U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
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MINUTES OF THE RECOMBINANT DNA ADVISORY COMMITTEE October 7-8, 1991

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The Recombinant DNA Advisory Committee (RAC) was convened for its forty-seventh meeting at 9:00 a.m. on October 7, 1991, in Building 31C, Conference Room 6, National Institutes of Health, 9000

Rockville Pike, Bethesda, Maryland 20892. Dr. Gerard J. McGarrity (Chair) presided. In accordance with Public Law 92-463, the meeting was open to the public. The following were present for all or part of the meeting:

Committee members:

Ronald M. Atlas, University of Louisville
John H. Barton, Stanford Law School
Al W. Bourquin, Ecova Italia
Michael F. Brewer, Dunn and Bradstreet Corporation
Nancy L. Buc, Weil, Gotshal and Manges
Alexander M. Capron, University of Southern California
Ira H. Carmen, University of Illinois
Roy H. Doi, University of California
Anna C. Epps, Tulane University
E. Peter Geiduschek, University of California, San Diego
Martin F. Gellert, National Institutes of Health
Robert Haselkorn, University of Chicago
William N. Kelley, University of Pennsylvania Medical Center
Brigid G. Leventhal, Johns Hopkins Hospital
Brian F. Mannix, Buckland Mill Associates
Gerard J. McGarrity, Coriell Institute for Medical Research
R. Scott McIvor, University of Minnesota
Barbara E. Murray, University of Texas
Leonard E. Post, Parke-Davis Pharmaceutical Division
Moselio Schaechter, Tufts University
Executive secretary:

Nelson A. Wivel, National Institutes of Health

A committee roster is attached (Attachment).

Ad hoc consultant:

LeRoy Walters, Georgetown University

Non-voting agency representatives:

Phillip Harriman, National Science Foundation
Daniel P. Jones, National Endowment of the Humanities
Henry I. Miller, Food and Drug Administration
Ralph E. Yodaiken, Department of Labor

National Institutes of Health staff:

W. French Anderson, NHLBI
Leon Baltrucki, NHLBI
Bobbi Bennett, OD
Robert Cowherd, NCI
Cindy Dunbar, NHLBI

Patrick Hwu, NCI
Christine Ireland, OD
Susan Jenks, NCI
Becky Lawson, OD
Lauren Linton, OD
Charles MacKay, OD
Steven Rosenberg, NCI
Paul Seder, OD
Mark Stewart, NHLBI
Theresa Wang, NHLBI
Debbie Wilson, OD
John Yannelli, NCI

Others:

Paul Aebersold, Food and Drug Administration
Frank Blanchard, Howard Hughes Medical Institute
Ken Blaylock, ABC News
Arindam Bose, Pfizer Central Research
Shannon Brownlee, U.S. News
Joseph Chen, U.S. Department of Agriculture
Yawen Chiang, Genetic Therapy, Inc.
Robert Cooke, Newsday
Carol Ezzell, Science News
Oscar Fisher, Hood College
Diane Fleming, Merck & Co.
Jeffrey Fox, Science Writer
Scott Freeman, University of Rochester Medical Center
Diane Gershon, Nature Magazine
T. Venkat Gopal, Otsuka Pharmaceuticals
Jennifer Grace, Hood College
Bill Greenwood, ABC News
Marianne Grossman, University of Michigan
Robin Herman, The Washington Post
Yasuo Iriye, Otsuka Pharmaceuticals
Bill Jenkins, ABC News
Attila Kadar, Food and Drug Administration
Michael Kaleko, Genetic Therapy, Inc.
Richard Knudsen, Center for Disease Control
Cheryl Ledford, Hood College
Fred Ledley, Howard Hughes Medical Institute
Karen Lim, Hood College
Craig McCune, University of Rochester Cancer Center
Michael McNeil, Center for Disease Control
Robert Moen, Genetic Therapy, Inc.
Lisa Morris, Genetic Therapy, Inc.
Cheryl Osborne, Viagene Inc.
Chris Plein, University of Missouri
Cathleen Schmidt, Hood College
Karen Schmidt, Science News

Tomiko Shimada, AAI, Inc.
Beth Smith, Hood College
Sharon Smith, Hood College
Sue Smith, Food and Drug Administration
Clarence Styron, Monsanto Company
Larry Thompson, The Washington Post
Paul Tolstoshev, Genetic Therapy, Inc.
George Wallrodt, Stenotech, Inc.
David Wheeler, The Chronicle of Higher Education
Lisa White, The Blue Sheet
James Wilson, University of Michigan
Ningsun Yang, Agricetus
James Zwiebel, Georgetown University Medical Center

I. CALL TO ORDER

Dr. McGarrity (Chair) called the meeting to order at 9:00 a.m., on October 7, 1991. He noted that due notice of the meeting was published in the the *Federal Register* dated September 3, 1991, and that a quorum was present. This committee serves as advisor to the Director of the National Institutes of Health (NIH). The Director has three options. She may accept the committee's recommendation, she may reject the recommendation, or she may send the committee's actions and votes back to the committee for furthe deliberation. Dr. McGarrity introduced the *ad hoc* consultant to the committee, Dr.LeRoy Walters, who is the Chair of the Human Gene Therapy Subcommittee (HGTS).

II. MINUTES OF THE MAY 30-31, 1991, MEETING

Dr. McGarrity called on Ms. Buc to comment on the minutes of the May 30-31, 1991, meeting of the RAC. Ms. Buc said she had read through the minutes, and they were accurate. She moved they be approved. Dr. McGarrity noted the second reviewer, Dr. Hirano, was not present. He asked Dr.Wivel to appoint another member to review the minutes and defer approval of the minutes until tomorrow morning, October 8.

III. PROPOSED AMENDMENT TO APPENDIX D OF THE *NIH GUIDELINES* **REGARDING HUMAN GENE TRANSFER PROTOCOL ENTITLED** *GENE TRANSFER FOR THE TREATMENT OF CANCER*

Dr. Gellert noted this proposal was considered by the HGTS at its meeting in July. The protocol is designed to kill ovarian cancer cells using gene therapy. The patient will be infused with tumor cells which have been transduced with the herpes simplex virus (HSV) thymidine kinase gene (TK). These cells are now exquisitely sensitive to killing by the drug, ganciclovir. However, there is a possibility that neighboring cells will also be killed.

Dr. Gellert said the investigators had performed animal experiments in which tumor cells were infused into mice. Shortly thereafter, the gene-modified tumor cells were introduced, and the mice were then treated with ganciclovir. Following the administration of ganciclovir, a small proportion of non gene-modified tumor cells were also killed. He said the mouse model did not meet the criterion of being the most appropriate animal model as is required in the *Points to Consider for the Design and Submission of Protocols for the Transfer of Recombinant DNA into the Genome of Human Subjects* because no attempt was made to treat mice that had a solid tumor mass. The experiments submitted by the investigator only dealt with cell suspensions. Entirely different effects might have been seen if mice with established ovarian tumors were treated. The proposed experiments should be done before this protocol is approved by the RAC.

Dr. Gellert said he would like a clarification of the rationale of this therapy. There are two killing mechanisms discussed that are now merged together. They need to be discussed separately. If TK- cells are mixed with TK+ cells, *in vitro*, and treated with ganciclovir, both populations of cells are killed. However, this may be irrelevant because when the animal experiments were performed in immune-deficient mice, such as nude mice or irradiated mice, no killing of the TK- cells was observed. Therefore, in the mouse, it appears that the killing mechanism is related to an immune system response. The *in vitro* experiments may have no relevance.

Dr. Gellert noted that the patients to be treated with the proposed therapy will have Stage I, II, or III cancer that have previously undergone treatment by surgery and/or chemotherapy, yet still have remaining tumor burden. He asked what the prospects of recovery for such patients might be if they were to receive chemotherapy of a different type. It is not made clear to the patient if they have other treatment options available.

Dr. Gellert noted that the HGTS requested information on the structure of the transduction vectors. Information on only one of the vectors was submitted. At that HGTS meeting, there was a question raised about possible mycoplasma contamination of the PA-1 cell line that will be transduced. The investigator has not supplied the requested information; they say that the sterility tests are pending. If the vector information and the mycoplasma testing of the cell line are not available, consideration of the protocol should be postponed.

Dr. Kelley noted that with regard to the expected likelihood of survival of the patients, the only patients to be treated should be those who have a relatively short life expectancy. He asked for a description of the imaging technique to be used in the measurement of pre-treatment tumor burden and called for an extensive discussion of the issues raised by the HGTS to ensure that they are resolved.

Dr. Geiduschek expressed concern about various aspects of the protocol. He questioned the relevancy of the mouse model to the therapeutic scheme. He noted the cell dosages in the model experiments are very different from those proposed for the Phase I human study. The choice of the PA-1 human ovarian cancer cell line for transfer of the HSV-TK gene has been criticized on two counts: (1) the safety of the cell line is not yet assured; and (2) to the extent that the immune response to the patients' ovarian tumors are important to the functioning of the "vaccine." The use of heterologous cells is questionable. By opting for the technical advantage of using the established cell line, the project appears to risk uninformative outcomes in the Phase I study. The postulated therapeutic mechanism is still unclear.

Dr. Geiduschek said the use of the term "cancer vaccine" in the informed consent document is problematic. Because the layperson assumes that vaccines work, the proposed treatment should not be described as a vaccine. With respect to the statement on benefits and risks, the investigators must be capable of making a prediction. The use of the phrase, "It is not possible to predict whether any personal benefit...," should be modified. These patients should be informed that they are participating in a procedure that may fail as a therapy; however, valuable scientific information relating to your disease may be obtained. The paragraph on risks and discomforts does not list the extra time that participating patients will have to spend in the hospital as one of the discomforts. With regard to compensation, the patients should be assured that under no circumstances will they accrue additional costs as a consequence of their participation in this study.

Dr. Geiduschek said unless these concerns are substantially answered by discussion at the meeting, he will recommend not to approve this protocol.

Mr. Brewer made reference to the section of the *Points to Consider* where references to the actual protocol were made rather than summarizing the pertinent information. Mr. Brewer said the *Points to Consider* is a public document intended for lay people, and it should summarize the requested information and not refer to the larger and more complex protocol document.

Mr. Brewer said that the patient selection criteria should be more focused concerning the possibility of benefits to the patients. In the laparotomy section of the informed consent document, the risks from anesthesia ought to be addressed separately from the possible risks of other procedures. In the voluntary participation portion of the consent form, it is clear that patients can withdraw at any time; however, clinical data still needs to be collected. It should be made clear that the data will be collected as a result of routine follow-up care in order to assure against any complications arising from participation, and that the patient should give specific consent for the collection of any other data. Lastly, it was not made clear by the investigator if the stop criteria are mostly qualitative or if there are some quantitative determinants used.

Dr. McIvor criticized the submitted vector information in the protocol and suggested that the RAC establish a standard for the presentation of vector information. The expectation should be that the investigators supply a print-out of the actual sequence, indicate who generated it, supply a standard retroviral map of the vector, and supply a description of how the vector was constructed. The RAC should also establish a standard regarding the safety testing of cell lines. The investigator should perform extensive helper assays as well as other assays to screen for infectious agents when this protocol uses a new cell line.

