RECOMBINANT DNA ADVISORY COMMITTEE

Minutes of Meeting

December 4–5, 2013

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

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

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
NATIONAL INSTITUTES OF HEALTH
RECOMBINANT DNA ADVISORY COMMITTEE
Minutes of Meeting[1]

December 4–5, 2013

The Recombinant DNA Advisory Committee (RAC) convened for its 136th meeting at 12:30 p.m. on December 4, 2013, at the Rockledge II Conference Center, 6701 Rockledge Drive, Bethesda, Maryland. Dr. Donald B. Kohn (RAC Chair) presided. In accordance with Public Law 92-463, the meeting was open to the public from 12:30 p.m. until 4:30 p.m. on December 4, 2013, and from 8:30 a.m. until 12:45 p.m. on December 5, 2013. The following individuals were present for all or part of the December 2013 RAC meeting.

Committee Members

Michael Atkins, Georgetown University School of Medicine
Saswati Chatterjee, City of Hope National Medical Center
William Curry, Harvard Medical School (pending)
Rebecca Dresser, Washington University School of Law
Norman Fost, University of Wisconsin, Madison
Marie-Louise Hammarskjöld, University of Virginia School of Medicine
Angelica Hardison, Georgia Regents University
Hans-Peter Kiem, University of Washington School of Medicine/Fred Hutchinson Cancer Research Center
Walter J. Koch, Temple University School of Medicine
Donald B. Kohn (RAC Chair), University of California, Los Angeles
David A. Ornelles, Wake Forest University School of Medicine
Joseph Pilewski, University of Pittsburgh *(Day 1 onsite; Day 2 via teleconference)*
Michael Sadelain, Memorial Sloan-Kettering Cancer Center (pending) *(Day 2 only)*
Marcella Sarzotti-Kelsoe, Duke University School of Medicine
Marshall Strome, St. Luke's–Roosevelt Hospital Center/New York Head and Neck Institute *(Day 2 only)*
Richard Whitley, University of Alabama at Birmingham
Dawn P. Wooley, Wright State University
Laurie Zoloth, Northwestern University

NIH Office of Biotechnology Activities (OBA)

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

Nonvoting Agency Representatives

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

NIH/OD/OBA Staff Members

Linda Gargiulo
Robert Jambou
Maureen Montgomery
Carolyn Mosby

[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.

Marina O'Reilly
Gene Rosenthal

Attendees

There were 44 attendees at this 2-day RAC meeting.

Attachments

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

I. Call to Order and Opening Remarks

Dr. Kohn, the RAC Chair, called the meeting to order at 12:30 p.m. on December 4, 2013. Notice of this meeting under the *NIH Guidelines for Research Involving Recombinant DNA Molecules (NIH Guidelines)* was published in the *Federal Register* on November 18, 2013 (78 FR 69097). Issues addressed by the RAC at this meeting included a report from the Gene Transfer Safety Assessment Board (GTSAB, a subcommittee of the RAC), and public review and discussion of five gene transfer protocols.

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

Dr. Corrigan-Curay reminded RAC members of the rules of conduct that apply to them as Special 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, September 11–12, 2013

RAC Reviewers: Dr. Cai and Ms. Hardison

Ms. Hardison reported that she had reviewed the minutes and had no comments. No changes to the document were suggested by other RAC members.

A. Committee Motion 1

Dr. Kohn asked the RAC to approve the minutes of the September 11–12, 2013, RAC meeting. The RAC voted unanimously by voice to do so.

III. Review and Discussion of Human Gene Transfer Protocol #1310-1260, Titled: A Three-Part, Multicenter, Open Label, Single Dose Study to Assess the Safety, Tolerability, and Efficacy of Intralabyrinthine (IL)–Delivered CGF166 in Patients with Severe Hearing Loss

Principal Investigator:	Hinrich Staecker, M.D., University of Kansas Medical Center
Presenter:	Lloyd Klickstein, M.D., Ph.D., Novartis
Sponsor:	Novartis
RAC Reviewers:	Ms. Dresser, Dr. Koch, and Dr. Ornelles
Ad Hoc Reviewer:	Konstantina Stankovic, M.D., Ph.D., Harvard Medical School

Drs. Fost, Strome, Whitley, and Zoloth were recused from consideration of this protocol due to conflicts of interest.

A. Protocol Summary

Sensorineural hearing loss and vestibular dysfunction result from destruction of inner ear sensory hair cells and/or auditory nerves. Humans are born with ~30,000 sensory hair cells. The organ of Corti, located within the mammalian cochlea, contains a mosaic of sensory hair cells and nonsensory supporting cells. The “hairs” of the hair cells, actually stereocilia, contact the tectorial membrane and sense fluid motion within the cochlea. The vestibular sensory hair cells are located in the semi-circular canals, the utricle and the saccule. Sensory cells can be damaged or destroyed by pharmacological agents (chemotherapy and aminoglycosides), loud noises, infections or simply aging and loss of these cells is permanent as they do not regenerate. Hearing loss is currently treated with a hearing aid or a cochlear implant in patients with severe impairment; patients are eligible for a cochlear implant when they have severe to profound hearing loss with the inability to discriminate 40% of speech. No pharmacologic agents exist to restore hearing function. Approximately 188,000 people worldwide have received cochlear implants. Cochlear implants improve auditory function but require invasive surgery, do not discriminate pitch, require ongoing maintenance and patient training; they also are expensive. Severe vestibular dysfunction patients may experience debilitating vertigo and nausea. There is no therapy other than rehabilitation to acquire environmental signals to compensate for vestibular dysfunction. There are no therapeutic options available to regenerate sensory hair cells to restore auditory or balance function and as such this represents a significant medical need.

The atonal gene, *ATOH1*, is a basic helix-loop-helix (bHLH) transcription factor that is required for initiation of the required gene regulatory pathway for differentiation of sensory hair cells from progenitors during prenatal development. The *ATOH1* protein is called Hath1 in humans and Math1 in rodents. *Atoh1* gene deletion in mice results in a complete absence of all hair cells in all regions of the ear. Moreover, *ATOH1* gene expression can force the supporting cells, which normally surround the sensory hair cells and lack sensory function, to trans-differentiate into new sensory hair cells. As such, in multiple preclinical rodent models a gene therapy approach has been developed to express ATOH1 in the inner ear to regenerate sensory hair cells that were lost due to administration of toxic doses of drugs and restore function; most studies to date used the rodent ATOH1, Math1. In these models, expression of the atonal gene by an adenoviral vector into the inner ear resulted in the transdifferentiation of supporting cells into functional hair cells that connected with proximal sensory neurons, consequently restoring auditory function and improving balance function.

CGF166 (Ad5.GFAP.Hath1.11D) is being developed by Novartis AG, in partnership with GenVec, Inc. (Gaithersburg, MD), as gene therapy for the treatment of patients with severe sensorineural hearing loss that does not respond adequately to hearing aids. CGF166 consists of an E1, E3, E4 deleted Adenovirus 5 (Ad5) vector engineered to deliver Hath1 expressed under the control of the GFAP (glial fibrillary acidic protein) promoter. This specific promoter restricts Hath1 expression in the inner ear to supporting cochlear cells, in the organ of Corti, vestibular supporting cells and glial cells in the spiral ganglion. As the GFAP promoter is not active in sensory hair cells, both the promoter selection and the limited persistence of adenoviral-mediated gene expression govern the transient expression of Hath1. Novartis conducted a series of preclinical studies with either Ad5.GFAP.Math1 (rodent analog) or Ad5.GFAP.Hath1 (CGF166) to demonstrate that the mouse or human atonal gene delivered in an adenoviral vector provides for a safe and localized trans-differentiation of supporting cells into functional hair cells. In all experiments CGF166 was administered into the perilymph fluid space of the inner ear (intralabyrinthine delivery). Vector biodistribution studies in rat indicate that CGF166 is well contained, with limited distribution outside the inner ear. Ex vivo experiments in rodent and human explant cultures as well as in vivo rodent models of hearing loss, where CGF166 was directly injected into the inner ear, showed that CGF166 regenerates hair cells that partially restored auditory function as well as improved vestibular function. As such, development of a regenerative approach using CGF166 offers the potential to regenerate sensory cells and restore auditory and vestibular function in patients. This therapy would address a large unmet medical need.

Novartis is planning to conduct a first-in-man study where single doses of CGF166 will be evaluated in sequential cohorts. The open-label, adaptive design will assess the safety, tolerability and efficacy of CGF166 in patients with severe sensorineural hearing loss. CGF166 will be delivered in the perilymph

fluid space of the inner ear via the external auditory canal in a surgical procedure which is a minor variation of the routine stapedotomy procedure performed by otologic surgeons and will be conducted under full anesthesia. The trial design uses staggered patient enrollment with continuous data reviews to limit unforeseen risk as much as possible. The trial is conducted in sequential three parts A, B and C. In part A an initial dose of CGF166 will be tested for safety; in part B the injection volume of CGF166 will be escalated; in part C the highest safe and tolerable volume determined in part B will be further evaluated to determine efficacy. Up to 45 patients may be enrolled in the trial.

B. Written Reviews by RAC Members

Ten RAC members voted for in-depth review and public discussion of this protocol. Key issues included the novelty of the investigational agent and the disease indication.

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

Ms. Dresser asked whether the optional five-year follow-up study is sufficient to ensure research participant safety and whether and how the investigators plan to contact original trial participants who fail to participate in the follow-up study if long-term risks are detected. With regard to the informed consent document, she noted overall that the form as presented to the RAC needs additional work before it will be ready for use. Ms. Dresser encouraged the investigators to have the form edited thoroughly to make it more informative to the layperson who will be considering joining this trial and to avoid technical terms that nonscientists will not understand. The form often refers to the investigational intervention as “treatment” and “medicine,” terminology that is inappropriate in a first-in-human study; she suggested substituting more accurate terms to avoid the misconception that the experimental intervention is of proven benefit. Ms. Dresser asked the investigators to describe what the follow-up trial would entail, requested that “withdrawal” from this trial be clarified if it occurs after gene delivery, and suggested that prospective participants would benefit from information about relevant animal data indicating possible risks to humans. She also suggested rewording parts of the informed consent document that might cause potential participants to infer the possibility of receiving direct health benefits from this first-in-human study, since such benefits usually do not accrue in early-phase trials. Ms. Dresser indicated several instances in the informed consent document that needed clarification or rewriting.

Dr. Koch noted that the protocol and related documents suffer from a lack of clarity and consistency with regard to the viral doses being administered, and he recommended including a table that spells out the exact viral load (in viral particles [vp]) to be administered. He requested that the investigators describe the overall procedure in more detail and how and when the virus would be administered, possibly by including a diagram. The device that will be used to inject the virus was not detailed, so Dr. Koch asked whether any formulation or device issues are of concern because of the small volumes; he also asked whether this device has been—or should be—tested in animal models or *in vitro* cells. He requested information from the investigators about whether the high inflammation and apparent immune responses seen in the primate studies would be countered by the use of steroids in the proposed clinical trial and, in general, requested more in-depth discussion of the preclinical data. Dr. Koch asked the investigators to explain the purpose of genotyping, delineate the specific targets for this research, and estimate the time frame for accruing the needed research participants. In addition, he suggested that the wording about withdrawal from “treatment” after gene delivery be changed, because participants cannot opt out after they have received the experimental product.

Dr. Ornelles suggested that the investigators explain more clearly the possible risks of participating in this study, even though the risks may be remote. He noted that the possibility of a malignant neoplasm is listed as a possible adverse event, which is appropriate since the wild-type adenovirus and the E1A and E4 products can cooperate to transform human cells of neural origin more efficiently than other human cell types although this risk would be low with the vector that lacks transforming genes. He asked the investigators to delineate the proposed procedures for following the research participants in the long term and to describe how chronic disturbances to the inner ear would be identified. In light of reports that sustained expression of ATOH1 in some cell types promotes transformation, Dr. Ornelles asked how

transient the expression from CGF166 is expected to be in humans and whether the investigators perceived any concerns about this expression. With regard to the preclinical studies with CGF166 in mice with experimentally induced hearing loss, he asked for clarification as to whether restoration of auditory function was derived from a dose titration of the mouse ATOH1-expressing vector alone or whether a vector expressing an unrelated gene had been used at the same concentration—and the certainty with which the investigators could conclude there was no beneficial effect of the vector backbone alone. Dr. Ornelles asked the investigators for additional information about the likelihood of an inflammatory response that could lead to labyrinthitis ossificans. He questioned whether a wild-type endogenous adenovirus that reaches the middle and inner ear following an upper respiratory infection could trigger a burst of viral vector replication in co-infected cells, which could lead to a profound inflammatory response with the risk of ossification. In addition, he asked how often an infection of the middle/inner ear following stapedotomy could be expected to occur. Dr. Ornelles expressed his belief that participants should be told in plain language that the experimental agent is derived from a virus and is a form of gene transfer, rather than calling the agent a “medicine.” Because opting out of this study is not possible once the agent has been delivered, he suggested that relevant wording be adjusted in the informed consent document.

Noting that the protocol is well written overall, *ad hoc* reviewer Dr. Stankovic requested that the investigators show monkey data, particularly viral distribution outside of the inner ear, that is relevant for the informed consent document and process. She suggested that, given the marked inflammatory response observed in the monkey, the investigators should consider giving perioperative steroids to the research participants. Preoperative evaluation of the participants should include a temporal bone CT scan to detect anomalies associated with perilymphatic gusher, leakage of cerebrospinal fluid. Regarding participants’ ability to tolerate injection through the oval window, Dr. Stankovic asked whether the injected volume could be decreased by concentrating the virus, and she requested descriptions of (1) how egress of fluid from the stapedotomy will be controlled for, (2) the drug delivery system, consisting of a delivery device and pump, (3) the anticipated rate of delivery (in terms of µl per minute or per hour), and (4) the stapedotomy plug. With regard to the informed consent document, Dr. Stankovic said that the assertion that a participant will be able to stop the study medication is inaccurate and should be reworded. The informed consent document should describe the risk of the virus reaching the brain (as occurred in 20 percent of rodents) or other tissues, such as lymph nodes in the neck, and, given the risk of the virus reaching the brain, the informed consent document should include wording about collection of cerebrospinal fluid (CSF). The informed consent document mentions that tinnitus may improve but should also mention that tinnitus could worsen, especially if hearing worsens. In addition, Dr. Stankovic stated that some of the language and sentence structure in the informed consent document is too complex to be understood by an average person, some terminology should be more precise, and other wording should be simplified.

