant were studied by transplant into three NSG mice. After eight weeks, the human cells in the bone marrow of the mice had an average vector copy of 0.35, and 20 percent of the human cells were positive for gp91phox, with a mean fluorescence intensity approximately half of that in control normal donor cells. Secondary transplants were not done with the NSG mouse recipients.

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

2. Responses to RAC Discussion Questions

Because RAC members did not raise any additional discussion questions relative to this protocol, responses were not necessary.

E. Public Comment

No public comments were offered.

F. Synopsis of RAC Discussion and RAC Observations and Recommendations

Ethical, Legal, Social Issues

- Gene transfer for CGD is not novel; trials using retroviral vectors have demonstrated clinical benefit, unfortunately with cases of myelodysplasia when the vector was inserted near known oncogenes. However, because this is the first use of a lentiviral vector with a novel promoter for gene transfer for CGD, it is not known whether this product will have equivalent efficacy to prior gene transfer trials. Therefore, in addition to the changes in the informed consent document made in response to the written reviews, the investigators should include a clear statement in the assent form that it is unknown whether this intervention will have clinical benefit.

G. Committee Motion 6

Dr. Fong summarized the RAC recommendations to be included in the letter to the investigators, expressing the comments and concerns of the RAC. Dr. Fong asked for a vote, and the RAC approved this summarized recommendation by a vote of 12 in favor, 0 opposed, 0 abstentions, and 7 recusals.

XV. Review and Discussion of Human Gene Transfer Protocol #1304-1231 titled: A Phase I Study of Intrathecal Administration of scAAV9/JeT-GAN for the Treatment of Giant Axonal Neuropathy

Principal Investigator:	Carsten Bonnemann, M.D., National Institute of Neurological Disorders and Stroke (NINDS), NIH
Additional Presenters:	Jahannaz Dastgir, D.O., NINDS; Steven Gray, Ph.D., University of North Carolina at Chapel Hill (UNC) Gene Therapy Center; R. Jude Samulski, Ph.D., UNC Gene Therapy Center
Sponsor:	Hannah's Hope Fund
RAC Reviewers:	Drs. Chatterjee, Chiocca, and Fost

A. Protocol Summary

Giant Axonal Neuropathy (GAN) is a rare chronic neurodegenerative disease characterized by enlarged axons with disordered microtubules and intermediate filaments, which is fatal by the third decade of life.

The disease pathology is due to homozygous loss-of-function mutations in the GAN gene, which encodes the protein gigaxonin, a protein needed for long-term nerve survival. Onset of symptoms is usually 3-4 years of age, with a slightly awkward gait. GAN is a progressive degeneration of the CNS characterized by loss of motor and sensory function and brain atrophy. The disease leads to complete loss of movement and ventilator dependence before causing death, typically by age 30. Currently, no established treatment exists for GAN.
The role of gigaxonin is to maintain the correct balance of certain structural components of nerves. In the absence of functional gigaxonin, these structural components accumulate and cause toxicity. Nerve cells within and around the spinal cord are particularly susceptible to this damage, which results in the breakdown of communication between the CNS and the peripheral nervous system and subsequent nerve death. This damage causes a progressive loss of control over the legs and arms, eventually leading to patients being wheelchair-bound and, later, quadriplegic; death follows this progression.

Intrathecal (IT) delivery of a self-complementary, adeno-associated virus serotype 9 (AAV9) expressing the GAN gene to the spinal cord and brain offers the potential of a minimally invasive and effective treatment for GAN, in the absence of any other options for these patients. In preclinical studies in cultured human cells and in mice that have GAN, introduction of a working copy of the gigaxonin gene restores the normal distribution of the structural proteins within cells that are a hallmark of GAN pathology. Delivery of the gigaxonin gene into mice that have GAN has improved their motor function, even when the mice were treated after the onset of their disease symptoms. To test whether this experimental therapy might be relevant to humans, monkeys were injected with a similar recombinant virus that carries a gene (green fluorescent protein [GFP]) that will make cells turn green if the gene is delivered successfully. In the monkeys, a single injection of this viral vector into the CSF surrounding the spinal cord resulted in widespread gene expression throughout the nervous system. This study demonstrated that the procedure would likely scale well to humans, suggesting that the encouraging results in mice might benefit patients with GAN.

This Phase I clinical trial aims primarily to test the safety of the procedure and product in humans. Participants at least 4 years old with minimal mutations will be enrolled first to reduce the likelihood of generating immune responses to the wild-type GAN gene. The primary endpoint will be safety, based on adverse events and standard laboratory safety evaluations. The secondary endpoints will include clinical assessment of motor and sensory function and possible rescue of disease pathology in peripheral nerves.

B. Written Reviews by RAC Members

Eleven RAC members voted for in-depth review and public discussion of this protocol. Key issues included that this study will test a novel transgene, vector, and delivery route for a new disease indication in children.

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

Dr. Chatterjee expressed a variety of concerns about this proposed study. Little is known about the disease, diagnosis is tricky, and outcome measures for the trial are yet undefined. Although the gigaxonin gene has been sequenced, Dr. Chatterjee noted that it is unclear whether the disease is solely a result of structural mutations or may have additional, especially regulatory, causes; if the latter, then gene transfer might not alleviate the symptoms. The available mouse exon knockout models do not accurately reflect the human disease in the level of severity and functional loss; the promoter used in the vector is extremely weak, and transgene expression, including the gigaxonin transgene, is extremely difficult to detect. Biodistribution analyses suggest widespread peripheral dissemination of the vector after intrathecal delivery, suggesting a major breach of the blood-brain barrier during intrathecal administration and raising questions regarding immune responses, pre-existing immunity, and participant selection. In the preclinical studies, Dr. Chatterjee noted that therapeutic benefit as a result of gene transfer was difficult to glean from the data provided by the investigators. Current estimates of the frequency of GAN appear to be 50 patients worldwide, so this trial would enroll approximately 10 percent of all GAN patients. For these research participants, it would mean they likely would be excluded from future trials

with possibly more-effective vectors. Dr. Chatterjee concluded that all of these issues raise concerns regarding the necessity to do a Phase I safety testing of a vector that may not adequately express the transgene in a disease in which patients are difficult to identify and outcome measures are as yet undefined. Specific comments and questions from Dr. Chatterjee included:

- How will GAN patients be identified for enrollment in this trial? Prior to enrollment, will the GAN gene of each prospective participant be sequenced to confirm that he or she actually has GAN and to identify the specific mutation?
- Are all known GAN mutations structural? What is the likelihood of additional regulatory mutants that probably would not be addressed through simple GAN cDNA transfer?
- The investigators should justify the choice of the weak JeT promoter used in the vector. If the transgene expression is essentially undetectable, then what would be learned from the trial?
- The investigators should describe precisely the toxicity of gigaxonin overexpression.
- Expression from the JeT promoter appears to drop between weeks 2 and 4 in almost all the nonhuman primate tissues examined. What are the implications for long-term transgene expression in humans?
- The exon-deleted mouse model of GAN does not appear to recapitulate faithfully the human disease, particularly since the disease severity and progression is a lot less in mice than in humans. Is it possible that the underlying genetic defect is significantly different between humans and mice?
- The data showing efficacy of gigaxonin transduction are sparse, with only two time points shown. Are the effects sustained?
- If intrathecal delivery breaches the blood-brain barrier in both mice and primates such that AAV is found widely at high frequencies in such locations as the liver, spleen, kidney, heart, lung, and biceps, then it is likely that circulating neutralizing antibodies could enter CSF and neutralize the vector.
- What are the potential consequences of a high level of peripheral transduction in GAN patients, especially if they are AAV seropositive?
- Why is the AAV frequency in most peripheral tissues, including the liver, spleen, and lymph nodes, much higher than that seen in the brain; what are the implications for therapy?
- Outcome measures and parameters for therapeutic benefit have not been defined and gigaxonin expression is hard to detect, making it difficult to assess safety if transgene expression is extremely minimal and to attribute any potential efficacy to gene transfer.

Dr. Chiocca suggested that measurement of infusion-site erythema and induration should be carried out on days 1 and 2 in addition to day 0. He asked the investigators to justify the need for a nerve biopsy. Dr. Chiocca requested explanation as to why specified CSF pressures or white blood cell counts would preclude further participation in this trial when clinical suspicions of increased intracranial pressure or CSF pleocytosis could be initial exclusions, thus avoiding invasive procedures once enrolled in the trial. He asked the investigators to provide more detail about the risks associated with gross overexpression of gigaxonin, the promoter for the study and its specificity for neurons, and which spinal cord neurons are targets. Dr. Chiocca noted that the investigators provided only preliminary results from the preclinical studies; in particular, the toxicity data in mice were noted as still in progress and therefore not currently available.