Dr. Post said that the mechanistic work and efficacy work should have been done with irradiated cells rather than live cells. He expressed concern that only one experiment was performed where transduced irradiated PA-1 cells were put into an animal. The only valuable information obtained from this experiment was the survival time of the mice.

Dr. Walters said the HGTS had provisionally approved the protocol with the stipulation that: (1) experiments be performed to ensure that the retrovirus is not producing any helper virus; and (2) the cell lines should be assayed for sterility. Dr. Wivel said there was a detailed discussion at that meeting about the particular cell line chosen. There was concern that PA-1 is a high-passage line that may carry mycoplasma.

Dr. Freeman responded that he plans to use Stage I, II, and III ovarian cancer patients who have been previously treated and have evidence of residual disease. These patients will be treated with irradiated gene-modified tumor cells that contain the TK gene. Patients will then receive three repeated doses of ganciclovir. The goal here is to evaluate safety and side effects, to determine a maximum cell dose, to evaluate the immunologic response, and to observe any clinical effects.

Dr. Freeman described the suggestions made at the HGTS meeting that he had since incorporated into the protocol. Dr. Leventhal had suggested that a laparoscopy should be performed before and after therapy in order to determine the extent of tumor burden. Dr. Parkman suggested that they thoroughly and carefully follow the immune status of these patients by their response to skin testing. The consent form has been revised.

Dr. Freeman said that the patients involved will have been initially diagnosed as Stage I, II, or III. Stage I patients may have as much as a 60% chance of long-term survival. If they relapse after having completed surgery and chemotherapy, there is no known treatment to prolong their survival. It is a prerequisite of the study that these patients have received conventional chemotherapy treatment with Cisplatin or Carboplatin since these are proven effective in the treatment of ovarian cancer. Therefore, patients must have been treated with the most effective drug prior to acceptance into this protocol. He noted that Stage

IV patients will not be used. Stage I, II, and III patients have disease confined to their peritoneal cavity, whereas Stage IV represents patients with metastatic disease. The population of patients chosen will not have large tumor masses, so they are not end-stage patients. Often the patient has disease detectable only by elevated levels of a serum marker.

Dr. Freeman described the mechanism of action of ganciclovir and the HSV-TK gene. Ganciclovir must be phosphorylated to be effective. Once phosphorylated, it cannot cross cell membranes, therefore, this compound cannot be injected directly into the patient. The vector to be used, STK, has an LTR promoted neomycin gene and a SV40 promoted thymidine kinase gene. It is hypothesized that putting this vector into tumor
cells will make them susceptible to killing by ganciclovir.

Dr. Freeman described his experimental animal model, the K-BALB murine fibrosarcoma cell line. Cells that do not contain the TK gene are not affected by ganciclovir treatment. However, the two cell lines that do have the TK gene were killed by ganciclovir treatment. Experiments performed with human cells yielded the same results. In both cases, the transduced cells were killed by ganciclovir at levels that are achievable in patients. In one animal model experiment, the TK+ tumor cells were injected subcutaneously and the mice treated with ganciclovir, which resulted in tumor regression. The possible effect of *in vivo* killing of these cells by ganciclovir on immunization was discussed. The investigator found that cell killing *in vivo* by ganciclovir provided the ability to reject subsequent tumor challenge. Since it is not possible to get the TK gene into all of the patient's cells, the TK+ tumor cells must have some effect on the resident, un-modified tumor cells. Dr. Freeman showed data from *in vitro* mixing experiments where there was a mixture of modified and unmodified cells, in which the entire population of cells were killed by ganciclovir. He hypothesized that the TK+ cells may die, break up, and form vesicles. These vesicles may contain a toxic metabolite of ganciclovir that may be transferred to nearby TK- cells. However, the data is not conclusive and subsequent experiments will be directed at identifying the mechanism by which TK- cells die.

Dr. Kelley said that it was important to determine the mechanism before proceeding to patients. If the killing of resident tumor cells requires cell to cell contact or contact with vesicles, then there have to be enough TK+ cells administered to the patient for effective therapy.

Dr. Freeman said that fluorescent activated cell sorting (FACS) analysis showed that fragments of the plasma membrane from TK+ cells were present in or on the TK- cells. He cautioned that more experiments are necessary to prove this mechanism. He proposed doing electron microscopic studies on the cells to determine where the membrane vesicles are localized.

Dr. Freeman restated that the TK+ cells can kill TK- cells *in vitro*. To assess this effect *in vivo*, TK+ and TK- cells were mixed and injected into animals subcutaneously and then treated with ganciclovir intraperitoneally. If at least 50% of the cells were TK+, the tumor did not grow.

Dr. Haselkorn asked if Dr. Freeman had ever treated the cells with ganciclovir before injecting them into animals. Dr. Freeman said he has not yet performed these experiments.

Dr. Freeman presented data from another animal model experiment which he said has some relevance to the patients he will treat. Because these patients have tumor confined to their peritoneal cavity, he would like to inject the TK+ cells directly so they will be in contact with all the TK- cells. Using this model, he accomplished that experiment. TK- cells were administered to the mice at day zero followed by the administration of TK+ cells and ganciclovir at a later time. Prolonged survival was observed.

Dr. Kelley said that as the animal experiments become more and more relevant to the human proposal, the ratio experiments become increasingly critical and should be presented to the RAC.

Dr. Leventhal said it would be helpful to have median survival depicted on all of the graphs to give an indication of how close to death these animals were when treatment commenced. She said that there was not enough information on the slides which made them extremely difficult to follow.

Dr. Freeman restated that he had three animal models: (1) The subcutaneous injection of the cells followed by treatment with ganciclovir. (2) The mixture of the TK+ and TK- cells prior to subcutaneous injection of the mixture on day zero and subsequent challenge intraperitoneally on day five with TK+ cells. In this experiment, the animal survival was prolonged. (3) The subcutaneous injection of TK- cells on day zero, and subcutaneous injection of TK+ cells on day one or day five. Dr. Kelley asked if there are visible tumor masses by day five, when the TK+ cells are administered. Dr. Freeman replied there were no visible tumor masses. Mr. Capron asked if he had control mice to look at the tumors at day five in the absence of the second TK+ cell injection. Dr. Kelley said the tumors were not visible at day five. Mr. Capron asked if autopsies are performed on the mice. Dr. Freeman replied he had not autopsied the mice at five days.

Dr. Freeman said that with the two mechanisms combined, the system will be more effective and lead to the elimination of a major portion of the intraperitoneal tumor. Perhaps the effect of the TK+ cells on the TK- cells along with the subsequent immune response may actually eliminate metastatic disease. He said he has established three separate *in vivo* animal models, as well as *in vitro* performed experiments, that indicate 10-50% of TK+ cells may lead to the elimination of nearby unmodified tumor cells and prolong survival of the animals.

Dr. Kelley said as the experiments have become increasingly relevant to the proposed human experiment, the precise ratio of TK+ to TK- cells has to be determined.

Dr. Freeman introduced his colleague, Dr. Craig McCune, to comment on animal models.

Dr. McCune said he is a medical oncologist who began to work with Dr. Freeman in the creation of the proposed clinical study. He acknowledged that the question of when to indicate a clinical trial is a difficult one. The committee must determine the scientific merit of what is being proposed, and evaluate the safety for the patient and the public at large. This is a potentially new approach for treatment of human cancer, and it should move forward rapidly. This approach should encompass both dimensions, the pursuit of animal studies and the treatment of patients.

Dr. Haselkorn asked about the intent of the investigators' use of the term "vaccine" in the description of the proposed therapy. Dr. McCune said the definition of vaccine in the dictionary is very broad. Vaccine refers to any approach by which the progress of a disease is impeded. Dr. Geiduschek said the phrase "by inoculation" is part of the definition. It is indeed broad, and it represents a process that was developed long before an understanding of immunology.

Dr. McCune said there are two mechanisms by which the therapy may work. One involves the local effects of the TK+ cells. The second is the contribution of the immune response as illustrated in the experiments using immunodeficient animals in which the unmodified, neighboring cells were not killed. In a broad sense, the therapy consists of the injection of an agent to produce a resistance to the disease in the patient. Dr. Haselkorn said that in the public's mind, there is a distinction between prospective and retrospective aspects of vaccination, which are confused in the protocol's nomenclature.

Dr. Freeman said that in an effort to determine if this treatment will be safe for patients, an experiment was performed in which irradiated TK+ cells and non-irradiated TK+ cells were assayed for their effects on neighboring TK- cells. If the TK+ cells were irradiated and exposed to ganciclovir, they were killed. In *in vitro* mixing experiments, murine fibrosarcoma cells were mixed with human irradiated TK- cells or human irradiated TK+ cells. Adding between 10-50% irradiated cells could affect the neighboring TK- cells. Clearly, irradiating the tumor cells prior to administration would improve safety. In animal model experiments, the murine TK- cells were injected intraperitoneally on day zero. On day one either irradiated murine TK- cells or irradiated murine TK+ cells were injected intraperitoneally into the mice in a 50:1 ratio. Survival was prolonged as in previous experiments. The intraperitoneal injection of human TK+ cells into a mouse that had received a prior intraperitoneal injection of tumor cells at a 50:1 ratio, also provided prolonged survival. He reminded the committee that animals may die because of the subcutaneous tumor growing at the site of the needle track as a result of the injection of tumor cells at day zero. These tumor cells have not been exposed to the intraperitoneally injected TK+ cells.

Dr. Leventhal asked about the status of the peritoneal cavity at the time of post-mortem. Dr. Freeman said he did not know because he had not performed autopsies on the dead animals. Dr. Leventhal asked if the experiments are limited to female mice. Dr. Freeman replied that only female mice were used.

Mr. Capron expressed his agreement with Dr. Leventhal. If the theory is that the intraperitoneal tumor cells are killed because of the treatment but the subcutaneous tumor cells grow because they are not reached by the treatment, it should be an easy matter to perform the autopsies to verify the hypothesis.

Dr. Wivel asked if it was known for a fact that the mouse does not have intraperitoneal tumor. Dr. Freeman replied that he did not know.

Dr. Leventhal asked if statistical analysis had shown a dose-response effect. Dr. Freeman replied that there was evidence of a dose-response effect.

Dr. Leventhal asked how many patients will have a chance of being cured by other therapies, how many animals with gross tumor have been studied, and what is the safety of the cell line. Dr. Freeman replied that few, if any patients, would recover with other therapies. Dr. Leventhal noted they had studied very few animals at day five.

Dr. Freeman, in answer to the safety question, stated that he can easily test for replication competent virus in the cell line PA-1. In terms of insertional mutagenesis, these cells are already tumor cells, so this is not an issue. The modified tumor cells administered to the patients will be irradiated first. Also, the irradiated cells contain the TK gene which is sensitive to ganciclovir. Overall, this is a fairly safe procedure in terms of retrovirology. During the HGTS meeting, he described how the PA-1 cells will be characterized for sterility. He will ensure these test results are negative before the cells are administered. The cells will be tested for mycoplasma.