C. RAC Discussion

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

- Dr. Hammarskjöld reminded the investigators that human adenovirus does not behave the same in animals—especially not in rodents—as it does in humans. One of the significant differences is that human adenovirus 5 does not replicate in rodent cells and is blocked early in expression. Dr. Hammarskjöld further asked whether the investigators plan to look for adenoviral immune responses and whether individuals would be excluded from participation in this trial if they have high pre-existing titers to adenovirus.

- Dr. Kohn asked whether the investigators plan to screen for mutations in the large number of genes that cause genetic-based hearing loss in order to meet the exclusion criterion for those with genetic causes.

- Dr. Kohn suggested that the investigators should recognize that any fluid that leaks out during vector administration will contain recombinant adenovirus and will need to be handled in the biohazardous waste disposal. The institutional biosafety committee will want to know how the

investigators plan to handle that material, which contains the vector even though it is replication defective.

D. Investigator Response

1. Written Responses to RAC Reviews

A five-year follow-up study for safety is standard in the adenoviral field when transient persistence of the viral episome is expected. The investigators plan to evaluate long-term persistence of clinical efficacy in this study. Research participants who decline to participate in the follow-up study, drop out, or are lost to follow-up will be sought by telephone and by contacting their referring physician. The five-year follow-up trial is a separate protocol that will entail yearly visits for assessment of safety and efficacy and will have a separate informed consent document.

The number of vector particles has been described either as the concentration per µL (5.5×10^8 vp/µL) or as the total number of vector particles in a single 0.5 mL vial of drug product (2.5×10^{11} vp). The number of vector particles has been expressed per µL inner-ear total fluid volume, where appropriate for dose scaling. To clarify the number of vector particles administered at each dosing volume, the investigators plan to include the vector particle amounts together with dosing volumes as part of the Treatment section of the clinical protocol.

GenVec, Inc., the partner of Novartis in the development of CGF166, has extensive experience with manufacturing adenovectors. The vector concentration is approaching the limits of the manufacturing process and formulation buffer; therefore, the current concentration is the highest feasible from a manufacturing and quality point of view.

The surgical procedure for this protocol is a minor variation of the stapedotomy procedure, a commonly used surgical procedure that is familiar to otologic surgeons. All principal investigators participating in this study have been trained on the surgical procedures and use of the delivery device using human cadavers.

CGF166 will be delivered using a new device that has been specifically designed for this application (manufactured by Unilife) and will be labelled “For Investigational Use Only” per FDA requirements. The device uses a specially developed 27G blunt stainless steel cannula fitted with a depth limiter to prevent over-insertion. The cannula was specifically designed not to fit tightly into the stapedotomy in order to minimize the risk of pressuring the labyrinth, which could cause injury. The cannula is fitted with a 30 inch small-diameter polyurethane tube that connects to a commercially available 250 µL borosilicate glass syringe, which will contain CGF166. The syringe is driven by an FDA-cleared syringe pump that provides a high level of accuracy (± 3 percent of intended dose). The performance of the combined devices has been tested following FDA requirements. In addition, studies were conducted to ensure that the device, including tubing and needle, and CGF166 are compatible. The device was used in the 7-day and 2-month rhesus monkey studies as well as being tested by the principal investigators on human cadaveric temporal bone specimens. A visual representation of the device was provided.

Three monkey studies were conducted by the Sponsor, with input from the FDA. Inflammation was observed; however, it was not considered to be related to administration of adenovector, but instead due to the extensive remodeling needed to allow access to the inner ear via the ear canal, which is significantly narrower in the monkey than in the human ear canal (2.5 mm vs. 7 mm).

A total of 45 research participants may be enrolled in this study; the number includes a potential increase of participants in part C of the study based on sample size re-estimation. In the first year, approximately eight participants will be recruited, followed by 20 the second year and up to 17 in the third year of the study. The principal investigators indicated that this rate of accrual is feasible.

Experiments in multiple species have shown that CGF166 administered by the IL route reaches the target-supporting cells in the inner ear, although the exact percentage of transduced cells has not been

determined. The rhesus monkey study conducted with Ad5.GFP showed immunohistochemical staining for green fluorescent protein (GFP) in the target tissues, specifically the organ of Corti in the cochlea and cells of the vestibule. Qualitatively, GFP-positive cells were distributed throughout the inner ear, but this was not quantitated to determine the percentage of positive cells. Several publications demonstrate that IL delivery of an adenoviral vector in rodents results in broad distribution in the organ of Corti and vestibular neuroepithelium.

The Ad5 adenovector backbone used in CGF166 has no unique elements and has been used safely in thousands of patients. Therefore, the Sponsor considers unforeseen risks with the use of this vector to be unlikely. As the surgical procedure is a minor variation of the well-known stapedotomy procedure, the risk of the surgery is mostly known. With respect to long-term follow-up of the research participants to allow identification of chronic disturbances, the study includes an MRI evaluation at the last study visit at six months to assess long-term effects on the inner ear. In addition, participants are encouraged to enroll in a five year follow-up study that includes annual clinic visits with hearing and balance assessments to assess long-term safety.

While supporting cells have the capacity to transdifferentiate in the presence of ATOH1 or CGF166, these cells are terminally differentiated and do not replicate. ATOH1 is a transcription factor that is downstream of multiple developmental pathways, including the Notch, Wnt, and Hedgehog pathways. Disruption of these developmental pathways in adults has been associated with potential for tumorigenesis, with down-regulation of the associated ATOH1 gene reported in colorectal cancers and Merkel cell carcinomas. The anti-oncogenic role of ATOH1 has also been seen in fruit fly and mouse models. These data suggest that, in adults, ATOH1 regulates and maintains normal cell differentiation and suppresses tumorigenesis. One identified exception to this pattern has been observed in medulloblastoma, an embryonal neuroectodermal tumor in children. Published data suggest that medulloblastoma tumor cells may respond to the transcriptional product of ATOH1 but that ATOH1 itself is not important in initiating medulloblastoma. Given that CGF166 will be administered to adults, it is highly unlikely that any cell types in the brains of this participant population would be at risk of being affected by ATOH1 expression. In the six month biodistribution study with the IL administration, limited and short-lived distribution of CGF166 occurred in the brains of a few rats: two of ten rats at six hours post-dose, 0 of ten rats at 24 hours post-dose, one of ten rats at Day four, 0 of ten rats at Day seven, one of ten rats at Day 14, and 0 of ten rats at Day 28. In the six month toxicology study in rats, CGF166 was also administered by the intra-cerebroventricular route in order to investigate the impact of direct delivery to the brain via the CSF, a matrix connected to inner-ear perilymph fluid. There were no signs of hyperproliferation as determined by histopathology in the brains of these animals.

All of the Ad5 vectors used in the preclinical monkey studies were developed using the GenVec GV11 backbone containing the E1, E3, and E4 deletions. The control was vehicle alone; a null-containing vector was not used in these studies. Previous data using a GFP vector showed no effect on the ability to regenerate hair cells in utricle explants. Although Ad5.GFP was used in the first two rhesus monkey studies, balanced salt solution was used in the final rhesus monkey study to focus the evaluation on the effect of volume administration into the inner ear, as was agreed upon by the FDA.

Inner ear mineralization, possibly a preclinical counterpart to labyrinthitis ossificans, was observed only in rodents that received both the kanamycin/furosemide treatment and a high dose (5.5×10^8 vp) of CGF166. Mineralization was not observed in the hundreds of normal rodents studied as part of the comprehensive preclinical program or in the control animals treated with kanamycin/furosemide only. Therefore, the mineralization was attributed to administration of adenovirus in the setting of active inner-ear inflammation due to the kanamycin/furosemide injury. While it is theoretically possible that co-infection of cells bearing the CGF166 vector with a wild-type adenovirus could enable vector replication, there are a limited number of case reports of inner ear infections with adenovirus in the scientific literature. Accordingly, the investigators cannot assess the expected frequency of inner ear inflammation and mineralization but expect it will be very low based on the preclinical data. To assess for this risk, the investigators propose to enroll participants who have some residual hearing function in the treatment ear and follow them with monthly clinical assessments. Any clinically significant change will be detected by a

decrease in hearing threshold or new symptoms. The study also incorporates a baseline and follow-up MRI at 6 months after dosing, to assess subclinical changes in inner-ear anatomy.

The rhesus monkey studies were designed with input from the FDA; they believed that the potential for toxicity of the administered injection volume and injection rate should be evaluated in a larger species. Additional studies were conducted to address the effects of volume administration on function of the inner ear in rhesus monkeys. The Sponsor had agreement from the FDA that all other toxicity evaluations—including biodistribution, systemic organ toxicity, and potential effects of brain exposure—would be evaluated in rodent safety studies. Therefore, no extra-auricular tissues were collected in the rhesus monkey studies.

The rat good laboratory practices (GLP) biodistribution study consisted of 55 female and 55 male rats (5 rats/gender at each time point; 11 time points). In addition, the GLP toxicology study evaluated the potential toxicities of CGF166 directly injected into the brain (intracerebroventricular [ICV]) at a dose of 1.5×10^9 vp. No toxicities were observed, as measured by hearing, balance, general health, clinical pathology, and histopathology. The investigators and Sponsor believe that the rat biodistribution and toxicology studies (by ICV administration) provide sufficient information for appropriate informed consent with respect to effects on the brain.

Although CGF166 is administered into the inner ear of both rats and humans, the approach to accessing the inner ear is markedly different. In the rat, the dose was administered via a dorsal post-auricular incision made to expose the cartilaginous external auditory canal and the dorsally located facial nerve. There was limited and short-lived distribution of CGF166 to the brain of a few rats. Based upon the anatomy of the ear in the rat, it may be possible for the CGF166 that was administered into the left ear of the rat to distribute to the right ear via the CSF. Due to the differences between the anatomy of the ear in rats and humans, the distribution of CGF166 from the injected ear to the contralateral ear in humans is unlikely. In humans, CGF166 will be delivered directly into the perilymph space of the inner ear through the ear canal, via a surgical procedure under general anesthesia, in order to reach the inner ear's supporting cells, which are the target cells. The overall surgical procedure is a minor variation of the routine stapedotomy procedure.

Experimental mice were treated with the toxin 3,3'-iminodipropionitrile, which significantly decreases the number of hair cells in the vestibular system with minimal effects on the cochlear hair cells; therefore, only the hair cells in the utricle were evaluated. The impact of CGF166 on recovery of cochlear hair cells was evaluated using the kanamycin/furosemide injury mouse model. This model showed recovery of sensory hair cells in the basal region of the cochlea, which correlated to functional recovery of hearing at 32 kHz.

Perilymph gusher refers to leakage of CSF from the stapes footplate during stapedotomy for conductive hearing loss or from the round window/cochleostomy during cochlear implantation. When stapes surgery is performed for mixed hearing loss, the incidence of a perilymph gusher is associated with males and progressive mixed hearing loss starting in childhood, as in X-linked deafness type 3. Recent neuroradiology literature suggests that MRI scans can detect abnormalities of the modiolus and malformations that would be associated with risk of gusher. By excluding patients with congenital and childhood progressive hearing loss and conducting a good-quality preoperative MRI scan, the incidence of gusher should be reduced.

Eligible patients include those who have bilateral vestibular dysfunction, those with normal vestibular function, and those with unilateral vestibular dysfunction in the experimental treatment ear. The investigators can only assess improvement in vestibular dysfunction in those research participants in whom it is present. In participants with unilateral vestibular dysfunction, the dysfunctional side would be selected as the experimental treatment ear to avoid the risk of causing bilateral vestibular dysfunction as an adverse effect of the procedure.

Patients with hearing loss caused by genetic or developmental disorders, as known at screening, will be excluded. The investigators are planning a retrospective analysis of known genetic causes of hearing loss. If there is variability of response to CGF166 for efficacy and/or safety, the investigators will

conditionally conduct an exploratory genetic analysis to see whether it is possible to identify a relevant responder subpopulation for efficacy or a susceptible population for safety concerns.

The current CGF166 vector concentration is the highest feasible from a manufacturing and quality point of view. The drug delivery system consists of an FDA-cleared Caesarea Medical Electronics T34 pump and an investigational Unilife delivery kit designed specifically for inner-ear drug delivery. The infusion rate will be 10 to 20 µL/min. The cannula of the delivery device was designed not to fit tightly into the stapedotomy, in order to minimize the risk of pressurizing the labyrinth, which could cause injury. During evaluation of the delivery system in human cadaveric temporal bone specimens, some variability was encountered in the volumes the labyrinth would accept before egress of fluid from the stapedotomy during the infusion; the compliance of living tissue is expected to be greater. The stapedotomy plug will consist of a small amount of fascia harvested from the earlobe prior to creation of the stapedotomy and drug delivery.

The investigators stated that no CSF samples would be collected during this trial. The anatomical difference between the cochlear aqueduct of rodents and humans suggests that brain exposure in research participants should be less frequent than in rodents. In addition, there were no clinical or histological findings in the rat toxicology study, in which the entire clinical dose was injected directly into the cerebral ventricles.

The Sponsor agreed to amend and update the informed consent document to reflect suggestions by the RAC reviewers.

