RECOMBINANT DNA ADVISORY COMMITTEE

Minutes of Meeting

September 19-20, 2002

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

CONTENTS

Note: The latest Human Gene Transfer Protocol List can be found at the Office of Biotechnology Activities' Web site at <www4.od.nih.gov/oba/rac/protocol.pdf>.

DEPARTMENT OF HEALTH AND HUMAN SERVICES
NATIONAL INSTITUTES OF HEALTH
RECOMBINANT DNA ADVISORY COMMITTEE
MINUTES OF MEETING[1]

September 19-20, 2002

The Recombinant DNA Advisory Committee (RAC) was convened for its 87th meeting at 1:00 p.m. on September 19, 2002, at the National Institutes of Health (NIH), Building 31, Sixth Floor, Conference Room 10, 9000 Rockville Pike, Bethesda, MD 20892. Dr. Theodore Friedmann (Chair) presided. In accordance with Public Law 92-463, the meeting was open to the public from 1:00 p.m. until 5:00 p.m. on September 19 and from 8:30 a.m. until 3:15 p.m. on September 20. The following individuals were present for all or part of the meeting.

Committee Members

W. Emmett Barkley, Howard Hughes Medical Institute
Baruch A. Brody, Baylor College of Medicine
James F. Childress, University of Virginia
Neal A. DeLuca, University of Pittsburgh
David L. DeMets, University of Wisconsin Medical School
Theodore Friedmann, University of California, San Diego
Thomas D. Gelehrter, University of Michigan Medical School
Linda R. Gooding, Emory University
Larry G. Johnson, University of North Carolina, Chapel Hill
Terry Kwan, TK Associates
Maxine L. Linial, Fred Hutchinson Cancer Research Center
Bernard Lo, University of California, San Francisco
Madison Powers, Georgetown University
David Sidransky, Johns Hopkins University School of Medicine
Robert D. Simari, Mayo Clinic and Foundation
Diane W. Wara, University of California, San Francisco

Office of Biotechnology Activities (OBA) Director
Amy P. Patterson, NIH

Executive Secretary
Stephen M. Rose, NIH

***Ad Hoc* Reviewers**
Donald M. Coen, Harvard Medical School (via teleconference)
Nikunj V. Somia, University of Minnesota, Twin Cities
Steven E. Wilson, University of Washington (via teleconference)
Jon A. Wolff, University of Wisconsin Medical School, Mirus Corporation (via teleconference)

Speakers
Kenneth G. Cornetta, Indiana University Cancer Research Institute
Nancy M.P. King, University of North Carolina, Chapel Hill (via teleconference)
A. Dusty Miller, Fred Hutchinson Cancer Research Center
Carolyn A. Wilson, U.S. Food and Drug Administration (FDA)

[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.

Nonvoting/Agency Representatives
Kristina C. Borror, Department of Health and Human Services
Stephanie L. Simek, FDA

NIH Staff Members
Steve Cheney, Office of the Director (OD)
Sussan Eftekhari, OD
Kelly T. Fennington, OD
Suzanne Goodwin, OD
Laurie Harris, OD
Robert Jambou, OD
Cheryl McDonald, OD
Catherine McKeon, National Institute of Diabetes and Digestive and Kidney Diseases
Maureen Montgomery, OD
Marina O'Reilly, OD
Alexander Rakowsky, OD
Gene Rosenthal, OD
Thomas Shih, OD
Allan Shipp, OD
Sonia I. Skarlatos, National Heart, Lung, and Blood Institute (NHLBI)
Sharon Thompson, OD
H. Eser Tolunay, NHLBI
Gisele White, OD

Others

More than 90 individuals attended this 2-day RAC meeting. A list of attendees appears in Attachment II.

I. Call to Order and Opening Remarks/Dr. Friedmann

Dr. Friedmann, RAC Chair, called the meeting to order at 1:00 p.m. on September 19, 2002. Notice of this meeting under the *NIH Guidelines for Research Involving Recombinant DNA Molecules* was published in the *Federal Register* on August 29, 2002 (67 FR 55415). The meeting involved discussion of four protocols, and retroviral vector packaging cell systems, presentation of the data management safety report and clinical updates, and an update on informed consent issues in human gene transfer research.

Dr. Rose referred the RAC members to the NIH Rules of Conduct and Conflict of Interest notice provided to them in their briefing materials.

II. Discussion of Human Gene Transfer Protocol #0207-545: A Phase I/II, Escalating-Dose, Open-Label Evaluation of the Safety, Feasibility, and Tolerability of Transgenic Lymphocyte Immunization Vaccine in Subjects With Histologically Proven Prostate Adenocarcinoma

Principal Investigator:	Maurizio Zanetti, M.D., and Frederick Millard, M.D., University of California, San Diego
Sponsor:	Cosmo Bioscience
RAC Reviewers:	Drs. Brody, Gooding, and L. Johnson
Ad hoc Reviewers:	None

Dr. Friedmann, who is employed by the same university as the investigator, recused himself from deliberations regarding this protocol. He did not participate in the preliminary review of the protocol, left the room during its discussion and transferred the chair responsibilities to Dr. Simari.

A. Protocol Summary

This protocol is a clinical evaluation of transgenic lymphocyte immunization (TLI) vaccination in research participants with histologically proven prostate adenocarcinoma. TLI involves the immunogenic properties of adult B lymphocytes, which can be rendered transgenic by a method of spontaneous transgenesis (i.e., a process that does not require the use of facilitating molecules or electrical/magnetic fields). B lymphocytes represent 10 to 15 percent of cells in the peripheral blood, and much is known about their lineage characteristics and their capacity to serve as antigen-presenting cells. B lymphocytes are made transgenic with non-viral plasmid DNA encompassing an immunoglobulin heavy (H) chain under control of a B-cell specific promoter. The plasmid DNA comprises an H chain gene, which is itself comprised of a rearranged murine variable (V) region and human constant region gene in genomic configuration. Inside the B lymphocyte, the H chain gene codes for an H chain polypeptide that provides the cell with a new antigen specificity to induce anti-tumor responses. The V region is re-engineered to code for selected epitopes of the tumor antigen telomerase reverse transcriptase (TRT), an enzyme overexpressed in the majority (>85%) of tumors in humans.

The protocol consists of intravenous (IV) administration of autologous lymphocytes transfected with nonviral (plasmid) deoxyribonucleic acid (DNA). The initial Phase I clinical study will involve up to 20 research participants with prostate cancer. For three months following immunization, participants' peripheral blood mononuclear cells will be collected at regular intervals to determine the longevity of transgenic lymphocytes *in vivo*. On the basis of studies in mice and consistent with the in vitro data, investigators anticipate that transgenic lymphocytes will be eliminated from the blood and lymphoid organs three weeks postinjection. This clinical trial is expected to generate important information about the safety of TLI and its applicability to vaccinating cancer patients.

B. Reviews by RAC Members and Ad Hoc Reviewers

Drs. Brody, Gooding, and L. Johnson submitted written reviews, to which the investigators responded in writing and during this meeting.

