DEPARTMENT OF HEALTH AND HUMAN SERVICES
NATIONAL INSTITUTES OF HEALTH
RECOMBINANT DNA ADVISORY COMMITTEE
MINUTES OF MEETING
June 12-13, 1997

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The Recombinant DNA Advisory Committee (RAC) was convened for its sixty-seventh meeting at 9:00

a.m. on June 12, 1997, at the National Institutes of Health (NIH), Building 31, Conference Room 6, 9000 Rockville Pike, Bethesda, Maryland 20892. Dr. Claudia Mickelson (Acting Chair) presided. In accordance with Public Law 92-463, the meeting was open to the public on June 12 from 9 a.m. until 5 p.m. and June 13 from 8:30 a.m. until 4:30 p.m. The following were present for all or part of the meeting:

Committee Members:

C. Estuardo Aguilar-Cordova, Texas Childrens Hospital
Joseph C. Glorioso, University of Pittsburgh
Leslie A. Leinwand, University of Colorado
M. Therese Lysaught, University of Dayton
M. Louise Markert, Duke University Medical Center
Kathleen M. McGraw, State University of New York at Stony Brook
R. Scott McIvor, University of Minnesota
Claudia A. Mickelson, Massachusetts Institute of Technology
Gail S. Ross, Cornell University Medical Center
Karen Rothenberg, University of Maryland School of Law
Bratin K. Saha, Emory University
Jon A. Wolff, University of Wisconsin Medical School

Executive Secretary:

Debra W. Knorr, National Institutes of Health

A committee roster is attached (Attachment I).

Non-Voting Representatives:

Philip Noguchi, Food and Drug Administration
Ralph Yodaiken, U.S. Department of Labor

Liaison Representative:

Daniel Jones, National Endowment for the Humanities

Ad Hoc Consultant:

Philip Bernstein, Nature Biotechnology

National Institutes of Health staff:

Diane Bronzert, NCI
Jan Casadei, NCI
Jay Greenblatt, NCI
Toby Hecht, NCI
Christine Ireland, OD
Heili Kim, OD
Becky Lawson, OD
Catherine McKeon, NIDDK

Gene Rosenthal, OD
Jeffrey Schlom, NCI
Thomas Shih, OD

Others:

Elham-Eid Alldredge, REDA International, Inc.
Victoria Allgood, GeneMedicine, Inc.
Robert Anderson, Food and Drug Administration
W. French Anderson, University of Southern California
Dale Ando, Chiron Corporation
Peter Ballard, The Blue Sheet
Roberta Binder, Public
Bridget Binko, Cell Genesys
Amy Bosch, Targeted Genetics Corporation
Andrew Braun, Massachusetts General Hospital
Jeff Carey, Genetic Therapy, Inc.
Rachel Carle, Genzyme Corporation
A. Antonio Championsmith, Cell Genesys, Inc.
Kenneth Culver, Codon Pharmaceuticals, Inc.
John Cutt, Schering-Plough Research Institute
Diane Fleming, American Society for Microbiology
Donald Gay, Chiron Corporation
Eli Gilboa, Duke University Medical Center
Dorothy Jessup, Public
Steven Kradjian, Vical, Inc.
H. Kim Lyerly, Duke University Medical Center
Andra Miller, Food and Drug Administration
Robert Moen, Baxter Healthcare Corporation
Mehrotra Priti, Food and Drug Administration
Tomiko Shimada, Ambience Awareness International, Inc.
Dominick Vacante, Magenta Corporation
Jeffrey Weaver, University of Maryland at Baltimore
Lisa White, The Blue Sheet

I. CALL TO ORDER AND OPENING REMARKS/DR. MICKELSON

Dr. Claudia A. Mickelson (Acting Chair) called the meeting to order and stated that due notice of the meeting under the *NIH Guidelines for Research Involving Recombinant DNA Molecules (NIH Guidelines)* was published in the *Federal Register* on May 22, 1997 (62 FR 28154). She noted that a quorum was present and outlined the order in which speakers would be recognized: (1) primary reviewers, (2) other RAC members, (3) *ad hoc* experts, (4) responses from the principal investigators (PIs), (5) other NIH and Federal employees, (6) the public who have submitted written statements prior to the meeting, and (7) the public at large.

Dr. Mickelson welcomed the *ad hoc* reviewer (Dr. Philip Bernstein) and the investigators (Drs. Eli Gilboa and H. Kim Lyerly) of the Protocol #9703-179. Dr. Mickelson stated that the goal of the RAC is to encourage and foster biomedical research while serving as a forum for the open discussion of the issues surrounding recombinant DNA research. She recognized the investment of time and effort that the investigators make for allowing public discussion of their research.

Dr. Mickelson noted that Drs. Glorioso, McGraw, Ross, Saha, and Samulski are completing their term of service to the RAC.

Dr. Mickelson noted that Forum 1997: 2nd Food and Drug Administration (FDA)/NIH Conference on Gene Therapy will be held on July 15-18, 1997, at the NIH, Bethesda, Maryland. She reminded the audience that copies of meeting materials of the RAC meeting are available from the Office of Recombinant DNA Activities (ORDA) upon request.

Dr. Mickelson stated that Dr. Lana Skirboll, NIH Associate Director for Science Policy, on behalf of Dr. Harold Varmus, the NIH Director, wants to thank Dr. Aguilar-Cordova for chairing the March 1997 RAC meeting and Dr. Mickelson for chairing the June 1997 RAC meeting. Dr. Varmus anticipates that a permanent Chair will be appointed by the September 1997 RAC meeting. Dr. Varmus expressed his appreciation for the RAC's sensitivity in handling the Lyerly protocol under unusual circumstances. Dr. Mickelson stated that both ORDA and the NIH Director's office discussed with the FDA the time delays surrounding the Lyerly protocol, and that mechanisms are being implemented that will improve communications with the FDA to avoid such problems in the future. Ms. Knorr, Acting Director of ORDA, stated that she received a positive response stating that FDA plans to implement procedures to notify ORDA immediately upon receipt of an IND involving human gene transfer.

Dr. Mickelson stated that Dr. Varmus is pleased with the discussion regarding the RAC recommendations on proposed changes to the *NIH Guidelines*, and that the RAC is poised to move forward. Dr. Mickelson noted that the proposed actions, i.e., relinquishing RAC and NIH Director approval of gene transfer protocols, etc., have not been published as a final action under the *NIH Guidelines*. Publication of these final actions is pending the completion of an Environmental Assessment and Finding of No Significant Impact. The decision to conduct an Environmental Assessment was made by the NIH Office of General Counsel. Dr. Mickelson noted that until the *NIH Guidelines* are formally amended, the current *NIH Guidelines* (January 1997) are still in effect with regard to the NIH oversight of gene transfer protocols.

Dr. Mickelson stated that Ms. Knorr had three items for RAC consideration later in the meeting. These were: (1) criteria for review of novel protocols and the framework for RAC discussion; (2) methods of streamlining a submission format between the NIH and the FDA; and (3) considering exempt experiments.

Other Comments

Ms. Rothenberg inquired if any RAC recommendations resulting from the RAC discussion at the December 1996 and March 1997 RAC meetings have been included in the *NIH Guidelines* (January 1997). Ms. Knorr responded that the proposed amendments have not been promulgated into the *NIH Guidelines*; the January 1997 version has been included in the meeting material as a reference. Dr. Mickelson noted that the final action will be published in the *Federal Register* after completion of the Environmental Assessment in the Fall 1997. Ms. Knorr noted that ORDA has sent a letter to the Chairs of Institutional Biosafety Committees (IBCs) and Institutional Review Boards (IRBs) informing them that until further notice the current procedures for submission of human gene transfer protocols is still in effect as published in the *NIH Guidelines* (January 1997).

Dr. Markert inquired if the motion passed by the RAC at its March 1997 RAC meeting to remove the requirements for prior IBC and IRB approvals from the protocol submission requirements of Appendix M can be decoupled from the *Proposed Actions* that requires an Environmental Assessment. The investigators would benefit if this amendment could be promulgated to the *NIH Guidelines* as soon as

possible. Dr. Markert indicated that she would propose a motion to decouple the IBC/IRB prior approval issue from the *Proposed Actions*. Dr. McIvor seconded the motion. Dr. Aguilar-Cordova suggested an friendly amendment to decouple the issue of relinquishing NIH Director approval of human gene transfer protocols from the whole package of the *Proposed Actions*; it appears to him that relinquishing NIH Director's approval is the only issue that requires an Environmental Assessment. Ms. Knorr stated that the NIH General Counsel would have to rule on the question of whether any of the suggestions is acceptable.

Ms. Rothenberg noted that the purpose of the motion would be to allow the issues that do not require an Environmental Assessment to move forward to the final action; she would support a motion to decouple the issues that do not need an Environmental Assessment. The motion would clarify the confusion of the gene therapy community about the status of the *Proposed Actions*, but she was not sure that the RAC has such an authority. Dr. Noguchi explained that the Environmental Protection Agency (EPA) had a concern with the July 8, 1996, *Proposed Actions* regarding NIH relinquishing IBC approval of human gene transfer experiments. In his opinion, the original EPA concern was a misunderstanding; under the *Proposed Actions*, IBC will still have the same authority for approval of human gene transfer protocols. Responding to Dr. McIvor's question of who should prepare the Environmental Assessment, Ms. Knorr stated that the ORDA is preparing the document; the NIH General Counsel will review and comment; and the NIH Director will approve the document. Dr. Markert emphasized that her motion does not relinquish IBC approval of protocols. Prior IBC approval will not be required when the protocol is submitted for RAC review; IBC approval will be still required before initiation of the clinical trial. Dr. Mickelson noted that the NIH General Counsel has to assess whether the change of NIH oversight has significant environmental impact. Dr. Lysaught was concerned that the interim solution of decoupling the prior approval issues would cause more confusion to the investigators; she would prefer that final actions be issued after completion of the Environmental Assessment. Dr. McIvor agreed and stated that he would withdraw his second to Dr. Markert's motion; Dr. McIvor said that the Environmental Assessment should move forward expediently. Dr. Mickelson noted that since the motion was not seconded, there is no need for a vote.

II. MINUTES OF THE MARCH 6-7, 1997, MEETING/DRS. MARKERT AND ROSS

Committee Motion 1

The RAC approved a motion made by Dr. McIvor and seconded by Dr. Ross to accept the minutes of the March 6-7, 1997, RAC meeting (with the incorporation of minor editorial changes), by a vote of 10 in favor 0 opposed, and no abstentions.

III. UPDATE ON DATA MANAGEMENT/ROSS

Dr. Ross noted that there are 184 protocols registered with ORDA including 30 gene marking protocols, 153 gene therapy protocols, and 1 non-therapeutic protocol. Of these protocols, 103 protocols are for cancer, 27 for monogenic diseases, 19 for human immunodeficiency virus (HIV) infection, and 4 for other diseases.

There has been 15 amendments submitted since the March 1997 meeting. Most of these amendments are for addition of sites, and addition or change of investigators.

Dr. Ross noted two significant adverse events: (1) Protocol (#9409-087) - patients receiving cell preparations (using fetal calf serum) encountered possible infusion reactions; (2) Protocol (#9503-103) - two Grade 3 events (hyperbilirubinemia and thrombocytopenia) and one ungraded event (chest tightness) were reported. All these events were deemed to be unrelated to gene transfer techniques. In addition, a patient death was reported for the multicenter brain tumor trial (Protocol #9608-157). Computer

tomography (CT) showed severe edema of the tumor bed and a small epiduralhematoma. Although the symptoms were possible complications of surgical resection, they appeared to be associated with injection of the vector producer cells; similar adverse effects have been previously discussed at the RAC meetings.

Since the March 1997 meeting, 10 protocols have been registered with ORDA: 9 of which are exempt from full RAC review and are listed below; and 1 of which Dr. Lyerly was allowed to treat subjects already entered into the study, however, enrollment of additional subjects was pending RAC discussion at this meeting.

9701-175

Lieberman, Frank; Germano, Isabelle; and Woo, Savio; Mount Sinai Medical Center, New York, New York; *Gene Therapy for Recurrent Glioblastoma Multiforme: Phase I Trial of Intraparenchymal Adenoviral Vector Delivery of the HSV-TK Gene and Intravenous Administration of Ganciclovir.*

NIH/ORDA Receipt Date: 1-22-97. Sole FDA Review Recommended by NIH/ORDA: 2-12-97

9702-176

Sanda, Martin G.; University of Michigan Urology Clinics, Ann Arbor, Michigan; *A Phase I/II Clinical Trial Evaluating the Safety and Biological Activity of Recombinant Vaccinia-PSA Vaccine in Patients with Serological Recurrence of Prostate Cancer Following Radical Prostatectomy.*

NIH/ORDA Receipt Date: 2-19-97. Sole FDA Review Recommended by NIH/ORDA: 5/13/97

9702-177

Verfaillie, Catherine; McIvor, Scott; McCullough, Jeff; and McGlave, Philip; University of Minnesota, Minneapolis, Minnesota; *Autologous Marrow Transplantation for Chronic Myelogenous Leukemia Using Retrovirally Marked Peripheral Blood Progenitor Cells Obtained after In Vivo Cyclophosphamide/G-CSF Priming.*

NIH/ORDA Receipt Date: 2-21-97. Sole FDA Review Recommended by NIH/ORDA: 3-14-97

9703-178

Belmont, John W.; Texas Children's Hospital, Houston, Texas; *Phase I Clinical Trial of TREV Gene Therapy for Pediatric AIDS.*

NIH/ORDA Receipt Date: 10-3-97. Sole FDA Review Recommended by NIH/ORDA: 3-31-97

9703-180

Netscher, David; Hand Clinic at the Veteran's Affairs Medical Center, Houston, Texas; *Phase I Single Dose-Ranging Study Of Formulated hIGF-I Plasmid In Subjects With Cubital Tunnel Syndrome.* Sponsor: Gene Medicine, Inc.

NIH/ORDA Receipt Date: 3-17-97. Sole FDA Review Recommended: 4-7-97

9703-181

Connick, Elizabeth; University of Colorado Health Sciences Center, Denver, Colorado, Deeks, Steven G.; University of California, San Francisco General Hospital, San Francisco, California, Scadden, David; Massachusetts General Hospital (East), Charlestown, Massachusetts, Mitsuyasu, Ronald; University of California, Los Angeles Medical Center, Los Angeles, California; *A Phase II Study of the Activity and Safety of Autologous CD4-Zeta Gene-Modified T Cells With or Without Exogenous Interleukin-2 in HIV Infected Patients.* Sponsor: Cell Genesys, Inc.

NIH/ORDA Receipt Date: 3-19-97. Sole FDA Review Recommended: 4-18-97

9703-182

Gardner, Phyllis; Stanford University's General Clinical Research Center, Palo Alto, California; *A Phase I/II Study of tgAAVCF for the Treatment of Chronic Sinusitis With Cystic Fibrosis.* Sponsor: Targeted Genetics Corporation

NIH/ORDA Receipt Date: 3-13-97. Sole FDA Review Recommended: 4-1-97

9703-183

Straus, Stephen E.; NIH, Bethesda, Maryland; *Administration of Neomycin Resistance Gene Marked EBV Specific Cytotoxic T-Lymphocytes To Patients With Relapsed EBV-Positive Hodgkin Disease.*

NIH/ORDA Receipt Date: 3-19-97. Sole FDA Review Recommended by NIH/ORDA: 3-25-97

9703-184

Belldegrun, Arie; University of California, Los Angeles, School of Medicine, Los Angeles, California; *A Phase I Study Evaluating the Safety and Efficacy of Interleukin-2 Gene Therapy Delivered by Lipid Mediated Gene Transfer (Leuvectin) in Prostate Cancer Patients.* Sponsor: Vical, Inc.

NIH/ORDA Receipt Date: 3-24-97. Sole FDA Review Recommended by NIH/ORDA: 5-21-97

IV. HUMAN GENE TRANSFER PROTOCOL #9703-179 ENTITLED: *A PHASE I STUDY OF ACTIVE IMMUNOTHERAPY WITH CARCINOEMBRYONIC ANTIGEN RNA-PULSED AUTOLOGOUS HUMAN CULTURED DENDRITIC CELLS IN PATIENTS WITH METASTATIC MALIGNANCIES EXPRESSING CARCINOEMBRYONIC ANTIGEN*

PI: H. Kim Lyerly, Duke University
Reviewers: Wolff, McIvor, Mickelson
***Ad hoc*: Philip Bernstein, Ph.D., Roger Pomerantz, M.D., John Coffin, Ph.D.,**
Mark Boyd, Ph.D, Thierry Heidmann, Ph.D.

Review--Dr. Wolff

Dr. Mickelson called on Dr. Wolff to present his primary review of the protocol submitted by Dr. H. Kim Lyerly of Duke University Medical Center, Durham, North Carolina. Dr. Wolff stated that the protocol is a Phase I clinical trial to investigate the antitumor immune response induced by infusion of autologous

dendritic cells exposed *in vitro* to ribonucleic acid (RNA) expressing the carcinoembryonic antigen (CEA). The purpose is to induce active immune response against metastatic cancers that are expressing CEA. There are precedents of protocols that used dendritic cells for the same purpose without gene transfer.

