U.S. DEPARTMENT OF HEALTH
AND HUMAN SERVICES
NATIONAL INSTITUTES OF HEALTH

MINUTES OF THE RECOMBINANT DNA ADVISORY COMMITTEE

February 4, 1991

TABLE OF CONTENTS

DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
NATIONAL INSTITUTES OF HEALTH

RECOMBINANT DNA ADVISORY COMMITTEE

MINUTES OF MEETING

February 4, 1991

The Recombinant DNA Advisory Committee (RAC) was convened for its forty-sixth meeting at 9:00 a.m. on February 4, 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
Al W. Bourquin, Ecova Italia
Ira H. Carmen, University of Illinois
Donald C. Carner, Carner, Ltd.
Anna C. Epps, Tulane University
E. Peter Geiduschek, University of California, San Diego
Martin F. Gellert, National Institutes of Health
Susan S. Hirano, University of Wisconsin
Donald J. Krogstad, Washington University
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
Robert F. Murray, Howard University
Leonard E. Post, Upjohn Company
Monica Riley, Marine Biological Laboratory
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:

George Duda, Department of Energy
Joel M. Dalrymple, Department of Defense
Phillip Harriman, National Science Foundation
Rachel E. Levinson, Office of Science & Technology Policy
Henry I. Miller, Food and Drug Administration
George P. Shibley, Department of Agriculture
Sue A. Tolin, Department of Agriculture

National Institutes of Health staff:

W. French Anderson, NHLBI
R. Michael Blaese, NCI
Elaine Blume, NCI
William Branson, DRR
Barrie Carter, NIDDKD
Kenneth Culver, NCI
MaryEllen Franko, NCI
Stephen Heyse, NIAMS
Christine Ireland, OD
Attan Kasid, NCI
Becky Lawson, OD
Heidi Monger, OD
Richard Morgan, NHLBI
William Polvino, NIGMS
Steven A. Rosenberg, NCI
Linda Schwab, NCI
Carol Shapiro, NCI
Debra Wilson, NINDS

Others:

J.C. Alexander
John A. Barranger, University of Pittsburgh
M. James Barrett, Genetic Therapy, Inc.
John Barry
John Barton, Stanford Law School
Arindam Bose, Pfizer Central Research
Malcolm K. Brenner, St. Jude Children's Research Hospital
Nancy Buc, Weil, Gotshal & Manges
Yawen Chiang, Genetic Therapy, Inc.
Thomas L. Copmann, Pharmaceutical Manufacturers Assoc.
Van DeSilva
Roy Doi, University of California, Davis
Jerome Donlon, Food and Drug Administration
Chris Evans, University of Pittsburgh
Carol Ezzell, BioWorld
Gershon W. Fishbein, Genetic Engineering Letter
Diane O. Fleming, Merck & Co., Inc.
Joseph R. Fordham, Nova Nordisk Bioindustrials, Inc.
Joe Glorioso, University of Pittsburgh
Robert Goldberg, Merck & Co., Inc.
Alan R. Goldhammer, Industrial Biotechnology Association
Venkat Gopal, Otsuka America Pharmaceuticals, Inc.
Keith Haglund, Medical Tribune
Lowell Harmison
Eric Hoffman, University of Pittsburgh
Patricia Hughes, Otsuka America Pharmaceuticals, Inc.
James Ihle, St. Jude Children's Research Hospital

Yasuo Iriye, Otsuka America Pharmaceuticals, Inc.
Mitsuo Itakura, Tokushima University of Japan
John E. Jaugstetter, Genentech, Inc.
Roger D. Jennings, British Embassy
Dorothy S. Jessop, Department of Agriculture
Attila T. Kadar, Food and Drug Administration
Daniel Kuebbing, Genetic Therapy, Inc.
Morris Levin, Maryland Biotechnology Institute
Michael T. Lotze, University of Pittsburgh
Joseph Palca, Science Magazine
Chris Plein, University of Missouri
Fran Pollner, Medical World News
Margaret McLaughlin, Office of Technology Assessment
William H. McMullen, Nova Nordisk Biochem, Inc.
Joseph Mirro, St. Jude Children's Research Hospital
Robert C. Moen, Genetic Therapy Inc.
Bruce Pitt, University of Pittsburgh
Camillo Ricordi, University of Pittsburgh
Paul Robbins, University of Pittsburgh
Marvin Rogul, Maryland Biotechnology Institute
Jude Samulski, University of Pittsburgh
Clarence E. Styron, Monsanto Company
Jane Suen, Centers for Disease Control
Martin Terry, Animal Health Institute
Larry Thompson, Washington Post
Paul Tolstoshev, Genetic Therapy, Inc.
Thomas Troost, Georgetown University
Joseph Van Houten, Pharmaceutical Research Institute
George Wallrodt, StenoTech, Inc.
John Whalen, National Inst. for Occupational Safety and Health
David L. Wheeler, Chronicle of Higher Education
Lisa White, Blue Sheet
Larry Zeph, Environmental Protection Agency

I. CALL TO ORDER AND INTRODUCTORY REMARKS:

Dr. McGarrity, Chair, called the meeting of the Recombinant DNA Advisory Committee (RAC) of the National Institutes of Health (NIH) to order at 9:00 a.m., February 4, 1991. The meeting was called pursuant to a *Federal Register* notice which, being 30 or more days prior to today's date, met requirements of the *NIH Guidelines for Research Involving Recombinant DNA Molecules*. The meeting would remain open to the public for its entirety, and that he expected the meeting to conclude within one day.

Dr. McGarrity noted a quorum was present and said every attempt would be made not to limit debate, but to keep the agenda moving, in hopes that all agenda items could be heard and discussed before members had to leave for travel purposes.

Dr. McGarrity stated that the RAC was advisory to the Director ofNIH and that any action taken by the committee was not binding on the NIH, but that Director may choose to accept, reject, modify, or defer any recommendations or advice provided by the RAC.

Dr. McGarrity noted that he intended to make every effort to abide by the distributed agenda with respect to time estimates for each item of business. He reminded the committee that in recognizing persons for comments he would use the following order: primary and secondary reviewers on each item as set forth in the agenda; other members of RAC; *ad hoc* consultants to the RAC; NIH staff members; members of the public who had submitted written comments; and finally, other members of the public.

Dr. McGarrity then called on Dr. Post to report on the next agenda item.

II. MINUTES OF OCTOBER 16, 1990 MEETING:

Dr. Post said the minutes were consistent with his recollection of the meeting with the exception of the fourth paragraph on page 13 of the minutes which deals with the modification to Appendix K of the *NIH Guidelines*. His recollection was that this agenda item was not voted on, but was turned back over to the subcommittee for further review. Dr. McGarrity said this was his recollection as well and asked the staff of the Office of Recombinant DNA Activities (ORDA) to ensure that the language in the minutes was consistent with the official transcript of the meeting.

Dr. McGarrity noted that the secondary reviewer was supposed to be Dr. Acosta, who had announced her resignation from the RAC due to increased job responsibilities in a new institution. Her resignation would be accepted with regrets and that Dr. McGarrity had written Dr. Acosta a letter thanking her for her service. In the absence of a secondary reviewer, Dr. McGarrity read the minutes and found a few housekeeping items which he would take up with ORDA staff. He asked for other comments on the minutes.

There being none he asked Dr. Post for a motion. Dr. Post moved that the minutes of the October 16, 1990, meeting of the RAC be accepted with staff making a review on the language in the paragraph describing the motion about Appendix K. Dr. Krogstad seconded the motion. There being no further discussion on the motion, the Chair put it to a vote and it passed unanimously by a vote of 15 in favor, 0 opposed, and no abstentions.

Dr. McGarrity noted the presence of the Chair of the Human Gene Therapy Subcommittee (HGTS), Dr. LeRoy Walters. Dr. McGarrity thanked Dr. Walters for his past efforts and welcomed him to the committee meeting.

Dr. McGarrity said that one of the contingencies of approval of human gene therapy protocols to this point is that investigators return to the RAC and give timely reports on their work to date, including any minor changes, modifications, or problems that the investigators may have witnessed. If major changes were envisioned, the investigators must return to the HGTS and the RAC for approval, but that minor modifications could be approved by Chairs of the HGTS and RAC in consultation with committee or subcommittee members without a formal vote of the RAC. Dr. McGarrity then called on Dr. W. French Anderson to present an interim report on the ongoing human gene therapy protocol on treatment of severe combined immunodeficiency syndrome (SCID) with the human adenosine deaminase (ADA) gene.

III. INTERIM REPORT ON THE HUMAN GENE THERAPY PROTOCOL ENTITLED "TREATMENT OF SEVERE COMBINED IMMUNODEFICIENCY DISEASE (SCID) DUE TO ADENOSINE DEAMINASE (ADA) DEFICIENCY WITH AUTOLOGOUS LYMPHOCYTES TRANSDUCED WITH A HUMAN ADA GENE":

Dr. Anderson began by noting his close working relationship with Drs. Rosenberg, Blaese, and Culver in the one human gene transfer protocol and the two human gene therapy protocols that have been approved to date, the N2/TIL protocol, the ADA protocol, and the TNFTIL protocol. Any future protocols

would continue to be a group effort. Therefore, it is appropriate that the Principal Investigator (PI) on each of the protocols present an update for the committee.

Dr. R. Michael Blaese noted that there were some new committee members present and he reviewed a bi of the rationale for the ADA protocol. The protocol had two major components: (1) an immune enhancement by giving children infusions of autologous T cells; and (2) genetic correction with the retroviral-mediated gene transfer of the ADA gene into theautologous T cells that are reinfused into the children.

Dr. Blaese said that there were many technical questions to be answered by the protocol. One question was whether routine apheresis could be performed on children repeatedly in order to collect sufficient numbers of cells for immunotherapy. This has proven to be an achievable and very simple procedure with no side effects noted that were associated with the collection of cells, and no anemia. Because of this success there has been no necessity to introduce central venous lines in order to performapheresis on a routine basis.

He noted the consistent success in culturing the T cells from peripheral blood samples and stimulating their proliferation, a question which had not been answered previous to the study. They also had reproducible ADA gene transfer and expression into these cells in tissue culture and that routine infusion of cultured T cells has not been limited by any side effects.

Dr. Blaese said that they had been able to demonstrate the feasibility of usingcryopreserved gene-modified cells. One child had been treated using this technique and that it has been demonstrated to be feasible.

He said one of the questions which had come up in the subcommittee discussions of this protocol had centered on the question of the half-life of the infused cells. He presented data to show that the persistence of these cells could be demonstrated and was quite dramatic in some cases. He further added that he would present data to show that infusions of interleukin-2 (IL-2) are not required for long-term persistence of these cells.

Dr. Blaese said there was limited data on the continued expression of the genes*in vivo* but that due to the protocol design of gradual escalation in cell dosages, all of the data are not complete. The long-term objective of the protocol was to develop improved patient immune function. A thorough evaluation of this would not be done until after the sixth infusion in patients, but that there are some preliminary data in this regard that he would present.

Dr. Blaese explained the protocol in detail, noting that it is divided into three parts or phases. The first part calls for early reinfusion of cells within a couple of weeks to limit the possibility of cells losing heterogeneity by being maintained in culture for long periods. The second part calls for cell selection to increase the proportion of cells expressing the human ADA gene. And the third part of the protocol is an attempt to escalate the dose of cells to determine if ultimately it would be possible to use this therapy to replace the requirement for PEG-ADA injections in the patients.

He presented data on the first patient showing a T cell growth curve beginning in the range of 50 million cells which was expanded in 1,000 fold in tissue culture over a 10-11 day period. Dr. Blaese showed that these polyclonal T cells could be grown up very rapidly in tissue culture. No change was noted in the patient's absolute T cell count for the first 20 days or so, but then her T cell count began to rise, until by th third infusion, it had increased dramatically. After the third infusion there was some difficulty with tissue culture contamination, and the fourth treatment was postponed until this problem was solved. During this

50 day period between the third and fourth infusions, the patient's lymphocyte count reverted to pre-therapy levels and rose again after the fourth infusion.