Dr. Brody asked about the rationale for the inclusion criteria and the designation of the study as "Phase I/II." He also questioned the design of the dose-escalation scheme, wondering why two participants were being enrolled per dose level, rather than the traditional three. He also asked about the relationship between the principal investigator (PI) (Dr. Zanetti) and the sponsor (Cosmo Bioscience).

Dr. Gooding expressed concern about the cytotoxic T lymphocyte (CTL) response to TRT peptides expressed by non-tumor cells, noting that induction of autoimmune destruction of hTRT expressing non-tumor cells, particularly hematopoetic stem cells could occur. She asked whether studies of autoimmunity in animal tumor model systems had been done. She also asked about the efficiency of spontaneous transgenesis including the transduction efficiency of B lymphocytes, the amount of plasmid DNA taken up by the cells and the levels of transgene expression.

Dr. L. Johnson also took note of the fact that the protocol's dose-escalation scheme is more consistent with a Phase I study and does not include the typical components of a Phase II trial. He expressed a further concern that the proposed range of doses of infused transgenic cells was rather limited in this dose escalation scheme. He suggested larger dose increases in half log increments. Dr. L. Johnson asked about the efficiency of spontaneous transgenesis in primary human B lymphocytes and the specificity of the proposed promoter. Regarding a statement in the informed consent document, he asked whether and how research participants who do not consent to the future therapeutic or commercial use of their white blood cells or DNA are able to continue participating in the study.

C. RAC Discussion

The following additional comments were made by other RAC members:

- Drs. DeMets and Gooding expressed a general concern about statistical issues and the design of Phase I trials, and they highlighted dose-escalation schedules and the applicability of animal data in determining human doses as issues that the RAC should consider in the future. Dr. Simek noted that when a protocol is submitted to the FDA, statistical issues, including the number of research participants per dose, are carefully evaluated.

- Ms. Kwan and Dr. Simari suggested that other changes to the protocol, in addition to its reclassification as a Phase I, might be needed for it to be consistent with a Phase I trial.

- Dr. DeLuca suggested removing non-essential sequence, such as the neomycin resistance gene, SV40 promoter and origin of replication from the plasmid.

- Dr. Borror expressed concern about the wording in the informed consent document that appeared to promise benefit. In particular, she noted the following quote, "By this procedure your immune system will be activated and taught to fight against all of your prostate cancer cells

D. Investigator Response

With regard to the question of whether the protocol would generate an autoimmune response, Dr. Zanetti explained that telomerase is present in many tissues and is expressed at very low levels in bone marrow, germinal center B lymphocytes, activated T cells, and the gonads. Telomerase expression in tumor cells is a thousand times higher. Dr. Zanetti indicated that the research participants in this protocol would be closely monitored for signs of inflammation of unexplained origin.

The B cell specific promoter is an IgH promoter and specificity has been demonstrated *in vitro* and in transgenic mice.

Regarding potential conflict of interest, Dr. Zanetti explained that he would not be acting in the role of PI for the protocol. Dr. Frederick Millard would be serving as PI.

In response to concerns about the phase of the trial, Dr. Zanetti indicated that the protocol had been re-designated Phase I and modified accordingly to reflect the change. However, assays to test for an immune response would still be performed along with toxicity studies.

With regard to the concern about two research participants per dose level rather than three, Dr. Zanetti explained that two participants per dose could be monitored more closely for quality control, in large part because the logistics allow both participants to be transfused at the same time. Dr. Sidransky responded that regardless of the number of participants per dose, any issues with quality control should be resolved to prevent potential problems with samples.

Dr. Zanetti agreed to modify the informed consent document so that clinical benefit would not be stated nor implied. The disclosure that a research participant's white blood cells or DNA may be used in the future for therapeutic or commercial reasons is required by the IRB and university's legal counsel. Dr. Zanetti indicated, however, that the following statement would be added to the document: " In case you check [No], we guarantee that any specimens obtained from you will not be used for therapeutic or commercial purposes and will be discarded after completion of the studies."

E. Public Comment

No public comments were offered.

F. RAC Recommendations

Dr. Simari summarized the following RAC recommendations:

- Research participants should be carefully monitored for inflammatory responses, including increases in complement and C-reactive protein levels and sedimentation rate, because an autoimmune response to other cell types that express telomerase reverse transcriptase is possible.

- The design of the dose escalation should be optimized for safety and statistical analysis and not for ease of product preparation. To achieve this, the investigator should obtain further statistical advice to refine the definition of desired end points, dose estimates and variability measures. To overcome errors inherent in estimating the numbers of cells administered, consideration should be given to using a broader dose range (perhaps two logs) with half log increments.

- Regardless of the number of participants at any dose level, product preparation should prevent the possibility of product confusion.

- Revisions are needed in the design of the protocol to reflect the fact that the study has changed from a phase I/II study to a phase I safety and toxicity study. This includes the requirement to enroll three research participants at each dose level.

- Non-essential sequence, including the SV40 promoter and origin of replication, and the neomycin phosphotransferase cDNA should be deleted from the plasmid in order to increase the safety of the gene transfer product.

- Because the protocol has been changed to a phase I safety study, the informed consent document should emphasize that the aim of the study is to assess the safety and toxicity of the product not its efficacy. Throughout the informed consent document, therefore, misleading statements about the study's potential benefits to the research participant should be deleted.

G. Committee Motion 1

It was moved by Dr. L. Johnson and seconded by Dr. Gooding that these recommendations expressed the comments and concerns of the RAC. Taken by roll call, the vote was 15 in favor, 0 opposed, 0 abstentions, and 1 recusal.

III. Discussion of Human Gene Transfer Protocol #0207-546: A Phase I/II, Double-Blind, Randomized, Placebo-Controlled Study To Assess the Safety and Efficacy of AMG0001 To Improve Perfusion in Critical Leg Ischemia

Principal Investigator:	Richard J. Powell, M.D., Dartmouth Medical School
Sponsor:	AnGes-MG, Inc.
RAC Reviewers:	Drs. Friedmann, Gelehrter, Powers, and Simari
Ad hoc Reviewers:	None

A. Protocol Summary

Critical leg ischemia (CLI) is the most severe manifestation of peripheral artery disease (PAD) in the legs. It causes significant morbidity for individual patients, and has high societal costs. Current treatment options are inadequate, particularly when replacement of blood vessels by surgery (revascularization) has failed or is not an option. Clinical outcomes of surgical repair of damaged arteries are also in need of improvement. Therapeutic angiogenesis attempts to promote the growth of new blood vessels for the treatment of disorders of inadequate tissue perfusion. The hepatocyte growth factor (HGF) plasmid has demonstrated potential for promoting new blood vessel growth in damaged tissue on the basis of in vitro and animal models and clinical studies.

HGF has been shown to be involved in the proliferation, mobility, and morphogenesis of various cells. It is also considered to be a humeral mediator of epithelial-mesenchymal interactions during embryonic development and organogenesis. HGF has potent angiogenesis activity that may result from a

combination of direct effects on endothelial cells and indirect effects, including paracrine up-regulation to vascular endothelial growth factor (VEGF) on vascular smooth muscle cells. While HGF and VEGF have many common features, HGF lacks the ability to increase vascular permeability. Endothelial cells express the HGF-specific receptor, c-met, whose expression is up regulated in response to hypoxia. Like VEGF, HGF stimulates the growth of endothelial cells without causing proliferation of smooth muscle cells. In addition, HGF has a typical signal sequence that allows it to be secreted from cells
This Phase I/II clinical trial will evaluate the effectiveness of HGF on individuals with CLI using various doses and several dosing regimens. The study will take approximately 2 years to complete, be conducted at approximately 20 sites around the country, and enroll approximately 110 research participants.

