

DRAFT

RECOMBINANT DNA ADVISORY COMMITTEE

Minutes of Meeting

September 14-15, 1992

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

1389

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

RECOMBINANT DNA ADVISORY COMMITTEE

MINUTES OF MEETING¹

September 14-15, 1992

The Recombinant DNA Advisory Committee (RAC) was convened for its fiftieth meeting at 9:00 a.m. on September 14, 1992, at the Bethesda Marriott Hotel, 5151 Pooks Hill Road, Bethesda, Maryland 20892. Dr. Barbara E. Murray (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:

John H. Barton, Stanford Law School
Al W. Bourquin, Ecova Italia
Nancy L. Buc, Weil, Gotshal, and Manges
Alexander M. Capron, University of Southern California
Ira H. Carmen, University of Illinois
Gary A. Chase, Johns Hopkins University
Patricia A. DeLeon, University of Delaware
Roy H. Doi, University of California
Krishna R. Dronamraju, The Genetics Foundation
E. Peter Geiduschek, University of California, San Diego
Robert Haselkorn, University of Chicago
Susan S. Hirano, University of Wisconsin
Donald J. Krogstad, Tulane University School of Medicine
Brigid G. Leventhal, Johns Hopkins Hospital
A. Dusty Miller, Fred Hutchinson Cancer Research Center
Barbara E. Murray, University of Texas
Robertson Parkman, Childrens Hospital of Los Angeles
Leonard E. Post, Parke-Davis Pharmaceutical Division
Moselio Schaechter, Tufts University School of Medicine
Marian G. Secundy, Howard University College of Medicine
LeRoy B. Walters, Georgetown University
Doris T. Zallen, VA Polytechnic Institute & State University

¹The RAC is advisory to the National Institutes of Health (NIH), and its recommendations should not be considered as final or accepted. The Office of Recombinant DNA Activities should be consulted for NIH policy on specific issues.

Executive secretary:

Nelson A. Wivel, National Institutes of Health

A committee roster is attached (Attachment).

Ad hoc consultants:

William N. Kelley, University of Pennsylvania Medical Center
Abbey S. Meyers, National Organization for Rare Disorders

Non-voting agency representatives:

Bernard Greifer, Department of Commerce
Henry I. Miller, Food and Drug Administration
Ralph Yodaiken, Department of Labor

Liaison Representative:

Daniel Jones, National Endowment for the Humanities

National Institutes of Health staff:

French Anderson, NHLBI
Michael Blaese, NCI
Charles Carter, CC
Lauren Chang, NHLBI
Chin-Shyan Chu, NHLBI
Ronald Crystal, NHLBI
Cindy Dunbar, NHLBI
Jay Greenblatt, NCI
Christine Ireland, OD
Susan Jenks, NCI
Cliff Lane, NIAID
Becky Lawson, OD
Susan Leitman, CC
John Miller, OPRR
Richard Morgan, NHLBI
Jack Raghub, NHLBI
Robert Walker, NIAID
Debra Wilson, OD

Others:

Paul Aebersold, Food and Drug Administration
James Barrett, Genetic Therapy, Inc.
Mark Berninger, Life Technologies, Inc.
Malcolm Brenner, St. Judes Childrens Hospital
Vickers Burdett, Duke University
Barrie Carter, Targeted Genetics
Kenneth Cartwright, Alteon, Inc.
Yawen Chang, Genetic Therapy, Inc.
Valentina Ciccarone, Life Technologies, Inc.
Albert Deisseroth, MD Anderson Cancer Research Center
Wanda DeVlaminck, Somatix Therapy, Inc.
Suzanne Forry-Schaudies, Genetic Therapy, Inc.
Jeffrey Fox, ASM News
Marianne Grossman, University of Michigan
Russell Herndon, Genzyme Corporation
Markus Hunkeler, Life Technologies, Inc.
John Jaugstetter, Genentech
Joel Jessee, Life Technologies, Inc.
Joseph Kozlovac, University of Maryland
Maryann Krane, Genzyme Corporation
Karen Lee, National Museum of American History
Peter Liljestrom, Karolinska Institute
Michael Lotze, University of Pittsburgh
Charles McCarthy, Retired NIH
Gerard McGarrity, Genetic Therapy, Inc.
Bonnie Mills, Baxter Health Care
Robert Moen, Genetic Therapy, Inc.
Ann Montgomery, FDC Reports
Richard Moscicki, Genzyme Corporation
Tapas Mukhopadhyay, MD Anderson Cancer Research Center
James Neel, University of Michigan Medical Center
Stephen Pijar, University of Maryland
Rex Rhein, Biotechnology Newswatch
Jack Roth, MD Anderson Cancer Research Center
John Russick, National Museum of American History
G. Terry Sharrer, National Museum of American History
Tomiko Shimada, Ambiance Awareness International
Friedrich Schuening, Fred Hutchinson Cancer Research Center
Jonathan Simons, Johns Hopkins University
Alan Smith, Genzyme Corporation

Tetsuo Taniguchi, The Asahi Shimbun
Allison Taunton-Ragby, Genzyme Corporation
Gary Temple, Life Technologies, Inc.
Larry Thompson, Science Magazine
Paul Tolstoshev, Genetic Therapy, Inc.
Katherine Trach, Life Technologies, Inc.
Bruce Trapnell, Genetic Therapy, Inc.
Thierry Velu, Brussels University
Michael Welsh, University of Iowa
David Wheeler, Chronicle of Higher Education
Clyde White, Carolina Biologicals

I. CALL TO ORDER

Dr. Murray (Chair) called the meeting to order. She noted that the notice of meeting was published in the *Federal Register* 15 days prior to September 14 as required by the *National Institutes of Health Guidelines for Research Involving Recombinant DNA Molecules (NIH Guidelines)*. The RAC serves as advisor to the NIH Director. The Director may accept, reject, or send the RAC's recommendations back to the committee for further deliberation.

Dr. Murray stated that a quorum was present and outlined the order in which speakers would be recognized. The primary and secondary reviewers will present their reviews of the protocol, followed by responses from the principal investigators of the protocols. The Chair will then recognize other RAC members, *ad hoc* consultants, other NIH and Federal employees, the public who have submitted written statements prior to the meeting, followed by the public at large. She welcomed Dr. Gary Chase of Johns Hopkins University as a new member of the RAC. She noted that a quorum was present.

II. MINUTES OF THE JUNE 1-2, 1992, MEETING

Dr. Murray called on Dr. Parkman to review the minutes of the June 1 and 2, 1992, RAC meeting. Dr. Parkman stated that the minutes of the June 1-2, 1992, RAC meeting were an accurate reflection of the committee's deliberations; however, he suggested several minor corrections. Drs. D. Miller and Geiduschek stated that they had additional minor corrections, and that they would submit these changes in writing.

A motion was made by Dr. Parkman and seconded by Dr. DeLeon to approve the minutes including the changes submitted by Drs. Parkman, D. Miller, and Geiduschek. Dr. Murray called for the vote. The minutes were approved by a vote of 17 in favor, 0 opposed, and no abstentions.

III. PROPOSED ADDITION TO APPENDIX D OF THE NIH GUIDELINES REGARDING A HUMAN GENE TRANSFER PROTOCOL ENTITLED: *A STUDY OF THE SAFETY AND SURVIVAL OF THE ADOPTIVE TRANSFER OF GENETICALLY MARKED SYNGENEIC LYMPHOCYTES IN HIV INFECTED IDENTICAL TWINS*/DRS. WALKER AND BLAESE

Review--Dr. Post

Dr. Murray called on Dr. Post to present his primary review of the protocol submitted by Drs. Robert Walker and R. Michael Blaese of the NIH, Bethesda, Maryland. Dr. Post provided a brief overview of the protocol. The protocol involves the *in vitro* culture of

lymphocytes from the identical twin of an human immunodeficiency virus (HIV) positive (+) patient. The lymphocytes will be separated into CD4(+) and CD8(+) subpopulations. Each of these subpopulations will be marked with one of two different retroviral vectors, G1Na or LNL6, and reinfused into the HIV infected twin. Transfection of the lymphocytes with the retroviral markers will determine survival of the infused cells. In addition, the investigators will examine any potential benefit resulting from the infusion of uninfected T lymphocytes.

Dr. Post inquired whether there is any real expectation that this protocol will provide any therapeutic effect. The infusion of activated T cells into HIV(+) patients may present an inherent safety issue because CD4(+) cells are the target of HIV replication. Since these patients are already immunodeficient, infusion of activated T cells may increase the pathogenicity of the disease. The investigators responded to these concerns stating that there is a clinical protocol already in progress in which patients have received CD8(+) cells and no untoward effects have been observed. However, these results do not predict the outcome of infusing large numbers of CD4(+) cells. Language has been incorporated into the informed consent document informing patients that if untoward effects are observed as a result of the T cell administration, the protocol will be terminated immediately. In addition, patients will be monitored for viral titers.

Dr. Post explained that the protocol presents a great deal of latitude with regard to the number of cells that will be infused. Peripheral blood mononuclear cells will be fractionated by CD4(+) selection and/or CD8(-) depletion. Patients will receive between 3×10^9 and 2×10^{11} fractionated cells.

Although the investigators propose to use the standard retroviral vectors, G1Na and LNL6, that have been reviewed numerous times by the RAC, it is unclear what assays will be performed to detect helper virus contamination. At the last RAC meeting, Dr. Anderson stated that the acceptable standard is currently to propagate the packaging cell line for two to three weeks after the vector has been harvested to demonstrate the lack of helper virus. Dr. Post stated that the Food and Drug Administration (FDA) has been conducting discussions regarding revised standards for monitoring helper virus. When he addressed concerns regarding vector safety testing, the investigators noted that the supplier of the vector, Genetic Therapy, Inc. (GTI), currently performs extended culturing of the packaging line following harvest as well as co-cultivation with an indicator cell line. It should be noted; however, that co-cultivation has not been accepted yet as a validated protocol for the detection of replication competent helper virus. He asked the investigators and Dr. D. Miller to respond to the importance of co-cultivation experiments.

There are no restrictions on the patient's stage of disease as an inclusion or exclusion criterion for this protocol; patients can have an asymptomatic HIV diagnosis or advanced

acquired immunodeficiency syndrome (AIDS). The investigators state that this latitude will yield valuable information regarding the various stages of the disease. There will be no restrictions on patients obtaining concurrent therapy. Patients will have the option to enroll in other experimental protocols. Overall, the investigators adequately responded to Dr. Post's concerns and he requested that they address the few remaining questions presented in his review. He recommended that the RAC approve the protocol.

Review--Dr. Dronamraju

Dr. Dronamraju inquired if the investigators will make a conscious attempt to randomize the patient population with regard to the stage of their disease, or is the twin population so limited that latitude is necessary in order to obtain a sufficient number of patients entering the protocol? Are there patients already available to enter the protocol? What will be the investigator's next step? What is the rationale for having not provided *in vivo* animal data to support the protocol?

Review--Dr. Carmen

Dr. Carmen stated that the protocol understates its prospects for broad theoretical contributions to the determination of how genetic factors trigger specific behavior patterns in humans. Identical twin literature has its own place on the natural science, neuroscientific, and social science research agenda. He recommended approval of this protocol because valuable information will be obtained regarding the relevant parameters of sexual orientation.

Other Comments

Dr. Parkman asked if the clinical protocol (without gene marking) involves the administration of CD4(+) and CD8(+) cells. If the clinical protocol is identical except for the addition of gene marking, then the RAC should only consider the gene marking portion of this protocol. If the analogous protocol uses only nonactivated CD4(+) and CD8(+) cells, then the entire protocol would fall under the purview of the RAC.

Dr. Parkman stated that no information was provided by the investigators regarding the transduction efficiencies in these fractionated subpopulations of lymphocytes. Since CD4(+) cells are being administered in the activated state, could they also become infected with HIV? The protocol states that if there is an overall increase in vital activation or decrease in CD4 counts, the protocol will be stopped. Will samples be obtained from the patients after the cells have been administered and cultured in order to determine the presence of HIV in gene marked cells? If there is evidence that gene marked cells have been infected, will the investigators stop or continue the protocol?

Dr. Zallen asked questions regarding the informed consent for this protocol. Is the uninfected donor aware of the HIV status of the infected twin prior to reviewing the informed consent document? The recipient consent form states that there will be no costs assigned for the procedures associated with this study. It states that immediate medical care will be provided in the event of physical injury resulting from participation in this study; however, there is no provision for free medical care. She asked the investigators to explain the discrepancy. The donor consent form should clearly state that the donor will have to donate cells at three different times.

Dr. Chase said that the protocol would provide useful information if the design was limited to several defined populations of patients rather than such a broad range of diagnoses.

Dr. Walters noted that although the investigators have stated that patients are eligible for a similar protocol without the gene marking aspect, this option has been omitted as an alternative therapy in the informed consent document.

As a follow-up to Dr. Zallen's comments regarding financial compensation for injury, Mr. Capron suggested that the investigators limit liability to non-negligent injuries so that the patients do not perceive themselves as waiving their rights to recover compensation if they are injured through negligence. Mr. Capron suggested revising the language in the informed consent document regarding this issue.

Dr. Haselkorn explained that the RAC recently reviewed another protocol involving HIV in identical twins that was submitted by investigators at Sloan-Kettering Memorial Cancer Center in New York. Would there be competition between investigators for the same groups of patients?

Presentation--Dr. Walker

Dr. Murray called on Dr. Walker to respond to the questions and comments of the primary reviewers and other members of the RAC.

Dr. Walker addressed the issue of disease stage. Currently, there is a list of approximately 24 identical twin pairs that are eligible to participate in this study. Approximately one-half to two-thirds of these twins have expressed interest in entering this protocol if it is approved by NIH. The fact is that there are very few individuals that fulfill the criteria of this study. Therefore, it is not possible to study the various disease stages in a controlled manner. However, important information will be obtained regarding the efficacy of administering activated T cells.

Dr. Walker explained that the concurrent protocol (approved by the National Institute of

Allergy and Infectious Diseases) differs from this proposal because it is only a clinical trial with no gene marking procedure and there is no fractionation of the T cells into CD4(+) and CD8(+) subpopulations. Therefore, it is relevant that the RAC review the entire protocol, not just the gene marking aspects.

Following infusion, a select number of patients will have samples collected and their cells will be sorted for CD4(+) and CD8(+) cells. These cells will then be cultured to analyze virus production. Daily assays will be performed to monitor p24 antigen levels and viremia during the first week following infusion and weekly thereafter for six weeks. Any significant increase in HIV production will be detected immediately.

Regarding cost to the individual patient participating in the protocol, Dr. Walker stated that NIH will assume all costs that are incurred after the screening visit. In the event of injury, the NIH will assume care for the patient to the extent that it is permitted. If a patient develops a chronic illness as a result of the therapeutic protocol, NIH would offer compensation and care on a case-by-case basis with the patient's home care provider.

In response to concerns regarding competition for the identical twin population between researchers at different institutions, Dr. Walker explained that inevitably there will be competition for a limited pool of patients. The decision to participate is always made by the patient. Currently, there is no communication with other laboratories with regard to this issue.

Presentation--Dr. Blaese

Dr. Blaese addressed the issue of transduction efficiency. Transduction efficiencies observed in whole populations of cells is similar to those observed with CD4(+) and CD8(+) subpopulations; however, there is variability between patients and between cultures. This result is the reason that latitude has been incorporated into the protocol regarding cell numbers.

Discussion

Dr. Parkman asked how they would distinguish the patient's autologous HIV infected cells from CD4(+) and CD8(+) that became infected following infusion? Will the patient's cells be selected in G418 in order to isolate and examine viral production in marked transduced cells? Dr. Blaese responded that these selection procedures would be performed. Dr. Parkman noted that this information was not included in the protocol.

In response to Dr. Zallen's comments regarding the informed consent process for the healthy donor twin, Dr. Walker said that it is essential that the donor twin is aware of

the HIV status of the infected recipient prior to obtaining the informed consent. Dr. Lane responded that in previous HIV twin studies, the infected twin always places the initial telephone call to the investigator performing the research. At that time, the investigator asks the patient for permission to discuss their HIV status with the healthy identical twin. The researcher then meets with the twins together and separately, providing each the opportunity to ask questions privately. Therefore, the informed consent issue raised by Dr. Zallen has never been a concern in previous protocols.

Dr. Lane also addressed the issue of liability. The NIH has standard language for informed consent documents. If the RAC is concerned about particular wording in these documents, perhaps the RAC should present this issue before the General Counsel and the NIH Director. Mr. Capron suggested that the RAC should advise the NIH Director that the language that addresses physical injury is misleading, and that there is no liability for such injury. The language should state that there will be no liability from injury that is not caused by negligence. Mr. Capron added that such a statement will not encourage litigation, only clarify the patient's rights.

Mr. Capron inquired as to the process by which patients will become informed about the availability of this protocol. Will information be disseminated in the medical community? Will there be a press release by the lay media? Are there special publications distributed only to patients who are HIV infected? He inquired about the process of determining the HIV status of the donor twin. If the proposed donor is tested and found to be HIV(+), how will this issue be dealt with? Dr. Walker replied that the NIH donor consent form addresses HIV testing policies. The prospective donor is informed that there is a remote chance that they will have an HIV(+) test, and this section is followed by an explanation of the implications of a positive test. Dr. Walker explained that the donor and recipient are counselled extensively prior to signing the informed consent document. In fact, both parties will have read and discussed the protocol with their primary physician prior to coming to NIH for screening.