Dr. Fost asked whether the monkeys were symptomatic before injection and whether they showed any clinical changes after injection. He noted that this trial is a first-in-humans test of this gene and of intrathecal injection of this vector, so the risks are unknown. Given a variety of factors with regard to participant selection, Dr. Fost opined that the risk/burden-to-benefit ratio appears significantly unfavorable and, therefore, he suggested that this early study be restricted to older participants who would be capable of understanding the issues and would be able to provide meaningful consent. With regard to extraction of CSF, he asked the investigators to clarify why additional CSF is needed, the amount needed (especially for younger participants), and what would be studied using those additional samples. Dr. Fost provided several suggestions for improvements and clarifications in the informed consent document,

including wording changes to avoid therapeutic misconception. He also noted that the protocol appeared to be incomplete and did not include an assent form.

C. RAC Discussion

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

- Dr. Badley asked about the safety data in terms of an inflammatory response and particularly meningitis with regard to injecting $1x10^{13}$ genomes of AAV into the CSF. Noting the hypothesis that intrathecal delivery might avoid many immune reactions and thus allow redelivery of the vector, Dr. Fong asked the investigators whether they have tried to re-deliver the vector in primates in the hopes of seeing additional gene transfer.
- Dr. Fong asked whether the investigators have given vascular agents that affect the choriod plexus to see whether there is any difference in gene delivery to the target cells or less delivery to the peripheral cells, with the goal of concentrating more vector within the CNS.
- Dr. Cai expressed concern that the small patient population might mean that a specific level of toxicity (20 percent, for example) would not be seen in the few participants in this Phase I trial.

D. Investigator Response

1. Written Responses to RAC Reviews

No other compounds are in development for GAN therapy. Thus, this trial represents the only available treatment option for any GAN patient in the next three to five years. The participant selection proposed by the investigators gives priority to older patients who may be too advanced in the course of their disease to participate in future trials. The vector and dose chosen for this Phase I trial are expected to be therapeutic based on the preclinical data, and the same dose is expected to be used in Phase II.

Prior to enrollment, all patients will have confirmatory genetic testing to identify the GAN mutations on both alleles.

It is possible that mutations in regulatory elements could lead to a loss of gigaxonin expression and the clinical presentation of GAN. All characterized mutations are within the gigaxonin coding sequence. At present, this clinical trial design requires a genetic diagnosis of GAN. If no mutations are identified in the gigaxonin gene or associated regulatory elements, gigaxonin gene transfer may not be justified. The current understanding of GAN genetics indicates loss of function as the genetic mechanism, which is the rationale for gene replacement in genetically proven GAN patients.

Gigaxonin is normally expressed at very low levels, and endogenous mRNA also can be challenging to detect. The promoter was chosen to allow packaging in self-complementary AAV but also to mimic normal expression levels. While expression is low, it is clearly present and detectable by immunohistochemistry, and this level of expression was sufficient to provide behavioral improvement in mice. From patient fibroblast and other experiments, the investigators have concluded that the expression afforded by the JeT promoter, while low, is appropriate to drive gigaxonin expression for the treatment of GAN. The exact level of gigaxonin needed is not known, but the investigators have performed functional tests that have demonstrated achievement of therapeutically relevant levels of expression with this promoter.

Evidence for overexpression-related toxicity of gigaxonin is weak and mostly constitutes a hypothetical risk. Gross overexpression of gigaxonin is expected to cause a deleterious loss of intermediate filaments, as seen in one study. The investigators expect that mild overexpression will be compensated for at the protein level, because gigaxonin serves as an adapter protein to target itself for ubiquitylation. No overexpression toxicity has been observed in mice.

Investigation into the drop in JeT promoter expression between weeks two and four revealed that it was due to a GFP-specific immune response, which was not seen in nonhuman primates injected with the scAAV9/JeT-GAN vector without GFP. The investigators have discovered persistent transgene

expression of gigaxonin in nonhuman primates increasing between weeks two and eight, whereas GFP expression was reduced during the same time. This possibility of a transgene-specific immune response against a foreign protein is the basis for Phase I exclusion of patients with complete null mutations.

The investigators explained that the knockout mouse models are the only animal models available for GAN. While the behavioral phenotype is very mild, it does replicate many aspects of the human disease, including the presence of intermediate filament aggregates, pathology in peripheral nerves, and white-matter abnormalities detectable by MRI. The difference in disease presentation in the mice could be explained by the much shorter axon length in mice compared to humans, because GAN is apparently an axon length–dependent axonopathy. The mice recapitulate enough aspects of the human disease to assess on a conceptual level whether gigaxonin gene transfer will be beneficial. Although the investigators would prefer to have a more relevant animal model, they believe that the absence of such an animal model should not preclude development and testing of an experimental treatment for an untreatable disease.

The mice were injected at 18 months of age and assessed at months 20, 21, 22, and 23. The original cohorts were nine treated knockout mice, six knockout mice receiving a GFP vector, and 15 normal mice, although a few mice died before the end of the study. The treated knockout mice trended better than the GFP-dosed mice starting at three months post-injection, but this was not significant at any time point. Analysis of the entire dataset revealed that the treated knockout mice showed a significant improvement over time, whereas the GFP-dosed mice did not. Additional data showed that mice injected at 12 to 14 months of age did significantly better than GFP-dosed mice at 19 months of age; however, the cohort sizes in this study were small.

The investigators' *in vitro* (human cells) and *in vivo* (knockout mouse) studies indicate that the low expression provided by the JeT promoter is sufficient to rescue the intermediate filament aggregate phenotype. Quantification of gigaxonin mRNA expression was difficult due to low expression levels, even in fibroblasts that were efficiently transduced and could be visually scored as having been rescued. If critical cells, such as motor neurons, were transduced but surrounding vascular tissue was not transduced, the amount of RNA in the motor neurons could be therapeutic, but the relative amount of RNA in a whole-tissue homogenate would be extremely low. For example, mRNA levels in mice that showed a behavioral improvement were still too low for accurate quantification. Gigaxonin RNA levels at a low level that is difficult to quantify does not mean those levels are subtherapeutic; in fact, the investigators explained that they have provided several lines of evidence that these levels of expression are sufficient to be beneficial.

The investigators explained that they have found no instances of neutralizing antibodies in the CSF even when relatively high titers were detected in the blood. They posited that high-vector frequency in peripheral organs could be due to a transfer of vector through the lymphatic system rather than the blood. This could be from CSF leakage from the injection site or vector transfer outside the CNS when CSF is reabsorbed. These speculative explanations highlight ways in which vector could be transferred to peripheral organs without being exposed to neutralizing antibodies in the blood.

The CNS, in particular the spinal cord and dorsal root ganglion, is the primary therapeutic target because the most prominent clinical manifestations in GAN patients can be referred to these cell types. Cell transduction in peripheral organs also could be therapeutically beneficial, given that gigaxonin is normally expressed in peripheral organ tissues as well as the CNS. If circulating neutralizing antibodies against AAV9 reduce gene transfer to peripheral tissues, then gene transfer to the CNS should still be successful. The research participant should not suffer any deleterious effects if circulating neutralizing antibodies clear the vector from the blood. In the investigators' GLP toxicity study in nonhuman primates, only very minimal signs of pathology were observed in the CNS and peripheral tissues, even though neutralizing antibodies against the capsid had risen.

Safety will be assessed within the context of this vector at this level of expression, and no claim of efficacy is implied. Although safety is the primary endpoint, the investigators also are interested in validating that gigaxonin is being expressed and exerting an effect as well as in determining whether any

meaningful therapeutic effect is realized. The investigators are using information from a natural history study of GAN patients to identify clear and reproducibly reliable motor function outcome measures and potential CSF biomarkers for this patient population.

The nerve biopsy is not being done to verify the diagnosis but as a baseline to assess a possible treatment effect on a biomarker—neurofilament accumulation in the peripheral nerve. The desire to document the effect of gigaxonin expression was the reason for inclusion of the peripheral nerve biopsy at baseline and 12 months post-gene administration. Data from an ongoing experiment in treated mice indicate selective preservation of large-diameter myelinated fibers and small-diameter unmyelinated axons in mice dosed after the onset of motor dysfunction at 18 months and examined at 24 months.