Dr. Post asked if they would do any animal safety tests on the final stock of cells, that will be administered to the patients after irradiation. Dr. Freeman replied that they had performed these tests, and the animals are doing fine. Dr. McCune added that the Food and Drug Administration (FDA) requires these general safety tests. Both guinea pig and mouse standard tests will be done on the final product.

Dr. Kelley asked for information regarding imaging, pre-treatment, and assessment of tumor burden. Dr. Freeman said they had decided to require laparoscopy before and after treatment. Tumor mass will be visualized. Hopefully, it will be possible to identify and mark where the mass is located to assess the effects of the therapy. This is a Phase I study where one looks for a dose effect.

Dr. McIvor said the committee should consider the generation of agents that are associated with the recombinant DNA process in the cell line. Helper assays must be performed to determine if there are other viruses generated, either from the packaging cell line or from the PA-1 cell line. If it is assumed that dying or dead cells are finding their way to the target tumor and are introducing a toxic metabolite of ganciclovir to these cells, there is the possibility that provirus from these cells will be introduced, i.e., secondary gene transfer process could occur *in vivo*.

Dr. Freeman said he had not checked to see if provirus is transferred. Dr. McIvor said that if a gene is being transferred into other tissues, the committee needs to be aware of that eventuality. This may create a safety problem if the gene is transferred into germ cells.

Dr. McGarrity provided a clarification on the safety testing requirements. This is the first time that a continuous cell line has been used in a protocol submitted to the RAC. Previous protocols have involved *ex vivo* manipulations. The cells are removed from the patient, manipulated, andreadministered to the patient. The committee has required helper cell lines to undergo a series of safety tests and evaluations. The same safety requirements apply to the continuous cell line that is to be used in this protocol. He stated that cell culture mycoplasma may induce interferon, activate macrophages, and induce IL-1, IL-2, or IL-6. This is the basis for safety concerns.

Dr. Freeman said he plans to perform the necessary tests. He asked if the tests have to be done in advance or if he can provide a list of these tests. Dr.McGarrity asked if Dr. Freeman could rule out a mycoplasma effect on all the data presented. Dr. McCune replied that the PA-1 cells were checked for mycoplasma.

Dr. Leventhal asked what tests the investigators propose to perform on the patients at the time of the second laparoscopy. Will the tests determine if any of the added material was transferred to the patient's tumor cells? If the treatment is failing, is it failing because the human ovarian tumor cells did not respond as expected *in vivo*, or because the ratio of the added cells to the resident tumor cells was ineffective? If there is a risk that the treatment will fail and the patients will die, there should be a provision that patients with gonads should be analyzed at post-mortem for the presence of the neo marker. Any other specific target tissues remaining should be assayed.

Dr. Freeman said he is hoping the laparoscopy will be useful in determining the tumor burden. Performing a tumor biopsy to check the cells for vector DNA, as well as checking the germ line for transfer, is a good idea. It might not be ethical to require that in advance. He does plan to obtain tumor samples for analysis. Dr. Leventhal said it is reasonable to ask for a collection of peritoneal fluid at the time of the laparoscopy. She asked about the type of cell analysis. Dr. Freeman said he would look for the vector DNA; and if it works in an *in vitro* model, he will look for the enzyme or theganciclovir phosphorylated compounds in the cells.

Dr. Kelley moved that the protocol be deferred. The committee has given the investigators very good advice, and they are to be complimented on developing an experimental model. On the other hand, there are a number of things that need to be done prior to proceeding to human experiments. DrGellert seconded the motion.

Dr. McGarrity called for further discussion. Mr. Capron asked for a summary of the points on which deferra rests. Dr. Kelley said while the animal model is a good one, there are many things that still need to be done. Particularly, an examination at autopsy to determine exactly what tumor cells have been killed needs to be done. Also, safety tests represent another major area where there are still some questions.

Measures of efficacy represent another area that bears discussion; such as, how to assess the patient's tumor burden before, during, and after therapy. Laparoscopy is a very subjective measure. Dr. Freeman said CAT scanning may also be performed.

Dr. Leventhal disagreed with the assessment by laparoscopy. She said that the most sensitive assay for residual tumor is the surgeon actually looking; it is better than a CAT scan. A patient who looked negative on CAT scan could be positive by laparoscopy, and certainly by laparotomy. However, one cannot require a patient to have two laparotomies in order to participate in an experimental protocol.

Dr. Leventhal said the committee would like to have some further evidence that tumor growing intraperitoneally can be cured with the proposed treatment. She suggested that Dr. Freeman treat the mice and sacrifice some untreated animals at the same time. This will show that the animal model is relevant. The investigator could start treatment at a measurable fraction of the residual survival of the individual mouse. If it has 15 days left to live, the mouse could be treated at five, six, or seven days. The investigator needs to observe untreated mice at five days to illustrate the outcome of the untreated mice. Dr. Leventhal asked what *in vivo* studies would be performed in that animal model, on the residual tumor cells, that might supply a way to evaluate the treatment. She expressed her concern that since the mechanism of the therapy is not understood, there may be a quantitative barrier in the human model because there is no end product to determine if the gene was transferred.

Dr. Haselkorn said that the system is a black box in the mouse. This black box will be transferred to humans, treating the humans as mice. Further investigation should be done on the immunological component of the mechanism. The hypothesis is that in the TK+ cell that is treated with ganciclovir, there is an accumulation of ganciclovir diphosphate, or ganciclovir triphosphate, in some kind of membrane enclosure which can be transferred to a susceptible cell. There have been no experiments that test that hypothesis directly. This experiment should be done before treating humans because it may not be necessary to administer whole cells; one may be able to merely inject the vesicles.

Dr. Leventhal said she would prefer to see the experiments done with whole cells before there is an elaborate effort made to fractionate the active principle.

Dr. McIvor proposed that the investigators examine the lymphocytes of the mice that have been treated to compare the levels of their cytotoxicity against the specific tumor target. This will help address the question of whether stimulation of an immune response is part of the mechanism. With respect to the prolonged survival curves, he questioned what ratio must be attained to save these animals and is it safe.

Dr. Geiduschek suggested that it might be possible to convey specific concerns about the protocol without having to discuss them around the table. He said he would be willing to convey them to Dr. Freeman and his coworkers directly.

Mr. Capron thought that the investigators were owed an explanation of what points are leading the committee to vote for a deferral. He said that the consent form needs to state that this is a study and not a vaccine treatment. The term vaccine is confusing to the ordinary person. It is a Phase I study of a biologic. In any case, the primary benefit to science is to determine its safety and to obtain a dose-response.

Dr. McGarrity put the motion to a vote. The motion was to defer this proposal until Dr. Freeman and his colleagues supply the requested information. The motion passed unanimously by a vote of 19 in favor, 0 opposed, and no abstentions.

IV. PROPOSED AMENDMENTS TO APPENDIX D OF THE *NIH GUIDELINES*

REGARDING HUMAN GENE THERAPY PROTOCOLS ENTITLED:
IMMUNIZATION OF CANCER PATIENTS USING AUTOLOGOUS CANCER CELLS MODIFIED BY INSERTION OF THE GENE FOR TUMOR NECROSIS FACTOR **AND** *IMMUNIZATION OF CANCER PATIENTS USING AUTOLOGOUS CANCER CELLS MODIFIED BY INSERTION OF THE GENE FOR INTERLEUKIN-2*

Dr. McGarrity announced he would disqualify himself from the review of these protocols. Recently, he has accepted employment from Genetic Therapy, Inc. (GTI). He noted that he had taken the position since the last HGTS meeting. While GTI is not mentioned in these protocols, Dr. Rosenberg has used vectors from them in the past. He turned the proceedings over to Dr. B. Murray.

Dr. B. Murray said there are two items that are relevant to this protocol: (1) In the original proposal, the NIH Institutional Biosafety Committee (IBC) requested additional studies using autologous cells lacking either the tumor necrosis factor (TNF) or the interleukin-2 (IL-2) genes. The NIH IBC has rescinded that request and has given formal approval to this protocol. (2) FDA approval has been granted for this protocol.

Dr. Leventhal said she would discuss the TNF protocol. In this experiment, Dr. Rosenberg plans to remove tumor from the patients that present with a diagnosis of melanoma, renal cell carcinoma, or colon cancer. He will attempt to grow these tumor cells in culture and, if successful, transduce these lines with the TNF gene. If the patients should relapse or their cancer progresses, these transduced cells will be injected subcutaneously into the thigh, and draining lymph nodes will be harvested 21 days later. Lymphocytes from these draining lymph nodes will be expanded and then reinfused into the patients along with IL-2. She pointed out that cell lines are cultivated for every patient who undergoes surgery at the NIH for primary therapy. These tumor cells will be cryopreserved and will be administered to the patient should he/she have recurrent tumor. Dr. Leventhal expressed concern that there would not be time, in a patient's rapidly progressing disease, to perform all of the tasks in the protocol.

Dr. Leventhal said the rationale for these experiments is that the TNF transduced cells are more immunogenic than untransduced cells. In the model experiments, the modified cells do not appear to grow as well as the unmodified cells, but an immunologic basis for this growth disparity is not completely proven. In Dr. Rosenberg's previous study with neo-modified TIL, he showed that the TIL home to tumor in three of five patients. However, it is not clear how many of the cells that are removed from the lymph node after subcutaneous injection of modified tumor cells, can be called TIL or TIL precursors. Dr. Leventhal questioned whether the tumor cells will grow in the patient after they are modified. If they do grow, they may endanger the patient because they are seeded with cells that make TNF constantly. This may render the patient symptomatic as TNF causes chills and fever. If the cells grow and remain in the patient forever, there would be the question of chronic toxicity. If the cells do not grow, will they be capable of stimulating the lymphocytes in the draining lymph nodes?

Dr. Leventhal recounted that due to these concerns the HGTS elected to approve the initial treatment of five patients with the stipulation that the local reaction be observed closely, and that measurements, descriptions, or photographs of the injection sites be submitted. The draining lymph nodes and other tumor sites should be studied by polymerase chain reaction (PCR) for signs of spreading transduced tumor cells and to study the trafficking pattern. The cells harvested from the lymph nodes should be studied to determine the cell types present. A toxicity reporting scale should be developed that provides more detail about the use of IL-2 and reinfused cells. She noted that Dr. Rosenberg had agreed to limit his initial treatment with this protocol to five patients and to perform PCR analysis of the draining lymph nodes near the cell injection site.

Dr. Haselkorn asked the investigators to discuss why the TNF-modified cells regress in the nude mouse. He asked if any experiments had been performed with animals that have established tumors rather than animals that have had tumor cells injected. With respect to the issue of safety, he asked why the investigators had not inserted the TK gene into the tumor cells that could then be killed by ganciclovir if necessary.

Dr. Carmen recounted the research activities that Dr. Rosenberg has presented to the RAC over the past several years. Dr. Carmen described the two protocols currently under the committee's consideration. With respect to these proposals, he noted that there is evidence that subcutaneous injections will be more effective than parenteral injections. There is evidence that the co-injection of modified and unmodified tumor cells can lead to the inhibition of growth for both types of cells. There is also evidence that IL-2 has much in common with TNF, both are known cytokines and can precipitate serious side effects that are treatable. While the reintroduction of modified tumor cells could cause tumor growth, appropriate counter measures are available if the patients have failed all other possible avenues of treatment.

