
RECOMBINANT DNA ADVISORY COMMITTEE

Minutes of Meeting

September 13 and 14, 2011

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[Note: The latest Human Gene Transfer Protocol List can be found at the Office of Biotechnology Activities' website at <http://oba.od.nih.gov/oba/index.html>.]

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

September 13-14, 2011

The Recombinant DNA Advisory Committee (RAC) was convened for its 126th meeting at 10:30 a.m. on September 13, 2011, at the Hilton Hotel and Conference Center in Rockville, Maryland. Dr. Yuman Fong (RAC Chair) presided. In accordance with Public Law 92-463, the meeting was open to the public from 10:30 a.m. until 5:45 p.m. on September 13 and 8:30 a.m. until 11:30 a.m. on September 14. The following individuals were present for all or part of the September 2011 RAC meeting.

Committee Members

Andrew D. Badley, Mayo Clinic and Foundation
Michael J. Buchmeier, University of California, Irvine
Tianxi Cai, Harvard School of Public Health
Saswati Chatterjee, City of Hope National Medical Center
E. Antonio Chioocca, Ohio State University Medical Center
Rebecca Dresser, Washington University Law School
Yuman Fong, Memorial Sloan-Kettering Cancer Center (RAC Chair)
Norman Fost, University of Wisconsin–Madison
Marie-Louise Hammarskjöld, University of Virginia School of Medicine
Joseph A. Kanabrocki, University of Chicago
Hans-Peter Kiem, University of Washington School of Medicine (*Day 1 only*)
Walter J. Koch, Thomas Jefferson University
Donald B. Kohn, University of California, Los Angeles
Anna C. Mastroianni, University of Washington School of Law (*via teleconference on Day 2 only*)
David A. Ornelles, Wake Forest University School of Medicine
Susan R. Ross, University of Pennsylvania
Marcella Sarzotti-Kelsoe, Duke University Medical Center
Marshall Strome, St. Luke's–Roosevelt Hospital Center/New York Head & Neck Institute
James R. Yankaskas, University of North Carolina at Chapel Hill

Office of Biotechnology Activities (OBA)

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

Additional Speakers

Rebecca Buckley, Duke University Medical Center
Richard O'Reilly, Memorial Sloan Kettering Cancer Center
David Williams, Harvard Medical School and Children's Hospital Boston
Krisztina Zsebo, Celladon Corporation

Non-voting Agency Representatives

Denise K. Gavin, U.S. Food and Drug Administration (FDA) (*Day 2 only*)
Daniel M. Takefman, FDA (*Day 1 only*)

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.

NIH/OD/OBA Staff Members

Linda Gargiulo
Chezelle George
Robert Jambou
Erin Luetkemeier
Maureen Montgomery
Marina O'Reilly
Gene Rosenthal

Attendees

There were 69 attendees at this 2-day RAC meeting.

Attachments

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

I. Call to Order and Opening Remarks

Dr. Fong, RAC Chair, called the meeting to order at 10:30 a.m. on September 13, 2011. Notice of this meeting under the *NIH Guidelines for Research Involving Recombinant DNA Molecules (NIH Guidelines)* was published in the *Federal Register* on August 31, 2011 (76 FR 54241). Issues addressed by the RAC at this meeting included a report from the Gene Transfer Safety Assessment Board (GTSAB, a subcommittee of the RAC), public review and discussion of three gene transfer protocols, updates/discussion of two clinical trials previously reviewed by the RAC, discussion of institutional biosafety committee (IBC) review of human gene transfer protocols, and discussion of two updates to the *NIH Guidelines* for work with defective viral genomes in tissue culture (Section III-E-1) and to Appendix B classification of human etiologic agents on the basis of hazard.

The 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 RAC Meeting June 7-9, 2011

RAC Reviewers: Drs. Koch and Roizman

Dr. Fong asked the RAC members for changes or additions to the June 2011 RAC meeting minutes. Hearing none, he asked for a motion to approve the minutes of the June 7-9, 2011, RAC meeting.

A. Committee Motion 1

Dr. Yankaskas moved and the motion was seconded that the RAC approve the minutes of the June 7-9, 2011, RAC meeting. Dr. Fong asked for any objections. Hearing none, he declared the minutes approved.

III. Discussion of Human Gene Transfer Protocol #1107-1119: An Open Label Phase I Study To Evaluate the Safety and Tolerability of GI-6301, a Vaccine Consisting of Whole, Heat-Killed Recombinant *Saccharomyces cerevisiae* (yeast) Genetically Modified to Express Brachyury Protein in Adults with Metastatic Carcinoma

Presenter: James L. Gulley, M.D., Ph.D., F.A.C.P., National Cancer Institute (NCI), NIH
Additional Presenters: David Apelian, M.D., Ph.D.; Claudia Palena, NCI; Timothy C. Rodelle, M.D., GlobelImmune, Inc.; Jeffrey Sloan, NCI
Sponsor: GlobelImmune, Inc.
RAC Reviewers: Drs. Fost, Sarzotti-Kelsoe, and Strome

A. Protocol Summary

Cancers occur when previously normal cells grow and divide in an uncontrolled fashion. Many human cancers have been shown to have increased quantities of proteins that control cell growth and division. These abnormally produced proteins are often required for the involved cell to become a cancer or for the cancer to spread from the original tumor to other parts of the body. One such over-produced “cancer protein” is called Brachyury. The Brachyury protein is normally involved in the development of an embryo but is not normally produced in the cells of an adult. However, Brachyury protein is produced in many human cancers including cancers of the gastrointestinal tract, bladder, kidney, ovary, uterus, breast, and testes.

Evidence exists that the immune system can fight cancer. One of the cells in the immune system, a killer T cell, can recognize cancer cells that are making proteins involved in cancer, including Brachyury. GlobelImmune has developed a novel immunotherapy product that seeks to stimulate killer T cells to fight cancers making the Brachyury protein. To do this, GlobelImmune scientists use recombinant DNA technology to modify ordinary baker’s yeast (the yeast commonly used to make bread or beer) such that the yeast produces the Brachyury protein. The yeast are then heat-killed and washed, resulting in a product composed of whole heat-killed yeast that contains the Brachyury protein inside. This product is called GI-6301.

No significant toxicities were observed in animal safety studies using GI-6301. GI-6301 has not yet been used to treat patients with cancer.

The purpose of the current study is to determine if GI-6301 can be administered safely to patients with cancers that have a high likelihood of producing the Brachyury protein in advanced stages of disease, to demonstrate immune responses to Brachyury, and to evaluate if there is a delay in cancer progression in research participants dosed with GI-6301.

B. Written Reviews by RAC Members

Seven RAC members voted for in-depth review and public discussion of the protocol. The Key issue was that Brachyury is a novel gene target.

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

Dr. Fost asked why the entry criteria are not limited to patients whose tumors are at the high end of probability of expressing Brachyury, and whether each individual tumor could be assessed for Brachyury expression as an entry criterion. Dr. Fost requested an explanation as to why the response reporting data will only include participants in the expanded cohort and why no statement as to the independence of the “Safety Monitoring Committee” is included in the protocol. He offered one wording change for the informed consent document and suggested creation of a half-page summary of that document written in sixth-grade language.

Dr. Sarzotti-Kelsoe suggested that data indicating lysis of B cells by Brachyury-specific T cells should be shown, and that expression of Brachyury on B cells should be discussed in the section on study design and should be considered in the safety monitoring testing. She asked the investigators to indicate what would be done if a research participant developed a strong response to self B cells. Regarding immunologic tests, Dr. Sarzotti-Kelsoe stated that the table in Appendix D, which indicates that blood will be obtained for immunologic assays, is confusing and should be explained more clearly. She asked the investigators to explain how long vials of GI-6301 would be stable at room temperature, as three different times were shown in the protocol. In the exclusion criteria, Dr. Sarzotti-Kelsoe requested clarification as to whether individuals with pericardial masses greater than 1 cm or greater than 2 cm would be excluded from participation in this trial and she asked why patients with vitiligo would be allowed to be enrolled in this trial. She requested an explanation as to whether “related vaccinia and fowlpox vaccines or antigen-specific peptides” are Brachyury-specific immune therapy approaches. To increase clarity, Dr. Sarzotti-Kelsoe offered four rewordings at various locations in the protocol.

Noting that this trial is well conceived, Dr. Strome also asked why the investigators were not limiting eligibility to subjects with tumors having increased expression of Brachyury, especially given that animal data shows the level of tumor cell lysis to be associated with the level of Brachyury expression. Based on the animal data, he suggested that selection be limited to locally advanced regional pathology; he also suggested excluding candidates with evidence of Epstein-Barr virus (EBV), given the association of EBV with Brachyury expression in B cells. Dr. Strome noted that the tolerability profile of yeast-based vaccines is overstated and should be modified, based on data from other trials using the same yeast platform in which DLTs occurred that possibly were related to product. He asked the investigators to explain more simply some of the terms used in the informed consent document, and requested a rewording of the statement that “several other large studies have been performed without serious side effects” to more accurately reflect the serious adverse events (SAEs) uncovered in other trials conducted by the investigators.

C. RAC Discussion

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

- Dr. Hammarskjöld asked the known function of Brachyury in adult humans.
- Dr. Badley suggested that the investigators consider altering their inclusion criteria from a history of acute EBV to ongoing EBV viremia because of the potential concerns of ongoing acute infections of B cells, particularly in the population of individuals who might have reactivation of EBV. He also suggested that the investigators consider enlarging their exclusion criteria to encompass all women of childbearing potential, because of the potential negative effects if women develop a sustained T-cell response to Brachyury and then become pregnant later on.
- Dr. Kiem asked whether other infections, such as cytomegalovirus (CMV), could increase the Brachyury levels.

D. Investigator Response

1. Written Responses to RAC Reviews

The investigators clarified that the gene is inserted into yeast that is then grown in large quantities and heat-killed. From results of their studies, the yeast particles that contain Brachyury protein are taken up by antigen-presenting cells (such as dendritic cells) that are activated by the yeast particles. These cells then present to the T cells, causing activation of Brachyury-specific T cells that can migrate to, recognize, and kill tumor cells expressing Brachyury.

Unlike analysis for other tumor antigens or markers, it is not possible to predict by analysis of primary tumors which patients would most likely benefit from targeting gene products of tumor cells that had undergone the epithelial-mesenchymal transition process and might be present at metastatic sites. In

matched samples, the investigators have shown a higher proportion of tumors expressing Brachyury in metastatic lesions than in the primary tumor. Therefore, the investigators do not believe it would be ethical to mandate biopsies of metastatic lesions prior to enrollment in this Phase I study; safety (primary endpoint) and immunogenicity (co-primary endpoint) do not require expression of Brachyury in tumors for analysis.

The NCI Safety Monitoring Committee (SMC) is an independent safety committee set up by the NCI leadership to monitor high-profile studies, including all gene transfer studies. It is independent of the NCI institutional review board (IRB), although the IRB receives SMC reports and vice versa. In an attempt to reduce redundant reporting, the SMC allows investigators to use relevant portions of the required annual reports to the IRB to supplement their SMC report.

In response to Dr. Fost's suggestion of including a half-page summary of the informed consent document in sixth-grade language, the investigators noted that the current language complies with standard NCI consent guidelines. They expressed their preference not to add another half page to this document. An IRB-approved synopsis will be available at the website clinicaltrials.gov.

Brachyury positivity detected in normal B-cell fractions could be attributed to the existence of EBV-infected B cells, which have been shown to constitute approximately one in 100,000 to one in 1 million B cells in peripheral blood mononuclear cells (PBMCs). The investigators do not believe that a reduction in EBV-infected B cells would pose any clinically significant danger to the research participant, and might be beneficial. Regarding the lysis of normal B cells *in vitro*, Brachyury-specific T cells have not been able to lyse normal B cells isolated from the peripheral blood of all five healthy individuals tested, while lysis was observed with the positive control H441 lung carcinoma cells.

Standard immune-suppression treatment would be given if the patient developed a strong response to self B cells. Each clinical situation is unique, but typically, if treatment is required, the investigators would start with systemic steroids and supportive treatment. The protocol employs standard language regarding this situation; the language has been approved previously by the appropriate regulatory agencies.

All three timepoints listed in the protocol for GI-6301 are correct as stated. The 24-hour timeframe reflects the product's stability, and the 8-hour limit after accessing a dose is an integrity issue (prolonged, repeated re-entry risks contamination). The investigators agreed to change the wording to improve clarity.

The investigators explained that it is customary to specify that patients with vitiligo, thought to be an autoimmune condition, are allowed to enroll on immune therapy studies at the NCI. There are no clinically significant consequences of progressive vitiligo; therefore, testing vaccines in research participants with advanced cancer who also have vitiligo poses no substantial increased risk.

After this initial safety/immunogenicity study is complete, the investigators hope to be able to treat individuals with locally advanced regional pathology with a Brachyury-targeted vaccine. Brachyury expression is also present on metastatic lesions, so clinical activity might occur in the patient population to be tested in the current Phase I study.

The investigators explained the DLT experienced by participants in previous studies using the heat-killed yeast vector to date, focusing on one individual in particular. Five participants had stable disease as their best response, lasting for 13+, 8+, 8, 4.5, and 4 months; two participants are still stable. The one participant who had a Grade 3 toxicity developed that toxicity after 3 months (seven vaccinations), and tolerated dosing well for 3 months with vaccines every 2 weeks. Six days after her seventh vaccine, she was admitted to a local hospital in Colorado, complaining of shortness of breath. A subsequent CT scan showed pleural and pericardial effusion, both of which had developed since her CT scan at the NCI approximately 10 days earlier. She was monitored and treated with supportive measures and empiric antibiotics for presumed infection, and a subsequent bronchoscopy with lavage and biopsy did not reveal any evidence of infection (from cultures) or lymphangitic spread of the cancer. When the participant's symptoms persisted, empiric high-dose steroids were given, after which her symptoms resolved within 2 days and a CT scan within 2 weeks demonstrated a significant resolution of pericardial and pleural

effusions. She is currently on off-label therapy for her disease and doing well without any residual pulmonary symptoms related to her adverse event. Since this event occurred beyond the first 29 days, it was not considered a DLT per the protocol. This was the only Grade 3 event possibly attributable to vaccine in the 25 participants tested, which is a much better safety profile than is seen with most approved therapies for patients with advanced cancer, where it is typical to experience grades 3, 4, or 5 toxicities in greater than 80 percent of patients.

Simplifications of language requested by reviewers have been implemented.

2. Responses to RAC Discussion Questions

Dr. Gulley explained that the investigators hope to accumulate more data during the Phase I trial, including analyzing additional tissues. Ideally, they would like to have a test that is reproducible and has limited false negatives and false positives, and that could identify metastatic lesions that express Brachyury. The investigators plan to look at Brachyury expression in a relatively noninvasive manner in circulating tumor cells.