B. Reviews by RAC Members and Ad Hoc Reviewers

Drs. Friedmann, Gelehrter, Powers, and Simari submitted written reviews, to which the investigator responded in writing and during this meeting.

Dr. Friedmann's comments focused on the following issues: 1) the inadequate description of *in vivo* pharmacokinetic properties of the vector; 2) the dearth of preclinical work aimed at characterizing the effects of plasmid administration on tumor-bearing animals; 3) the use of overly optimistic language in parts of the protocol and informed consent document; and 4) an inconsistency between Federal adverse event reporting requirements and the protocol's proposed reporting schedule.

Dr. Gelehrter noted that the PI is appropriately cautious about the risks to research participants, especially those with PAD and either diabetic retinopathy or occult neoplasms, if this therapy is effective in stimulating angiogenesis systemically. He expressed concern about the paucity of information on the biology of HGF and on preclinical studies demonstrating the efficacy of this approach. Dr. Gelehrter suggested that given concerns about the potential for HGF to affect the fibrinolytic and coagulation pathways, it might be reasonable to monitor the research participants' coagulation parameters. He noted that virtually no data were presented to suggest that the surrogate marker to be used—transcutaneous partial oxygen pressure ($TcPO_2$)—correlates with other evidence of enhanced angiogenesis. Dr. Gelehrter asked the investigator to explain why the naked plasmid approach was selected for this protocol.

With respect to study design, Dr. Powers questioned whether the requirement that subjects be fifty years of age or older be justified. Regarding the informed consent document, Dr. Powers said that as currently worded the description of possible benefits of participation in the study is too optimistic and potentially misleading, and he suggested that all references to "treatment" and "therapy" be deleted and the term "gene transfer" be used in order to make clear that therapeutic benefits are not expected. In addition, the disadvantages and risks associated with flexible sigmoidoscopy should be discussed; and that the financial relationship between the investigators and AnGes, Inc. needs to be clarified.

Dr. Simari asked the investigators to speak to the theoretical concerns that are raised by the understanding of the biology of HGF and how these been addressed in preclinical and clinical studies. He asked about the basis for the weekly dosing interval and the pre-clinical and clinical data that support its safety and usefulness. With respect to the enrollment criteria, he asked whether the investigator or attending physician will be deciding that a potential participant is a "poor candidates for surgical therapy", and thereby, eligible for enrollment. He also asked whether the protocol's cancer screening criteria are in accord with current federal and American Cancer Society age-appropriate guidelines. Like Dr. Powers, Dr. Simari suggested that references in the informed consent document to 'treatment' or 'therapy' be deleted. He also questioned whether the investigators had potential for conflicts of interest.

C. RAC Discussion

During the discussion, the following additional comments were made and questions raised:

Dr. Simari asked whether anyone has been able to affect $TcPO_2$ in a preclinical model using the HGF plasmid.

Dr. Gelehrter noted that hind limb ischemia models are more consistent with large vessel ischemia than the condition of the participants in the protocol. He questioned whether there were animal models that replicate both large and small vessel disease. Dr. Gelehrter asked whether HGF might have anti-angiogenic effects rather than or in addition to the hoped-for angiogenic effects given that HGF is similar to plasminogen in its structure and that the internal portion of HGF has been shown to have anti-angiogenic effects,

Dr. Friedmann asked whether transgene expression had been detected in non-target tissues and if so, what its duration was. He asked about the use of this growth factor in tumor-bearing animal models.

Dr. Sidransky suggested that because the HGF molecule is putatively active through the c-met oncogene, the investigators could consider using a mouse tumor-bearing model such as renal cell carcinoma or heptocellular carcinoma, that would express c-MET on the surface, to assess whether the HGF would cause an increase in tumor growth.

D. Investigator Response

Regarding studies of $TcPO_2$ in a nonhuman animal model, Dr. Powell explained that the probes used are relatively large. These probes must adhere well to the skin to produce meaningful readings, which in animal models is difficult to achieve because most animals have fur.

With respect to Dr. Simari's concerns about patient recruitment, referral and selection, Dr. Powell responded that the other vascular surgeons in the group practice, not the investigators, would decide whether potential participants are eligible for the study.

With regard to Dr. Gelehrter's question about possible anti-angiogenic effects, Dr. Powell indicated that none of their animal is suggestive of an anti-angiogenic process. In fact, arteriograms and laser doppler velocimetry studies in the experimental models have shown flow increases that would suggest a pro-angiogenic effect.

To address Dr. Gelehrter's question about whether there are animal models, that replicate both the large and small vessel disease seen in humans, Dr. Powell noted that while TcPO2 studies are impractical in animals, imaging studies tend to be reproducible. However, in humans TcPO2 studies are fairly sensitive, but imaging studies are difficult to time to capture collateral flow. Dr. Annex added that use of animal models allows muscle to be excised and examined in detail. Dr. Powell indicated that the hind limb ischemia model is a subacute model because the animal is treated for ten days to two weeks after the initial ischemic event, and, therefore, they are not looking at reperfusion of an acute ischemic limb. Dr. Powell suggested that an atherosclerotic non-human primate model would be the best other animal model but that studies with such a model are long-term and very expensive. Another model such as the Watanabe rabbit model would probably not represent a classic atherosclerotic lesion but could be considered.

Dr. Powell indicated that they were concerned about avoiding the unintentional transfection of non-target tissues, and chose to use naked plasmid, which has lower transfection efficiency, for that reason.

Regarding possible tumorigenicity, Dr. Powell stated that they do not have data in a tumor-bearing, animal model, but data are available from other investigators who have examined, in an *in vitro* assay, the effects of HGF on hepatocellular carcinoma cells. This data suggest that HGF had a negative effect on hepatocellular carcinoma proliferation.

Dr. Annex indicated the American Cancer Society screening criteria would be used in the protocol.

In response to Dr. Friedmann's question, Dr. Powell explained that in the higher dose groups, the plasmid could be detected at low levels locally for up to 14 days. These results were obtained from tissues harvested from the injection site, and suggested that the plasmid was in the skeletal muscle. However, immunochemistry had not been conducted to determine whether the expression was in the skeletal muscle or endothelial cells within the muscle section. Dr. Morashita noted that other investigators have found that intramuscular injections of the plasmid resulted in local expression in skeletal muscle cells only. Dr. Annex noted that the only way to examine this in humans would be to do a muscle biopsy at the exact site of injection. However, such a procedure would not be safe in participants with critical limb ischemia.

E. Public Comment

No public comments were offered.

F. RAC Recommendations

Dr. Friedmann summarized the following RAC recommendations:

- There is very little information in the protocol submission on the potential angiogenic and tumorigenic properties of human hepatocyte growth factor (HGF). In addition, the protocol submission included few data on the biodistribution of the vector. Because levels of circulating human HGF could be below the detection limit for the assay and vector biodistribution could potentially lead to high local concentrations, the investigators should consider studying the investigational agent in appropriate tumor-bearing animal model to assess whether it is associated with an increase in tumor growth.