2. Responses to RAC Discussion Questions

Dr. Klickstein explained that the investigators tested the consequences of pre-existing antibodies on the ability of the adenovirus to transduce. Animals were immunized with adenovirus, and the investigators looked at the ability of an IL delivery of the vector to transduce cells. They did not look at the specific antibodies recognized by the virus; they looked generally at an antiviral titer. Normally there is no immunoglobulin in the perilymph fluid of the inner ear, so local inflammation in that privileged spot is not likely; however, Dr. Klickstein explained that the investigators plan to use imaging to look for it, in part because no one has ever done so. They will not exclude participants with pre-existing titers to adenovirus because of the contradictory preclinical results: In experiments conducted in guinea pigs with adenoviral gene delivery, pre-existing immunity interfered with efficacy; in experiments that Dr. Staecker conducted in mice, pre-existing immunity did not interfere with efficacy; and in experiments with rats, the investigators were unable to determine an effect. Dr. Klickstein stated that the investigators will measure pre-existing immunity but will not use it as an inclusion or exclusion criterion. They will conduct an exploratory analysis of efficacy as a function of immunity.

Regarding screening for genetic causes of hearing loss, Dr. Klickstein said that a commercial panel exists for such screening, but it is continuously evolving as additional information becomes available. Therefore, the investigators have decided to exclude individuals who have known genetic causes, but they will not conduct a genetic analysis for all the known and hypothesized causes of genetic hearing loss. However, they might be able to obtain that data at a later time and use it to analyze variation and clinical response as an element of a later pharmacogenetic component.

E. Public Comment

Kellee Kim, EcoR1 Capital, asked whether the investigators plan to examine prospectively the genetic profiles of research participants to determine genetic differences and, possibly, subpopulations. Dr. Klickstein responded that the investigators intend to exclude individuals with known genetic causes of hearing loss. They plan to profile all potential causes of hearing loss after the study has been completed, followed by a retrospective assessment of responders versus genetics. Although the investigators recognize that such data will be underpowered for meaningful conclusions, the data will provide general direction for future studies.

Regarding the preliminary mouse studies, Mike Pellum asked how long after the ototoxic injury the therapeutic virus had been administered. Dr. Klickstein responded that the virus was delivered ten days after the injury.

F. Synopsis of RAC Discussion and RAC Observations and Recommendations

Clinical and Trial Design Issues

- As noted in the summary of this protocol, the gene transfer agent will be delivered via stapedotomy. During this surgical procedure, there will likely be some egress of fluid from the inner ear that may contain recombinant virus. Since the adenoviral vector is classified as a Risk Group 2 agent in the *NIH Guidelines*, this material will need to be disposed of in a manner that is consistent with the requirements for Biosafety Level 2 material specified in the *NIH Guidelines*.

Ethical/Legal/Social Issues

- As discussed at the December RAC meeting, the informed consent document will incorporate the revisions outlined in the written reviews. In particular, the document will be modified to indicate clearly that this is a gene transfer approach, that once the vector is surgically administered it is not possible to remove the agent, and, in general, technical language present in the draft informed consent document will be simplified.

G. Committee Motion 2

Dr. Kohn summarized the RAC recommendations that would be included in the letter to the investigators, expressing the comments and concerns of the RAC. Dr. Kohn asked for a vote on these summarized recommendations, which the RAC approved by a vote of 11 in favor, 0 opposed, 0 abstentions, and 3 recusals.

(Dr. Pilewski chaired the following portion of the RAC meeting because Dr. Kohn was recused from discussion of this protocol due to a conflict of interest.)

IV. Review and Discussion of Human Gene Transfer Protocol #1310-1262, Titled: A Phase I Study of Ad-RTS-hIL-12, an Adenoviral Vector Engineered to Express hIL-12, in Combination with Veledimex, an Oral Activator Ligand, in Subjects with Recurrent or Progressive Glioblastoma or Grade III Malignant Glioma

Principal Investigator:	Antonio M. Omuro, M.D., Memorial Sloan-Kettering Cancer Center
Presenters:	John Barrett, Ph.D., ZIOPHARM Oncology, Inc.; E. Antonio Chiocca, M.D., Ph.D., Brigham and Women's and Faulkner Hospital and Dana Farber Cancer Institute *(via teleconference)*; François Lebel, M.D., ZIOPHARM Oncology, Inc.; Mary Matthew, ZIOPHARM Oncology, Inc.
Sponsor:	ZIOPHARM Oncology, Inc.
RAC Reviewers:	Drs. Curry, Pilewski, and Zoloth

Dr. Kohn was recused from consideration and discussion of this protocol due to a conflict of interest.

A. Protocol Summary

Malignant gliomas are the most common type of primary brain tumors. Glioblastoma is the most frequent type of malignant glioma and is associated with particularly aggressive disease and a dismal prognosis. The current standard of care focuses on surgical resection to remove as much of the tumor as possible, followed by a combination of radiation therapy and temozolomide chemotherapy. Even with aggressive treatment, patients with glioblastoma experience only modest improvements in survival. The median

overall survival of newly diagnosed patients is between 11 months and 17 months, and the 2-year survival rate is between 15 percent and 27 percent. Once the tumor recurs following initial treatment, few treatment options exist, because surgical intervention is limited by the infiltrative nature of the disease and the lack of clear margins delimiting the tumor. Given the poor overall prognosis and lack of effective treatments, new treatment approaches for malignant gliomas are needed.

Tailoring immunotherapy for brain tumors is particularly compelling. It is well documented that cytokines enhance the immune response and induce antitumor activity. Specifically, interleukin-12 (IL-12) is a potent anticancer cytokine that enhances both innate and adaptive immune responses and facilitates the activation of immune cells, including natural killer cells and cytotoxic T cells, and cytokines such as interferon-γ and tumor necrosis factor. However, systemic delivery of IL-12 is not feasible because it induces severe toxicity.

The investigators have developed an experimental system that can activate the expression of IL-12 directly into the tumor in a controlled manner. The system is composed of two parts: Ad-RTS-hIL-12 is the adenoviral vector encoding the IL-12 gene under control of the RheoSwitch Therapeutic System (RTS)® regulated promoter, and veledimex is the activator ligand that signals Ad-TShIL-12 to produce IL-12 in a dose dependent manner. The investigators have shown that they can safely inject Ad-RTS-hIL-12 directly into melanoma tumors and give oral veledimex capsules to start the production of IL-12. In addition, because Ad-RTS-hIL-12 expresses IL-12 only in the presence of veledimex, this system offers the flexibility to turn off the expression of IL-12 by stopping the veledimex capsules, if serious side effects develop.

The investigators propose a clinical trial to test the safety and tolerability of this experimental treatment in patients with recurrent malignant gliomas. A fixed dose of Ad-RTS-hIL-12 will be injected directly into the brain tumor using stereotactic surgery. Once the participants recover from surgery, they will receive veledimex capsules, by mouth, for 14 consecutive days. The dose of veledimex will be systematically increased in sequential cohorts (three participants in each cohort) to test for toxicity. The first participant in each cohort will be thoroughly monitored for 21 days (14 days while taking veledimex capsules and seven days after taking the last veledimex capsule) before the next two participants in that same group will be dosed. Any serious safety issues will be reviewed by a Safety Review Committee (SRC), and a recommendation will be made with regard to continuation of the trial. If no significant safety issues arise, the second and third participants in each cohort will be dosed. Overall, up to 39 individuals will participate in this trial.

The primary endpoint is safety and tolerability of Ad-RTS-hIL-12 and veledimex in participants with recurrent malignant gliomas. Other endpoints include effects of treatment with Ad-RTS-hIL-12 and veledimex, antitumor response, pharmacokinetics, immune response, and survival. The data collected from this study will provide important information that will define the safety profile and dosing of Ad-RTS-hIL-12 and veledimex and help the investigators design subsequent clinical trials for patients with malignant gliomas.

B. Written Reviews by RAC Members

Eight RAC members voted for in-depth review and public discussion of this protocol. Key issues included the reports of four serious adverse events (SAEs) in three participants in another protocol using this system, along with reports of hypotension, fever, and neutropenia from a different protocol. While recurrent or progressive glioblastoma has a poor prognosis and there is a need for new therapeutics, the safety of intracranial administration of this agent in a population that may be more susceptible to neurological complications deserves further discussion.

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

Although sufficient experience exists with adenoviral injection into brain tumors, Dr. Curry stated that little to no data exists on intracranial IL-12 expression in humans, although preclinical data cited in the protocol and in the literature are suggestive of efficacy without toxicity. He noted that the preclinical study using

intracranial GL261 cells in syngeneic mice resulted in two unexplained animal deaths, and he asked whether those mice were treated with Ad-RTShIL-12/veledimex and, if so, whether they underwent full necropsy. Dr. Curry asked the investigators whether *in vitro* or *in vivo* evidence exists of a dose-response effect using this vector, this transgene, and this inducer; whether the investigators could estimate or explain how glioma cells respond to infection and RTS-hIL-12 induction; and the kinetics of IL-12 expression when veledimex is withheld. Because it appeared that the planned escalation scheme was not fully considered in the protocol, he wondered whether veledimex or adenoviral dosing (or both) would be de-escalated if the first dose level were to show unacceptable toxicity. Dr. Curry requested that the investigators update the RAC with details of the SAEs in the two prior protocols to examine the use of this inducible system. Additionally, he asked the investigators to explain what they would do if postoperative MRI reveals a gross total resection and adenovirus has been administered.

Dr. Pilewski requested information about the tropism of adenovirus type 5 for brain tissue and the expression of adenovirus receptor in the brain, along with the implications of previous studies of IL-12 therapy for intracranial tumor. He asked the investigators to clarify the dose for cohort 1, because two different daily doses—80 mg and 160 mg—were described within the protocol; he suggested that the lower dose be used, depending on the results of the studies of pharmacokinetics across the blood-brain barrier. Dr. Pilewski offered six additional suggestions for improving this protocol:

- The use of prophylactic antibiotics for sepsis prevention would be inappropriate, given the stated goal of determining safety and toxicity.
- The use of CYP450 3A4 inhibitors should be prohibited under all circumstances prior to the first dose of veledimex, given the secondary objective of defining tolerability and pharmacokinetics.
- An Investigator's Brochure for veledimex should be provided to allow for assessment of the adverse events and risk/benefit, and of inclusion/exclusion criteria, particularly for renal and liver function, baseline hematology, and CYP450 3A4 inhibitor interactions.
- Rather than allowing the medical monitor to have sole authority to define dose-limiting toxicity (DLT), an independent data and safety monitoring committee would be useful in this study, given the high likelihood of adverse events and deaths in a very ill population with a dismal prognosis.
- Examination of tissue at any point after vector injection would be worthwhile to enhance understanding of transgene expression and immune responses; therefore, post-mortem studies should be planned.
- The informed consent document should clarify (1) that the study will escalate the dose until reaching the maximum tolerated dose; (2) how costs related to study participation will be covered; and (3) the full range of adverse events in prior studies with this vector and veledimex.

Dr. Zoloth commented on various ethical issues to improve the protocol. Overall, she noted that participants with advanced glioblastoma are facing death in approximately six months, so they are an extremely vulnerable population; therefore, complete honesty must be maintained in obtaining consent for their participation so that potential participants can answer for themselves the ethical question as to whether they wish to spend that time in a rigorous Phase I clinical trial to test the safety of gene transfer. Great care must be taken to avoid the impression that this intervention is designed to be curative, and Dr. Zoloth suggested several improvements in the informed consent document to achieve that goal:

- Potential participants should be told more clearly that they are facing the prognosis of death within six or seven months; current wording in the document implies that that may not be the case.
- Discussion of adverse effects that might be terrifying, including "delirium," should be included. Participants need to know about the problem of adverse effects in similar studies, especially since the prospect of significant states of severe mental confusion might be confused with disease progression.
- Explanation of the animal deaths in a similar study should be included.
- Data about the use of hIL-12 in human brains should be included and clearly explained.
- The role of the Sponsor is problematic in that the company will have complete control over two aspects of the trial that require external and impartial oversight: the use of a medical monitor to

assess side effects and dose-related toxicity, and the evaluation of whether possible injuries attributable to side effects are caused by the drug protocol and costs will be covered by the company.
- Clear and simple language should be used; currently, that is not the case throughout the informed consent document.

C. RAC Discussion

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

- Dr. Pilewski commented about the relationship between adverse events/dose response and dose in the previous trials. He expressed concern about the number of adverse events encountered at the 160 mg dose; although a causal relationship may not be present, a lot of adverse events occurred in the breast and melanoma trials. This patient population is a sick population, many of whom may not tolerate adverse events, and deaths are likely to occur within a short period of time within the study. He thanked the investigators for addressing those issues.

- Noting that this protocol proposes a relatively high fixed dose of vector, Dr. Hammarskjöld asked the investigators how they determined that 1×10^{12} vp is likely to be a safe dose and to explain their rationale for using that number of particles as the fixed dose for this protocol.

- Dr. Atkins asked the investigators how they will ensure that the vector is injected only into the tumor and not into the brain. He suggested that there might be a risk of extra toxicity if the vector injection follows the tumor resection.

- Dr. Atkins inquired about how steroid use might influence the findings of this study and the effect of steroid use on eligibility criteria. He expressed concern that, because the investigators plan to allow participants to use steroids, they might find a dose that is safe in the presence of steroids but might not be safe if administered to individuals not on steroids. Dr. Curry stated that a research participant on steroids could experience a beneficial effect and/or a muted toxicity. In immunotherapy trials to date, there has been an expectation that a steroid could be used at a minimal or medium dose (such as 2 mg twice a day of dexamethasone).

- Dr. Atkins asked how many days of the activator a research participant must receive in order to be classified as not having a DLT.

- Dr. Fost commented that the likelihood of clinical benefit from a Phase I gene transfer trial is less than one percent, so he suggested rewording portions of the informed consent document to reflect that it is highly unlikely that benefit would accrue to participants of this trial.

- Dr. Corrigan-Curay stated that the *NIH Guidelines* contains a requirement that the informed consent document discuss that, at the time of a participant's death, the principal investigator will approach the family about autopsy. This is not a requirement for autopsy; it is a requirement to raise the issue and forewarn the family that the topic will be broached.

