RECOMBINANT DNA ADVISORY COMMITTEE
Minutes of Meeting
December 15-16, 1997

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

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The Recombinant DNA Advisory Committee (RAC) was convened for its sixty-ninth meeting at 9:00 a.m. on December 15, 1997, at the National Institutes of Health (NIH), Building 31, Conference Room 10, 9000 Rockville Pike, Bethesda, Maryland 20892. Dr. Claudia Mickelson (Chair) presided. In accordance with Public Law 92-463, the meeting was open to the public on December 15 from 9:00 a.m. until 7:00 p.m and December 16 from 8:30 a.m. to 6:00 p.m. The following were present for all or part of the meeting:

Committee Members:

C. Estuardo Aguilar-Cordova, Texas Childrens Hospital
Dale G. Ando, Cell Genesys, Inc.
Jon W. Gordon, Mt. Sinai School of Medicine
Jay J. Greenblatt, National Institutes of Health
Michael M.C. Lai, University of Southern California
Leslie A. Leinwand, University of Colorado
M. Therese Lysaught, University of Dayton
Ruth Macklin, Albert Einstein College of Medicine
M. Louise Markert, Duke University Medical Center
R. Scott McIvor, University of Minnesota
Claudia A. Mickelson, Massachusetts Institute of Technology
Karen Rothenberg, University of Maryland School of Law
Inder M. Verma, The Salk Institute
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:

F. William Dommel, Jr., Office of Protection from Research Risks
Daniel D. Jones, U.S. Department of Agriculture

Philip Noguchi, Food and Drug Administration

National Institutes of Health staff:

Melissa Ashlock, NHGRI
Bobbi Bennett, OD
Kristina Borror, OD
Diane Bronzert, NCI
Jeffrey Cohen, NIAID
Cheryl Corsaro, NHGRI
Greg Downing, OD
Joseph Gallelli, CC
Harold Ginsberg, NIAID
Roberta Haber, NIDDK
Christine Ireland, OD
Julie Kaneshiro, OD
Robin Kawazoe, OD
Richard Knazek, NCRR
Becky Lawson, OD
Carol Manning, NCI
Catherine McKeon, NIDDK
Mikel Miller, OD
Monique Mansoura, NHGRI
Pearl O'Rourke, OD
Gene Rosenthal, OD
Aiman Shalabi, NCI
Thomas Shih, OD
Sonia Skarlatos, NHLBI
Lana Skirboll, OD
Stephen Straus, NIAID
Harold Varmus, OD

Others:

Victoria Allgood, GeneMedicine, Inc.
Robert Anderson, Food and Drug Administration
W. French Anderson, University of Southern California
Kameron Balzer, Genentech, Inc.
Steven Bauer, Food and Drug Administration
Bridget Binko, Cell Genesys, Inc.
Flavia Borellini, MA BioServices
Xandra Breakefield, Massachusetts General Hospital
Judi Buckalew, Senator Kay Bailey Hutchinson's Office (Texas)
Jeff Carey, Genetic Therapy, Inc.
Ira Carmen, University of Illinois
Joy Cavagnaro, Human Genome Sciences
Yung-Mien Chang, Genetic Therapy, Inc.
Yara Cheikh, Cheikh
Daniela Cirillo, Stanford University
Hillel Cohen, Merck Research Laboratories

Ronald Crystal, Cornell University
Kenneth Culver, Codon Pharmaceuticals, Inc.
John Cutt, Novartis Pharmaceuticals Corporation
Barbara Davies, Pro-Neuron, Inc.
Laura Dely, FDA Week
Ronald Dorazio, Genetix Pharmaceuticals, Inc.
Anne Dunne, Strategic Results, LLC
Matthew During, University of Auckland, New Zealand
Ross Durland, GeneMedicine, Inc.
Suzanne Epstein, Food and Drug Administration
Mitchell Finer, Cell Genesys, Inc.
Gary Gamerman, Fenwick and West
Joseph Glorioso, University of Pittsburgh
Elissa Grabowski, Dewe Rogerson, Inc.
Tina Grasso, GenVec
Stephen Hoffman, Naval Medical Research
Russell Howard, Maxygen
Lee Huang, Rhone-Poulenc Rorer
Jeffrey Isner, Tufts University
John Jafari, Genetic Therapy, Inc.
Joan Keiser, Parke-Davis Pharmaceutical Research
Imre Kovesdi, GenVec
Larry Kowal, Genetic Therapy, Inc.
Steven Kradjian, Vical, Inc.
Mike Kulkarx, Aurx, Inc.
LaVonne Lang, Parke-Davis Pharmaceutical Research
Michael Langan, National Organization for Rare Disorders
Fred Ledley, Variagenics, Inc.
Brian Ledwith, Merck Research Laboratories
Russette Lyons, Genetic Therapy, Inc.
Carol Marcus-Sekura, Biotechnology Assessment Services, Inc.
Reuben Matalon, University of Texas Medical Branch
Gerard McGarrity, Genetic Therapy, Inc.
Andra Miller, Food and Drug Administration
Margaret Moore, NeuroVir, Inc., Canada
Carolyn Nagler, PSI International
Luigi Naldini, Cell Genesys, Inc.
John Norman, Vical, Inc.
Marina O'Reilly, Genetic Therapy, Inc.
Sheryl Osborne, NeuroVir, Inc., Canada
Jeffrey Ostrove, NeuroVir, Inc., Canada
Amy Patterson, Food and Drug Administration
Nick Pelliccione, Schering-Plough Corporation
Janet Peterson, University of Maryland
Anne Pilaro, Food and Drug Administration
Leonard Post, Parke-Davis Pharmaceutical Research
Toni Putnam, Public
Andrew Quon, American Association of Medical Colleges
Rafel Rieves, Food and Drug Administration
Bernard Roizman, University of Chicago

Joseph Rokovich, Pangaea Pharmaceuticals, Inc.
Todd Rosengart, Cornell University
Dean Rupp, Yale University
Margaret Samyn, Parke-Davis Pharmaceutical Research
Victor Santamarina, Genetic Therapy, Inc.
Jack Schaumberg, GeneMedicine, Inc.
Mercedes Serabian, Food and Drug Administration
Tomiko Shimada, Ambience Awareness International, Inc.
Stephanie Simek, Food and Drug Administration
Thomas Smart, GenVec
Frank Tufaro, NeuroVir, Inc., Canada
Andrew Uprichard, Parke-Davis Pharmaceutical Research
Dominick Vacante, Magenta Corporation
Edward Wagner, University of California at Irvine
Lisa White, The Blue Sheet
Carolyn Wilson, Food and Drug Administration
Chris Wysocki, Genetic Therapy, Inc.
Grant Yonehiro, GenVec

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

Dr. Claudia A. Mickelson, Chair of the Recombinant DNA Advisory Committee (RAC), called the meeting to order. She stated that due notice of the meeting, and the proposed actions under the *NIH Guidelines for Research Involving Recombinant DNA Molecules (NIH Guidelines),* were both published in the *Federal Register* on October 16, 1997 (62 FR 53908) and on November 19, 1997 (62 FR 61862).

Dr. Mickelson welcomed Mr. F. William Dommel, J.D., Director of Education, Office of Protection from Research Risks (OPRR), as a non-voting agency representative; and Inder M. Verma, Ph.D., Professor, Laboratory of Genetics, The Salk Institute, La Jolla, California, as a new RAC member.

Dr. Mickelson stated that the *Environmental Assessment and Finding of No Significant Impact* was completed, and that a notice of the availability of this document was published in the *Federal Register* on November 4, 1997 (62 FR 59720). She noted that the final action to promulgate the amendments to the *NIH Guidelines* regarding NIH oversight of human gene transfer research was published in the *Federal Register* on October 31, 1997 (62 FR 59032). Under the new *NIH Guidelines* the RAC no longer has approval authority for human gene transfer protocols. The RAC can review novel protocols and it can make recommendation(s). Any RAC recommendations may be forwarded to the NIH Director, the principal investigator, the sponsoring institution, and other Department of Health and Human Services components, as appropriate.

Dr. Mickelson stated that the proposed actions to be considered by the RAC are amendments to the *NIH Guidelines* regarding: (1) Institutional Biosafety Committee (IBC) approval of experiments involving transgenic rodents, (2) Appendix K regarding large scale production of human gene transfer vectors, (3) Section III-D-6 regarding experiments of more than 10 liters of culture, (4) Appendix M-I regarding the timing issue of IBC and Institutional Review Board (IRB) approvals, and (5) Appendix M-I regarding submission deadline of human gene transfer protocol submission to the NIH Office of Recombinant DNA Activities (ORDA).

Dr. Mickelson noted that two additional actions have been promulgated under the *NIH Guidelines*. These are the amendments to Appendix B, *Classification of Human Etiologic Agents on the Basis of Hazard*,

regarding classification of Flexal, Sabia, and Equine morbillivirus; and the amendments to eliminate submission requirements for a separate document of point-by-point responses to Appendix M, *Points to Consider in the Design and Submission of Protocols for the Transfer of Recombinant DNA Molecules into One or More Human Subjects (Points to Consider)* and the vector sequence diskettes. She reiterated that the *Federal Register* publication on October 31, 1997 (62 FR 59032) details the new RAC oversight function regarding human gene transfer research.

Dr. Mickelson noted that a RAC Forum on New Technologies will be conducted during the first day of the RAC meeting. The RAC Forum is meant to complement the Gene Therapy Policy Conference, and will address novel technologies of human gene transfer research.

II. RAC FORUM ON NEW TECHNOLOGIES

NOTE: Because of the highly technical nature of information presented during this forum, the minutes for the first day contain substantial amounts of verbatim language from the presentation materials. These minutes do not contain citations that would normally be used for such verbatim language.

II-A. Herpesvirus Vectors

Dr. Mickelson called on Dr. McIvor to introduce speakers for the RAC Forum on herpesvirus vectors. Dr. McIvor said that Dr. Roizman will briefly discuss the biology of herpesviruses and then describe his studies on replication-competent vectors. Dr. Wagner will present the subject of herpesvirus gene expression and latency, which is relevant to the safety issue of using these vectors. Dr. Breakefield will discuss her work on amplicon vectors. Finally, Dr. Glorioso will present his work on replication-defective vectors and diseases that are potentially treatable by herpesvirus vectors. The presentations will provide a better understanding of herpesvirus vectors to the RAC in preparation for reviewing forthcoming gene transfer protocols using these vectors.

II-A-1. Biology of Herpesviruses & Replication-Competent Vectors

Presentation -- Dr. Roizman

Dr. Roizman stated that he is a professor at the University of Chicago and is a consultant to Aviron, Inc. for vaccination against Herpes simplex virus (HSV) and to NeuroVir, Inc. for design of viruses for cancer therapy. His presentation consisted of two parts. Part 1 was on general properties of HSV. There are two types of HSV: HSV Type 1, which usually affects people above the waist; and HSV Type 2, which usually is viewed as causing infection to genital organs and is transmitted by physical contact. The viruses remain latent in sensory ganglia; on reactivation, the viruses may cause recurrent lesions and disease. The Herpesviruses can cause a wide range of diseases including significant morbidity in newborns, adult encephalitis, and recurrent lesions in the eye (which is a major cause of blindness). Herpesviruses encode 84 different proteins. More than half of the genes may be deleted without affecting the ability of the virus to replicate in tissue culture. This phenomenon is the basis for construction of replication-competent vectors.

Dr. Roizman explained the natural course of infection from these viruses. HSV Type 1 usually infects children at a very young age and causes dermatitis. The virus immediately enters nerve endings, and it is transported by retrograde transport to a sensory neuron where it establishes latency. In part of the infected population (10 to 40%) the virus reactivates. In individuals where reactivation occurs, the virus is transported to a site at or near the portal of entry. A flare-up during pregnancy may result in significant disease in the newborn child. If the reactivated virus enters the central nervous system, it will cause

encephalitis in adults.

Estimates of annual incidence in the U.S. population are: 500,000 new cases of genital infections, 10,000,000 people with recurrent infections, 500,000 episodes of herpes keratitis. Twenty to twenty-five percent of all U.S. deliveries are by caesarian section and approximately 10% of these are justified by fear of transmission of HSV to newborns. Yearly there are approximately 1,000 newborn herpes infections. Additionally, there are an unknown number of episodes of severe disease in immunocompromised individuals.

Dr. Roizman showed a schematic representation of the replication of HSV in susceptible cells. After entry of the virus by fusion with the cell surface membrane, the capsid containing DNA is transported to the nuclear port where it releases the DNA directly into the nucleus. The linear viral DNA is circularized, and it is transcribed by cellular enzymes to make viral proteins. There are three rounds of transcription of the viral genome. The first round is to make regulatory proteins, the second round is to make enzymes and factors which replicate viral DNA, and the third round is to make structural proteins of the virus. The virus particles are then assembled and are released out of the cell.

The viral genome has an unusual structure of inverted repeat sequences. There are 84 different genes encoding proteins, and five of them are repeated twice.

For over 15 years, Dr. Roizman's laboratory has developed and practiced a technique for making deletions of the virus genome. He found 45 of the viral genes can be deleted while still permitting viral replication. The remaining viral genes are essential for viral replication. He has constructed a series of minimally to maximally attenuated viruses.

There are 37 minimal essential genes. Four are for virus entry, envelope formation, and exocytosis; two are for regulation of gene expression; seven are for replication of viral DNA; eight are for capsid proteins including the protease; six are for packaging of DNA; and ten are for virion assembly and other functions. There are 47 supplemental essential (accessory) genes (they can be deleted in tissue culture infection but not for natural infection). Eleven are for entry in polarized cells, sorting, and exocytosis of virus in cells with fragmented and dispersed Golgi apparatus; five are for regulatory functions; four are for blocking host response; seven are for augmenting the precursor pool of DNA or for repairing DNA; one is for degrading cellular mRNA and facilitating viral regulatory cascade; 17 are for functions unknown; and two are suppressed during productive infection.

Dr. Roizman summarized his 1997 model to understand and categorize various viral gene functions. The first category contains standard functions: multiply in dividing cells, ensure gene expression, replicate viral DNA, assemble components into virions, and egress from the infected cell. The second category contains accessory genes that allow multiplication in any cell, augmentation of nucleic acid precursors, "turbocharged" gene expression, stimulation of the exocytic pathway, shut-off cell response to infection (block apoptosis, block response to double stranded RNA, block presentation of antigenic peptides), and enable the virus to remain latent.

In the second part of Dr. Roizman's presentation, he summarized his work on genetic engineering and characterization of attenuated HSV. He described two of the constructs in detail: R7020, a prototype of the first generation attenuated HSV; and R3616, a prototype of the second generation attenuated HSV.

The basis for constructing R7020 is deletion of internal inverted repeats, and U_L24, U_L55, and U_L56 from the HSV-1 genome. Since this construct was intended to be a vaccine against both HSV-1 and HSV-2 infection, three HSV-2 genes were included (genes encoding glycoproteins G, D, and I). The virus was

constructed at the University of Chicago in 1984. Pre-clinical studies were done at Institut Pasteur, France during 1984-1990. Phase I clinical studies were conducted at the University of Grenoble, France in 1990.

In this construct, Dr. Roizman said that it is important to retain thethymidine kinase gene so that an antiviral drug may be used in case adverse events are observed. There are several issues to be resolved for this first generation attenuated virus.

The first issue is genetic stability. Many of these viruses, when put under acute selective pressure, can develop mutations that compensate for the deletion and enable the virus to perpetuate. It is important to put the construct under maximum stress and maximum selective pressure to test for genetic stability. R7020 was inoculated into mouse brain and then amplified in cell culture. After nine passages, no change of viral genome was observed. Dr. Roizman noted that several other virus constructs had regained virulence after serial passages.

The second issue is to test the virus for its attenuation.Pathogenicity of the virus was tested in animal models, i.e., mouse (encephalitis), guinea pig (eye and vagina infections), rabbit (eye infection), and Aotus monkey and marmoset (viral infection). Latency, protection from virus challenge, and virus reactivation were also investigated. Dr. Roizman summarized his conclusions from the studies of R7020. (1) R7020 infection is not fatal in mice, guinea pigs, rabbits, and in the extremely susceptible non-human primates (owl monkeys and marmosets). (2) The virus appears to establish latent infections. However, recovery of the virus from sensory ganglia is extremely low. (3) R7020 protected mice, guinea pigs, rabbits, and non-human primates from lethal infection. In two highly susceptible non-human primates, it did not prevent the establishment of latency by the challenge virus. (4)Immunosuppression of owl monkeys before or after the administration of R7020 did not lead to virus reactivation or dissemination from the site of immunization.

Dr. Roizman summarized the conclusions from the Phase I clinical trials performed by French investigators. R7020 was tested in human volunteers. A dose up to 2.2×10^5 $TCID_{50}$ injected subcutaneously did not cause any side reaction in seronegative volunteers. In HSV-1 seropositive volunteers, lower amounts of virus caused mild to moderate local and systemic reactions which were not reminiscent of lesions caused by HSV infection. Approximately half of the seropositive volunteers shed wild-type virus in their saliva at some point during the study. R7020 was not detected in the saliva or any other samples collected from either seropositive or seronegative volunteers. Antibody titers to HSV remained unchanged in seropositive volunteers given R7020. Increases in antibody titers were noted only in seronegative volunteers receiving two injections of 2.6×10^4 $TCID_{50}$ or one injection of 2.2×10^5 $TCID_{50}$. The latter group also showed demonstrable neutralizing antibody.

Dr. Roizman described R3616, a prototype of the second generation of attenuatedHSV. The basis of this construct is deletion of the $\gamma_1$34.5 gene. It was constructed at the University of Chicago in 1989, and animal studies were conducted at the University of Alabama and the University of Chicago. The studies demonstrated that the function of $\gamma_1$34.5 gene is in protein synthesis to enable the virus to multiply itself.

Dr. Roizman concluded his presentation by pointing out several issues important for evaluating herpesvirus vectors. (1) Viral thymidine kinase should not be deleted from genetically engineered viruses. (2) Safety studies should include genetic stability, morbidity and mortality in rodents and Aotus monkeys (lack of symptoms in Aotus monkeys may indicate over attenuation in humans),and reactivation in immunosuppressed Aotus monkeys. (3) It is feasible to construct replication-competent viruses for human administration.

Other Comments

Dr. Verma asked if a repeat infection can occur in monkeys after inoculation with HSV. Dr. Roizman responded that wild-type virus will kill the very susceptible monkeys and the attenuated virus will establish latency. In rabbits under special conditions, repeat infection may occur.

Dr. Gordon inquired if the viral genome ever integrated into cellular chromosomes. Dr. Roizman responded that in normal infection the viral genome does not integrate into cell chromosomes. Integration may occur, however, if a piece of viral DNA is transfected in cell culture.

From a safety consideration perspective, Dr. McIvor asked at what frequency a thymidine kinase minus mutant would be generated in an infected animal. Dr. Roizman responded that it may arise at a very low frequency, i.e., 10^{-5} to 10^{-6}; and that the frequency would depend on experimental conditions. Dr. Straus added that the deletion mutants resistant to the antiviral drug Acyclovir do occur in clinical situations in immunosuppressed patients, but not in normal individuals.

Dr. Wolff inquired if animal studies are predictive of safety in humans, and if there are any concerns about high multiplicity of infection that might be used in gene therapy protocols. Dr. Roizman responded that safety concerns should take into consideration the route of virus administration, i.e., direct brain injection in animal studies vs. direct intratumoral injection in human subjects. Dr. Roizman noted that the Aotus monkey is a good model for safety testing.

Ms. Rothenberg asked about the purpose of the Pasteur Institute study involving human volunteers. Dr. Roizman responded that the design was to test 40 volunteers in an escalating dose study to determine if the attenuated virus could be used for vaccination (live wild-type viruses were tested in humans previously). The study involved both seronegative and seropositive individuals.

Dr. Straus inquired about the potential of vaccine strains to recombine with existing endogenous latent HSV-1 or HSV-2. Dr. Roizman responded that separate considerations should be given to genetically engineered viruses both without gene inserts and with gene inserts. For a construct without an insert, the consequence of recombination would be generation of the wild-type virus. For a construct with an insert, the probability for the recombinant virus to retain the insert is very low and, most likely, a wild-type virus will emerge. Dr. Roizman considered that the safety risk generally would be very low, but it needs to be assessed.

Dr. Aguilar-Cordova asked about the size limit of the insert. Dr. Roizman responded that a maximum of 7 kb may be inserted into the wild-type virus, but in a deleted virus 25 kb or more of DNA may be inserted into the viral genome.

Dr. McIvor inquired if there is a public health concern in utilizing the replication-competent viruses. Dr. Roizman responded that in experiments with Sentinel monkeys (not Aotus), and in guinea pigs and mice, the inoculated virus disappeared without horizontal spreading.

Dr. Matthew During (Auckland University, New Zealand) inquired if the genetically engineered virus may be over-attenuated for use in humans. Dr. Roizman responded that the Aotus monkey is very susceptible to HSV infection and it is easy to over-attenuate the virus so that it still survives in the Aotus monkey but will not survive in humans.

Dr. Mickelson thanked Dr. Roizman for his presentation.

II-A-2. Regulation of Herpesvirus Gene Expression & Latency

Presentation -- Dr. Wagner

Dr. Wagner noted that the work to be described has been contributed by the following scientists: M. Rice, G. Devi-Rao, J. Aguilar, N. Pande, M. Petroski, and P. Lieu of his laboratory (University of California-Irvine), and D. Bloom and J. Hill on studies of virus latency.

Dr. Wagner stated that productive replication of HSV Type 1, in its natural host or in tissue culture, involves a complex interaction of a number of cellular and virus-induced regulatory proteins directed towards orchestrating the regulated cascade of expression of upwards of 100 individual transcripts, each controlled by its own contiguous promoter. In contrast, latent infection of neurons requires cell-mediated restriction of this productive cascade resulting in the viral genome being transcriptionally quiescent, except for the expression of the latency associated transcript (LAT). This state is reversible in that stress-induced reactivation results in renewed replication of virus (presumably resident in neurons) at the site of initial infection. Such reactivation can result in grave sequelae, although it rarely does.

A major factor in the regulated expression of HSV genes is the regulation of transcription of individual transcripts mediated by the promoter controlling them. The most fundamental division in the transcriptional program is between latent and productive infection in the first place. There is also major restriction in transcription functioning during productive infection. A subset of viral genes important in priming the cell for viral genome replication, as well as accessory functions, is expressed abundantly prior to maximal levels of viral DNA synthesis. These early genes are shut off at later times. Conversely, viral genes involved in virion structure, assembly, and egress are transcriptionally quiescent or silent prior to viral DNA replication and are transcribed at high rates following this marker.

The molecular factors determining programmed expression of viral genes during productive and latent infection can best be investigated in the context of the viral genome. Viral transcription is mediated by cellular transcription machinery, and the latent/productive cycle "decision" is clearly mediated by specific interactions between host cell transcription factors and their cognate targets on the viral genome. Further, there is no evidence of significant or irreversible alterations in this machinery as productive infection proceeds. Factors involved in the differential expression of viral genes can operate at one or several levels within the infected cell. The most general or global factors would involve virus-mediated control of template availability as well as access to specific subsets of the cell's transcriptional machinery. Those architectural features of specific viral promoters that mediate their transcriptional activity at various times during the replication cycle constitute the most specific level of transcriptional control. Local environmental factors lie between these two extremes, and may have either specific or non-specific effects upon the ability of a given promoter to be expressed at a particular time during replication.

Dr. Wagner's laboratory has constructed and described a number of recombinational "cassettes" designed to introduce viral promoters and defined modifications thereof into the viral genome in order to study the *cis*-acting factors involved in the differential transcription of the viral genome during virus infection. Numerous controlled experiments comparing the expression of reporter genes controlled by various viral promoters and their wild-type cognates demonstrate that gross genomic location does not affect the kinetic properties of HSV promoters during productive infection. Similarly, with the promoters that he has investigated, Dr. Wagner finds no local effects upon relative levels of promoter activity as compared to the wild-type transcript. These conclusions are based upon studies of replicate isolates of recombinant viruses that have been rigorously tested by polymerase chain reaction (PCR) analysis to preclude wild-type contamination.

Detailed architectural analysis of model HSV promoters representing various kinetic classes reveals some class-specific features. Most notable among these are the lack of any *cis*-acting elements downstream (3') of the TATA box for early promoters. Further, these promoters contain a variety of general cellular transcription factor binding sites upstream of the TATA box. In contrast, strict-late promoters (which have essentially no transcriptional activity in the absence of viral genome replication) have promoters that extend from the TATA box through a putative "INR" sequence at the cap site to include a downstream element (called DAS by Dr. Wagner for *down-stream activating* sequence). The "INR" sequence is a transcriptional element located at the transcriptional start site. Late promoters that are measurably active in the absence of viral DNA replication have a more variable architecture but all appear to contain the "INR"-like sequence, a feature that is missing from early promoters.

A very simple model for the early-late switch in productive cycle transcripts might involve early promoters being "strong" and, thus, measurably active from limited numbers of viral templates, while late promoters would be "weak" and require high template copy numbers for measurable expression. This model can be eliminated with a number of experimental tests, but one of the most striking features is the demonstration that strict-late and leaky-late promoters are only slightly less active when used as templates in *in vitro* transcription assays.

Dr. Wagner suggests that the absence of transcription factor binding sites downstream of the TATA box of early promoters is important in the shut-off mechanism of such promoters following viral genome replication. In this context, it may well be significant that the "TFIID" complex has a footprint on template promoters which extends from the TATA box to a point about 30 bases downstream of the transcription start site, the very region where HSV DAS elements are found.

One important conclusion from these results is that there are no promoter elements that are unrepresentative of cellular elements. Further, the functional architecture of the viral promoters reveals that they represent classes of cellular promoters. Detailed characterization of one particular late promoter (controlling expression of the U_L38 gene) suggests that there are additional cellular DNA binding proteins which can serve to stabilize the interaction between the basal pre-initiation machinery and the viral template. In particular, Dr. Wagner has found that the DNA-binding component (Ku) of cellular "DNA-PK" is a very strong candidate for the cellular protein which binds DAS. Dr. Wagner has purified this protein to homogeneity using preparative DNA binding to DAS, and has shown that DAS is essentially identical to the "NRE1" element which binds this protein. "NRE1" also known as "nuclear response element 1" is a transcriptional factor binding site. Further, affinity purified Ku activates transcription from the U_L38 promoter. Finally, elements similar to DAS are found in a number of other HSV promoters of similar kinetics.

Based on the results obtained to date, Dr. Wagner concludes that template restriction and differential promoter recognition are major factors in the regulation of HSV gene expression. Even though the global activation of transcription by viral regulatory proteins is a major feature of HSV productive infection, the major feature of regulation of gene expression is the selective ability of the virus to restrict transcription to specific architectural motifs at various times following infection. The mechanism for this remains obscure, but must involve the global reorganization of the nucleus that is seen occurring concomitant with viral genome replication.

Turning to latent phase gene expression, which is representative of the most transcriptionally restricted stage of infection, only a single transcript is expressed -- the latency associated transcript, or LAT. This transcript does not express a protein during latent phase infection, yet its continued expression is clearly correlated with efficient reactivation in several *in vivo* models, especially rabbits. The mechanism of LAT action remains obscure, but it is clear that its role in reactivation is mediated by a very limited region of the

9 kb region, which is transcribed during latent infection. This region extends no more than 350 bases and is within the first 650 bases of the transcript. Of course, other functions besides reactivation may reside in LAT, but this is the only one for which Dr. Wagner has any biological marker at the present time. Whatever its mechanism of action, it does not appear to involve a simple restriction in levels of virus present in the latently infected neuron, a situation which may not hold true with all animal model systems. All available evidence indicates that LAT does function in the neuron.