Dr. Wolff stated that his initial rationale in recommending full RAC discussion was that this protocol is the first use of nonviral RNA molecules for gene transfer experiments. Therefore, it is useful to discuss the ramification of RNA transfer as a means of gene therapy in terms of the central dogma of molecular biology, i.e., genetic information transfer from the gene to RNA and then to protein. Dr. Wolff said that several complexities should be considered for a full evaluation of the possible risks associated with the RNA transfer protocol.

(1) *The possibility that the introduced RNA species is packaged in a virus and is spread within the patient's body or to other individuals.*

It has been demonstrated that non-retroviral cellular RNAs can be packaged and encapsidated in retroviral particles and then infect another cell. Then the transferred RNA can be reverse transcribed and integrated into the cellular genome. The non-retroviral cellular RNAs do not have to contain retroviral *cis* sequences in order to proceed through this process. This process has been termed "retrofection." It has been demonstrated that if the introduced RNA is packaged into a retroviral particle along with another RNA, recombination between the two RNA species can occur. Additionally, recombination between viral and non-viral RNA species can occur in other RNA viruses, i.e., coronavirus, poliovirus, and Sindbis virus.

Human cells contain a variety of human endogenous retroviruses that produce retroviral particles. However, these particles are not fully infectious retroviruses; and the retroviral sequences have multiple defects such as stop codons. For example, the T47D human mammary carcinoma cell line produces human endogenous retrovirus (HERV) particles that contain active reverse transcriptase activity; virus production is increased by steroid hormones. Also, the production of intracisternal A-type retroviral particles (endogenous murine defective retroviruses) in BL6 melanoma is inhibited by transfection with major histocompatibility complex class 1 genes which suggest that expression of a foreign gene could influence the production of HERVs. Do human dendritic cells produce HERVs, and does their stimulated growth for 7 days *in vitro* (in the presence of granulocyte-macrophage colony stimulating factor and interleukin-4) and CEA gene transfection affect their level of HERVs? Dendritic cells can be infected with HIV, but the proposed study will not be performed on HIV-positive patients.

The issue of germ-line transmission is related to the question of whether HERVs or other viruses would be produced containing the exogenous RNA. It has been reported that DNA sequences occasionally appear in new genomic locations, both in germ-line and somatic cells. In many instances, there is good evidence that these molecules have transposed via RNA intermediates. Given that germ-line transmission has not been demonstrated in mammals receiving replication-defective retroviral vectors, and the very small chance of viral encapsidation of the introduced RNA, the chance of germ-line transmission with RNA in the context of the present protocol should approach nil.

(2) *The possibility that the introduced RNA species is reverse transcribed and becomes integrated into the chromosome of germ-line cells.*

There is a very small but definite risk that RNA can be reverse transcribed in normal cells, and the cDNA can be incorporated into the genome. In one published study, it occurred at an absolute frequency of 5×10^{-8} events per cell per generation. This frequency was obtained from a foreign gene introduced as DNA. The major concern of such a process would be if the protocol involves the use of an oncogene.

(3) *The possibility that expression of the transferred RNA can have a harmful effect on the target cell.*

Several questions could be asked. (i) Could CEA expression affect the neoplastic potential of the dendritic cells? A study found that stable expression of human CEA on the surface of rat L6 myoblasts blocked their ability to differentiate into mature myotubes and enabled the cells to maintain their proliferation potential. High serum CEA levels have been associated with cancer progression. Dr. Wolff remarked that many anticancer therapies increase the risks of secondary cancers. (ii) Could CEA expression in the transferred dendritic cells raise the serum levels of CEA and affect the metastatic potential of the endogenous tumors? Intravenous injection of 40 µg of CEA protein enhanced the metastasis of human colorectal carcinoma cells to the liver and the lung in athymic nude mice. However, it is unlikely that the gene transfer methods employed in this protocol will affect the serum levels of CEA protein in the patients. On the other hand, the dendritic cells could migrate to the liver or other tissues and affect the local tissue concentrations of CEA which could enable metastasis. (iii) Could CEA expression in the dendritic cells lead to their aberrant migration which could produce harmful effects? Previous studies have suggested that CEA affects cell to cell contacts; and that this effect could lead to aberrant homing of the dendritic cells, tissue inflammation and destruction, or to an autoimmune process. The issue of autoimmunity has been raised by the investigators. (iv) Could CEA expression enable infection of the dendritic cells with a virus contaminating its growth media and lead to an infection in the patients? For example, after transfection of COS-7 cells with the human CEA gene, the cells were able to be infected with two different strains of mouse hepatitis virus and produced the virus. Mouse hepatitis virus infections in mice have served as a model for demyelinating disease such as multiple sclerosis. It is not unprecedented for a cell surface molecule to be a receptor for more than one type of virus. Even if the dendritic cells are not grown in mouse-derived products, other products could be contaminated with other viruses that use CEA as a receptor. Dr. Wolff asked the investigators to explain what type of testings will be performed for detecting viral contaminants.

In summary, Dr. Wolff stated that the use of nonviral RNA for gene transfer is a novel method. It raises theoretical concerns that should be discussed. The concerns are testable with appropriate assays, and the RAC should discuss which of these tests are worthy of conducting in terms of minimizing the risks to the patients and to the general public.

Review--Dr. McIvor

Dr. McIvor raised several concerns in his written review, and he noted that these concerns are addressed by the investigators in their written response.

(1)This proposal represents the first time that recombinant RNA-treated cells will be used in humans for therapeutic purposes. While it might not be expected that exposure of dendritic cells to the recombinant RNA would be capable of effective long-term genetic modification, the possibility exists that the RNA could be converted (reverse transcribed) into DNA and incorporated into the genome of the target cells. The questions for the investigators are: (a) Is there any evidence for reverse transcription of the RNA into DNA in the treated cells? (b) Is there any evidence for stable integration of the RNA sequences (in DNA form) in the target cells? The investigators responded in writing that there is no evidence for reverse transcription nor for integrations. In view of the fact that very little CEA RNA is present in the transfected dendritic cells, it would be extremely difficult to devise an experimental approach to detect a low level of reverse transcription. Dr. McIvor asked the investigator if any attempt has been made to detect reverse transcription.

(2)If there is evidence for reverse transcription and integration of the RNA sequences in the target cells,

the primary concern is tumorigenesis. Have samples of RNA-pulsed human and/or murine dendritic cells been evaluated in long-term culture for evidence of tumorigenesis? The investigators responded in writing that they have attempted to maintain RNA transfected dendritic cells in culture, but they were not successful in establishing any immortalized cells. Dr. McIvor was satisfied with the response.

(3)The therapeutic RNA will be generated by *in vitro* transcription, and will be characterized by spectrophotometric analysis, electrophoresis, northern blot analysis, western blot analysis, and functional analysis (i.e., ability of pulsed dendritic cells to stimulate CEA-specific T-cell response). *In vitro* transcribed RNA is often contaminated with many short transcripts. What will be the standards of acceptance in each of these analysis? Have release criteria been established? Dr. McIvor noted that the investigators have not responded adequately to his questions in the written response.

(4)In response to Appendix M-II-B-2-b-(4), it is stated that "The RNA product contains the coding sequences for the human CEA antigen and includes also: a 5' cap, upstream "Kozak" sequences to facilitate initiation of translation, and a 100 to 200 nucleotide long polyA tail at the 3' end." However, the description of the RNA preparation includes only a transcription step using T7 RNA polymerase with no mention of capping or polyadenylation reactions. This is in contrast to the preclinical work described in the paper by Boczkowski, et. al., which includes capping and polyadenylation steps. Will the *in vitro* transcribed RNA be capped and polyadenlylated? If they will not be carried out, how will the RNA be translated since a cap is required for efficient formation of a ribosomal complex of translation? The investigators responded in writing that the RNA will be capped and polyadenylated. Dr. McIvor said that the investigators have described a procedure for polyadenylation, but a procedure for capping is not included in the protocol.

(5)Because of the notorious susceptibility of RNA to ubiquitous ribonucleases, it is surprising that naked RNA will be used for introduction into the dendritic cells without any other supplements to promote RNA stability or to facilitate transfer across the cell membrane. How stable is the RNA in the mixtures with dendritic cells during the RNA transfer reaction? Is there any molecular evidence that the RNA crosses the cell membrane under these conditions? The investigators responded in writing that they have not determined directly the stability of the RNA during the transfection procedure. Dr. McIvor asked the investigator to elaborate on this point.

(6)Is the RNA translated into CEA protein in the exposed dendritic cells? Has there been any analysis of CEA protein synthesis from the newly introduced RNA in these cells? Will any sort of analysis for CEA protein synthesis be carried out in the RNA-exposed dendritic cells to be used in the therapeutic trials? The investigators responded in writing that they have not been able to biochemically detect expression of CEA protein in human dendritic cells transfected with CEA RNA. Dr. McIvor said that preclinical studies have shown that immune response was stimulated by dendritic cells exposed to CEA RNA, but expression of the CEA protein in the dendritic cells has not been demonstrated.

(7)What is the evidence from preclinical studies that cytotoxic T lymphocyte (CTL) response specifically targets CEA on the surface of cells? Is cytotoxicity blocked by antibody against CEA? The investigators responded in writing that CEA specific T cells recognize peptides representing CEA CTL epitopes on the surface of target cells. Dr. McIvor was satisfied with the response.

(8)What is the correlation between the dendritic cell doses to be studied in the clinical trial and the cell doses tested in experimental animals under conditions where a successful antitumor immune response was elicited? Considering the comparative doses, does it appear likely that an immune response will be detectable in the patients infused with RNA-pulsed dendritic cells? Dr. McIvor asked the investigators to address this question.

(9)The description of studies to assess anti-tumor response was not adequately detailed. Will the assessment of tumor regression or progression be gauged primarily on radiologic or other results? The investigators responded in writing that tumor measurements will be made of evaluable lesions, radiographically or biochemically. Dr. McIvor asked the investigators to elaborate on the biochemical analysis.

(10)Will the presence of residual CEA RNA, CEA protein, or dendritic cells be analyzed in samples obtained from the patient at various times after dendritic cell infusion? Dr. McIvor was concerned about the tumorigenesis of the residual CEA molecules and if this risk should be described in the Informed Consent document.

Review--Dr. Mickelson

Dr. Mickelson made several comments on this protocol.

(1)The investigators offer *in vitro* human dendritic cell data and reprints of animal and human lymphoma studies. Close reading of these papers and the data in the appendices of the submission indicated that while tumor cell lysis was antigen specific, there appeared to be a high background of non-specific CTL response in some studies. Is the response a potential side effect of the infusion of cultured dendritic cells? Would the side effects be detrimental to patients if it is as great as it is in the animal models and in the *in vitro* human dendritic cell data presented in the appendices? This point should be clarified in the text, at least as part of the Risk discussion in Appendix M-II-B-2-b. Will this background of non-CEA related activity be assayed?

(2)The researchers stated that CEA RNA is expected to persist within the dendritic cells for only a few days. No data is presented or referenced. This point should be tested to verify the duration and fate of CEA RNA in "pulsed" dendritic cells.

(3)The cDNA sequence contains a small deletion, possibly resulting from a splicing error, that deletes one amino acid codon. Obviously, the investigators expect no effect on efficiency or effectiveness either in intracellular translation, protein degradation, or range of antigenic peptides due to this small deletion. It should be stated that this deletion is not expected to affect CEA peptide/antigen presentation by "pulsed" dendritic cells.

(4)It is unclear what percent of *in vitro* lytic activity constitutes a successful "pulsed" dendritic cells preparation. Stated criteria are viability, percent of cell population characterized by presence or absence of certain cell surface markers, but *in vitro* activity is not mentioned. Do the investigators propose to use "pulsed" cell cultures that are not active in the *in vitro* assays? If not, the level of *in vitro* immune stimulation is necessary before deciding to continue with the cell infusion.

(5)Within the Informed Consent document, it stated that "failure to stimulate the immune cells in the test tube may occur 50% of the time." It is unclear from the rest of the paragraph whether in these cases the dendritic cell cultures will be used if the *in vitro* test is negative. If the *in vitro* test is negative, it should be clearly stated that dendritic cells that fail to stimulate immune cells *in vitro* will not be administered to patients. Or, that regardless of *in vitro* immune stimulation results, uninfected autologous "pulsed" dendritic cell cultures will be given to patients if requested. Whatever the case, this point should be stated in the study design.

(6)In some places, the Informed Consent document states that Schering-Plough will have data access, in

other spots this statement is left out. If the company is to be granted data access, it should be stated uniformly throughout the document. Additionally, if Schering-Plough is funding some portion of this Phase I trial, that should be stated under the section on *Sources of Research Funds*.

(7)For the purpose of the RAC, the study should have a target enrollment of 18 patients. In the text, the number of patients is described as 3 at dose level 1, 3 at level 2, with 6 at the highest cell dose. While elsewhere in the protocol the number is given as 18.

(8)What criteria will be used to determine what information is in the public domain versus protected. Who will make that decision? Section M-V-A-B should be clarified.

Dr. Mickelson agreed with Dr. McIvor that the investigators should develop lot release criteria for the transfected dendritic cells.

Review--Dr. Bernstein

Dr. Bernstein (Nature Biotechnology) stated that most of his concerns are related to the issues of RNA being reverse transcribed and integrated within the genome, RNA being captured by endogenous retroviruses, and the stability of transfected RNA within the cell. It has not been addressed whether decreasing stability of RNA would affect the CTL response. Dr. Bernstein asked the investigators to clarify if the CEA deleted with an amino acid residue would affect tumorigenesis and its presentation by antigen-presenting cells to generate efficient CTL response. Has the wild-type CEA been studied with regard to the immune response question? Alternatively, the CEA RNA could be modified to minimize the potential risk of homologous recombination while keeping its immunogenic activity.

Reviews--Drs. Pomerantz, Coffin, Boyd, and Heidmann (presented by Dr. Wolff)

Dr. Mickelson called on Dr. Wolff to summarize the written reviews by Drs. Coffin, Pomerantz, Boyd, and Heidmann.

Dr. Coffin (Tufts University) wrote that while the study is a "pilot" experiment, he saw no reasonable source of gene-therapy-associated risk associated with the proposal on the following grounds: (1) The chance of integration of reverse transcribed DNA is small; (2) The exogenously added RNA is a small fraction of total cellular RNA and any additional contribution from added RNA to integration is insignificant; (3) The risk from integration of CEA is small since the integrated DNA is likely to be nonfunctional; even if CEA is functional, it is not an oncogene; and (4) The risk about encapsidation by endogenous retrovirus is not significant since the frequency of retrovirus transferring cellular RNA is very low. Dr. Coffin was distressed by the political pressure brought to bear by the relatives of the patients who have been led to believe that the protocol represented a chance for a "cure" of cancer. He was concerned about the informed consent process.

Dr. Pomerantz (Thomas Jefferson University) wrote that there is small but definite frequency that transduced mRNAs may be reverse transcribed and integrated (approximately 10^{-8}). He was not deeply concerned that this risk is significant to humans. Most gene therapy protocols that use retroviral vectors lead to integration as well; integration by RNA transfection would be in far fewer cells than integration with retroviral vectors. Dr. Pomerantz stated that RNA encoding an oncogene would be a concern but he was not aware that CEA is an oncogene. The potential for interaction of transduced RNA with human endogenous retroviruses is a theoretical risk. In summary, Dr. Pomerantz was not convinced that this protocol would be a significant risk in the patient population described or a clear risk to other humans.

Dr. Boyd (Allegheny University) wrote that there is no documented information regarding the stability of naked RNA in the tissue culture media. There is no demonstration that the RNA enters the cell, and no evidence that the RNA is translated. These effects of transfected RNA are all inferred from assays of efficacy of dendritic cells to function as antigen presenting cells. The theoretical basis is very weak. Gamma interferon has been shown to be induced by double stranded RNA, and induction of gamma interferon would be a potential explanation for the activation of dendritic cells to become more potent antigen presenting cells. Regarding the safety issue, Dr. Boyd does not believe that there is any risk of the RNA sequences being reverse transcribed and re-integrated into the genome.

Dr. Heidmann (Institut Gustave-Roussy, France) wrote that the possibility indeed exists that RNA molecules are reverse transcribed and integrated into the genome. His study demonstrated that in cultured human or murine fibroblasts, the measured frequency of these events are approximately 10^{-7} events per cell per generation. The frequency of re-integration is proportional to the ratio of "pulsed" RNA to total cellular RNA; the expression of the re-integrated cDNA is depend upon whether it is integrated within a chromosomal region that provides a promoter for its expression (with a probability lower than at 10^{-3}). Consequently, he estimated that the probability that a re-integrated cDNA from a pulsed RNA molecule to be finally expressed in some dendritic cells should be less than 10^{-9}. He stated that the proposed protocol should have much less chance of integration than any other protocols involving the direct transfer of the corresponding DNA including the retrovirus-mediated gene transfer. He suggested that it should be checked if the pulsed RNA molecules contain any cryptic promoter, such a cryptic promoter has been demonstrated in N-*myc* pseudogene. The presence of such promoter elements would significantly increase the likelihood of an expression of the re-integrated cDNA.