D. Investigator Response

1. Written Responses to RAC Reviews

The Sponsor has reported the two SAEs of delirium in a single research participant. All trials in which Ad-RTS-hIL-12 or activator ligand were used, either alone or in combination, were searched for adverse events of delirium and/or changes in mental status. Six studies were identified, including four healthy-volunteer studies, the currently enrolling melanoma study ATI001-101, and the ATI001-201 breast cancer study. Safety and clinical databases were reviewed, and 925 adverse events from 161 research participants across the clinical studies were reviewed. Delirium, somnolence, confusion, or mental status

changes were reported in six participants in three clinical studies, with events consisting of eight adverse events and five SAEs. The events of somnolence in the volunteer studies were all mild and of short duration. The time of onset was within 15 minutes for one participant, a time at which absorption of veledimex from the gastrointestinal (GI) tract was just starting. The other participant experienced a similar event but only upon the second dosing, suggesting that a relationship to veledimex was unlikely, because the event did not occur during initial dosing. A participant who received placebo experienced a similarly timed event, again suggesting a lack of relationship to veledimex. Three participants were identified in both databases who respectively experienced confusion, altered mental status, or delirium; those three individuals developed SAEs but not necessarily as their initial clinical presentation. They were seriously ill participants with multiple confounding factors, and their confusion, altered mental status, or delirium were not isolated signs or symptoms but were part of a more complex syndrome in all cases.

A summary of events involving changes in hypotension, fever, and neutropenia indicated that a dose response to the frequency of adverse events was observed, including for related SAEs. These observations are subject to change because both studies (ATI001-101 and ATI001-201) in which these SAEs occurred are still open and actively recruiting participants. Three SAEs of pyrexia (two from the melanoma trial and one from the breast cancer trial) were identified, two SAEs of hypotension were from the melanoma trial, and one SAE of neutropenia and one SAE of febrile neutropenia were from the melanoma trial; these SAEs were considered by the investigators to be “related” or “possibly related.” One research participant with an SAE of fever also experienced malignant neoplasm progression, pancytopenia, altered mental status, and septic shock, and died as a result of neoplasm progression.

Regarding the two unexplained mouse deaths, the investigators responded that the study report indicates that the two animals died due to handling; no necropsy was performed. Deaths occurred in mice that were injected with Ad-RTS-mIL-12 and veledimex; however, they were not the only animal deaths in that experiment. Mice in the control groups that received no Ad-RTS-mIL-12 or veledimex also died and, in that same experiment, four mouse groups that received Ad-RTS-mIL-12 and/or veledimex resulted in no mouse deaths. The investigators explained further that, in preclinical studies in which mice are housed in groups, random deaths are common due to laboratory personnel handling of the animals or animals fighting within the cage.

Both *in vitro* concentration-response and *in vivo* dose-response studies have been performed, and results demonstrate that the localized delivery of IL-12 encoded by the replication-incompetent adenoviral vector, and controlled by the combination of Ad-RTS-IL-12 with the oral activator INXN-1001, is an effective immunotherapeutic strategy. The investigators’ results show that this therapy induced localized controlled production of IL-12 that correlated with an increase in tumor cytotoxic T cells and memory T cells, leading to the desired biologic response of tumor growth inhibition.

The investigators’ current plan for this Phase I study is to begin dosing at 40 mg/day, administered as a divided dose of 20 mg AM/PM. Assuming an average body weight of 70 kg, the resultant dose corrected for body surface area would be 21 mg/m^2/day, at the minimally effective dose level. At this dose level, no adverse clinical signs were observed and, in the Sponsor’s Phase I study in melanoma, a dose of 100 mg (53 mg/m^2) had biologic effects and was devoid of DLT. If toxicity is observed, the investigators plan to de-escalate the activator ligand as appropriate.

The Sponsor explained that it is inherently difficult to predict individual patient survival time. A recent study that evaluated survival time estimates among cancer patients with brain metastases undergoing radiosurgery reported that neurosurgeons, radiation oncologists, and medical oncologists were not able to accurately predict individual patient survival. Because no validated algorithms can accurately predict survival among the high-grade glioma population, the Sponsor will rely on the investigators’ expertise for that information.

The investigators acknowledged that the use of prophylactic antibiotics to prevent infections after neurosurgical procedures remains a topic of debate. The protocol allows the use of prophylactic antibiotics in consideration of the treating physician and neurosurgeon and the standard practice and experience they have developed with this patient population at their institutions. Common practice

includes administration of an intra-operative antibiotic and continued antibiotic support for 24 hours. Standard short-duration antimicrobial prophylaxis for neurosurgical procedures is permitted, according to various professional guidelines. Because this trial aims to accrue participants at four to eight institutions, this provision will allow the neuro-oncology treatment team to care for their patients as per their standard practice. The protocol is permissive; it does not require or prohibit perioperative antibiotic use. The use of antimicrobials for treating new active infection would have to be graded and recorded as an adverse event.

The investigators explained the two modifications to the protocol that will help better gauge and monitor participant safety. The first modification asserts a lower starting dose (40 mg of veledimex) and a subsequent dose escalation of 40 mg, 80 mg, 100 mg, and 120 mg, daily. A safety review of the Sponsor's ongoing melanoma and breast cancer trials revealed that the majority of SAEs occurred at the 160 mg/day veledimex dose, so the planned 120 mg maximum dose for this trial should be well tolerated. Since this is a two-part study drug, however, the veledimex dose is relevant only if it can be present with Ad-RTS-hIL-12 (at the injection site). The mouse model data demonstrate that veledimex crosses into the intracranial compartment, although the investigators acknowledged that they will be certain of this occurrence only when they measure veledimex distribution in human brain tissue. The second modification entails that one veledimex dose (40 mg) is given before a planned resection, so that tumor, plasma, and CSF can be collected and veledimex distribution analyzed.

The Sponsor does not intend to give the medical monitor sole authority to define DLTs or evaluate toxicity assessments; that responsibility and authority remains with the investigators. Each individual investigator retains the right and responsibility to determine the causality of any observed adverse event or SAE and to determine whether they wish to continue study participation, regardless of whether the Sponsor concurs. The SRC remains the Sponsor's preferred vehicle for decisionmaking, because it is composed of the individuals with relevant expertise to uphold participant safety and to evaluate whether the investigational agents have the potential to contribute to clinical benefit. The SRC depends on a collaborative effort between the investigators and the Sponsor.

Tissue examination after vector injection is worthwhile to help understand the investigational agents. The dosing period will last 14 days, after which participants will be followed for a maximum of 2 years. Postmortem studies or postmortem tissue collection were not included as part of the protocol primarily because it is not known whether research participants will be placed on other therapies or clinical trials after this study. However, the Sponsor will ask the neurosurgeon to collect tumor tissue, CSF, and plasma as they become available during the course of standard or palliative intervention.

The Sponsor agreed that patients should be well informed regarding their prognosis and their choice of treatment options after disease progression or recurrence. It is the responsibility of the treating physician (investigator) and the clinical care team to provide such information to patients, because those individuals better understand the patient and the medical history. The investigators explained that they have modified the informed consent document and added additional language on this topic in a manner that will prompt potential participants to discuss this topic with their physician or surgeon. Providing specific survival estimates is not recommended, because such estimates apply to specific populations and likely are not accurate for the individual patient, who might misinterpret such data.

The investigators acknowledged that the protocol and the informed consent document have been modified to include updated safety/side effect information from ongoing trials. Additional safety data from the melanoma and breast cancer ongoing clinical trials have provided further information about the potential side effect profile, and additional preclinical mouse model data have provided a rationale for dose selection.

To be transparent and compassionate toward an individual who participates in any of the Sponsor's clinical trials, the Sponsor has modified the informed consent document to accurately reflect that it is unknown whether the investigational agents in this trial will provide any benefit, but that the investigational agents are likely to result in side effects. Patients are alerted to have this discussion with their physician

and/or surgeon before they decide to participate in this study, and they are cautioned to consider the risks, possible benefits, and impact on their quality of life.

On the topic of injuries directly attributed to study medication, the investigators reported that the informed consent document was modified to clarify that the Sponsor will pay reasonable and necessary treatment costs for injuries directly related to the study drug, in accordance with the study plan.

The Sponsor agreed to amend and update the informed consent document to reflect suggestions by the RAC reviewers.

2. Responses to RAC Discussion Questions

Regarding the vector dose, Dr. Lebel acknowledged that 1 x 10^{12} vp is a somewhat high dose; however, the investigators have been reassured by their experience in humans, in which that dose has not resulted in toxicity associated with viral replication. They have seen some transaminitis, fever, and thrombocytopenia, but all were of short duration and were reversible upon discontinuing the oral ligand. This fixed dose was chosen based on extrapolation of the level of efficacy in animal models, taking into account that a mouse tumor is about 100 times smaller than a human tumor.

Dr. Chiocca acknowledged that it is not possible to ensure that the vector will be injected only into the tumor and not into the brain; injection usually results in at least some reflux, which the investigators try to minimize by performing the injection slowly. After more than ten years of practice injecting adenoviral vectors as well as oncolytic adenoviruses in this manner, no significant issues have arisen. Dr. Lebel added that the vector could be “turned off,” by withdrawing the oral ligand, if the vector were to be injected at the wrong site.

Regarding steroid use, Dr. Lebel explained that the protocol provides some guidance to the study centers, noting that many of the centers already have their own guidelines on the use of steroids. The investigators realize that they will have to allow some steroid use, and they are still tweaking the specific language in the protocol to minimize the exposure without compromising participant safety. They have initiated animal studies to determine the impact of high-dose steroid in mice and, although final results are not yet available, preliminary data indicate that steroids do not affect efficacy. Dr. Chiocca added that the extent of steroid use by a trial participant is a clinical judgment that needs to be made on a case-by-case basis.

Dr. Lebel clarified that 14 days constitutes the full duration of activator administration. A research participant who goes through that full course is classified as not having a DLT.

Regarding autopsy request wording, Dr. Lebel agreed to add the appropriate language in the informed consent document.

E. Public Comment

No comments from the public were offered.

F. Synopsis of RAC Discussion and RAC Observations and Recommendations

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

- The protocol states that the safety population includes research participants who receive the Ad-RTS-hIL-12 and/or the inducer, veledimex. However, in the 3 + 3 safety cohort design, the RAC recommends that progression to the next cohort only occur after three research participants have received both the Ad-RTS-hIL-12 and veledimex, as that is the intended investigational intervention.

- The protocol will enroll participants who are undergoing resection of their tumor, and it is expected that this cohort of participants is more likely to require lower doses of steroids compared with those who are not resected but instead receive stereotactic, intratumoral injection. To understand whether steroid use influences the safety of this approach, and to avoid escalating to a higher dose before that question has been explored for each population, it may be helpful to have two separate safety cohorts—one for resected patients, who are likely to have their steroids weaned after surgery, and a second cohort of those who have progressive, nonresectable disease. In addition, the investigators should consider an exclusion criterion that limits the maximum dose of steroids on entry in order to obtain a more uniform population for this Phase I safety study.

- The investigators should consider removing the language regarding withdrawal of participants who have had a total gross resection and who received the adenoviral vector, as these individuals likely have residual tumor and should be evaluated for safety.

- This protocol will have an SRC that will include investigators, subinvestigators, and the Sponsor's medical monitor. The protocol submitted for RAC review appeared to give the medical monitor, an employee of the company, the sole authority to determine whether there was a DLT and whether the study should be modified or terminated due to safety issues. The investigators clarified in their responses that the medical monitor alone will not make those decisions; any such decision will be made in collaboration with the SRC. Ultimately, however, the Sponsor will have the final responsibility to modify or terminate the study. The investigators also correctly note that independent safety monitoring committees are unusual in Phase I studies. However, given that the research participants for this trial will be quite ill, making attribution of adverse events (especially neurological toxicities) potentially more difficult, the investigators should consider structuring the SRC to be more independent of the company.

Ethical/Legal/Social Issues

- The prognosis for these patients is unfortunately extremely poor, with life expectancy measured in months. While the investigators would not move forward with this protocol if they did not believe that this experimental therapy will potentially have clinical benefit, the reality of drug development is that few agents tested in Phase I become a therapy. There needs to be very clear communication to these potential participants that personal benefit is unlikely and that their choice is whether they would like to participate in this early trial to add to the body of knowledge that may lead to much needed therapies for this disease. The Sponsor and investigators should review the language from *NIH Guidance on Informed Consent for Gene Transfer Research* (http://oba.od.nih.gov/oba/rac/ic/appendix_m_iii_b_1_d.html) for examples of how to describe potential benefit for different phases and designs of trials.

- The neurological toxicities—mental status changes and delirium—that may be of particular concern to these patients should be highlighted in the informed consent document.

G. Committee Motion 3

Dr. Pilewski summarized the RAC recommendations to be included in the letter to the investigators, expressing the RAC's comments and concerns. Dr. Pilewski asked for a vote on these summarized recommendations, which the RAC approved by a vote of 13 in favor, 0 opposed, 0 abstentions, and 1 recusal.

(At this point, Dr. Kohn resumed chairing the December 2013 RAC meeting.)

V. Review and Discussion of Human Gene Transfer Protocol #1310-1253, Titled: Phase I/II Study of the Treatment of Metastatic Cancer that Expresses MAGE-A3 Using

Lymphodepleting Conditioning Followed by Infusion of HLA DP0401/0402 Restricted Anti-MAGE-A3 TCR-Gene Engineered Lymphocytes and Aldesleukin

Principal Investigator: Steven A. Rosenberg, M.D., Ph.D., National Cancer Institute (NCI), NIH
RAC Reviewers: Ms. Dresser, Dr. Kohn, and Dr. Sadelain

Dr. Atkins was recused from consideration and discussion of this protocol due to a conflict of interest.