The reason for the continued expression of the LAT promotor (a weak promotor during productive infection) during latent infection is not clear. Experiments with "ICP4-null" virus infection demonstrate, however, that the basis for this promoter weakness is not simply the presence of a strong "ICP4" binding site at the LAT cap which can be expected to suppress transcription during productive infection. Whatever the basis for neuronal-specific expression of LAT, it does involve a complex interaction between a number of *cis*-acting elements in the promoter and the basal transcriptional machinery of the differentiated neuron.

Dr. Wagner has carried out an exhaustive set of PCR-based analysis of viral LAT promoter mutants. From these analyses, it is clear that there is no evidence of other gene expression during latent infection in the absence of this promoter. This is important given that there are other viral transcripts which encode potential regulatory functions that are co-linear with LAT, and which use the same polyadenylation signal for termination. Further, it is clear that stress-induced reactivation of HSV in rabbits (or mice) leads to a transient expression of productive cycle transcripts. Dr. Wagner does not yet know whether this "wave" of transcription is a normal productive cycle cascade or one in which the primary expression of the immediate early gene products is abrogated. He sees evidence of normal alpha gene transcription in his studies, but others have reported that this is not seen in some murine reactivation studies.

One problem in such studies is that the extreme sensitivity of PCR-based transcription analysis can result in detection of very limited transcript expression. For example, infection of an "ICR4-null" virus in rabbit skin cells can lead to minimal expression of a late transcript even when infection has not progressed beyond the immediate early stage. This is hardly surprising given that the viral promoters are of cellular architecture, and template restriction may not be absolute. Despite the questions concerning the nature of the productive cascade induced by stress in latently infected neurons, it is clear that there is transcription in the R_L and elsewhere in the viral genome in the absence of LAT expression in latent infections. The role of such transcription in reactivation is not at all clear. Dr. Wagner suggests that LAT-negative virus may express less productive cycle transcripts upon stress-induced reactivation, but appropriate internal controls are difficult in animal studies and PCR-quantitation is not fully reliable.

Whatever the basis for LAT expression in neurons, the understanding of factors controlling it is of obvious importance in the consideration of and development of neuronal-expression systems based on HSV. A number of open questions remain and must be answered using recombinant viruses in the appropriate animal models. These include continued study of the mechanism of LAT-mediated reactivation. Two questions are notable: whether LAT can function in ectopic positions in the HSV genome, and whether it functions in *cis*- or *trans*-. Indeed, it is not yet fully established that the neuron is the sole site of LAT-mediated enhancement of reactivation.

Other direct questions include whether the LAT promoter is exploitable for use in vectors designed to provide continuous expression of an appropriate gene in the neuron. It is a weak promoter, and some of its continued expression may be a result of that weakness. It is not self-evident at this time that high level expression of any gene in neurons is compatible with latent phase restriction of transcription from the remainder of the viral genome. This will be an important question to answer in the future.

Other Comments

Dr. Gordon inquired about the recombinational "cassettes" promoters. Dr. Wagner responded that it is difficult to construct a viral promoter that will be regulated properly in terms of the early and late switch.

Dr. Lai asked why late genes are not expressed early in infection. Dr. Wagner explained that although the basic properties of transcription factors remain unchanged during the entire course of viral infection, the repertoire of transcription factors may be changed following viral DNA replication as the viral templates are replicated. Essentially, there is an exclusion of certain viral transcription factors from the replication centers late in infection.

Dr. Gordon inquired if the latency promoters may be relocated to result in sustained expression. Dr. Wagner responded that there are reports in the literature of such experiments, but it is difficult to get well-controlled gene expression with promoters in ectopic positions. Dr. Aguilar-Cordova asked if the LAT promoter also functions outside the context of the viral genome. Dr. Wagner said that there are no convincing data to demonstrate that the LAT promoter can function outside the viral genome. Dr. McIvor asked if LAT could be used as a diagnostic marker for latency. Dr. Wagner said that it is a complicated question because LAT expression cannot be definitively correlated with latency.

Dr. McIvor asked if early promoters would be more effective than using late promoters at providing high level gene expression for the purpose of constructing a gene transfer vector. Dr. Wagner said yes, that it is a reasonable hypothesis. Long-term gene expression *in vivo* in animals is, however, more complicated.

Dr. Lai asked if the LAT promoter needs other viral genes to function in neurons. Dr. Wagner explained that the LAT promoter seems to function on its own within the context of the viral genome.

Ms. Rothenberg inquired about the safety concerns of putting theherpesvirus vectors in humans. Dr. Wagner responded that a major problem is that it is not known how to clear the virus once it establishes a latent infection in the human brain. Unforeseen events could reactivate this virus. An animal model is necessary to evaluate the long-term consequence of such vector administration. Ms. Rothenberg asked how much time would be needed to acquire such safety data before human experimentation. Dr. Wagner responded that he does not have a definitive answer for that question.

Dr. Roizman noted that most people already harbor HSV Type 1 infection. Dr. Wagner agreed that most people have latent HSV infection.

Dr. Mickelson thanked Dr. Wagner for his presentation.

II-A-3. Herpes Simplex Virus (HSV) - Amplicon Vectors

Presentation -- Dr. Breakefield

Dr. Breakefield stated that HSV amplicon vectors are DNA plasmid constructs packaged in HSV-1 virions. These plasmid constructs bear two non-coding elements of HSV-1: the origin of DNA replication (ori_S), and the packaging/cleavage signal (*pac*). The advantages of this system are high infectivity for most cell types, including dividing and non-dividing cells; a large transgene capacity, typically 10 - 15 kb and potentially up to 150 kb; no expression of viral genes by the vector; relatively stablevirion particles; and ease of construction.

Recent advances that have increased the potential therapeutic use of these vectors include the ability to package vectors free of helper virus, the incorporation of other viral elements which promote the stability of transgene sequences in the host cell nucleus, and the use of regulatable promoters. In most studies to date, vectors have been packaged using replication-defective HSV helper virus in cells transfected with the missing essential viral genes, thereby generating both packaged vectors and helper viruses (vectors generated typically at 10^8/ml in a 1:1 ratio with helper virus). In the helper virus-free system, cells are transfected with a set of overlapping cosmids that span the HSV genome but which are deleted in *pac* signals so that they provide all viral functions. The resulting virus genome cannot be packaged in virions (vectors generated typically at 10^7/ml with no helper virus).

Regulatory issues in terms of virus stocks include the stability of constructs, the load of helper virus, and possible contamination with replication-competent virus. HSV vectors have not yet been tested in humans and many issues remain, including: toxic inflammatory responses to virus proteins; reactivation of latent wild-type virus; and in the case of encephalitis the effectiveness and complications of potential antiviral therapy. In general these problems can be avoided by using helper virus-free amplicon vectors, which should prove especially useful for gene delivery to neurons for the treatment of pain, nerve damage, and focal epilepsy.

Dr. Breakefield noted several key points in her presentation regarding HSV amplicon vectors:

1. Basics

Elements of design

·HSV origin of DNA replication - ori_S
·HSV packaging signal - *pac*

Packaging in HSV virions

·With helper virus deleted in essential immediate early genes and complementary cell line
·Without helper virus using a set of overlapping cosmids spanning the HSV genome, and deleted in *pac* signals

2. Variations

Combination with HSV recombinant virus vectors

·Piggy-back system
·Companion vectors

Incorporation of other viral elements

·Retroviral elements (long terminal repeat (LTR) flanked transgenes, *gag*/*pol*/*env*) to generate retrovirus vectors by endogenous cells *in vivo*
·Epstein-Barr virus (EBV) elements (*oriP, EBNA1*) to establish replicating episomes in host cells
·Adeno-associated virus (AAV) elements (inverted terminal repeat (ITR) flanked transgenes, *rep* gene) to allow for replication, amplification, and genomic integration in host cells

Control of transgene expression

·CRE = element to excise intervening sequences between *lox* sites and thus turn genes on in transgenic animals

Cell specific and regulatable promoters

·*Tet* system, glucocorticoid induction, and tyrosine hydroxylase

3. Future developments

Larger transgene inserts (up to 150 kb)
More efficient packaging or concentration of particles to achieve higher titers
Modification of virion

· Delete or modify toxic tegument genes
· Add ligands to glycoproteins to achieve cell specific targeting

4. Examples of therapeutic applications

Systemic applications

· Targeting liver, lung, and spleen after intravenous injection
· Protein replacement (larger promoters and genes)
· Vaccination
· Cancer therapy

Nervous system applications

· Peripheral nervous system - pain and nerve regeneration
· Central nervous system - neural degeneration, epilepsy, stroke, and brain tumors

5. Regulatory and safety issues

Regulatory issues for vector stock

· Stability of constructs, i.e., percentage of vectors containing the authentic construct
· Frequency of recombinations to generate replication-competent virus, i.e., relative content of wild-type virus
· Relative number of transducing particles per defective particles (important because "empty" virions still have some toxicity)
· Relative ratio of amplicon vectors to helper virus in helper virus packaging systems and piggy-back/companion systems
· Titers of amplicon vectors (especially important in nervous system because volume of vehicle can be toxic)

Safety issues *in vivo*

· Helper virus-free amplicon vectors intrinsically "safer" than recombinant virus vectors but with many of

the same concerns
· Possibility of reactivation of endogenous, latent wild-type virus with subsequent encephalitis or systemic viremia (How effective will systemic antiviral drug treatment be in blocking this toxicity? What toxicity can result from these antiviral drugs?)
· Possibility of shedding wild-type or recombinant virus to the environment
· Will pre-existing antibodies and cytotoxic T cells directed to HSV antigens cause toxic inflammatory responses? Will they markedly compromise the efficiency of gene delivery? (Patients should be tested for HSV antibodies before and during therapy as well as for HSV sequences in the central nervous system, blood, and urine. This testing should be accomplished by PCR analysis.)

Other Comments

Dr. Verma asked what percentage of the cells will be transduced at the injection site when injecting a small volume of virus (5 or 10 µl)? Dr. Breakefield responded that the efficiency of transduction can be increased by multiple injections at a given site, with efficiency approaching ten virus particles per neuron. Dr. Verma asked why the gene expression level goes down to 30 to 40% of the starting level as a function of time. Dr. Breakefield responded that there is no acute toxicity at the low multiplicity of infection. The gene expression level probably comes down due to some unknown configurational change of the DNA promoter, making it less accessible to transcription factors (as in most cases using the cytomegalovirus promoter).

Dr. Aguilar-Cordova asked if the ori_S sequences of the amplicon vector replicate with cell division. Dr. Breakefield responded that her amplicon vector does not replicate in phase with cell division. Other variations, using the EBV *oriP*, have been shown to replicate in phase with cell division for up to 20 cell generations.

Dr. Lai asked if the amplicon vectors replicate in target cells. Dr. Breakefield said that normally the virus in latency does not replicate unless it is reactivated.

Dr. Noguchi inquired if tandem repeats of several ITR sequences will prolong the duration of gene expression. Dr. Breakefield responded that she has not yet tried this kind of experiment. Some stable expression may be due to integration.

Dr. Roizman noted that in the case of wild-type HSV, the copy number of viral genome per neuron is relatively high, i.e., ten to 100 copies per cell.

Dr. McIvor inquired about the appropriate test to assess contamination of replication-competent virus in the amplicon vector preparations. Dr. Breakefield responded that the plaque-forming assay makes it relatively easy to detect any contaminating replication-competent viruses. Dr. Glorioso remarked that a very sensitive PCR assay (sensitivity of one in 10^9) can detect any recombinant viral mRNA that is not supposed to be produced by the vector.

Dr. Verma inquired why the *rep* gene of AAV is included in the amplicon vector since *rep* produces a toxic protein. Dr. Breakefield responded that at a multiplicity of infection of up to 10, no toxicity has been observed in the presence of *rep*. She speculated that *rep* under its own promoter control may be shut off after infection. Dr. Glorioso asked if there is any excision of the AAV sequences in the construct with *rep*. Dr. Breakefield answered yes, that the amplicon vector sequences can be excised and packaged at relatively high titer in the AAV particles.

Dr. Gordon inquired whether the frequency of integration depends on the presence of ITR and *rep* sequences of AAV. Dr. Breakefield responded that an amplicon vector with no AAV sequences has very low stable gene expression. Inclusion of both ITR and *rep* sequences greatly increases the frequency of stable gene expression in human cells.

Dr. Mickelson thanked Dr. Breakefield for her presentation.

II-A-4. Replication-Defective Herpesvirus Vectors and Diseases Potentially Treatable Using Herpesvirus Vectors

Presentation -- Dr. Glorioso

Dr. Glorioso stated that he would limit his presentation to the use of HSV vectors for cancer gene therapy. The studies to be presented were conducted by his laboratory at the University of Pittsburgh with collaboration of Drs. Neal DeLuca and David Fink.

Dr. Glorioso stated that using herpesvirus vectors has many advantages. The virus has been tested as a vaccine in humans in France and has a natural tropism to infect the central nervous system (CNS), a property that is useful for latent infection to express genes in CNS.

Dr. Glorioso summarized the advantages of HSV vectors as follows: compatibility with large gene inserts, very high titers, infecting a variety of cells, infection not dependent on cell division, natural tendency for persistence, and long-term gene expression in neurons.

Dr. Glorioso noted that there are a number of safety issues which need to be addressed in order to use HSV vectors for human gene transfer. Impediments to the use of HSV vectors are: cytotoxicity, antiviral immunity, inappropriate transgene expression, and targeting. Cytotoxicity and antiviral immunity may be advantageous to cancer therapy, but are problems of concern for long-term expression in other target cells. Inappropriate transgene expression and targeting is of concern for long-term expression, but is less of a concern for short-term vaccination purposes.

Dr. Glorioso outlined several potential therapeutic strategies of using HSV vectors for cancer gene therapy. The first strategy is cell killing by direct cytopathic effect of virus infection of tumor cells or to transduce the thymidine kinase (TK) gene to tumor cells to render tumor cells susceptible to prodrugs, e.g., ganciclovir. This strategy may be used in combination with other genes to induce inflammation or to improve tumor antigenicity. Another strategy is to use HSV vectors to deliver cytokine genes as *ex vivo* or *in vivo* cancer vaccine gene therapies. One *ex vivo* approach is to transduce dendritic cells to present the tumor antigens to the immune system.

Dr. Glorioso has concentrated his efforts to develop HSV vectors defective for replication. These vectors have been constructed by Dr. Neal DeLuca by removing the ICP4 gene essential for viral replication. ICP4 is an immediate early gene, and without it no other late viral genes can be expressed.

Dr. Glorioso described in detail the first generation HSV vectors. These viruses are highly cytotoxic and the infected cells only survive under very low multiplicities of infection, i.e., 0.1 or 1 virus per cell. In tissue culture experiments, there is a very good "bystander" effect in cell killing of uninfected cells with gap junctions. In a rat brain tumor model (employing the 9L human glioblastoma cell line), Dr. Glorioso did not observe any significant antitumor effect. He speculated that the vector is too cytotoxic to allow adequate expression of the TK gene.

A second generation HSV vector has been developed to reduce cytotoxicity of the virus. Additional genes other than ICP4 (ICP22 and ICP27) have been deleted. The triple deletion virus can be propagated in packaging cells with the complementing genes. These viruses have improved transgene expression due to reduced toxicity. The infected cells survive up to 14 days post infection and are transiently arrested in the G1 phase.

Dr. Glorioso presented data to demonstrate antitumor effects using the construct derived from second generation HSV vectors. A construct expressing tumor necrosis factor α (TNF-α. Ganciclovir has a synergistic effect in cell killing.

Dr. Glorioso described experiments on nude mice carrying transplanted L929 cells. There was a dramatic antitumor effect after injection of HSV vectors expressing both TNF-α and the thymidine kinase following ganciclovir treatment. The survival rate of the treated tumor-bearing mice was improved. Similar antitumor results were obtained from experiments with nude mice carrying U87 brain tumor cells injected into the brain.

To improve the tumor cell killing by the "bystander" effect, Dr. Glorioso constructed a virus expressing the gap junction protein, connexin 86. This construct augmented the bystander cell killing of cells lacking the gap junction protein, e.g., U87 cells. Dr. Glorioso said that animal experiments with a vector expressing three genes (TK, TNF-α, and connexin) are ongoing.

Dr. Glorioso said that the other strategy of cancer gene therapy is to introduce multiple genes that might improve immunogenicity of tumor cells as tumor vaccines. He has constructed a virus to express interleukin-2, human B7.1 co-stimulator, and the granulocyte macrophage colony stimulating factor (GM-CSF). This construct expresses the transgenes for up to seven days in cell culture. Using ß-galactosidase as a surrogate antigen for the animal experiments, Dr. Glorioso demonstrated immunological responses and antitumor effects using this triple gene vector.

Dr. Glorioso noted that HSV vectors are also useful for transducing dendritic cells as a means of effective antigen presentation.

In conclusion, Dr. Glorioso stated that HSV is a versatile vector for brain tumors and other tissue applications. HSV vectors can be constructed to be nontoxic and to be produced with no replication-competent virus. In addition, HSV vectors can express multiple transgenes with complementary functions. These HSV vectors will be useful for cancer therapy and vaccine applications that require only transient gene expression.

Other Comments

Dr. Aguilar-Cordova asked if a comparison study has been performed in the 9L mouse tumor model to compare the efficacy of TK expression from adenovirus vectors vs. HSV vectors. Dr. Glorioso responded that adenovirus can be produced with virus titers several logs higher than the HSV vectors. No direct comparison can be made at dosage levels used for adenoviruses.

Dr. Gordon inquired if there is any concern that the bystander effect will kill normal cells if the vector expressing connexin is administered to organs other than the brain, e.g., the liver. Dr. Glorioso did not believe it would pose any serious problem for the liver.

Dr. Lai asked Dr. Glorioso to explain how a TNF molecule expressed intracellularly, without binding to the extracellular domain of the receptor, can exert its cell killing effect. Dr. Glorioso responded that he is

conducting an experiment with a cell lacking the TNF receptor in order to answer such a question. Neutralizing antibody to TNF, however, does not inhibit the cell killing effect.

Dr. Roizman noted that using his highly attenuated HSV vector expressing TNF, he found that it is highly cytotoxic even at a low multiplicity of infection. Dr. Glorioso said that he did not observe such cytotoxicity with his own construct, but that he had no ready explanation for the different results.

Dr. McIvor inquired about the frequency of generating replication-competent viruses in the virus stocks and in the *in vivo* animal experiments. Dr. Glorioso responded that replication-competent viruses have never been detected in *in vivo* experiments. In tissue culture of cells containing complementing genes with homologous sequences, the frequency of recombination is one in 10^6 to 10^7 if complementing one essential gene and one in 10^{12} if complementing two essential genes. He said that the viruses with multiple gene deletions are very safe.

Dr. Breakefield asked whether the virus can still enter latency if three immediate early genes are deleted, and she inquired about the transduction efficiency using such a virus. Dr. Glorioso responded that the virus can enter latency. Transduction efficiency is good by direct infection of neurons, but is otherwise poor because the virus cannot replicate itself. Viruses with three or four early genes deleted express very little late viral gene products.

Dr. Aguilar-Cordova inquired if the complementing genes are stably transfected into the packaging cell lines. Dr. Glorioso responded yes. The genes are toxic to the cell and are expressed at a low level. Upon HSV infection, it activates the immediate early gene promoters to express the complementing genes at higher levels. Dr. Aguilar-Cordova inquired about potential safety concerns of using HSV for human trials. Dr. Glorioso responded that the degree of safety would depend on the specific application. For cancer gene therapy, an inflammatory response is beneficial.

Dr. Wolff inquired about pre-clinical safety testings in animal models. Dr. Glorioso explained that safety testing is a complicated issue. Toxicity of direct injection into the brain of animals needs to be assessed. Vaccination studies of animals, such as rhesus monkey, are complicated since these monkeys harbor viruses very similar to HSV. The Aotus monkey seems to be a good primate model.

Dr. Mickelson thanked Dr. Glorioso for his presentation.

II-A-5. Points to Consider for Safety Issues of HSV Vectors

Presentation -- Dr. Straus

Dr. Straus made a short presentation regarding the nine major points for consideration of the safety issues of HSV vectors, i.e., replication, stability, virulence, latency, shedding, reactivation, recombination, effectiveness of antiviral therapy, and seroconversion.

The first question is whether there is replication-competent virus in the virus stocks. The second issue is if the virus construct is genetically stable. The third issue is virulence in normal or immunocompromised hosts at different sites of injection. The fourth issue is latency. PCR analysis is a sensitive assay to detect latency. The fifth issue is virus shedding. It is an important issue for replication-competent viruses. The sixth issue is reactivation. The virus can be reactivated under certain conditions. The seventh issue is recombination. This is more of a concern for HSV constructs with transgene inserts because after recombination with wild-type viruses already present in the host, recombinant replication-competent

viruses with transgenes might emerge. The eighth issue is effectiveness of antiviral therapy.HSV vectors need to retain the TK gene for the effective use of antiviral drugs directed against this viral enzyme. Finally the ninth issue is seroconversion. Immunological responses to the products of virus genes and transgenes are important for ethical, safety, and therapeutic considerations.

Other Comments

Dr. Noguchi noted that safety issues such as latency, reactivation, and recombination are very complicated. He asked if there are any good suggestions for addressing these issues. Dr.Straussaid latency can be readily assessed by sensitive PCR analyses of LAT by *in situ* hybridization or by reverse transcriptase-PCR. Similar analyses can be developed for reactivation and recombination.

Dr. Straus considered a safety study to infect animals with high titers of replication-competent virus, and to recover the virus to infect animals already carrying a latent virus to see if there is any difference. Dr. Roizman said that he has conducted such an experiment. Normally one cannotsuperinfect the ganglia twice with the same wild-type virus. With attenuated virus, one cansuperinfect the ganglia but the wild-type virus efficiently outgrows the attenuated virus.

Dr. Ando inquired if radiation or prednisone will reactivate the latent virus similar to the stress-induced reactivation. Dr. Straus said that a variety of factors can reactivate the latent virus, e.g., fever, emotional stress, cytotoxin, prednisone, radiation, etc.

Dr. Lai inquired if DNA integration is an issue. Dr. Straus said most HSV vectors do not integrate into cellular chromosomes under normal infection. Dr. Roizman agreed.

Dr. McIvor inquired about the route of normal virus spread. Dr. Straus explained that HSV is normally spread by direct contact with mucus membranes or by contact with breaks in the skin of virus shedding individuals. The spreading pattern may be different if the vector is administered by direct brain injection or by intravenous injection.

Dr. Mickelson thanked Dr. Straus for his presentation.

II-B. Lentivirus Vectors

Dr. Mickelson called on Dr. Ando to introduce the speakers. Dr. Ando stated that Dr.Verma plans to present an overview of lentiviral vectors and describe the rationale for developing this type of retroviral vectors, as well as the molecular genetics and biology of lentiviruses. Dr. Naldini will describe in detail the development of replication-defective lentiviral vectors, and discuss manufacturing issues related to the development of vectors for clinical trials. Dr. Ando said thatlentiviral vectors should be considered as a second generation of human gene transfer vectors based on retroviruses. The first generation vectors are based on murine retroviruses.

Presentation -- Dr. Verma

Dr. Verma stated that the main attractions of thelentiviral vectors are their potential for sustained expression of transgenes. The model system chosen for his studies involves lentiviral vectors expressing the blood-clotting protein Factor IX for the treatment of hemophilia B. A dog model with Factor IX deficiency is available for pre-clinical studies. Factor IX is a secreted protein with extensive post-translational modifications. The post-translational modifications normally occur in the liver. If the protein is expressed as a transgene in other target cells, the protein must be appropriately modified in

order to be biologically active.

Dr. Verma said that, in his studies, Factor IX was first expressed withmurine retroviral vectors. In *ex vivo* mouse experiments, fibroblasts were transduced with the virus and the transduced cells were injected into the leg muscle of mice. Unexpectedly, it was found that Factor IXexpressionwas initially very high but it dwindled precipitously to a level approximately one tenth of the initial level. This observation pointed out a serious problem involving the use of retroviruses as a gene delivery vehicle in *ex vivo* gene transfer applications. Similar phenomenon of transcriptional shut-off has been observed in the transduction of myoblasts. Modifying the vector and replacing the viral LTR with other promoter/enhancers resulted in improved transgene expression in the mouse myoblast experiments. When similar experiments were performed with the dog model, however, transcriptional shut-off was again observed.

At first Dr. Verma speculated that the problems were associated with *ex vivo* gene transfer and subsequent transplantation of transduced cells back to the animals. About six years ago he attempted direct *in vivo* gene delivery using adenoviral vectors. The adenoviral construct expressing Factor IX was injected into mice intramuscularly. He showed data with nude mice demonstrating high levels of transgene expression. When the same experiment was performed with theimmunocompetent mice, antibodies directed against the Factor IX protein were detected. When the experiment was performed in the hemophiliac dog model, he observed an initial therapeutic effect of shortening the blood clotting time following a single injection of the recombinant adenovirus. Sustained expression, however, was not obtained due to immune responses developed against the virus. The antiviral immune responses also prevented repeat applications of the adenoviral vector.

Dr. Verma showed histological data demonstrating lymphocyte infiltration of the muscle tissue at the injection site and destruction of muscle cells transduced by the virus. He noted that the immune response is directed toward the viral proteins rather than thetransgene products because vectors expressing the homologous Factor IX of the same animal species, which is not immunogenic, elicited similar cytotoxic T cell responses. Antiviral immune responses were also observed with inactivated adenoviruses. Apparently, the virus particle per se is capable of inducing the response, and it is independent of viral replication. He noted that long-term transgene expression is problematic with adenoviral vectors.

To overcome the shortcomings of the retroviral and adenoviral vectors, Dr.Verma explored the potential of gene transfer vectors based onlentiviruses. Lentiviruses have the unique capability to infect nondividing cells. This is because in the viral cycle the proviral DNA, which is made in the cytoplasm after the virus enters the cell, has the ability to cross the nuclear membrane to enter the cell nucleus where it is integrated into the chromosomes of the host cells.

With slide illustrations, Dr. Verma explained the construction of the lentiviral vectors. Like the simpler retroviruses, the human immunodeficiency virus (HIV) has the*gag, pol,* and *env* genes, but it also carries genes for five accessory proteins termed*tat, rev, vpu, nef,* and *vif*. The *env* gene was substituted with the vesicular stomatitis virus (VSV) G protein to widen the host range of the virus. Most other accessory viral genes were deleted and the transgene was expressed under the control of the cytomegalovirus (CMV) promoter.

Dr. Verma presented data showing that the lentiviral vector is capable of infecting nondividing cells. He noted that the titers oflentiviral vectors in the supernatants is about 10^5 to 10^6 virus particles per ml, approximately the same as that obtained with murine retroviral vectors. In tissue culture,lentiviral vectors are capable of infecting cells arrested at the G1/S phase of cell cycle whereas as amurine retrovirus is ineffective. Viral infection is dependent on the integrase, whichfacilitates viral DNA integration, because a mutant defective in integrase is unable to infect cells either dividing or nondividing. A HIV-based vector is

also capable of infecting macrophages.

Dr. Verma showed data demonstrating lentiviral infection by direct *in vivo* administration to rodents. The virus was concentrated to a titer of 10^8 to 10^9 virus particles per milliliter, and subsequently injected into the brain to determine if nondividing neurons would be transduced. Using a confocal microscope, he demonstrated that the transgene was expressed in neurons using a lentiviral vector, whereas a murine retroviral vector expressed its transgenes only in glial cells but not in neurons. In terms of transduction efficiency, he noted that nearly 80 to 85% of the cells were transduced within 2.5 to 3 mm of the injection site. Again, the long-term expression is dependent on viral DNA integration. A sustained long-term expression of up to six months or even one-and-a-half years has been observed. A sustained expression of over six months was similarly observed when the lentiviral vector was injected into rodent liver, muscle, eye or pancreatic islet cells.