Dr. Walker stated that there has been an HIV infected identical twin registry in existence since the early 1980s, and this database serves as the primary source from which patients are recruited. In addition, there is an extensive mailing list of practitioners in the U.S. that receive HIV announcements of protocols. Occasionally, researchers advertise in local and national publications. Dr. Dronamraju inquired as to the number of identical twin pairs that are currently on the registry. Dr. Walker stated that there are currently 24 pairs of twins on the registry. Dr. Dronamraju asked about a similar registry for nonidentical twins. Dr. Walker answered that this population has never been a research interest. Dr. Dronamraju asked the investigators to expand on their statement that approximately one-half to two-thirds of these patients have expressed interest in participating in this protocol. Dr. Walker said that this statement is based on telephone conversations with these patients lasting an average of 30 minutes.

Dr. Dronamraju asked the investigators for an estimation of the number of patients available for the various stages of diseases. Dr. Lane explained that with regard to staging of infection, staging procedures were developed before extensive knowledge of HIV, the role of CD4(+) cells, and techniques for tagging the disease by examining CD4(+) counts were understood. Rather than approaching HIV research by examining discreet stages, it is more efficacious to gather data for future interpretation regarding disease stage.

Dr. Parkman asked if there is a minimum level of transduction that will be informative. If a large number of patients exhibit transduction levels that are less than informative, will they be excluded from participating in the protocol? Dr. Blaese explained that although transduction levels have proven to be variable, they have always been able to transduce at a minimum level of 0.5%. Therefore, all patients would be eligible since there has never been a clinical population that was not transducible at this level. The level of marking will be carefully evaluated. Patients entering this protocol will receive approximately 20-fold more lymphocytes than patients receive in the adenosine deaminase (ADA) protocol. Transduced cells have been detected in the ADA gene therapy patients as soon as one day following infusion. There should be detectible levels of transduced cells based on the ADA protocol data. Dr. Parkman explained that there is a basic biological difference between the ADA and HIV protocols. Presumably in the ADA protocol, introduction of the neomycin resistance (neo^R) gene confers a selective advantage. Dr. Parkman stated that in HIV(+) patients, the majority of cells are not infected with HIV. Therefore, these cells are not any different from the normal noninfected cells within patients. Is there a minimum level that would be valid? Dr. Blaese responded that although there probably is a minimum value, there is no capacity to assay transduction levels in a timely fashion prior to infusion.

Ms. Buc reiterated the concerns raised by Dr. Zallen regarding the donor informed consent document and assessing the HIV status of the donor twin. Although the investigators have communicated the process by which the donor and recipient are solicited and counselled prior to obtaining their informed consent, the entire process should be included as part of the informed consent document. The current informed consent document does not accurately reflect the process as it currently exists. In addition, the section in the informed consent document that describes the lymphopheresis procedure should be inserted prior to the explanation of travel. The lymphopheresis is a major procedure, and its importance and description should not be understated. With regard to HIV testing of the donor, Dr. Lane explained that both the donor and recipient twin will have consulted a referring physician and the donor will have had a recent HIV test prior to coming to the NIH. In addition, NIH will retest for HIV infection of the donor if the RAC recommends repeat testing.

Dr. Parkman referred to a sentence in the informed consent document stating that the

potential benefit of the patient would be improved treatment of HIV infection using a new therapeutic approach. This statement is overly optimistic since it is not known if the procedure will provide any potential benefit to the patient. Dr. Walker disagreed with this conclusion noting that the ADA data suggests that lymphocyte transfer offers a potential benefit to patients. Potential is the key word. Dr. D. Miller suggested that the therapeutic benefit comments should focus on the gene transfer aspects of the protocol.

With regard to the vector safety testing requirements, Dr. D. Miller suggested that the RAC should focus on the issue of long-term cultivation of packaging cell lines. Is long-term cultivation for two to three weeks following harvest of vector supernatant the most sensitive test that can be performed to detect the presence of helper virus? This post-harvest culture criteria would be an acceptable standard for the RAC to adopt. Although the RAC could require investigators to co-cultivate supernatants with a cell line that would rescue helper virus, Dr. D. Miller suggested that such a requirement is probably not necessary for the RAC to request. Dr. Blaese noted that the FDA had a meeting today regarding revised standards for helper virus testing and suggested that Dr. Tolstoshev of GTI could comment on the current standards that are being employed by their company. Dr. Blaese stated that there is no indication from any experiments that have been performed to date that the current techniques or technologies for detecting helper virus contamination are not sufficient.

Dr. Tolstoshev commented on the issue of helper virus testing. GTI, which supplies many investigators with retroviral vector supernatants, has adopted the standard of culturing all packaging cell lines for three weeks following harvest of the vector supernatant and to monitor for helper virus during this period. If the RAC and FDA agree that these assay standards are adequate, then GTI has an abundance of vector material to provide for current trials. GTI has incorporated additional safety modifications to increase the specificity of helper virus assays. Regarding co-cultivation assays, these assays have not yet been validated. Dr. Parkman asked if aliquots were frozen from past production runs? Dr. Tolstoshev responded that cells were not generally frozen from past runs. Dr. Post asked if the FDA has an official position on standards for helper virus testing? Dr. Henry Miller of the FDA stated that the Center for Biologics is discussing the relevant issues today, and that he would rather not comment on the issue at this point in time. Dr. Post asked Dr. H. Miller if he could present an update of the FDA meeting during the afternoon RAC session. Dr. H. Miller answered that he would try to obtain the relevant information and report back to the RAC.

Dr. Chase said that the investigators have stated that there are 24 discordant identical twin pairs and that they represent a relatively complete catchment of the U.S. population. Is this assertion supported by formal computations? Dr. Lane explained that registry may not be the most appropriate term to use. Actually, there are probably

several thousand HIV patients who have an identical twin. The registry referred to those patients with a twin who have contacted the investigators regarding their interest to participate in select experimental protocols. Twenty-four actually represents a fraction of the total eligible population.

Dr. Secundy asked for clarification regarding the extent to which sections of the informed consent document are deliberated that are not directly related to gene marking. Dr. D. Miller stated that the RAC should only discuss issues that pertain to the use of recombinant DNA in a patient. In the case of the current protocol, the RAC should probably not consider the donor twin consent issues since the donor will not be undergoing any recombinant DNA procedure. Mr. Capron said that since the RAC is advisory to the NIH Director, it is obligated to discuss any problems that manifest during the review process, whether they are recombinant DNA issues or not. Dr. Wivel noted that the final control over informed consent documents resides with the local Institutional Review Board (IRB) irrespective of RAC recommendations. However, the RAC should not be discouraged from discussions it views as relevant.

Dr. Walters suggested that since this protocol actually consists of two separate protocols, the adoptive transfer of syngeneic lymphocytes that has the potential for therapeutic effect and gene marking of CD4(+) and CD8(+) fractionated T cells, perhaps the informed consent document should be divided into two distinct sections. The gene marking of fractionated cells is not a Phase I or Phase II study, only a method for monitoring their survival after they have been transferred for therapeutic purposes. The informed consent might be clarified for the recipient if it was presented as a gene marking form and a therapeutic form. Dr. Leventhal agreed with Dr. Walters suggestion.

Committee Motion

A motion was made by Dr. Post and seconded by Dr. Parkman to approve the protocol with the following stipulations: (1) the investigators may use the vector supernatants currently in storage; however, any future vector preparations will be tested by long-term culturing of the packaging line following vector supernatant harvest, (2) that the informed consent document should be divided into two separate documents, one for gene marking and the other the therapeutic aspects, and (3) the section of the donor informed consent document describing the lymphopheresis procedure should be moved before the section describing the required travel schedule.

Mr. Capron suggested an additional stipulation to include a sentence in the recipient informed consent document stating that there will be no waiver of liability for negligent injury. Obviously, the consent form will have to be approved by the IRB. Dr. Lane stated that inclusion of such a statement would create potential problems with the NIH

General Counsel and the IRB. Drs. Post and Parkman said that they would not accept the stipulation presented by Mr. Capron as a part of their motion for approval. However, Dr. Post recommended that the statement could be offered in the form of advice. Ms. Buc suggested that the RAC review the Code of Federal Regulation (45CFR46) that addresses IRB regulations as a future agenda item. If the RAC proposes changes to the current regulations, these changes could be presented in terms of advice to the NIH Director. Dr. Murray reminded the RAC that Dr. McCarthy, formerly from the Office for Protection from Research Risks, is on today's agenda to present information regarding this subject.

Dr. Murray called for a vote. The motion to approve the protocol with stipulations passed by a vote of 19 in favor, 0 opposed, and no abstentions.

Drs. Parkman and Post recommended that the issue of providing separate informed consent documents for the gene marking and clinical procedures should be placed on the agenda for the next RAC meeting as amendment to the *Points to Consider in the Design and Submission of Protocols for the Transfer of Recombinant DNA into the Genome of Human Subjects (Points to Consider)* of the *NIH Guidelines*.

IV. PROPOSED ADDITION TO APPENDIX D OF THE *NIH GUIDELINES* REGARDING A SEMLIKI FOREST VIRUS HELPER EXPRESSION SYSTEM/DR. TEMPLE

Review--Dr. Schaechter

Dr. Murray called on Dr. Schaechter to present his primary review of the proposal submitted by Dr. Gary Temple of Life Technologies, Inc., Gaithersburg, Maryland. Dr. Schaechter reviewed the proposal to conduct experiments with a Semliki Forest Virus (SFV)-SQL helper expression system at Biosafety Level (BL) 2. Dr. Schaechter explained that the gene expression vector system was initially developed in Sweden. It would be sold and commercially distributed as a kit. SFV is endemically found in Africa and is capable of causing disease. Past experience with laboratories working with this virus has resulted in one death; thus, the level of containment was originally elevated from BL2 to BL3. This agent is not harmless, symptoms include headaches and fever.

The expression vector kit being proposed consists of a virus in which foreign DNA can be cloned. The virus portion of the expression vector contains several point mutations that render it incapable of replication alone. In order for the virus to replicate, it must come in contact with a replication competent helper virus. Data demonstrates that the incidence of viral replication occurs at a relatively low frequency. Dr. Schaechter noted that written reviews were provided by two experts in this area, Dr. Robert Johnston from the University of North Carolina and Dr. Sondra Schlessinger from Washington University, St. Louis, Missouri. Both of these *ad hoc* reviewers stated that the data

submitted by the investigators are insufficient to conclude that this expression system can safely be used by laboratory workers.

The actual protocol for obtaining gene expression with the proposed system is complex, involving 16 separate procedures. Some of these steps are extremely intricate, including electroporation, creating cDNA, mRNA, etc. There is a high likelihood that some investigators may not be able to get the system to work. In that case, investigators may tamper with the system beyond the procedures that have been outlined. In some instances, laboratory workers who are not knowledgeable enough to understand all of the complexities and requirements of particular biosafety levels may use the kit.

Although this expression system may not be extremely hazardous to the investigators who purchase it, there remains the likelihood of pathogenicity if not handled under the proper containment conditions.

Review--Dr. Hirano

Dr. Hirano stated that she was in agreement with the comments made by Dr. Schaechter, in particular, the possible generation of infectious viral particles. The investigators have not supplied sufficient data to support the safety of this expression vector system despite the inclusion of three safety features that have been engineered into the system.

Other Comments

Dr. Post stated that he would like more background information regarding the classification of SFV as a BL3 agent. Ms. Buc requested that the investigators provide information about the disclosure of information to the purchaser. What kinds of warnings will be issued? What information will be included with the kit? Is there a manual?

Dr. D. Miller asked if people who travel to Africa are capable of becoming infected by this agent and transport it back to the U.S? Dr. Schaechter explained that this is not an issue of concern.

Presentation--Dr. Temple

Dr. Temple presented a summary of new data that the RAC members did not review prior to the meeting. He explained that this new data may address the issue of generation of replication competent virus using this system. These experiments were designed to determine the combination frequency of the helper virus with the vector. "Leakiness" of viral particles occurs at a rate of 1 in 10^6 cells. The term "leakiness" refers to uptake by any means, including: (1) passive endosomal uptake, (2) exogenous

or endogenous protease activity that activates the virus, or (3) a genetic revertant. In the case of the SQL mutation, the estimated frequency of reversion is approximately 10^{-12} . It should be noted that a second site revertant would probably occur at a higher frequency.

Dr. Temple presented plaque assay data demonstrating that when equal numbers of infectious units of the Helper 1 package virion and cells (10^6) were combined, approximately 700 to 10,000 plaque forming units were observed. This corresponds to the number of units that would result from one or two recombinant viruses. Therefore, 1 in 10^6 recombinant particles are detectable using this assay. Additional plaque assays demonstrated no infectious particles using the SQL helper virus. In fact, recombination plus suppression of the SQL effect was required for the generation of replication competent virus. He showed *in vivo* experiments in which 24 newborn and 40-day-old mice received intracerebral or intranasal injections of greater than 10^8 infectious units of the packaged SQL helper virus. No evidence of replication competent virus was demonstrated.

Dr. Temple stated that earlier data indicated that the recombination frequency of this virus is approximately 10^{-6} , and the frequency of "leakiness" is approximately 10^{-6} . However, both events would have to occur in order for replication competent virus to emerge. Therefore, even if the kit is used under circumstances other than those specifically outlined, there is a wide margin of safety. Because this is an efficient system, it is in high demand by many researchers. It is very important that its use be approved at the BL2 level of physical containment. Researchers using this system at the BL2 level will be required to sign an acknowledgement that they have been adequately informed of the procedures necessary to minimize replication competent virus emerging and that they are knowledgeable of BL2 requirements. An independent confirmation will also be required regarding the principal investigator's (PI's) expertise and qualifications by the Institutional Biosafety Committee. In addition, the PI will be requested to sign an agreement that he/she will not distribute the kit to other investigators without prior approval. Although there is no guarantee that the investigator will adhere to these principles, this process provides a degree of safety.

Discussion

Dr. Schaechter explained that because this kit will not fall under FDA regulations, the RAC is in the rare situation of deciding the disposition of this material on an advisory basis, not a statutory basis. Dr. Murray asked the investigators to respond to Dr. Schaechter's concern regarding other regulatory bodies that would provide approval of this kit. Dr. Temple stated that there is no other regulatory body whose approval is required.

Dr. Doi noted that the investigators stated that this kit was already in use by some

researchers. Approximately how many laboratories have used this expression system? What level of physical containment has been employed? Dr. Temple introduced his co-investigator, Dr. Liljestrom, to respond to Dr. Doi's question. Dr. Liljestrom said that the system has only been used in about ten laboratories because of safety concerns. All of these laboratories have a great deal of expertise working with SFV.

Dr. Krogstad spoke in regard to the earlier question about transmission. Just because no data exists regarding the issue of mosquito transmission, one should not rule out transmission by mosquito vectors. It is appropriate for the RAC to make their decisions based on the nature of the virus and on the appropriate containment for protecting it from exposure to the outside. There is inadequate evidence regarding the vector population in the U.S. and its competence.

As a point of clarification, Dr. D. Miller asked Dr. Liljestrom if the term "leakiness" referred to the ability to infect cells, but not necessarily to replicate. Dr. Liljestrom agreed to the interpretation of the term "leakiness". Dr. D. Miller noted that the data presented earlier suggested that the SQL mutant could convert to a pathogenic virus at a relatively high frequency, i.e., 1 in 10^7 particles. It is conceivable that an investigator may be working with as much as 10^9 particles using this kit. Therefore, there is a high probability that replication competent helper virus will be generated using this system. Dr. Liljestrom disagreed with Dr. D. Miller's statement, noting that the recombination frequency would have to be combined with the reversion frequency to estimate the frequency of replication competent particles. Dr. Schaechter stated that the data is not convincing. It represents the actual conditions of the experiment to be performed; therefore, the data does not accurately reflect the likelihood of detecting recombinant particles. One experiment does not reflect the degree of variability that would be observed from multiple tests. The data reflects imprecise measurements. Dr. Temple agreed that they have not performed strict reconstruction experiments. Instead, they are only able to conclude that they can detect a single or several recombinational events when equal number of cells to viral particles are used.

Dr. D. Miller said if helper virus is detected occasionally, what is the persistence of this virus on cell surfaces? How stable are these viruses in the laboratory environment? Dr. Temple responded that there is no data regarding the persistence of the viruses on open surfaces. Dr. D. Miller asked if inactivation experiments were performed. Dr. Liljestrom stated that SFV is a membrane virus; therefore, it is probably very labile. Virus particles would probably be dead in one to two days since detergents or 1% hypochlorite inactivate animal membrane viruses in seconds; contamination of membrane viruses could be cleaned up readily.

Dr. Temple responded to the question regarding the physical containment classification of BL3. The classification was based on the one reported death attributed to contact

with this virus. This virus is endemic to Central Africa and some areas of Switzerland. Generally, the symptoms are mild and nondifferentiable from the acute phase of other febrile illnesses such as acute influenza and early stage malaria. Although SFV is thought to be relatively safe, BL3 containment was designated because there could be some low incidence of fatal infection.

Dr. Post said that it is unclear as to why there is a different containment classification for SFV than Sindbis virus. There are a number of investigators who are currently using Sindbis vector systems as BL2 containment levels. Why have the investigators chosen to develop an SFV expression system instead of the Sindbis vector system? Dr. Liljestrom explained that his laboratory has extensive experience with SFV, and that in his opinion, Sindbis vectors do not function properly. Sindbis titers can not be obtained as high as those with SFV, probably because Sindbis does not replicate as efficiently. Dr. Temple noted that both of these are pathogens, but the only difference is that Sindbis has never had any fatalities associated with it.