A. Protocol Summary

Studies in experimental animals have demonstrated that the cellular rather than the humoral arm of the immune response plays the major role in the elimination of murine tumors. Much of this evidence was derived from studies in which the adoptive transfer of T lymphocytes from immune animals could transfer resistance to tumor challenge or in some experiments, the elimination of established cancer. Thus, most strategies for the immunotherapy of patients with cancer have been directed at stimulating strong T cell immune reactions against tumor-associated antigens.

In contrast to antibodies that recognize epitopes on intact proteins, T cells recognize short peptide fragments (8-18 amino acids) that are presented on surface class I or II major histocompatibility (MHC) molecules and it has been shown that tumor antigens are presented and recognized by T cells in this fashion. The molecule that recognizes these peptide fragments is the T-cell receptor (TCR). The TCR is analogous to the antibody immunoglobulin molecule in that, two separate proteins (the TCR alpha and beta chains) are brought together to form the functional TCR molecule. The goal of this protocol is to transfer MAGE-A3 reactive TCR genes into normal peripheral blood lymphocytes (PBL) derived from cancer patients and to return these engineered cells to patients aimed at mediating regression of their tumors. This trial is similar to previous Surgery Branch TCR gene transfer adoptive immunotherapy protocols that have used HLA-Class I restricted TCRs except that in this trial we will use an HLA-Class II (DP0401/0402) restricted (MAGE-A3-DP4) TCR reactive with a MAGE-A3 epitope expressed on the tumors of research participants with melanoma as well as research participants with common epithelial malignancies.

The study will begin by evaluating the safety of escalating doses of cells from 10^7 to 10^{11}. Once a safe dose has been confirmed, research participants will be enrolled into the phase 2 portion of the trial using the cell dose found to be safe in phase 1 of this protocol. In order to complete the dose escalation phase and both phase 2 cohorts, a total of up to 25+82=107 research participants may be required (25 + 2 strata with a maximum of 41 apiece). Research participants who are HLA-DP4 positive and who have metastatic cancer that expresses MAGE-A3 antigen and who are refractory to standard therapy will enroll in this study. The primary objective of this study will be to determine a safe dose of the administration of autologous CD4 cells transduced with an anti-MAGE-A3-DP0401 restricted (MAGE-A3-DP4) TCR and aldesleukin to research participants following a nonmyeloablative but lymphoid depleting preparative regimen; to determine if this approach will result in clinical tumor regression in research participants with metastatic cancer expressing MAGE-A3-DP4; and to determine cells and the toxicity profile of this treatment regimen. The secondary objective of this study is to determine the *in vivo* survival of the TCR gene-engineered cells.

B. Written Reviews by RAC Members

Eight RAC members voted for in-depth review and public discussion of this protocol. In light of previous deaths using this target, the protocol was selected for in-depth public review to discuss the data supporting the safety of this approach.

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

With regard to risk/benefit issues, Ms. Dresser asked the investigators whether *in vitro* studies or other preclinical investigations provide additional evidence for the belief that this TCR will not produce either the serious toxicities observed in previous studies or new serious toxicities. She also asked whether additional monitoring measures could improve the research team's ability to detect toxicities earlier than

in previous studies so that SAEs could be avoided. Ms. Dresser offered six recommendations for improving the informed consent document, including changing the wording of phrases that refer to this investigational intervention as treatment or therapy, rewording the material on possible benefits to make it more informative, and adding a reference to laboratory testing that addresses the evidentiary basis for believing that this TCR will not produce the side effects seen in earlier studies.

Dr. Kohn stated that the major issue for this new study is the safety risk of the proposed approach. He asked whether the proposed class II restricted TCR is expressed in CD4+ Tregs in the transduced CD4+ cell product and whether an inhibitory effect might occur. Dr. Kohn requested that the investigators explain the proposed mechanism by which CD4+ cells lead to tumor elimination—whether they are expected to be directly cytolytic or whether they activate other effectors. He requested information about the time course of the appearance of the severe toxicities in the affected research participants in the prior MAGE-A3 trials, and suggested that the enrollment be staggering accordingly to allow novel toxicities to be observed before subsequent participants at the next dose level are enrolled.

With regard for TCR specificity, Dr. Sadelain asked for further information about the algorithm for searching for related or mimicking peptides for MAGE-A3 and for MAGE-A6, and whether alanine scanning or an equivalent method had been used to search for peptides with possible cross-reactivity. Dr. Sadelain requested additional information about whether the 6F9 antibody redirects CD8+ T-cell cytotoxicity or the CD8+ T-cell proliferation and whether it redirects CD4+ Tregs. He asked the investigators to explain why a suicide gene is not included in this study, and how research participants will be monitored to recognize unexpected T-cell reactivity. In addition, Dr. Sadelain asked whether the informed consent document will refer explicitly to the difficulty of predicting TCR reactivity, especially in the setting of an MHC haplotype differing from that of the original TCR donor.

C. RAC Discussion

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

- Dr. Fost asked the investigators to state the percent of gene transfer studies of cancer that have become proven therapies.

- Dr. Atkins commented that the TCR trials have shown that 10 to 20 percent of research participants have been durable, complete responders. Dr. Kohn added that the response percent for all approaches to cancer treatment is approximately one percent. However, when investigators look at a few therapies that are the leading strategies, a significant fraction of those research participants are surviving, which indicates that not all cancer research participants in gene transfer trials can be described with the same statement of no prospect or low prospect of benefit.

- Ms. Dresser commented that cancer patients receiving approved therapies often are given a probability statement regarding the potential effectiveness of the therapy. However, enrollees in gene transfer trials merely receive a statement saying that the investigators do not know whether the experimental treatment will be beneficial. She suggested that a more informative probability statement would be helpful, given that some people do benefit although many do not.

D. Investigator Response

1. Written Responses to RAC Reviews

The investigators explained that they have performed alanine scans and have tested all peptides with similarity to the DP0401 peptide that they are targeting, and none was reactive. Data on these tests are shown in Table 1 in the protocol.

The dose escalation schema for this protocol is designed to minimize the likelihood of serious adverse outcomes, and the investigators will carefully monitor research participants for indications of off-target toxicities.

In response to concerns about the language used in the informed consent document that might result in misinterpretation as to the chance for direct benefit from this protocol, the investigators explained that the language as written is consistent with the NCI template language for oncology protocols and is the same language as used in all NCI Surgery Branch gene transfer protocols. The principal investigators and research nurses take great care during the consenting process to ensure that potential participants understand that they are participating in research and that there may be no proven benefit. In addition, the language regarding possible benefits is consistent with the NCI institutional review board template. The experimental treatment procedures are described in detail to all participants during the consent process and at many subsequent points throughout the screening and evaluation period; the level of detail is tailored to the understanding and interest of each participant and their family.

The investigators reported that they have revised the informed consent document to include information regarding the cardiac toxicities in previous trials and why they do not anticipate cardiac toxicities in this trial.

The investigators clarified that, after the rapid expansion, the infused product does not contain FoxP3-positive Treg cells.

With regard to the time course of the appearance of severe toxicities in the prior MAGE-A3 trials, the investigators responded that, in their experience, most severe toxicities present by day seven following cell infusion.

The investigators explained that this proposed vector does not include a suicide gene, an approach that has been reviewed and approved by the FDA. Through use of the proposed dose escalation schema, the investigators will closely monitor research participants for any indication of off-target toxicities.

The difficulty of predicting TCR reactivity, especially in the setting of an MHC haplotype differing from that of the original TCR donor, is a challenging concept to explain in a document written at an eighth-grade level. The general risks, including death, are presented in the written document. At several time points during the informed consent process, both the principal investigator and the research nurse will review and explain the risks and benefits and the toxicities to the research participant and their family, at a level that is readily understood.

The Sponsor agreed to amend and update the informed consent document to reflect suggestions by the RAC reviewers.

2. Responses to RAC Discussion Questions

Dr. Rosenberg stated that, to date, no gene transfer studies of cancer have become proven therapies, although several studies are heading toward that kind of approval. Four companies are attempting to commercialize this type of technology, but none have completed the venture to date. He predicted that this approach—T cells that can react against targets on cancer cells—will be an important part of the future of cancer treatment and reminded the RAC that this field is relatively new, with the first study conducted in 2006.

Dr. Rosenberg said that the objective response rate to CD19 is 80 percent in research participants with diffuse large B-cell lymphomas and that encouraging results have been published in acute lymphocytic leukemia as well. This treatment is slowly winding its way through the regulatory environment that will enable it to have more widespread application. Dr. Rosenberg predicted that anti-CD19 and anti-NY-ESO-1 would be the first two gene transfer cancer treatments to be widely applicable. He explained that the search for targets is the major problem confronting the extension of this kind of cancer immunotherapy.

E. Public Comment

No comments from the public were offered.

F. Synopsis of RAC Discussion and RAC Observations and Recommendations

The Committee noted that all of the scientific and clinical questions had been addressed in the written reviews. Only one recommendation related to the informed consent document remained:

Ethical/Legal/Social Issues

- The Surgery Branch has a large portfolio of trials using gene-modified TCRs against a number of cancers. There have been some very impressive results, including long-term remissions in patients with metastatic cancer. That being said, this trial is new and although it builds on this considerable experience, few Phase I studies yield successful therapies. Therefore, it is important that prospective research participants understand that clinical benefit in a Phase I study is unlikely. The use of words such as “therapy” or “treatment” in the informed consent document can mislead potential participants. More neutral terms, such as “intervention” or “investigational agent,” that are less likely to suggest therapeutic benefit are preferred. Similarly, language such as “we do not know whether you will benefit from this intervention” can be misconstrued as indicating a possibility of benefit that may be greater that can be expected from a first-in-human trial. The investigators should consider revising statements regarding benefit to prevent any unwarranted therapeutic misconception.

G. Committee Motion 4

Dr. Kohn summarized the RAC recommendations to be included in the letter to the investigators, expressing the RAC’s comments and concerns. Dr. Kohn asked for a vote on these summarized recommendations, which the RAC approved by a vote of 12 in favor, 0 opposed, 1 abstention, and 1 recusal.

VI. Day 1 Adjournment

Dr. Kohn adjourned Day 1 of the December 2013 RAC meeting at 4:30 p.m. on December 4.

VII. Day 2 Opening

Dr. Kohn opened Day 2 of the December 2013 RAC meeting at 8:30 a.m. on December 5.

VIII. Gene Transfer Safety Assessment Board Report

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

A. GTSAB Report

Dr. Kohn presented the GTSAB report for the fourth quarter of 2013. Within the past three months, the OBA received 29 protocol submissions, 24 of which were not selected for public review at this RAC meeting. Of the 24 protocols not selected for public review, 14 were oncology protocols, four were for muscle disorders, two were for eye disorders, and one each was for HIV, wound healing, sickle cell disease, and amyotrophic lateral sclerosis. In these 24 protocols, seven used plasmid, six used lentivirus, four used adeno-associated virus (AAV), two each used pox viruses and RNA transfer, and one each

used retrovirus, attenuated measles virus, and attenuated *Salmonella enterica*. Information about these trials will be available on the OBA website after this RAC meeting.

The GTSAB reviewed initial and follow-up reports on 46 SAEs from 19 protocols. After analyzing these events, the GTSAB concluded that none warranted public discussion at this RAC meeting.

During this quarter, the OBA received notification from investigators that three protocols, all of which had been reviewed publicly by the RAC, were newly open to enrollment:

- OBA Protocol #1006, reviewed by the RAC in December 2009: Treatment of Subjects with Adenosine Deaminase (ADA) Deficient Severe Combined Immunodeficiency (SCID) with Autologous Bone Marrow CD34+ Stem/Progenitor Cells After Addition of a Normal Human ADA cDNA by the EFS-ADA Lentiviral Vector
- OBA Protocol #1214, reviewed by the RAC in June 2013: A Phase I Randomized, Double-Blind, Placebo-Controlled Study of a Multi-Antigen DNA Vaccine Prime Delivered by In Vivo Electroporation, rVSV Booster Vaccine in HIV-Infected Patients Who Began Antiretroviral Therapy During Acute/Early Infection
- OBA Protocol #1236, reviewed by the RAC in September 2013: Infusion of Allogeneic, Third-Party CD19-Specific T Cells (CD19RCD137+ T Cells) in Patients with Refractory CD19+ B-Lineage Malignancies

B. RAC Discussion

No discussion occurred.

C. Public Comment

No public comments were offered.

IX. Review and Discussion of Human Gene Transfer Protocol #1310-1263, Titled: A Phase IIa Randomized, Multicenter, Double-Blind, Placebo-Controlled, Sequential Dose Escalation Study to Assess the Safety, Tolerability, Pharmacokinetics, and Pharmacodynamics of AG014 Administered Orally in Subjects with Moderate to Severe Active Ulcerative Colitis

Principal Investigator:	William Sandborn, M.D., University of California, San Diego
Additional Presenters:	Bernard Coulie, M.D., Ph.D., ActoGeniX; Pieter Rottiers, Ph.D., ActoGeniX; Lothar Steidler, Ph.D., ActoGeniX
Sponsor:	ActoGeniX N.V. (Belgium)
RAC Reviewers:	Drs. Sarzotti-Kelsoe, Strome, and Zoloth
Ad Hoc Reviewer:	Vijay Yajnik, M.D., Ph.D., Harvard Medical School

A. Protocol Summary

Inflammatory bowel disease (IBD) is a family of chronic disorders that involves inflammation of the digestive tract. Characterized by a tendency for chronic or relapsing immune activation and inflammation within the GI tract, IBD has two major idiopathic forms: Crohn's disease (CD) and ulcerative colitis (UC). UC is a chronic, relapsing inflammatory disease of the rectum and colon characterized by an increased stool frequency and bloody diarrhea; the most common symptoms are intestinal bleeding, diarrhea, and cramping abdominal pain. UC onset typically occurs between 15 and 25 years of age but can also begin in young children. The worldwide annual incidence of UC is 0.5 to 25 per 100,000 people, with the highest rates in developed countries and the lowest in developing countries.