Other Comments

Responding to inquiries from Drs. Ross and Lysaught, Dr. Wolff summarized the reviews for the public members. He said that the first question is if the RNA transfer will lead to long-term expression of CEA and whether long-term expression has any harmful effect. The use of RNA is to provide a short term expression due to its instability within the cell. The second question is the possibility that transfected RNA could be reverse transcribed into DNA and integrated within the genome. The chance for such events is small but definitely exists. A cryptic promoter within the transfected RNA would increase the chance of its expression when integrated.

Dr. Aguilar-Cordova commented on the risk related to reverse transcription and integration of the transfected RNA. He considered this risk to be rather small compared with other CEA protocols utilizing retrovirus or plasmid DNA as vectors for gene transfer; these latter vectors have much more chance of integration within the genome. The CEA RNA transfer protocol is not novel in the sense that protocols using retroviral or plasmid vectors all express the CEA gene through transcription into a messenger RNA (mRNA) that encodes the CEA protein.

Dr. Glorioso stated that this protocol is one of the safest protocols that have been reviewed by the RAC. The use of retrovirus to mediate gene transfer poses much more risk of integration and expression. He found that there is scientific merit to use RNA transfer to dendritic cells to stimulate antitumor immune response. Simple experiments with dendritic cells can distinguish if the antitumor immune response induced by these antigen presenting cells is specific to CEA, e.g., as a control experiment using a CEA RNA construct with a stop codon to prevent its translation into an active CEA protein.

Dr. Saha said that the issue of whether the immune response is specific to CEA can be readily addressed by simple experiments, e.g., using a RNA molecule encoding the antisense of the CEA gene. The immunological assays are far more sensitive than biochemical assays for detecting the expression of

CEA protein in the "pulsed" cells. Even if the antitumor immune response is not induced specifically by CEA RNA, it is still a useful technique.

Ms. Rothenberg asked the investigators to explain from the patients perspective why this "novel" protocol would be perceived as a cure for his or her cancer.

Dr. Bernstein noted that introduction of a "naked" RNA to a cell to produce a protein product is a novel application; because of this degree of novelty, several questions related to this application should be discussed.

Dr. Lysaught understood that the safety issue is probably not significant for this protocol; the RAC discussion in a public forum of a novel application of gene transfer will serve the purpose of providing comfort for the public knowing that relevant issues have been raised and discussed.

As a point of clarification, Dr. Glorioso said that he did not imply that the protocol is a "cure" for cancer, but it would be an exciting scientific finding if immune response can be stimulated by the RNA transfer method.

Dr. Noguchi commented on the issue of "naked" RNA. A "naked" RNA of the polio virus is capable of infecting target cells.

Dr. Lysaught raised her concern about the informed consent process. In this case, emotional and psychological harms have been inflicted on the patients; the patients have been led to believe that the protocol is to cure their cancer.

Ms. Knorr noted that RAC discussion of a novel protocol may not necessarily have reflected concern about the safety issues of that protocol; holding the public discussion would give the RAC a chance to address concerns about potential uses of that application, e.g., using a RNA encoding an oncogene. Following the April 22, 1997, telephone conference call, Dr. Lyerly was allowed to treat the subjects already entered into the study because the RAC considered this protocol to be safe. The RAC requested to discuss further enrollment of additional subjects and other potential applications involving naked RNA at this meeting.

Investigators Response--Drs. Gilboa and Lyerly

Dr. Gilboa stated that he will give a brief overview of the scientific rationale of the clinical protocol and will address the safety concerns raised by the RAC.

Dr. Gilboa noted that the protocol will be conducted at the Center for Genetics and Cellular Therapies, Duke University Medical Center. The Center is devoted to translation research from discovery to clinical reality. The protocol is a collaborative effort between two laboratories. His own laboratory involved concept development and the developmental work of immune models for *in vitro* human systems; Dr. Lyerly's laboratory involved the translation of those concepts into a clinical setting.

The purpose of the protocol is to induce an immune response in cancer patients to eradicate the metastatic disease of an existing cancer and to provide a long-term immunological memory that could protect the patient from recurrence of the disease. Most of the patients will have prior treatments and are clinically healthy in a state of remission. Immunotherapy of cancer is based on the observation that most forms of human cancer are sufficiently distinct from normal tissue so that they can be recognized by the immune system. Most, if not all tumors express tumor rejection antigens. CD8+ T cells are best equipped

to recognize tumor cells and initiate the cascade of events leading to their destruction. It is important for gene therapy to use genetically modified tumor vaccines in which specific genes were introduced (e.g., interleukin-2 or granulocyte-macrophage colony stimulating factor) to enhance the immunogenecity *in vivo*. This strategy has been well validated in animal studies, but the results when translated in humans are disappointing. Against this background, Dr. Gilboa has developed an alternative strategy to use dendritic cells as tumor vaccines.

Dendritic cells occupy a central role in the immune system, i.e., to present antigen to naive T-cells in the lymphoid organs, and as such, to control the initiation of all T-cell dependent responses. For immunotherapy of cancer, dendritic cells were loaded with tumor antigens and were used as vaccines to stimulate a tumor specific T-cell response in the cancer patient.

One advantage of dendritic cells is that these cells can be generated from progenitor cells that are present in peripheral blood, and thus clinical trials are feasible. The protocol is a dose-escalating trial using induction of CTL as a biological endpoint. Most of the safety issues regarding preparation of clinical grade cell cultures have been developed with the advice from FDA. Testing of murine viruses will be considered as suggested by some RAC members.

Dr. Gilboa showed data from murine model studies that dendritic cells loaded with RNA isolated from B16/F10.9 tumor cells induce regression of metastases in mice and CEA specific CTL responses.

Dr. Gilboa showed a slide to indicate that there are a growing list of human tumor antigens being identified, several for melanoma and a few for other cancers. The main advantage of immunization with defined tumor antigens is that there is no need for tumor tissue from the patient. The disadvantages include limited use for cancers where candidate tumor antigens have been isolated, and there is no clear evidence that it mediates effective tumor rejection. Another approach is to use unfractionated tumor antigens. The advantage of immunization with unfractionated tumor antigens is its wide clinical applicability, but the disadvantages are low concentration and reduced efficacy, requirement of tumor tissue, and increased risk of autoimmunity.

Dr. Gilboa stated that their strategy to combine the advantages of the above mentioned approaches and to overcome the disadvantages is to develop an alternative approach of immunotherapy of cancer, i.e., dendritic cell-based vaccines using tumor antigens in the form of RNA. Dr. Gilboa provided three reprints of his animal studies showing that vaccination with dendritic cells loaded with unfractionated tumor materials in the form of peptides isolated from the tumors is effective in inducing tumor immunity, and he observed better responses than when using gene-modified tumor cells. However, the limitation is the difficulty of preparing antigens from each cancer patient. To overcome this limitation, Dr. Gilboa considered immunotherapy with the use of dendritic cells based vaccines using tumor antigen in the form of RNA. The transfected RNA will be translated into the protein products in the cell to induce T-cell mediated immunity. This hypothesis has been successfully tested in animal models. Dr. Gilboa showed animal data demonstrating that dendritic cells pulsed with RNA are potent antigen-presenting cells *in vitro* and *in vivo*.

To extend the animal studies to humans, Dr. Gilboa showed data on experiments using human dendritic cells. He observed induction of secondary and primary CTL responses *in vitro* using dendritic cells pulsed with peptides and RNA.

Responding to the question of CEA specific immune responses, Dr. Gilboa stated that extensive animal, and preclinical *in vitro* human cell studies, demonstrated that CEA specific immune responses are induced.

Responding to the question of "naked" RNA pulsing of dendritic cells, Dr. Gilboa stated that it is a unexpected observation that pulsing dendritic cells with CEA RNA alone, without a lipid complex, is effective in inducing CEA specific CTL responses. Biochemical evidence for entering the cell and translation of RNA within cells has not been obtained due to lack of a sensitive assay.

In summary, Dr. Gilboa stated that vaccination with RNA transfected dendritic cells is a very powerful means of cancer immunotherapy. The unique advantage of using the approach of RNA as opposed to the conventional approaches of using peptides or proteins is that it offers the opportunity of treating all cancer patients from whom right tumor antigens are not available. The RNA can be isolated from a single tumor cell, the process can be automated, and it is a cost effective means of treating cancer patients.

After finishing his scientific overview of the protocol, Dr. Gilboa addressed specific comments made by RAC members.

Responding to the question of equating transient expression with safety, Dr. Gilboa agreed thatsuch a statement is overstating the safety issue. There is no risk-free procedure for treating the patients. The question is the magnitude of the risk. He compared the risk of the protocol to others that have been approved by the RAC, and he concluded that the risk is minimal to neglible. He noted that the statements of the consultants are in keeping with his opinion about safety. He referenced Dr. Coffin's statement, "I think a general discussion of RNA based gene transfer protocol would be worthwhile, but I do not think that this rather innocuous proposal should be held up for this reason."

Dr. Gilboa used a slide illustration to explain the normal flow of genetic information from DNA, the gene, to RNA, and then to protein in the cell. The cell has approximately 100,000 species of mRNAs, and they are transiently present in the cell. The CEA RNA to be transfected into dendritic cells is identical to one of the mRNA species. The investigators are adding a small fraction of total mRNA to a small fraction of total body cells; the additional risk of the procedure is minimal. Dr. Gilboa noted that all gene transfer protocols involve transcription of the DNA into mRNA, and all would entail the same risk of integration and other concerns of direct transfer of a mRNA species. In summary, Dr. Gilboa stated that the safety concerns are minimal to negligible by comparison to other gene transfer protocols. The protocol is not novel in the sense that CEA RNA is similar to other natural mRNA.

Dr. Gilboa did not consider the RNA transfer protocol as a "gene" transfer protocol. It is erroneous to refer to the "naked" CEA RNA as a RNA vector. A vector or a vehicle in the context of gene transfer is a piece of DNA or RNA that would carry the genetic information to be persistence in target cells. The CEA RNA, which he is using in the protocol, is not a vector but rather a species of mRNA similar to a protein molecule used for other therapeutic purposes.

Dr. Mickelson called on Dr. Lyerly to address the clinical issues. Dr. Lyerly stated that Dr. Jeffrey Schlom of the National Cancer Institute, NIH, who has participated in the majority of CEA clinical trials, is present today and is available to answer any questions from the RAC.

As a point of clarification, Dr. Lyerly noted that the present protocol is emanated from his collaboration with Dr. Gilboa and it is not sponsored by industry. Schering Corporation only provides the cytokines used in the *in vitro* generation of the dendritic cells.

Dr. Lyerly presented with slide illustrations the rationale and the supporting data of the proposed protocol. His clinical trial group concentrate their study to develop broadly based platforms for immunotherapy involving T-cell mediated immune response. They have received close to 300 inquiries to enter the study,

and rejected most of the patients if there is any form of conventional therapy that might benefit the patients. They have a proactive patient advocacy group closely integrated within the clinical research unit. Dr. Lyerly pointed out that it is this open nature of the clinical trials that he voluntarily submitted his protocol for RAC review even though he initially considered the protocol not a gene transfer protocol.

Dr. Lyerly noted that although the usefulness of CEA in cancer treatment is not yet known, CEA has a long history of safety in human clinical use. With regard to the question of CEA being an oncogene, Dr. Lyerly stated that Dr. Schlom did several studies in which long-term high expressing cell lines were established, and these cells had never demonstrated any growth and metastatic characteristics of cancer cells. CEA has been studied as a possible tumor marker. The high serum level of CEA reflects a large tumor burden and poor prognosis for cancer patients.

Why choose the CEA as a tumor antigen to develop the dendritic cell immunotherapy? Dr. Lyerly stated that his rationale is to choose an antigen that is very common in a large number of cancer patients. Over half a million patients a year will develop cancers that express CEA, and thus provide a large body of patient population to select patients who have failed conventional therapies and would be eligible for the present protocol. In animal models, Dr. Schlom has demonstrated the proof of principle that T-cell directed immune response against CEA does provide protection against tumor progression and enhances survival benefit. Several ongoing clinical protocols based on the same principle use vaccinia virus, ALVAC vector, or plasmid to transduce the CEA gene for immunotherapy of cancers. No major CEA specific side effects have been observed in these clinical trials; however, immune reactions to the vector backbones have been detected. The compounding features of the immune response against the vectors have prompted the investigators to pursue the alternative strategy of using the CEA RNA transfected dendritic cells.

Dr. Lyerly stated that dendritic cells are isolated by a set of phenotypic markers and that strict criteria of sterility, fungus, microplasma, etc., are applied to their preparation. The functional analysis is not included in the lot release criteria due to lengthy time frames required for the analysis; the required *in vitro* stimulation of patient's autologous T-cell often takes 4 to 6 weeks to validate the results. In the 7 patients treated with dendritic cells, functional analysis was performed even if it is not defined as a lot release criterion.

Dr. Lyerly explained the reason that the lot release criteria are not included in the Informed Consent document is that not all patients will have successful dendritic cells prepared from them to undergo the present treatment; his IRB has advised him against inclusion of such sophisticated criteria in the Informed Consent document.

Dr. Lyerly presented data from a patient infused with dendritic cells pulsed with CEA peptides. From the data, T-cell response that was specific for CEA expressing human leukocyte antigen (HLA)-A2 positive cells was observed. A stringent assessment of immune response to the vaccination strategy has been developed.

Dr. Lyerly stated that the protocol involving CEA-peptide pulsed dendritic cells has a restriction on the entrance criteria, i.e., only patients with HLA-A2 haplotype are eligible. One advantage of the CEA-RNA protocol is that all patients with CEA-expressing tumors will be eligible regardless of their HLA-A2 status; most caucasians are HLA-A2 positive, but the ethnic minority populations are often HLA-A2 negative.

The RNA-pulsed dendritic cell approach has many advantages including its wide applicability in the future, ability to modify the RNA for its stability and trafficking pattern in the cell, and to encode multiple antigens.

As a point of clarification, Dr. Lyerly stated that the present protocol is not a therapeutic trial in the sense that therapy is not the primary endpoint of the study. However, the tumor burden will be studied by the radiographic method and by observing biochemical evidence of serum CEA levels.

Dr. Lyerly used slide illustration to explain the eligibility criteria and the study schema. With regard to the lot release criteria of RNA preparations used to pulse the dendritic cells, Dr. Lyerly said that if the RNA shows a single band on Northern blot analysis, active *in vitro* translation into CEA protein, and capability to induce CEA-specific CTL response, the preparation will be eligible to be used for patient's dendritic cells. The assay to monitor for antinucleotide antibody response is being developed.

Other Comments

Dr. Saha stated that he is satisfied with the murine data demonstrating the CEA-specific immune response. He inquired if the CTL response could be due to the contaminating CEA DNA rather than to RNA *per se*, and why "naked" RNA is more effective than RNA/lipid complex in inducing the response. Dr. Gilboa responded that the CTL response was specifically blocked by the antisense RNA oligonucleotide directed to the target RNA sequences. He did not compare CTL induction plus or minus lipid in the immature dendritic cell experiments; his current laboratory work is directed toward elucidating the mechanism of RNA uptake in this cell system.

Dr. Saha asked why there is HLA-A2 restriction in the peptide-pulsing protocol. Dr. Lyerly responded that the peptide is a HLA-A2 binding peptide; the alternative of using the whole CEA protein for this purpose is less successful. Pulsing with CEA RNA circumvents this problem.

Dr. Wolff emphasized that he considers this protocol to be safe; but due to the novelty of the protocol, it is necessary for the RAC as a watchdog to publicly raise the concerns and those concerns must be addressed by the investigators. With regard to the question of whether the protocol should be considered as a gene therapy protocol and whether the RNA is indeed a vector or a vehicle, Dr. Wolff said that RNA as distinct from protein contains genetic information and is capable of reverse transcription and recombination. For this reason, RNA transfer protocol should be considered as a gene transfer protocol. Dr. Gilboa agreed to the statement; however, he does not consider the RNA protocol poses any more risks than protocols associated using other gene transfer procedures. Ms. Knorr clarified that the *NIH Guidelines* define human gene transfer research proposals as studies involving the deliberate transfer of recombinant DNA or DNA or RNA derived from recombinant DNA into human subjects.

Dr. Wolff stated that the lot release criteria of the RNA preparations should be more rigorously defined than just demonstrating a single band on Northern blot analysis or staining the polyacrylamide gel. The contaminating RNA or DNA should be ruled out. Dr. Gilboa would accept any RAC advice on how best to characterize the RNA. He stated that he was careful to eliminate DNA contamination by treating the sample with DNase and to minimize any chance of contamination from bacterial RNA by using highly purified enzymes in the RNA synthesis mixtures. Dr. Wolff was concerned that there is no clear lot release criteria and procedures for validation of the products. Dr. Saha suggested bacterial transformation as a sensitive means to assess DNA contamination. Dr. Gilboa agreed.