Dr. Blaese said that the polymerase chain reaction (PCR) was used to determine how many of the peripheral T cells contained the ADA gene. Nothing was seen until prior to the second infusion when there was an increase in cell count and detectable numbers of gene-containing cells appeared in the peripheral blood. However, following the second infusion they disappeared and then reappeared. One hour after the third infusion the cells produced a very strongPCR signal, this has remained at a relatively high level, and seems to persist as long as 40 days.

He said that the peripheral T lymphocytes were surviving for at least twice as long as the cells in the previous N2/TIL protocol, but that there were no data available to confirm how long they will survive. The number of cells that the patients had received in this protocol is only one-tenth the number that were infused in the N2/TIL protocol. Therefore, it is obvious that there are differences in the characteristics of a tissue lymphocyte such as the TILs and the circulating peripheral T cells that are being used in this protocol.

Dr. Blaese presented data showing evidence of immune reconstitution in the patients as measured by antibody responses to isohemagglutinin which was the strongest evidence that the therapy was having an effect on the patient's immune system.

Dr. McIvor asked for clarification on thequantitation of the PCR results relating to the actual numbers of cells in the blood stream that actually contain the ADA gene. Dr. Blaese said he could not quantify it at this point because the scales being used for the PCR analysis were not adequate. The investigators were eager to determine those numbers. Cells are being banked so that after the first phase of the protocol is complete, they can be run as a group and quantified.

Dr. Krogstad asked what type of T lymphocytes were surviving in culture and in the patient. Dr.Blaese said that the first patient had a predominance of CD8 cells in peripheral blood at the start of therapy and that with each cycle of growth in culture there has been a progressive increase in CD8 numbers over time

Dr. Atlas asked whether there was any opinion on the part of the investigators as to why there was seemingly a lag period between the first and second infusions and then the steady decline during the period after the third infusion when no cells were being administered. Dr. Blaese was unclear as to the difference. However, there was speculation about the possibility that the treatment was blocking hepatic receptors, although there was no data in this regard and that they will continue to look at this as the protocol continues.

Dr. McGarrity asked if the PEG-ADA treatment relates in any way to the predominance of CD8-positive cells. Dr. Culver responded by saying that the second patient was slightly CD8 predominant, however, she was within normal range for a CD4:CD8 ratio and had varied over the course of the last few years in this regard.

Dr. McIvor asked if the investigators thought that the cells being infused were replicating. Dr.Blaese said he did not know. However, when they looked for activation antigens on the peripheral T cell population, a significant proportion still expressed HLA-DR and the IL-2 receptor at 2-3 weeks post-infusion. Therefore, either new cells are being recruited or cells are persisting because they begin to express these receptors while still in tissue culture. His guess would be that they are persisting in an activated state.

Dr. McGarrity asked about the number of patients now under study. Dr.Blaese said the first patient was

started on September 14, 1990 and the second patient was started on January 31, 1991. Dr Blaese said another patient would probably be enrolled within the year. Dr. McGarrity asked if there had been any significant changes in the protocol. Dr. Blaese said no significant changes had been made.

IV. INTERIM REPORT ON THE HUMAN GENE THERAPY PROTOCOL ENTITLED "THERAPY OF PATIENTS WITH ADVANCED CANCER USING TUMOR INFILTRATING LYMPHOCYTES TRANSDUCED WITH THE GENE CODING FOR TUMOR NECROSIS FACTOR":

Dr. Rosenberg presented an update on the TNFTIL protocol. The first two patients were treated six days prior to the meeting on January 29, 1991, and both were doing well. There was very little to report since they were only six days into the protocol but he would update the committee on what had transpired since the last RAC meeting.

Dr. Rosenberg said that the protocol had come about as a result of attempts to develop biologic therapies for cancer patients based on adoptive transfer of immune lymphocytes with anticancer activity. He cited his previous research using lymphokine activated killer (LAK) cells administered in combination with IL-2 which produced disease regression in patients with melanoma and kidney cancer. One woman treated in 1985 remains disease-free at this time, and supports the theory that adoptive transfer of LAK cells and IL-2 in a small subset of patients can result in quite meaningful clinical effects.

He said that the early trials with LAK cells had prompted a search for a more effective cell type and that studies with tumor infiltrating lymphocytes (TILs) in mice served as a basis for attempts at human gene modification. These TILs were shown to be approximately 50 times as effective in mediating tumor regression in mice and thus a study of the effects of TILs in humans was undertaken. He summarized the data from the first 50 patients treated with TIL therapy.

Dr. Rosenberg said the response rate of TIL treatment in the first 50 patients was twice that seen when LAK cells and IL-2 were administered, and that 38% of patients responded with an objective regression of their cancer (melanoma). The responses varied in duration from a few months to well over a year, but that there was room for improvement in the response rate.

He said that a central aspect of the use of TILs for genetic modification resulted from studies showing that TILs accumulated in tumor deposits while not remaining in normal tissue. This resulted in the hypothesis that TILs could be used to deliver reagents to the tumor site that might increase the effectiveness of the TILs at those sites. This was the basis for the first protocol in which TILs with a neomycin-resistance gene were inserted into patients to study the long-term survival and distribution of TILs, the results of which had been presented previously to the RAC. This experiment, in which 10 patients were treated, showed that these TILs consistently circulated in the patients for three-four weeks, but that some patients had N2 TILs in their circulation out to 60 days post infusion. In fact, these cells were found in circulation up to 189 days in one patient.

He noted that the patients all had advanced malignancy at multiple sites including liver, lung, brain and subcutaneous tissue and that their life expectancies did not exceed 90 days. Two of the first five patients treated had objective responses. He cited one woman, 26 years of age, who had multiple tumor deposits in her lungs and soft palate who had undergone complete regression of her melanoma, and continues in complete regression now 18 months post-treatment.

Dr. Rosenberg said that autopsies had been performed on three patients who had succumbed to their disease over 200 days post-transfer. Two of such autopsies had been analyzed and no evidence for residual cells was seen by PCR assay.

Dr. Rosenberg stressed that there were no side effects noted in any patient due to the gene transfer. No evidence exists that any patient was ever exposed to replication-competent virus, either in cells tested, or in studies performed on lymphocytes or serum of patients after transfer.

He said that the second phase of these studies was an attempt to insert genes that might improve therapeutic efficacy of the TILs, using the gene for tumor necrosis factor (TNF).

Dr. Rosenberg said that tests in mice had shown TNF to be remarkably effective as an antitumor agent. However, in a study of 39 patients receiving intravenous TNF, as well as in over a dozen other studies performed around the world with TNF in humans, no antitumor effects were shown at all with TNF. A possible explanation for this is that humans cannot tolerate a large enough dose of TNF to produce the antitumor effects. Humans can tolerate a systemic injection of only 8-10 mg/kg of TNF, whereas the effects in mice were shown with a dose of 400 mg/kg. Therefore the TNF TIL protocol is an attempt to bypass the toxicity to normal cells and allow for a high accumulation of TNF at the localized tumor site.

Dr. Rosenberg said that preclinical evidence for the possibility of this experiment succeeding was provided by transducing the TNF gene into mouse and human tumor cells and then transferring these into syngeneic mice or human tumors which spontaneously regressed due to local accumulation of TNF being produced by the tumors. A further study was done at the behest of the NIH Institutional Biosafety Committee that showed that anti-TNF antibody could abrogate the regression of the tumor, thus demonstrating indeed it was the TNF production by these tumor cells that led to the regression. Studies also were performed showing that local injection of TNF into tumors could lead to tumor regression.

Dr. Rosenberg said a two-gene construct was developed for the protocol using the TNF gene promoted by the retroviral LTR and the neomycin resistance gene with the SV-40 promoter, although the initial protocol did not call for selection of these cells. Studies were performed which showed that when these genes were inserted into TILS, secretion of TNF by these transformed cells was increased 100-fold.

Dr. Rosenberg said that TNF was very toxic, causing severe hypotension and low blood pressure in patients receiving it intravenously in high doses. Therefore, the investigators were cautious in selecting an appropriate starting level of TNF which was calculated to be .07 mg/kg based on a 70 kg human receiving 10 TNF-transduced TILs. It had been shown previously that a human can withstand a dose of 8 mg/kg of soluble TNF without toxic consequence, but since TILs can accumulate in liver and other organs, this technique may result in different local toxicity. Further, humans had previously been exposed to 1.2 mg/kg of TNF as a result of LAK cell therapy. Therefore, the chosen level was significantly lower than that already shown to be safely tolerated in humans.

Dr. Rosenberg said the protocol calls for infusion of escalating doses of transduced TILs at 3-week intervals in combination with IL-2 given at 180,000 iu/kg every 8 hours. The dose escalation then would be escalated to reach 2-3 X 10 cells, at which level one would predict enough TNF would be produced at the tumor to cause tumor regression. Subsequent stages of the protocol would then call for administration of selected cells and then higher doses of IL-2.

Dr. Rosenberg said that the protocol had undergone a modification as a result of a request from the Food and Drug Administration (FDA) to lower the initial starting dose to 3 X 10 cells and to eliminate co-administration of IL-2. However, the FDA said that if this dose escalation was used, a weekly escalation was permissible rather than once every three weeks. When a maximum tolerated dose is reached, the dose is reduced to one-third of this and then a new triweekly escalation using this one-third dose, in combination with IL-2, is started. This modification was brought about by the desire on the part of

the investigators to use a vector fromGTI, but because the safety studies were carried out with a Cetus-produced vector, the FDA had thought it necessary to drop the starting levels.

Therefore, the protocol had begun with a weekly dose of 3 X 10TNFTIL cells, and that the first 2 patients had received these doses. On February 8, 1991, the first escalation to 3 X 10 cells will take place. This will cause a delay of about 5 weeks in terms of getting the doses ofTNFTIL in combination with IL-2, but that it is being done in the interest of patient safety.

Because of the extensive disease in the patients currently under treatment, Dr. Rosenberg thought the experiment would put the investigators in a position to make a comment on the effectiveness of the cells. However, because 40% of patients respond toTILs without the TNF gene it will require a larger series of patients to see whether or not this modification represents an improvement. He hoped to treat 50 patients with the TNFTIL cells over the course of the next year.

Dr. McIvor asked if there was any change in the amount of expression the investigators were getting from the transduced TILs before they are infused into the patients. Dr. Rosenberg said that they were not capable of getting the gene into all of theTILs being produced by the patients and that it was difficult to do, but by following cell cycle kinetics of the TILs and adding the vector exactly when the cells were in S phase, the transduction efficiency was being improved.

Dr. McIvor asked if negative control samples were being taken from non-tumorous tissues to indicate homing to tumor relative to normal tissue. Dr. Rosenberg said that they had tried hard to get this data but that so far the only option to get normal tissue has been at autopsy. Biopsies have been taken from two patients who had died. Using PCR there was no finding ofTILs in normal tissue in one case, but in the other case there was a positive finding in one side of the renal cortex. This was thought to be due to contamination and that PCR is being repeated. There was no evidence of continued survival in normal tissues at a sensitivity of 1 in 100,000 cells.

Dr. Atlas noted that the original protocol was designed to determine a maximum tolerated dose, and he wondered if the change in the protocol, starting at a lower dose and increasing the dose twice as fast, will affect the ability to determine what the real maximum entry dose level should be. Dr. Rosenberg said this was a good point but noted that toxic effects ofTNF would be expected to be seen hours after infusion, although some might take longer because of slower clearance from the liver. The patients were being closely monitored as far as central venous oxygen saturation, systemic vascular resistance, and cardiac output, and to date no changes in any parameter have been noted. To date, the patients had been treated on an out-patient basis because of the lack of any signs of deleterious effects. However, when the doses are escalated to higher levels he anticipated they would need to remain hospitalized.