Dr. Post added that pathogenicity is an important issue and reminded the RAC that there are a number of organisms classified for use at the BL2 level of containment that have proven to be fatal, e.g., *Vaccinia*. Dr. Post said it is unclear how the classification of pathogenic organisms in Appendix B of the *NIH Guidelines* was assigned. Apparently, the difference between a BL2 and BL3 classification is subjective. Dr. Wivel noted that Appendix B is largely based on Centers for Disease Control (CDC) data which considers a number of factors. The basis for most of the pathogenic classifications is based on data published in, *Biosafety in Microbiological & Biomedical Laboratories* published by the NIH and CDC. This document categorizes microorganisms based on the severity of illness, risk of infection, and lability of the infectious agent and focuses on human and animal pathogens. Dr. Temple noted that the NIH/CDC manual states that SFV can be safely handled for most laboratory uses as BL2; however, this classification was made prior to the fatality associated with its use. The latest edition of this manual was published in 1988. Mr. Barton inquired as to whether a distinction could be made regarding BL2 and BL3 classification if an organism is indigenous to the U.S. or not. Dr. Wivel answered that while this may be a consideration, classification is based in part on the availability of a vaccine against a particular microorganism.

Dr. Haselkorn asked the investigators to review the practical differences between BL2 and BL3 physical containment. Dr. Wivel explained that for BL3 containment, there is a requirement for negative air flow and that an autoclave be in the laboratory, not down the hall. Dr. Murray directed the members of the RAC that the definitions could be found on page 16974 of the (57 FR 19512). Dr. Geiduschek asked the investigators if the issue of commercialization is linked to a BL2 classification versus BL3. Dr. Temple said that Life Technologies, Inc., has no intention of distributing the kit unless it has a BL2 designation.

Ms. Buc commented that this is the first time that the RAC has been requested to reduce the physical containment level for a pathogenic expression vector system. Since RAC will likely receive more requests, the RAC should establish a *Points to Consider* document that outlines critical questions for investigators to respond to prior to seeking RAC approval for expression systems. A document of this nature would allow for a more disciplined and methodical review of these proposals.

Dr. Parkman stated that this viral vector expression system should be reviewed based on whether the viral modification can be safely handled at BL2. However, there is the more general question of whether the parent virus, Semliki, is more suitably classified as a BL2 agent instead of BL3. Probably, it would have been more appropriate for the investigators to request a reclassification of the parent organism as opposed to the modified virus.

Dr. Leventhal inquired about the data submitted from the laboratory that reported the one fatal case associated with SFV. The investigators state that antibodies to the virus were detected in four laboratory workers. How many were screened? What percentage does this represent? Dr. Temple responded that there is no extensive data regarding the incidence or frequency of symptomatic infections associated with the virus.

Dr. Hirano asked if any of the laboratories that have already worked with this expression system have worked at BL3 containment. Dr. Temple explained that all of the laboratories, both in the U.S. and abroad, have used the system at BL2. Dr. Hirano noted that all of the data was generated using the baby hamster kidney system. Have experiments been performed on other cell lines to test if there is a higher probability of generating infectious particles? Dr. Liljestrom responded that the system has not been tested on any other cell lines.

Committee Motion

A motion was made by Dr. Schaechter and seconded by Dr. Hirano to defer approval of Dr. Temple's request to lower the physical containment level from BL3 to BL2 for the SFV vector expression system. Dr. Murray called for a vote. The motion to defer approval passed by a vote of 17 in favor, 1 opposed, and 2 abstentions.

Dr. D. Miller suggested that the investigators should perform experiments to determine the frequency of infectious recombinants under the conditions of the expression system, i.e., 10^9 particles. Dr. Krogstad added that it would be useful to consult with investigators working with similar viruses at BL2 and establish the incidence of seropositivity among laboratory workers.

V. PRESENTATION: FINANCIAL OBLIGATION OF RESEARCH INSTITUTIONS TO

PATIENTS/DR. MCCARTHY

Dr. Wivel said that recently the RAC has had numerous discussions regarding the limits of liability on the part of sponsoring institutions that support human gene therapy protocols. Many of the issues that have been raised are generic to all clinical research, focusing of the phraseology of informed consent documents. In light of these discussions, Dr. Charles McCarthy, former Director of the Office of Protection from Research Risk, NIH, was asked to provide background information regarding the *Code of Federal Regulations* and the process of liability with regard to clinical research.

Presentation--Dr. McCarthy

Dr. McCarthy explained that much of the RAC's concern with regard to gene therapy is about injury that may occur to research subjects, not as a result of negligence, but as the result of unforeseen consequences of the research. In the history of clinical research to date, there have been virtually no cases of this kind although there have been several lawsuits relating to negligence.

Dr. McCarthy said that due to concerns about this issue, the Secretary's Task Force on Compensation of Injured Research Subjects was established as an advisory body to the Secretary of the Department of Health and Human Services (DHHS) for several years. The conclusion of the Task Force was that there is an obligation to provide compensation for subjects injured in the course of research where the injury itself is not related to the disease or condition from which the subject is suffering. Under Secretary Califano, legislation was proposed whereby injured subjects would be compensated under the Worker's Compensation Act. Individuals injured as a result of Federally funded research would be considered Federal employees for the purposes of compensation. However, the same week that the legislation was to be signed, Secretary Califano was fired. The proposed legislation was never signed. This issue was then taken up by the Ethics Advisory Board which also disbanded before a recommendation was developed. Next, the issue was passed to the President's Commission for the Study of Ethical Problems in Medicine and Biomedical and Behavioral Research. The Commission was also of the opinion that there is an ethical obligation to compensate injured research subjects. Nevertheless, the obligation was viewed as a consideration, not an absolute obligation. The Commission recommended that NIH should conduct a study to determine the cost of such a program. The NIH committee found that because the incidence of research related injury not attributable to negligence was so low, it was not possible to obtain data regarding the proposed cost of a nationwide program in a reasonable period of time. Therefore, the statistics on this issue are largely lacking. The prevailing opinion of experts today is that injuries can and do occur on rare occasions where the injury is not the result of direct negligence, but rather of unforeseen consequences of the research. Since it is not likely that DHHS will develop a program

for such injuries in the near future, the Office of Protection from Research Risks and the President's Commission endorsed the recommendation of the Ethics Advisory Board. Namely, research subjects should be informed as to whether or not a research institution is prepared to provide compensation in the event of unforeseen injury. Consequently, informed consent documents are required to state if compensation for injury that may be research related will be provided. Although several institutions in the U.S. carry insurance to cover such compensation, the majority do not regard themselves as obliged to provide compensation in the event of research related injury.

The *Code of Federal Regulations* does not permit exculpatory language. That is, a subject cannot waive his/her rights to bring lawsuits or seek compensation in the event of injury or harm. Therefore, subjects always have the option of bringing lawsuits against the sponsoring institution. Anecdotally, minor injuries related to research are always treated free of charge by the sponsoring institution. Long-range chronic injuries are not likely to be compensated.

Discussion

Dr. Parkman noted that a further complication to this issue is randomized clinical trials in which the patient may agree to participate in a protocol but not have a choice regarding the type of therapy that will be received. Would the institution be required to cover injury related to the standard treatment because it was included as a part of the experimental randomization?

Dr. Krogstad asked if there have been any instances in which non-negligent injury that has caused serious injury or death has been disputed and gone on to litigation in which the courts have had to make a judgement. Dr. McCarthy stated that he was not aware of any such cases where negligence was not an issue. Mr. Capron added that there have been a number of lawsuits resulting from the absence of informed consent. For example, at the University of Chicago, women were given diethylstilbestrol (DES) experimentally for the prevention of miscarriage without their knowledge. While the women receiving DES had no complications from the drug, untoward effects were observed a generation later in their offspring. As for the case of non-negligence, most attorneys would probably advise against litigation because the recovery is likely to be small.

Mr. Capron noted that one of the drawbacks of the Worker's Compensation system for the payment of non-negligent research related injury is that a typical Worker's Compensation claim is made by a normal subject. In the case of research subjects with disease, one must consider treating the natural course of their disease as a contributing factor. Sponsoring institutions may end up paying for events resulting from illness in addition to the treatment.

Ms. Buc said that in the few reported cases of litigation, almost all of the patients have been denied recovery. If the informed consent document is clear and accurate, there should be no negligence on the part of the investigator or the sponsoring institution.

Mr. Capron explained that one of the conclusions of the task force is that the reason for the low incidence of injury claims is probably due to the level of care that research subjects generally receive. Under normal circumstances, patients usually receive better care in the research setting than under the care of a primary physician. Negligence can occur by withholding something that is desirable, applying something that is desirable in an incorrect way, or by giving something that is undesirable. Mr. Barton stated that he is not as confident about the low number of liability cases as other RAC members. It is this fear of liability that is serving as a major deterrent to development of major research products, such as vaccines. Occasionally legislation is submitted to shift the responsibility for liability to the Federal Government to cap liability claims; however, other than the Swine Influenza legislation, none of these efforts have been realized.

Dr. McCarthy addressed the responsibilities of IRBs and the sponsoring institutions. The IRB is advisory to the institution except for the case when a protocol has been rejected. In that case, the IRB has veto power over research involving human subjects. The IRB/institution relationship is analogous to the RAC/NIH Director. Theoretically, the NIH Director would have the authority to approved a protocol that has been rejected by the RAC. However, such an outcome is unlikely.

Dr. Chase inquired if subjects are compensated for lost wages during the time that they participate in a research study and would receipt of such a payment alter their capacity to recover damages in a lawsuit. Dr. McCarthy responded that payment to a participant in a research study does not prevent them from initiating litigation. With regard to compensation for participation in a study, the *Code of Federal Regulations* are very vague stating that the amount of money offered to a subject should not be coercive. This amount of compensation is largely a judgement call by the IRB as to whether it is coercive with respect to the patient population. NIH generally compensates for travel expenses and lost wages during treatment.

Dr. Zallen asked if the Task Force, President's Commission, or any of the various committees ever made a distinction between therapeutic and non-therapeutic research. Dr. McCarthy said that a distinction was attempted; and at the time of the study, no injuries had been attributable to non-therapeutic research. Often, the distinction between therapeutic and nontherapeutic is unclear. For that reason, the terminology was changed to refer to research that is directly intended to benefit the patient versus not intended to benefit the patient. Dr. Zallen noted that the issue of compensation for research related injury was discussed at the November 21-22, 1991, Human Gene Therapy Subcommittee (HGTS) meeting. She and Ms. Buc drafted a resolution

embodying their concerns, and suggested that the resolution should be submitted for consideration at the next RAC meeting. The resolution recommends that the NIH establish uniform standards for the payment of medically related costs for injuries arising out of non-therapeutic biomedical research. Dr. Walters said that he supported Dr. Zallen's proposal.

Dr. Leventhal suggested that Data Management reporting should also be included as an agenda item for the next RAC meeting.

VI. PRESENTATION: RADIATION AND MUTATION RATES IN HUMAN POPULATIONS/DR. NEEL

Dr. Wivel explained that the HGTS formed a working group to discuss germ line gene therapy issues. These discussions resulted in a proposal to invite a series of experts to speak on issues that are relevant to the area of germ line gene therapy. While germ line gene therapy cannot be considered an imminent procedure, it is not premature to begin to discuss these issues in the event that this type of therapy becomes a reality. Since the RAC voted to merge the subcommittee with the parent committee, it is appropriate that these presentations be made to the RAC. He presented the first in this series of expert speakers, Dr. James Neel, a population geneticist from the University of Michigan.

Presentation--Dr. Neel

Dr. Neel noted that a relevant issue for the discussion of germ line therapy is an analysis of spontaneous and induced genetic mutations. Spontaneous mutation data reveals the frequency of DNA mutations in the absence of external factors, whereas induced mutation data reveals the magnitude of the response to known perturbing factors. Conclusions can be drawn regarding the homeostatic properties of the genome. Despite considerable research, there is a significant amount of information that needs to be obtained about the frequency of human germ line mutation rates.

Functional genes have a mutation rate between 1 to 2×10^{-5} per gene per generation. With 50,000 functional genes, a newly fertilized egg has two to four point mutations in these functional genes. At the DNA level, the frequency of spontaneous mutation for nucleotides is 1 to 2×10^{-8} per generation. With 3×10^9 nucleotides in the haploid genome, this corresponds to 30 to 60 mutations per gamete and 60 to 120 per zygote. Many of these mutations occur in DNA with little functional consequence, but the remainder will occur in DNA whose integrity must be maintained.

Purines and pyrimidines are constantly being displaced from the DNA. Purines are displaced at the rate of 3×10^{-11} per second, suggesting remarkable stability for any specific site. Mammals lose approximately 10,000 purines from their DNA every 20

hours by spontaneous hydrolysis. Unless precisely replaced, each loss could result in a mutation. DNA is constantly exposed to mutagenic chemical radicals, resulting from indigenous and exogenous metabolism. These effects must be repaired exactly or a mutation will result. To meet these challenges, organisms have developed a complex array of fail safe mechanisms. Experiments demonstrate that *E. coli* is capable of repairing over 95% of the damage that occurs to its DNA.

Dr. Neel presented a slide demonstrating the presence of rogue cells in human peripheral blood. Although the most abnormal of these rogue cells are not capable of undergoing mitosis, the less abnormal ones could be capable of cell division. It is postulated that these cells occur in other tissues of the body and may be the starting point of oncogenesis. Data suggests that these abnormal cells are the result of transposon activation.

Dr. Neel addressed the effect of severe external perturbation, such as exposure to atomic bombs, on DNA. He noted a 46 year victim follow-up to the Hiroshima and Nagasaki bombings. This follow-up includes a complex epidemiological analysis of the individuals effected as well as their offspring. These data have been matched to control individuals with respect to sex and year of birth. Rosters (cohorts) exist that include data on approximately 30,000 offspring of the exposed individuals. In this group, the rate of untoward pregnancies and mortality exclusive of cancer is far below the level of statistical significance. Protein studies reveal no evidence of increased mutation rates. In addition, these data suggest that there was no effect on the physical development of these offspring. No change in the expected sex ratio was observed. No increased incidence of inheritable or non-inheritable tumors was observed. In summary, it has not been statistically demonstrated that parental exposure to the nuclear radiation resulting from these bombings has adversely affected the attributes of the children of the exposed individuals.

Dr. Neel stated that for the past 40 years, data regarding the genetic implications of radiation have been guided by *in vivo* murine research. Presently, mutational geneticists are discovering that the human data derived from the acute exposure to radiation does not correspond to data derived in mice. The doubling dose of acute radiation for mice is approximately 0.4 severs. Humans are five times less sensitive to acute radiation than mice to radiation. For exposure to chronic radiation, the doubling dose is 1.0 severs for mice and 4.0 severs for humans. It is difficult to compare the human and murine findings. Therefore, mutation geneticists hypothesize that the only murine data that would compare to the human situation would be specific locus (phenotype) testing. Following this method of analysis, it was found that when the estimates of the acute dose in mice are converted to chronic dose by a dose rate factor of three, a doubling dose of 4.0 severs is obtained. This result very closely estimates the human scenario. However, there are errors associated with these estimates.

Regarding germ line gene therapy, Dr. Neel added that despite the overwhelming scientific advances towards genome research in the last 30 years, understanding the anatomy and interactions of the genome is still in its infancy. He stated that in his opinion, it would represent intellectual arrogance for scientists to seriously consider embarking on human germ line gene therapy. The implications and lessons of somatic cell gene therapy will not be completely known for at least 30 years. Recent data suggests that "shotgun" injection of DNA into transgenic mice results in serious genetic defects for 10% of the population; this frequency may actually be higher. For this reason, germ line therapy carries an unacceptable level of risk at this time.

Dr. H. Miller inquired about the following. If data demonstrates that only 10% of the animals acquired developmental abnormalities and since the patients who would be eligible for such therapy have profound life threatening diseases, is this level not acceptable for risk to the patient? Dr. Neel cautioned Dr. H. Miller that modifications incorporated through germ line gene therapy remain in the gene pool, therefore, affecting future generations. Although there may be an immediate gain, the inserted gene may be a "genetic time bomb."

Dr. Parkman acknowledged that the RAC is not in a position to consider germ line gene therapy as a primary form of treatment for life threatening diseases at this time. However, the RAC must consider the possibility that the genetic material administered for somatic cell therapy could inadvertently become incorporated into the germ line of that patient.

Dr. Walters asked Dr. Neel to comment on the prospects for technical breakthroughs in homologous recombination and site specific integration of new genes. Dr. Neel explained that he could not visualize any introduction of a gene so precise that there would be no residual change in the nucleotide composition of that particular segment of DNA.

Dr. Krogstad inquired about the frequency of crossing over and reduction division that occurs in a single generation. Dr. Neel noted that when crossing over is not precise, there is potential for a mutation to occur. Experiments to address this issue are just beginning and are very complex to perform in higher eukaryotes. It is known that crossing over is sometimes imprecise, e.g., hybrids between the beta and delta locus. However, the extent to which there is imprecise crossing over within one locus is unclear.

Dr. Haselkorn asked about the basis for different mutation rates among different genes and the "real life" equivalent of five severs. Dr. Neel responded that a large gene, e.g., neurofibromatosis, has a higher mutation rate than a smaller gene. In addition, there are certain nucleotide sequences that are susceptible to mutation by methylation. Dr. Neel explained that during the reproductive cycle, an individual is exposed to

approximately 10 Roentgen units of chronic radiation, from both spontaneous and industrial exposure. The doubling dose for acute radiation is about 1/40th of the chronic dose. One important issue is that risk of mutation is principally at the time of replication. Controlled experiments in which cell cycles are regulated in culture suggest that mutations occur when nucleotide substitutions are made at the time of cell division.

Dr. DeLeon asked Dr. Neel to comment on the fact that the egg is responsible for about 10% of chromosomal abnormalities. Dr. Neel said that at least 10% of newly fertilized eggs carry a major chromosomal abnormality, and that most of these are eliminated early in pregnancy. These abnormalities can be derived from either the father or the mother. Sperm cells transmit a large number of abnormalities.