If participants have had biopsies of metastatic lesions, Dr. Gulley stated that the investigators will make every effort to obtain those biopsies. Dr. Apelian added that, in Phase II and Phase III, the investigators want to explore how to treat patients who have a tumor type of known-high Brachyury risk before they have detectable Brachyury expression, because the goal is to arrest disease progress as opposed to targeting the primary tumor.

Dr. Palena explained that the function for Brachyury is well known during development as being essential for the formation of the mesendoderm. No reports were found in the literature about the function of Brachyury in any adult tissue, until her colleagues and she published their first report illustrating the expression of Brachyury as a mediator of the epithelial tumor transition in tumor cells. That mediator function is the only known/reported function in adult tissue.

Dr. Gulley offered assurance that the investigators have not seen any other infections, such as CMV, increase the Brachyury levels.

E. Public Comment

No public comments were offered.

F. Synopsis of RAC Discussion and RAC Observations and Recommendations

Clinical and Trial Design Issues

- Brachyury expression may vary in tumor tissue as the tumor cells acquire the ability to migrate and metastasize. As a result, initial tumor biopsies done months prior to enrollment may not accurately reflect the Brachyury expression at the time the research participant is enrolled. However, there are tumors that are known to have a relatively higher expression of Brachyury compared to other tumors. The inclusion criteria currently states that efforts will be made, as much as possible, to enroll patients with tumor types with known increased expression of Brachyury, but there is no explanation regarding how this will be accomplished. It is possible to preferentially select patients with tumors that are known to highly express Brachyury, e.g., breast, lung, or pancreatic cancers. If such an enrollment strategy is planned, then the inclusion criteria should specifically indicate those tumors that will be preferentially enrolled.
- EBV-infected B cells appear to overexpress Brachyury. In patients chronically infected with EBV, the number of EBV-infected B cells is in the range of 1×10^5 to 1×10^6 cells. However, it is unclear whether the number of Brachyury-expressing B cells might be higher in the period following acute infection or in those with ongoing viremia. Therefore, it might be prudent to preclude enrollment of

research participants for a reasonable time period after an acute infection or for those with known viremia.

- As Brachyury is critical in fetal development, it would be prudent not only to exclude pregnant or breastfeeding women, but also any female of childbearing potential, because the vaccine leading to a T-cell response against Brachyury could have implications for future pregnancies.

Ethical, Social, and Legal Issues

- This is a complex study and a half-page summary of the study, to supplement the current informed consent document, would be useful.

G. Committee Motion 2

Dr. Fong summarized the RAC recommendations to be included in the letter to the investigators, expressing the comments and concerns of the RAC. Dr. Yankaskas moved that these comments be approved by the RAC, and Dr. Badley seconded the motion. The RAC voted to approve these summarized recommendations by a vote of 17 in favor, 0 opposed, 0 abstentions, and 0 recusals.

IV. Discussion of Proposed Amendment to OBA Protocol #950: Gene Therapy for SCID-XI Using a Self Inactivating (SIN) Gammaretroviral Vector

Presenter:	Sung-Yun Pai, M.D., Harvard Medical School and Children's Hospital Boston
Sponsor:	David A. Williams, M.D., Harvard Medical School and Children's Hospital Boston
Other Discussants:	Bobby Gaspar, M.B.B.S., Great Ormond Street Hospital, United Kingdom (<i>via teleconference</i>); Luigi Notarangelo, M.D., Harvard Medical School and Children's Hospital Boston (<i>via teleconference</i>)
<i>Ad hoc</i> Reviewers:	Rebecca H. Buckley, M.D., Duke University Medical Center (<i>via teleconference</i>), and Richard O'Reilly, M.D., Memorial-Sloan Kettering Cancer Center (<i>via teleconference</i>)

A. Protocol Update — presentation by Dr. Pai

Dr. Pai presented an overview of this gene transfer study for X-linked severe combined immunodeficiency (X-SCID), reviewed the rationale of the RAC recommendation for excluding research participants less than 3½ months old, showed new data to support evidence that the investigators should offer enrollment to research participants younger than 3½ months, and proposed changes to the informed consent document and the protocol of a new multi-institutional international trial encompassing five sites in London, Paris, Boston, Cincinnati, and Los Angeles.

Two previous trials in London and in Paris treated individuals with X-SCID with gene transfer. A total of 20 research participants were treated, resulting in successful immune reconstitution in 18 of the 20. T-cell counts rose promptly in the 18 individuals at day 90, day 120, and day 180. Two participants failed to reconstitute; one survived after bone marrow transplantation and the other did not. During longterm followup, five participants developed T-cell leukemia due to insertional oncogenesis and one participant in Paris, died of this complication. Out of the 18 participants with successful reconstitution, currently 17 of the 18 have had successful gene transfer and are alive and well without infection and with no evidence of SCID. The vector used in those two trials was a traditional long-terminal-repeat (LTR) driven gamma retroviral vector of an MFG backbone driving the expression of IL-2RG or gamma-C from the viral elements.

The Trans-Atlantic Gene Therapy Consortium designed a new vector with the hopes of retaining efficacy while improving the safety profile for future trials. The new vector has had a number of key modifications

in order to improve safety, including removal of the viral LTRs to reduce transactivation of neighboring genes, removal of all gamma retroviral coding regions, the use of a cellular weak elongation Factor 1 alpha short promoter to drive transgene expression placed in the internal position, and a number of other modifications to improve expression and titer. Extensive preclinical studies were conducted to assess the efficacy and safety of this vector. The result of three separate tests with mice led Dr. Pai and colleagues to conclude that the new vector had the potential to be safer compared to the vector used in previous trials, from an insertional oncogenesis standpoint.

The investigators then embarked on a trial in which autologous bone marrow of purified CD34 cells was harvested from eligible participants with the Clinimax system and subjected to three rounds of transduction after appropriate cytokine pre-stimulation; the cells are then infused without conditioning, which is a format similar to unconditioned transplants for SCID. Dr. Pai showed efficacy data from the three research participants enrolled in this trial so far, all of whom showed nascent immune reconstitution.

Recent published data compared the outcomes of patients with SCID in which there are two affected members in the family. The investigators also queried a multi-institutional registry to identify the most similar patient population to the X-linked SCID gene transfer trial participants. They concluded that survival after gene transfer is currently very similar to both single-institution data and multi-institution data. In addition, with regard to the incidence of graft-versus-host disease (GVHD), Dr. Pai and colleagues gathered multi-institution registry data that showed that approximately 22 percent of patients who had early haploidentical transplant had acute GVHD and approximately 9 percent had chronic GVHD; gene transfer avoids GVHD entirely.

B. Protocol Update — presentation by Dr. Williams

Dr. Williams stated that the data presented by Dr. Pai argue that parents of patients younger than 3½ months with SCID should be offered the opportunity to participate in a gene transfer trial. Based on extensive preclinical data, the investigators expect the current vector to be at least as safe as the previous vector and likely safer. The survival data for the current and previous gene transfer trials in X-SCID compares favorably with survival data of allotransplants in infants younger than 3½ months. In addition, no pre-autologous transplant chemotherapy is used in this protocol. Preliminary data shows that all three research participants dosed to date experienced a response to infusion, with a rise in their T cells and NK numbers. Two of the three participants showed a clear response therapeutically and one participant showed a complete resolution of infection. No GVHD has been reported in participants receiving autologous, gene-corrected cells in this trial, the previous trial, or any other gene transfer trial.

The investigators are requesting to amend the protocol to eliminate the restriction that a child must be older than 3½ months to be offered participation in this trial. In the informed consent document under the section of how X-SCID is usually treated, the investigators propose to add the point that donors may be parents or closely matched people outside the family, but the results are not as successful as a fully matched brother or sister.

Stem cell transplants using parents as donors can be done with or without chemotherapy. In some institutions, haploidentical transplants are performed with chemotherapy ablation because of the experience in many centers that these children reconstitute their immune systems more fully with than without ablation. The investigators propose to add the sentence, "Sometimes the transplant does not fully fix the immune system and those patients then need lifelong infusion of immunoglobulin or antibodies."

Dr. Williams presented the investigators' other additions to the informed consent document:

- According to published and unpublished studies, survival after stem cell transplant from a parent is approximately 60 percent to 80 percent for children older than 3½ months and 85 percent to 90 percent for children younger than 3½ months at the time of transplant.
- Approximately 20 percent of children receiving these types of transplants may develop GVHD that might require treatment with strong and prolonged immune suppression medicine.

- If the child is younger than 3½ months, an alternative therapy such as stem cell transplant should be considered.

C. Presentation by Dr. Buckley

Dr. Buckley related her and colleagues' experience with transplants involving X-SCID patients. The transplants are done without pretransplant conditioning or posttransplant GVHD prophylaxis; followup time is currently up to 29.4 years. During the past three decades, 17 patients had a matched sibling and 151 patients received half-matched transplants from their mothers or fathers; the overall 29-year survival rate is 74 percent for the haploidentical matches and 100 percent for the human leukocyte antigen (HLA) identical matches. The survival rate of patients who were transplanted before age 3½ months has been 94 percent, whereas only 69 percent of those transplanted later have survived.

In 75 percent of the transplanted patients who died, the cause of death was viral infections presented when the patient was referred for treatment. The leading causes of death were cytomegalovirus (CMV), adenovirus, and Epstein-Barr virus (EBV) infection, but other types of viruses also are problematic for these patients. Survival data on the effect of age at transplantation show a steady decline in survival rate after the first 150 days. It is posited that early transplantation occurs prior to the viral infections that are the leading cause of death, thus resulting in a greater survival rate. Because of this data, in January 2010 the Secretary's Advisory Committee on Heritable Disorders of Newborns and Children unanimously recommended adding SCID to conditions routinely screened-for at birth. DHHS Secretary Sebelius approved this recommendation in May 2010 and included related T-cell lymphopenias as other conditions that would be screened-for secondarily, endorsing both screenings as a national standard. Currently six States and one Territory screen for SCID; a progress report from May 2011 indicated that 12 cases of SCID had been discovered by newborn screening in the one year since that screening began.

The advantages of doing a T-cell-depleted nonablated parental marrow transplant, as opposed to a chemoablative transplant, include the following:

- The donor is usually immediately available.
- It is not necessary to wait for the patient to get over infections or become stable.
- It can be done in neonates.
- If the patient is well, it can be done as an outpatient transplant.
- The side effects of chemotherapy and GVHD prophylactic drugs are avoided.

Of the 48 SCID infants transplanted by Dr. Buckley, 34 were neonates (younger than one month old); ten were ten days old or younger at transplant, and one was a 31-week premature baby who was transplanted at seven days old. The donors were mothers, fathers, and in one case an HLA-identical sibling. None of the patients were infected at the time of transplant and none received pretransplant chemotherapy. Except for the marrow cell infusion, the infants were outpatients. The transplanted infants were admitted overnight for the cell infusion, then discharged to an apartment and followed in the clinic every one to two weeks until T-cell function developed. They did not have central lines or GVHD prophylaxis, and a majority of these infants were breastfed. All 12 of the infants who were transplanted at ten days old or younger are still surviving. In addition, treating patients who are transplanted at younger than 3½ months old is significantly more cost effective — at approximately one-fourth the cost — compared to treating individuals who are transplanted after 3½ months, primarily because of the higher cost in older patients for treating infections, many of which require admission to intensive care units.

Long-term outcome over three decades for all 168 patients transplanted by Dr. Buckley and colleagues shows sustained good T-cell function as well as phytohemagglutinin (PHA) response and thymic output, sustained through 21 years, that is not statistically different from what is observed in normal individuals. Clinically, there are statistically significant differences between those who were treated early versus those who were treated later, with survival being much better in those who were transplanted early and with many more patients who reported no problems in the group of those who were transplanted early. A higher number of those who were transplanted late required booster transplants, and the growth retardation is slightly higher in those who were transplanted late.

Regarding the possible advantages of gene transfer compared to T-cell depleted haploidentical transplants, Dr. Buckley noted that originally it was reported that all lineages were transduced but in longterm followup that does not appear to be the case. Claims that gene transfer does not require a donor search is also true for haploidentical transplants, as at least one parent is usually available so no search is required. Neither pretransplant chemotherapy nor posttransplant GVHD prophylaxis is required for either treatment approach; however, a higher risk for malignancy exists for those who receive gene transfer.

Dr. Buckley summarized that a diagnosis of SCID is a pediatric emergency and the potential exists to diagnose this condition routinely at birth. If a rigorously T-cell depleted stem cell transplant from a relative can be done in the first 3½ months of life without chemoablation or without posttransplant immunosuppressive drugs and before infections develop, success is highly probable. Nonablated T-cell depleted haploidentical marrow transplantation provides lifesaving therapy for all forms of SCID, but Dr. Buckley conceded that it is not a perfect treatment.

D. Presentation by Dr. R. O'Reilly

Dr. R. O'Reilly presented information about the transplants he and his colleagues have performed; his data is similar to Dr. Buckley's data. Approximately 80 children, some dating back to 1980, who received haplotype disparate transplants, show an overall 70 percent disease-free survival rate.

In studies conducted by Dr. R. O'Reilly and colleagues, the transplants have involved a lectin-separated marrow graft. X-SCID patients can be durably and consistently engrafted without chemotherapy. However, the limitation with a transplant in X-SCID is that the donor T cells will engraft but the patients are left with host B cells that do not function well; as a result, these patients need gamma globulin prophylaxis. Treating these patients nonablatively with chemotherapeutic regimens such as busulfan, cyclophosphamide, or thiotepa with fludarabine can result in engraftment of donor stem cells. When that regimen is followed, the result is consistent engraftment of donor T cells and donor B cells and reconstitution of the T-cell and the B-cell systems, with patients who are examples of this success for more than 30 years.

Data from Dr. R. O'Reilly's experience with regard to GVHD in transplanted SCID patients shows an eight percent incidence of acute GVHD and no chronic GVHD. Currently, haplotype-disparate transplants are nearly comparable to HLA-matched unmodified marrow transplants if the new approaches and technologies are used, thus not conferring an advantage to gene transfer. The promise of gene transfer providing B-cell reconstitution is enticing but there are significant concerns about whether or not progenitor cell populations that can give rise to corrected B cells are being transferred. Dr. R. O'Reilly's biggest concern is that 5 of 20 research participants who have received gene transfer for this disorder have developed leukemia, and a 25 percent risk of leukemia in this population constitutes a major risk. The issue of GVHD is a smokescreen; if appropriate T-cell depletion is used, it is not a problem.

With regard to the proposed changes in OBA Protocol #950, Dr. R. O'Reilly noted that the new data showing reductions in insertions within LMO2 and in terms of clonality are favorable. The early T-cell reconstitution looks about the same as what has been seen in prior trials but the expression is less. Because of the risk of developing leukemia with the prior vectors within three to four years, Dr. R. O'Reilly suggested using gene transfer approaches for patients who are at greater risk as a result of a marrow transplant or who have other pressing circumstances.