Patients with GAN are not known to have an increased risk of elevated intrathecal CSF pressures or pleocytosis, but the investigators would prefer to check for these possibilities prior to gene administration. This check would be precautionary and as a baseline for comparison if the individual were to have an adverse reaction to vector administration. If these values are abnormal, the investigators would not proceed with gene administration. The investigators explained that they would have to insert an IV line for anesthesia if the research participant is unable to tolerate the lumbar puncture or nerve biopsy procedure under local anesthetic. Ideally, they plan to perform the nerve biopsy prior to the lumbar puncture and gene administration because it would be best if any complications from vector administration did not take place while the sural nerve is exposed and being harvested.

The investigators reported that the toxicology in mice at 7 days and 28 days has been completed, and no instances of vector-related toxicities were observed.

The vector is not restricted to the CNS following intrathecal administration, but the ratio of CNS-to-periphery delivery is considerably more favorable than for an IV administration. While this biodistribution profile may increase the risk for an immune response against the vector or transgene, the extent of peripheral tissue gene transfer is not expected to pose a significant health risk resulting from gigaxonin overexpression. Because gigaxonin normally is expressed in all tissues, the peripheral biodistribution could provide an additional benefit to research participants. The investigators' GLP toxicology studies in mice and nonhuman primates support the conclusion that there is no overexpression-related toxicity arising from the peripheral tissue gene transfer.

The investigators explained that the biggest potential risk to research participants is an immune response to the expressed wild-type gigaxonin protein. For this reason, they do not want enrollees in the first part of this study to be gigaxonin-naïve. Patients expressing at least some full-length gigaxonin off at least one allele would meet the inclusion criteria for this Phase I trial.

The only animal models for GAN are three separately derived knockout mouse strains. All nonhuman primate studies were conducted in normal asymptomatic animals solely for the purpose of testing vector biodistribution and/or vector-related toxicity. None of the nonhuman primates in these studies displayed any clinical changes following administration of scAAV9/JeT-GAN, except for one animal that required euthanasia due to self-mutilation behavior, an occurrence that was determined unlikely to be related to the test article.

The investigators stated that the preclinical studies outline strong evidence showing (1) the molecular mechanism by which gigaxonin gene transfer can fully correct cellular pathology, (2) the ability of the delivered gigaxonin gene to resolve pre-existing pathological intermediate filament aggregates following gene transfer in knockout mice, and (3) the ability of the delivered gigaxonin gene to provide benefit in motor function to knockout GAN mice even after the onset of symptoms. These studies show that if the gigaxonin gene is delivered to a GAN cell, it can rapidly restore the function of that cell. The investigators pointed out that their studies in mice, pigs, and nonhuman primates demonstrate the ability of AAV9 to broadly target neurons and glia throughout the CNS, including efficient targeting of motor and sensory neurons in the spinal cord and dorsal root ganglia; these areas are highly dysfunctional in GAN patients and that dysfunction is a major contribution to patients' deteriorating health and quality of life. In light of

this information, the investigators contend that a considerable likelihood exists for benefit to GAN patients, along with a reasonable probability of conferring meaningful clinical benefit.

With regard to risk, the GLP safety studies conducted in mice and nonhuman primates showed no evidence for potential adverse effects beyond the potential for an immune response against the vector or against the transgene product if gigaxonin is recognized as a foreign antigen. The investigators expressed cognizance about the considerable unknown risk associated with this first intrathecal injection of AAV9 vectors in humans coupled with this first administration of the gigaxonin gene in humans. However, the normal disease course of GAN is well understood, and, in the face of no other options for therapeutic intervention, the investigators disagreed that the risk/benefit ratio (risk of doing nothing versus risk of treatment versus potential benefit of treatment) should be considered “very unfavorable.” However, they agreed that the risk/benefit ratio is more favorable for older patients who are more advanced in their disease course, and they noted that the selection process for this study favors inclusion of older patients first, particularly adults capable of providing their own informed consent.

Although this is the first intrathecal administration in humans, the investigators noted that there have been previous AAV vector administrations to the CNS in humans.

Currently, in the United States, there are 18 patients with GAN who range in age from four to 29 years old. Subtracting end stage patients, null patients, and one patient who would be excluded from Phase I due to celiac disease leaves 11 potential U.S. research participants for this trial. Seven of the 11 are awaiting the results of gene sequencing. Assuming those seven patients are not null, the 11 U.S. patients range in age from four to 11 years old. The sponsor is also in contact with 13 families worldwide; four potential research participants are at least 18 years old and likely would meet the inclusion criteria. Additionally, the sponsor was told that 20 to 25 GAN patients in Europe have not yet been contacted. As soon as this proposed trial has met all regulatory requirements and is posted as “active” on www.clinicaltrials.gov, the investigators plan to recruit GAN patients in other countries through Orphanet and Eurodis. The investigators pledged that every effort would be made to enroll participants over the age of 18.

A functional gain in motor and sensory systems is unlikely to be beneficial to a GAN patient who has lost cognition. Therefore, the investigators agreed to perform baseline neuropsychological assessments on potential participants to establish a baseline level of competence regarding whether they are willing to participate in this study.

The lumbar punctures will aid investigation of secondary outcome measures for this study. Because gigaxonin is an intracellular protein, there is no way to assess directly whether successful gene transfer is occurring. Because this is the first time AAV9 vectors will be administered into the CSF of a human, the investigators recognize that this proposed trial carries a scientific burden to verify that successful gene transfer has occurred. These investigations are crucial to inform the design of future gene transfer approaches for GAN and other diseases. Excluding postmortem analysis, no practical and safe way exists to directly measure gigaxonin expression. However, it is likely that targets of gigaxonin, such as neurofilaments, can be quantified in the CSF as biomarkers. Given the novel nature of this study, the investigators acknowledged the difficulty of predicting the kinetics or duration of any potential change in biomarker abundance. Originally, they planned for regular lumbar punctures to assess this, but they have changed the schedule of follow-up lumbar punctures because of the potential for unacceptable discomfort and risk to the study participants. Annual lumbar punctures will remain included after the first year for five successive years if biomarkers have been identified within the CSF in order to assess whether the notable biomarker quantity has decreased, stabilized, or increased over time.

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

2. Responses to RAC Discussion Questions

Regarding the safety data for injecting the proposed amount of vector, Dr. Samulski explained that none of the studies conducted to date in primates, rodents, pigs, and humans have shown problems related to inflammatory response or meningitis. However, only the animal studies featured intrathecal injection. Dr. Bonnemann indicated that the investigators are planning to provide research participants with a one-time bolus of high-dose methylprednisolone—a relatively standard procedure for study drug administration into the CSF—to avoid a primary meningitic reaction to this new antigen. They plan to monitor participants carefully, and they would treat a meningitic reaction (that is not immunologically mediated as a strong autoimmune phenomenon) by providing immunosuppression therapy.

Dr. Gray indicated that the investigators would like to conduct an experiment to show whether redelivery of the vector in primates would produce additional gene transfer, but they have not yet done so.

In response to RAC questions about additional issues to explore, Dr. Gray explained that the investigators want to keep this Phase I trial simple and to introduce as few variables as possible. Once this approach has been established as safe, the investigators will add on additional variables for subsequent-phase trials.

Responding to statistical concerns about the very small patient population, Dr. Bonnemann acknowledged that fact and explained that the investigators are looking primarily for any immunological effects on the CNS. Subsequent studies will be ongoing safety studies, in addition to their other purpose(s), to attempt to fill in the safety information that will be sparse given the small patient population.

Dr. Bonnemann stated that the mouse model is appropriate with respect to biomarkers. However, he indicated that the impact of this neurological disease is functionally very different in humans than in mice.

E. Public Comment

The following individuals provided public comment to the RAC in support of this protocol (listed below in order of appearance):

- Lori Sames
- Mary Pizzurro *(via video, presented by Ms. Sames)*
- Matt Sames
- Steve Grube
- Jody Ketcham *(via letter, read by Ms. Sames)*
- Sharon King
- Reza Shabazz *(via letter, read by Mr. Sames)*
- Magaen Bates *(via email, read by Ms. Sames)*

The parents described the experiences of their children, their advocacy efforts, and support for this trial. Testimony of the above individuals is included, verbatim, in Appendix A.

Dr. Fong thanked the parents for attending this RAC meeting and sharing their stories. He stated that members of the RAC share the belief that gene transfer will make a difference in neurologic and other diseases. He also explained that the RAC ensures that gene transfer trials are designed in the best way to gather data so that the field moves forward and can affect these diseases as quickly and effectively as possible. Dr. Fong further thanked the parents for being advocates for their children and for forming foundations to combat these rare diseases.