In terms of immune response, Dr. Verma noted that in the rodent experiments, no CD4 or CD8 cell infiltration occurred at the injection site (3×10^7 virus particles were injected). Repeated injections at the same site resulted in transgene expression at the same level, observed after the first injection.

Dr. Verma demonstrated that a lentiviral vector is capable of transducing a variety of different cell types within the retina of the eye if a proper promoter is chosen. He attempted to correct a retina gene deficiency in a strain of mutant mice.

Dr. Verma noted that lentiviral vectors with deletion of several accessory viral genes retain most of the desirable properties of the vectors. He said that most of the pre-clinical studies have been performed by transient transfection of viral DNA, but suitable virus packaging cell lines have been developed recently. The packaging cell lines contain a tetracycline inducible gene encoding the VSV G protein, which is cytotoxic to the cells.

Dr. Verma noted that the dog model of Factor IX deficiency has many limitations, and he has constructed a gene knock-out mouse model by deleting the gene encoding the catalytic domain of Factor IX. The knock-out mouse model should prove to be useful for the study of gene therapy of hemophilia. He emphasized that animal research is vital to medicine.

In conclusion, Dr. Verma outlined some of the future challenges of gene transfer research: (1) Gene delivery system. An efficient and safe gene delivery system is needed. (2) Transgene expression. Much more needs to be learned about gene expression and regulation. (3) Immune problems. Both humoral and cellular immune responses against viral and transgene products are important. (4) Cell biology. Much of the stem cell biology remains a big challenge.

Dr. Mickelson thanked Dr. Verma for his presentation. She noted that Dr. Harold Varmus, the NIH Director, was in the audience during the presentation.

Presentation -- Dr. Naldini

Dr. Naldini said that his work on lentiviral vectors began while he was in Dr. Verma's laboratory at the Salk Institute. His presentation covered the technical details of the development of lentiviral vectors and the production of the vectors for human gene transfer research.

Dr. Naldini stated that lentiviral vectors are replication-defective, hybrid viral particles made by: (1) a minimal set of core proteins of HIV-1, (2) the envelope protein of an unrelated virus, either VSV or the

amphotropic murine leukemia virus, and (3) a genome of the transfer vector containing an expression cassette for the transgene flanked by *cis*-acting sequences of HIV-1 without any viral gene. The use of a heterologous envelope protein broadens the tissue tropism of the vector, and makes the generation of wild-type virus impossible during vector production.

The lentiviral vector was initially developed by Luigi Naldini, Didier Trono, and Inder Verma at the Salk Institute for Biological Studies, La Jolla, California, in collaboration with the laboratories of Fred Cage also at the Salk Institute, and Richard Mulligan at the Whitehead Institute, Cambridge, Massachusetts.

After injection into the brain of adult rats, the vector efficiently transduced neurons and achieved long-term expression of the transgene in the absence of detectable pathology. Animals analyzed ten months after a single administration of the vector, the longest time tested so far, showed no decrease in transgene expression and no sign of tissue pathology or immune reaction.

For use in clinical applications, it is important to prove the biosafety of lentiviral vectors and to generate a producer system suitable for certification and scale-up. Dr. Naldini's laboratory has defined a minimal set of sequences necessary for efficient transduction *in vitro* and *in vivo*. This minimal set of sequences is comprised of the following: (1) An HIV-derived packaging construct in which the viral *cis*-acting sequences and the *env*, *vif*, *vpr*, *vpu*, and *nef* genes, all critical virulence factors, have been deleted (minimal core packaging construct). (2) A separate construct encoding a heterologous envelope. (3) A transfer vector containing minimal HIV-derived *cis*-acting sequences, and the 5' LTR with or without the enhancer/promoter in the U3 region. The 3' LTR was deleted (self-inactivating vector). The removal of five viral genes and LTR sequences essential for HIV pathogenesis eliminates the possibility that replication-competent retroviruses (RCRs) might arise during vector production. After direct injection *in vivo*, the vector produced by the minimal packaging construct was as efficient at delivering transgenes into neurons as one derived from a wild-type construct. After injection into the portal vein of rats (in collaboration with M. Kay of University of Washington, Seattle, Washington), integration and dose-dependent transduction of hepatocytes was observed without signs of liver or serum pathology.

The minimal packaging construct was stably introduced into 293 and 293G cells (expressing the VSV G protein). The 293G packaging cell clones were used to generate stable producers by infection with the transfer vector. Upon induction of VSV G protein expression, these cells released high titer vectors.

Dr. Naldini is developing assays to screen for the presence of replication-competent and partial recombinants in lentiviral vector stocks. Because the only features of the parental virus shared by any RCR would be those dependent on the *gag* and *pol* genes, RCR monitoring is performed by HIV p24 Gag immunocapture assay or HIV-1 *pol* RNA detected by PCR analysis. Any RCR that emerged would be sensitive to anti-HIV drugs that target reverse transcriptase or the viral protease.

The availability of efficient vectors made from only a minimal set of HIV sequences, stable producer systems, and sensitive assays to screen for recombinants should now pave the way to the clinical testing of lentiviral vectors.

Dr. Mickelson thanked Dr. Naldini for his presentation.

Other Comments

Dr. McIvor asked if the genes of the newly introduced proviral DNA in the cell nucleus are expressed without integration. Dr. Naldini showed data on the vector construct carrying an inactivating mutation of the integrase gene. In *in vivo* experiments, there is no gene expression. When infecting HeLa cells in

tissue culture, the mutant virus produced unintegrated proviral DNA and there was short-term gene expression at low levels but it disappeared upon cell division. The stable expression of transgenes requires proviral DNA integration. Dr. Verma confirmed that long-term gene expression comes from the integrated proviral DNA.

Dr. Aguilar-Cordova asked about the minimal *cis*-acting sequences required for the packaging cassette. Dr. Naldini responded that the nucleotide sequences at the beginning of U3 of the LTR are strictly required, but the five viral genes most associated with HIV pathogenesis can be deleted. Dr. Verma explained that certain *cis*-acting sequences can be removed, but *gag/pol* and integrase genes are essential and these genes have to be in a *cis* configuration. Dr. Verma emphasized that this feature is a key point in the construction of lentiviral vectors.

Dr. Lai inquired if the error frequency of HIV polymerase is of concern. Dr. Verma responded that once the proviral DNA is integrated, transcription will result from the cellular polymerase. Dr. Lai asked why there is no immune response when the vector is expressing its transgenes at a high level in muscle cells. Dr. Verma responded that there is no immune response to the Factor IX protein. Antigenicity is dependent on the type of transgene product.

Dr. Wolff inquired about the animal model used to test for HIV vector toxicity. Dr. Naldini responded that this question requires a great deal of discussion. In his opinion, using the simian immunodeficiency virus to test for toxicity in monkeys is not directly relevant to HIV. Dr. Wolff asked if there is any toxicity associated with inactivated viruses similar to that observed with inactivated adenovirus particles. Dr. Verma responded that the virus titer of the HIV vectors tested for toxicity is not high enough to elicit a cytotoxic T-cell response.

Dr. McIvor asked for further clarification regarding the integrase negative mutants. The lack of viral DNA integration may be due to a defect in the transport of the proviral DNA into the cell nucleus rather than due to failure of integration per se. Dr. Naldini agreed that the data do not allow such a distinction. Dr. McIvor asked if a wild-type virus can rescue the lentiviral vector upon superinfection. Dr. Naldini responded affirmatively.

Dr. Glorioso inquired whether there are certain cell types that the lentiviral vectors are unable to infect. Dr. Verma responded that, in terms of relative efficiency of infection, the brain is the best; the liver, the muscle and the eye are good; other target tissues such as hematopoietic cells and the lung are relatively poor. Dr. Verma noted a species difference, e.g., expression in rats is better than in mice.

Dr. McIvor asked Dr. Verma to elaborate on the data on hematopoietic cells. Dr. Verma said that in *in vitro* studies, lentiviral vectors appear to be better than the murine retroviral vectors. Experiments on *in vivo* reconstitution of the transduced cells are in progress.

Dr. Mickelson inquired if there is any concern about germ line infection. Dr. Verma responded that such issues need to be investigated.

Dr. Wolff asked if there are any pre-clinical studies using large animals. Dr. Naldini said that most studies have been conducted with rats or mice.

Dr. Gordon asked a follow-up question on germ line infection. He inquired if any experiments have been performed with early pre-implantation embryos. Dr. Verma said that such experiments are needed and that chick embryo experiments are ongoing. Dr. Gordon was concerned about the hazard of inadvertent infection of the pre-transplantation embryos in *in vitro* fertilization clinics. Dr. Naldini responded that safety

studies aimed at *in vivo* delivery of the lentiviral vectors are being conducted. Dr. Verma noted that all the lentiviral experiments are conducted in Biosafety Level 3 facilities to avoid inadvertent infection.

Dr. Aguilar-Cordova inquired about the safety considerations of production facilities, particularly for a vector with a broad host range. Dr. Verma responded that lentiviral vectors are debilitated viruses and have little environmental concern. No RCR have been observed in any of the production batches of the lentiviral vectors.

Dr. Lysaught inquired if there is any concern about virus shedding, e.g., rescuing the vectors in the HIV-infected patients. Dr. Naldini said that sensitive assays for RCR may detect any rescue or shedding of lentiviral vectors. Dr. Lysaught inquired if there is any concern about the high mutation rate of HIV. Dr. Verma explained that the reason for replacing the HIV envelope with the VSV G protein is to broaden the host , rather than due to concern of mutation of the HIV envelope gene. Dr Naldini noted that the high HIV mutation rate is due to its high rate of viral replication. The lentiviral vector can only have one round of reverse transcription when administered to the target cells.

Dr. Leinwand inquired if there are any data comparing the stable gene expression between the lentiviral vectors and the adenoviral vectors at the same dosage level. Dr. Verma explained that such comparison is not meaningful because the adenoviral vectors are used at a much higher dosage, and there is no significant gene expression from adenoviral vectors at the titers used for lentiviral vectors.

Dr. Lai asked if the VSV G has any tropism for T cells and if there are any cytopathic effects. Dr. Verma said that the virus can infect T cells, and in the short-term it does not appear to be cytotoxic to T cells. Dr. Naldini said that there is a dose-dependent cell fusion effect.

Dr. Mickelson noted that more than 80% of the HIV genome has been deleted from the lentiviral vectors, and yet the vectors still retain the properties of transducing cells at high efficiency. Dr. Verma said that the advantage over the murine vector is that the lentiviral vectors can infect nondividing cells. Dr. Naldini explained that the mechanism may be related to the nuclear localization signal of the HIV integrase, and nuclear transport of the viral DNA may involve the complex with the *gag/pol* gene products.

Dr. During (Auckland, New Zealand) asked about the sustained gene expression in brain under the transcriptional control of the CMV promoter. Dr. Verma said that, in reference to the murine retroviral vectors, the lentiviral vectors are capable of infecting nondividing cells whereas the murine viruses are not capable of doing so. The lentiviral vectors can be effectively administered by direct *in vivo* injection whereas the murine vectors depend on cell division. Therefore, direct infection by murine retroviral vectors *in vivo* is not effective. However, both types of vectors exhibit transcriptional shut-off following transplantation in *ex vivo* applications.

II-C. Human Artificial Chromosomes

Presentation -- Dr. Ashloc

Dr. Ashlock presented an overview of the basic strategy for artificial chromosome construction, and th technical safety problems of utilizing this new technology for human gene transfer research.

Dr. Ashlock noted that the study of artificial chromosomes will define the minimal sequence requiremer for functional human chromosomal elements, and will provide improved understanding of chromosome behavior. Another objective of the study is to achieve stable gene transfer.

The minimal functional elements of human artificial chromosomes are thought to be similar to those required for yeast artificial chromosomes (YACs), i.e., centromeres , telomeres, and origins of replic YACs have proven very useful in introducing full length genes under the control of their own regulator elements to study gene expression and gene function. However, the functional elements of human chromosomes are less well defined. The origin of replication remains to be defined. The centromere i thought to be alpha satellite sequences, and the telomere is the best characterized of the three elements. The function of the telomere is to protect the ends of the chromosomes during chromosome replication.

The potential advantages of human artificial chromosomes for gene transfer include the large capacity for DNA inserts, the ability to replicate and segregate independently like human chromosomes without the risk of insertional mutagenesis, and the stability of long-term gene transfer in replicating cell

Dr. Ashlock showed a slide illustrating the strategy of constructing a human artificial chromosome. I consists of telomeres, the origin of replication, a target gene insert, and a selectable marker. The construc will be introduced into a somatic cell during mitosis, and it will segregate like other chromosomes during cell division.

Dr. Ashlock noted two approaches for artificial chromosome construction: the top-down approach and t bottom-up approach. In the top-down approach, a chromosome is fragmented and it is transduced to a together with a plasmid vector containing a sequence homologous to the centromere , a selectabl marker, and a telomere sequence. A minimal functional artificialchromosome can then be selected. Th human artificial chromosome generated by this approach is not very stable and rearrangements frequently occur. It is a difficult task to characterize a chromosome generated by this method.

Dr. Ashlock described a bottom-up approach, which has recently been used successfully by J. J Harrington *et al*. to construct a human artificial chromosome (*Nature Genetics, 15*, 345-355, 1997). Harrington *et al*. used synthetic DNA to mimic the centromere sequence. The investigators also include pieces of natural DNA from human chromosomes. These DNA pieces were injected into cells where they self-assembled into chromosomes with the help of cellular enzymes. The transfected cell clones wer analyzed to see if they contained *de novo* chromosomes. In one experiment that lasted six months, genes on an artificial chromosome were still present and functioning in daughter cells after the parent cell had divided about 240 times.

Dr. Ashlock described the strategy used in her own work employing the YAC as a basis for makin human artificial chromosome. The YAC was retrofitted with human chromosomal elements. Sh presented data of her work on construction of human artificial chromosomes.

Dr. Ashlock noted that there are formidable technical obstacles remaining to be overcome in order t make this new technology useful for human gene transfer applications. It is too soon to tell when this new technology will be developed as a therapeutic modality. The safety issues will be redefined as the technology advances. The immediate potential benefit of this new technology is to learn more about human chromosome structure and function, and gene expression studies in tissue culture settings.

Other Comments

Dr. Gordon inquired about the stability of human artificial chromosomes in the absence of selection. Dr. Ashlock responded that, in her study, they are quite stable for up to 150 generations in the HT108 human tumor cell line.

Dr. Noguchi asked if some portion of cellular DNA has to be deleted before the extrachromosome can

introduced into the HT1080 cells. Dr. Ashlock said that she is conducting studies in mouse cells t answer this question.

Dr. Yung Chang (Genetic Therapy, Inc.) asked if there is any concern about homologous recombination between the artificial chromosome and endogenous chromosomes. Dr. Ashlock responded that the iss has not been fully investigated to date.

Dr. Lysaught asked if a chromosome without the centromere or the telomere can be reproduced in Dr. Ashlock said that extra chromosomes associated with certain human diseases have been investiga to identify the minimal essential elements.

Dr. Mickelson inquired about the species specificity for centromeres and telomeres. Dr. Ashlock exp that mammalian telomere sequences are the same between species. However, the alpha satellite sequences of the centromere from the human cells are not stable in murine ce

II-D. "Gene Shuffling"

Presentation -- Dr. Howard

Dr. Howard of Maxygen , Santa Clara, California stated that his presentation has two goals in mind. Th first goal is to provide information to the RAC on a new technology for the creation and evolution of DNA sequences in genes, and the potential commercial exploitation to produce new therapeutic proteins and develop new gene transfer vectors or vaccines. The second goal is to begin a dialogue with the RAC in terms of safe development of this new technology.

Dr. Howard noted that the biological properties embodied in a pharmaceutical protein, a DNA based vaccine, or a gene therapy vector are many and complex and generally not completely understood. Recombinant techniques generate improved forms of DNA by testing variants, usually generated by site-specific or random mutagenesis, and selecting those that express a desired trait. "Molecular breeding," also called DNA shuffling, is a new way for generating desired traits that parallels evolution in nature.

In molecular breeding, diversity is generated through a combination of random, low-level mutagenesis and recombination, without *a priori* assumptions on what part(s) of a gene, plasmid, or virus might b important for the desired phenotype. Maxygen , Inc, has demonstrated the ability to recombine and exp genetic diversity in suitably homologous genes from different organisms, regardless of how closely related the organism might be. A library of "shuffled" genetic material is generated and subjected to a selection or a screen to identify a population of variants that express the desired properties. The genetic material from 20-100 improved variants is "shuffled" again and submitted to reiterative rounds of screening/selection with increasing criteria for success. The quality of the output from molecular breeding is directly related to the properties of the selection or screen.

The technology has greatest competitive value when a suitable assay can be designed to identify the desired phenotype and there is little or no mechanistic understanding to guide a rational approach on how to achieve this goal. This holistic and empirical approach to generation and identification of novel DNA variants is being applied to the generation of novel DNA vaccine plasmids, novel gene therapy vectors, new viral disease models, novel cytokines, novel vaccine antigens, and novel enzymes for gene therapy. The RAC is invited to consider the implications of this technology for the safe discovery and use of novel therapeutics to meet important medical challenges.

In conclusion, Dr. Howard showed a slide illustrating the difference between his company's molecular breeding technology and the technology involved in other classical animal and plant breeding or classical biochemistry and molecular biology approaches. He highlighted the major advantages of molecular breeding as follows: holistic, *a priori* understanding of mechanism is not required but can be exploited, rapid/inexpensive, focused selection/screening, tremendous breath of diversity generated, unexpected and multiple solutions, diversity generated by *in vitro* analogs of natural processes by mutation and recombination, pre-existing natural diversity exploited, and backcross exploited.

Other Comments

Dr. Verma inquired about the stability of the modified molecules. Dr. Howard responded that the inhere process of selection in *E. coli* is selection for stability and, in several cases, the modified molecules are stable in mammalian cells as well. Dr. Verma asked if the modified molecules are more immunogenic. Howard responded that immunogenicity can be increased for vaccine uses and reduced for enzym products that are intended for industrial uses.

Dr. Lai asked if there is any statistical basis for predicting that favorable mutations will be generated, e.g., modified interferon. Dr. Howard said that there is no statistical prediction of the outcome. In his studies of alpha interferon, one in eight of the modified molecular species has better biological activities than the parental molecule.

Dr. Leinwand asked that if there is any improvement with genes that are highly conserved among spec Dr. Howard responded that they have molecularly-evolved highly-conserved genes, e.g., antibodies, thymidine kinase , beta galactosidase

Dr. McIvor inquired if a microbial screening system rather than a mammalian system is needed to screen large numbers of modifications. Dr. Howard stated that he has performed mammalian cell screening of a membrane protein using a fluorescence-activated cell sorter. Screening in *E. coli* is much more efficient.

Dr. Gordon inquired if there is any strategy to find out what other characteristics may inadvertently occur ir addition to the properties intended by the selection process. Dr. Howard mentioned that, in some experiments, a particular enzyme was bred for a particular specificity and serendipitously obtained a new specificity. In another instance with a herbicide degradation pathway a new specificity against another herbicide was obtained serendipitously. For therapeutic purposes, it is important to perform pre-clinical safety testing of the new products before any human testing.

Dr. Verma inquired about the experiment with HIV. Dr. Howard explained that the experiment is to d shuffling in *E. coli* and then replicate the new virus in a double transgenic mouse either *in vitro* or *in vivo* in order to obtain HIV that will infect the mouse model.

Dr. Noguchi asked about the use of this technology to modify organisms. He asked whether more virulent organisms could be created and, secondly, whether empty gene transfer vectors (such as adenovirus) could be generated. Dr. Howard responded that the first issue is a much broader question that has more than just scientific implications. In response to the second issue, he said this methodology could be employed, as an alternative method, to create a useful vector without presumption about which genes may be deleted.

Dr. Wolff asked if molecular breeding could be used to create a pathogen that is more powerful than its naturally occurring strains. Dr. Howard responded that the experimental settings of molecular breeding have the potential to generate commercial products or pathogens more powerful than that generated by

nature. Dr. Wolff inquired if all these molecular breeding experiments should be performed under the Biosafety Level 3 physical containment facilities. Dr. Howard responded that these important questions have been discussed by his IBC. In terms of viruses, there is an enormous difference in the selective pressure faced by a virus growing in the relatively undemanding environment of cell culture. Life in the real world is more demandingbecause the virus must survive in a specific environment and within a ho providing a series of constraints and selective pressure. In gene shuffling of a virus, only a few properties are selected. Dr. Howard stated that he would welcome any RAC comments regarding the safety issues.

Dr. Mickelson noted that in the HIV experiment the requirement for transmission within the population may be different than in the laboratory situation. Dr. Howard agreed that biosafety is his major concern, an that his company has highly-trained personnel to handle pathogenic agents. Dr. Noguchi sounded a note of caution in the development of this new technology.

III. Food and Drug Administration (FDA) Presentation: Discussion of the Risks of Gonadal Distribution and Inadvertent Germ Line Integration in Patients Receiving Direct Administration of Gene Therapy Vectors

III-A. Industry Perspective

Presentation -- Dr. Ledle

Dr. Mickelson called on Dr. Fred Ledley (formerly of Variagenics and GeneMedicine , Houston, T present industry perspectives regarding the issue of gonadal distribution of vector sequences. Dr. Le stated that his presentation was not intended to represent the viewpoints of either company on this issue, but rather to frame the topics for the subsequent discussion.

Dr. Ledley said that gene therapy research is going to produce new and better therapies for commo diseases, e.g., cancer, vascular disease, and infectious diseases. The intent of industry-sponsored research is to cure these common diseases. Eventually gene therapies should be made applicable and acceptable in routine clinical practice. At that time gene therapy likely will be indistinguishable to the patients as just another conventional therapy. The gene therapy products should be pharmaceutically-acceptable and approvable by FDA. In his view, they are not different from the protein products made from recombinant DNAs

In terms of approval of gene therapy products, Dr. Ledley said that there must be an empirical discussi rather than a theoretical discussion. The assessment of toxicity in gene therapy products should adhere to precedents used in the assessment of toxicity in conventional drugs and biologics. The key principle in the laboratory is to use validated tests, and in the clinic to have a quality informed consent process. There should be no difference in clinical ethics for an end-stage disease and an non-end-stage disease. In his opinion, the informed consent is more valid from a patient who has no end-stage disease.

With regard to the issue of bio-distribution to the gonads of gene transfer vectors, Dr. Ledley stated tha is not surprised that such DNA sequences can be detected in many tissues using the highly sensitive techniques such as PCR analysis. Some vectors such as retroviruses are designed to be integrate within the chromosomes of host cells. Nonviral vectors are mostly designed to be episomal . Find plasmid DNA vector sequences in gonads by the highly sensitive PCR assays may not necessarily hav any functional significance, e.g., vertical transmission.

Dr. Ledley emphasized his view that the RAC is not a committee charged to review the scientific merit. believes that the primary purpose of the RAC is to evaluate the risk and benefit ofapplications of th

recombinant DNA technology. It is important to acknowledge that there are risks associated with these applications, and one should avoid unrealistic expectations when the technology is applied to treat patients. Attempts should be made to quantify the risks by laboratory and animal studies. Eventually, the human risks should be evaluated in clinical trials. The risk of gene therapy products should be assessed using the standards accepted for conventional pharmaceuticals. No higher standards should be used for gene therapy products than for conventional drugs and biologics. Gene therapy products should be allowed to compete effectively with other conventional products.

Other Comments

Dr. Mickelson inquired if any vector sequences have been detected in any clinical trials. Dr. Ledley responded that there is no example of human data. In animal experiments, DNA sequences administered as plasmid DNA/liposome complexes have been detected in gonads but there was no evidence of integration in germ cells.

Dr. Gordon noted an experiment reported in the literature involving a Moloney murine leukemia viru was deliberately introduced into murine embryos, which resulted in animals harboring the integrate proviral DNA. He noted that some of these animals displayed chronic viremia , but that no viral DNA ever detected that reintegrated into additional integration sites other than the original integration event. He suggested that the risk of viral DNA integration is relatively low. Dr. Ledley agree

Ms. Rothenberg inquired if any follow-up autopsies have been performed in human gene transfer trials to resolve the issue of vector DNA distribution in gonads. Dr. Ledley said that he does not have suc information.

Dr. Verma noted that protein products such as Factor IX do not have the same issue of gonad distribution of vector DNA sequences. Dr. Ledley noted that Factor IX from human blood has othe contamination issues involving HIV.

Dr. Macklin was concerned that most of the Informed Consent documents she has reviewed for IRBs a not satisfactory. Dr. Ledley agreed that this is an area that needs improvemen

Mr. Dommel noted that Federal regulations protecting human subjects are meant to protect livin individuals, and in most cases do not grant authority to require autopsy for participants of clinical trials. However, an IRB may require autopsy for a particular experiment if there is anticipated benefit fro performing such autopsy. Dr. Ledley noted that the patients or their families have the right to withdra their permission for autopsy.

Dr. Gary McGarrity (Genetic Therapy, Inc.) noted that his company has presented autopsy dat demonstrating that no vector sequences have ever been detected in any autopsy. Dr. Ledley said tha such empirical data is more important than a theoretical discussion.

Dr. French Anderson (University of Southern California, Los Angeles) stated that in his collaborative study with Dr. Steven Rosenberg (Protocol #8810-001), involving ne^R gene marking of tumor infiltrating lymphocytes, extensive autopsies were performed. No evidence ofvector sequences in gonads was fou in either the testes of male subjects or the ovaries of female subjects.

III-B. Gonadal Distribution and Risks of Inadvertent Germ Line Alterati

Presentation -- Dr. Bauer

Dr. Bauer stated that, in the past, gene therapy treatment generally has been restricted to patients with serious or life-threatening conditions. Sponsors of gene therapy clinical trials are increasingly interested in gene therapy for less serious disease, for earlier intervention before manifestations of disease, and for augmentation or enhancement. In contrast to earlier studies, patients in these proposed studies may be of child-bearing age. Multiple pre-clinical safety studies have shown unexpected persistence of nucleic acid vectors in animal gonadal tissu

According to Dr. Bauer, the FDA has to weigh the risks and benefits of each Investigational New Drug (IND) application in the context of the disease and the patient population that will be subjected to gen therapy. Most of the studies to assess bio-distribution of vectors have been performed in animals, and in some instances gonadal distribution has been detected using a variety of vectors, formulations, an routes of administration. Dr. Bauer said that the purpose of his presentation was to make the public aware of this observation, and to stimulate public discussion. The FDA's concern is the potential risk for inadvertent germ line alteration.

Dr. Bauer noted that there are technical considerations related to the use of PCR methods for detectin the vector sequences. The advantage of this technique is that it is highly sensitive (detecting 1 vector copy in 1 µg of cellular DNA). The major disadvantage is the potential for false positive and false negative results. Generally, only a small portion of tissue is analyzed. The FDA encourages sponsors to use state-of-the-art techniques to perform the PCR analysis, e.g., spiking studies, discrimination of fals positives, and quantitative assays with internal controls and analysis of PCR reaction kinetic

Dr. Bauer noted several unresolved issues with regard to gonadal bio-distribution, e.g., question regarding the source of PCR signal (germ cells, stroma , or blood cells), the state of the vector (ep integrated), and potential developmental effects related to vector DNA integration.

Dr. Bauer discussed risk/benefit considerations with regard to the issue of gonadal vector distribution. I stated that gene therapy holds enormous potential to treat the cause of disease, provide endogenous production of therapeutics, and provide healthy individuals with enhancement and augmentation. Individual risks involve tumorigenicity , toxicity, and teratogenicity . Due to the presence of vec sequences in germ cells, potential public health risks involve germ line alteration and horizontal transmission. He noted that, based on limited supporting data, the magnitude of the potential risks is unknown and generally is regarded as low.