Dr. Wolff inquired if any viral testings will be performed to assess the viral contaminants in the dendritic cell cultures. Dr. Lyerly responded no. Dr. Wolff was concerned that monoclonal antibodies of murine origin used in the cell cultures might have murine virus contamination. Dr. Lyerly responded that the murine monoclonal antibodies are clinical grade products that are FDA-approved for human use. With regard to the serum used for cryopreservation of dendritic cells, Dr. Lyerly said that patient's own plasma will be used for the dendritic cells. With regard to the magnetic columns used for cell isolation, Dr. Lyerly

said that all the equipment is commercial products approved by FDA for human use.

Dr. Wolff inquired if the investigators plan to inject the RNA-transfected dendritic cells into the nude mice to assess the neoplastic potential. Dr. Lyerly responded that dendritic cells do not survive *in vitro* long enough to permit this type of experiment. Dr.Schlom added that many preclinical studies have been conducted in nude mice with colon carcinoma cells transfected with CEA; no increase in growth rate was observed. In terms of transformation potential of CEA, Dr.Schlom stated that there are hundred of reports in the last 20 years concluding that CEA is not a prognostic indicator of any transformation potential in humans. Dr. Wolff asked if such a study has been performed with dendritic cells. Dr. Gilboa responded that in dendritic cells cultured *in vitro*, he has never been able to established any immortalized cell line from these cell cultures or to observe any cell growth in semi-solid agar medium which indicates potential transformation.

Dr. Wolff inquired if any sensitive assay, e.g., luciferase reporter gene, has been used to detect CEA protein expression in RNA-transfected dendritic cells. Dr. Gilboa responded that using other reporter genes, he was unable to detect the CEA protein.

Dr. Wolff asked why there is a low level of non-specific CTL responses observed in the murine experiments. Dr. Gilboa responded that he has no explanation for the phenomenon.

Dr. Ross expressed her appreciation of the informative presentation made by Drs.Gilboa and Lyerly. Gene transfer with "naked" RNA is a novel approach, and the discussion served to reassure the public about the safety and the promising approach that has potential to benefit the patients in the future.

Dr. Bernstein asked if any transgenic mouse model has been established for CEA expression. Dr. Schlom responded that two transgenic mice have been developed for CEA and none have developed more tumors than wild-type mice.

Dr. Bernstein stated that the RNA transcribed *in vitro* is very similar to mRNA, but it is not the same as the natural mRNA. Dr. Gilboa agreed and stated that the subtle difference does not affect its persistence in the cell.

Dr. McIvor asked if the investigators have performed experiments to see if any RNA is reverse transcribed and integrated within the cellular genome. Dr. Gilboa responded that such experiments have not been conducted. The frequency for such events would expectedly be very low and beyond detection by the most sensitive assays, e.g., polymerase chain reaction (PCR).

Dr. McIvor noted that the protocol describing Northern blot analysis of RNA is incorrect, and that only ethidinium bromide stained gel will be examined. Dr. Gilboa agreed. With regard to Dr. McIvor's question of the capping step in the enzymatic synthesis of RNA, Dr.Gilboa said that the RNA synthesis mixture contains excessive GTP analog and the RNA synthesized will be automatically capped. There is no need for an additional capping step. Regarding the question of how long the RNA persists in the pulsed cell culture and whether it actually enters the cells, Dr.Gilboa said that there is no direct evidence for the occurrence of these events. The presence of CEA protein within transfected cells is inferred from the observation of CEA-specific CTL responses induced by the transfected cells.

Dr. Mickelson stated that most of her concerns have been addressed by the investigators.

Ms. Rothenberg stated that Dr. Coffin as well as other RAC members were concerned about the political pressure and misunderstanding in the part of the patients and their families that the study represents a

cancer cure. Ms. Rothenberg asked the investigators to respond to this concern for the record. She understood that there were 2 patients already entered the trial since the RAC telephone conference on April 22, 1997. Dr. Lyerly clarified that 3 patients had been enrolled into the study before he received the written letter from the RAC. Dr.Lysaught asked the investigators to explain the informed consent process as to why the patients were led to believe that theprotocolwas their last chance at a cure of their cancers, and their lives might be in danger by waiting 2 more months until RAC discussion.

Dr. Lyerly responded that the people responsible for enrolling the patients are not administratively in his own department. There are more than 300 inquiries for this trial and most of them have been rejected if there are alternative conventional therapies. The issue is a generic one that is common to most clinical trials. Why did patients conceive this trial as therapeutic? Dr. McGraw offered a possible psychological explanation from the patient's perspective. The patients may be given a proper Informed Consent document stating that the protocol is a research and safety study. After a great deal of effort is made to participate in the protocol and with no other hope for their cancer, terminally ill subjects may have a tendency to distort reality to fit what they want to believe. Drs.Lysaught and Wolff commented that it takes more than this normal psychological reaction to prompt the patients and their families to take the political actions, i.e., writing letters to the U.S. President and members of Congress. Ms. Rothenberg asked why this case became so political. Dr. Ross asked why the patients believed that the RAC was delaying their participation in the trial. Dr. Lyerly responded that the unusual circumstances the patients first being told that the protocol had received FDA approval, and then later being informed that it was waiting for RAC discussion. This delay might have prompted the patient's families to take political actions in order to start the treatment. Ms. Rothenberg said she appreciated Dr.Lyerly's explanation regarding the unusual scenario of the events. Dr. Lyerly noted that the patients and their families took the political actions without his prior knowledge. He said he consulted with his IRB to see if the patients had been properly informed that there will be no expected therapeutic benefit from the protocol. Dr.Lyerly emphasized that he will always maintain compliance with the *NIH Guidelines* and the recommendations of the RAC. Ms. Rothenberg thanked Dr. Lyerly for his explanation.

Dr. Ross stated that the issue may be not so generic. The response of the patients in this case may be specific, because the strategy of the protocol may appear to be compelling the patients to believe they might receive therapeutic benefits from this trial. Dr.Lyerly agreed, but the scenario of the patient being told "yes" then "no" in participating in the trial may have aggravated the situation. Dr.Lysaught said that the use of the term "treatment" in the Informed Consent document might have conveyed a mixed message to the patients. Dr. Lyerly noted that patients with a disease that is completely dominating their life sometimes feel a loss of control and are willing to undergo some form of treatment that may have a devastating impact on them.

Dr. Ross was concerned that the RAC will no longer review the Informed Consent document on a case-by-case basis. She noted that the Informed Consent document of thecubital tunnel syndrome protocol (#9703-180) referred to the gene therapy vector as a "drug" instead of a recombinant DNA agent; the patients might be led to believe it is a mainstream medical treatment. Ms.Knorr noted that Dr. Varmus, the NIH Director, does not favor the RAC reviewing Informed Consent documents on a case-by-case basis. She said she is proposing to have the RAC play a proactive role in educating the gene therapy community on proper informed consent processes for gene transfer trials.

Dr. McIvor stated that the unfortunate events surrounding this protocol are due to unusual circumstances, and a similar event is unlikely to occur in the future.

Dr. Aguilar-Cordova noted that there was a letter written by a patient to a U.S. Senator stating "...DrLyerly is caught in the middle of what appears to be a political power struggle and that he (Dr.Lyerly) clearly

continues to feel strongly that continued treatment would be appropriateandnecessary...." Dr. Aguilar-Cordova stated that the patient's perception may be stronger than Dr.Lyerly's understanding of their feelings.

Dr. Aguilar-Cordova asked the investigators to clarify whether the CTL immune response they observed is due to the induction of interferon. Dr.Gilboa responded that the CEA-specific CTL response could not be due to the non-specific effect of interferon. Dr.Lyerly stated that indirect experimental evidence on the time course of induction suggests the effect was not mediated by interferon.

Dr. Noguchi stated that FDA defines gene therapy vectors as biologics and drugs. He said that FDA gets similar political pressure from patients, and this issue is generic. The patients need someone to talk to if they are denied treatment. Dr.Lysaught noted that the patient's letter suggested that the patient had discussed the issue with the investigators. Dr. Lyerly denied that he had prior knowledge about the written letters to the President and members of Congress. Dr. Noguchi agreed that the patients could have taken the political actions without consulting with the investigators.

Dr. Anderson stated that he agrees with Dr. Noguchi's comment on referring to gene therapy vectors as drugs. Dr. Anderson said that the RAC has contributed enormously in the past by reviewing the Informed Consent documents and formulating the specific guidelines for gene transfer protocols, and now is the time for the RAC to move on. Ms.Knorr noted that the RAC can still provide general educational advice about the Informed Consent documents.

Dr. Mickelson invited comments from members of the public in the audience.

Dr. Allgood (GeneMedicine, Inc.) said that her company sponsored thecubital tunnel syndrome protocol that refers the gene transfer vector as a drug. The protocol is written in keeping with the FDA submission definition of biologics and drugs. Dr. Allgood mentioned that Appendix M of the *NIH Guidelines* use the terms "treatment" and "gene therapy" as well; and that she considers it a semantic issue.

Dr. McIvor made a motion to endorse continued enrollment of patients on the protocol. Dr. Aguilar-Cordova seconded the motion.

Dr. Saha inquired if the RAC has resolved the issue of whether RNA transfer protocols are under the RAC purview. Dr. Mickelson responded that RNA transfer protocols are considered as gene transfer studies. Dr. Saha asked if synthetic oligonucleotides are included in this definition. Dr. McIvor clarified that oligonucleotides are not recombinant DNA or DNA or RNA derived from recombinant DNA and are not included in the gene transfer definition.

Dr. McIvor stated that the RAC has raised concerns and the investigators have responded to those issues There is very low risk associated with the RNA transfer procedure, and the preclinical studies are encouraging. For these reasons, he made the motion to allow the protocol to continue enrolling patients.

Committee Motion

A motion was made by Dr. McIvor and seconded by Dr. Aguilar-Cordova to endorse continued enrollment of patients on the protocol submitted by Dr. H. KimLyerly of Duke University Medical Center, Durham, North Carolina. The motion passed by a vote of 9 in favor, 0opposed,and 1 abstention.

Note: The RAC held a telephone conference call on April 22, 1997 to resolve issues surrounding premature enrollment of subjects on this protocol. The RAC sent a letter to Dr.Varmus recommending that

Dr. Lyerly be allowed to treat subjects already entered into the study. The RAC requested to discuss further enrollment of additional subjects and other potential applications involving naked RNA at this meeting.

Protocol Summary

Dr. H. Kim Lyerly, Duke University Medical Center, Durham, North Carolina, may conduct a gene transfer experiment involving RNA-pulsed dendritic cells on 18 patients, _ 18 years, with metastatic adenocarcinoma expressing CEA. Autologous peripheral blood precursor-derived dendritic cells will be pulsed by co-incubation with RNA encoding CEA. The CEA RNA is transcribed from the cloned plasmid DNA, pGEM:CEA. The CEA RNA-pulsed autologous cultured dendritic cells will be infused in patients with metastatic malignancies. Because dendritic cells play a pivotal role in initiating T cell responses *in vivo*, the investigator proposes to explore the use of dendritic cells to initiate CEA-specific antitumor responses. The primary objective of the study is to determine the safety and dose limiting toxicity of intravenous injections of autologous, cultured, dendritic cells pulsed with CEA RNA. The secondary objectives are: (1) evaluation of cellular immune response to the CEA protein, and (2) evaluation of clinical and biochemical responses to the treatment and the duration of such responses.

V. APPRECIATION OF RETIRING MEMBERS

Dr. Mickelson presented plaques and letters of appreciation for members completing their terms of service to the RAC. These retiring members are Drs. Saha, Ross, McGraw, Glorioso (not present at the meeting), and Samulski (not present at the meeting). Dr. Noguchi thanked the retiring RAC members who, in a forthright and civilized manner, have contributed to the public discussion of gene therapy issues that immensely helped FDA in its credibility.

VI. DISCUSSION ON GENETIC VACCINES AGAINST CANCER-RELATED ANTIGENS AND ONCOGENE PROTEINS/WOLFF

Dr. Mickelson called on Dr. Wolff to lead the discussion on genetic vaccines against cancer-related antigens and oncogene proteins. As a result of the submission and discussion of Dr. Lyerly's protocol involving the use of an *in vitro* RNA transcript encoding carcinoembryonic antigen for the immunization of cancer patients, Dr. Wolff stated that the RAC should anticipate submission of protocols involving oncogenes, such as a mutated p53 tumor suppressor gene or a *ras* oncogene, along the same line of approach. Dr. Lyerly' protocol uses a cancer-related antigen to elicit an antitumor immune response. Dr. Wolff anticipated a similar approach being used to devise cancer vaccination with a mutated oncogene that has a potential for causing a second cancer. A genetic vaccine using a virus vector or naked DNA or RNA to deliver the oncogene proteins within the cell is likely to stimulate the cell-mediated immune response to tumors. Gene transfer may be performed by *ex vivo* transduction of target cells and injection of those cells to patients. Alternatively by an *in vivo* approach, the vaccines may be directly injected into patients, e.g., injection of a DNA vaccine into muscle or into skin by a gene gun. These vaccinations will be first used therapeutically for cancer patients; but eventually they may be considered as a prophylactic vaccine available to the general population to prevent cancer. Several questions should be asked by the RAC: What is the potential risk of transient expression? What is the potential for integration? Would such applications be acceptable for prophylactic use? Are there any animal models to evaluate these risks?

Dr. Wolff noted a written review by Dr. Robert A. Weinberg, Ph.D. (Whitehead Institute, Massachusetts Institute of Technology, Cambridge, Massachusetts). Dr. Weinberg responded to several issues: (1) The introduction of RNA into patients should not under any realistic conditions pose any long-term threats to the health of the patient in terms of permanently altering the genotype or phenotype of a patient's cell. (2)

The introduction of DNA and associated genes into patients, in contrast, poses the very real possibility if not probability of integrating the DNA within the patient's cell genome. (3) The risk associated with introducing oncogenes into patients should be considered on a case-by-case basis in which the identity of a gene and its known/proposed cell physiologic functions is taken into account, e.g., activated *ras* oncogene would be a concern. (4) Dr. Weinberg anticipates that the protocols in the short run would be used for treatment of patients with diagnosed tumors, but he considers the prospects for preventing cance are very remote at present.

Other Comments

Dr. Noguchi noted two issues to be addressed: (1) the gene therapy itself might be tumorigenic, and (2) animal models to evaluate the safety issues. The proposal is a double-edged sword and very few animal models are available to evaluate these issues.

Drs. Saha, Ross, and Aguilar-Cordova noted that the RAC discussion of the adenovirus E1A as a potential oncogene for gene therapy of cancer is related to the present topics (Protocol 9512-137). In this study, the E1A is being used as a tumor suppressor gene for patients with metastatic breast or ovarian caner that overexpresses HER-2/neu oncogene. In addition the oncogenic potential of a mutated p53 tumor suppressor gene has been discussed in Protocols 9403-031 and 9406-079 for the treatment of patients with non-small cell lung cancer.

Dr. Wolff stated that the RAC should develop the safety criteria for trials using a potential oncogene. Dr. Aguilar-Cordova stated that the evaluation of the safety tests should be reviewed on a case-by-case basis when such protocols are submitted to the RAC; because it will be difficult for the RAC to address globally these safety issues before protocol submission. Drs. Lysaught and Saha agreed that case-by-case review will be useful in addressing specific oncogene issues. Any of these safety issues can trigger full RAC review of the protocols. Dr. Aguilar-Cordova noted that if more than three RAC members noticed such a problem, full RAC review will occur. Ms. Knorr noted that this issue of novel and non-novel protocol categories may be discussed in tomorrow's session.

Dr. Wolff was concerned about the potential spread to the general population of the viral vectors used for gene therapy of cancer that express oncogenes. Unlike patients in a controlled research laboratory environment, the patients in the general population have potential to spread these agents to other persons. Drs. Aguilar-Cordova and Lysaught agreed that such a safety issue will be reviewed by the RAC when such protocols are submitted. Dr. Mickelson noted that the safety issue of amphotropic retrovirus or adenovirus expressing an oncogene will be discussed by the IBC which reviews the protocol.

The consensus of RAC discussion on the safety issues of genetic vaccines against cancer-related antigens and oncogene proteins was that any such protocols would be assessed based on risk versus benefit on a case-by-case basis.

VII. DISCUSSION ON TRANSGENICS/SAHA

Dr. Saha stated that Section III-C-4, *Experiments Involving Whole Animals*, of the *NIH Guidelines* stipulates that all transgenic animal experiments are subject to IBC approval before initiation. Responding to a letter dated April 22, 1997, from an IBC representative, Dr. George Gutman of the University of California at Irvine, California, the RAC discussed the possibility of changing this requirement to initiation of the experiments simultaneous with IBC notification. In order to dealing with an increasing number of proposals involving the production or use of transgenic mice, almost all of which would be approved with Biosafety Level 1 (BL1) physical containment, Dr. Gutman suggested to allow such experiments to be

initiated simultaneous with IBC notification, provided they do not have any factors requiring more careful attention, e.g., toxic molecules or infectious agents.