The purpose of this Phase IIa study is to investigate the safety and tolerability of AG014 administered orally during a 42-day period in research participants with moderate to severe active UC. The pharmacokinetics (PK) and pharmacodynamics (PD) of AG014 will be analyzed on blood, stool, and

biopsy samples. These analyses will be used to explore how the drug is processed (PK) and whether the drug has a beneficial effect on the disease (PD).

AG014 is made up of genetically engineered bacteria called *Lactococcus lactis (L. lactis)*, which is commonly used to produce dairy products, including buttermilk and cheeses. To make AG014, the DNA of *L. lactis* has been engineered in the laboratory to secrete the active component of certolizumab (a monoclonal antibody directed against tumor necrosis factor alpha [TNF-α]). AG014 has been evaluated in many animal studies, in which it was shown to be safe and to reduce inflammation. This is the first time that AG014 will be used in humans.

During the study, research participants will receive AG014 or placebo twice daily for 42 days. Three different doses of AG014 will be studied and compared with placebo: 15 participants will receive the lowest dose level of AG014 or placebo (two capsules per day), 15 participants will receive the intermediate dose level of AG014 or placebo (four capsules per day), and 30 participants will receive the highest dose level of AG014 or placebo (eight capsules per day).

Throughout the study, safety will be evaluated by collection and recording of adverse events, including SAEs, clinically significant laboratory abnormalities, the presence of anti-certolizumab antibodies in serum, and presence of the bacteria in whole blood. Physical examination including vital signs (pulse, blood pressure, and temperature) will be performed, and a stool diary (stool frequency, consistency, and presence of gross blood) will be collected. Blood samples, stool samples, and colon biopsies (tissue samples) will be used to evaluate PK and PD parameters.

Information obtained from this exploratory research study may improve the treatment and understanding of UC. The final goal of the development program is to establish AG014 as a therapeutic option for inducing and maintaining remission in IBD patients.

B. Written Reviews by RAC Members

Seven RAC members voted for in-depth review and public discussion of this protocol. Key issues included the use of a novel transgene expressing a TNF-α inhibitor. In addition, the trend toward worsening of UC symptoms in a previous trial using the same vector expressing IL-10 deserves further discussion.

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

Dr. Sarzotti-Kelsoe noted that the investigators had presented a well-organized set of *in vitro* and animal studies but expressed concern that none were GLP studies, which would have ensured the uniformity, consistency, reliability, reproducibility, quality, and integrity of these preclinical tests. She stated that she found several inconsistencies in the descriptions of the animal studies, and she requested data to support the investigators' assertions that no bacteria or certolizumab were detected in systemic circulation and no gross pathological changes were demonstrated in the GI tissues, kidney, liver, spleen, mesenteric lymph nodes, or pancreas of the dosed animals. Dr. Sarzotti-Kelsoe requested further information about which cytokines, chemokines, or biomarkers will be investigated as secondary outcome measures, where these studies would be conducted, what type of controls would be included, and what these studies are expected to uncover. She also requested clarification regarding the criteria for study discontinuation, specifically whether symptoms suggesting clinically significant bacteremia attributable to sAGX0354 would be determined by three consecutive positive blood cultures or one positive blood culture by polymerase chain reaction (PCR). In addition, she asked whether the investigators had yet selected the clinical dose for this study based on the outcome of the preclinical studies in the pig PK model. She noted that it is difficult to produce antibodies with correct folding of heavy and light chains in bacteria, and asked for any evidence that AG014 would produce fragment antigen binding (Fab) of true immunoglobulin.

Dr. Strome asked the investigators to explain their rationale for proposing a Phase IIa trial for this first-in-human study and to provide justification for staggering entry within groups as a way to mitigate toxicity concerns about entering research participants at the same time. He noted the need for caution in the proposed study, due to the fact that no good animal model for human UC currently exists. Dr. Strome expressed concern about the results of a trial of 60 participants performed outside of the United States, using a similar construct administered differently, in which three episodes of worsening colitis occurred. Given that Asacol (mesalamine) has been shown to be more effective in treating UC when given both systemically and rectally and with the potential for continued systemic TNF in this protocol, Dr. Strome suggested that the investigators administer a small systemic amount of certolizumab in combination with the construct and that the investigators include an experimental arm administering certolizumab via a transrectal route alone. He acknowledged that, in human UC, the epithelial production of TNF could be reduced with the investigators' construct but asked what would be the fate of TNF derived from cells in the lamina propria, which would have the potential to contribute to a continued presence of circulating TNF. Dr. Strome raised concerns about the selection criteria, given that 90 percent of UC patients seem to do relatively well using the existing therapeutic strategies. He noted that surgery might be preferable for some UC patients, because it would be curative with regard to cancer prevention and UC treatment, although advanced unresponsive disease might benefit from this proposed new strategy. Dr. Strome suggested that teenagers not be allowed to participate in this trial, because certolizumab is not currently approved for use in either children or teenagers. He also suggested that UC patients who must continue using steroids should not participate in this trial, because the immunosuppressive nature of steroids could skew data by complicating interpretation of efficacy and/or masking side effects.

Dr. Zoloth noted the many issues experienced by teenagers affected by this disease and said she believed that population would be especially vulnerable. Therefore she suggested that this first-in-human study enroll a small number of research participants and limit the participant population to individuals in their mid- and late 20s. Because moderate disease can be controlled effectively with conventional therapy and could remit spontaneously, Dr. Zoloth asked the investigators to discuss their rationale for enrolling patients with moderate disease in this study, and she asked whether the results from another trial involving certolizumab, to be concluded in December 2013, would affect the design of the current trial. Because UC has psychological components, she asked whether psychological support or follow-up or a cross-group controlled component would be included in this study, and whether the differential factor of home environment would be evaluated. In addition, she requested that the investigators discuss any plan for lifelong follow-up of the cohort, given that UC is a lifelong condition unless the patient undergoes surgical removal of the colon. With regard to public health, Dr. Zoloth lauded the investigators for their attention to the reality that members of the public are likely to be concerned by the phrases "genetically altered bacteria" and "inserted into the intestinal tract." However, she said that asserting that there is no danger of the bacteria entering the public sphere is not sufficient as a plan and, noting that accidents will happen even if they are statistically unlikely, she asked the investigators to describe how they plan to take responsibility for this event if it should occur.

Ad hoc reviewer Dr. Yajnik expressed concern that the investigators presented no data on the characterization of the Fab fragment other than its ability to neutralize human TNF. Throughout the document, the Fab fragment is referred to as certolizumab (an FDA-approved drug with the trade name of Cimzia that is not approved for treating UC), but AG014 has secondary and tertiary DNA structures that appear to be distinct from certolizumab, an issue that needs to be clarified because it could influence clinical trial selection, as it may be perceived by potential participants as "taking Cimzia via a new mechanism." Studies in mice support the theory that neutralizing TNF in the mouse lumen is protective from disease, but whether secreted anti-human TNF by *L. lactis* can treat inflammation is still not known. Dr. Yajnik requested that safety data be presented from the two ActoGeniX studies on *L. lactis*. Regarding the clinical trial design, he recommended a follow-up period of four to eight weeks after Day 42 in order to gather information on *L. lactis* colonization, delayed adverse effects, and durability of response in responders. Dr. Yajnik also recommended that the investigators consider conducting stool microbiome analyses, because *L. lactis* or anti-TNF could alter the microbial community structure, and he noted that there was no plan for continued medication for research participants who respond to the experimental medication. He asked the investigators to explain why rectal formulations are not being considered in this study.

C. RAC Discussion

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

- Dr. Sadelain asked whether the antibody is human and, when given systemically, whether immune responses develop.

- Dr. Sarzotti-Kelsoe suggested that the investigators describe AG014 as “certolizumab-like” or that they say that it is biosimilar to certolizumab, to address the concern about participants mistakenly believing that AG014 actually is certolizumab.

- Dr. Strome suggested several alterations to the protocol: (1) that the investigators use rectal administration first, (2) that a patient’s steroid dose be stabilized for one month before entry, and (3) that the Mayo Score for entry into this protocol be raised above six. He also asked to see the Phase I data before casting a vote on this study.

D. Investigator Response

1. Written Responses to RAC Reviews

Before initiating this Phase IIa clinical trial, the Sponsor reported that they have planned a Phase I study in healthy volunteers to be performed in Europe. This first-in-human, two-part study will evaluate safety and pharmacokinetics. The purpose of the first part of the study is primarily to determine safety and PK of AG014 administered once on a single day (four capsules of AG014) and includes collecting feces and blood for PK and safety analysis. The purpose of the second part is to determine the safety and PK of AG014 administered for four days (four capsules of AG014 twice daily from day one to day three, and four capsules of AG014 on day four) and includes collection of feces, blood, and colon biopsies. Phase I study participants will receive a total of 32 capsules of AG014. Safety evaluations in this healthy volunteers study will include documentation of adverse events, clinically significant laboratory abnormalities, clinically significant physical examination findings, changes in vital signs, levels of intestinal pro-inflammatory cytokines, and presence of sAGX0354 in whole blood. Together with the non-GLP repeat-dose toxicity study in colitic mice, this study will support the safe administration of AG014 to IBD patients and will contribute to the selection and justification of the clinical dose for the Phase IIa study.

At necropsy, all pigs sacrificed were weighed and blood was collected. The blood samples of all animals treated with AG014 capsules were plated (using live AG014 bacteria) and examined by quantitative PCR (qPCR) to detect *L. lactis*. Both analyses were negative, indicating no dissemination of live or dead bacteria into the circulation, and no certolizumab levels above the limit of detection were recovered from the serum of AG014-treated animals. Upon collection of the GI tissues, no gross pathological changes were noted in any of the AG014-treated pigs. No significant differences could be demonstrated in the weight of the kidney, spleen, stomach, small intestine, cecum, and colon of untreated piglets as compared with AG014-treated animals.

No ideal biomarkers have been identified to date that predict safety and anti–TNF-α responsiveness in IBD, but some molecular biomarker candidates have been proposed. Identification and monitoring of such biomarkers in blood, colon mucosa, and feces may provide knowledge in terms of safety and pharmacological effects of TNF-α blockage, as may clinical and endoscopic findings. In order to identify any immune-related parameters that may predict safety and/or anti–TNF-α responsiveness, even before clinical and/or endoscopic findings could be observed, blood, colon biopsy, and fecal samples will be collected and used to analyze the levels of a broad range of cytokines, chemokines, adhesion molecules, and markers of activation—all relevant in the context of TNF-mediated inflammation and/or clinical activity in UC.

In case of clinically significant bacteremia that can be attributed to sAGX0354, as determined by three consecutive positive blood cultures, study treatment for that participant will be discontinued. However, in

case of clinical sepsis confirmed by one positive blood culture attributed to sAGX0354, the entire study will be stopped. Any clinically significant bacteremia, regardless of the number of blood cultures used to identify the event, will be documented as an AE or SAE if it fits the criteria of such an event. However, a single positive culture (or multiple positive cultures) would not be a reason to halt the trial or to document an AE or SAE in the event of non–clinically significant bacteremia. The number of cultures outlined in the protocol was selected to ensure that if a participant develops clinical symptoms of bacteremia, there is sufficient evidence to document the cause and potential relationship to the bacteria used in the test product (sAGX0354). Given that the UC population will have damaged mucosa, asymptomatic transient bacteremia with a species not known to have invasive properties, such as *L. lactis*, would not be of concern; however, since this is a clinical trial, it is necessary to conduct multiple cultures to ensure that the bacteria causing the infection are identified properly.

The investigators have excluded patients with fulminant UC; eligible patients will have moderate to severe disease. Because the investigators will be randomizing the 60 participants, it is highly likely that the extent of disease activity will be similar in the dosed and placebo groups.

The dose has not been selected yet. Dose selection will be defined based on a combination of the toxicology study using the surrogate *L. lactis* strain, the European Phase I AG014 study in healthy volunteers, the colitis mouse PD study using the surrogate *L. lactis* strain, and the pig intestinal loop PD study using AG014.

The staggered approach to entry and treatment was developed in response to FDA review and at their recommendation. At the pre-IND meeting, the FDA explained that, to help ensure participant safety, the Sponsor should not dose more than one participant per day. In response to FDA query, the Sponsor stated that the exposure time was roughly equal to the intestinal transit time. The staggered treatment design along with evaluation of AG014 in the preceding European Phase I healthy volunteer study should ensure adequate safety monitoring for the study participants.

Regarding the clinical trial using certolizumab (Cimza) for IBD that is scheduled to conclude in December 2013, the Sponsor explained that the full data set of this study will not be public, so it will not be possible to determine the reasons for limited efficacy or nonsuperiority. In any case, except for mode of action, AG014 has a fundamentally different treatment profile compared with Cimzia in characteristics such as formulation, drug substance, oral administration, dosing frequency, local delivery, and PK profile. As a consequence, the outcome of the Cimzia study will have little predictive value for the outcome of the Phase IIa study with AG014.

Although the investigators agree that there is no good animal model for human UC and that animal pharmacology data need to be interpreted with caution, TNF-α has been clinically validated as a relevant target for IBD treatment. Extensive pig PK studies with AG014 formulated in the clinical dosage form demonstrated that, after capsule dissolution in the distal small intestine, the full extent of the colon contains equal amounts of living bacteria from ten to 30 hours after oral administration. Levels of certolizumab can be detected in cecum and colon and correlate with the number of bacteria present.

TNF-α is expressed primarily in the lamina propria of the gut of CD and UC patients. Binding and neutralizing the biological activity of TNF-α at this site of inflammation will lead to an effective management of intestinal inflammation and as such has the potential to control the levels of circulating TNF-α. The current capsule formulation is designed to target AG014 to the site of inflammation at the lower part of the small intestine as well as throughout the entire colon, making it suitable for delivery at the inflamed mucosa of UC patients.