Mr. Capron asked if the current thrust of DNA research directed towards these questions still remain to be answered. Dr. Neel said more intensive investigation is needed using animals with short life spans. The carcinogenic implications of DNA therapy could be better addressed in animals with shorter life cycles. Mr. Capron asked if the 10% of abnormalities that occur result in neoplasms or growth deformities. How is fetal wastage taken into consideration in this figure? Dr. Neel said that genetic abnormalities occur in 10% of transgenic mice having a gene inserted into their germ line. Dr. Anderson stated that the actual percentage could be higher than estimated. Dr. Neel explained that there is a range of abnormalities reported ranging from homozygous lethal *in utero* effects to physical abnormalities.

Dr. Leventhal said that the data indicated that the offspring of individuals exposed to ionizing radiation were normal compared to control groups. Is there any evidence that future generations have a higher likelihood of being abnormal? Is there a protective effect of the radiation damage so that damaged cells are incapable of reproducing? Dr. Neel stated that the data suggests that the homeostatic properties of the genome are better than originally hypothesized. With regard to future generations, there is evidence of recessive hidden damage as evidenced by the presence of abnormal proteins. These studies are currently in progress at laboratories at Hiroshima and Nagasaki. Dr. Leventhal asked if there is a reason to suspect that the mechanism of alteration of DNA by gene therapy is more likely to result in transmissible damage than any other external source? Dr. Neel stated that cells have a coping mechanism that has evolved to compensate for radiation and chemical damage. Because there are multiple retroviral footprints in the human genome, a copying mechanism also exists to a degree. However, it is unclear what the cost to the population will be of randomly inserting genetic material into our genome. Dr. D. Miller interjected that there is a large body of data regarding the integration of transposable elements into genes in disease states such as Factor 8. Can any predictions be made regarding how often these integrations occur? Dr. Neel responded that data derived from *Drosophila* experiments suggest that 50% of all mutations are the result of retroviral movement.

Dr. Walters noted that Dr. Neel had published the first scientific article in the *American Journal of Human Genetics*, published in 1949, and thanked Dr. Neel for addressing the RAC. Dr. Walters asked Dr. Neel to address Mueller's "load of mutations" theory, and whether these mutations could ever be reversed through technology or if humans must learn to cope with this inevitable condition. Dr. Neel answered that Mueller envisioned the human species as precariously balanced between an increase in the mutation rate and biological collapse. Dr. Neel stated that the prospects for the human condition are significantly better than those proposed by Mueller.

Dr. Neel stated that he is not opposed to the approval of somatic cell gene therapy experiments for the treatment of disparate diseases of the type that the RAC has already approved. However, for protocols such as ADA, these children must be evaluated indefinitely. Sometimes the short-range gain is offset by long-term loss. An example of this scenario is the children who were cured of leukemia by intensive radiation and chemotherapy. Twenty years later, 20% of these individuals have induced secondary tumors. Dr. Murray thanked Dr. Neel.

VII. PROPOSED ADDITIONS TO APPENDIX D OF THE NIH GUIDELINES REGARDING THREE HUMAN GENE TRANSFER PROTOCOLS ENTITLED: (1) PHASE I/II STUDY OF THE USE OF RECOMBINANT HUMAN INTERLEUKIN 3 STIMULATED PERIPHERAL BLOOD PROGENITOR CELL SUPPLEMENTATION IN AUTOLOGOUS BONE MARROW TRANSPLANTATION (ABMT) IN PATIENTS WITH BREAST CARCINOMA OR HODGKIN'S DISEASE, (2) EVALUATION OF THE USE OF RECOMBINANT HUMAN GRANULOCYTE COLONY STIMULATING FACTOR (G-CSF) STIMULATED PERIPHERAL BLOOD PROGENITOR CELL SUPPLEMENTATION IN AUTOLOGOUS BONE MARROW TRANSPLANTATION IN PATIENTS WITH LYMPHOID MALIGNANCIES, AND (3) A TRIAL OF G-CSF STIMULATED PERIPHERAL BLOOD STEM CELLS FOR ENGRAFTMENT IN IDENTICAL TWINS/DR. SCHUENING

Review--Dr. Geiduschek

Dr. Murray called on Dr. Geiduschek to present his primary review of the three gene transfer protocols submitted by Dr. Friederich Schuening of the Fred Hutchinson Cancer Research Center, Seattle, Washington. Dr. Geiduschek provided a brief overview of these three protocols involving ABMT of CD34(+) subpopulations of cells that have been transduced with a marker gene encoding for neo^R. The investigators have presented a large amount of data derived from large animal models. These *in vivo* data suggest that there are wide fluctuations in the frequencies of clonal populations. Dr. Geiduschek stated that with the original review of these protocols, he had a number of concerns; however, Dr. Schuening responded to all of these questions satisfactorily. The one issue that remains to be resolved is that of safety testing and what constitutes a

quantitative and information based system for assuring the safety of transducing particle suspensions.

Dr. Geiduschek stated his concerns regarding the issue of helper virus testing. Standards should be established for these assays to ensure that the probability of contamination by one helper virus particle is less than 10^{-x} , where "x" is a suitable number greater than two or three. Currently, the S⁺/L⁻ test does not meet this requirement. There is an extended S⁺/L⁻ assay that may be more sensitive than the standard test; however, this extended assay was not performed by Dr. Schuening.

Dr. Geiduschek explained that the investigators cultured the packaging cell line following harvest of the vector supernatant for several weeks to ensure that no helper virus particles were present in the original preparation. This culturing procedure is a more sensitive method for the detection of replication competent helper virus than the standard S⁺/L⁻ assay because the entire preparation is tested, not just a fraction. In addition, long-term culture relies on the dynamics of helper virus appearance in a population resulting from an initial contamination. If the dynamics of this long-term culture procedure are established with regard to the specification of cell line, constructs, and producers, then this procedure should yield the appropriate safety criteria for monitoring helper virus contamination. The necessary reconstruction experiments have not yet been performed to determine these specific criteria. This issue should be settled in a quantitative and reliable manner. Since Dr. Schuening will obtain his vector supernatants from a source other than the one that has supplied investigators of previous approved protocols, perhaps the RAC should base its decision on safety data submitted in response to established safety criteria rather than relying on the track record of a particular supplier.

Review--Dr. Krogstad

Dr. Krogstad said that he had some of the same initial concerns regarding the helper virus assay that were raised by Dr. Geiduschek, but the important issues were discussed previously. The study proposes to treat 20 patients over a four year period. If there are subgroups of patients with various diagnoses, how will the investigators evaluate this data? If the disease process of a particular group has an impact on the outcome of the experiment, the data may be difficult to interpret. Dr. Schuening responded earlier that all of these patients should have marrow activity restored; therefore, similar positive results should be obtained for all groups with regard to the marking study. From a theoretical point, it would be preferable to employ a technique that would allow one to distinguish between marked cells that have and have not replicated their DNA. In response to concerns about quantitative PCR, Dr. Krogstad noted that the investigators will respond to this point during their presentation.

Review--Dr. Walters

Dr. Walters asked the investigators to hypothesize about the fate of the gene marked cells if they survive and contribute to reconstitution. The investigators propose to use the LN retroviral vector. How does LN compare to the LNL6 vector that has already been approved for use in other human gene therapy protocols? How many animals have been studied in the *in vivo* experiments to date? What is the transduction efficiency of human cells by the proposed retroviral vector? With regard to the informed consent document, Dr. Walters stated that it was clear and targeted directly to the gene marking aspect of the protocol.

Other Comments

Dr. Parkman said that the RAC needs to define its criteria for a vector, regardless of the supplier of the vector. A set of vector standards needs to be established. These standards may evolve as assays are refined, and their level of sensitivity is increased. Since the safety standards established by the RAC will continue to evolve, it is critical that the RAC maintain consistency, fairness, and objectivity in its review. If Dr. Schuening already has vector supernatants that meet the same safety standards as were required for Drs. Walker and Blaese, then they should be allowed to use the preparations that already exist. Have Dr. Schuening's preparations already been approved by the FDA? If Dr. Schuening's vector is not analogous to the vector proposed in the protocol review this morning, would the new extended S⁺/L⁻ assay standard be appropriate?

In response to Dr. Parkman's comment, Dr. Geiduschek stated that the issue of equity between commercial enterprises is not the dominant one. The question is how to assure safety within agreed guidelines. Dr. Parkman responded that the S⁺/L⁻ assay has been the gold standard for the RAC up to this point. GTI has provided numerous lots of vector supernatants screened by this assay method. The issue is whether the RAC should use a different standard for safety between two protocols reviewed at the same meeting; this issue is about fairness. The fact that a better assay exists should not influence the review of this protocol. If the RAC decides that the extended S⁺/L⁻ assay is now the standard, then that should be a requirement for protocols presented at future RAC meetings. Dr. Geiduschek added that Dr. Schuening is using a vector and supplier different from those used in the Walker-Blaese protocol.

Dr. Zallen said that the informed consent document states that the gene marking protocol will not directly benefit the patient; however, the patient will be responsible for the cost of the therapy. First, the gene marking protocol is not considered therapeutic. Second, Dr. Schuening agreed that the statement about payment by the patient would be removed. She asked the investigators to respond to these discrepancies in the informed

consent document.

Dr. Post requested that the investigators provide further information regarding co-cultivation of the cells with the producer cell lines. This procedure may complicate the issue of helper virus contamination. What is the stroma that will be in the long-term bone marrow culture?

Dr. Murray called on Dr. Paul Aebersold of the FDA to present a brief summary of the FDA meeting held in the morning regarding safety testing for replication competent retroviral vector supernatant preparations.

Presentation--Dr. Aebersold

Dr. Aebersold of the Division of Biological Investigational New Drugs, FDA, noted that protocols previously approved by the RAC and FDA were approved prior to recent observations in monkeys that developed lymphomas. This new data suggests that replication competent retroviruses possibly could be pathogenic in primates. The FDA is concerned about the implications of these findings and whether it should initiate testing beyond previous requirements.

On the other hand, when dealing with industrial scale production of biological agents, it is not possible to exhaustively test entire production lots. For example, a production run of 200 flasks would require a final scale up to 20,000 flasks in order to obtain a final S⁺/L⁻ readout of the entire preparation. Quality control testing is not exhaustive testing, only representative aliquots. Therefore, quality control testing can never assure that no replication competent viruses exist. The focus of the FDA's discussions is how to sample a production run for testing, what assays will be performed on these aliquots, the percentage of producer cells that should remain in long-term culture, and whether the producer cells should be cultured with a permissive cell line for retroviral replication, including xenotropic viruses. It has been proposed that the co-cultivation assay procedure may be a more sensitive method for detecting helper virus contamination; however, this result has not been verified in a side-by-side comparison. In the future, the FDA will probably require supernatants to be assayed on a cell line that is sensitive to xenotropic viral replication such as the *Mus dunni* murine cell line which supports xenotropic virus replication. In addition, the FDA may require co-cultivation testing of an aliquot of the producer cells. However, neither of these assays would absolutely assure that there are no replication competent viruses in a clinical lot. Although the FDA will probably be initiating more stringent standards for the assay of replication competent helper viruses, it is unclear what action will be taken on existing clinical lots.

Dr. Post inquired as to the FDA's requirements on existing protocols. Dr. Aebersold stated that he could not respond to Dr. Post's question because this issue is not a simple

one. Investigators who submit a protocol to the FDA today are not aware of the newly developed standards. There will have to be a transition period that has not been established yet to deal with the clinical lots that are already in existence. The producer cell from these lots are no longer in existence and the supernatants are cryopreserved and ready for use. Investigators cannot retrospectively go back and obtain a sample of the cells since they are no longer available.

Dr. Post asked what the requirements were under the old system of safety standards. Dr. Aebersold responded that S⁺/L⁻ testing and amplification on NIH 3T3 cells were required for all supernatant preparations. Dr. Parkman asked if investigators will be required to keep an aliquot of the producer cells in long-term culture to verify the lack of helper virus contamination. Dr. Aebersold answered that there will be a requirement to culture the producer cells.

Ms. Buc stated that it is inconceivable that the RAC is deliberating on an issue that was not an issue for approving a protocol this morning. There needs to be a sense of consistency. Dr. Parkman said the important issue is whether Dr. Schuening has a vector preparation already in storage that has been approved by the FDA. If they do not have a lot with FDA approval, then this discussion is irrelevant because the FDA will employ the revised requirements for subsequent lots.

As a point of clarification, Dr. Krogstad explained the potential differences for concern between Drs. Walker and Blaeses' protocol and Dr. Schuening's protocol. The discussion about Drs. Walker and Blaeses' protocol was predicated on economics and the wasting of a substantial investment of a lot. The issues encompassing Dr. Schuening's protocols focus more on the safety aspect. If the RAC decides that there is a significant difference in the level of safety between these two protocols, safety should take precedence over economics. Dr. Parkman stated that economics was not a consideration with regard to his earlier comments. The relevant issue is the same testing criteria for both protocols.

Presentation--Dr. Schuening

The objective of the marking protocol is to determine whether peripheral blood progenitor cells contain pluripotent hematopoietic stem cells. Insights will also be obtained regarding the best method for mobilizing these peripheral blood stem cells in order to achieve optimal results. Data obtained from this study will indicate if peripheral blood contains long-term repopulating cells which would establish their potential use for autologous and allogeneic transplants or as long-term carriers of therapeutically relevant genes.

Patients eligible for the gene marking protocol are those who are participating in the

clinical protocol involving ABMT for non-myeloid neoplasia. The inclusion criteria are limited to patients with non-myeloid neoplasms, e.g., breast carcinoma, to prevent the possible mobilization of leukemic cells. These patients have received growth factors that will mobilize their peripheral blood progenitor cells. These progenitor cells are then harvested and stored for transplantation. Patients participating in the gene marking portion of the study, will have 25% of their stored peripheral blood progenitor cells transduced with the neo^R gene. These transduced cells will be transplanted into the patient in addition to the nontransduced cells. Patients will be monitored over time to detect the presence of gene marked cells following transplantation to determine if the administration marked pluripotent stem cells result in continued expression of the neo^R gene in both myeloid and lymphoid populations.

Dr. Schuening presented preclinical *in vivo* data obtained using the canine model to address the issue of continued gene expression in both myeloid and lymphoid cells. Long-term gene expression has been observed out to 3½ years following transplantation of transduced marrow cells in irradiated animals. Two different methods of transduction were used. One method used a co-cultivation procedure in which marrow cells were cultured with the vector producing cells for 24 hours and subsequently incubated in a long-term marrow culture with vector supernatant for an additional four days. Preliminary *in vitro* data suggest that expanding the exposure time to the vector-containing supernatant increases transduction efficiency. The animals were pretreated with cytoxan to enrich for nondifferentiating and stem cells. Cytoxan also stimulates the stem cells to cycle which is an important prerequisite for retroviral transduction.

In recent *in vivo* experiments, animals underwent bone marrow aspiration and their marrow cells were cryopreserved. Following bone marrow harvest, the animals received growth factor to stimulate the early progenitor cells. Six days later, the animals were leukapheresed, and the cells were enriched for Class II antigen positive (+) cells which have been shown to contain the stem cell fraction in the canine model. These enriched cells were cocultivated for 24 hours with vector producing cells followed by continued incubation with long-term marrow cells for 11 days. These cultures were replaced with fresh vector containing media every other day. The animal was then lethally irradiated and both transduced and untransduced cells were readministered. Three animals have been transplanted to date using a modified protocol that is very similar to the proposed human experiments. 1×10^7 nontransduced cells and 1×10^6 transduced cells were transplanted per kilogram. All three of these animals engrafted; one death occurred due to infectious complications. The remaining two animals have survived out to 180 days post-transplant. Drug resistant colonies from both lymphocytes and marrow cells have been obtained out to 22 weeks in these two animals. These data suggest that pluripotent stem cells have been transduced in these animals.

Dr. Schuening presented *in vitro* human data indicating that 24 hour co-cultivation of

marrow cells with vector producing cells results in 5-24% neo^R colonies. This data corresponds to the information obtained from the canine model. In addition, peripheral blood progenitor cells were obtained from a patient participating in the ongoing therapeutic trial in which the patient received G-CSF for the mobilization of progenitor cells. Following mobilization, CD34(+) cells were selected. This selected fraction of cells was then transduced by 24 hour co-cultivation with vector producing cells. Fractionation increases the efficiency of transduction because the number of target cells has been greatly reduced.

The goal of the protocol is to determine if genetically marked peripheral blood repopulating cells contribute to long-term hematopoietic reconstitution after autologous marrow transplantation, and specifically, to monitor the quantitative differences in long-term contribution by marked peripheral blood repopulating cells based on the particular growth factor administered prior to bone marrow harvest. One protocol uses interleukin (IL) 3 and the other uses G-CSF for mobilization of early progenitor cells. Important information will be derived from this protocol regarding which growth factor is the best choice for mobilization in ABMT.

Dr. Schuening described the human protocol. Twenty days prior to transplantation, the patient's marrow will be harvested and cryopreserved. Subsequently, the patient undergoes cytokine treatment for the mobilization of peripheral blood progenitor cells. Four days following cytokine treatment, the patient is leukapheresed and the cells are enriched for CD34(+) cells. Twenty-five of these CD34(+) cells will then be transduced using the 24 hour co-cultivation with producer cells followed by long-term marrow culture. Vector containing supernatant will be replaced in these long-term cultures every other day in order to extend the exposure time to the retroviral vector thus, increasing the transduction efficiency. The LN vector is safer than the LNL6 vector because the *env* sequences have been eliminated, reducing the possibility of homologous recombination leading to helper virus production. However, there have been no instances of helper virus production with the LNL6 vector to date. If the RAC decides that insufficient safety data has been submitted with the LN vector, then the LNL6 vector will be used. Following the preparatory regimen, the patient will receive the transduced and untransduced marrow cells in addition to the transduced CD34(+) cells. After hematopoietic recovery, marrow and peripheral blood cells will be assayed by polymerase chain reaction (PCR) for the presence of the neo^R gene as well as the development of neo^R colonies.