Dr. Bauer noted an important aspect of the risk/benefit analysis is the public acceptance of mutagenic or teratogenic therapies in the development of drugs for oncologic (e.g., chemotherapies) and infecti diseases (e.g., thalidomide), and for cosmetic uses (e.g., Accutane). He believes that there is societa acceptance of the germ line risks weighed against patient benefit, and that patients can make their own decisions regarding individual risks.

Dr. Bauer noted that the FDA response to the unknown risks of gene therapy is made in the context that gene therapy has been restricted to somatic gene therapy and to life-threatening disease, in a setting where reproduction is unlikely. In these protocols, the informed consent process has acknowledged the potential for inadvertent germ line alterations and has requested that patients practice birth control during treatment. The FDA encourages sponsors to collect data through patient follow-up and monitoring of patient body fluids, semen samples, and requesting autopsies.

Dr. Bauer noted that the future of gene therapy is moving toward treatment of chronic, non life-threatening

diseases and to early interventions, e.g., before the onset of overt disease, and administration to infants *in utero*. He stated that the FDA is seeking discussion of issues raised by observations of gonadal bio-distribution in pre-clinical animal studies used in support of gene therapy.

Presentation -- Dr. Pilar

Dr. Pilaro stated that the purpose of her presentation was to seek input from the public and the RA regarding distribution of foreign genes detected in gonadal tissues after inadvertent targeting via multip routes of administration in the treatment of patients with non life-threatening diseases. She discussed a potential "decision tree" approach to conducting specific targeted studies.

Dr. Pilaro noted that the FDA regulatory requirements for approval of gene therapeutic agents are n different than regulatory requirements for other therapeutics in terms of product characterization, purity, potency, safety issues, and clinical trial design. The pre-clinical studies will need to address issues related to product labeling, e.g., genotoxicity and mutagenicity , long-term toxicity and carcinogenici reproductive effects.

Dr. Pilaro posed several questions to be answered by pre-clinical studies: (1) What is the relationship o the dose to the biologic activity? (2) What is the relationship of the dose to the toxicity? (3) Does the route and/or schedule of administration affect activity or toxicity? (4) What risks can be identified for the clinical trials? She pointed out critical parameters in pre-clinical studies related to species selection, route of administration, dose selection, and disease indication. The issues to be concerned about are vector dissemination, vector or transgene toxicity, host immune response, and the intrinsic pathology of th disease to be treated.

Dr. Pilaro stated that reproductive studies of gene therapeutic agents need to address issues such a transmission to gonadal tissue, persistence, integration, the affected cell types, product class, patien population, and duration of treatment. Pre-clinical evaluation of reproductive effects on gene therapy will be expected when: (1) the gene product affects physiologic processes related to fertility and maintenance of pregnancy, (2) the vector is used in pregnant females, or (3) there are concerns on the effects of the vectors on a fetus and the potential effects of fetal development on the vector.

Issues raised by Dr. Pilaro at the 1996 and 1997 FDA/ NIH Gene Therapy Conferences included: (1 safety studies should be required to demonstrate a lack of toxicity to germ cells when positive gene transfer is seen? (2) At what phase in development of a gene therapy protocolshould full scal reproductive toxicity studies be initiated? (3) What evidence is necessary to determine if or when reproductive studies are needed?

Dr. Pilaro offered an "decision tree" approach to address issues of gonadal distribution. If PCR a shows that the vector does distribute to the gonads, then the next step is to identify what type of cells are taking up the DNA. If the gene is present in germ cells, its intracellular localization should be determined, i.e., episomal or integrated. Any passage of vector sequences to the progeny should be examined. Th crucial question is whether there is detectable gene expression in the progeny and whether there is any developmental or teratogenic effects on the progen

In summary, Dr. Pilaro stated that pre-clinical testing requirements for developmental effects of gen therapies should be addressed depending on the results of the bio-distribution studies. Factors to be considered are patient population and acceptability of the risk to the reproductive systems of the patient population. The need for animal studies will depend on the information available. Questions to be asked include whether the vector is integrated and passed on to progeny. The FDA strives for a rational

scientifically-based approach to problem solving based on the best available technology, careful design of the experiments, and judicious use of animals.

Other Comments

Dr. McIvor asked for additional information about the observation of gonadal distribution of vecto sequences. Dr. Pilaro responded that there are data showing the sequences persisted up to three mon however, she emphasized the proprietary nature of these data. Dr. Noguchi explained that gonadal bio-distribution for a significant duration after vector administration has been observed in a variety of experiments involving different vectors, different routes of administration, and even with therapeutic proteins.

Dr. Gordon stated that he is unable to comment on the issue without seeing the data. In his estimation, the probability that the transduced DNA was actually in sperm cells or fertilized eggs is very small. Dr Noguchi noted that rigorous experimental data are needed to assure that there are no untoward effects on the germ line. Dr. Gordon said that it will take long-term observation (50 or more years) to assure that no vector sequences are inadvertently transmitted through the germ line in animal models or human subjects. Dr. Pilaro noted that the F's "decision tree" approach is intended to address this difficult issue in term of incremental risks.

Dr. Macklin noted that the risks of inadvertent germ line intervention are uncertain. She was concerned about the relative risks vis-a-vis intentional germ line alteration, which the RAC will not entertain a present. Dr. Pilaro stated that the FDA shares Dr. Macklin's concern.

Dr. Verma suggested that an obvious method to look for germ line alteration by retroviruses is to do retrospective study of HIV-infected persons to see whether there is any germ line transmission of the viral sequences. HIV patients have tremendous rounds of viral infection of body cells.

Dr. Aguilar-Cordova noted that it appears to be more useful to discuss what type of experiments should be designed to address the risk of germ line integration, e.g., by retroviruses. Dr. Noguchi agreed.

Dr. Wolff inquired if the vector sequences have proven to be associated with germ cells rather than just persistence in gonads. Dr. Pilaro responded that such data have not yet been submitted by the sponso

Dr. Gordon noted that there are literature reports regarding infection of germ cells in animals. Retroviruses are capable of infecting pre-implantation embryos with low efficiency, and post-implantation embryos are at risk of retroviral infection. For studying primordial germ cells, Dr. Ralph Brinster's experiments in transplanting spermatogonial cells from the testes of a donor to a recipient animal is a useful model t evaluate germ line integration by vectors.

Dr. Ando noted that it is a difficult technical challenge to assess vertical transmission of vector sequences in animal studies. In human subjects enrolled in retroviral gene transfer, at least 100 patients have been studied for the presence of retroviral vectors in sperm. The results are negative.

Dr. Leinwand suggested that *in situ* PCR analysis of the autopsy samples may be useful. Dr. Pilaro responded that *in situ* PCR is a useful technique; however, this method is not as sensitive as "nested PCR on gonadal tissue. Responding to Dr.'s Pquestion of whether the germ cells can be easily separated from gonads to perform the study, Dr. Epstein (FDA) said that cell separation is not a simple procedure. Dr. Gordon noted that this type of procedure is laborious, but it can be performed. Dr. Noguchi noted that the gene therapy community has not yet committed its resources to obtain such data.

Dr. McIvor stated that PCR and *in situ* hybridization are very useful to address the issue of gonadal cel bio-distribution. For mice with sustained vector persistence, breeding experiments can be performed to address transmission to the progeny. Dr. Gordon noted that to be statistically significant such experiments would require the use of millions of mice.

Dr. Markert remarked that the discussion is very difficult without actual data. She was concerned abou how best to inform gene therapy investigators and their Institutional Review Boards.

Mr. Michael Langan (National Organization for Rare Disorders) stated that the RAC has the responsibi to address the issue of potential germ line transmission in order to allay the fear of patients enrolling in the gene transfer protocols. Inadvertent germ line transduction is a safety issue, and it should be within the scope of FDA regulation.

Dr. Lysaught said that the germ line question is an important informed consent issue that should b resolved with systematic scientific studies.

Dr. Mickelson suggested that the gene therapy research community should consider a consortium approach, and perhaps pool collective resources to fund the experimental studies needed to address this issue. She noted that the Environmental Protection Agency has taken a similar approach for certain difficult environmental issues.

Dr. Aguilar-Cordova asked if the RAC can make a recommendation to the NIH Director to consider funding mechanism, e.g., Request for Application, to support this kind of study.

Dr. Naldini stated that the vector sequences appear to be present in a very small fraction of gonadal He said the probability of finding transmission to the progeny is very small. Dr. Gordon expressed similar concern about the ability to design meaningful experiments that would allay the fear about potential germ line alteration of patients of gene transfer protocols.

Dr. Mickelson suggested using the phrase "unknown risks" in the Informed Consent document to convey the potential germ line risk to patients.

Dr. Verma inquired about the mechanism to obtain the necessary data to permit scientific review of it validity concerning this important issue.

Dr. Noguchi said that it is a long process to seek release of proprietary information. He suggested independent public studies are another avenue to obtain data to resolve this issue. Dr. Noguchi said that such an undertaking needs support from the RAC. Dr. Lysaught stated that conclusions from such stud are important in informing the patients regarding the germ line risks.

Dr. Mickelson suggested that the RAC consider adopting a motion to write a letter recommending funding for studies involving gonadal biodistributi

Dr. Wolff inquired if the issue of sexual transmission of the vectors has been raised. Dr. Noguchi responded that it is another question not fully addressed.

Dr. Markert asked if the IBCs and IRBs should be properly informed about concerns of gonada bio-distribution. Dr. Bauer said that the data submitted to the FDA cannot resolve the questions of whether the germ cells are transduced

Dr. Macklin said that IRBs can require some sort of disclosure in the Informed Consent document, an that IRBs can restrict the enrollment of persons childbearing potential."

Ms. Knorr suggested that the RAC consider sending a letter to all IBCs and principal investigat requesting pertinent data regarding gonadal distribution of vector sequences. Dr. Mickelson agreed to suggestion.

Dr. Epstein noted that the RAC should not be too alarming. The gonadal issue pertains to only certai vectors and the investigators and sponsors of those protocols should know about the data.

Dr. Brian Ludwig (Merck Research Laboratories) stated that he has conducted pre-clinical studies to address these issues with naked DNA vaccines by intramuscular injection. A low level of plasmid DNA sequences was detected in testes and ovaries within one to seven days after injection. There was no evidence of DNA integration. This information was conveyed to the participants of the study. He cautioned that different types of vectors should be evaluated separately and that the general recommendation is of limited value.

Committee Motion 1

A motion was made by Dr. Macklin and seconded by Dr. Lysaught to send a letter to all IBCs principal investigators requesting the following information: (1) pre-clinical data demonstrating gonadal distribution and/or inadvertent germ line alteration in pre-clinical studies, and (2) clinical follow-up data demonstrating gonadal distribution and/or inadvertent germ line alteration. The motion passed by a vot of 12 in favor, 0 opposed, and no abstentions.

IV. Call to Order/Mickelson

Dr. Mickelson called the meeting to order for the second day of the RAC meeting. She noted that when reviewing human gene transfer protocols, the RAC no longer has approval authority and any RAC recommendations may be summarized in a letter to the principal investigator with a copy being sent to the IBC. The IBC may review the investigator's response to determine if it is acceptable.

V. Minutes of the September 12, 1997, Meeting/Ando, Greenblat

Committee Motion 2

The RAC approved a motion made by Dr. Greenblatt and seconded by Dr. Lysaught to accept the m of the September 12, 1997, RAC meeting (with the incorporation of minor editorial changes), by a vote of 13 in favor, 0 opposed, and no abstentions.

VI. Update on Data Management/ Greenblat

New Protocols

Dr. Greenblatt noted that to date there have been a total of 222 protocols registered with ORDA inc 30 gene marking, 190 gene therapy, and two nontherapeutic . Gene therapy protocols include 21 fo infectious disease (all HIV infection), 32 monogenic diseases, 132 cancer, and five for other diseases. Since the September 12, 1997, RAC meeting, the following 18 protocols have been recommended by

ORDA for sole FDA revie

9705-189

Belani , Chandra P., University of Pittsburgh Medical Center, Pittsburgh, Pennsylvania; *Phase I Study of Percutaneous Injections of Adenovirus p53 Construct (Adeno-p53) for Hepatocellular Carcin*

NIH / ORDA Receipt Date: 5-27-97. Sole FDA Review Recommended by NIH / ORDA :

9706-192

Belldegrun , Arie , and Figlin , Robert., UCLA School of Medicine, Los Angeles, California; *A Phase I Study in Patients with Locally Advanced or Recurrent Adenocarcinoma of the Prostate Using SCH5850 (rAd /p53) Administered by Intratumoral Inject* Sponsor: Schering-Plough Corporation

NIH / ORDA Receipt Date: 6-9-97. Sole FDA Review Recommended by NIH / ORDA :

9706-193

Marshall, John L., Vincent T. Lombardi Cancer Research Center, Georgetown University Medical Center, Washington, D.C.; *A Pilot Study of Sequential Vaccinations with ALVAC-CEA and Vaccina-CEA wit addition of IL-2 and GM- CSF in Patients with CEA Expressing Tu* Sponsor: National Cancer Institute-Cancer Therapy Evaluation Program

NIH / ORDA Receipt Date: 6-18-97. Sole FDA Review Recommended by NIH / ORDA :

9706-195

Conry , Robert M.;The University of Alabama at Birmingham, Birmingham, Alab *A Phase I Trial of a Recombinant Vaccinia-CEA (180 Kd) Vaccine Delivered by Intradermal Inj Injection Versus Subcutaneous Jet Injection in Patients with Metastatic CEA -Expressing Adenocar* Sponsor: Drug Regulatory Affairs Branch, CTEP , Division of Cancer Treatment, Diagnosis and Centers, NCI,

NIH / ORDA Receipt Date: 6-26-97. Sole FDA Review Recommended by NIH / ORDA

9706-197

Conry , Robert M.; University of Alabama at Birmingham, Birmingham, Alabama; *Phase Ib Trial o Intratumoral Injection of a Recombinant Canarypox Virus Encoding Human B7.1 (ALVAC-hB7.1) or Combination of ALVAC-hB7.1 and a Recombinant Canarypox Virus Encoding Human Interleukin-1 (ALVAC-hIL-12) in Patients with Surgically Incurable Melanoma* Sponsor: National Cancer Institute-Cancer Therapy Evaluation Program

NIH / ORDA Receipt Date: 6-30-97. Sole FDA Review Recommended by NIH / ORDA

9707-198

Venook , Alan and Warren, Robert S., University of California, San Francisco, California and Fisher George; Stanford University, Palo Alto, California; *A Phase I/II Study of Autologous CC49-Zet*

Gene-Modified T Cells and α-Interferon in Patients with Advanced Colorectal Carcinomas Expressing the Tumor-Associated Antigen, TAG-72 Sponsor: Cell Genesys , In

NIH / ORDA Receipt Date: 7-7-97. Sole FDA Review Recommended by NIH / ORDA :

9707-199

Park, Chan H.; Samsung Medical Center, Seoul, Korea; Kim, Sunyoung ; Seoul National University Seoul, Korea; Lotze , Michael; Tahara , Hideaki; and Robbins, Paul; University of Pittsburgh, Pittsbu Pennsylvania; *IL-12 Gene Therapy Using Direct Injection of Tumors with Genetically Engineered Autologous Fibroblas*

NIH / ORDA Receipt Date: 7-22-97. Sole FDA Review Recommended by NIH / ORDA :

9707-204

Hickstein , Dennis; University of Washington School of Medicine, Seattle, Washington *Retrovirus-Mediated Transfer of the cDNA for Human CD18 into Peripheral Blood Repopulating Cells Patients with Leukocyte Adherence Deficiency*

NIH / ORDA Receipt Date: 7-31-97. Sole FDA Review Recommended by NIH / ORDA :

9708-205

Simons, Jonathan W.; Johns Hopkins Oncology Center, Baltimore, Maryland; *Phase I/II Study of Allogeneic Human GM- CSF Gene Transduced Irradiated Prostate Cancer Cell Vaccines in Pati Prostate Cancer*

NIH / ORDA Receipt Date: 8-19-97. Sole FDA Review Recommended by NIH / ORDA

9708-206

Flowers, Mary E. D. and Riddell, Stanley; Fred Hutchinson Cancer Research Center, Seattle, Washington; *Infusion of Polyclonal HyTK (hygromycin phosphotransferase and HSV thymi gene)- transduced Donor T Cells for Adoptive Immunotherapy in Patients with Relapsed CML af Allogeneic Stem Cell Transplant: Phase I-II Clinical Tri*

NIH / ORDA Receipt Date: 8-19-97. Sole FDA Review Recommended by NIH / ORDA :

9708-208

Schwarzenberger , Paul; Louisiana State University Medical Center, New Orleans, Louisiana *The Treatment of Malignant Pleural Mesothelioma with a Gene-Modified Cancer Vaccine: A Phase I Stu*

NIH / ORDA Receipt Date: 8-25-97. Sole FDA Review Recommended by NIH / ORDA :

9709-210

Gonzales, Rene; University of Colorado Cancer Center, Denver, Colorado and Hersh , Evan; Arizon

Cancer Center, Tucson, Arizona; *Compassionate Use Protocol for Retreatment with Allovectin-Immunotherapy for Metastatic Cancer by Direct Gene Trans* Sponsor: Vical , In

NIH / ORDA Receipt Date: 9-8-97. Sole FDA Review Recommended by NIH / ORDA :

9709-212

Gonzales, Rene; University of Colorado Health Sciences Center, Denver, Colorado; and Hersh , Evan M Arizona Cancer Center, Tucson, Arizona; *Phase I Study of Direct Gene Transfer of HLA-B7 Plasmid DNA/ DMRIE /DOPE Lipid Complex (Allovectin-7) with IL-2 Plasmid DNA/ DMRIE /DOPE Lipid Comp (Leuvectin) as an Immunotherapeutic Regimen in Patients with Metastatic Mela* Sponsor: Vical , In

NIH / ORDA Receipt Date: 9-18-97. Sole FDA Review Recommended by NIH / ORDA :

9709-213

Deeks , Steven G.; University of California, San Francisco General Hospital, San Francisco, Californ *Phase II Study of Autologous CD4-Zeta Gene-Modified T Cells in HIV-Infected Patients with Undecta Plasma Viremia on Combination Antiretroviral Drug Thera* Sponsor: Cell Genesys , In

NIH / ORDA Receipt Date: 9-22-97. Sole FDA Review Recommended by NIH / ORDA :

9709-214

Breau , Randall L.; University of Arkansas for Medical Sciences, Little Rock, Arkansas; Clayman , Ga The University of Texas MD Anderson Cancer Center, Houston, Texas; Yoo , George H.; Wayne Stat University/Barbara Ann Karmanos Cancer Center, Detroit, Michigan; Medina, Jesus E., University o Oklahoma Health Sciences Center, Oklahoma City, Oklahoma; Murphy, Barbara S., Vanderbilt University Medical Center, Nashville, Tennessee; Goodwin, W. Jarrard , University of Miami Hospitals and Clinics Miami, Florida; Weber, Jeffery S., University of Southern California, Los Angeles, California; Schuller David E., Ohio State University Medical Center, Columbus, Ohio; and Bukowski , Ronald M., Th Cleveland Clinic Foundation, Cleveland, Ohio; *A Phase II Multi-Center, Open Label, Randomized Study to Evaluate Effectiveness and Safety of Two Treatment Regimens of Ad5CMV-p53 Administered by Intra- Tumoral Injections in 78 Patients with Recurrent Squamous Cell Carcinoma of the Head and N (SCCHN* Sponsor: Gencell (Division of Rhone- Poulenc Rorer Pharmaceutic

NIH / ORDA Receipt Date: 9-22-97. Sole FDA Review Recommended by NIH / ORDA :

9710-217

Logothetis , Christopher J.; University of Texas MD Anderson Cancer Center, Houston, Texas *Tolerance and Efficacy Study of Intraprostatic INGN 201 Followed by Pathological Staging and Pos Radical Prostatectomy in Patients with Locally Advanced Prostate Cancer* Sponsor: Introgen Therapeutics, Inc.

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

9710-218

Krishnan, Amrita and Zaia , John, A.; City of Hope Medical Center, Duarte, California *High Dose Chemotherapy and Autologous Peripheral Stem Cell Transplantation for HIV Lymphomas: A Phase I Study of Comparative Marking Using a Ribozyme Gene and a Neutral Ge* Sponsor: Ribozyme Pharmaceuticals, Inc.

NIH / ORDA Receipt Date: 10-6-97. Sole FDA Review Recommended by NIH / ORDA :

9710-219

Pagliaro , Lance C.; The University of Texas MD Anderson Cancer Center, Houston, Texas *A Phase I Trial of Intravesical Ad-p53 Treatment in Locally Advanced and Metastatic Bladder Ca*

NIH / ORDA Receipt Date: 10-21-97. Sole FDA Review Recommended by NIH / ORDA :

The following eight protocols are still under review: 9708-207, 9708-209, 9708-211, 9709-215, 9709-216, 9710-220, 9711-221, and 9711-222.

Amendments to Protocols

Dr. Greenblatt noted that ORDA has received 21 amendments to protocols since the September meeting. In a letter dated September 25, 1997, Drs. David Williams and Kenneth G. Cornetta , Indian University, responded to a RAC inquiry regarding the rationale for changing the retroviral vector used in Protocol 9701-173 due to detection of RCR . Dr. Williams stated that the switch of vectors with differen promoters was not related to the RCR issue; RCR was found only upon cocultivation of produce and it was not present in the supernatants.

Adverse Events

Dr. Greenblatt noted the submission of four adverse event reports since the September RAC meeting. Protocol 9611-169, a patient had severe rigors after injection of Leuvectin , a lipoplex express interleukin-2 into the liver tumor mass. The adverse event was probably due to Leuvectin injection. I Protocol 9608-157 of the Phase III trial involving HSV-TK / Ganciclovir for glioblastoma , a pa developed local cystic encapsulation and perifocal edema of the brain tumor surgical cavity. The adver events were possibly related to both the surgical procedure and xenograft implantation. In Protoco 9512-137, involving E1A gene therapy for patients with breast or ovarian cancer overexpressing Her-2/ *ne*, a patient developed fever after DNA lipoplex intraperitoneal infusion. The adverse event have been due to extensive tumor burden, but theinvestigator could not rule out the study drug as th cause. In the same protocol, a patient had severe abdominal pain, nausea, and fever after infusion of the DNA lipoplex . The investigators believed the adverse event was related to the study dru

Dr. Gordon noted that in Protocol 9512-137 the adverse events are very similar and they both seem to be related to the study drugs. Dr. Greenblatt agree

One progress report was received regarding Protocol 9701-172. This report stated that two of the patients failed to mobilize adequate CD34 cells for transduction. Gene transduction efficiencies for these two patients, based upon PCR analysis of colonies, was 11 percent and 22 percen

Committee Motion 3

A motion was made by Dr. Leinwand and seconded by Dr. Markert to accept the Data Managem Report. The motion passed by a vote of 13 in favor, 0 opposed, and no abstentions.

VII. Amendment to Institutional Biosafety Committee Approvals of Experiments Involving Transgenic Rodents Under Section III of the *NIH Guidelin*: Aguilar-Cordova

Dr. Aguilar-Cordova noted that the issue of transgenic rodents was discussed in previous RAC meetings (March 6-7, 1997; June 12-13, 1997; and September 12, 1997). Section III-D-4, *Experiments Involving Whole Animals*, of the *NIH Guidelin* stipulates that all transgenic animal experiments are subject to IBC approval before initiation. In a letter dated April 22, 1997, Dr. George Gutman (an IBC representative o the University of California, Irvine, California) inquired whether the *NIH Guidelin* could be amended so that experiments involving production and use of transgenic mice under Biosafety Level 1 containment could be initiated simultaneous with IBC notification.

The RAC discussed this issue during its June 1997 meeting, recommending that this requirement be changed to allow initiation simultaneous with IBC notification. The RAC agreed that the requirement for IBC approval prior to initiation is unnecessary, and recommended that the *NIH Guidelin* should be amended so that: (1) the generation of transgenic rodents at the Biosafety Level 1 containment (not all animals) can be initiated simultaneous with IBC notification, and (2) the purchase and use of transgenic rodents should be exempt from the *NIH Guidelin*. A motion was made that these proposed changes to the *NIH Guidelin* should be published in the *Federal Register* for consideration at the September 12, 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-E, *Experiments that Require IBC Notice Simultaneous with Initiation*; and (2) the purchase and use of transgenic rodents should be exempt from the *NIH Guidelin*. The motion passed by a vote of 9 in favor, 0 opposed, and no abstentions.

On September 10, 1997, a letter was received from the American Biological Safety Association requesting that the public comment period for the proposed actions under the *NIH Guidelin* published in the *Federal Register* on August 20, 1997 (62 FR 44387) be extended for an additional 60 days.

During the September 12, 1997 RAC meeting, the RAC was scheduled to vote on the issues surrounding the amendments to IBC approvals of experiments involving transgenic rodents. The RAC decided to modify the language of the proposed actions and publish the revised version in the *Federal Register* for additional public comment, as requested by the American Biological Safety Association. The RAC accepted the proposed actions with the deletion of the two words "and use" from the language that reads: "the purchase and use of transgenic rodent..." A motion was made to accept the amendments to the *NIH Guidelines* with regard to: (1) the generation of transgenic rodents at the Biosafety Level 1 containment (not all animals) can be initiated simultaneously with IBC notification, and (2) the purchase of transgenic rodents should be exempt from the *NIH Guidelin*. The motion passed by a vote of 11 in favor, 0 opposed, and no abstentions.

The proposed actions were published in the *Federal Register* on October 16, 1997 (62 FR 53908). On December 2, 1997, a letter was received from C. Geoffrey Davis, Ph.D., Vice President, Research, Abgenix, Inc., Freemont, California, requesting to add two words, "or transfer," to the language of th proposed action published in the *Federal Register* regarding the purchase *or transfer* of transgenic rodents to be exempt from the *NIH Guidelin*. In a letter dated December 5, 1997, Richard C. Knudsen, President, American Biological Safety Association, endorsed the proposed action and requested insertion of a statement, "(See Appendix G-III-M, *Footnotes and References of Appendix G*)," to aid individuals in determining the suitability of Biosafety Level 1 containment for their constructs. Appendix C-VI, *The Purchase of Transgenic Rodents,* is proposed to read:

"The purchase of transgenic rodents for experiments that require BL1 containment (See Appendix G-III-M, *Footnotes and References of Appendix G*) are exempt from the *NIH Guidelin*"

The RAC discussed the proposed actions. Dr. Gordon asked for clarification regarding how the biosafety level of a transgenic animal experiment is determined. Dr. Mickelson said that in practice the biosafety level is determined by the Animal Care Committee in conjunction with a biosafety officer. The RA accepted the proposed actions with the amendments requested by Abgenix , Inc. and the America Biological Safety Association.

Committee Motion 4

A motion was made by Dr. Aguilar-Cordova and seconded by Dr. Markert to accept the language with t amendments requested by Abgenix , Inc. and American Biological Safety Association, of the propose action published in the *Federal Register* on October 16, 1997 (62 FR 53908). The motion passed by a vote of 13 in favor, 0 opposed, and no abstentions.