The goal of the study is to evaluate the effect of the *L. lactis* construct secreting certolizumab as a stand-alone therapy primarily in terms of assessing safety and tolerability of AG014. Additional systemic treatment with certolizumab (Cimzia) would be a confounding variable in the proper assessment of safety and tolerability of AG014 in this initial study in UC patients. AG014 is being developed to be a safe and effective alternative to systemically administered anti-TNF-α therapy rather than as adjunct therapy. The

lack of systemic exposure in the case of AG014 treatment is one of the key drivers for the development of this potential advancement in UC treatment.

The investigators acknowledged that Dr. Strome's suggestion of transrectal administration is an excellent one as an additional route of administration for left-sided UC. This approach will be considered in further development of AG014, including as part of the European Phase I study in healthy volunteers.

Patients do not find surgery for UC either acceptable or attractive: Ileocolectomy and pouch procedures to treat active disease carry high morbidity and high complication rates. Consequently, surgery is generally used in patients who have failed available treatment options. A Mayo Clinical Score between six and 12 will almost always interfere with patient-reported quality of life and is accepted by clinical investigators and regulators to define UC patients who are eligible for studies evaluating experimental therapies.

Steroids are a common therapy for patients with moderately to severely active UC. The resulting improvement in symptoms is highly relevant to patients and fills an unmet clinical need. In the trial design, the investigators have included stable-dose steroids during induction that they believe will not confound their analysis.

AG014 may offer an effective oral therapy that will reduce the risk of toxicity associated with systemically administered TNF-α inhibitors. The inclusion of patients 18 years old and older defines an adult study population able to make independent decisions to participate in a clinical study. The inclusion criteria are consistent with initial studies evaluating new therapies in patients with UC, offer a therapeutic option to the age group affected by this disease, and allow the study results to be generalizable to the adult patient population.

In response to a reviewer's request to provide a rationale for enrolling the proposed patient population, the investigators explained that recent (unpublished) medical evidence suggest that IBD patients have lower rates of hospitalization and disease-related complications if biologic therapy like AG014 is initiated early in the disease course, irrespective of symptom activity. AG014 is being developed to provide a safe medical treatment of UC that inhibits the immune attack on the bowel mucosa, with the hope of potentially limiting the need for systemic immunosuppressants and biologic therapies. The investigators hope that this approach will prevent development of complications more effectively than conventional medical therapy. All participants will be informed of the availability of conventional therapy as part of the informed consent process.

Given the study's early phase, small sample size, and relatively short treatment/follow-up, prolonged follow-up, psychological support, or a cross-group controlled component are not currently planned. Differential factors generally documented in UC studies include gender, age, educational level, work status, and tobacco and alcohol use; the investigators do not plan to evaluate the impact of home environment in this early-phase study. A four-week follow-up visit after the last dose of study drug has been included in the protocol design, with the intent to gather data about *L lactis* colonization, adverse events, and durability of response. In addition, formal study follow-up will be completed with telephone contact 40 weeks after dosing. Frequently, study participants are followed by their gastroenterologist after study; however, in similar studies, lifelong follow-up of the study cohort has not been approved or requested by reviewing regulatory authorities.

TNF-α is largely expressed in the lamina propria of the gut of patients with CD and UC, but circulating TNF-α is also detected in some of those patients. As there is no systemic exposure to AG014 and hence to the secreted anti–TNF-α antibodies, it is not probable that AG014 would have a direct effect on other systemic inflammatory conditions mediated by circulating TNF-α. However, in case AG014 successfully dampens intestinal inflammation, it is possible that AG014 could indirectly affect plasma TNF-α concentrations and its (systemic) inflammatory cascade.

The likelihood of any public health hazard occurring should be carefully considered and anticipated, and the Sponsor has already taken responsibility for avoiding any survival and accumulation of its recombinant strain by providing an inbuilt environmental containment strategy. This strategy

encompasses careful selection of the host organism, in that the parent organism, from which sAGX0354 was derived, does not accumulate or survive in the environment. In addition, a simple and robust inherited “suicidal” system is included that is based on removal of the thyA gene, which encodes thymidylate synthase, a unique enzyme in the biosynthesis of thymidine. The absence of thymidine leads to induced death of sAGX0354.

Neutralization of TNF-α is the key biological activity required for this clinical experiment, a result that was achieved through recombinant expression of genes that encode certolizumab. The data show that the neutralizing capacities of *L. lactis* derived from certolizumab and the commercial product are highly comparable, implying that binding to TNF-α is highly similar. However, use of the recombinant *L. lactis* should not be confused by patients or their physicians with the use of the commercial product Cimzia. To avoid any such confusion, the Sponsor agreed to add an appropriate explanatory sentence to the Investigator’s Brochure.

The *in vivo* data obtained with the surrogate *L. lactis* strain secreting the anti-murine TNF antibody fragment (MT1-MT1) support the assertion that *L. lactis*-delivered anti-TNF antibodies are highly effective in neutralizing TNF-α in the intestine and thereby reducing signs of enterocolitis. Those studies demonstrated that *L. lactis* gets trapped and resides in the inflamed lamina propria and as such delivers the anti-TNF antibodies actively in the underlying lamina propria, in close proximity to TNF-α–secreting and –responsive cells. Since the intrinsic features of the *L. lactis* bacteria are similar between the surrogate strain and AG014, the investigators anticipate that a similar process of mucosal delivery will take place for AG014. The data obtained with AG014 in the pig intestinal loop model further support the hypothesis that anti–hTNF-α secreted in the GI tissues is bioactive and effective in neutralizing the hTNF-α induced upregulation of intestinal pro-inflammatory markers.

Microbiome composition is a useful readout in assessing IBD. Therefore, stool samples will be collected and stored in such a way that microbial composition analysis can be performed at a later stage. Request for this analysis will be added to the informed consent document.

Until the safety and efficacy of AG014 in the treatment of UC is further studied and established, the investigators do not plan to continue study participants on AG014 therapy following study participation.

The current capsule formulation is designed to target AG014 to the site of inflammation at the lower part of the small intestine as well as over the entire colon, making it suitable for delivery at the inflamed mucosa of UC patients.

The Sponsor agreed to amend and update the informed consent document to reflect suggestions by the RAC reviewers.

2. Responses to RAC Discussion Questions

Dr. Sandborn explained that the antibody is human and that immune responses could develop. The systemically administered pegylated product does have some immunogenicity, and the antibodies can be neutralizing and could affect the PK. This could occur with the systemic product, and the investigators plan to look for it.

Dr. Rottiers confirmed that the investigators have several examples demonstrating that what *L. lactis* is delivering in the GI tract does not induce immunogenicity, as they have proved in healthy animals and in animals with full-blown colitis, in which the antibody is delivered into a mix of pro-inflammatory cytokines. Using this and other examples, the investigators stated that the GI tract is prone to induce tolerance rather than immunity, and they are confident that use of a human antibody fragment or an immunogenic antigen will not result in immunogenicity. To be certain, they plan to monitor the participants’ blood for this possibility.

E. Public Comment

No public comments were offered.

F. Synopsis of RAC Discussion and RAC Observations and Recommendations

Clinical and Trial Design Issues

- This Phase IIa study will be conducted following a European Phase I study to evaluate the safety of AG014 in healthy volunteers. The safety of this vector in the UC population is unclear, because, in a previous study of *L. lactis* expressing IL10 in UC research participants, the bacteria apparently did not survive the transit to the colon. In addition, the Phase I study will not be able to provide information on the biological activity of AG014 in research participants with UC. Prior to moving to a much larger Phase IIa study using oral administration, the investigators may wish to perform a small, initial proof-of-concept study in a limited number of UC research participants, using rectal administration to establish that these bacteria secrete the active component of certolizumab, are tolerated, and have the expected biological activity on the mucosa. If such a design is not implemented, it would be useful to include in the protocol a discussion of the rationale for moving from a Phase I in healthy volunteers to a Phase II in UC patients using oral rather than rectal administration.

- According to the inclusion criteria in the protocol, research participants with a Mayo Clinic Score greater than or equal to 5 would be eligible for this study. This could include patients who have 1 to 2 stools a day, with blood streaking, but moderate disease on endoscopy. The investigators should consider whether this first-in-human trial should be limited to research participants with more symptomatic disease, such as those with a Mayo Clinic Score of at least 6. The rationale would be that the disease is still having a significant impact on these research participants' quality of life even though they are receiving standard of care.

- Although this disease affects teenagers and young adults, in this initial study it would be helpful to consider whether a more mature population could be enrolled, limiting research participants to those at least 21 years old.

- The protocol specifies that "Concurrent treatment with prednisone, or equivalent glucocorticoids (≤ 20 mg/day) is acceptable as follows: a) minimum dosing period of 4 weeks prior to screening, b) stable dose for 2 weeks prior to screening, and c) expected to remain on a constant dose during this study." It may be more prudent to require a stable dose of steroids for at least 4 weeks, as this may be more predictive of ability to maintain a stable dose throughout the trial.

- The criteria for early withdrawal should include a stopping rule for worsening colitis. While it is likely that fluctuations in colitis symptoms will occur during the course of the trial, there should be criteria that can be developed to identify those research participants whose worsening of colitis during the trial, using clinical and/or endoscopy criteria, constitutes an adverse event that warrants withdrawal from the trial.

Ethical/Legal/Social Issues

- The informed consent document states, "To make AG014, the DNA of *L. lactis* has been engineered in the laboratory to secrete certolizumab." The transgene product expressed by these bacteria will not be pegylated, as certolizumab is, and will likely differ in secondary and tertiary structure. Therefore, it may be more accurate to state that the bacteria secrete "the active component" of certolizumab. The investigators should consider modifying the informed consent document and protocol to clarify this distinction.

- The informed consent document should clearly articulate that bleach should be used to clean up any spillage of body fluids that may contain the vector.

G. Committee Motion 5

Dr. Kohn summarized the RAC recommendations to be included in the letter to the investigators, expressing the comments and concerns of the RAC. Dr. Kohn asked for a vote on these summarized recommendations, which the RAC approved by a vote of 11 in favor, 0 opposed, 2 abstentions, and 0 recusals. Two RAC members abstained from voting because they did not agree with one or more of the recommendations regarding the initial safety study using rectal administration, the need to raise the Mayo score for inclusion, and the need to recruit participants who are older than 21 years of age.

X. Review and Discussion of Human Gene Transfer Protocol #1310-1266, Titled: A Phase I, Open-Label Clinical Trial Evaluating the Safety, Tolerability, and Immunogenicity of Intradermally Administered ID-LV305 in Patients with Locally Advanced or Metastatic Cancer Expressing NY-ESO-1

Principal Investigator:	Neeta Somaiah, M.D., University of Texas, MD Anderson Cancer Center
Additional Presenters:	Richard Kenney, M.D., FACP, Immune Design Corp.; Jan ter Meulen, M.D., Dr. habil., DTM&H, Immune Design Corp.
Sponsor:	Immune Design Corp.
RAC Reviewers:	Drs. Fost, Hammarskjöld, and Wooley

A. Protocol Summary

Development of effective therapeutic vaccines for cancer requires generating appropriate immune responses to achieve effective tumor cell killing. The Sponsor is proposing a Phase I clinical study to evaluate whether a cancer therapeutic product candidate (ID-LV305) that has been modified from a lentivirus to stimulate the immune system is safe, can increase immune activity against the tumor, and has the potential to cause a clinical response. This immunotherapeutic approach is based on the observation that human tumor cells make a variety of antigens that are not found in normal tissues. A protein called NY-ESO-1, which is detected only in certain testicular cells in healthy individuals, is found in many types of cancer and can be used as a specific target for immune system activation.

The ID-LV305 therapeutic cancer vaccine candidate was engineered to induce broad and strong immune responses in patients with NY-ESO-1–positive cancers. The vaccine is directed toward specific immune cells in the skin called dendritic cells (DCs) that play a key role in instructing T cells to kill the tumor cells and lead to resolution of the cancer. Lentivirus-based vaccine vectors offer an attractive approach to inducing strong T-cell responses because they can enter DCs, activate them, and then cause expression and presentation of a tumor-associated antigen, such as NY-ESO-1. Potential targets include cancers of the lung, breast, ovary, and kidney, as well as melanoma and certain sarcomas. In addition to being able to bind only to certain specialized host immune cells, ID-LV305 has been designed with several redundant molecular safety features to inhibit replication and integration. Together, these features make ID-LV305 conceptually safer than other third-generation lentivirus vectors currently being used for gene transfer in humans.

This clinical study proposes to evaluate the safety and immunogenicity of ID-LV305. Research participants with a variety of advanced carcinomas will be included if their tumors express NY-ESO-1. Safety will be monitored with review of findings at each stage before progression to higher doses. The study will be conducted in centers experienced in this type of clinical effort, and the safety features incorporated in ID-LV305 should further minimize the risks associated with the use of a lentivector. When available, post-treatment tumor biopsies will help clarify the mechanisms of treatment effect, and potential benefit will be explored by evaluating tumor responses and tumor progression with long-term follow-up. To assess long-term safety, peripheral blood samples will be evaluated for persistence of the vector genome for several years. The results from this Phase I study will make further development of ID-LV305 possible, either alone or in combination with other agents that can synergistically stimulate an effective immune response to treat multiple types of cancer.

B. Written Reviews by RAC Members

Six RAC members voted for in-depth review and public discussion of the protocol. Key issues included the multiple novel elements of this vector system, including the first use of an integration-deficient lentiviral vector and modified Sindbis virus envelope.

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

Dr. Fost noted that one of the risks of the proposed product is that it could cause HIV screening tests to return false positives, although a confirmatory Western blot test would demonstrate that HIV-related proteins are not present. Research participants will be informed of this risk. He asked about the implications for humans of the observation that the product did not produce immunogenicity in macaques. Dr. Fost requested additional information about (a) the number and type of SAEs in participants in a prior trial who received NY-ESO protein with or without adjuvants, (b) the constitution of the data monitoring committee (DMC) and its independence from the Sponsor, and (c) whether the investigators would be allowed to publish results after a defined interval. He suggested several wording changes in the informed consent document to minimize the possibility of therapeutic misconception and to allow participants to select from three choices for which their samples could be used for additional research.