This issue is coming forward at this time because so many States now are conducting neonatal testing for evidence of SCID, and these patients are being referred regularly to expert centers for therapy.

E. Discussion

Dr. Fong framed the questions for the RAC as (1) Is there now enough information to include research participants younger than 3½ months, and is the informed consent document, as modified, stated

appropriately? (2) What data should be gathered that would allow a later comparison study to determine that gene transfer is comparable? (3) Within professional societies, what will be the advice if both approaches are considered effective therapies?

Noting that no issue remains about outcomes for patients younger than 3½ months and that insertional oncogenesis concerns appear to have been addressed, Dr. Williams explained that Dr. Pai is requesting that parents and families have an opportunity to understand the relative upsides and downsides of both therapies and, in an informed way, make a decision for themselves as to whether the patient should be enrolled in a gene transfer trial. There is no implication that haploidentical transplantation is not a desirable approach, but if a patient is being offered ablative or subablative chemotherapy, an alternative to that — gene transfer without chemotherapy — is attractive for many families who should be allowed to consider that option.

Dr. R. O'Reilly proposed and Dr. Williams agreed that the informed consent document should be modified to state that there is no assurance that recipients of gene transfer will experience B-cell reconstitution to a degree any greater than what is accomplished with a haplotype disparate marrow transplant.

Dr. Gaspar explained the approach that has been taken by United Kingdom regulators, which is that these two approaches “are in equipoise.” At the moment it is not possible to differentiate between the two; each has its own advantages and risks. Because differentiation between the two is currently not possible, both opportunities to choose essentially equivalent forms of treatment should be given to the patients, along with sufficient information and independent counseling for guidance. Parents should have the opportunity to look at both forms of treatment and to look at the risks and benefits of each. Given the data presented at this RAC meeting, Dr. Gaspar suggested that families in the United States also should have this opportunity.

Dr. Buckley responded that the first case of leukemia did not appear until after three years in the French trial, and because that timepoint has not yet been reached with this new gene transfer vector, the risk is not yet known, even though theoretically the new vector should hold less risk. She noted that the informed consent document emphasizes GVHD but the real risk of GVHD remains unclear. She also suggested that the statement in the informed consent document that the patient might need immunoglobulin replacement for the rest of his/her life if given a haplotransplant should either be modified or removed, because the same risk exists with gene transfer.

Dr. R. O'Reilly stated that the informed consent document needs to be modified to indicate that gene transfer and a good haploidentical transplant might yield the same functional effects, and that a risk of GVHD exists with the allotransplant and a risk of leukemia exists with gene transfer.

Dr. Williams clarified his and Dr. Pai's opinion that the data supports offering either trial — haplotype disparate grafting or gene transfer — to the families and letting the families decide, with the appropriate changes in the informed consent document. These trials are reviewed by the RAC, the FDA, and the local IRB at five different sites; the informed consent document is completely detailed with respect to the risk of insertional mutagenesis. In addition, all of the trial sites have independent consent monitors, appointed by the IRBs locally, who make sure that the information that has been provided is fair and is understood by the families.

In response to Dr. Fost's query, Dr. Williams explained that each center uses a different method of depleting T cells. Because there is no standardized method, the degree of T-cell depletion varies — and the degree of T-cell depletion has downstream implications for the incidence of GVHD.

Dr. Gaspar explained that the data from Europe is mixed and extends back many years to when T-cell depletion was not as rigorous. He noted that any haploidentical transplant carries a risk of GVHD, albeit low, that is unpredictable even with the best T-cell depletion.

Dr. Notarangelo stated that an opportunity now exists in the United States to do a head-to-head comparison of gene transfer versus bone marrow transplantation. The Primary Immunodeficiency

Treatment Consortium consists of 14 major U.S. centers and a number of minor centers, at which gene transfer and bone marrow transplantation are both considered, which will result in data from each patient enrolled. Dr. R. O'Reilly added that the lessons learned from these studies in terms of the construct being used and the information obtained will shape the future of gene transfer as it is applied to all genetic diseases.

Dr. R. O'Reilly noted that the large numbers of U.S. newborns being tested for SCID will result in the possibility of testing whether this new vector can reduce or eliminate the risk of leukemic transformation while correcting the disease. The transplant approach offers a definitive treatment and the gene transfer approach is potentially definitive. As long as the data is being updated as it becomes available, the informed consent document is clear that a hypothesis about leukemic transformation is being tested, and the informed consent document contains language about the risk of GVHD, the trial using this new vector should go forward. He noted that results with adenosine deaminase SCID patients have been "spectacular."

Dr. Williams explained that the investigators have designed this new protocol to be identical to the previous protocol so that they could rigorously compare the risk of insertional mutagenesis in the previous vector with the risk associated with this new vector. They did not change anything in the protocol other than the vector.

Dr. Corrigan-Curay agreed to distribute the current informed consent document.

F. Public Comment

No public comments were offered.

G. RAC Recommendation

After reviewing Dr. Pai's data, together with the data and opinions of two transplantation experts, the RAC concluded that it is reasonable to offer gene transfer to an infant less than 3½ months of age, even if that infant has the option of a related haploidentical transplant. However, the RAC recommends that the informed consent document and process be revised in order to present the risks and benefits of both procedures, clearly and accurately. It is especially important that parents understand that, while overall survival may be relatively equivalent, there are potential differences in event-free survival. Haploidentical transplant may carry a risk of GVHD, although that risk is low if rigorous T-cell depletion is performed, while gene transfer carries a risk of leukemia that could be as high as 25 percent based on available clinical data. Although Dr. Pai's compelling preclinical data indicates that the modified vector might lower this risk, it is not known yet whether this vector will lower the risk in the clinical setting.

The current informed consent document (activation date October 6, 2010) will need to be edited and reorganized to present the two options in a balanced manner. For example, it does not discuss stem cell transplants as an alternative treatment until page 12. Including this discussion earlier in the informed consent document will help parents understand and consider both options before being immersed in the details of the gene transfer approach. The description of the transplant as an alternative does not provide detailed data on potential efficacy in young children. Therefore, the document should state clearly that some centers have achieved longterm survival rates of greater than 90 percent in infants who are less than 3½ months of age, with low risk of GVHD. It is reasonable to add, however, that some centers have better results than others and to offer to identify those centers that have the best results. In any discussion of the risk of GVHD, it likewise should be noted that some centers have developed techniques that greatly reduce the risk of a serious case of GVHD. Finally, it is misleading only to highlight the lack of development of B-cell function in patients who receive bone marrow transplants, since the longterm followup studies of the Paris and London gene transfer trials also showed lack of correction of the defect in B cells.

Regarding the risk of insertional mutagenesis leading to leukemia, the informed consent document should state clearly that one child enrolled in an X-SCID gene transfer trial died, as is stated in the discussion of

the gene transfer trial for chronic granulomatous disease. This point is implied but not stated in the current informed consent document. Moreover, in summarizing the safety of the modified vector, the statement “Therefore we do not know for sure whether it is actually safer” might be more objective and accurate if revised to say “Therefore we do not know whether it is actually safer.”

These are just examples from the current informed consent document of areas that the IRB might want to review carefully to ensure that parents are provided all the necessary information on the relative risks and benefits of gene transfer versus a related haploidentical transplant, so as to make an informed decision.

H. Committee Motion 3

Dr. Fong orally summarized the RAC recommendations, which centered on approval of dropping the age limitation from the inclusion/exclusion criteria alongside an appropriate informed consent document. Dr. Yankaskas moved for approval; there was no second to his motion. The RAC voted to approve this summarized recommendation by a vote of 12 in favor, 0 opposed, 1 abstention, and 0 recusals. (Dr. Fost was in favor of the proposal but abstained until he has had the opportunity to review the informed consent document.)

V. Updates to the *NIH Guidelines: Proposed Changes to Section III-E-1 — Experiments with Defective Viral Genomes in Tissue Culture* and Appendix B — Classification of Human Etiologic Agents on the Basis of Hazard

Presenters: Drs. Kanabrocki and Ross (representing the RAC Biosafety Working Group [BWG])

A. Presentation by Dr. Ross with Regard to Section III-E-1

Dr. Ross explained that Section III-E-1 of the *NIH Guidelines* pertains to research with partial genomes of eukaryotic viruses in tissue culture. Currently, Section III-E-1 allows investigators to initiate research with partial viral genomes in tissue culture at BL-1 containment upon registration of their experiment with their IBC, which is required to review and approve the containment. Under this section, the virus has to contain less than two-thirds of the genome from any family of viruses, and no helper virus can be present.

The purpose behind Section III-E is to facilitate initiation of low-risk research in tissue culture. The BWG proposed to retain the notion that only “low-risk” research should fall under Section III-E-1, and to change the criterion of a “defective” virus from a quantitative definition (less than two-thirds of the genome) to a functional definition. Work with defective viruses under this section should be restricted to non-complementing cells, which present the lowest possible potential for rescue of a replication-competent virus. The key biosafety consideration for working with defective viruses in tissue culture is whether a replication-competent virus could be rescued. Rescue events are dependent on viral (or cellular) replication and are more likely to occur in the presence of helper virus, to a lesser extent in complementing cells, and to the least extent in non-complementing cells.

RAC review of biosafety considerations for research with synthetic nucleic acids led to discussions of Section III-E-1. A question arose as to whether synthetic techniques might be used to generate a functional virus containing less than two-thirds of a genome. There was also recognition that rescue of a replication-competent virus could occur in the absence of helper virus through mechanisms that involved the recovery of autonomous viral replication functions through recombination of nucleic acid sequences contained in a defective virus with those present in complementing cell lines.

In 2009, the OBA published a proposal in the *Federal Register* to amend Section III-E-1 by changing the criterion regarding the size of the virus genome deletion from one-third to one-half and, in addition to demonstrating the absence of any helper virus, the principal investigator would be required to provide evidence that the resulting nucleic acids in the tissue culture cells are not capable of producing a replication-competent virus. Public comment on this proposal raised two issues: that research has been

conducted safely for many years under this section with viruses that contain more than one-half of the genome but less than two-thirds (e.g., viral replicon particles of Venezuelan Equine Encephalomyelitis [VEE]) and, rather than a quantitative standard based on deletion size, the current understanding of virus biology might allow for a reduction in containment based on functional impairment.

After further consultation with the RAC, the OBA proposed additional amendments in 2010 that would retain the proposed criterion of allowing work under this section if only one-half of the genome was present. In addition, the revisions

- Clarified that this only applied to Risk Group (RG) 3 and RG4 viruses, because research with less than one-half of the genome of a RG2 virus is already exempt from the *NIH Guidelines*.
- Included functional criteria that would allow reduction of containment to be based on the removal of one or more viral genes that are essential for cell-to-cell transmission, even if these genes accounted for less than one-third of the genome. Removal of such genes should prevent the propagation of virus and its ability to cause disease.
- Clarified that containment for research with retroviruses including lentiviruses with the potential to transduce human cells and integrate should not be less than biosafety level (BL) 2.

The new proposed language for Section III-E-1 was published in the *Federal Register* at 75 FR 21008 on April 22, 2010. It stated that an investigator could initiate work at BL1 containment in tissue culture upon notification of the IBC if no more than half of the eukaryotic viral genome is present or if there is a complete deletion in one or more essential viral capsid, envelope, or polymerase genes required for cell-to-cell transmission of viral nucleic acids. In addition, the investigator must provide the IBC with evidence to demonstrate a complete deletion of the nucleic acid sequence such that these functions cannot be rescued through homologous recombination. In both situations there must be evidence that the resulting nucleic acids are not capable of producing a replication-competent virus in a cell line that would normally support replication of the wildtype virus, and that no helper virus is present. In addition, a minimum of BL2 containment is required for experiments with retroviruses including lentiviruses that have the potential to transduce human cells and cause insertional mutagenesis.

Although no public comments were offered in response to the April 2010 *Federal Register* notice, the review of research to create a defective RG4 agent raised concerns about the advisability of working with these viruses under lower containment prior to IBC review. The possibility of a rare event resulting from homologous and/or non-homologous recombination could result in the rescue of a potentially lethal virus at lower containment. Because non-homologous recombination events are independent of nucleic acid sequence similarity, the amount of sequence removed from a viral genome has no influence on the potential for rescue of a replication-competent virus; therefore, a quantitative deletion standard is not a reliable measure of biosafety.

The BWG conducted a biosafety analysis and recommended that RG4 viruses should not be included under Section III-E-1 because most research with RG4 viruses will need to be conducted at BL4, and lowering of containment for the RG4 viruses should be based on data that is reviewed by the OBA and the IBC before work begins. Work with defective RG3 viruses in non-complementing cell lines would also not be included under Section III-E but would qualify for a reduction in containment to BL2, and in certain circumstances to BL1, after IBC review of appropriate biological safety data. Initiation of research should not proceed until there has been an independent review of safety data to ensure that the data documenting the absence of replication-competent virus and the low probability of a rescue event are scientifically valid.

For RG3 viral agents, the BWG noted that:

- Containment for research in tissue culture with all RG3 defective virus constructs will be reviewed under Section III-D and maintained at BL3 unless the IBC authorizes the lowering of containment to BL2 for experiments performed exclusively in tissue culture cells that cannot complement the deleted viral functions. The tissue culture system used at BL2 containment must not allow for cell-to-cell transmission of a defective virus.

- The IBC may lower containment to BL2 following completion of a risk assessment that should include an analysis of the data gathered to examine the presence of replication-competent virus.
- BL1 containment with BL2 practices may be considered if the specific experimental conditions or procedures cannot be performed within a BL2 facility, e.g., the required specialized equipment is located in a BL1 environment.
- Unlike most experiments reviewed by the IBC under Section III-D, this change will grant the IBC authority to lower containment for experiments using defective RG3 viruses in non-complementing cell lines.
- Under the proposed Section III-E, investigators may initiate work with RG2 viruses in non-complementing cell lines at BL1 containment upon registration with the IBC.
- The rationale for allowing containment to be lowered to BL1 without IBC review of the experiment is based on the differences between BL2 and BL1 containment for tissue culture experiments that primarily involve the implementation of biosafety practices; no specialized equipment is mandatory in most cases.

The BWG offered a proposed Section III-E-1, which would state that, upon registration with the IBC, recombinant nucleic acids from a RG2 eukaryotic virus may be used in cells in tissue culture at BL1 if there is a complete deletion in one or more essential viral capsid, envelope, or polymerase genes required for cell-to-cell transmission of viral nucleic acids, and if the tissue culture system used is not capable of complementing the deleted viral functions. A minimum of BL2 containment is required for experiments with retroviruses including lentiviruses that have the potential to transduce human cells and cause insertional mutagenesis.