F. Synopsis of RAC Discussion and RAC Observations and Recommendations

<u>Preclinical Issues</u>

- Readministration of AAV vectors is often not feasible because the immune response to the initial administration might prevent effective delivery to the target tissue and expression of the

transgene. In this protocol, the relative immune privilege of the CNS might allow for the readministration of the vector in the event the initial administration does not result in adequate gene transfer. Because the study procedures for this protocol include CSF collection during the first year, this may present an opportunity for vector readministration. To evaluate the effect of readministration, the investigators should consider conducting vector readministration studies in nonhuman primates either in advance of this trial or preceding a subsequent trial.

Clinical and Trial Design Issues

- Although intrathecal administration to the CNS results in vector delivery primarily to a relatively immune-privileged site, the animal studies showed widespread systemic dissemination of vector. Therefore, the investigators should consider initially enrolling several research participants who do not have pre-existing AAV9-neutralizing antibodies to minimize further the risk of an immune reaction against the vector and destruction of vector-transduced cells in the CNS and periphery.

Ethical, Legal, Social Issues

- Despite the impressive preclinical studies and the experienced investigative team for this protocol, Phase I studies are far more likely to fail than to result in a new therapy. In any protocol for a fatal disease with no available therapeutic options, the risk of overestimating the potential benefits and minimizing the risks is a natural tendency. To counteract any potential therapeutic misconception, the informed consent document and process should clearly state that it is unlikely that the research participant will receive any benefit.

G. Committee Motion 7

Dr. Fong summarized the RAC recommendations to be included in the letter to the investigators, expressing the comments and concerns of the RAC. Dr. Fong requested a vote, and the RAC approved these summarized recommendations by a vote of 17 in favor, 0 opposed, and 3 abstentions. Dr. Fong asked the public to note that all three bioethicists on the RAC—Ms. Dresser, Dr. Fost, and Dr. Zoloth—abstained from this vote because they supported an additional recommendation to enroll initially older adult or adolescent subjects who would be capable of providing assent or consent.

XVI. Review and Discussion of Human Gene Transfer Protocol #1303-1214 titled: A Phase I Randomized, Double-Blind, Placebo-Controlled Study of a Multi-Antigen DNA Vaccine Prime Delivered by In Vivo Electroporation, rVSV Booster Vaccine in HIV-Infected Patients Who Began Anti-retroviral Therapy During Acute/Early Infection

Principal Investigator:	Michael C. Sneller, M.D., National Institute of Allergy and Infectious Diseases (NIAID), NIH
Additional Presenters:	Tae-Wook Chun, Ph.D., NIAID; Michael Egan, Profectus Biosciences, Inc. *(via teleconference)*
Sponsor:	NIAID
Pharma Support:	Profectus Biosciences, Inc. (vaccine); Ichor Medical Systems (device)
RAC Reviewers:	Drs. Badley, Cannon, and Zoloth

A. Protocol Summary

The advent of combination antiretroviral therapy (cART) has dramatically improved the clinical outcome in human immunodeficiency virus (HIV)-infected individuals through sustained reduction in viral replication. However, it has become clear that cART alone cannot eradicate HIV in infected individuals, likely in part due to the persistence of viral reservoirs in peripheral blood and various tissue compartments. Consequently, a major thrust of HIV research over the past several years has been to develop therapeutic strategies that can eliminate persistent viral reservoirs and boost host immunity to control viral replication upon discontinuation of cART. Therapeutic HIV vaccination is one approach that could

potentially achieve these goals through vaccine-induced improvement in HIV-specific immune responses and/or by direct reactivation of HIV-specific CD4+ memory T cells that harbor latent HIV. An effective therapeutic vaccine could augment immunologic control of HIV infection and potentially obviate the need for chronic cART.

The current study is an exploratory randomized, 2-arm (1:1), double-blind, placebo-controlled trial evaluating the safety and efficacy of an HIV-1 multi-antigen plasmid DNA (HIV-MAG pDNA) vaccine prime in combination with an interleukin-12 plasmid DNA (IL-12 pDNA) adjuvant delivered by in vivo electroporation followed by a recombinant vesicular stomatitis virus vector containing the HIV-1 *gag* gene (rVSV HIV *gag*) booster vaccine in subjects on cART who started therapy during acute or early HIV infection.

Subjects will be randomized to receive placebo or the HIV-MAG pDNA (3000 µg) vaccine prime and IL-12 pDNA adjuvant (1000 µg) at week 0, 4, 12, and 36, and the rVSV HIV *gag* booster vaccine (1x107 plaque-forming units) at week 24 and 48. The HIV-MAG pDNA vaccine prime and IL-12 pDNA adjuvant will be administered as two intramuscular (IM) injections, one into each deltoid, with electroporation using the Ichor TDS device, while the rVSV HIV *gag* booster vaccine will be administered as two conventional IM injections, one into each deltoid. After the week 56 visit, all subjects will undergo an analytical treatment interruption to determine if the vaccination strategy results in an improved immune control of viral replication, as evidenced by a blunted or absent rebound in HIV plasma viremia. All subjects will be followed through week 96 for safety and efficacy parameters.

The study population includes HIV-infected adults who began cART during acute or early infection. Subjects must be receiving an effective cART regimen, with a CD4 cell count of >450 cells/mm3 at screening, and they must have documented viral suppression below the limit of detection for >one year. The rationale for testing the study vaccine regimen in this subject population is because these individuals may have a relatively preserved immune function, which could be augmented by therapeutic vaccination.

B. Written Reviews by RAC Members

Twelve RAC members voted for in-depth review and public discussion of this protocol. Key issues included the safety of this combination of vectors is currently being tested in healthy volunteers; however, the decision to enroll subjects who started cART early in infection raises several issues. Recent data indicate that patients who initiate cART during early infection may achieve long-term remission even after interruption of cART. If this is indeed the case, it raises questions regarding the design of this trial, because participants in both arms may be able to maintain viral suppression off cART for more than the 16-week interruption. In addition, because these individuals may have achieved long-term control of the virus, an additional concern is whether vaccination with these agents could inadvertently “break” such control or seed or increase the latent HIV virus reservoir. Thus, this trial was found to warrant in-depth review and public discussion due to the selection of the study population, the incorporation of an analytical treatment interruption to determine differences between the placebo and active arm, and concern about whether any vaccination strategy in this subject population may inadvertently interfere with infection control.

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

Dr. Badley stated that this clinical trial proposes a logical intervention supported by significant preclinical data. He noted that macaque studies using IL-12-enhanced HIV-MAG pDNA followed by an rVSV gag booster resulted in improved magnitude and breadth of T-cell responses, and he asked about the effect of vaccination on simian immunodeficiency virus (SIV) disease progression and whether an analytic treatment interruption (ATI) was performed in vaccinated versus unvaccinated macaques. Dr. Badley suggested that, before moving forward with this trial, the investigators consider the results of the ACTG 5281 trial, which has completed enrollment and is using HIV-MAG DNA vaccine with IL-12 pDNA adjuvant in HIV-infected participants. Because it is likely to be difficult to identify which research participants control viremia due to early treatment versus those who control viremia due to the impact of vaccination, Dr. Badley suggested performing two ATIs—one before vaccination and one after vaccination—that would

allow a direct comparison of viral set points to be compared before and after vaccination. He noted that an increasing body of evidence indicates that control of HIV is associated with different genotypes and that different genotypes respond to vaccinations differently, which raises the possibility that response to human therapeutic vaccination for HIV also may be genotype-specific. Therefore, Dr. Badley suggested performing HLA typing of all participants enrolled in the study and stratifying the analysis of response to treatment by individual genotypes.

Dr. Cannon focused her review on two features of this proposed trial: (1) the nature of the patient group to be recruited, who started on cART at an early stage of infection and who are therefore hypothesized to have better preservation of immune function such that they may respond better to a therapeutic vaccination, and (2) the use of an ATI as a means to evaluate outcome between the vaccinated and placebo groups. She noted that some early-treated patients are now known to control HIV spontaneously when withdrawn from cART, although the rate at which this occurs is disputed. However, it has been suggested that the likelihood of such post-treatment control in these patients could increase with longer periods of cART; therefore, Dr. Cannon asked whether there is a risk that ATI in this patient population could “reset the clock” such that a patient would lose the opportunity for a more favorable outcome if withdrawn from cART too early. (She noted this concern as being especially relevant for the placebo control group.) She asked the investigators to justify the need for a control group rather than using historical data from other ATIs in early-infected HIV patients. Dr. Cannon summarized her concerns as whether the trial design offers a risk that may be unacceptable to the placebo group; she defined that risk as loss of a “privileged status” that could allow a favorable outcome or the ability to participate in other interventions in the future. She suggested that this complex issue be addressed more fully in the informed consent document.