VIII. Amendment to Appendix K, Physical Containment for Large Scale Uses of Organisms Containing Recombinant DNA Molecules

Requestor: Gerard McGarrity , Genetic Therapy, In

Reviewer: McIvor

In a letter dated November 5, 1997, Gerard J. McGarrity , Ph.D., Senior Vice President for Developmen Genetic Therapy, Inc., Gaithersburg, Maryland, requested amendments to Appendix K, *Physical Containment for Large Scale Uses of Organisms Containing Recombinant DNA Molecules*, of the *NIH Guidelines* to clarify the containment requirements for large scale production of viral vectors for gene therapy. In part, the letter stated:

"For the longer term, we believe it is most appropriate to revise the relevant portions of Appendix K to enable application of large scale to viral vectors. We request that RAC address this issue and propose the following language be added to the end of Sections K-III-C, K-IV-C and K-V-C of Appendix K:

'Culture fluids that contain viable organisms or viral vectors intended as final product may be removed from the primary containment equipment by way of closed systems for sample analysis, further processing or final fill. '

*"*We propose the following language be added to the end of the first sentence of Sections K-III-F, K-IV-F and K-V-F:

'...except when the culture fluids contain viable organisms or vectors intended as final product as described in Section K-III-C (or K-IV-C or K-V-C respectively) above.'

"We believe these additions maintain the original concept of Appendix K while addressing the needs of specific product types."

Dr. McIvor said that the language of the proposed amendment as stated in Dr. McGarrit letter is acceptable. He noted that the language that reads: "...by way of closed systems..." may limit the investigator's option of how to sample and analyze the culture fluid, but said he could not think of a better

substitute for this wording. Dr. McIvor noted another point for discussion is the ten liter threshold for the applicability of Appendix K for a large scale experiment.

Dr. Aguilar-Cordova agreed that the ten liter threshold is an artificial number. He noted that adenoviral preparations are much more concentrated than supernatants containing retroviruses, i.e., adenoviruses in 50 ml have the same infectious units as retroviruses in 50 liters.

Dr. Mickelson said the issue of a ten liter threshold in Appendix K is a separate issue from the proposed actions. Ms. Knorr said such an amendment would require publication of a new proposed action for fut RAC consideration. Dr. Aguilar-Cordova noted that Appendix K needs an extensive revision in the future.

Dr. Mickelson explained that the statement that reads: "...by way of closed systems...," is to allow removal of samples for analysis without compromising the integrity of the larger batch of materials in the bioreactors.

Dr. Ando noted that there are two reasons for requiring a closed system, i.e., sterility of the culture and safety of the operator. Dr. McIvor noted that IBCs should have oversight on these safety measures. H said that experiments at higher biosafety levels, e.g., BL3, would still need the requirement for "samplir by way of a closed system."

Dr. McIvor made a motion to accept the language proposed by Dr. McGarrity . He noted that the othe issues pertaining to Appendix K may be revisited in the future. Dr. Ando seconded the motion.

Dr. Gordon noted that for future revision of Appendix K one should consider how much danger a procedure poses rather than the volume of the preparations. Ms. Knorr agreed that it should be based the biohazard classification of the agents.

Dr. McGarrity explained that his proposed language of closed systems is to keep the spirit of the curre Appendix K; most situations are amenable to closed system handling. He noted that he was a RAC member, and a member of the large-scale subcommittee, when Appendix K was added to the *NIH Guidelines*.

Committee Motion 5

A motion was made by Dr. McIvor and seconded by Dr. Ando to accept the language of the proposed action published in the *Federal Register* on November 19, 1997 (62 FR 61862) for the amendment to Appendix K, *Physical Containment for Large Scale Uses of Organisms Containing Recombinant DNA Molecules*. The amendment would allow production and harvest of biologically active viral vectors intended as the final product. The motion passed by a vote of 13 in favor, 0 opposed, and no abstentions.

IX. Amendment to Section III-D-6, Experiments Involving More than 10 Liters of Culture

Requestor: Richard Knazek ,

Reviewer: Mickelson

Dr. Mickelson called on Ms. Knorr to present the background of the amendment to Section III-D-6 *Experiments Involving More than 10 Liters of Culture*.

In a letter dated November 6, 1997, Richard A. Knazek , Medical Officer, Clinical Research, Nationa Center for Research Resources, NIH , requested an amendment to Section III-D-6, *Experiments Involving More Than 10 Liters of Culture*, of the *NIH Guidelines*. Dr. Knazek proposed an addition of statement,"When more than 10 liters of culture media is to be produced within a GMP -accredited fa for subsequent clinical use, the level of appropriate containment shall be determined by the IBC affiliated with the institution where the investigator will perform the clinical manipulation of the vector." He stated that the purpose of this amendment is to prevent an additional layer of bureaucracy from impeding the implementation of an appropriately reviewed and approved gene therapy protocol.

Ms. Knorr said she believes that Dr. Kna amendment derived from a misinterpretation of Appendix M-I, *Submission Requirements -- Human Gene Transfer Experiments*. The recently amended submission requirements state that "Institutional Biosafety Committee approval must be obtained from each institution at which recombinant DNA material will be administered to human subjects (as opposed to each institution involved in the production of vectors for human application)." Ms. Knorr said she believes Dr Knazek intended the statement to mean that the IBC of the vector production site should not be require review human gene transfer protocols if such protocols have been previously reviewed and approved by the National Gene VectorLaboratories (NGVL). She said that the proposed change of Section III-D- be consistent with his interpretation. Ms. Knorr noted that the IBC approval issue was discussed at th March 6-7, 1997, RAC meeting.

Dr. Mickelson noted that the RAC minutes do not indicate that vector production sites will be exempt from IBC oversight. The new language clarifies that the IBC approval from clinical trial site(s), rather than fro production site(s), should be submitted to ORDA . Dr. Mickelson said that local IBC oversight should maintained for the vector production sites.

Dr. Aguilar-Cordova agreed with Dr. Mickelson that the IBC of the vector production site should provide oversight for the facility, but that this IBC does not need to review all the clinical protocols performed at other clinical sites.

Ms. Knorr suggested that any exception to Section III-D-6 that would not need IBC approval can be sta in a note to this section; the present wording of the proposed action does not convey the intention of this proposal.

Dr. McIvor stated that IBCs should have purview over the biosafety issues at their institutions. Mickelson agreed that even for the NGVL , where most protocols have been reviewed by other steerin committees, the IBC should retain oversight of the facility.

Dr. Gordon was concerned that removal of IBC oversight of vector production will cause confusion. He did not favor the proposed amendment.

Dr. Aguilar-Cordova was sympathetic to the intent of the proposed amendment to simplify IBC oversight. For example, a vector production site does not need to seek IBC approval every time a minor change is made to tailor a vector to a particular protocol. Dr. Mickelson responded that each IBC can work out a procedure to simplify its approval policy. Ms. Knorr noted that each institution has the authority to decid how its IBC should exercise its oversight.

Dr. Victoria Allgood (GeneMedicine , Inc.) asked to clarify whether Section III-D-6 covers experime involving plasmid DNA intended for gene therapy. Dr. Mickelson responded affirmatively.

Dr. Gordon reiterated his proposal that Appendix K should be revised in the future with regard to the ten

liter criteria used for its applicability.

Committee Motion 6

A motion was made by Dr. Leinwand and seconded by Dr. Aguilar-Cordova to disapprove the propose action published in the *Federal Register* on November 19, 1997 (62 FR 53908) for the amendment to Section III-D-6*, Experiments Involving More than 10 Liters of Culture.* The proposed action is to include a statement to Section III-D-6, "When more than 10 liters of culture media is to be produced within a GMP -accredited facility for subsequent clinical use, the level of appropriate containment shall b determined by the IBC affiliated with the institution where the investigator will perform the clinical manipulation of the vector." The motion passed by a vote of 13 in favor, 0 opposed, and no abstentions.

X. Human Gene Transfer Protocol #9708-209 entitled: *Systemic and Respiratory Immune Response to Administration of an Adenovirus Type 5 Gene Transfer Vector (Ad$_{GV}$CD.10)*

PIs: Ben-Gary Harvey, Cornell University Medical College

Ronald G. Crystal, New York Hospital-Cornell Medical Center

Reviewers: McIvor, Lai, Macklin

Ad hoc: Harold Ginsberg, NI

The protocol was recommended for full RAC review due to the novelty and safety risk of administering a recombinant adenoviral vector, via a bronchoscope, directly into the lungs of healthy subjects.

Review -- Dr. McIvor

Dr. Mickelson called on Dr. McIvor to present his primary review of the protocol. Dr. McIvor stated that the purpose of this clinical trial is to study the immune response in healthy volunteers against an adenovirus vector (Ad$_{GV}$CD.10) delivered intrabronchially . $_{GV}$CD.10 contains the *E. coli* cytosine deaminase ge and is a E1A minus, partial E1B minus, and partial E3 minus vector. The adenovirus vector would be administered in a single dose for the purpose of the first part of the study, and in multiple doses in the late part of the study.

The study seeks to address four questions: (1) whether adenovirus-specific immunoglobulins develo which would prevent adenovirus entry into lung cells, (2) whether adenovirus-specific cytotoxic lymphocytes (CTL) develop that recognize autologous targets infected with the adenovirus type 5 (vector type), (3) whether humoral and cellular immune responses in the lung differ from those in the bl and (4) whether bronchial administration of adenovirus vector elicits inflammation either locally or systemically.

Fifteen healthy subjects would be entered into the study (with the possible addition of five more). Patients will receive 2 x 10^7, 2 x 10^8, or 2 x 10^9 plaque forming units (pfu) of$_{GV}$CD.10 by bronchoscopy . A various times after administration, humoral and cellular immunity against$_{GV}$CD.10 will be evaluated in the blood and in samples collected from the lung by bronchoalveolar lavage (BAL) (a total of five procedures would be undertaken for each subject). Gene transfer would be assessed by quantitative PC and by fluorescence *in situ* hybridization.

In additional studies, the Ad_{GV}CD.10 vector would be administered at increasing doses to the same individual at two-week intervals (total of nine subjects). Immune response would be assessed in these subjects by testing for anti-Ad5 and Ad2 antibodies, and for cellular immune response (T-cell proliferation and CTL responses) in blood and in BAL samples. Inflammatory responses would be determined b assessing the level of inflammatory cytokines. It is anticipated that these analyses in these volunteers would provide an assessment of the systemic and local immune/inflammatory response to Ad_{GV}CD.10 as a representative adenovirus vector, thereby providing valuable information for future administration of adenovirus vectors for therapeutic purposes.

Dr. McIvor offered his critique of the protocol. Adenovirus vectors have become a frequently utilized vehicle for delivery of therapeutic genes and gene therapy trials. However, the utility of adenovirus vectors for delivery of genes *in vivo* is limited by immune and inflammatory reactions. In this protocol the investigators have designed a trial in healthy subjects to evaluate the immune and inflammatory response to a model adenovirus vector, Ad_{GV}CD.10. The vector would be administered to the bronchial epithelium to test for anti-vector immunity. The proposed studies would provide a good complement to animal studies to evaluate immune responses to the vector, and the effectiveness of vector modifications in minimizing the anti-vector immune response.

Preliminary results in support of the proposed studies came from previous human studies citing the lack of adverse response in healthy subjects injected intradermally with$_{GV}$CD.10, in cancer patients administered Ad_{GV}CD.10 in liver tumors, and in the intrabronchial administration of a cystic fibrosi transmembrane conductance regulator (CFTR) adenovirus vector to individuals with cystic fibrosis (Thus, intrabronchial administration of$_{GV}$CD.10 in healthy subjects is not likely to result in an adverse reaction; however, evaluation for inflammatory response will be a part of the proposed studies. Previous studies have already generated results on immune responses to adenovirus vectors administered *in vivo*. It is not known, however, whether results from adenovirus vector administration in patients with CF is an accurate reflection of the pulmonary immune response.

Safety considerations were discussed by Dr. McIvor. As summarized earlier, and based upon previous adenoviral studies, the researchers do not anticipate that administration of Ad_{GV}CD.10 will pose a significant risk to the participants in this study. Evaluation for inflammatory reactions constitutes an important part of the proposed study. Possibilities for *in vivo* generation of recombinant virus and virus spread are remote. The protocol includes collection of sperm samples to evaluate the possibility of vector spread to germ cells.

Effectiveness was discussed by Dr. McIvor. Because the proposed protocol does not contain a therapeutic endpoint, "efficacy' in this case is interpreted as the likelihood that the proposed procedure will provide interpretable results of the immune response to an adenovirus vector administered to the lung. The protocol is well-designed to accomplish this aim and to provide results on the immunogenicity of this type of adenovirus vector administered to the human lung. It should be noted that the immune response to other types of adenovirus vectors may be different, and would require additional studies for immune response evaluation.

Dr. McIvor stated that the investigators needed to address some additional points of concern. One point not raised in the protocol or in the Informed Consent document is the possibility that a robust immune response to the planned adenovirus vector administration may compromise the effectiveness of future adenoviral vector treatments. A second point is that there is no mention in the protocol of evaluating the immune response to cytosine deaminase , a foreign protein. He asked if this will be evaluated, and ho

Regarding the *Points to Consider* document, Dr. McIvor noted that the document submitted with the protocol was written for the intradermal $_{GV}$CD.10 administration protocol (#9701-171). This has now been rectified. (As an aside, D. McIvor noted that the responses to the issues raised in the "Points to Consider" document must be complete and should not make reference to material that is not readily available to RAC reviewers.)

Review -- Dr. Ginsberg

Dr. Ginsberg stated that this is a very well-written proposal, and that he has no serious concerns. It does seem unlikely that at a dose of 10^9 pfu that there will not be a moderate amount of pulmonar inflammation. Moreover, he would like to recommend to the investigators that they re-insert the E3 gene encoding the 19 kd protein, which has been clearly demonstrated to reduce cell surface expression of Class I major histocompatibility gene, and therefore, to reduce CTL expression against adenoviral infected cells. Dr. Ginsberg noted that the protocol does not mention chest X-rays.

Review -- Dr. Lai

Dr. Lai noted that this protocol was submitted as an addendum to the previous protocol (#9701-171). This previous protocol is similar in design with the exception of the use of intradermal administration of th same vector. The previous protocol was approved by the RAC after public discussion. The discussion concerned the use of healthy individuals; the potential systemic toxicity related to the virus; the potential for germ line infection; and the scientific rationale for administering the vector intradermally to evaluat immune responses in the lung.

The current study will provide critical information about one of the major problems in the use of adenovirus vectors: the immune response to the virus. Because the adenovirus vector in this amended protocol will be administered into the lung, which is the likely route of administration of these vectors in many future gene therapy protocols, Dr. Lai feels that the scientific rationale for this addendum is much stronger than for intradermal administration. The present approach would provide data that are mor relevant to future clinical applications. Thus, the data obtained in this amended protocol regarding local and systemic immune responses following intrabronchial administration will be more usefu

On the other hand, administration of an adenovirus to its natural target organs in healthy individuals using an invasive procedure poses even higher potential risks to the individual than intradermal administratio The previous RAC review (March 1997) addressed most of the issues regarding the use healthy individuals, the use of this particular vector, and the use of bronchoscopy . Several questions from th previous RAC discussion, however, remain germane to this protocol. Before proceeding with this more invasive procedure, the investigators should relate their experiences with patients who have received the adenovirus vector intradermally and those CF patients who have received it intrabronchially . These should be examined by the RAC.

Dr. Lai noted four additional questions: (1) Is there evidence of systemic spread of the virus? Has the possibility of viral integration been examined? Has germ line transmission been examined? Did BAL provide enough cells for examination of the immunological and inflammatory parameters as proposed in this protocol? (2) Bronchoscopy will likely damage the epithelial surface of the bronchi. Will th administration of adenovirus to the damaged bronchial surface cause increased risks? (3) Repeated bronchoscopy will likely cause chronic epithelial cell inflammation. Will this affect the immunologica parameters? (4) How is efficacy to be evaluated in the second part of the study?

Review -- Dr. Macklin

Dr. Macklin stated that her review examined ethical aspects of the protocol. She discussed four areas of concern: the use of healthy subjects, recruitment of subjects, benefits to subjects, and payments to subjects.

The Use of Healthy Subjects. The protocol justifies the use of healthy subjects as the study population for two basic reasons. The first reason is the need to acquire baseline information about anti-adenovirus vector immunity independent of complicating features that exist in patients who have a disease. Second, in healthy individuals all the relevant immune parameters can be assessed in cells recovered from BAL, which is not possible in individuals with CF.

Subjects will be recruited from the New York metropolitan area and "elsewhere" (no indication of why "elsewhere" might be needed, or where that might be). Pregnancy is an exclusion criterion, as is an HIV+ status. The protocol states that HIV testing will follow the requirements of New York state law, including a separate consent form for HIV testing. Both the protocol and the Informed Consent document mention HIV testing and that counseling will be provided in the event the individual tests positive. However, New York State law requires pre-test counseling for HIV, as well as post-test counseling.

Ethical questions regarding recruitment. Dr. Macklin stated several specific ethical concerns regarding recruitment: (1) The protocol does not specify how subjects will be recruited or who will be approached. The protocol simply says that "accrual will be random." The clinical protocol does not mention whether any notices or advertisements are to be posted; however, the response to Appendix M-II-3 mentions IRB approved advertisements will be used. (Submission of Appendix M was for the original study involving intradermal administration of the vector, not the present addendum.) (2) Where and how will potentia subjects be approached? (3) When and where, and by whom, will informed consent be obtained? (4) Before reading the consent form, how will subjects learn that they will receive $950 for their participation?

Safety Risks to subjects. For the purpose of ensuring safety, both Part A and Part B are designed to use ascending doses. After Part A is completed, Part B will be initiated. Previous studies on CF patients have shown that mild, reversible local inflammation of the lung is observed by histologic evaluation; however this does not seem to be clinically apparent.

Risks to subjects appear to be considerably higher from fiber optic bronchoscopy with BAL and brushin than from the adenovirus vector. These risks include fever, bleeding, allergic reactions, and cardiac arrhythmias. Rare cases of fatalities stemming from overdose caused by a topical anesthesia have been reported, but the investigators say they will reduce that risk by careful control of the amount of anesthesia. Subjects will be asked whether they have had any allergic reactions in the past to the types of medication used as a local anesthetic in this study. But what if they have never experienced administration of lidocaine to their nose and throa

The investigators have performed thousands of bronchoscopies . In this context, the protocol claims tha bronchoscopy , bronchoalveolar lavage , and brushing are safe procedures. Despite this claim, th risk of bronchoscopy has frequently been determined to be more than a minor increase over minimal ri Some IRB members consider these procedures to be in the "significant" risk category. The risks of bloo drawing, radiation from a single chest X-ray, and pulmonary function tests are typically considered to be minimal.

Benefits to subjects. Dr. Macklin stated that she has three ethical concerns about benefits to subjects: (1) There are no direct benefits to subjects. (2) The protocol mentions "financial compensation" as a benefit, but payment for participation should *not* be construed as a benefit for purposes of assessing the

benefit-risk ratio of a research protocol. (3) Also mentioned is the "theoretical benefit of possibly being immunized against future infection with adenovirus type 5." Dr. Macklin said she was unable to assess whether this "theoretical" benefit has any likelihood of being an "actual" benefit.

Payment to subjects. Individuals will be paid $150 following each of six bronchoscopies and $50 for th skin biopsy. The maximum an individual who completes the study may receive is $950, plus an optional $25 for male subjects who submit a sperm sample. Some IRBs place a ceiling on the amount subject can earn from any one experiment, in order to minimize the possibility of payment constituting an undue inducement. One view about payment to subjects is that the monetary amount should not be pegged to the degree of risk the subjects will face. A different view holds that the higher the risk, the higher the monetary amount should be. Dr. Macklin said that paying $950 for a single study seems high.

Dr. Macklin made several specific comments on the Informed Consent document, which have been accepted by the investigators in their written response.

Other Comments

Dr. Ginsburg noted that since most people have been infected with adenovirus type 5 during childhood, no practical benefit of immunization with the vector should be expected.

Dr. Markert said that the Informed Consent document should include an explanation as to why sper samples will be collected. Dr. Gordon agreed.

Dr. Wolff was not convinced that the use of the cytosine deaminase transgene is relevant to the trea of CF patients in the future.

Dr. Aguilar-Cordova noted that the first generation adenovirus vectors have been found to have limited success in CF patients, and he asked the investigators to explain the significance of testing such vectors. Dr. Lai said he has the same question.

Dr. Lysaught noted that the Informed Consent document does not state the need for birth control and t risk of germ line transmission, and should provide an adequate explanation regarding the major purpose of the study, i.e., to study immune responses to adenoviral vectors.

Dr. Verma noted that there are similar questions regarding the immune response to the vector in th subsequent protocol to be discussed. Dr. Mickelson said that those issues will be discussed in the context of the next protocol. Ms. Knorr noted that the RAC may make a recommendation for each specifi protocol, and may also make general recommendations regarding the use of specific vectors. Dr. Lysaught said that both specific and general issues need to be addresse

Dr. Aguilar-Cordova questioned the significance of the proposed study from two perspectives: (1) the use of the first generation adenovirus vectors, which have been found to be unsatisfactory, and(2) the available data from previous vaccine studies of adenoviruses conducted by the U.S. Army in 1970's. Dr. Ginsberg noted that those studies involved a live virus vaccine administered intranasally

Dr. Wolff said that the study would be more significant if the vector used is the one to be used in future CF clinical trials.

Dr. Markert said that the RAC recommendations may be sent to the IBC and IRB , and the issue potential germ line risk can be stated in a letter.

Investigator Response -- Dr. Crystal

With regard to the question of studying the first generation adenovirus vectors in the present protocol, Dr. Crystal said that a similar question was raised during the RA's review of his previous protocol (#9701-171). Dr. Crystal stated that there is no evidence that the later generation vectors have proven more effective in humans; however, most of the studies have been conducted in animals. According to his observation, the animal studies do not predict what will be found in humans. Dr. Crystal said that it is important to study the first generation vectors to obtain background information as a basis for designing future vectors.

Dr. Crystal said that Dr. Ginsberg's suggestion to re-insert the E3 gene coding for the 19kd protein may yield a better vector with less immunogenicity , but that he cannot predict the outcome. Dr. Crystal's strategy is to finish the present series of experiments and to use the information as a guide to develop future vectors. Responding to Dr. Ginsberg's question of chest X-ray, Dr. Crystal said that an X-ray will be taken to evaluate lung inflammation following vector administration.

Responding to Dr. Lai's inquiry about the data from ongoing protocols, Dr. Crystal said that he has completed the first part of the intradermal protocol (#9701-171); there are no safety issues in all cohort to the highest dose. Humoral responses have been observed in all but one subject at the lowest dos cohort. No CTL responses have been detected with the vector; however, CTL responses have b observed with wild-type adenovirus. In the CF trials, Dr. Crystal said that most studies have been completed. There is no safety issue, and there is a transient (three day) local correction of the mRNA defect at the site of vector administration. There is transgene expression following the second repea administration, but none after at the third administration. There are no systemic neutralizing antibodies and CTL response in the bloo

With regard to the issue of monetary payments to the subjects, Dr. Crystal said that the payment schedule is consistent with payments in the NIH intramural clinical research program. He said such payments ar intended to compensate volunteers for the discomfort caused by several of the procedures, such as blood drawing and bronchoscopy

In terms of the theoretical benefit of immunization, Dr. Crystal clarified that, even though a humoral immune response is stimulated, administration of an adenoviral vector does not protect individuals against adenovirus infection.

In response to Dr. Macklin's comments on the lidocaine anesthesia during bronchoscopy , Dr. Crysta that an allergic reaction is extraordinarily rare but he would agree to add such a statement of the potential risk in the Informed Consent document. Dr. Macklin has suggested replacing phrases such as "your doctors" or "your attending physicians" with the phrase "the researchers" because the subjects are healthy individuals rather than patients. Dr. Crystal responded that he has made these changes except in "boiler plate" language dictated by his IRB

Dr. Crystal agreed to include an explanation in the Informed Consent document with regard to sperm donation.

Responding to the question of using the cytosine deaminase transgene instead of the CFTR ge Crystal said that for healthy subjects it is more prudent to use a heterologous transgene such as cy deaminase . This is because there is a theoretical risk of an autoimmune response in using th homologous CFTR gene product. Dr. Crystal emphasized that the major interest of the protocol is to st

the response to the vector *per se* rather than the transgene . Dr. Wolff asked why the investigators did n choose to use an empty vector without any gene insert. Dr. Crystal said that he has considered such an option, and said the NGVL would welcome such a recommendation from the RA

Responding to Dr. Lysaught's suggestion, Dr. Crystal agreed to include statements in the Informed Consent document regarding the need for birth control and regarding the methodologies for immune parameters.

Other Comments

Dr. Lai inquired about the number of subjects who have been enrolled in the intradermal protocol. Dr Crystal said six subjects have been entered for the single dose part of the protocol, and said he is beginning to start the second multiple dose study. All except the first subject have developed neutralizing antibodies in serum and in the lung. Dr. Lai asked if it is still prudent to enroll additional patients for the intradermal protocol. Dr. Crystal responded that adding additional subjects is worthwhile to complete th study and to help understand compartment responses to the vector, i.e., the skin vs. the lung.

Dr. Verma stated that in animals adenovirus vectors induce massive CTL responses and sev histological tissue damage at the injection site. He asked how the animal data can be reconciled with the human data showing no CTL response. Dr. Crystal responded that animal experiments are not totall predictive for humans, and said that is exactly the reason to perform studies on humans to evaluate the vectors.

Ms. Rothenberg asked how the healthy subjects have reacted to being subjected to "gene therapy." In response, Dr. Crystal said that he has not observed any strong concern expressed by the six subjects treated so far. All of these subjects have been recruited through an advertisement put through by the IRE and most of them have never participated in other clinical trials. Ms. Rothenberg noted that using healthy subjects for gene transfer is quite novel, and the data obtained will be unique.

Dr. Ginsberg stated that pathology obtained in mice is much milder than in cotton rats, a situation similar to humans. He said researchers should not disregard the animal studies. Dr. Verma noted that the lack CTL response in humans is strikingly different from the animal data. Dr. Crystal stated that he has use cotton rats, mice, pigs, etc., and obtained results similar to those reported by Dr. Ginsberg.

Dr. Lysaught noted the immune responses to adenovirus vectors have been observed in several othe protocols, and they are not drastically different from the animal data. Dr. Crystal responded that his anima data are consistent with other experiments in which the adenovirus vectors are administered to the lung of an animal; there are neutralizing antibodies and CTL in the blood. In humans, there are no neutralizin antibodies after four repeat administrations of a dose of $10^{8.5}$ pfu every two weeks. There is no C response to the vector, although wild-type virus containing the E1 gene does induce a CTL response These human data are different from animal studies.

Dr. Leinwand inquired if there is any difference in immune responses from seropositive ver seronegative individuals. Dr. Crystal said that he found no correlation of neutralizing antibodies o preexisting infection with immune response to the vector. Dr. Leinwand asked if the CTL response been examined in histological tissue sections. Dr. Crystal responded that the CTL response was tested the blood by the conventional methodologies; however, it has not been examined locally in skin biopsy. Dr. Leinwand suggested that the lack of CTL response in the blood may be due to dilution. It is accurate to state that animal studies are absolutely irrelevant to the human situations. Dr. Crystal agreed. Dr. Leinwand suggested that using an empty vector is better than a vector with a heterologous g

insert. Dr. Crystal said that he would transmit the RAC recommendation to the NGVL for thei consideration.

Dr. Aguilar-Cordova noted that it is much more difficult to assay for CTL response in humans than i animals. The lack of CTL in circulating blood is no indication that there is a fundamental differenc between the human and animal data. Dr. Crystal responded that one of the reasons for the different results may be due to dose differences, i.e., mice have relatively higher doses of vectors per body weight than humans.

Dr. Gordon emphasized that the public should not be left with an impression that animal studies are irrelevant to human trials. In terms of the vectors, both the empty vector and vectors with gene inserts are relevant since immune responses may be different. Dr. Crystal stated that he agrees with the RAC comments that animal studies are important for human trials.