Dr. Carmen asked what criteria Dr. Rosenberg will use to determine which patients will receive the gene for TNF and which patients will receive the gene for IL-2. He noted the NIH IBC had recommended a pilot study which would test the subcutaneous insertion of autologous tumor, putting aside the recombinant DNA phase of the protocol. The HGTS considered such studies unnecessary by vetoing the insertion of unmodified tumor cell lines and substituting its own safety measures. Using only cells that were transduced with cytokine genes in these experiments seems to be a drastic revision.

Mr. Barton asked if it is possible to administer TNF together with the tumor cells, rather than using the TNF-modified tumor cells to control the dosage of the TNF more precisely. He questioned whether the TNF and the IL-2 protocols are actually as parallel as one might expect, given the significantly greater toxicity of the TNF. The number of patients seems high considering the uncertainties. In the consent form, it should be made clear how the patients are to be involved in the cost sharing. With regard to risks, it should be noted that once the genetically engineered tumor cells have been administered to the patients, the dosages of IL-2 or TNF may not be controllable. The hypothesis should be clearly explained in the consent form as well as how the treatment might be a positive step forward.

Dr. McIvor responded to Dr. Carmen's concerns that injection of transduced cells alone, versus transduced cells combined with untransduced cells. In animal models, only transduced cells elicited an increased immune response. Therefore, we must assume that they are expressing IL-2 or TNF at increased levels which stimulates this immune response and an anti-tumor response against these cells. The injection of untransduced cells along with transduced cells would be much riskier and may not stimulate the same response. This is the logic behind eliminating the co-injection of untransduced cells.

Mr. Capron asked the investigators to explain an aspect of the consent document whereby the tense used in different portions of the form make it difficult to know where the patients are in the process of entering into the experiment.

Dr. Wivel reiterated that the HGTS had asked Dr. Rosenberg to give a report after five patients, and to use the PCR assay for gene-modified cells in specimens taken from the regional lymph nodes or from other biopsies of subcutaneous tumor masses.

Dr. Rosenberg responded that there have been no safety problems in his previously approved protocols. The protocol currently under review is an attempt to immunize patients against their own autologous tumors by using gene-modified tumor cells. It is based on previous animal experiments with adoptive transfer of lymphocytes as well as published experiments where gene-modified cells expressing

cytokines have been shown to become more immunogenic. He showed experimental data where attempts were made to raise cytolytic cells against weakly immunogenic tumor cells. These experiments were minimally successful. If one transduces the gene for IL-2 into the tumor cells, however, highly tumor-specific cytolytic cells can be generated. It is the CD8+ cells that have shown effectiveness and are probably the cytolytic cells based on adoptive transfer in model systems. The major point is that one can stimulate cytolytic cells against a weakly immunogenic tumor by immunizing with gene-modified cells.

Dr. Rosenberg stated that he plans to use the same retroviral vector that had been approved for use in the previous TNF TIL experiments. The vector contains the gene for TNF and the neomycin resistance gene. It has been shown that the TNF gene has been inserted into the tumor lines and that the tumor lines then produce message and secrete protein. When animals are injected with the gene-modified tumor cells, the tumor grows for eight to nine days and then spontaneously regresses. It is the TNF production by the tumor that leads to this regression. The assessment of the immunologic nature of this phenomenon is that it is mediated by T cells, presumably the cells that are specifically cytolytic. Animal experiments have indicated that when adoptively transferred, these T cells can mediate anti-tumor effects.

Dr. Rosenberg said the protocol attempts to take advantage of that observation in two ways: (1) by immunizing patients with the gene-modified cells as a form of active immunization; and (2) by subsequently harvesting draining lymph node cells to be used for adoptive immunotherapy. He showed data from animal experiments illustrating that the genetic modification of the tumor cells causes them to spontaneously regress while immunizing the mouse against tumor rechallenge and stimulating the generation of tumor-specific killer cells. The treatment scheme is to resect the tumor as part of standard treatment. All of the patients who will be treated have advanced cancer that have failed all standard treatments. When tumor is resected from the patient, as part of the standard treatment or as part of a TIL protocol, these tumor cells will be established in culture for studies of the immunologic reaction of that patient against his/her own tumor. Once the tissue culture line has been established, the cytokine gene is introduced, the activity is established, safety measures are performed, and then the therapy part of the protocol will be performed. The gene-modified cells are injected subcutaneously. The draining lymph nodes are removed three weeks later. Then lymph node cells are grown in IL-2 and used for therapy. Only terminally ill patients for whom there is a previously established cell line with the gene in it will be eligible for the protocol. Only at this time will the informed consent form be presented to the patient. The patient will be asked to enter this protocol only when they have no other treatment options available.

Dr. Rosenberg addressed the question raised about the nude mouse experiments. If this phenomenon is immunologically mediated, why do these tumors not grow in immune deficient mice? While immune deficient nude mice still have immune responses and have T cell precursors, they can develop normal immune functions. Most human tumors transplanted into a nude mouse will not grow because crossing the species barrier is an enormous immunologic insult. Even a nude mouse can reject most human tumor implants. It is only highly virulent tissue culture lines that can grow regularly in nude mice. The vectors to be used, which were approved, have genes encoding for neomycin resistance and TNF. Dr. Rosenberg said he could make constructs containing the TK gene, that might give an added level of safety, but he stressed that it would be a lot more difficult to get a three gene construct to work, because of the promoter inhibition that is observed. He said his laboratory attempts to put both the TNF and the IL-2 genes into the patient's tumor cells. It is not always successful. The best producer is the one that is used. If it happened in one patient that the TNF gene was transduced into a line and the IL-2 gene transduced into another, the question would arise as to which one to administer. The decision would depend on how many patients had gotten TNF and how many had gotten IL-2, since they are limited to five patients for each group. With respect to the issue of giving unmodified tumor cells, the NIH IBC had made that a strong request. He agreed with the subcommittee that it is not a good idea and the IBC has since removed that stipulation. Only the gene-modified tumor cells will be administered. The TNF cannot be administered

separately from the tumor cells because the half-life of theTNF is about four minutes. However, when the tumor cell manufactures TNF, there is a continuous bathing of the tumor cells withTNF. There is a concentration of all of TNF's immune regulatory properties at the site of the tumor; it is a very different situation.

Dr. Rosenberg addressed the question raised about the cost. At theNIH there is no charge for patient care. The costs would come out of the laboratory budget. The patients would not be asked to pay anything. He thought that the consent form explains the nature of the protocol and the potential risks in simple language, but can be changed if necessary.

Dr. Post asked if there exists animal data to show that it is better to useTNF than IL-2. Dr. Rosenberg said he is in the process of trying to compare them in the animal models, but there is no question that in both systems one can raise highly cytolytic cells with specificity for tumor antigens.

Dr. McIvor asked if the preclinical data in animals has been done with clones of tumor cells that have been transduced and then selected to secrete an optimized level of cytokines. The proposal calls for establishing a heterogeneous transduced cell population where there will be a wide variety of levels of expression from one cell to the next within the population. Dr. Rosenberg replied that they have criteria for selection of the bulk population. If possible, a heterogeneous population will be used to get as many of the antigens that would be present on the original tumor as possible. The human cells have not been selected, but the bulk populations do make a large amount ofTNF. There has to be a minimum, and there is no need to administer cells that are not making enough TNF. Hopefully, correlations can be drawn as to how much is needed to see an anti-tumor effect. If there are some non-TNF producing cells in the mixture, they should be eliminated by the immune system.

Dr. Leventhal moved that the treatment of five patients on the protocol withTNF-modified tumor cells be approved with further approval contingent on Dr. Rosenberg reporting back to the RAC on the side effects in these first five patients. Dr. McIvor seconded the motion.

Dr. B. Murray called for further discussion. There being none, the motion was put to a vote. The motion passed by a vote of 17 in favor, 0 opposed, and 1 abstention.

Dr. Leventhal asked Dr. Rosenberg to summarize the difference between the IL-2 study and theTNF study.

Dr. Rosenberg said the protocols are virtually identical with the exception of the few paragraphs that substitute the word TNF for IL-2. Since the tumors do not grow in either case, it is not expected to be any significant toxicity due to the production of the cytokine by the tumor. There are no potential problems with IL-2 that would be different than those ofTNF, but it is impossible to predict relative efficacy.

Dr. Kelley asked if the investigators were pursuing both experiments simultaneously to enhance the likelihood of a positive outcome. Dr. Rosenberg replied that was his intention. Dr. Leventhal asked if there are positive outcomes in both treatments. What are the future plans for doing this double-armed experiment? Dr. Rosenberg said he would vigorously pursue them both or exploit animal models to help develop improvements. Dr. Leventhal said that if there are positive results from the first five patients, a specific outline should be submitted of their future plans. This should include a statistical plan of what the next step will be in the further study of these materials in patients.

Dr. Leventhal moved to approve Dr. Rosenberg's proposal to treat five patients with IL-2 modified tumor cells with the stipulation that he report back to the committee on local and systemic toxicity and efficacy of

the treatment. Dr. Kelley seconded the motion.

Dr. B. Murray put the motion to a vote. The motion passed by a vote of 17 in favor, 0 opposed, and 1 abstention.

Dr. B. Murray returned the Chair to Dr.McGarrity.

Dr. McGarrity announced that if the discussion of the next agenda item finishes early, there has been a request that the committee begin the discussion of the agenda item for tomorrow regarding the future role of the HGTS.

V. PROPOSED AMENDMENT TO APPENDICES B-I-B-1 AND B-I-B-2 OF THE *NIH GUIDELINES* **REGARDING THE BACTERIAL ORDER** *ACTINOMYCETALES*

Dr. Schaechter stated that the proposal deals with the molecular manipulation of organisms which are quite important to industry, in the production of antibiotics, as well as other uses. However, there is concern that in this group of organisms,*Actinomycetales*, there are some known pathogens and a large number of suspected pathogens. In May 1991, the Mid Atlantic Biological Safety Association, represented today by Dr. Fleming, asked the committee to remove the gram-negative bacteria in the group of *Actinomycetales* from the *NIH Guidelines* for two reasons: (1) This group of organisms was originally classified as fungi. *Actinomycetales* is now classified as bacteria. (2) The list was quite inclusive. In the current *NIH Guidelines*, Appendix B-I-B-2, Fungal Agents, lists *Actinomycetes*, including *Nocardia* species, *Actinomyces* species and *Arachnia propionica*. It is not clear which are included and which are excluded under the term "*Nocardia* species" and "*Actinomyces* species." It could be interpreted as all inclusive or partially so. The Mid Atlantic Biological Safety Association has proposed that the list include only known pathogens in order to make the list inclusive as opposed to exclusive. The problem was that the committee was given numerous classification lists and did not know which ones were considered likely pathogens.