Dr. Zoloth noted the informed consent document is carefully written and thorough. She suggested that the risks of mutated viral forms and of stopping analytic treatment should be highlighted as quite serious, separated from the list of minor risks, and explained as such. Second, it should be made clear that a remission or reduction in viral load could be due to many factors, not just the success of this trial. Third, the fact that the risk of mutation is completely unknown and untested in humans needs to be more thoughtfully discussed. Fourth, the document should more clearly emphasize that without cART, some proportion of research participants will rebound quickly with a high viral load, therefore making transmission to sexual partners more likely.

Dr. Zoloth asked the investigators to discuss the larger public health implications of this trial, including the possibility of a mutated HIV. Because the risk of stopping analytic treatment in control and treatment groups for 16 weeks is a nontrivial issue with serious risks to each participant, she stated her appreciation for the thoroughness and detail of the adverse events section and the checklist for participants. Dr. Zoloth stated that the patient population from which this trial will draw is particularly vulnerable and will have options for (perhaps years of) successful therapeutic intervention and control of disease. To become part of this trial, given the double-blind nature of the trial design and the four-month treatment withdrawal, will require courage and altruism. She cautioned that care and attention to these problems are critical in the informed consent discussion and document, as well as at regular intervals throughout the trial.

C. RAC Discussion

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

- Dr. Fong suggested that the investigators conduct a smaller, safety-only trial and then, if safety is proven, proceed to a larger trial that would include a placebo arm.

- Dr. Badley noted that the investigators’ options are to use a placebo group, to design the trial with a control within the same patient population, or to use data from historical controls, all of which present significant concerns. Provided that research participants are sufficiently informed of the theoretical risks of ATI in a placebo-treated group and that they are sufficiently informed about the 50/50 chance they will be placed in that group, he agreed it would be acceptable to proceed with

the two-arm trial design as presently proposed. Parenthetically, he expressed uncertainty as to whether he would choose to enroll in such a trial as a participant.

- Dr. Strome opined that whether an individual would enroll in this trial as currently constituted should be the determining factor as to whether this trial should move forward.

- Dr. Cannon stated that she would be more comfortable with this trial design if the potential participants were not early-treated individuals. There is something fundamentally different about early–ART-treated people; knowledge in the field and from the literature is not clear on this issue. She further stated that it should be possible for the investigators to obtain good information without requiring a placebo group because of the plethora of historical data. She requested that the informed consent document include information that "early-treated individuals are different (and how they are different is unknown), but an ATI could alter your ability to benefit from some future treatment. Half of all participants will not receive the experimental treatment, as they will be assigned to the placebo group."

- Dr. Zoloth stated that she would support the current trial design only with a strong informed consent document that included wording similar to what Dr. Cannon suggested.

- Dr. Fost pointed out that HIV patients have historically volunteered by the thousands for placebo-controlled trials during the past 25 years. Dr. Kiem added that typically HIV patients are well informed and highly motivated.

- Dr. Kiem asked the investigators at what point a participant in the placebo group would be placed back on ART.

- As a statistician who believes in randomized trials, Dr. Cai opined that, with 15 participants in each of two arms and given the likely viral load heterogeneity of these individuals, the investigators might not necessarily benefit much from the randomization unless the signal/effect is clear.

D. Investigator Response

1. Written Responses to RAC Reviews

Vaccination with an SIV pDNA prime vaccine, rVSV-SIV gag boost vaccine, did not alter the kinetics or magnitude of the viral rebound after withdrawal of cART relative to unimmunized control animals. However, in that study, the animals were not started on ART until they reached their post-infection viral set point, a different prime-boost protocol was used, and the study was conducted in a different species with a different virus and vaccine. Therefore, these results in this animal model do not preclude potential efficacy in human HIV.

The ACTG 5281 trial has completed its enrollment using HIV-MAG DNA vaccine with IL12 pDNA adjuvant in HIV-infected participants. The investigators stated that data from this trial may be available in late June or early July 2013.

In the design of this proposed trial, the investigators considered the possibility that a few individuals who began therapy early in infection might be able to control plasma viremia, at least transiently, without cART. Results from other studies suggest that ATI by itself can provide some degree of "autoimmunization" that can affect plasma viral rebound. To remove this possible confounding factor, the investigators took the same approach as was used in ACTG A5181 and have proposed a single ATI, with the difference in peak plasma viremia post-ATI as the endpoint.

The investigators will be testing HLA type for all study participants.

Because this is a Phase I safety study and the first time this vaccination regimen has been used in HIV-infected humans, the investigators prefer to take a conservative approach and not include participants with a history of any AIDS-defining condition.

There is no credible evidence to suggest that, for individuals treated during early infection, a linear relationship exists between duration of cART and the likelihood of achieving immunologic control of viremia. In none of the published observational cohort studies of this early-treated patient population has the duration of cART prior to treatment interruption been shown to correlate with the estimated frequency of post-treatment control of plasma viremia. Data from the nontreated arm of the French ANRS PRIMO Cohort cast doubt on the role early cART plays in the "post-treatment control" phenomenon.

The investigators stated their strong belief that a placebo group is needed and justified. The primary endpoint of this study is safety, and a placebo group is fundamental and necessary to the accurate evaluation of adverse reactions in safety studies. Second, there is not enough historical data on the expected magnitude of rebound plasma viremia at 16 weeks post-ATI in this patient population to make a placebo group unnecessary. An estimated approximately 5 percent of individuals who began treatment during early infection may be able to control HIV viremia for prolonged periods of time following treatment interruption. It is likely that a slightly higher percentage of such individuals may be able to control or partially control viremia for shorter periods of time, such as the 16-week ATI planned for this study. Therefore, a placebo group is necessary to determine whether the vaccine is having any significant effect on the magnitude of the rebound in plasma viremia at 16 weeks post-ATI (the secondary endpoint of this study). Third, the investigators believe that the ATI poses no special risk to the placebo group.

Given the estimated low frequency of individuals who may be "post-treatment controllers" (4 to 5 percent) and that only 15 individuals will be randomized to the placebo arm of this study, the investigators noted that it is statistically unlikely that more than one individual who is destined to become a "controller" would be randomized to the placebo arm. For that one participant to suffer any harm from the 16-week ATI in the study, several assumptions would need to be made that are not supported by available clinical data. First, a linear relationship would have to exist between the duration of cART therapy and the chances of becoming a post-treatment controller; this assumption is not supported by the published data. Second, the individual would have to have been not treated "long enough" with cART, a possibility that cannot be evaluated since it is not known what "long enough" is or if duration of cART treatment matters at all. Third, the ATI would somehow have to interfere with a research participant who has not been treated "long enough" becoming a post-treatment controller by "setting the clock back," an assumption that is not supported by clinical or experimental data. These three theoretical possibilities must be weighed against severely compromising the study design by eliminating the placebo group and thereby preventing important information from being obtained from this study.

RAC reviewer concerns regarding performing an ATI in this patient population are not unique to the recombinant DNA vaccine used in this study. Because ATI is the only way to assess clinically relevant efficacy of immune-based interventions, this concern would apply to any study testing the efficacy of any immune-based experimental therapy in this patient population. If performing placebo-controlled trials involving ATI is considered "unacceptable" based on theoretical concerns not supported by clinical data, then the investigators stated it would be nearly impossible to assess the efficacy of any immune-based therapy in this patient population, which is the population most likely to respond to such interventions.

The investigators explained that this protocol and its informed consent document have been closely reviewed and approved by a number of regulatory committees and agencies including the NIAID Scientific Review committee, the NIAID IRB, and the FDA. These regulatory bodies carefully considered the potential risks related to the study design (including the treatment interruption) and how these potential risks were presented in the informed consent document.

The sentence in the consent that reads "…*or vaccine DNA mixing with your own DNA*" is describing a potential general risk for all DNA vaccines, not just HIV DNA vaccines—that the plasmid DNA theoretically could integrate into host DNA and potentially alter host genetic sequences. This possibility is theoretical and has never been shown to occur in the hundreds of plasmid DNA vaccine studies

conducted within the past 20 years. No real danger exists of the plasmid DNA in this vaccine combining with the HIV DNA and producing a mutant super-HIV.

Research participants in the placebo arm might potentially derive benefit from participating in this trial due to factors other than receiving the vaccine. For example, a small proportion of HIV-infected individuals are able to control their infection for many years without cART. Starting such individuals on cART during early infection will interfere with detecting their ability to immunologically control HIV replication; the only way to identify such individuals is to stop cART and see whether they are able to control plasma viremia in the absence of medications. During the ATI phase of the study, some individuals (in both arms) may discover they are “immunologic controllers” and may be able to remain off cART for years, which is valuable information that would constitute a potential benefit of participating in this study.