Dr. Saha agreed that the transgenic mice have been increasingly used in numerous experiments (including purchase and use of such mice), e.g., by himself and in his institutions without knowing that such experiments require IBC approval under the current *NIH Guidelines.* Dr. Saha noted that there are numerous transgenic mice available for investigational use; they are simply being treated as another mouse strain by their providers, e.g., Jackson Laboratory in Maine. Dr.Saha stated that two issues need to be addressed: (1) production versus purchase and use, and (2) separate consideration of laboratory rodents versus larger livestocks, e.g., sheep and cattle. The production and use of transgenic livestock is not a simple issue of research purpose, because it involves additional issues surrounding the production of food and pharmaceuticals. Dr. Saha suggested separating these different categories of transgenic animal experiments in different sections of the *NIH Guidelines* that require different levels of oversight, i.e., transgenic mice vs. transgenic animals in general, and generation or production versus purchase or use of such animals.

Ms. Knorr noted that the issue of transgenic animals reflects in general that the *NIH Guidelines* need extensive revisions to update their usefulness. In terms of transgenic mice experiments, they can be moved from Section III-C for experiments that require IBC approval to Section III-D for experiments that need IBC notification simultaneous with initiation. Dr. Saha stated that the amendment should include only the transgenic mice that require BL1 containment; transgenic mice that produce toxin or infectious agents requiring higher levels of containment should not be included in this reclassification. Ms. Knorr pointed out that the issue of generation versus purchase or use should be addressed. After RAC discussion of these issues, proper languages of amendments to the *NIH Guidelines* will be published in the *Federal Register* in order to vote on this amendment at the next RAC meeting.

Dr. Mickelson noted that purchase and use is a separate issue from conducting recombinant DNA experiments to generate the transgenic animals.

Dr. Aguilar-Cordova made a motion to remove the generation of transgenic mouse experiments that require BL1 containment from Section III-C to Section III-D, i.e., experiments requiring IBC notification simultaneous with initiation. Dr. Markert made a friendly amendment to exempt all such experiments from the *NIH Guidelines.* Dr. Wolff was concerned about the public safety of transgenic experiments, e.g., adding a receptor molecule for an infectious agent. Ms.Knorr and Dr. McIvor suggested to keep the generation of transgenic mouse experiments under Section III-D, and the purchase and use of such mice under Section III-E, *Exempt Experiments.* Dr. Markert accepted the friendly amendment to her amendment.

Dr. Mickelson said that in the long-term, the RAC should form a subcommittee to revise the entire *NIH Guidelines*. Dr. Ross agreed. Dr. Leinwand stated that the long-term goal of overhauling the *NIH Guidelines* should not preclude the current amendment dealing with transgenic mice.

Dr. Aguilar-Cordova restated his motion: (1) The generation of transgenic mice at the BL1 containment can be initiated simultaneous with IBC notification, and (2) the purchase and use of transgenic mice should be exempt from the *NIH Guidelines.* Dr. Ross suggested that all rodents including rats should be included. Dr. McIvor said that transgenic rodents include animals generated by the gene knock out experiments. Dr. Saha stated that he supported the motion; the IBC needs to determine if the experiment is a BL1 experiment and is automatically to have an oversight of the transgenic experiments.

Dr. Mickelson said that the motion would propose changes to be published in the *Federal Register.*

Therefore, at the next RAC meeting, the RAC may vote to approve this amendment to the *NIH Guidelines*.

Dr. Diane Fleming noted that there is a book entitled, *Occupational Health and Safety in the Care and Use of Research Animals* published by the National Research Council of the National Academy of Sciences on the subject of animal care and animal handlers, and it has information about various committees and agencies having the oversight responsibilities of experimental animals. To order this book, call 1-800-624-6242 or 202-334-3313, or via the internet at http://www.nap.edu/bookstore.

Committee Motion

A motion was made by Dr. Aguilar-Cordova and seconded by Dr. Leinwand to propose an amendment to the *NIH Guidelines* for publication in the *Federal Register* for consideration at the September 1997 RAC meeting. The proposed action would allow: (1) the generation of transgenic rodents that require Biosafety Level 1 containment to be included under Section III-D, *Experiments that Require IBC Notice Simultaneous with Initiation*; and (2) the purchase and use of transgenic rodents should be exempt from the *NIH Guidelines*. The motion passed by a vote of 9 in favor, 0 opposed, and no abstentions.

This recommendation will be published in the *Federal Register* for public comment and voted on at the September 1997 RAC meeting.

VIII. PRESENTATION REGARDING DEFINITION OF STANDARDS FOR VIRAL VECTOR QUANTIFICATION/AGUILAR-CORDOVA

Presentation -- Dr. Aguilar-Cordova

Dr. Aguilar-Cordova made a presentation of standards for characterization of viral vectors for human studies in terms of purity and strength. He focused his discussion of standardization of adenoviral vector quantification. A commonly accepted standard is required for meaningful comparison of data obtained from different clinical trials.

Characterization of viral vectors involves two aspects. (1) Purity. The vectors should be free from contamination by adventitious agents including replication-competent viruses (RCV). (2) Strength. The data should show active concentration for toxicity and efficacy. This information is critical when conducting a dose escalation study. Characterization of viral vectors is significant for comparison between studies in terms of dose-related efficacy and toxicity.

Dr. Aguilar-Cordova focused his discussion of standardization of adenoviral vector quantification. Similar standardization is applicable to other virus vectors, e.g., retroviruses,adeno-associated virus, etc. The major factors for detection of adenovirus include virus particle numbers and infectious units. Most of the gene therapy protocols use the infectious units. Dr. Aguilar-Cordova said that infectious units is a qualitative measure rather than a quantitative measure of vector strength.

Dr. Aguilar-Cordova showed a slide to illustrate a typical setup to determine the virus titer. The setup involves a tissue culture dish, tissue culture media containing virus dilution, and the target cells to assay for the virus titer.

Adenovirus particles can be very quantitatively determined by a physical method of reading the ultraviolet absorption at the 260 nm wavelength with a spectrophotometer. One OD_{260} unit equals to 1.1×10^{12} virus particles. This number multiplied by the dilution factor is the virus particle number.

The infectious unit is a biological assay of the virus strength. It is subject to many biological factors affecting the assays, such as compatibility of virus and target cells in terms of receptors for the virus, viability of virus and target cells, and the product effect of the insert genes. The same batch of virus preparation when assayed in different laboratories showed that the infectious units could vary as much as 100 times.

The infectious unit is subject to many physical factors affecting the assays. These physical factors are distance between virion and target cells, concentration of virus and target cells, and duration of the assays. All these physical factors affect the probability of a virus colliding with a target cell. Collision of a virus particle with its target cell is subject to factors such as Brownian motion of the particle, the concentration gradient, and other external forces. The Fick's laws of diffusion defines the rate of diffusion. A typical titer setup involves a mixture of vectors in liquid medium, which is placed on top of the target cells. After a period of incubation, one counts the number of virions by counting how many foci or patches of abnormal cells caused by the biological effects of the virions on the target cells. The virions are limited by how they move around in the liquid medium by Brownian motion, gravitational force, and concentration gradient.

There is a mathematical formula to describe a single hit detection in static conditions. The validity of the mathematical prediction was tested with virus titer experiments in Dr. Aguilar-Cordova's laboratory. A significant variability existed depending on the actual assay conditions, e.g., the volume of media, external centrifugal forces, etc. Dr. Aguilar-Cordova improved the prediction validity of the mathematical formula to describe a single or multiple hit detection with virion displacement. The multiple hit equation (referred to as N.A.S. titer) gave the best calculation of the virus titer.

In summary, Dr. Aguilar-Cordova said that infectious units are very variable measures of the virus strength. Unfortunately, they have been used in many protocols to describe the vector dose.

Dr. Aguilar-Cordova stated three conclusions: (1) hydro-physical effects of these biological assays can be mathematically modeled, (2) infectious units closely approximate virus particle units (determined by OD_{260}), and (3) infectious units are qualitative, whereas, virus particle units are a quantitative measurement of the virus dose. Dr. Aguilar-Cordova noted that there are many protocols using different measures for describing the virus dose, and it is important to keep in mind the variability of virus units in order to meaningfully interpret the data from different clinical trials.

Other Comments

Dr. Wolff asked if the protein components of virus particles would affect the measurement of virus particles by ultraviolet absorption at 260 nm, e.g., contamination by incomplete particles lacking the DNA component. Dr. Aguilar-Cordova responded if a virus preparation is purified by cesium chloride gradient centrifugation, most of the incomplete particles would be eliminated. OD_{260} measures mostly the ultraviolet absorption of the DNA component.

Dr. Aguilar-Cordova used a slide illustration to describe the schema of quality control tests of an adenovirus vector preparation. These assays include general sterility, microplasma, tumorigenity, adventitious viruses, karyology, and electron microscopy.

Dr. Saha asked if the centrifugation step is important in the virus titer assay. Dr. Aguilar-Cordova responded that if one uses his N.A.S. titer formula to calculate the hydrophysical conditions of the assay, centrifugation effect or the volume effect has minimum contribution to the vector titer. Dr. Noguchi

remarked that FDA would welcome public discussion on how best to quantify virus titers for interpretation of data obtained from different clinical trials. OD_{260} is a good measurement of adenovirus quantitation, but the situations of other virus systems are more complex. Dr. Aguilar-Cordova agreed that physical assays such as OD_{260} cannot be readily applied to retroviruses; furthermore, these viruses are biologically more fragile.

IX. DISCUSSION ON FLOW OF INFORMATION BETWEEN RAC MEMBERS/McIVOR

At the March 1997 RAC meeting, Dr. McIvor asked whether it would be preferable to have a few RAC members read the whole submission package and make a determination whether that protocol would require RAC review, or alternatively, have all RAC members review only the summary information of every protocol provided by ORDA to make such a determination. Since the March 1997 meeting, Dr. McIvor found that the present practice of having all RAC members review only the summary information of each protocol is working well, and he would recommend continuation of the current practice of identifying protocols that require full RAC review.

In the current practice, RAC members' comments regarding the necessity of RAC review of a given protocol is immediately circulated among all RAC members before the final tally of RAC votes are counted at the end of the 15 working-day period. Dr. McIvor was concerned that individual RAC member may be easily persuaded by the recommendations of other RAC members regarding the necessity for full RAC review; therefore, he suggested that initial recommendations about necessity for full RAC review should not be circulated.

Dr. Lysaught stated such a feedback process is very helpful to nonscientific members of the RAC with regard to clarification of risks and safety issues of a protocol. She used the Lyerly protocol as an example to make her point. She initially did not realize the novel aspect of the study until it was pointed out by the feedback from a comment made by another RAC member, and she changed her vote afterwards.

Dr. Markert agreed with Dr. Lysaught that she would consider other RAC member's opinion before making her final vote on a given protocol. She suggested that ORDA should e-mail as opposed to facsimile transmission to circulate comments and recommendations in order to expedite the review process.

Dr. Saha emphasized that each RAC member's initial opinion should be made independently based on the individual's own judgment of the protocol. However, the final vote could be taken after comments of other RAC members have been circulated.

Dr. McIvor noted that the rationale for requiring a full RAC review of a protocol is whether the protocol represents a novel approach, e.g., RNA transfer procedure of Lyerly protocol. He considered the criterion is not just the issue of whether the protocol is safe. The summary sheet provides adequate information for making such a determination. Dr. Lysaught said that the safety issue is equally important; another p53 protocol is not novel but the safety issue of potential oncogenicity of mutant p53 still poses a safety concern.

Dr. Aguilar-Cordova noted that a different opinion of each RAC member is valuable; RAC members are capable of making their own decision of whether to change their votes; the final decision is a committee decision. Dr. Wolff noted that the feedback is useful for a committee to reach a consensus. Ms. Knorr noted that the current practice of circulating RAC comments stemmed from a discussion at the December 1996 RAC meeting. At that meeting, Dr. Straus, a former RAC member, recommended immediate circulation of the comments requesting RAC review to all members. Dr. Mickelson said that she found

reading other members' comments very helpful. Dr. Noguchi noted that the process is similar to FDA review. Each RAC member represents his or her own expertise; therefore, the initial opinion from each RAC member is important. The key is to have all members make a statement first, and then they could change their mind after reading other members' comments. Dr. Saha agreed with Dr. Noguchi.

Several RAC members used the example of the Lyerly protocol to illustrate how each individual reached a final decision on whether the protocol would require RAC review. A question was raised whether the comments should be circulated after ORDA has received all the initial comments, or whether the comments should be circulated as soon as they are received by ORDA. Due to the time constraint of 15 working days, such a two-step process would be impractical. Dr. Aguilar-Cordova said that if a RAC member does not want to be influenced by other member's opinion, he or she can just ignore the comments circulated by ORDA.

Dr. Wolff said that the RAC should consider the issue of what is considered a novel protocol that would require RAC review.

Ms. Knorr noted that to send RAC comments by e-mail will expedite the review process in the future. ORDA will develop a format of the summary sheet that could be sent out by e-mail. Dr. Mickelson noted a RAC consensus of the discussion is to circulate comments and recommendations by e-mail in order to expedite the review process.

After completing discussion on flow of information between RAC members, Dr. Markert stated that she is considering to bring up the topic of decoupling RAC submission from FDA submission in order to allow investigators to submit their protocols to the RAC at an earlier stage of protocol development. Ms Knorr noted that Dr. Andra Miller of the FDA has made similar suggestions, and that discussion will take place later on in the meeting during the session on streamlined submission. The current *NIH Guidelines* require investigators to submit their protocols simultaneously to FDA and ORDA under the consolidated NIH/FDA review procedure.

X. DISCUSSION OF CRITERIA FOR REVIEW OF NOVEL PROTOCOLS AND A FRAMEWORK FOR RAC DISCUSSION/ KNORR

Dr. Mickelson called on Ms. Knorr to present a proposal outlining major revisions to Appendix M, *The Points to Consider in the Design and Submission of Protocols for the Transfer of Recombinant DNA Molecules into the Genome of One or More Human Subjects (Points to Consider)*. The proposal is the first attempt to revise the *Points to Consider* and to streamline the submissions in harmony with Investigational New Drug (IND) submission to FDA. Dr. Mickelson welcomed RAC comments regarding the proposal.

Presentation -- Ms. Knorr

Ms. Knorr emphasized that her presentation is to serve as a place to start the RAC discussion of the topic. There are three agenda items to be discussed: (1) Criteria for review of novel protocols and the framework for RAC discussion. The goal is to define categories of protocols based on degree of "novelty" or potential risk, e.g., novel, non-novel, and exempt. (2) Streamlined submission format for NIH and FDA submission. The goal is to define submission requirements for each specific "category" of protocols. The definition may be based on the degree of "novelty." (3) Defining the protocols to be exempt from submission under the *NIH Guidelines.* For discussion of novel protocols, the goal is define the framework or parameters of RAC discussion of such protocols.

With regard to criteria for review of novel protocols, the RAC should first define the three categories of

protocols that require different submission and reporting requirements. These are: (1) novel protocols that require ORDA submission, RAC discussion and recommendations, and the standard reporting requirements; (2) non-novel protocols that require ORDA submission and the standard reporting requirements; and (3) exempt protocols that require only the adverse event reporting.

The first issue for RAC discussion is how to define the criteria for determining a "novel" protocol. Ms. Knorr proposed two models for the definition: (1) Define what is "novel." This is an *exclusive* definition that would result in a discussion of only those protocols captured by the definition, all other protocols would be excluded. (2) Define what is "non-novel." This is an *inclusive* definition that would result in a discussion or evaluation of the need for a discussion of all protocols that fall outside a defined category of "non-novel" protocols.

Ms. Knorr outlined the possible criteria for defining novel protocols. These criteria include new vector type, new vector production method, new functional or marker gene, new delivery method, new route of administration, new *ex vivo* or *in vivo* target cell, new indication, and new treatment group.

Ms. Knorr gave examples of the new vector types that have been used before or will be used in the near future. These vector types are: (1) DNA/Virus category that include retrovirus, adenovirus, adeno-associated virus, *Herpes simplex* virus, vaccinia virus, canarypox virus, fowlpox virus, and lentivirus. (2) RNA/Virus category that include antisense RNA using retrovirus, hammerhead ribozyme using retroviral vector, and hairpin ribozyme using retroviral vector. (3) DNA. (4) RNA. (5) Plasmid DNA in DNA/liposome complexes (lipoplexes).

The category of new vector production methods include: (1) New physical methods, e.g., cellular microinjection, electroporation, particle-mediated gene transfer, and calcium phosphate precipitation of DNA. (2) New transduction methods, e.g., co-cultivation with vectorproducingcells, and incubation with viral supernatant. (3) New packaging cells, e.g., PA317, psi-crip, D-17, PG-13, and 293. (4) New helper virus used, e.g., adenoviruses and herpesviruses.

The category of new functional or marker genes include: (1) Functional genes, e.g., adenosine deaminase, tumor necrosis factor, cytokines, low density lipoprotein receptor, *Herpes simplex* virus thymidine kinase, cystic fibrosis transmembrane conductance regulator, p53, K-*ras* antisense RNA, granulocyte-macrophage colony stimulating factor, multi-drug resistance-1, gamma interferon, HIV-1IIIB envelope protein, glucocerebrosidase, HLA-B7/beta-2 macroglobulin, HIV *tar* antisense RNA, HIV *rev* M10 antisense RNA, insulin-like growth factor-1 antisense RNA, and HIV hairpin ribozyme, CD4-Zeta chimeric T-cell receptor, alpha-1 antitrypsin, carcinoembryonic antigen, Fanconi anemia complementation group C, c-*fos* antisense RNA, c-*myc* antisense RNA, iduronate-2 sulfatase, human vascular interleukin-7, transdominant REV/antisense TAR, p47 *phox*, prostate specific antigen, B7.1(CD80), MART-1, gp100, and E1A. (2) Marker genes, e.g., neomycinphosphotransferase (neo^r), and hygromycin phosphotransferase.