Dr. McGarrity asked the committee members how long they thought that it would be necessary to continue to receive these interim reports from the investigators on approved protocols. He also asked whether there needed to be some clarification as to what is a "minor" modification or change so that neither the investigators nor the committee are put in the position of being surprised by something in the future.

Dr. Walters said the issue of how long interim reports should continue had not been discussed by the HGTS. He noted that theHGTS did receive correspondence between the FDA and Dr. Rosenberg relative to the changes that had been made to the protocol.

Dr. McGarrity asked where the delays had come in the final approvals necessary to begin theTNFTIL protocol, and whether they were due to technical difficulties with the FDA, or internalNIH delay. Much mention had been made of delays, and he thought it was good for the committee to be aware of the

situation in this case. Dr. Rosenberg said the FDA approved the protocol on January 8, 1991. Dr. McGarrity noted that this was considerably longer than the 30 days in which the FDA is required to act on a submission. Dr. Rosenberg said they did respond within 30 days but their response asked for more data on potential toxicities and changes in doses. Negotiations between the investigators and the FDA resulted in the protocol not being approved by the FDA until January 8, 1991.

Dr. McGarrity thanked Dr. Rosenberg for his update on the TNFTIL protocol and noted for the record that a written update was also submitted on the neomycin-resistant TIL protocol.

Dr. Walters said for the first few human gene therapy protocols, it may be worthwhile to continue to get updates at each meeting of the RAC, primarily on issues of safety. It will be much longer before any efficacy data will be available. However, since this research is so novel, these reports may help the RAC and HGTS in their ability to monitor these protocols.

Dr. McGarrity thanked all the investigators and wished them continued success in their research efforts. He then called on Dr. McIvor to present the next agenda item.

V. PRECLINICAL DATA (ANIMAL MODELS) FOR HUMAN GENE THERAPY PROTOCOLS:

Dr. McIvor noted that the HGTS had approved 5 protocols, two of which would be before the committee today for consideration, and that the RAC had already approved three such protocols. However, in all cases there seemed to be good reasons why good preclinical data could not be provided with the protocols. He was concerned that a major policy was being set as a result of the case-by-case consideration of these protocols, and it was time the committee considered the role and expectations of preclinical studies in human gene transfer protocols, separate and apart from any specific protocol being discussed.

Dr. McIvor said it was apparent to anyone considering human gene therapy that experiments in animals are of the utmost importance to demonstrate the feasibility of proceeding to human trials. This approach sets an extensive precedent in the evaluation of new clinical therapies for human disease. However, Dr. McIvor was concerned that, unless the RAC takes the time to clarify its expectations in this regard, investigators submitting protocols in the future will consistently fall short in supplying such data.

Dr. McIvor said that perhaps the *Points to Consider* document should be modified by incorporation of a phrase such as "results demonstrating the efficacy and feasibility of the proposed procedure using the most relevant animal and/or cell culture model systems should be included." This would be a stronger statement than what is currently contained in the *Points to Consider*, which simply asks the investigator to describe results obtained in preclinical studies. He asked that this item be placed on the agenda for the next RAC meeting and said he would welcome comment from the rest of the committee on this issue.

Dr. Anderson said that it was true that the protocols that had come forward to this point had not had substantial animal studies on efficacy, but added that this was because of the lack of animal models. In contrast there were substantial adequate safety studies performed in animals to address issues of safety and that it was only the efficacy studies which were lacking.

Dr. McIvor understood this but noted that efficacy was an issue to be considered in determining feasibility. He was afraid that the message being sent to investigators was that there is no necessity to do animal work.

Dr. McGarrity asked Dr. Walters if this issue could be brought before the HGTS at its next meeting. Dr.

Walters ensure that it was placed on the agenda for discussion at the next meeting.

Dr. McGarrity then called a 15 minute recess for coffee and asked the committee to reassemble at 10:30 a.m.

Dr. McGarrity called the committee back to order after the morning recess and called on Dr. Gellert to present the next agenda item.

VI. PROPOSED ADDITION TO APPENDIX D OF THE "NIH GUIDELINES" REGARDING A HUMAN GENE TRANSFER PROTOCOL ENTITLED "THE ADMINISTRATION OF INTERLEUKIN-2, INTERLEUKIN-4, AND TUMOR INFILTRATING LYMPHOCYTES TO PATIENTS WITH MELANOMA":

Dr. Gellert said that this proposal was very similar to the first approved protocol using a neomycin resistance gene to mark TILs in melanoma patients. The main difference was that the cells would be cultured in IL-4, and that IL-4 would also be administered to patients. He said that Dr. Lotze had given assurance that IL-4 had already been used at NIH to expand cells in culture. However, data on such experiments had never been reviewed by the RAC, and Dr. Gellert suggested that it may be useful to have information on any significant differences that had been noted when using the IL-2/IL-4 cell expansion.

Dr. Gellert questioned the rationale contained in the protocol for dose escalation which called for escalation to continue unless 100% of patients reached Grade IV toxicity and asked for further discussion on this issue. Further, the protocol was unclear as to the doses of cells to be administered in this protocol. One of the new features of the protocol was supposed to have been that the fraction of gene-marked cells given would be greater than in previous protocols, using 50% of marked cells. However, the protocol contained a statement to the effect that a previous protocol had already been conducted at a 50% level. Furthermore, the protocol implied that in one experiment 100% gene-marked cells would be used but at a very small dose. He asked for clarification of this statement.

He was not particularly concerned about using high proportions of transduced cells containing the neomycin resistance gene, but rather he was concerned about the manipulations necessary to transduce the cells and select for G418 resistance. In such cases, it would be difficult to ensure that the cells surviving such manipulations would have the same properties as the initial cells and whether their tumor homing and tumor killing capabilities would be the same.

As far as the possibility of insertional mutagenesis, Dr. Gellert said that the protocol called for extensive testing of the cells *in vitro* before reinsertion. He did not know of any way of testing this that would be relevant. By using a retroviral vector there will be no way of controlling the location of gene insertion and therefore genes could be activated or inactivated at any position in the genome.

Dr. Carmen said his review was more from a layman's standpoint, since his background was in social science, and that he would split the review into issues of assessment of the research design and informed consent. He agreed that the protocol was very similar to the Anderson-Blaese-Rosenberg protocol involving the Neo-marked TILs, but that he was confused by the fact that there were 5 mini-protocols under the umbrella of this overarching protocol that involved patients suffering from cancers other than melanoma. Furthermore, that in response to a question from Dr. Parkman, Dr Lotze had replied that,

> "The main goal of our series of protocols is to evaluate the ability of combinations of IL-2 and IL-4 to enhance the antitumor efficacy of the adoptive transferred TILs."

Dr. Carmen said that this sounded more like a Phase II trial than a Phase I trial. Therefore, it seemed that a risk-benefit assessment must be performed and he went about it in the following two steps:

a. Assessment of the TIL cell expansion, *in vitro*, in the presence of both IL-2 and IL-4, followed by an application of retrovirally mediated genetic transduction to a small portion of the TILs; and,

b. Assessment of the introduction, *in vivo*, of the mutant artifacts in conjunction with IL-2 and IL-4.

Dr. Carmen said that after using this assessment technique he thought that, judged solely as a gene transfer exercise, the protocol seemed reasonable and appropriate, using the Anderson-Blaese-Rosenberg regimen, to track the lymphocytes being used. As a TILs cell therapy exercise, the benefits to the patients were speculative, but the risks to the patients, given their terminal status, were minimal.

Dr. Carmen said that when the Anderson-Blaese-Rosenberg protocol to treat patients with severe combined immune deficiency (SCID) patients was before the RAC, he had asked the investigators to include in their informed consent documents disclosures which would identify the contents and purposes of all alien DNA employed as recombinants which the investigators agreed to supply. In the protocol before the committee, nothing is included which tells the patient either what the gene is that is being employed or information about the retroviral vector. He identified this as the only deficiency in the informed consent document.

Dr. Walters said that when the protocol was approved by the HGTS, one of the stipulations was to include the definition of how the homing experiments were to be carried out. A revised informed consent form was to be supplied and this was to include the following changes:

1. That a list of co-investigators and associate investigators be deleted from the informed consent document;

2. That the investigator add, at the beginning, a statement of the purpose of the study, highlighting that there will be gene marking of some of the cells that will be infused into the patient with an emphasis on what is different in this case from the kind of therapy that otherwise would be given;

3. Editorial changes in the discussion of the issue of confidentiality;

4. Editorial changes in the discussion of the issue of long-term withdrawal from the protocol to include the patient's right to withdraw, but also stressing the importance of staying with the study on a long-term basis;

5. Removal of an error introduced by word processing which caused two sentences to be juxtaposed relating to potential side effects; and,

6. Clarification of issues as to what the patients would be paying for and what costs would be borne by the study, as well as any additional costs which may accrue to patients as a result of their participation in the experimental portion of the study.

Dr. Walters noted that the subcommittee had given approval for the proposal to come directly to the RAC via the primary and secondary reviewers for the subcommittee.

Dr. McIvor asked if Dr. Walters found the methodology for the homing experiments to be sufficiently clarified. Dr. Walters had not seen anything written that updated the protocol in any way, and he supposed that the investigators would be addressing this in their comments.

Dr. Geiduschek said that despite the fact that only a small portion of this protocol came directly under the auspices of the RAC, it was important that the investigators appropriately inform the patients that the gene marking aspect of the protocol is of no conceivable benefit to the patient. Further, it appeared that the patients were defraying a substantial portion of the cost of the study. He was worried about the long-term consequences of allowing such a protocol to go forward without more detailed consideration by the committee.

Dr. McGarrity noted that traditionally prior to 1981 RAC review was entirely scientific in scope. However, after the publication of the National Academy of Sciences' document entitled *Splicing Life*, it became apparent that ethical issues needed to be considered in review of human gene therapy protocols. Dr. Walters noted that it was the President's Commission on Bioethics that produced the report, *Splicing Life*. Although it contained a thorough discussion of the philosophical aspects of human gene therapy research, it was the result of a request by the then-Chair of the RAC, Mr. Robert Mitchell, that the RAC began discussing human gene therapy research. As a result of this discussion, the *Points to Consider* document was developed to look at the scientific issues as well as social and ethical considerations such as informed consent, privacy, and confidentiality.

Dr. McGarrity then summarized what had been requested from Dr.Lotze thus far. Further issues for discussion were gene transduction efficiency, informed consent, and patient costs. He also noted that on page 16 of tab 1413, the final paragraph, as it was stated:

> "Although we plan to use LNL6 for the protocol submitted, if a superior vector becomes available we would use it. We would make this substitution only with the approval of the FDA and notification of the RAC."

Dr. McGarrity said that such a change would require not only notification of the RAC but approval of the RAC.

Dr. Anderson said that this particular wording was taken directly from the N2/TIL protocol and was a result of HGTS insistence that the investigators switch to the LNL6 vector, rather than using the N2 vector, despite lack of FDA approval of the LNL6 vector. This had set a precedent, which he had included in several documents after that point, that the replacement of a superior vector is not even a minor amendment that requires approval. The intention was to allow use of safer vectors as they are developed, and to be able to substitute them with FDA approval.

Dr. McGarrity said this particular substitution of vectors had been the basis for the development of the rapid review process for minor changes. In light of this new process now being in place it would be the one that should be followed. Dr. Anderson said that several other protocols, including Dr. Brenner's, contain the exact same wording. Since it is recognized now that such a vector substitution is a minor

modification, the wording will have to be modified in these protocols.