Treatment with cART reduces but does not eliminate the risk of transmitting HIV, which is why all HIV-infected patients, regardless of whether or not they are on cART, are encouraged to practice safe sex. Although there may be an increased risk of transmitting HIV during the ATI phase, this is not certain, because some participants (whether they receive the vaccine or placebo) may not experience a rebound in plasma viremia during the 16-week ATI. The statement about this situation in the informed consent document was written to reflect accurately the known facts regarding HIV transmission on and off cART and to appropriately stress the need for using safe sex practices at all times to reduce possible HIV transmission.

The participants in this trial would not be newly diagnosed with HIV. To be eligible for this study, participants must have documented suppression of plasma viremia for greater than one year on cART. Because it generally takes three to six months of treatment to achieve complete suppression of plasma viremia, the minimum time between HIV diagnosis and being eligible for this study would be more than one year.

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

2. Responses to RAC Discussion Questions

Dr. Sneller explained that placebo-group participants would be placed back on ART if (1) a 30 percent drop in CD4 count occurs compared to the baseline count or if the CD4 count drops below 350, although he doubted either would happen; (2) viral load exceeds 50,000 on two determinations, which he stated is a conservative lower viral load; or (3) any AIDS-defining condition develops, which is unlikely to happen in the 16 weeks of ATI.

Dr. Sneller stated that most of the viremic rebound observed in one of the other studies showed the peak viral loads appearing at between weeks 17 and 20. The ATI for this proposed trial will end at week 16.

After consulting with the statistician working with the investigators, Dr. Sneller and his colleagues agreed to control for the likely viral load heterogeneity of potential participants.

E. Public Comment

Dr. Fong read into the record a letter from Robert Reinhard, a global health consultant from San Francisco, California. In the letter, he suggested that treatment interruption may not be necessary in this early Phase I trial and that a placebo group is not necessary in this early-phase trial and adds risk to a vulnerable group. In regard to treatment for research-related injury, he noted that the IAS HIV Cure Ethics Working Group recommends that participants in HIV cure research should receive appropriate medical care for study-related physical injuries at no cost to them. That form of protection in all biomedical trials is also included in currently circulating draft versions to the Declaration of Helsinki scheduled for adoption in 2014. This protection is particularly important if a placebo arm does indeed remain as part of this study design or if unintended consequences of the recombinant rVSV remain in the system, or, as one reviewer noted, concerns remain with mutated virus. He also stated that some inclusion/recruiting screen would be

a useful element of enrollment to determine that a participant is infected with a clade B virus for which the vaccine was designed and not infected with a form of the virus common in other region.

Dr. Fong then read into the record three additional comments e-mailed from Mr. Reinhard while he listened to the RAC discussion of this proposed protocol. Those comments pertained to reseeding the viral reservoir during treatment interruption, the use of placebo for safety comparisons, and whether placebo participants should be asked to undergo electroporation.

F. Synopsis of RAC Discussion and RAC Observations and Recommendations

Ethical, Legal, Social Issues

- This Phase I trial will enroll research participants who were treated early in the course of their infection. They will undergo a 16-week treatment interruption in both the vaccination and the placebo groups. A structured treatment interruption is not extremely risky but does entail some risk, since transient immune function changes may have longer-term effects. The informed consent document should explicitly inform potential participants that, because they were treated early in the course of their infection, data supporting the safety of treatment interruption in previous trials of HIV-infected patients may not be predictive of safety in a different population, such as early-treated patients; they will be monitored carefully. The informed consent document should clearly communicate to potential participants that early treatment of HIV might alter the natural course of HIV infection, although these differences are not well understood and the data are just emerging.

- The informed consent document states under the section titled Alternatives, “You may choose not to participate in this study and continue to receive care from your current physician(s).” For this early-treated population, there may be a number of alternative clinical trials that are specifically interested in these patients, and this alternative should likewise be mentioned.

- The section titled Counseling Subjects on Safe Sexual Practices includes a statement that there may be an increased risk of transmitting HIV (for example, to a sexual partner who does not have HIV) during treatment interruption. This section goes on to emphasize the importance of safe-sex practices even during HIV treatment to avoid implying that those practices are only necessary during treatment interruption. The research participants also should be informed that the potential for an increased risk of transmission is due to the fact that treatment interruption may lead to an increase in HIV viral loads.

G. Committee Motion 8

Dr. Fong summarized the RAC recommendations to be included in the letter to the investigators, expressing the comments and concerns of the RAC. Dr. Fong asked for a vote, and the RAC approved these summarized recommendations by a vote of 18 in favor, 0 opposed, and 0 abstentions. (Drs. Fost and Zoloth voted by proxy.)

XVII. Closing Remarks and Adjournment

Dr. Fong thanked the RAC members and the OBA staff and adjourned the June 2013 RAC meeting at 3:15 p.m. on June 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.)

_____
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: _____

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

Attachment I:
Recombinant DNA Advisory Committee Roster

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Attachment II:
Public Attendees

Sara Albert, NIAID
Ira Berkower, FDA
Anthony Conley, NIAID
Stacy Constable, Tacere Therapeutics, Inc.
Susanna Day
Sandra Donkervoort, NINDS
Kathleen Gittens, NIAID
Steve Grube, parent
Sandra Bridges Gurgo, NIAID
Sean Healy, CBS News
John D. Heiss, NINDS
Greg Keating, NINDS
Sharon King, parent
Lloyd Glickstein, Novartis
Meganne Leach, NINDS
Pev Lindell, Tacere Therapeutics, Inc.
Barry Manjam, Duke University
Bonnie J. Mills, Tacere Therapeutics, Inc.
Kelly Obermayer, Hannah's Hope Fund
Keyna Parsi, Tacere Therapeutics, Inc.
Carla Pettinelli, NIAID
Sarah Read, NIAID
Lori Sames, Hannah's Hope Fund
Matt Sames, Hannah's Hope Fund
Curt Scribner, RRD International
Val Sevastita, NIAID
Thomas Stanton, CBS News
Vuillert, Carole, NINDS
Ellen Wertheimer, NIAID
Hallie Whalen, NINDS
S. Rachel Witten, FDA
J. Fraser Wright, Children's Hospital of Philadelphia and Tacere Therapeutics, Inc.

Attachment III:
Abbreviations and Acronyms

AAV	adeno-associated virus
Ad-Endo	adenovirus expressing endostatin
ALL	acute lymphoid leukemia
AML	acute myeloid leukemia
ART	antiretroviral therapy
ATI	analytic treatment interruption
AZT	zidovudine
cART	combination antiretroviral therapy
CD	cytosine deaminase
CGD	chronic granulomatous disease
CHC	chronic hepatitis C
CNS	central nervous system
CSF	cerebrospinal fluid
CT	computed tomography
DLTs	dose-limiting toxicities
EP	electroporation
FDA	Food and Drug Administration, HHS
5FC	flucytosine
5FU	5-fluorouracil
GAN	giant axonal neuropathy
GCC	guanylyl cyclase C
G-CSF	granulocyte colony stimulating factor
GFP	green fluorescent protein
GI	gastrointestinal
GLP	“Good Laboratory Practices”
GTSAB	Gene Transfer Safety Assessment Board
GVHD	graft-versus-host disease
HCV	hepatitis C virus
HHS	U.S. Department Health and Human Services
hGCC	human guanylyl cyclase C
HGG	high-grade glioma
HLA	human leukocyte antigen
HPV	human papillomavirus
HSCs	hematopoietic stem cells
HSCT	hematopoietic stem cell transplantation
HSV	herpes simplex virus
IC	intracranial
IC-IT	intracranial-intratumoral
ICV	intracerebral-ventricular
IM	intramuscular
IMRT	intensity-modulated radiation therapy
IND	Investigational New Drug
IRB	institutional review board
IV	intravenous
MDS	myelodysplastic syndrome
miRNA	micro RNA
MLV	murine leukemia virus
MRI	magnetic resonance imaging
NAA	N-acetylaspartate
NCCN	National Comprehensive Cancer Network
NIAID	National Institute of Allergy and Infectious Diseases, NIH

NIH	National Institutes of Health
NINDS	National Institute of Neurological Disorders and Stroke, NIH
NK	natural killer
NSG	NOD SCID gamma
OBA	Office of Biotechnology Activities, NIH
OD	Office of the Director, NIH
PBMCs	peripheral blood mononuclear cells
PBSC	peripheral blood stem cell
RAC	Recombinant DNA Advisory Committee
RT-PCR	reverse transcription polymerase chain reaction
SAE	serious adverse event
shRNAs	short hairpin RNAs
SIV	simian immunodeficiency virus
UNC	University of North Carolina at Chapel Hill
WAS	Wiskott-Aldrich Syndrome
WASp	Wiskott-Aldrich Syndrome protein
XCGD	X-linked chronic granulomatous disease

Appendix A:
Public Testimony on Human Gene Transfer Protocol #1303-1231

[This testimony is provided verbatim, as reported in the transcript of the June 2013 RAC meeting.]