A proposed new section under III-D would state that experiments with defective recombinant nucleic acids from a RG3 eukaryotic virus in cells in tissue culture will usually be conducted at BL2 containment if there is a complete deletion in one or more essential viral capsid, envelope, or polymerase genes required for cell-to-cell transmission of viral nucleic acids, and the tissue culture system used is not capable of complementing the deleted viral functions. BL1 containment with BL2 practices may be considered if the specific experimental conditions or procedures cannot be performed within a BL2 facility, e.g., the need to use specialized equipment located in BL1 environments. A minimum of BL2 containment is required for experiments with retroviruses including lentiviruses that have the potential to transduce human cells and cause insertional mutagenesis.

Under the proposed Section III-E for RG2 or III-D for RG3, to qualify for a reduction of biocontainment, the following data must be provided:

- Results of experimental assays demonstrating that the defective virus cannot be transmitted from cell to cell when transfected in a cell line that would normally support cell-to-cell transmission of the intact virus. Such documentation should include evidence that the number of virus-infected cells does not increase on multiple serial passages of the transfected cells. Experimental assays should be designed to detect the presence of replication-competent virus and should be appropriately controlled for sensitivity and limits of detection.
- Demonstration that the cells lack helper viruses for each specific family of defective virus being used. If helper virus is present, review will proceed under Section III-D-3, Experiments Involving the Use of Infectious Animal or Plant DNA or RNA Viruses or Defective Animal or Plant DNA or RNA Viruses in the Presence of Helper Virus in Tissue Culture Systems.

The potential impact on current research was considered by the BWG. Comments from researchers using VEE attest that some investigators are working in complementing cell lines with defective RG3 viruses (e.g., VEE replicons) at BL1 or BL2 containment under the current Section III-E-1. If the recommended changes were implemented, under the revised *NIH Guidelines* this work in complementing cell lines would require BL3 containment. Investigators would be allowed to ask the OBA to lower containment for such research by submitting supporting documentation, and the IBC then could perform a risk assessment taking into account the OBA recommendation.

Implementation of this proposal will require publication of a *Federal Register* notice and request for public comment on the proposed changes to Sections III-D-3 and III-E-1 of the *NIH Guidelines*.

B. RAC Discussion and Comments

Dr. Hammarskjöld asked whether a definition of a complementing cell line would be provided, or whether guidance would be left to the IBCs. Dr. Ross responded that the expectation is that a complementing cell line has a gene from a particular class of viruses that will complement the missing function. It will be the responsibility of the investigator to demonstrate to their IBC that they are not using a complementing cell line. One of the requirements is to show data that demonstrates an inability for the virus to replicate in the cell being used.

Dr. Buchmeier queried as to whether this recommendation would affect existing attenuated viruses, especially those with a long history of safety in humans. Dr. Ross explained that this recommendation deals only with viruses with deletions in essential genes; Dr. Corrigan-Curay added that it deals only with tissue culture and a wild type virulent virus that is being deleted.

Dr. Jambou reiterated that research that removes 50 percent of the genome of a RG2 virus is not covered by the *NIH Guidelines*.

C. Committee Motion 4

Dr. Yankaskas moved for approval by the RAC of the BWG recommendations for changes to Section III-E-1 and III-D of the *NIH Guidelines*, and the motion was seconded. The RAC approved these recommendations by a vote of 18 in favor, 0 opposed, 0 abstentions, and 0 recusals.

D. Presentation by Dr. Kanabrocki with Regard to Appendix B

Dr. Kanabrocki presented the conclusions of another BWG work product — the classification of human etiologic agents on the basis of hazard as enumerated in Appendix B of the *NIH Guidelines*. Appendix B specifies the risk group classification of an agent based on its ability to cause disease in healthy adults and the availability of treatment for that disease. The risk group of an organism is a key component of the risk assessment to determine containment under the *NIH Guidelines*; therefore, it is important to list an organism in its appropriate risk group. IBCs can increase containment for experiments under the *NIH Guidelines* and, in general, IBCs must consult with the OBA to lower containment.

The BWG proposes the following organisms to be added to the list of RG2 bacteria:

- *Coxiella burnetii* Nine Mile strain, plaque purified clone 4
- *Francisella tularensis* subspecies *novicida*, Utah 112; *holarctica* LVS, and biovar *tularensis* strain ATCC 6223 (also known as strain B38). For research involving high concentrations of these attenuated *F. tularensis* strains, BL3 practices should be considered.
- *Yersinia Pestis*, *pgm*(-) (lacking the 102 kb pigmentation locus) and *lcr* (-) (lacking the LCR plasmid)
- Chikungunya vaccine strain 181/25
- Junin virus candid #1 vaccine strain
- Venezuela equine encephalitis vaccine strain V3526
- Japanese encephalitis virus strain SA 14-14-2

The BWG suggests that descriptions of the following vesicular stomatitis virus (VSV) non-exotic strains that are RG2 organisms should be clarified: VSV-Indiana 1 serotype strains (e.g., Glasgow, Mudd-Summers, Orsay, San Juan) and VSV-New Jersey serotype strains (e.g. Ogden, Hazelhurst).

The BWG proposes the following organisms to be added to the list of RG3 viruses:

- SARS-associated coronavirus
- Chikungunya virus
- West Nile Virus

A notice about this proposed updating of Appendix B was published in the *Federal Register* on July 25, 2011 (76 FR 44340) and public comment ended on September 9, 2011. One comment was received, from the American Biological Safety Association (ABSA), which stated that “OBA should consider adding additional information to Section II-A-3 covering the assignment of Risk Group to commonly used attenuated strains.” Dr. Kanabrocki explained that Section II-A-3 provides a framework for conducting a comprehensive risk assessment. In response to ABSA’s comments, the OBA will add a reference to Appendix B in the last sentence of the first paragraph of Section II-A-3 that will read: “Certain attenuated strains or strains that have been demonstrated to have irreversibly lost known virulence factors may qualify for a reduction of the containment level compared to the Risk Group assigned to the parent strain (see Appendix B, *Classification of Human Etiologic Agents on the Basis of Hazard* and Section V-B, *Footnotes and References of Sections I-IV*).”

Assuming the RAC concurs with these proposed changes to Appendix B and Section II-A-3 of the *NIH Guidelines*, a Final Notice will be published in the *Federal Register* to implement these changes.

E. RAC Discussion

In response to Dr. Hammarskjöld’s question, Dr. Kanabrocki clarified that Appendix B lists pathogens by risk group, and the attenuated strains would appear as specific strains. Therefore, if investigators bring other attenuated strains to their IBC, the IBC will need to obtain guidance from the OBA.

Dr. Fong asked who decides the classifications of pathogen strains. Dr. Kanabrocki explained that, in the United States, these classifications are the responsibility of committees at the NIH and the Centers for Disease Control and Prevention. The characteristics that are weighed for classification are communicability, whether therapies are available, the severity of the disease, and whether the pathogen causes disease in healthy adult humans.

Dr. Buchmeier commented that this updating of Appendix B is long overdue, and that interpreting the various inconsistent documents has been a source of problems for many years. Updating is important because the rate of pathogen discovery is greater than it has ever been. Dr. Kanabrocki added the importance of this updating being an ongoing effort.

F. Committee Motion 5

Dr. Fong requested a motion to approve the BWG proposals to update the classification of human etiologic agents on the basis of hazard as enumerated in Appendix B of the *NIH Guidelines*. Dr. Buchmeier moved for approval by the RAC, and the motion was seconded. The RAC voted to approve these recommendations by a vote of 18 in favor, 0 opposed, 0 abstentions, and 0 recusals.

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

Principal Investigator:	E. Antonio Chiocca, M.D., Ph.D., Ohio State University Medical Center
Presenters:	Debra Gessner, M.S., Tocagen, Inc., Douglas Jolly, Ph.D., Tocagen, Inc.; Dan Pertschuk, M.D., Tocagen, Inc.
Sponsor:	Tocagen Inc.
RAC Reviewers:	Drs. Kohn, Ross, and Yankaskas

Dr. Chiocca was recused from discussion of this protocol due to a conflict of interest.

A. Protocol Summary

There is an ongoing, intensive search for novel therapies to improve the prognosis of patients with the most common and aggressive form of primary brain cancer; high grade glioma (HGG; Grade III or IV glioma). Gene transfer is one such approach. Early gene-transfer studies with replication incompetent vectors showed this approach to be generally safe, but ineffective due to limited transduction of the tumor. More recently gene transfer has been attempted with oncolytic, replicating viruses. However these viruses are rapidly cleared by the immune system due to the inflammatory response generated to the virus and its lytic process. Toca 511 uses a retroviral replicating vector (RRV) to overcome the limitations of previous gene transfer protocols. This platform has the following advantages: 1) vector only infects dividing cells, 2) virus stably integrates into the genome of the tumor cells allowing for long-term control of the tumor, 3) virus is not intrinsically oncolytic, and 4) virus has been engineered to express the prodrug-activator enzyme, cytosine deaminase (CD), that catalyzes the intracellular conversion of the antifungal drug, flucytosine (5-fluorocytosine, 5-FC) to the cytotoxic drug 5-fluorouracil (5-FU). In both xenograft and syngeneic intracranial mouse tumor models the Toca 511/5-FC combination was able to significantly increase the survival of treated animals. The goal of the current clinical development program is to demonstrate the safety and efficacy of Toca 511 administered intratumorally to subjects with recurrent HGG followed by cyclic treatment with the oral prodrug 5-FC. The vector used in this gene-transfer platform is a retroviral replicating vector derived from a cloned Moloney murine leukemia virus (MLV). The original ecotropic envelope gene has been replaced with an amphotropic envelope gene enabling the virus (referred to as amphi-MLV) to infect human cells. A modified, yeast-derived cytosine deaminase (CD) gene has been inserted into this vector. The vector and CD gene construct is classified as a prodrug-activator form of gene transfer. The final formulation of the vector/CD construct is referred to as Toca 511.

A Phase 1 clinical, ascending dose study evaluating the safety and tolerability of single doses of Toca 511 administered intratumorally via stereotactic, transcranial injection and followed by repeated cycles of orally administered 5-FC has previously been reviewed by NIH RAC (Protocol 0904-976) and is currently enrolling subjects. In this ongoing study, three subjects in the first dosing cohort have received Toca 511 injected once into the tumor and followed three-four weeks later by six days of oral 5-FC. 5-FC was repeated monthly until tumor progression. There were no DLTs or product-related SAEs reported for the first dosing group, and overall safety and tolerability were excellent. The new proposed clinical study is similar to the ongoing clinical study, but will evaluate the safety and clinical effects of ascending doses of Toca 511 administered to subjects with recurrent HGG at the time of resection, followed by treatment with repeat cycles of oral 5-FC. This study will evaluate intracavitary injection of a single dose of Toca 511 administered by multiple injections into the walls of the resection cavity at the time of craniotomy and tumor resection. Up to four dose levels of Toca 511 will be studied, with three subjects per dose level, and six subjects will be enrolled at the maximum tolerated dose. Approximately seven weeks (\pm one week) later, subjects will begin treatment with oral 5-FC for eight days. On the 6th, 7th or 8th day of dosing the trough 5-FC serum concentration will be determined and the dose of subsequent 5-FC cycles adjusted to maintain the concentration in the therapeutic range. If tolerated, these eight-day courses of 5-FC will be repeated approximately every eight (\pm one week) weeks until study completion. All three subjects in a dosing cohort must complete at least one cycle of 5-FC before dose escalation can occur. Subjects will undergo MRI scanning approximately every eight weeks. Tumor response will be assessed using the Macdonald criteria. Safety assessments will include flucytosine blood levels, monitoring of blood, saliva and urine for virus, clinical chemistries and hematology at selected time points, and recording of adverse events throughout. All subjects will be followed for six months in this study. All subjects who receive Toca 511 treatment may elect to roll into a continuation protocol that will record long-term follow up for safety and viral biodistribution.

B. Written Reviews by RAC Members

Eleven RAC members voted for in-depth review and public discussion of the protocol. Key issues included the potential safety concerns raised by detection of vector DNA and RNA sequence in the blood.

As 5-FC is not administered until eight weeks after vector administration, vector replication and integration in the early weeks of the study might increase the risk of lymphomagenesis in the proposed dose escalation study.

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

Dr. Kohn noted that this study is well developed, with a well-designed vector dosage escalation schema, intraoperative verification of the presence of residual tumor, clinical monitoring for absorption of the 5-FC prodrug, and monitoring for vector adverse effects, especially the risks of insertional oncogenesis. He applauded the premise that the safety monitoring results from the prior trial of the same vector would be considered in guiding safety and dose-escalation deliberations for this trial. Dr. Kohn suggested that the occurrences and causes of insertional oncogenesis should be updated and upgraded in the protocol and in the informed consent document. He asked how brain mass would be computed, and suggested that the long-term follow-up study that would be offered to the research participants should monitor them for replication-competent retrovirus, as it is a potential complication that could arise late. He requested that the investigators discuss what will happen to participants whose tumors progress on study — whether they will continue to receive courses of 5-FC and whether efforts would be made to continue to monitor for virus levels in blood, urine, and saliva. Noting that the informed consent document was clear, readable, and thoroughly detailed in the study's description, Dr. Kohn suggested adding leukemia and lymphoma to the list of severe potential complications.

Dr. Ross asked the investigators to provide the data regarding the loss of viral sequences coinciding with the appearance of antiviral antibodies, as well as the evidence that research participants are mounting an antiviral humoral immune response. She queried as to whether the investigators were concerned that 5-FC might not control spread among PBMCs. She noted that two of three participants who received Toca 511 and then subsequently had re-excision of their tumor upon progression showed evidence of virus spread in the tumor despite multiple courses of 5-FC treatment, and asked whether the investigators believe these tumor cells had insufficient expression of the CD gene. Dr. Ross suggested that evidence of persistent viral infection (not necessarily accompanied by evidence of tumor progression) should be a criterion for offering antiretroviral drug therapy. While the investigators provide good evidence that retroviral (azidothymidine [AZT]) treatment of tissue culture cells is sufficient to prevent virus spread, Dr. Ross noted that the investigators appear not to have tested this theory in an *in vivo* model. She asked whether the investigators have demonstrated in their xenograft model that the vector spreads among tumor cells, whether GBM cells are infected efficiently by Toca 511, and whether the investigators have examined the vector insertion sites at multiple progression sites to show that the purported Toca 511 spread in tumors is not tumor cell spread. Because of the uncertainty regarding horizontal transmission, Dr. Ross suggested recommending or requiring barrier contraception for all research participants and their partners until there is no evidence of virus, rather than the 1-year time period currently described in the protocol.

Because the escalating doses of Toca 511 might increase the likelihood of virus persistence and shedding, Dr. Yankaskas asked the investigators to provide current data from the glioma dog studies and any human data at higher Toca 511 doses. He also requested that the investigators describe the time course of neoplastic complications in mice (because the lymphoma and leukemia risks in mice could differ from those in humans) and other animals that might define a risk period in the human studies, and whether the followup period would encompass the longest likely risk period. Dr. Yankaskas also suggested two clarification changes to the informed consent document.