- The role of the principal investigator in determining which potential participants are eligible for enrollment in the study should be clarified. The protocol should clearly delineate that the principal investigator will not be involved in any of the following: determining which participants are eligible for enrollment in the study versus continuing to receive standard care; providing a presentation of the protocol to prospective research participants; the consent and enrollment process

G. Committee Motion 2

It was moved by Dr. Gelehrter and seconded by Dr. Wara that these recommendations expressed the comments and concerns of the RAC. Taken by roll call, the vote was 16 in favor, 0 opposed, 0 abstentions, and 0 recusals.

IV. Informed Consent Working Group Update/Dr. Brody and Ms. King (via teleconference)

Dr. Brody described the working group's effort to develop draft guidance, which will include examples of appropriate and problematic language, that could be used by investigators when crafting informed consent documents. They are also considering encouraging informed consent documents for gene transfer to be streamlined so that basic concepts are explained in an appendix rather than in the main text. The working group's current plan is to divide up sections among its members, decide upon a format useful for investigators, participate in a conference call in November 2002 to craft and critique specific language, meet the day before the December 2002 RAC meeting, and present its work to the entire RAC at a future meeting.

A. RAC Discussion

Dr. Lo urged greater emphasis on the entire consent process rather than only on the consent document. Dr. Lo suggested conferring with investigators to determine what they need and what type of format would be most useful to them. Dr. Brody agreed and further suggested that input from research participants was also needed.

Dr. Simek suggested streamlining the informed consent document to make it easier for potential research participants to read. Dr. Brody suggested that this might be possible by pairing a short document with a longer appendix providing additional information.

Ms. Kwan suggested that the working group consider consumer protection laws as a model to offer potential research participants a waiting period before signing the informed consent documents to allow time for consultation with primary health care providers.

B. Public Comment

Dr. Ruth Ryan Lessard, Introgen Therapeutics, Inc., suggested that institutional review boards and institutional biosafety committees also be consulted regarding the content and format of the guidance. These boards have much more input into crafting the informed consent documents than do investigators.

V. Day One Adjournment/Dr. Friedmann

Dr. Friedmann thanked the participants and adjourned the first day of the September 2002 RAC meeting at 5:00 p.m. on September 19, 2002.

VI. Day Two Opening Remarks/Dr. Friedmann

Dr. Friedmann opened the second day of the September 2002 RAC meeting at 8:30 a.m. on September 20, 2002.

VII. Discussion of Human Gene Transfer Protocol #0206-539: A Phase I/II Evaluation of the Safety and Efficacy of a Matrix-Targeted Retroviral Vector Bearing a Dominant-Negative Cyclin G1 Construct as Adjunctive Intervention for Superficial Corneal Opacity/Corneal Scarring

Principal Investigators:	Jonathan C. Song, M.D., W. French Anderson, M.D., and Erlinda M. Gordon, M.D., University of Southern California; and Peter J. McDonnell, M.D., University of California, Irvine
Sponsor:	None
RAC Reviewers:	Drs. Childress, DeMets, and Wara
Ad hoc Reviewers:	Nikunj V. Somia, Ph.D., University of Minnesota, Twin Cities, and Steven E. Wilson, M.D., University of Washington (via teleconference)

A. Protocol Summary

The purpose of this protocol is to test the safety and potential efficacy of a gene transfer intervention in reducing the incidence of blurred vision (also known as “haze”), a complication of laser eye surgery caused by an aberrant wound healing response. Shortly after laser treatment, the activated corneal fibroblasts (known as keratocytes) proliferate and migrate to the anterior compartment of the cornea (the outer covering of the lens of the eye). These activated keratocytes synthesize collagen and other extracellular matrix proteins that reflect incident light and, thereby, generate the corneal haze. Targeting the cellular events related to keratocyte proliferation after laser treatment may be a potential way to modulate the wound healing response and the occurrence of corneal haze. In studies in a rabbit model, a targeted vector (Mx-dnG1), which was further modified to seek out injured tissue was found to be more effective in reducing the occurrence of “haze” than a non-targeted vector with the same gene. The purpose of this clinical study is to evaluate the *in vivo* efficacy and safety of a matrix-targeted anti-proliferative retroviral vector bearing a mutant cell cyclin G1 construct for prevention of corneal haze development after excimer laser phototherapeutic keratectomy for treatment of corneal scarring.

The clinical protocol will enroll 9 to 15 eligible participants who are scheduled for laser eye treatment for scarring of the cornea. Each research participant will receive a specific dose that will be administered as eyedrops every 30 minutes to 1 hour for approximately 12 hours, for 3 consecutive days after surgery. Three increasing dose levels will be given. Participants will be evaluated at specified intervals for a period of 1 year following the gene transfer intervention.

B. Reviews by RAC Members and Ad Hoc Reviewers

Drs. Childress, DeMets, and Wara and *ad hoc* reviewers Drs. Somia and Wilson submitted written reviews, to which the investigators responded in writing and during this meeting.

Dr. Childress focused on the informed consent document. His major concern was the overstatement of potential benefits in the protocol and the informed consent document. He pointed out several misleading or unclear statements in the informed consent document and also suggested that a request for autopsy be included.

Dr. DeMets noted that preliminary data for the use of this vector in treating corneal opacity and scarring are from one small study in a rabbit model and that information about potential side effects is limited. He was concerned that the trial design seemed incomplete since the protocol does not address the rationale for the sample size, the precision needed to establish the maximum tolerable dose (MTD), or the toxicities that might be monitored to determine the MTD. He suggested that the protocol lacks well-defined end points, which could introduce bias in the post trial analysis. He urged the investigators to correct this deficiency before the protocol is begun.

Dr. Wara outlined eight issues of concern: 1) the use of a phase I/II study prior to the determination of a MTD; 2) the study's apparent insufficient statistical power to address efficacy and a subject cohort that is too diverse to address either safety and or efficacy questions; 3) poor delineation of end points and inadequate attention to toxicity criteria; 4) use of eyedrops, which are difficult to quantitate, will limit the ability to determine the MTD; 5) insufficient preclinical data which limit the determination of the initial and maximum doses to be tested in this Phase I trial; 6) unclear trial design, especially the role of the "allocation number" and the sequence of events; 7) overstatement of potential benefit in the protocol and throughout the informed consent document; and 8) inadequate discussion of the potential risk to participants, particularly in regard to the possible expression of the transgene in participants' eyelids and elsewhere in the body.

Dr. Somia pointed out the following concerns: 1) insufficient preclinical safety data and uncompelling preclinical efficacy data; 2) the frequency of eyedrop administration and whether repeated application of the vector would result in a difference in gene transduction or rescue from opacity; 3) the possibility of a potential inflammatory reaction to the vector; 4) whether higher rates of haze in the rabbit make it an inadequate animal model of the condition; 5) issues related to vector design, preparation, and production.