Video testimony of Mary Pizzurro, presented by Lori Sames

Ms. Sames: This video was provided by Mary Pizzurro, and these are her two boys, Mike and Joey.

(Video played. In the video, Ms. Pizzurro described the lives of her two sons, Mike and Joey, their support for the GAN trial, and desire that late stage patients not be excluded from phase I or phase II trials.)

Testimony of Matt Sames

"Mr. and Mrs. Sames, your daughter has a rare disease. It is terminal, there's no treatment, no clinical trial and no organization to turn to. Make Hannah comfortable and enjoy your time with her." That was the news we received a little over five years ago, and our lives were changed forever.

To watch your perfect, angelic little girl slowly slip away is painful beyond description. At first she just needed a hand walking, and now she uses a walker for short distances before she gets exhausted and she asks to be carried. Her eyesight is deteriorating, and her breathing is becoming more labored. She loves to sing and perform for people, but her vocal cords are becoming more compromised. Within the past year, her smile and her facial expressions have become dulled, and the brightness that she used to bring into every room has dimmed.

A few months ago, my now nine-year-old Hannah, she asked me if she was going to die from GAN. And we explained that, no, she isn't. She isn't going to die from GAN because we have worked tirelessly for the past 64 months raising money, running 36 events each year. She isn't going to die from GAN, because we have become the world's only charity focused on treating this horrible disease. And there are 42 other GAN families counting on us. She isn't going to die from GAN, because our scientific team has worked around the clock for 62 months to develop a gene delivery procedure that we believe in.

And she isn't going to die from GAN because it's her destiny to change the world, to keep families together and to save children for generations. She's our daughter. She's not going to die from GAN.

Testimony of Steve Grube

I'm the very proud father of two little girls. I am also a pediatric infectious diseases physician. My little three-year-old sits right now on the floor. She's ataxic but without the formthotics, she can still run without falling too much.

At age 4½, her sister was diagnosed with a neuromuscular disorder. At age five, she could walk short distances; 5½, she was using a wheelchair. She'll be eight this month. At age seven, we were told that her carbon dioxide-dependant respiratory drive is gone; that with the next cold she gets, she will likely need oxygen; and that will suppress her oxygen-dependent drive, and she in all likelihood will stop breathing spontaneously at seven. She learned recently that GAN is fatal, and she told me, "Daddy, I don't want to die."

I'm aware of the science. I think they have done fantastic work. I have the objective knowledge to know the success rate of Phase I clinical trials in a whole host of diseases and that at seven, it is unlikely, despite her advanced progression, that she would probably be included as one of the few patients to meet criteria and be included. But this would set the foundation for what will follow for another Phase I that succeeds, for a Phase II that's efficacious, for treatment that could save her life. Her birthday, later this month, cannot be her last.

Testimony of Jody Ketcham, presented by Ms. Sames

I'm going to read a letter written by Jody Ketcham, Casey's mom.

"This is one of the most difficult letters I've had to write. Two months ago, my 21 year-old daughter, Casey, passed away of acute respiratory failure. She spent a majority of the last four months of her life in the ICU with pneumonia. Casey was diagnosed with GAN at the age of 6, and as courageous and full as her life was, it was not free of challenges in her early years.

"She was hospitalized when she was 6 with a serious case of pneumonia and pleurisy, and again, at the age of 11, in order to have rods placed in her back to correct scoliosis. Intestinal and urinary issues were also an issue in her early years.

"From that point, deterioration in her health continued. She lost the ability to walk when she was 12. Her vision began to worsen, and her fine motor skills slowly disappeared over time. At the age of 20, pressure sores became a concern, and just before her 21st birthday, she began to experience seizures and had a feeding tube placed. Pulmonary issues were an ongoing health concern throughout her life, and vigilance was critical to keep her healthy. Thankfully, cognition never seemed to be affected.

"Another noteworthy aspect of this journey is that insurance did not cover any home health aids until she turned 21. I was a single parent, widowed, for the last seven years of her life, making it very difficult to sustain an income. Somehow through all of these challenges, she maintained her spirit, and we were blessed by and so grateful for the many ways in which she blessed our lives.

"Casey taught us all the most profound lessons about life and love. She was fully aware of the efforts of Hannah's Hope Fund, completed the initial round of natural history studies at Columbia, and was hoping to be the first to participate in the study. Sadly, that was not meant to be.

"As her parent, I have read through the feedback of the RAC reviewers and the responses, and do not have any concerns. GAN is a devastating disorder from the onset, and it was Casey's desire to take part in this effort from the moment she heard about it."

Comments from Ms. Sames on Jody Ketcham's testimony:

I've met Jody several times now. She lives in New Jersey, we live in upstate New York. We meet halfway at a coffee shop, and at our first meeting when we were preparing binders to hand to philanthropists with our business plan for how we're going to tackle GAN, I said to her, "Jody, this is largely a disease in children. Because we very quickly need to go into a pediatric setting, I feel FDA is going to require that we go into nonhuman primates." And at first she thought that that was going to add to the timeline, and she said to me, "Lori, I don't want to take the time for a primate study. Let Casey go first. Let her be the large study that would help them feel better about the safety profile of this, pro or con, as to whether or not this could then go into a child."

So I wanted to share that message. We chose, and thankfully we were able to raise the funds to do both rodent and nonhuman primate simultaneously. So the addition of primates added nothing to our timeline.

Testimony of Ms. Sames

I have a couple of things I want to say before I go into what I had prepared to say. Our daughter that Matt spoke about is 9. He just gave you a synopsis of where she is, clinically, today.

Twice a day when I go to put on her AFOs [ankle foot orthoses], and at night when I take them off, I stretch her Achilles tendons. And we do toe exercises and foot exercises, and she still has the ability to squeeze my finger with her toes. She still has innervated muscle fibers, distal. She's 9. Some days I feel nothing, but I curve them with my thumb. Other days she can do it, really impressively. It may have to do with a cartoon she was watching on TV when I tried to get her to do it the day before.

One of our scientific advisers, every now and then, over the past 5 years, would remind me, we're so focused on trying to stop progression, rarely do I ever let myself go to the world where, oh my gosh, this worked beyond our wildest dreams. She'll remind me, "Lori, right now, Hannah's axons may just be dysfunctional." She may not have nerve death yet. We don't know. I know no pediatric neurologist knows. You can't biopsy the motor neurons in a patient.

So we moved forward with the best information that we have. As a passionate mom, I'm highly critical. When myself, Jude, and Steve thought this was ready for an IND, I needed to be certain we had the money to do this once; we raised it completely grass roots. And, thank God, NIH is stepping up at this point and is going to house our child at the Clinical Center here in Bethesda.

So I brought Guangping Gao from UMass to New York City, and Paola Leone, who I believe was here speaking yesterday, to New York City with Steve. For a full day in a conference room, he presented both the efficacy and tox data that we had. I challenged them. I said, "Paola, Guangping, please, from the onset, scrutinize this data. At the end of that day I want you to be able to tell me whether you feel this is ready for a human trial. Have we done due diligence?"

From day one, when Hannah was diagnosed, we decided to fight. She was diagnosed in March 2008. In August 2008 we held the first symposium on GAN in Boston. We had 22 research scientists together—E3 ligase experts, ubiquitin protease experts, anyone that had published anything on GAN was present to look at the data and prioritize their therapeutic approaches. Dr. Gray was there at that meeting, and at that point he didn't know if they had a vector that could transduce the blood-brain barrier and impact motor neurons.

So, we've come a long way, but five years ago Jude Samulski told me, "Lori, the day you inject a vector in Hannah is the day you need to be prepared to bury her." My husband and I talk about this, mostly in the evenings when the kids are in bed. When do we decide—given that we've gotten all regulatory clearances? Hannah's nine. When do we decide it's time for Hannah?