The category of new delivery methods include: injection of viral supernatant, injection of vector-producing cells, injection of vector-containing cells, direct vector injection, liposome-mediated delivery, and hydrogel coated angioplasty balloon.

The category of new routes of administration include: aerosol administration, bone marrow transplant, intraarterial injection, intradermal injection, intrahepatic injection, intrajoint injection, intramuscular injection, intranasal administration, intraperitoneal injection, intrapleural injection, intratumoral injection, intravenous injection, intraventricular injection, intraventricular catheter, respiratory tract administration, and subcutaneous injection.

The category of new *ex vivo* or *in vivo* target cells include: primary tumor cells, tumor cell lines, CD4+ or CD8+ peripheral blood lymphocytes, tumor infiltrating lymphocytes, CD34+ bone marrow, peripheral blood, or cord blood cells, granulocyte colony stimulating factor mobilized bone marrow cells, hepatocytes, placental cells, umbilical cord cells, fibroblasts, cytotoxic T cells, EBC-specific T cells, respiratory, nasal, or maxillary sinus epithelial cells, muscle cells, synovial cells, and vascular endothelial cells. These target cells may be autologous, allogeneic, or syngeneic, and in the future may be xenogeneic.

The category of new indications include: (1) disease/disorder including cancer, HIV, monogenic diseases, rheumatoid arthritis, and cardiovascular disease; (2) gene marking; (3) study of biologic response, e.g., cellular and humoral immunity to vector; (4) "health enhancement," e.g., augment low density lipoprotein receptors, growth hormone, etc; (5) genetically-modifiedxenografts; and (6) germ-line.

The category of new ethical issues include: (1) issues related to gene transfer, e.g., reproductive considerations, autopsies, potential risks, benefits, side effects, or discomfort to subjects, family, or health care workers, and follow-up requirements; and (2) issues not related to gene transfer, e.g., subject privacy and confidentiality, informed consent process, financial compensation to subjects or investigators, and inclusion/exclusion criteria (age and gender).

The category of new treatment groups include: adults (greater than or equal to 18 years), pediatric (3 months to 18 years), newborn (less than or equal to 3 months), and *in utero*.

The next area to be discussed is the submission, review, and reporting requirements of gene transfer proposals. Ms. Knorr noted that the *Points to Consider* is outdated and needs extensiverevision. The goal is to make it more "user friendly." Ms.Knorr suggested revising Appendix M, *Points to Consider*, as follows: (1) consolidate appendices of the *NIH Guidelines;* (2) define sub-categories of experiments and relate the submission, review, and reporting requirements according to the sub-categories; (3) expand the guidance section for investigators, IBC, and IRB; (4) update questions for investigator response; and (5) ask specific questions for novel applications.

Appendix M is proposed to be consolidated into three subject areas: (1) preamble; (2) guidance; and (3) submission, review, and reporting requirements (as defined by category). The current preamble has nine sections. The current preamble regarding historical background cites: (1) *"Splicing Life" - President's Commission for the Study of Ethical Problems in Medicine and Biomedical and Behavioral Research (1982),* and (2) *Human Gene Therapy - Office of Technology Assessment--Background Paper (1984)*. The proposed background section would include the following additional items: *First Human Gene Transfer Protocol Approved (1988), "Points to Consider" - NIH Guidelines Amended (1990), "Sunsetting" of the Human Gene Therapy Subcommittee (1992), Accelerated Review (1994),* and *Consolidated Review (1995)*.

The current policy statements of the preamble include: (1) *"Civic, religious, scientific, and medical groups have all accepted, in principle, the appropriateness of gene therapy of somaticcells in humans for specific diseases,"* (2) *"The RAC will not at present entertain proposals for germ-line alterations..."*, and (3) *"The purpose of somatic cell gene therapy is to treat anindividuals patient, e.g., by inserting a properly functioning gene into the subject's somatic cells."* The proposed preamble would add the following statements: *Policy regarding the use of normal subjects, Policy regarding "health enhancement" gene transfer, and Policy regarding in utero gene transfer.*"

With regard to the submission requirements, Ms.Knorr stated that the current requirements for all

protocols are: abstracts (scientific and non-technical), local committee approvals (IBC and IRB), responses to Appendix M-II, *Description of the Proposal*, clinical protocol, Informed Consent document, appendices, curriculum vitae, and vector sequence disks. Ms. Knorr proposed to amend the submission requirements based on protocol categories, i.e., novel, non-novel, and exempt protocols. The submission requirements of "novel" protocols would include responses to Appendix M-II, *Description of the Proposal* (a working group to be established to revise questions in anticipation of novel applications, e.g., lentiviruses, *in utero*, etc.), clinical protocol, and Informed Consent document. The submission requirements of "non-novel" protocols would be clinical protocol and Informed consent document. No submission would be required for "exempt" protocols. A working group will be established to revise definition of "exempt" protocols. The current "exempt" definition is, *"Human studies in which the induction or enhancement of an immune response to a vector-encoded microbial immunogen is the major goal, such an immune response has been demonstrated in model systems, and the persistence of the vector-encoded immunogen is not expected, may be initiated without RAC review if approved by another Federal agency."*

The current Appendix M-II, *Description of the Proposals* includes three sections for which investigators must answer all questions for each clinical trial: *Appendices M-II-A, Objectives and Rationale of the Proposed Research, M-II-B, Research Design, Anticipated Risks and Benefits, and M-II-C, Selection of Patients.* Ms. Knorr proposed revising Appendix M-II as knowledge is gained about potential "novel" applications of gene transfer, e.g., lentiviruses, herpesviruses, and *in utero* applications.

Currently there are three sections providing guidance to local IRBs on social, legal, and ethical issues, i.e., *Appendices M-III, Informed Consent, M-IV, Privacy and Confidentiality, and M-V, Special Issues.* The proposed guidance sections would translate the knowledge that has been gained from previous studies, e.g., retrovirus vectors, adenovirus vectors, plasmid DNA, etc., into guidance for local IBCs and IRBs. The advantage of the proposed safety guidance is to provide an educational tool to assist local IBCs in reviewing "non-novel" and "novel" human gene transfer protocols. The guidance might include recommendations regarding biological containment conditions (e.g., standards for aerosol administration of adenovirus vectors), recommendations regarding assays for the detection of adenoviral shedding, and recommendations regarding assays for the detection of replication-competent viruses. The advantage of the proposed informed consent guidance is to provide an educational tool to assist local IRBs in reviewing "non-novel" and "novel" human gene transfer protocols, e.g., issues relating to health care workers and family members, e.g., potential viral shedding, inadvertent exposure during aerosol administration, reproductive considerations that may be specific to a particular application of gene transfer, guidance for the use of normal subjects. The advantage of the proposed study design guidance is consistent with goals and objectives of the NIH Director regarding gene transfer studies.

With regard to framework for RAC discussion of novel protocols, Ms. Knorr noted that at December 9, 1996, RAC meeting, Dr. Brian Smith, suggested a series of recommendations addressing different aspects of the protocol to be reviewed, i.e., safety, scientific merit, and ethical issues.

As far as the reporting requirements are concerned, no change is proposed. The reporting requirements include adverse events, protocol modifications, and annual Data Management Report.

Other Comments

Dr. Anderson stated that he was the primary author of the original *Points to Consider*, and he agreed that now is the time to overhaul this historical document.

Dr. McIvor recalled that the requirement for the investigators to respond to the questions in the *Points to*

Consider by point-by-point was instituted in 1991. At that time, the RAC was concerned about the inconsistency of the information contained in the protocols submitted for RAC review. Dr. McIvor suggested that the point-by-point response to the *Points to Consider* be eliminated from the submission requirements; however, the questions raised in the *Points to Consider* should be addressed by the investigators in the clinical protocols.

Dr. Aguilar-Cordova agreed with Dr. McIvor and suggested that the RAC accept a copy of the IND submission to the FDA in lieu of a separate submission documentation for the RAC. Dr. Noguchi supported the uniform submission format for both the NIH and the FDA; he suggested that the NIH and the FDA develop a joint guidance document for the investigators regarding gene transfer studies.

Ms. Rothenberg inquired how relinquishing the protocol approval authority of NIH will impact on the revision of the *Points to Consider.* Ms. Knorr noted that with NIH relinquishing protocol approval, the local IBC and IRB will play greater roles in protocol review. Providing guidance in the *Points to Consider* will help investigators write better protocols for local review. Ms. Rothenberg was concerned about ethical review, and whether it is a major emphasis of the FDA review. Ms. Knorr noted that the RAC will still be reviewing novel protocols including ethical aspects, and the recommendations will be welcome by the FDA and the NIH Office for Protection from Research Risks (OPRR).

Ms. Rothenberg stated that the first priority is to define the RAC's role in the overall oversight of human gene transfer protocols. Dr. Lysaught noted that RAC should be concerned with ethics and safety. Dr. Aguilar-Cordova noted that there is a feedback loop within the reporting requirements for the investigators to report to ORDA any changes of protocols as a results of RAC and/or FDA review. Ms. Knorr noted that ORDA will make a broader use of the ORDA homepage to provide a wider use of the *Points to Consider* as an educational tool.

Dr. McIvor noted that a uniform submission format for both NIH and FDA is a long range goal, and the requirement of point-by-point response to the *Points to Consider* should be eliminated in the meantime. Ms. Knorr noted that the RAC may make such a recommendation, and that it would be published in the *Federal Register* and voted on by the RAC at the next RAC meeting. Dr. Aguilar-Cordova noted that most of the responses to the *Points to Consider* are included in the IND submission to FDA. Ms. Knorr pointed out the IND content and format required by 21 CFR 312.23 contains most of the information required by the RAC. However, chemistry, manufacturing, and control information of the drug products is not required. Dr. Lysaught stated that she is not prepared to vote on such a motion today.

Dr. Noguchi said that he supports a uniform format, and he suggested forming a working group to work ou the details.

Dr. Markert noted that accepting a subset of the IND application for RAC submission would simplify the task of preparing the documentation of gene transfer studies by the investigators. Dr Markert stated that the RAC's concerns about gene therapy studies goes further than the novelty aspect of the study. Issues such as normal subjects and the use of oncogenes may have precedents, but there are controversial aspects that require RAC review.

Ms. Rothenberg noted that the context of the study that affects risk/benefit analysis is important rather tha individual elements of the protocol. Ms. Knorr suggested that rather than trying to define "novelty," the RAC may choose to define a category of protocols that are "non-novel" and exempt those protocols from RAC review. Ms. Rothenberg stated that it is very difficult to define such a category of non-novel protocols at the present time. Dr. McIvor agreed that he is uncomfortable with defining any particular vector, gene, route of administration, etc., as "non-novel," because they could arise in some kind of context that might

raise a concern.

Dr. McIvor preferred to continue the current practice of having all the RAC members examine the summary information of protocols to determine if a given protocol should be reviewed by the RAC. The investigators are expected to prepare the entire submission package for RAC review. Ms. Rothenberg remarked that a "novel" protocol is difficult to define; however, you know a "novel" protocol when you read it.

Dr. Wolff said some kind of guidelines will help RAC members make such a distinction. Dr. Wolff was asking if novelty is a sufficient reason to trigger a RAC review, or it should have a safety concern. He noted that the Lyerly protocol is an example of a protocol that is novel but has a low degree of safety concern. Dr. McIvor said that personally he considers novelty *per se* should trigger review in order to determine the risks and benefits of such a study. Dr. Mickelson agreed that evaluating risk/benefit issues requires RAC discussion.

Dr. Noguchi remarked that xenotransplantation raises safety and ethical concerns, but it is not due to a strict sense of novelty. Similar xenotransplantation has been applied to many brain tumor protocols employing the strategy of injecting murine cells producing the retrovirus expressing the herpes simplex virus-thymidine kinase gene into the human brain. With regard to Ms. Rothenberg's concern of relinquishing RAC approval authority, Dr. Noguchi said that the RAC still has strong voice in the area of human gene transfer protocols.

Dr. Aguilar-Cordova agreed with Dr. McIvor that novel protocols need to be discussed regardless of the perceived risk. He suggested having a category of protocols that would warrant RAC discussion, but due to the low risk factor would be allowed to proceed with the trial prior to RAC discussion.

Responding to Dr. Noguchi's comment, Ms. Rothenberg noted that the IND submission content and format do not have items that relate to the ethical issues. Ms Knorr stated guidance of ethics and informed consent will be an important part of the revised *Points to Consider*, and that the Informed Consent document should be included in the submission requirement to the RAC. Ms. Rothenberg noted that in the human cloning report issued by National Bioethics Advisory Commission (NBAC), there is a reference to the value of the RAC as an effective oversight body outside the legislative venue, e.g., moratorium on germ-line gene alteration and being a leader in ethical discussion of human gene transfer protocols. Ms. Knorr pointed out that the proposed preamble of the *Points to Consider* will highlight policy statements such as moratorium of germ-line alteration. Dr. Noguchi emphasized that the RAC has important functions to perform in the area of ethical issues, and that the NBAC can only address very broad issues.

Dr. Lysaught noted that three major issues that the RAC needs to finalize: (1) the criteria to trigger the RAC review; (2) establishing a subcommittee to revise the *Points to Consider*; and (3) accepting Dr. Smith's framework for RAC review, i.e., safety, scientific merit, and ethical issues.

Definition of Recombinant DNA

Dr. Leinwand noted that recombinant DNA or DNA or RNA derived from recombinant DNA are within the scope of the *NIH Guidelines* whereas synthetic oligonucleotides are not. Dr. Leinwand failed to see a rationale for this distinction.

Ms. Knorr stated that the RAC relinquishing approval of protocols, the RAC needs to revisit the definition of the vaccine experiments stated in Appendix M-IX. This category is exempt from submission and reporting requirements of Appendix M.

Dr. Wolff noted that there is a need to revisit the definition of recombinant DNA. New technology allows alteration of genome not just by inserting new genes but by recombination using a single strand oglionucleotide technique. Dr. Leinwand agreed that definition of recombinant DNA should include such a new technology of gene correction.

Dr. Noguchi was concerned about broadening the scope of the *NIH Guidelines*. He noted that synthetic oligonucleotide is a very broad and a rapidly expanding area of industry research and development. Expanding this area under the purview of the *NIH Guidelines* will be a cause of concern to the industry.

Dr. Saha made two comments: (1) Regarding the issue ofoligonucleotide, the scope of the *NIH Guidelines* is clear. Synthetic oglionucleotide expressed through a recombinant DNA vector system is within the scope of the *NIH Guidelines*, whereas administration of syntheticoligonucleotide directly to humans is not. The latter application is similar to the administration of other pharmaceuticals. (2) Regarding the issue of ethical review by FDA, Dr. Saha suggested including the ethical items of Appendix M, *Points to Consider*, into the IND submission requirements. Dr. Noguchi suggested a joint FDA/NIH guidance document for human gene transfer research to deal with these issues.

Dr. Leinwand was concerned that in the Lyerly protocol using a RNA transfer technique, if the RNA was derived from a synthetic nucleic acid, it will not be covered by the *NIH Guidelines,* and that such a distinction is not reasonable. Drs. Saha and Aguilar-Cordova agreed that in view of technological advancement capable of synthesizing a large piece of DNA or RNA chemically, the definition of recombinant DNA needs to be revisited. Dr. Noguchi noted that the intention of altering the human genome is a major concern rather than a specific means. Dr. Wolff stated that experiments such as introduction of an oligonucleotide that would affect permanent change of a cell, or creating a polynucleotide capable of self replication, would be within the RAC purview. Ms. Knorr noted Section I-B, *Definition of Recombinant DNA Molecules*, that defines recombinant DNA experiments for the purpose of the scope of the *NIH Guidelines*. The issue is of far reaching significance and needs a working group to address the issue.

Ms. Rothenberg inquired how the *NIH Guidelines* would apply to the issue of human cloning. Dr. Mickelson noted that the pertinent issue is genetic intervention. Dr. McIvor explained that cloning is the introduction of a cell nucleus into a fertilized embryo cell from which the nucleus has been extruded. Cloning *per se* is not a recombinant DNA issue, but the genetic intervention of the process would be under the *NIH Guidelines*. Ms. Rothenberg said that genetic manipulation of human cloning is a concern. Ms. Knorr noted that xenotransplantation, e.g., humanized pig, raises a similar concern.

Revision of Appendix M

Dr. McIvor stated that at the next RAC meeting, two issues should come to closure: (1) elimination of point-by-point response to the *Points to Consider;* and (2) identifying the novel protocols during a case-by-case evaluation by RAC members.