Dr. Wilson's main concern was that the study cohort's small number and heterogeneity would make efficacy difficult to demonstrate. He suggested that a multicenter trial with a focus on one or two types of research participants would be a more statistically sound design and enable the parameters of corneal opacity to be analyzed more fully. He suggested that the incidence of clinically significant haze may be overstated in the protocol. In addition, the availability of mitomycin C as an alternative topical treatment for corneal haze should be mentioned in the informed consent document.

C. RAC Discussion

Several other concerns were raised by RAC members:

Dr. Sidransky noted that the presence of retrovirus in the lower eyelid is of concern, in part because of the potential for SV40 large T-antigen expression due to the SV40 promoter in the plasmid. He suggested that this be studied by obtaining cell scraping from the lower lid and analyzing them using PCR.

Dr. Gooding asked whether participation in the study would necessitate the postponement of corneal transplant treatment if it were needed, and if so, how long would the wait be and what would its implications be for the participant.

Dr. Linial encouraged the researchers to consider conducting long term studies (up to 6 months) in the rabbit model to gain a better understanding of how long the vector is present.

Dr. DeLuca asked whether the researchers had assessed vector distribution at the trigeminal ganglia of the experimental rabbits. In animal studies of latent herpes infection which involves applying the virus to scarified cornea, the herpes virus gains access to terminal nerve endings, and is transported back to the ganglia. Dr. DeLuca suggested that in similar fashion, the vector could be transported to the trigeminal ganglia. Preclinical studies to determine whether the trigeminal ganglia are affected would be important.

Following on Dr. DeLuca's point, Dr. Friedmann indicated that there is a likelihood that some of the retroviral vector would get to the trigeminal ganglia, a site that harbors herpes virus. He asked about the consequences of an interaction between the retroviral vector and herpes virus.

Dr. Wara reiterated the concerns about the need for a well-defined study population and stringent inclusion and exclusion criteria. For example, ocular herpes infection causes a greater inflammatory response and different healing process than scarring due to other conditions. Responses to the vector may differ based on the origins of the participant's corneal scarring.

Dr. DeMets questioned the small number (13) of animals involved in the preclinical research and the advisability of mounting a clinical study at this point.

Dr. Brody emphasized the need for additional research participants at each dose level. He pointed out that because the threshold for toxicity in the study should be extremely small given the availability of alternative treatments.

Dr. Somia suggested that the researchers look specifically for the SV40 T-antigen expression in vectors using real-time PCR. He also suggested that gene-marking experiments be carried out to determine the level of transduction in the human eye, with a single dose and repeated doses.

D. Investigator Response

Regarding the suggestion of a multicenter trial, Dr. Song noted that using postrefractive surgery patients from multiple centers would provide the appropriate participant numbers needed for a larger efficacy study. However, he explained that for a safety and toxicity study such as the proposed protocol, using a smaller and more heterogeneous group of research participants is more appropriate.

Dr. Song agreed that scraping of the conjunctiva of all research participants is more appropriate than a full eyelid biopsy. He noted that, if ulceration or any other changes in the conjunctiva or eyelid occurred during the study, those areas would be biopsied.

In response to Dr. Gooding's query about transplantation, Dr. Song stated that this research protocol offers an alternative to corneal transplantation, and he explained that there are no detrimental effects in delaying corneal transplantation.

Dr. Song explained that the response in the rabbit model appears to be similar to the response seen in humans, although the rabbits heal more aggressively. This characteristic actually assists researchers in determining whether a significant response and the presence of vector will occur in humans and is a reason rabbit models are used in this research.

In response to Dr. Wara's concerns, Dr. Song acknowledged that ocular herpes infection leads to aggressive healing and more corneal haze and offered to exclude herpetic disease.

With regard to Dr. DeMets' question about the adequacy of the preclinical studies, Dr. Anderson explained that the researchers thought that the number of animals tested was sufficient but agreed to consider conducting more preclinical studies or adding research participants per dose level if the RAC believes either to be necessary. Dr. Anderson also noted that a vector identical to the study vector has undergone many safety tests, although not in eyedrop format. Given this safety record, the researchers believe that human trials can be accomplished safely. Dr. Anderson indicated that long-term animal studies will be conducted concurrently with the Phase I clinical trial and that the animals will be maintained for an amount of time necessary to obtain long-term data before proceeding to a Phase II trial. With regard to the number of participants in each dose level, Dr. Anderson agreed to rethink the issue in consultation with statisticians at the University of Southern California.

Dr. Anderson agreed to Dr. Somia's suggestion that screening be done for the SV40 T-antigen expression in vectors using real-time PCR.

In response to Dr. Friedmann's concerns about the interaction between the retrovirus and the herpes virus, Dr. Anderson stated that he was unaware of any such interaction. Dr. Linial pointed out that such an interaction has been seen in birds, with Marek's virus.

Dr. Gordon explained that immunohistochemical staining did not show any mononuclear infiltration in the histopathologic tissue sections but that the researchers will perform the CD4 and CD8 evaluations suggested by RAC members.

E. Public Comment

No public comments were offered.

F. RAC Recommendations

Dr. Friedmann summarized the following RAC recommendations, suggestions, and comments:

- The intended questions(s) to be studied must be more precisely defined because the objectives of the protocol will affect the size of the study population. For example, a better definition of the baseline rate of complications following laser surgery is necessary in order to be able to discern whether additional complications occur with the administration of the study agent. Once the intended questions are defined, the investigator should confer with clinical statisticians about the number of participants that will be needed to address the questions.

- To optimize the interpretation of results and assessment of toxicities, enrollment should be limited to research participants whose corneal defects have similar causes and mechanisms. Potential gene transfer and disease interactions such as underlying inflammatory responses that may skew the evaluation of toxicity should be considered in the selection of a more homogeneous research participant population. Problems in accruing a sufficient number of participants could be addressed through a multi-site study.

- Concerns were expressed about the insufficient supporting data from the preclinical models. The following specific inadequacies were noted: the number of preclinical studies is small; the number of animals that received the proposed product is very small; long-term studies are lacking; focused studies are lacking on transduced cells, the distribution of cells, the level and duration of transgene expression, and the effect on the cell of the expression of a cell cycle inhibitor that leads to apoptosis. Further animal studies and studies using cultures of human eye tissue should be considered..

- Serious concerns were expressed about the presence of SV40 sequences in several of the plasmids used for vector production and the possibility that they could lead to recombination events and the

expression of SV40 large T antigen. Vector preparations should be carefully examined for such recombination events.

- Questions were raised about the purity of the vector preparation given the presence of potentially toxic materials, such as sodium butyrate. To every extent possible, vector preparations should be thoroughly purified to remove such agents.

- Based on findings from animal studies, conjunctival scrapings of the participants' lower eyelids should be tested for the presence of vector. If the vector is found in this material, further analysis should be done to determine which, if any, cells were transduced.

G. Committee Motion 3

It was moved by Dr. Wara and seconded by Dr. Sidransky that these recommendations expressed the comments and concerns of the RAC. Taken by roll call, the vote was 13 in favor, 0 opposed, 0 abstained and 1 recused.