Dr. Schaechter explained that the *Actinomycetales* have been difficult to classify by taxonomists; consequently, there have been a lot of name changes. These organisms are known for causing a number of diseases. The current proposal by Dr. Fleming is based on a consultation with experts at the Centers for Disease Control (CDC), and with Dr. Beaman at the University of California at Davis who discussed a German assessment of risk in this group. The results are a list of *Actinomycetales* considered to have "proven pathogenicity" by the experts at the CDC. However, this list does not make recommendations. It includes agents of proven pathogenicity, of suspected pathogenicity, and of opportunistic organisms in immune compromised hosts. It is not clear where to draw the line. Possibly the RAC will have to create a working group which is charged not only with providing information, but also making recommendations to the RAC.

Dr. McGarrity drew the committee's attention to a letter from Dr Brinckerhoff. Dr. Schaechter summarized the letter for the group. In the letter, Dr Brinckerhoff commented on the confusing nature of the submitted information as well as the lack of recommendations from the experts. She felt that the proposal is diffuse and unorganized. Those individuals, who are directly interested in reclassifying these organisms, should do so based on the information available. They should present the proposed reclassification to the committee for discussion and approval. Their proposal should have a concise and up-to-date listing of the organisms, the category into which they fall, and the appropriate references.

Dr. B. Murray said it would have been much easier and more straightforward to have had a proposal that listed the specific organisms that are safe. Currently, it is too open because many of these organisms

have suspected pathogenicity.

Dr. McGarrity thanked Dr. Knudsen and Dr. McNeil for coming from the CDC to comment on the issue.

Dr. Knudsen said he is the new Chief of Biosafety at the CDC in the Office of Health and Safety. The table was meant to provide information, not to make any recommendations. The difficulty arises as to how to define a pathogen, a suspected pathogen, and an opportunistic pathogen, and an appropriate place in the *NIH Guidelines*. The *Actinomycetes* are a good example of a group which contains these three kinds of pathogens. The regulations on how to handle these are somewhat obtuse. He introduced Dr. Mike McNeil, who is an expert on *Actinomycetes*.

Dr. McNeil said he is a Medical Epidemiologist in the Mycotic Diseases Branch at the Division of Bacterial and Mycotic Diseases at the CDC. He stated that there are not a great number of animal studies which are needed to determine the pathogenicity of the organisms. The weakness in trying to classify these organisms is that there may be no reports for some of these organisms. Also, the taxonomy is shifting. In the table, the proven pathogens are the few organisms listed for which there are citations to animal studies. The suspected pathogens are designated if there is evidence in the literature ofhistologic invasion by the organism; or if in CDC review of clinical isolates, it has been observed that these organisms have been isolated from normally sterile sites. The list of opportunists includes those where there is a citation or where there is a patient mentioned as having acquired immunodeficiency syndrome, or another immune compromising condition. The anaerobic *Actinomyces* have not addressed the aerobic ones. The anaerobic organisms are considered to be normal flora although they certainly can cause infections. The interest really is in the aerobic *Actinomyces*.

There was a discussion as to the extent the animal model criteria used by the various contributors to classify the pathogens presented in the table.

Dr. Fleming noted that the other experts polled were not particularly concerned about animal models. Dr. Beaman, one of the consultants contacted about this issue, is an expert on *Nocardia*. He felt that animals should be used to test some of the *Nocardia*. However, many of them were pathogenic in man, whether or not they had ever been shown to be pathogenic in an animal model.

Dr. Wivel thought that some confusion may arise from the fact that there are clinical reports of lesions in human patients in which any of a variety of organisms may be isolated from that lesion. The problem is that those organisms may be passenger agents which are not the cause of the lesion. Therefore, that would be placed in the "suspected" category unless it could be documented in an animal model.

Dr. McNeil said that in the suspected group compiled by the CDC, they have identified human cases where there is clinical evidence to suggest the agent caused the infection. That group also contains reports about clinical isolates referred to CDC from various sources. These organisms are ubiquitous. They are acquired by both normal and immunocompromised hosts. The relative transmission is either by inoculation or, much more commonly, by inhalation. If people in industry are to grow these organisms up in large quantity, there may also be dose effects to consider.

Dr. McGarrity said that perhaps it would be desirable to appoint an advisory group to bring this discussion to a conclusion.

Dr. Schaechter asked where in the latest *Classification of Etiological Agents* do the *Actinomycetes* fall. Dr. McNeil said they are handled at a level between I and II, albeit in an experienced laboratory. Dr. Schaechter asked if they have been used in large-scale applications without any known ill effects. Dr.

McNeil replied that it is his understanding, given a lack of reporting, that they have been used in large-scale applications without any known untoward effect.

Dr. Haselkorn suggested the *NIH Guidelines* require that one cannot do an experiment before demonstrating that the organism to be used is not pathogenic. Dr.Schaechter said it would not be easy to show the pathogenicity. Dr. Post said it is an overkill for an organism that has been used for decades.

Dr. B. Murray said it would be easier if the five specific organisms that are now being used, and have been used for a long period of time, were considered separately. There is a whole variety of these organisms described in the clinical literature. For instance, there are many reports on *Actinomadura madurae* of association with tumor.

Dr. Fleming said that in 1988, the organism *Streptomyces lividens* was brought before the committee by SmithKline Beecham. They were asked to go back to the CDC for reference as to whether it was pathogenic for humans. This last year, several of these organisms were brought to her attention by some of the groups in her organization. These organisms had been modified with recombinant DNA techniques to produce a secondary metabolite for use as an antibiotic or anti-tumor agent. She said that it seemed feasible to ask the committee to look at this whole group of organisms.

Ms. Buc said there is a hazard question and a taxonomy question. Perhaps the committee should only address the hazard question. The organisms should be grouped by hazard.

Dr. Kelley moved that this issue be sent to an organization, like the American Society of Microbiology (ASM), to ask for advice. Dr. Post seconded the motion.

Dr. Atlas said that they should establish a working group to bring in consultants from the appropriate organizations and come back with a recommendation to the RAC.

Mr. Barton added that the working group should be free to assess the species that are most important commercially. Ms. Buc agreed and suggested that the group of companies propose the ten safest *Actinomycetes* for exemption. Dr. Post asked if the companies could come up with a list of organisms they want reclassified, and possibly a list of organisms that are already being used safely under good industrial large-scale practices.

Dr. Fleming said that some of those organisms are included in the back of the index of the *Industrial Biosafety Manual*; there is a list of *Streptomyces* and *Nocardia* that have been used for the production of antibiotics and anti-tumor agents. There are not many pathogens on the list, which is the reason for the requested exclusion of those organisms from Appendix B.

Mr. Capron said that the proposed working group should be given some instruction as to whether they should try to avoid risks or try to encourage the development with some tolerance of a level of risk and uncertainty. If this is largely a group of organisms that is not very pathogenic, but has certain known pathogens, then the listing only the latter in Appendix B would make sense. If this is a black box where little is known, that introduces another set of problems.

Dr. Schaechter suggested a substitute motion that would charge the working group with examining the question and to come up with the pros and cons. This working group will report back to the RAC at its next meeting. Dr. Post seconded the substitute motion.

Dr. Kelley said he was willing to accept the charge to the working group, and withdrew his motion to refer

to the ASM.

Dr. McGarrity restated the motion to refer the issue to an *ad hoc* working group within the RAC.

Dr. Schaechter said that it is essential that the motion include not just fact finding. The working group has to be charged with making recommendations as well as constructing a proper scenario for the RAC to be able to make a decision. Mr. Capron said that the working group would include people from CDC, people from academia, people from industry, as well as several people from the RAC with this expertise.

Dr. Schaechter restated the motion as a recommendation to create an *ad hoc* working group with outside consultants to determine the pros and cons of including known pathogens and excluding non-dangerous members of the *Actinomycetes*. Dr. Kelley seconded the motion.

Dr. McGarrity put the motion to a vote. The motion passed by a vote of 19 in favor, 0 opposed, and no abstentions.

VI. FUTURE ROLE OF THE HUMAN GENE THERAPY SUBCOMMITTEE

Dr. McGarrity said he would give a summary review of this afternoon's discussion of this topic at the appropriate time in the agenda tomorrow, October 8.

Dr. Walters summarized the subcommittee's discussion during the July 29-30, 1991, meeting. Two working groups of the subcommittee were established. One working group, chaired by Dr. Parkman, was to look at how the question of the possible germ line effect of somatic cell gene therapy should be approached by the subcommittee. The second working group, chaired by Dr. Leventhal, was charged with assessing how the subcommittee has done thus far and how its activities could be better coordinated with the parent committee. This working group would talk with investigators who have been through the review process to ask how the process could be more helpful and to obtain frank comments on the *Points to Consider*. Also, it would consider recommendations for streamlining the review process including combining the functions of the subcommittee and the parent RAC.

Dr. Leventhal said that the major focus of her working group was to ask the investigators to supply the results of the protocols so that the subcommittee could decide whether there were categories of protocols that no longer needed to be reviewed by the committee because they were safe. The end result of knowing what has happened to the patients might very well be the streamlining of the review process. However, the emphasis is more on results of the approved protocols.

Dr. Post said that there were good examples of why the HGTS does not really function as a subcommittee of the RAC. He noted that Dr. Freeman had repeatedly argued that, "Well, the subcommittee approved this." He did not get approval by the RAC, and the reasoning that led the subcommittee to approve this proposal is not clear. There are really two, quite separate, reviews that are not linked in a subcommittee/committee relationship. Two reviews are not necessary as illustrated by the fact that both Dr. Freeman and Dr. Rosenberg seemed to present the same material to the RAC that they had presented to the subcommittee. The *Science* article (August 9, 1991, volume 253) quoted one gene therapy company as saying they were thinking of bypassing the RAC review because it was so cumbersome. The article stated that if duplication of review continues, scientists would be better off spending their time and resources doing research rather than flying in for RAC and HGTS meetings. The RAC should think very seriously of trying to streamline the process into one single review at this level. The RAC could be reconstituted in recognition that it would have gene therapy as its primary mission. Outside consultants could also be used to cover other issues before the RAC. There could be some type of a pre-meeting

review where the primary and secondary reviewers would consult in the study section format with the investigators.

Dr. Atlas agreed with Dr. Post. Today's events were good examples wherein the investigators gave a presentation of the research to the RAC that was previously presented to the HGTS. The subcommittee did not say, "Here is what we reviewed, here is what we thought were the key issues, here is why we recommended this or that." The RAC had to listen to the original data and ask questions which may or may not have been previously asked. If the subcommittee is retained, it should give input from its review in the form of a report to avoid a duplication of efforts.

Dr. Bourquin reemphasized that most of the real expert review of the protocols before the RAC is done in the subcommittee. There is a different review that occurs in the RAC due to the composition of the committee. He endorsed combining the two committees because the in-depth review that the subcommittee performs is needed in the RAC. With the emphasis for the RAC being human gene therapy the function of the subcommittee must be acquired by the RAC through restructuring to provide expertise in that particular area. Issues like the *Actinomycetes* should be handled by the creation of a working group of experts composed of committee members and *ad hoc* consultants to make a recommendation to the RAC.