In response to Dr. Walters question regarding the fate of the marked peripheral blood repopulating cells, Dr. Schuening responded that based on the *in vivo* canine model, data suggests that peripheral blood derived stem cells behave similarly to bone marrow derived stem cells. Following infusion, peripheral blood repopulating cells will migrate to the bone marrow which will lead to hematopoietic recovery. Despite the *in vivo*

murine evidence that peripheral blood derived stem cells reconstitute similar to marrow derived stem cells, this result has not been proven in the human system.

With regard to the differences between LNL6 and LN, Dr. D. Miller will respond to this question. In response to the question about the number of animals transplanted to date, three dogs have been transplanted. The plan is to transplant a total of six animals in order to provide a firm base for the human study.

Dr. Schuening addressed the earlier question about cost associated with the treatment. He explained that the informed consent document has been changed so that the form clearly states that patients will not be responsible for the cost of the gene transduction procedure.

The most efficient transduction procedure is obtained by co-cultivation with lethally irradiated vector producing cells and subsequent incubation in a long-term marrow culture system fed every other day with vector containing supernatant. Earlier experiments indicate that 24 hour co-incubation with irradiated producer cells alone results in short-term expression of the marker gene. This recent data suggests that long-term exposure is necessary for the transduction of committed progenitor cells because stem cells replicate very rarely. The long-term exposure increases the likelihood of transduction.

Dr. Post inquired if cell types other than the CD34(+) selected peripheral blood cells are present in the long-term marrow culture system. Dr. Schuening explained that the long-term marrow culture system consists of an adherent cell layer that is established from the patient's marrow cells at the time of harvest. Dr. Post asked if the adherent cells will also be administered to the patient. Dr. Schuening replied that the adherent cells also would be returned to the patients. Dr. Post inquired about the potential transduction of the adherent cells by the neo^R gene. Dr. Schuening said that the fibroblasts will probably be transduced by the retroviral vector. Dr. Post was concerned that there would be additional stem cells derived from the marrow culture that would be transduced in addition to the peripheral blood stem cells. It was unclear how the investigators will distinguish between the two cell types. Dr. Schuening answered that the adherent layer also will be irradiated; therefore, the marrow culture cells are incapable of being transduced.

Dr. Parkman noted that following co-culture of the progenitor cells with the producer cells, the cells will be trypsinized and added to the autologous stroma. In that event, murine cells are now mixed with human cells. Although most of the irradiated murine cells will die, there exists the possibility that a few mouse cells will remain and be administered to the patient. Dr. John Belmont has reported that transduction with autologous stroma and cell free supernatants is as efficient as the co-culturing method.

He asked Dr. Schuening if he had data in his model to confirm this hypothesis. Is the co-culture step necessary since there is the potential of added risk? Dr. Schuening responded that his *in vitro* data suggest that the co-cultivation step is necessary. Cells were transduced with and without co-cultivation with producer cells and gene expression was monitored for six weeks. Neo^R expression was much longer with the co-cultivation procedure.

Dr. Walters asked for further information regarding the cause of death in one of the animals. Dr. Schuening said that the animal died of infectious complications, i.e., sepsis, resulting from the transplantation procedure. It is not unusual for animals to die in the early phase post transplantation because they are immunosuppressed as a result of the chemotherapy regimen. Dr. Walters asked how long the remaining two animals will be studied before sufficient data is obtained to initiate the human protocol. Dr. Schuening stated that all of the animals would be monitored for helper virus during their lifetime. However, monitoring of the marked peripheral blood cells will not be monitored for this period of time. A reasonable estimate is that the peripheral blood would be assayed in these remaining animals for one year following transplantation to demonstrate that pluripotent stem cells have been transduced prior to initiating the human protocol. By the time FDA approval is obtained, the animals will have been followed for approximately one year.

As a point of clarification, Dr. Parkman explained that the actual objective of the protocol is to determine the most optimal cytokine for mobilizing repopulating cells in these patients. Other investigators have already demonstrated that lethally irradiated patients could be reconstituted with peripheral blood stem cells. In terms of efficacy, all patients are obtaining their own bone marrow; so unless there is a flaw in the cryopreservation process, all patients will engraft. Seventy-five percent of the stem cells readministered to the patient will be untransduced; therefore, the clinical impact of the gene marking is minimal. Dr. Geiduschek inquired if the only variable between the protocols submitted by Dr. Schuening is the choice of mobilizing cytokine, then explain the choice of different patient populations and different target tumors. Dr. Schuening replied that he was not entirely in agreement with the statement made by Dr. Parkman. Previous data does not indicate whether long-term recovery is due to the transplanted peripheral blood derived cells or to endogenous recovery resulting from the temporary support provided by these cells. This question has never been answered formally in the human setting. IL-3 and G-CSF are the two cytokines proposed for use in these protocols; however, additional protocols will be submitted in the future to examine a broader range of cytokines.

Dr. Leventhal said that these protocols are not designed to answer the cytokine question, because they are intended to target two separate tumor populations. Why are the investigators looking at so many variables, i.e., different patient populations, different

chemotherapy histories, and different cytokines for each group? At what point do the investigators plan to use a single patient population using three different mobilizing agents as the variable? Dr. Schuening responded that in the long run, this approach would be the best for obtaining definitive answers. However, with the patient recruitment levels for these therapeutic protocols, it is not possible to have sufficient numbers of patients to examine these variables in a systematic approach. The gene marking protocol must be approved as an addition to these already existing protocols. To date, no difference has been observed in the time of recovery from autologous transplantation between the various types of tumors. Dr. Leventhal noted that the protocol design would be improved if patients with various tumor types were given one particular cytokine versus one cytokine to one tumor type. Dr. Leventhal requested clarification as to the tumor type versus the specific cytokine that will be used. Dr. Schuening said that G-CSF will be administered to breast cancer and Hodgkin's patients, whereas IL-3 will be administered only to lymphoma patients. Dr. Murray noted that the protocol titles do not correspond to this fact.

Dr. Chase said that experimental design dictates that multiple treatments for multiple diagnosis does not require increased numbers of patients, merely the proper allocation of patients. Therefore, this is not a resource question, it is a question regarding experimental design.

Dr. Parkman said that the RAC must focus on the fact that this protocol is a gene marking study, independent of preexisting therapeutic studies. Dr. Leventhal said that if this trial were to be truly randomized, then representative patients from each tumor type should be assigned for each particular cytokine. Dr. Parkman explained that if the investigators are required to make this change, then they will have to change the design of their basic therapeutic protocols.

Dr. Murray called on Dr. D. Miller² to present background information regarding the LN vector. Dr. D. Miller stated that LN is a modest modification of the LNL6 vector that has been approved for use by the RAC. LNL6 has an envelope sequence that could allow for homologous recombination with a packaging DNA or RNA. With LN, all of these *env* sequences have been deleted. LN is almost identical to the G1Na vector which has an additional linker on the end. Therefore, LN should be the safest vector. LN is produced by Targeted Genetics, Inc. This company also supplied the vector used by Dr. Philip Greenberg of Seattle, Washington. Dr. Greenberg's protocol was previously reviewed and approved by the RAC and the FDA.

²Dr. D. Miller is a co-investigator on this protocol. His remarks are in response to reviewers comments. He temporarily relinquished his position as a committee member during this protocol.

The LN vector has probably been safety tested more extensively than any of the other vectors reviewed to date. LN has been "ping-ponged" between various helper virus sensitive cell lines up to eight times. No detectable helper virus was demonstrated in these experiments. One of the older vectors, N2, produced helper virus within two passages. In addition, LN producing cell lines have been cultured for several weeks following supernatant harvest and no detectable helper virus was observed. Targeted Genetics, Inc., assays are only representative of helper virus.

Dr. Murray inquired if the investigators were assaying for helper virus by the extended S⁺/L⁻ assay. Dr. D. Miller said that although they are not performing the extended S⁺/L⁻ assay, their safety testing is actually more stringent than other assay methods. The packaging cells are kept in continuous culture for up to four weeks following vector harvest. With this method, any replication competent particles originally present should be amplified to extremely high levels that would be readily detected by PCR. Dr. D. Miller noted that they have recently started to perform the *Mus Durni* assay also on vector preparations. The viral stocks have been prepared, and assay are in storage. Helper virus has never been demonstrated with the PA317 cell line or in the co-cultivation and long-term marrow studies.

Dr. Carmen submitted revised language for incorporation into the informed consent document. The word "bacterial" should be inserted prior to the word "gene" so that the patient will understand the source of that gene.

Dr. Leventhal asked if the twin protocol requires autologous bone marrow (ABM) reinfusion or does it apply only to peripheral blood stem cells. Dr. Shuening answered that the twin study pertains only to the infusion of peripheral blood stem cells.

Dr. Geiduschek inquired as to the number of transducing particles that are produced per producer cell. Dr. D. Miller responded that between one and ten particles of virus are produced per cell per day. This number of particles translates to approximately between 10⁶ and 10⁷ colony forming units (CFUs) per milliliter. There are approximately 25 milliliters per flask.

Committee Motion

A motion was made by Dr. Geiduschek to approve the protocol subject to a redefinition of the safety of the LN particles that verify that no helper virus is present in the equivalent of 300 milliliters of supernatant. Dr. Post suggested that the wording be changed to less than one particle per 300 milliliters. The investigators will be obligated to provide evidence of this data without actually testing all 300 milliliters of supernatant. This data should be obtained by extended culture of the producer cell.

Dr. Parkman seconded the motion only to speak against the safety testing requirements proposed by Dr. Geiduschek. Dr. Parkman said that the RAC cannot change the standards by which they review protocols at the same meeting. Although it is likely that the FDA may change their vector testing requirements for the presence of replication competent virus, the RAC should remain consistent in its requirements for testing at this time.

Dr. Doi asked the time-frame that would be required to perform the additional experiments suggested by Dr. Geiduschek. Dr. D. Miller said that they do not have the option of returning to the stocks and reassaying for helper virus. Those cells no longer exist, the vector supernatant stocks are already in storage. In the future when more supernatants are produced, there will be the option to perform the suggested assays.

Dr. Post asked if the NIH 3T3 amplification assay would increase the level of sensitivity to detect one particle per 300 milliliters. Dr. D. Miller stated that the NIH 3T3 assay would not provide this information; however, one culture flask which makes approximately 300 milliliters of supernatant was passaged, cultured long-term, and assayed for replication competent helper virus. Dr. Krogstad asked about the degree of certainty that one infectious particle will be detected by this method. Dr. D. Miller explained that at the time of supernatant harvest, the producer cells are confluent. If one viral particle is present at this stage, that particle should replicate to detectable levels within several weeks following trypsinization and reculture of these cells. Dr. D. Miller described experiments performed with PA317 cells in which a confluent culture was spiked with helper virus. Within two weeks, the number of helper virus particles increased to 10^7 particles. This level of virus is readily detectable by PCR.

Dr. D. Miller said that investigators will have to provide data according to the standards established by FDA. The RAC should discuss the issue of helper virus. Although FDA will ultimately develop the required testing standards, this issue should be conducted in public. The RAC provides such an open forum to discuss this issue unlike the FDA. Dr. Aebersold added that the FDA's *Points to Consider in Somatic Cell Gene Therapy* document was established based on public review and comments. FDA reviews gene therapy confidentially; however, the document which provides the basis for this review was established publicly. Dr. Post asked Dr. Aebersold how long these public meetings would continue. Dr. Aebersold responded there is no expectation that FDA's public meetings will be abandoned.

Amendment--Mr. Barton

Mr. Barton moved that the motion made by Dr. Geiduschek should be amended to delete the requirement for the special review of the safety of the vector. The amended motion would merely approve the protocols with the expectation that the FDA will set

the safety testing standards. The amendment was seconded by Dr. Post.

Dr. Carmen asked what the impact of this amendment would be on the RAC review process with regard to safety testing requirements. Mr. Barton stated that the RAC would be sending the message to FDA that they will bear the ultimate responsibility for establishing these standards. Dr. Carmen said that the RAC should not be in the business of sending messages to the FDA and that the RAC should establish and conduct its own agenda as it deems necessary. Mr. Barton agreed that the issue of vector safety is certainly within the purview of the RAC. The RAC should not relinquish its responsibility to discuss this issue; however, the RAC should not try to second guess the final FDA standards.

Dr. Parkman suggested that the RAC should vote on Dr. Schuening's protocols based on the standards that were used earlier in this meeting. In addition, Drs. D. Miller and Geiduschek should proposed a recommendation as the vector safety experts on the RAC to develop a set of standards that investigators should meet prior to RAC review.

Dr. Secundy asked if there is a reason to believe that the vector safety standards previously established by FDA are currently inadequate. Dr. Parkman responded that there is no evidence of inadequacy. The purpose of this discussion is that assays are evolving that provide a greater level of sensitivity than existed previously. These tests are not better, just more sensitive. Dr. Secundy asked if the current standards are quantitatively and statistically inadequate as opposed to inadequate for detecting health risks. Dr. Secundy said that if the current standards are in any way inadequate, she would not support the approval of this protocol based on ethical standards.

Dr. Parkman said that there is no clinical evidence that current standards are inadequate and provided background information regarding the vector safety concerns. There is data from one investigator demonstrating that monkeys developed lymphomas as a result of helper virus contamination of the retroviral vector preparation used for gene transfer. No patients developed lymphomas. Dr. Walters said that he supports Mr. Barton's amendment.

Dr. Murray called on Mr. Barton to restate the proposed amendment. Mr. Barton stated that the amendment will revise Dr. Geiduschek's motion to read approval of the protocols as submitted with the expectation that the FDA will report back to the RAC about the helper virus safety tests and the approval of the protocol. Dr. Murray called for a vote. The amendment to revise Dr. Geiduschek's motion passed by a vote of 12 in favor, 4 opposed, and 2 abstentions³.

³Dr. D. Miller abstained.

Mr. Capron moved that the revised motion for approval should be restricted to the two protocols which involve a fully autologous system. Dr. Parkman seconded the motion. Dr. Murray stated that the newly amended motion would be to approve the two autologous bone marrow transplantation protocols allowing the FDA to established the required vector testing standards.

Dr. Parkman suggested a friendly amendment to this motion. The language should be changed to read "utilizing an FDA approved vector." Although the FDA must ultimately approve these vectors, the RAC should not defer its responsibility. Ms. Buc agreed and said that the RAC's purpose is to set its own standards for what is appropriate and should not defer to FDA. Dr. Wivel reminded the committee members that the RAC review process is independent and parallel to the FDA review process. Mr. Barton accepted this change as a friendly amendment. Dr. Parkman stated that based on this clarification, he withdrew the friendly amendment to Mr. Barton's motion.

Dr. Leventhal called the question. Dr. Murray stated that the motion now on the table is to approve the two autologous protocols as written with the expectation that the FDA will convey to the RAC its standards for approval. The motion to approve the two autologous protocols passed by a vote of 14 in favor, 2 opposed, and 2 abstentions³.

Dr. Parkman asked if any patients have been enrolled in the standard protocol utilizing peripheral blood stem cells from identical twins. Dr. Schuening stated that the first patient is currently being treated. Dr. Leventhal asked why the autologous patients receive G-CSF for seven days and the normal donors in this twin protocol receive G-CSF for five days when peripheral blood collection begins on day four. Dr. Schuening said that he is not the PI on these clinical protocols and referred the RAC to the background section of the protocol for the rationale of the various procedures. The optimal time to harvest peripheral blood progenitor cells is between four and seven days. It is unclear at this time what the most optimal day is for harvest.

Dr. Leventhal asked if there is any evidence that the gene marking process may slow down the repopulation process. Dr. Schuening explained that the only difference between this marking protocol and the ongoing clinical protocol is that the donors will undergo a fourth leukapheresis in order to obtain enough cells for the marking procedure.

Dr. Walters asked how many patients worldwide have had their peripheral blood stem cells transferred to another patient. Dr. Parkman said that one recent report cited 25 lymphoma patients. Dr. Leventhal added that there is extensive data regarding autologous stem cell transplant following chemotherapy. There is currently no data

³Dr. D. Miller abstained.

regarding the ability to reconstitute following G-CSF administration. Dr. Schuening noted that his laboratory has conditioned 12 patients with G-CSF, harvested autologous peripheral blood cells, and transplanted the stem cells. All twelve of these patients recovered within the expected time frame. Dr. Leventhal asked if these patients received chemotherapy conditioning. Dr. Schuening stated that these patients did not receive chemotherapy, only G-CSF.

A motion was made by Dr. Krogstad and seconded by Dr. Parkman to approve the identical twin protocol. Dr. Murray called for a vote. The motion passed by a vote of 11 in favor, 2 opposed, and 5 abstentions.³

VIII. ADDITION TO APPENDIX D OF THE NIH GUIDELINES REGARDING THE INTRODUCTION OF A GENE CODING FOR TETRACYCLINE RESISTANCE INTO PORPHYROMONAS GINGIVALIS/DR. PROGULSKE-FOX

Review--Dr. Schaechter

Dr. Murray called on Dr. Schaechter to present his primary review of the proposal submitted by Drs. Progulske-Fox and Keierleber of the University of Florida, Gainesville, Florida. Dr. Schaechter presented an overview of the proposal. The investigators are requesting to clone the gene encoding for tetracycline resistance into *Porphyromonas gingivalis*. The basis for this request comes from Section III-A-3 of the *NIH Guidelines* which states, *Deliberate transfer of a drug resistance trait to microorganisms that are not known to acquire it (2) if such acquisition could compromise the use of the drug to control disease agent in human or veterinary medicine or agriculture.* Since this request falls within this category, RAC, NIH, and IBC approval are required. This experiment should be approved because it carries a negligible risk. Although transfer of tetracycline markers to this organism (also known as *Bacteroides gingivalis*) has not been demonstrated, analogous transfers have been performed among similar *Bacteroides* from the human mouth.