VIII. Retroviral Vector Packaging Cell Systems: Current Status

A. Retroviral Packaging Cell Development and Safety Issues/Dusty Miller, Ph.D., Fred Hutchinson Cancer Research Center

At the request of the RAC chair, the safety issues associated with the use of different types of packaging cell lines used to generate retroviral vectors were discussed. Dr. Miller reviewed safety modifications to the design of packaging cell lines. In the earliest cell lines, the packaging signal was deleted from the helper virus. Because there was considerable sequence homology with the vector, a single recombination event could generate replication competent retrovirus (RCR). The second generation packaging cell lines, such as PA317, had multiple deletions in the helper construct so that two recombination events would be required to generate RCR. More recently, split packaging lines have been developed in which the gag/pol and env sequences are separated onto two plasmids which in theory, would work to further decrease the probability of RCR generation. The type of vector used with a particular packaging cell line needs to be considered also to minimize potential sequence overlap. When used with an appropriate vector, even some earlier packaging cell lines did not generate detectable RCR.

PA317 has been used most frequently, but several other packaging cell lines have also been used to generate vectors for clinical gene transfer protocols. While RCR has been detected in supernatant lots produced by several different packaging cell lines, OBA has not received reports that RCR has been detected in treated participants. Dr. Miller suggested that available data, rather than theoretical assumptions, should be considered when evaluating the use of a particular packaging cell line.

B. Replication-Competent Retrovirus and Different Packaging Lines for Retroviral Vector Manufacture/Carolyn A. Wilson, Ph.D., FDA

Dr. Wilson reviewed the FDA guidance of October 2000 that recommended RCR testing at multiple steps during vector manufacture and long term clinical follow-up of research participants for RCR. The FDA sent a letter on March 6, 2000, to sponsors of gene transfer clinical trials requesting information about rejected lots of vector. RCR had been identified in lots using PA317, and in lots from two split packaging cell lines. She also presented data on the experiences of the National Gene Vector Laboratory experience with different packaging cell lines. RCR occurred in 75 percent of lots made with PA317, and 25 percent of Am12 lots. The few lots made with psi-CRIP and PG13 did not produce RCR. The FDA's Biological Response Modifiers Advisory Committee (BRMAC) discussed these results and advised the FDA not to prohibit use of particular packaging cell lines such as PA317 but to consider the packaging cell line in context with the vector. However, the FDA recommends that sponsors consider using lines other than PA317. As a general matter, the FDA encourages investigators to continue to publish RCR test results and to permit the FDA to discuss RCR testing results in public. A cumulative database of

RCR testing results and methods will enhance the FDA's ability to provide future guidance on these topics.

C. Comments by Kenneth G. Cornetta, M.D., Indiana University Cancer Research Institute (via teleconference)

Dr. Cornetta stated that the National Gene Vector Laboratory (NGVL) is no longer accepting PA317-based cell lines, primarily because investigator efforts to reduce RCR with use of this line have been insufficient. However, the NGVL would consider the use of PA317 cell lines with vectors designed to minimize overlapping homology. He noted that it is difficult to make specific recommendations to the RAC regarding the use of packaging cell lines since many new lines are being developed using different envelopes. Dr. Cornetta suggested instead that emphasis be placed on ensuring proper use of assays for detecting RCR, including choice of controls and detection sensitivity.

D. RAC Discussion

Dr. Friedmann asked for advice about the kind of questions the RAC should ask investigators who bring a proposal to the RAC using a PA317-based retrovirus. Dr. Miller noted that the FDA ensures that vector products for use in research participants are screened properly for the presence of helper virus. The RAC will need to be concerned in the future about novel packaging cell lines or vector systems, such as lentiviral vectors, and to assist investigators in developing appropriate assays.

Dr. Sidransky asked what additional studies researchers would have to conduct if they decide to switch packaging cell lines. Dr. Wilson responded that some minimal pharmacologic toxicology data would need to be presented to the FDA. Dr. Pilaro further explained that the FDA would require a bridging study, either *in vitro* or *in vivo*, to show that the vector is behaving comparably to the vector produced with the original cell line. For ongoing clinical trials, investigators would be able to start at a dose previously determined to be safe and begin the dose escalation of the new material from that point.

IX. Data Management Report/Drs. Simari and Wara

Dr. Simari reported that, in the quarter from May 1 to August 1, 2002, 226 serious adverse events (SAEs) were reported to the OBA, of which 190 were initial reports and 36 were followups. Of the 190 initial reports, 17 were classified as "A1" (serious, possibly associated, and unexpected). These 17—plus six followup reports—were reviewed in detail by the appropriate RAC members and were determined to be similar to SAEs seen previously in these kinds of studies. As a result, no further public discussion was considered necessary.

Dr. Wara reported on protocol amendments and annual reports. Annual reports or amendments were submitted by 49 PIs. Reported changes involved investigators, sites, sponsors, completions of protocols, notifications of publications, minor amendments, and the addition of requests for autopsy to informed consent documents.

X. Minutes of the June 20-21, 2002, RAC Meeting/Dr. Linial

Dr. Linial noted that the minutes of the June 2002 RAC meeting had been reviewed and that minor changes had been suggested and incorporated.

A. Committee Motion 4

Dr. Linial moved and Dr. Wara seconded that the RAC approve as revised the June 2002 RAC meeting minutes. Taken by roll call, the vote was unanimous in favor, with no abstentions.

XI. Discussion of Human Gene Transfer Protocol #0207-544: A Phase I Study To Evaluate the Safety and Pharmacokinetics of Pro-1, a Liposome-Encapsulated Thymidine Kinase Gene Formulation, in Patients With Stage IV Metastatic Melanoma

Principal Investigator:	John A. Thompson, M.D., University of Washington
Other Investigator:	Ian MacLachlan, Ph.D., Protiva Biotherapeutics, Inc.
Sponsor:	Protiva Biotherapeutics, Inc.
RAC Reviewers:	Drs. DeLuca, Lo, and Sidransky
Ad hoc Reviewers:	Donald M. Coen, Ph.D., Harvard Medical School (via teleconference), and Jon A. Wolff, M.D., University of Wisconsin Medical School, and Chief Scientific Officer, Mirus Corporation (via teleconference)

Dr. Linial, who is employed by the same university as the investigator, recused herself from deliberations regarding this protocol. She did not participate in the preliminary review of the protocol, and left the room during its discussion.

A. Protocol Summary

Each year, 56,000 cases of melanoma are diagnosed in the United States, accounting for 1-2 percent of all cancer deaths. The incidence of melanoma is increasing faster than any other cance; 1 in 75 individuals will develop melanoma during their lifetimes. Currently, there is no completely effective treatment available for this type of cancer.

This protocol will evaluate the safety of intravenous injection of an experimental gene transfer product, Pro-1, followed two days later by administration of the oral antiviral drug, Valtrex (valcyclovir). Pro-1 is a product consisting of DNA coding for thymidine kinase (tk) surrounded by a small lipid sphere, which is composed of four separate lipid products. The hypothesis is that the Pro-1 product will preferentially bind to tumor cells, and with the addition of valcyclovir, bring about tumor cells deaths.

The purpose of the protocol is to assess the safety of Pro-1 in combination with Valtrex in individuals with metastatic melanoma. Its objectives are to determine the safe dose of Pro-1 and how long it persists in the blood. No previous studies of Pro-1 have been conducted in humans; however, preclinical studies suggest that Pro-1 is safe and can act against melanoma.