Mr. Mannix said the question of improving the working relationship between the subcommittee and the parent committee, with respect to human gene therapy proposals, should be separated from the question of what to do with the rest of the *NIH Guidelines* issues. The more urgent business is deciding how to handle the human gene therapy proposals. Combining the committees and meeting more often than three or four times a year is a good idea. In the longer term, the question of what to do with the rest of the *NIH Guidelines* and the other proposals that do not involve human subjects will have to be addressed.

Dr. Epps agreed with the combination of the two committees. She was also concerned that too much time is allowed for the presentations. The RAC is often not being advisory but educating the individuals. Those who were expert in certain areas certainly were very generous with the information that they provided. However, the RAC should not rush into dissolving the subcommittee. Dr. Leventhal should complete her working group assignment.

Dr. Kelley said one way is to approach this issue with some general principles to which the RAC would agree. One principle would be that efforts should be made to facilitate development of the field of gene therapy. A second principle might be that one should have only one scientific review at this level. Another principle might be that a scientific review should include experts in the specific area involved. Another principle, that is more debatable, is that whatever mechanisms are developed, they should be time-tested at the NIH so that it is not a completely different approach than the one that the NIH is accustomed to using.

Dr. Leventhal said the subcommittee had started giving contingent approval to a number of protocols to save time. However, there has to be a way to ensure that the second committee and the investigator knows what is expected for approval. A good way is the reporting of the deliberations of the scientific review body back to the investigators so that they can present clear answers to those questions. She proposed that the primary reviewer of the protocol take notes during the discussion and clarify the requirements for approval in a letter to the investigator. That letter then becomes part of the record.

Mr. Capron said it is possible to have a sequence in which a protocol comes before the subcommittee; and if it falls into one of the categories where there is little reason for concern, the members could approve it. If there are unresolved questions, those are then stated for the investigator who has the opportunity at

the RAC meeting to come forward with provisional approval and satisfy the points of concern that were raised by the HGTS. He added that it is clear from today's proceedings that the requirements stated in the *Points to Consider* need to be clarified for RAC and the investigators. The *Points to Consider* are a means of indicating the categories of information which this committee is likely to want to know. They are not guidelines. They are not regulations. They are simply categories. Another issue of concern that has been on everybody's mind is the potential for inheritable changes and for genetic enhancements in the field of gene therapy. The subcommittee is thinking about what it is going to do to inform itself, the RAC, and the American public, about responding to these basic issues. It would be valuable to have two groups working on these issues as well as the *NIH Guidelines* issues.

Dr. McIvor said that as a member of both the HGTS and the RAC, he is sensitive to the problem of relaying information from the subcommittee meetings to the parent committee. He said that the current system works.

Mr. Capron asked Dr. Kelley to consider reformulating his first principle. The Atomic Energy Commission got itself into a lot of trouble by trying to promote and regulate the field. There are a lot of people around, in private industry and at the NIH, who can promote this field. Perhaps the notion should be that the RAC does not intend to unduly, improperly, or unnecessarily obstruct the field. Dr. Kelley agreed with the reformulation.

Dr. Gellert suggested that when proposals start going through both rounds of review without any changes, then the subcommittee will be plainly redundant. As yet, there have not been any that are even close. The double review is not yet redundant. If there is an effort to amalgamate the committees or reduce the role of the subcommittee, it is very likely that applications will come to the full committee twice with no particular saving of effort.

Dr. B. Murray stated that even though a protocol might come to the same committee twice, at least everybody is exposed to the full discussion. There could be some advantage to having it come to the same committee twice instead of two different committees in the same time interval.

Dr. Geiduschek said that the need for two non-overlapping groups to do the review implies a lack of confidence in the reviewers. Even with minutes, having the first review and the second review done by non-overlapping committees results in wasted effort. A single group doing the review, even multiple times, is better than the situation as it exists.

Ms. Buc expressed concern about having the interaction between the investigator and either of the committees be in the form of *post hoc* letters which are not part of the public process. There is a value to the discussions being public; the minutes are the way to solve that problem.

Dr. Epps suggested the development of an interim solution. Mr. Barton suggested they ask one of the overlapping members between the subcommittee and the RAC to give a short summary of the critical issues before the HGTS.

Dr. Kelley said that the RAC and the HGTS may never have enough expertise to review some of the protocols as long as the field is as broad as it is right now. The notion of using *ad hoc* consultants is very common and very familiar to the NIH. As long as proposals cover many different fields, the process would be well served to try and put *ad hoc* consultants into the mechanism.

Dr. Walters suggested a new kind of document to come out a short time after the subcommittee meeting, a one or two page statement of the critical issues in the review process of a particular protocol.

Dr. Leventhal reiterated that the primary reviewer of the protocol at the subcommittee level could write a letter to the investigator stating what needs to be done to have the protocol approved. She added that the investigator could respond in writing the second time and not come and make the entire presentation all over again.

Dr. McGarrity summarized the committee's statements. In the interim period, the members of the RAC would like to streamline the process by getting minutes out as quickly as possible, and sending a summary of specific questions out to the investigators as quickly as possible, having them respond in writing. If possible, the same reviewers will be used for both reviews if there are two reviews; this will provide continuity. Dr. McIvor suggested that the chair of the committee write the letter to the investigators Dr. McGarrity also suggested this could be done by the Office of Recombinant DNA Activities (ORDA).

The meeting ended its first day's session at 5:05 p.m. on October 7, 1991.

VII. PROPOSED AMENDMENT TO APPENDIX D OF THE *NIH GUIDELINES* **REGARDING A GENE THERAPY PROTOCOL ENTITLED:** *GENE THERAPY OF FAMILIAL HYPERCHOLESTEROLEMIA*

Dr. McGarrity reconvened the committee. He called on Dr. McIvor to begin the discussion.

Dr. McIvor noted that he was the secondary reviewer for Dr. Wilson's protocol at the subcommittee meeting in July. He said he would give a summary of the proceedings of that meeting and how the investigators have answered particular questions about the protocol. The proposed treatment is for patients with familial hypercholesterolemia (FH) which is characterized by severely elevated levels of cholesterol in the bloodstream. These partients are at risk of severe liver coronary disease. The protocol involves the surgical resection of a portion of the patient's liver, and the isolation ofhepatocytes from this tissue. The hepatocytes are then transduced with the retroviral vector, which is designed for expression of the low-density lipoprotein (LDL) receptor. These patients are defective or deficient in expression of the LDL receptor; this is the molecular basis for the disease. After transduction, thehepatocytes are reinfused into the patient through the portal vein. Thesehepatocytes will seed the liver and may function to lower the level of cholesterol in these patients, and reduce their risk of coronary disease. The proposed therapy uses autologous cells and therefore, is less risky than anorthotopic liver transplant, the currently available therapy.

Dr. McIvor noted that the investigators have done extensive experiments in an animal model, the Watanabe hyperlipidemic rabbit, in which they have demonstrated a long-term reduction in the level of serum cholesterol. They have essentially performed the same experiment that is being proposed for humans. The vectors to be used are safer than the ones that have been proposed in the previous protocols, because the enhancer elements in the long-terminal repeats have been inactivated. The packaging cell line is one that splits the protein coding sequences into two separate pieces resulting in less chance of generating replication competent virus. The PA317 cell line, used by others, expresses both of the retroviral protein coding genes from the same segment of DNA. The only possible risk would be associated with the expression of the LDL receptor. There could be a possible immune response in patients that previously have not expressed any LDL receptor. The investigators have indicated that they will follow these patients closely for any possible immune response.

Dr. McIvor said the efficiency of repopulating the liver with these infused cells was determined by doing RNA protection studies on animals that had received transduced hepatocytes. The investigators were able to estimate the frequency with which hepatocytes were repopulating in the liver. In terms of the molecular and metabolic evaluation, the investigators will be doing molecular tests such as PCR and *in*

situ hybridization. They may do RNase protection if there is sufficient sample available from biopsies. In terms of the anticipated efficacy, there can be a five-fold variability of LDL receptor expression from one transduced hepatocyte to the next. The gene transfer frequency in the hepatocyte population is at most 5%. Finally, the frequency with which the cells repopulate the liver is as much as 2%. All three of these issues factor into the efficiency that one can express this gene in a patient and, therefore, affect the disease.

Dr. McIvor noted that patient selection was discussed at the subcommittee meeting. The treatment may involve children. The question of whether to include individuals who could not give their consent needs to be addressed. There was also some discussion on limiting the study to only the receptor negative population. The subcommittee decided to leave it open to all of these patients, although it was the receptor negative patients who were most likely to benefit from the procedure. The protocol was approved by the subcommittee with additional information to be provided about quality control data on the virus and the cells post-transfection, and clarification of the stopping rules. A revised consent document was also requested.

Dr. Doi said that some of the strong points of the protocol were as follows. The preclinical results with the Watanabe rabbit model seemed very promising. The recombinant autologous hepatocytes were associated with the 30-40% decrease in serum cholesterol which persisted for about four months. No immune response to the recombinant LDL receptor was noted. Although there was a higher than normal level of LDL receptor expression, it did not seem to affect the physiology of the cells. There was relatively little, or no, rejection of infected allogeneic hepatocytes, which will allow long-term treatment in the absence of immunosuppressive therapy. The investigator worked out some aspects of the experimental design with a single baboon. The technical aspects of partial hepatectomy and catheter placement seem to work well with no postoperative difficulties. There are two kinds of patients, those who are homozygous for the abnormal LDL receptor genes and those who are heterozygous. The homozygous patients usually die at about age 12; so it is critical to treat these patients. In summary, this is a well thought-out protocol based on solid preclinical data and that the probability of success seems high.

Mr. Capron noted that patients are to be brought to the University of Michigan to participate in this protoco from around the country. He asked whether the information that accompanies the consent form is provide at the time that they are already in Michigan. He asked for clarification of the timing of the information, the timing of the consent process, the patient's transportation to Michigan, and the availability of other treatments at the University of Michigan in addition to gene therapy. The *Points to Consider* submitted by the investigator to the RAC insist that subjects not withdraw after the liver is resected but before the cells have been reinfused. The reason for concern is that the patient would have taken a surgical risk and received no benefit. Clearly this is a paternalistic judgement on the part of the researchers. If someone chooses to withdraw at this point, he/she must be free to withdraw. He expressed concern, as he had at the subcommittee meeting, that this research, which is still at a very early stage of gene therapy, should not be performed with children. This is a disease which does express itself in children but also expresses itself in adults. The committee has to recognize the fact that this remains experimental, and the researchers say there is only a small possibility that there will be any benefits. This is a learning process. Learn first with people who can consent to participate in that context. If children are going to be treated, older children should be asked to sign the consent form because it is not appropriate to have an older child participate solely on parental permission. The data from the protocol indicates that 25% of the patients die by the age of 11. However, there are patients who are critically ill and would be suitable as subjects who are 18 and over. The age of consent in most states is 18 years.