Dr. McGarrity called on Dr. Lotze to present the protocol and respond to the questions put forward by the committee. Dr. Lotze said he would try to discuss comments that arose both in the meeting of the HGTS and in this meeting.

Dr. Lotze said that this research represents a costly investment to the institutions embarking on it. The estimated cost of merely growing up cells for adoptive transfer is in the range of $20,000 to $30,000 dollars per patient. Dr. Lotze said his comments should be taken in light of these factors.

He said that the original protocol submitted to the RAC called for administration of IL-2 with TILs and that in the time that had elapsed between the original submission and today, the protocol had undergone several iterations in an attempt to improve on the therapy. The introduction of IL-4 into the protocol was one attempt to enhance the growth of TILs and is something which has been shown to increase growth and activity of TILs. Approximately 20-30 patients had received TILs grown in combinations of IL-2 and IL-4 and that there were no differences in terms of responsiveness. He identified one patient who had received transduced TILs, a portion of which were grown in combinations of IL-2 and IL-4.

Dr. Lotze said there were two approaches to improving this therapy:

1. The introduction of cytokines, such as TNF, which can improve the efficacy of the cells; and,

2. Methods to improve the ability of the cells grown in tissue culture to be able to home to tumor sites more efficiently and perhaps engraft and persist for longer periods of time.

Dr. Lotze said his protocol was seeking to use the first approach, to use cytokines which are believed to be important for improved T cell growth and activity.

He said there was substantial information gained from treating over 100 patients at the National Cancer Institute (NCI) with IL-4 alone, or IL-2 plus IL-4. In fact, the major goal of this protocol is to obtain tumor specimens repeatedly in an attempt to assess whether TILs have targeted to the sites in ways that have been anticipated.

Dr. Lotze said it had become apparent that there are toxic effects associated with this type of therapy. Because of the terminal nature of the patients who will be entered into the protocol, and the fact that they have failed all other known therapies, the risk to the patients is minimal.

In responding to questions as to the actual dosage of cells to be used, Dr. Lotze admitted that the protocol was somewhat confusing. He explained that they would take approximately half of the TILs initially, attempt to transduce them, and insert the neomycin phosphotransferase (NPT) gene. The goal is then to give no more than 10% of the transduced TILs along with non-transduced TILs. He had assayed TILs into which the NPT gene was inserted and there was no difference in terms of cytolytic activity. The question of how the cells would react *in vivo* is another matter and can only be answered by giving a pure population of such cells, which is not the intent of the protocol. In previous studies it has been possible to transduce upwards of 50% of the cells, but only 20% end up becoming marked with the NPT gene.

Dr. Lotze said that the issues regarding tumorigenesis were difficult to resolve, but there had never been an identifiable tumor caused in subhuman primates using this identical vector, even when giving

concentrated cultures of this virus intravenously. There were no instances of tumor production by the virus in murine models. This does not mean that it cannot occur and noted that it was still of concern to the investigators, even to the point of their evaluating the potential use of other vectors that can be very precisely inserted. The vector they were exploring was a adeno-associated virus vector which inserts on chromosome 19. The vector has not been available yet for study but is currently being evaluated. He underlined that the vector they are planning to use is the identical vector that has been used in patients previously and as yet there is no evidence of tumorigenesis in animals.

Dr. McGarrity asked whether the cost estimate of $20,000-$30,000 for adoptive transfer was based on just working with the TILs and disregarded the marked gene. Dr. Lotze said that this is just the cost associated with the TILs and there is no cost charged for marking. None of the very experimental components of the therapy would be charged to the patient, including the gene marking.

Dr. Krogstad asked what the patients would be billed for in these studies. Dr. Lotze said that his institution had a long history of involvement in testing novel therapies such as liver transplantation and that it had championed the use of biologic agents. Every attempt is made to obtain payment from third party payers prior to administration of therapy to patients. A large infrastructure has evolved to assist patients so that no unreasonable costs are borne by the patient. Such costs are handled on a case-by-case basis.

Dr. Krogstad was concerned with the continuing struggles relative to payment by third party payers. He wondered whether this would not be such an impediment to human gene therapy that it may in fact bring into question whether it can be performed outside the confines of theNIH. Such circumstances could limit the transfer of this technology from experimental to applied research and into general practice.

Dr. Lotze remarked that cautions were well founded but that he wanted to keep these issues separate from the direct issues surrounding approval of the protocol by the committee. He reiterated that no costs associated with the experimental therapy, including the administration of TILs and gene marking of TILs, would be borne by the patients.

Dr. Gellert said that one issue that still remained was the fact that third party payers may pay for the cost o direct treatment, but that they often refuse to pay for the cost of complications that result from experimenta treatments, and he asked for Dr.Lotze's comments on this. Dr. Lotze said this was a problem outside the confines of a Federally supported institution such as the NIH, and that it was a societal issue that must be addressed. Even the NIH has a proviso in its informed consent to the effect that there is no guarantee that even the Federal government will make payment for injuries incurred as a result of experimental therapies. This was not his area of expertise, but he believed his protocol was reflective of the standard approach that is taken both inside the NIH and in academia, in dealing with the conduct of experimental therapies.

Dr. Lotze wanted to make it clear that both a bacterial gene and amurine virus were being used in this protocol. He asked that he be allowed to forward a revised protocol to the RAC by the end of the week incorporating all the changes that had been discussed at the HGTS and here in this meeting.

Dr. Post had two questions for Dr. Lotze. One question related to G418 selection. He asked whether this had been done before in TILs. His second question dealt with mention in the protocol of transduction of bone marrow cells.

Dr. Lotze said that the reference to bone marrow cells was a word processing error which had crept into the protocol and had been removed from the current version of the protocol. However, the issue of G418 selection is deceptive in that sometimes cells that contain the gene will grow up in G418; other times

these cells are less well protected from the effects of G418 and they do not grow. The intent of the protocc is to select in G418 to maximize the chance they would be able to detect the cells in the peripheral blood. A goal of the protocol would be to attempt to carry out parallel cultures. That is,TILs selected in G418 and TILs which were not selected. He would attempt to infuse cells that were cultured in G418 since they would be more likely to carry the marker gene and provide better information. However, because of G418's toxicity to mammalian cells, this may not be possible. Thus, the investigators would like to be able to give selected transduced cells but also have the possibility to give unselected suitably marked cells as well.

Dr. Gellert was not clear on the issue of the Grade IV toxicity. He asked if it was really the intent to stop escalation only if 100% of the patients encountered Grade IV toxicity, and what would be expected to be seen at higher dose levels.

Dr. Lotze said that the protocol defined Grade IV toxicity as "platelet counts that fall below 25,000 per cubic millimeter." This is a level of toxicity which precludes further administering the therapy until the platelet count comes back to normal, because of problems associated with bleeding. However, this would not prevent going ahead and retreating the patient after the platelet count had returned to normal.

He said the reason for a step-wise dose escalation was to introduce an additional level of safety into the protocol. It was known that humans could tolerate the higher levels of IL-2/IL-4 safely and that there had not been any deaths in patients receiving this combination at the high or low doses. TILs do not add substantively to the toxicity associated with IL-2 administration. However, since TILs have not been given to patients along in combination with IL-2/IL-4 at the higher dose it is thought safer to introduce a lower dose escalation to err on the side of maximum safety. The decision to proceed will be based on, once again, patient safety and if no severe toxicity is associated with TIL administration with IL-2/IL-4 at the lower level, then the dose would be escalated to the higher level.

Dr. Anderson said that although Grade IV toxicity is deemed to be associated with life-threatening non-reversible toxicity, in cancer patients this is not the rule. Commonly it is a result of accrued IL-2 toxicity over time and once administration of IL-2 is stopped, the patient's symptoms reverse and within 24 hours the patients are back to near normal.

Dr. Lotze emphasized that there appear to be dose-response relationships in cancer that show that increases in treatment are associated with enhanced response rates. This is true also for the use of IL-2 single agent treatment.

Dr. Walters called for further discussion on the issue of how the homing experiments will be performed. Dr. Lotze said the HGTS had raised the question of why these studies were being carried out. The reason for doing the studies was to test questions ofTILs homing and persistence. The consent document as well as the revised protocol clearly states that biopsies will be done repeatedly and in fact is the major goal of the protocol. Dr. McIvor said that the subcommittee had voted to approve this protocol with the idea in mind that it would come to the RAC with all of the details spelled out on the homing and persistence experiments. Further, he did not feel any direct preclinical data had been presented to show that the cell marking experiment could work.

Dr. Lotze reiterated that the major goal of the protocol was to track the cells and try to see if they migrate into tumor sites better than in blood or skin. That despite what the protocol seemed to say, this was the major goal. As far as preclinical data, Dr. Lotze said that information was provided in terms of his previous experience with IL-2 and IL-4 in patients, which was recently published in *The Journal of Experimental Medicine*, which shows that IL-4 in addition to IL-2 causes enhanced growth ofTILs and allows for a

decrease in the non-specific cytolytic activity. Animal model data for this protocol was no better or worse than that for any protocols so far approved. The major problem again was the ability to transduce TILs in the mouse model in the same way as with humans cells. Dr. McIvor said that if preclinical data did in fact exist, it was not included in the protocol and that it should be.

Dr. R. Murray brought up a procedural question dealing with the method in which the subcommittee had approved the protocol. The subcommittee provided the investigator with a detailed list of conditions under which it would approve the protocol in order for the RAC to review it at this meeting. What he had before him was the same exact protocol as was received at the HGTS meeting, and that the reviewers had in fact been asked to review the same protocol without substantive changes. Historically there had been a problem when a protocol was forwarded to the RAC for consideration without approval of the HGTS, and he concluded that this situation was being repeated in this case.

Dr. McGarrity said that the subcommittee had approved the protocol to go forward to the RAC with supplemental data. It was the committee's decision to determine whether this supplemental data had in fact been supplied.

Dr. R. Murray said that his impression was that this data was to have been supplied in writing so that the reviewers could view the complete revised protocol, including the supplemental data, in order to form a judgement on the merits of the protocol.

Dr. B. Murray noted that the question was not whether the HGTS and the RAC were following its own procedures, but whether the investigators had followed through on their promise to supply supplemental data before the protocol was reviewed. She said that apparently they may not have complied with the stipulations placed on them by the HGTS.

Dr. Lotze responded that the subcommittee had given the protocol a very fair hearing and that most of the changes they requested were really for clarification rather than substantive changes. Since he had never received any formal comments from the subcommittee and since he had heard that the protocol had been submitted to the full RAC, he thought the explanations and clarifications given were sufficient. He had made pen and ink changes to his own document, and he was aware that these were not generally available to the reviewers and the committee in their copies. He was aware that the RAC would have the right, based on what he now understood of the approval being granted with stipulations, to table discussion on the protocol and await the data, but that he and his group were anxious to push ahead with the protocols.

Dr. Wivel noted that a portion of the transcript of the meeting of the HGTS was sent to Dr. Lotze via FAX detailing the six questions which were the basis for the provisional approval of the protocol. Dr. Lotze said he had never seen this document.

Dr. McIvor said the question then before the committee was whether or not it was possible to undertake an adequate scientific review of the proposal with the material submitted.

Dr. McGarrity asked for a recommendation from the floor, either in the form of a motion or in the form of friendly advice, in order to come to closure on this agenda item.

Dr. Anderson regretted that he had not thought to ask Dr. Lotze if he had received the comments from the HGTS meeting. Dr. Anderson noted it was the expressed intent of the subcommittee that a revised protocol be available for the RAC to review. The revised protocol was available back in Pittsburgh. If there was a procedure in place whereby the protocol could be temporarily approved, pending a review by the

primary and secondary reviewers of the revised protocol to ensure the verbal statements had been carried over into the written version of the protocol.