Dr. Verma noted that, in his animal experiments, CTL induced by adenoviral vectors resulted in tis damage, e.g., in liver and muscle. The interesting observation in humans is that the CTL -induced tissu damage is minimal.

Dr. Lai stated that the value of animal data from the pre-clinical studies to human trials should be appreciated. He asked if any immune response has been detected in the lung in the CF protocols. Dr. Crystal responded that the CF patients, the number of lymphocytes in the lung lavage are very low and due to the interference of inflammatory cells present in the lavage , the immune reaction in the lung of t CF patients is difficult to examine. Dr. Lai agreed that it is valuable to study the lungs of the healthy subjects, and it is more relevant to choose the same vector intended for CF patients. Dr. Crystal agreed.

Dr. McIvor proposed ending the discussion. Dr. Mickelson noted that the RAC is satisfied with Dr. Crystal's responses, and there is no need for a committee motion. Dr. Noguchi said that the FDA has approved this protocol; however, Dr. Crystal has elected to wait until after the RAC discussion to commence the protocol.

Dr. Gordon supported Dr. McIvor's suggestion to end the discussion without implying approval or disapproval.

Dr. Lysaught inquired if any recommendation should be transmitted to the NIH Director. Ms. Knorr that the RAC minutes and its summary are available to the public as well as to the NIH Director. Dr Mickelson suggested that the RAC may consider sending a letter to the IBC and IRB if there are seriou concerns. Dr. McIvor said that the RAC may transmit such concerns to the NIH Director and othe interested parties; however, he does not sense such concerns for this protocol. Ms. Knorr asked if ther any formal recommendation regarding the use of an empty vector. Dr. McIvor said that the RAC made such a suggestion and Dr. Crystal accepted the recommendation.

Dr. Markert made a motion to send a letter to the IBC stating that the RAC concerns have bee satisfactorily addressed by Dr. Crystal. Dr. Verma noted that the RAC discussed the issues and did no come to a resolution in order to make a formal recommendation. Dr. Verma agreed with Dr. McIv suggestion simply to end the discussion. Dr. Markert stated that her intent is to provide feedback to th IBC with regard to this protocol. Ms. Rothenberg cautioned that any formal action taken by the RAC will set a precedence for a future procedural issue of the RAC process. She said she does not sense that the RAC has serious concerns regarding the present protocol.

Dr. Wolff suggested sending the minutes of the RAC discussion to the IBC and IRB . Ms. Rothenber

supported Dr. Wolff's suggestion. Dr. Markert agreed to withdraw her motion, but noted that the minute will not be officially accepted until the next RAC meeting. Mr. Dommel suggested sending a copy of th transcript instead. Dr. McIvor was not sure that the IBC would be able to make their decision based on such materials. Dr. Gordon said that the RAC will discuss the procedural questions in a later session of the meeting. Drs. Aguilar-Cordova and McIvor noted that it is a moot point for any RAC actions since the protocol has received approval from the IBC, IRB , and the FD

Dr. Verma suggested ending the RAC discussion of this protoco

Protocol Summary

Drs. Ben-Gary Harvey and Ronald G. Crystal, Cornell Medical Center, New York, New York, will conduct gene transfer experiments on a total of 15 healthy male or female subjects, age>18 years. The rationale of this protocol is to characterize the local and systemic immune response of healthy individuals to intrabronchial administration of a replication-deficient adenovirus type 5 gene transfer vecto ($Ad_{GV}CD.10$) carrying the gene coding for the *E. coli* enzyme, cytosine deaminase . The propose protocol is identical to an ongoing protocol (#9701-171) where the vector is administered by the intradermal rout

The study will be divided into two parts. Part A focuses on single administration and Part B focuses on repeat administration of the vector. The objectives are: (1) to determine if adenovirus-specific immunoglobulins will develop which would prevent the entry of adenovirus into the cell of the lung o repeat administration, (2) to determine if the adenovirus will evoke cytotoxic T-cells, which will recogniz autologous cells infected with adenovirus vectors of the same or different serotypes, (3) to determine if humoral and cellular immune response, evoked by respiratory tract adenoviral administration in the lun differs from that in blood, and (4) to determine if bronchial administration of an adenovirus vector evokes local or systemic inflammation. The RAC ended discussion of this protocol without any recommendations.

XI. Human Gene Transfer Protocol #9711-221 entitled: *Phase I Study of Direct Administration of a Replication-Deficient Adenovirus Vector (Ad_{GV}VEGF121.10) Containing the VEGF121 cDNA to th Ischemic Myocardium of Individuals with Life Threatening Diffuse Coronary Artery Disease*

PI: Ronald G. Crystal, New York Hospital-Cornell Medical Center

Reviewers: Verma , Gordon, Lysau

Ad hocs Harold Ginsberg, NIH ; William Kraus, Duke Universi

The rationale for full RAC review of the protocol included: the first administration of an adenoviral vector to the ischemic myocardium of patients with coronary artery disease, the invasiveness of the vector administration procedure during open-chest heart surgery, the use of patients in the "compassionate use" group, and the concern of potential myocarditis induced by the adenoviral vecto

Review -- Dr. Verm

Dr. Mickelson called on Dr. Verma to present his primary review of the protocol submitted by Dr. Ronal G. Crystal (The New York Hospital-Cornell Medical Center, New York, New York). In his overview, Dr. Verma stated that the overall goal of this protocol is to evaluate the adenovirus vector delivery of cD encoding an isoform (121) of human vascular endothelial growth factor (VEGF) directly to ische

myocardium of individuals with life threatening myocardial or coronary artery disease (CAD). Pre-clinical information on animal model systems is provided. Dr. Crystal has proposed using a first generation adenoviral vector (Ad5, E1A$^-$, partial E1B$^-$, and E3$^-$) as the means of delivery.

The major advantages of potential gene therapy of CAD are: (1) It provides the equivalent of a "sustained-release capsule," providing high concentrations of a therapeutic protein for a sustained period. (2) While animal models of hind limb ischemia do show induction of angiogenesis with a single intraarterial bolus of the VEGF protein, intramuscular administration for limb ischemia requires repe doses over several days as does intracoronary administration for myocardial ischemia. Thus the ability introduce genes for a sustained period of several days with adenoviral vectors is desirable. (3) Gene transfer can be designed to provide regional delivery of high concentration of VEGF to the ischemic lim or ischemic myocardium.

In his critique of the protocol, Dr. Verma stated that he had several concerns. The current proposal i based on a number of observations included as publications, manuscripts in press, or submitted. All of these observations lead to the general conclusion that angiogenic growth factors, administered either a recombinant protein or by gene transfer, may augment blood flow toischemic myocardium. Thes strategies appear to improve myocardial perfusion by promoting the development of collateral blood vessels that serve as endogenous bypass routes capable of circumventing obstructed blood vessels. It should, however, be stated that actual documentation of the collateral vessels in coronary circulation*in vivo* is difficult in animal model systems. In situations where direct DNA was injected, only samples from human systems are known thus far. Therefore, the animal study can provide only limited conclusions.

Dr. Verma had some concerns regarding the vectors to be used. The primary concern related to the ab of the adenovirus vector to induce CTL . The investigators are cognizant of this concern, and repeatedl mention the fact that the expression will be for only a short term. Dr. Verma's main concern on this point was that, even though the expression is for a short term, it is likely to elicit the CTL against viral protein and perhaps even the transgene product. There will be ten injections of 100 µl each in the heart, and th injections will be separated by 1.5 to 2 cm. The overall area covered will be about 150 to 220 cm^2. If these cells are infected efficiently, then a large number of these cells will eventually be removed or killed by the CTL , which will create considerable damage to the heart. The investigator states that the function of th heart will not be affected. Unfortunately, stated Dr. Verma , there is absolutely no proof from th histological data to support this statement. It would have been very beneficial if there were any histological samples showing that the injected areas were not damaged. This concern becomes more serious for many myocardial patients who already have fibrosis because further fibrosis due to inflammatory response CTL could be very detrimental. This concern clearly needs to be resolved o answered before any efficacious clinical outcome can be determined.

Other concerns include: (1) A number of patients are going to be carrying antibodies against adenovirus and, therefore, may not be susceptible to infection by the adenovirus vector. (2) The repeat injections are problematic. (3) There is the additional question of whether VEGF if made in an uncontrolled fashion ca have the potential to cause damage. (4) It is not really clear if the technology used in animal systems is sufficient to determine whether one can actually detect the dissemination of the virus. It is highly probable that the virus is disseminated quite extensively. It will be essential to do PCR to determine whether oth sites have been transduced due to viral disseminatio

Dr. Verma had a number of concerns regarding the methodology to be used regarding the bypass surg itself. First, the site selected for direct myocardial injections is of concern. Aside from the fact that the thickness of the right ventricle wall is one-third to one-fourth that of the left ventricle, what is the rationale for performing one of the three injections into the right ventricle in Part A? Second, the investigator states

that the rationale for targeting directly to the right ventricle for myocardial injection in Part A is that injury of this myocardial area is the least likely part of the heart to be adversely affected by the adenovirus VEGF gene transfer. What is the evidence for this rationale? Third, there are several questions regarding the basic science of the pre-clinical studies, e.g., the kinetics of induction of the production of VEGF , th magnitude of production of VEGF , or other effects that excessive VEGF may have on localized ar

In summary, Dr. Verma stated that this is a highly qualified group of investigators who are proposing t use adenoviral vectors, and these investigators have substantial experience. Dr. Verma believes however, that the proposal is somewhat premature. There are still serious issues of " vectrology " that n to be resolved, i.e., lack of histological data showing that heart muscles are not damaged by adenovirus vector injection.

Review -- Dr. Gordon

This is a protocol to introduce an adenoviral vector encoding VEGF , a known stimulant of angiogenesis directly into the myocardial muscle of patients with diffuse vessel narrowing. Three groups of patients will be studied. Group A consists of individuals undergoing bypass, but with one or more vessels not amenable to bypass. These patients will be used to determine toxicity levels of the virus. Group B consists of patients with inoperable disease who will undergo elective thoracotomy for gene insertion Group C consists of patients undergoing bypass who will be studied for efficacy of the gene transfer procedure. Gene insertion will be accomplished by direct injection of 100 microliters of virus-containin solution into ten sites in the myocardium, with an effort made to target areas that are unlikely to benefit from a bypass. Dr. Gordon identified a number of issues relevant to this protocol that the investigators should address, including:

In Group A, increasing doses of virus will be administered until "toxicity" is observed. What kind of toxicity is anticipated? If arrhythmias are included, then extension of the "highest nontoxic dose" to group B could be dangerous. In this latter group, the controlled conditions available in the setting of bypass will not prevail. This point should be discussed.

Dr. Gordon stated that Group B is of particular concern. First, no control group is planned. While it is entirely understandable that such a group is logistically and ethically problematic, the investigator should discuss this issue. It is important in this regard that patients in Groups A and C are not truly comparable to those in Group B. Not only do they have operable disease, they have had an operation. Another issue with Group B relates to risk factors that preclude admittance to the study. The application states that patients with recent myocardial infarction, malignant arrhythmias, or chronic heart failure will be excluded. Dr. Gordon said it is highly unlikely that these patients will have no history of myocardial infarction or failure or arrhythmias, and it is far more likely that they will be on medication to alleviate chronic heart failure and/or arrhythmia predisposition. The investigators should discuss what level of medical intervention for such conditions would be considered too great for admittance to the study. The history of arrhythmias is particularly troubling because insertion of the hypodermic needle could precipitate such an event. Also to be excluded are those who have undergone transplantation. While this exclusion makes sense, the investigators should also reassure the RAC that patients who are likely to undergo transplantation in the near future will not be admitted to the study. While transplantation is not a perfect therapy, it at least has some record of success. A cardiac disaster in a study patient who is likely to undergo transplantation soon would be unfortunate indeed.

Dr. Gordon said there is a concern regarding evaluation of efficacy of treatment in Groups A and C. These patients will have undergone bypass surgery immediately prior to the gene insertion; therefore, we expect their cardiac function will improve regardless of whether gene therapy takes place. Even though an effort

will be made to introduce the vector into ischemic areas not predicted to benefit from the bypass, collatera circulation may improve after bypass and give a misleadingly-positive result. The investigators should discuss this issue, and should also address analysis of cardiac perfusion studies in these patients. Based on these concerns, Dr. Gordon said he wonders if meaningful statistics can be obtained.

Dr. Gordon said that under normal circumstances the injection protocol described here is likely to be safe, especially in patients already in the operating room. However, patients in Groups A and C will be treated with anticoagulants and will have low platelet counts. Predisposition to bleeding will persist for many hours after surgery. If some bleeding occurs at the injection sites, intramuscular hematomas could form which could affect the delivery of virus to target cells. Moreover, viral delivery may not adequately model the use of such therapies in patients such as those in Group B, who will not receive anticoagulants. The investigators should discuss this point, with particular reference to any available animal studies.

Review -- Dr. Lysaugh

Dr. Lysaught provided a detailed written review, to which the investigators had previously responded i writing, and she summarized the highlights of her review. She stated that, in patients who are already compromised by the disease, the investigators need to address the possibility of inflammation in response to the adenovirus. She was particularly concerned about the Group B part of the protocol regarding the "compassionate use" of gene therapy. She suggested delaying initiation of this part of the study until efficacy of the treatment has been demonstrated. She asked the investigators to provide long-term animal data on toxicity. Dr. Lysaught asked the investigators to elaborate upon the clinical endpoints to provid quantitative estimates of efficacy, i.e., any measurable differences as a result of gene therapy that would result in significant improvement for the patients. Dr. Lysaught asked the investigators to clarify the iss of vertical transmission of adenovirus vectors to offspring.

Dr. Lysaught raised several points regarding the Informed Consent document, which have bee addressed by the investigators in their written response. The potential costs of an adverse event is not clearly stated for Group B patients. There is no mention of reproductive considerations for men and women. The likelihood of significant media attention is not clearly stated. She noted that significant media attention resulted from a related study of VEGF treatment of peripheral artery disease (Protoco #9409-088, Dr. Jeffrey Isner). In that study, an article in the *Dayton Daily News* stated, "Scientists tinkering with gene therapy think they have found a way to make bad hearts grow their own bypasses."

Dr. Lysaught raised a point about the inclusion criteria. She asked investigators to clarify the criterio regarding patients with "life threatening" diffuse coronary artery disease. She noted a correspondence between Dr. Crystal and his IRB regarding the change of the protocol title from individuals with diffuse coronary artery disease" to "…individuals with life threatening diffuse coronary artery disease." She asked if the title imply that there are two categories of coronary artery disease, life threatening vs. non life threatening?

Review -- Dr. Ginsberg

Dr. Ginsberg provided his critique of the protocol. He stated that this is a very well written proposal, and in most aspects well thought-out. He found several important problems that require RAC discussion.

Part A is designed to determine the highest nontoxic dose to be used in Parts B and C. In this protocol, however, it is unclear how this safe, nontoxic dose relative to production of inflammation will be determined in these volunteers. There is no mention at any place in this document of the danger of the adenovirus vector producing inflammation, i.e., myocarditis , including the section on "Risk-Benefi

Consideration." Indeed, the document states that "The risks for the delivery of saline are identical to those associated with delivery of the vector."

Animal studies have been performed with this vector; however, no histological data are presented to indicate the degree of inflammation produced. There is one statement that, when greater than 10-fold of the proposed dose was used in animals, the administration produced only 'minimal to mild inflammation which did not lead to any illness or death.' No actual data were shown, however, and clearly the animals were not suffering from severe coronary disease as will the patients involved in this study. It is well known that any vector that has the extent of E3 deletion, as in this vector, will produce considerable inflammation in the lung after pulmonary instillation. Therefore it appears there is an absence of any data which could lead one to believe that myocarditis would not be produced after inoculation with the proposed vector Clearly there are other possible vector constructs that would possibly cause much less inflammation.

Evidence was presented recently (November 11, 1997) at the American Heart Association Scientific Sessions in Orlando, Florida, indicating that an adenovirus can cause life-threatening myocarditis . Th investigators from John Hopkins University and from Baylor University reported examination of autopsied heart tissue from 13 patients. Seven of the patients had myocarditis and six did not. Virus was detecte five of the seven heart patients (71.4%), but in none of the control hearts. Adenovirus DNA was identified in the afflicted hearts in addition to evidence that Coxsackie B virus (an enterovirus) was present, whic has always been considered the only viral etiologic agent of myocarditis . These data imply that th adenovirus is an etiologic agent in a significant proportion of adult cases of viral myocarditis . In fact adenovirus and the enterovirus could work together to induce this condition. The type of adenoviru involved was not identified, and whether it involves a single type or multiple types is unknown. These data strongly indicate, however, that type 5 adenovirus should not be inoculated into the human heart unti extensive studies in animals have been pursued.

Written Review -- Dr. Kraus

Dr. Mickelson noted a detailed written review provided by Dr. Kraus, to which Dr. Crystal has responded in writing. Dr. Kraus noted that there seems to be insufficient justification for the use of an adenovirus vector for delivery of the cDNA . Others are studying administration of naked DNAs using similar me and for similar purposes. The added value of the use of viral vectors over naked DNA injections is not clear. He asked how the investigators are going to assess a successful therapeutic intervention? The clinical endpoints are not well defined and are non-specific. He stated that direct needle injections into the heart, with likely localized inflammatory responses and scarring, are likely to lead to local anisotropy and be pro-arrhythmic with potentially devastating consequences in individuals with underlying myocardial ischemia. He asked whether preliminary *in vitro* and *in vivo* safety data obtained in appropriate models are sufficient. Dr. Kraus has a major issue with the way in which medical costs of this study are being passed on to study subjects. He stated that all of the study costs in excess of "usual care" associated with the surgery itself should be borne by the study sponsor.

Other Comments

Dr. Aguilar-Cordova asked if the animal studies have been performed with pre-immunized animals as they are more likely to develop severe reactions to adenoviral administration. He suggested that the dose should be given in viral particle units as well as their relation to the infectious units. He asked how the clinical outcome will be assessed.

Dr. McIvor was concerned about the Group B patients for whom the only reason to do the surgery is for vector delivery; it is justified only if there is evidence for the efficacy of this treatment. He said that the risk

of surgery compounds the risk of vector delivery, and he asked the investigators to elaborate on the surgical risk.

Dr. Macklin said that no Phase I study can justify the use of the term "compassionate use." As a point of clarification, Dr. Noguchi said that there is no such terminology in the FDA legal language. The FDA has emergency IND authority to be applied case-by-case for a situation where there is no other potentia therapy available. Dr. Macklin noted that "compassionate use" is a term used by IRBs under certai conditions.

Dr. Lai noted that the protocol is quite different from any protocol reviewed by the RAC so far; the surgical procedure is very invasive and the risk of adenovirus-induced myocarditis is very high. He asked if th FDA has approved this protocol. Dr. Noguchi said he wants to hear the RAC discussion of this protocol.

Ms. Rothenberg stated that the protocol could be unethical. More data showing efficacy is needed before proceeding to Group B patients. Patients in Groups A and C are going in for necessary cardiac surgery, and it is unethical to impose the additional risk of gene transfer unless the investigators have a better understanding of the potential benefits. She was concerned about the Informed Consent document regarding the costs and compensation associated with adverse effects. She said relative risks and benefits are not easily understood by the patients.

Dr. Gordon inquired if there is any animal model to investigate the likelihood that induction of neo- vascularization in one part of the heart will actually reduce the blood supply to the ischemic part o the heart. Neo- vascularization in this type of setting may actually be harmful to the patient

As a point of clarification, Ms. Rothenberg said that she did not mean to imply that Dr. Crystal is performing an unethical protocol. She asked Dr. Crystal to explain how his IRB evaluated the relativ risks and benefits of this protocol, and how the determination was made to accept those risks for the subjects in his institution.

Dr. Markert was concerned that the Informed Consent document does not state any of the risks associ with potential myocarditis and arrhythmias. In terms of the animal studies, attempts should be made t develop an animal model with a heart condition similar to the coronary artery disease in patients to be treated in the protocol. In terms of costs, she understands that heart patients are charged a single fee for the entire procedure.

Dr. Leinwand asked the investigators to elaborate on the issue of potential myocarditis induced adenoviruses.

Dr. Mickelson asked the investigators to explain the risk of left thoracotomy to be performed on the Gro B patients in terms of the relative risks and benefits. She noted that patients should be pre-screened for any neoplasias so that there is no potential risk of neo- vascularization with small tumors. Dr. Mark that she has consulted with cardiac surgeons at her institution and they think that a left thoracotomy is appropriate procedure for this protocol.

Drs. Mickelson, McIvor, and Lysaught asked the surgeon to explain the risk relative to other types o bypass heart surgery.

Investigator Response -- Drs. Crystal and Rosengar

As a point of reference for this proposed gene transfer protocol, Dr. Crystal made a brief video

presentation of the procedure called " Transmyocardial Revascularizaton (TMR)", which has performed on 3,000 individuals worldwide. Dr. Crystal introduced Dr. Rosengart , a cardiac surgeon and co-investigator.

TMR is a procedure that creates channels in the myocardium using high energy lasers. The mechanis of action is unclear and it relates to channel patency and/or angiogenic response. It is the only avai therapy for patients with significant coronary ischemia unsuitable for angioplasty or bypass. It requires left anterior thoracotomy (6-10 cm). In TMR , between 25 and 50 laser-induced 1 mm diameter channel opened in left ventricle.

Dr. Crystal pointed out that the entrance criteria of the Group B patients are identical to these for patients treated with TMR , and the identical left thoracotomy procedure is performed. The video presentat showed that between 25 to 50 holes of 1 mm size are punched with a laser beam, resulting in burning tissue. Dr. Crystal said that one should keep the reference point of the TMR procedure in mind durin deliberation of the present protocol.

Dr. Crystal presented animal experiment data. The studies involved 64 rats, 20 dogs, 65 pigs, and 50 mice with regard to the amount of inflammation and necrosis induced by the adenovirus vector, Ad_{GV}VEGF121.10. An empty vector, AdCMV.Null , and saline were used as controls for thes experiments.

Dr. Crystal described the pig experiments. A metal sleeve constrictor was placed over the left circumflex coronary artery to induce artery occlusion. An adenovirus vector or saline control was administered to the heart three weeks later. Dr. Crystal showed myocardial inflammation data three days following direct myocardial injection of the clinical grade vector. At the highest dose planned for the protocol (10^9 pfu) only mild inflammation resulted from injection of the vector and equaled the level of inflammation caused by saline. The amount of myocardial necrosis was minimal. Similar results were observed 28 days following direct myocardial injections. Dr. Crystal noted that occlusion of the coronary artery*per se* caused mild inflammation.

Dr. Greenblatt asked if these experiments were performed on pre-immunized animals. Dr. Crystal sai pigs are not pre-immunized. Dr. Ginsberg asked whether similar experiments have beenperformed wit cotton rats. Dr. Crystal responded that rats are too small for this kind of experiment. Dr. Aguilar-Cordova asked how many sections of the heart have been injected, and what methods are used to determine where the vector was administered. Dr. Crystal responded that multiple sections of the heart are examined for pathology. He agreed with Dr. Aguilar-Cordova that it is difficult to determine exactly where the administration of vector or saline was performed. In addition, Dr. Crystal said that results from their group on dogs using an adenovirus containing a marker gene have been published. In these studies, a single 100µl injection of the vector carrying a marker gene was performed. Expression of the marker gene 1.5 cm from the injection site was 10% of the expression observed at the injection site.

Dr. Crystal stated that the rat experiments were performed with pre-immunized and non-immunized animals with similar results. He noted that in the pig experiments, neutralizing antibodies were very low; there was no problem with repeat administration.

In terms of arrhythmias, Dr. Crystal noted that he has not observed any arrhythmias in the animal experiments. He pointed out that in the TMR procedure, which appears to be more severe than injectio of vectors, there have been no fatal arrhythmias.

Dr. Crystal explained his rationale for performing the Group B study. The study is designed to obtain

preliminary data on efficacy. In Group A and C patients, such data are not available due to the bypass surgery to be performed at the same time. If gene transfer is successful, the Group B non- bypassable patients might benefit from the treatment. Patients who have both non- bypassable and bypassable have a higher mortality than patients who have only bypassable areas. In the TMR study with a sim group of patients, no fatality has been reported.

In terms of statistical evaluation of the efficacy data, Dr. Crystal said that similar methodologies have been developed for the published pig study. In the human study, standard scoring systems for tests such as angiography, nuclear medicine, and echo cardiography will be evaluated by two internal individuals an one outside individual; the study will be blinded. In addition, there will be a safety monitoring board, with experts from around the country, to evaluate the safety data on a continuing basis.

Dr. Crystal noted that he has not seen any hematomas in the pig study. He said that Dr. Rosengart experienced cardiac surgeon; the heart was cooled down during surgery and no hematomas wer observed.

In terms of "compassionate use," Dr. Crystal noted that this phrase is an IRB term meaning that th patients have no other alternative therapies. He agreed to remove the term "compassionate use" from the protocol.

With regard to long-term efficacy, Dr. Crystal said the animals have been observed for ten months and no efficacy data has been collected; in another study with adipose tissue, he observed neo- vascularization for a period of up to three months.

In terms of gonadal distribution of the vector, Dr. Crystal noted there were only background level detected in his animal studies. In the Informed Consent document, the importance of using barrier contraception will be included. With regard to inclusion of fertile females in this study, Dr.Crystal note that such recommendations were made by the RAC for his other protocols previously approved by the RAC.

With regard to the question of compensation in case of a potential adverse reaction, Dr. Crystal noted that it is a difficult issue both for the RAC and for the various institutions. He observed the standard practice in this protocol. Dr. Lysaught noted that the Informed Consent document contains two parts, and the cost such treatments are stated in one part of the document and not in the other part. Dr. Crystal agreed to amend the document.

In terms of patient confidentiality, Dr. Crystal said that any patient's identity will not be disclosed in this study. Dr. Lysaught said that in the Informed Consent document, the patients should be advised about likelihood of media interest particularly with regard to the present protocol. Dr. Crystal agreed to include such a statement in the Informed Consent document.

With regard to the change of the protocol title to include "life threatening" in the title, Dr. Crystal noted that this recommendation was made by his IRB to indicate that the coronary artery disease patients have serious disease that is life threatening.

Responding to Dr. Ginsberg's review, Dr. Crystal agreed that myocarditis caused by adenovirus infecti has been reported in the literature. He noted that inflammation is associated with adenoviruses retaining the E1 region. Dr. Crystal stated that studies in animals with the Ad$_{GV}$VEGF121.10 vector used doses more than ten times greater than the highest proposed dose for this protocol. Even with this relatively high dosage, these studies have shown only minimal to mild inflammation, which did not lead to any illness or

death. In the context of theoretical concerns regarding the adenovirus, it is important to note that doses equivalent to the highest dose to be used in the present protocol have been administered repetitively by way of aerosol administration to the bronchi of individuals with cystic fibrosis without adverse effects.

Dr. McIvor asked the surgeon to describe the surgical procedure. Dr. Rosengart explained that tw different surgical procedures will be performed. In the first procedure, for the patients with bypass surgery, a medium sternotomy will be performed. A skin incision from the top to the bottom of the breast bone i made; then the breast bone is divided with a saw. The second procedure, the left thoracotomy for th Group B patients, is 'minimally invasive" cardiac surgery in which an horizontal incision of five to seven inches is made between the rib bones. A chest tube is then inserted. Dr. Crystal noted that the State of New York publishes statistics for every cardiac surgeon in the state showing the number of operations performed, the number of deaths, and expected mortality. He said Dr. Rosengart has a high professior reputation.

Responding to Dr. Gordon's question regarding evaluation of efficacy of the treatment in patients in Groups A and C, Dr. Crystal cited a porcine study that he conducted with Dr. C. A. Mack; similar studies will be performed with patients in Groups A and C. The echo cardiography used in these studies is functional measure of the myocardium both at rest and exercise; the data suggest functional restoration.