Dr. Kelley noted for the record that Dr. Wilson got his Ph.D. in his laboratory and is a close personal friend. With regards to the treatment of children, there are a number of pediatricians on the subcommittee

who thought that it is not appropriate to exclude children if there is reason to believe that they would be good candidates for the protocol. Secondly, there was concern expressed at the subcommittee meeting about restricting the kinds of patients who would be able to participate in this protocol. Too much restriction would make the treatment less likely to be successful. Thirdly, there was a discussion that children may be better candidates since their average life expectancy is only 10 or 12 years; the sick patients are children. Fourthly, there was discussion that the livers from the younger children might actually respond better to this therapy than the livers of older patients.

Dr. Miller stated that it would be interesting to look at an analogous situation, that of human growth hormone deficiency. In his work at the FDA, he faces a dilemma when overseeing the testing of the recombinant human growth hormone. In patients who are completely deficient in the gene for growth hormone, neutralizing antibodies are produced to the recombinant human growth hormone. It is likely that a spectrum of immune responses to the LDL receptor protein will also occur in the FH patients. That has implications for the question of whether to use receptor deficient or receptor negative patients. Those who are receptor negative are likely never to have seen the LDL receptor protein and are more likely to mount an immune response. However, negative patients are likely to have the greatest clinical benefit and whose mortality and morbidity would be the greatest. He suggested that the investigators include both groups because of the potential knowledge to be gained. However, keep them statistically separate so that any differential effects, both in efficacy and in immune response, can be observed.

Dr. Leventhal said this disease should be thought of as a childhood disease, i.e., like Adenosine Deaminase (ADA) deficiency. If one insisted on doing ADA deficiency experiments in adults, one would never be able to do the experiments. The risk in a child with FH, who has a lot of stigmata of the disease at age 4, of having this procedure done is a great deal less than by the time the child reaches the age of 12 or 13, when coronary artery disease is pronounced. The risk of anesthesia and the procedure itself goes up with each year that one lives with the disease. The committee could place a medical restriction on the protocol that the serum cholesterol be a certain level for a certain number of years before the procedure can be initiated. Such a disease severity criterion instead of age criterion is reasonable.

Dr. Wilson responded that FH is a devastating disease that has encouraged the development of gene therapy technology. He described a patient, Stormy Jones, a young girl who had FH. Homozygous FH is extremely rare; there are approximately 100
patients in the United States. This homozygous group has on average elevated cholesterol levels of around 700 mg/dl. There is no therapeutic treatment of choice for homozygous FH at this time. One approach is to purge the blood of LDL by bringing the patient in every other week for plasmapheresis. The level of LDL goes down temporarily and then returns to baseline. This is done for the life of the patient. There is also orthotopic liver transplantation. There is significant morbidity associated with this procedure although some decrease in cholesterol levels has been obtained. These conditions suggested a model for gene therapy in that selective reconstitution of LDL receptor expression in the liver could produce some level of metabolic correction.

Dr. Wilson described the experimental strategy. A portion of the liver from a FH patient would be removed and cultures of the hepatocytes established. The recombinant retrovirus able to efficiently transduce these cells would be generated, the cells transduced, and transplanted back into the patient. He cautioned that normal levels of LDL receptor activity cannot be reconstituted in the patient. However, the procedure should provide some level of correction. He described the preclinical animal experiments. In the animal model for FH, the Watanabe rabbit, a portion of the liver was removed, the hepatocytes were transfused with a recombinant retrovirus that expresses the rabbit LDL receptor gene, and introduced back into the rabbit. LDL receptor expression was reconstituted in about 20%, or as many as 50%, of the transduced hepatocytes as shown in RNAse protection analysis. The level of correction varied between a 175 mg/dl

to a 450 mg/dl decrease in cholesterol levels. It seemed to be proportional to the level of baseline cholesterol in the rabbits, which averages approximately 600 mg/dl.

Dr. Wilson then described experiments using human hepatocytes. Interestingly, hepatocytes obtained from the youngest patient yielded the highest level of gene transfer. As to the issue of the age of the patients used in the study, his observation in virtually every animal experiment, as well as in the limited human cell experiments, is that the hepatocytes isolated from a younger specimens are more capable of incorporating the retrovirus. He noted that the entire vector to be used in the study is being sequenced in an appropriate FDA-approved laboratory. The packaging cell line is Y-CRIP. It differs from the packaging cell lines used by others in that both functions necessary to form a virus are contained on separate DNA molecules. This would make it less likely that a recombinant replication competent virus would be formed.

Dr. Wilson noted that the real risks of the procedure are not related to recombinant DNA but are related to the surgical procedures and manipulations. The patient is only subjected to one operative procedure. During the liver resection, a catheter that exitspercutaneously will be placed in the inferior mesenteric vein which leads into the portal circulation. The cells will be infused into the catheter which will be subsequently removed; this can be done at the bedside. After a dialogue with the referring physician, it will be decided if the patient would be a possible candidate. The patient would be brought to the University of Michigan four to six weeks before the procedure for a two to three day visit in the University Clinical Research Center at the Center's expense. The patient will be informed about the procedure. Dr. Wilson said he will personally talk to patients about the protocol. There will be a non-invasive evaluation of their eligibility; and if selected, therapy will be offered. After therapy, the patient will be discharged within ten days, and there will be subsequent follow-up with respect to the metabolic analysis.

Dr. Wilson said that experiments had been also performed in one baboon. The goals of the baboon experiment were to demonstrate the feasibility of the surgical and logistic issues of the procedure. They also wanted to look at issues of short-term toxicity, specifically issues that relate to harvesting and growing the cells as well as any complications of the reinfusion and of the catheter insertion. The therapy was administered, and the animal subjected to a laparotomy. The animal was checked for patency of the portal circulation, and a liver biopsy was performed. In the long-term follow-up, the animal was clinically normal, and the chemistry and hematology were within normal limits.

Dr. Wilson addressed patient selection issues. First, the receptor status is checked. If the patients are receptor-negative, they have a poor prognosis. Whereas if they are receptor-defective, some patients have a poor prognosis; but it is difficult to stratify that group. Thus, their disease status will be carefully evaluated. The patients will be evaluated by history and physical exam, echo with Doppler, and an exercise test to identify those who have absolute contraindications. At that point if they have acceptable risks, they would be considered as candidates for therapy.

Dr. Leventhal asked if there is a reasonable alternative therapy for this group of patients. Dr. Wilson said most of these patients are followed by a lipid center and are being managed either byplasmapheresis, drugs, or both. In terms of his own clinical interest, he would like to have the opportunity to manage some homozygotes by whatever means appropriate. Treatment has to be individualized; there is no standard therapy because nothing seems to work. If and when a liver transplant becomes indicated, it would be offered to the patients.

Mr. Capron said there is a potential problem whenever rare diseases are studied, and people are transported to a center for only that purpose. He asked what could be done to have more of the education and the real decision-making, including Dr. Wilson's conversations with the patient's, occur in the patient's local clinical center before coming to the University of Michigan. Dr. Wilson replied it is possible, given the

quality of some of the local clinicians, but it is equally possible that the patient may gain additional insight from in conversations with the investigators at their first meeting in Michigan. Mr. Capron said that it is likely that once a patient comes to the University of Michigan at the university's expense, the patient may not feel the freedom to decline treatment. Ms.Buc asked if the investigators will also pay the for the patient to return home if it is decided not to continue the therapy. Dr. Wilson said that the full cost of all pre-evaluations will be paid by the university.

Dr. McGarrity said he hoped that patients would be able to walk away, think about it, talk to other people who can help make a decision, return to continue the educational process, officially enroll, and sign the consent form. Dr. Wilson said he is quite comfortable with Dr.McGarrity's suggestion of giving the patient time to decide.

Dr. Doi asked about the half-life of a hepatocyte cell that has been transfused back into the animals. Dr. Wilson said it has been a very difficult question to address, but he had read estimates of anywhere between one month and a year.

Dr. Leventhal asked about long-term follow-up for the baboons. She noted that Dr. Wilson is planning to biopsy the human patients at three months, and asked what else he is planning for the patient's follow-up.

Dr. Wilson replied that the long-term follow-up for the baboons will consist of clinical evaluations, chemistries, hematology, tests for replication competent virus, and probably onepercutaneous liver biopsy for gross histopathology, as well as some specific immunocytochemical staining for viral antigens. The patient long-term follow-up is essentially metabolic with one percutaneous liver biopsy.

Dr. Leventhal noted that in a couple of committee reviews, there were concerns about the possible carcinogenesis of this procedure. She asked if there was any experimental basis for that concern. Dr. Wilson said it is theoretically possible. The simple cultivation *in vitro* and potential insertional mutagenesis could potentially predispose the reinfused cells to carcinogenesis. He stressed that these are theoretical concerns. In his experience in hepatology, it has been very difficult to transform hepatocytes since they are terminally differentiated cells. Carcinogenesis in rodent and rabbit experiments has not been observed.

Dr. Doi asked about the relatively short hepatocyte half-life and the need for subsequent infusion of modified hepatocytes. Dr. Wilson said they could repeat the treatment. The investigator's goal is to cryopreserve cells and simply reintroduce them. What poses a scientific hurdle is the ability to expand hepatocytes *in vitro*. This has not been done. It is currently possible, however, tocryopreserve hepatocytes and reinfuse them.

Dr. Atlas asked what the investigators plan to do if there is an immunologic response in the receptor-negative patients. Dr. Wilson said that the potential effect of the therapy involves taking a receptor-negative patient from zero to 5%. The receptor-negatives, whatever their age, are more importan in terms of potential benefit. A younger group of patients is preferable because it is easier to isolate, propagate, and more importantly totransduce younger hepatocytes. This is true in the limited human experiments, as well as in the animal studies. The analysis of the immunologic consequences is one of the most important aspects in the treatment of these patients. This information would be extremely helpful in the subsequent design of other experiments. He pointed out that the University of Michigan's transplant immunologists will participate in the protocol because it is possible that the receptor-negatives will develop an immune response.

Ms. Buc asked if the reduction of cholesterol levels from 900 mg/dl to 700 mg/dl would be of any benefit to

the patient particularly when the remaining level would still be so high. Dr. Wilson stressed that the therapy is not going to be a cure. But pharmacology andplasmapheresis, the currently available treatments forFH, only accomplish a transient decrease that eventually goes back to baseline. The hope is that the proposed therapy will diminish the baseline and allow the patients to better participate in the other therapies. The problem with drug treatment in the receptor-negative patients is that the pharmacology is based on up-regulating the LDL receptor. These homozygous patients do not have receptor, but gene therapy could give them a receptor level of approximately 5%.

Dr. Gellert asked Dr. Wilson to comment on the issue of telling patients that they should not withdraw after resection and before reinfusion. Dr. Gellert asked Dr. Wilson if he was comfortable with withdrawing that as part of the design. Dr. Wilson said a statement has been included indicating explicitly that the patient can withdraw at any time. Dr. Gellert said that there is an explicit statement that it would be critical for patients not to withdraw at this particular time. He said that a patient should always be free to withdraw regardless of the fact that they have taken the risk and are not deriving benefit. He asked Dr. Wilson to modify the protocol to reflect that stance. Dr. Wilson said he would make the changes.

Dr. Leventhal moved to approve the protocol, for the treatment of three patients as requested by Dr. Wilson. Dr. Kelley seconded the motion.