Dr. Gellert was confused by references to a revised protocol, and thought it was unfortunate that some sor of miscommunication had resulted in the revised protocol not being available for review. However, the committee had gotten into trouble before by approving a protocol and then taking a telephone vote to confirm informal agreements made at the meeting. Dr.Gellert then made a motion to postpone a vote on this protocol until such time as the revised protocol has been made available for review. This could be done by the next meeting of the RAC.

Dr. B. Murray seconded the motion. She added that she was in attendance at the HGTS meeting, and that she believed it was evident that the subcommittee was asking that the revised protocol be made available to reviewers prior to the meeting of the RAC.

Dr. Carmen asked what the consequences of a 4-month delay would be. Dr.Lotze said his primary concern was that a large group of investigators had been formed at the University of Pittsburgh, who were enthusiastic about working on the protocol. Further, patients had indicated an interest in receiving immunotherapy based on recent publicity in both local and national media. The investigators would move along with all other aspects of the protocol outside the purview of the RAC. He was concerned that this delay could result in preventing all the information being gleaned from these protocols due to the inability to perform the gene marking experiments.

Dr. Atlas asked how many of the questions that were being asked dealt with portions of the protocol dealing with gene marking versus the question of IL-2/IL-4 administration.

Dr. Walters read the motion made by Dr. Epstein at the November 30, 1990, meeting of the HGTS. His motion was stated as follows:

> "I move that we give this protocol provisional approval, contingent upon the investigator bringing to the RAC an amended protocol defining how the homing experiments will be done, and an amended consent form to be consistent with that."

He said this text clearly indicates a request for information on being able to determine in a semiquantitative way, the extent of specific homing of gene-marked TILs to tumor.

Dr. Harriman of the National Science Foundation asked whether it was possible to have the revised protocol FAXed from Pittsburgh over the luncheon recess so that the committee could have the option to be able to deal with it during the meeting. Dr. Lotze agreed to do this if the committee thought this were an option.

Dr. R. Murray clarified that it was unfair to limit review only to the portions dealing with recombinant DNA, in that it was a portion of an overall protocol which could not be taken out of context. It would be necessary to see a revised complete protocol.

Dr. McIvor suggested that if the protocol is deferred, that it be sent back through the HGTS, so that at the next RAC meeting a much more thorough evaluation could be presented in terms of the changes that were being requested.

Dr. Walters felt a bit guilty in that, as a reviewer, he had not begun his review earlier at a time when he could have advised Dr. Lotze that he needed to submit the revised protocol. Dr. Lotze said that it was his own responsibility and that if the committee would wish to see the revised protocol, he could have it FAXed over the lunch hour.

Dr. R. Murray felt it not necessary for the protocol to go back to the HGTS. However, he offered a substitute motion to Dr. Gellert's motion to defer. Dr. R. Murray moved that he wished to "change Dr. Gellert's motion to say that we would review the materials as soon as they were available" and to exclude the words "next meeting" in Dr. R. Gellert's motion.

Dr. McGarrity asked Dr. Gellert if he would accept such a change in wording. Dr. Gellert agreed provided the changes were such that they could be reviewed in a finite time.

Dr. R. Murray reminded the Chair that his motion was made as a substitute motion, rather than a friendly amendment, although if Dr. Gellert were to reword his motion he would withdraw the motion for a substitute motion.

Dr. Atlas seconded Dr. R. Murray's motion.

Dr. McIvor asked if the revised protocol included a detailed proposal on how the homing experiments are going to be done with the clinical materials collected from the experiments. Dr. Lotze replied that his recollection was that the revised protocol contained a paragraph stating that the investigators would obtain blood, skin biopsies and tumor biopsies, when possible, at fixed intervals.

Dr. Geiduschek pointed out that this seemed to be a technically difficult problem to assess, and he expressed a hope that the expertise around the table was sufficient to be able to quickly assess the revised protocol. He asked if it was possible to assess homing using blood, skin and tumor biopsies. Dr. Lotze said that it was possible in light of experiments performed by Dr. Rosenberg in which he was able to define homing of TILs to sites of tumor with radiolabeled markers using blood and skin as controls. Since radionuclide markers dissipate quickly, there is no ability for long-term testing.

Dr. R. Murray called the question on the substitute motion.

Dr. Anderson called for a point of order on whether such a motion was consistent with the Administrative Procedures Act which required voting to occur in a public meeting. Dr. R. Murray said that the intent of his motion was that the vote on the protocol be delayed until later in this meeting pending receipt and review of the revised protocol. If the reviewers were unable to comment on the revised protocol during today's meeting, then it would become necessary to delay any vote until the next meeting of the RAC. Dr. McGarrity underlined that in either case it would be voted on in a full, open meeting of the RAC.

Dr. McGarrity called for a vote on Dr. R. Murray's request to call the question on the substitute motion. The vote was unanimous, 16 in favor, 0 opposed, and no abstentions, to call the question. Dr. McGarrity then called for a vote on the substitute motion offered by Dr. R. Murray. The motion passed by a vote of 13 in favor, 2 opposed, and 1 abstention.

Dr. R. Murray clarified that the sense of his motion was that the reviewers must be able to clearly identify the responses to the concerns expressed. If it were not possible to do that during today's meeting, a vote on the protocol would not be taken until the next meeting of the RAC or such later time when the materials were made available and review had been completed.

Mr. Mannix noted that the RAC was governed by the Federal Advisory Committee Act, not the Administrative Procedures Act, as alluded to by Dr. Anderson. Dr. Anderson said he had misspoken, and that he had quoted the wrong act.

Dr. McGarrity suggested that the revised protocol be copied in sufficient quantities as to allow all members of the RAC to be able to comment on it. However, he requested that the primary and secondary reviewers be given the first copies available so they could begin their review in a timely manner. He then adjourned the committee for its lunch recess and asked members to return promptly at 1:00 p.m.

Dr. McGarrity called the committee to order from its lunch recess at 1:10 p.m., and called on Dr. McIvor to present the next agenda item.

VII. PROPOSED ADDITION TO APPENDIX D OF THE "NIH GUIDELINES" REGARDING A HUMAN GENE TRANSFER PROTOCOL ENTITLED " AUTOLOGOUS BONE MARROW TRANSPLANT FOR CHILDREN WITH ACUTE MYELOGENOUS LEUKEMIA (AML) IN FIRST COMPLETE REMISSION: USE OF MARKER GENES TO INVESTIGATE THE BIOLOGY OF MARROW RECONSTITUTION AND THE MECHANISM OF RELAPSE":

Dr. McIvor said the problem that was being addressed in the protocol was the high rate of tumor relapse in the treatment of acutemyelogenous leukemia (AML) by autologous bone marrow transplant. In the treatment, marrow is first obtained from theleukemic patient. Next, the patient undergoes chemotherapy to eliminate tumor cells in the body, and then the marrow isreinfused to reconstitute the patient's hematopoietic system. Dr. McIvor said some patients remain disease-free for extended periods following such treatment but that the tumor regenerates in a large proportion of cases. The issue was whether the chemoradiotherapy was insufficient to eliminate tumor in the patient's body, or whether the regeneration of tumor was made possible by cells emanating from the reinfused bone marrow.

Dr. McIvor said the investigators planned to address this question by exposing the marrow to a retroviral vector prior to reinfusion. If integrated retroviral provirus was found in the tumor, this would be evidence for an infusion-derived regeneration of tumor. If identical integrants were found in both normal andtumorous material post-transplant, this would implicate the involvement of stem cells in AML.

Dr. McIvor said the disease is very serious and results from such a study would provide information which may improve therapy. Further, the overall approach was well thought out and*in vitro* preclinical data were provided to demonstrate that gene transfer into humanleukemic cells is capable of colony formation. Dr. McIvor had some questions about the protocol, namely:

1. Assessment of safety risks needed to be looked at since the retroviral vector will be introduced into marrow, including stem cells, which would be capable of long-term reconstitution in the patient and there is a chance that the newly introduced gene sequence could persist in the patients for the rest of their lives.

2. On the issue of efficiency of gene transfer there needed to be further clarification on how the investigators would scale up from the preclinical *in vitro* experiments to an entire human bone marrow transplant protocol. The investigators had provided details in Appendix A in this regard, but that clarification was still needed on the differences between the procedure and the conditions used to generate the *in vitro* data.

3. In regard to the molecular analysis of the regenerated tumor, questions still exist concerning evidence that tumor cells can be transduced, injected into animals and recovered for analysis,

which was not discussed despite the fact that a rat model for human AML has been established and studied extensively.

Dr. McIvor said that in the absence of*in vivo* results on tumor cell tagging and recovery, it was quite likely that marked cells could not be found in regenerated tumor and therefore the results of the experiment will be uninformative. However, because of the small risk associated with the procedure and the fact that useful information may be generated, he could recommend approval of the protocol with an addition to the informed consent document. He asked that the following statement be inserted into the informed consent document:

> "I understand that while useful information might be generated from my participation in this study, it is also possible that no useful information will be generated."

He said this was necessary to properly inform people participating in the study that there is a possibility that no meaningful information may result if tumor tagging does not occur. Further, he urged that the wording on the fourth line of page 111 of the mailing be changed to reflect that the virus containing the marker has been "**extensively** disabled," rather than "**strongly** disabled." Dr. McIvor spoke with Dr. Childress in relation to the issues of the informed consent document and that Dr. Childress had been in complete agreement with him.

Dr. McGarrity noted that Dr. Childress was absent because of the necessity to deal with departmental business at his institution. Dr. Childress sent a FAX which stated:

> "My review of the materials is not thorough enough or careful enough to justify a written evaluation. I is too impressionistic. LeRoy Walters will, I'm sure, cover thoroughly and carefully what I may have been able to provide."

And with that, Dr. McGarrity called on Dr. Walters. Dr. Walters said that Dr. Epstein had moved to approve this protocol and that Dr. McIvor had seconded the motion and that five stipulations had been placed on the approval, two dealing with the consent form and assuring that the desirability of long-term follow-up was mentioned, as well as issues surrounding publicity and privacy issues. The investigators were also asked to prepare an assent form for children who were old enough to understand what was going on in the study. The final two points, raised by Dr. McIvor, concerned the efficiency of theretrovirally-mediated gene transfer into human bone marrow cells and analysis of the origin and character of the regenerated tumor. Dr. Walters said the final vote to approve the protocol with the 5 stipulations was 10 in favor, 0 opposed, and 1 abstention.

Dr. Walters reviewed the revised informed consent document and that the two points on long-term follow-up were addressed. The assent form was also included in the revised protocol. He believed the assent form needed to include the 14-year follow-up to more closely mirror the informed consent document, but otherwise it appeared the investigators had complied with the 5 stipulations, subject to Dr. McIvor's critique on the issue of gene transfer into bone marrow.

Dr. Geiduschek asked about the issue of what costs of this proposal would be borne by the patients.

Dr. McGarrity called on Dr. Brenner to make a brief presentation. Dr. Brenner thought that Dr. McIvor had summarized the aims of the study reasonably well. Dr. Brenner was concerned over whether the marked gene will enter the stem cells is not a question that can be answered definitively at the present time. There was no evidence that this would occur. As to the issue of whether the conditions of transduction used in the *in vitro* preclinical studies would match exactly the conditions in the patients, he said that they would. At present, they had not scaled this up to cell numbers representing full marrow conditions. If approval for the protocol was granted that they would perform a preliminary experiment on stored marrow to make sure that scale-up is possible. In all other respects, the methodology was exactly the same and there was no use of growth factors contemplated for the experiment.

Dr. Brenner noted that in regards to the issue of the terms "extensively" and "strongly" in the informed consent document, the original wording had been "extensively." However, due to a rule that the informed consent document be worded at a fifth grade reading level, the word was changed via a computer program to concur with a fifth grade vocabulary.