Our barometer is, when do we decide Hannah has cancer? When is a rare blood cancer in a child—or let's not even call it a rare one—when is leukemia imminently fatal in an untreated child with leukemia? I don't think anyone has the answer to that. When was death imminent for Casey? And Mike and Joey, it's his next pneumonia. So Casey had a severe bout of pneumonia at the age of six. We lost Naomi in Scotland when she was eight from respiratory problems and complications from GAN. When is death imminent for a GAN patient? It's their next bout of pneumonia, whether they're 1ten or whether they're 20.

So as parents, we take in the information, we know the ultimate risk that this could cause their death. We know chemotherapy drugs are toxic.

A kid in our school district at home had a blood cancer, and he died from a brain bleed that was a known side effect of his chemotherapy. His parents chose to give him the chemotherapy, knowing it could kill him, but his tomorrow was too horrific so they chose to do something. And that's the choice every one of us needs to have for our children.

Thank you very much. There are more to follow, I believe.

Testimony of Sharon King

Hello. I'm Sharon King, and I want to thank you for allowing me this chance to speak on behalf of this work for GAN. Taylor's Tale is a 501(c)3 not-for-profit organization with the mission to provide funding for Batten disease research and to advocate for increased awareness and support of the rare disease community. Since its founding in 2007, Taylor's Tale has raised awareness and more than half a million dollars to support research. I'm president of Taylor's Tale, but, more importantly, I'm Taylor's mom.

When my child was born in 1998, she was a perfect baby in every way. Taylor was a bonus child, so she had two much-older siblings; in fact, my first child was driving. We all adored her. Her older siblings adored her just about as much as her parents. She met or exceeded her developmental milestones. Everything about our Taylor seemed indeed perfect. But little did we know that she had a health time bomb lurking in her body.

That awful reality emerged when Taylor began to demonstrate problems at school and to have problems with her night vision. After nearly two years of testing and incorrect diagnoses, we finally received the devastating news that our sweet Taylor suffered from a form of Batten disease, an inherited neurodegenerative disease that strikes children, and in some very rare forms adults, and is currently not preventable or treatable.

It's been almost seven years since Taylor's diagnosis, and during that time we've watched her go from a child who was either laughing, singing, dancing, 24/7, to a very lovely teenager who's totally blind. She struggles to speak. She walks with a walker, and she's quickly losing all her independence.

At the age of ten, Taylor joined her Girls on the Run group at her school, and she ran that year two 5Ks. She completed them. The second one she actually improved her time. She had lost a good deal of her vision by that point, so she ran with the help of a running buddy, an older student at the school. That was five years ago. Today she is struggling to walk. Batten disease works quickly to steal our children.

I could choose to give up and to enjoy the time I have left with my child. But I won't, because if I do, there will always be another Taylor, and how does anyone live without hope? I just don't know.

More than 2½ years ago, I heard about this work for GAN and I have followed it since that time. This February, Taylor's Tale joined an international coalition of families and organizations to fund two years of a project entitled Global Gene Transfer for Batten Disease, at the University of North Carolina Gene Therapy Center. We are incredibly excited about this work using the same vector and the same method of administration as for GAN, and you can be sure that we're preparing for next steps as the GAN results start to come in.

The Batten community isn't the only single-gene disorder closely watching this work. There are so many families out there who are searching for real treatments for our children, and we see the potential that this can offer. As parents, we try to do the very best that we can for our kids. We realize the risk of participating in a trial like this, but we live every day with the knowledge that the consequence of doing nothing for our children is sure and certain death.

Given the chance, informed parents will make the decision that is best for them and for their child. I refuse to accept a life without hope for my daughter, Taylor, and that journey led me to Dr. Samulski, Dr.

Gray, and this GAN project. Hope is a really incredible thing, and I'm here, and there is a community of parents out there with me that's ready, right there with me, to help move it forward.

Testimony of Reza Shabazz, presented by Mr. Sames

This is a letter from an M.D. at the University of California, Reza Shabazz. He is the brother of a young man who has GAN. It says:

"To whom it may concern: I wanted to thank Mr. and Mrs. Sames for literally devoting their lives to find a cure for GAN patients. My brother's a 26 year-old GAN patient who's been on a vent for several years. He lives at home with me and my parents, and we take care of him 24/7. It's true that he has no motor function left below his neck; however, he is a happier person than a lot of people I know around me.

"I am a physician, and I follow every detail of his health care. He wakes up every day smiling, hopeful about life, and lives his life more than anybody I know. He goes out in his wheelchair and participates in family and social events. And it is true, he's on a vent; however, my parents and I take such good care of him, but thankfully he's been out of the hospital for three years now. Slowing down his disease progression is a miracle and equal to a cure for us. Although he spends a lot of time in bed, he's always happy and he truly enjoys his life. His memory and cognition is better than an average person's.

"As an interventional radiologist, I take care of a lot of terminally ill patients, and I know when to let a patient just be comfortable and not try heroic measures to buy them a few more days, and when to try everything we can to save them. I have read about this research, and the safety and all the risks and benefits, and I hope my brother gets a chance to have this treatment done. This is a fatal disease and anything that might slightly increase his chance of living will be a miracle to him and my family.

"A lot of times, patients are not willing to continue with treatments, and families who are not able to accept the reality push for it. However, my brother is still fighting this battle, and he's so hopeful that this research will help him fight GAN.

"As a doctor, as a brother and as a human being, I beg of you to consider all these terminal patients, and at least give them a chance to slow down the progression of this disease.

"Regards, Reza Shabazz, M.D."

Testimony of Meaghan Bates, presented by Ms. Sames

I received another note via e-mail late last evening from Meaghan Bates. She asked me to read this. Her son is Lawton, and he was the one who was being helped to bed with the G-tube.

"On December 16, 2006, our family's world came to a devastating halt when our son, Lawton, was diagnosed with Giant Axonal Neuropathy at the age of eight. He is now just months away from turning 15, which should be a milestone birthday for him. He should be excited. He should be excited about getting his Learner's Permit and learning to drive a car, a big step toward independence. But instead, he will drive his power chair for a few more years until he can no longer do so by himself.

"He should be experiencing the best years of his life, free of worry of the future, but instead, he is experiencing loss after loss. He can no longer walk. He can no longer dress himself. He can no longer feed himself. He can no longer take himself to the bathroom. He can no longer shower himself. He has no independence whatsoever and has only more losses ahead, unless there is an effective treatment for Giant Axonal Neuropathy.

"On behalf of Lawton and our family, I plead with you to please give Lawton and other kids with Giant Axonal Neuropathy a chance for a different future. Please be a part of the miracle that changes these kids' lives and gives them a chance at a future, because without this clinical trial, they have no chance at a life at all. Meaghan Bates, mom of Lawton."

Closing Comments by Ms. Sames

I think, lastly, I would just like to close. One continuous conversation that we've had is—and Jude alluded to this—when a comment or a suggestion about the new generation of vectors coming along. When do we push the go button? I'm excited to hear that the next generation is going to be even better hopefully than AAV9. AAV9 to my knowledge is not even in a human yet. We can continue to chase great science for decades and never inject a human.

I pray this team at UNC, which I know they'll do, will continue to chase those great vectors. We're not done. Hannah's Hope Fund is not going anywhere. We know this Phase I is a Phase I safety study in a

vector that's never been put in a human, and for a vector that's never put in the CSF. But it's a start of a bright future for the field of gene therapy. Thank you.

There are many groups closely looking at the safety of this trial, and they already have some preclinical studies under way for their various indications. I know that it's a goal, and it's one of the charters, of the American Society of Gene and Cell Therapy. How do we make this process cookie-cutter safe but cookie-cutter and affordable for other groups?

I'm hoping that the RAC in your private meetings in the months and years to come, if this Phase I AAV9 intrathecal trial is shown safe, what's the recommendation of the RAC collaborating with FDA/CBER for the minimal requirements to come present the protocol to you? Not everyone has money for monkey studies. They can cross-reference our biodistribution data, the nonhuman primate, and move much more quickly and save a ton of money.

How robust is a rodent study, and does it have to be under GLP? These are the questions that we're all being asked by other groups who are closely watching our trial. So I really hope perhaps with CBER, the RAC, the American Society of Gene and Cell Therapy, a guidance can be developed. It needs to be worked on now.

I've had several parents text me this morning with comments like "knock 'em dead" and "break a leg" for this big presentation. And my text response was, "I've got your back. I've got your kid's back." I'm going to try to advocate today not just for GAN, but for those of us who are trying hard to raise the funding to initiate some type of gene therapy project for their indications.

So I think it's very important. I think the time has come when we need to look at how do we do this in a safe manner, with reasonable timeframes and with reasonable amount of cost. Thank you all very much.