The use of tetracycline for the treatment of oral gingivitis is rooted in habit. It is doubtful that antibiotics in any way interfere with the course of this disease. The treatment of choice is surgical; and if antibiotics are used, there are other drugs from which to choose. Therefore, the transfer of tetracycline resistance to this organism mimics a natural phenomenon, and the acquisition of this trait is not likely to compromise the use of this drug to control human disease.

Review--Dr. Krogstad

³Dr. D. Miller abstained.

Dr. Krogstad agreed with the statements provided by Dr. Schaechter and added that there is no evidence that tetracycline is necessary for the treatment of the clinical entity.

Review--Dr. Murray

Dr. Murray concurred with the positive assessment of the proposed experiment offered by the other primary reviewers.

Committee Motion

A motion was made by Dr. Schaechter and seconded by Dr. Haselkorn to approve the proposal. Dr. Murray called for a vote. The motion passed by a vote of 15 in favor, 0 opposed, and 1 abstention.

IX. ADDITION TO APPENDIX D OF THE NIH GUIDELINES REGARDING A HUMAN GENE TRANSFER PROTOCOL ENTITLED: *USE OF RETROVIRAL MARKERS TO EVALUATE THE EFFICACY OF PURGING AND TO DISCRIMINATE BETWEEN RELAPSE WHICH ARISES FROM SYSTEMIC DISEASE REMAINING AFTER PREPARATIVE THERAPY VERSUS RELAPSE DUE TO RESIDUAL NEOPLASTIC CELLS IN AUTOLOGOUS MARROW FOLLOWING PURGING IN PATIENTS WITH CHRONIC LYMPHOCYTIC LEUKEMIA (CLL)*/DR. DEISSEROTH

Review--Dr. Doi

Dr. Murray called on Dr. Doi to present his primary review of the protocol submitted by Dr. Albert Deisseroth of MD Anderson Cancer Center, University of Texas, Houston, Texas. Dr. Doi presented his review of this protocol. This protocol is similar to other leukemia and leukemia remission studies that have been reviewed and approved by the RAC and NIH. The investigators will use *in vitro* gene marking with either the LNL6 or G1Na vectors to test the efficacy of purging methods and to determine whether relapse after autologous transplantation results from residual systemic disease or from neoplastic cells remaining in the ABM preparations used for the transplantation of CLL patients.

ABM and peripheral blood stem cells will be harvested and stored after induction of remission. The ABM cells then will be reinfused into the patient to restore marrow function after intensive systemic and *in vitro* preparative therapy. The investigators will gain insights regarding the necessity of preparative therapy from this study. In some CLL patients, the remission period is greater than one year, suggesting that relapse from leukemic cells contaminating the ABM preparation infused after preparative therapy. Patients demonstrating a short remission period suggest that relapse may be due to residual systemic disease.

Introduction of the neo^R gene into the ABM cells will allow the investigators to identify the source of relapse, therefore, providing an opportunity to improve the systemic and/or *in vitro* purging techniques. Additional data may be obtained regarding the clonality of relapse, efficiency of normal versus neoplastic cell separation procedures, and efficiency of systemic preparatory therapy.

Both the LNL6 and G1Na vectors have been reviewed and approved for use by the RAC in previously submitted protocols. The difference between this protocol and previous studies is the use of bone marrow and peripheral blood stem cells. PCR analysis will be performed to confirm stem cell transduction with the neo^R gene. Neo^R colonies will be positively selected in G418 medium. The investigators will perform assays that readily distinguish the two vectors.

CLL patients will be eligible to participate in this study based on the ability to transduce and grow their ABM cells. Although data was not provided regarding the frequency of transduction in CLL cells in the original submission, Dr. Deisseroth has indicated that this data now exists.

Dr. Doi said that he inquired about the retention of the LNL6 and the G1Na genomes, and that Dr. Deisseroth had responded to his question satisfactorily. Dr. Doi said that Dr. Deisseroth also responded to the concern that if the conventional dose of chemotherapy does not reduce the percentage of abnormal cells below 0.01%, what is the probability of improving the *in vitro* purging methods.

Dr. Doi stated that the protocol is designed to answer questions other than those addressed by the previously approved acute myelogenous leukemia (AML) and chronic myelogenous leukemia (CML) protocols, and the study is experimentally sound. Therefore, the protocol should be approved.

Review--Dr. Bourquin

In Dr. Bourquin's absence, Dr. Doi summarized Dr. Bourquin's review. Since the LNL6 and G1Na vectors were previously approved, there is no objection to their use. Although this protocol does not provide an immediate benefit to patients participating in this study, the information obtained from this protocol will provide a great potential for benefit to patients in the future. The consent form is well presented, and the patients will be adequately informed of the potential risks. In addition, the patient information, manuals and video presentation provides comprehensive explanation of the study.

Review--Ms. Meyers

Ms. Meyers was also unable to attend the meeting. Dr. Doi summarized Ms. Meyer's

review. The language in the informed consent document should be modified to ask permission to perform an autopsy rather than requiring that an autopsy be performed. Dr. Deisseroth had responded to Ms. Meyers' concerns regarding the importance of the gene marking/ABM research satisfactorily. However, she mentions an increasing concern among the genetic disease consumer group that funding for genetic research is inadequate, whereas funding for cancer research is excessive in comparison. Concern about these perceptions is important from a public policy standpoint for NIH. Many protocols reviewed by the RAC require the patient to pay for procedures that are expected to have no therapeutic value, this requirement raises moral and ethical issues that have not been adequately addressed. The bone marrow marking studies may provide answers to important scientific questions that are applicable to many diseases. Unfortunately, many patients with hereditary life-threatening disorders will not live long enough to benefit from the answers. This research would appear less biased if protocols were presented at future RAC meetings involving diseases other than AIDS and cancer. Finally, she was concerned about what could be done to encourage the submission of other types of protocols?

Dr. Parkman noted that this CLL protocol differs from previously submitted protocols in that CD34 cells, stem cells, will be positively selected in addition to the negative selection of the CD19(-) B cells. However, the CD19 selection process was inadvertently omitted from the final protocol. A description of the complement lysis procedure should be included in the final version of the protocol. He inquired about the relevant efficiency of the two types of purging used.

Dr. Post asked for clarification regarding which particular vector, LNL6 or G1Na, will be used to transduce either the marrow or peripheral blood cell populations. Dr. Haselkorn asked the investigators to expand on the magnetic bead selection procedure. Dr. Murray inquired about the status of Dr. Deisseroth's previously approved protocols. Have the studies been initiated? How many patients have been enrolled? Dr. Murray called on Dr. Deisseroth to present a summary of the proposed experiment and to respond to the RAC's questions and comments.

Presentation--Dr. Deisseroth

Dr. Deisseroth explained that this protocol is designed to determine the origin of relapse in the ABM transplant setting. Two similar, yet distinguishable, safety modified vectors will be used to evaluate the independent multiple steps used to remove abnormal cell populations. These two steps are CD19(-) selection and CD34(+) selection. The negative selection process is designed to remove the unwanted CLL cells, whereas the positive selection procedure retains the normal cells.

This CLL protocol differs qualitatively from other protocols in that it involves the

aforementioned selection procedures to purge the ABM which will be used to restore hematopoietic function after preparative therapy. Using the retroviral markers to evaluate each stage of the process, the impact of the purging will be determined. The use of these markers to evaluate the purging process will save both time and patients because the only alternative to studying the efficacy of purging is to observe the clinical outcome of survival and remission duration. CLL is a unique feature of this protocol. CLL represents a disease in which both the peripheral blood and the bone marrow are contaminated with abnormal cells. One patient eligibility requirement of this protocol is that a patient diagnosed with CLL must have an expected survival of two years without nonconventional therapy.

Two events make initiation of this protocol possible. First, the chemotherapeutic regimens have been developed that can reduce the number of leukemia cells in the patient to a level that permits further purging of the marrow to render it sufficiently free of disease for use in the autologous setting. Transplantation alone cures 40% of CLL patients in the allogeneic setting. However, only a small percentage of patients are eligible for allogeneic transplantation because of age and donor availability. This protocol is designed to provide an alternative therapy option to those patients who are not eligible to receive allogeneic therapy. The second event that makes initiation of this protocol possible is that methods now exist for selecting and purging the cell population, namely CD34(+) selection of normal cells using the Cell Pro® column, and CD19(-) selection which removes abnormal cells. The two retroviruses, LNL6 and G1Na, will be used to mark these selected populations independently.

Conventional dose chemotherapy will be used to irradiate patients of bulk systemic disease and to induce remission. ABM and peripheral blood cells will be stored, fractionated, and transduced with the retroviral vectors. Systemic therapy will be administered, and the patients will be transplanted with the selected, transduced cells.

Dr. Deisseroth presented data in which ABM cells were harvested from CLL patients after induction of remission by fludurabine and transduced. These ABM cells were transduced at a frequency of approximately 1-3%. The question of whether or not CLL cells are marked is not answered in this setting. In order to demonstrate CLL marking, ABM cells were harvested from patients who were not in remission; therefore, leukemic cells were predominant. Normal transduced cells were mixed with the transduced CLL cells at a 1:1 ratio. This mixture of transduced cells was then sorted by Fluorescence Activated Cell Sorting (FACS) into two populations, normal myeloid and CD34(+)/CD19(-), and then grown in a culture system that selectively promotes growth of these cells. RNA was extracted and reverse transcriptase and sequence amplification was performed for detection of the neo^R gene. In four out of five patients, marked neoplastic cells were detected by PCR. Dr. Parkman asked how many cells were used for the PCR assay. Dr. Deisseroth answered that between 10³ and 10⁴ cells were used.

Dr. Parkman noted that the real question of interest is the transduction efficiency. Marker genes are readily detected in neoplastic cells by PCR when the starting population is 50%. What results are obtained when a patient is in remission where the frequency of neoplastic cells could be 2-3 logs lower? Will the neo^R be detectable? Dr. Deisseroth explained that the goal of the protocol is to assess the presence or absence of the markers at the time of relapse. At relapse, the ratio of normal to leukemic cells will be 1:1. Dr. Parkman stated that since most CLL cells are not cycled, the transduction efficiency could be 1 in 1,000 cells. The probability of transduction could be 1-2 logs less than predicted. Dr. Deisseroth said that CLL is a disease in which cells accumulate rather than result from a highly proliferative population. However, there is a low frequency of CLL cells which are proliferating. At the time that the CLL population will be transduced with the vector, the culture conditions will promote proliferation in this small fraction of cells. It is this population of proliferating cells which contribute to relapse.

Dr. Parkman inquired about the incubation conditions. Dr. Deisseroth explained that the cells will be transduced in Dexter culture without hydrocortisone on stromal monolayers. Basically, the Whitlock-Witte culture system. The ABM cells are incubated on stromal monolayers that have been irradiated with and without hydrocortisone. Hydrocortisone promotes the growth of myeloid cells while the absence of hydrocortisone promotes lymphoid cell growth. The retroviral vector will be added to the cells at a ratio of 10 infectious particles per cell for 72 hours.

Dr. Deisseroth explained that both FACS analysis and fluorescent *in situ* hybridization (FISH) will be used to distinguish abnormal cells from normal cells. FISH analysis requires the use of a DNA probe that is specific for chromosome 12. The presence of differentiation antigens allows for the selection of abnormal cells by FACS and analysis of the marker gene by FISH and PCR.

Dr. Deisseroth explained the cell fractionation process. The positive selection of normal cells is accomplished by incubation with the CD34 monoclonal antibody conjugated to biotin and passage through an avidin column. The biotin binds to avidin and the CD34(+) cells remain in the column. This process enriches for early progenitor cells by two logs, and there is a three log reduction in neoplastic cells because neoplastic cells do not possess the CD34 antigen. The negative selection procedure eliminates CD19(-) leukemic cells. Magnetic beads conjugated to CD19(-) are incubated with the enriched CD34(+) cell population at 4°C resulting in a two log reduction in the abnormal cell population. Therefore, the overall enrichment frequency of the normal cells is two logs, and the depletion of abnormal cells is five logs.

Dr. D. Miller asked if CD19 binds CLL progenitor cells. Dr. Deisseroth explained that the progenitor for CLL has never been isolated. Dr. D. Miller asked if a colony forming

assay exists to detect CLL progenitors. Dr. Deisseroth said that he was unaware of such an assay. Dr. D. Miller expressed concern about the frequency of gene marking. If the frequency is 0.1%, then the analysis will be difficult. Is there no method of measuring the frequency of marking? Dr. Deisseroth responded that since there is no technique for growing these progenitors, no estimate of the marking percentage can be provided. Dr. D. Miller inquired if PCR analysis of DNA rather than RNA would provide data regarding the frequency of transduction. Dr. Deisseroth said that RNA quantitation was chosen for PCR analysis because the sensitivity of the assay would be increased and the DNA could be analyzed. Dr. D. Miller noted that RNA is extremely difficult to quantitate, and it is difficult to estimate the number of cells.

In response to Ms. Meyers concerns, Dr. Deisseroth stated a large number of bone marrow marking protocols are necessary because each type of leukemia is different, both in terms of biology and the questions that are addressed in each of the protocols. CML is a disease which results in cells that have unique cell surface changes, and these cells repopulate both the peripheral blood and bone marrow. There is no method for separating CML cell populations by differentiation antigens as is the case with CLL. Another reason to initiate a variety of bone marrow marking protocols is that patients who are eligible for bone marrow transplantation demonstrate a 10 to 30% mortality rate depending on whether they receive autologous or allogeneic transplantation. The application of gene marking to this protocol provides the opportunity to improve on these transplantation therapies. Most importantly, the study of hematopoietic reconstitution coupled with *in vitro* fractionation of marrow and retroviral marking provides important information that will lead to the initiation of therapy not only for hematopoietic neoplasms, but also for solid tumors and other human disease. Therefore, the information obtained from these marking protocols not only addresses the clinical problems associated with specific types of leukemia, but provides essential information regarding the use of bone marrow cells as a conduit through which to introduce therapeutic molecules into the systemic circulation.

The importance of focusing on gene transfer in leukemia and cancer before other diseases is that cancer provides the clinical setting for solving the technical aspects of vector modification of somatic cells. Investigators can learn how to isolate early progenitor cells from marrow, monitor gene marking, obtain data that will yield immediate therapeutic implications relevant to the immediate disease, as well as prepare the foundation for procedures that will be necessary to provide molecules to the systemic circulation that can provide therapeutic benefit to patients with genetic diseases. These protocols set the stage for gene therapy of genetic diseases.

Dr. Haselkorn inquired about the status of Dr. Deisseroth's previously approved protocols. Dr. Deisseroth said that the first approved protocol designed to establish the origin of relapse in CML has been initiated. Two patients have been transplanted with

gene marked marrow cells and have recovered from hematopoietic reconstitution with CD34(+) cells. Gene marking data is currently being collected. Dr. Haselkorn asked if any of the other investigators with approved gene marking protocols have observed patient relapse. Dr. Deisseroth said that other investigators have reported relapse. Dr. Haselkorn asked if purging is introduced as a part of the CLL protocol, there must be a sense that relapse is due to the transducing cells. Dr. Deisseroth stated that data obtained from Dr. Brenner's protocols for AML indicate that at the time of relapse, patients exhibited gene marked leukemic cells suggesting that cells present in the marrow contribute to the evolution of relapse in AML. Dr. Deisseroth asked Dr. Brenner to confirm these results.

Dr. Brenner said that a total of 13 patients have entered the gene marking protocols at St. Jude Children's Hospital in Memphis, Tennessee, seven with neuroblastoma and six with AML. Two AML patients and no neuroblastoma patients have relapsed. One relapsed AML patient had no distinctive leukemic markers, but there were large numbers of blasts which produced malignant appearing colonies that were marked with the neo^R gene. The second relapsed patient had two distinctively leukemic clone markers CD56 and CD34, and a distinctive translocation. The translocation produces a unique leukemic transcript. This cell population was purified on its phenotypic basis, cultured, and colonies selected. The resulting colonies contained both the leukemic transcript and the neo^R gene. Therefore, for the first AML patients remission has demonstrated that the reinfused marrow contributes to relapse. The effect of purging and frequency are still unknown at this time.

Dr. Leventhal asked for clarification regarding the first relapsed AML patient. Dr. Brenner explained that the first patient had CD34(+) blasts in their circulation at the time of relapse that were separated by FACS and grown up to colonies. Two percent of the blast colonies expressed the neo^R gene. It is unlikely that these are normal progenitor cells contaminating the population. Dr. Deisseroth noted that Dr. Brenner's results were obtained without any additional fractionation procedures. The CLL protocol includes two steps to remove the abnormal leukemic cells.

Dr. Parkman said that the title of the protocol indicates that peripheral blood cells will be transduced and administered to the patient; however, it is not discussed in the protocol. Dr. Deisseroth concurred that peripheral blood would not be included in this protocol. Dr. Deisseroth clarified the fractionation and labelling procedure as requested by Dr. Parkman. Ninety percent of the ABM cells will be fractionated over the CD34 column. This CD34 enriched population of cells will then undergo CD19 negative selection. This double fractionated population of cells will be transduced with G1Na. The remaining 10% of unfractionated cells will undergo CD19(-) selection only prior to transduction with LNL6. If lymphoid cells contain both markers at the time of relapse, then CD34(+) fractionation is of no benefit in the selection process.

Dr. Parkman asked if there are any specific losses that resulted from the double depletion process. Dr. Deisseroth explained that the percentage loss of myeloid cells is between 20 and 50% during the second selection step. Are the depleted cells early progenitor cells being lost or an excess of nonreconstituting cells? The double selection/double marking protocol was designed to answer this question.

Mr. Capron noted that the suggested language offered by Ms. Meyers regarding patient autopsy does not appear in the revised informed consent document. Dr. Deisseroth agreed that Ms. Meyers' suggested language would appear in the final version of the document.