C. RAC Discussion

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

- Dr. Fong asked whether and for how long barrier contraceptive would be recommended.
- Because he was concerned about the contacts of these research participants and the community in which they live, Dr. Fong suggested that the investigators consider assaying for infectious

particles in additional bodily fluids. He noted that the investigators described the universal precautions (Class 2 isolation) under which these research participants would be kept while in the hospital, but data showed that the viremia occurs after they are out of the hospital and have returned home. Knowing which body compartments and which fluids are involved in shedding is important.

- Dr. Fong asked how the investigators decide when to administer antivirals. He strongly suggested that some standard operating procedure be used that is not based only on DNA or RNA copies in the blood but also a clinical parameter or an infectious particle parameter.
- Dr. Badley asked the investigators which antiretroviral drug(s) they plan to administer and how they will monitor for resistance. Noting that using AZT monotherapy is likely to result in resistance and that AZT is one of the more toxic antiviral agents, he encouraged the investigators to use *in vitro* tests to determine which of the current antiretroviral agents have activity, pick the best two/three/four of those in consultation with someone who treats human immunodeficiency virus (HIV) and knows how to administer those drugs, and administer antiretroviral therapy that is likely to be durable — meaning administering three or more drugs at once.
- Noting that the investigators plan to test for infectious particles in blood, saliva, and urine, Dr. Hammarskjöld suggested (instead or in addition) that the investigators test lymph nodes, vaginal fluid, and semen, especially if there is a concern about sexual transmission.
- Dr. Strome expressed concern that some of the risks to the research participants might be overstated, given that the lifespan for these individuals is approximately 6 months past enrollment in this clinical trial. The emphasis on viral risk should be with the contacts, not with the participants.
- Dr. Fong summarized his concerns by stating that this replication-competent integrating virus is not found in nature, so adequate data is needed to figure out where it is in the human body, when it appears, and whether it is transmissible to someone nearby.

D. Investigator Response

1. Written Responses to RAC Reviews

Currently, the potential for oncogenesis in retroviral vector transduction protocols is believed to be a function of the vector elements (e.g., promoters), vector configuration, the transgene, the clinical setting, and the type and number of cells transduced. Oncogenesis (e.g., leukemia/lymphoma) has been observed with transduction of hematopoietic stem cells after cultivation with cytokines, in patients with severe immunodeficiency and typically when the patients are young and the transgene has conferred a survival advantage on the transduced cells. The clinical situation associated with this protocol shares none of these characteristics, and the investigators therefore believe that the risk of vector-induced oncogenesis in study participants is low. The informed consent document has been amended to better reflect what is now known, and the protocol and Investigator's Brochure will be amended similarly.

All participants who receive Toca 511 will be advised that they will be requested to participate in the longterm followup (LTFU) protocol, Tg 511-09-01. In this protocol, quantitative polymerase chain reaction (qPCR) on whole blood and quantitative reverse-transcription polymerase chain reaction (qRT-PCR) on plasma will continue to be performed on the following schedule:

- Year 1: If either test is positive in the initial protocol, monthly testing will continue until negative. If testing is negative in the initial protocol, retesting will be every 3 months; if testing becomes positive in the LTFU study, testing will revert to monthly until negative and then will be conducted every 3 months.

- Years 2-5: If either test is positive, monthly testing will continue until negative; if testing is negative, retesting will be performed every 6 months.
- Years 6-15: If either test is positive, monthly testing will continue until negative; if testing is negative, retesting will be performed every 12 months.

Urine and saliva specimens will be collected monthly as long as either the qPCR on whole blood or the qRT-PCR on plasma is positive. This testing algorithm was based on preclinical data showing that mice did not shed vector unless high levels of signal were present in the blood. PCR signal in blood was the most sensitive predictor of shedding in the mouse.

Regarding what will happen to participants whose tumors progress on study, the investigators explained that studies in immune-competent mice indicate that tumors continue to grow after the first course of 5-FC, but subsequently shrink and disappear after the fourth cycle. Documentation in humans indicates that radiographic response might significantly lag behind true tumor response, so investigators will keep participants in the study and on 5-FC despite early radiographic evidence of progression. Following true clinical or radiographic progression, participants will be encouraged to enter the LTFU study and, in this protocol, are allowed to continue to receive 5-FC. The LTFU study will monitor blood, urine, and saliva.

IRBs have requested that potential side effects be listed by severity and expected frequency. The informed consent document lists severe side effects as occurring in approximately 1 percent to 3 percent of participants. As requested by the RAC at the presentation of the investigators' prior protocol, the occurrence of lymphoma/leukemia in mice and in X-SCID patients is discussed in the current informed consent document.

The Sponsor has not proposed that 5-FC would control infection; rather the investigators note that, in mice administered Toca 511, lymphomas were not observed following a single course of 5-FC. The timing of the appearance of the vector sequences in patients' blood is similar to that seen after intracranial injection of vector into mice in various studies. Based on published studies in monkeys and human intravenous dosing of nonreplicating retroviral vectors, the investigators expect some vector uptake into white blood cells and that the immune system would control viral replication systemically. This expectation appears to be corroborated by the data from the first dosing cohort in the intratumoral injection protocol, Tg 511-08-01 (RAC Protocol #0904-976).

Based on the systemic control of Toca 511 seen in B6 mice while there is ongoing tumor-specific viral replication and therapeutic efficacy as well as published data of control of amphotropic murine leukemia virus in monkeys, it is likely that the immune system controls spread of the vector outside of the immune-privileged environment of the tumor. This appears to be corroborated by the data from dosing cohort 1 in the ongoing Tg 511-08-01 intratumoral administration clinical study. Thus, the investigators continue to believe that it is appropriate that antiretroviral therapy only be recommended when research participants have persistent, high-level infection outside of the tumor coupled with no apparent clinical benefit.

The investigators report identifying three possible explanations for the presence of vector in the tumor after 5-FC treatment: 1) CD is underexpressed or not expressed in the tumor, 2) CD is expressed but the tumor has become 5-FU resistant, or 3) residual vector is the consequence of the integrating nature of the vector and the fact that 5-FU does not kill non-dividing cells. Under-expression of CD seems unlikely, given that primary GBM and GBM cell lines readily become transduced and express the transgene. 5-FU resistance is observed during systemic treatment of cancers with 5-FU. The local conversion of 5-FC to 5-FU is expected to result in much higher local concentrations of 5-FU than those normally reached by infusion or bolus administration of 5-FU, which should limit the emergence of 5-FU resistance. Since the locally produced 5-FU is rapidly inactivated by dihydropyrimidine dehydrogenase as it diffuses from the tumor, it is possible to safely have high 5-FU concentrations in the tumor without side effects in other tissues. The integrating nature of the vector and the inability of 5-FU to kill non-dividing cells predicts persistence of vector in the tumor after 5-FC treatment. After Toca 511 infection in the tumor, multiple courses of 5-FC are administered. The use of multiple courses of 5-FC is based on the observations that, in a human xenograft model, cyclic administration of 5-FC appears more effective for survival than does a

single course of 5-FC. In the investigators' preclinical syngeneic mouse model, multiple courses of 5-FC were confirmed to increase survival.

The investigators explained the presumed mechanism of action of Toca 511 and 5-FC. After intratumoral administration, the vector only infects and integrates in replicating cells. Subsequently during oral courses of 5-FC, the 5-FC is absorbed, travels through the blood, crosses the blood-brain barrier, and enters the tumor where it is converted into 5-FU only in the Toca 511 transduced cells. 5-FU is an anti-cancer drug that has an S-phase cell killing profile — it only kills replicating cells. Since not all tumor cells divide during a short course of 5-FC, not all infected cells are killed, explaining the presence of vector sequences in the resected tumors after three courses of 5-FC. The transduced and surviving cells continually produce Toca 511 by virus budding without the cells themselves being lysed. This endogenously produced Toca 511 infects remaining replicating tumor cells even after one or more courses of 5-FC. This proposed mechanism of action is supported by the human resection data showing persistence of the vector genome and production of RNA in the tumor. In immune-competent mouse models, evidence of dose-proportional efficacy is seen over a Toca 511 intracranial dose range of 1.7×10^3 TU/g brain to 4.7×10^6 TU/g. The Toca 511 dose evaluated in the first three research participants in the ongoing clinical trial (2.6×10^3 TU/g) is at the low end of this range, so the investigators are not surprised that net tumor regression has not been observed.

Given the real and imminent risk of the study participants dying from their tumor in six to eight months, the investigators believe that the risk/benefit ratio does not favor automatically administering antiretroviral drugs to participants with persistent viremia who demonstrate a clinical or radiographic improvement or stabilization after Toca 511 and 5-FC administration. Based on the current understanding of virus-associated oncogenesis, the potential for this event to occur in this clinical protocol is very low.

The investigators have tested the effect of AZT on Toca 511 viral levels in the blood of infected mice. In addition, published experiments show that AZT can inhibit vector spread in a mouse subcutaneous tumor model.

The investigators stated that they have not observed a reduction in survival in any of the control animal groups that were treated only with vector in mouse tumor models compared to controls with tumor not treated with vector.

The investigators did not perform studies with mice of the opposite sex because fighting and biting by male mice could cause parenteral transmission of the vector that would confound interpretation of the sexual transmission data. Studies were performed with viremic female mice co-housed with vector naïve females to determine if the vector could be transmitted by saliva, excreta, or casual contact. In these studies, virus was not observed to be transmitted horizontally. However, because semen and vaginal secretions might be infectious, the investigators will recommend condom use for sexually active men and women in this clinical study. At the suggestion of a RAC reviewer, the investigators agreed to change the informed consent document to require condom use for at least six months or until vector sequences are no longer detected, whichever is longer.

The Toca 511 vector preparations used in all experiments are viral particles from a permanent producer line, originally infected with transiently produced virus, so no CMV promoter is present in Toca 511. The glioblastoma infection experiments were performed with LTR-driven infectious vector preparations demonstrating efficient infection and spread.

The investigators shared the most current data from humans and animals:

- In the ongoing clinical study, Protocol #0904-976 (Tg 511-08-01), three participants have received intratumoral Toca 511 in the next dosing cohort (9.5×10^3 TU/g), with no product-related adverse effects observed to date.
- In addition to mice, the biodistribution of Toca 511 vector has been examined in two dose groups of healthy (nontumor-bearing) male beagle dogs after intracranial vector administration, in two

healthy (nontumor-bearing) male beagle dogs after intravenous (IV) administration, in a single healthy (nontumor-bearing) female mixed-breed dog after intracranial vector administration using convection enhanced delivery (CED) followed by subsequent 5-FC treatment, and two male client-owned dogs with naturally occurring malignant gliomas that both received intratumoral Toca 511 administration by CED followed by multiple courses of oral Toca FC (5-FC extended release). The biodistribution study of intracranial injection of Toca 511 in healthy male beagle dogs showed no detectable virus at any timepoint to 180 days in blood or in potential shedding samples (saliva, feces, urine, skin swab, and semen) at multiple timepoints to Day 180. The results show that at no point during any of these studies were any Toca 511 vector DNA sequences detected outside of the injected brain tumor, with the exception of the blood sample from the two dogs receiving Toca 511 intravenous. Despite lack of detectable vector sequences in the peripheral blood or in any shedding samples to date, tumor samples obtained from both dogs with glioma were positive for vector sequences, and further analyses for the one dog tested showed presence of an intact CD gene. Both patient dogs that have received Toca 511 have shown conversion to seropositivity for antivector antibody. The second dog was positive at multiple timepoints then dropped to low but detectable levels at ten months after vector injection. The two healthy intravenous dogs also had large antibody responses to the vector infusion.

- Previous studies with replication-competent amphotropic-Moloney murine leukemia virus hybrid vectors in monkeys with IV or intraperitoneal administration have shown some low level persistence of viral sequences, immune responses to the virus, and no pathogenicity out to a mean of more than 2 years. For Toca 511, the BALB/c mice, chosen as the animals most susceptible to infection and pathogenesis, show lymphoma at 10 percent to 20 percent at or close to 180 days after intracranial injection of Toca 511. No vector-related lymphomas were observed after even a single course of 5-FC treatment, including both with and without intracranial brain tumor implants. No vector-related lymphomas/leukemias have been observed in any experiment with Toca 511 and B6C3F1 mice.

2. Responses to RAC Discussion Questions

Dr. Pertschuk stated that barrier contraceptive would be required of all participants (men and women) for six months or until the virus is not detected, whichever is longer. At present, the investigators are still encountering antibodies up to week 18.

Regarding assaying for infectious particles, Dr. Pertschuk explained that this virus appears to have a predilection for T cells or lymphatic tissue, and it would most likely be spread through parental means or sexual transmission. This is a RG2 virus that is not known to cause human disease; given that HIV is a RG3 virus and no serious precautions are taken with HIV patients (other than advising them not to donate blood, share needles, or have unprotected sex), the recommendation about intimate contacts is reasonable but protecting the population from this RG2 virus in other ways does not appear necessary. The investigators inform research participants that they should not share razors or needles, donate blood, or get tattoos, nor should they engage in unprotected sex.

Dr. Pertschuk stated that the investigators intend to model their plans for antiviral administration and monitoring after what has done in response to HIV infection. At present, their intent is to start AZT monotherapy in the case of exposure from a research participant to healthcare worker or research staff. The investigators would assess the severity of the exposure and the status of the research participant's viremia. If the participant has no detectible virus, the course of treatment of the exposed individual likely would not be as aggressive as with HIV; however, if the participant had virus in their blood, it is likely that a full course of post-exposure prophylaxis would begin.

Dr. Jolly accepted the RAC's suggestion to consider using multidrug antiretroviral therapy if needed.

E. Public Comment

No public comments were offered.

F. Synopsis of RAC Discussion and RAC Observations and Recommendations

Preclinical Issues

- In the event that persistent viremia is detected in a research participant, the protocol proposes treatment with anti-retroviral therapy using AZT based on AZT's efficacy against HIV and its ability to reduce Toca 511 replication in a murine model. However, because of the high rate of resistance that can occur, AZT is not commonly used as a monotherapy to treat HIV. Moreover, in the mouse studies, while AZT reduced virus load it did not eliminate it. Additionally, AZT's side effect profile is not as favorable as alternative antivirals used to treat HIV. Additional studies, in consultation with infectious disease experts, should be considered to determine the optimal antiviral treatment, including the possible use of a multidrug regimen.
- The protocol proposes to use a replication-competent retroviral vector because such a vector should be able to infect preferentially dividing tumor cells, resulting in improved delivery of the therapeutic gene throughout the tumor. However, data from *in vivo* models are lacking regarding vector spread through the tumor. While the vector was detected at multiple sites in the resected tumors in two of three research participants dosed to date in the ongoing trial (OBA #0904-976), it is not clear whether this is due to spread of the replicating vector or spread of the transduced tumor cells in which the vector has integrated. Use of a replication-competent virus increases the risk of viremia and potentially increases both the risk of transmission to close contacts as well the risk of insertional mutagenesis. To balance these risks, it is important to demonstrate in an *in vivo* model that the replication-competent vector does indeed spread throughout the tumor as compared to a replication-incompetent vector. Vector spread should be demonstrated in a xenograft model or by tumor clonality analysis of vector insertion sites.