Dr. Hammarskjöld requested that the investigators clarify (a) the composition and function of the DMC, (b) how peripheral blood samples will be tested for potential integration into chromosomal DNA if persistence is detected, and (c) that participants cannot truly withdraw from this kind of trial and that it may not be in their best interest not to participate in the follow-up period that will monitor potential adverse events. Even if the vector has been designed not to integrate, she advised testing for integration if persistence is detected. Dr. Hammarskjöld suggested wording changes in the informed consent document to clarify several concepts, increase accuracy of descriptions, reduce redundancy, and minimize the possibility of therapeutic misconception.

Dr. Wooley asked the investigators to delineate and clarify specifics regarding the modified Sindbis virus glycoprotein, the proposed dose, the life expectancy of the DCs in the body after they are transduced with the lentivector, and whether the vector could transduce human stem cells. She asked whether integration could be reduced further by treating the research participants with an HIV integrase inhibitor. With regard to the informed consent document, Dr. Wooley suggested that the investigators clarify that ID-LV305 is derived from HIV and correct and clarify several other statements, such as those about integration efficiency and binding to and infection of the DCs. In addition, because the NY-ESO-1 protein is expressed on testicular cells, she asked the investigators to discuss whether it is possible that young boys, especially those who are prepubescent, could be particularly vulnerable to a harmful effect if unexpected horizontal transmission occurs.

C. RAC Discussion

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

- Noting that the trials he has conducted with lentiviral vectors all indicate that the vector is derived from HIV, Dr. Kohn opined that potential participants need to be informed that HIV is the origin of the vector. At the September 2013 RAC meeting, investigators from one trial reported that a lentiviral vector had triggered a false-positive HIV test; this finding is another reason to inform potential participants when a vector is derived from HIV.

- Dr. Zoloth added that the informed consent document should mention that HIV is the virus that causes AIDS, along with an explanation about why using this lentiviral vector is safe. Dr. Wooley explained further that an ELISA test would come back as positive for HIV and a Western blot test would come back as indeterminate for HIV. Research participants need to have explained to them that they will get injected with viral protein and they likely will have an immune response to it but that tests on that immune response will report back as falsely positive for wild-type HIV. Dr.

Wooley suggested language such as, “May cause some HIV screening tests to become positive, but later tests would confirm that the full HIV was not there.”

D. Investigator Response

1. Written Responses to RAC Reviews

Successful transduction of any cell type with the Sponsor’s lentivirus vector depends on the presence of specific receptors for attachment and entry, as well as the permissiveness of the cell for reverse transcription and translation of the introduced genetic material (cancer antigen). Without successful transduction, no immune response to the cancer antigen will be induced in any organism. Because animals have evolved various restriction mechanisms to lentivirus infections, the permissiveness to transduction varies greatly between species and can only be determined experimentally. Therefore, the absence of induction of an immune response in one species has no a priori predictive value for the immune response in another species. Because the investigators have shown that the Sponsor’s vector successfully transduces human DCs, the induction of an immune response in humans is expected. The absence of an immune response in macaques only demonstrates the inadequacy of this animal species as a preclinical model for evaluating the immunogenicity of the lentivirus vector.

The functions of the DMC are described in the protocol, and its constitution will be fully characterized in its charter to include the Sponsor, the investigators, and an independent medical expert. The investigators agreed to change the DMC’s name to Safety Monitoring Committee in the amended protocol to avoid causing confusion. In addition, the investigators noted that the 2013 draft FDA guidance, “Considerations for the Design of Early-Phase Clinical Trials of Cellular and Gene Therapy Products in Phase I Studies,” states that the need for an independent DMC is not expected at this stage of product development.

The Sponsor explained that they do not intend to suppress publication of findings, and the investigators are free to submit their data once the primary results are published. However, the Sponsor includes an intent to “review and approve” manuscripts before submission to allow for consideration of intellectual property concerns. A formal publication plan for the primary results will be discussed with the principal investigators and established before the start of the trial.

The investigators intend to develop a qPCR approach to detect the presence and (if positive) number of copies of the LV305 genome, consistent with FDA guidance regarding the detection of persistence of vector sequences in samples from research participants who have received a gene transfer agent. If sequential samples are positive, further testing will be required to assess the possibility of integration.

Even though benefits are uncommon in Phase I trials, the Sponsor explained that they would not be developing this product or exposing cancer patients to the proposed vector if they did not expect the potential benefits to outweigh the risks. Rather than predicting the certainty of a lack of benefit as suggested by the RAC, the Sponsor preferred to delete the relevant sentences from the informed consent document.

Regarding the ability of research participants to withdraw from this study, the Sponsor explained that the wording used is in all of their informed consent documents and is derived from the Belmont Report, which established that research participants always have the right to withdraw completely from studies at any time during their involvement. The fact that this is a study of a particular type of product does not abrogate that right. However, the Sponsor assured the RAC that the principal investigators will explain the importance of follow-up in the context of gene transfer in order to monitor potential adverse events and to assess the possibility of vector persistence.

Vector potency is measured by two means: the quantitation of vector genomes by qRT-PCR (expressed as vector genomes, or particles, per mL) and transduction of live cell cultures, which is followed by qPCR of vector-encoded sequences (expressed as infectious units per mL). The Sponsor will continue to develop and refine the infectivity assay and, concurrent with the manufacture of additional lots to

establish process capability and consistency, intends to report a particle-to-infectivity ratio for each batch of ID-LV305 prior to the initiation of Phase II trials.

The intended dose levels of 5×10^8, 5×10^9, or 5×10^{10} vector genomes will be administered to each research participant at each visit. Therefore, with the successful completion of a three-injection regimen as specified in the protocol, each participant will receive a total of either 1.5×10^9, 1.5×10^{10}, or 1.5×10^{11} vector genomes, depending on the cohort in which he or she has been enrolled.

Dendritic cells are nondividing cells whose lifespan is highly dependent on anatomical location and cellular subtype. Following HIV infection *in vitro*, human myeloid DCs are capable of surviving for up to 45 days. Langerhans cells that have become infected with HIV have a half-life of about 15 days. However, since ID-LV305 does not bind to Langerhans *in vivo*, the investigators do not anticipate transduction of Langerhans cells *in vivo*. The biodistribution studies of LV305 in mice show that the detection of vector sequences as measured by qPCR between days 21 and 42 decreases to the level of detection at the injection site. Assuming the signal stems from transduced DCs, this result indicates that transduced DCs have a lifespan of up to six weeks in the mouse. This estimate is in agreement with several published reports. In addition, no experimental evidence suggests that the transduced DCs could become quiescent and reactivated later, beyond the cells' natural lifespan.

The ability of ID-LV305 to transduce human stem cells has not been evaluated. Bone marrow-derived CD34+ cells do not express DC-SIGN and are thus not expected to be transducible.

A prototype version of the construct was obtained from the laboratory of David Baltimore at the California Institute of Technology. This prototype was then further modified by the Sponsor in order to generate the platform construct that is the basis for ID-LV305. Recombinant and synthetic techniques were used to generate the constructs. For the former, techniques such as cloning and PCR mutagenesis were utilized; for the latter, synthetic sequences were synthesized using for-fee services. All of the plasmids were completely sequenced with 4x coverage to confirm the original intended sequences.

The Sponsor agreed with the RAC reviewers that ID-LV305 has a defective viral genome and described the resulting particle as a "replication-incompetent virus." They explained that ID-LV305 has been engineered so that it is incapable of setting up a productive infection within the host. That concept is somewhat challenging to describe in an informed consent document in lay terms, although the Sponsor concurred that participants need to understand that the experimental product is derived from HIV.

The chance of horizontal transmission is severely limited, as ID-LV305 is a nonreplicating vector. Mucosal surfaces are not likely to be contaminated with virus. Preclinical studies are under way to demonstrate the lack of shedding, and the GLP biodistribution study indicates no significant presence of the vector beyond the site of injection. The few positive signals seen on Day 1 post-injection in scattered tissues are likely due to a small amount of circulating cells, and no subsequent signal was detected. Successful horizontal transmission would require some form of injection for the vector to infect DCs and set up an immune response, which is not likely. Testicular spermatogonia are in a site protected from the immune system, so cytotoxic T-lymphocytes that might be generated by vector transduction of DCs would not be expected to have a harmful effect on testicular tissues, even in the primary vaccinee.

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

2. Responses to RAC Discussion Questions

Dr. Kenney stated that the proposed lentivirus-based vector is nonreplicating. It infects and then dies. The consequence of a potential horizontal transmission is therefore much less risky. Even if an intravenous horizontal transmission occurs in the testicular cells, the chances of a dose that would be high enough to generate an immune response are very low.

Dr. Kenney acknowledged that Dr. Wooley's comments about possible shedding stimulated the investigators to conduct a preliminary experiment to look for shedding. When swabbing the site after 30 minutes, they detected some virus, a finding they will pursue and that likely will result in recommending standard precautions.

E. Public Comment

No public comments were offered.

F. Synopsis of RAC Discussion and RAC Observations and Recommendations

Preclinical Issue

- While this vector is designed so as not to integrate into the genome, data indicate there is still some low level of integration. The investigators should consider a preclinical study using a PCR assay to determine whether the use of an integrase inhibitor would further reduce vector integrations. In addition, it would be helpful to establish that this vector is unable to transduce hematopoietic stem cells through receptors other than DC-SIGN. Although it is not critical that these studies be performed prior to moving forward with this clinical study, they may provide data useful during product development.

Clinical and Trial Design Issues

- It is important that both positive and negative results of clinical trials be published. The publication policy as written in the protocol states that "All publications…must be reviewed and approved in writing by Immune Design, in advance of submission," which appears to allow the Sponsor to prevent certain publications. The Sponsor should consider expanding upon this policy, perhaps using as a template other established publication policies, to articulate more clearly the intent, stated during the RAC meeting, to allow publication once primary results are available.

- To inform dose determination and increase safety in the trial, it is important to determine the ratio of defective particles to vectors capable of transducing the dendritic cells in the released product.

Ethical/Legal/Social Issues

- This is a Phase I trial, and although the preclinical results regarding the possibility of clinical benefit are encouraging, statistics across all studies suggest that direct benefit to individuals in Phase I trials is uncommon. Therefore, it is important that research participants understand that direct benefit is not expected in an initial safety trial. The Sponsor and investigators should review the language from "NIH Guidance on Informed Consent for Gene Transfer Research" (http://oba.od.nih.gov/oba/rac/ic/appendix_m_iii_b_1_d.html) for examples of how to describe potential benefit for different phases and designs of trials.

- The informed consent document should clarify that withdrawal from the study refers to withdrawal from study follow-up and procedures but that the product itself cannot be withdrawn because it has the ability to persist.

- The informed consent document contains the following statement: "ID-LV305 is a virus-like particle (a vector) that has been 'engineered' from a special type of virus (called a lentivirus) in a way that keeps the vector from reproducing in your body." This is a complex statement, and it fails to mention that the vector is derived from HIV-1. While it is understandable that the Sponsor does not want to alarm potential participants and is concerned that participants could mistakenly think there is a risk of HIV infection from this vector, participants should nonetheless have this information. Given that the informed consent document already addresses the risk of a positive HIV test, the RAC recommends a simple statement regarding the source of ID-LV305 that clarifies that this product is a viral vector containing sequences derived from the HIV-1 virus.

- The informed consent document states that, “ID-LV305 includes genes that may cause some HIV screening tests to become falsely positive.” This finding is a result of detection of certain HIV sequences present in the viral vector and, potentially, immune responses to the HIV viral proteins in the viral vector particles. Technically, the test is not “falsely positive,” as it is detecting the sequences and immunoglobulins it was designed to detect. It might be more accurate to state that if a research participant receives ID-LV305, a test to screen for HIV virus may be positive but that this result reflects the presence of the viral vector and not HIV infection.

G. Committee Motion 6

Dr. Kohn summarized the RAC recommendations to be included in the letter to the investigators, expressing the comments and concerns of the RAC. Dr. Kohn asked for a vote on these summarized recommendations, which the RAC approved by a vote of 12 in favor, 0 opposed, 0 abstentions, and 0 recusals.

XI. Closing Remarks and Adjournment

Dr. Kohn thanked the RAC members and the OBA staff and adjourned the December 2013 RAC meeting at 12:45 p.m. on December 5, 2013.

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

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

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

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

Date: _____

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

Attachment I:
Recombinant DNA Advisory Committee Roster

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Attachment II:
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(This list includes only individuals who are not identified elsewhere in this document.)

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Attachment III: Abbreviations and Acronyms

AAV	adeno-associated virus
CD	Crohn's disease
CSF	cerebrospinal fluid
DCs	dendritic cells
DLT	dose-limiting toxicity
DMC	data monitoring committee
Fab	fragment antigen binding
FDA	Food and Drug Administration, HHS
GFP	green fluorescent protein
GI	gastrointestinal
GLP	good laboratory practices
GTSAB	Gene Transfer Safety Assessment Board
HHS	U.S. Department of Health and Human Services
IBD	inflammatory bowel disease
ICV	intracerebroventricular
IL	intralabyrinthine
IL-12	interleukin-12
IV	intravenously
L. lactis	*Lactococcus lactis*
NCI	National Cancer Institute
NIDCD	National Institute on Deafness and Communication Disorders
NIH	National Institutes of Health
NIH Guidelines	*NIH Guidelines for Research Involving Recombinant DNA Molecules*
OBA	Office of Biotechnology Activities, NIH
OD	Office of the Director, NIH
PCR	polymerase chain reaction
PD	pharmacodynamics
PK	pharmacokinetics
RAC	Recombinant DNA Advisory Committee
qPCR	quantitative polymerase chain reaction
SAEs	serious adverse events
SRC	Safety Review Committee
TCR	T-cell receptor
TNF-α	tumor necrosis factor alpha
UC	ulcerative colitis
vp	viral particles