In terms of the cost to the patients, Dr. Crystal stated that the General Clinical Research Center will cover the costs of safety studies. The other costs involving angiograms, nuclear medicine,echo cardiograph and treadmill studies will be covered by the investigators. These are not the responsibility of the patient.

Dr. Mickelson inquired if the illness of Group B patients might subject them to higher risks than Groups A and C, and therefore they might take a longer time to recover from the surgery. Dr. Rosengart respond that the small incision of thoracotomy is very well tolerated by most patients. Dr. Crystal added that the are identical to the patients receiving TMR

Ms. Rothenberg asked the investigators to clarify the intent of the study for Group B patients, i.e., research vs. therapy. Dr. Rosengart responded that, as a surgeon taking care of patients in the context of research protocol, he believes the procedure potentially may benefit the individual patients.

Ms. Rothenberg observed that the Group B patients have the same inclusion criteria as the TMR study Because of this, she asked how the surgeon would advise the patients to choose which protocol in which to enroll. Dr. Rosengart responded that, at this point, TMR is not a proven therapy to be offered as option. Dr. Crystal said that the investigators and his IRB have discussed the issue. Dr. Aguilar-Cordov had a similar concern of how to choose between TMR and the gene transfer protocol to enroll the sam population of patients. Dr. Crystal did not provide a direct answer to this question.

Dr. Lysaught was concerned that this protocol would be presented to patients as potentially beneficial Parts A and C are strictly research protocols and patients would agree to do this because they want to contribute to the research endeavor. She noted there is a distinction between "potential" for benefit and "probability" for benefit; there should be some efficacy data before proceeding on the Part B study. Dr. Crystal said he would welcome any comments from the RAC as to how best to inform the patients.

Dr. Leinwand inquired if using the clinical grade vector is the factor responsible for lack of inflammatio seen in the pig experiments. Dr. Crystal responded that he showed his own data with the clinical grade vector; he cannot explain why it differs from other published studies involving adenovirus-induced inflammation. As to Dr. Leinwan's question regarding vector persistence, Dr. Crystal explained that VEGF expression disappeared between day 7 and day 14, and the vector sequences were diminished

close to background levels by day 28. The transient expression of VEGF is an advantage for neo- vascularization application. Dr. Crystal noted that the mechanism for the disappearance of adenov DNA is very complex, and it is a subject under active investigation probably involving immunological reactions as well as apoptosis of the host cells.

Dr. Gordon asked how the size of incision of the thoracotomy compares with pericardium window an pericardiectomy . Dr. Rosengart said the size of the incision is in between these two procedures. Gordon asked if there is any risk of neo- vascularization of the pericardium. Dr. Crystal said that any su risk will be assessed by measuring VEGF levels within the pericardium during the first few days while t patient still has a catheter inserted.

Responding to Dr. Lysaught's question of long-term follow-up, Dr. Crystal said that he has changed the Informed Consent document to reflect a lifetime follow-up rather than one year.

Dr. Leinwand asked Dr. Crystal to clarify if pre-screening will be performed for malignancies. Dr. Crysta explained that most tumors make some VEGF and he has considered the pros and cons of requirin pre-screening. He has come to the conclusion of not requiring pre-screening because it may pose an additional burden on the patients and will yield false positive results.

Dr. Verma asked why transgene expression has been observed only for a short period of time if the no inflammatory response to the virus. Dr. Crystal said that the mechanism for this phenomenon is complex and should be investigated further.

Dr. McIvor asked about the risk of the surgical procedures. Dr. Rosengart responded that the risk o sternotomy is the same as the risk for bypass surgery; the main risk is infection, with a mortality rate o about 1 percent. The mortality rate for thoracotomy is less than 1 percent. Dr. McIvor asked ho investigators will assess efficacy in Group B patients since there is no control group. Dr. Crystal responded that Group B patients will serve as their own controls; several functional tests will assess the effects of neo- vascularization . Groups A and C have a "watershed" effect from the bypass surgery that confound the efficacy assessment.

Dr. McIvor said he sensed a remaining disagreement regarding the risk of myocarditis . He questione why the investigators are moving forward with an invasive procedure where there is potential risk of an inflammatory response. Dr. Crystal responded that his decision to move forward is based on the animal studies. He plans to proceed with caution, and to give the patients the best possible Informed Consent document describing the potential risk of " myocarditis

Dr. Aguilar-Cordova made four specific recommendations: (1) data should be provided on pre-immunized animals regarding toxicity of the adenovirus vector, (2) a scenario should be described to make the outcome of the protocol interpretable, (3) the Group B patients should be given the choice of TMR or th gene transfer procedure, and (4) the adenovirus dose should be expressed in both virus particle units and in infectious units.

Dr. Noguchi made an observation regarding various results reported in the literature about adenovirus toxicity. He said that the FDA requires that the same vector preparations to be used in humans be tested first in animals. It is likely that the purer clinical grade vectors may induce less inflammatory response than the research grade vectors used in many animal studies.

Dr. Greenblatt stated that Phase I studies are designed to study toxicity, and any efficacy assessment secondary. He said that myocarditis reported in the literature is due to replication-competent adenoviru

and the vector used in the protocol is replication-deficient.

Dr. Lysaught said she feels that the protocol is premature in that many issues about the adenovirus ve are still outstanding; she would prefer to postpone the study until other adenovirus protocols have provided more safety data. Dr. Verma expressed the same feeling that there is a certain degree o underlying concern remaining with most RAC members. Dr. Crystal responded that his reputation is at stake if something untoward happens. He noted a gulf between the clinical investigators and those who have not had the clinical experience in making a decision as to when to proceed with a clinical trial.

Dr. Markert stated that she would be more comfortable if the pig toxicity data were obtained fro pre-immunized animals, and if the interval between the first and the second patients was longer than two weeks in order to allow a toxicity assessment to be performed on the first patient. Dr. Crystal responded that from his data based on repetitive administration of adenovirus vectors in animals, there is no inflammation detected with his clinical grade vector. There is a theoretical concern, but his data show that the clinical grade vector is safe.

Dr. Macklin stated that she is not convinced by the entire discussion that the risks could be justified by the anticipated benefits of this protocol.

Dr. Aguilar-Cordova noted that a clinical grade adenovirus vector has been found to cause inflammatory reaction in the brain of cotton rats; it is more severe in pre-immunized animals than in non-immunized animals.

Dr. Lai said that the RAC no longer has approval authority; however, he suggested taking a straw vote on this protocol.

Dr. McIvor asked if there is a way to assess if the vector is causing an inflammatory reaction. Dr. Crystal responded that echo cardiography will assess heart functio

Dr. Gordon stated his assessment of the protocol. He said that the risks to the patients are not terrible considering the seriousness of the disease. He was concerned whether the protocol is properly designed to obtain definitive results, particularly with Part B of the study. Myocarditis is his concern, but he is no certain how serious its impact will be on the patients. Dr. Crystal said that this study is primarily an early Phase I study. Part A will assess the toxicity before the protocol progresses to Parts B and C to evaluate efficacy. Dr. Gordon asked whether the investigators have considered delivering VEGF via a plasmi DNA vehicle. Dr. Crystal responded that it is not relevant to the discussion of this protocol.

Dr. Mickelson called on comments from the public.

Dr. Matthew During (Auckland University, New Zealand) noted that the RAC was deliberating on whether a Phase I study should have an efficacy endpoint. He supported Dr. Crystals response that the gene transfer intervention in the context of heart surgery is relatively trivial. There must have been some degree of inflammation since the virus is cleared from the body in short time. The question of the risk of inflammation to the patients will be answered by the human trial, and additional animal experiments will not provide any significant data. He noted an ethical dilemma that the trial cannot be justified without any benefit to the patients. As a point of clarification, Dr. Lysaught said that for adult patients there does no have to be a benefit to the patients. There has to be a significant advance in knowledge to balance out the risk for adult patients who can consent for themselves; it is different for children.

Dr. Victoria Allgood (GeneMedicine , Houston, Texas) asked if there is any risk of germ line transmi

for patients with reproductive potential. Dr. Crystal said that he is not concerned with this risk since adenoviral persistence is short term.

Dr. Aguilar-Cordova said that he would recommend writing a letter to the investigators summarizing the RA's concerns about the protocol. He offered to draft a motion for RAC consideration after the lunch break. Dr. Crystal said that he can respond to RAC comments today. Dr. Verma preferred to reconven the discussion after the lunch break to decide on a course of action.

After the lunch break, Dr. Mickelson reconvened the meeting to discuss the format by which to make a formal recommendation to Dr. Crystal.

Dr. Aguilar-Cordova made four recommendations as follows: (1) to present the data including histopathological studies of intracardiac injection of VEGF121.10 in pre-immunized animals, (2) to provide a scenario of possible outcome and the interpretation of the possible results, (3) to provide a clarification of the methodologies used to determine infectious units and the vector particle to infectious units ratio, and to describe dosing based on vector particles, and (4) to provide patients in Part B with choice of other investigational procedures such as TMR

Dr. Mickelson noted that Dr. Crystal has agreed in an informal discussion at the lunch break to incorporate these suggestions to his protocol.

Dr. Verma noted that the protocol is a Phase I study using a replication-deficient adenoviral vector; mo of the concerns raised regarding " vectrology " of adenoviruses are more general in nature. Dr. Ver suggested that the investigators proceed with the study of Group A patients to obtain toxicity data, and then report back to the RAC to allay its concerns before progressing to Group B and C patients. Dr. Crystal responded that he agrees with the sequence of proceeding first with Group A, and then B and C. However, he has some concerns about reporting back to the RAC before initiating later parts of the protocol, e.g., concerns regarding proprietary information generated from the clinical trial.

Dr. McIvor proposed sending a letter to the IBC and IRB conveying the RAC concern: inflammator reaction generated by administration to the heart, the study design to determine if there is any adverse effects, and questions concerning whether there would be any efficacy of the treatment.

Dr. Markert agreed with the idea of sending a letter to the IBC and IRB ; however, she said she sens divided opinion among RAC members.

Dr. Gordon was concerned about sending a letter to the local committees; he would prefer to send a summary of the RAC discussion instead. He said that inclusion of the Group B patients is not appropriate for a Phase I study.

Dr. Macklin asked for clarification on whether the RAC recommends delaying the Group B and Group C portions of the study. Dr. Gordon said that in his view Group B is a therapeutic investigation and it is in a different category of Phase I protocols. Dr. Crystal stated that he respectfully disagrees with Dr. Gordon's statement. He feels that Group B consists of patients with no alternative therapies, and that the animal data suggests potential efficacy of the treatment.

Dr. Markert noted that the autopsy data will be very valuable in determining the extent of myocarditis any, caused by the vector injection. Dr. Crystal agreed to include a request for autopsy in the Informed Consent document.

Dr. Greenblatt said he sensed a divided opinion in the RAC discussion; he was not sure the RAC has t authority to send a letter to the IBC or IRB with specific recommendations regarding this protocol. Ms Knorr explained that IBCs are directly under the authority of the NIH *NIH Guidelines* and it is appropriate for the RAC to send a letter to them. The RAC may make its recommendations to the NIH Director and those recommendations can be passed on to the IRB . Dr. Verma was concerned that might misunderstand and interpret the letter to mean that the RAC is questioning IRB approval of th protocol. Dr. McIvor said that the purpose of the letter is to convey RAC concerns to the IRB

Dr. Mickelson noted that a RAC recommendation of a general policy issue may be transmitted to the NIH Director; other avenues may be considered for sending concerns regarding specific protocols.

Dr. Crystal stated that the RAC should focus on a more general issues and allow the FDA to deal with specific issues of the protocol, just like other clinical trials involving new drug development.

Dr. Noguchi stated that the track record of the RAC is to focus on issues that deserve public discussion. Any unexpected toxicity from a gene transfer protocol may be brought to the attention of the RAC for public discussion. He was not sure what is expected of an IBC and IRB in response to RAC concerns i letter is sent to them.

Ms. Rothenberg (speaking via telephone) suggested that the RAC letter should be addressed to the FDA for its consideration in deciding whether to approve the protocol.

Dr. Macklin noted that it is unfair to Dr. Crystal to use his protocol to debate the new role of the RAC in the oversight of human gene transfer research. She suggested tabling the discussion of the protocol until the RAC has a chance, in a later session, to deliberate how to handle protocol reviews. Dr. Crystal said that he is uncomfortable with the possibility that the RAC might discuss his protocol in a later portion of the meeting during his absence.

Dr. Gordon said that discussion of the protocol should be separated from a general discussion of RAC procedures. He suggested holding in abeyance any decision to write letters to anybody. The RAC discussion will be conveyed in published minutes. Although he has some reservations regarding the protocol, he favors going forward with this clinical trial and said he feels the RAC does not appear to have reached a consensus with respect to the protocol.

Dr. Markert suggested a straw poll of RAC membe individual opinions.

Dr. Verma agreed that the FDA has the approval authority; therefore, RAC concerns should be directe the FDA.

Ms. Knorr reminded the RAC that it should make a motion to end the discussion, or to take any actio regarding the protocol.

Dr. Lysaught made a motion to table the discussion, and to have the RAC discuss the general procedu issue of how to communicate with other agencies and to disseminate the results of RAC deliberations. Dr. Mickelson explained that the motion is to end the discussion. Dr. Verma seconded the motio

Committee Motion 7

A motion was made by Dr. Lysaught and seconded by Dr. Verma to end the discussion of Proto

9711-221. The motion passed by a vote of 11 in favor, 0 opposed, and 1 abstention.

Protocol Summary

Dr. Ronald G. Crystal, The New York Hospital-Cornell Medical Center, New York, New York, will conduct gene transfer experiments on a total of 59 male or female patients, age 18 to 85 years, with life threatening diffuse coronary artery disease. The vector, Ad_{GV}VEGF121.10, is an $E1A^-$, partial $E1B^-$, and $E3^-$ adenovirus type 5 expressing the human VEGF121 cDNA under the control of the C promoter/enhancer. Ad_{GV}VEGF121.10 will be administered directly to the ischemic myocardium at the time of open thoracotomy. Group A (n=9) is a dose ranging study to determine the highest safe dose to used in Groups B and C. Group B will use that dose in a compassionate use group (n=10) and Group C will use that dose in a blinded, controlled (vs saline) study (n=20 vector, n=20 saline) superimposed o their coronary bypass surgery. The objectives are: (1) to determine the dose-dependent safety/toxicity of direct administration of Ad_{GV}VEGF121.10 to the ischemic myocardium, and (2) to demonstrate whether direct administration of Ad_{GV}VEGF121.10 to the myocardium will induce growth of collateral blood vessels, improve coronary blood flow, and improve cardiac function in the region of ischemia.

XII. Amendment to Appendix M-I, Submission Requirements--Human Gene Transfer Experiments Regarding the Timing of Institutional Biosafety Committee and Institutional Review Board Approvals/ Marker

During the June 12-13, 1997, RAC meeting, the following motions were approved by the Committee: (1) A motion was made that Appendix M-I, *Submission Requirements -- Human Gene Transfer Experiments,* should be amended to require investigators to submit documentation verifying that a human gene transfer protocol has been submitted to an appropriate IBC. Evidence of IBC notification shall be provided at the time the protocol is submitted to ORDA. (2) A motion was made to delete the requirement at the time o ORDA submission of IBC and IRB approvals from Appendix M, *Submission Requirements -- Human Gene Transfer Experiments,* of the *NIH Guidelin*

On September 10, 1997, a letter was received from the American Biological Safety Association requesting that the public comment period for this proposed action under the *NIH Guidelin*published in the *Federal Register* on August 20, 1997 (62 FR 44387) be extended for an additional 60 days.

During the September 12, 1997 RAC meeting, the RAC was scheduled to vote on the proposed actions to delete prior IBC and IRB approvals from the submission requirements, and to require investigators o sponsors to provide evidence of protocol submission to the IBC. Considering therequest by the Americ Biological Safety Association to extend the public comment period, the RAC decided to modify the language of the proposed actions and to publish the revised version in the *Federal Register* for an additional 60 days. A motion was approved by the RAC to amend the proposed actions published in the *Federal Register* on August 20, 1997, regarding the submission requirements as follows: "The RAC recommends that final approvals from the IBC and IRB should be withheld until after ORDA provide IBC and IRB with RAC concerns (if any), and (1) ORDA notification that the protocol is exempt from RAC review, or (2) ORDA notification that the protocol has triggered full RAC review. Human gen transfer protocols shall not be initiated prior to submission of final IBC and IRB approvals to ORD

The proposed actions were published in the *Federal Register* on October 16, 1997 (62 FR 53908). Three letters were received by ORDA in response to the *Federal Register* notice.

In a letter dated December 5, 1997, Richard C. Knudsen, (President, American Biological Safety

Association), endorsed the proposed actions. In addition, the letter requested: (1) to add a requirement of proof of submittal to an IRB as well as the IBC, and (2) to delete the designation as a note and to inclu the statement as a second paragraph of Appendix M-I, "The RAC recommends that final approvals from the IBC and IRB should be withheld until after ORDA provides the IBC and IRB with RAC conce any), and (1) ORDA notification that the protocol is exempt from full RAC review, or (2) ORDA notifi that the protocol has triggered full RAC review. Human gene transfer protocols shall not be initiated prior to submission of final IBC and IRB approvals to ORD

In a communication dated December 9, 1997, Mr. Robert Lanman, NIH Legal Counsel, addressed th issue of whether having the RAC as the first level of review for gene transfer protocols would affect the structural framework of the *NIH Guidelin* For all other experiments subject to the *NIH Guidelin*, the IBC makes the primary assessment of containment. In Mr. Lanm's view, the proposed change, althoug legally authorized, would change the basic structure of the *NIH Guidelin* in that local review would follow NI's review. This change raises the question of whether gene therapy research is so unique that justifies a different review structure (primacy of national review rather than local review).

In a letter dated December 8, 1997, Nicholas J. Pelliccione , Ph.D. (Senior Director, U.S. Regulator Affairs, Schering Corporation, Kenilworth, New Jersey) disagreed with the proposed actions that the final IBC and IRB approvals should be withheld until after ORDA provides the IBC and IRB wit concerns (if any). Dr. Pellicciori's view was that this recommendation, if adopted, would create undue delays to the process of initiating proposed clinical studies and would appear to be an encroachment of the RAC into the purview of the FDA. The FDA is responsible for the assurance of the safe conduct of investigational drug studies.

RAC Discussion

Dr. Markert outlined the current review process of human gene transfer protocols. The investigator submit their protocols to the IBC and IRB , and it takes one to three months for their approval. Th investigators then submit their protocols to ORDA , which takes three weeks to three months for RA review. At the same time investigators submit their protocols to the FDA, which has 30 days in which to decide if a protocol should be allowed to proceed. Dr. Markertstated that her proposal would allo investigators to submit their protocols to the IBC, IRB , and ORDA concurrently. After approval from IBC and IRB , and the 's decision on whether to conduct a full RAC review, the investigators will be able to submit their IND application to the FDA. Dr. Markert said that her proposal would speed up overall review process.

Dr. Markert proposed new language regarding Appendix M-I *Submission Requirements--Human Gene Transfer Experiments.* Item 3 of Appendix M-I is to be amended with inclusion of the statement, "...letter stating that the protocol has been submitted to the local IBC and IRB . If available, submit the records o their deliberations pertaining to your protocol." Items 5 and 6 of Appendix M-I are to be amended to require submission of the clinical protocol and Informed Consent document *as submitted to* the IBC and IRB , rather than *as approved by* the IRB and IBC. A note is to be included in Appendix M-I stating: **Note:** The rationale of (3) above is to allow the investigator to proceed with the RAC submission before final IR and IBC approval. The RAC anticipates that full local IRB and IBC review will occur in parallel with th RAC review process, thus expediting the review process."

Dr. Markert explained that this revised proposal would allow the local committees to retain all the appro authority and would not create "primacy of national review rather than local review", as stated in Mr. Lanman's communication. In response to Dr. Knudsen's letter, Dr. Markert included in Item #3 of he proposal the requirement for submission to the IRB . Dr. Markert noted that in response to Dr. P'ell

letter, a note to Appendix M-I of the proposed actions that conditions IBC and IRB approvals pending RAC notification of exempt or review decision has been deleted. A new note is proposed to indicate that the rationale of the amendment is to expedite the review process.

Dr. Noguchi said that Dr. Markert's explanation has clarified many issues regarding the proposed actions. Dr. Noguchi stated that the FDA has formally recognized IBC approval in the context of xenotransplantation protocols, and he supports extending such recognition to other areas including human gene transfer protocols. He said that the FDA prefers that its approval be the last stage in the chain of the approval processes, thus preventing the situation where an FDA approved protocol is waiting for RAC discussion.

Dr. Macklin noted that IRBs frequently require changes of a protocol. She asked, if the RAC is reviewin the protocol before IRB approval, how would any changes be communicated to the RAC? Dr. Marke responded that in her own institution, the investigators submit their protocol to various committees, and after revision of the protocols in accordance with all the suggestions, the revised protocols together with a cover letter explaining all the changes are sent to all the respective committees. Dr. Markert said that h proposal gives the investigators an option to submit their protocols in a parallel fashion to different review bodies.

Ms. Knorr noted that Mr. Lanman stated the viewpoint as the General Counsel of NIH . The RAC wo benefit from prior IBC and IRB review in that the local bodies have an intimate knowledge of thei investigators and research projects. She asked Mr. Dommel to make a comment from an OP perspective.

Mr. Dommel noted that projects must have prior IRB approval in order to be considered for NIH He was concerned about sending any indication to institutions that they could delay IRB review pendin reviews by another committee.

Dr. Aguilar-Cordova noted that an IRB frequently gives conditional approval pending FDA approval, an he does not think that Dr. Markert's proposal is contradictory to the current practice. Mr. Dommel w concerned about the statement that "final" IRB approval should await RAC notificatio

Dr. Mickelson stated that at present the RAC reviews only a small fraction of protocols at public meetings, and the changes have little impact on the RAC review process. Local approvals assure that the protocols submitted to the RAC are well written and complete. She was concerned that changing the timing of local review would result in the RAC reviewing many premature protocols. Dr. Mickelson noted that prior approvals by the local committees do not preclude the RAC from sending recommendations to either an IBC and/or an IRB

Dr. Markert explained that the intention of her proposal is to expedite the review process. She would b happy to review and to offer advice to investigators of protocols still in the development process. Dr. Markert explained that in her institution, the IRB grants conditional approval of protocols pend decisions about the availability of funding and changes recommended by other review bodies.

Mr. Dommel re-stated his point that scientific review at NIH of an application will not occur in the ab of an IRB approval. This process is to maintain the primacy of local review and to assure that al regulations regarding human subjects are met.

Dr. Macklin said that the issue of whether the RAC will send letters to the IBC and IRB should b addressed first. The timing issue is only relevant if the RAC decides to send such letters.

Dr. Gordon said that the RAC should not be in a position to hold up any protocol. Public discussion of a protocol and RAC recommendation, if any, may be transmitted to the IBC and IRB even if the protoco already has received approvals from the local bodies.

Ms. Knorr noted as a separate issue the RAC may encourage investigators to submit their applications ORDA before they file IND applications with the

Dr. Noguchi noted that a difficult situation arises when the RAC informs an IRB of RAC concerns. Such letter has a connotation of disapproval, and it carries the authority of the NIH imprimatur. Dr. Lysaugh that if the letters are standardized, and are sent to the IBCs and IRBs for all protocols registered w ORDA , there will not be any misunderstanding regarding approval or disapprova

Dr. Macklin said that the RAC needs to decide either to abandon protocol review and focus on broader issues, or to continue protocol review and to convey RAC concerns to all interested bodies.

Dr. McIvor stated that the FDA is expecting the feedback from the RAC regarding public review of novel protocols. Dr. Macklin said that the RAC is able to fulfill this role of discussing the novel protocols without taking the task of reviewing each protocol as a "super" IRB . Dr. Lysaught said that the IBC and IR benefit from letters summarizing any comments made by the RAC.

Dr. Gordon suggested that the RAC should forgo formal "review" of protocols; and instead flag those protocols that require public discussion. He noted that discussion of Dr. Crystal's protocol is an example. The RAC raised concerns and, with contributions from the investigators and the FDA, publicly discussed the protocol without a binding power of approval or disapproval. Ms. Knorr noted that if the RAC decide to address broader issues and to use protocol registration as a means to identify issues deserving of public discussion, this would be in line with the initial changes proposed by the NIH Director. *Ad hoc* experts may be included to help focus on issues.

Dr. Gordon made a motion to send a letter to the NIH Director stating that the RAC would like to modif the protocol review procedure so that protocols will no longer be looked at from the "review" standpoint.

Ms. Rothenberg questioned whether the motion will accomplish anything. The RAC flags novel protocols for public discussion; the recommendation from the RAC discussion should be transmitted to the FDA. She noted that RAC discussion of ethical issues is particularly important to the FDA. The motion concerns mostly the semantical issue of the meaning of the word "review." Dr. Noguchi said that RAC discussion not limited to ethical issues, e.g. RAC discussion of Dr. Crystal's protocol addressed the issue of vector-induced inflammation. He noted the difficulty of selecting a few "novel" protocols for public discussion without looking at issues other than ethics. Ms. Rothenberg said that the other alternative is for the FDA to use the RAC in an advisory capacity for issues that require RAC discussion.

Dr. Lysaught disagreed with Dr. Gordon's motion. She noted that ethics and science are intertwined; risk and benefit issues must be evaluated in a scientific context, e.g., risks of inflammation vs. benefit to a patients. She said that good policy should be developed on a case-by-case basis in an incremental fashion.

Dr. McIvor said that the current procedure for protocol oversight is working well. He said the larger context of changing the process should not be discussed today, but should be addressed in the future.

Dr. Gordon said that the RAC should be concerned about highly unusual ethical issues posed by novel

gene therapy strategies, rather than being concerned about standard ethical issues of most routine protocols.

Mr. Steven Kradjian (Vical Inc., San Diego, California) noted that the timing issue of IBC and approvals has been discussed and voted by the RAC in the last few meetings. He asked how would NIH implement the amendments. Ms. Knorr responded that the RAC needs to vote on the *proposed actions* and the changes suggested by Dr. Markert . The NIH Director will consider whether to accept the m and publish it as an Action under the *NIH Guidelin*. As a point of clarification, Dr. Markert said that th major differences of her proposal today is that: (1) the letter should state that the protocol has been submitted to the IRB in addition to the IBC, and (2) thenote of withholding IRB and IBC final app pending a RAC recommendation has been replaced by a note stating that the rationale of the proposal is to allow investigators to proceed with RAC submission before final IRB and IBC approval

Committee Motion 8

A motion was made by Dr. Gordon and seconded by Dr. Lai to remove procedures under the *NIH Guidelines* regarding human gene transfer research that resemble "reviewing" of individual clinical protocols. The motion failed by a vote of 3 in favor, 5 opposed, and 2 abstentions.

Dr. Markert made a motion to accept the proposed actions published in the *Federal Register* regarding the timing issue of IRB and IBC approvals with the changes indicated in a draft proposal she presented to the RAC today. She noted deletion of the statement that the IBC must condition its approval on receipt of RAC notification regarding the protocol. Dr. Ando seconded the motion.

Dr. Lysaught noted that taking out the IBC statement changes the whole context of the proposed actions.

Dr. Gordon opposed the motion. He said that the entire issue of protocol review should be addressed in the future. Dr. Macklin agreed with Dr. Gordon that this issue should be considered at a future time.

Dr. Ando said that the motion is to take one step at a time. Under the current *NIH Guidelines,* investigators have to wait for IRB and IBC approvals before they can submit their protocols to ORDA. Dr. Markert explained that the reason to delete the IBC condition statement is that it is controversial for the RAC to ask an IBC to withhold its approval. Dr. McIvor said he favors the motion.