Dr. Secundy said that the written protocol varies considerably from the protocol that has been verbally presented to the RAC. Will a vote for approval endorse the written or verbal proposal? The written protocol would be revised to reflect the changes that the investigators have presented orally. Dr. Murray stated that such a decision would have to be approved by the RAC. Dr. Parkman asked Dr. Deisseroth if the written protocol accurately reflects the procedures. Dr. Deisseroth said that the strategies that were presented orally accurately reflect the design of the protocol. Dr. Parkman suggested that the CD19 depletion procedure should be incorporated into a revised protocol. Dr. D. Miller added that approval of the protocol should be contingent on review and approval of the revised protocol by primary reviewers to ensure that questions presented have been answered satisfactorily.

Dr. Krogstad asked what fraction of patient relapses are likely to be interpretable in terms of their source. Dr. Deisseroth answered that the number of marked cells in the lymphoid population in each category at the time of relapse is approximately 500 cells. If the marking frequency is 1%, the probability of the test failing to detect a relapse is probably between .004 and 4%. The probability of not detecting a marked relapse is low combined with projected number of patients; the probability of all ten patients not providing an interpretable answer is extremely low. Dr. D. Miller asked if this hypothesis is based on the assumption that all 500 cells will grow. What if the relapse is clonal? Dr. Deisseroth stated that relapse is not likely to be clonal. Patients will have to be screened and selected to include those with relatively poor prognoses, because this patient population yields a higher labelling frequency.

Committee Motion

A motion was made by Dr. Parkman and seconded by Dr. Secundy to approve the protocol contingent on the following stipulations: (1) submission of a revised outline of the cell selection and gene marking procedures for review and approval by Drs. Bourquin, Geiduschek, Parkman, and Walters and Ms. Buc; and (2) the informed consent document will be revised to reflect minor language modifications. Dr. Murray

called for a vote. The motion passed by a vote of 17 in favor, 0 opposed, and 4 abstentions.

Discussion

Ms. Buc addressed the issue of RAC review and approval of multiple versions of similar protocols. It is not undesirable to repeat the early stages of experiments in which the outcome will answer a scientifically important question. Dr. Parkman noted that in the case of the various leukemia studies, numerous protocols are necessary to answer the important issues. Data cannot be extrapolated from one disease to another. Dr. Deisseroth added that scientific method, especially in the clinical setting, depends on the reproducibility of any finding. There is a need to proceed, not in a sequential fashion with one investigator addressing one item, but for multiple investigators to address important questions in parallel that resolve current scientific dilemmas. It is important that each protocol should be viewed as a new step in the evolving field of gene therapy.

X. ADDITION TO APPENDIX D OF THE NIH GUIDELINES REGARDING A HUMAN GENE TRANSFER PROTOCOL ENTITLED: *CLINICAL PROTOCOL FOR MODIFICATION OF ONCOGENE AND TUMOR SUPPRESSOR GENE EXPRESSION IN NON-SMALL CELL LUNG CANCER (NSCLC)/DR. ROTH*

Review--Dr. D. Miller

Dr. Murray called on Dr. D. Miller to present his primary review of the protocol submitted by Dr. Jack Roth of MD Anderson Cancer Center, University of Texas, Houston, Texas. Dr. D. Miller presented an overview of this protocol designed to reverse the transformation of cancer cells by introduction of the tumor suppressor gene, p53, and the antisense Kirsten-ras (K-ras) gene to inhibit transformation. Dr. Roth has presented data demonstrating that cells that have reverted back to a normal phenotype exhibit a bystander effect that converts other cancer cells to a more normal state.

Dr. D. Miller had concerns regarding the proposed retroviral vectors. These vectors are basically derived from the standard LNSX vector which contains a long terminal repeat (LTR) driving the neo^R gene plus an SV40 promoter for the expression of the second gene. The K-ras vector contains a β -actin promoter with the K-ras gene in reverse orientation. Therefore, β -actin would drive K-ras in a counter clockwise direction. An SV40 early promoter drives the K-ras in the positive direction which could result in K-ras protein. An LTR in the 5' end drives neo^R that could make an antisense message to the K-ras antisense message; therefore, creating a sense message. There is the potential for hybridization of these various messages and the vector. In addition, the K-ras could mutate to an oncogene. The mutation rate of retroviruses is probably in the order of 1 in 10⁴ base pairs per generation. The issue of whether K-ras is capable of mutating to

form an oncogenic virus is critical. The investigators can perform assays that would determine the oncogenic potential of these vectors. If no transformation is observed using 10^8 particles, the vector is relatively safe. The p53 vector contains the β -actin promoter in reverse orientation that drives p53. He questioned if p53 could acquire mutations that make it an oncogene, and what would be the frequency of such an event.

Regarding the bystander effect, Dr. D. Miller stated that the data demonstrates that there is a bystander effect capable of reverting cancer cells to normal morphology is not compelling. Data suggests that there is growth with all of the mixtures of cell types at seven days. The data would be more convincing if these cultures were extended over a longer period of time.

Review--Dr. Hirano

Dr. Hirano stated that she had two major concerns with this protocol. Patients will have their lung tumor cells transduced by the direct *in vivo* injection of retroviral vectors into the tumors. Preliminary data suggests that a transduction efficiency of 50 to 70% will be necessary to obtain an antitumor effect. The protocol specifies that this level of transduction will be accomplished by direct injection of 10^6 CFUs into the patient daily for five consecutive days. The treatment will be repeated monthly provided there is no evidence of disease progression. Since this vector will be injected directly into a patient for the first time, the RAC should determine whether the preclinical data addresses the issues of toxic or adverse side effects.

The investigators used the LNSX vector for the preliminary experiments, but their packaging cell line was different from the one that they are proposing to use, PA317. Have any experiments been performed to demonstrate that high titer vector can be produced from PA317? Who will produce the vector supernatants?

There is *in vitro* data with the antisense K-ras which suggests that the transduction efficiency can be increased as a function of the number of cycles of transduction. Is there analogous data for the p53 vector? In the investigator's response it was stated that PCR will be used to determine transduction efficiencies *in vivo*. Is there transduction efficiency data in the murine model where animals have been injected with the human cell line, HL60, and subsequently challenged with vector supernatant?

Review--Mr. Capron

Mr. Capron asked if there will be some degree of risk imposed on other persons exposed to the patient, i.e., hospital personnel and family members. The investigators indicate that there will be a 48-hour period that the patient may be capable of shedding virus. Are the precautions that will be taken adequate and is there a possibility that this virus

might be transferred as an aerosol?

What is the relationship between the patient selection process and the informed consent process? Should the informed consent document be separated into two sections, one for the initial tumor screening process, and one for the protocol?

Other Comments

Dr. Parkman noted that patients develop antibody responses to foreign proteins. While this response will not be a concern for the initial injections, there is the possibility that inflammatory responses will occur from the monthly injections due to the production of antibodies to foreign antigens. Since most of these patients will already have some degree of tracheal obstruction, what could be the potential effect of an inflammatory response in these patients? Will patients be monitored for the presence of circulating antibodies to these antigens? Will fetal calf serum be used which could stimulate an immune response?

Dr. Leventhal asked Dr. Roth to address why he chose to use a viral construct instead of direct injection of the antisense oligonucleotide. Dr. Post asked if the investigators had performed antisense experiments to determine if the observed effects are the result of a less specific nonproliferative effect.

Presentation--Dr. Roth

Dr. Roth explained that this protocol is designed to treat NSCLC patients who are refractory to conventional surgery, radiation therapy, and chemotherapy and exhibit significant obstruction of their airway by tumor, such that it is a life threatening process. Using topical anesthetic, the bulk tumor will be removed by endoscopy and laser techniques. Following tumor resection, the residual microscopic tumor cell bed will be injected with retroviral vector supernatants directed against specific genetic abnormalities within the tumor cells. Data suggests that these vectors are capable of reversing the effect of the dominant oncogene (K-ras) and promoting the effect of the tumor suppressor genes (p53).

Dr. Roth discussed the proposed K-ras antisense mechanism. The dominant oncogene family is activated primarily by point mutations, amplifications, or chromosomal rearrangement. In the case of K-ras, there is a specific base mutation which allows a single allele of that gene to become transformed and produce a hyperactive protein. The K-ras antisense construct is specifically designed to reduce the concentration of the hyperactive K-ras protein and reverse some of the features of the transformed phenotype. A retroviral construct will be used as opposed to an antisense oligonucleotide because higher levels of long-term expression can be achieved. Data

demonstrates that these retroviral constructs are capable of effecting long-term expression out to six months, whereas oligonucleotides are subject to degradative processes with extremely high concentrations and frequent infusions required.

Dr. Roth presented *in vivo* murine data demonstrating that introduction of the normal p53 gene into cells that have a mutant p53 gene reverses the critical features of the malignant phenotype. Data was presented which was derived from experiments with immunosuppressed nude mice. When human lung cancer cells were injected into these mice, extensive local growth of the tumor occurred. However, when mice with three day established tumors were treated with three intratracheal injections of the retroviral supernatant, tumor burdens were significantly reduced. This very closely replicates the proposed clinical protocol. Data was presented demonstrating that injection of *in vitro* transduced cells greatly reduced the size of the tumor burden in mice; however, this data is not directly applicable to the proposed *in vivo* human experiments.

Dr. D. Miller noted that an antitumor effect was observed when mice were treated with 10^4 *in vitro* LNSX transduced cells. What effect would be observed if these animals were injected with five times this number of cells? Dr. Roth explained that data suggests that 10^4 transduced cells is the threshold for the murine experiments. He stated that 10^4 transduced cells prevents tumors in approximately 50% of the mice, and increasing this number to 10^5 cells will prevent 80 to 100% of the tumors.

In vivo data was presented demonstrating that mice receiving the retroviral supernatant alone exhibited a significant reduction in tumor development; and in the few animals that did develop tumor, the volume of tumor was small in comparison to control mice. Microscopic examination of mice that did not develop tumors indicated no evidence of cancer cells.

Dr. Roth addressed the issue of the effect of the transduced cells on the nontransduced cells known as the bystander effect. Based on transduction efficiency data one would expect 30 to 50% reduction in the growth of unselected tumor cells transduced with LNSX/p53. However, an 80 to 90% reduction in growth was observed suggesting that the transduced tumor cells may have an effect on untransduced tumor cells. This observation led to more controlled mixing experiments in which 50% transduced cells, either p53 or K-ras, were mixed with nontransduced cells. Data demonstrates that this mixed population of cells exhibit a growth pattern identical to a population of cells that have been transduced and selected in G418.

Dr. Roth hypothesized that the transduced cells could possibly be releasing a factor that affects the nontransduced population. Evidence for this hypothesis is derived from the supernatant experiments in which untransduced cells were mixed with culture supernatant of p53 transduced cells. A 50% reduction in growth was observed with the

addition of the culture supernatant. Culture supernatant from LNSX transduced cells had no effect on the growth on nontransduced cells. Additional evidence of the bystander effect is seen in the morphologic changes observed in nontransduced tumor cells. Untransduced tumor cells grow in a three-dimensional configuration, not in monolayers as is observed with normal cells. However, when untransduced tumor cells are cultured with the p53 retroviral vector supernatant, morphological events occur similar to those of programmed cell death and apoptosis, i.e., formation of blebs or vacuoles. This observation is in contrast to the appearance of cells that normally express p53. The extreme morphological changes observed when cells are transduced with p53 may correlate with the release of factors into the supernatant.

Dr. Roth responded to Dr. D. Miller's question regarding the stability of the viruses and potential for oncogenic transformation. Both the H358a and H460a cell lines have been maintained in culture for as long as six months that have been transduced with both the p53 wild-type and the antisense K-ras genes, and these genes have been continuously expressed with no evidence of oncogenic transformation. Dr. D. Miller stated that if these cells contained a mutant p53 gene, the mutation would not be detected. The usual assay for transforming virus within a preparation is to perform an assay on a monolayer cell line. Dr. Roth said that these experiments had been performed and that no focus formation has been observed when one milliliter of supernatant containing 10^7 CFUs were cultured with NIH 3T3 cells for three weeks. The same results were observed for both vectors. Dr. D. Miller asked if the wild-type K-ras virus was used as a positive control for these experiments. Dr. Roth said that this control was not included.

Dr. Roth responded to questions regarding transduction efficiencies. The packaging cell line currently being used produces viral titers of greater than 10^6 CFUs, without the addition of co-cultivation techniques. Semi-quantitative PCR demonstrates between 30 and 50% transduction. The protocol specifically states that patients will not be treated unless this level of virus production is obtained. GTI will be producing the retroviral supernatant and performing the necessary quality control measures. MD Anderson's IRB has already approved the LNSX vector for use in the clinical trial; therefore, LNSX will be the initial vector. In the event that a superior vector is identified, a request will be made to the RAC for a minor modification to the protocol.

Regarding possible toxic side effects of the protocol, Dr. Roth stated that no toxic effects have been observed in mice that have been injected with retroviral supernatant containing 350 times the number of viral particles proposed for the human protocol. Some of these animals have survived for six months with no specific manifestations of acute toxicity.

Dr. Roth stated that the MD Anderson IRB has consulted with Dr. Goodrich, an infectious disease expert. Both Dr. Goodrich and the IRB have concluded that the

precautions outlined in the protocol are more than adequate. In fact, the detailed precautions are greater than those taken for other infectious diseases such as tuberculosis or HIV. Normal tissue, germ cells, peripheral blood lymphocytes, and bronchial mucosa will be assayed for neo^R.

Patients requiring an additional biopsy beyond the one that is routinely required prior to the protocol will provide informed consent prior to entering the protocol and prior to having the additional biopsies performed. Between 80 and 90% of eligible patients will have had previous biopsies that will be evaluable for determining whether the mutations exist. Mr. Capron asked how many biopsy specimens would probably have to be screened before identifying 14 eligible patients. Dr. Roth explained that the prevalence of p53 mutations in NSCLC is between 50 and 70%. The prevalence of K-ras mutations is approximately 20%. Since the two mutations do not necessarily occur together, it is estimated that 7 of every 10 patients screened would possess both mutations.

Dr. Leventhal asked what experiments would be performed on any recurrent tumor tissue. Dr. Roth explained that patients will be bronchoscoped at frequent levels so the tumor can be biopsied at the time of recurrence. The tissues will be examined for retroviral integration by semiquantitative PCR analysis in addition to gene expression. Dr. Leventhal suggested that the investigators should screen for new mutations, such as p52 in addition to the original mutations.

Dr. Geiduschek stated that the data presented with regard to the bystander effect did not address the critical issue. The key experiment that would confirm this effect would determine that the genotype is transferred while the growth curve is not affected. Cells were never removed between day seven and nine to analyze the genotype distribution. Dr. Roth explained that as a result of Dr. Hirano's initial review, these experiments were initiated. Data suggests that when a population of cells is mixed and then selected for neo^R, 46% of the cells still express neo^R. Therefore, the ratio of the two cell types after nine days would be the same as it was at the beginning of the experiment.

Dr. Geiduschek inquired if the *in vivo* experiments in which mice were preinnoculated with tumor three days prior to treatment accurately reflects the human situation. Some of these NSCLC patients have had established tumors for a long period of time. Dr. Roth explained that there is always the difficulty of extrapolating animal models to the human situations. This particular murine model was designed because it most closely resembles the clinical manifestation observed in humans. The life span of mice is too short to approximate the human condition. However, the tumor cells injected into the mice are rapidly growing human NSCLC cells.

Dr. H. Miller stated that one might argue that at the time of bronchoscopy, a physician has an obligation to resect as much tumor as possible. However, if it is entirely resected,

the injection target would be lost. He asked Dr. Roth if he had any reservations about not resecting the entire lesion. Dr. Roth said that in actuality, the tumor will be resected as much as possible and noted that this standard surgical technique results in 100% recurrence. It is impossible to resect these tumors completely. Dr. H. Miller asked if the procedure will be one of irrigating the resected area rather than actual injection into the remaining tumor bed. Dr. Roth stated that the area would be irrigated.

Dr. H. Miller asked about the stopping rule for progression of metastatic disease. Dr. Roth responded that if local control of tumor is observed, and there is progression of metastatic disease, the patient will have to be removed from the protocol.

Dr. D. Miller said that the vector used for the preclinical studies was prepared by cocultivation with packaging cells, and an extremely high viral titer was obtained. The protocol stated that PA317 cells will be used; this differs from the preclinical experiments. If the new packaging line produces lower viral titers as the investigators have indicated, will the *in vivo* experiments be repeated using the new vector? Dr. Roth said that the efficacy of the new vector will be monitored to ensure that similar titers are obtained. Dr. D. Miller stated that GTI may not have experience with producing viral titers of 10^7 CFUs continuously. Dr. McGarrity stated that GTI consistently produced vectors which produce viral titers of 10^7 CFUs. Dr. D. Miller asked if the animal experiments will be repeated. Dr. Roth said that some of the animal experiments will be repeated once the packaging cell line is established; however, the *in vitro* experiments correlate very well with the animal model data. It is reasonable that only the *in vitro* experiments will have to be repeated with the new packaging cell line.

Dr. D. Miller asked if normal cells express the same genes as the transduced cells, why don't normal cells secrete the bystander factor and suppress neighboring tumor cells? Dr. Roth said that cells lose their transformed phenotype but they are not normal, i.e., they do not flatten out. Many investigators are currently conducting experiments to biochemically characterize the factor that produces the bystander effect. It is not known what the actual transcriptional events are at this time.

Dr. Dronamraju asked if any primate data exists. Dr. Roth said that it is not possible to develop this type of tumor model in primates or canines because of the complexities of having to identify the specific oncogenes involved and the necessity to use an immunosuppressed model if human tumor cells are used.

Dr. Post asked if experiments have been performed on NSCLC cells that do not possess either the K-ras or p53 mutations. Dr. Roth said that they have performed these experiments on several cell lines, and no effect has been observed on growth.