Clinical and Trial Design Issues

- If viral RNA is detected in the serum, it is important to confirm using an appropriate available assay that it is associated with infectious virus. In addition, given the detection of viremia in research participants enrolled in the ongoing trial (OBA #0904-976), it is important to develop assays for viral detection in other bodily fluids, in addition to urine and saliva, and to incorporate these assays in that trial so that such data can be used to inform the risk/benefit assessment for this trial. As one risk of transmission is through sexual contact, it would be ideal to understand whether viremic individuals shed the virus in vaginal fluids or semen. However, given the clinical condition of the research participants enrolled, this may not be feasible.
- The protocol states "Subjects with evidence of persistent viremia (>30,000 copies/mL by RT-PCR on two occasions separated by at least 1 month) and clinical or radiographic evidence of tumor progression despite multiple courses of 5-FU should be considered for treatment with antiretroviral medication." Evidence of persistent viremia alone should be a criterion for offering antiviral therapy and the risks and benefits of taking antiretroviral therapy should be explained to the participants at that time. In addition, the definition of persistent viremia (e.g., whether cell-associated virus or free virus in the plasma/sera) should be clarified in the protocol.
- As retroviral vectors have been associated with leukemia due to insertional mutagenesis, research participants will be monitored for the development of secondary tumors. The protocol should also include information regarding the plan for analysis of tumors for vector insertion sites.

Ethical, Social, and Legal Issues

- The informed consent document includes information regarding the cases of leukemias seen in the X-SCID gene transfer trials that were attributed to retroviral vector insertional mutagenesis. The discussion should also include information about the leukemia and myelodysplastic cases reported in the Wiskott-Aldrich Syndrome and chronic granulomatous disease trials. The OBA recognizes that there are important differences between the current trial and the trials in which insertional mutagenesis has been seen. For example, in all of the previous trials the vector was used to transduce hematopoietic stem cells. In addition, the risk of insertional mutagenesis and secondary tumors must be balanced against the fact that the population being treated in this trial has an expected lifespan of 6 to 8 months with standard treatments. Nonetheless, it is still important that participants be fully informed regarding the risks, and the informed consent document should clearly state that despite these differences in trial design the risk of insertional mutagenesis with this vector is unknown.
- The risks of viral persistence and shedding and the potential for transmission through sexual contact should be clearly stated in the informed consent document. Research participants should agree to use barrier contraception for up to 6 months or until viremia clears. To help ensure that the risk of vector transmission to close contacts is clearly understood, the participants should indicate their acknowledgement of this risk by initialing the consent form section that discusses it or by another comparable mechanism.

G. Committee Motion 6

Dr. Fong summarized the RAC recommendations expressing the comments and concerns of the RAC. Dr. Yankaskas moved that these comments be approved by the RAC, and Dr. Badley seconded the motion. The RAC voted to approve these summarized recommendations by a vote of 16 in favor, 0 opposed, 1 abstention, and 1 recusal. (Dr. Strome abstained until he has the opportunity to review the exact wording of the recommendations.)

VII. Discussion of IBC Review of Human Gene Transfer Protocols

Presenter: Dr. Corrigan-Curay

A. Presentation

Dr. Corrigan-Curay presented background on the role of the IBC in reviewing human gene transfer trials, shared some feedback from investigators regarding IBC review especially in multisite trials, discussed the various options currently offered under the *NIH Guidelines* to have one IBC conduct multiple reviews of human gene transfer trials, and stated the current options and challenges. For low biosafety risk trials, the OBA is considering a streamlined review process and would like the RAC to begin thinking about what aspects need to be looked at further in order to develop proposals.

IBCs identify and manage biosafety issues raised by human gene transfer trials. They ensure that the informed consent document incorporates information regarding risks that arise from the biological nature of the agent. They look at the preclinical animal data that supports the safety of a vector, and there is ongoing reporting to IBCs, similar to that for IRBs, to identify new biosafety issues through analysis of adverse event reports. If a protocol comes before the RAC, it is the IBC's responsibility to ensure that the RAC recommendations are considered by the PI.

IRBs and IBCs have joint oversight, with slightly different foci. The IRB focuses on risk/benefit assessments relative to the individual research participants and other ethical issues. The IBC focuses more broadly on the risk to the environment and to public health, to close contacts, and to health care workers; IBCs also look at the risk to the individual participant, adequacy of facilities, standard operating procedures, training of personnel, and who is delivering the vector. IBCs carry out all the requirements of the *NIH Guidelines* and the containment level, thus reviewing trial design and biosafety issues, and they ensure compliance with the *NIH Guidelines*. The *NIH Guidelines* include specific requirements for IBCs:

members need expertise in assessing risk to the environment and to the public health, and they need to know about the institution, its commitments, and its standards for biological safety and physical containment.

IBCs can have no fewer than five members, and many IBCs are composed of more than five members. Appropriate recombinant DNA expertise is needed, so reviewing human gene transfer trials requires expertise in that area or a way of getting that expertise to the IBC. At least two local members who are not affiliated with the institution are required under the *NIH Guidelines*; this requirement differs from other local oversight bodies that review clinical trials.

Local IBC members can be representatives of community interests with respect to health and protection of the environment, such as state or local public health officials or representatives from an environmental authority or other local government body; individuals with medical, occupational, or environmental expertise; or individuals who “represent community attitudes,” such as a teacher, clergy, community organizer, or local resident. The *NIH Guidelines* require nonaffiliated members who can represent local interests, because the risk tolerance for research may vary by community. They provide a mechanism for transparency and local public input, and may be of particular importance for international trials to ensure that local and cultural norms are taken into account.

Regarding review of multisite trials, feedback from some investigators indicates that a number of human gene transfer trials are conducted using vectors for which there is considerable clinical experience and for which biosafety risks are well characterized; this may be particularly true for “off the shelf” products that do not have to be reconstituted at the site and can be administered using standard precautions. Multiple individual IBC reviews of low-risk trials add little benefit to protect public health, and such reviews can be costly to set up and administer and can cause delays in initiating important research. Dr. Corrigan-Curay noted that a mechanism to streamline the review of low biosafety risk trials is needed to facilitate research, especially for multisite trials.

The three situations in which a human gene transfer trial requires IBC review are as follows:

- If the NIH funds a trial directly, in which case every site for that trial must have an IBC review the trial whether inside or outside the United States.
- If the trial is being conducted at an institution that receives NIH funding.
- If NIH funding supports the vector development up to the point of conducting a clinical trial and the original investigator remains involved in that trial.

Dr. Corrigan-Curay reviewed a hypothetical situation in which a cancer vaccine trial’s sponsor receives NIH funding and wants to conduct a trial at ten U.S. institutions, nine of which already have IBCs in place. The tenth site could designate one of the other institution’s IBCs as their IBC of record but they must still appoint two unaffiliated local members who participate fully in review of the trial. Another option for this 10-site trial would be to construct one IBC to review all sites of this trial; however, each site would still need to appoint two unaffiliated local members who would be fully involved. The central IBC must have knowledge of each trial site’s facilities, standard procedures, training and expertise of personnel involved in research, and other local matters pertinent to that site. Using a central IBC for a multisite trial becomes more complicated as the number of sites increases.

Potential alternatives for discussion include the following:

- Develop mechanisms that facilitate a shared or central IBC, and eliminate the need for local unaffiliated members when reviewing a human gene transfer trial that poses extremely low risk to public health and the environment. Institutions could be permitted to share local nonaffiliated members even if the sites are geographically distant, but different considerations might be necessary for U.S. versus international trials.
- After the initial review of the first or second trial using a product determined to have a low biosafety risk, provided there are no serious adverse events that led to a change in the

recommendations from the IBC regarding the trial design, the *NIH Guidelines* could offer IBCs the option of conducting an administrative review. However, questions arise as to how much clinical trial experience would be required, how an administrative review would be structured, and what infrastructure needs to be in place to review the trial if the proposed site does not have an IBC in place.

Dr. Corrigan-Curay stated that the next step is to establish a RAC Working Group to develop proposals for consideration by the full RAC. Those findings would be reported out by the Working Group at a future RAC meeting.

B. RAC Discussion and Comments

Dr. Buchmeier elaborated on another problem that might be difficult to get a local institution to accept — some institutions look at the RAC review as a way of protecting themselves from liability risk, so getting them to accept outside members to fulfill that role might be difficult. Dr. Corrigan-Curay acknowledged that this issue has surfaced in relation to IRB reviews.

Dr. Fost noted that a move toward IBC centralization parallels changes in IRBs; for example, both the NCI and the Veterans Administration have central IRBs. The current IBC system is outmoded, and expertise could be better utilized if applied centrally.

Regarding community input, Dr. Fost described his experience, which is that community members on IBCs and IRBs rarely contribute. He asked whether any data exists from the past 20 years about whether community members on local IBCs have made significant contributions to their committees.

Dr. Hammarskjöld commented that the quality of community members varies widely, but that her experience with community members has been positive. However, the workload of reviewing the one gene transfer protocol that came to the IBC was burdensome — for community members and faculty members alike.

Dr. Chiocca noted that these issues are being brought forward as more gene transfer trials are moving to Phase III, which is the phase most likely to incorporate multi-institutional sites.

Dr. Kanabrocki suggested discussing expedited review possibilities.

Dr. Kohn volunteered to participate on this Working Group.

Dr. Corrigan-Curay requested that RAC members email her if they are willing to join this Working Group. The OBA will also recruit from the BWG. One or two meetings of this new group will be held before the December RAC meeting.

C. Public Comments

Julie Ledgerwood, D.O., a physician and Deputy Chief of Clinical Trials Core of the Vaccine Research Center at the National Institute of Allergy and Infectious Diseases (NIAID), introduced herself as an intramural investigator, an intramural NIAID IRB member, and an investigator who has presented to her local IBC many times in the past 8 years. She represented a number of her NIAID colleagues who were unable to attend this RAC meeting.

In the past year, the NIAID has considered this issue extensively. Consideration began as an intramural and extramural working group and ended at the NIAID executive committee. The NIAID would be in favor of further consideration of any of the ideas posited by the RAC during its discussion about these issues. The executive committee at NIAID considered some of those but voted unanimously for a different option that was not mentioned. Dr. Ledgerwood read an excerpt from the alternative policy that was developed by the NIAID working group:

NIAID proposes that an alternative policy option be considered for application of a very specific case of clinical trials of recombinant gene based vaccine constructs that are non-transmissible, that are therefore exempt from Appendix M-I so they are exempt from RAC review, and this alternative policy option reflects the accumulated knowledge concerning the safety of these agents and is consistent with the November 2007 FDA guidance for industry on the matter.

For well-characterized agents that only require standard universal precautions, the IBC's responsibilities, which include reviewing containment levels and ensuring compliance with the institution's health surveillance requirements and reporting, are also stringently fulfilled through the FDA IND review and IRB reviews and through good clinical practice. Therefore, a local IBC review process may not add as much in that setting to the conduct of the trial.

By unanimous approval the NIAID clinical research subcommittee and the executive committee of NIAID, we recommend that the OBA consider discontinuing the requirement for IBC review in this very specific case of human recombinant gene based vaccine trials that meet the criteria delineated in Appendix M-VI-A, which means trials that are exempt from Appendix M-I.

Dr. Ledgerwood explained that this option might only require an update to the OBA's interpretation of the Appendix M-VI-A requirements in the FAQ on vaccine exemption, such that non-integrating and non-replicating gene-based vaccine clinical trials would no longer need IBC review at every site for every study for every trial phase.

VIII. Day 1 Adjournment

Dr. Fong, RAC Chair, adjourned Day 1 of the September 2011 RAC meeting at 5:45 p.m. on September 13, 2011.

IX. Day 2 Call to Order and Opening Remarks

Dr. Fong, RAC Chair, called to order Day 2 of the September 2011 RAC meeting at 8:55 a.m. on September 14, 2011.

X. Gene Transfer Safety Assessment Board Report

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

Dr. Kohn reported that the OBA received 14 protocol submissions in the past three months, 11 of which were not selected for public review at this RAC meeting. Of the 11 protocols not selected for public review, ten were oncology protocols and one was for hemophilia. In these 11 protocols, seven used plasmid vectors, two used lentivirus vectors, and two used vaccinia virus vectors.

Eleven protocols submitted Appendix M followup information indicating their enrollment. Of trials that had initiated enrollment in the past 3 months, two protocols had been reviewed by the RAC at previous public meetings and provided responses to RAC recommendations:

- Protocol #922, reviewed by the RAC in June 2008, involves adoptive immunotherapy for CD19-positive B lymphoid malignancies using the sleeping beauty transposon to express a CD19-specific chimeric antigen receptor in autologous *ex vivo* expanded T cells. At the time, a recommendation was made, given the possibility the transposon system could cause insertional mutagenesis, an evaluation for clonal expansion should be undertaken. At the time of cryopreservation of cells for culture 21 days after electroporation and culture, two million cells will

be cultured for 14 days to evaluate for clonal expansion using flow cytometry. Changes were also made to the definition of the dose-limiting toxicity in the informed consent document, in response to RAC discussions.

- Protocol #1016, reviewed by the RAC in March 2010, is a Phase II study to determine the efficacy and safety of allogeneic human chondrocytes expressing TGF-B1 in patients with Grade 3 chronic degenerative joint disease of the knee. The RAC discussions at the time raised a concern regarding the lack of data as to whether the gene-modified cells would remain in the joint, adhere to the cartilage, and secrete TGF-B1 in the joint. New data was submitted by the investigators from *ex vivo* cell adhesion studies in rabbit and human knee tissue demonstrating that transduced cells adhere to the cartilage surface. In human cartilage tissue obtained from arthroplasty surgery, 40 percent to 60 percent of cells remained on the knee tissue. The RAC also raised a concern regarding the number of deaths observed in preclinical animal studies. In response, the investigators provided further analysis of longterm mice, rabbit, and goat studies, which analysis did not reveal a difference in mortality between controls and injected animals. Also noted during the RAC discussions was that the two allogeneic lines could have a potential to induce an immune response; this was not modeled in preclinical studies that used xenogenic rather than allogeneic cells. A recommendation was made to monitor for immune response by evaluating for preexisting antibodies in T cells and performing serial blood samples. To address this concern, additional analyses were done on data from the U.S. Phase I study to examine HLA antigen expression levels of the allogeneic chondrocyte cells and the participants' immune responses. The chondrocytes exhibited significantly decreased levels of class 1 HLA antigens compared to control peripheral blood mononuclear cells, and antibody analysis from participants in the Phase I study did not show an increase in antibodies against donor-specific HLA antigens. In addition, cytokine analysis showed increased cytokine levels in half the participants at various timepoints; however, given the inconsistent trends in the onset, duration, and levels of cytokine increase, these data do not support a conclusion that the cytokine elevations indicate an immune response to the gene-modified allogeneic chondrocytes.

