

**NATIONAL INSTITUTES OF HEALTH
PUBLIC CONSULTATION
SYNTHETIC NUCLEIC ACIDS AND THE
NIH GUIDELINES FOR RESEARCH INVOLVING RECOMBINANT DNA MOLECULES**

**Hyatt Regency Crystal City
Arlington, Virginia**

**June 23, 2009
8:30 am – 5:30 pm**

Agenda

- 8:30 am **Welcome and Opening Remarks**
- Jacqueline Corrigan-Curay, J.D., M.D.
Acting Director, Office of Biotechnology Activities, National Institutes of Health (NIH), Bethesda, MD
 - Howard Federoff, M.D., Ph.D.
Recombinant DNA Advisory Committee Chair and Executive Vice President and Executive Dean, Georgetown University Medical Center, Washington, DC
- 8:45 am **[Introduction to the Proposed Revisions to the NIH Guidelines for Research Involving Recombinant DNA Molecules \(NIH Guidelines\)](#)**
- Stephen Dewhurst, Ph.D.
University of Rochester Medical Center, Rochester, NY
- 9:00 am **[Panel I – Basic Research Involving Synthetic Nucleic Acids](#)**
- Co-moderators:**
- Claudia Mickelson, Ph.D.
Massachusetts Institute of Technology, Cambridge, MA
 - Jane Flint, Ph.D.
Princeton University, Princeton, NJ

Background

Section I-B Basic Research with Recombinant and Synthetic Nucleic Acids

The proposed revisions to the *NIH Guidelines* are intended to clarify the applicability of the *NIH Guidelines* to research with synthetic nucleic acids and provide principles and procedures for risk assessment and management of such research. Although synthetic biology utilizes different techniques than traditional recombinant methods, the ultimate product may be the same. The biosafety considerations in most cases are related to the product being produced more than the technique used. Synthetic nucleic acids that cannot replicate will be exempted from the *NIH Guidelines* unless they are used in human gene transfer (see Section III-C-1 of the *NIH Guidelines*). This exemption is proposed so that the *NIH Guidelines* apply to synthetic nucleic acid research in a manner consistent with the current oversight of basic and preclinical recombinant DNA research. Currently oversight is limited to recombinant molecules that replicate or are derived from such molecules. The biosafety risks of using such constructs in basic and preclinical research are believed to be low. In contrast, the risks of administering non-replicating synthetic nucleic acids in the clinical setting are considered equivalent to those with replication incompetent vectors and this research is not exempted.

Discussion Questions

1. Is there a sufficient distinction between the risks of basic and preclinical research with replicating vs. non-replicating synthetic nucleic acid molecules to warrant the exemption?
 - a. What are the risks with the use of replication-incompetent, integrating vectors in the laboratory?
 - b. At the lower doses typically used in laboratory experiments, are the risks to the laboratory worker of such non-replicating, synthetic nucleic acids sufficiently low as to warrant exemption from the *NIH Guidelines*?

2. Since the increased risk associated with human gene transfer is in part related to the administration of higher doses, should the exemption be limited to experiments involving the handling of low quantities or doses of nucleic acids? What quantity would be expected not to pose a biosafety risk?
3. Are there examples of non-replicating, synthetic nucleic acid research that should not be exempt due to greater potential risks, in particular expression cassettes for oncogenes or toxins?

Panel Discussion

Institutional Biosafety Committee

- Carolyn Keierleber, Ph.D.
The Scripps Research Institute, La Jolla, CA

Academic Investigators

- Drew Endy, Ph.D.
Stanford University, Stanford, CA
- John Glass, Ph.D.
Synthetic Biology Group, Venter Institute, Rockville, MD
- Kristala Jones Prather, Ph.D.
Massachusetts Institute of Technology, Cambridge, MA

Public Comment

11:00 am **BREAK**

11:15 am [**Panel II – Human Gene Transfer Research Involving Synthetic Nucleic Acids**](#)

Moderator:

- Howard Federoff, M.D., Ph.D.
Georgetown University, Washington, DC

Background

Currently human gene transfer experiments involving non-replicating recombinant molecules are captured by the *NIH Guidelines* when derived through recombinant technology that has steps involving replication (e.g., replication incompetent vectors, RNAi or antisense RNA expressed from vectors derived from replicating systems). The potential safety risks for human gene transfer with synthetic non-replicating nucleic acids are not fundamentally different than for non-replicating recombinant vectors. The distinction between laboratory research and human gene transfer for non-replicating synthetic nucleic acids is based on the difference in the potential health risk between inadvertent exposure during basic or preclinical work and deliberate clinical gene transfer. The doses and routes of administration used in human gene transfer increase the risks. The risks to be considered for human gene transfer are not limited to the ability of the vector to replicate, and include transgene effects, insertional mutagenesis, and unanticipated immunological responses. Human gene transfer also raises scientific, medical, social and ethical considerations that warrant special attention and public discussion.