Mr. Barton moved that the treatment be restricted to adults. DrLeventhal refused to accept the amendment, because she said that the treatment should be available to patients on the basis of symptoms, not age.

Mr. Barton stated concern about small children not being of age to consent to these experiments where risk is involved. There is an adequate adult patient population available for the initial three treatments in which expected and unexpected risks can be assessed. After the first three patients, the age limit could be removed.

Dr. Leventhal said that the risks of the procedure are much lower for the patient who is symptomatic with angina pectoris than the risk of death, liver transplant, or coronary artery bypass. It is a deprivation, not a protection, to disallow a patient that needs treatment.

Dr. McIvor asked for Dr. Wilson's viewpoint on patient availability. Dr. Wilson said he thought it would be possible to find three patients who were over 18. He stressed that they would not be the patients most likely to benefit from this procedure. It is much easier to efficiently complementhepatocytes from a younger patient. Patients with the most aggressivefulminant disease are the younger patient population who are receptor-negative.

Ms. Buc said recognizing that there are risks, the question is not really who is most likely to benefit but from whom it is appropriate to elicit consent. Unless the younger children are different in kind, not just in degree from the older children, then the benefit alone should not be the only factor in deciding to start with the younger patients. Dr. Wilson said he could enroll adults, but he reiterated that the chance of demonstrating efficacy in that group is not as high as it is with the younger group.

Dr. McIvor said that this protocol varies considerably from some of the previous protocols in that it could provide a therapeutic effect. He said he would like to see the investigator given a chance to find the circumstances under which efficacy will most likely be demonstrated, therefore, the experiment is most likely to work. He said he was in favor of not restricting this protocol to adults.

Dr. Kelley suggested a compromise that would encourage the investigators to seek older patients if

possible. This is an important experiment, and it would be a shame to see it delayed for a length of time awaiting that particular requirement.

Dr. Schaechter said the biology of this situation, as well as the practicalities, demand that the work be done with children.

Dr. Miller said he would be surprised if the FDA accepted a limitation to adults on the basis of these consent arguments. Dr. Carmen agreed.

Dr. B. Murray added that because of the risk of other experimental therapies, such as liver transplantation or heart-lung transplantation, she would be opposed to the age limit restriction.

Mr. Capron asked if the receptor-deficient patients are included in the protocol for comparative reasons. Will there be any clinical benefit to those patients? Dr. Wilson replied that the expectation is that they would get benefit.

Dr. McGarrity put the motion to restrict the patient selection to the adult population of 18 years of age or older to a vote. The motion failed by a vote of 3 in favor, 13 opposed, and 1 abstention.

Dr. McGarrity put the motion to allow Dr. Wilson to treat three patients to a vote. The motion passed by a vote of 16 in favor, 0 opposed, and 1 abstention.

Dr. McGarrity added that the action signifies a new attack on yet another genetic disease, and represents an important advance in human gene therapy of genetic diseases. He wished Dr. Wilson the best and commented that his group had done an outstanding job in the presentation of their material.

VIII. MINUTES OF MAY 30-31, 1991, MEETING (CONTINUED)

Dr. McGarrity asked the committee to move to the next item, the approval of the minutes from the previous RAC meeting. He noted that Dr. McIvor had volunteered to review them.

Dr. McIvor said he had read the minutes for the May 30-31 RAC meeting and seconded their approval with some typographical corrections.

Dr. Post said he was listed as reviewing Dr. Brenner's consent form, and that is incorrect.
Dr. McGarrity put the motion to approve the minutes to a vote. The motion passed with a vote of 15 in favor, 0 opposed, and no abstentions.

IX. FUTURE ROLE OF THE HUMAN GENE THERAPY SUBCOMMITTEE

Dr. McGarrity summarized the major points of the previous discussion of this topic. Over the years the RAC has characterized itself as undergoing a continuing evolution. The development and evolution of the *NIH Guidelines* includes areas such as industrial scale-up, industrial practices, and environmental release. There is a general consensus that something must be done to streamline the review process of gene therapy proposals. There have been a variety of comments on this issue. Also, there have been a number of comments addressing the matter of a rapid reporting of the minutes so that they can get back to the group in a timely fashion. He asked Dr. Walters if he would begin the discussion as Chair of the HGTS.

Dr. Walters noted that eight gene transfer protocols and seven gene therapy protocols have been reviewed by the HGTS. Six protocols were approved by the subcommittee and forwarded to the RAC on the first cycle of review. Three protocols went through two cycles of review and three protocols required three cycles of review. Two protocols were deferred, either by the HGTS or the RAC. There was one protocol that was deferred by the subcommittee, and then not pursued by the principal investigator. In the last two HGTS meetings, seven new protocols were submitted. Five were approved on the first round and two were deferred.

Dr. Walters suggested a parallel review process rather than a sequential review process as a way to streamline review procedures. He suggested simultaneous submission of protocols to the local Institutional Review Board (IRB), IBC, ORDA (for consideration by the HGTS and the RAC), and FDA. The subcommittee could provide a forum for the consideration of issues like germ line intervention.

Dr. Leventhal said one of the benefits to parallel processing of protocols would come in the final processing of the consent form. This is really the responsibility of the IRB; the RAC spends more time on consent forms than is within its purview.

Mr. Capron said he wrote a motion that the RAC endorse the process established by the HGTS to reexamine how each committee handles various aspects of gene transfer experiments, including those with expected therapeutic effects as well as marking. The RAC would look forward to the results of the working groups on germ line therapy chaired by Dr. Parkman and the follow-up of approved gene transfer protocols chaired by Dr. Leventhal. The RAC should establish a working group to develop a set of principles to guide its operations and future formulation of guidelines, along the lines that Dr. Kelley suggested. Barring major new developments, the RAC should not further debate the issue of merging itself with the HGTS during the coming year, pending the recommendations of the three working groups. In the interim, the following procedures could be employed to facilitate the effective and efficient review of protocols involving human subjects.

3A. Immediately after the review of the protocol by the HGTS, the primary reviewer (working with the committee Chair and the Executive Secretary) will prepare a summary of the points needing further attention, which will be submitted to the principal investigator.

3B. Such statements will also be promptly circulated to members of the subcommittee, and any points that they identify as having been omitted from a summary will be added to the list and conveyed to the principal investigator.

3C. As a standard routine matter, the principal investigator will be asked to provide a written summary and copies of any slides regarding material
presented orally at a HGTS meeting that were not in prior written submissions to ORDA.

3D. If a protocol is deferred, the summary of the prior discussion, along with minutes of the meeting, will be submitted to the HGTS prior to its next review of the protocol.

3E. Once a protocol has been fully or provisionally approved by the HGTS, it will be placed on the agenda at the next meeting of the RAC, whose members will be provided with any summary statements of the HGTS's consideration of the protocol, relative minutes, and the written material submitted by the principal investigator to cover points presented orally.

Dr. Geiduschek seconded the motion.

Dr. Leventhal proposed, with respect to her working group's activities, that the term gene transfer be replaced by "follow-up the protocols already approved." Mr. Capron said he would substitute the phrase "follow-up the human subject protocols already approved."

Dr. Post said that Mr. Capron's suggestions would help the relationship between the RAC and the HGTS, but asked why they should freeze the discussion for a year. Dr. Post proposed that this motion taken up at the next RAC meeting after the recommendations from Dr.Leventhal's and Dr. R. Murray's working groups.

Mr. Capron suggested that the phrase "during the coming year" be changed to "pending taking actions."

Dr. Anderson expressed his concern that the primary area of attention is shifted from what is best for the development of the field of gene therapy to having the best bureaucratic procedure. The point is that there really is no need for two separate reviews.

Dr. Leventhal said that the RAC would very much like to make it easier for the investigator to understand, in a timely fashion, what comments had been made at the meeting. The charge of her working group is to develop a regular format for follow-up and feedback of active protocols. However, the RAC needs to be considerate of the investigators and not subject them to an overabundance of reporting. The second charge of the working group is the possible development of categories of protocols will exist that do not need to come to this committee anymore because there have been no problems to date treating a certain number of patients.

Dr. Miller agreed with Dr. Anderson that the dual review is regressive, superfluous, and slow. Given that the RAC has little review other than gene therapy protocols, it seems self evident that the committee should reconstitute itself to be primarily concerned with gene therapy.

Dr. B. Murray said that she did not see the need for two committees considering what has been done recently. The review of the protocols could be facilitated if the committees were merged. Dr. McIvor did not agree that the process by which protocols moved through the system would speed up. There has not been a protocol that would have gone through the system requiring only one review. But what is absolutely apparent from the proceedings at this meeting is that there is a lot of repetitiveness. This argues strongly in favor of a single review. He reiterated a suggestion made by Dr. Epps that a working group could informally discuss some of the issues with the investigator; and make a series of provisions, if necessary. The protocol could be given conditional approval and then sent to the RAC for formal review.

Dr. McGarrity said his understanding is that the subcommittee is looking at the future activities of the RAC. A motion could be passed to ask the HGTS to examine it's future relationship with the full committee.

Dr. Schaechter proposed that the Chair call the question.

Dr. McGarrity called a vote to call the question on Mr. Capron's motion. The motion to call the question passed by a vote of 8 in favor, 3 opposed, and 2 abstentions.

Ms. Wilson stated that Mr. Capron's motion had been changed in item number one where the phrase "gene transfer experiments" was changed to "protocols involving human subjects " the second time it

appears. In item number three, "during the coming year, pending taken actions" has been changed to "pending having taken action on" the recommendation. In current number three, where it begins "and as an interim matter," it would become item number four.

Dr. Geiduschek said there was also a change in item number two where it says "The RAC establish a working group." That would change to "The RAC assigned to Dr. R. Murray's working group."

Dr. McGarrity put the motion with the wording as amended to a vote. The motion passed by a vote of 10 in favor, 1 opposed, and 2 abstentions.

Dr. Leventhal said that Dr. Freeman was owed timely feedback, as well as the subcommittee, as to what the concerns were in his protocol that led to its being deferred, what information he must have, and where he should bring it. Dr. McGarrity said ORDA's staff would take care of conveying the information to Dr. Freeman.

Dr. Post moved that the RAC request that the three working groups of the HGTS have some form of recommendation available at the next RAC meeting so that they can be discussed further at that time. Dr. Atlas seconded the motion.

Dr. McGarrity put the motion to a vote. The motion passed by a vote of 10 in favor, 0 opposed, and 3 abstentions.

X. FUTURE MEETING DATE OF THE RECOMBINANT DNA ADVISORY COMMITTEE

Dr. McGarrity noted that the next meeting of the RAC will be February 10-11, 1992. A letter from Dr. Deisseroth was received in which he proposes a minor change in his protocol. It was referred to the primary reviewers on the RAC, and Dr. Walters is referring it to the primary reviewers on the subcommittee.

XI. ADJOURNMENT

Dr. McGarrity adjourned the meeting at 1:00 p.m. on October 8, 1991.

Nelson A. Wivel, M.D.
Executive Secretary

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

Date: 2/10/92

Gerard J. McGarrity, Ph.D.
Chair
Recombinant DNA Advisory Committee
National Institutes of Health