Eighteen SAEs from 14 protocols were reviewed by the GTSAB, including initial and followup reports. After analysis of these events, the GTSAB concluded that one report warranted additional public discussion.

The OBA was informed of an unexpected toxicity in a trial that uses a leukemia vaccine that consisted of a combination of irradiated K562 cell lines transduced by a plasmid encoding the transgene for GM-CSF mixed with irradiated autologous leukemia cells. This is a Phase I trial for patients with hematologic malignancies in the setting of allogeneic transplant. The reported toxicity involved an unexpected sustained and severe leukocytosis, and several months after this leukocytosis was first detected the research participant died of what appears to have been possible complications of infection and sepsis. Analyses are ongoing regarding the etiology of the leukocytosis and any contribution of the gene transfer vaccine to the leukocytosis and the subsequent clinical events. No conclusions are available at this time and the trial was placed on hold by the investigators.

To analyze if similar events had been reported, OBA's GeMCRIS system was analyzed and 24 studies that employ a tumor vaccine using irradiated GM-CSF secreting K562 cells were identified. At least 20 of these trials reported enrollment of research participants and are complete, and approximately 300 participants have been dosed. Forty-four studies that use a tumor vaccine consisting of irradiated tumor cells transduced with GM-CSF were also identified, and at least 34 of these trials reported enrollment of participants and are complete. Approximately 1,400 participants have been dosed in these studies. The OBA screened GeMCRIS for SAEs submitted on these protocols in which the participant developed an unexpected leukocytosis after receiving one or more vaccinations. This analysis identified one event out of all participants; the leukocytosis was self-limiting and the participant clinically did well. Cytokine analysis in that case indicated a rise in GM-CSF levels after the vaccination at the time the leukocytosis developed.

The sponsor of the Phase I trial with the recent adverse event will present his assessment of the case at the December 2011 RAC meeting. The OBA will inform investigators working with similar products and their IBCs of any new information as it is developed.

Dr. Kohn discussed one favorable result from a gene transfer trial, OBA protocol #793. The research participant, who was highlighted in *The New England Journal of Medicine* paper in August 2011, had advanced refractory, p53-deficient chronic lymphoid leukemia. This individual achieved a complete remission that was ongoing at least ten months after infusion with second-generation chimeric antigen receptor (CAR) T cells; the participant also experienced loss of normal B cells, which also express the target antigen CD19, which is being managed by intravenous immune globulin. In their subsequent article in *Science Translational Medicine* (August 10, 2011), the investigators provided additional data on expansion, persistence, and function of the CAR-modified T cells in all three research participants dosed to date. Their studies indicated that the persisting CAR-modified T cells consisted of both central and effector memory T cells, which could lead to a persistence of the cells. Dr. Carl June from the University of Pennsylvania has been invited to present his findings at the December 2011 RAC meeting.

Dr. Kohn reminded RAC members about the meeting on December 15-16, following the December 2011 RAC meeting, on RNA oligonucleotides as an emerging clinical application. The meeting is open to the public and an agenda for this meeting can be found on the OBA's website at http://oba.od.nih.gov/rdna/rdna_symposia.html#CONF_001.

XI. Discussion of Human Gene Transfer Protocol #1107-1117: A Phase I/II Safety, Pharmacokinetic, and Pharmacodynamic Study of APS001F with Flucytosine and Maltose for the Treatment of Advanced and/or Metastatic Solid Tumors

Principal Investigator: John J. Nemunaitis, M.D., Mary Crowley Cancer Research Center (*via teleconference*)
Presenter: Barry Anderson, M.D., Ph.D., Theradex, Inc.
Sponsor: Anaeropharma Science, Inc.
RAC Reviewers: Dr. Badley, Dr. Buchmeier, Ms. Mastroianni (*via teleconference*), and Dr. Ornelles

A. Protocol Summary

Most human solid tumors contain regions of acute or chronic hypoxia, mainly due to insufficient and unusual angiogenesis. Tumor hypoxia is a prognostic factor commonly associated with aggressive tumor growth and poor survival rate. These microenvironments cause decreased uptake of chemotherapeutic drugs. Though many drugs and technologies exploiting tumor hypoxia have been explored in clinical trials, no effective anticancer drugs or methods based on tumor hypoxia have been developed to date. A critical factor in the success of this strategy is the need to develop an adequate delivery of anticancer drugs targeting regions of tumor hypoxia. One approach to improve the treatment of hypoxic solid tumors is to deliver most of the anticancer agent directly to the tumor site, thus concentrating the effect on the tumor and avoiding toxicity to normal tissues. Some bacteria have the interesting property of accumulating preferentially within tumors following IV administration in animals, reaching very high numbers in the tumor compared to normal tissues if they are obligate anaerobes (requiring an oxygen-poor environment for survival).

Bifidobacterium longum isolated from human feces is a normal flora in the intestine and is a non-pathogenic obligate anaerobe. APS001F is live recombinant *Bifidobacterium* expressing the cytosine deaminase (CD) enzyme that can be given safely via IV to mice and rats with implanted tumors. These bacteria maintain their property of preferentially accumulating within the tumors. APS001F is also not shed from the body in stool or urine as determined in the rat, which indicates that it is unlikely to spread to the environment, to health care workers, or to other people.

5-fluorocytosine (5-FC), a commercially available anti-fungal agent, has been used for approximately 50 years and has a good safety profile in humans. 5-FC is converted to 5-fluorouracil (5-FU) by the CD enzyme expressed within APS001F. 5-FC has been used in many clinical trials in combination with bacteria or virus modified to express the CD gene. When 5-FC is administered orally in the presence of APS001F, the CD enzyme converts 5-FC to 5-FU. Since APS001F is selectively targeted to tumor tissues where there is an inadequate oxygen supply, APS001F facilitates the local conversion of 5-FC to 5-FU in the tumor environment resulting in tumor-specific exposure to 5-FU, which will provide antitumor activity while minimizing exposure of normal tissue. Maltose injection is effective as a nutrient source for the growth of APS001F; it was approved in Japan 40 years ago and is used generally to provide carbohydrate during or after an operative procedure and in diabetes mellitus.

This clinical study proposes to test APS001F in combination with 5-FC and maltose in subjects with advanced cancer who have exhausted all effective treatment options. The cycle of bacterial injection, 5-FC treatment, and maltose will be repeated every 28 days if the tumor shows signs of stabilization or shrinkage.

B. Written Reviews by RAC Members

Eleven RAC members voted for in-depth review and public discussion of the protocol. Key issues included the novelty of IV administration of this recombinant bacterium in combination with 5-FC and maltose.

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

Dr. Badley's primary concern with this protocol was safety. He noted that the statement that *B. longum* is nonpathogenic is not accurate, as IV administration of any bacterium, whether live or dead, could have negative physiologic consequences, citing two human case reports of sepsis induced by live *Bifidobacterium* species and toxicology studies performed on animals by the sponsor. Because infectious complications from bacteremias vary according to the host risk profile, Dr. Badley asked whether the sponsor had performed preclinical safety studies of animals with such conditions as neutropenia, lymphopenia, bicuspid aortic valves, sclerotic cardiac valves, known deep-vein thromboses, prosthetic joints, and indwelling central venous catheters in order to further understand the safety profile of administration of *Bifidobacterium*. Given that the underlying rationale of using *Bifidobacterium* administration as a probiotic is for its effects on immune function, he stated that it would be of interest to determine the effects of this *Bifidobacterium* species on immune function following IV administration and specifically to determine the effects on tumor-specific cellular immune responses. Although the maximum tolerated dose is described as being a DLT clearly attributable to the experimental treatment, Dr. Badley asked for more details as to what those limiting toxicities will be, including the degree of fever, hypertension, leukopenia, and/or transaminitis. Because the investigators acknowledge that APS001F might be detected in sites other than the tumor sites where it is intended to locate, he asked that individuals who have bacteremia, bacteruria, or colonization of APS001F in the gut be excluded from receiving 5-FC treatment so that the effects of 5-FU would not occur in those unintended sites. He requested that the investigators clarify how APS001F would be detected, within the tumor as well as in other sites, suggesting the use of PCR to do so. Dr. Badley also suggested that the investigators consider excluding eight specific patient populations and that the informed consent document should include "death" and "shock" as known risks and side effects related to APS001F.

Dr. Buchmeier remarked that the investigators and sponsor made attempts to reach the layperson with their descriptions of common procedures. He found the lack of consideration of any possibility of adverse effects associated with *B. longum* to be surprising in view of the fact that a PubMed search using the terms *Bifidobacterium* and bacteremia or septicemia turns up numerous reports of association of *B. longum* with sepsis in infants and in liver transplant patients, and association with abdominal wounds. Although these adverse events are not the norm, Dr. Buchmeier noted that the patient population chosen for the present study also does not represent the norm, so it is essential that the protocol consider and plan for unexpected outcomes. If participants develop anti-*B. longum* antibody and the bacteria is opsonized and removed from the system, he asked how the investigators plan to monitor this reaction.

Dr. Buchmeier specified several concerns about the informed consent document, and noted that it is extremely long and detailed and would benefit from a summary of the important timepoints and a schedule of procedures.

Ms. Mastroianni commended the investigators for their attention to detail, their responsiveness to Appendix M, and the preparation and clarity of the informed consent document. She stated that she did not have any specific comments for revision or clarification.

Dr. Ornelles noted no major concerns with this protocol, although some of the underlying mechanisms remain uncertain. A better understanding of possible mechanisms influencing bacterial “homing” could significantly improve this therapeutic approach. Because *B. longum* is restricted to the hypoxic portion of tumors, he believed it possible that this experimental approach might be unsuited for patients with multiple, well-oxygenated metastases. Filter mating experiments were used to show that the plasmid in *B. longum* does not transfer between unrelated species of bacteria or other strains of *Bifidobacterium*; however, these experiments are limited in their ability to replicate potential conditions under which bacteria could conduct horizontal gene transfer. Dr. Ornelles noted that it might be possible for the *E. coli* plasmid in *B. longum* with spectinomycin resistance to be transferred to other bacteria in the research participant. He wondered whether the toxicity of maltose in the rat model raised concerns for its use at the levels required for this protocol, and he queried as to why *Bifidobacteria* (or the CD-bearing plasmid) is so dependent on maltose for an effect. Dr. Ornelles asked why dogs are most sensitive to exposure to the study agent and whether that fact provides useful information regarding the dosing of APS001F in humans, beyond setting a lower no observed adverse effect level (NOAEL).

C. RAC Discussion

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

- Dr. Hammarskjöld asked whether the investigators had considered an upper age limit for participation in this trial.
- Dr. Badley asked whether patients who were considered brittle diabetics would be allowed to enroll in this trial, given that such individuals would get dosed with 10 percent maltose and that maltose effects glucose tolerance and insulin secretion.
- Dr. Strome noted that behavior consequences accrue the more agents such as 5-FU are administered to patients. While the reason for those changes is not understood, the changes can begin to lead to changes in appetite and other potentially toxic behaviors. The potential exists for additional organ damage, particularly to renal function and hepatic function.
- Dr. Fong asked the investigators to explain their rationale for allowing subjects to participate in this trial who have been treated previously with 5-FU and were 5-FU failures.

D. Investigator Response

1. Written Responses to RAC Reviews

B. longum has the potential to cause a sepsis-like event depending on the amount of bacteria administered and the subject’s physiological condition and immunocompetence. Cases reported in the literature strongly suggest the importance of considering the subject’s immune status and history of surgeries prior to treatment. Although APS001F infusion caused a number of adverse events in preclinical toxicology studies, the duration and severity of symptoms was a function of the APS001F dose. The investigators intend to initiate dosing with APS001F at 3×10^4 cfu/m²/day, which is 1,000-fold lower than the NOAEL in the most sensitive species (dog); dose escalation will be gradual. Since individual variation in the responses to APS001F infusion is anticipated, the research participants will be hospitalized for the three days of APS001F infusions and must recover from significant clinical toxicities prior to discharge.

Participants will be advised to avoid direct contact with immunocompromised people, the elderly, and infants.

Because the investigators agree that the proposed approach might have hazards for certain cancer patients, they agreed to exclude individuals with indwelling catheters and known bicuspid aortic valves, known AV malformation, and known history of deep-vein thrombosis. The toxicology study using APS001F combined with 5-FC and maltose in tumor-bearing nude mice resulted in no observable serious symptoms like sepsis. However, the investigators agreed to exclude individuals with the disorders suggested by Dr. Badley because they have not performed preclinical safety studies for those indications.

The investigators currently have no plan to conduct specific studies evaluating the effect of APS001F on the host immune system, including tumor-specific cellular immune responses.

Presence of APS001F in the tumor and blood will be measured by culture only. These measurements will be conducted to allow pharmacodynamic and pharmacokinetic analysis. The proposed culture method has the ability to detect the presence of "live" APS001F bacteria, which provides the most useful information. While PCR is useful in detecting the presence and residue of bacteria, it is problematic for analyzing bacteria in fecal samples due to assay interference by intestinal flora.

A short description of the bone scan procedure will be included in the informed consent document. Individuals under consideration for this trial would only undergo a bone scan procedure if bone scans had been previously used for routine monitoring of their tumors. The investigators will use whatever diagnostic test (e.g., CT scan, MRI, bone scan, or ultrasound) had been followed routinely in the past for each individual.

The protocol provides guidance to investigators for treating research participants with antibiotics to control potential infectious complications associated with *Bifidobacterium* infusions. The protocol also provides information to the investigator about how to proceed if anti-APS100F antibodies are detected.

B. longum (APS001F) is an obligate anaerobic bacteria and cannot survive for a prolonged period in the aerobic conditions of normal organs and in blood. *B. longum* is non-motile; there are no reports that they are able to traffic to tumor sites actively or to move to sites in response to chemo-attractants. Therefore, the investigators believe that the *Bifidobacterium* are retained in the hypoxic site of tumors and replicate at the site. The therapeutic concept of APS001F combined with 5-FC is to target the hypoxic areas of solid tumors and produce active drug at those sites.

During the development of the plasmid, extra sequences that are not necessary for plasmid function were eliminated to prevent unexpected gene expression, as had been recommended by the FDA. As a result, the plasmid harbored by APS001F consists of just three units: a CD-expression unit, a spectinomycin-resistant unit, and a plasmid-replication unit that is only active in *Bifidobacterium*. The replication unit of the plasmid was originated from a wild *Bifidobacterium* strain, and it is likely to work and replicate only in *Bifidobacterium*. Therefore, even if the plasmid were to be transferred to other bacteria, the plasmid would not be able to replicate. Repeated filter mating experiments were used to ensure that the plasmid in APS001F does not transfer among unrelated species of bacteria or other strains of *Bifidobacterium*.