Dr. Parkman said that the investigators have fulfilled the requirement of providing the

most appropriate animal model. Models are not the same as wild-type human disease.

Dr. Roth stated that only serum free supernatants will be used for treatment of patients. Therefore, an immune response to fetal calf serum is not a concern.

Dr. D. Miller suggested that data should be provided demonstrating K-ras induced foci and this rate of transformation should be compared with the supernatant that will be used for the clinical protocol. If 10^7 CFUs corresponds to 10 milliliters of supernatant, then the investigators should demonstrate that there is no transforming virus in this volume or 100 milliliters. Dr. Post suggested that mixing experiments should be included.

Committee Motion

A motion was made by Dr. D. Miller and seconded by Dr. Krogstad that the protocol be approved contingent on the review and approval of the following information by Drs. D. Miller, Hirano, and Geiduschek: (1) submission of data demonstrating the transforming potential of 100 milliliters of retroviral supernatant analogous to the preparation that will be used for the clinical protocol, (2) submission of data obtained from *in vitro* mixing experiments, (3) submission of *in vitro* data demonstrating that the new vector preparations have activity, and (4) incorporation of minor changes in the informed consent document as noted by Drs. Carmen and Hirano. Dr. Murray called for a vote. The motion passed by a vote of 18 in favor, 0 opposed, and no abstentions.

XI. ADDITION TO APPENDIX D OF THE NIH GUIDELINES REGARDING A HUMAN GENE TRANSFER PROTOCOL ENTITLED: *A PHASE II TRIAL OF THE BAXTER NEUROBLASTOMA BONE MARROW PURGING SYSTEM USING GENE MARKING TO ASSESS EFFICACY/DRS. BRENNER AND MILLS*

Review--Dr. Haselkorn

Dr. Murray called on Dr. Haselkorn to present his primary review of the protocol submitted by Dr. Malcolm K. Brenner of St. Jude Children's Research Hospital, Memphis, Tennessee, and Dr. Bonnie J. Mills of Baxter Healthcare Corporation, Santa Ana, California. In Mr. Barton and Dr. Brinckerhoff's absences, Dr. Haselkorn summarized their comments in addition to his own remarks. This Phase II protocol of the Baxter neuroblastoma bone marrow purging system, based on magnetic bead separation of cell populations using gene marking to assess efficacy. This protocol is very similar to the neuroblastoma protocol submitted previously by Dr. Brenner. The patient's bone marrow is harvested and separated into two fractions, purged and unpurged. The purged fraction will undergo separation through the magnetic bead column. Each fraction will then be marked with distinguishable retroviral vectors. At

the time of relapse, samples will be collected and assayed for the presence of the vectors in order to determine the efficacy of the purging procedure.

All three of the primary reviewers noted that purging is a preferable procedure and were concerned about readministering unpurged bone marrow to these patients. Dr. Haselkorn asked Dr. Brenner to respond to this issue.

Other Comments

Dr. Murray encouraged Dr. Brenner to submit written documentation as a follow-up to his previously approved protocols. Dr. Murray noted that written data has not been forthcoming from many investigators, and this information is critical to assessing the progress of these protocols.

Dr. Parkman asked Dr. Brenner to discuss the standard of care at St. Jude for patients undergoing ABMT for neuroblastoma.

Presentation--Dr. Brenner

Dr. Brenner explained that the first gene transfer patient was treated at St. Jude exactly one year ago. Reports have been written regarding the two patients who have relapsed and the 13 patients who have been transplanted without relapse. These written reports will be forwarded to the Office of Recombinant DNA Activities (ORDA).

Regarding the administration of unpurged marrow, Dr. Brenner said that it is not known whether relapse is caused by residual disease remaining in the marrow. Currently, his laboratory is attempting to solve this question with the AML protocol.

The standard of care for neuroblastoma autologous transplant patients at St. Jude is to administer marrow that has not been purged. No data exists demonstrating that patient survival is increased in patients receiving purged marrow as opposed to unpurged marrow. This situation may be partially due to the fact that engraftment in these patients is somewhat slow. Relapse may be through a mixture of disease in the patient and residual disease in the harvested marrow.

Discussion

Dr. Parkman inquired about the specificities of the monoclonal antibodies that will be used in the purging system. Dr. Mills said that the antibodies that were selected for targeting neuroblastoma cells were chosen based on their high level of reactivity with a broad panel of neuroblastoma specimens. Data obtained from Dr. Don Hempstead's laboratory demonstrated that these antibodies reacted with 274 out of 275 neuroblastoma

patient specimens. Statistical analysis indicates that 98% of all neuroblastoma tumors will react with one of these antibodies. Dr. Parkman asked if it is an inclusion criterion that the patient must demonstrate reactivity with one of the antibodies on the panel. Dr. Brenner said that this positive antibody screening is not an inclusion criterion; all neuroblastoma patients are eligible for this protocol.

Dr. Parkman inquired if these antibodies had an effect on hematopoietic progenitor cells, specifically, pluripotent hematopoietic stem cells. Dr. Brenner said that data suggests that these antibodies cause a slight reduction in the number of CFUs; however, patients have been treated with these antibodies and successfully engrafted in less than 43 days. Dr. Parkman asked if the period of engraftment is delayed as compared to patients receiving unpurged marrow. Dr. Brenner said that engraftment with unpurged marrow occurs within the same timeframe; however, no side-by-side comparisons have ever been performed.

Dr. Zallen noted that some patients may enter this protocol from Mt. Sinai Hospital. She asked if the statement in the St. Jude informed consent document regarding non-negligent physical injury will be included in the informed consent of Mt. Sinai. Dr. Mills said that the statement is applicable to all patients entered into this protocol, regardless of the institution.

Dr. Walters asked Dr. Brenner to expand on several issues. How is the study funded? How will data be disclosed that emerges from this protocol? Are there any restrictions on the disclosure of data? Dr. Brenner said that he is not aware of any restrictions on the disclosure of data. With regard to funding, the antibodies and purging devices will be supplied by Baxter. The cost of the treatment will be met by St. Jude or third-party carriers if they will provide payment. If third-party carriers do not reimburse the costs, then St. Jude will cover all costs associated with the treatment. The retroviral vector supernatants will be supplied by GTI.

Committee Motion

A motion was made by Dr. Haselkorn and seconded by Dr. Krogstad to approve the protocol. Dr. Murray called for a vote. The motion passed by a vote of 19 in favor, 0 opposed, and no abstentions.

XII. OTHER COMMITTEE MATTERS

Dr. Murray stated that Dr. Anderson has indicated his intention to submit a report that will be included as an agenda item for the December RAC meeting. The report will detail the safety issues surrounding retroviral supernatant testing.

Dr. Parkman suggested an additional agenda item should be included for the next RAC meeting; namely, the issue of separation of the therapeutic and gene marking informed consent documents.

Ms. Buc requested that the issue of data reporting and how to enforce compliance should be added to the next RAC agenda. Mr. Capron asked if the concerns that investigators have regarding the release of data and possible threat to publication in a peer reviewed journal is a legitimate concern. Dr. Anderson said that this issue is not bogus. Although in principle *Science* and the *New England Journal of Medicine* have agreed, that the presentation of data at an open forum such as the RAC will not interfere with subsequent publication of data in these journals, the reality is that reviewers evaluate data and assign priority based on the importance of the paper. In certain instances, a journal could put a hold on the publication of this information. Dr. Krogstad said that this situation is one of those unusual circumstances in which the RAC has a monopoly. If the RAC decides that it requires pertinent data to make intelligent decisions regarding the approval of future protocols, then the committee members must maintain their position regarding the procurement of data.

Dr. Murray read a letter, dated June 11, 1992, that was forwarded to all investigators who have received approval to initiate human gene transfer/therapy trials. The letter requested that meeting abstracts, IRB annual reports, reports of adverse effects, FDA annual reports, and published scientific papers should be forwarded to ORDA. The response has been minimal. Dr. Secundy asked if this request could become a requirement. Ms. Buc noted that the list of requirements was compiled with much forethought; and that every listed document is one that has to be created for other purposes, not solely for the RAC. The committee has tried to make compliance as easy as possible for investigators.

Ms. Buc said that data reporting is critical because it will lead the RAC in two directions. First, it helps to enforce the requirements that the RAC has already established. Second, the RAC may begin to categorize certain types of experiments based on proven standards of safety. Eventually, experiments labelled as safe could qualify for a streamlined review process. The RAC cannot make these decisions without solid data.

Dr. Anderson stated that there are basically two types of data: safety and efficacy. Safety data is immediately available to everyone. Fortunately, there have been no side effects from any gene therapy protocol in the world. With regard to side effects, the *Points to Consider* require that any side effects must be reported immediately to the RAC. Efficacy is the difficult issue. Investigators are fearful that submission of efficacy data will be made available to the press. The standard for judging a successful protocol for scientists is a published manuscript. These investigators will publish as quickly as

significant data is obtained. Dr. Leventhal reminded Dr. Anderson that all of these investigators are required to file IRB reports prior to publication. Dr. Leventhal noted that published manuscripts do not describe what was proposed in the protocol, only the outcome. For example, if an investigator treats more patients than he/she was given approval for, then the RAC needs to be apprised of this information.

Committee Motion

Dr. Walters moved that the investigators should be required to provide the requested information for review prior to the December RAC meeting. Dr. Secundy seconded the motion. Dr. Leventhal suggested that protocols should be rescinded if this information is not forthcoming. Dr. D. Miller suggested a friendly amendment to the motion that the reporting requirements in the *Points to Consider* should be changed from semi-annual reporting to read, "reports not received within 12 months of the initial start date of the experiment may result in cancellation of its approval." Dr. Walters noted that to change the *Points to Consider* would require public notice prior to RAC discussion and approval as a minor amendment to the *NIH Guidelines*. Dr. D. Miller withdrew his amendment to the motion.

Dr. Leventhal suggested that a second letter should be sent to investigators of approved protocols as a follow-up to the initial request letter dated June 11, 1992. This follow-up letter should specify that if the requested information is not received, the approval of the protocol will be reconsidered. In addition, the submitted information is due to ORDA by November 1, 1992. Drs. Walters and Secundy agreed to accept Dr. Leventhal's suggestion as a friendly amendment to his motion. Dr. Murray called for a vote. The motion passed by a vote of 20 in favor, 0 opposed, and no abstentions.

XIII. ADDITION TO APPENDIX D OF THE NIH GUIDELINES REGARDING A HUMAN GENE THERAPY PROTOCOL ENTITLED: GENE THERAPY OF CANCER: A PILOT STUDY OF IL-4 GENE MODIFIED ANTITUMOR VACCINES/DR. LOTZE

Review--Dr. Leventhal

Dr. Murray called on Dr. Leventhal to present her primary review of the protocol submitted by Dr. Michael T. Lotze, University of Pittsburgh, Pittsburgh, Pennsylvania. Dr. Leventhal presented a brief overview of the protocol. Systemic IL-4 therapy has resulted in no significant antitumor responses as hypothesized. For this reason, the investigators propose to administer a mixed vaccine of IL-4 transduced autologous fibroblasts and autologous tumor to multiple biopsy sites in order to provide local IL-4 therapy rather than systemic. The protocol is designed to administer this cell mixture in a dose-response manner such that the number of tumor cells is constant and the number of fibroblasts will be variable.

Dr. Leventhal stated concerns about the eligibility requirement that patients must have an anticipated life expectancy of less than six months. It is not ethical to permit a patient with such advanced disease to participate in an experimental protocol which offers no therapeutic benefit. Also, there has not been a satisfactory response with regard to defining criteria for failure versus success.

Dr. Leventhal stated that she had initial concerns regarding the cost of the certain procedures to the patient; however, Dr. Lotze has specified that there will be no cost to the patient for additional evaluations.

Review--Dr. DeLeon

Dr. DeLeon said that the protocol does not include the details of the clinical assessments that will be performed after the vaccine has been administered. Dr. Lotze has provided a revised section to the protocol to outline these procedures. Another concern is that in a population size of 20 patients with four histological diagnoses, it will be difficult to measure therapeutic efficacy.

Review--Dr. Zallen

Dr. Zallen stated that the investigators have adequately responded to her questions regarding patient recruitment and conduct of the informed consent process. There is a section of the informed consent document that is still of some concern regarding the requirement that the patient is responsible for costs associated with the thorascopic removal of lung metastases. She asked Dr. Lotze to explain why a patient will be required to pay for a procedure that is necessary to obtain tumor cells that will be used to develop the vaccine. This procedure is part of the experimental process.

The right-to-withdraw section of the informed consent document should be revised so that it is written in the first person singular. All references to the term "therapy" should be omitted from the informed consent document since this procedure has not been proven to be therapeutic. Several paragraphs had inadvertently been deleted from the revised protocol, and this language needs to be reinserted.

Dr. Zallen asked if there is the possibility that a patient will develop an immune response against the autologous fibroblasts. Could insertion of the IL-4 gene result in the overproduction of a normal antigen that would elicit such a response.

Other Comments

Dr. Haselkorn suggested that the word "vaccine" should be removed from the protocol since this is an inappropriate term.

Dr. Parkman asked if the size of biopsy that has been specified will be adequate to produce a sufficient number of fibroblasts for the injection. If an insufficient number of autologous fibroblasts is obtained, will allogeneic fibroblasts be used?

Dr. Parkman inquired as to why the investigators did not choose to transduce tumor cells alone. For this protocol, tumor cells will have to be grown to a certain extent. Why is it not possible to continue to expand these tumor cells such that there is a sufficient number of cells to transduce? Injection of tumor cells alone would obviate the need for fibroblasts and eliminate concerns regarding an immune response against fibroblasts.

Dr. D. Miller stated that he did not receive a 3½" diskette of the vector sequence to run through GenBank. The sequence must be screened for open reading frames, etc., prior to initiating this protocol. He asked what the effect of the irradiated transduced cells would be on neighboring normal cells.

Presentation--Dr. Lotze

In response to Dr. Zallen's question, Dr. Lotze explained that the cost of the thoracoscopic procedure will be incurred by the University of Pittsburgh unless it is performed for an entirely different purpose, such as for diagnostic purposes. Dr. Lotze said that he would revise the cost section such that the cost of the thoracoscopy will be provided as a procedure that is of no cost to the patient.

With regard to the issue of induction of an autoimmune response, Dr. Lotze said that there is a large body of data demonstrating that cytokine administration does not induce an autoimmune response, with the exception of IL-2 induced thyroiditis. If symptoms occur, patients would require treatment to suppress the autoimmune phenomenon, i.e., steroid administration.

Dr. Parkman added that if an immune response occurs, it may be only for the period of time during which there is increased IL-4 production. Since the fibroblasts are irradiated, the production of IL-4 will be self-limiting. Dr. Zallen asked if treatments administered for an autoimmune response would interfere with other antitumor therapies that patients might receive. Dr. Lotze responded that the patients eligible for this protocol have exhausted all traditional therapies for treatment of their tumor. Dr. Parkman stated that the reverse situation would be effected. If a patient participating in this protocol would require concurrent administration of steroids, this treatment would interfere with the effect of the experimental therapy.

Dr. Lotze addressed the issue of using transduced tumor cells versus fibroblasts. Although one would hypothesize that a patient would benefit from cytokine production made by the cell type that is being targeted, local delivery of cytokines is normally

provided by lymphocytes, not tumor cells.

Dr. Lotze agreed to omit the term "vaccine" from the protocol. Dr. Parkman suggested replacing this term with IL-4 transduced fibroblasts or simply cells. The reality is that the IL-4 transduced fibroblasts are functioning as an adjuvant, not a vaccine.

Dr. Lotze responded to questions regarding the generation of adequate numbers of fibroblasts for each patient. He described the Hayflick phenomenon in which fibroblasts have a limited number of cell divisions. Although the number of possible generations is approximately 50, this number of fibroblasts is sufficient to provide for this therapy. In the event that a patient does not provide enough fibroblasts, he/she would not be eligible for this protocol. These patients may then be eligible for the tumor infiltrating lymphocyte protocol.

In response to Dr. D. Miller's question, tumor cells will not be grown up for this experiment. The protocol specifies that tumor suspensions will be prepared from enzymatically digested primary tumors. The tumors will not be cultured. He agreed to submit the pertinent vector sequence information to ORDA.

Dr. D. Miller asked if local administration of IL-4 would produce the same results as IL-4 transduced fibroblasts. Is delivery the problem? Dr. Lotze responded that a variety of vehicles exist for delivery of these agents. Experiments are now being performed to determine whether polyethylene glycol can be used as a delivery vehicle for IL-4 since this protein is not rapidly cleared. Dr. Parkman explained that a pump could be inserted locally; however, this pump may be a potential source of infection in these already immunosuppressed patients. Dr. Leventhal said that even if the investigators were successful at implanting a pump for secreting IL-4, it would be difficult to deliver identical amounts to different sites continuously. It would be impossible to interpret the results of such a dose-response experiment designed in this way.

Committee Motion

A motion was made by Dr. Parkman and seconded by Dr. Leventhal to approve the protocol contingent on: (1) submission and review of the vector sequence on a 3½" diskette in ASCII format and (2) the term "vaccine" will be removed from the protocol. Dr. Murray called for a vote. The motion passed by a vote of 19 in favor, 0 opposed, and no abstentions.

XIV. FUTURE MEETING DATE OF THE RECOMBINANT DNA ADVISORY COMMITTEE

Dr. Murray noted that the next meeting of the RAC will be December 3-4, 1992. The meeting will be held at NIH, Building 1, Wilson Hall.

XV. ADJOURNMENT

Dr. Murray adjourned the meeting at 1:25 p.m., on September 15, 1992.

Nelson A. Wivel, M.D.
Executive Secretary

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

Date: _____

Barbara E. Murray, M.D.
Chair
Recombinant DNA Advisory Committee
National Institutes of Health