B. Reviews by RAC Members and Ad Hoc Reviewers

Drs. DeLuca, Lo, and Sidransky and *ad hoc* reviewers Drs. Coen and Wolff submitted written reviews, to which the investigators responded in writing and during this meeting.

Dr. DeLuca highlighted four issues of concern: 1) the need for more rigorous biodistribution studies; 2) the lack of toxicity studies with Pro-1 plus Valtrex and the need to clarify the toxicity studies that were presented; 3) the specificity of the preclinical efficacy studies (all of which used a dose of Pro-1 plus Valtrex in excess of that proposed for this clinical study); and 4) the need to disclose in the informed consent document the fact that Valtrex has not been approved by the FDA for this application.

Dr. Lo expressed concerns about safety of the study, in view of the evidence of toxicity in mouse studies, which included abnormal liver function and decreased platelet counts. He suggested modifying the informed consent document to include the possibility of liver and bone marrow toxicity as a potential risk and to delete statements that might imply effectiveness.

Dr. Sidransky expressed concern about the need for more extensive biodistribution studies. He suggested that following clearance of the product from the bloodstream, MRI studies be performed to analyze plasmid absorption and transgene expression in tumor and non-tumor tissue. He recommended that all research participants have skin biopsies of both tumor and normal skin to compare levels of Pro-1 present, and that target expression be determined by testing ribonucleic acid or protein levels in tissue and serum. Dr. Sidransky suggested that further studies using larger numbers of animals might be

needed because the highest human dose proposed for this protocol is only one log less than the MTD in the mouse.

Dr. Coen discussed six issues: 1) the safety of the stabilized plasmid-lipid particle (SPLP)-encapsulated thymidine kinase (*tk*) gene; 2) the use of valcyclovir vs. ganciclovir in terms of potential efficacy; 3) the potential for the plasmid DNA to integrate into chromosomal DNA;4) the need for more thorough, longer term toxicological studies focusing on dividing cells and organ systems; 5) the need for pharmacokinetic studies in mice, and possibly other animals, to determine the product's persistence; and 6) the questionable predictive value of mouse studies for humans and whether a canine model was preferable.

Dr. Wolff focused on issues related to particle characterization, efficiency of gene expression, and toxicity. He noted the lack of information about Pro-1's specific formulation (this information was deemed proprietary and therefore was not disclosed to the *ad hoc* reviewers). He questioned the potential for benefit, noting that benefit would depend on the efficiency of the gene transfer.

C. RAC Discussion

Several concerns were raised by RAC members in addition to those expressed by the primary reviewers:

- Dr. Coen encouraged the investigators to include Valtrex or IV acyclovir in the toxicology studies, instead of solely using ganciclovir.

- Dr. Lo asked whether the presence of SPLPs in the small intestine might be related to the temporary transient weight loss seen in the experimental mice. He asked whether there was damage to the epithelium in the small intestine or to the mucous membranes, which might have compromised the animal's ability to take in or absorb adequate nourishment. Dr. Lo suggested that the adequacy of the preclinical evidence of the product's potential benefit was in question and that additional animal studies might be needed.

- Dr. Sidransky requested additional information about the biodistribution data showing that SPLPs could be recovered from a number of organs. Dr. Sidransky suggested additional testing of the product's antitumor effects using a skin test for T-cell mediated immunity.

- Dr. L. Johnson requested additional information about changes in the spleen volume in the experimental animals. He asked whether a similar event had occurred in human research participants, using other plasmid-based vectors.

D. Investigator Response

In response to Dr. Wolff's request for more information about the specific formulation of Pro-1, Dr. MacLachlan explained that while this proprietary information was not available to the *ad hoc* reviewers, members of the RAC reviewed it. A battery of release tests will be applied to the material used in the clinical trial as well as to the material used in the preclinical testing. The systems meet all normal quality-control standards, including FDA guidelines for residual solvents, endotoxins, and sterility. He noted that the contents of the lipids are listed in the protocol.

Dr. MacLachlan indicated that assays for expression of the kanamycin resistance gene in mammalian cells have not been conducted, but no toxicity associated with the transfection of this plasmid in mammalian cells has been seen.

Regarding the preliminary biodistribution study, Dr. MacLachlan explained that the data illustrating the biodistribution of the SPLP following IV administration illustrated accumulation in approximately 10 tissues, not including skin or muscle. This accumulation accounted for 85 percent of the total injected dose, which is deemed a reasonable result for a preliminary biodistribution study.

Dr. MacLachlan reported that no toxicology studies are planned in animals other than mice. The toxicology program proposed was adopted after extensive discussions with the FDA and is considered adequate to support a Phase I clinical trial.

Dr. MacLachlan agreed to explore the use of Valtrex administered in an oral gavage or IV acyclovir, as suggested by Dr. Coen. Dr. Pilaro explained that the FDA accepted ganciclovir use in animal models because this drug has a potentially more severe adverse event profile and provided important safety data.

With respect to Dr. DeLuca's concern about the potential to transfect nontargeted tissue, Dr. MacLachlan explained that studies are ongoing. The investigators have developed a quantitative PCR assay for DNA in the gonads and other tissues, and they will focus on demonstrating DNA clearance from those tissues.

With regard Western blot analysis and the availability of antibodies, Dr. MacLachlan indicated antibodies to thymidine kinase were not commercially available. If monoclonal antibodies were available to perform this type of analysis, the researchers would consider using them. Dr. Coen suggested a potential source for these reagents.

Dr. MacLachlan explained that the strong, nonspecific antitumor effect (as an adjunct to the gene-specific therapeutic effect) associated with the systemic administration of plasmid DNA is believed to be mediated primarily by immune activation accompanying the systemic delivery of cytidine-guanosine dinucleotide motifs. The investigators have used several different techniques to demonstrate the specificity of the antitumor effect by treating the animals in the absence of prodrug and by utilizing frame-shift mutants and other techniques to dissect the mechanism of action. In experiments where the number and total amount and the number of doses of DNA were lowered, a dose-dependent antitumor effect has been achieved. Even though they do not have evidence that the mechanism will work in humans, the investigators expect to see some nonspecific efficacy associated with the systemic delivery of plasmid DNA.

Dr. MacLachlan and Dr. Thompson indicated that, although many of the participants are expected to have the type of tumor that can be biopsied, inclusion criteria for this protocol do not require it. Where possible, the investigators plan to perform punch biopsies and carry out the analysis suggested by Dr. Sidransky. Dr. Thompson said that the investigators would consider whether to include punch biopsies of normal tissue as well.

In response to Dr. Lo's concern about the potential for damage to mucous membranes in the gut, Dr. MacLachlan explained that histological analysis has not been conducted on the gut of the experimental animals. The investigators' working hypothesis is that the considerable weight loss is an effect related to a general syndrome that results in behavioral changes in the animals. They stop eating and drinking and become dehydrated. Dr. MacLachlan stated that the investigators plan to conduct extended toxicology studies, including analysis of the gut as well as extensive histological analyses of all relevant tissues.