Dr. Markert stated that she would be in favor of a motion to allow investigators to submit a subset ofIND applications to the RAC, and the RAC could evaluate the novel or controversial issue raised by each protocol during a case-by-case evaluation of the summary information provided byORDA. Dr. Markert preferred to establish a separate category of RAC review in which a novel protocol is allowed to proceed with clinical trial prior to a full RAC discussion provided that such a protocol is found to have low risk and high scientific merit. The Informed Consent document should continue to be required forORDA submission.

Dr. Wolff was concerned about how to assess safety risk of novel protocols without a full RAC discussion. Dr. Saha said that "controversial" may be a better term than "novel" to describe a protocol that requires RAC discussion. The Lyerly protocol is a new application; however, all the elements of the protocol have been applied to other protocols, and they are not novel. Dr. McIvor noted that the unfortunate circumstances leading to the RAC review of the protocol were unusual, and that allowing the investigators to submit their protocols for RAC screening in a early stage of protocol development might resolve this problem.

With regard to Lyerly protocol, Dr. Noguchi said that initially the FDA reviewed this protocol as a variation of many other tumor vaccine studies and did not consider it to be a gene transfer protocol. The safety issue of any protocol receives extensive review by the FDA, and this particularly protocol was found to be safe by the FDA as well as by the RAC. Dr. Markert agreed with Dr. Noguchi that a protocol should proceed if FDA determines that it is safe. Dr. Saha said that FDA assessment of safety issues of protocols is adequate. Ms. Knorr noted that RAC members may be reluctant to vote for RAC review of a protocol if it will hold up the clinical trial, and that the RAC may be more concerned if a protocol raises a serious ethical issue.

Ms. Rothenberg did not agree with investigators allowed to proceed with a clinical trial before a RAC review of a protocol. An after-the-fact review of a protocol is a good scenario. From a public perception, this timing really undermines the RAC's credibility in reviewing a protocol objectively. She found the recent RAC review of Crystal (Protocol #9701-171) and Lyerly protocols to be unsatisfactory. Dr. Markert said that holding up a protocol that is found to pose low risk from scientific viewpoint is unnecessary. Ms. Rothenberg noted that there is a possibility that such a protocol might raise a troubling ethical issue, e.g., offensive patient recruitment strategy. Dr. Lysaught noted that if a protocol is interesting to discuss and does not raise any serious safety and ethical issues, the protocol should be allowed to proceed. As an example, Dr. Lysaught said she would hold up the Crystal protocol but not the Lyerly protocol. Dr. Markert agreed.

Ms. Rothenberg was concerned about how to avoid an incident such the Crystal protocol from occurring in the future. Dr. Markert said that allowing the investigators to submit their protocols before IND submission to the FDA would avoid this kind of scenario. Ms. Knorr noted that ORDA will attempt to highlight troubling issues in its summary sheet to alert the RAC of such problems. Ms. Rothenberg agreed.

Public Comments

Dr. Mickelson invited the public in the audience to make comments.

Ms. Binko (Cell Genesys, Inc.) inquired about the reference to the IND submission requirements. Ms. Knorr responded those requirements are listed in 21 CFR 312.23, *IND Content and Format*. A subset of the submission may be submitted to the RAC except those sections containing proprietary information, e.g., formulation of drugs, and perhaps trials on Phase III or even Phase II IND applications. Ms. Binko stated that her personal preference is to prepare a separate set of documents for the RAC that does not contain any proprietary information rather than to extract a subset from IND submission. From the company's point of view, IND submission is primarily aimed at product development. She would prefer to have flexibility rather than being required to submit specified certain sections of the IND be submitted to ORDA. Ms. Knorr agreed.

Dr. Aguilar-Cordova suggested establishing a subcommittee to revise Appendix M, *Points to Consider*.

Dr. Greenblatt (National Cancer Institute (NCI), NIH) asked if Dr. McIvor's suggestion to eliminate the point-by-point responses to Appendix M will be considered at today's meeting. Dr. Mickelson responded any recommendation made by the RAC today will be published in the *Federal Register* and will be voted on at the next RAC meeting.

Committee Motion 4

A motion was made by Dr. Aguilar-Cordova and seconded by Dr. McIvor to establish a subcommittee to revise Appendix M, *Points to Consider*, of the *NIH Guidelines*. The motion passed by a vote of 9 in favor, 0 opposed, and no abstentions.

Recommendations from the subcommittee will be published in the *Federal Register* for public comment and voted on at the September 1997 RAC meeting.

Committee Motion 5

A motion was made by Dr. McIvor and seconded by Dr. Saha to eliminate the point-by-point response to Appendix M-II, *Description of the Proposal*, provided that the questions raised in Appendix M-II must be addressed in the clinical protocol. The motion passed by a vote of 8 in favor, 0 opposed, and 1 abstention.

This recommendation will be published in the *Federal Register* for public comment and voted on at the September 1997 RAC meeting.

Ms. Rothenberg abstained, stating that the motion is not completely satisfactory to her.

Dr. Markert suggested that investigators or sponsors be allowed to submit their protocols to the RAC prior to their submission to FDA. Dr. Glorioso supported such a suggestion.

Dr. McIvor warned that decoupling of RAC submission from the FDA, IRB, and IBC might encourage some people to submit a premature proposal to the RAC, that was not based on solid scientific studies. Dr. Noguchi stated that the RAC has a responsibility to review proposals submitted to it. Dr. Aguilar-Cordova noted that Dr. Markert's suggestion would allow the RAC to review the protocol in early stage. Dr. Wolff agreed.

Dr. Anderson stated from his experience as a journal editor that people will submit any unsubstantiated proposal if there is no safeguard of quality. Dr. Anderson suggested that the RAC submission should be coupled with IRB and IBC submission in order to prevent receiving premature proposal.

Dr. Miller noted that simultaneous submission requirements to both the FDA and the NIH is problematic, and that some investigators submitted their NIH submission package to FDA before filing their IND. Responses to Appendix M contribute only a subset of IND requirements.

[Dr. Mickelson presented a plaque and a certificate to Dr. Glorioso for his service to the RAC. Dr. Glorioso was not present earlier in the meeting when such presentation was made to other retiring members.]

XI. A STREAMLINED PROTOCOL SUBMISSION/KNORR

Dr. Mickelson called on the RAC to continue the discussion of the procedures for protocol submission and review.

Dr. Markert proposed changes to Appendix M-1, *Submission Requirements - Human Gene Transfer Experiments.* The proposed wordings of Appendix M-1 were distributed to the RAC. Dr. Markert proposed to decouple the submission of Appendix M to the FDA in order to allow the investigators to submit their proposals to the RAC before filing for IND application to the FDA. The requirement of point-by-point responses to Appendices M-II through M-V, *Description of the Proposal, Informed Consent, Privacy and Confidentiality, and Special Issues*, will be deleted from the submission requirements.

Dr. Mickelson noted that Dr. Markert is tabling her motion until later on in the discussion.

Dr. Wolff said that while the RAC is considering decoupling Appendix M submission to the FDA, it should coincide with IBC and IRB submission as suggested by Dr. Anderson. As a point of clarification, Dr. W noted that at the March 1997 RAC meeting, the RAC has accepted two motions: (1) Remove the prior IR and IBC approvals from the submission requirements of Appendix M-I. (2) The protocols should be submitted to ORDA with simultaneous notification to IBC. Final IBC and IRB approvals should submitted to ORDA upon receipt of the following: (a) NIH notification of exemption from full discussion, or (b) subsequent to full RAC discussion (if applicable). Human clinical trials should not be initiated prior to submission of final IBC and IRB approvals to the N

Dr. Anderson stated that for quality control, simultaneous submission to IRB and IBC should be require The RAC may accept the application prior to IRB and IBC approval

Dr. Saha inquired if the ORDA maintains mailing lists of IBC and IRB . Ms. Knorr responded t required to be registered with ORDA according to th*NIH Guidelin*, and ORDA has an up-to-dat listing of IBCs . ORDA has obtained a list of IRBs from OPRR . The local institutions can be n regarding RAC recommendations and the *NIH Guidelin* amendments. Responding to a question from Dr. McIvor regarding the current *NIH Guidelin* requirements, Ms. Knorr said that currently prio approvals of IRB and IBC are required according to submission requirement

Dr. Noguchi stated the decoupling of NIH submission from that of the FDA is an excellent ide

Dr. Anderson inquired if ORDA will be able to dismiss an inappropriate protocol administratively fro RAC review. Ms. Knorr responded that ORDA cannot readily make such a decision, because s guidance from the RAC is needed. Ms. Knorr noted that it is essential that the IBC, which is directly un the *NIH Guidelin*, simultaneously receive the protocol as a check and balance in terms of the safety issues of the protocol. Dr. Aguilar-Cordova questioned if there is such a necessity for submission to IBC as a quality control. Dr. Anderson responded affirmatively. As a journal editor, he frequently receives unsubstantiated genetic engineering proposals from certain individuals; and a screening procedure is needed to keep these unsubstantiated protocols from the RAC agenda.

Dr. Markert suggested a new category of RAC recommendation regarding the summary sheet provided ORDA for necessity of RAC review. The new category is for premature protocols for which the RAC wi defer consideration. Dr. Anderson agreed.

Dr. Markert said that another category of RAC recommendation should be protocols that require full RA discussion with the investigators allowing to proceed before RAC discussion. Dr. McGraw was concerned that this category may allow protocols to proceed simply based on safety concerns but no ethical safeguards. Dr. Aguilar-Cordova cited the Lyerly protocol as an example of this category of nove protocols, i.e., low safety and ethical concerns. Dr. McGraw said that without a RAC discussion, it is difficult to assess the safety of this protocol. Drs. Aguilar-Cordova and Markert said that safety issues v

be addressed by the IBC and FDA, and this new category provides RAC some flexibility in dealing with an unusual protocol which is novel andscientifically interesting but has low safety concerns. Dr. Leinv did not see a need for RAC to have this category. Scientifically interesting protocols can be discussed in any scientific meeting, and the RAC should consider only those protocols that have raised safety and ethical concerns. Ms. Rothenberg agreed with Dr. Leinwand

Ms. Knorr noted that she is concerned that IBC might give stamp approval if IBC approval comes afte RAC review. IBC has a primary responsibility to ensure the safety of the protocol. Ms. Rothenberg said that she has a similar concern with decoupling IRB approval, because the local institutions may b subject to economical pressure to give stamp approval of protocols. Dr. Markert said that the major be of eliminating the prior IBC and IRB approvals is to streamline the oversight process and not hold up protocol for several additional months. Dr. McIvor said that another benefit is to enable the RAC to review a protocol at an earlier stage of its development. Ms. Rothenberg said that it is not RA's responsibility t tutor the investigators on how to write the Informed Consent document. IRB should approve the Inform Consent before it is submitted to the RAC.

Regarding Dr. Marke's proposal for a new category of RAC discussion of the protocol with the investigators allowing to proceed, Drs. Noguchi and Aguilar-Cordova said that the Lyerly protoco represents a very unfortunate and unusual circumstance, and there is no need for this new category. If there is a novel approach of gene transfer procedure, the RAC can discuss such issues separately from the protocol, thus not delaying impeaching on its initiation.

Dr. Markert agreed to withdraw her suggestion regarding a new category of protocols that would requir full RAC discussion with the investigators allowing to proceed. She made a motion to include three categories of RAC recommendation based on the summary sheet provided by ORDA as: (1) protocol that do not need full RAC review, (2) protocols that do require full RAC review, and (3) premature protocols that will not be considered by the RAC. Dr. Aguilar-Cordova seconded the motion.

There was discussion about what terminology should be used to describe the third category protocols. Dr. Noguchi suggested the category is for protocols with insufficient information. Dr. Markert agree

Dr. Glorioso stated that the RAC should accept all submitted protocols; protocols should not be rejecte due to insufficient information. Dr. Mickelson said that from the RA's past experience, none of the protocols would fit into the new category. Nonetheless, all protocols should have prior approvals from local institutions.

Dr. Glorioso suggested that affiliation with an institution and simultaneous notification to IBC will preve submission of premature protocols to the RAC. Dr. Aguilar-Cordova agreed. Dr. Glorioso stated that th major reason that he is not in favor of Dr. Mark'er motion is that the RAC should not be in a position of deciding on whether the science is ready for a given application to humans. Dr. Aguilar-Cordova withdrew his second to the motion. Dr. Markert agreed to withdraw her motio

Dr. Aguilar-Cordova proposed an alternative measure to deal with a premature proposal. He suggested coupling the RAC submission with simultaneous IBC submission; this requirement would limit proposals to those investigators having institutional affiliation. Dr. McIvor noted that the investigators should provide ORDA with evidence of protocol submission to IBC rather than simultaneous submission to both IBC a ORDA on the same da

Ms. Rothenberg still had concerns with eliminating prior approvals from IBC and IRB . Dr. Glorioso s that the RAC should be a public forum to deliberate novel protocols, but the approval authority of

protocols should be vested in the FDA and local committees. Dr. Noguchi said that the local IRB has th final authority to permit a protocol to be initiated even if it has FDA approval.

Committee Motion 6

A motion was made by Dr. Aguilar-Cordova and seconded by Dr. Glorioso that the RAC should no review any gene transfer protocol until the investigator has provided ORDA with evidence of protoco submission to the IBC. IBC notification is needed in order to avoid the circumstances in which the RAC might review a protocol that has not been submitted to the IBC. The motion passed by a vote 8 in favor, 1 opposed, and no abstentions.

This recommendation will be published in the *Federal Register* for public comment and voted on at the September 1997 RAC meeting.

Ms. Knorr asked what would constitute "evidence" of protocol submission to IBC. Dr. Leinwand sug a letter from the IBC. Ms. Binko suggested that a copy of investigat submission letter to IBC should be sufficient. Dr. Glorioso stated that it should be a letter from the IBC acknowledging receipt of th application. Dr. Allgood (GeneMedicine , Inc.) said they have difficulty in obtaining a letter from their IBC. Dr. Saha stated that a copy of submission letter to IBC should be acceptable. Dr. Mickelson said letter from IBC is necessary. Ms. Rothenberg said since the RAC has already eliminated prior approval requirement, there is no difference if the letter is from the investigator or IBC. A submission letter to IBC is acceptable, and ORDA can follow-up to verify the submission if there is any question. Dr Aguilar-Cordova agreed. Ms. Knorr said that IBC chairs should be consulted in terms of what woul constitute "evidence" of submission.

Dr. Mickelson called on Dr. Markert to state her motion regarding the submission requirements. Dr Markert proposed to eliminate the requirement of prior IRB and IBC approvals, and point-by-po responses to Appendix M-II through M-V from the current Appendix M-I, *Submission Requirements - Human Gene Transfer Proposals*. In addition, a letter would be required stating that submission has been made to the IBC.

Ms. Rothenberg was concerned about no prior IRB review and no screening of Informed Consen document. Dr. McIvor said that the RAC's initial question should be whether a protocol is novel and woul require RAC review.

Ms. Knorr suggested that the protocol should include discussion of issues in Appendix M-II through M- although no point-by-point response is required. She inquired that if curricula vitae and vector sequence diskettes should continue to be required for submission.

Dr. Markert suggested to delete the requirement for sequence diskettes. Dr. Saha agreed that sequence diskettes are not needed, but he would retain the curricula vitae. Dr. McIvor and Ms. Rothenberg said that there is no additional burden to include curricula vitae of the investigators.

Dr. McIvor inquired if the FDA reviews the vector sequence diskettes. Dr. Miller responded that the FDA does not conduct independent review of the DNA sequences and such sequence analysis should be performed by the investigators or sponsors. Dr. Aguilar-Cordova said that none of thecurrent RA members is reviewing the diskettes information, and that the requirement is superfluous. Dr. Markert sa that sequence diskettes can be deleted from the submission requirements. Dr. McIvor was concerned about a novel vector. Dr. Leinwand said that if there is such a need to review the sequence, the RAC m request such information from the investigators. Dr. Aguilar-Cordova said that FDA requires vector

sequence analysis from the investigators. Dr. McIvor stated that DNA sequences should be requested if a protocol requires full RAC review. Dr. Markert said that such a request could be put forward by any RA member, but it does not need to be submitted for every protocol.

Dr. Greenblatt pointed out that NCI files IND for products developed by commercial companies, and these companies file separate drug master files with FDA. Some companies consider vector sequence information proprietary, and they are reluctant to submit such information to the RAC.

Dr. Allgood asked if the RAC would accept curriculum vitae without page limitation. Dr. Mickelso responded that they should be in a biographical sketch format with 2 page limit.

Committee Motion 7

A motion was made by Dr. Markert and seconded by Dr. Leinwand to delete prior IBC and IRB a responses to Appendix M-II through M-V, and vector sequence diskettes from Appendix M-I, *Submission Requirements -- Human Gene Transfer Experiments*. The RAC accepted the submission requirements as follows:

Appendix M-I, Submission Requirements -- Human Gene Transfer Experiments

Investigators must submit the following material to the Office of Recombinant DNA Activities, National Institutes of Health/ MSC 7010, 6000 Executive Boulevard, Suite 302, Bethesda, Maryland 20892-7010 301-496-9838 (see exemption in Appendix M-IX-A, *Footnotes of Appendix M*). Proposals will be submitted in the following order: (1) scientific abstract--1 page; (2) non-technical abstract--1 page; (3) protocol--20 pages including discussion of issues in Appendix M-II through M-V; (4) Informed Consent document prepared for IRB submission (see Appendix M-III, *Informed Consent*); (5) letter stating that submission has been made to the IBC; (6) appendices (including tables, figures, and manuscripts); and (7) curricula vitae--2 pages for each key professional person in biographical sketch format.