Dr. Brenner said that at St. Jude's Hospital all patients are cared for regardless of ability to pay and that all costs incurred, regardless of the manner in which they are incurred, are fully covered. Dr. Brenner also concurred with Dr. Walter's comment regarding inclusion of the 14-year follow-up in the assent document and said that this would be revised and included.

Dr. Post asked what the prognosis was for the children who would take part in the protocol and whether there was a likelihood that a significant percentage of them would completely recover. Dr. Brenner said the initial reason for the protocol was to improve the percentage of full recoveries from what is now 35-45%. There is some evidence that if bone marrow is purged, survival is prolonged. However, there is also evidence that a proportion of patients die from the effects of the purging and that it is important to find out if purging is removing leukemic cells or having possible immunological effects on the marrow.

Mr. John Barton noted that, under the *Federal Guidelines for Research With Children*, a child cannot be asked to undergo a procedure with anything more than minimal risk unless the child is going to directly benefit from the procedure. He asked whether there was a way to confirm, either in animal models or in adults, whether this will benefit these children. Dr. McIvor asked why the experiments were being done in children, rather than adults, since the disease also occurs in adults.

Dr. Brenner asked Dr. Mirro, an associate at St. Jude's, to explain why the investigators believed it important to offer children the chance to benefit from this. Dr. Mirro said that this question was also brought up in the subcommittee discussion of the protocol and that the reasoning was that the procedure may be most beneficial in children because they are more likely to be able to tolerate an intensification of an ablative procedure, should the procedure show that it is residual leukemic populations in the patient's bone marrow that are responsible for the tumor regeneration. If it is found that the marrow is contaminated with leukemic cells and purging is required, children would have the greatest benefit of any transplant protocol since they are able to tolerate the ablative procedures. Children were really the ideal population for testing such a clinical approach.

Mr. Mannix asked if children as a whole would benefit, or whether individual benefit is anticipated to be derived from the protocol. Dr. Mirro said that since children are most likely to undergo transplantation (allogeneic or autologous), they will benefit on the whole. However, the second part of the question dealing with individual benefit is critical and the hope of the investigators is to be able to modify transplant protocols based on knowledge gained as to the necessity for purging the bone marrow. Purging delays engraftment and exposes the children to possible opportunistic infection in the meantime. If purging is

found necessary to prevent recurrence of the tumor, that this was a necessary risk, but that if it is found unnecessary, then it would save a great deal of risk to future transplant patients.

Mr. Mannix asked whether there was a chance that the children undergoing the protocol would benefit in subsequent treatment from the results of this experimental treatment as a result of assuming the risks involved. Dr. Mirro said that since most children will not undergo a second autologous transplant they will not benefit directly. Dr. Brenner added that some future protocols that are being planned would contain second autografts if the procedure is acceptable and therefore could result in benefit to these patients.

Dr. Anderson explained that there are three categories of risk:

1. Any additional risk because of the clinical procedure;

2. The production of a recombinant virus that could be pathologic for the patient or for the health care professionals involved; and,

3. Risk that the results of the random insertion may result in potential cancer production.

He said since no additional clinical procedures were to be employed beyond what is already done in routine transplant, there is no additional risk from the first category. There has been considerable engineering of the vector and it has been shown not to present a major risk of producing pathologic retroviruses. However, a series of studies is being done to detect such an event should it take place. This leaves only the unanswered question of the risk of oncogenesis because of random insertion. In safety studies in monkeys, now approaching 70 monkey years in duration, there has been no evidence of a single tumor produced by a retrovirus.

Dr. Walters added that during the HGTS discussion Dr. Mirro had addressed three points as to why children were included in the study. They were:

1. There is less myeloid dysplasia in children than adults, therefore making them better candidates for the therapy;

2. The terrible prognosis for children with AML; and,

3. The fact that if purging is going to work, it is more likely to work in children than in adults.

Dr. Walters said the subcommittee agreed that there were good reasons to do the study in children, although in general they thought they would prefer beginning with adults. When comparing the risks of the disease and the risks of standard therapy, the risks associated with cell marking are minimal.

Dr. McIvor asked Dr. Anderson if he had ever done the calculations on the likelihood ofinsertional mutagenesis. Dr. Anderson tried, but there are many assumptions that are necessary for each particular cancer. Dr. McIvor was looking for information on how many integration events in a cell population would be necessary before the activation of two oncogenes could be observed. Dr. Ihle said that several experiments had been done in this regard and that the frequency of activating of an oncogene is something on the order of 10. Therefore, the frequency of activating two oncogenes would be 10. In

regard to these experiments, the vast number of cells will not reconstitute in the long-term, and only a very small percentage of stem cells would contribute to cancer. It would be very difficult to calculate the risk of oncogenesis in such experiments.

Dr. Carmen suggested the following wording be inserted in the informed consent document under the heading of Bone Marrow Transplant Procedure, found on page 31 of the protocol (page 109 of the mailing):

The current sentence beginning, "If you agree, one-third of the marrow will be treated with..." should be reworded as follows:

> "If you agree, one-third of the marrow will be treated with a marker bacterial gene, attached to a disabled mouse virus, to carry it into the cell."

Further, on page 33 of the protocol (page 111 of the mailing) under the heading of Marrow Marking, the third line, insert the word "mouse" between the words "the" and "virus" to make the sentence read:

> "For example, although the mouse virus containing the marker has been greatly disabled and is considered harmless, it is conceivable that changes might occur in the cells in which it is placed, which would allow the virus to recover, grow and potentially even cause cancer."

Dr. Walters said that one other piece of information was received via FAX from Dr. Mulligan during the morning and it states:

> "I've read the response of Dr. Brenner,*et al.*, to my comments on the clinical protocol....and believe they have adequately addressed my concerns. My vote would now be to approve the protocol."

Dr. McGarrity asked if Dr. Brenner had any comments on Dr. Carmen's suggestions on the informed consent. Dr. Brenner said it looked fine and would be incorporated in the informed consent document.

Dr. McIvor moved that the protocol be approved with Dr. Carmen's revisions with the one revision which he suggested, which was that the informed consent document include the following statement:

> "I understand that while useful information might be generated from my participation in this study, it is also possible that no information will be generated."

Dr. Carmen seconded the motion.

Dr. Walters asked whether the investigators had accepted the addition of the 14-year follow-up to the assent document, or whether it was necessary to include this in the motion. Dr.McGarrity asked if Dr. McIvor would include this in his motion. Dr. McIvor said he was agreeable to this friendly amendment. Dr. Carmen also concurred.

There being no further discussion on the motion, Dr. McGarrity called for a vote on the motion. The motion passed unanimously by a vote of 16 in favor, 0 opposed, and no abstentions.

Dr. McGarrity asked for guidance from the committee on how they wished to proceed with the afternoon's agenda in as much as they had received the revised protocol via FAX from the University of Pittsburgh. Dr. Gellert said that since the reviewers had just received the document, time did not allow for an adequate informed review of the revised protocol while still completing the rest of the agenda. Dr. Lotze said there were only five minor points, and that he had underlined them all in his copy. It would require five minutes or so to go over them. Dr. Schaechter believed it was worthwhile to try to attempt to resolve the five questions and suggested scheduling a coffee break, during which time the reviewers could sit together and possibly come up with some consensus.

Dr. McGarrity wanted to be fair to the investigators on this protocol, but also believed a thorough review was imperative. If this was possible, without jeopardizing the rest of the afternoon's agenda, that he agreed to call for a short coffee break during which time the reviewers could meet and discuss the revised protocol. Dr. Wivel noted that Conference Room 9 would be available for the primary reviewers to use if they wished. Dr. McGarrity called for a brief recess and asked the committee members to reassemble at 2:10 p.m.

Dr. McGarrity called the committee back to order at 2:10 p.m. and asked Dr. Gellert to give the group a sense of the discussions that had taken place relative to the revised protocol.

VI. (Cont'd) PROPOSED ADDITION TO APPENDIX D OF THE "NIH GUIDELINES" REGARDING A HUMAN GENE TRANSFER PROTOCOL ENTITLED "THE ADMINISTRATION OF INTERLEUKIN-2, INTERLEUKIN-4, AND TUMOR INFILTRATING LYMPHOCYTES TO PATIENTS WITH MELANOMA":

Dr. Gellert said that he and Drs. McGarrity, McIvor, and Carmen had reviewed both the original and revised protocols over the coffee break and it was thought that there are still some problems that need further work before the protocol can be approved. The main concern was stipulation by the HGTS that the homing experiments and their analysis needed clarification, was not yet completely addressed. The revised protocol did not contain an adequate technical description of what will be done. A further point was that the consent form should explicitly state that the gene tagging will offer no therapeutic benefit to the patient. Additionally, there are still problems with the description of how payment will be handled and what costs the patient will bear in regard to the protocol. Finally, there was very sparse inclusion of preclinical data and that the data on the results of treatment of patients with cells cultivated in IL-2/IL-4, which were reported by Dr. Lotze, still were not included in the protocol.

Dr. McIvor added that there was concern over the specific type of protocol to be used and the awareness of the patient. Dr. Gellert said that since this was a compendium of five different protocols, only one of which was of concern to the RAC, some clarity needed to be provided in how the patients will be sorted into the various protocols and to what extent the patient has a choice in which protocol he participates in.

Dr. R. Murray said that there were portions underlined on pages 3 and 7 of the revised protocol regarding the absence of benefit from the gene marking and payment for development of cells. Dr. Gellert said that this did not address payment for injuries resulting from the protocol and this was his main concern. The third party payers often sever their connection with the treatment and throw the responsibility back on the patient for payment in these instances and clarification is needed as to who will pay for treatment of such injuries.

As far as the lack of benefit from the gene insertion, Dr. Gellert said that he had overlooked this and this fulfilled that criterion.

Dr. Walters said that if the only issue was that of payment for injury caused by the protocol, it would not be sufficient cause to delay approving the protocol since this is a universal problem that all institutions must deal with. However, the issues of preclinical data were more substantive issues in his mind.

Dr. McGarrity felt that a formal motion was necessary, but that it was apparent that the RAC could not approve this protocol on the basis of the revised documentation. He underlined that Dr. Lotze should present documentation addressing the points mentioned by Dr. Gellert first to the HGTS and then, if the subcommittee was satisfied with the information supplied, the protocol could be brought back before the RAC at its next meeting.

Dr. Lotze would do his best to supply the required information and to bring the protocol back before the RAC.

Dr. McGarrity then noted that because of scheduling conflicts, he would reverse the order of presentation of the next two agenda items. He called on Dr. Riley to present what was originally noted on the agenda as Item IX.

VIII. REVISION OF APPENDIX K OF THE "NIH GUIDELINES" REGARDING ESTABLISHMENT OF GUIDELINES FOR LEVEL OF CONTAINMENT APPROPRIATE TO GOOD LARGE SCALE PRACTICES (GLSP):

Dr. Riley noted that at the last full RAC meeting, the Subcommittee on Large Scale Practices was asked to reconvene to discuss several matters. The revision of Appendix K was the result of letters from the Industrial Biotechnology Association and the Pharmaceutical Manufacturers Association requesting guidance for large scale practices for organisms requiring lower levels of containment than called for in Biosafety Levels 1, 2, and 3, currently embodied in Appendix K.

Dr. Riley said the subcommittee agreed that such a lower level would be useful and should be called "Good Large Scale Practices" (GLSP), analogous to the term "Good Large Scale Industrial Practices" for handling volumes of 10 liters or higher of organisms considered to be safe and not having an increased hazard due to carrying recombinant DNA molecules.

Dr. Riley said that tab 1412 contained the proposed revisions to Appendix K, beginning on page 5. She noted that during the course of the subcommittee's work a grid was developed by ORDA staff which incorporated all levels of containment from GLSP through BL3-LS for cross-reference to Appendices K and G.