Regarding changes in spleen volume, Dr. MacLachlan responded that, in some of the animals treated at the higher doses, a two-fold to three-fold increase in spleen volume was seen approximately 1 week after treatment. This effect has been observed in the development of other liposomal drugs and is typically associated with a sequestration of the liposomal drug delivery system by macrophages that accumulate in the marginal zone of the spleen. Dr. MacLachlan indicated that this effect is not manifested clinically. In general, the doses used clinically are lower than the doses required to elicit this effect on the spleen in animals.

In response to Dr. Sidransky's concern, Dr. MacLachlan described the preclinical studies conducted to assay the mitogenicity and immunogenicity of the product, administered in multiple doses. In all of these experiments, no specific mitogenicity associated with the treatment was seen.

Drs. Wolff and Coen noted apparent irregularities in the data presented and references to other studies in the literature. Dr. MacLachlan indicated that he would reconfirm the accuracy of the information presented in the protocol. While also noting discrepancies, Drs. Friedmann and DeLuca emphasized the need to focus on which future studies to conduct.

E. Public Comment

No public comments were offered.

F. RAC Recommendations

Dr. Friedmann summarized the following RAC recommendations, suggestions, and comments:

- The investigator's responsiveness to some of the issues raised during the initial review of the protocol were acknowledged, especially the addition of a statement in the informed consent document that Valtrex is licensed by the Food and Drug Administration as an anti-viral medication but it has not been approved for use in combination with a transgene construct.

- The preclinical work with SPLP vector has concentrated on a product with reporter gene constructs that may have different properties than the product proposed to be used in the protocol (SPLP combined with the transgene, thymidine kinase, with and without Valtrex). The following additional studies should be conducted on the product proposed to used in the protocol:

    - Preclinical studies to determine the biodistribution and elimination profiles.
    - Plasmid biodistribution studies using qRT-PCR and protein (tk) detection methods to determine thymidine kinase transgene expression in both target tumor tissue and non-tumor tissues.
    - Studies to determine anti-tumor activity and potential adverse events that may occur due to transduction of non-tumor tissue and the level of transduction necessary to detect a thymidine kinase expression effect.
    - Studies to determine the optimal methods to monitor thymidine kinase expression. Methodologies using antibody to thymidine kinase for detection and activity assays, or PET scanning, should be evaluated. If levels of tk expression are too low for detection using these assays, qRT-PCR should be evaluated. The sensitivity and specificity of the qRT-PCR assay should be determined and documented.
    - Studies analyzing the efficacy of co-administration of the Pro-1 product and valcycovir. When necessary for studies in certain species, intravenous acyclovir may be substituted. Toxicity studies of Pro-1 combined with other forms of guanine nucleotide analogue, such as ganciclovir, should be considered.

- *In vitro* studies should be conducted to assess the potential for inadvertently inducing resistance to antibiotic Kanamycin in research participants.

- Gastrointestinal toxicity should be monitored in the participants to better understand the significant weight loss seen in animals receiving the higher doses of the product.

- In order to further characterize the distribution of the Pro-1 product, the participants' melanoma skin lesions as well as adjacent non-tumor skin should be biopsied and tested.

- Additional preclinical studies should be considered to evaluate the proposed use of valcyclovir several days after injection of the Pro-1 product.

- The need for the use of valcyclovir, as opposed to the use of ganciclovir or acyclovir, should be discussed further with the FDA.

G. Committee Motion 5

It was moved by Dr. Sidransky and seconded by Dr. Lo that these recommendations expressed the comments and concerns of the RAC. Taken by roll call, the vote was 13 in favor, 0 opposed, 0 abstentions, and 1 recusal.

XII. Closing Remarks and Adjournment

Dr. Friedmann thanked participants and adjourned the meeting at 3:15 p.m. on September 20, 2002.

…/s/…

Stephen M. Rose, Ph.D.
Executive Secretary

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

Date: …/s/…

Theodore Friedmann, M.D.
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Attachment I
Committee Roster

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Attachment II
Attendees

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Brian Annex, Duke University
Masayuki Aso, AnGes MG, Inc.
Andrew Byrnes, FDA
Jeff Carey, GenVec
Joy A. Cavagnaro, Access BIO
Joann C. Delenick, private citizen
James H. Gipson, Pacific Financial Research
Erlinda M. Gordon, University of Southern California
Stephen Grant, FDA
Joanne Hawana, *The Blue Sheet*
Erik K. Henricson, Children's National Medical Center
Andrew G. Hinson, AnGes, Inc.
Beth Hutchins, Canji, Inc.
Connie J. Kohne, GenStar Therapeutics
Hitoshi Kotani, AnGes, Inc.
Susan Leibenhaut, FDA
Ruth Ryan Lessard, Introgen Therapeutics, Inc.
Bi Lilia, FDA
Lee-Cheng Liu, AnGes, Inc.
Chris Lockey, AnGes, Inc.
Zhifeng Long, AnGes, Inc.
Richard E. Lowenthal, AnGes, Inc.
Ian MacLachlan, Protiva Biotherapeutics, Inc.
J. Tyler Martin, Sr., Valentis
Cardinali Massimo, FDA
Peter J. McDonnell, University of California, Irvine
Maritza C. McIntyre, FDA
Ryuichi Morishita, Osaka University
Mark J. Murray, Protiva Biotherapeutics, Inc.
Norifumi Nakamura, AnGes, Inc.
Nelia Padilla, Protiva Biotherapeutics, Inc.
Phil Pendergast, Ohio State University
Anne M. Pilaro, FDA
Richard J. Powell, Dartmouth Medical School
Bill Raschke, GenStar Therapeutics
Tina S. Rogers, Southern Research Institute
Joseph Rokovich, Edwards Lifesciences LLC
T. Shimada, Ambience Awareness International, Inc.
Jonathan C. Song, University of Southern California
Daniel M. Takefman, FDA
Argaw Takele, FDA
Sarina Tanimoto, AnGes MG, Inc.
John A. Thompson, University of Washington
Melissa A.B. Tice, Schering-Plough Research Institute
Maurizio Zanetti, University of California, San Diego

Attachment III
Abbreviations and Acronyms

BRMAC	Biological Response Modifiers Advisory Committee, FDA
CBER	Center for Biologics Evaluation and Research, FDA
CLI	critical leg ischemia
DNA	deoxyribonucleic acid
dnG1	dominant-negative cyclin G1
FDA	U.S. Food and Drug Administration
HGF	hepatocyte growth factor
IV	intravenous
MTD	maximum tolerable dose
NGVL	National Gene Vector Laboratory
NHLBI	National Heart, Lung, and Blood Institute
NIH	National Institutes of Health
OBA	Office of Biotechnology Activities, NIH
OD	Office of the Director, NIH
PAD	peripheral artery disease
PCR	polymerase chain reaction
PI	principal investigator
RAC	Recombinant DNA Advisory Committee
RCR	replication-competent retrovirus
SAE	serious adverse event
SPLP	stabilized plasmid-lipid particle
SV40T	simian virus 40 T-antigen
$TcPO_2$	transcutaneous partial oxygen pressure
tk gene	thymidine kinase gene
TLI	transgenic lymphocyte immunization
TRT	telomerase reverse transcriptase
VEGF	vascular endothelial growth factor