The motion passed by a vote of 7 in favor, 0 opposed, and 1 abstention.

This recommendation will be published in the *Federal Register* for public comment and voted on at the September 1997 RAC meeting.

Ms. Rothenberg abstained due to her concern that it is difficult for a non-scientific RAC member to determine that a given protocol is non-novel and thus exempt from RAC review.

XII. DISCUSSION ON EXEMPT GENE THERAPY PROTOCOLS/ KNOR

Ms. Knorr noted that Appendix M-IX, *Footnote of Appendix M,* exempts protocols from both requirements submission and reporting to ORDA . The exempt protocols are mostly for recombinant vaccine studies humans. In 1994, Appendix M-IX-A was modified to limit exemption to protocols involving "microbial" encoded immunogens . Appendix M-IX-A states, "Human studies in which the induction or enhancemen of an immune response to a vector-encoded microbial immunogen is the major goal, such an immun response has been demonstrated in model systems, and the persistence of the vector-encoded immunogen is not expected, may be initiated without RAC review if approved by another Federa agency." In the last couple of RAC meetings, it was suggested that Appendix M-IX-A needs to be revisited in order to decide whether the exemption should be narrowed in order to capture more vaccine protocols for the purpose of registration and reporting, or it should be broadened to exempt a wider categories of gene transfer studies.

Ms. Rothenberg inquired who is making the determination that protocols are exempt under Appendix M-IX. Ms. Knorr noted that investigators, sometimes in consultation with ORDA staff, determine if th protocols are in this exempt category.

Dr. Greenblatt noted that he wrote a letter dated April 18, 1997, to ORDA regarding the submission cancer vaccine protocols. Dr. Greenblatt explained the historical origin of the amendment of Appendi M-IX-A regarding the registration of cancer vaccine protocols. In 1993, Drs. Schlom and Mike Hamiltor NCI conducted a recombinant vaccinia CEA study that was determined to be exempt under Appen M-IX-A by ORDA . Subsequently, Appendix M-IX-A was amended in 1994 by the RAC to limit vaccin exemption to vectors encoding microbial immunogens , and the cancer vaccine protocols are under th RAC purview. NCI-Cancer Evaluation Program (NCI- CTEP) has since sponsored many cancer vaccin studies using poxvirus vectors. Dr. Greenblatt noted a need to clarify the definition of this category o vaccine protocols since under FDA regulation they are submitted through the Office of Vaccines rather than as gene transfer protocols through the Division of Cellular and Gene Therapies.

Dr. Greenblatt noted that from July 1995 to January 1997, NCI- CTEP submitted 9 protocols to OF the submission included only the clinical protocols and Informed Consent document. In a letter dated April 18, 1997, Dr. Greenblatt requested that ORDA and the RAC simplify the submission requirements these cancer vaccine protocols as opposed to the complete documentation required under Appendix M-I, *Submission Requirements - Human Gene Transfer Proposals.* This request was prompted by the incidence in connection with submission of Protocol 9704-185 by Dr. Robert Conry of the University o Alabama at Birmingham.

Dr. McIvor noted that under Appendix M-IX-A, vaccine studies for infectious diseases are exempt, and cancer vaccines are not included in this definition. Dr. Aguilar-Cordova noted that the vector, ALVAC-hIL-12, of Protocol #9704-185, is not intended to elicit immune response to interleukin-12, and thus the study is not included in the exempt protocols defined by Appendix M-IX-A. Ms. Knorr stated th since the December 1996 RAC meeting, the role of the RAC in terms of its oversight of human gene transfer protocols has been clarified. ORDA has subsequently sent a letter to the Chairs of the IBCs IRBs to remind the investigators to the requirements stipulated by Appendix M-I, *Submission Requirements - Human Gene Transfer Proposals,* and Dr. Conry was asked to comply with the comple submission requirements.

Dr. Markert suggested a wider use of the ORDA home page on the Internet to provide the investiga and sponsors with updated information regarding the RAC. She stated that the RAC should consider revisiting the definition of exempt protocols under Appendix M-IX-A.

XIII. DISCUSSION ON HUMAN GENE THERAPY ABROAD/MICKELSON

Dr. Mickelson gave an overview of the Korean interleukin-12/cancer gene therapy trial. Two recent articles in *Nature* [Vol. 387, page 6] on May 1, 1997, and *Science* [Vol. 276, page 1035] on May 16, 1997, prompted an inquiry by the RAC about the level of collaboration between University of Pittsburgh and Korean researchers. The study is the first human gene transfer trial to be conducted in Korea. Using a protocol originally developed by Dr. Michael Lotze (University of Pittsburgh), skin fibroblasts from th patient were treated with a retrovirus containing the gene for interleukin-12. Cells containing the gene were injected into the patient after lethal irradiation. Korean investigators will treat the patient using this protocol. In a letter dated May 20, 1997, Dr. Mickelson inquired if the protocol has been registered with ORDA in compliance with the *NIH Guidelines*.

In a letter dated May 30, 1997, ORDA informed Dr. Lotze that the NIH General Counsel has cor that this trial is a collaboration (based on multiple criteria); therefore, the trial is subject to compliance under the *NIH Guidelin*. The trial has been placed on hold in Korea until the protocol is submitted to the RAC.

Dr. Mickelson noted that this case is an apparent oversight on the part of the investigators, and that they have cooperated fully and intend to submit the protocol to ORDA in the immediate future. Ms. Rothenb noted that similar cases of international collaboration will occur with increasing frequency in the near future.

Dr. Glorioso confirmed that this particular case should be considered as a collaborative effort, becaus the vector is produced by the NIH -funded General Clinical Research Center at University of Pittsburgh Dr. Markert remarked that the present case illustrates an important issue for the RAC. Ms. Knorr nc South Korea is convening a conference in September 1997 to develop guidelines for the oversight of human gene transfer trials.

XIV. DISCUSSION ON FUTURE GENE THERAPY POLICY CONFERENCE (GTPC TOPICS/ MARKER

Dr. Markert stated that she has consulted with Dr. Noguchi regarding future topics for the Gene Therap Policy Conferences (GTPC). She identified lentiviruses and herpesviruses as potential subject GTPC . The use of lentiviruses , e.g., HIV-1 and -2, as gene transfer vectors involves many safety ethical issues that warrant public discussion. Lentiviruses have an advantage of infecting nondividi target cells. However, there are many issues of concern, i.e., infecting germ-line, spreading to health care workers and the public, the function of many regulatory genes of the virus, the required safety testings , There is much preclinical work underway with the development of lentivirus vectors, and it is timely t discuss these issues and to provide investigators with guidance.

Dr. Saha inquired if any recommendations have been made regarding GTPC topics. Ms. Knorr that at the March 1997 RAC meeting, the RAC recommended that the first GTPC should be held on th use of normal subjects and/or enhancement gene transfer. These recommendations resulted from discussion of Dr. Crystal's normal subjects protocol. The RAC should make recommendations regarding the priority of the topics, and the NIH Director will make the final decisio

Dr. Aguilar-Cordova inquired if protocols using herpesvirus vectors are forthcoming. Dr.Glorio responded that these vectors are being considered for the treatment of brain tumors. Dr. Miller stated that pre- IND meetings have been already held for clinical trials of herpesviruses but not yet for lentivir

Dr. Aguilar-Cordova noted that herpesvirus vectors should take precedence, and he would propose motion to recommend to the NIH Director to convene a GTPC on the use *Herpes simplex* virus as a vector for human gene transfer studies.

Committee Motion 8

A motion was made by Dr. Aguilar-Cordova and seconded by Dr. Saha to recommend to the NIH D to convene a Gene Therapy Policy Conference (GTPC) on the use *Herpes simplex* virus as a vector for human gene transfer studies. The motion passed by a vote of 7 in favor, 1 opposed, and no abstentions.

Discussion

Ms. Rothenberg noted that there is a session on lentivirus vectors at the forthcoming Forum 1997 Gen
Therapy Conference organized by the FDA and the NIH on July 15-18, 1997, at Bethesda, Maryland. S
inquired why there is no session on herpesvirus vectors if these issues are imminent. Dr. Miller respon
that herpesviruses was discussed in last y's Forum including one approximately a month ago by NCI.
Ms. Knorr asked if it would be possible to get transcripts from the upcoming FDA conference. Dr. Mille
responded that she is unsure whether there will be a transcript; however, the slides will be published. Ms.
Rothenberg said focusing on novel vectors is very critical but wondered whether it should be a policy
conference.

Ms. Rothenberg inquired what kind of policy issues should be discussed with regard to these vectors. Dr.
Aguilar-Cordova said that the RAC should focus on the critical issues of safety concerns. Ms. Knorr no
that the RAC should consider a policy statement to be included in the *Points to Consider* of the *NIH
Guidelines*, and that the RAC should provide guidance to the investigators regarding the use of these
vectors. Dr. Wolff suggested that the discussion should be focused on a specific protocol. It would be
beneficial to request investigators who are considering these types of protocols to submit a pre- IND o
pre-RAC protocol to focus the GTPC discussion. Dr. McIvor was unsure whether the *Points to Consider*
should include what assays need to be conducted for a particular new vector. He emphasized that the
RAC needs to educate itself with regard to the biology of the new vector, the risks associated with the new
vector, how to evaluate the potential efficacy, and what target disease of the new vector.

Ms. Rothenberg noted that at the last RAC meeting, the FDA said that if a protocol was submitted for
genetic enhancement and was deemed to be safe, the FDA would most likely approve such a study
(absent any policy relevant to such proposals). She pointed out that maybe both gene enhancement and
novel vectors are priorities. She said that she was concerned about public perception that the RAC is not
focusing immediately on the ethics issue, but she supported focusing on novel vectors. She asked if the
recommendation to convene a GTPC on gene enhancement has been proposed to Dr. Varmus , the
Director, as a result of the March 1997 RAC meeting. Ms. Knorr stated that the recommendation has b
forwarded to the Dr. Varmus

Ms. Knorr asked if the RAC wanted to invite submission of a mock protocol or an early protocol for th
herpesvirus to provide some context for a discussion, and whether the discussion should be in a RA
meeting or in a policy conference forum. Dr. Wolff said he liked the concept of a mockprotocol an
indicated that either forum was acceptable. Ms. Rothenberg said this idea was proposed approximately a
year ago for *in uter*, but no mock protocols were received by the RAC. It is risky for investigators to be
willing to submit a mock protocol for public critique.

Dr. Mickelson noted that the RAC has one motion put forward to recommend herpesvirus vectors as
next topic for GTPC . Dr. Markert proposed a friendly amendment to add lentivirus vectors as a s
priority topic.

Dr. Anderson recalled that in late 1986, Dr. Emmett Barkley of the NIH (now at the Howard Hughe
Medical Institute) sent a memorandum at the request of the Human Gene Therapy Subcommittee (HGTS
asking investigators to submit a mock protocol. Dr. Anderson said his group and Dr. Michael Blaese
submitted a pre-clinical data document to focus the HGTS discussion. He said that he propose
submitting a mock protocol for *in uter*, but withdrew it after he sensed from ORDA that the RAC did no
want to discuss *in uter* in the absence of other protocols. He noted that in an *in uter* symposium in
August 1996 in Reno, Nevada, there was a consensus of investigators in the field to wait until the safety
issues have been resolved for the clinical trials involving *in uter* stem cell therapy before proposing any
in uter gene therapy study. He stated he is still planning on submitting a mock protocol on *in uter* when
he and others in this area of research feel that it is appropriate timing. *In uter* protocols are in the near

future, and it would be useful to have a GTPC on this subject. However, normal controls an enhancement is a much higher priority.

Dr. Anderson said submitting a mock protocol requires an investigator willing to submit it, because it is a lot of work with almost nothing to gain and everything to lose. But it is very useful if what you want is a public discussion of a particular problem, and you cannot have that type of discussion without a mock protocol. It needs a case and a protocol stating what experiment the investigator plans to perform so the RAC can have a useful discussion.

Dr. Glorioso said he is aware of a protocol that is imminent involving an *ex vivo* approach by placing a cytokine into tumor cells. He did not know if the protocol lends itself to a contextual discussion. It is a different set of issues than using a replicating virus in the brain to kill tumor cells, which raises very complex issues. How replication is ultimately controlled and what impact that has on viral pathogenesis or on resident viruses, and there may be potential recombination. Those are difficult but important issues tha need to be discussed by the RAC.

Ms. Knorr asked what is the best mechanism for soliciting mock protocols. Dr. Wolff said that investiga who are contemplating research on gene enhancement or herpesviruses would have something to gai by discussion of a mock protocol in such a forum. Dr. Anderson said an academic institution might be motivated, but he believes a company would not risk negative feedback on mock protocols, because it could affect the company's ability to raise money. Ms. Rothenberg said that might be a good reason to focus on gene enhancement and normal subjects first, because this topic for a conference would be less risky in the commercial context and could be tried out in a policy conference. Dr. Glorioso said normal gene enhancement are very controversial, and the RAC may not want that type of public controversy now Gene therapy right now is about treating disease, and it will stay in that area until results of research are observed. Dr. Anderson asked Dr. Glorioso if he considered baldness a disease, and Ms. Rothenber asked if Dr. Glorioso considered being short a disease. Dr. Glorioso said these are good points. Ms asked where the line is drawn between health enhancement and social enhancement. Dr. Glorioso sai he thinks this issue will eventually come up, but not in the near future.

Dr. McIvor was skeptical that anybody would be willing to submit a mock protocol. At the same time, the RAC has to prepare itself for new vectors and become educated on issues of biological safety and efficacy. There is a lot to be gained by having a one-day policy conference on herpesviruses, as a example. Dr. Glorioso said there is a great complexity in the herpes simplex alone, because there are least 35 genes, and alteration of such viral genes might contribute to virus replication and the host cell function.

Dr. Wolff expressed his desire to make the GTPC different from the other scientific meetings. H suggested requesting submission of abbreviated protocols or a summary statement, rather than the whole protocol. It could be for a disease, patient group, or type of virus. Therefore, it could be discussed by experts in the basic science. Dr. McIvor said he hoped the GTPC would have standard format of startir with the science and biology of the particular system, moving to issues regarding translation discussing safety issues, how to quantify measurements, and concluding with an informational document that states what information will be needed to evaluate the protocol when it is reviewed by the RAC. Ms. Knorr not that the RAC recommendation can be incorporated into Appendix M, *Points to Consider*. Dr. Glorioso noted that it may not be feasible to formulate a general guidance document due to the complexities of the biology of herpesvirus infection. Dr. Aguilar-Cordova said that GTPC will deal with the general prob first, and specific issues related to a particular protocol will be reviewed on a case-by-case basis. Dr. Markert agreed that a GTPC is to identify a variety of potential safety iss

Ms. Knorr stated that both of the following recommendations will be forwarded to Dr. Varmus: (1) b normal subjects and/or enhancement of gene transfer, and (2) herpesviruses. She said the RA previously recommended that one RAC member co-chair the GTPC, and she said Dr. Varmus wo likely move fairly quickly on both issues. Dr. Glorioso and Ms. Rothenberg said Dr. Varmus may wa decide who will chair the GTPC. Dr. Glorioso suggested recommending several topics to Dr. Varn him to make the final decision on the GTPC topic and chai

XV. CLOSING REMARKS/MICKELSON

Dr. Mickelson suggested several topics for the September 1997 RAC meeting. These items are: (1) update the preamble of Appendix M, *Points to Consider*, (2) develop guidance for new applications, new vectors, etc., (3) reevaluate the definition of recombinant DNA of the *NIH Guidelines*, (4) invite a representative from the the OPRR to discuss the transgenic animal use issues, and (5) form subcommittee for revision of Appendix M, *Points to Consider*.

Dr. Braun suggested that a discussion of RA's responsibility other than oversight of human gene transfer protocols will be useful. Ms. Knorr noted that th*NIH Guidelin*need to be revisited and updated with regard to other biosafety issues and host-vector system

XVI. FUTURE MEETING DATES/MICKELSON

The next meeting of the RAC will be on September 11-12, 1997, at NIH, Building 31C, Conference Ro 6, Bethesda, Maryland

[Executive Secretary Note: The first Gene Therapy Conference will be held on September 11, 1997, Bethesda Holiday Inn, Bethesda, Maryland. The next meeting of the RAC will be on September 12, 1997, at NIH, Building 31C, Conference Room 6, Bethesda, Maryland

XVII. ADJOURNMENT/MICKELSON

Dr. Mickelson adjourned the meeting at 2:55 p.m. on June 13, 1997.

Debra W. Knor
Executive Secretary

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

Date: June 13, 1997

Claudia A. Mickelson, Ph.D.
Acting Chair
Recombinant DNA Advisory Committee
National Institutes of Health