Dr. Riley suggested that the discussion be divided into two issues, one concerning the new lower GLSP level and the other a discussion of the grid. She noted that there were some specific recommendations from the subcommittee that call for higher containment in some cases. She added that correspondence had been received and dealt with the reactions of companies and associations to changes that affect the higher containment levels. Dr. Riley called for comments from other members of the subcommittee prior to a full committee discussion.

Dr. Hirano said she wished to discuss the grid first. She said that it nicely summarized the differences between GLSP and BL1-LS, BL2-LS, and BL3-LS, but in doing so it had created some confusion. She said the subcommittee suggested the following changes to the grid appearing on page 9 of tab 1412:

1. The wording for Criterion 20 should read "Access to the workplace." and under the column marked "GLSP" should read "NR" (not required); under the column marked "BL1-LS" it should read "G-II-A-1-a"; under the column marked "BL2-LS" it should read "G-II-B-1-a"; and the column marked "BL3-LS" would remain the same, "K-III-L."

2. Items 21 through 42 would be eliminated from the grid because they do not pertain to a controlled access area for the categories of GLSP, BL1-LS and BL2-LS.

3. In lieu of items 21 through 42 in the grid, these will be a text statement that the requirements and characteristics of a controlled access area are elaborated in Appendix K, Part V.

Dr. Schaechter cautioned that the wording of "not required" for some of the criteria between 21 and 42 was incorrect. Dr. Riley suggested that a statement be made that "requirements and characteristics of a controlled access area are elaborated in Appendix K, Part V." Dr. Hirano said this would make the grid clear.

Dr. Post noted that the whole issue of good large scale practice was delegated by the RAC to the Institutional Biosafety Committees (IBCs) in 1988 and that some IBCs have, in fact, been doing the review. Thus, this proposal is merely to make it official and define the conditions at the level of the RAC rather than to leave it for the IBCs to do this on an *ad hoc* basis.

Dr. Miller noted that Item Number 33, referring to the use of the Universal Biohazard Sign, was useful enough and misinterpreted enough that it should be left in to be explicit. Dr. McGarrity asked if Dr. Miller if he meant that it should be left in to say that it was not required. Dr. Riley said she did not agree, and that to be consistent there should be no emphasis placed on one criterion versus another.

Dr. Miller added that there were frequently references to a "closed system," and that oftentimes the FDA deals with fermentations which are used for producing yogurt and wine which are not closed fermentations, and he wondered if this was going to be misinterpreted.

Dr. Atlas asked how the subcommittee thought about responses received from Merck as to the handwashing and HEPA filtration. Dr. Riley said that is what caused Dr. Hirano to find the inconsistencies which resulted in her comments as to the changes in the grid. She said that no one is mandated to wash his or her hands, but that it is mandated to have facilities for handwashing. Yet there is no requirement for handwashing facilities in a controlled access area. This brought about the changes that Dr. Hirano had outlined.

As far as the HEPA filtration, two letters had been received raising the question of whether it was wise for the subcommittee to recommend treatment of exhaust air from controlled access areas. She noted that as Appendix K now stands, HEPA filtration is not required for closed containment facilities as long as the air is not directed into sensitive places such as near air intakes. She said, however, that in discussing this issue at its meeting in December the subcommittee members had thought that if there was a requirement to change clothes before entering a facility, there must be some expectation of a level of pathogenic organisms in the air. Therefore, air should be filtered before discharge. The other side of the argument is that in these instances one is dealing with closed systems in which there is not supposed to be any escape. Therefore, why filter large quantities of air that circulate in large laboratory spaces which are part of a closed system?

Dr. Gellert said his impression was that one of the most frequent occurrences to be expected would be a spill, in which case aerosols and sprays of material would go into the air and that these could be disseminated before any air supply could be shut off. Therefore, it would not be unreasonable to require some sort of filtration.

Dr. Riley moved adoption of the subcommittee recommendations to Appendix K of the *NIH Guidelines*, including the modification outlined by Dr. Hirano. Dr. Gellert seconded the motion. There being no further discussion on the motion, Dr. McGarrity called for a vote. The motion passed by a vote of 15 in favor, none opposed, and 1 abstention.

Dr. McGarrity thanked Dr. Riley and her subcommittee and then called on Dr. Schaechter to begin discussion of the next agenda item.

IX. AMENDMENT TO APPENDIX B-I-B-1 OF THE "NIH GUIDELINES" REGARDING "SALMONELLA TYPHIMURIUM" LT2:

Dr. Schaechter said that this item came as a request from Dr. Robert LaRossa of Dupont on September 25, 1990, to downgrade levels for work with *Salmonella typhimurium* strain LT2 from BL2 to BL1. It was specific for the LT2 strain, and not for all *S. typhimurium*. It was hard to see why this request had come to the RAC since all *Salmonella* are classified as Class 2 agents by the *CDC-NIH Guidelines for Biosafety in Microbiological and Biomedical Laboratories* . This should be treated in the context of the pathogenesis of the organism rather than as a recombinant DNA recipient.

Dr. Schaechter said that this strain was very old and should be compared with *E. coli* K-12, but that the information that really exists on it in regards to its relative pathogenesis is small since no human experiments have been done comparable to those with *E. coli* K-12. The strain has been used before and that nothing much has happened, although this does not mean the strain is not pathogenic. In fact, there have been repeated anecdotal reports of workers contracting enteric fever from ingestion of the strain. Dr. Schaechter said that while this strain is possibly debilitated, it is still not something he would want to swallow or have in the workplace environment. Therefore, it should be worked on with proper precautions. The difference between working on something at BL1 versus BL2 were not that great and should not impede the investigators in their work.

Dr. Schaechter said that the use of rough strains which are further debilitated could constitute a reason to downgrade the containment, but that such strains are messy to work with; this would be a possible out for the investigator if there is a valid reason for working at a lower safety level.

Dr. Schaechter was not in favor of the request and would like to leave *S. typhimurium* at the BL2 safety level.

Dr. B. Murray said she agreed with Dr. Schaechter and that she would be hesitant to reclassify potentially pathogenic organisms and have people work with them with less concern for safety. She recommended that the organism be left at the BL2 level.

Dr. Krogstad said that the articles which had been supplied by the investigator proposing the lowering of biosafety level were based on molecular and genetic studies not aimed at an examination of virulence and not addressing the fundamental question of why the level should be reduced. In light of the lack of evidence demonstrating a lack of virulence in human volunteers, he believed it inappropriate to downgrade the safety level.

Dr. Krogstad moved that the committee recommend leaving the containment at the BL2 level. Dr. Schaechter seconded the motion. There being no further discussion on the motion, Dr.McGarrity called for a vote. The motion passed by a vote of 13 in favor, 0 opposed, and 1 abstention.

Dr. McGarrity then called on Mr. Mannix to present the next agenda item.

X. REPORT FROM THE PLANNING SUBCOMMITTEE IN CHARGE OF REVIEWING COMMENTS RECEIVED DURING THE REGIONAL HEARINGS CONDUCTED BY THE RECOMBINANT DNA ADVISORY COMMITTEE CONCERNING THE FUTURE ROLE OF THIS COMMITTEE:

Mr. Mannix said that the report that he prepared (tab 1420a) was only a draft and that he had not had an opportunity to talk with all subcommittee members. In this respect, the views represented were his own. Further, the recommendations contained in his report were an effort on his part to put in the form of recommendations, items that were discussed and which the Planning Subcommittee would like to have evaluated by the RAC. The first recommendation was the only one on which the Planning Subcommittee took a vote and is ready for action by the RAC. All others are for discussion and further research, not recommendations for action to be taken.

Mr. Mannix said that the final clarification was that the report did not recommendsunsetting the *NIH Guidelines* or the RAC, but recommends transforming the RAC and refocusing it on human gene therapy. The most that the draft report recommends is looking around to see if there is another appropriate organization with the means for keeping the *NIH Guidelines* current, in readable form, and available to researchers.

Dr. R. Murray was grateful to Mr.Mannix for taking the time to compile this report since Dr. R. Murray had missed some of the discussion during the meeting due to a scheduling conflict. He agreed with some of the points made by Mr. Mannix, but that he understood other members of the subcommittee differed in their impressions of some of the discussions that took place at the meeting. This report was a good point of departure for discussion. The reports of the subcommittees that had been presented so far show that there is still a need for the RAC, but that there could be discussion of how it should be structured and wha should be its focus.

Dr. B. Murray said that the report reflected MrMannix's opinion, rather than a consensus opinion of the subcommittee, and that she did not feel a consensus was reached on many items. She said she thought that all the items that were presented in the report were discussed and that there were very few specific recommendations to make. One issue that was believed to be generally agreed upon was removing environmental release from RAC oversight on the basis that it was covered by other agencies. She asked for clarification as to whether this indeed was the case.

Dr. Wivel perceived that the question was whether the RAC should consider keeping the trigger in place to require notification of the RAC for environmental release until such time as the United States Department of Agriculture (USDA) Guidelines were promulgated. It was clear that the Environmental Protection Agency (EPA) and the USDA would be the major players in planned release experiments. Dr. Wivel said that clearly the regulatory agencies would have to take some responsibility for environmental release, whether or not the NIH chose to remain in the area. Further, ifNIH is to stay in the review process there are certain requirements relating to the National Environmental Policy Act (NEPA) that involve monitoring and testing, as well as the possibility of public hearings. The NIH is not well positioned to undertake such activities since this agency lacks any regulatory authority.

Dr. Sue Tolin of the USDA noted that on February 1, 1991, the proposed *USDA Guidelines for Research Involving Planned Introduction into the Environment of Organisms with Deliberately Modified Genetic Traits* was published for a 60-day comment period. She said it describes the principles for assessing research safety with specific organisms and designing confinement measures to promote safety. She noted that the deadline for comment on these guidelines is April 2, 1991, after which the USDA will consider the comments received. She emphasized that this document in no way includes a triggering mechanism for regulation and implementation. Another document being worked on separately and will be forthcoming.

Dr. Miller asked if these guidelines were aimed only at USDA-funded research. Dr. Tolin responded that since there is no implementation phase they are not pointed toward regulation. She said they are principles for assessing safety which are intended to be similar to *Points to Consider*, to aid principal investigators in designing research so that it can be conducted safely outside contained facilities. She noted that the document encourages institutions to utilize IBCs to aid in safety evaluation and confinement design. She said it was written primarily for USDA-funded research but could be viewed as not being exclusively pointed at it.

Dr. Miller pointed out that one of the problems that confounds regulatory agencies is the ability to create exemptions. This could bring the NIH back into the picture because it would not be under the jurisdiction of another agency if it falls into a regulatory exemption. Other mechanisms do exist for overseeing non-recombinant organisms that range from little risk to high risk and deal with field trials of plants and domesticated foodstuffs. Such mechanisms seem to function reasonably well.

Dr. Shibley of USDA said that for some time the Animal and Plant Health Inspection Service (APHIS) has been doing environmental assessment, looking at both conventional products and products containing recombinant DNA.

Dr. Hirano asked how often ORDA received requests from researchers or IBCs relevant to environmental release. Dr. Wivel said that most inquiries occur with respect to Appendices P and Q, since people are aware that these documents are in the process of being promulgated. Most investigators are using the 1987 *Federal Register*, which contains the draft language of Appendices P and Q, as an unofficial guide to those experiments.

Dr. R. Murray said that this was one area where there seemed to be unanimity among the members of the committee and the subcommittee. He moved that the RAC consider deleting this trigger mechanism for environmental release.

Dr. McIvor seconded the motion.

Dr. McGarrity noted that this would then be published in the *Federal Register* as an action to be taken by the RAC at its next meeting. There being no further discussion on the motion, Dr. McGarrity called for a vote. The motion passed by a vote of 12 in favor, 0 opposed, and 2 abstentions.