Discussion Questions

1. Research with non-replicating, synthetic nucleic acids expressed by plasmids or vectors is covered by Appendix M and subject to RAC review and reporting, but similar research with identical synthetic nucleic acids, which do not fall under the current definition of recombinant nucleic acids, would not be. Potential advantages of both classes of nucleic acids being subject to the *NIH Guidelines* include:
 - a. Provide the RAC and OBA with a more complete nucleic acid transfer data base particularly in regard to safety information;
 - b. Provide maximum degree of safety for research participants;
 - c. Provide a common public forum for other governmental review bodies, or be advisory to them, and stimulate liaisons between these different review groups; and
 - d. Enhance scientific expertise by providing scientists greater access to information.

Given these potential advantages for inclusion, should all human nucleic acid transfer be consistently covered under the amended *NIH Guidelines* or are there classes of research with certain non-replicating synthetic nucleic acids that should be exempt and why?

2. Several public comments noted that short oligonucleotides differ from other forms of gene transfer in the following:
 - Inability to express a transgene;
 - Inability to replicate in vivo;
 - Inability to integrate in cellular genome or modify genomic sequences; and
 - Transient duration of action.

However, biosafety concerns do exist for clinical trials involving synthetic nucleic acids (e.g., off-target gene suppression, saturation of miRNA processing systems, activation of immune responses). Should these biosafety and clinical safety considerations bring this class of nucleic acids under the *NIH Guidelines* for human gene transfer?

3. If there are classes of non-replicating synthetic molecules that should be exempt from the *NIH Guidelines* due to lower potential risks (e.g., antisense RNA, RNAi, etc.) what criteria should be applied to define such classes?
4. Would an exception based on function, for example an exemption for non-replicating, synthetic nucleic acids that transiently modify the function of RNA but cannot modify the genome of a cell and do not integrate, be reasonable?
5. If a distinction is to be made between short synthetic oligonucleotides and longer nucleic acid molecules such as expression cassettes or plasmids that may be chemically synthesized, is it possible to identify a size threshold (e.g., 40 nt) for lower risk research that should be exempt?
 - a. Does the size alter the potential function or toxicities?
 - b. Would the same size apply for different types of short oligonucleotides (e.g., siRNA, shRNA, miRNA, antisense, ribozymes, immune stimulators, etc.)?
 - c. Would the use of a number of nucleotides be too arbitrary?
6. Should an exception combine a size and functional definition?
7. Are there certain experiments with short oligonucleotides that should not be exempt because they may result in a long-term effect? For example, induced pluripotent stem (iPS) cells are known to cause teratomas in animal models and human fetal neural stem cells have apparently caused brain tumors to develop in a human subject (Amariglio *et al.*, PLoS Med. 2009, 6(2):e1000029). Though iPS cells are currently reprogrammed using recombinant viral vectors, would the potential future use of synthetic oligonucleotides (e.g. microRNAs, siRNA, antisense nucleic acid etc.) to control the differentiation of stem cells (either induced, embryonic or other) for therapeutic applications in human subjects present sufficient risk to warrant RAC review and public discussion?

Panel Discussion

Institutional Biosafety Committee

- Peter Besmer, Ph.D.
Memorial Sloan-Kettering Cancer Center
- Edouard Cantin, Ph.D.
City of Hope, Duarte, CA
- Henry V. Huang, Ph.D.
Washington University School of Medicine, Saint Louis, MO

Academic Investigators

- Scott Q. Harper, Ph.D.
Nationwide Children's Hospital, Columbus, Ohio
- Phillip Zamore, Ph.D.
University of Massachusetts Medical School, Worcester, MA

Industry Investigators

- Richard Geary, Ph.D.
Isis Pharmaceuticals Inc., Carlsbad, CA
- Akshay K. Vaishnav, M.D., Ph.D.
Alnylam Pharmaceuticals, Inc., Cambridge, MA

Public Representation

- Terry Kwan, M.S. Ed.
Brookline, MA

Public Comment

- 12:30 pm **LUNCH**
- 1:15 pm **Panel II – *Continued***
- 2:15 pm **BREAK**
- 2:30 pm **[Panel III – Revisions to Section III-A-1. Major Actions Under the *NIH Guidelines*](#)**

Co-moderators:

- Louis Kirchhoff, M.D.
University of Iowa, Iowa City, IA
- Dennis Dixon, Ph.D.
National Institutes of Allergy and Infectious Diseases, NIH, Bethesda, MD

Background

Section III-A-1-a

Under the *NIH Guidelines*, certain experiments involving the deliberate transfer of a drug resistance trait to microorganisms, if such acquisition could compromise the ability to treat or manage disease agents in human and veterinary medicine, or agriculture, must be reviewed by RAC and approved by the NIH Director. In the current *NIH Guidelines*, if the microorganism is "known to acquire the trait naturally", then transfer of the drug resistance does not need to be reviewed. The proposal would delete this language because a microorganism's ability to acquire the trait naturally may not be determinative of the safety and public health implications of the research. In addition, some in the research community have erroneously interpreted this section as applying only when the drug resistant trait is against first-line treatments. Therefore, the revised section clarifies that even if an alternative drug or drugs exist for the control or management of disease, it is important to consider how use of the drug resistance marker might affect the ability to control infection by resistant microorganisms in certain groups or subgroups for which alternative treatments may not be readily available. Affected groups or subgroups may include, but are not limited to: children, pregnant women, and people who are either allergic to effective alternative treatments, immunocompromised or living in countries where the alternative effective treatment is not readily available.

Discussion Questions

- 1) Should the lack of documented resistance to the drug in the community be the primary criterion for determining what needs to be reviewed?
 - a) What if there are only 1 or 2 antibiotics available and there is only low level of resistance?
 - b) What if the drug resistance marker is for a drug used primarily to treat children?
- 2) At what point does one consider that an organism "can acquire the trait naturally?" Is a single case report sufficient? What if there is no documented resistance in the United States?

- 3) Are there other objective criteria that could be used to better capture those experiments that have potentially significant public health implications?
- 4) How do we address special populations, e.g., pregnant women, children, and health care systems with more limited resources?

Panel Discussion

Institutional Biosafety Committee

- Andrew B. Onderdonk, Ph.D.
Harvard Medical School, Boston, MA

Principal Investigators

- Ron Atlas, Ph.D.
University of Louisville, Louisville, KY
- William R. Bishai, M.D., Ph.D.
Johns Hopkins School of Medicine, Baltimore, MD
- Stanley Maloy, Ph.D.
San Diego State University, San Diego, CA
- Louis Rice, M.D.
Case Western Reserve University, Cleveland, OH
- Alfredo Torres, Ph.D.
University of Texas Medical Branch, Galveston TX

Public Comment

4:00 pm **BREAK**

4:10 pm [Panel IV – Revisions to Section III-E-1. Experiments Involving the Formation of Recombinant DNA Molecules Containing No More than One-Half of the Genome of any Eukaryotic Virus](#)

Co-moderators:

- Stephen Dewhurst, Ph.D.
University of Rochester School of Medicine and Dentistry
- Bernard Roizman, Sc.D.
The University of Chicago, Chicago, IL

Background

Section III-E-1

Under the current *NIH Guidelines*, tissue culture experiments with viruses (with all viruses from a single family being considered identical) containing < 2/3 of the genome can be initiated at Biosafety level (BL) 1 upon registration with the Institutional Biosafety Committee. Concerns were raised that this level of oversight may not be adequate for research with potential synthetic biology agents derived from multiple segments of nucleic acid from a family of viruses. In addition, some wild type viruses may be functional with less than 2/3 of the genome present. Therefore, a recommendation was made to propose changing the 2/3 of the genome criteria to 1/2 of the genome to reflect the current understanding of the biology of certain viruses. Since under Appendix C-1, recombinant molecules from Risk Group 1 and 2 viruses that contain less than 1/2 of any eukaryotic genome are exempt from the *NIH Guidelines*, the revised section would only apply to tissue culture work with Risk Group 3 and 4 viruses containing less than 1/2 of the genome. In addition, the new section requires that the principal investigator must provide evidence that the preparation(s) are free of replication competent virus.

Discussion Questions

1. Is the reduction from 2/3 to 1/2 the genome more appropriate from a biosafety perspective or are there many experiments involving Risk Group 3 or 4 viruses which contain more than 1/2 of the genome but less than 2/3 that can safely be done at BL1?

2. As this section allows initiation of the experiment simultaneously with IBC registration, is the requirement to obtain evidence that the resulting nucleic acid in these cells are not replication competent nucleic acids sufficiently clear that it could be implemented by Principal Investigators in a consistent manner?

5:10pm **Wrap-up and Concluding Remarks**

- Howard Federoff, M.D., Ph.D.

5:30 pm **ADJOURNMENT**