Maltose injection has been used for more than 40 years in patients undergoing surgery and in diabetic patients in Japan. A large safety database exists, and maltose infusion is considered to have a benign safety profile. Maltose is also used as an excipient for biologic drug products.

Bacteria including *Bifidobacterium* require an energy source for survival and replication. Maltose was selected for this experimental treatment approach because it has some advantages compared to glucose. In the investigators' experiments, maltose appears to have a significant effect on the localization, growth, and persistence of APS001F in the tumor sites. The administration of maltose in combination with APS001F allows for very high levels of intra-tumoral colonization to be sustained for weeks, resulting in an extended duration of local 5-FU production after oral 5-FC administration.

It is unknown why dogs are more sensitive to maltose, compared to rats and monkeys.

The investigators agreed to incorporate the requested wording changes to the informed consent document.

2. Responses to RAC Discussion Questions

Regarding the possibility of adding an upper age limit to the exclusion criteria, Dr. Nemunaitis explained that the investigators generally do not exclude potential participants merely on the basis of chronological age. Instead, they assess the performance and health of the individual and decide about participation based on those results.

Dr. Nemunaitis explained that a person considered a brittle diabetic would not likely be permitted to enter this trial if the person needed insulin management. Each research participant will need to be healthy and in good medical standing.

With regard to individuals who were 5-FU failures being allowed to participate in this trial, Dr. Nemunaitis clarified that patients generally get 5-FU base regimens at a later stage of their cancer, oftentimes at a palliative stage. With localized expression of 5-FU, it might be possible to deliver a higher concentration locally to the tumor site. Therefore, the investigators might be able to observe a possible response in a participant who has previously been exposed — but did not respond — to 5-FU.

Dr. Hartso briefly described a general toxicity study conducted in the tumor-bearing mice that used a dose approximating the highest dose proposed in the clinical trial. The only adverse event seen in these mice occurred directly after dosing as a small amount of respiratory distress that cleared within one hour. Cytokines were not examined in this model.

At Dr. Fong's suggestion, Dr. Nemunaitis agreed to write a standard operating procedure for how to deal with abscesses that might form — or might already have been formed — on tumors, including whether and at what point antibiotics would be administered and whether and at what point an abscess would be drained.

E. Public Comment

No public comments were offered.

F. Synopsis of RAC Discussion and RAC Observations and Recommendations

Preclinical Issues

- The biodistribution of this bacterium has been studied in a tumor-bearing animal. Several days after administration, this recombinant bacterium was found to localize preferentially to the tumor in comparison to normal tissue. It is important to understand further how this distribution occurs over time, using, for example a fluorescently-modified *B. longum*. Such a study could be performed in mice.
- The investigators have generated *in vitro* data suggesting that the strain of *Bifidobacterium* to be used is susceptible to several parenteral antibiotics. It is important to know whether these antibiotics are also effective in the animal models, including in those animals in whom a systemic inflammatory response syndrome occurred.

Clinical and Trial Design Issues

- Significant increases in serum levels of tumor necrosis factor (TNF) alpha were observed in some of the animals who received the highest dose of the recombinant *B. longum*. The death of some animals at these higher doses is concerning, as these elevated TNF-alpha levels might indicate

an inflammatory and cytokine response to the bacteria. Adequate realtime sampling for proinflammatory and immunomodulatory cytokines, including gamma interferon, should be included to facilitate analysis of any possible toxicity and to inform dose escalation. As *B. longum* is known to be immunomodulatory, storage of serum and PBMCs should be considered so that further analysis of these immunomodulatory properties can be examined if the data on safety support additional clinical studies.

- Repeated IV infusion of this bacterium will likely result in a cognate immune response. It is important to understand the kinetics of any antibody response that is seen, and whether the antibody response might contribute to an inflammatory response.
- Colonization of these bacteria in large necrotic tumors could lead to the development of an abscess that likely could not be managed by antibiotics alone. A written standard operating procedure or guideline to address this situation would help ensure uniform management and that any intervention occurs at an appropriate time.
- In order to monitor for bacteremia, blood cultures will be collected at several timepoints after infusion. In addition to the planned collections at days 1 and 3, it is recommended that a blood culture be obtained at Day 6 instead of at Day 8, which should allow sufficient time for any bacteria to be cultured prior to the first administration of 5-FC on Day 8. As the sensitivity of blood cultures depends in part on the amount of blood collected, standard procedures should be established to ensure uniform blood culture collection.
- As some research participants will receive maltose, consider whether the protocol should specifically exclude certain participants with difficult-to-control diabetes.

Ethical, Social, and Legal Issues

- The informed consent is quite long and complex and would benefit from a flowchart or other schematic to enhance understandability. This flowchart/schematic should highlight the key risks of this protocol as well as provide an outline of the protocol procedures.

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. Yankaskas moved that these comments be approved by the RAC, and Dr. Badley seconded the motion. The RAC voted to approve these summarized recommendations by a vote of 18 in favor, 0 opposed, 0 abstentions, and 0 recusals.

XII. Discussion of Results of OBA Protocol #0610-809: A Phase I/II Randomized, Double-Blinded, Placebo-Controlled Dose Escalation Trial of Intracoronary Administration of MYDICAR® (AAV1/SERCA2a) in Subjects with Heart Failure

Presenter: Krisztina Zsebo, Ph.D., Celladon Corporation

A. Background and Presentation

Dr. Zsebo discussed results from a Phase II trial in advanced heart failure patients. Mydicar® (AAV1-SERCA2a) targets endstage heart failure, which is a significant health care burden. At the end stage of the disease, the patient's options are heart transplant or a left ventricular assist device (LVAD), which is essentially a mechanical pump. The majority of costs to the health care system in treating heart failure are due to repeated inpatient hospitalizations. The goal of this research is to reduce hospitalizations and the need for these invasive procedures. Sarcoplasmic reticulum ATPase deficiency (SERCA2a) is central to the progression of heart failure.

Mydicar® is an AAV1-based vector expressing SERCA2a as a genetic enzyme replacement therapy. SERCA2a deficiency results in abnormal Ca^{2+} handling and a deficient contractile state. The two main pharmacological effects of the product are an increase in contractility of the cardiomyocytes as well as transduction of the coronary endothelial cells upregulating the production of nitric oxide and causing relaxation, which promotes increased coronary blood flow. The product is administered once for approximately ten minutes by direct intracoronary infusion performed in the cardiac catheterization lab, which is a relatively simple outpatient procedure in which the research participant is mildly sedated. There is no balloon occlusion and no injection or local trauma to the tissues.

Dr. Zsebo reviewed some of the preclinical pharmacology and toxicology studies. Substantial improvement was seen in a number of parameters over placebo — improvement in two measurements of contractility, increase in cardiac output and end systolic volume. Dose response studies in the sheep model showed positive effects that correspond roughly to the proposed mid-dose level in the human trial. The investigators also conducted a pivotal toxicology and biodistribution study in mini-pigs; at three times the high dose proposed for the human trial, there were no signs of toxicity, clinical pathology, or histopathology.

The Phase II trial was a small-dose escalation, open-label study of patients with N.Y.H.A. Class 3/Class 4 heart failure of ischemic or non-ischemic etiology. Inclusion criteria were designed to select for more advanced participants, including those with low ejection fraction and low maximum oxygen consumption; individuals with any measurable amount of anti-AAV1 antibodies were excluded. The study was a randomized double-blind, placebo-controlled trial of 39 participants at three dose levels plus placebo, with on-study observation for 12 months and longterm followup for 2 years. Participants were relatively elderly, mostly male, and relatively representative of the typical heart failure systolic population plus about half were ischemic cardiomyopathy patients.

The most striking result was that Mydicar® reduced the incidence of serious cardiovascular clinical events including cardiovascular death, LVAD implantation, cardiac transplant, worsening heart failure, myocardial infarction, and need for chronic IV inotropic medications. In the Mydicar®-treated research participants in the low-dose and mid-dose groups, there was a delay in the onset of the events that was not sustained, and in the high-dose group a substantial reduction in overall events was observed. For the 12-month period, one participant in the high-dose group received a transplant; this individual was antibody positive at baseline (although negative at initial screening).

The investigators are currently working with the FDA to come to consensus on a Phase III endpoint. They are proposing to look at a model of time to recurrent heart-failure-related hospitalization. Data from the Phase II trial using that proposed endpoint show the high-dose group with a hazard ratio of 0.12, or an 88 percent risk reduction of heart-failure-related hospitalizations, with a p value of .003.

Dr. Zsebo discussed the clinical events in the Phase II study. The primary endpoint at six months included safety and positive concurrent trends without clinically significant worsening in heart failure symptoms, exercise tolerance, serum biomarkers, cardiac function, and duration of heart failure hospitalizations. She presented data to show the individual components of the endpoint: serum biomarker N-terminal pro b-type natriuretic peptide (NT-ProBNP) (the high-dose participants are stable), the six-minute walk test as a measure of exercise performance (the high-dose participants are stabilized), quality of life as assessed by the Minnesota Living with Heart Failure Questionnaire (the high-dose participants showed improvement or stabilization), cardiac function via echocardiography (the high-dose group was stable or improving), and left ventricular ejection fraction (not much difference between the high dose and placebo). She noted that, at the end stage of this disease, ejection fraction as a marker of heart failure progression is not a reliable endpoint.

Mydicar® demonstrated an excellent safety profile. Recent analysis showed nine deaths in the trial — four in placebo, four in low dose, one in mid dose, and one in high dose. Throughout the 12 months of study no changes over time occurred in cardiac enzymes, serum chemistry, hematology, vitals, and heart rate, and no ECG or ICD interrogation. ELISPOT assay results showed a few asymptomatic positives but

no clinically significant changes. Based on three of the four cases of tissue inflammation, the investigators hypothesized that a heightened immunological state increased the reactivity of the T cells.

The investigators did not see a T-cell response, which was surprising given that other AAV1 studies as well as other AAV serotype studies have resulted in T-cell responses. They posited that this result stemmed from their efforts to eliminate tissue damage during administration, their attempt to minimize the peak serum levels by using a slow infusion over time as opposed to a large bolus administration, and the fact that the route of administration went through the coronary sinus into the lungs, a site of the human mononuclear phagocyte system.

Dr. Zsebo summarized the findings to date. In this Phase II study of research participants with advanced heart failure, Mydicar® was found to be safe and associated with benefit in clinical outcomes, symptoms, functional status, NT-ProBNP, and cardiac structure, and these encouraging results support further studies to determine the value of genetically targeted enzyme replacement of SERCA2a in advanced heart failure.

B. RAC Discussion

In response to Dr. Badley's query, Dr. Zsebo stated that the investigators saw no difference in outcome between ischemic and non-ischemic forms of the disease. The participants in the high-dose group generally responded well except for the one individual who required a transplant. In addition, many animal models of this disease show no difference in outcome.

Because the beneficial effects appears to be waning at about the 1-year timepoint, Dr. Badley wondered if the investigators are considering retreatment, and whether they have seen a *de novo* antibody response that might preclude subsequent administration. Dr. Zsebo responded that all the research participants who received Mydicar® seroconvert and the most recent data through the end of the first year of follow-up indicate that there is not a steep drop-off in beneficial effect. She indicated that Mydicar® is a one-time treatment because of the high antibody levels that develop, which prohibit retreatment.

Dr. Zsebo stated that a substantial number (but not all) of the research participants were on the transplant list upon enrollment in this trial. In some cases, participants were dosed and then did well enough to be taken off that list.

In response to Dr. Kohn's question, Dr. Zsebo explained that screening potential participants for the presence of antibody, using a very sensitive assay, results in excluding approximately 50 percent of the population.

C. Public Comment

No public comments were requested or offered.

XIII. Closing Remarks and Adjournment

Dr. Fong thanked the RAC members and the OBA staff, and adjourned the September 2011 RAC meeting at 11:30 a.m. on September 14, 2011.

[Note: Actions approved by the RAC are considered recommendations to the NIH Director; therefore, actions are not considered final until approved by the NIH Director.]

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

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

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: RAC Roster
Recombinant DNA Advisory Committee

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Attachment II Public Attendees

[This list includes only those individuals who are not elsewhere identified.]

Wilson Bryan, FDA
Karen Byers, Dana Farber Cancer Institute
Debra Gessner, Tocagen
Linda M. Griffith, NIAID
Harry Gruber, Tocagen
James Hodge, NCI
Ying Huang, FDA
Nancy Jones, NIAID
Julie Ledgerwood, NIAID
Jinhua Lu, FDA
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Luigi Notarangelo, Children's Hospital Boston
Sung-Yun Pai, Children's Hospital Boston
Claudia Palena, NCI
Tom Rodell, GlobalImmune
Jeffrey Schlom, NCI
Takele Argaw, FDA
Ramjay Vatsan, FDA

Attachment III Abbreviations and Acronyms

5-FC	5-fluorocytosine
5-FU	5-fluorouracil
ABSA	American Biological Safety Association
AZT	azidothymidine
BL	biosafety level
BWG	Biosafety Working Group
CAR	chimeric antigen receptor
CD	cytosine deaminase
CED	convection enhanced delivery
CMV	cytomegalovirus
DLT	dose-limiting toxicity
EBV	Epstein-Barr virus
FDA	Food and Drug Administration, U.S. Department of Health & Human Services
GBM	glioblastoma multiforme
GTSAB	Gene Transfer Safety Assessment Board
GVHD	graft versus host disease
HIV	human immunodeficiency virus
HLA	human leukocyte antigen
IBC	institutional biosafety committee
IRB	institutional review board
IV	intravenous
LTFU	longterm followup
LTR	long terminal repeat
LVAD	left ventricular assist device
MRI	magnetic resonance imaging
NCI	National Cancer Institute
NIAID	National Institute of Allergy and Infectious Diseases, NIH
NIH	National Institutes of Health
<i>NIH Guidelines</i>	<i>NIH Guidelines for Research Involving Recombinant DNA Molecules</i>
NOAEL	no observed adverse effect level
NT-ProBNP	N-terminal pro b-type natriuretic peptide
OBA	Office of Biotechnology Activities, NIH
OD	Office of the Director, NIH
PBMCs	peripheral blood mononuclear cells
qPCR	quantitative polymerase chain reaction
qRT-PCR	quantitative reverse-transcription polymerase chain reaction
RAC	Recombinant DNA Advisory Committee
RG	risk group
SAE	serious adverse event
SERCA2a	sarcoplasmic reticulum ATPase deficiency
SMC	Safety Monitoring Committee, NCI
TNF	tumor necrosis factor
VEE	Venezuelan Equine Encephalomyelitis
VSV	vesicular stomatitis virus
X-SCID	X-linked severe combined immunodeficiency