Dr. McGarrity agreed with Dr. B. Murray in that Dr. McGarrity thought that tab 1420a was a representation of one person's perspective on the meeting of the subcommittee. It contained some factual misrepresentations of what was actually was said by people during the public hearings. He pointed out that Dr. Fredrickson did not recommend sunsetting the *NIH Guidelines*, but had been speaking in favor of the RAC removing itself from reviewing environmental release experiments. Dr. McGarrity complimented Mr. Mannix on his efforts to summarize the meeting but said his recollections often did not match those found in the document.

Mr. Mannix responded that in attempting to write down his perspectives with the aim of being able to either reconvene the subcommittee or discuss them before presentation to the RAC. However, he was late in getting it around to people and therefore Dr. McGarrity was correct in characterizing this as the perspective of one individual.

Dr. Schaechter said that, regardless of the consensus of views, this document was useful as a point of departure for discussing the issues contained in it. One of the crucial points in the document was whether the RAC ought to be reformed as a committee more akin to the HGTS. Dr. Schaechter asked to hear other opinions on this matter.

Dr. Wivel noted that one of the major issues, when looking through all the comments was that of the question of changing the definition of "recombinant DNA." Opinions seemed to be split evenly on whether this should be done. He asked for a short discussion by the committee on how it thought about this issue after hearing and reading the public debate on it.

Dr. Wivel noted that the issues were, on the one hand, that there were no new, unique risks associated with the new technologies that would justify changing the definition on grounds of safety issues. Increasing the breadth of the definition was seen as creating increased bureaucratic paperwork. On the other hand, there is concern that there are new ways being developed to introduce DNA into cells which require no oversight at all, and there needs to be a means to assess whether they involve sufficient risk to require continued oversight as they develop and evolve.

Dr. B. Murray said that she would be happy leaving the definition unchanged for microorganisms, but that she had a problem with leaving it unchanged for instances where recombinant DNA is used for human gene interventions.

Mr. Carner said that this issue was one which needed to be looked at in the broader sense of the future role of the RAC. Concerns over the future role of the RAC was the impetus for the public hearings and tha input was sought on how the public viewed the committee, but that this was too important a question to resolve on the basis of public input and that a more in-depth consideration of the future role of the RAC was indicated.

Dr. Miller said that one of the recurrent themes in the discussion of redefining "recombinant DNA" was that of a process versus product based approach to the oversight of recombinant DNA research. This issue should be one which the RAC should focus on, rather than looking at technique-based triggers for RAC oversight.

Dr. McGarrity asked whether the committee would recommend to the Director,NIH, that any human gene therapy proposal involving recombinant DNA by definition, or techniques that would achieve the same objective, should be reviewed by the RAC. Dr. Walters noted that RAC had agreed to review recombinant DNA introduced into human beings as well as DNA or RNA derived from recombinant DNA, which already expanded the purview of the committee. Such a determination would be the next step in this line of thinking.

Dr. Wivel said that for such a determination to be made it would constitute a change in the definition of "recombinant DNA," and would require publication in the *Federal Register* for comment and would follow the same process as any proposal to modify the *NIH Guidelines*.

Mr. Mannix said that rather than having two different definitions of "recombinant DNA." It would be simpler

to change the RAC's charge to include all types of human genetic intervention. Dr. Schaechter disagreed with this approach, noting that it would merely make the RAC into a HGTS and would create a vacuum as far as guidelines for the rest of recombinant DNA research. Dr. Schaechter called attention to the strong public support of the RAC as a place for public discussion of issues of recombinant DNA research, reflecting the trust that the public had placed in it. He did not want the RAC to lose this position as a public forum.

Mr. Mannix said that in reading the transcripts of the public hearings, he became aware of the extent of support for maintaining the *status quo* because the RAC has worked reasonably well. However, he also noted the absence of many people coming out and saying there were any real risks to worry about. At NIH today there is a need for an AIDS Advisory Committee and a need for a committee to oversee human gene therapy. However, there is no longer a need for a committee to oversee recombinant DNA research that does not involve human subjects. Therefore, the RAC should reorient itself and its traditions and should expand its purview in the area of human gene therapy. It would be far more direct to say that with respect to human gene intervention all such experiments should come before the RAC, regardless of the actions necessary for ensuring that this is done.
Dr. B. Murray said that if the definition were broadened and made more consistent, it would not result in an increased workload or review because what is really being looked at is product, not process. She said that if the *NIH Guidelines* are left in place there needs to be some sort of committee responsible for revising them when special requests and new information become available. She said she would be happy to see a complete revision of the definition and would anticipate seeing mostly human gene therapy proposals coming before the RAC, with the occasional request to downgrade something as has been the case in the last year.

Dr. R. Murray believed Dr. Walters was trying to point the committee in the direction whereby "recombinant DNA" is not redefined, but instead the purview of the RAC is refocused not on how DNA is derived but instead on the use of the DNA. Dr. R. Murray suggested that the RAC then include in its purview, experiments which derive the DNA from PCR techniques, but only where the introduction of the DNA poses a potential hazard or in cases involving human gene therapy, human manipulation, or any introduction into humans.

Dr. R. Murray said that if this were to be the case, the RAC would be focusing purely on the application, rather than the process, and this may introduce some complications but that it would then focus on the areas of concern which have changed over time.

He also pointed out that the use of the term "genetic intervention" would be an appropriate term for what the RAC would include in its oversight. This would not exclude the committee from looking at areas where review may be needed, due to some novel approaches of dealing with DNA that are as yet unknown.

Dr. McGarrity asked whether the committee believed that this was something that the HGTS should be asked to consider at its next meeting and then returned to the RAC with recommendations at its next meeting. Dr. R. Murray said that more discussion was clearly necessary in this area. However, it would require substantive discussion and if this was not possible within the time constraints of the HGTS and the RAC, then may be a good idea to reconvene the Planning Subcommittee to continue the discussion.

Dr. Geiduschek said that the ORDA staff would have to play a significant role in providing a detailed assessment of the consequences of broadening any definition or expanding the purview of the RAC. It was important to be aware of all the implications of a particular expansion in relation to the activities of the RAC. He cautioned that enough time be set aside for these discussions to allow for these far-ranging implications to be discussed in-depth.

Dr. McGarrity asked Dr. Wivel for an assessment of the agenda for the April meeting of the HGTS. Dr. Wivel said that Dr. Lotze's protocol from today will undoubtedly be on the agenda and there was the possibility of the need to discuss a protocol that may be submitted from Baylor College of Medicine dealing with hepatic cells. Dr. Brenner also may be resubmitting a protocol which was deferred earlier by the subcommittee. Dr. Walters added that another issue was the letter from Mr. Alexander Capron which was received just prior to the previous meeting of the HGTS and which in fact deals with some of the issues of what the purview of the subcommittee should be if vectors and cells become drug delivery systems. This could be linked with the discussions of purview before the committee today.

Dr. R. Murray noted that the issue of redefining "recombinant DNA" had constantly been surfacing over the last couple of years and it was time that the committee decide whether it wanted to pursue it and, if so to deal with it in a thorough manner and come to a conclusion on the issues.

Dr. McIvor said that the issues of the purview over human genetic engineering and the definition of "recombinant DNA" were two separate issues and that could be dealt with separately. He did not know exactly what is to be done with the definition, what should be included, and why it should be changed. He was not sure the definition should be changed since it pretty well covers everything under the purview of the RAC.

Dr. Schaechter said that if Mr. Mannix's proposal was to simply transform the RAC into a Human Gene Therapy Advisory Committee then much of what was being discussed was not germane. He asked whether there was sentiment for this type of a proposal among other members of the committee, or whether this was simply the viewpoint of Mr. Mannix. He was confused by the concept of dealing with the definition of "recombinant DNA," and then saying that the committee is only going to deal with human gene therapy.

Dr. R. Murray said it was important that the RAC continue since that appeared to be the consensus from comments received during the public hearings. What Mr. Mannix was putting forward was a discussion of how the RAC should continue, in what format, or under what umbrella, and that to discuss dissolving the RAC would be ignoring the message sent by the public.

Dr. McGarrity added that the public had strongly urged that the *NIH Guidelines* not be allowed to sunset. It is his position as Chairman to ensure that the committee is aware that if it decides to sunset the *NIH Guidelines* it is doing so against the recommendations of the public.

Dr. R. Murray agreed that the *NIH Guidelines* should not be sunsetted, but that perhaps the NIH was not the proper place to have them continue and be updated. Dr. R. Murray also said that the current "double review" of human gene therapy protocols by the HGTS and the full RAC was beneficial in that it allowed two different groups of people to express their views on the merits of such proposals. He underlined two issues most important were:

1. Should the NIH continue to be the source of the RAC? and,

2. Should review of human gene therapy protocols and human gene use continue to be done by the RAC or separate from it?

Dr. R. Murray said that if these issues were decided then it would be easy to come to grips with the

definition of "recombinant DNA," because in one case the committee would not have to worry about the definition, and in the other case, any use of DNA from any source, no matter how derived, would have to be considered.

Dr. Carmen said that the best statement he had read on the subject came from Joan Bennett of the American Society for Microbiology who said,

> "You should assume responsibility for these new techniques (microinjection, electroporation); however, the RAC should continue to direct its attention to areas having a degree of expected hazard and exempt non-hazardous experiments from review."

Further, Dr. Carmen had no feeling from the public hearings that the RAC should become a clone of the HGTS. He was unsure how happy the public would be with such a turn of events.

Dr. Post said the impression he received from the hearings was that not only did the public not want to see the *NIH Guidelines* sunsetted, but there was the feeling that the RAC had been an efficient way to deal with recombinant DNA research. There was no feeling that this activity should be turned over to a regulatory agency to administer.

Dr. Post wished to argue against the RAC becoming just a Human Gene Therapy Advisory Committee in that human gene therapy may evolve to the point that it is nothing more than an activity similar to environmental release, and may be turned over completely to the FDA to regulate. He did not know what the future held for the RAC, but that it had evolved over the years and had continued to serve a vital role since its inception.

Dr. McGarrity asked for opinions on whether this issue should be put on the agenda for theHGTS. Mr. Carner did not see any reason for trying to rush discussion on these critical issues and that perhaps the Planning Subcommittee would be a better place to discuss this since it is much more broad-ranging than merely issues of human gene therapy. Dr. Hirano agreed that it should go back to the Planning Subcommittee for discussion.

Dr. Miller endorsed Mr. Carner's comments and suggested that the group to discuss these matters should be broadened and suggested that Dr. Donald Fredrickson be asked to Chair a small group to consider these issues more broadly. Dr. Miller noted that often the dynamics of a group do not lend themselves wel to changing the *status quo* very drastically.

Dr. R. Murray suggested setting up a retreat where this would be the only item on the agenda and small groups of people could look at sub-issues and combine thinking on all aspects of the problem. Small group of four-five individuals was inconsistent with the importance and far-reaching consequences of these decisions. He suggested a special meeting of the RAC dedicated to this one topic.

Dr. Wivel noted that this would require having an additional meeting of the RAC with a single agenda item and asked if everyone would agree to committing time to this endeavor. There was general agreement from the committee on this issue.

Dr. McGarrity recommended that all members take the opportunity to read the minutes of the last RAC meeting regarding the regional hearings and to review Mr. Mannix's summary as well as the summary of the regional hearings that was prepared by Dr. Wivel.

XI. FUTURE MEETINGS OF THE RAC AND THE HUMAN GENE THERAPY SUBCOMMITTEE:

Dr. McGarrity called the committee's attention to the schedule of future meetings and noted that the next meeting of the full RAC would be May 31, 1991, and the next meeting of theHGTS was scheduled for April 5, 1991.

XII. ADJOURNMENT:

Having concluded the agenda and there being no further business to be discussed, Dr. McGarrity adjourned the committee at 3:55 p.m., on February 4, 1991.

Nelson A. Wivel, M.D.
Executive Secretary

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

Date: 5/30/91

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