Dr. Lysaught was concerned that the motion has significantly changed the context of the language stated in the published proposed actions, which was passed by a RAC vote at the September, 1997 RAC meeting.

Dr. Nicholas J. Pelliccione (Schering Corporation) stated that he supported Dr. Markert's proposal to delete the IBC condition language. Under the language of the proposed action, investigators cannot initiate their studies until the IRB and IBC receive RAC notification.

Committee Motion 9

A motion was made by Dr. Markert and seconded by Dr. Ando to remove the requirements of prior IBC and IRB approvals at the time of protocol submission to ORDA. The Appendix M-I, *Submission Requirements -- Human Gene Transfer Experiments,* are amended to read (changes from the current *NIH Guidelines* indicated in bold print):

"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-VIII-A,*Footnotes of Appendix M*). Proposals shall be submitted to NIH/ORDA in the following order: (1) scientific abstract; (2) non-technical abstract; (3) letter stating that the protocol has been submitted to the local Institutional Biosafety Committee (IBC) and Institutional Review Board (IRB). If available, submit the records of their deliberations pertaining to your protocol (submission for Institutional Biosafety Committee approval must be made to each institution at which recombinant DNA material will be administered to human subjects (as opposed to each institution involved in the production of vectors for human application and each institution at which there is*ex vivo* transduction of recombinant DNA material into target cells for human application)); (4) Responses to Appendix M-II through M-V,*Description of the Proposal, Informed Consent, Privacy and Confidentiality, and Special Issues* (the pertinent responses can be provided in the protocol or as an appendix to the protocol); (5) clinical protocol (as **submitted to** the local Institutional Biosafety Committee and Institutional Review Board); (6) Informed Consent document--as submitted to the Institutional Review Board (see Appendix M-III, *Informed Consent*); (7) appendices (including tables, figures, and manuscripts); and (8) curricula vitae--2 pages for each key professional person in biographical sketch format. Investigational New Drug (IND) applications shall be submitted to the FDA in the format described in 21 CFR, Chapter I, Subchapter D, Part 312, Subpart B, Section 23,*IND Content and Format.* Submissions to The FDA should be sent to the Division of Congressional and Public Affairs, Document Control Center, HFM-99, Center for Biologics Evaluation and Research, 1401 Rockville Pike, Rockville, Maryland 20852-1448.

"**Note: The rationale of (3) above is to allow the investigator to proceed with the RAC submission before final IRB and IBC approval. The RAC anticipates that full local IRB and IBC review will occu in parallel with the RAC review, thus, expediting the review process."**

The motion passed by a vote of 7 in favor, 1 opposed, and 2 abstentions.

Dr. Mickelson noted that Dr. Lysaught opposed, and Dr. Macklin and Ms. Rothenberg abstained.

XIII. Human Gene Transfer Protocol #9708-211 entitled:*Gene Therapy for Canavan Disease*

PI: Margretta Seashore, Yale University

Reviewers: Wolff, Markert, Juengst

Ad hoc:Reuben Matalon, University of Texas, Galveston
John Barranger, University of Pittsburgh

The protocol was recommended for full RAC review due to its novelty and several concerns, which included the first protocol for the treatment of an inherited metabolic disorder by gene transfer into the brain, a different type of liposome administered to the brain, new vector, new disease, the requirement for intracranial surgery, and a patient population involving children.

Review -- Dr. Markert

Dr. Mickelson called on Dr. Markert to present her primary review of the protocol submitted by Drs. Margretta R. Seashore, Albert B. Deisseroth, and Paola Leone, (Yale University, New Haven, Connecticut). Dr. Markert stated that besides the three key investigators on this project, Dr. Matthew

During, of New Zealand, has done extensive research of *in vivo* gene transfer in the CNS.

Canavan disease is a progressive leukodystrophy that leads to severe psychomotor delay, retardation, and premature death. It presents in the first few months of life. Children have hypotonia and fail to meet developmental milestones. During the second and third year, the children become increasingly spastic and regress developmentally. They usually die between the ages of four and ten. The disease is caused by mutations in the gene aspartoacylase (ASPA). The mechanism of neurologic damage is thought to be the accumulation of N-acetylaspartate (NAA), which normally would be metabolized by aspartoacylase. Aspartoacylase is expressed in the brain, which is why there is pathology in the CNS. This enzyme is present in many other tissues, such as fibroblasts. It is not clear why other tissues are not affected. All therapies at the present time are supportive.

The investigators propose injection of the aspartoacylase cDNA into the cerebral spinal fluid (CSF) in an attempt to halt the progression of the disease and possibly reverse some of the symptoms. The cDNA will be put into a liposome mixture and will be injected into patients using an Ommaya reservoir. The ASPA cDNA is in a plasmid that also contains the CMV early promoter which has been previously used in clinical studies, the SV40 poly A site, and adeno-associated virus inverted (145 bp) terminal repeats. The gene is injected as a cationic liposome-polymer-DNA complex. The hope is that the gene will be incorporated into the cells lining the brain. The vector is made under Good Laboratory Practice conditions by Qiagen in Germany. The vector is given with intravenous (IV) mannitol injection (<1 g/kg/24 hrs IV) to facilitate ventricular and parenchymal diffusion. New patients will receive 5 cc (this dose has previously been given to two patients); the two previously treated patients will receive 10 cc. The investigators propose to treat 13 new patients and to re-treat the first two patients treated for this disease. All other future doses will be reviewed by the local IRB and the FDA.

P>The primary outcome being studied is safety. No statistical tests will be done. However, a number of tests (psychometric testing, evoked potentials, nuclear magnetic resonance (NMR) spectroscopy, neurologic assessments, parental reports, CSF fluid analyses, and routine blood tests) will be performed prior to the treatment and at 1, 3, 6, 9, and 12 months after surgery. The investigators may re-treat patient after six months depending upon NAA levels.

There is no animal model for Canavan disease. Rats and primates have been treated with the proposed vector infused into the CSF. In assessing expression of the cDNA in these animal studies, the investigators used reverse transcriptase polymerase chain reaction of RNA prepared from tissue obtained from various aspects of the brain. They did not use*in situ* PCR, which would have given an estimation of the percentage of cells expressing the cDNA.

Two subjects received this vector in a previous study. Both patients exhibited a decrease in the metabolite, NAA, in the brain; the first patient more than the second.

Dr. Markert made several specific critiques and they were responded to previously in writing by Dr. Seashore.

Review -- Dr. Wolff (presented by Dr. Markert)

Dr. Wolff stated that the Yale University Human Investigation Committee has done an outstanding job of raising many important issues concerning this protocol. Nonetheless, it still remains unclearwhether the proposed gene transfer technique will enable sufficient expression to affect the clinical course. Although

animal models do not exist for Canavan disease, this issue of gene transfer efficiency should be able to be adequately and clearly addressed by quantitating the expression of a surrogate reporter gene using the proposed vector system.

Dr. Wolff made several specific comments. He summarized that the efficiency of gene transfer is the critical unresolved issue for this proposal. The plight of these children with Canavan disease is a very difficult situation. He noted, however, as stated in the summary of the Yale IRB evaluation, "the committee had strong views that simply doing a procedure because it would not harm the patients was an insufficient reason for doing it."

Review -- Dr. Juengst (presented by Dr. Markert)

Dr. Juengst wrote a very detailed review, to which Dr. Seashore responded previously in writing. Specifically, Dr. Juengst raised several issues regarding subject selection and recruitment and the Informed Consent document, as follows:

Subject Selection and Recruitment. In response to question I-C-3 of Appendix M regarding recruitment procedures, the investigators report that: "We are in contact with the Canavan Foundation and the Canavan Research Fund. These charitable trusts will let families know of the experimental trial." The protocol includes no evidence that these organizations are prepared or equipped to provide this service, or any details about how it would be done. More importantly, the protocol neglects to explain why such a procedure is a "fair and equitable" method of recruiting subjects, as Part I-C of Appendix M requests.

Informed Consent. The answers to the questions under Part I-D-3, D-4, and D-5-a of Appendix M are non-responsive. The questions do not ask about the subjects' current knowledge on these topics, but about what they will be told by the investigators. One of the concerns in recruiting subjects for this study is the possibility that, in fact, there are already enough families desiring admission to the study on the basis of hearsay to make additional subject recruitment a moot point. This is a concern because, to the degree that these families' interest is based in a hope of therapeutic benefit, the educational challenge of providing parents with a realistic understanding of the Phase I nature of the trial will increase. If the investigator or the project's oversight committee comes to believe that all the subjects being recruited for this study are committed to participation in advance of the study's own informed consent process, are there ways of reaching and recruiting new families whose views of the merits of this research are more disinterested?

Privacy and Confidentiality. What will parents be told about the privacy risks involved in their creating and keeping a video record of their children's behavior? These tapes will be very attractive to the news media if the study should become newsworthy. Will the parents be able to retrieve them from the investigators once submitted? Will the tapes be destroyed after examination by the investigators?

Dr. Juengst raised several specific issues regarding the Informed Consent document, which have been responded to in writing by the investigators.

Review -- Dr. Matalon

Dr. Matalon raised several concerns. He stated that the understanding of the natural history of Canavan disease is grossly misrepresented and indicated that the principal investigators do not have an accurate idea how these children behave and how they progress. The investigators suggest that children with "more mild juvenile forms" survive to the second decade. This statement indicates that the investigators have not had enough experience with the natural history of Canavan disease. This problem is critical and

can lead to erroneous conclusions. Many Canavan patients, without treatment, survive beyond the first decade of life. The investigators need a better measure if survival is going to be used as evidence of changing the course of the "mild" or "juvenile" form of the disease. The protocol promises that a small fraction of transduced cells might correct the defect and cited a publication by Toft*et al*. of juvenile form of Canavan disease. Dr. Matalon said he does not consider that the paper by Toft*et al*. deals with Canavan disease patients.

The efficiency of gene transfer does not seem to be adequately addressed. The studies suggest psychometric tests, etc. These tests are not adequate due to the fact that children without treatment gain skills as they grow older. This issue is either not known to the investigators or it has not been addressed. This flaw is very serious in the design because claims can be made of "success" with no real substantive improvement. These same concerns are also true for parental response and neurological evaluations. If these evaluations are not completed and reviewed by those who have experience with Canavan disease, the results can be misleading. The question of NMR spectroscopy is of concern. Sampling errors can lead to "fluctuation" in NAA levels. This testing needs to be rectified by allowing different centers to examine and evaluate the data.

Dr. Matalon was disappointed that the investigators are not measuring NAA levels in urine. Since NAA is synthesized by the grey matter of the brain, reduction of its levels should be reflected in the urine. Also, N-acetylaspartylglutamate (NAAG) needs to be measured; if treatment is indeed helpful, the level of NAAG should reduced.

The magnetic resonance imaging (MRI) studies on the two previously-treated children with Canavan disease show lack of evidence of improvement that was claimed for one child. Children with Canavan disease without treatment may show myelin formation in the subcortical white matter as they grow older.

The evaluation of gene expression needs to be improved. Direct evidence of human ASPA expression is lacking. Without proof of expression of ASPA, circumstantial evidence is of no help.

Dr. Matalon noted that the benefits described in the Informed Consent document create a false hope of a real cure.

Review -- Dr. Barranger (presented by Dr. Markert)

Dr. Barranger stated that the study brings up a perplexing issue in translation science, i.e., when is a clinical trial of gene transfer premature in a uniformly fatal neurodegenerative disorder? Evidence of gene delivery and expression is incomplete in the pre-clinical studies supporting this protocol. Furthermore, as no animal model exists for Canavan disease, there is no direct indication that the approach is likely to be efficacious. Having alluded to the scientific difficulties with this protocol, Dr. Barranger would concur that the proposed study can be judged to be anacceptable risk for children dying from Canavan disease. The safety of the approach is clear from the earlier study in two children.

Other Comments

Dr. Markert noted that primary endpoints were not stated in the protocol, but were provided by the investigators in their written response.

Dr. McIvor asked the investigators to clarify whether there is a genotype/phenotype correlation of disease severity. He noted that in other hereditary monogenic disease, e.g., lysosomal storage disease, a low level gene expression may be enough to ameliorate the symptoms; he asked if any attempt has been

made to try bone marrow transplantation. He asked a technical question of the reproducibility of forming DNA complexes. Dr. Matalon stated that Canavan disease is different from the lysosomal storage disease in that the ASPA is primarily found in the white matter while the substrate, NAA, is synthesized by neurons in the grey matter. The enzyme is not expressed in white blood cells and, therefore, a bone marrow transplant will not be useful.

Dr. Mickelson inquired about ASPA distribution. Dr. Matalon said that the enzyme is found in specific tissues (kidney, lung, and skin fibroblasts), but the substrate for the enzyme is present only in grey matter. Dr. Mickelson inquired about methods of tracking gene expression in patients by measuring enzyme levels before and after gene transfer, and a more quantitative method of assessment of the children. Dr. Matalon responded that a brain biopsy will answer these questions; it is part of the diagnostic procedure of the disease.

Dr. McIvor inquired about the data of NAA levels before and after gene transfer in the two patients treated in New Zealand.

Dr. Lysaught asked for a clarification about the efficiency of gene transfer in the animal studies, and if the expression is long term.

Investigator Response -- Dr. During

Dr. During noted that Dr. Dean Rupp (IBC Chair of Yale University) was in the audience. Dr. During noted that this protocol has been extensively reviewed by the Yale IBC and that most of the questions raised by the RAC have been raised by the Yale IBC as well.

Responding to the question of statistical analysis of patient data, Dr. During said that it is less valuable for this protocol since this is not a rigorous double blinded controlled efficacy study. In terms of the NAA levels in the brain, two children were repeatedly scanned with MRI and NMR spectroscopy using their baseline data as a control. Because of the variability of the measurements, a large group of patients is needed to obtain conclusive results. Moreover, the data from different clinical trial sites cannot be directly compared due to variability of the biochemical measurements. Dr. During noted that the Children's Hospital in Philadelphia has more experience with the spectroscopy technique to assess the children, and all patients will be scanned there. Dr. During said that the major rationale to choose Canavan disease for study is that brain levels of NAA provide a surrogate marker to assess gene transfer.

Dr. During said that he has considered a variety of vectors for the treatment of Canavan disease, e.g., adenoviruses, adeno-associated virus, HIV vectors, HSV vectors, etc. The reason that he chose nonviral plasmid DNA complexes is related to minimal toxicity and safety. There is very little systemic immune response to the administration of a DNA complex to the brain. The plasmid DNA is not detected in any tissues outside the brain.

Responding to the question of how to type the disease of varying severity and to evaluate the efficacy of the treatment, Dr. During said that natural progression of disease may be affected by intervention depending on how aggressive the treatment is. He said that natural history of the disease is that when there is minimal intervention, most patients will die before ten years of age. He acknowledged that there is huge variability in the clinical symptoms of the disease and those individuals who have more enzyme expression due to treatment will do better.

Responding to the question of genotype/phenotype correlation, Dr. During said that there is very little data in the literature. In one gene mutation in one family, there was a significant phenotypic variability. For this

reason, genotype cannot be used as a predictor of phenotype or as an inclusion criterion. Dr. During hoped that the present study will provide some information about the natural history and genotype/phenotype association for Canavan disease. He noted that Canavan disease is very rare.

Responding to the question of urinary NAA levels, Dr. During said that he has researched the literature for a better objective measurement of gene transfer other than brain NAA levels. He noted that the urinary levels are too variable to be useful; excretion of metabolites from the brain is not a simple diffusion phenomenon. Dr. During said that NMR spectroscopy is the best measurement available to assess brain NAA levels.

Dr. During said that the endpoints of the study are clear biochemical measurements with some degree of reproducibility and validity. He noted that it is not practical to perform needle biopsy to measure the NAA level in the brain; it has ethical and technical problems.

Dr. During stated that the data from the two children treated in New Zealand are being prepared for publication. The manuscript includes specific quantitative data on the measurement of NAA levels before and after gene transfer; before treatment, both children had elevated levels of NAA in the frontal cortex. The NAA levels came down within normal range in one month, and in one child, it remained normal for 12 months. He noted evoked responses, psychometric tests, and behavioral tests were all improved. He admitted that there are no really good clinical measurements to assess the benefits of gene transfer.

Dr. Gordon asked if there are any data to predict that correcting the enzyme deficiency will improve the clinical status of the patients. Dr. During drew a diagram to illustrate his point. The two children previously treated are at a plateau period of the development of neurological function. Dr. During agreed with Dr. Matalon that the normal developmental process poses a problem of interpreting the data. He said that the only way is to do a double-blinded control study. He noted that the earlier the intervention is given, the more likely that there will be a regenerative capacity within the brain.

Dr. Lai asked what cell types are most susceptible to the disease. Dr. During responded that oligodendrocytes which form myelin are most crucial in this disease. Dr. Lai inquired if the gene transfer will target those cells. Dr. During said that gene correction may not need to be in those oligodendrocytes to be effective; however, the scientific question has not been resolved. Dr. Lai asked if the gene needs to be put in the brain at all. Dr. During responded that NAA, like other acids, does not easily pass through the blood-brain barrier; therefore, the gene transfer needs to occur in the brain.

Dr. Macklin expressed her concerns about the children to be treated under this protocol. She said that children are incapacitated from the standpoint of being able to give informed consent. The risks to children in this protocol are more than minimal risks. In addition, she does not consider that the protocol provides direct benefit to children. Dr. Macklin stated that the benefit to risk ratio is unfavorable in this protocol, and she was concerned that the Yale IRB approved the protocol. Dr. During responded that this is an experimental intervention which carries some potential benefit and that he is not promising any cure. Most neurological treatments do not reverse the disease, but they do slow disease progression. He said that the surgical procedure is not a high risk procedure for most neurosurgeons. Dr. Macklin stated that the risk of surgery and many other procedures to follow-up on the intervention are more than minimal risks to the incapacitated subjects from the standpoint of an IRB.

Dr. McIvor asked investigators to clarify the exact nature of the risk to these patients as a result of their going through this procedure. Dr. During said that the mortality risk of drilling a hole and putting a catheter into the brain is about 1 percent. He said that the mortality risk of placing a reservoir in the brain is about 5 percent, mostly due to infection.

Dr. During noted that the protocol has gone through many ethical reviews including four or five reviews in New Zealand, in addition to the IRBs of Yale University and Thomas Jefferson University.

Dr. Lysaught noted that in the brain tumor protocols involving HSV-TK/ganciclovir treatments there are adverse events reports of infection due to placement of an Ommaya reservoir. Dr. Noguchi explained that, in this protocol, the risk of placing an Ommaya reservoir should be less than that in the brain tumor protocols because the brain tumor needs to be debulked before placing the reservoir in those protocols.

Dr. McIvor asked about cellular transport of the substrate, NAA, to other cells expressing the enzyme, ASPA. Dr. During responded that the mechanism of cellular transport is still under active investigation and it is not well understood at present. Dr. McIvor asked about the NAA levels in healthy and Canavan disease patients. Dr. During said that he has collected NAA data from 22 children but not all of them will be enrolled into the protocol, and these data cannot be used as NAA levels for a "control" group. Dr. McIvor asked about the reproducibility of the procedure to prepare the DNA complexes. Dr. During responded that they have a standard operating procedure for making the DNA preparations, but they are still developing procedures for potency measurements of the products.

Dr. Mickelson asked the investigator to clarify whether all the NMR spectroscopy scans will be performed at Philadelphia Children ’s Hospital. Dr. During said that the NMR scan is part of the inclusion criteria and all children will be scanned at Philadelphia Children ’s Hospital. Dr. During said that he is planning to initiate the protocol at both Yale and Thomas Jefferson Universities, and that all patients will have an initial NMR evaluation at Philadelphia.

Dr. Gordon asked how the protocol will proceed if the outcome is not toxicity, but if there is no dramatic clinical improvement of patient status. Dr. During said that he expects the study will demonstrate safety, but stated there is a big question whether the protocol can progress to a Phase II efficacy study. In the meantime, he is doing the pre-clinical studies in the laboratory in order to find a more efficient gene delivery system for the treatment of Canavan disease.

Dr. Mickelson inquired if any effort has been made to develop an animal model for Canavan disease. Dr. During said that he is collaborating with Dr. Matalon to develop a gene knock-out mouse model. Dr. Matalon said that he started on this project after he moved to Texas.

Dr. Markert stated that she is not concerned about children undergoing the surgical procedure of placing the Ommaya reservoir and sampling of the CSF because these procedures are well tolerated by children. She suggested involving a biostatistician in the protocol to help study design. Dr. During said that he has consulted with a biostatistician in New Zealand, and he will make efforts for such consultation at Yale and Thomas Jefferson Universities.

Dr. Mickelson thanked Dr. During for his responses to RAC questions and concluded the discussion of the protocol.

Protocol Summary

Drs. Margretta R. Seashore, Albert B. Deisseroth, and Paola Leone (Yale University, New Haven, Connecticut) will conduct gene transfer experiments on 15 patients with Canavan disease. Canavan disease is an autosomal recessive leukodystrophy caused by mutations in the ASPA gene. The loss of ASPA activity leads to an elevation in the brain concentration of NAA and spongiform degeneration of oligodendrocytes leading to neurodevelopmental retardation and childhood death. The children will

undergo a surgical procedure for the implantation of a cerebrospinal fluid reservoir and intracranial administration of a plasmid vector, pAAVaspa. Plasmid pAAVaspa contains the 145 base ITR from the adeno-associated virus, and expresses the human ASPA cDNA under the control of the cytomegalovirus promoter/enhancer. The plasmid DNA is condensed using protamine then encapsulated in a liposome-polymer-DNA complex with the lipids, 3ß[N-(N', N'-dimethylaminoethane)carbamyl]cholesterol (DC-Chol) and dioleoylphosphatidylethanolamine. The study is to evaluate the safety and efficacy of the gene transfer procedure.

XIV. Amendment to Appendix M-I, Submission Requirements--Human Gene Transfer Experiments Regarding Deadline Submission for RAC Review/McIvor

Dr. McIvor requested a proposed action regarding the deadline for submission of human gene transfer protocols that will require public discussion at the RAC meetings. He stated that in order to give the RAC sufficient time to review a protocol and the investigators to respond to primary reviewers written comments, Appendix M-I, *Submission Requirements - Human Gene Transfer Experiments*, of the *NIH Guidelines*, is proposed to be amended to include a statement regardingthe submission deadline. Submission material will be accepted by ORDA at any time. However, if a protocol is recommended for full RAC review, the submission material must have been received in ORDA a minimum of eight weeks prior to the next scheduled RAC meeting.

Other Comments

Ms. Knorr noted that the deadline submission criteria was previously deleted from the *NIH Guidelines* at the time NIH and the FDA initiated the consolidated review process for human gene transfer protocols.

Dr. Lysaught noted that the deadline submission of eight weeks applied only to "novel" protocols that require full RAC discussion. Dr. McIvor said that it is a reasonable time frame required for RAC review. Dr Markert noted that the eight week period included the first 15 working days for the RAC to make its decision whether a protocol would require full RAC discussion. Dr. Mickelson said that a minimum of eight weeks is needed if *ad hoc* consultants are needed to review a protocol. Ms. Knorr noted that the RAC is facing a situation where a protocol might have been initiated prior to full RAC discussion. Dr. Mickelson noted that the RAC no longer approves protocols and it is less of an issue in the context of discussion, even if the protocol has enrolled patients. Dr. Markert agreed that the eight week submission deadline is reasonable.

Dr. Gordon stated that in the light of the RAC relinquishing protocol approval authority, the RAC will be able to review any controversial protocol at anytime without the constraint of finishing the review before protocol initiation. Dr. Mickelson agreed that without this constraint the RAC will be able to broaden its horizon to deal with broader issues, e.g., *in utero* or even germ line gene transfer. Dr. Gordon said that review of the Canavan disease protocol is a good example of how the RAC's ethical review added an extra dimension to the scientific issues of gene transfer research. Dr. Mickelson agreed.

Dr. McIvor made a motion to adopt the language published in the *Federal Register* to reinstate the deadline submission of eight weeks. Dr. Mickelson seconded the motion.

Dr. Lysaught agreed that as long as the RAC is reviewing protocols, a deadline submission is needed. Dr. McIvor stated that the deadline will help ORDA to inform the investigators that a protocol submitted after the deadline will not be discussed at the forthcoming RAC meeting. Dr. Gordon noted that the RAC can revisit this issue if it is decided to forgo protocol review altogether.

Dr. Victoria Allgood (GeneMedicine, Inc.) noted that the eight week deadline could potentially delay RAC discussion of a given protocol for as much as five months. She asked if further public comment is needed before the RAC voted on this issue. Ms. Knorr noted that the proposed action was published in the *Federal Register* two weeks prior to the RAC meeting; no public comment has been received in ORDA. Dr. Lysaught noted only novel protocols that require full RAC discussion will be affected by the deadline; all other protocols will be exempted from full RAC discussion within 15 working days. Dr. Noguchi said that if the RAC decides to give up individual protocol review, the deadline is no longer needed. Dr. McIvor said that as long as the RAC is still reviewing protocols, a submission deadline is needed. Dr. Allgood was concerned about a situation where a protocol, in her view, is just using the same vector to express a new geneand yet the RAC might decide on full discussion of such a protocol; in this case, the protocol would experience significant delay.

Dr. Sterling (GeneMedicine, Inc.) asked if the RAC could elaborate on the criteria of what kind of protocols would be considered as "novel" and therefore require full RAC discussion. Dr. Mickelson said that, as an example, the rationale for requiring RAC review for the protocols reviewed at this meeting was communicated to the investigators/sponsors as well as to individual RAC members. Dr. Mickelson agreed that, in the future, such rationale should be made clearer to the public audience as well.

As a point of clarification, Dr. Gordon said if a protocol has already received approvals from the IRB, IBC, and the FDA, it can be initiated before full RAC discussion. The FDA may decide to give approval before RAC discussion, or may opt to wait until a public RAC discussion. In terms of what constitutes a novel protocol, the RAC can only make such determination when they review the protocol information.

Dr. McIvor noted that from the protocols reviewed at this meeting, it is quite clear to the investigators what kind of protocols might trigger full RAC discussion.

Committee Motion 10

A motion was made by Dr. McIvor and seconded by Dr. Mickelson to accept the proposed action published in the *Federal Register* on November 19, 1997 (62 FR 61862) regarding deadline submission for RAC review. A note to Appendix M-I, *Submission Requirements--Human Gene Transfer Experiments*, is amended to read:

"**Note:** Submission material will be accepted by NIH/ORDA at any time. However, if a protocol is recommended for full RAC review, the submission material must have been received in NIH/ORDA a minimum of eight weeks prior to the next scheduled RAC meeting."

The motion passed by a vote of 6 in favor, 0 opposed, and 2 abstentions.

Dr. Mickelson noted that Drs. Gordon and Greenblatt abstained.

XV. Chair's Closing Remarks/Mickelson

Dr. Mickelson noted that the RAC Forum on New Technologies was very exiting and interesting. The RAC needs to further deliberate on its future role in view of the recent changes adopted for its oversight of human gene transfer research.

Dr. French Anderson recommended that *in utero* gene transfer research be considered as the topic for the next Gene Therapy Policy Conference.

XVI. Future Meeting Dates/Mickelson

The next meeting of the RAC will be on March 10, 1998, at NIH Building 31C, Conference Room 10. On March 9, 1998, the second Gene Therapy Policy Conference will be held at the Bethesda Marriott Hotel, in Bethesda, Maryland.

XVII. Adjournment/Mickelson

Dr. Mickelson adjourned the meeting at 5:30 p.m. on December 16, 1997.

Debra W. Knorr
Executive Secretary

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

Date: 12/16/97

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